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The Hong Kong Polytechnic University

Department of Health Technology and Informatics

Epidemiology and Molecular Characteristics of *Staphylococcus aureus* and Coagulase-negative Staphylococci with Reduced Antiseptic Susceptibility in Hong Kong

Zhang Ming

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

May 2013

DECLARATION

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ABSTRACT

Over the past decade, there has been increasing efforts aimed at the prevention of hospital infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). One of the main preventive measures has been the use of antiseptic agents for decontamination of surfaces and hand hygiene. Guidelines recommend using antiseptics for decolonization of high risk groups and routine cleansing of all patients when basic interventions have failed to reduce rates of MRSA infection to acceptable levels. Frequently touched surfaces should also be decontaminated with disinfectant.

However, recently there has been increasing concern that increased use of biocides may contribute to the emergence and/or selection of resistant pathogens. Staphylococcal strains harboring genes coding for increased resistance to quaternary ammonium compounds (QACs) and other disinfectants (*qac* genes) as demonstrated by increased minimum bactericidal concentrations (MBCs) to antiseptics as described in several studies and there is evidence that carriage of these genes may be increasing. Widespread use of biocides may impose selective pressure and contribute to the emergence of bacteria with decreased antiseptic susceptibility. In addition, there is evidence that use of biocides may contribute to the emergence of cross-resistance and co-resistance between widely used biocides

and antibiotics. However, the distribution of *qac* genes in *S. aureus* and CNS has only been investigated in clinical isolates, with most focus on MRSA, and little is known about the frequency of *qac* genes in colonizing strains in the population and the environment. There is a need for more studies to determine colonization and contamination rates with strains harbouring *qac* genes in various populations and locations as well as for evidence for the development of cross-resistance to methicillin and non-beta-lactam antibiotics. Residues of biocides increase bacterial exposure, which may lead to selection of mutant strains possessing broad spectrum efflux pumps displaying reduced susceptibility to both biocides and antibiotics.

Determination of reduced susceptibility to biocides is somewhat difficult as a convenient gradient method for MIC determination had not been developed for antiseptics. Spiral gradient endpoint (SGE) technique, is a gradient method for MIC determination which is simple, rapid and cost-effective and has been shown to be suitable to determine antibiotic MICs for fastidious organisms and for determination of vancomycin resistance. SGE has the advantages of employing a concentration gradient and is suitable for a wide range of substances and overcomes the problem of large increments experienced in conventional dilution methods.

The purpose of this research was to provide significant and original contributions to the knowledge base of antiseptic resistance genotypes in *S. aureus* and coagulase-negative staphylococci in selected populations and environments in Hong

Kong. The need for an accurate and simple method for determination of antiseptic MICs was addressed by development and evaluation of SGE for these agents. Finally, it aimed to determine the effects of increased and prolonged exposure to disinfectants on levels of antiseptic resistance. Overall, this provided a framework for effective detection of reduced antiseptic susceptibility, guidance for antimicrobial selection in treatment of *S. aureus* and MRSA carrying antiseptic resistant genes, improving infection control, and contributing to better clinical outcomes.

This is the first study of *qac* gene prevalence in staphylococci in Hong Kong. It examined a wide variety isolates including colonizing strains from nurses, the general population, and the elderly residents in both nursing homes and the community as well as clinical isolates and those contaminating the public environment and hospital surfaces. The study aimed to determine if the health care environment increased risk of strains carrying antiseptic resistance genes. The increased proportion of antiseptic resistance gene positivity in *mec*A positive isolates suggests co-selection of these genes, contributing to survival of MRSA in community and health care facilities including hospital and nursing homes. Use of antiseptics may be selecting for antibiotic-resistant strains and assisting their survival in the environment. In order to carry out further studies on increasing biocide resistance, a simple, flexible test for determining the MIC of a biocide or biocide mix is needed. This study evaluated SGE for determination of MICs of antiseptics and showed that SGE offers a precise and accurate method for biocide

MIC measurement. The induction study demonstrated that biocide exposure could indeed select for strains with increased tolerance at sub-MIC concentrations, being disinfectant reduced susceptibility isolates.

In conclusion, this study has demonstrated the wide dissemination of strains carrying antiseptic resistance genes in both the community and healthcare facilities in Hong Kong. Rates of carriage of these genes were higher in healthcare settings possibly reflecting increased disinfectant exposure. There is a need for further investigation of the importance of reduced antiseptic susceptibility and its relevance in infection control.

PUBLICATIONS ARISING FROM THIS THESIS

Journal Articles

1. M Zhang, M O'Donoghue, T Ito, K Hiramatsu, MV Boost. Prevalence of antiseptic resistance genes in *Staphylococcus aureus* and coagulase-negative staphylococci colonizing nurses and the general population in Hong Kong. *Journal of Hospital Infection*, 78(2):113-7, 2011.

2. M Zhang, M. O'Donoghue and MV Boost. Characterization of Staphylococci contaminating automated teller machines in Hong Kong. *Epidemiology and Infection*, 19:1-6, 2011.

3. Ye HF, Zhang M, O'Donoghue MV Boost. Are *qac*G, *qac*H and *qac*J genes transferring from food isolates to carriage isolates of staphylococci? *Journal of Hospital infection,* 78(2):113-7, 2011.

Conference Poster Presentations

1. M. Zhang, M. Boost, M. O'Donoghue. Prevalence of antiseptic resistance genes in staphylococcus and coagulase-negative staphylococci from ATM machine in Hong Kong. International Fourth Ditan International Conference on Infectious Diseases. Beijing. International Journal of Infectious Diseases, Volume 14, supplement 2, July 2010, S28.

2. M. Zhang, M. Boost, M. O'Donoghue, E. Tong, K. Hiramatsu. Comparison of prevalence of antiseptic resistance genes in *Staphylococcus aureus* and

coagulase-negative staphylococci from nurses and the general population in Hong Kong. 20th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 10 - 13 April 2010, Vienna, Austria.

3. M. Zhang, M. Boost, M. O'Donoghue Coagulase-Negative Staphylococci colonizing ATM machines in Hong Kong. The 4th International Congress of the Asia Pacific Society of Infection Control (APSIC09), 5-9 July, 2009 Macau SAR, China.

4. M. Zhang, M. Boost, M. O'Donoghue *qac*A and *smr* genes in *Staphylococcus aureus* and coagulase-negative staphylococci colonizing nurses in Hong Kong. The 7th International Symposium on Antimicrobial Agents and Resistance. 18-20 March, 2009, Bangkok, Thailand.

5. O'Donoghue M, Zhang M, Ho J, Boost M. (2012) Do carriage rates of *Staphylococcus aureus* and MRSA differ between elderly homes resident and elderly attendees of day care centers? 15th International Congress on Infectious Diseases, June 13-16, 2012, Bangkok, Thailand.

6. Ho J, O'Donoghue M, Zhang M, Boost M. (2012) Antiseptic resistance genes in *Staphylococcus aureus* from the elderly. 15th International Congress on Infectious Diseases, June 13-16, 2012. Bangkok, Thailand.

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ABBREVIATIONS

AAP: Accumulation-Associated Protein

ABC: ATP-Binding Cassette Family

AD: Agar Dilution

Agr: Accessory Gene Regulator

ALCE: ALC Elderly Home

AST: Antimicrobial Susceptibility Test

ATMs: Automated Teller Machines

Bap: Biofilm-Associated Protein

BHA: Brain Heart Agar

BHI broth: Brain Heart Infusion Broth

BlaZ: β-lactamase Structural Gene

BC: Benzalkonium Chloride

BSI: Blood Stream Isolates

CA-MRSA: Community-Associated Methicillin Resistant *Staphylococcus aureus*

CAT: Chloramphenicol Acetyltransferase

ClfA/B: Clumping Factor A and B

CLSI: Clinical Laboratory and Standards Institute

CIs: Confidence Intervals

CNS: Coagulase-Negative Staphylococci

CV: Coefficient of Variation

CTPC: Cetylpyridinium Chloride

CTAB: Hexadecyltrimethylammonium Bromide

CHG: Chlorhexidine Gluconate

DA : Clindamycin

DC: Daycare Center

DHFR: Dihydrofolate Reductase

E : Erythromycin

Eb: Ethidum bromide

ECCMID: European Congress of Clinical Microbiology and Infectious Diseases

EC: End Point Concentration

EGFR: Epidermal Growth Factor Receptor

EN: Experienced Nurse

EUCAST: European Committee on Antimicrobial Susceptibility Testing

Ets : Exfoliative Toxins

EtOH : Ethyl Alcohol

FbpA : Fibrinogen Binding Protein

FnbA/B : Fibronectin-Binding Protein A and B

FD: Fusidic Acid

FN: Fresh Nurse

GEN: Gentamicin

GlcNAc: N-Acetylglucosamine

HA-MRSA: Methicillin-Resistant *Staphylococcus aureus*

HAIs: Hospital-Acquired Infections

hVisa: Heteroresistant VISA

HSQE: HSQ Elderly Home

IR: Inverted Repeat

MATE: Multidrug Toxic Compound Extrusion Family

MBC: Minimal Bactericidal Concentration

MFS: Major Facilitator Super Family

MH: Mueller-Hinton Broth

MIC: Minimal Inhibitory Concentration

MLST: Multi Locus Sequence Typing

MSCRAMM: Microbial Surface Components Recognizing Adhesive Matrix Molecules

MSSA: Methicillin Susceptible *Staphylococcus aureus*

MRSA: Methicillin/Multi- Resistant *Staphylococcus aureus*

MRSE: Methicillin resistant *S. epidermidis*

ORFs: Open Reading Frames

P: Penicillin

PBP: Penicillin-Binding Protein

PCR: Polymerase Chain Reaction

PTSag: Pyrogenic Toxin Superantigens

PVL: Panton-Valentine leucocidin

PVP: Povidone

PSM: Phenol-Soluble Modulin

PHMBs : Polyhexamethylbiguanides

PMF : Proton Motive Force

PSMR: Paired Small Multidrug Resistance Proteins

qac gene: Quaternary Ammonium Resistance Gene

QAC: antiseptic resistant

*QAC*s: Quaternary Ammonium Compounds

RCHE: Residential Care Homes for the Elderly

RND: Resistance Nodulation Division Family

S. aureus : Staphylococcus aureus

SA : *S. aureus*

SAD : Serial Agar Dilution

sarA : Staphylococcal Accessory Regulator

SasG : Surface protein G

SCC*mec* : Staphylococcal Cassette Chromosome *mec*

SE : Staphylococcal Enterotoxins

SE-like toxins *:* SEI toxins

S. epidermidis : *Staphylococcus epidermidis*

SGE : Spiral Gradient Endpoint

SMR: Small Multidrug Resistance Family

SMP: Small Multidrug Pumps

Spa: Staphylococcal Protein A

SSIs: Skin and Soft Tissue Infections

SSSS: Staphylococcal Scalded Skin Syndrome

SSP: Staphylococcal Surface Proteins

SUG: GroEL Mutation Protein

Staphylococcus species: *Staphylococcus* spp.

SXT: Co-Trimoxazole

TE: Tetracycline

TEC: Trailing End Point Concentrations

TMS: 14-transmembrane-segment

TNFR-1: Tumour Necrosis Factor Receptor-1

TP: Trailing End Point

TRIS: Tris (hydroxymethyl) aminomethane

TSS: Toxic Shock Syndrome

TSST-1: Toxic Shock Syndrome Toxin 1

UTI: Urinary Tract Infection

VISA: Vancomycin-Intermediate *S. aureus*

WTA: Wall Teichoic Acid

YLE: YL Elderly Home

PREAMBLE

The research described in this thesis involves the health care and infection control aspects of antiseptic resistance of the common bacteria *Staphylococcus aureus.* Worldwide emergence of antimicrobial resistance in Staphylococci is a serious and increasing problem, especially in the hospital. In order to prevent the spread and growth of harmful pathogens in both the hospital and the community, as well as to improve practices of infection control, biocides are widely used in disinfectant preparations in healthcare settings to decontaminate surfaces, disinfect the hands of hospital personnel, and treat patients colonized by *S. aureus*. However, increasing evidence implies that widespread use of the biocides may impose selective pressure and contribute to the emergence of bacteria with decreased antiseptic susceptibility. Concern has also been raised that the use of biocides may contribute to development of antibiotic resistance among microbes, and the potential emergence of cross-resistance and co-resistance between widely used biocides and antibiotics.

The major aim of this study was to investigate the epidemiology of antiseptic resitance. This has been done by means of four investigations involving comparisons of rates of resistance between healthcare and community settings and at risk and standard populations. In Chapters 3, 4, 5 and 6 chapters, the epidemiology of antiseptic resistance genes carriage isolates of *S. aureus* and coagulase-negative staphylococci in Hong Kong were investigated by determine the prevalence of

isolated carrying the QAC genes from selected populations and environments. In the remaining chapters, the accuracy of a rapid screening tool to determine MICs of antiseptics to *S. aureus* using the spiral gradient endpoint (SGE) technique was evaluated and the effects of increased and prolonged exposure to disinfectants on levels of antiseptic resistance were determined by means of in vitro testing of strains with antiseptic resistance genes using both the gradient technique developed.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Staphylococcus aureus has long been recognized as a major human pathogen. Today, methicillin-resistant *S. aureus* (MRSA) is a common nosocomial pathogen in hospitals worldwide. There has been increasing evidence that owing to dramatically increasing prevalence of multiple-drug-resistant strains, coagulase-negative staphylococci (CNS), acting as opportunistic pathogens, can also be frequent and important causes of disease (Carbon, 2000).

Considerable efforts have been made in recent years to prevent the spread and growth of harmful pathogens in both the hospital and the community settings to improve practices of infection control. One of the most important practices is increased emphasis on use of disinfectants. These include quaternary ammonium compounds (QACs), e.g., benzalkonium chloride (BC), cationic biocides, e.g chlorhexidine (CHG), and biocides containing bisphenol ether, e.g. triclosan, are widely used in disinfectant preparations in healthcare settings to decontaminate surfaces, disinfect the hands of hospital personnel, and treat patients colonized by *S. aureus* (McDonnell & Russell, 1999). Even when infection control practices were rigidly implemented, current measures have failed to control the spread of MRSA.

MRSA is widely dispersed in the hospital environment and has been isolated from numerous locations including catheters and disinfectant soap dispensers (Brooks et al., 2002; Aarestrup & Hasman, 2004).

There is increasing evidence that widespread use of the biocides may impose selective pressure and contribute to the emergence of staphylococci with decreased antiseptic susceptibility. Such organisms have been isolated from clinical samples and settings (Alam et al., 2003a, 2003b). In addition, the large quantities of chemotherapeutic agents employed in clinical practice have resulted in the development and spread of antibiotic resistance determinants among bacterial populations. Concern has been raised that the use of biocides may contribute to development of antibiotic resistance among microbes, and to the potential emergence of cross-resistance and co-resistance between widely used biocides and antibiotics (Koljalg et al., 2002; Walsh et al., 2003).

1.2 Classification of *Staphylococcus S*pecies

Staphylococcus spp. is catalase-positive, gram-positive cocci. Under the microscope, they appear round and arranged in grape-like clusters. Most species are harmless and reside normally on the skin and mucous membranes of humans and other organisms. The genus *Staphylococcus* includes at least forty species, of which nine have two subspecies and one has three subspecies (Harris, 2002). Staphylococci isolated from humans and other primates include *S. aureus*, *S. epidermidis*, *S. capitis*, *S. caprae*, *S. saccharolyticus*, *S. warneri*, *S. pasteuri*, *S. haemolyticus*, *S. hominis*, *S.*

lugdunenis, S. auricularis, S. saprophyticus, S. cohnii, S. xylosus, and *S. simulans*. To classify *Staphylococcus* species, one of the main criteria is the ability to produce coagulase, which is an enzyme causing clotting of plasma. The first group, known as the coagulase positive staphylococci includes several species, of which the most important in human disease is *S. aureus*; the second group, known as coagulase negative staphylococci (CNS), comprises of many diverse species which colonize both humans and animals.

1.3 Colonization and Infections with *S. aureus*

Although staphylococci are widespread in nature, they are mainly isolated from the skin, skin glands, and mucous membranes of mammals and birds. They may also be isolated from the mouth, blood, mammary glands, intestinal, urinary, and upper respiratory tracts of these hosts, as well as from environmental sites and objects contaminated by the hosts (Murray, 2003).

S. aureus colonizes the skin and nasal mucosae of human beings. Extra-nasal sites including the hands, skin, perineum, and pharynx also frequently harbour *S. aureus*, whilst the gastrointestinal tract, vagina, and axillae are less frequently colonized (Armstrong-Esther and Smith, 1976; Wertheim et al., 2005b,). *S. aureus* nasal carriage and hand carriage are strongly correlated (CO., 1965). Hands are the main vector for transmitting *S. aureus* from surfaces to the nasal niche, for instance, by nose picking (Wertheim et al., 2005a). Although multiple body sites can be colonized in human beings, the anterior nares of the nose are the main ecological

niche in humans for *S. aureus* (Wertheim et al., 2005a).

Carriage of *S. aureus* in the nose plays an important role in both epidemiology and pathogenesis. The prevalence of *S. aureus* nasal carriage varies according to the population studied. In a review by Kluytmans et al., (1997) the mean nasal carriage rate of *S. aureus* was 37.2% although the range of rates was wide. However, in a later report, Kluytmans and Wertheim (2005a) observed that approximately 25% of humans carried *S. aureus* in their nasal cavities.

Longitudinal studies have identified at least three *S. aureus* nasal carriage patterns in healthy individuals including persistent carriage, intermittent carriage, and non-carriage. The staphylococcal colonization rate in adults is approximately 40% at any given time. About 20% (range 12–30%) of individuals are considered to be persistent *S. aureus* nasal carriers, approximately 30% are intermittent carriers (range 16–70%), and about 50% (range 16–69%) non carriers (Kluytmans et al., 1997; Nouwen et al., 2004). In comparison to adults, children are more prone to be persistent carriers and many people change their carriage pattern between the ages of 10 and 20 years (Kluytmans et al., 1997; Wertheim et al., 2005a).

Persistent carriers have higher *S. aureus* loads, with a higher risk of developing a *S. aureus* infection, and are often colonized by the same strain over long time periods. However, intermittent carriers may carry different strains over time (Wertheim et al., 2005a). As variation of their colonizing strains is higher in intermittent carriers than

in persistent carriers, this implies that the basic determinants of persistent and intermittent carriage in humans are different (Nouwen et al., 2004). The biology of *S. aureus* nasal carriage remains unclear. It has been reported that host genetic and environmental factors, including cell-wall lipotechoic acid, hormonal status, and antimicrobial activity of nasal secretions could influence the carriage of *S. aureus* (Peacock et al., 2001; Weidenmaier et al., 2004,). It has also been reported that there appear to be differences in adherence between *S. aureus* strains and nasal epithelial cells from different individuals. *S. aureus* from carriers has a greater affinity for nasal epithelial cells than those from non-carriers. Additionally, *S. aureus* is more prone to adhere to nasal epithelial cells from patients with eczema than to cells from patients without eczema (Kluytmans et al., 1997). Nouwen et al. (2004) followed persistent nasal carriers and non-carriers inoculated with a mixture of different *S. aureus* strains, and found the majority of non-carriers and nearly all persistent carriers returned to their original carrier state, and demonstrated that the human factor is an important determinant of nasal carriage. The vaginal carriage rate of staphylococcal species is approximately 10% in premenopausal women with an even higher rate during menses. Staphylococcal species commonly colonize neonates on the skin, perineum, umbilical stump, and gastro-intestinal tract (Wertheim et al, 2005a).

Nasal carriage is major risk factor for *S. aureus* infection, especially for MRSA. Although commensal carriage of MRSA remains low in healthy individuals (from 0.2% to 2.8%) in most countries, it brings about a greater risk for subsequent infection than methicillin-susceptible *S. aureus* carriage (Kluytmans & Wertheim, 2005). It has

been demonstrated that nasal colonization or carriage with *S. aureus* frequently precedes *S. aureus* infection and rates of infection are three-fold higher in nasal carriers. Despite this, it does not mean that all carriers will eventually be infected by *S. aureus*. The risk of carriers acquiring infection may be associated with the antibiotic resistance pattern and virulence factors of their *S. aureus* strains as well as their health status (Kluytmans & Wertheim, 2005).

S. aureus is one of the five most common causes of nosocomial infections, and a frequent cause of post surgical wound infections. It can cause a range of illnesses from minor skin infections, such as pimples and impetigo, to serious diseases such as pneumonia, meningitis, acute endocarditis, and scalded skin syndrome (SSSS). It is reported that untreated *S aureus* bacteraemia carries a mortality rate that exceeds 80%. It has been suggested that the increased mortality among patients with *S aureus* bacteraemia is associated with thrombocytopenia (McClelland et al., 1999; Gafter-Gvili et al., 2011). It has been observed that the mortality rate of *S. aureus* bacteraemia in elderly persons is markedly higher than that of younger patients (Reunes et al., 2011).

1.3.1 Colonization and Infection with MRSA

MRSA are strains of *S. aureus* that are resistant to beta-lactam antibiotics, which include the penicillins (penicillin, methicillin, oxacillin) and the cephalosporins. Institutions, such as hospitals and nursing homes, remain the most common sources of MRSA strains, but recently colonization has become increasingly prevalent in the

community leading to community-acquired infections (Cimolai, 2006).

The first MRSA harbouring a staphylococcal cassette chromosome *mec* I (SCC*mec* type I), which results in resistance to methicillin, was isolated in the UK in 1961, soon after the introduction of methicillin into clinical practice (Kayser, 2009). From the 1960s to the early 1970s, MRSA spread to other European countries, and then became pandemic with isolations reported as widespread as Australia, Japan, and the USA. In 1982, an MRSA strain harbouring a new mec type, SCC*mec* type II, was isolated in Japan and emergence of type III occurred in the late 1980s. According to the SENTRY Antimicrobial Surveillance Program investigation (1997 to 1999), the MRSA prevalence in clinical isolates from hospitalized patients varied widely: 23% in Australia, 26% in Europe, 32% in the USA, 35% in Latin America, 40% in South America, and 67% in Japan (Deurenberg & Stobberingh, 2008). MRSA isolates, initially reported in Japanese teaching hospitals, spread into community hospitals in the 1990s. The SENTRY study showed that even in Europe the MRSA prevalence in clinical isolates between countries varied considerably, from about 1% in Scandinavia to 45% in Southern Europe. MRSA infections still remain rare even in the health care setting in Denmark, Sweden, the Netherlands, Norway, and Finland, which has been attributed to strict control and widespread surveillance programs (David & Daum, 2010).

Since the mid-1990s, there has been an increasing incidence of MRSA infections in populations without exposure to the health care system. This led to the recognition

of new MRSA strains, named as community-associated MRSA (CA-MRSA). The first report of CA-MRSA isolation from healthy individuals was in 1993 from Western Australia (Udo et al., 1993). CA-MRSA prevalence remained low worldwide in the 1990s. However, in the past decade, these CA-MRSA strains appear to have disseminated rapidly among the general population and CA-MRSA has emerged not only in the community, but also in healthcare facilities (Chambers et al., 2001; Etienne, 2005; Gould et al., 2009a).

Molecular typing techniques have been used to distinguish CA-MRSA strains from health care-associated MRSA (HA-MRSA). HA-MRSA strains carry one of the relatively larger SCC*mec* types *(*types I, II, or III) and seldom carry the genes for the Panton-Valentine leukocidin (PVL). In contrast, CA-MRSA isolates carry smaller SCC*mec* elements, such as SCC*mec* type IV, V or VII and are characterized by the presence of the PVL toxin. They are resistant to fewer non-beta-lactam classes of antimicrobials in comparison to HA-MRSA (Deurenberg & Stobberingh, 2008).

More recently, the distinction between HA-MRSA and CA-MRSA has become blurred, as CA-MRSA has started to replace HA-MRSA in healthcare facilities in some countries (Klevens et al., 2006). In a study carried out in a medical center in Houston, USA, It was reported that 60% of nosocomial MRSA isolates were CA-MRSA (Gonzalez et al., 2006). The majority of UK isolates harbour SCC*mec* type IV, but most of them are considered to be hospital associated (Cooke & Brown, 2010). Gould et al., (2009a) reported a hospital neonatal unit CA-MRSA outbreak in Scotland and eight cases of

skin and soft tissue infections were due to ST5 CA-MRSA SCC*mec* IV. In addition, a subset of CA-MRSA clones has been found in some countries including Denmark, Norway and The Netherlands, where there is usually a low incidence of HA-MRSA (Bartels et al., 2007; Stam-Bolink et al., 2007, Feng et al., 2008, Larsen et al., 2009). These have now been characterized as live-stock associated MRSA, and have been shown to cause infections in persons occupationally-exposed to pigs and veal calves (Wulf & Voss, 2008; Van Cleef et al., 2010).

It was reported that the mean rate of MRSA in clinical isolates of *S. aureus* in China was over 50%, and that over 80% of hospital isolates in Shanghai in 2005 were MRSA (Wang et al, 2008c). Another study based on a nationwide antimicrobial resistant surveillance network covering 17 hospitals in 15 cities throughout China revealed that 62.9% of clinical isolates of *S. aureus* were methicillin resistant (Yu et al., 2008). Recently, Li et al. (2013) reported 68.1% clinical isolates were MRSA. It is therefore urgent to implement strategies to control transmission of MRSA.

In Hong Kong, the first documented CA-MRSA infection occurred in May 2004 when a 50-year-old man with no identifiable risk factors presented to a local casualty department with a carbuncle on his back (Ho et al., 2004). Since January 2007, all practicing doctors in Hong Kong were required to report both confirmed and suspected cases of CA-MRSA infection to the Department of Health. Reported cases of CA-MRSA in Hong Kong increased in the two years since its addition to the list of notifiable diseases (Ho et al., 2008a). Continued monitoring of the situation is

obviously required.

Although many studies have investigated rates of MRSA colonization, the majority have focused on hospital patients at admission (Verrall et al., 2013; Torres et al., 2013), elderly subjects in residential facilities (Eveillard et al., 2008; Mody et al, 2008; Pfingsten-Würzburg et al., 2011), or special at-risk groups such as HIV positive subjects (Peters et al., 2013) or dialysis patients (Schmid et al., 2013). Outbreaks in hospitals may sometimes be attributed to MRSA acquired in nursing homes (Bradley, 1997; Kerttula et al., 2005).

A report of a four year study of colonization of residents of UK care homes showed 9-22% carried MRSA (Horner et al., 2013). Verrall et al. (2013） reported 41% MRSA colonization in hospital patients at admission from nursing homes compared with only 6.0% for non-nursing home residents. These studies provide strong evidence for nursing homes acting as reservoirs of MRSA (Ludden et al., 2013). Two studies conducted on elderly home residents in Hong Kong have shown colonization rates of 2.8% and 5.1% (Ho et al, 2007b; 2008b). The difference is prevalence may reflect different conditions at the homes sampled or the more extensive sampling method employed in the second study.

Other groups such as prisoners (Baillargeon, 2004; Aiello et al., 2006) and homeless persons and illicit drug users (Gilbert et al., 2006) may also have an elevated risk of MRSA colonization. In Texas jails and prisons, the proportion of MRSA among prison

patients with *S .aureus* infections increased from 25% (864/3520) in 1998 to 66% (5684/8633) in 2002 (Baillargeon et al., 2004)

Sportsmen and military personnel may have increased risks of colonization and infection with MRSA due to sharing of personal items and equipment. Outbreaks of MRSA in footballer players have been investigated (Begier et al., 2004; Kazakova et al., 2005).

The rates of MRSA colonization in healthy subjects are usually lower but may be elevated in healthcare workers such as nurses (Elie-Turenne et al., 2010; Jannati et al., 2013) or emergency staff such as ambulance drivers and firemen (Amiry et al., 2013) in whom rates of 4.6% and 6.4% have been recently reported. Albrich et al. (2008) reported the average MRSA prevalence in health care workers was 4.6%. A similar result was reported by Wang et al. (2004) who found an 8.5% rate of carriage of MRSA 8.5% in health care workers in Taiwan. A colonization rate of 3.0% was observed in medical students in China (Du et al., 2011). Asymptomatic nasal carriage of MRSA in healthcare workers could increase the risk of outbreaks of MRSA in the hospital, by serving as a reservoir for MRSA cross-transmission (Albrich & Harbarth, 2008).

However, rates in less exposed subjects are generally lower. Only 1.6% of new medical students (Bettin et al., 2012) and 2% of military recruits in the US (Whitman et al., 2012) were colonized with MRSA. The overall MRSA colonization rate in the

US has been estimated to be less than 2% (Nair et al., 2011). A recent study of community carriage in Italy reported only 1% of subjects carried MRSA (Monaco et al., 2013). A study of medical students before exposure to patients in China over a three year period revealed MRSA colonization rates from 0.95 – 1.7% (Du et al., 2011). In Hong Kong, the colonization rate in healthy subjects remains low (approximately 1%) (O'Donoghue & Boost, 2004; Boost et al., 2008a)

1.4 Colonization and Infection with Coagulase Negative Staphylococci

CNS forms an important part of the normal flora of the skin and gastro-intestinal system mucosal membranes. Certain CNS types are associated with specific body sites such as *S. capitis* on the head (Nizet & Klein, 2011).

CNS rarely causes disease and clinicians frequently regard isolates as contaminants of microbiological cultures. They are most frequently associated with infectious processes in immuno-compromised patients or patients using intra-venous catheters. There is an increasing trend of CNS being associated with significant infections. Thirty percent of all nosocomial blood stream infections are attributed to CNS, with *S. epidermidis* being the most commonly isolated CNS in nosocomial infections. *S. epidermidis, S. haemolyticus, S. lugdunensis, and S. saprophyticus* are the most common species associated with human infections. Between 55-75% of nosocomial isolates of CNS are resistant to methicillin (McCann et al., 2008; Frank et al., 2008). In a study conducted in Naples between January 1996 and December 1998, overall 30.4% neonatal infections were attributed to *S. epidermidis.* It was the primary

causative pathogen in bloodstream infections (39.8%), skin infections (29.8%), and meningitis (58.3%) (Villari et al., 2000). Similarly, *S. epidermidis* was responsible for 33.5% of nosocomial blood stream infections in Ethiopia (Rasheed & Awole, 2007), and nosocomial bacteraemia due to *S. epidermidis* appears to be a rapidly increasingly problem worldwide (Rogers et al., 2009a). *S. epidermidis* is the most prevalent pathogen in clinical infections and comprises 65-90% of all CNS isolated from human sources (Vuong & Otto, 2002; Van Mellaert et al., 2012).

Amongst other CNS, the pathogenicity and virulence of *S. lugdunensis* is more similar to *S. aureus* than to *S. epidermidis*. *S. lugdunensis* not only cause skin and soft tissue infections, but is also involved in severe infections with high mortality, such as endocarditis. *S. lugdunensis* is part of the normal skin flora and colonizes the lower abdomen and extremities, occupying different sites from those of *S. aureus* (Frank et al., 2008; Rogers et al., 2009a).

S. saprophyticus colonizes the inguinal and perineal areas, and is an important opportunistic pathogen in human urinary tract infections, especially in young, sexually active women (Otto, 2004). It forms part of the normal rectal flora of 10% of females, and is second only to *Escherichia coli* as a cause of acute urinary tract infection in female outpatients (Raz et al., 2005). It has also been proposed as an agent of nongonococcal urethritis in males or a cause of other sexually transmitted diseases, prostatitis, wound infections, and septicaemia (Otto, 2004; Rogers et al., 2009a).

S. haemolyticus is the second most frequently reported CNS species in human infections, and is involved in native-valve endocarditis, septicaemia, peritonitis, urinary tract infections, as well as wound, bone and joint infections (Huebner & Goldmann, 1999; Rogers et al., 2009b).

1.5 Pathogenesis and Virulence factors of *S. aureus*

As stated above, *S. aureus* is the major pathogen among the staphylococcal species, because, unlike most CNS, it possesses a range of virulence factors which increase its pathogenicity. The pathogenic factors of *S. aureus* are grouped into three areas: the surface-associated virulence factors, the extracellular virulence factors and the regulation of virulence factors.

1.5.1 Surface-Associated Virulence Factors

Surface-associated virulence factors are of vital importance in helping *S. aureus* avoid host defence and promote colonization. *S. aureus* produces wall teichoic acid (WTA), a surface-exposed staphylococcal polymer, which is a major constituent of the cell envelope. WTA is composed of 40 ribitol phosphate repeating units modified with N-acetylglucosamine (GlcNAc) and D-alanine (Endl et al., 1983, Weidenmaier et al., 2008). WTA can provide a binding site for cationic antimicrobial peptides and proteins (Weidenmaier et al., 2005; Koprivnjak et al., 2008). A study using a WTA-deficient *S. aureus* mutant has shown that WTA confers resistance to antimicrobial fatty acids, produced by human sebaceous glands, by preventing fatty acid binding to the bacterial cell. WTA deficient mutants are impaired in their ability

to adhere to nasal cells, and completely unable to colonize cotton-rat nares. This suggests that WTA is essential for nasal colonization and mediates interaction with human nasal epithelial cells (Weidenmaier et al., 2004).

Staphylococcal Protein A (Spa) is a 40-60 kDa ‘microbial surface component recognize adhesive matrix molecules’ (MSCRAMM) is another surface protein which was originally isolated from the cell wall of *S. aureus*. Spa surface-associated virulence factor is encoded by *spa* and its regulation is controlled by DNA topology, cellular osmolarity, and a two-component system called ArlS-ArlR. Spa appears to play an important role in the success of *S. aureus* as a human pathogen. It can bind to a variety of ligands, including the Fc region of IgG, von Willebrand factor, tumour necrosis factor receptor-1 (TNFR-1), the Fab-heavy chains of the VH3 subclass of IgG and the epidermal growth factor receptor (EGFR) (Loughman et al., 2005; O'Seaghdha et al., 2006).

By binding to the Fc portion of immunoglobulin, Spa can assist *S. aureus* in the evasion of phagocytosis by neutrophils (Foster, 2005). Moreover, Spa binds to von Willebrand factor, which is involved in supporting platelet adhesion in the early stages of thrombosis (O'Seaghdha et al., 2006). Spa also binds to the EGFR to regulate TNFR-1 availability (Gómez et al., 2007). It has been shown that Spa is crucial in the pathogenesis of both septic arthritis and pneumonia in a murine model. Palmqvist et al. (2002) reported Spa could bind directly to TNFR-1 in lung epithelial cells and cause pro-inflammatory signaling in the pathogenesis of staphylococcal

pneumonia. In a recent study, it was shown that Spa could bind directly to osteoblasts, and this interaction prevents proliferation, induces apoptosis, and inhibits mineralization of cultured osteoblasts (Claro et al., 2011).

Spa can be used for reliable and accurate typing of MRSA. The *spa* typing technique uses the sequence of a polymorphic 24bp variable-number tandem repeat within the 3' coding region of *S. aureus*-specific *spa.* Each new base composition of the polymorphic repeat found in a strain is assigned a unique repeat code. The repeat succession for a given strain determines its *spa* type. *Spa* typing is a single-locus typing technique and offers a sub typing resolution comparable to more expensive laborious techniques such as multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). The technique is now widely used for sub typing of *S. aureus* in hospital and outbreak settings (Koreen et al., 2004).

1.5.2 Extracellular Virulence Factors

A wide range of extracellular virulence factors are produced by *S. aureus* to help it to colonize and cause disease.

1.5.2.1 Pyrogenic Toxin Superantigen (PTSag) Family

The PTSag family is a group of exotoxins secreted by *S. aureus* and *Streptococcus pyogenes*. PTSAgs have super antigen activities that can induce toxic shock syndrome (TSS) and a range of symptoms related to food poisoning. The PTSag exotoxins include TSST-1, the staphylococcal enterotoxins (SE) (SEA, SEB, SEC, SED, SEE, SEH,

SEI) (Balaban & Rasooly, 2000) and the streptococcal pyrogenic exotoxins (SPEA, B, C, F, G, H, J) and streptococcal superantigen (Bohach et al., 1990; Dinges et al., 2000).

The SEs are a family of heat stable enterotoxins that function both as potent gastrointestinal toxins and superantigens which stimulate non-specific T-cell proliferation. SEA and SEB were first characterized in 1959 and 1960 respectively (Bergdoll et al., 1959; Casman, 1960). To date, 22 different SEs have been described, which are designated SEA to SElV2 in the chronological order of their discovery except for SEF which was later renamed TSST1 (Casman, 1960; Thomas et al., 2007; Ono et al., 2008). The recognized enterotoxins are: enterotoxin A (SEA), B (SEB), C1 (SEC1), C2 (SEC2), C3 (SEC3), D (SED), E (SEE), G (SEG), H (SEH), I (SEI), J (SElJ), K (SElK), L (SElL), M (SElM), N (SElN), O (SElO), P (SElP), Q (SElQ), R (SER), S (SES), T (SET), U (SElU), and U2 and V. (Hennekinne et al., 2012). The proteins named SE were named based on their emetic activity after oral administration in a primate model. Those designated as SE-like toxins (SEl) either demonstrated no emetic properties on testing or have not yet been tested in primate models (Lina et al., 2004; Thomas et al., 2007).

TSST-1 is a 22KDa exotoxin generated by *S. aureus*. It can not only trigger monocytes and T cells to synthesize cytokines including Interleukin-1 (IL-1), TNF, IL-2, and interferon (IFN-γ), but also stimulates T cell activation and proliferation. TSS caused by TSST-1 is a rapid-onset illness characterized by fever, hypotension, rash, vomiting, diarrhoea, and multiple organ failure and can be fatal without proper treatment.

TSS-related *S. aureus* infection can be broadly divided into menstrual (involving vaginal infection in menstruating women) and non-menstrual cases. Ninety percent of menstrual TSS cases are related to tampon use and involve TSST-1; non-menstrual cases are evenly split between those caused by TSST-1 and those associated with SEs (Iwatsuki et al., 2006).

Staphylococcal food poisoning which is the second most common cause of reported food-borne illness and is attributable to enterotoxin production (Bohach et al., 1990; Balaban & Rasooly, 2000). Although many genes code for staphylococcal enterotoxins, most outbreaks are associated with enterotoxins A – E (SEA-SEE) from *S. aureus*, with SEA causing most outbreaks followed by SED and SEB (Chiang et al., 2008). More recently, studies have reported that certain CNS strains are able to produce enterotoxins, suggesting enterotoxigenic potential for CNS (Zell et al., 2008; Even et al., 2010). However, to date only coagulase positive strains have been evidenced in food poisoning incidents, of which most are associated with *S. aureus.*

Most research has focused on SEs produced by *S. aureus* from food sources (Cha et al., 2006; Rall et al., 2008), with few reports on their presence in clinical isolates (Mehrotra et al., 2000; Becker et al., 2003). Environmental contamination with strains carrying enterotoxin genes could increase the food poisoning risks of the general population.

The role of *S. aureus* in food poisoning has been recognized for many years, with the

first description of Staphylococcal food poisoning being related to cheese contamination in Michigan (USA) in 1884 reported by Vaughan and Sternberg (Dack, 1956). Over 185,000 food-borne illnesses are estimated to be attributable to staphylococcal food poisoning each year in the United States (Mead et al., 1999). In 2006, The European Food Safety Authority (EFSA) reported that SEs were detected in 4.1% (236/5807) of food poisoning outbreaks and *S. aureus was* ranked as the fourth most common causative agent. In 2008, EFSA reported that SEs were involved in 5.5% of all notified food poisoning outbreaks (Hennekinne et al., 2012). MRSA strains can also cause food-poisoning as cases involving community-acquired MRSA carrying SEC have been reported (Jones, 2002).

Enterotoxin-producing strains caused a total of 910 staphylococcal food poisoning outbreaks in Hong Kong between 2001 and 2009. They accounted for 18.5% of food poisoning outbreaks associated with bacterial causes, making *S. aureus* the third commonest food poisoning outbreak agent (www.chp.gov.hk). The food types most commonly associated with staphylococcal food poisoning were siu-mei, lo-mei (Chinese pork products) and chicken. For example in 2006, a large outbreak was associated with a Siu-Mei/ Lo-Mei food stall in Hong Kong. Other implicated foods include poultry and other meat, bakery products and desserts. Poor personal hygiene of food handlers and improper storage of cooked food were identified as the major contributing factors, especially in large outbreaks involving more than 20 persons (www.chp.gov.hk).

1.5.2.2 Other Exotoxins

Virulence factors of *S. aureus* also include exfoliative toxins (ETs). These toxins are mediated by two genes: *eta* and *etb*. *Eta*, encoding 242 amino acids, is located in the genomic sequence of a temperate phage, allowing conversion ET-A non-producing strains into ETA producers. The *etb* gene, encoding 246 amino acids, is located on a plasmid and can also transfer horizontally. ET-A and ET-B share approximately 40% amino acid homology (Ladhani et al., 1999; Yamaguchi et al., 2000).

The extremely specific serine proteases of ETs recognize and cleave desmosomal cadherins only in the superficial layers of the skin, resulting in SSSS characterized by skin exfoliation. SSSS appears most commonly in infants and young children, frequently occurring as epidemics in hospital nurseries (Stanley & Amagai, 2006). Studies have reported little difference in the prevalence of ETA between MRSA and MSSA, with about 10% of MRSA being *eta* positive and 3% MSSA carrying either *eta* or *etb* (Sila et al., 2009; Bukowski et al., 2010), Research from Japan has shown that *etb*-positive community associated MRSA may cause SSSS in healthy adults (Noguchi et al., 2006). ETA is more common in Europe, USA and Africa, where more than 80% of toxin-producing strains carry ETA. However, ETB producing strains are more prevalent than those expressing ETA in Japan (Bukowski et al., 2010).

Panton-Valentine Leukotoxin (PVL) is a pore-forming toxin secreted by strains of community-associated methicillin-resistant *S. aureus* (CA-MRSA). PVL is a bicomponent cytokine consisting of LukS-PV and LukF-PV and belongs to the

synergohymenotropic toxin family. LukS-PV and LukF-PV assemble to form PVL in the membrane of host defence cells and is able to destroy leukocytes (Voyich et al., 2006). The bicomponent PVL toxin is also associated with severe necrotizing pneumonia in children (Gillet et al., 2002). PVL may increase the expression of Spa, a key pro-inflammatory factor for pneumonia (Labandeira-Rey et al., 2007).

1.5.3 Regulation of Virulence Factors

Whilst virulence of *S. aureus* is determined by cell wall associated proteins and secreted toxins, the expression of the gene encoded virulence factors is regulated by mechanisms which act mainly at the transcriptional level.

Virulence factor production is mainly regulated by two-component regulatory systems, such as *agr* (accessory gene regulator) and *sar* (Staphylococcal accessory regulator) systems. These systems, which include a sensor histidine kinase and a response regulator protein, are sensitive to the environment. The most important regulatory system controlling virulence factor expression in *S. aureus* is the *agr* system which functions as a repressor of transcription of a number of cell wall associated proteins (protein A, coagulase, fibronectin binding protein) as well as an activator of several exoproteins (e.g. TSST-1, leukotoxins, ETA and ETB) during the post-exponential growth phase. Additionally, the *agr* system controls the majority of SE expression, with the expression of both *seb* and *sec* being *agr*-dependent, although *sea* and *sej* expression are agr independent. The *agr* system works together with the DNA-binding proteins, such as the *sar* system, which also regulate virulence

factor expression. The regulatory systems are sensitive and specific to environmental changes and signals, inducing infection, or providing protection to the bacterium (Bronner et al., 2004).

1.6 Pathogenesis and Virulence Factors of Other Staphylococci

Virulence factors of staphylococci especially those of *S. aureus,* aid their ability to cause infections. The major difference between *S. aureus* and CNS is the production of coagulase by *S. aureus*. However, although *S. aureus* is the major pathogen, there is little evidence to show the pathogenicity of coagulase. The virulence factors of other staphylococci are discussed below.

1.6.1 Virulence Factors of Coagulase Negative Staphylococci

There is an increasing rate of opportunistic infections and infections associated with medical devices caused by CNS. This increase is mainly attributed to the spread of antibiotic resistance among CNS and the increasing use of medical devices over recent years. The pathogenic potential of CNS is primarily due to their capacity to form biofilms on indwelling medical devices and the factors contributing to CNS biofilm formation have received considerable attention. Biofilms can protect bacteria from antibiotics and the immune system. CNS also produce several lipases, proteases, and other exoenzymes, which possibly contribute to the persistence of CNS in the host and may degrade host tissue. *S. epidermidis* is believed to account for most infections caused by CNS, although other CNS including *S. saprophyticus* and *S. haemolyticus* have also been isolated from infections (Nizet & Klein, 2011). Several

virulence factors have been associated with these infection processes which are described below.

1.6.1.1 Adaptation to a Specific Habitat

S. epidermidis is well adapted the human skin and mucous membranes. It is known to withstand very high salt concentrations, allowing it to become the predominant species on the human skin. It can endure high osmolarity, relatively low pH, and changeable moisture and temperature. Sequencing of the genome of one strain, *S. epidermidis* RP62A, has shown that there are several genes involved in osmoregulation, including genes with similarity to those for Na+/H+- and glycine betaine transporters (Otto, 2004).

S. saprophyticus has a unique adhesion protein, UafA, which allows it to adhere to human ureteral epithelial cells. In addition, *S. saprophyticus* can encode several transport proteins to adjust to osmotic and pH changes and also produces plentiful urease which allows it to proliferate in urine (Huebner & Goldmann, 1999; Rogers et al., 2009a).

1.6.1.2 Factors Involved in Biofilm Formation

Biofilm formation requires two steps: the rapid adherence of bacterial cells to a polymer surface, followed by the accumulation of microcolonies as multilayered cell clusters (Agarwal et al., 2010). Proteins secreted by *S. epidermidis* have been linked to initial attachment to plastic surfaces. Surface-associated protein, AtlE encoded by

*alt*E, is reportedly involved in the direct attachment to hydrophobic surfaces. It was demonstrated that *S. epidermidis* transposon mutants devoid of AtlE production lacked the ability to adhere to polystyrene microtitre plates (Heilmann et al., 1996).

Other protein factors have been linked to the attachment to plastic, including two very large staphylococcal surface proteins of *S. epidermidis*, SSP-I (280 kDa) and its degradation product SSP-2 (250 kDa) (Vuong et al., 2000). The biofilm-associated proteins (Bap), coded by *bap* with a predicted molecular mass of 238.5 kDa, also contributes to the adherence of staphylococci on polystyrene surfaces and subsequent biofilm formation (Otto, 2004).

CNS can attach to matrix proteins (e.g. fibrinogen, fibronectin, and collagen) on host tissues or in a conditioning film around prostheses. Bacterial proteins that bind to host matrix proteins are called MSCRAMMS. These serve as important virulence factors as attachment constitutes a crucial step of bacterial colonization, and include fibrinogen-binding protein (Fbe), biofilm-associated protein (Bap), and fibronectin-binding protein (Embp) (Otto, 2004).

Adherence of clinical isolates of both coagulase-positive and negative staphylococci to biomaterials is mediated by surface-bound fibronectin. In *S. epidermidis,* Fbe is encoded by *fbe* gene and has a molecular mass of 119KDa. It functions as an adhesion and binds to the beta-chain of fibrinogen. Studies show that antibodies against Fbe can significantly increase macrophage phagocytosis and block adhesion

to fibrinogen-coated surfaces and implanted catheters, which suggests that this protein is a major factor mediating adhesion to fibrinogen in *S. epidermidis* (Nilsson et al., 1998).

The most important process of biofilm formation consists of the accumulation of bacteria in multi-layered structures. The formation of cell clusters is closely related to production of a polysaccharide antigen mediated by polysaccharide intercellular adhesion (PIA) (Mack et al., 1996b; Agarwal et al., 2010). PIA is an unbranched homopolymer of beta-1, 6-linked N-acetylglucosamine residues, of which 15-20% is de-acetylated (Mack et al., 1996a). It is produced by the intracellular adhesion operon consisting of *ica*A, *ica*D, *ica*B, and *ica*C genes (O'Gara & Humphreys, 2001; Götz, 2002).

The 140 kDa extracellular accumulation-associated protein (AAP), expressed predominantly under sessile growth conditions, is also implicated in biofilm accumulation on polymer surfaces. It is speculated that the role of this protein might be to link PIA to the bacterial cell surface. AAP is proposed to play a role in anchoring PIA to cell surfaces, because mutants lacking AAP produce PIA that is only loosely attached to the cell surface. However, the specific mechanism which links PIA to the bacteria is not yet known. Several studies have suggested that *sar* could up-regulate the production of a biofilm by influencing *ica* gene transcription and PIA production (Hussain et al., 1997; O'Gara & Humphreys, 2001; Agarwal et al., 2010).

Biofilm formation is under positive regulation by genes including those responsible for clumping factor, fibronectin binding protein A, and coagulase, and negative regulation a and b haemolysin, enterotoxin B, SP1A (a serine protease), cystinoprotease (SP1B), the metallo-protease, Aur staphopain, and leucotoxin D (Agarwal et al., 2010). Rbf protein, coded by *rbf* with a molecular mass of 190 kDa, also contributes to multicellular aggregation during biofilm formation and is involved in the induction of biofilm formation by NaCl and glucose (Agarwal et al., 2010).

1.6.1.3 Exoenzymes and Toxins

S. epidermidis has been reported to produce three lipases, GehC, GehD, and Geh-1 (Farrell et al., 1993). Lipases enable the bacteria to persist in the fatty secretions of human or mammalian skin, and possibly interfere with phagocytosis. It has been found that the lipase GehD of *S. epidermidis* can bind to collagen, suggesting a novel role for lipase in virulence (Bowden et al., 2002).

Phenol-soluble modulin (PSM), present in *S. epidermidis,* is a complex with at least three secreted amphiphilic peptides with inflammatory properties (Mehlin et al., 1999). PSM production is strictly positively regulated by the *agr* gene (Klingenberg et al., 2007). It was postulated that low *agr* activity favours biofilm formation and the ability of *S. epidermidis* to colonize catheter material, whilst high *agr* activity increases the invasive capacity of *S. epidermidis* and inhibits biofilm formation. It has been inferred that the surfactant-like PSMs, under regulation of agr, inhibit the later stages of biofilm formation and lead to biofilm dispersion (Klingenberg et al., 2007).

In addition to those mentioned above, *S. epidermidis* produces other exoenzymes, such as fatty acid-modifying enzyme, which inactivates bactericidal lipids present on the skin (Chamberlain & Brueggemann, 1997).

1.7 Antibiotic Resistance Determinants

Antibiotic resistance in staphylococci has been shown to be both chromosomally and plasmid mediated. The mutation of chromosomal loci of staphylococci is associated with resistance to several antibiotics, including streptomycin, rifampin, fusidic acid, mupirocin, and novobiocin (Wichelhaus et al., 2001; O'Neill & Chopra, 2006). Plasmids play an important role in the acquisition of antimicrobial resistance genes, which can be transferred to the bacterial chromosome. For example, plasmids can act as vectors to transpose genetic elements harbouring antimicrobial resistance genes, which can translocate either from one plasmid to another or from a plasmid to a chromosomal site (Kleckner, 1981). The emergence of multi-resistant staphylococci can be attributed to the clustering on plasmids, or the chromosome, of various transposons carrying different resistance determinants (Saunders, 1984).

1.7.1 β-lactam Antibiotics

β-lactam antibiotics are bactericidal, and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. Such inhibition is due to the covalent binding of the antibiotic to one or more penicillin-sensitive enzymes, the penicillin-binding proteins (PBPs). The first reports of penicillin resistance in staphylococci involved the production of an inducible extracellular β-lactamase

(penicillinase), which inactivates the antibiotic by hydrolysis of the β-lactam ring. The β-lactamase structural gene (*blaZ*), responsible for penicillinase production, is present on Tn*552*-like transposons that are carried by a diverse group of β-lactamase/heavy-metal resistance plasmids (Chambers, 1997).

1.7.2 Methicillin

The introduction of methicillin in 1960 was rapidly followed by development of resistance. Though these strains did not become widespread until several years later, the resistance is attributed the expression of a low-affinity PBP, termed PBP2a, in staphylococci which causes the organism to be resistant to both β-lactamase-sensitive and β-lactamase-resistant penicillins (Chambers & Sachdeva, 1990).

The *mec*A gene is located on a mobile genomic island, known as SCC*mec*. This gene is 2.1 kb in length, encoding for the 78-kDa PBP 2a (or PBP2') which causes the bacterium to be resistant to β-lactam antibiotics (Ito et al., 2001; Ma et al. 2002). The β-lactam antibiotics, binding to the native PBPs present in the cell wall of methicillin-sensitive *S. aureus* (MSSA), result in the disruption of the synthesis of the peptidoglycan layer and cell death. However, β-lactam antibiotics cannot bind to the foreign PBP2a in MRSA, and peptidoglycan layer synthesis is not disrupted, allowing the growth of MRSA (Berger-Bächi & Rohrer, 2002).

The *mecA* gene is regulated by the repressor MecI and the trans-membrane

β-lactam-sensing signal-transducer MecR1, which are both divergently transcribed. MecI represses both the transcription of *mecA* and *mecR1–mecI* in the absence of a β-lactam antibiotic. In the presence of a β-lactam antibiotic, MecR1 is auto-catalytically cleaved and the metallo-protease domain becomes active. This metallo-protease cleaves MecI, which, in turn, combines with the *mecA* operator region, launching the transcription of *mecA,* and the subsequent production of PBP2a (Ito et al., 2001; Ma et al. 2002).

SCC*mec* is a mobile genetic element carried on the chromosomes of MRSA isolates and consists of the *mecA* gene, which encodes PBP2a, and intact or truncated sets of the divergently transcribed regulatory genes, *mecR1* and *mecI* (Ito et al., 2001; Ma et al. 2002). SCC*mec* elements can act as mechanisms to capture foreign DNA segments allowing for survival in a toxic environment. The uptake of resistance determinants to other antibiotics leads to the multi-resistance of MRSA strains.

SCC*mec* elements have been characterized based on structural differences and, to date, eleven types of SCC*mec* (type I to XI) are recognized based on the *mec* gene complex, the *ccr* gene types, and integration site sequence , which serves as a target for ccr-mediated recombination, and flanking direct repeat sequences (www.sccmec.org/Pages/SCC_TypesEN.html) (Ito et al., 2009; IWG-SCC 2009; Li et al., 2011; Shore et al., 2011; García-Álvarez et al., 2011) (Fig 1.1 , Table 1.3). The size of SCC*mec* elements ranges from 20.9kb to 66.9 kb. Presence of SCC*mec* type I (34.3 kb), IV (20.9–24.3 kb), V (28 kb), VI (20.9 Kb), VII (35.9 kb), VIII (32.17 kb), IX (43.68), X

(50.8kb) or XI (30kb) is usually associated with β-lactam resistance only. Other resistance determinants can integrate into SCC*mec* type II (53.0 kb) and III (66.9 kb), resulting in resistance to multiple classes of antibiotics. For instance, integrated plasmids, e.g. pUB110, pI258 and pT181, and two transposons, e.g. Tn*554* and ΨTn*554* have been observed in these SCC*mec* types. The *ant* (4')-1 gene located in the integrated plasmid pUB110 confers resistance to several aminoglycosides, including kanamycin, tobramycin, and bleomycin, but not to gentamicin. pI258 encodes resistance to penicillins, while pT181 confers resistance to tetracycline. Transposon Tn*554* harbours the *ermA* gene which confers resistance to macrolides, lincosamides, and streptogramins (MLS). In addition to their presence on SCC*mec*, these resistance genes may also be found at other sites in *S. aureus* e.g.Tn*554* on plasmids and the genome. This situation is reported in SCC*mec* type IV isolates (Ito, 2001, 2003; Leclercq, 2002; Robinson, 2004).

It has been shown recently that SCC*mec* type III is a composite element that consists of two SCC elements, i.e. SCC*mec* type III and SCC*mercury*, harbouring *ccrC*, pI258 and Tn*554*. Furthermore, SCC*mec* can carry several insertion sequences, such as IS*431* and IS*1272*, as well as the genes responsible for the regulation of *mecA* transcription, i.e. Δ*mecR1* (SCC*mec* type I, IV, V, VI and VII), or *mecR1* and *mecI* (SCC*mec* types II and III) (Ito, 2001, 2003; Leclercq, 2002; Robinson, 2004; Ito et al., 2009; García-Álvarez et al., 2011; Li et al., 2011; Shore et al., 2011).

1.7.3 Resistance to non β-lactam Antibiotics

1.7.3.1 Chloramphenicol

Chloramphenicol is a bacteriostatic agent that binds to the bacterial 50S subunit and inhibits the transpeptidation step in protein synthesis. Acetyltransferases that inactivate chloramphenicol are the most common resistance mechanisms for this antibiotic. Chloramphenicol acetyltransferases (CAT), are inducible detoxification enzymes which mediate resistance to chloramphenicol, and are located in rolling-circle (RC) plasmids within the size range of 2.9 to 5.1 kb, (Levy et al., 2004, Levy & Marshall, 2004). There are two genetically unrelated CAT enzymes, but both types of enzymes function as homotrimers (Alekshun & Levy, 2007). Resistance to chloramphenicol may also be associated with efflux of the drug by both specific membrane-associated transporters and by multidrug transporters (Roberts & Schwartz, 2009). Resistance may also occur due to mutations which reduce the expression of outer membrane proteins, mutations in the 23S rRNA, inactivation of chloramphenicol by 3-O-phosphotransferases or target site modification by a 23S rRNA methylase (Roberts & Schwartz, 2009).

1.7.3.2 Tetracycline

Tetracycline inhibits protein synthesis by binding to the 30S ribosomal subunit and preventing association of aminoacyl-tRNA with its acceptor site. Tetracycline resistance can be mediated by tetracycline efflux proteins. There are now more than 20 different tetracycline efflux proteins recognized which are separated into six groups, containing either 12- (TetA-E in gram-negative bacteria) or 14- (TetK and TetL

in gram-positive organisms) putative transmembrane-spanning segments (Alekshun & Levy, 2007). Presence of tetracycline inactivates repressors of these genes, leading to expression of the tetracycline efflux systems.

There are two mechanisms of tetracycline resistance in *S. aureus*: active efflux via *tet*(*K*) and *tet*(*L*) and ribosomal protection via *tet*(*M*). Tetracycline induces synthesis of Tet(K) and Tet(L) by translation attenuation and re-initiation respectively. Tet(K) and Tet(L) efflux proteins belong to the major facilitator superfamily and contain 14 transmembrane segments. Tetracycline efflux in *S. aureus* strains is most commonly mediated by *tet* (*K*) (Alekshun & Levy, 2007).

1.7.3.3 Aminoglycosides

Aminoglycosides bind with the 30S ribosomal subunit and disrupt the translocation of peptidyl-tRNA to inhibit protein synthesis. Resistance to aminoglycosides is due to enzymatic modification of their amino groups preventing binding with ribosomes (Shaw et al., 1993). These enzymes may N-acetylate (acetyltransferases) phosphorylate (phosphotransferases), or adenylate (nucleotidyltransferases) aminoglycosides. Several aminoglycoside-modifying enzymes encoded by genes on transferable elements, are widespread in clinically relevant organisms and confer resistance to aminoglycosides (Davies and Wright, 1997). The acetyltransferases are capable of modifying tobramycin, gentamicin, netilmicin, and amikacin, the nucleotidyltransferase proteins alter the activity of tobramycin, and the phosphotransferases affect amikacin susceptibility (Alekshun & Levy, 2007).

Many of the aminoglycoside-modifying enzymes are found on integrons and other mobile genetic elements where they associate with additional resistance determinants. Resistance to gentamicin, amikacin and kanamycin, is mediated by AAD and APH activities catalyzed by a single bifunctional protein encoded by *aacA-aphD*. This gene is carried by the IS*256*-flanked composite transposon Tn*4001*, found on large staphylococcal multiresistance plasmids such as pSK1 and pSK41, or on the chromosome (Lyon et al., 1984; Rouch et al., 1987; Alekshun & Levy, 2007).

1.7.3.4 Macrolides, Lincosamides, and Streptogramins (MLS)

Macrolide antibiotics such as erythromycin, lincosamides such as clindamycin, and streptogramin antibiotics bind to the 50S ribosomal subunit and cause dissociation of the peptidyl-tRNA during elongation to stop protein synthesis (Gale et al., 1981). There are a number of inactivating enzymes acting on the MLS antibiotics. Esterases work on 14- and 15-membered macrolides; the hydrolases confer resistance to streptogramin B drugs; the acetyltransferases affect streptogramin A antibiotics; and the nucleotidyltransferases induce resistance to the lincosamides (Alekshun & Levy, 2007). In staphylococci, inducible resistance to the MLS antibiotics is commonly encoded by the rRNA methylase gene, *erm(C)*, present on small multicopy plasmids (Shivakumar & Dubnau, 1981; Alekshun & Levy, 2007).

MsrA (macrolide-streptogramin resistance), an ABC efflux protein, confers resistance to 14- and 15-membered macrolides and streptogramin B antibiotics in streptococci and staphylococci, but does not affect susceptibility to clindamycin (Li & Nikaido,

2004). Recently, several new resistance deteminants to the MLS antibiotics have been described in staphylococci. These include VgaA, which is related to MsrA and located on plasmids, confers resistance to streptogramin A antibiotics and lincosamides. VgaB, similar to VgaA, affects pristinamycin (a mixture of streptogramin A and B antibiotics) susceptibility (Chesneau et al., 2005; Alekshun & Levy, 2007).

1.7.3.5. Sulphonamides and Trimethoprim

The sulphonamides target dihydropteroate synthetase, which catalyzes the conversion of p-aminobenzoic acid to dihydropteroic acid. Trimethoprim, which was introduced in 1968, has a high affinity for dihydrofolate reductase (DHFR) and competitively inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid. These agents, affecting the same biosynthetic pathway for folate production, act synergistically. In clinical *S. aureus* isolates that are resistant to sulphonamides, mutations in the gene encoding dihydropteroate synthetase reduces its affinity for these drugs. Mutations in the gene encoding dihydrofolate reductase result in over-expression of an enzyme with a reduced affinity for trimethoprim and confer high-level trimethoprim resistance in *E. coli* and *H. influenzae* (Huovinen et al., 1995). In contrast, intermediate-level trimethoprim resistance in *S. aureus* is chromosomally mediated via mutations in the *dfrB* gene, which encodes DHFR (Hampele et al., 1997). High-level resistance is plasmid mediated mostly due to a unique DHFR encoded by the *dfrA* gene, resulting in reduced affinity for trimethoprim (Tennent et al., 1988; Alekshun & Levy, 2007).

1.7.3.6 Glycopeptides

Glycopeptide antibiotics, which include vancomycin, are an antibiotic class composed of glycosylated non-ribosomal peptides that inhibit cell wall synthesis. They bind with high affinity to the D-Ala-D-Ala C-terminus of late peptidoglycan precursors (*N*-acetylmuramic acid and *N*-acetylglucosamine peptide subunits), inhibiting their incorporation into the peptidoglycan matrix. Heteroresistant vancomycin-intermediate *S. aureus* (hVISA) reduces susceptibility to vancomycin and appears to be an adaptive multifactorial response to sub lethal vancomycin exposure that is often associated with thickening of the cell wall (Sieradzki & Markiewic, 2004; Cui et al., 2006; Gould et al., 2011). *S. aureus* isolates with intermediate resistance are termed VISA. The genetic changes causing the hVISA/VISA phenotype have yet to be clearly determined although several genes have been associated with vancomycin intermediate resistance, such as the *vraSR* operon and the two-component sensor gene *graS* (Howden et al., 2008). Co-resistance to daptomycin in VISA strains has been reported (Van Bambeke et al., 2008). In addition, there is evidence that development of rifampin resistance caused by mutation in *rpo*B gene may also be associated with development of VISA (Matsuo et al., 2011). High level resistance to vancomyin is mediated by the *van*A gene, but this is rare with less than 20 confirmed cases worldwide, the majority in the United States (Howden et al., 2008).

1.8 Biocides

1.8.1 Introduction to Biocides

"Biocide" is an active chemical molecule able to control the growth of micro-organisms or kill micro-organisms incorporated in products, which include antiseptics, disinfectants and preservatives. Antiseptics are biocides that destroy or inhibit the growth of microorganisms in or on living tissue (hand washes and surgical scrubs). The term antisepsis indicates the treatment or prevention of an infection by application of antiseptics to skin and mucosa. Disinfectants are similar to antiseptics but are generally used on inanimate objects or surfaces. Disinfection is an operation aimed at preventing infections, which is used in the decontamination process of patient-care devices, and environmental surfaces. Preservatives are used for the prevention of growth or survival of microorganisms in formulated products, including food, medical, and pharmaceutical products (McDonnell & Russell, 1999; McDonnell, 2007). Antiseptics and disinfectants are used widely in hospitals and health care settings, and are of great importance in infection control and infection prevention (Rutala & Weber, 1999). Biocides are also used in the community such as in homes, day-care centres, nursing homes, and food-service sites (Beumer et al., 2000). A summary of biocidal products, their active substances and their uses is shown in Table 1.1. Of these biocides, three groups are commonly used in antisepsis, the biguanides, the quaternary ammonium compounds (QACs), and the phenylethers which include triclosan.

1.8.2. Cationic Biocides

Cationic biocides have been widely used for over half a century as effective agents against micro organisms. They are an indispensable part of infection control practice and are helpful in preventing nosocomial infections.

There are two important regions in the molecular structures of surfactants (surface-active agents) which are characteristic of their action, the hydrophobic group and the hydrophilic group. According to the charge of the hydrophilic group, it is classified as a cationic, anionic, non-ionic or an ampholytic (amphoteric) compound. Among these, cationic agents are some of the most useful antiseptics and disinfectants (McDonnell & Russell, 1999).

The quaternary ammonium compounds (*QAC*s), diamidines, biguanides, cationic dyes and nuclear stains are commonly used cationic biocides. There is increasing use of these agents in the community owing to the enhanced awareness of hygiene. Table 1.2 shows the structure of CHG and benzalkonium chloride.

The outermost surfaces of bacterial cells often carry a negative charge, which is associated with the cell wall components teichoic acid and polysaccharide. The presence of divalent cations such as Mg^{2+} and Ca^{2+} can stabilize the cell. The cationic biocides have high binding affinity for bacterial cells, as these agents carry a strong positive charge and a hydrophobic region, which interacts with the cell surface and integrates into the cytoplasmic membranes (Gilbert & Moore, 2005). Several factors affect the antimicrobial activity of these biocides, such as concentration, pH, organic

load and temperature. Of these, concentration is most important. It is presumed that at lower concentrations biocides target only specific sites of the microbial cell despite the biocide having multiple target sites (Maillard, 2002).

1.8.2.1 QACs

The QACs are cationic surfactants in which four carbon atoms are linked directly to a nitrogen atom by covalent bonds. The general structure of QACs is demonstrated in Table 1.2. The four side-groups, namely R1, R2, R3 and R4, can be alkyl or heterocyclic; while the nitrogen group can be part of a ring system. Since the functional portion of the molecule is positively charged, QACs attach to negatively charged surfaces. For a QAC to have a high antimicrobial activity, at least one of the side-groups must have a chain length of 8-18 carbons (Fraise et al., 2004).

Commonly used QACs include cetyltrimethylammonium bromide (known as CTAB), and benzalkonium chloride (BC) (Table 1.1). CTAB is a mixture of n-alkyltrimethyl ammonium bromides where the n-alkyl group is between 8-18 carbons long. BC is a mixture of n-alkyldimethylbenzyl ammonium chlorides in which the n-alkyl groups can be of variable length with the greatest bactericidal activity attributed to alkyl derivatives of 12-14 carbons long. Compounds with n-alkyl chain lengths of $n<=4$ or $n>=18$ are virtually inactive against bacteria (Gilbert & Moore, 2005).

QACs, broad spectrum low-level disinfectants, have been used as biocides since the 1930s (Russell, 2002a). They are widely used as disinfectants and antiseptics for

control of bacterial growth in domestic households, health care settings, the food industry, and veterinary applications. They are most commonly used for clinical purposes including pre-operative disinfection of unbroken skin, application to mucous membranes, and disinfection of non-critical surfaces. Modified QACs are also present in contact lens multipurpose solutions. Apart from their antimicrobial properties, QACs are also excellent for hard-surface cleaning and deodorization but they have limited efficacy against spores, some Mycobacteria, and certain viruses (McDonnell & Russell, 1999).

QACs are membrane-active agents, the primary targets of which are bacterial and fungal cell walls and membranes. Lower concentrations (0.0005%) of QACs such as are used in contact lens solutions, are active against Gram-positive bacteria. Cationic agents are quickly absorbed and penetrate into the cell wall, where they react with phospholipids components in the cytoplasmic membrane, causing membrane distortion and cell lysis under osmotic stress (Kanazawa et al., 1995). Additionally, CTAB is reported to have an effect on proton motive force (PMF) in *S. aureus* causing discharge of its hydrogen ion component. It is reported that higher concentrations (0.0033%) of QACs are lethal to Gram-negative rods (Fraise et al., 2004), as a result of damage to the outer membrane, followed by uptake of the antiseptic agent into the cell and subsequent intracellular changes (McDonnell & Russell, 1999).

1.8.1.1 Biguanides

Biguanides, containing the $C_2H_5N_7$ ligand, were first synthesized in the early 20th century. They are broad-spectrum bacterial biocides and function actively against Gram-positive and Gram-negative bacteria. In general, biguanides are more active against Gram-positive bacteria, even in the presence of organic materials, such as blood or serum. They have limited activity against fungal agents (McDonnell & Russell, 1999).

CHG, first synthesized in 1950, possesses a high level of antibacterial activity, low mammalian toxicity, and a strong affinity for binding to skin and mucous membranes. CHG is 1, 6-di (4-chlorophenyl-diguanido) hexane, a cationic bisbiguanide (See Table 1.2). It is a strong base and insoluble in water (0.008% wt/vol at 20°C). The water solubility of CHG salts varies widely. The most commonly utilized compound, soluble CHG digluconate, cannot be isolated as a solid and is manufactured as a 20% wt/vol aqueous solution. The concentration of CHG used in antiseptics is 2% to 4% (Kampf & Kramer, 2004). The antimicrobial activity of CHG is pH dependent with the optimum range of 5.5 to 7.0 corresponding to the pH of skin and mucous membrane. Additionally, its activity is greatly reduced in the presence of organic matter (Russell, 1993).

The mechanism of action of biguanide antiseptics against bacteria is similar to that of the QACs in that they target the anionic sites of the cell membrane and cell wall of bacteria, especially acidic phospholipids and proteins (Chawner & Gilbert, 1989).

Biguanides penetrate these sites and integrate into the cytoplasm thereby causing loss of structure and function. Biguanides interact with the Gram-negative cell envelope leading to rapid and specific penetration of the negatively charged bacterial outer membrane, with strong adsorption to phosphate containing compounds. Since the integrity of the outer membrane is impaired, biguanide is able to penetrate into the inner membrane by direct insertion or binding to the phospholipids. The permeability of the inner membrane is thus increased, leading to bacteriostasis or complete loss of membrane function, causing a bactericidal effect (McDonnell, 2007). CHG is the most important bactericidal agent in this group (Denyer & Stewart, 1998). It has been shown that the uptake of CHG by *E. coli* and *S. aureus* is very rapid (Hugo & Longworth, 1966), but is dependent on the CHG concentration and pH.

The antiviral activity of CHG is variable (McDonnell & Russell, 1999), but seems to include both lipid-enveloped (Park & Park, 1989) and non-enveloped viruses (Springthorpe et al., 1986).

In hospitals, the use of CHG showers and bed baths has been proposed as a measure to control antimicrobial resistant bacteria, especially in intensive care units, and can be effective in preventing carriage, and the subsequent development of healthcare-associated blood stream infections (BSI) (Vernon et al., 2006; Derde et al., 2012; Karki et al., 2012). It was reported that the introduction of such decolonization measures brought about a 70% reduction in hospital-wide MRSA infections within 2 years in three US hospitals (Viviani et al., 2005). In another study, the application of

CHG gluconate to the nasopharynx and the oropharynx of cardiac surgery patients resulted in a significant (58%) reduction in *S aureus* carriage, which was associated with a decreased incidence of nosocomial infection compared with patients who received a placebo preparation (Batra et al., 2010). Studies have demonstrated that perioperative decolonisation with mupirocin or CHG gluconate, or the two agents together can significantly reduce risk of development of deep surgical-site infections (Bode et al., 2010; Walsh & Kirshner, 2011).

Decolonisation is less popular in the USA than the UK because of the fear of increasing mupirocin and other resistance occurrence (Immerman et al., 2012). In the UK, decolonisation is widely recommended, including for recurrent infections, elderly and immuno-suppressed individuals, or where multiple family members are affected (Edgeworth, 2011).

The increased use of CHG in ICUs does, however, raise concerns about selecting for resistance. In an ICU study performed in the UK, a CHG-based antiseptic protocol failed to prevent transmission of MRSA strains (ST239-TW) carrying *qac*A/B genes. ST239-TW MRSA body site colonization was not reduced compared to a reduction observed for MSSA (ST22 and ST36 strains) (Batra et al., 2010).

The polymeric biguanides are a newer class of heterodisperse mixtures of polyhexamethylbiguanides (PHMBs). PHMBs have wide applications for surface disinfection and water sanitization, particularly as an alternative to chlorine and

bromine in swimming pools and spas. As they have low toxicity and a broad spectrum of bactericidal and fungicidal activity at low concentrations, PHMBs are used in soft contact lens disinfecting solutions (Santodomingo-Rubido, 2007).

1.8.1.2 Diamidines

The diamidines consist of a small group of antimicrobial agents with similar structure. Propamidine and the halogenated dibromopropamidine are the most widely used diamidino compounds. The diamidines are biocides with a broad spectrum activity against bacteria and fungi. The typical MICs of diamidines against Gram-positive bacteria range from 0.2 to 0.25mg/L. They can be used directly in the eyes depending on the concentration, so are used in contact lens solutions and eye drops. They also function as a component in creams or ointments for prevention of infection or topical treatment of wounds (McDonnell, 2007).

Although the exact mechanism of diamidines remains unclear, it is considered to be similar to other cationic biocides. It has been reported that diamidines can disrupt and cause damage to cell surfaces of *Pseudomonas aeruginosa* and *Enterobacter cloacae* (Richards et al., 1993). Diamidines can also penetrate into the cytoplasm, bringing about denaturation and coagulation of proteins and enzymes. There is specific inhibition of various enzymes, including membrane-associated and intracellular proteins (Fraise et al., 2004).

1.8.1.3 Cationic Dyes

Antimicrobial dyes have been used as antiseptics and for water disinfection under certain conditions. Cationic dyes consist of three groups: the acridines, the triphenylmethane group, and the quinones. The acridines were first introduced as systemic and topical antimicrobials in the early 1900s. Their use decreased after the introduction of antibiotics. The acridine derivatives were used in the treatment of infected wounds.

In the triphenylmethane group, the most important compound is crystal violet. It was previously used as a local antiseptic for application to wounds and burns. Typical applications include localized treatment with a dye tincture or addition of a few drops of a dye preparation to a water sample, which is then used for skin or mucous membrane washing (McDonnell, 2007). Quinones are natural dyes, which give colours to many forms of plant and animal life, and can be used as agricultural bactericides and fungicides.

In general, the acridines can function effectively against Gram-positive organisms and Gram-negative organisms and they are not inactivated by serum. Acridines compete with H^+ ions for anionic sites on the bacterial cell and are more effective at alkaline than acid pH (Fraise et al., 2004). Mutation and indirect selection of bacteria lead to resistance to these agents (Thornley & Yudkin, 1959).

Acridines focus on the nucleic acids of bacteria. The positively charged structure of

acridine dyes specifically targets the nucleotide base pairs. They also intercalate between base pairs in the double helix, and this binding to DNA causes blocking of replication, gene expression and protein synthesis (Wainwright, 2001).

1.9 Bacterial Resistance to Biocides

A culture is recognized as “resistant” to a biocide when it is not inactivated by the in-use concentration of the biocide, or the biocide concentration used can inactivate other strains of that organism (Russell, 2003). “Resistance” is a relative word. Terms like “reduced susceptibility” and “tolerance” were suggested to be more appropriate, because reduced susceptibility to biocides does not necessarily correlate with failure of the product (Eccles, 2000).

There have been reports of bacterial resistance to biocides since the 1950s. In health care settings, resistance to compounds such as bisphenol, triclosan (Sasatsu et al., 1993) and quaternary ammonium compounds (Romao et al., 2005) has been reported. Low level CHG resistance in MRSA strains to QACs in was first reported in 1983 (Townsend et al., 1983). It was found to be mediated by a quaternary ammonium resistance gene (*qac*A) located on a gentamicin resistance plasmid (Cookson et al., 1991). Bacteria resistant to biocides have also been reported from other settings such as food production, cosmetics, and animal husbandry (Gilbert & McBain, 2003; Akinkunmi & Lamikanra; 2011; Buffet-Bataillon et al., 2012).

It has been suggested the increasing trend for reduced susceptibility to biocides is

associated with incorrect use of biocides. Chronic exposure to a biocide-containing environment may be associated with mutational changes of organisms, and such transformation may lead to changes in susceptibility to antibiotics (McBain et al., 2002).

It was reported that presence of BC could induce the expression of *qac*A and *qac*B (Paulsen et al., 1998). Since the initial observations of resistance to QACs in clinical isolates of *S. aureus* (Townsend et al., 1983), reports have now extended to include staphylococci in the agriculture and food industries (Leelaporn et al., 1995; Heir et al., 1999b; Bjorland et al., 2001; 2003; 2005;).The QAC genes are located on plasmids of the pSK1 plasmid families (Lyon et al., 1984). The pSK1 families include pSK4, pSK23, pSJ24, pWBG53 and pWG50 (Townsend et al., 1983; 1984; Lyon & Skurray, 1987). It has been claimed that the spread of resistant staphylococci has been increased by the use of QAC-based and biguanide antiseptics (Reverdy, 1992, Sasatsu et al., 1994, Buffet-Bataillon et al., 2012; Ho et al., 2012). However, to date there has been limited investigation of carriage of QAC genes in human or animal colonizing strains of staphylococci or of environmental isolates.

Reduced susceptibility to biocides has been documented in several other species of bacteria including *Pseudomonas aeruginosa* (Maillard, 2007), *Mycobacterium chelonae* (Maillard, 2007), *E. coli* (Braoudaki & Hilton, 2004), *Serratia* spp. (Sheldon & Eliopoulos, 2005). The reduced susceptibility of these organisms is due to the inability of the biocides to reach their targets in the cells. Potenski et al. (2003)

reported that following exposure to biocides, mutant *Salmonella* showed multiple resistances to antibiotics. It is necessary to establish the relationship of resistance between biocides and antibiotics.

1.10 Mechanisms of Resistance to Biocides

Cellular changes to the cell wall or envelope or expression of efflux mechanisms may lead to resistance to biocides (Russell, 2002b; Kosmidis et al. 2012). To gain access to the cytoplasm of the cell, biocides need to penetrate into the cell wall and membrane. Bacteria can limit nutrient uptake and reduce their growth rate in order to protect themselves against biocides (Gilbert et al., 1990). Development of biofilms can effectively prevent access of biocides into the bacterial cell.

The expression or overproduction of an efflux system is an important mechanism in both antibiotic and biocide resistant organisms. Many antibiotics or biocides can induce efflux pumps in both Gram-positive and Gram-negative bacteria. Under chronic sub-lethal exposure, bacteria may select hyper-expressing mutants of such efflux pumps to defend the cell against environmental toxicants (McBain et al., 2002; Kosmidis et al. 2012; Ciusa et al., 2012).

Resistance to antiseptics is attributable to the presence of multidrug transporters. Multidrug transporters include several proteins: the major facilitator super-family (MFS), the small multidrug resistance family (SMR), the multidrug toxic compound extrusion family (MATE), the resistance nodulation division family (RND) and the

ATP-binding cassette family (ABC). The MFS, SMR and RND families are PMF (proton motive force)-dependent multidrug efflux systems (Paulsen et al., 1996b). QAC resistant determinants *qac*A/B and *smr* belong to the MFS and the SMR family, respectively.

1.10.1 Families of Multidrug Transporters

1.10.1.1 Major Facilitator Superfamily (MFS)

The MFS is the largest group of secondary active transport proteins and are found in both microorganisms and higher organisms (Saier et al., 1999). It had been known for some time that the MFS functions in the uptake of sugar, but it was later shown to also be involved in drug resistance (Henderson & Maiden, 1990).

MFS is composed of several families, specific for sugars, drugs, metabolites, and anions, and is known as the uniporter-symporter-antiporter family. This family consists of a large number of membrane-bound transport proteins that are present in all classes of living organisms. The MFS proteins consist of at least 400 amino acid residues and function in a variety of cellular processes, including the uptake of essential ions and nutrients and export of toxic compounds (Brown & Skurray, 2001). According to sequence comparisons and topological predictions, MFS can be classified into more than 29 clusters of transporters. A relationship exists between each phylogenetic family and the class of compound transported. Although there are considerable structural differences between MFS proteins, they share a similar secondary structure of 12- or 14-transmembrane segment (TMS). Three MFS families possess drug efflux systems, namely DHA (drug H+ antiporter) 1-3, which is grouped

into members with 12 (DHA-1 and 3) TMS families and 14 (DHA-2) TMS member families (Paulsen et al., 1996b; Saier et al., 1999).

The 14-TMS family includes the plasmid-mediated *qac*A multidrug efflux protein, QacA (staphylococci), Gram-negative multidrug efflux emrB (*E. coli*), and Gram-positive efflux proteins TetK and TetL (Bacillus *stearothermophilus* and *S. aureus*) (Paulsen et al., 1996b). The 12-TMS family includes vesicular amine transporters from higher eukaryotes involved in neurotransmission, which can also mediate multidrug resistance, and some other PMF dependent efflux proteins, such as TetB (Paulsen et al., 1996b).

The first of a number of chromosomally-encoded staphylococcal MFS family drug transporters to be identified and characterized was the NorA family of multidrug resistance proteins (Hassan et al., 2007). A Japanese study suggested that NorA over expression could be the cause of significant antiseptic resistance in 19 of 98 MRSA strains (Noguchi et al., 1999).

1.10.1.2 Small Multidrug Resistance (SMR) Family

The SMR protein bacterial multidrug transporter family consists of proteins that are typically 110 amino acid residues in length with four predicted TMS. They do not exhibit sequence homology with the 12-14 TMS family (Leelaporn et al., 1995; Paulsen et al., 1996b).

In comparison with other multidrug transporter proteins, the SMR protein family has been found to transport lipophilic compounds, primarily QACs as well as a variety of antibiotics, antiseptics, and detergents. In a similar way to MFS proteins, SMRs can also function in drug efflux via an electrochemical proton gradient. Therefore, they are classified as proton-dependent multidrug efflux systems.

The best-characterized SMR family transporter is QacC in *S. aureus* which is located on a range of both conjugative and non-conjugative staphylococcal drug resistance plasmids. SMRs confer resistance to a range of monovalent cationic antimicrobials such as QACs and Ethidium Bromide (EtBr) as well as beta-lactam antibiotics (Fuentes et al., 2005). The conserved charged Glu-13 residue in TMS1 of Smr appears to be essential for activity of the efflux system, since even conservative substitution with asparagine effectively abolishes transport activity (Grinius & Goldberg, 1994).

1.10.1.3 Resistance Nodulation Division Family (RND)

Although examples of the RND family exist in all kinds of living organisms, these proteins are mainly involved in the drug resistance mechanisms of gram-negative bacteria (Paulsen et al., 1996b). The RND family proteins include transporter protein AcrB in the inner membrane, periplasmic accessory protein AraA, and outer membrane channel protein TolC (Koronakis et al., 2004). RND multidrug efflux systems display much wider substrate specificity than the MFS or SMR multidrug efflux proteins (Paulsen et al., 1996b).

1.10.1.4 ATP-binding Cassette Family (ABC)

The SMR and NOD family transporters are driven by PMF, while the ABC families utilize ATP as an energy source (Davidson & Chen, 2004). There are 48 ABC transporters described in humans and 80 in Gram-negative bacteria (Saier, 2000). In Gram-negative bacteria, ABC transporters mediate secretion of their protein substrates across both membranes simultaneously, bypassing the periplasmic space (Binet et al., 1997). They also function critically in nutrient uptake and in secretion of toxins and antimicrobial agents (Davidson & Chen, 2004).

1.10.1.5 Multidrug Toxic Compound Extrusion Family (MATE)

The MATE family contains multidrug pumps identified in Gram-negative bacteria, which utilize both PMF and ATP as their energy source. The first MATE transporter, NorM, was found in *Vibrio parahaemolyticus.* Homologues of NorM have been identified in other gram-negative bacteria. Subsequently, MATE pumps were identified in Gram-positive organisms including *Clostridium difficile* and *S. aureus* (He et al., 2004; Dridi et al., 2004; Kaatz et al., 2005).

1.11 QAC Genes of Staphylococci

Several QAC genes have been identified from various staphylococcal species and (Heir et al., 1995; 1998; 1999a; 1999b; Bjorland et al., 2003; 2005; 2007; Longtin et al., 2011; Lepainteur et al., 2013). The majority of QAC genes are plasmid-borne and the gene determinants belong to two membrane transporter families: MFS family which includes *qac*A, *qac*B and SMR family which includes *smr*, *qac*G, *qac*H, *qac*J.

1.11.1 *qac*A/B Genes

1.11.1.1 *qac*A Gene

The *qac*A gene, which belongs to MFS, was first described in the *S. aureus* plasmid pSK1 (Tennent et al., 1985) and subsequently on the β–lactamase/heavy metal resistance plasmid pSK57 (Gillespie et al., 1986) and is suggested to be homologous with the antibiotic resistant gene *tet*. It encodes the QacA membrane protein which is composed of 514 amino acids with a predicted size of 55kDa by sequence analysis (Rouch et al., 1990). Membrane topological analysis, utilizing alkaline phosphatase and beta-galactosidase fusions as reporters of subcellular location, demonstrated that 14 alpha trans membrane segments (TMS) make up the QacA protein (Brown & Skurray, 2001).

The presence of the *qac*A gene confers resistance to a wide range of monovalent and divalent cationic antimicrobials, and lipophilic compounds, but no resistance has been reported to trivalent cations or to anionic compounds (Tennent et al., 1989; Littlejohn et al., 1992; Mitchell et al., 1998). It mediates resistance by exporting compounds by means of the transmembrane electrochemical proton gradient, driven by PMF (Rouch et al., 1990; Paulsen et al., 1996a).

Several studies have investigated the mechanism of multidrug resistance of QacA, based on investigation of a wide variety of substrates. It was observed by means of fluorescent competition assays that QacA-mediated efflux of the monovalent cation, EtBr, can be competitively inhibited by the presence of other monovalent cations,

such as BC, and non-competitively by that of divalent cations, such as propamidine (Brown & Skurray, 2001). Based on these findings, it was suggested that monovalent substrates either possess a common binding site or have unique but overlapping binding sites and divalent cations bind elsewhere. It was concluded that QacA has at least two unique substrate-binding sites (Mitchell et al., 1999; Brown & Skurray, 2001). This indicated that critical sites or residues in Qa*c*A play an important role in transport. It has been demonstrated that the presence of an aspartic acid residue at amino acid position 323 was related to resistance to divalent cations (Paulsen et al, 1996a).

Both domain and residues of QacA are critical to transporters in that substrate interactions occur within the TMS. In addition to the acidic residue at position 323 for resistance to divalent cations, other amino acids at the TMS are also of considerable importance. The presence of a negative charge at position 34 in TMS1 is of significance to QacA mediated export and it appears that arginine at position 114 in TMS4 is highly conserved within the protein of the DHA2 family (Brown & Skurray, 2001).

Hassan et al. (2006) studied the importance of the 10 intra-membranous proline residues of QacA to determine if these were essential for the formation of structures required for the QacA substrate transport mechanism. Although several proline-substituted QacA mutants failed to confer high-level resistance to selected QacA substrates, no single proline mutation, including those at conserved positions,

significantly inhibited QacA protein expression or QacA mediated resistance to all representative substrates, suggesting that these residues are not essential for QAC substrate transport (Hassan et al., 2006).

In contrast, highly conserved tryptophan residues, within the QacA transport protein are reported to be functionally important. The nine tryptophan residues of the staphylococcal QacA multidrug efflux protein were investigated by amino acid mutation and function analysis (Hassan et al., 2008). Three tryptophan residues (W58, W149, and W173) were revealed to have an important function, especially W58. Alanine substitution (W58A) at this site, which is likely to be located at the extracellular interface of TMS 2, abolished all detectable QacA-mediated resistance and transport function. However second-site suppressor analyses identified several mutations located on TMS 3 or 12 and 13 which could rescue the function of the W58A QacA mutant. This demonstrated that long-distance functional associations exist between residues on opposite sides of the membrane and in distal N- and C-terminal regions of the QacA polypeptide. This is similar to the observation of suppressor relationships between residues in corresponding regions of 12-TMS MFS proteins. Side chain aromaticity at position 58 is not essential for QacA-mediated transport function and W58 is unlikely to participate in important stacking of cation bonds with QacA substrates or adjacent amino acid side chains. However, both the W58A and W58C QacA mutants lack a resistance function. It was proposed that a bulky side chain at position 58 plays an important role in promoting the correct packing of TMS 2 with surrounding helices (Hassan et al., 2008).

Seven tyrosine residues in QacA have been studied for their transport function by examining the phenotypic effect of conservative (aromatic) and non-conservative (non-aromatic) substitutions for these residues. Three tyrosine positions, 63, 410 and 429, were demonstrated to be of significance to QacA-mediated transport and resistance to the majority of tested substrates. A tyrosine residue at amino acid position 63 was found to be highly conserved, and was presumed to function as a drug transporter such as for proton translocation or essential intra-molecular contacts. Tyrosine residues in QacA at position 410 and 429 appear to mediate a QacA-specific function, possibly in composition or by stabilizing part of the QacA drug binding region (Wu et al., 2008).

1.11.1.2 *qac*B Gene

The *qac*B gene was first detected from the *S. aureus* plasmid pSK23 (Lyon and Skurray, 1987) and found to have high structural similarity to *qac*A. The staphylococcal QacB multidrug efflux protein encoded by the *qac*B gene, confers low or no resistance to divalent cation drugs, such as diamidines and biguanides (Paulsen et al., 1996a), but does confer a high level of resistance to a wide range of monovalent cationic dyes and QACs, in a similar way to the QacA protein. However, although QacA and QacB have similar binding affinities and are presumed to possess identical binding sites for monovalent cations, QacA utilizes an independent high affinity binding site for the recognition of divalent cations which is absent in QacB (Mitchell et al., 1999).

According to sequence analysis, the structural difference between *qac*A and *qac*B amounts to only seven nucleotide substitutions (Paulsen et al., 1996a). The acidic residue, aspartic acid (D) at residue 323, in TMS 10 in the QacA polypeptide is replaced by an uncharged alanine residue at the same site in the QacB protein (Paulsen et al., 1996a). This strengthens the evidence that the region functions as the high affinity binding site for divalent cations in QacA, resulting in the phenotypic differences in activity between the proteins. Paulsen et al. (1998) proposed that the *qac*A gene evolved from *qac*B, in that the *qac*B determinant has been detected in staphylococci initially isolated in the 1950s, which considerably predates the earliest isolates positive for *qac*A which were from the 1980s (Littlejohn et al., 1992; Leelaporn et al., 1994; Paulsen et al., 1998). Although this evolutionary process has not been confirmed, the increasing widespread dissemination of QacA may be attributable to selection pressure prevailing in the clinical environment (Brown & Skurray, 2001).

1.11.1.3 QacR, a Multidrug Export Regulatory Protein

Expression of the *qac*A gene carried on pSK1 is controlled by a regulatory protein QacR encoded by a 188 amino acid gene (Grkovic et al., 1998). QacR belongs to the family of regulatory proteins, which includes TetR. The N-terminal ends of QacR possess an α-helix-turn-α-helix (HTH) DNA binding domain, which is same as that of TetR. However, QacA possesses significantly different C-termini, presumed to be associated with the binding of inducing compounds (Rouch et al., 1990; Aramaki et al., 1995; Brown & Skurray, 2001). There is a single amino acid difference at position

104 between QacR polypeptide on pSK1, in which it is tyrosine, and on plasmid pSK23, in which cysteine is present (Brown & Skurray, 2001).

The site of QacR binding is a large inverted repeat (IR) located immediately adjacent to, and downstream from the *qac*A and *qac*B promoters (Grkovic et al., 1998) and IR1, which overlaps the *qac*A promoter sequence (P*qac*A), containing the *qac*A region protein (Brown & Skurray, 2001). In the absence of QacA substrates, QacR binds IR1 and down regulates *qac*A transcription (Grkovic et al., 2002). It was shown that the active DNA-bound form of QacR may be tetrameric, unlike TetR which binds to its DNA-target sequences with high affinity as a dimer (Brown & Skurray, 2001). TetR can only interact with a limited range of structurally similar tetracycline-based compounds, while QacR can directly interact with a range of structurally toxic organic cations through binding to IR1. It was shown that QacR does not bind to or auto-regulate its own promoter (P*qac*R) (Brown & Skurray, 2001).

1.11.2 The *smr* Gene Family

On the basis of SMR sequence alignments and phylogenetic analysis, the SMR family is grouped into three subclasses: the small multidrug pumps (SMP), suppressors of groEL mutation proteins (SUG), and paired small multidrug resistance proteins (PSMR). The proteins encoded by the *smr* gene (formerly *qac*C), *qac*G, *qac*H and *qac*J all belong to the SMP group (Bay et al., 2008).

The *smr* gene was first identified in *S. aureus* on the pSK41 plasmid (Littlejohn et al.,

1991). *qac*G and *qac*H were initially isolated from staphylococci in the food industry (Heir et al., 1998; 1999a), while *qac*J has been mainly isolated from staphylococci of equine origin (Bjorland et al., 2003). All SMR proteins are of a similar size (-11-12kDa) having a four trans-membrane helix topology with a highly conserved key residue Glu-14 (Muth & Schuldiner, 2000). The SMR protein families use PMF to drive drug efflux (Paulsen & Skurray, 1993) and are classified as proton-dependent multidrug efflux systems.

The SMR proteins are well known for their ability to confer resistance to a range of QACs and cationic dyes. Substrates of SMR proteins include QACs such as BC, cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride (CTPC) and inter-calating dyes such as EtBr, and crystal violet (Bay et al., 2008). Although most Smr proteins confer host resistance to a range of QAC and cationic dyes, some Smr family members possess unique resistance profiles extending resistance to other compounds. For example, QacJ confers a higher level resistance to BC than QacG and QacH (Bjorland et al., 2003), and QacH positive strains have a higher MIC to EtBr (Heir et al., 1998). Regulatory control mechanisms have not been identified for Smr gene expression (Grkovic et al., 2002).

1.11.2.1 *smr* Gene

The *smr* gene, which is identical to *qac*C/*qac*D, has been reported to be located on large (>20kb) conjugative plasmids (e.g. pSK41, pTZ22, pTZ 20) or on small (<3kb) rolling circle replication non-conjugative plasmids including pSK89, pSK108, pST827,

and pNVH99 (Littlejohn et al., 1991; Leelaporn et al., 1995). Some medium size plasmids may also carry the *smr* gene. A new 5.55kb plasmid carrying the *smr* gene, pSP187 in *Staphylococcus pasteuri*, was reported in 2007 (Bjorland et al., 2007b). Smr protein, encoded by *smr,* contains 107 amino acids and consists of four transmembrane (TM) α-helices with short hydrophilic loops. Although *smr* is widely distributed in a variety of staphylococcal plasmids, these plasmids normally include open reading frames (ORFs). That is they consist of a multidrug resistance determinant and a replication gene. The ORF of *smr* is conserved, displaying little genetic diversity (Alam et al., 2003a).

The Smr protein can mediate multidrug export as well as working as a multidrug exporter (Paulsen et al., 1995). It has been shown that the *smr* gene in *S. epidermidis* mediates resistance both to EtBr and to several beta-lactam antibiotics (Fuentes et al., 2005). This was the first report of a SMR pump being involved in resistance to beta-lactam antibiotics. Site-directed mutagenesis on *smr* indicated that the replacement of Glu-24 with Asp enables bacteria to display greater resistance to EtBr without affecting the expression level of the protein (Grinius & Goldberg, 1994).

1.11.2.2 ***qac*G Gene**

As mentioned above, the *qac*G gene was first detected in 1999 from a 2.3 kb staphylococcal plasmid pST94 from a food industry isolate (Heir et al., 1999a). The 107 amino acid protein, QacG, encoded by *qac*G shows 69.2% similarity with SMR and 45% similarity to QacE. QacG differs from the SMR protein in 33 of 107 amino

acid positions (Heir et al., 1999a).

QacG confers resistance to EtBr and QACs via a proton dependent efflux. There is little difference in MIC values of BC and EtBr between *qac*G isolates and *smr* isolates. QacG uses the same resistance mechanism as the SMR protein (Heir et al., 1999a) and the hydrophobic amino acid in the QacG and SMR proteins share similar locations (Heir et al., 1999a).

1.11.2.3 *qac*H Gene

The first description of *qac*H was in 1998 in a strain of *Staphylococccus saprophyticus* isolated from a poultry processing plant (Heir et al., 1998). The *qac*H gene present on the 2.4kb plasmid p2H6, encodes the QacH protein containing 107 amino acids. It shows high homology with the SMR protein family, having 78% similarity to Smr and 70% to QacG (Heir et al., 1998). It also has significant similarity to other members of the SMR protein family, including QacE (41%). QacH protein confers resistance to QACs with high-level resistance to EtBr and low-level resistance to proflavine, which differs from Smr and QacG (Heir et al., 1998). Although site-directed mutagenesis of the *smr* gene by replacing the Asp-24 residue with Glu-24 affects resistance to EtBr, this mutation on the *qac*H gene did not produce a similar result and it was suggested that other residues and/or domains of QacH may be responsible for the altered phenotypic characteristics (Heir et al., 1998).

1.11.2.4 *qac*J Gene

The *qac*J gene was first identified in Norway in 2003, located on a 2.65kb plasmid PNVH01 from equine strains of *S. aureus*, *S. intermedius,* and *S. simulans* (Bjorland et al., 2003). PNVH01 belongs to the PC194 family of rolling circle replication plasmids and contains two ORFs, named repNVH01, and *qac*J. The *qac*J gene encodes the QacJ protein which also contains 107 amino acids. Homology analysis indicated that QacJ is a new group of the SMR protein family, with 72.5% similarity to Smr, 82.6% to QacG, and 73.4% to QacH. In comparison to Smr, QacJ confers increased resistance to BC, but mediates the same level of resistance to CTAB (Bjorland et al., 2003). This indicates that an amino acid substitution affects substrate specificity rather than a transcriptional change caused by a mutation in the *qac*J promoter, as a promoter mutation would be likely to lead to a proportional increase or decrease in resistance to both BC and CTAB (Bjorland et al., 2003).

1.11.2.5 *qac*E and *qac*EΔ1

*qac*E and *qac*EΔ1 were isolated from an integron on a 51.4 kb plasmid R751, which was original identified from *Klebsiella aerogenes* (Jobanputra & Datta, 1974; Paulsen et al., 1993). The two ORFs, ORF1 and ORF4, on the 3’conserved segment of the integron, were designated as *qac*E and *qac*EΔ1, respectively (Paulsen et al., 1993). Analysis revealed the proteins QacE and QacEΔ1 encoded by these two genes shared a certain degree of homology with QacC, and that these two proteins mediate resistance to disinfectants by PMF (Jobanputra & Datta, 1974). Strains containing either *qac*E or *qac*EΔ1 are resistant to both intercalating dyes and QACs, but the

presence of *qac*E confer higher resistance levels. Neither confers resistance to either diamidines or biguanides (Jobanputra & Datta, 1974).

1.12 Epidemiology of QAC Genes in *Staphylococcus* spp.

The distribution of the antiseptic resistance genes in *S. aureus* has been investigated in clinical isolates from several countries (Wang et al., 2008a; Vali et al, 2009, Longtin et al., 2011; Ho et al., 2012; Karki et al.,2012; Lepainteur et al., 2013). Compared to *S. aureus*, the epidemiology of antiseptic resistance genes in CNS has received little attention in human isolates, although Leelaporn et al. (1994) detected *qac*A and *smr* in human CNS clinical isolates. Recently, *qac*G, *qac*H, and *qac*J genes were reported in *S. haemolyticus* human clinical isolates (Correa et al., 2008). The epidemiology of antiseptic resistance genes according to the source of isolates is summarized in Table1.3

1.12.1 QAC Genes in *Staphylococcus* spp. of Human Clinical Isolates

1.12.1.1 Research in Asia

Noguchi et al (1999) were the first to analyze the distribution of antiseptic resistance genes in clinical isolates. *qac*A/B and *smr* were detected in 10.2% and 20.4 % of MRSA isolates collected in Japan in 1992, respectively. The results suggested that QAC genes were not widespread in MRSA (Noguchi et al., 1999). More recently, an investigation of clinical MRSA isolates collected in Japan between 1998-1999 revealed 44.3% *qac*A/B and 3.4% *smr* positive strains (Noguchi et al., 2005).

In a further study in Japan using strains isolated between 1999 and 2004, the prevalence of *qac*A/B genes was considerably lower in MRSA clinical isolates from patients with impetigo and SSSS (1.3%) than in MRSA isolates from patients with other diseases, (45.9%,) (Hidemasa Nakaminami, 2008). As a result, MRSA isolates from the patients with impetigo and SSSS were more susceptible to antiseptic agents. The MIC of BC, determined by the agar doubling dilution method, for the MRSA isolated from patients with impetigo and SSSS ranged from 2-4 mg/L, while the MIC range for the strains from patients was 1-7 mg/L. The MIC of CHG was 1 to 4 mg/L. It was concluded that a lower distribution rate of *qac*A/B genes resulted in higher susceptibilities to cationic antiseptic agents in MRSA isolated from patients with impetigo and SSSS (Hidemasa Nakaminami, 2008).

Alam et al (2003a, 2003b) also investigated *qac*A/B and *smr* distribution in 522 *S. aureus* clinical isolates from a Japanese hospital. The *qac*A/B gene was detected in 32.6% of MRSA and 7.5% MSSA, whereas *smr* was present in only 3.3% MRSA and 5.9% MSSA. Coexistence of *qac*A/B and *smr* was only found in two strains of MSSA.

A similar study was performed in Taiwan using a total of 240 nosocomial MRSA isolates obtained in 1990, 1995, 2000 and 2005. There was an increasing trend for *qac*A/B gene carriage rate in MRSA from no detection in 1990 to 16 *qac*A/B positive isolates (26.7%) in 1995 which all belonged to a single clone. In 2005, there were 20 *qac*A/B positive isolates (33.3%), which belonged to seven different clones, showing that the *qac*A/B gene had spread in the hospital (Wang et al., 2008b).

A study In China in 2008, reported *qac*A/B in 61.6% of clinical MRSA isolates (Wang et al., 2008a). This result was much higher than the 5% reported in a study conducted in China in 1999 suggesting that prevalence of these genes conferring antiseptic resistance may be increasing (Noguchi et al., 2005). There is no data on the prevalence of these genes in isolates from Hong Kong.

1.12.1.2 Research in Europe

In a study of European *S. aureus* isolates, 42% harboured *qac*A/B genes. Comparison of rates in MRSA and MSSA showed 62.6% MRSA were positive compared with 12% MSSA. Far fewer strains harboured *smr* genes (5.8%), with no significant difference in carriage rates in MRSA (6.4%) and MSSA (5%). *qac*A/B and *smr* genes were detected concomitantly in five isolates (1%) (Mayer et al., 2001).

In UK, examination of clinical MRSA isolates revealed 8.3% carried *qac*A/B, 44.2% *smr*, 3.3% *qac*H, and 36.7% *nor*A, but *qac*G gene was not detected (Vali et al., 2008). In another study of clinical isolates of *S. aureus* including 38 HA-MRSA, 25 CA-MRSA, 25 MSSA, and 6 VISA (intermediate resistance to vancomycin) strains, CA-MRSA isolates did not carry *qac* genes and *qac*G, H and J were not detected in HA-MRSA. Ten HA-MRSA isolates, one MSSA isolate and one VISA isolate carried *smr*. One VISA isolate carried *qac*A and *smr* (Smith et al., 2008).

1.12.1.3 Research in Other Areas

Similar studies have been carried out in Australia, Argentina, and Brazil. Antiseptic

resistance genes were first reported in CNS isolates in Australia, who reported 50 % carried only *qac*A, 10% carried only *smr*, and the remaining 40% had both *qac*A and *smr* (Leelaporn et al., 1994). A study in Argentina, first reported the presence of *qac*G, *qac*H, and *qac*J in *S. haemolyticus* of human origin with 24% of isolates carrying *qac*A/B, 100% *smr*, 52% *qac*G, 47% *qac*H, and 19% *qac*J. It was concluded that *S. haemolyticus* could act as a reservoir of resistance determinants and serve as a donor to more virulent Staphylococci (Correa et al., 2008). In Brazil, it was reported that 80% of MRSA isolates collected in 2002 and 2003 from three government hospitals carried *qac*A/B genes (Miyazaki et al., 2007). There appears to be considerable geographical variation in prevalence of QAC genes.

1.12.2 QAC gene in *Staphylococcus* spp. of Animal Origin

S. aureus and CNS are common causes of bovine and caprine intramammary infections (Bjorland et al., 2005; Behiry et al., 2012) and are associated with various infectious diseases in horses, particularly skin and bone disorders (Shimizu et al., 1991). Data about the antiseptic resistance genes of *S. aureus* and CNS in animals is limited, and has focused mainly on bovine, caprine, and equine isolates, although a recent study revealed the presence of *smr* and *qac*G in MRSA isolated from pork carcasses (Wong et al., 2013).

Bjorland et al. (2001) reported the presence of the *smr* gene in bovine *S. aureus* isolates, whilst *qac*A was found in both bovine and feline *S. haemolyticus* isolates (Anthonisen et al., 2002). *qac*H was first detected in *S. saprophyticus* from a poultry

processing plant (Heir et al., 1998) and *qac*J was found in equine *S. aureus, S. simulans* and *S. intermedius* (Bjorland et al., 2003). Bjorland et al. (2005) also identified QAC genes in isolates from 21% of isolates from cattle (*qac*A/B, *smr*, *qac*G and *qac*J) and 10% from goats (*qac*A/B, *smr*) and concluded that both the intra- and inter-species spread of QAC plasmids and strains has lead to the widespread distribution of Staphylococci carrying QAC genes.

1.12.3 QAC Genes in *Staphylococcus* spp. of Environmental Origin

In the food industry, the presence of Staphylococci resistant to disinfectants is of special concern because of the need to avoid contamination and spoilage. The presence of antiseptic resistant Staphylococcus spp. hosting *qac*A/B and *smr* from different food production environments and food products has been reported (Heir et al., 1999b). Additionally, *qac*G and *qac*H were found in food and food processing plants (Heir et al., 1999a; Bjorland et al., 2003). Following identification of 24 CNS isolates, mainly *S. epidermidis* and *S. saprophyticus*, hosting QAC genes, it was concluded that Staphylococci with reduced antiseptic susceptibility appeared to be widely distributed in food environments (Heir et al., 1999a). However, other environmental isolates have not been characterized for QAC gene carriage.

1.13 Risk Factors for QAC Gene Carriage in *Staphylococcus* spp.

Although the risk factors for QAC gene carriage have not been investigated in detail, some factors discussed below could potentially be associated:

1.13.1 Occupation

Nurses play an important role in transmission of pathogens such as MRSA as they may become colonized and act as a reservoir for the organism. They can act as an interface between hospitals, long-term care facilities, nursing homes, and the community acting as reservoirs for cross-infection, or may themselves become colonized or infected (Muder et al., 1993; Eveillard et al., 2004; Vonberg et al., 2006; Hughes et al., 2011; Stone et al., 2012). Obviously, nurses are more likely to be in contact with both pathogens and biocides. An increasing prevalence of antiseptic-resistant Staphylococci has been detected in clinical isolates in recent years (Smith et al., 2008; Wang et al., 2008a; Vali et al., 2008; Sheng et al., 2009; Longtin et al. 2011; Ho et al 2012a, b; Karki et al.,2012; Lepainteur et al., 2013; Horner et al., 2012a). It is likely that nurses could become contaminated and spread antiseptic-resistant Staphylococci in hospitals and in communities. This may be an unexpected and important challenge for disease prevention and control in public health.

1.13.2 Age

Owing to ageing and decreasing ability of the immune system, the elderly are prone to suffer from more infectious diseases compared to the general population. MRSA is an important cause of morbidity and mortality among elderly people in hospitals and nursing homes. Like nurses, the elderly also play an important role in transmission of MRSA. It is believed that most MRSA isolates in nursing homes are acquired by the

elderly when they are admitted to affiliated hospitals (von Baum et al., 2002). Conversely, outbreaks in hospitals may sometimes be attributed to MRSA acquired in nursing homes (Bradley, 1997; Kerttula et al., 2005; Ludden et al., 2013, Verrall et al., 2013). In the Netherlands, there is increasing concern that nursing homes may be reservoirs of MRSA (Goettsch et al., 2000). It is possible that QAC genes may be more common in Staphylococci from the elderly than in the general population.

1.13.3 Environmental Factors

Until recently, the role of the environment in the spread of MRSA was not well recognized, and many national guidelines provide few details on environmental decontamination regimens (Sexton et al., 2006). There is increasing evidence that the environment may play a significant role in spread of pathogens. Environmental contamination with endemic MRSA may pose a risk both to patients subsequently in the same room and to other concurrent patients by being transmitted by healthcare workers in the absence of optimal hand hygiene (Huang et al., 2006; Drees et al., 2008). Large quantities of biocides are used for disinfection and decontamination, and simulation studies have shown that low levels of biocide in the environment after usage may lead to increased selective pressure for disinfectants and antibiotic resistance (McBain et al., 2003; Randall et al., 2004). However, to date there has been very limited reporting of QAC genes in organisms isolated from the environment (Baquero et al., 2008; Kosmidis et al., 2012). Environmental

contamination with biocide may increase the risk for antiseptic resistance gene carriage in *Staphylococcus* spp.

1.13.4 Relationship between Antibiotic Resistance and Carriage of QAC genes in *Staphylococcus* spp.

There is increasing concern that use of biocides might not only reduce biocide effectiveness but also change susceptibilities to antibiotics, although this has not been demonstrated in a clinical setting. It has been demonstrated in an in vitro experiment that multidrug resistance affected by efflux pump activity was over expressed in *S. aureus* after repeated exposures to sub lethal concentrations of biocides and dyes (Huet et al., 2008). Fraise (2002) suggested that genetic linkages may exist between biocide resistance genes and antibiotic resistance genes, possibly being carried on the same plasmids or acquired concurrently. Certain biocides could select for antiseptic resistant strains and maybe co-select antibiotic resistant strains. There is convincing evidence that common mechanisms that confer resistance to biocides and antibiotics are present in bacteria and that bacteria can acquire resistance through the integration of mobile genetic elements. These elements carry independent genes conferring specific resistance to biocides and antibiotics. Epidemiological studies are lacking to show the occurrence of the genes in the general population and risk factors for carriage.

1.14 Assessment of Bacterial Susceptibility to Biocides

Although QACs have been used since 1930, there are few reports about apparent

resistance to these agents. Minor changes in minimum inhibitory concentrations (MIC) have been described associated with the presence of various genes in bacteria, but these changes do not affect their activity as they are far below at-use levels (Gilbert & McBain, 2003). The changes in MIC may be associated with changes in acidic phospholipid content of the bacterial membrane (Wright & Gilbert, 1987) or be connected with acquisition, or hyper expression of multi-drug efflux pumps (e.g. *qac* genes) (Heir et al., 1999a).

Determination of MICs has been used in many studies as an indicator of changes in bacterial susceptibility to a biocide (Russell & McDonnell, 2000). Several reports have shown that the level of resistance can increase through selection if the bacterium is repeatedly exposed to a low concentration of biocide or increasing concentrations of a biocide (Thomas et al., 2000; Walsh et al., 2003; Maillard, 2007). Using MIC to determine the resistance to biocides is debatable, because in the clinical environment, higher concentrations of biocides are used and MICs are usually not elevated to a relevant level. Even bacterial strains showing a significant increase in MICs to biocides remain susceptible to in-use concentrations of the same biocides (Thomas et al., 2005; Lear et al., 2006). Determination of MIC change alone does not demonstrate clinical relevance. As increased in vitro MICs do not correlate with reduced effectiveness, MIC determination may not be as relevant for antiseptics as for antibiotics.

The determination of minimum bactericidal concentrations (MBCs) is a more

appropriate methodology that allows comparison of lethality between a standard and resistant strains. The determination of the lethality of a biocide requires use of a neutralizing agent or removal of the biocide to prevent overestimation of biocidal effects.

The determination of the inactivation kinetic is an alternative method to assess bacterial resistance. Following exposure to a biocide, the inactivation curve of the organism to the biocide may be determined, which can provide information about the nature of bacterial resistance.

1.15. Conclusions

This review has demonstrated that there has been an increase in prevalence of QAC genes in Staphylococcus spp. in both clinical isolates and isolates from food and animals. This increased prevalence has been associated with increased use of antiseptics and may be selected for by presence of antiseptic residues. The association between antibiotic resistance and antiseptic resistance has been demonstrated. However, many of the studies performed have suffered from bias due to relatively small sample sizes. In particular, little attention has been paid to risk factors for colonization with strains with QAC genes such as occupation in healthcare or increased age. In addition the presence of these genes in environmental isolates has not been well investigated. There have been no studies on rates of QAC gene carriage in clinical isolates in Hong Kong. Further work is also needed to develop alternative methods to detect increases in resistance to antiseptics as dilution

methods usually involve large increments between dilutions at higher concentrations. Availability of a suitable gradient method could help to more accurately determine changes in MIC due to increased antiseptic exposure.

Table 1.1 Summary of the active substances in biocidal products

Biocide type	Example(s)	General examples of use(s)
Alcohols	Ethanol	Hand sanitising
Aldehydes	Formaldehyde	Virucidal agent
	Formaldehyde-releasing agents	Topically; irrigation solutions
	Glutaraldehyde	Endoscope disinfection
	Orthophthalaldehyde	Endoscope disinfection
Biguanides	Chlorhexidine	Antiseptic, disinfectant, pharmaceutical preservative
	PHMB (polymeric)	Swimming pool disinfection, contact lens solutions
Cationic Dyes	Acridines, Ethidium bromide	Topical antimicrobials
Diamidines	Propamidine	Eye drops, Contact lens solutions
Halogens	Sodium hypochlorite, NaDCC industrial	Disinfection of blood spillages; sanitization compounds
	Povidone-iodine	Pre-operative skin cleansing
Isothiazolones	Chloromethyl and methyl derivatives	Preservatives (cosmetics, pharmaceuticals)
Peroxygens	Hydrogen peroxide	Antiseptic, disinfectant, fumigation
	Peracetic acid	Endoscope disinfectant
Phenylethers	Triclosan	Body washes, dental hygiene
Phenols and cresols	Dettol, Lysol	Preservatives, disinfectants
Quaternary Ammonium Compounds (QACs)	BC, CTAB	Skin disinfection; preoperative disinfection; antiseptics; pharmaceutical preservatives
	Cetrimide/cetylpyridinium	
Vapour phase sterilants	Ethylene oxide	Low-temperature sterilization of thermostable materials
PHMB = polyhexamethylene biguanide; NaDCC = sodium dichlorocyanurate; BC = benzalkonium chloride; CTAB = cetyltrimethylammonium bromide.		

(Table modified from Russell, 2003; Bloomfield & Exner, 2003).

Table 1.2 Chemical Structures of Common Cationic Biocides (Adapted from McDonnell and Russell, 1999)

Biocide regents	General Structure	Examples	Chemical structures
Quaternary Ammonium Compounds (QACs)	$R^3-N^+(R^1)(R^2)-R^4$	Benzalkonium chloride (BC)	$C_6H_5-CH_2-N^+(CH_3)_2-C_nH_{2n+1}\ Cl^-$ (n = 8 to 18)
Biguanides	$R_2N-C(=NR)-NR-C(=NR)-NR_2$;	Chlorhexidine	

Table 1.3 Epidemiology of QAC genes in *Staphylococcus* spp.

Origins	Article	Isolate Source	Isolates	Result
Human	Leelaporn et al., (1994)	Australia	Clinical 40 CNS	50% (20/40) only *qacA*, 10 % (4/40) contained only *smr*, 40% (16/40) both *qacA* and *smr* by DNA hybridization analysis.
	Mayer et al., (2001)	Europe	Clinical 497 SA	*qacA/B in* 42% SA; *qac*A/B in 63% MRSA and 12% MSSA; *smr* in 5.8% SA, 6.4% MRSA and 5% MSSA.
	Alam et al., (2003)	Japan	Clinical 552 SA	In 552 SA, *qac*A/B in 32.6% MRSA and 7.5% MSSA; *smr* in 3.3% MRSA and 5.2% MSSA
	Noguchi et al.,(2005)	Japan	Clinical 413 MRSA	*qac*A/B (47.9%); *smr* (3%)
	Noguchi et al., (2006)	Japan	Clinical MRSA	Less *qacA/B* genes (1.3%, 1/76) from patients with impetigo and SSSS than from patients with other diseases (45.9%, 95/207)
	Miyazaki et al., (2007)	Brazil	Clinical 74 MRSA	59 (80%) *qacA/B* gene
	Wang et al., (2008)	Taiwan	Clinical 240 MRSA	Carriage rate for *qac*A/B increased from 0 in 1990 to 26.7% in 1995 (1 clone only). By 2005, 33.3% and 7 clones.
	Wang et al., (2008)	China	Clinical 131 MRSA	*qacA/B* 80 (61.6%)
	Vali et al., (2008)	UK	Clinical 120 MRSA	*qacA/B* (8.3%), *smr* (44.2%), *qacH* (3.3%), *norA* (36.7%), *qac*G (0%)
	Smith et al .,(2008)	UK	Clinical 94 SA	CA-MRSA no qac genes, *qac*A/B in 26.3% (10/38) HA-MRSA and 66.7% (4/6) VISA, *smr* in 5.26% (2/38)HA-MRSA ,4% (1/25) MSSA and 16.7 (1/6) VISA.No *qac*G H J
	Correa et al., (2008)	Australia	Clinical 21 *S.haemolyticus*	24% (5/21) *qacA/B* gene, 100 % (21/21) *smr* gene, 52 %(11/21) *qacG* gene, 47 %(10/21) *qacH* gene and 19 %(4/21) *qacJ*
Animal	Heir et al., (1998)	Norway	CNS	*qac*H in *Staphylococcus. saprophyticus* from a poultry processing plant
	Bjorland et al., (2001)	Norway	SA	*smr* gene in bovine *S. aureus* isolates
	Sundet et al., (2002)	Norway	CNS	*qac* A in bovine and feline *S. haemolyticus* isolates

	Bjorland et al., (2003)	Norway	SA, CNS	*qac*J in equine *S. aureus, S. simulans* and *S. intermedius*
	Bjorland et al., (2005)	Norway	CNS	QAC genes in 21% (27/127) of cattle herds (*qac*A/B, *smr, qac*G, and *qac*J) and 10% (13/12.7) goat herds (*qac*A/B, *smr*)
Food	Heir et al .,(1999)	Norway	CNS	*qac*A/B and *smr* from different food production environment and food products has been reported
	Heir et al., (1999)	Norway	CNS	*qac*G were found in food and food processing plants

CHAPTER 2

AIMS OF THE STUDY

2.1 Aims

The aims of this research study were structured to provide significant and original contributions to the knowledge base of antiseptic resistance genotypes in *S. aureus* and coagulase-negative staphylococci in selected populations and environments in Hong Kong in order to allow better understanding of the epidemiology of antiseptic resistance and provide a reference base for future monitoring of temporal trends. Data generated in the study would also allow for comparison with the overseas situation and allow for better formulation of control measures. Secondly, this research aims to provide a framework for improved effective detection of antiseptic resistance by development of a gradient technique for MIC determination, to improve antimicrobial selection in treatment of *S. aureus* and MRSA carrying QAC genes, as well as to improve clinical outcomes and infection control. Finally, the study aimed to determine the effects of increased and prolonged exposure to disinfectants on levels of antiseptic resistance by means of in vivo testing of strains with QAC genes using both the gradient technique developed and a standard method. To achieve the above aims, the proposed research has the following

objectives:

1. To determine the distribution and rates of QAC genes in *S. aureus* and coagulase-negative staphylococci in selected populations and environments in Hong Kong;
2. To investigate if there is an increased risk of presence of QAC genes in colonizing staphylococci of health care workers , represented by nurses, and the elderly compared to the rates in the general population;
3. To investigate presence of QAC genes in staphylococci contaminating frequently contacted sites in the community and the hospital environment.
4. To determine if the presence of QAC genes is associated with an increased rate of antibiotic resistance in colonizing and contaminating strains of *S. aureus* and coagulase-negative Staphylococci.
5. To optimize and evaluate the SGE method for determining the MIC of antiseptics for *S. aureus* in comparison with standard methods.
6. To determine if exposure to antiseptic leads to an increase in MICs of exposed strains by means of an induction study using sub-inhibitory concentrations of antiseptic. Changes in susceptibility would be measured using both the standard method and SGE.

CHAPTER 3

PREVALENCE OF STAPHYLOCOCCI WITH ANTISEPTIC RESISTANCE GENES IN CARRIAGE ISOLATES FROM NURSES AND THE GENERAL POPULATION IN HONG KONG

3.1 Introduction

Methicillin-resistant *S. aureus* (MRSA) is a common nosocomial isolate in hospitals causing human disease worldwide (Stefani & Varaldo, 2003). There is increasing evidence that owing to dramatically increasing prevalence of multi-drug-resistant strains, CNS acting as opportunistic pathogens can also be a frequent and important cause of disease (Carbon, 2000).

To prevent the spread and growth of pathogens in the hospital and the community, antiseptics, such as CHX and BC, are widely used in decontamination of surfaces, hand disinfection, and decolonization of patients with *S. aureus.* Guidelines recommend using surface antiseptics for decolonization of high risk groups and

routine cleansing of all patients when basic interventions have failed to reduce rates of MRSA infection to acceptable levels. Whilst rigid implementation of infection control practices have led to reductions in MRSA infection rates (Thompson et al., 2009), its continued presence in the hospital has been evidenced by isolation from numerous locations, including catheters and disinfectant soap dispensers (Brooks et al., 2002).

Staphylococcal strains harbouring plasmid-encoded genes conferring increased minimum bactericidal concentrations (MBCs) to antiseptics have been reported. These antiseptic resistance (QAC) genes, including *qac*A/B and *smr*, were present in 32.6% of clinical isolates of MRSA in Japan (Alam et al., 2003a) and in 63% of European (Mayer et al., 2001), 62% of Chinese (Wang et al., 2008a), and 80% of Brazilian isolates (Miyazaki et al., 2007). It appears that carriage of these genes is becoming more common in MRSA, as a longitudinal study from Taiwan reported an increasing trend of strains positive for QAC genes. No QAC gene-positive strains were detected in 1990 but, by 1995, 26.7% were *qac*A/B positive, rising to 33.3% by 2005 (Wang et al., 2008b).

There is increasing evidence that widespread use of biocides may impose selective pressure and contribute to the emergence of bacteria with decreased antiseptic susceptibility. Similarly, selective pressure from antibiotics employed in clinical practice has resulted in the development and spread of antibiotic resistance determinants. The use of biocides may contribute to the emergence of

cross-resistance and co-resistance between widely used biocides and antibiotics (Koljalg et al., 2002; Walsh et al., 2003). Mobile genetic elements such as plasmids and transposons may allow rapid spread of resistance among species. QAC determinants have been shown to be located adjacent to antibiotic resistance genes on transferable genetic elements such as pST6, and transposon Tn4002 (Sidhu et al., 2001a).

The distribution of QAC genes in *S. aureus* and CNS has been investigated in clinical isolates from several countries, with most focus on MRSA (Alam et al., 2003b; Noguchi et al., 2005; 2006b; Wang et al., 2008a). However, little is known about the frequency of QAC genes in colonizing strains in the general population. Nurses may be colonized by hospital strains, which may be more likely to have QAC genes. In this study, the prevalence of QAC genes in colonizing strains of *S. aureus* and CNS from nurses and the general public was determined and risk factors for their carriage investigated. The association between the presence of QAC genes and antibiotic resistance in isolates was also determined.

3.2 Materials and Methods

3.2.1 Epidemiological Investigation

3.2.1.1 Sample Size for the Study

A cross-sectional study of nasal colonization rates with *S. aureus* and CNS harbouring QAC genes in nurses was performed. The frequency of carriage of *S.*

aureus is about 20% in Hong Kong, which is similar to levels reported elsewhere (O'Donoghue & Boost, 2004; Kluytmans & Wertheim, 2005a; Boost et al., 2008a), but the prevalence of QAC genes in carriage isolates has not been investigated. QAC genes include *qac* A-J, with *qac* A/B and *smr* being more common in human isolates than other QAC genes (Noguchi et al., 1998; 1999; Alam et al., 2003a). With reference to Mayer et al.'s (2001) results, in which the *qac* A/B gene carriage rate in *S. aureus* isolates was 42%, while that of *smr* was only 5.8%, the carriage rate of *S. aureus* having a QAC gene in nurses was estimated as 5% based on the lower rate of *smr.* A sample of 202 nurses was estimated based on a *S. aureus* carriage rate of 20% and assumed 5% carriage rate of QAC genes in *S. aureus* and CNS within 3% error with 95% confidence level according to the formula:

$$N=\mu a^2\pi\ (1-\pi)/\delta^2.$$

It was planned to compare rates of QAC gene presence in all *S. aureus* and a random selection of 200 CNS isolated from 775 nasal swabs collected in a recent study of the general population as a control group assuming the carriage rate to be similar.

3.2.1.2 Nurses

Nurses from ten different hospitals in Hong Kong were invited to join the project. These subjects were attending Hong Kong Polytechnic University as students of a part-time Masters degree. The nurses in this study were designated as either "fresh" nurses with bachelor degree (newly graduated nurses with < 2 years nursing experience in the hospital) and "experienced" nurses (working time ≥2 years). Their

health status was good without obvious infections.

3.2.1.3 The General Population

The general population samples were randomly selected from isolates of dog owners who took their dogs to animal clinics and families of students attending the Hong Kong Polytechnic University. QAC gene positive levels in *S. aureus* isolated from these subjects (n=186) and a random selection of 200 CNS isolated were compared with those of carriage isolates of nurses.

3.2.1.4 Questionnaire for Nurses

A questionnaire was used to investigate factors associated with risk of carriage of QAC positive strains. This questionnaire, which was completed by the subjects at the time of sample collection, provided information including demographic details (age, sex), long term health conditions, years of working experience, antibiotic use in past six months, and contact with MRSA patients in the last three months, and antiseptic usage in daily life. The questionnaire is shown in Appendix II. The general population did not complete the questionnaire.

3.2.1.5 Confidentiality and Ethical Considerations

The study was approved by the Human Subjects Ethics Sub-Committee of The Hong Kong Polytechnic University. All the invited subjects were fully informed of the significance, advantages, the detailed procedures, and the potential risks of this project. 90% of those invited took part in the project. Written informed consent was

obtained from all subjects enrolled in the study. The consent form is shown in Appendix I and V. Questionnaires, which did not include the name of the participant using only a code for identification, were kept in a locked filing cabinet accessed only by the investigator.

3.2.2 Sample Collection

The subjects were given clear oral and written instructions on the use of a sterile cotton-tip swab (Transwabs, Medical Wire & Equipment Co Ltd, Corsham, UK) for sampling the nares. The swab was pre-moistened with sterile saline before inserting approximately 1-2 cm into one nostril. The swab was rotated against the nasal mucosa and then gently withdrawn. All swabs were inoculated into transport medium and kept at 4 ^{o}C until sample collection was completed (O'Donoghue & Boost, 2004). Cultures were performed in the laboratory within eight hours of collection.

3.2.3 Laboratory Investigation

3.2.3.1 Bacterial Strains, Culture Media and Growth Conditions

Swabs were placed into Brain Heart Infusion broth (Oxoid, Basingstoke, UK) with 5% NaCl and incubated at 37 ^{o}C for 24 h. The additional salt was used as a selective agent for *Staphylococcus.* Broths were subcultured onto mannitol salt agar (Oxoid) and incubated for 48h. *S. aureus* strains fermented mannitol and produced bright yellow colonies, whilst other staphylococci produced pink or light yellow colonies. If

more than one colony type of CNS was present, the two predominant types were sub-cultured.

3.2.3.2 Isolation and Identification of Staphylococci

All organisms with staphylococcal morphology were identified by catalase test and tube coagulase test, and confirmed by 16S rRNA genes (Martineau et al., 2001). Tube coagulase test, the most widely used criterion for the identification of pathogenic staphylococci associated with acute infections, was performed, using plasma containing EDTA which was obtained from local hospitals and had been tested for clotting capability and lack of infectious agents.

A 0.1 mL aliquot of an overnight broth culture of each isolate was mixed with 0.5 mL of plasma in a glass tube, and the mixture was incubated at 37 ^{o}C overnight. After 24 h, the tubes were tilted to observe clot formation. Any degree of clot formation was recognized as a positive result. Tube coagulase test negative strains were reported as CNS. *S. epidermidis* was differentiated from other CNS by PCR (Martineau et al., 2001) (Table 3.1 and 3.2).

3.2.4 Distinguishing *S. aureus* from other Coagulase Positive Staphylococci

The Staphaurex® Plus test (Murex Biotech Ltd, Dartford, Kent, UK) is an agglutination test that detects the presence of bound coagulase (clumping factor) and protein A. The test reagent is composed of latex particles coated with purified human IgG and fibrinogen.

A small portion of an isolated colony was emulsified in a drop of the latex reagent on a black card, which was then rotated and observed for the presence of clumping within 30 secs. This clumping represents the presence of clumping factor which is only present in one coagulase positive species, *S. aureus.* This allows *S. aureus* to be differentiated from other coagulase positive species including *S. intermedius, S. pseudintermedius,* and *S. schleiferi* subsp. *coagulans ,* which primarily colonize other animals, but may sometimes be found in human nares (Fitzgerald & Penades, 2008).

Confirmation of *S. aureus* was by means of demonstration of the *fem*A gene. *femA* has been suggested to have a role in cell wall metabolism and is reported to be present in all *S. aureus* isolates. *S. aureus* identified by Staphaurex were confirmed by *femA* gene detection (Mehrotra et al., 2000). Strains were stored in 80% (v/v) glycerol at -80 ^{o}C and sub-cultured twice before use. Each strain was inoculated into BHI broth and incubated at 37 ^{o}C for 16-18 hours before DNA extraction.

DNA Extraction: In order to determine the *fem*A gene by PCR, 500 µL of bacterial suspension was placed into 1500 µL Eppendorf tube, and then centrifuged at 10,000rpm for five mins. The pellet were re-suspended in 400 µL of lysis buffer (Table 3.3) in an Eppendorf tube and incubated at 37 ^{o}C for 40 mins. The Eppendorf tubes were then placed in boiling water for 10 mins, transferred onto ice for 10 mins, before centrifuging at 5031 g for 10 mins. A 200 µL portion of supernatant was withdrawn for measurement of DNA concentration and adjustment to 100 ng/µL DNA before storage at -20 ^{o}C. This extract was also used to confirm methicillin

resistance by detection of the presence of *mec*A (Section 3.2.8).

The presence of *fem*A was detected by amplification of the extract. The reaction master mix for *femA* contained: 100 ng of extracted DNA, 3 µL reaction buffer (Promega, Madison, USA), 3 mM $MgCl_2$ (Promega), 0.24 mM dNTPs (Promega), 0.4 µM of each primer (Invitrogen) and 0.5 unit of Taq polymerase (Promega) in 25 µL total volume (Table 3.4). Amplification of *femA* was performed using 30 cycles consisting of 2 mins of denaturation at 94 ^{o}C, 30 s of annealing at 57 ^{o}C and one min of extension at 72 ^{o}C (Table 3.5). The PCR products were visualized by electrophoresis in 2% agarose followed by staining with EtBr and compared with products amplified from a control strain, ATCC *S. aureus* 25923.

3.2.5 Detection of QAC Genes

Presence of QAC genes in *S. aureus* and CNS isolates was detected using a conventional PCR method.

3.2.5.1 Extraction of Plasmid DNA

The lysis solution for the extraction of plasmid DNA was prepared using the following formula: 10 µL 500 U/mL lysostaphin (Sigma-Aldrich, St. Louis, Mo.), 10 µL 5000 U/ml lysozyme (Sigma-Aldrich, St. Louis, Mo.), 4 µL 0.5 M EDTA, 2 µL 1 M Tris-HCl and 174 µL MilliQ water. Colonies from fresh overnight cultures were transferred to the lysis solution, vortexed to homogenize the bacterial suspension

and incubated with agitation at 37 ^{o}C for one hour. The mixture was centrifuged and the supernatant collected and transferred to fresh Eppendorf tubes.

A 400 µl aliquot of TENS solution (0.5 mL 1M Tris, 0.5 mL 0.1 M EDTA, 5 mL 1 M NaOH, 2.5 mL 10% SDS and 41.5 mL distilled water) was added into each Eppendorf tube, and mixed gently by inversion 8-10 times, followed by addition of 150 µL potassium acetate and immediate mixing. The contents were placed on ice (4 ^{o}C) for ten minutes, centrifuged at 11,200 g for 3-5 minutes, and the supernatant transferred to a new Eppendorf tube. 1.0 mL 100% chilled ethyl alcohol (EtOH) was added into each new tube and mixed gently. The tubes were placed on ice for 15 minutes, and then centrifuged at 11,200 g for 10-15 minutes. 1.0 mL 70% EtOH was added into each tube, mixed gently, and centrifuged at 11,200 g for 3-5 minutes. The supernatant was discarded and the precipitate was air dried at room temperature. 50 µL MilliQ water was added into the tube and the contents were stored at -20 ^{o}C for later use.

3.2.5.2 Polymerase Chain Reaction

Primers were purchased from Invitrogen (Carlsbad, CA, USA). The primer sequences for *qac*A/B, *smr*, *qac*G, *qac*H, and *qac*J genes used for the PCR had been previously published and are shown in Table3.6.

The reaction master mix for *qac*A/B and *smr* contained: 100 ng of extracted plasmid DNA, 3.0 µL reaction buffer (Promega, Madison, USA), 1.5 mM $MgCl_2$ (Promega), 0.2

mM dNTPs (Promega), 0.1 µM of each primer (Invitrogen) and 0.5 unit of Taq polymerase (Promega) in 15 µL total volume (Table 3.7). Amplification of *qac*A/B and *smr* was performed using 30 cycles consisting of 30 s of denaturation at 95 ^{o}C, 30 s of annealing at 53 ^{o}C and one min of extension at 72 ^{o}C (Table 3.8). The PCR products were visualized by electrophoresis in 2% agarose and compared with products amplified from control strains: *S. aureus* TS77 (with *qacA/B* genes) and *S. aureus* L20 (with *smr* genes), which were kindly donated by Professor K. Hiramatsu, Juntendo University Tokyo, Japan.

The reaction master mix for *qac*G, *qac*H and *qac*J contained: 100 ng of extracted plasmid DNA; 3 µL reaction buffer (Promega); 1.5 mM $MgCl_2$ (Promega); 0.2 mM dNTPs; 0.2 µM of each Primer (Invitrogen) and 0.5 unit of Taq polymerase (Promega,) in a 15 µL total volume (Table 3.9). DNA was amplified using the following PCR conditions: initial denaturation at 95 ^{o}C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30s, annealing for 30 s at 55 ^{o}C, and extension at 72 ^{o}C for 60s. Amplification was completed with an extension step of 72 ^{o}C for 5mins (Table 3.10). PCR products were analyzed by electrophoresis in 2% agarose. Positive control strains for *qac*G (RN4220), *qac*H (RN4220) and *qac*J (RN4220) were kindly provided by Dr. J Bjorland, Oslo, Norway.

3.2.6 Antiseptic Susceptibility Test

The MICs and MBCs of BC (Sigma-Aldrich Ltd, USA) and CHG (Sigma-Aldrich) were determined by broth micro-dilution method following the procedure recommended

by CLSI (CLSI, 2005) with concentrations ranging from 0.25-256 mg/L for both QAC gene positive and negative isolates of *S. aureus* and CNS from nurses and the general population.

Strains were sub-cultured onto nutrient agar and incubated at 37 ^{o}C. After overnight incubation, one or two colonies were picked from the agar and subcultured in 10 mL Mueller-Hinton (MH) broth (Oxoid), and incubated at 37 ^{o}C for 24 h. The turbidity of the broth was approximately equal to 0.5 McFarland standards10^{8} CFU/mL). A 5µL aliquot of the broth culture was added to an Eppendorf tube containing 495 µL of MH broth to obtain a concentration of 10^{6} CFU/mL. Using a 96-well microtitre plate, 90 µL of MH broth was added into each of the wells of row 2 to row 12. 180 µL CHG and BC with a working concentration of 128 mg/L was added into the first row of the microtitre plate and double dilution was performed to achieve serial concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 mg/L. Broth without any disinfectant was used as a positive control. 10µL of the adjusted culture (10^{6} CFU/mL) was inoculated into wells 1-11 to achieve a final concentration of 10^{5} CFU/mL. Row 12 of the plate contained a mixture of 90 µL of MH broth and 10µL of 128 mg/L disinfectant, and was regarded as a negative control. The lowest concentration totally inhibiting bacterial growth after 24 h incubation at 37 ^{o}C was considered the MIC. 10 µL from each well with no growth observed was mixed with 90 µL of neutralizer (Dey-Engley broth, Sigma-Aldrich, USA) and held for 10 min. This mixture was then spread on nutrient agar plates. The agar plates were incubated at 37 ^{o}C for 16-18 hours and the MBCs calculated as the concentration

producing a 99.9% kill (Fuursted et al., 1997). MIC/MBC determinations were performed in duplicate.

3.2.7 Antibiotic Susceptibility Testing for *S. aureus* and CNS

Susceptibility to a range of antibiotics was determined by disc diffusion for all *S. aureus* isolated from nurses and the general population, following CLSI guidelines (CLSI, 2009), except for fusidic acid, when British Society for Antimicrobial Chemotherapy guidelines were used (Andrew et al., 2001).

Fresh overnight colonies were inoculated into 0.9% saline to prepare a 0.5 McFarland standard suspension. A sterile cotton swab was dipped into the inoculum suspension, then streaked over the surface of MH agar three times, with the plate rotated approximately 60° each time to ensure even distribution of the inoculum. Antibiotic disks (Oxoid, UK) were individually placed on the surface of agar by sterile forceps, ensuring the centre to centre distance was at least 24mm. Six antibiotic discs were placed on each 90 mm agar plate. A total of 12 agents were tested: penicillin G (1 U), oxacillin (1 mg), tetracycline (30 mg), erythromycin (15 mg), fusidic acid (10 mg), ciprofloxacin (5 mg), chloramphenicol (30 mg), clindamycin (2 mg), gentamicin (10 mg), quinupristin-dalfupristin (15 mg), linezolid (30 mg), and trimethoprim–sulphamethoxazole (1.25/23.75 mg). The plates were incubated at 37 ^{o}C for 24 h. The zone diameters were automatically measured using the Mastascan Elite (Mast Group Ltd, Bootle, UK), and were reported as susceptible, intermediate, and resistant according to CLSI guidelines (CLSI, 2009). To detect

vancomycin non-susceptibility, isolates were tested by an MIC method, using a standard Etest or agar dilution on MH agar. Isolates with vancomycin MICs >2 mg/L are likely to be resistant.

3.2.8 Identification of Methicillin-Resistance in Staphylococci and Genotyping of MRSA

The presence of the *mecA* gene which defines methicillin-resistance in Staphylococci was determined for all *S. aureus* and CNS (Ryffel et al., 1990). Amplification of *mec*A (448bp) was performed using DNA extracted as described above and performed as shown in Tables 3.11 and 3.12 (Sakoulas et al., 2001) followed by visualization on agarose gel. *S. aureus* ATCC 25923 and N315 were included as reference negative and positive strains respectively.

A multiplex PCR was used to type the SCC*mec*, using a previously described method (Zhang et al., 2005) (3.13 and 3.14). Control strains were included on each occasion: SCC*mec* I: NRS100, SCC*mec* II: NRS 22, SCC*mec* III: NRS 65, SCC*mec* IVa; NRS123, SCC*mec* IVb: ATCC 1762, SCC*mec* IVc and SCC*mec* IVd (kindly donated by Prof. H de Lencastre, University of Lisbon), and SCC*mec* V: ATCC2094.

3.2.9 Statistical Analysis

Information obtained was analyzed and comparisons made between the populations. Statistical analyses were performed using SPSS system for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). All categorical variables were analyzed by

Chi-square statistic or Fisher's exact test. Odds ratios (OR) with 95% confidence intervals (CIs) were used to identify risk factors of univariate analysis. Stepwise multiple logistic regression analysis was used to adjust for the confounding bias between groups. Risk factors identified as significant ($p < 0.05$) in the univariate analysis were included in multivariate stepwise (forward) analysis to determine independent risk factors for nurses to carry QAC genes. Mann-Whitney U-test was used to compare the MBC and MIC results. $p <0.05$ was considered statistically significant.

3.3 Results

3.3.1 Demographics of Nurses

Between June 2008 to March 2009, samples were obtained from 254 nurses. Five samples were excluded because no bacteria were cultured from the swabs provided, thus leaving 249 participants. The nurses were divided into two groups: 157 (63.1%) nurses with at least two years working experience in the hospital (EN) and 92 (36.9%) fresh nurses (FN) who were recent graduates who had practiced in the hospital for about one year.

3.3.1.1 Age and Sex Distribution

The ages of the nurses ranged from 22 to 47 with a mean of 25 years. The age range for the EN was 23 to 47 with a mean of 27 while FN nurses ranged from 22 to 26 years with a mean of 23. Of the 249 nurses, 84.3% (210) were female and 15.7 %

(39) male (Table 3.15).

3.3.1.2 Health Conditions

Of the 249 nurses, 93.2% (232) were healthy and 6.8 % (17) reported they suffered from underlying conditions and chronic illnesses (Table 3.16).

3.3.2 Investigation of Antibiotic Use and MRSA Exposure in the Nurses

About 40% of nurses reported they had used antibiotics in the past 6 months, while 85 % reported that they had contact with known MRSA patients in last 3 months (Table 3.16).

3.3.3 Investigation of Antiseptic Usage by Nurses

The frequency of use of four kinds of antiseptic products was investigated. Hibiscrub® (Molnlycke Health Care, Dunstable, UK) which contains CHG in the form of an alcoholic solution. Povidone-iodine (PVP-I) is a stable chemical complex of polyvinylpyrrolidone (PVP) and elemental iodine. It contains from 9.0% to 12.0% available iodine. Alcohol hand rub and antiseptic soap were also commonly used.

It was found that 50.6% nurses always used alcohol hand rub while 28.6% used it very often. Hibiscrub® was always used by 35.7% nurses and very often by 24.5% nurses. Similarly, 36.1% nurses always used antiseptic soap and 26.5% nurses used soap very often. Povidone iodine usage was low and 69.9% nurses rarely or never used it and only 6.8% nurses always used it (Table 3.17 and Fig. 1).

3.3.4 Colonization with *S. aureus* and CNS in Nurses and the General Population

Colonization in nurses: A total of 404 CNS were isolated from nurses with 197 CNS isolates from EN and 207 from FN. *S. aureus* was isolated from 20.5 % (51/249) nurses, of whom 21% (33/157) EN and 19.6 % (18/92) FN (Table3.18). There was no significant difference in the colonization rate with *S. aureus* between EN and FN (p>0.05).

Colonization in the general population: The carriage rate of *S. aureus* in general population was 24% (186/775). Of the approximate 700 CNS from the general population, 200 isolates were randomly chosen. There was no statistical difference in *S. aureus* colonization rates between nurses and the general population (P>0.05).

Of the total 604 CNS, 82.4% (528) strains were determined to be *S. epidermidis* by PCR, with 338 strains from nurses and 190 from the general population. The frequency of *S. epidermidis* from nurses and the general population was 83.7% (283/338) and 95% (190/200), respectively. Of the 338 *S. epidermidis* from nurses, 159 were from EN and 179 from FN (Table 3.18).

3.3.5 Antibiotic Susceptibility

3.3.5.1 Antibiotic Susceptibility in *S. aureus*

Sensitivity testing of the 51 *S. aureus* isolates from nurses and 186 from the general

population revealed resistance was frequent to penicillin, erythromycin, and tetracycline with isolates displaying 81.7%, 28.7% and 27.5 % resistance, respectively. Rates of resistance to oxacillin, ciprofloxacin, gentamicin, clindamycin, chloramphenicol, quinupristin/dalfopristin, and linezolid were less than 10%, while resistance was rare to trimethoprim–sulphamethoxazole and fusidic acid. No *S. aureus* were detected with resistance to vancomycin (Table 3.19).

Resistance rates were compared between nurses' isolates and those of the general population. Isolates from nurses were more likely to be resistant to oxacillin, ciprofloxacin, trimethoprim–sulphamethoxazole, clindamycin, and chloramphenicol compared with those the general population (p<0.05). For other antibiotics, although the rates of resistance of strains from nurses were a little higher, the differences did not reach significance (Table 3.19).

3.3.5.2 Antibiotic Susceptibility in CNS

Sensitivity testing of the 404 CNS from nurses and 200 from the general population revealed that overall 68.5%, 23.1%, 29%, and 33.8% were resistant to penicillin, oxacillin, clindamycin, and erythromycin, respectively. There was less resistance to trimethoprim–sulphamethoxazole, tetracycline, fusidic acid and chloramphenicol with 11.8%, 14.9%, 10.3%, and 16.2% of the isolates testing resistant. Less than 10% of strains displayed resistance to ciprofloxacin (8.1%), gentamicin (6.1%), quinupristin/dalfopristin (4%), and linezolid (0.3%). None of the isolates appeared

to be resistant to vancomycin (Table 3.19).

Comparison of antibiotic resistance in CNS isolates from nurses and the general population, indicated that isolates from nurses were significantly more resistant to oxacillin, penicillin, clindamycin, erythromycin and chloramphenicol than those from the general population ($p<0.05$). For other antibiotics, the somewhat higher resistance rates in nurses did not reach significance (Table 3.19).

3.3.5.3 Comparison of Resistance Rates between *S. epidermidis* and other CNS

Of the total 604 CNS isolates, 82.4% (528/604) strains were *S. epidermidis*. There were no differences in the antibiotic susceptibility rates between the *S. epidermidis* isolates and other CNS ($p>0.05$) (Table 3.20).

3.3.6 MRSA and MRCNS Colonization in Nurses and the General Public

MRSA Colonization in nurses: 8/51(15.7%) *S. aureus* isolates displaying phenotypic resistance to oxacillin were confirmed as MRSA by presence of the *mecA* gene. Three of the isolates harboured SCC*mec* type IVa and five SCC*mec* type V. Seven of these isolates were carried by EN, only one FN being colonized with MRSA. The overall carriage rate of MRSA in nurses was 3.2 % (8/249), with 4.46% (7/157) in EN and 1.09% (1/92) in FN (Table 3.21).

MRSA colonization in the general population: only four subjects were colonized with MRSA, two being SCC*mec* type IVa and two SCC*mec* type III. The colonization rate in the general population was 0.52%. The difference in MRSA colonization rates between the nurses overall and the general population was statistically significant (P=0.001), but the rates for FN and the general population were not statistically different (Table 3.21).

MRCNS in nurses and general population: Methicillin resistance was significantly more common in CNS from nurses with 28.9% (117/404) isolates being MRCNS compared to 11% (22/200) in the general population (p<0.001). MRCNS rates were similar in the nursing isolates with 27.3% (56/205) in FN and 31.0% (61/199) in EN.

The rate of methicillin resistance in *S. epidermidis* (120/528; 22.7%) was similar to that of CNS overall (23%) and other CNS (19/76; 25%). Of the methicillin resistant *S. epidermidis* (MRSE), 48 strains originated from EN (26.5%), 51 from FN (32%), and 21(11.5%) from the general public. Overall 29.3% (99/338) of *S. epidermidis* from nurses were MRSE which was significantly higher than the rate in the general public (p<0.01). The rates of methicillin resistance in other CNS were 10% (1/10) in the general public and 10.8% (5/46) in FN, but significantly higher in EN (65%, 13/20) (p<0.01).

3.3.7 Prevalence of *qac*A/B and *smr* in *S. aureus* and CNS Isolates from Nurses and the General Population

The overall frequency of both *qac*A/B and *smr* was significantly higher in CNS strains than *S. aureus,* with 42.4% CNS (256/604) compared to 17.7% (42/237) *S. aureus* positive for *qac*A/B ($p<0.001$) and 14.9% CNS (90/604) carrying *smr* compared with only 6.8% (16/237) *S. aureus* ($p=0.001$).

Carriage rates of *qac*A/B in *S. aureus* differed significantly between nurses and the general public with 41.2 % positive nursing isolates compared to only 11.3% in those from the general population isolates (OR 5.5, 95% CI: 2.7 - 11.2, $p<0.001$). However, the difference in prevalence of *smr* in *S. aureus* isolates from the two populations, 11.8% (6/51) *S. aureus* isolates from nurses compared to 5.4% *smr* gene positive isolates from general population, did not reach significance. (OR 2.3, 95% CI: 0.6 - 6.8, $p=0.11$).

Differences between the populations were also seen in prevalence of both *qac*A/B and *smr* in CNS isolates. In the general population, 13.5% (27/200) CNS isolates harboured *qac*A/B and 8.5 % (17/186) *smr*, while in nurses, *qac*A/B was detected in 43.3% and *smr* in 18.3 %. The differences in rates for both *qac*A/B and *smr* genes between nurses and general population reached statistical significance (OR 8.4, 95% CI: 5.4- 13.2, $p<0.001$ for *qac*A/B gene; OR 2.4, 95% CI: 1.4 - 4.2, $p = 0.002$ for *smr* gene) (Table 3.22).

There was no overall difference in the frequency of either *qac*A/B or *smr* between *S. epidermidis* and other CNS (Table 3.23). However, further stratified analysis revealed a difference in the *qac*A/B gene prevalence between *S. epidermidis* and other CNS in isolates from nurses (*p*=0.044, OR 1.72, 95% CI: 1.01 - 2.92), but this difference was not observed in the strains from general population group. However, no difference was detected in the prevalence of *smr* between the *S. epidermidis* and other CNS isolates for either group (*p*>0.05) (Table 3.23). Six strains of *S. aureus* and 37 of CNS were found to harbor both *qacA/B* and *smr*.

3.3.8 Prevalence of *qac*A/B and *smr* in MRSA and MRCNS Isolates

Presence of *qac*A/B was significantly more likely in both MRCNS and MRSA than in methicillin sensitive strains. The frequency of carriage of QAC genes in all MRCNS was higher, with 66.9 % (93/139) for *qac*A/B and 23.7% (33/139) for *smr* in MRCNS in comparison to 33.6 % (167/467) for *qac*A/B (OR 3.7, 95% CI: 2.5-5.6, *p* <0.001*)*) and 12.3% (57/467) for *smr* in MSCNS (OR 2.2, 95% Cl: 1.4 - 3.6, *p*<0.001). Likewise, MRSA carried significantly more *qacA/B* than MSSA (*p*=0.003). However, the difference did not reach significance for *smr* in which 16.7% MRSA and 6.2 % MSSA carried the gene (OR 3.0, 95% Cl: 0.6 - 15.1, *p* =0.16) (Table 3.24).

3.3.9 Prevalence of *qac*G, *qac*H, *qac*J in *S. aureus* and CNS Isolates from Nurses and the General Population

Of the total isolates of *S. aureus* (237) and CNS (604), only four isolates carried *qac*G,

one *qac*H, and four *qac*J. Only one CNS strain, a *S. epidermidis* from the general population, harboured *qac*J. The remaining isolates of *S. aureus* were one (1.96%) *qac*J positive from nurses and two (1.08%) from the general population; four *qac*G positive (2.15%), and one *qac*H positive (0.54%) from the general population. The presence of these three genes, *qac*G, *qac*H and *qac*J (8/237) in *S. aureus* was significantly higher than CNS (1/604) (p<0.001) (Table 3.25). One *qac*G positive isolate also harboured *qac*A/B and another *qac*G positive isolate contained *smr* concomitantly.

3.3.10 Risk Factors for Carriage of *qac*A/B or *smr* in Staphylococci from Nurses

Only the *S. aureus* and CNS from nurses were included in this part of analysis. Association between isolates with the QAC genes (*qac*A/B or *smr*) and sex, working experience (EN vs. FN), underlying conditions and chronic illnesses, antibiotic usage, contact with MRSA patients, presence of *mec*A gene, and antiseptic usage was investigated.

Factors identified by univariate analysis to be associated with the presence of QAC genes were the co-existence of *mec*A gene (OR: 2.5), recent contact with MRSA patients (OR: 2.0), shorter working experience (FN) (OR: 0.6), and antibiotic use in the past 6 months (OR: 1.51) (Table 3.26). The carriage of QAC genes in isolates of *S. aureus* and CNS was not associated with sex (OR 1.14, 95% Cl: 0.68-1.92, p=0.638) or presence of underlying conditions and chronic illnesses of nurses (OR 0.94, 95% Cl: 0.46-1.95, p=0.872). Association between use of CHG for hand hygiene and

presence of resistance genes did not reach significance but there was a significant trend for increased use (Table 3.26).

After adjustment for confounding by logistic regression analysis, the risk factors that remained significant were contact with MRSA positive patients, presence of the *mec*A gene in the isolate, and years of working experience (Table 3.27). Use of antibiotics did not reach significance in the multivariate model (p=0.309).

3.3.11 MICs and MBCs of Antiseptics and *qac*A/B or *smr* Carriage

Tests were performed on all QAC gene-positive *S. aureus* isolates. For CNS, all 36 QAC gene-positive strains from the general public and 80 from nurses (40 strains randomly selected from fresh and experienced nurses) were included. Thirty negative isolates each of *S. aureus* and CNS (15 each from nurses and the general public) were also tested. (Table 3.29)

There was no difference in MIC or MBC for BC or for MIC for CHG between the nurse isolates and those of the general population. However, there was a significant difference in MBC for CHG between the two groups (p<0.001) (Table 3.29).

The MIC ranges and the MICs at which 50% and 90% isolates were inhibited (MIC_{50} and MIC_{90}, respectively) are shown in Table 3.29 as well as MBC range and the MBC_{50} and MBC_{90}. Isolates with QAC genes had higher MICs and MBCs to BC and CHG, and a wider range of MICs and MBCs, in comparisons to the isolates without

($p<0.05$). The results showed that reduced antiseptic susceptibility for staphylococci was commonly associated with presence of QAC genes encoding efflux proteins. Presence of both *qac*A/B and *smr* together resulted in an elevated MIC_{50} and MBC_{50} to BC in *S. aureus* isolates compared to either of these genes alone, but did not alter MICs or MBCs of CHG. There were no significant effects of this co-carriage on MICs or MBCs of CNS isolates.

3.3.12 MICs and MBCs of Antiseptics and Presence of *qac*G, H, and J

The MICs of CHG and BC for *qacG/H/J* positive strains were determined and are shown in Table 3.31. The MICs of *qacG/H/J* positive strains against BC ranged from 4 to 8 mg/ L, two times higher than that of negative control strains (2 mg/L).

For CHG, the MICs of *qacG/H/J* positive strains ranged from 2 to 4 mg/ L, in contrast to 2 mg/L observed for the negative controls. The MICs of BC and CHG of a *S. aureus* strain carrying *qacG* and *qacA/B* concurrently were 8 and 4 mg/ L, while that of another strain harbouring both *qacG* and *smr* were 8 and 2 mg/ L, respectively (Table 3.31), showing that presence of two QAC genes increases the level of resistance.

3.3.13 Antibiotic Resistance and QAC Gene Carriage

3.3.13.1 Correlation between Antibiotic Resistance and *qacA/B, smr* Carriage in *S. aureus*

Antibiotic susceptibility patterns of *S. aureus* isolates were analyzed for correlation

between QAC gene carriage and antibiotic resistance. Resistance to oxacillin, penicillin, ciprofloxacin, trimethoprim–sulphamethoxazole, clindamycin, and tetracycline resistance were all statistically associated with presence of QAC genes. ($p<0.05$) (See Table 3.32).

3.3.13.2 Correlation between Antibiotic Resistance and *qacA/B* and *smr* Carriage in CNS Strains

Association between antibiotic resistance and presence of QAC genes was investigated in CNS isolates. Based on the Fisher exact analysis, resistance to oxacillin, penicillin, clindamycin, tetracycline, erythromycin, and chloramphenicol was significantly associated CNS isolates having QAC genes ($p<0.05$) (Table 3.32).

3.3.13.3 Correlation between Antibiotic Resistance and *qacG, H, J* Carriage

Susceptibility testing of the eight strains of *S. aureus* and single positive S. *epidermidis* carrying *qac*G, H, and J genes revealed that they were all susceptible to quinupristin/dalfopristin, linezolid, ciprofloxacin, and vancomycin. The *qacH* strain was susceptible to all antibiotics except penicillin. Resistance to penicillin was observed in all *qac*G and H strains and three of the *qac*J strains. Resistance to tetracycline, gentamicin, erythromycin, clindamycin, fusidic acid, co-trimoxazole, and chloramphenicol was frequently detected in *qac*G strains, but there was less resistance to antibiotics in *qac*J positive strains, which displayed resistance only to clindamycin, erythromycin, gentamicin, fusidic acid, and tetracycline (Table 3.33).

Comparison of resistance patterns between *qacG/H/J* positive strains and QAC negative isolates, revealed significant differences in the following antibiotics: gentamicin (p=0.007), fusidic acid (p=0.02), clindamycin (p=0.006) and tetracycline (p=0.02), suggesting that presence of *qacG/H/J* genes is associated with resistance to these antibiotics (Table 3.33).

Comparison of antibiotic resistance patterns and MIC values of BC and CHG can be seen in Table 3.34. MIC values of BC and CHG ranged from 4-8 mg/L and 2-4 mg/ L, respectively. One strain carrying *qacA/B* and *qacG* concomitantly was resistant to penicillin, clindamycin, fusidic acid, erythromycin and chloramphenicol, while another isolate carrying *qacG* and *smr* concomitantly was resistant to penicillin, tetracycline, fusidic acid and erythromycin. Both of the MIC values of BC for *qacA/B + qacG* and *smr + qacG* isolates were 8 mg/ L. and MICs of CHG for these two strains were 2 mg/L and 4 mg /L, respectively.

3.4 Discussion

This study focused on the epidemiology of QAC genes in colonizing *S. aureus* and CNS from nurses and the general population and investigated the risk factors for carrying QAC gene positive strains in nurses. This is the first report of QAC genes in colonizing strains of *S. aureus* and CNS from healthy subjects, though QAC genes have previously been reported in clinical isolates (Vali et al., 2009; Smith et al., 2009).

3.4.1 Colonization with *S. aureus* in Nurses and the General Population

Carriage of *S. aureus* in the nose plays an important role in transmission of infection. Carriage rates of *S. aureus* from nasal cavities in nurses and the general population were 20.5% and 24% respectively, which was similar to previous studies (Kluytmans & Wertheim, 2005a; Boost et al., 2008a,). Nasal carriage of *S. aureus* in the nurses and the general population in Hong Kong was not significantly different.

MRSA carriage was low in the general population and in FN. The comparatively shorter exposure time or limited exposure to more serious patients may explain the lower MRSA colonization rate in FN. The 4.5% MRSA colonization rate in EN, was similar to the average 4.6% prevalence reported in health care workers (Albrich & Harbarth, 2008) though lower than the rate of 8.5% reported in Taiwan (Wang et al., 2004). The higher MRSA carriage rate may be due to more exposure to seriously ill patients, but poor rates of adherence to hand hygiene guidelines and precautions or the contamination of the hospital environment may also contribute to their colonization.

The majority of nurse MRSA isolates harboured SCC*mec* types typical of community-associated MRSA (CA-MRSA), which supports the evidence that the CA-MRSA has transferred into the hospitals in Hong Kong. Asymptomatic nasal carriage of MRSA in healthcare workers may increase the risk of outbreaks of MRSA in the hospital, by serving as a reservoir for MRSA cross-transmission (Albrich &

Harbarth, 2008).

In Hong Kong, practicing doctors are legally required to report both confirmed and suspected cases of CA-MRSA infection to the Department of Health. It is likely Hong Kong is still at the early phase of the CA-MRSA epidemic. Therefore, it is important to study its epidemiology and monitor changes in the molecular types of MRSA for timely and effective control measures.

3.4.2 Colonization with CNS in Nurses and the General Population

CNS rarely causes disease and clinicians frequently regard isolates as contaminants. However, there is an increasing trend of CNS being associated with significant infections. *S. epidermidis* is the most prevalent species and comprises 65-90% of all CNS isolated from human sources (Rogers et al., 2009a). In our investigation, 82.4% of CNS strains were *S. epidermidis*.

Rates of methicillin resistance in CNS have not previously been reported in Hong Kong, and colonization with MRCNS was considerably more frequent in nurses (29.9%) than in the general population (11%). In our study, 30.6% EN carried MRCNS. However, the prevalence of MRCNS in nurses was lower than rates reported from clinical isolates in Europe (60 -70% (Stefani & Varaldo, 2003) and the USA (58%) (Sahm et al., 1999).

Nurses play an important role in transmission of pathogens including CNS. So their

carriage of MRCNS may act as an important reservoir in the hospital. A study in Korea also showed a significant difference in colonization rates of nurses with MRCNS, 60%, compared with 13% in a non-health care worker control group (Lee et al., 2000). Nurses can act as an interface between hospitals, long-term care facilities, nursing homes and the community and act as reservoirs, or may serve as victims of pathogen cross-transmission (Muder et al., 1993; Vonberg et al., 2006). Therefore, in order to prevent MRCNS transmission, long term monitoring, especially of MRSE, in healthcare workers should be performed.

3.4.3 Prevalence of QAC Genes

Previous research has mainly focused on the prevalence in of QAC genes in *S. aureus* and MRSA clinical isolates (Mayer et al., 2001; Alam et al., 2003b; Noguchi et al., 2006; Smith et al., 2008; Wang et al., 2008a; Vali et al., 2008; Sheng et al., 2009), although a few have determined rates in carriage isolates of CNS, including colonizing strains from healthy subjects (Leelaporn et al., 1994; Sidhu et al., 2002; Anthonisen et al., 2002).

Our study showed that nurses appeared to be more likely to be colonized with CNS and *S. aureus* harbouring QAC genes than the general public. Selective pressure from antiseptic residues may allow for persistence of such strains and enhance horizontal and vertical spread of QAC genes (Leelaporn et al., 1994; Sidhu et al., 2002; Anthonisen et al., 2002).

In vitro experiments, have shown that sub-inhibitory concentrations of biocides are able to induce *qacA/B* gene expression (Smith et al., 2008) and that QAC genes could potentially be transferred to pathogens under selective stress (Dantas et al., 2008). Whilst current MICs to QACs do not reach levels which would allow survival at in-use concentrations, even moderately increased antiseptic resistance may allow persistence and spread of these less susceptible Staphylococci in the presence of residual disinfectant.

Currently, rates of staphylococci with QAC genes are lower in general population isolates, but these levels may increase due to transfer of strains from the hospital to the community accompanied by increasing usage of antiseptics at home. Long term monitoring of rates of QAC gene positive strains and their levels of resistance is necessary.

In our investigation, only nine isolates were found to be carrying one of the *qacG*, *H* and *J* genes. Only one *S. aureus* strain containing *qacJ* gene was detected in isolates from nurses (1.91%), whereas two *qacJ* positive *S. aureus* and one *qacJ* positive S. *epidermidis* were found in isolates from the general public. *qacJ* was initially reported in equine *S. aureus, S. simulans,* and *S. intermedius* (Bjorland et al., 2003), which suggested that spread of *qacJ* may be due to both dissemination of a *qacJ* containing *S. aureus* clone and plasmid transfer within and between various staphylococcal species. In the environment, residual concentrations of biocides may play an important role in the transfer of QAC genes in *S. aureus*.

qacG or *qacH* genes were not detected in *S. aureus* from nurses, and the prevalence of *qacG*, *H* and *J* in *S. aureus* from the general population were only 1.03%, 0.26% and 0.78%, respectively. This suggested that prevalence of these genes remains low and a larger sample size would be needed for further study. *qac*H was first detected in *S. saprophyticus* from a poultry processing plant (Heir et al., 1998). Isolates harbouring *qacG,* and *qacJ,* but not *qacH* were reported from dairy cattle herds (Bjorland et al., 2005). Liu et al. (2009) reported the presence of *qacH* gene in <1% of clinical isolates of *S. aureus* and Vali et al. (2008) in 3.3% MRSA in UK. However, another UK study failed to detect any isolates harbouring *qacG*, *H* and *J* genes (Smith et al., 2008).

3.4.4 Risk Factors for Carriage of QAC Gene Positive Staphylococci by Nurses

In our investigation, presence of *qac*A/B was significantly more likely in both MRCNS and MRSA than in methicillin sensitive strains. The frequency of carriage of QAC genes was higher in MRCNS than MRSA. The significant association between presence of *mec*A and QAC genes is similar to reports in clinical isolates (Alam et al., 2003b; Hidemasa Nakaminami, 2008). Alam et al. (2003a, 2003b) found 32.6% MRSA and 7.5% MSSA carried *qac*A/B in comparison to 3.3% MRSA and 5.9% MSSA containing *smr* in *S. aureus* clinical isolates from a Japanese hospital. Mayer et al. (2001) reported 62.6% of MRSA and 12% of MSSA harbouring *qac*A/B and 6.4% of MRSA and 5% MSSA with *smr* genes in European isolates. Our study found no statistically significant difference for carriage of the *smr* gene between MRSA and

MSSA isolates. Our study also demonstrated that contact with MRSA infected patients may lead to increased carriage of QAC gene positive strains.

3.4.5 Antibiotic Resistance and QAC Gene Carriage

In our investigation, both *S. aureus* and CNS isolates from nurses were more resistant to non-beta-lactam antibiotics than those from the general population, although this difference did not always reach significance. Isolates from both nurses and the general population carrying QAC genes (*qac*A/B, *smr*, and *qacG*/H/J) were generally more resistant to antibiotics, with resistance to penicillin, ciprofloxacin, trimethoprim–sulphamethoxazole, clindamycin, erythromycin, and tetracycline significantly associated with presence of QAC genes. This is consistent with reports in clinical isolates (Sidhu et al., 2001a; 2002). The results are compatible with there being a selective advantage to isolates carrying both antibiotic resistance and QAC genes and imply that the presence of QAC genes in *S. aureus* and CNS results in co-selection of antibiotic resistant bacteria.

Studies have shown that the β-lactam antibiotic resistance gene, *bla*Z, as well as *bla*I and *bla*R which control *bla*Z gene expression, may be present on several different staphylococcal transposons (e.g. Tn552) on large plasmids or on the chromosome. Genetic linkage between QAC and antibiotic resistance genes, including *bla*Z, has been reported in clinical and food industry isolates (Sidhu et al., 2001a; 2002). The multi-resistance plasmid, pMS62, harbours QAC genes, *bla*Z, and the tetracycline

resistant determinant, *tet*K, conferring resistance to antiseptics, penicillin, and tetracycline. *tet*K was also found on small transmissible plasmids (e.g. pT181) which could integrate into the chromosome of staphylococci.

Genetic linkage between QAC genes and other antibiotic resistance genes *erm*C, *dfr*A, and *aacA-aphD*, conferring resistance to erythromycin, trimethoprim, and gentamicin-kanamycin-tobramycin, has also been reported (Sidhu et al., 2001a; 2002). QAC genes and *aacA-aphD* determinants have been observed on pMS62.

Isolates resistant to erythromycin and susceptible to clindamycin with a D shape around a clindamycin disk are considered positive for inducible resistance. It is not yet clear whether co-existence of QAC genes can contribute to the prevalence of inducible clindamycin resistance. However, in this study the significantly higher clindamycin resistance rate found in QAC gene positive strains indicated a possible relationship between these genes and clindamycin resistance determinants. The presence of similar resistance genes and mobile elements in several staphylococcal species suggests that plasmid exchange occurs frequently in nature. Transfer of resistance determinants from CNS is likely to play an important role in the maintenance and dissemination of resistance determinants in the more pathogenic *S. aureus*.

In our study, resistance to a greater number of antibiotics was observed in strains harbouring more than one QAC gene, and these isolates also exhibited relatively

elevated MICs to BC and CHG. Exposure to sub-inhibitory doses of biocides selects for up-regulation of efflux pumps capable of transporting these compounds, as well as some antibiotics out of the cell and contributes to reduced biocide susceptibility. The increased resistance level of some staphylococcal strains may be associated with regulation of QAC gene expression, presence of resistance genes other than QAC genes as well as individual genomic diversity. Since resistance to biocides is plasmid-mediated, this raises concern that biocide exposure could contribute to spread of antibiotic resistance by selection and dispersal of plasmids mediating resistance to both antibiotics and biocides.

3.4.6 MICs of Benzalkonium Chloride and Chlorhexidine

As a result of efforts to control increasing occurrence of hospital infections, there may be overuse and incorrect use of biocides, which is similar to the inappropriate use of antibiotics. A large amount of evidence implies that an association exists between bacterial resistance and use of biocides, leading to development of strains which are more tolerant to antiseptics, and consequently increasing difficulties for infection control and imposing a heavy economic load for society.

In this study, QAC gene positive isolates had both wider ranges and higher mean MICs and MBCs to BC and CHG, in comparison with QAC gene-negative isolates ($p<0.05$). It confirmed previous observations that reduced biocide susceptibility in staphylococci is associated with QAC genes (Smith et al., 2008). Our results implied

that bacteria harbouring QAC genes have a selective advantage, conferring a reduction in susceptibility to BC and CHG which can enhance their survival in the environment.

Some isolates harbouring QAC genes in our study showed cross resistance to BC and CHG. If disinfectants use the same pathway to reach the target site of bacteria (e.g. porins) or other similar mechanism of action (e.g. inhibition of protein synthesis), or have similar resistance mechanisms (e.g. reduction in permeability), it can result in biocide resistance to one antimicrobial agent being accompanied by cross resistance to another agent. Therefore, it may be advisable to change disinfectant types used regularly, in order to avoid cross resistance. Nevertheless, in practice, disinfectants with similar components are often applied and this may promote the development of bacterial cross resistance.

The MICs of *S. aureus* strains with *qac*G/H/J to BC were similar. The proteins encoded by these three genes all belong to the SMR protein family. Two isolates, one with *qacA/B + qacG* and one with *smr + qacG*, had higher MICs against BC and CHG than the mean MIC values. Over-expression of resistance genes or inter-actions between two different resistance determinants may contribute to the increased resistance levels.

3.5 Limitations and Recommendations of the Study

In this study, the general population was represented by 775 healthy adults but as

they did not complete the questionnaire, both demographics and information for disinfectant usage was unavailable. It is, however, very likely that the nurses are exposed to more disinfectants as a result of occupational exposure. It is difficult to calculate the actual exposure time of disinfectants even in the nurses group. Their working time and experience may be associated with cumulative exposure time to antiseptics. However, their working conditions might affect the exposure time to antiseptics because nurses in charge of patients are more exposed than nurses responsible for administration. Working time and working department were not included in the questionnaire, which might lead to missing risk factors for carriage of QAC gene positive staphylococci.

The general population samples were randomly selected from isolates of dog owners who took their dogs to animal clinics and families of students attending the Hong Kong Polytechnic University. Dog owners would be likely to use more antiseptics in daily life to prevent the transmission of diseases, which may represent one of the highest exposed groups of general population. Although it could bring about bias and reduce the power to detect the difference, a large difference between nurses and the general population selected in this study was still found in our study, suggesting that the prolonged daily exposure of nurses to antiseptics may be a greater selective pressure than less sustained domestic use.

During the sample collection period, pandemic influenza H1N1 flu infections were occurring in Hong Kong over the whole year. In order to prevent the development

of secondary infections and prevent shortages of nurses, antibiotic were widely used in nurses, which could lead to the very high reported use of antibiotic usage by nurses in the past three months (40%).

Most nurses are female and sex could influence carriage acting as a confounding factor because differences exist in the proportion of males and females between the nurses and the general public, even though this was not shown as significant in the analysis. It is not clear whether sex would affect bacterial colonization and distribution of resistance genes in bacteria. There is evidence that staphylococcal colonization is higher in males (Kuehnert et al., 2006; Van Howe & Robson, 2007), although this was not observed in our study.

Dilution based MIC methods have been used extensively to determine resistance levels to antibiotics and disinfectants. The major advantage of this method is that it is easily performed and many strains or antibacterial agents can be tested simultaneously. However, MIC and MBC methods do depend on good technique to prevent carryover and contamination. Although the procedure was standardized, it is still hard to totally eliminate all experimental variation, even though all testing was performed by one operator, using the same instruments.

In conclusion, use of antiseptics may be selecting for antibiotic-resistant strains and assisting their survival in the healthcare environment. The association between *mec*A and *qac*A/B/*smr* may contribute to survival of MRSA in the hospital

environment. The increased prevalence of QAC genes in isolates from nurses indicates that the hospital environment exerts selective pressure for carriage of these strains. The increased proportion of QAC gene positivity in *mec*A positive isolates suggests co-selection of these genes, as does the increased risk of carriage of QAC gene positive strains by those in recent contact with MRSA positive patients. Although *qac*A/B and *smr* do not confer antiseptic resistance at concentrations used, they may pose an infection control risk by allowing persistence of bacteria in areas with low level antiseptic residues.

Table 3.1 Master mix for amplification of *Staphylococcus* spp. and *S. epidermidis* (modified from Martineau et al., 2001)

Reagents	Concentration	Volume (µL)
Buffer	5 ×	5
d NTP	2 mM	2.5
$MgCl_2$	25 mM	3
Primers		
Se 705-1F 5'-ATC AAA AAG TTG GCG AAC CTT TTC A-3'	10 µM	1
Se705-2R 5'-CAA AAG AGC GTG GAG AAA AGT ATC A-3'	10 µM	1
TStag765 R 5'-TIA CCA TTT CAG TAC CTT CTG GTA A-3′	10 µM	0.5
Tstag 442F 5'-GGC CGT GTT GAA CGT GGT CAA ATC A-3′	10 µM	0.5
Taq polymerase (5 U/µL)		0.1
DNA template	100 ng/µL	1
Sterile water	make up to 25 µL	

Table 3.2 PCR conditions for amplification of *Staphylococcus* spp. and *S. epidermidis* (modified from Martineau et al. 2001)

Temp ^oC	Time	No. of Cycles
4	15 min	1
94	3 min	1
95	30 s	40
55	30 s	
72	30 s	
72	3 min	7

Table 3.3 Lysis buffer formula for DNA extraction

Reagents	Vol (µL)
Lysostaphin	20
Lysozyme	20
Tris HCl	4
EDTA (0.5 mM)	8
Water	348

Table 3.4 Master mix for amplification of *fem*A gene (132bp)

(Modified from Mehrotra et al., 2000)

Reagents	Vol (µL)
10X Buffer	3
dNTP (2 mM)	3
$MgCl_2$ (25 mM)	3
Primer (10 µM)	
*fem*AF5'-AAAAAAGCACATAACAAGCG-3’	1
*fem*AR5'-GATAAAGAAGAAACCAGCAG-3’	1
DNA (100 ng/µL)	1
Taq polymerase (5 U/µL)	0.1
Sterile water	make up to 25 µL

Table 3.5 PCR conditions for amplification of *fem*A gene

(Modified from Mehrotra et al., 2000)

Temp ^{o}C	Time	No. of Cycles
94	5 min	1
94	2 min	30
57	30 sec	
72	1 min	
72	7 min	1

Table 3.6 Primers for QAC genes (modified from Noguchi et al., 2005; Smith et al., 2008 for *qac*G, H, J)

Primer	Sequence (5'→3')	Amplicon size (bp)	Reference sequence (GeneBank accession No.)
qacA-F	5'-GCA GAA AGT GCA GAG TTC G CCA-3'	361	pSK1 (X56628)
qacA-R	5'-GTC CAA TCA TGC CTG-3'		
smr-F	5'-GCC ATA AGT ACT GAA GTT ATT GGA-3'	195	pTZ20 (X15574)
smr-R	5'-GACTACGGTTGTTAAGACTAAACCT-3'		
qacG-F	5'-TTTCGTTTGGAATTTGCTTT-3'	194	pSKI265 (Y16944)
qacG-R	5'-AATGGCTTTCTCCAAATACA-3'		
*qac*H-F	5'-CAATAGTCAGTGAAGTAATAGGCAGTG-3'	295	pSKI265 (Y16945)
qacH-R	5'-TGTGATGATCCGAATGTGTTT-3'		
*qac*J-F	5'-GGCCAACATTAGGCACACTTA-3'	232	pSKI265 (AJ512814)
qacJ-R	5'-TGACTTGATCCAAAAACTTTAAGA-3'		

Table 3.7 Master mix for amplification of *qac*A/B or *smr*

(Modified from Noguchi et al. 2005)

Reagents	Concentration	Volume(µL)
Buffer	5×	3
d NTP	2 mM	1.5
$MgCl_2$	25 mM	0.9
Primers		
F	10 µM	0.15
R	10 µM	0.15
Taq polymerase (5 U/µL)		0.1
DNA template	100 ng/µL	1
Sterile water	Add up to 15µL	

Table 3.8 PCR conditions for amplification of *qac*A/B or *smr*

(Modified from Noguchi et al., 2005)

Temp ^{o}C	Time	No. of Cycles
95	5 min	1
95	30 s	
53	30 s	
72	30 s	30
72	5 min	7

Table 3.9 Master mix for amplification of *qac* G, *qac*H or *qac*J

(Modified from Smith et al. 2008)

Reagents	Concentration	Volume(µL)
Buffer	5×	3
d NTP	2 mM	1.5
$MgCl_2$	25 mM	0.9
Primer		
F	10 µM	0.2
R	10 µM	0.2
Taq polymerase (5 U/µL)		0.1
DNA template	50 ng/µL	2
Sterile water	Add up to 15 µL	

Table 3.10 PCR conditions for amplification of *qac*G, q*ac*H, and *qac*J

(Modified from Smith et al. 2008)

Temp ^{o}C	Time	No. of Cycles
4	15 min	1
95	5 min	1
95	30 s	
55	30 s	
72	30 s	30
72	5 min	7

Table 3.11 Master mix for amplification of *mec*A (448bp) (Sakoulas et al., 2001)

Reagents	Vol (μL)
10 X Buffer	3
dNTP (2 mM)	3
$MgCl_2$ (25 mM)	6
Primer (10 μM)	
*mec*AF5'-CTCAGGTACTGCTATCCACC-3'	1
*mec*AR5'-CACTTGGTATATCTTCACC-3'	1
DNA (100 ng/μL)	1
Taq polymerase (5 U/μL)	0.1
Sterile water	make up to 25 μL

Table 3.12 PCR conditions for amplification of *mec*A (Sakoulas et al., 2001)

Temp ^oC	Time	No of Cycles
95	5 min	1
94	30 sec	30
51.5	30 sec	
72	30 sec	
72	10 min	1

Table 3.13 PCR master mix for SCC*mec* typing (Zhang et al., 2005)

Reagents	Vol (μL)	Product (bp)
10 X Buffer	2.5	-
dNTP (2 mM)	2.3	-
$MgCl_2$ (25 mM)	2.3	-
Primer (5 μM)		
SCC*mec* IF 5'-GCTTTAAAGAGTGTCGTTACAGG-3'	0.2	613
SCC*mec* IR 5'-GTTCTCTCATAGTATCACGTCC-3'	0.2	
SCC*mec* IIF 5'-CGTTGAAGATGATGAAGCG-3'	0.2	398
SCC*mec* IIR 5'-CGAAATCAATGGTTAATGGACC-3'	0.2	
SCC*mec* IIIF 5'-CCATATTGTGTACGATGCG-3'	0.2	280
SCC*mec* IIIR 5'-CCTTAGTTGTCGTAACAGATCG-3'	0.2	
SCC*mec* IVaF 5'-GCCTTATTCGAAGAAACCG-3'	0.5	776
SCC*mec* IVaR 5'-CTACTCTTCTGAAAAGCGTCG-3'	0.5	
SCC*mec* IVbF 5'-TCTGGAATTACTTCAGCTGC-3'	0.5	493
SCC*mec*IVbR 5'-AAACAATATTGCTCTCCCTC-3'	0.5	
SCC*mec* IVcF 5'-ACAATATTTGTATTATCGGAGC-3'	0.4	200
SCC*mec* IVcR 5'-ACAATATTTGTATTATCGGAGAGC-3'	0.4	
SCC*mec* IVdF 5'-CTCAAAATACGGACCCCAATACA-3'	1.4	881
SCC*mec* IVdR 5'-TGCTCCAGTAATGCTAAAG-3'	1.4	
SCC*mec* VF 5'-GAACATTGTTACTTAAATGAGCG-3'	0.3	325
SCC*mec* VR 5'-TGAAAGTTGTACCCTTGACACC-3'	0.3	
*Mec*A 147F 5'-GTG AAG ATA TAC CAA GTG ATT-3'	0.1	147
*Mec*A147R 5'-ATG CGC TAT AGA TTG AAA GGA T-3'	0.1	
DNA(50 ng/μL)	2	-
Taq polymerase (5 U/μL)	0.2	-
Sterile water	Make up to 25 μL	-

Table 3.14 PCR conditions for SCC*mec* typing (Zhang et al., 2005)

<table>
<tr><th>Temp °C</th><th>Time</th><th>No. of Cycles</th></tr>
<tr><td>94</td><td>5 min</td><td>1</td></tr>
<tr><td>94</td><td>45 Sec</td><td rowspan="3">10</td></tr>
<tr><td>65</td><td>45 Sec</td></tr>
<tr><td>72</td><td>1.5 min</td></tr>
<tr><td>94</td><td>45 Sec</td><td rowspan="3">25</td></tr>
<tr><td>55</td><td>45 Sec</td></tr>
<tr><td>72</td><td>1.5 min</td></tr>
<tr><td>72</td><td>10 min</td><td>1</td></tr>
</table>

Table 3.15 Age and sex distribution of nurses

Group	Total			Female			Male		
	N	Age		N	Age		N	Age	
		Range	X±SD		Range	X±SD		Range	X±SD
EN	157	22-47	26±4	125	23-42	28±5	32	22-47	27±4
FN	92	22±26	23±1	85	22-25	24±1	7	22-26	23±1
Total	249	22-47	25±3	210	22-42	27±5	39	22-47	25±4

EN: experienced nurse; FN: fresh nurse

Table 3.16 Risk factors for colonization of nurses

Factor		N	Percentage (%)
Underlying conditions and chronic illness	Yes	17	6.8
	No	232	93.2
	Total	249	100
History of antibiotic use in past 6 months	Yes	100	40.2
	No	149	59.8
	Total	249	100
Contact with known MRSA patients in last 3 months	Yes	212	85.1
	No	37	14.9
	Total	249	100

Table 3.17 Antiseptic used for hand hygiene by nurses (n=249)

Antiseptic commonly used in the last 3 months for hand hygiene	Rarely and never (<25%)	Sometimes (25-49%)	Very often (50-75%)	Always (>75%)
	N (%)	N (%)	N (%)	N (%)
Alcohol hand rub	12 (4.8)	40 (16.1)	71 (28.5)	126 (50.6)
Soap	53 (21.3)	40 (16.1)	66 (26.5)	107 (45.5)
Hibscrub (chlorhexidine)	45 (18.1)	54 (21.7)	61 (24.5)	89 (35.7)
Povidone iodine	174 (69.9)	42 (16.9)	16 (6.4)	17 (6.8)

Fig 3.1 Investigation of antiseptic use for hand hygiene by nurses

Table 3.18 Isolates of *S. aureus* and CNS/ *S. epidermidis* from nurses and the general population

Group	Source	No.	No. *S. aureus*	*S. aureus* Colonization rate (%)	No. CNS	*S. epidermidis* colonization rate (%)
Nurses	EN[a]	157	33	21	197	80.7
	FN[b]	92	18	19.6	207	86.5
	Total	249	51	20.5	404	83.7
General population		775	186	24	200	93.5

a: Experienced nurse; b: Fresh nurse

Table 3.19 Comparison of resistance rates in staphylococcal isolates from nurses and the general population

Antibiotic	Susceptibility	All *S. aureus* (n=237) N (%)	Group: Nurses N (%)	Group: General population N (%)	*p* value	All CNS (n=604) N (%)	Group: Nurses N (%)	Group: General population N (%)	*p* value
Oxacillin	R	17 (7.2)	10 (19.6)	3 (3.8)	**0.001***	139 (23.1)	117 (29.1)	22 (11)	**<0.001***
	S	220 (92.8)	4 (80.4)	179 (96.2)		465 (76.9)	285 (70.9)	180 (89)	
Penicillin	R	194 (81.9)	45 (88.2)	149 (80.1)	0.222	414 (68.5)	309 (76.5)	105 (52.5)	**<0.001***
	S	43 (18.1)	6 (11.8)	37 (19.9)		190 (31.5)	95 (23.5)	95 (47.5)	
Ciprofloxacin	R	12 (5.1)	8 (15.7)	4 (2.2)	**0.001***	49 (8.1)	37 (9.2)	12 (6.0)	0.207
	S	225 (94.9)	43 (84.3)	182 (97.8)		555 (91.9)	367 (90.8)	188 (94)	
Trimethoprim–sulphamethoxazole	R	3 (1.3)	5 (9.8)	8 (4.3)	**0.01***	71 (11.8)	52 (12.9)	19 (9.5)	0.283
	S	234 (98.7)	46 (90.2)	178 (95.7)		533 (88.2)	352 (87.1)	181 (90.5)	
Gentamicin	R	13 (5.5)	11 (21.6)	10 (5.4)	0.160	37 (6.1)	26 (6.4)	11(5.5)	0.721
	S	224 (94.5)	40 (78.4)	176 (94.6)		567 (93.9)	378 (93.6)	189(94.5)	

Clindamycin	R	21 (8.9)	14 (27.5)	31 (16.7)	**0.001***	175 (29)	145 (35.9)	30(15)	**<0.001***
	S	216 (91.1)	37 (72.5)	155 (83.3)		429 (71)	259 (64.1)	170 (85)	
Tetracycline	R	14 (27.5)	3 (5.9)	2 (1.1)	0.106	90 (14.9)	62 (15.3)	28 (14)	0.717
	S	192 (81.0)	48 (94.1)	184 (98.9)		514 (85.1)	342 (84.7)	172 (86)	
Fusidic acid	R	5 (2.1)	5 (9.8)	0	0.068	62 (10.3)	45 (11.1)	17(8.5)	0.393
	S	232 (97.9)	46 (90.2)	186 (100)		542 (89.7)	359 (88.9)	183 (91.5)	
Vancomycin	R	0 (0)	18 (35.3)	50 (26.9)	-	0 (0)	-	-	-
	S	237 (100)	33 (64.7)	136 (73.1)		604 (100)	-	-	
Erythromycin	R	68 (28.7)	0 (0)	14 (7.5)	0.294	204 (33.8)	162 (40.1)	42 (21)	**<0.001***
	S	169 (71.3)	51 (100)	172 (92.5)		400 (66.2)	242 (59.9)	158 (79)	
Chloramphenicol	R	14 (5.9)	4 (7.8)	16 (8.6)	**0.045***	98 (16.2)	78 (9.3)	20 (10)	**0.003***
	S	223 (94.1)	47 (92.2)	170 (91.4)		506 (83.8)	326 (80.7)	180 (90)	
Quinupristin /Dalfopristin	R	20 (8.4)	1 (2)	11 (5.9)	1.0	24 (4)	15 (3.7)	9 (4.5)	0.661
	S	217(91.6)	50 (98)	175 (94.1)		580 (96)	369 (96.3)	191 (95.9)	
Linezolid	R	12 (5.1)	2 (3.9)	0(0)	0.470	2 (0.3)	2 (0.5)	0	1.000
	S	225 (94.9)	49 (96.1)	186(100)		602 (99.7)	402 (99.5)	200 (0)	

R: resistant; S: sensitive; * Difference is statistically significant (*p*<0.05); Statistical analysis: Chi-Square tests or Fisher's exact test

Table 3.20 Comparison of antibiotic resistance in *S. epidermidis* and other CNS isolates

Antibiotics	Susceptibility	All CNS (n=604) N (%)	Group		*p* value
			Non S. epidermidis (n=76) N (%)	*S. epidermidis* (n=528) N (%)	
Oxacillin	R	139 (23.0)	19 (25)	120 (22.8)	0.663
	S	465 (77.0)	57 (75)	408 (77.2)	
Penicillin	R	414 (68.5)	52 (68.4)	362 (68.6)	1.00
	S	190 (31.5)	24 (31.6)	166 (31.4)	
Ciprofloxacin	R	49 (8.1)	8 (10.5)	41 (7.8)	0.374
	S	555 (91.9)	68 (89.5)	487 (92.2)	
Trimethoprim–sulphamethoxazole	R	71 (11.8)	7 (9.2)	64 (12.1)	0.57
	S	533 (88.2)	69 (90.8)	464 (87.9)	
Gentamicin	R	37 (6.1)	7 (9.2)	30 (5.7)	0.57
	S	557 (93.7)	69 (90.8)	498 (94.3)	
Clindamycin	R	175 (29.0)	25 (32.9)	150 (28.4)	0.420
	S	429 (71.0)	51 (67.1)	378 (71.6)	
Tetracycline	R	90 (14.9)	14 (18.4)	76 (14.4)	0.388
	S	514 (85.1)	62(81.6)	452 (85.2)	
Fusidic acid	R	62 (10.3)	6 (7.9)	56 (10.6)	0.550
	S	542 (89.7)	70 (90.8)	472 (89.2)	
Vancomycin	R	0 (0)	-	-	-
	S	604 (100)	76(100)	528 (100)	
Erythromycin	R	204 (33.8)	33(43.4)	171 (32.4)	0.069
	S	400 (66.2)	43(56.6)	357 (67.6)	
Chloramphenicol	R	98 (16.2)	13(17.1)	85 (16.1)	0.868
	S	506 (83.8)	63(82.9)	443 (83.9)	
Quinupristin /Dalfopristin	R	24 (4.0)	4 (5.3)	20 (3.8)	0.528
	S	580 (96.0)	72 (94.7)	508 (96.2)	
Linezolid	R	2 (0.5)	0	2 (0.4)	1.0
	S	602 (99.5)	76(100)	526 (99.6)	

R, resistance; S, sensitive; Chi-Square tests or Fisher's exact test

Table 3.21 Comparison of isolation rates of MRSA from nurses and the general population

Group	Sources	No. of person	No. of *S. aureus*	No. of MRSA	Colonization rate for MRSA (%)
Nurses	EN	157	33	7	**4.46***
	FN	92	18	1	1.09
	Total	249	51	8	**3.2***
General population		775	186	4	0.52

* Statistically significant compared to general population, $p<0.05$

Table 3.22 Prevalence of *qac*A/B and *smr* in isolates of *S. aureus* and CNS from nurses and the general population

QAC gene	Species	Group	Positive N (%)	Negative N (%)	*p* value	OR	95% CI
*qac*A/B	CNS	Nurses	229 (56.7)	175 (43.3)	**<0.001***	**8.4**	**5.4 -13.2**
		General Population	27 (13.5)	173 (86.5)			
	S. aureus	Nurses	21 (41.2)	30 (58.8)	**<0.001***	**5.5**	**2.7-11.2**
		General Population	21 (11.3)	165 (88.7)			
smr	CNS	Nurses	73 (18.1)	331 (81.9)	**0.002***	**2.4**	**1.4 - 4.2**
		General Population	17 (8.5)	183 (91.5)			
	S. aureus	Nurses	6 (11.8)	45 (88.2)	0.1	2.3	0.8 - 6.8
		General Population	10 (5.4)	176 (94.6)			

Statistical analysis: Chi-Square tests; * Difference is statistically significant.

Table 3.23 Comparison of the prevalence of *qac*A/B and *smr* between *S. epidermidis* and other CNS from nurses and the general population

Group	QAC gene type	CNS type	Positive N (%)	Negative N (%)	*p* value	OR	95% CI
Nurses	*qacA/B*	SE	199 (58.1)	30 (45.5)	**0.044***	**1.72**	**1.01 - 2.92**
		Other CNS	139 (41.1)	36 (54.5)			
	smr	SE	61 (18)	12 (18.2)	0.979	0.991	0.5 - 1.96
		Other CNS	277 (82)	54 (81.8)			
General population	*qacA/B*	SE	27(44.2)	0 (0)	0.364	-	-
		Other CNS	163 (85.8)	10(100)			
	smr	SE	17 (8.9)	0 (0)	0.323	-	-
		Other CNS	173(91.1)	10 (100)			
Total CNS	*qacA/B*	SE	226 (42.8)	30 (39.5)	0.621	-	-
		Other CNS	302(57.2)	46 (60.5)			
	smr	SE	78 (14.8)	12 (15.8)	0.863	-	-
		Other CNS	450 (85.2)	64 (82.4)			

SE: *S. epidermidis* ; *****Difference is statistically significant ($p<0.05$);

Statistical analysis: Chi-Square tests or Fisher's exact test

Table 3.24 Association between presence of QAC genes *qac*A/B and *smr* and methicillin resistance in isolates of *S. aureus* and CNS

QAC gene	Species	Positive N (%)	Negative N (%)	*p* value	OR	95% CI
*qac*A/B	MRSA[a]	6(50.0)	6 (50.0)	**0.003***	**5.3**	**1.6 -17.2**
	MSSA[b]	36 (16.0)	189 (84.0)			
	MRCNS[c]	93 (66.9)	46 (33.1)	**<0.001***	**3.7**	**2.5-5.6**
	MSCNS[d]	163 (35.1)	302 (64.9)			
smr	MRSA[a]	2 (16.7)	10 (83.3)	0.16	3.01	0.6-15.1
	MSSA[b]	14 (6.2)	211(93.8)			
	MRCNS[c]	33 (23.7)	106 (76.3)	**0.001***	**2.2.**	**1.4-3.6**
	MSCNS[d]	57 (12.3)	408 (87.7)			

[a] Methicillin resistant *S. aureus;* [b] Methicillin sensitive *S. aureus;* [c] Methicillin resistant Coagulase-negative Staphylococci*;* [d] Methicillin sensitive Coagulase-negative Staphylococci *S*tatistical analysis: Chi-Square tests; *statistically significant

Table 3.25 Distribution of *qac* G/H/J in staphylococci from nurse and the general population

Staphylococci	Group	Number of isolates	Number of *qacG* (%)	Number of *qacH* (%)	Number of *qacJ* (%)
S. aureus	Nurses	51	0	0	1 (1.91)
	General population	186	4 (2.15)	1 (0.54)	2 (1.07)
	Total	237	4 (1.69)	1 (0.42)	3 (1.27)
CNS	Nurses	404	0	0	0
	General population	200	0	0	1 (0.50)
	Total	604	0	0	1 (0.16)

Table 3.26 Univariate analysis of risk factors for carriage of QAC in isolates of *S. aureus* (n= 51) and CNS (n= 404) from nurses

Variable	QAC gene Positive N (%)	QAC gene Negative N (%)	*p* value	OR	95% CI
Sex					
Female	238 (63.0)	140 (37.0)	0.17	0.7	0.4- 1.2
Male	42 (54.5)	35 (45.5)			
Nurse					
Experienced nurse	127 (55.0)	104 (45.0)	**0.003 ***	**0.6**	**0.4- 0.8**
Fresh nurse	153 (68.3)	71 (31.7)			
Antibiotic use history in the past 6 months					
Yes	156 (65.8)	81 (34.2)	**0.054 ***	**1.5**	**1.0- 2.1**
No	124 (56.9)	94 (43.1)			
Contact MRSA patients in the past three months					
Yes	263 (62.9)	155 (37.1)	**0.042**	**2.0**	**1.0-3.9**
No	17 (45.9)	20 (54.1)			
*mec*A gene carriage in isolates					
Positive	95 (76.0)	30 (24.0)	**<0.001***	**2.5**	**1.6-3.9**
Negative	185 (56.1)	145 (43.9)			
Underlying conditions and chronic illness					
No	258 (61.4)	162 (45.2)	0.8	1.1	0.5-2.2
Yes	22 (62.9)	13 (37.1)			
Hibiscrub commonly used in					

the last 3 months for hand hygiene				
Rarely and never (<25%)	40 (65.6)	21(34.4)		
Sometimes (25-49%)	58 (67.4)	28 (32.6)		
Very often (50-74%)	68 (59.6)	46 (40.4)		-
Always (>75%)	114 (58.8)	80 (41.2)	0.477	
Soap commonly used in the last 3 months i for hand hygiene				
Rarely and never (<25%)	61 (62.2)	37 (37.8)		
Sometimes (25-49%)	38 (56.7)	29 (43.3)		-
Very often (50-74%)	93 (67.9)	44 (32.1)		
Always (>75%)	88 (57.5)	65 (42.5)	0.256	
Alcohol hand rub commonly used in the last 3 months for hand hygiene				
Rarely and never (<25%)	12 (54.5)	10 (45.5)		
Sometimes (25-49%)	54 (67.5)	26 (32.5)		
Very often (50-74%)	95 (68.8)	43 (31.2)		-
Always (>75%)	119 (55.3)	96 (44.7)	**0.041***	
Povidone iodine commonly used in the last 3 month for hand hygiene				
Rarely and never (<25%)	171 (58.6)	121 (41.4)		
Sometimes (25-49%)	78 (70.3)	33 (29.7)		
Very often (50-74%)	21 (60.0)	14 (40.0)		-
Always (>75%)	10 (58.8)	7 (41.2)	0.190	

*Difference is statistically significant; Statistical analysis: Chi-Square tests or Fisher's exact test

Table 3.27 Adjusted Odds Ratio of risk factors for carriage of QAC genes in *S. aureus* and CNS isolates

Variables	*p* value	aOR	95% CI
Fresh nurses vs experienced nurses	**0.02***	**0.6**	**0.3-0.9**
Presence of *mec*A	**<0.001***	**2.8**	**1.7-4.7**
Contact with MRSA patients in the past three months	**0.01***	**2.8**	**1.3-6.2**

aOR, adjusted odds ratio; CI, confidence intervals;

*difference is statistically significant ($p<0.05$)

Table 3.28 Comparison of MICs and MBCs of BC and CHG for *S. aureus* and CNS from nurses and the general population

Source	MIC of BC (mg/L)				MBC of BC (mg/L)			
	Range	MIC_{50}	MIC_{90}	*p* value	Range	MBC_{50}	MBC_{90}	*p* value
Nurses	0.5-8	2	4	0.731	0.5-32	4	8	0.054
General population	0.5-64	2	4		1-128	4	16	

Source	MIC of CHG (mg/L)				MBC of CHG (mg/L)			
	Range	MIC_{50}	MIC_{90}	*p* value	Range	MBC_{50}	MBC_{90}	*p* value
Nurses	0.5-4	2	4	0.099	1-16	8	16	**<0.001***
General population	0.5-32	2	4		0.5-32	2	8	

*Difference is statistically significant ($p<0.05$); Statistical analysis: Mann-Whitney-U test.

Table 3.29 Comparison of MICs and MBCs of BC for *S. aureus* and CNS with and without QAC genes from nurses and the general population

Isolates	QAC genes present	N	MIC of BC (mg/L)				MBC of BC (mg/L)			
			Range	MIC_{50}	MIC_{90}	*p* value [a]	Range	MIC_{50}	MIC_{90}	*p* value [a]
S. aureus	*qacA/B*	36	0.5–4	2	4	**<0.001***	0.5–16	4	16	**<0.001***
	smr	10	2–4	2	4	**<0.001***	2–16	4	8	**0.008***
	*qac*A/B + *smr*	6	2–16	4	16	**0.002***	2–16	8	16	**0.029***
	Negative	30	1–2	1	2	–	1–8	2	4	–
CNS	*qacA/B*	48	0.5–64	2	4	**<0.001***	0.5–128	4	8	**<0.001***
	smr	31	0.5–8	2	4	**<0.001***	1–32	2	8	**<0.001***
	*qac*A/B + *smr*	37	0.5–64	2	4	**<0.001***	0.5–64	4	8	**<0.001***
	Negative	30	0.5–4	1	2	–	0.5–8	1	4	–
Total	*qacA/B*	84	0.5–64	2	4	**<0.001***	0.5–128	4	16	**<0.001***
	smr	41	0.5–8	2	4	**<0.001***	1–32	4	16	**<0.001***
	*qac*A/B + *smr*	43	0.5–64	2	4	**<0.001***	0.5–64	4	16	**<0.001***
	Negative	60	0.5–4	1	2	–	0.5–8	2	4	–

[a] Comparison to isolates without QAC genes ; ***** Difference is statistically significant ($p<0.05$); Statistical analysis: Mann-Whitney-U test.

Table 3.30 MICs and MBCs of CHG for *S. aureus* and CNS with and without QAC genes from nurses and the general population

Strains	QAC genes present	N	MIC of CHG (mg/L)				MBC of CHG (mg/L)			
			Range	MIC_{50}	MIC_{90}	*p* value [a]	Range	MIC_{50}	MIC_{90}	*p* value [a]
S. aureus	*qacA/B*	36	1–8	2	8	**0.008***	1–8	2	8	**0.018***
	smr	10	1–2	2	2	**0.039***	2–8	4	8	**0.003***
	*qac*A/B + *smr*	6	1–4	2	4	**0.042***	1–8	4	8	NS
	Negative	30	0.5–2	1	2	–	1–4	2	4	–
CNS	*qacA/B*	48	0.5–8	2	4	**<0.001***	0.5–16	8	16	**<0.001***
	smr	31	0.5–4	2	4	**<0.001***	0.5–16	8	16	**<0.001***
	*qac*A/B + *smr*	37	1–32	2	4	**<0.001***	2–32	8	16	**<0.001***
	Negative	30	0.5–4	1	2	–	0.5–8	2	8	–
Total	*qacA/B*	84	0.5–8	2	4	**<0.001***	0.5–16	4	16	**<0.001***
	smr	41	0.5–4	2	2	**<0.001***	0.5–16	8	16	**<0.001***
	*qac*A/B + *smr*	43	1–32	2	4	**<0.001***	1–32	8	16	**<0.001***
	Negative	60	0.5–4	1	2	–	0.5–8	2	8	–

[a] Comparison to isolates without QAC genes;***Difference is statistically significant ($p<0.05$); Statistical analysis: Mann-Whitney-U test.

Table 3.31 Minimum inhibitory concentrations of benzalkonium chloride and chlorhexidine for isolates with *qac*G,H or J

Gene	Isolate	MIC (mg/L)	
		BC	CHG
qacG + qacA/B	*S. aureus*	8	4
qacG + smr	*S. aureus*	8	2
qacG	*S. aureus*	4	2
qacG	*S. aureus*	4	2
qacH	*S. aureus*	4	2
qacJ	*S. aureus*	4	2
qacJ	*S. aureus*	8	2
qacJ	*S. aureus*	4	2
qacJ	S. *epidermidis*	4	2
qacA/B control strain	*S. aureus*	4	2
smr control strain	*S. aureus*	4	2
qacG control strain	*S. aureus*	4	2
qacH control strain	*S. aureus*	4	2
qacJ control strain	*S. aureus*	4	2
qac negative control	*S. aureus* ATCC 25923	2	2

Table 3.32 Relationship between QAC gene (*qac*A/B, *smr*) carriage and antibiotic resistance in *S. aureus* and CNS isolates

Antibiotic	Susceptibility	Number of *S. aureus* isolates with QAC gene (%)			Number of CNS isolates with QAC gene (%)		
		Positive	Negative	*p* value	Positive	Negative	*p* value
Oxacillin	R	10 (58.8)	7 (41.2)	**<0.001***	101 (34.6)	38 (12.2)	**<0.001***
	S	41 (22.0)	179 (81.4)		191 (65.4)	274 (87.8)	
Penicillin	R	47 (24.2)	147 (75.8)	**0.031***	225 (77.1)	189 (60.6)	**<0.001***
	S	4 (9.3)	39 (90.7)		67 (22.9)	123(39.4)	
Ciprofloxacin	R	7 (58.3)	5 (41.7)	**0.005***	29 (9.9)	20 (6.4)	0.136
	S	44 (19.6)	181 (80.4)		263 (90.1)	292(93.6)	
Trimethoprim–sulphamethoxazole	R	3 (100)	0 (0)	**0.001***	37 (12.7)	34 (10.9)	0.529
	S	48 (20.5)	186 (79.5)		255 (87.3)	278 (89.1)	
Gentamicin	R	5 (38.5)	8 (61.5)	0.16	23 (7.9)	14 (4.5)	0.09
	S	46 (20.5)	178 (79.5)		269 (92.1)	298 (95.5)	
Clindamycin	R	9 (42.9)	12 (57.1)	**0.023***	113 (38.7)	62 (19.9)	**<0.001***
	S	42 (19.4)	174 (80.6)		179 (61.3)	250 (80.1)	
Tetracycline	R	16 (35.6)	29 (64.4)	**0.011***	55 (18.8)	35 (11.2)	**0.012***
	S	35 (18.2)	157 (81.8)		237 (81.2)	277 (88.8)	
Fusidic acid	R	3 (60)	2 (40)	0.068	32 (11)	30 (9.6)	0.595
	S	48 (20.7)	184 (79.3)		260 (89)	282 (90.4)	
Vancomycin	R	0 (0)	0	–	-	-	-
	S	51 (21.5)	186 (78.5)		292 (100)	312(100)	
Erythromycin	R	18(26.5)	50 (73.5)	0.239	124 (42.5)	80 (25.6)	**<0.001***
	S	33 (19.5)	136(80.5)		168 (57.5)	232(74.4)	
Chloramphenicol	R	2 (14.3)	12 (85.7)	0.740	60 (20.5)	38 (12.2)	**0.006***
	S	49 (22)	174 (78)		232(79.5)	274 (87.8)	
Quinupristin /Dalfopristin	R	5 (25)	15 (75)	0.776	10 (3.4)	14 (4.5)	0.539
	S	46 (21.2)	171 (78.8)		282 (96.6)	298 (95.5)	
Linezolid	R	1 (8.3)	11 (91.7)	0.470	0	2(0.6)	0.5
	S	50(22.2)	175 (77.8)		292(100)	310 (99.4)	

R: resistance; S: sensitive; ***Difference is statistically significant ($p<0.05$); Chi-Square tests or Fisher's exact test

Table 3.33 Antibiotic resistance in *qac*G, *qac*H and *qac*J positive isolates of *S. aureus*

Antibiotic	*qacG*		*qacH*		*qacJ*		Total	
	N	Resistance Rate (%)	N	Resistance Rate (%)	N	Resistance Rate (%)	N	Resistance Rate (%)
Penicillin	4	100	1	100	3	75	8	88.89
Quinupristin/ Dalfopristin	0	0	0	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0
Ciprofloxacin	0	0	0	0	0	0	0	0
Co-trimoxazole	1	25	0	0	0	0	1	11.11
Gentamicin	2	50	0	0	1	25	3	33.33
Fusidic Acid	2	50	0	0	1	25	3	33.33
Vancomycin	0	0	0	0	0	0	0	0
Erythromycin	3	75	0	0	2	50	5	55.56
Clindamycin	2	50	0	0	3	75	5	55.56
Tetracycline	3	75	0	0	1	25	4	44.44
Chloramphenicol	1	25	0	0	0	0	1	11.11

Table 3.34 Comparison of antimicrobial resistance patterns in *qac*G, H and J positive staphylococci and MICs to BC and CHG.

QAC gene	Species	Antimicrobial resistance patterns*	MIC (mg/L)	
			BC	CHG
qacG	*S. aureus*	P, GEN, DA, TE	4	2
qacG	*S. aureus*	P, GEN, SXT, TE	4	2
qacG + smr	*S. aureus*	P, DA, FD, E, CHL	8	2
qacG+ qacA/B	*S. aureus*	P, TE, FD, E	8	4
qacH	*S. aureus*	P	4	2
qacJ	*S. aureus*	P	4	2
qacJ	*S. aureus*	P, DA, E	4	2
qacJ	*S. aureus*	DA, TE, FD	8	2
qacJ	S. *epidermidis*	P, DA, GEN, E	4	2

*BC-Benzalkonium chloride; CHG-Chlorhexidine digluconate; P-Penicillin; GEN-Gentamicin; DA-Clindamycin;TE-Tetracycline; SXT-Co-trimoxazole; FD-Fusidic acid; E-Erythromycin

CHAPTER 4

PREVALENCE OF ANTISEPTIC RESISTANCE GENES IN STAPHYLOCOCCAL ISOLATES FROM COMMUNITY AND HOSPITAL ENVIRONMENTS

4.1 Introduction

Methicillin-resistant *S. aureus* (MRSA), for many years an important hospital associated pathogen, has more recently emerged in the community. This has led to increased public concern about the risks of infection, including from contamination of the environment (Miller & Diep, 2008).

Sampling of household contacts of infected subjects frequently yields low rates of colonized subjects (Zafar et al., 2007) and thus the possibility of contracting the organism from an environmental source must be considered. This has lead to studies investigating contamination of sites frequently touched in the hospital and in the community. Hands may be contaminated with *S. aureus* by nose picking or

touching the nasal area (Wertheim et. al., 2006) and the organism transferred to commonly contacted items such as Automated teller machines (ATMs). Hospital surfaces may be contaminated by patients' isolates as well as their own normal flora. An earlier report in Hong Kong had shown that there was a relatively higher isolation rate of *S. aureus* from ATMs than from other commonly accessed items in the environment (Boost et al., 2008b). This suggested that ATMs may serve as a good marker of community contamination.

The importance of decontamination by cleaning to reduce the risk of MRSA/*S. aureus* infection has long been stressed, and aggressive environmental cleaning has been a key component of the successful "search-and destroy" policy to minimize MRSA infection in the Netherlands (Vriens et al., 2002; Meek, 2004). However, there is concern about emerging resistance to antiseptics used as decolonization and decontamination agents. Staphylococcal strains harbouring genes that increase resistance to QACs and other disinfectants (QAC genes) by conferring increased MBCs to antiseptics have been demonstrated in clinical isolates of MRSA (Smith et al. 2008; Vali et al., 2008) and there is evidence that carriage of these genes may be increasing (Wang et al., 2008). The distribution of QAC genes in *S. aureus* and CNS has been investigated in clinical isolates, with most focus on MRSA (Smith et al., 2008; Vali et. al., 2008). However, little is known about the frequency of these genes in *S. aureus* isolated from the environment.

Staphylococcal food poisoning is the second most common cause of reported bacterial food-borne illness and is attributable to enterotoxin production. Contamination with strains carrying enterotoxin genes could increase the food poisoning risks of the general population. As both QAC and enterotoxin genes are present on plasmids, it is possible that selection for antiseptic resistance may also select for enterotoxin production (Sidhu et al., 2001a; 2002).

In this study, we investigated the frequency of *S. aureus* and MRSA contamination of the public environment, using ATMs as a marker. We also determined the prevalence of QAC genes in contaminating staphylococci and of enterotoxin genes in strains of *S. aureus* isolated from the ATMs. Biocide susceptibilities of the strains with QAC genes were also determined.

In addition, in recent years, there has been a dramatic increase in prevalence of antibiotic resistant pathogens. This has been accompanied by increased concern about the role of the environment in the transmission of nosocomial infections (Otter et al., 2011; Kassem, 2011). Hospital patients can obtain organisms from many sources including the environment. Several reports have demonstrated that persistence of pathogens in the environment may serve as a significant reservoir increasing the risk of hospital-acquired infections (Sexton et al., 2006; Hardy et al., 2006; Otter et al., 2011; Kassem, 2011). Spread of infectious agents can be mediated by dissemination by a vector, contact, air turbulence, and contact with medical equipment, furniture, or material directly or via the hands of a health

worker. Staphylococci are major contaminants of contact surfaces in clinical settings (Otter et al., 2011; Kassem, 2011). Additionally, CNS are important reservoirs for genes that contribute to the evolution of MRSA in both community and hospital settings. The association between environmental surfaces contaminated with MRSA and hospital infection rates is not fully understood (Sexton et al., 2006; Hardy et al., 2006). Although several studies elsewhere have investigated contamination of the hospital environment, there is limited information with regards to hospital environmental contamination in Hong Kong.

In this study, the rates of MRSA contamination of the hospital environment, as well as the prevalence of QAC genes in contaminating staphylococci, were determined. Furthermore, comparison between isolates from the public environment, represented by the ATMs, and hospital environment was carried out.

4.2 Materials and Methods

4.2.1 Epidemiological Investigation

4.2.1.1 Epidemiological Investigation of ATMs in Hong Kong

A cross-sectional study was performed to investigate the contamination rates of ATMs with *S. aureus* and CNS harbouring QAC genes. A minimum sample size of 202 ATMs was estimated based on a *S. aureus* contamination rate of 20% and an assumed 5% carriage rate of QAC genes in *S. aureus* and CNS with 3% error and 95% confidence intervals. This was based on a pilot study of contamination of frequently contaminated items in the community (Boost et al., 2008b). A total of 400 ATM

terminals situated throughout Hong Kong were randomly sampled between May 2009 and May 2010. Samples were collected from the three main geographical regions (Hong Kong Island, Kowloon, and New Territories), which were further classified to 18 districts (Table 4.6). Samples were collected by swabbing three designated buttons of each ATM with saline-moistened transport swabs, which were placed into transport medium (Transwabs, Medical Wire & Equipment, Corsham, England) and cultured within two hours of collection.

4.2.1.2 Epidemiological Investigation of the Hospital Environment

Representative sites in the hospital environment were selected to carry out the investigation. The samples were collected by nurses, all of whom were trained in the sampling method, and were required to strictly follow sampling instructions, in order to avoid cross-contamination. The swabs were moistened before collection of the sample. To standardize areas sampled, plastic templates with an open area of 10 square centimeters were placed over the sampling site and the swab pressed over the area revealed.

Sample collections were performed in a large district hospital from three sources: the bed rails from orthopaedic wards, six commonly contaminated surfaces of hospital operating theatres, and the blood pressure cuffs used in medical wards. Sample collections in the operating theaters were carried out between operations following limited cleaning. Bleach (5% hypochlorite solution) was used for cleaning of blood present in operation theaters. In other situation, sterilized water was

applied.

The 56 samples from the bed rails were collected were collected on 14 occasions over a 7-day period, between June and July 2009. Forty samples from twenty sphygmomanometer cuffs were sampled between March and June 2010. Five operating theatres, defined as contaminated or dirty were chosen, for investigation: these included one theatre for gastrointestinal surgery, two for emergency surgery (acute trauma surgeries), and two for orthopaedic surgery (fractures, amputations, tumours). Operative wounds based on the degree of microbial contamination were classified according to whether the surgical procedures are clean or dirty, according to the guidelines of the US National Research Council group (Table 4.7) (Berard & Gandon, 1964; Gottrup et al., 2005). Three hundred samples were collected between September 2010 and February 2011. The samples were collected from six selected items: the computer keyboard, the suction catheter container, the operating table control handset, jelly pad, operator button on the anaesthesia machine, and the door handle. Samples were taken on non-consecutive days once a week for five months in dirty operating theaters.

4.2.2 *S. aureus* and CNS Isolation and Identification

All swabs were transferred to the laboratory within eight hours, placed into 5% salt Brain Heart Infusion broth and incubated overnight (Oxoid, Basingstoke, UK). Broths were subcultured onto *S. aureus* selective agar (SA Select, Biorad, Marnes-la-Coquette, France) and incubated for 24 hours. S. aureus typically grows

with a deep pink pigmented colony on this agar. All organisms with staphylococcal morphology were identified by Gram stain and catalase test, and confirmed by their 16S *rRNA* genes (Martineau et. al., 2001). Tube coagulase test negative strains were reported as CNS. *S. aureus* was confirmed by a positive Staphaurex Plus test (Murex Biotech, Dartford, UK) and presence of *fem*A gene (Mehrotra et. al., 2000). *S. epidermidis* was differentiated from other CNS by PCR (Martineau et al., 2001). The detailed procedures are described in the Chapter 3.

4.2.3 Antibiotic Susceptibility Testing

Susceptibility to a range of antibiotics was determined by disc diffusion for all *S. aureus* isolated from ATMs and the hospital environment, following CLSI Guidelines (CLSI; 2009), except for fusidic acid, when British Society for Antimicrobial Chemotherapy guidelines (Andrew et al., 2001) were used. Vancomycin susceptibility was determined by agar dilution. The detailed procedures are as described in Chapter 3. Any strain showing resistance to cefoxitin was tested for presence of *mec*A by PCR and, if present, a multiplex PCR was used to type the SCC*mec*, as previously described (See Chapter 3)

4.2.4 Genotyping of MRSA

The repeat-containing region of the staphylococcal protein A gene (*spa*) was amplified, sequenced and *spa* type determined using Ridom Spa Server (spa.ridom.de) (Shopsin et al., 1999; Harmsen et al., 2003). Genomic DNA was purified from each isolate as a template. The reaction master mix contained: 100ng

genomic DNA; 2.5 unit of AmpliTaq Gold DNA polymerase, 2 mM MgCl2, 0.5 µM 1095F forward PCR primer (5-AGACGATCCTTCGGTGAGC-3), 0.5 µM 1517R reverse primer (5-GCTTTTGCAATGTCATTTACTG-), 0.35 mM total dNTPs, to 25 µL of PCR master mixture (Table 4.1). DNA amplification was carried out in a ABI thermocycler as follows: an initial 10 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 60 °C, and 45 s at 72 °C; and a final extension at 72 °C for 10 min. (Table 4.2) DNA sequences were obtained with an ABI 377 sequencer (Applied Biosystems, Foster City, Calif.) Sequence type was determined using designated software with the help of Dr A. Moodley (University of Copenhagen).

4.2.5 Detection of QAC Genes

Detection of the QAC genes, *qac*A/B, *smr* and *qac*G, H, and J, was performed as described in Chapter 3.

4.2.6 Antiseptic Susceptibility Testing

The MICs and MBCs of the isolates to BC and CHG were determined in duplicate by broth micro-dilution with concentrations ranging from 0.5-256 mg/L for both QAC gene positive and negative isolates from ATMs. The lowest concentration totally inhibiting growth after 24 h incubation at 37 ^{o}C was considered the MIC. The MBCs were determined as the concentration producing a 99.9% kill and performed after addition of neutralizing reagent to the wells showing no growth of organisms in MIC. The details were as described in Chapter 3. The MICs and MBCs were not determined for the hospital isolates as it was assumed that these would be similar

to nursing isolates.

4.2.7 Detection of Enterotoxin Genes

The detection of *sea-see* genes was performed using the multiplex PCR method described by Mehrotra et al. (2000), using DNA extracted by the phenol chloroform method. The reaction master mix contained: 100 ng of extracted DNA, 5.0 µL reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM (each) of *sea*, *seb*, *sec* ,and *see* primers, 0.4 µM (each) of *sed* primer, and 2.5 unit of Taq polymerase in 50 µL total volume. DNA amplification was carried out in a ABI thermocycler as follows: initial denaturation at 94 °C for 5 min, 35 cycles of amplification (denaturation at 94°C for 2 min, annealing at 57 °C for 2 min, and extension at 72 °C for 1 min), ending with a final extension at 72 °C for 7 min. Reference strains NRS 111 (harbouring *sea*, *see* genes), NRS158 (harbouring *seb* gene), NRS113 (harbouring *sec* gene), and NRS382 (harbouring *sed* gene) were included as positive controls. Amplicons were visualized on agarose gel with EtBr. (Table 4.3, 4.4, 4.5)

4.2.8 Statistical Analysis

Statistical analyses were performed using SPSS system for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Association of categorical variables was determined by Chi-square statistic or Fisher's exact test. Mann-Whitney U-test was used to compare the MBC and MIC results.

4.3 Results

4.3.1 ATM Results

4.3.1.1 Distribution of Staphylococcal Contamination

Of the total of 400 ATM terminals that were randomly sampled between May 2009 and May 2010, 135 (33.8%) were located on Hong Kong Island, 130 (32.4%) in Kowloon, and the remaining 135 (33.8%) in the New Territories. The detailed distribution of sampling of ATMs is listed in Table 4.6.

4.3.1.2 Staphylococcal Contamination of ATMs

Over the one year sampling period, *S. aureus* was detected on 15.5% (62/400) of ATMs. The ATM contamination rate with *S. aureus* in different districts ranged from 5.9 - 20%, but there was no significant difference in rates between the different sites (p>0.05) (Table 4.6).

Almost all ATMs (95.3%) (381/400) were contaminated with CNS. Contamination rates for Hong Kong Island (97%), Kowloon (93.8%) and New Territories (94.8%) were not significantly different (p>0.05) (Table 4.6). Of the CNS detected, 67.2% (256/381) were *S. epidermidis.*

4.3.1.3 MRSA and MRCNS Contamination of ATMs in Hong Kong

Only two of the 62 (0.32 %) *S. aureus* isolates were MRSA as confirmed by presence of *mecA*. Of these, one which was isolated from an ATM in Wan Chai in Hong Kong Island, harboured SCC*mec* type IVa, and was spa type t091, and the second, SCC*mec*

type V and spa type t1081 was detected from an ATM in North District in the New Territories. *mecA* was present in 15.3% (60/391) CNS isolates with 18% (40/256) *S. epidermidis* strains being resistant to methicillin.

4.3.1.4 Antibiotic Susceptibility of *S. aureus* from ATMs

Resistance rates were determined for *S. aureus* isolates based on sensitivity testing against fourteen antibiotics including penicillin, gentamicin, clindamycin, fusidic acid, erythromycin, chloramphenicol, ciprofloxacin, tetracycline, Linezolid, vancomycin trimethoprim–sulphamethoxazole, quinupristin/Dalfopristin, Imipenem. All isolates were susceptible to linezolid and vancomycin.

High levels of resistance were observed to penicillin (87.5 %), tetracycline (22.6%), fusidic acid (33.9%) and erythromycin (29 %), with less resistance to chloramphenicol (3.2%), ciprofloxacin (6.5%), trimethoprim–sulphamethoxazole (3.2%), gentamicin (8.1%), clindamycin (6.5%) and imipenem (1.6%). Rates of resistance to non-beta lactams were similar to those recently reported for nasal *S. aureus* isolates from Hong Kong (Boost et. al., 2008a), except for fusidic acid resistance which was somewhat higher.

4.3.1.5 Prevalence of QAC Genes in *S. aureus* and CNS

Of the total 62 *S. aureus* strains, 7 (11.3%) harboured *qac*A/B, only one (1.6%) *S. aureus* carried the *smr* gene, and two (3.2%) harboured *qacG* gene. For CNS, 99 isolates harboured *qacA/B* (26.9%) and 53 *smr* (14%, 53/381). Two CNS (0.5%)

harboured *qacG*, whilst *qacJ* was present in only one isolate (0.25%). Simultaneous presence of *qacA/B* and *smr* was detected in 18 CNS strains (4.7%). There was a significantly higher prevalence of *qacA/B* and *smr* in CNS ($p<0.05$) than in *S. aureus.*

4.3.1.6 QAC Genes and Methicillin Resistance in *Staphylococcus* Spp.

Comparison of the presence of methicillin resistance revealed that a significantly higher percentage of MRCNS (27/60; 45.0%) than MSCNS (72/321; 22.4%) isolates carried the *qac*A/B gene ($p<0.001$, OR 2.8; 95% CI: 1.6-5.0). Likewise, there was a higher prevalence of *qacA/B* (100%, 2/2) in MRSA compared to MSSA (8.3%, 5/60). ($p=0.011$). No similar association was found for QAC genes *smr*, *qac*G, *qac*H, *qac*J and methicillin resistance ($p>0.05$) (Table 4.8)

4.3.1.7 Antibiotic Resistance and QAC Gene Carriage

Association between antibiotic and antiseptic resistance was investigated. Using the Chi-square test, resistance to tetracycline was significantly more frequent in QAC gene positive (50%, 5/10) than gene negative *S. aureus* (17.3%, 9/52) ($p=0.024$, OR 4.8, 95% CI: 1.1-20.0). No relationship was found between other antibiotics and presence of QAC genes (Table 4.9).

4.3.1.8 Relationship of QAC Gene Carriage with MICs and MBCs of BC and CHG

Biocide MICs and MBCs were performed for all positive *S. aureus* (7 *qac*A/B, 1 *smr*) and 58 CNS (20 *qac*A/B only, 20 with *smr* only, and 18 *qac*A/B + *smr*), and for 40 gene negative controls (20 each *S. aureus* and CNS). The MIC/MBC range,

MIC_{50}/MBC_{50}, and MIC_{90}/MBC_{90}, are shown in Table 4.5a,b. Isolates with QAC genes had higher mean MICs and MBCs to both BC and CHG, with a wider range of MICs and MBCs, than isolates without QAC genes ($p<0.05$) (Table 4.10a, 4.10b).

4.3.1.9 Association between QAC and Enterotoxin Genes in *S. aureus*

Enterotoxin genes were present in 37.1% (23/62) *S. aureus* isolates, with 14.5% (9/62) harbouring *sea*, 12.9% (8/62) *seb*, 11.3% (7/62) *sec,* and 12.9% (8/62) *see* gene. Both *sea* and *see* were detected in 6 isolates, *sea* and *sec* in two. Only one isolate carried *sea*, *sec*, and *see* simultaneously. No isolates were found to have *sed*.

Three *S. aureus* strains with *qac*A/B and one with *qac*G also carried *seb.* Other enterotoxin genes were not detected in *S. aureus* harbouring QAC genes. Presence of s*eb* appeared to be associated with carriage of QAC genes in *S. aureus* (p=0.005, OR 8, 95% CI: 1.6-40.6). There was no association between presence of other enterotoxin genes and QAC genes. The MRSA isolates did not harbour enterotoxin genes. No association was found between enterotoxin genes and antibiotic resistance in *S. aureus* (Table 4.11).

4.3.2 Hospital Results

4.3.2.1 Staphylococcal Contamination of the Hospital Environment

Between 2009 and 2010, sample collections in the hospital were performed at three locations: the bed rails of orthopaedic wards, the surfaces of operation theatres and

blood pressure cuffs. A total of 336 positive isolates yielded 232 CNS and 104 *S. aureus*. Hospital bed rails yielded 285 isolates consisting of 137 CNS and 48 *S. aureus*. Of the 102 isolates from hospital operating theatres 61 were CNS and 41 *S. aureus*. The 49 isolates from the pressure cuffs consisted of 45 CNS and 15 *S. aureus*. Of the total CNS isolates, 46.1% (107/232) were *S. epidermidis*.

4.3.2.2 MRSA and MRCNS Contamination of the Hospital Environment

The *mec*A gene was present in 44.0% (102/232) CNS and 55.0% (57/104) of *S. aureus*. There was no significant difference in rates of methicillin resistance between *S. aureus* and CNS (p=0.066).

Of the isolates from hospital bed rails, 43.8% (60/137) CNS were methicillin resistant and 79.2% (38/48) *S. aureus* were MRSA. *S. aureus* was more likely to be resistant to methicillin than CNS (p<0.001). However, in the hospital operating theatres, there was no significant difference in methicillin resistance rates between *S. aureus* (39.0%, 16/41) and CNS (41.0%, 25/61) (p=0.843). In contrast, resistance to methicillin was less common in the *S. aureus* isolates from the pressure cuffs with 20% (3/15) being MRSA, but 68% (23/34) CNS strains displayed methicillin resistance.

There were significant differences in the rates of methicillin resistance of *S. aureus* in isolates from bed rails, operating theatre, and pressure cuffs (p<0.001) with *S. aureus* from the bed rail (79.2%) statistically more likely to be methicillin resistant

than from the operating room (39.0%) (*p*<0.001, OR 6.0, 95% CI: 2.3 -15.2) and from the pressure cuffs (20%) (*p*<0.001, OR 15.3, 95% CI: 3.4-16.7). But there was no significant differences in MRCNS between isolates from these sites (*p*=0.696).

4.3.2.3 SCC*mec* Typing

Of the total MRSA isolates, 8.8% (5/57) were SCC*mec* II, 43.9% (25/57) SCC*mec* III, 7% (4/57) SCC*mec* IVa, and 40.4% (23/57) were SCC*mec* V (Table 4.7). Thus, over half, 52.7%, MRSA were HA-MRSA consisting of types SCC*mec* II and III, while the remaining 47.4% (27/57) were CA-MRSA types, SCC*mec* IVa and V. There was no significant difference in the proportion of HA-MRSA and CA-MRSA in the hospital isolates (*p*>0.05). The SCC*mec* type of MRSA was not associated with the different hospital sites (*p*>0.05) (Table 4.12).

4.3.2.4 Susceptibility Testing

Susceptibility testing was performed for all 104 *S. aureus* isolates from hospital surfaces against the same antibiotics used for isolates from ATMs. All isolates were susceptible to vancomycin. Rates of resistance of *S. aureus* to penicillin, ciprofloxacin, tetracycline, gentamicin, fusidic acid, erythromycin, oxacillin, and imipenem were high, but resistance was less common to chloramphenicol, clindamycin, linezolid trimethoprim–sulphamethoxazole, and quinupristin/dalfopristin (Table 4.13).

4.3.2.5 Susceptibility Patterns of MRSA

The relationships between susceptibility to non-beta lactam antibiotics of the various SCC*mec* types were investigated and are shown in Table 4.9. Susceptibility rates differed significantly between types for ciprofloxacin, clindamycin, tetracycline, fusidic acid and imipenem. There was no difference in antibiotic susceptibility patterns between CA-MRSA and HA-MRSA in our study except for imipenem resistance (p =0.004) (Table 4.15).

4.3.2.6 QAC Genes in *S. aureus* and CNS from Hospital Isolates

Of the total isolates, 36.2% (84/232) CNS and 46.2% (48/104) *S. aureus* were *qac*A/B positive but this difference did not reach significance (p=0.084) (Table 4.16). Of the isolates from bed rails, 38.4% (71/185) were *qac*A/B positive, which were similar to the 39.2% (40/102) from the operating theatre and 42.9% (21/49) from the pressure cuffs. The carriage of *qac*A/B in staphylococci was not associated with the sampling sites in the hospital (p=0. 085) (Table 4.17).

Overall, 15.7% (37/232) CNS and 7.7% (8/104) *S. aureus* were *smr* positive. *S. aureus* harboured significantly more *smr* than CNS (p=0.040) (Table 4.16). Of bed rail isolates, 11.4 % (20/185) were *smr* positive, in contrast to 17.6% (18/102) of isolates from the operating theatre and 14.3% (7/49) from pressure cuffs, but carriage of *smr* in CNS and *S. aureus* was not significantly associated with the hospital sampling site (p=0.621) (Table 4.17). A total of 21 strains contained both *qac*A/B gene and

smr gene, including 19 CNS and two *S. aureus*.

Two *qac*G positive strains were found in *S. aureus* (1.9%, 2/104), one from an operating theatre and one from a pressure cuff. No *qac*G was found in CNS isolates (Table 4.16, 4.17). Patient bed rails yielded two *qac*J positive isolates of *S. aureus* (1.9 %, 2/104) and the two operating theatre CNS isolates carried *qac*J (0.9%, 2/232). *qac*H was not detected. (Table 4.16, 4.17)

4.3.2.7 Prevalence of QAC Genes in MRSA and MRCNS Isolates

There was a statistical association between presence of *qac*A/B and methicillin resistance in these isolates. Methicillin resistant strains harboured significantly more *qac*A/B (48.4%, 77/159) than methicillin sensitive strains (31.1%, 55/177) (p=0.001, OR 2.1, 95% CI: 1.3-3.3). There was no association between other QAC genes (*smr*, *qac*G, *qac*H, *qac*J) and methicillin resistance (Table 4.18).

Compared to MSSA (29.8%, 14/47), there was a statistically higher proportion of *qac*A/B in MRSA (59.6%, 34/57) (p=0.002, OR 2.9, 95% CI: 1.0-8.1), but the difference in CNS *qac*A/B carriage between the MRCNS and MSCNS did not reach significance (p>0.05). There was no statistically significant relationship between *smr*, *qac*G, *qac*H, or *qac*J between MRSA and MSSA or between MRCNS and MSCNS (Table 4.18.)

4.3.2.8 Antibiotic Resistance and QAC Gene Carriage

The antibiotic susceptibility rates of the 104 *S. aureus* isolates from the hospital environment were analyzed for correlation between QAC gene carriage and antibiotic resistance. Resistance to ciprofloxacin, gentamicin, tetracycline and erythromycin was significantly more frequent in QAC gene positive than gene negative isolates (p<0.05) (Table 4.19).

4.4 Discussion

4.4.1 Contamination of Surfaces in the Community

The most important niche for *S. aureus* is the anterior nares (Wertheim et al., 2005a) and colonization at this site increases the risk for clinical infection (Kluytmans et. al., 1997; von Eiff et al., 2001). However, investigations of outbreaks of MRSA in footballer players failed to isolate the strain from the nares of any of the infected players (Begier et al., 2004; Kazakova et al., 2005). Several studies have demonstrated that although cases of CA-MRSA have increased rapidly in the community, rates of MRSA colonization in non-health care workers in the community remain low (Baggett et al., 2004; Nair et al, 2011).

This is the first report of isolation of MRSA from an inanimate surface in the community environment. Until recently, the role of the environment in the spread of MRSA was not recognized, and most national and other guidelines provide few details on environmental decontamination regimens (Jernigan et al., 2003; Muto et

al., 2003). Recently, CA-MRSA has emerged in the Hong Kong community (Ho et. al., 2007a; 2007b), but there is little information on community sources of the organism contributing to its spread. This study showed that only 0.5% of sites examined were MRSA-contaminated. This was to be expected, since colonization rates with MRSA remain low in non-healthcare exposed persons in Hong Kong (Boost et. al., 2008a). However, ATMs are contacted by numerous users over the course of a day and a contaminated machine could act as a source of the organism which can contaminate hands and potentially lead to colonization of the subject. The two MRSA isolated in our study were typical of CA-MRSA (SCC*mec* type IVa, spa type t091 and SCC*mec* type V, *spa* type t1081). Both of these types have been reported in clinical isolates in Hong Kong (Ho et al., 2008b; 2009) and in mainland China (Chen et al., 2010; Geng et al., 2010; Wu et al., 2010), as well as elsewhere (Rijnders et al., 2009). This finding was in contrast to the pilot study, which showed that ATMs were frequently contaminated with *S. aureus*, but did not yield MRSA (Boost et. al., 2008b). This may be due to the small sample size or improvements in use of selective and enrichment methods. The isolation rate of *S. aureus* from ATMs was also somewhat higher in the current study (15%) than in the earlier pilot (11.9%) (Boost et. al., 2008b).

Both *S. aureus* and CNS harboured QAC genes, though the rate was higher in CNS. However, as these genes are plasmid-mediated, there is potential for spread between staphylococcal species. The isolates from ATMs had a significantly higher rate of positivity of QAC genes than isolates of staphylococci from healthy subjects

not employed in health care (Chapter 3). In that study, *qac*A/B was present in 12.5% of isolates and *smr* in 7%, compared to 23.9% and 12 % respectively in the current study (*p*=0.001, OR 1.9, 95% CI: 1.3 -2.7, for *qac*A/B; *p*=0.016, OR 1.8, 95% CI:1.1 - 2.9 for *smr*). This higher rate suggests that QAC gene positive strains may have a survival advantage in the environment especially if low levels of disinfectant residues are present on ATMs. Such frequently contacted items in the environment could act as a source for dissemination in the population of strains positive for QAC genes. As has been shown in other studies, presence of *qac*G, H and J remains rare in human staphylococcal isolates and appear to be mainly associated with food (Bjorland et. al., 2005, Smith et. al. 2008).

The presence of QAC genes has been associated with increased antibiotic resistance and the possibility of co-selection suggested, in particular with respect to methicillin resistance (Smith et.al. 2008; Vali et. al., 2008). Both MRSA isolates in this study carried QAC genes. Association between tetracycline, gentamicin and erythromycin resistance determinants and presence of QAC genes has been reported previously (Sidhu et. al., 2002), as these genes may be present on the same plasmids as QAC genes. These associations were reflected in higher rates of resistance in QAC positive isolates in this study. The coexistence of *mec*A and QAC genes may contribute to environmental survival of MRSA and MRCNS, by allowing persistence in areas with low level antiseptic residues increasing the risk of spread of MRSA in the community.

Large quantities of biocides are used for disinfection and decontamination, in particular in cleaning during times of outbreaks of respiratory infections. This often increases exposure of organisms to residues of disinfectants in turn increasing selective pressure for carriage of QAC genes. Isolates harbouring QAC genes had reduced susceptibility to BC and CHG compared to gene negative strains.

There were a total of 910 staphylococcal food poisoning outbreaks reported in Hong Kong between 2001 and 2009. They accounted for 18.5% of bacterial food poisoning outbreaks, making *S. aureus* the third commonest food poisoning agent (Scientific Committee on Enteric Infections and Foodborne Diseases, 2011). This is the first report of *S. aureus* harbouring enterotoxin genes isolated from a common access site in the community. Most research has focused on enterotoxin-producing *S. aureus* from food sources (Cha et al., 2006; Rall et al., 2008) or clinical isolates (Mehrotra et. al., 2000; Becker et. al., 2003). Although the frequency of enterotoxin-positive *S. aureus* in these community isolates (37.1%) was lower compared with strains from food sources (68.4%) (Rall et. al., 2008), it exceeded that of clinical isolates (23%) reported recently (Reza & Yazdchi, et al., 2009). This suggests that community sources may be significant in spread of enterotoxin-positive strains which could transfer to foods if hygiene measures are not strictly enforced. Enterotoxin *seb* and QAC genes were found to co-exist in some isolates, but there was no association between QAC genes and those for other enterotoxins. It is possible there was co-selection for *seb* and QAC genes, but their co-existence may just reflect selection for disinfectant resistant strains at the site.

Although there have been no reports linking community exposures such as ATM use directly with food poisoning, the results showed that such frequently contacted items could act as reservoirs for organisms causing food-poisoning. As use of an ATM is generally not perceived as a risky behaviour, many people would not consider themselves as “contaminated” and be willing to prepare or consume food without washing their hands after contact with a machine. In addition, spread by fingers to the nose increases the risk of carriage of such strains and their later dissemination during food preparation or to others.

Although environmental cleaning may have an important role to play in control of infection, if disinfectant agents are used, it is essential that this is done in accordance with guidelines covering correct in-use concentration and regular rinsing of wiping cloths. Failure to adhere to correct practice results in low efficiency of disinfection and possible selection of more disinfectant tolerant organisms. The potential for these more resistant organisms to harbour other virulence factors, such as antibiotic resistance determinants and enterotoxins, increases the need for both proper disinfection procedures and surveillance of organisms in the community.

In Hong Kong, wiping of surfaces with disinfectant solutions has become common since the 2003 SARS outbreak. However, there have been no published reports on its effect on bacterial contamination at these sites and the procedures for

performing this disinfection are poorly monitored. This may lead to residues of low concentration disinfectant which selects for QAC positive strains.

4.4.2 Hospital Environment

According to the WHO definition, a nosocomial infection, also known as “hospital-acquired infection” (HAIs), is an infection not present and without evidence of incubation at the time of admission to a healthcare setting (WHO 2002). Multiple risk factors have been shown to influence the development of HAIs (Weidenmaier et al., 2004; Sheng et al., 2007). However, the role of contamination of hospital surfaces has become increasingly recognized as a possible cause of infections in recent years.

Pathogen-contaminated surfaces are generally not directly associated with transmission of infections to humans. The main concern is still transmission indirectly via hand contact with the contaminated surface. However, many studies have demonstrated the relationship between surface contamination and hospital infections. Hardy et al. (2006) found patients developed MRSA infections during their stay in ICU, which suggested that the MRSA was directly acquired from the surrounding healthcare environment. In a study by Dancer et al. (2006), patients were suggested to have acquired MRSA in an ICU department, due to a reduction in numbers of trained nurses and hygiene failures predominantly involving hand-touch sites. Mahamat et al., (2011) reported MRSA rates in a hospital significantly decreased after application of infection control interventions, but with an increase

in MRSA rates after stopping the hypochlorite disinfection, which demonstrated environmental contamination was important in the transmission of MRSA.

Environmental surfaces can be categorized into medical equipment surfaces (e.g. x-ray machines, instrument carts, pressure cuffs, and dental units) and housekeeping surfaces (e.g. floors, walls, tabletops, bedside rails) (Sehulster & Chinn, 2003). Computer keyboards, door handles, jelly pads, suction catheter containers, and bed-control handsets are classified as high touch surfaces in the hospital (Sehulster & Chinn, 2003).

Other studies for hospital surfaces have been carried out (Faires, et al., 2012; 2013). Faires (2013) reported that in patient rooms and in the ward environment, specific locations were identified as being contaminated with MRSA. The most commonly contaminated items were chair backs, bed rails, over bed tables and privacy curtains. Overall 2.5% of surfaces in the ward environment were contaminated with MRSA.

In this study, the bed rails, the surfaces in operating theatres, and blood pressure cuffs were selected as indicators of contamination of hospital surfaces. Use of these sites can indicate both staphylococcal contamination levels and reflect the effectiveness of hospital cleaning, and could also reveal the MRSA load in different hospital environments.

Of total strains isolated from the hospital, 44% of CNS harboured the *mec*A gene

and 55% of *S. aureus* was MRSA. *S. aureus* from the bed rails was statistically more likely to be methicillin resistant than from the operating theatre and from the blood pressure cuffs. The MRSA positive rate in the *S. aureus* from the hospital environment was much higher than that observed from the ATMs (0.5%).

Several studies have demonstrated that pathogens can survive for considerable periods on the surfaces of environmental objects, which can potentially to lead to cross infection in the hospital. Staphylococci possess a striking ability to persist on inanimate objects (Kramer et al., 2006). *S aureus* can survive on stainless steel for about 4 days and on vinyl and wood for as long as 3 months (Noyce et al., 2006). In other studies, MRSA was shown to survive for between 7 days to 7 months on inanimate surfaces, and MRCNS lasted for 14 to 90 days on various surfaces in the hospital (Makison & Swan, 2006; Neely & Maley, 2000). It was recently shown that use of copper alloy for hospital surfaces can reduce survival of staphylococci (Schmidt et al., 2012).

A study by Boyce et al. (1997) indicated that in a university-affiliated teaching hospital, 27% samples from surfaces in rooms of infected patients yielded MRSA and objects such as blood pressure cuffs, as well as the floor and the over bed tables ,were frequently contaminated .

In our study, 75% of *S. aureus* isolates from the bed rails were MRSA. Brady et al. (2007) found that MRSA colonized 12.9% of hospital bed-control handsets. However,

only 1% of bed-control handsets were contaminated by MRSA in a study in an American hospital (Young et al., 2005). Bed rails are commonly touched by both healthcare personnel and patients, leading to frequent presence of MRSA on the bed rails, providing a potential surface for the transfer of bacteria between individuals and surfaces. Therefore, bed rails act as a reservoir to transmit MRSA, especially in wards with patients carrying or infected with MRSA.

In our study, 39% of *S. aureus* isolated from the operating theatres in the hospital were methicillin resistant, which was much higher than the methicillin resistance rate of *S. aureus* from the public environment in this study. The operating theatres should have a high hygiene standard, but contamination with *S. aureus* and MRSA may increase risk for post surgical infections because staff may frequently touch the items during the operating procedure which can serve as sources to disseminate the bacteria to the health care staff. The high rates of use of operating theatres reduces time available for cleaning and increases risk of survival of contaminating organisms.

The six high-contact items in operating theatres investigated were highly contaminated by staphylococcal species and should be considered to pose an infection risk. Much indirect evidence has suggested that high-contact items can act as reservoirs for infection of patients in epidemic situations (CDC, 2008). Surgical site infections account for 15% of all nosocomial infections (Watanabe et al., 2008). *S. aureus*/MRSA causes far more surgical site infections than any other type of

bacteria with an increasing trend in recent years (Anderson et al., 2007; Bode et al., 2010). Surgical site infections not only lead to increased costs and prolonged duration of hospitalization, but also result in poorer health outcomes (Watanabe et al., 2008; Reichman & Greenberg, 2009).

The MRSA isolates consisted of an almost equal proportion of HA-MRSA (52.6%) and CA-MRSA (47.4%). In the investigated hospital, SCC*mec* III and SCC*mec* V were predominant types. This indicates CA-MRSA has transferred from the community to this hospital. The study of nurse colonization also reported that the majority of the MRSA isolates harboured SCC*mec* types associated with CA-MRSA.

Surface disinfection is one of the basic measures for hospital hygiene and infection control, and a large volume of disinfectants and detergents are used to prevent transmission of pathogens. In hospitals, the advantages of environmental surface disinfection in hospital have been described for more than 20 years. *S. aureus* including MRSA has been used as an indicator organism for assessment of surface hygiene. Hand washing is recognized as the most important factor for prevention of colonization and infection with MRSA spread between the hands of hospital staff and patients. However, although various infection control practices have been carried out, overall levels of infection rates have remained high suggesting failures in effectiveness in current infection control practices (Boyce, 2007; Otter et al., 2011).

In this study, some of the selected inanimate objects are non-critical devices which come into contact with skin, such as blood pressure cuffs, or are items that do not make contact with patients, such as environmental surfaces (Spaulding, 1968). Thus, lower level disinfection was often used for decontamination. For example, QAC solutions are widely applied in clinical settings. This extensive use of biocides in the hospital environment is likely to select for less antiseptic susceptible bacteria.

To date, there are few reports concerning QAC gene prevalence in staphylococci isolated from the hospital environment, most reports being related to clinical isolates from patients. This study indicated QAC genes are common in isolates from the hospital environment and that *S. aureus* and CNS harboured these genes in approximately equal proportions.

A significantly higher proportion of isolates from the hospital environment were positive for *qac*A/B (39.3%) than isolates from the ATMs (23.9%), ($p<0.05$). For *smr*, prevalence of carriage was generally similar in isolates from the hospital and the environment. This higher rate of *qac*A/B in the hospital suggests that positive strains may have a survival advantage if low levels of disinfectant residues are present, as in the hospital environment. Frequently contacted items in the environment could act as a source for dissemination of strains positive for QAC genes.

The prevalence of *qac*G/H/J genes in the hospital environment was lower than

other QAC genes with only six positive isolates detected. As has been shown in other studies, presence of *qac*G, H and J remains rare in human staphylococcal isolates and appears to be mainly associated with food (Bjorland et al., 2005; Smith et al., 2008).

There was no difference in the proportion of QAC gene positive isolates from the different sites in the hospital, suggesting that staphylococci carrying these genes are already widely disseminated in the hospital environment. This study provides evidence of a high incidence of staphylococcal contamination of hospital surfaces which may increase risk of nosocomial infections. It also identified several potential inanimate objects, present in all hospital environments that could act as markers of environmental contamination and applied in further studies to evaluate effectiveness of interventions to reduce contamination.

Environmental infection control in health care facilities is of great importance in reducing the risk of cross-infection. Infection control failure is often due to incorrect compliance with recommended procedures. Improper disinfectant use in the hospital environment could select for *Staphylococcus* spp. carrying QAC genes and could lead to failures in infection control in health care settings. Thus, it is suggested that rigid adherence to disinfection protocols is of vital importance in infection control. Appropriate and prudent use of biocides is paramount to prevent the emergence of antiseptic resistance and cross-resistance to antibiotics.

4.5 Limitations and Recommendations

A few sites in the environment were selected to represent the overall distribution of contaminating staphylococci, and the proportion of those isolates harbouring QAC resistance determinants. Obviously, this cannot provide the whole picture of the hospital environment or public environment, because these environments are extremely complex and include many kinds of surfaces with varying degrees of contamination. Many factors can also affect survival such as humidity, temperature and cleaning schedules. This study, using representative sites in the hospital and community showed there was widespread contamination with *S. aureus* and MRSA with a high percentage of those strains containing QAC genes.

As, only one hospital was included in the study, further investigations involving other hospitals should be performed involving a carefully designated selection of surfaces to be sampled taking into account cleaning schedules, amount of contact and other factors. The sample sizes must be well considered to balance sampling to prevent bias in order to determine an accurate estimate of the prevalence.

Poor compliance with hand hygiene and underestimating the role of hospital environmental surfaces are infection control gaps. Cleaning procedures are not standardized in hospitals, which increases the risks of spread of antiseptic resistant *Staphylococcus* spp. Studies that evaluate cleaning protocols and the effects of disinfectant use on surfaces on the survival of QAC positive strains are urgently needed.

Table 4.1 Master mix for amplification of *spa* gene

(Modified from Shopsin et al. 1999; Harmsen et al., 2003)

Reagents	Final Concentration
10 X Buffer	1 X
dNTP (2 mM)	350 µM
$MgCl_2$ (25 mM)	2 mM
Primer (10 mM)	
spa-F	0.5 µM
spa-R	0.5 µM
DNA (100 ng/µL)	100 ng
Taq polymerase (5U/µL)	2.5 unit
Sterile water	make up to 50 µL

Table 4.2 PCR conditions for amplication of *spa* gene

(Modified from Shopsin et al. 1999; Harmsen et al., 2003)

Temp ^{o}C	Time	No. of Cycles
95	10 min	1
95	30 s	
60	30 s	
72	45 s	30
72	10 min	7

Table 4.3 Master mix for identification of enteroxin gene

(Modified by Mehrotra et al., 2000)

Reagents	Final Concentration
10 X Buffer	1 X
dNTP (2 mM)	0.2 mM
$MgCl_2$ (25 mM)	1.6 mM
Primer (10 µM)	
sea-F	0.2 µM
sea-R	0.2 µM
seb-F	0.2 µM
seb-R	0.2µM
sec-F	0.2 µM
sec-R	0.2 µM
sed-F	0.4 µM
sed-R	0.4 µM
see-F	0.2 µM
see-R	0.2 µM
DNA (100 ng/µL)	100 ng
Taq polymerase (5U/µL)	2.5 unit
Sterile water	make up to 50 µL

Table 4.4 PCR conditions for amplication for entertoxin gene

(Modified by Mehrotra et al., 2000)

Temp ^oC	Time	No. of Cycles
94	5 min	1
95	2 min	
57	2 min	
72	1 min	35
72	3 min	7

Table 4.5 PCR primer for amplification of entertoxin gene (Mehrotra et al., 2000)

Gene	Primer	Oligonucleotide sequence (5'- 3')	Location within gene	Size of amplified product (bp)
sea	GSEAR-1	GGTTATCAATGTGCGGGTGG	349–368	102
	GSEAR-2	CGGCACTTTTTTCTCTTCGG	431–450	
seb	GSEBR-1	GTATGGTGGTGTAACTGAGC	666–685	164
	GSEBR-2	CCAAATAGTGACGAGTTAGG	810–829	
sec	GSECR-1	AGATGAAGTAGTTGATGTGTATGG	432–455	451
	GSECR-2	CACACTTTTAGAATCAACCG	863–882	
sed	GSEDR-1	CCAATAATAGGAGAAAATAAAAG	492–514	278
	GSEDR-2	ATTGGTATTTTTTTTCGTTC	750–769	
see	GSEER-1	AGGTTTTTTCACAGGTCATCC	237–257	209
	GSEER-2	GATAAAGAAGAAACCAGCAG	1556-1575	

Table 4.6 Staphylococcal isolates from ATMs in different districts in Hong Kong

Region	District	*S. aureus* isolates		CNS isolates	
		N	(%)	N	(%)
Hong Kong Island	Central and Western	6/32	18.8	30	93.8
	Wanchai	7/35	20	35	100
	Eastern	6/39	15.4	37	94.9
	Southern	5/29	17.2	29	100
Kowloon	Yau Tsim Mong	2/29	6.9	27	93.1
	Sham Shui Po	4/22	18.15	20	90.1
	Kowloon	5/31	16.1	28	90
	Wong Tai Sin	5/25	20	24	80
	Kwun Tong	4/23	17.3	23	100
New Territories	Tsuen Wan	2/15	13.3	14	93.3
	Tuen Mun	2/18	11.1	17	94.4
	Yuen Long	2/18	11.1	17	94.4
	North	2/19	10.5	18	94.7
	Tai Po	3/13	23.1	13	100
	Sai Kung	2/13	11.1	12	66.7
	Sha Tin	4/20	20	19	9.0
	Kwai Tsing	1/17	5.9	17	100
Overall		62	15.5	381	95.3

Table 4.7 Classification of operative wounds based on degree of microbial contamination (Spalding 1968)

Classification	Criteria
Clean	Elective, not emergency, non-traumatic, primarily closed; no acute inflammation; no break in technique; respiratory, gastrointestinal, biliary and genitourinary tracts not entered.
Clean-contaminated	Urgent or emergency case that is otherwise clean; elective opening of respiratory, gastrointestinal, biliary or genitourinary tract with minimal spillage (e.g. appendectomy) not encountering infected urine or bile; minor technique break.
Contaminated	Non-purulent inflammation; gross spillage from gastrointestinal tract; entry into biliary or genitourinary tract in the presence of infected bile or urine; major break in technique; penetrating trauma <4 hours old; chronic open wounds to be grafted or covered.
Dirty	Purulent inflammation (e.g. abscess); preoperative perforation of respiratory, gastrointestinal, biliary or genitourinary tract; penetrating trauma >4 hours old.

Table 4.8 Association between presence of QAC genes and methicillin resistance in isolates of *S. aureus* and CNS from ATMs

QAC gene	Species	Positive N (%)	Negative N (%)	*p* value [a]	OR	95% CI
*qac*A/B	MRSA	2 (100)	0 (0)	**0.011***	**-**	**-**
	MSSA	5 (8.3)	55 (91.7)			
	MRCNS	27 (45.0)	33 (55)	**<0.001***	**2.8**	**1.60-5.0**
	MSCNS	72 (22.4)	249 (77.6)			
smr	MRSA	0 (0)	2 (100)	0.854	-	-
	MSSA	1(1.7)	59 (98.3)			
	MRCNS	8 (13.3)	52 (86.7)	0.888	-	-
	MSCNS	45 (14.1)	276 (85.9)			
*qac*G	MRSA	0 (0)	2 (100)	0.793	-	-
	MSSA	2 (3.3)	58 (96.7)			
	MRCNS	0 (0)	60 (100)	0.665	-	-
	MSCNS	1 (0.3)	320 (99.7)			
*qac*H	MRSA	0	2 (100)	-	-	-
	MSSA	0	60 (100)			
	MRCNS	0	60 (100)	-	-	-
	MSCNS	0	321 (100)			
*qac*J	MRSA	0	2 (100)	-	-	-
	MSSA	0	60 (100)			
	MRCNS	0	60 (100)	0.665	-	-
	MSCNS	1 (0.3)	320 (99.7)			

MRSA: Methicillin resistant *S. aureus;* MSSA: Methicillin sensitive *S. aureus;* MRCNS*:* Methicillin resistant coagulase-negative staphylococci*;* MSCNS: Methicillin sensitive coagulase-negative staphylococci*;* [a] Statistical analysis: Chi-Square tests or Fisher's exact test; * statistically significant

Table 4.9 Relationships between antibiotic susceptibility and QAC gene carriage of *S. aureus* isolates from ATMs (n = 62)

Antibiotic	Number resistant (%)		Presence of QAC genes in *S. aureus* isolates (%) Positive	Negative	*p* value
Penicillin	54 (87.1)	R	8 (14.8)	46 (85.2)	0.604
		S	2 (25.0)	6 (75.0)	
Ciprofloxacin	2 (3.2)	R	1 (50.0)	1 (50.0)	0.299
		S	9 (15.0)	51 (85.0)	
Trimethoprim–sulphamethoxazole	4 (6.5)	R	1 (25.0)	3 (75.0)	0.618
		S	9 (15.5)	49 (84.5)	
Gentamicin	5 (8.1)	R	0 (0)	5 (100)	0.306
		S	10 (17.0)	47 (83.0)	
Clindamycin	4 (6.5)	R	0 (0)	4 (100)	0.365
		S	10 (17.2)	48 (82.8)	
Tetracycline	14 (22.6)	R	5 (35.7)	9 (64.3)	**0.024***
		S	5 (10.4)	43	
Fusidic acid	21 (33.9)	R	2 (9.5)	19 (90.5)	0.312
		S	8(19.5)	33 (80.5)	
Vancomycin[#]	0 (0)	R	0	0	-
		S	10 (19.2)	52 (80.8)	
Erythromycin	18 (29)	R	2 (11.1)	16 (88.9)	0.492
		S	8 (18.2)	36 (81.8)	
Chloramphenicol	2 (3.2)	R	0	0	-
		S	10 (16.1)	52 (83.9)	
Imipenem	1 (1.6)	R	0 (0)	1 (100)	0.658
		S	10 (16.4)	51 (85.6)	
Linezolid	0 (0)	R	0	0	-
		S	10 (19.2)	52 (80.8)	

R: resistant; S: sensitive; # Sensitive = MIC <2.0, **p*<0.05

Table 4.10a MICs and MBCs of BC for *S. aureus* and CNS from ATMs with and without QAC Genes

Isolates	QAC Genes Present	N	MIC of BC (mg/L)				MBC of BC (mg/L)			
			Range	MIC_{50}	MIC_{90}	*p* value [a]	Range	MIC_{50}	MIC_{90}	*p* value [a]
S. aureus	*qac*A/B	7	4-8	8	8	**0.001***	8-32	16	32	**0.048***
	smr	1	8	-	-	0.095	16	-	-	0.571
	Negative	20	2-4	4	4	-	4-32	8	32	-
CNS	*qac*A/B	20	4-8	4	8	**<0.001***	4-64	8	64	**0.004***
	smr	20	2-16	4	8	**0.003***	4-64	8	32	**0.013***
	*qac*A/B + *smr*	18	1-8	4	8	**0.001***	8-64	16	64	**<0.001***
	Negative	20	0.25-8	2	4	**-**	1-32	4	16	-
Total	*qac*A/B	27	4-8	4	8	**<0.001***	4-64	8	64	**0.005***
	smr	21	2-16	4	8	**0.020***	4-64	8	32	0.068
	*qac*A/B + *smr*	18	1-8	4	8	**0.004***	8-64	16	64	**<0.001***
	negative	40	0.25-8	2	4	-	1-32	8	16	-

[a] Comparison to bacteria without QAC genes; * $p<0.05$ statistically significant

Table 4.10b MICs and MBCs of CHG for *S. aureus* and CNS from ATMs with and without QAC Genes

Strains	QAC genes present	N	MIC of CHG (mg/L)				MBC of CHG (mg/L)			
			Range	MIC_{50}	MIC_{90}	*p* value [a]	Range	MIC_{50}	MIC_{90}	*p* value [a]
S. aureus	*qac*A/B	7	2-4	4	4	**0.041***	4-16	16	16	0.219
	smr	1	4	-	-	0.095	32	-	-	0.095
	negative	20	2-4	2	2	-	4-16	8	16	-
CNS	*qac*A/B	20	2-4	4	4	**<0.001***	2-16	8	8	**0.047***
	smr	20	2-4	2	4	**0.001***	4-32	8	32	**0.003***
	*qac*A/B + *smr*	18	1-4	2	4	**0.003***	1-32	8	32	**0.048***
	negative	20	0.25-2	2	2	-	1-32	4	16	-
Total	Only *qac*A/B	27	2-4	4	4	**<0.001***	2-16	8	16	0.244
	Only *smr*	21	2-4	2	4	**0.001***	4-32	8	32	**0.026***
	*qac*A/B + *smr*	18	1-4	2	4	**0.018***	1-32	8	32	0.636
	negative	40	0.25-4	2	2	-	1-32	8	16	-

[a] Comparison to bacteria without QAC genes; Statistical analysis: Mann-Whitney-U test;

* *$p<0.05$* statistically significant

Table 4.11 Relationship between presence of enterotoxin and QAC genes in *S. aureus* from ATMs

Enterotoxin gene	+/-	Presence of QAC genes (%)		*p* value	OR (95% CI)
		Positive	Negative		
sea	+	0 (0)	9 (17.3)	0.155	-
	-	10 (100)	43 (82.7)		
seb	+	4 (40)	4 (7.7)	**0.005***	**8 (1.6-40.6)**
	-	6 (6)	48 (92.3)		
sec	+	0(0)	7 (13.5)	0.218	
	-	10 (100)	45 (86.5)		
sed	+	0	0	-	-
	-	10 (100)	52 (100)		
see	+	0 (0)	8 (15.4)	0.184	-
	-	10 (100)	44 (84.6)		

* $p<0.05$ statistically significant

Table 4.12 Distribution of SCC*mec* types in MRSA in hospital isolates

Sample site	SCC*mec* type				
	II N (%)	III N (%)	IVa N (%)	V N (%)	Total N (%)
Patient bed rail	2 (5.26)	14 (36.8)	4 (10.2)	18 (47.4)	38 (100)
Operating theatre	3 (18.8)	9 (56.2)	0 (0)	4 (25)	16 (100)
Pressure cuff	0 (0)	2 (66.7)	0 (0)	1 (33.3)	3 (100)
Total	5 (8.8)	25 (43.9)	4 (7.0)	23 (40.4)	57 (100)

Table 4.13 Antibiotic susceptibility of *S. aureus* from the hospital environment

Antibiotic	Resistant N (%)	Sensitive N (%)
Oxacillin	57 (54.8)	47 (45.2)
Penicillin	98 (94.2)	6 (5.8)
Ciprofloxacin	59 (56.7)	45 (42.3)
Trimethoprim–sulphamethoxazole	10 (10.6)	94 (89.4)
Gentamicin	44 (42.3)	60 (51.7)
Clindamycin	10 (9.6)	94 (90.4)
Tetracycline	58 (55.8)	46 (44.2)
Fusidic acid	18 (17.2)	86 (82.8)
Vancomycin[#]	0 (0)	104 (100)
Erythromycin	70 (67.3)	34 (32.7)
Chloramphenicol	2 (1.9)	102 (90.1)
Quinupristin/Dalfopristin	8 (7.7)	96 (92.3)
Linezolid	2 (1.9)	102 (90.1)
Imipenem	26 (25)	78 (75)

[#] Sensitive = MIC <2.0

Table 4.14 Susceptibility of MRSA harbouring varying SCC*mec* types

Antibiotic	N (%) of MRSA resistant to antibiotics					
	II	III	IVa	V	Total	*p* value
Penicillin	5 (100)	25 (100)	4 (100)	23 (100)	57 (100)	-
Ciprofloxacin	3 (60)	22 (80)	2 (50)	23 (100)	50 (87.8)	**0.007***
Trimethoprim–sulphamethoxazole	0 (0)	4 (50)	2 (50)	4 (17.4)	10 (17.5)	0.260
Gentamicin	2 (40)	15 (60)	2 (50)	19 (82.6)	38 (66.7)	0.154
Clindamycin	1 (20)	6 (24)	2 (50)	0 (0)	9 (15.8)	**0.027***
Tetracycline	3 (60)	15 (60)	0 (0)	17 (73.8)	35 (61.4)	**0.048***
Fusidic acid	2 (40)	0 (0)	2 (50)	3 (13)	7 (12.3)	**0.003***
Vancomycin[#]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
Erythromycin	4 (80)	24 (96)	4 (100)	21 (91.3)		0.564
Chloramphenicol	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
Quinupristin /Dalfopristin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
Linezolid	0 (0)	2 (8)	0 (0)	0 (0)	2 (3.5)	0.448
Imipenem	2 (40)	16 (60)	2 (50)	4 (11.4)	24 (41.2)	**0.013***

* Difference is statistically significant; [a] Statistical analysis: Chi-Square tests or Fisher's exact test ; [#] Sensitive = MIC <2.0.

Table 4.15 Comparison of susceptibilities between HA-MRSA and CA-MRSA

Antibiotic	N (%) of MRSA resistant to antibiotics				
	HA-MRSA		CA-MRSA		p value
	Resistant	Sensitive	Resistant	Sensitive	
Penicillin	30 (100)	0 (0)	27 (100)	0 (0)	-
Ciprofloxacin	25 (83.3)	5 (16.7)	25 (92.6)	2 (7.4)	0.288
Trimethoprim–sulphamethoxazole	4 (13.3)	26 (86.7)	6 (22.2)	21 (77.8)	0.378
Gentamicin	17 (56.7)	13 (43.3)	21 (77.8)	6 (22.2)	0.091
Clindamycin	7 (23.3)	23 (76.7)	2 (7.4)	25 (92.6)	0.100
Tetracycline	18 (60)	12 (40)	17 (6.3)	10 (93.7)	0.891
Fusidic acid	2 (6.7)	28 (93.3)	5 (18.5)	22 (81.5)	0.173
Vancomycin[#]	0 (0)	30 (100)	0 (0)	27 (100)	-
Erythromycin	28 (93.2)	2 (6.7)	25 (92.6)	2 (7.4)	0.913
Chloramphenicol	0 (0)	30 (100)	0 (0)	27 (100)	-
Quinupristin/Dalfopristin	0 (0)	30 (100)	0 (0)	27 (100)	-
Linezolid	2 (6. 7)	28 (93.3)	0 (0)	27 (100)	0.172
Imipenem	18 (60)	12 (40)	6 (22.2)	21 (67.8)	**0.004***

*Difference is statistically significant; Statistical analysis: Chi-Square tests or Fisher's exact test; [#] Sensitive = MIC <2.0.

Table 4.16 Prevalence of QAC genes in *Staphylococcus* spp. from hospital environments

QAC Gene		Source			*p* value
		CNS N (%)	*S. aureus* N (%)	Total N (%)	
*qac*A/B	Negative	148 (63.8)	56 (53.8)	204 (60.7)	**0.084**
	Positive	84 (36.2)	48 (46.2)	132 (39.3)	
smr	Negative	195 (84.1)	96 (92.3)	291 (86.6)	**0.040***
	Positive	37 (15.9)	8 (7.7)	45 (13.4)	
*qac*G	Negative	232 (100)	102 (98.1)	334 (99.4)	**0.034***
	Positive	0 (0)	2 (1.9)	2 (0.6)	
*qac*J	Negative	230 (99.1)	102 (98.1)	332 (98.8)	0.407
	Positive	2 (0.9)	2 (1.9)	4 (1.2)	

* Difference is statistically significant; Statistical analysis: Chi-Square tests or Fisher's exact test

Table 4.17 Distribution of QAC gene positive *S. aureus* and CNS from various hospital sites

QAC Gene		Source				
		Patient Bed Rail % (N)	Operating Theatre % (N)	Pressure Cuff % (N)	Total	*p* value
*qac*A/B	Negative	61.6 (114)	60.8 (62)	57.1 (28)	60.7 (204)	0.085
	Positive	38.4 (71)	39.2 (40)	42.9 (21)	39.3 (132)	
smr	Negative	88.6 (165)	82.4 (84)	85.7 (42)	86.6 (291)	0.621
	Positive	11.4 (20)	17.6 (18)	14.3 (7)	13.4 (45)	
*qac*G	Negative	100 (185)	99 (101)	98 (48)	99.4 (334)	0.213
	Positive	0 (0)	1 (1)	2 (1)	0.6 (2)	
*qac*J	Negative	98.9 (183)	99 (101)	98 (48)	98.8 (332)	0.836
	Positive	1.1 (2)	1 (1)	2 (1)	1.2 (4)	

* Difference is statistically significant; Statistical analysis: Chi-Square tests or Fisher's exact test

Table 4.18 Prevalence of QAC genes in methicillin resistant and sensitive hospital isolates

QAC gene			Methicillin susceptibility			*p* value
			Sensitive N (%)	Resistance N (%)	Total N (%)	
Total	*qac*A/B	Negative	122 (68.9)	82 (51.6)	204 (60.7)	**0.001***
		Positive	55 (31.1)	77 (48.4)	132 (39.3)	
	smr	Negative	156 (88.1)	135 (84.9)	291 (86.6)	0.385
		Positive	21 (11.9)	24 (15.1)	45 (13.4)	
	*qac*G	Negative	175 (98.9)	159 (100)	334 (99.4)	0.179
		Positive	2 (1.1)	0 (0)	2(0.6)	
	*qac*J	Negative	177 (100)	155 (97.5)	332 (98.8)	0.049
		Positive	0 (0)	4 (2.5)	4 (1.2)	
	*qac*H	Negative	177 (100)	159 (100)	336 (100)	-
		Positive	0 (0)	0 (0)	0 (0)	
S. aureus	*qac*A/B	Negative	33 (70.2)	23 (40.4)	56 (53.8)	**0.002***
		Positive	14 (29.8)	34 (59.6)	48 (46.2)	
	smr	Negative	44 (93.6)	52 (91.2)	96 (92.3)	0.649
		Positive	3 (6.4)	5 (8.8)	8 (7.7)	
	*qac*G	Negative	45 (95.7)	57 (100)	102 (98.1)	0.116
		Positive	2 (4.3)	0 (0)	2 (1.9)	
	*qac*J	Negative	47 (100)	55 (96.5)	102 (98.1)	0.195
		Positive	0 (0)	2 (3.5)	2 (1.9)	
CNS	*qac*A/B	Negative	89 (68.5)	59 (57.8)	148 (63.8)	0.095
		Positive	41 (31.5)	43 (42.2)	84 (36.2)	
	smr	Negative	112 (86.2)	83 (81.4)	195 (84.1)	0.324
		Positive	18 (13.8)	19 (18.6)	37 (15.9)	
	*qac*G	Negative	130 (100)	102 (100)	232 (100)	-
		Positive	0 (0)	0 (0)	0 (0)	
	*qac*J	Negative	130 (100)	100 (98)	230 (99)	0.109
		Positive	0 (0)	2 (2.0)	2 (0.9)	

* Difference is statistically significant; Statistical analysis: Chi-Square tests or Fisher's exact test

Table 4.19 Association between antibiotic resistance and QAC gene carriage in *S. aureus*

Antibiotic	R/S	Presence of QAC genes (%) Positive	Negative	*p* value
Cefoxitin	R	39 (67.2)	18(39.1)	**0.004***
	S	19 (32.8)	28(60.9)	
Penicillin	R	58 (100)	45(97.8)	0.442
	S	0 (0)	1 (2.2)	
Ciprofloxacin	R	40 (69)	19 (41.3)	**0.005***
	S	18 (31)	27 (58.7)	
Trimethoprim–sulphamethoxazole	R	8 (13.8)	2 (14.3)	0.105
	S	50 (86.2)	44 (95.7)	
Gentamicin	R	39 (67.2)	5 (10.9)	**<0.001***
	S	19 (32.8)	41 (89.1)	
Clindamycin	R	8 (13.8)	2 (4.3)	0.105
	S	50 (86.2)	44 (95.7)	
Tetracycline	R	41 (70.7)	17 (37)	**0.001***
	S	17 (29.3)	29 (63)	
Fusidic acid	R	11 (19)	7 (15.2)	0.616
	S	47 (81)	39 (84.8)	
Vancomycin[#]	R	58 (100)	46 (100)	-
	S	0 (0)	0 (0)	
Erythromycin	R	47 (81)	23 (50)	**0.001***
	S	11 (19)	23 (50)	
Chloramphenicol	R	2 (3.4)	0 (0)	0.203
	S	56 (96.6)	46 (100)	
Imipenem	R	13 (22.4)	13 (28.3)	0.494
	S	45 (77.6)	33 (71.7)	
Linezolid	R	2 (3.4)	0 (0)	0.203
	S	56 (96.6)	46 (100)	
Quinupristin /Dalfopristin	R	3 (5.2)	5 (10.9)	0.279
	S	55 (94.8)	41 (89.1)	

* Difference is statistically significant; Statistical analysis: Chi-Square tests or Fisher's exact test; [#] Sensitive = MIC <2.0; R: Resistant. S: Sensitive.

CHAPTER 5

PREVALENCE OF QAC GENES IN CLINICAL ISOLATES OF *S. AUREUS*

5.1 Introduction

S. aureus is a major cause of bloodstream infections. It is also a leading cause of health-care-associated pneumonia, and surgical-site infections (McClelland et al., 1999; Gafter-Gvili et al., 2011). Staphylococcal infections are often caused by the patients' colonizing strains. Blood stream infections tend to be more common in patients with co-morbidities and reduced immunity. These patients may have been hospitalized on several occasions. Surgical sites infections are often associated with breaks in infection control as well as colonization.

Biocides are used to decrease or eliminate nosocomial pathogens on skin and mucosa of patients and health care workers. CHG and QACs are commonly used skin disinfectants in the hospital. To further prevent spread of pathogens, thorough, rigorous infection control regimes, including hand and surface disinfection and decolonization of patients with *S. aureus,* are performed. Therefore, the emergence of resistance to these decolonization agents would be of serious concern. Already, mupirocin resistance is recognized, and increasing CHG resistance in MRSA has

become an emerging threat (Edgeworth, 2011; Horner et al., 2012a).

It has been reported that MRSA strains carrying QAC genes can have decreased susceptibility to CHG (Batra et al., 2010). The distribution of QAC genes in *S. aureus* and CNS has been investigated in clinical isolates from several countries, with most focus on MRSA (Alam et al., 2003b; Miyazaki et al., 2007; Wang et al., 2008a). However, this problem has not been investigated in Hong Kong.

In this study, the prevalence of QAC genes in clinical isolates of *S. aureus* from both bloodstream and skin and soft tissue infections was determined and the results compared with those from the studies described in Chapters 3 and 4. Finally, the association between the presence of QAC genes and antibiotic resistance was determined.

5.2 Materials and Methods

5.2.1 Sample Size

A cross-sectional study of the prevalence of QAC genes in clinical isolates of *S. aureus* was performed. Of the QAC genes, *qac* A/B and *smr* are more commonly reported in human isolates (Noguchi 1998; 1999; Alam et al., 2003a), but the QAC gene carriage rate in *S. aureus* clinical isolates has varied between studies and sample sources. Considering the high MRSA prevalence in Hong Kong, with methicillin resistance in clinical isolates exceeding 30%, it was estimated that the rate of QAC genes in clinical *S. aureus* isolates would be 20%. It was estimated that

a minimum sample size of 70 isolates of *S. aureus* would be needed to determine the prevalence rate assuming a 20% carriage rate of QAC genes with 1% error and 95% confidence level according to the formula $N=\mu a^2 \pi (1- \pi)/\delta^2$.

5.2.2 Sample Collection

Clinical isolates from hospital patients with skin and soft tissue infections (SSI) and bloodstream infections (BSI) were collected from a large district hospital in Hong Kong between June and November 2011. After confirmation of the identification of *S. aureus*, all isolates were stored at -80°C. The strains were subcultured twice on blood agar before use.

5.2.3 Confirmation of *S. aureus*

Although the clinical isolates had been identified as *S. aureus* in the hospital, presence of *fem*A gene was detected by PCR to confirm the isolates as *S. aureus*. The method was performed as described in chapter 3.2.3

5.2.4 Detection of QAC Genes

The detection of QAC genes (*qac*A/B, *smr*, *qac*G, *qac*H, *qac*J) was performed as described in Chapter3.

5.2.5 Antibiotic Susceptibility Testing

Susceptibility to a range of antibiotics was determined by disc diffusion for all clinical isolates, following CLSI guidelines (CLSI, 2009) except for fusidic acid, when

British Society for Antimicrobial Chemotherapy guidelines were used (Andrew et al., 2001).The details of the procedure as the same as described in chapter 3. Susceptibility to vancomycin was determined by standard agar dilution.

5.2.6 Identification and Genotyping of MRSA

All S*. aureus* isolates were screened for presence of *mec*A gene to confirm methicillin resistance. The method was as described in Chapter 3. SCC*mec* types were determined for the MRSA strains as described in Chapter 3.

5.2.7 Statistical Analysis

The rates of QAC positivity between MSSA and MRSA, SSI and BSI isolates and HA-MRSA and CA-MRSA were compared. The proportion of clinical isolates harbouring QAC genes was compared with those from the hospital environment and general population isolates. Statistical analyses were performed using SPSS system for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). All categorical variables were analyzed by Chi-square statistic or Fisher's exact test.

5.3 Results

5.3.1 Isolates

A total of 300 clinical isolates were collected. These consisted of 150 isolates from patients with SSI (50 MSSA and 100 MRSA), and 150 from BSI (50 MSSA and 100 MRSA).

5.3.2 Genotypes of Clinical Isolates of MRSA

The distribution of SCC*mec* types of MRSA clinical isolates are shown in Table 5.1. Of the total 200 MRSA isolates, 12.5% were SCC*mec* type II, 73% type III, 6.5% type IV, and 5% type V with 3% untypeable. HA-MRSA types (II and III) were predominant accounting for 85.5% of the isolates with only 12% confirmed as CA-MRSA (types IV and V). Comparison of the SCC*mec* types of BSI and SSI isolates revealed the distribution was very similar (Table 5.1) and there was no significant difference in their distribution ($p>0.05$).

5.3.3 Antibiotic Susceptibility of *S. aureus* from Clinical Isolates

Susceptibility testing was performed for all 300 *S. aureus* against a range of non-beta lactam antibiotics and the rates of resistance are shown in Table 5.2. There were significant differences in resistance rates between methicillin sensitive and resistant strains for all antibiotics other than fusidic acid. Comparison of patterns of resistance of HA-MRSA and CA-MRSA revealed little difference between these types (Table 5.3).

5.3.4 QAC Genes in Clinical Isolates of *S. aureus*

Of the total 300 *S. aureus*, 28.3% (85/300) strains were *qac*A/B positive, 4.3% (13/300) *smr* positive, and 6.7% (20/300) *qac*J positive. No *qac*G, or *qac*H positive strains were detected (Table 5.4). Carriage of *qac*A/B was detected in a significantly higher percentage of *S. aureus* from SSI (38.7%; 58/150) than BSI (18.0%; 27/150) isolates ($p<0.001$, OR 2.9, 95% CI: 1.7-4.9), but rates of carriage of *smr* were similar

in SSI (4.0%) and BSI isolates (4.7%) (*p* = 0.777). Carriage of *qac*J also did not differ significantly between SSI (4.7%) and BSI (8.7%) isolates (*p*=0.165) (Table 5.4).

5.3.5 Association between *mec*A and QAC Genes

Only 2% of MSSA were positive for *qac*A/B, compared with 41.5% MRSA (*p*<0.001, OR 37.8, 95% CI: 8.3-145). In contrast, rates for presence of *smr* were similar with 6% MSSA and 3.5% MRSA isolates positive (*p*=0.316). The *qac*J gene was only detected in methicillin resistant isolates (10%, 20/200) (*p*<0.001) (Table 5.5). However, the distribution of QAC genes between typical HA-MRSA and CA-MRSA were similar for *qac*A/B and *smr* (*p*>0.05, but *qac*J was more common in CA-MRSA isolates (*p*=0.028) (Table 5.6).

There was no difference in the *qac*A/B rate for MSSA from SSI and BSI (*p*>0.05) both having only 2% (1/50) positive strains. However, MRSA from SSI carried more *qac*A/B (57%) than isolates from BSI (26%) (*p*<0.001, OR 3.8, 95% CI: 2.1- 6.8). In contrast, presence of *smr* did not differ significantly between SSI and BSI isolates with 6% (3/50) MSSA and 3% MRSA (3/100) from SSI positive and 6% (3/50) MSSA and 4% MRSA (4/100) from BSI positive for this gene. Similarly, there was no difference in the *qac*J gene prevalence from SSI 7% (7/100) and BSI (13%, 13/100) (*p*= 0.157).

5.3.6 Antibiotic Resistance and QAC Gene Carriage

All clinical isolates were screened for antibiotic susceptibility and analyzed for

correlation between carriage of genes for antiseptic and antibiotic resistance. Based on the Chi-square analysis, the frequency of tetracycline, gentamicin, ciprofloxacin and erythromycin resistance was significantly higher in the *S. aureus* isolates with QAC genes than without ($p<0.05$) (Table5.7).

5.4 Discussion

5.4.1 Clinical isolates of *S. aureus* from Hong Kong

MRSA is an important causative organism of both SSIs and BSIs locally. In European clinical settings, SSI was the most frequently observed MRSA infection, in a report involving 79% of European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) delegates (Dryden et al., 2010) and the incidence of BSI caused by MRSA ranged from 0.2 in Sweden to 24.4 in Portugal per 100,000 patient-days. Meta-analysis of the data indicated that MRSA BSIs are related to an almost two-fold higher mortality than those due to MSSA (Tacconelli, 2010).

In this study utilising 300 recent isolates, a large district hospital in an urban area of Hong Kong was chosen as the sentinel hospital to carry out the research. MRSA typing revealed that the predominant type from BSIs was HA-MRSA, SCC*mec* type III (73%), followed by SCC*mec* II (13%). Similar results were observed in MRSA from SSIs.

Studies have shown that certain *S. aureus* genotypes are more likely to be associated with clinical syndromes or severe infections (Wang et al., 2010). In most

reports, CA-MRSA has been isolated mainly from SSI (98%), although severe invasive infections caused by these strains, such as pyomyositis, osteomyelitis, necrotizing fasciitis, severe pneumonia, and sepsis, have also been reported (Wang et al., 2010).

The results of this study did not support this finding, as the percentages of HA-MRSA and CA-MRSA were similar in SSI and BSI isolates. Although previous studies have reported that HA-MRSA was associated with hospital acquired BSI, there is increasing evidence that CA-MRSA has become an important cause of community-acquired BSI, possibly leading to infective endocarditis, with a high risk of mortality (Chi et al., 2010; Ponce-de-León et al., 2010; Campanile et al., 2011). It is suggested that CA-MRSA BSI may be exacerbations of local infections, whereas HA-MRSA BSI occurs most frequently as a complication of intravascular catheter use (Naber, 2009).

Recently, CA-MRSA has spread into hospitals and begun to replace traditional HA-MRSA in nosocomial infections worldwide (Carey et al., 2010; Okesola, 2011). A study from the USA showed that although MRSA BSI rates in the hospital were relatively stable during 2000–2006, CA-MRSA strains have replaced traditional HA-MRSA strains, with the proportion of CA-MRSA increasing from 24% to 49% (Popovich et al., 2008). In Korea, CA-MRSA strains were also reported to have emerged as a major cause of BSIs in healthcare settings (Park et al., 2009). A study of CA-MRSA strain types causing hospital outbreaks between 2003 and 2010 in

several countries including North America, Switzerland, Greece, and the UK revealed that CA-MRSA USA300 clone has emerged as a significant cause of HAIs (Otter & French, 2011). In Denmark, the overall incidence of CA-MRSA isolates increased tenfold from 1999 to 2006, exceeding cases of HA-MRSA isolates by 2006 (Larsen et al., 2009).

5.4.2 Antibiotic Treatment for MRSA Infection and Drug Resistance in Clinical Isolates

There are several antibiotics available with reliable activity against CA-MRSA strains: vancomycin, linezolid, tetracyclines (doxycycline and minocycline), co-trimoxazole, fusidic acid and occasionally quinolones. Inexpensive oral agents, such as clindamycin, co-trimoxazole, and fusidic acid are also commonly recommended for treatment of CA-MRSA infections.

For minor infections such as incision, an oral antibiotic such as a tetracycline, clindamycin or trimethoprim–sulfamethoxazole can be used; however, serious SSIs with a high risk of bacteraemia or endocarditis should to be treated with glycopeptides, daptomycin or linezolid (Cunha, 2005; Gould et al., 2009b; Morgan, 2011).

Vancomycin has been the gold standard treatment for severe MRSA infections for many years. Increasing MICs to ≥2 mg/ml (‘vancomycin creep’) has led to increased dosages to achieve higher serum levels in severe invasive infections (Deleo et al.,

2010, Gould, et al., 2011). Daptomycin has replaced vancomycin as the antibiotic for serious/systemic MRSA infections in several countries. Daptomycin is also effective against MSSA (Wilcox, 2011). However, cross-resistance between daptomycin and vancomycin has been reported (Boucher & Sakoulas, 2007).

In our study, 17.5% HA-MRSA and 26.6% CA-MRSA were resistant to clindamycin, which is similar to reports elsewhere (Dryden et al., 2010). In addition, there was a high rate of fusidic acid resistance (34.5 %,) showing that attention must be paid to its use. The resistance rate may reflect increased use of this agent for treatment of local skin infections. Linezolid resistance in staphylococci is still rare and only 1.5% MRSA were resistant to this agent which was consistent to the other investigations (Jones et al., 2008). Recently, new mechanisms of resistance to linezolid mediated by the *cfr* gene have been reported (Witte & Cuny, 2011) and close monitoring of linezolid resistance is necessary (Rossolini et al., 2010; Morales et al., 2010).

Although in most studies, CA-MRSA has been reported to be more likely to be sensitive to a wider range of these antibiotics than HA-MRSA (David et al. 2010), there was no statistical difference in the drug resistance between HA-MRSA and CA-MRSA based on SCC*mec* types isolated from this study. MRSA sensitivities vary geographically and evolve continuously. CA-MRSA strains were all susceptible to trimethoprim, vancomycin, clindamycin, and linezolid in England and Wales during 2004–05 (Nathwani et al., 2008). Results of a survey of CA-MRSA and MSSA isolates from SSIs in Hong Kong revealed little difference in susceptibilities to rifampin,

minocycline and clindamycin (99%, 98%, 75% respectively) and only slightly more resistant to erythromycin in CA-MRSA (77%) than MSSA (70%) (Ho et al., 2008b). The levels of resistance observed in clinical isolates were much higher in the current study.

5.4.3 Use of Antiseptics to Prevent MRSA Transmission

Decolonization of patients can reduce the bacterial load leading to a reduction of endogenous infections and transmission of antibiotic-resistant bacteria, such as MRSA and vancomycin resistant enterococci (Climo et al., 2009). The two most commonly used decolonization agents are mupirocin for nasal carriage and CHG for skin.

5.4.4 QAC Gene Prevalence in *S. aureus* Hospital Isolates

CHG is a safe and effective antiseptic, widely used to prevent healthcare associated infections (Milstone et al., 2008). It is used in hand washing and skin antisepsis prior to blood culture collection and the insertion of vascular catheters. Other infection control applications of CHG include oropharyngeal antisepsis to prevent ventilator-associated pneumonias; impregnated catheter site dressings; impregnated catheters, and oral decontamination (Chaiyakunapruk et al., 2002; Timsit et al., 2009). However, it has been suggested that the frequency of CHG use and prevalence of bacteria with reduced susceptibility to CHG are associated (Lee et al., 2011; Horner et al., 2013).

This study revealed that over 40% of MRSA isolates harboured *qac*A/B. This proportion was somewhat lower than that reported elsewhere with *qac*A/B present in 60% of European strains, 47.9% to 62.6% in Japan , 61.6% in China, and up to 80% in Brazil (Alam et al., 2003a; Noguchi et al., 2005; Miyazaki et al., 2007; Wang et al. 2008a; Vali et al., 2008). An increasing trend of *qac*A/B carriage in clinical isolates of MRSA was observed in Taiwan (Wang et al., 2008b).

Although the majority of reports focused on MRSA, those reporting prevalence of *qac*A/B in MSSA described lower carriage rates, with *qac*A/B genes present in only 12% of European MSSA isolates and 7.5% in Japan (Mayer et al., 2001) These findings are in accordance with this study in which only 2% MSSA carried *qac*A/B.

In contrast, prevalence of *smr* in MRSA and MSSA did not differ significantly, with 6% of MSSA and 3.5% MRSA being positive. This is again similar to other reports as Mayer et al. (2001) reported that 6.4% MRSA and 5% MSSA were *smr* positive and Alam et al. (2003a) observed *smr* in 3.3% MRSA and 5.9% MSSA. In another Asian studies, *smr* were present in only 1.9% of MRSA isolates (Noguchi et al., 2005). However, some studies reported much higher prevalence of *smr* in MRSA, including 44.2% in UK (Vali et al., 2008) and in 31.6% from India (Noguchi et al., 2005).

This study found no difference in the frequency of the *qac*A/B between HA-MRSA and CA-MRSA ($p>0.05$). This indicates that plasmids harbouring *qac*A/B had been horizontally transmitted between MRSA clones, becoming widespread in Hong Kong

clinical isolates. A previous report showed that HA-MRSA seemed to harbour more *qac*A/B than CA-MRSA (Smith et al., 2008), but this may have reflected lower exposure of CA-MRSA strains due to their more recent introduction to the hospital environment at the time of sample collection.

There are limited reports describing the frequency of other QAC genes in clinical isolates. In this study, 6.7% *S. aureus* and 10% MRSA were *qac*J positive, but no *qac*G or *qac*H positive strains were detected. In the study of isolates from hospital surfaces (Chapter 4), two isolates of *S. aureus* carried *qac*G and *qac*J, but *qac*H gene was not detected. It appears that *qac*H remains rare in the hospital and these three genes isolated from animal and food sources (Heir et al., 1999a; Bjorland et al., 2003) occur only at a low prevalence in isolates from human sources. *qac*G, *qac*H, and *qac*J have been reported in *Staphylococcus haemolyticus* human clinical isolates from Argentina, with 52% carrying *qac*G, 47% *qac*H, and 19% *qac*J, and suggested that *S. haemolyticus* could act as a reservoir of resistance determinants for more virulent Staphylococci (Correa et al., 2008). However, their presence may reflect local conditions as there is a large beef cattle industry in Argentina. *qac*H was detected in 7.1% QAC tolerant *S. aureus* from a teaching hospital in Shanghai (Liu et al., 2009) and was present in 3.3% of clinical MRSA isolates in UK (Vali et al., 2008). In contrast, Smith et al. (2008) failed to detect *qac*G, H, and J in UK MRSA isolates. Thus as exposure to animal or food sources may differ, prevalence of these genes may vary more than *qac*A/B and *smr.*

Although overall isolation rates were low, this study found CA-MRSA carried more *qac*J than HA-MRSA, suggesting that *qac*J may be transmitted from the community to the hospital. *qac*J has not been previously documented in a clinical isolate of *S. aureus* from human sources, although this gene was present in one CNS isolate from the community (Chapter 3). As these genes may now be becoming more common in human isolates, their presence should be monitored in both clinical isolates and the hospital environment.

Most studies reporting the presence of QAC genes in clinical isolates do not provide further information about their source. In this study, the isolates were differentiated into SSIs and BSI, which may be helpful in determining the epidemiology of QAC genes in *S. aureus* clinical isolates. This study showed isolates from SSIs contained significantly more *qac*A/B genes than those from BSIs. This may reflect use of antiseptic agent for skin disinfection, with biocide residue pressure selecting for strains with QAC genes. There was no difference between SSI and BSI isolates in rates of other QAC genes. However as prevalence of these genes was lower, it may be more difficult to differentiate between rates in SSIs and BSIs. In contrast to these findings, a study in Japan found that the rate of *qac*A/B genes was remarkably lower in MRSA isolates from patients with impetigo and SSSS compared with those from other infections whilst presence of *smr* was rare in all MRSA isolates (Hidemasa Nakaminami, 2008). This suggests that geographical differences may exist between countries and that the prevailing MRSA clones may affect the result.

Several laboratory-based studies have investigated a possible association between biocide use and antibiotic resistance (Berg et al., 1998; Sidhu et al., 2001a; 2001b; 2002; Perez-Roth et al., 2010). This study provided further evidence that antiseptic–antibiotic co-resistance in *S. aureus* isolates, especially for MRSA, was common. The frequency of oxacillin, tetracycline, gentamicin, ciprofloxacin, and erythromycin resistance was significantly higher in clinical isolates of *S. aureus* with QAC genes. Similar results were observed in the studies of carriage organisms of nurses and the hospital environment.

QAC genes are harboured on plasmids such as pST6, pSK4, and pSK41, which also carry genes encoding resistance to gentamicin, penicillin, kanamycin, tobramycin, penicillin, and tetracycline (Berg et al., 1998; Sidhu et al., 2001a; 2001b; 2002; Perez-Roth et al., 2010). This has led to increasing concern that the indiscriminate use of biocides would reduce their effectiveness and simultaneously alter susceptibility to antibiotics. Some in-vitro studies have indicated that following use of biocides, strains showed higher MICs to antibiotics. MRSA mutants resistant to BC showed increased resistance compared to parent strains to various β-lactam antibiotics including cloxacillin, and moxalactam (Akimitsu et al., 1999).

This study did have some limitations. Firstly, the data were derived from only one Hong Kong hospital and may not be generalizable to all hospitals. As sample in our study were collected from BSIs and SSIs only, clinical isolates from other sources including urinary tract infection and ventilator associated pneumonia need to be

considered in the further expanded studies.

In conclusion, surveillance of clinical isolates for presence of QAC genes is necessary as evidence of increasing tolerance or resistance might lead to a reconsideration of CHG use in hospitals. Presence of *qac*A/B in MRSA clones associated with both higher resistance to CHG and other antiseptic agents and increased antibiotic resistance, might limit the choice of drugs for treating MRSA infections and present challenges for infection control, underlining the need for well designed systems for combined surveillance of biocide and antibiotic resistance.

Table 5.1 The distribution of SCC*mec* type of MRSA from the hospital patients

Sources	N (%) of SCC*mec* type							
	II	III	IVa	IVb	IVc	IVd	V	TOTAL
Skin & Soft tissue infections	12 (12)	73 (73)	2 (2)	1 (1)	1 (1)	0 (0)	7 (7)	96 (96)
Blood stream infections	13 (13)	73 (73)	4 (4)	2 (2)	0 (0)	3 (3)	3 (3)	98 (98)
Total	25 (12.5)	146 (73)	6 (3)	3 (1.5)	1 (0.5)	3 (1.5)	10 (5)	194 (97)

Table 5.2 Susceptibility of *S. aureus* clinical isolates

Antibiotics	*S. aureus* (n=300) Resistant N (%)	MSSA (n=100) Resistant N (%)	MRSA (n=200) Resistant N (%)	Difference between MRSA and MSSA (*p* value) [a]
Ciprofloxacin	178 (59.3)	13 (13)	186 (82.5)	**<0.001***
Trimethoprim–sulphamethoxazole	40 (13.3)	1 (1)	39 (19.5)	**<0.001***
Gentamicin	114 (38)	6 (6)	108 (54)	**<0.001***
Clindamycin	41 (13.7)	5 (5)	36 (18)	**0.002***
Tetracycline	153 (51)	20 (20)	133 (66.5)	**<0.001***
Fusidic acid	92 (30.7)	23 (23)	69 (34.5)	**0.042**
Erythromycin	161 (53.1)	19 (19)	142 (71)	**<0.001***
Chloramphenicol	17 (5.7)	8 (8)	9 (4.5)	0.288
Quinupristin/Dalfopristin	6 (2)	5 (5)	1 (0.5)	**0.017***
Linezolid	4 (1.3)	1 (1)	3 (1.5)	1.0

* Difference is statistically significant; [a] Statistical analysis: Chi-Square tests or Fisher's exact test

Table 5.3 Comparison of antibiotics susceptibility between HA-MRSA and CA-MRSA

Antibiotic	N (%) of MRSA Resistant to Antibiotics		
	HA-MRSA	CA-MRSA	Difference between HA-MRSA and CA-MRSA
	Resistant	Resistant	(*p* value) [a]
Ciprofloxacin	143 (83.6)	16 (69.6)	0.100
Trimethoprim–sulphamethoxazole	37 (21.6)	2 (8.7)	0.146
Gentamicin	55 (94)	12 (52.2)	0.800
Clindamycin	30 (17.5)	6 (26.6)	0.322
Tetracycline	117 (68.4)	12 (52.2)	0.121
Fusidic acid	60 (35.1)	7 (30.4)	0.659
Erythromycin	121 (70.8)	17 (73.9)	0.754
Chloramphenicol	8 (4.7)	1 (4.3)	0.944
Quinupristin/Dalfopristin	0 (0)	0 (0)	-
Linezolid	3 (1.8)	0 (0)	0.522

[a] Statistical analysis: Chi-Square tests or Fisher's exact test

Table 5.4 Prevalence of QAC genes in *S. aureus* isolated from different sources

QAC genes		Sources			*p* value [a]
		Skin & Soft Tissue Infections N (%)	Blood stream infections N (%)	Total N (%)	
*qac*A/B	Negative	92 (61.3)	123 (82)	215 (71.7)	**<0.001***
	Positive	58 (38.7)	27 (18)	85 (28.3)	
smr	Negative	144 (96)	143 (95.3)	287 (94.7)	0.777
	Positive	6 (4)	7 (4.7)	13 (4.3)	
*qac*J	Negative	143 (95.3)	137 (91.3)	280 (93.3)	0.165
	Positive	7 (4.7)	13 (8.7)	20 (6.7)	

* Difference is statistically significant; [a] Statistical analysis: Chi-Square tests or Fisher's exact test

Table 5.5 Frequency of QAC gene in MSSA (n=100) and MRSA (n=100) isolates

QAC genes		Source			*p* value [a]
		MSSA N (%)	MRSA N (%)	Total N (%)	
*qac*A/B	Negative	98 (98)	117 (58.5)	215 (71.7)	**<0.001***
	Positive	2 (2)	83 (41.5)	85 (28.3)	
smr	Negative	94 (94)	193 (96.5)	287 (95.7)	0.326
	Positive	6 (6)	7(3.5)	13 (4.3)	
*qac*J	Negative	100 (100)	180 (90)	280 (93.3)	**0.001***
	Positive	0 (0)	20 (10)	20 (6.7)	

* Difference is statistically significant; [a] Statistical analysis: Chi-Square tests or Fisher's exact test

Table 5.6 Frequency of QAC gene in HA-MRSA and CA-MRSA isolates

QAC genes		MRSA types			p value [a]
		HA-MRSA N (%)	CA-MRSA N (%)	Total N (%)	
*qac*A/B	Negative	96 (56.1)	15 (65.2)	111 (57.2)	0.409
	Positive	75 (43.9)	8 (34.8)	83 (42.8)	
smr	Negative	164 (95.9)	23 (100)	187 (96.4)	0.323
	Positive	7 (4.1)	0 (0)	7 (3.6)	
*qac*J	Negative	158 (92.4)	18 (78.3)	176 (90.7)	**0.028***
	Positive	13 (7.6)	5 (21.7)	18 (9.3)	

* Difference is statistically significant; [a] Statistical analysis: Chi-Square tests or Fisher's exact test

Table 5.7 Relations between QAC gene carriage and antibiotic among *S. aureus* isolates

Antibiotic	R/S	Presence of *qac* genes in *S. aureus* isolates (%)		*p* value [a]
		Positive	Negative	
Oxacillin	R	103 (92.8)	97 (51.3)	**<0.001***
	S	8 (7.2)	92 (48.7)	
Ciprofloxacin	R	90 (81.1)	88 (46.6)	**<0.001***
	S	21 (18.9)	101 (53.4)	
Trimethoprim–sulphamethoxazole	R	17 (15.3)	23 (12.2)	0.439
	S	94 (84.7)	166 (87.8)	
Gentamicin	R	72 (64.9)	42 (22.2)	**<0.001***
	S	39 (35.1)	147 (77.8)	
Clindamycin	R	15 (23.5)	26 (13.8)	0.953
	S	96 (86.5)	163 (86.2)	
Tetracycline	R	79 (71.2)	74 (39.2)	**<0.001***
	S	32 (28.8)	115 (60.8)	
Fusidic acid	R	36 (32.4)	56 (29.6)	0.611
	S	75 (67.6)	133 (70.4)	
Erythromycin	R	80 (72.1)	81 (42.9)	**<0.001***
	S	31 (27.9)	108 (57.1)	
Chloramphenicol	R	3 (2.7)	14 (7.4)	0.089
	S	108 (97.3)	175 (92.6)	
Linezolid	R	3 (2.7)	1 (0.5)	0.113
	S	108 (97.3)	188 (99.5)	
Quinupristin/ dalfopristin	R	1(0.9)	5 (2.6)	0.297
	S	110(99.1)	184 (97.4)	

* Difference is statistically significant; [a] Statistical analysis: Chi-Square tests or Fisher's exact test; R: Resistance; S: Sensitive

CHAPTER 6

PREVALENCE OF ANTISEPTIC RESISTANCE GENES IN ISOLATES OF *S. AUREUS* AND COAGULASE-NEGATIVE STAPHYLOCOCCI FROM THE ELDERLY IN HONG KONG

6.1 Introduction

Hong Kong is facing the challenge of an aging population. In 2006, the percentage of the Hong Kong population over 65 years old reached 12.4% (Tse & Leung, 2007). The proportion of the elderly are forecasted to reach 21.9% by 2026 according to the Census 2001 Report. Approximately 60,000 elderly people resided in nursing homes, accounting for about 7% of the population aged 65 years old and above in 2005. (http://www.swd.gov.hk/tc/index/site_pubsvc/page_elderly/sub_residentia/). Recent census data showed that the percentage of the population aged 65 and over showed an increasing trend with the proportion rising from 11% in 2001 to 13% in 2011. http://www.census2011.gov.hk/pdf/Feature_articles/Trends_Pop_DH.pdf.

MRSA has been recognized since the 1980s as a major nosocomial pathogen causing problems in hospitals and other healthcare institutions worldwide. MRSA is also a problem for older people resident in nursing homes. Nursing homes are ideal places to spread MRSA, because the elderly live in close proximity with the majority having underlying health problems such as pressure sores, and chronic illness. Residents are frequently admitted to hospital and then discharged back into nursing home, which may become a reservoir for MRSA providing the potential for an outbreak and further hospital outbreaks when affected nursing home residents require hospital treatment. All these factors increase the risk of the elderly acquiring MRSA, and so increase morbidity and mortality (O'Sullivan & Keane, 2000; Grundmann et al., 2002; Vovko et al., 2005; Tacconelli et al., 2006; Hughes et al., 2011; Stone et al., 2012).

Baldwin et al. (2009) reported that >20% residents in the nursing homes were colonized with MRSA and >36% MRSA carriage rates in older people have been reported in long-term care facilities in France and the USA (Horner et al., 2012b). In comparison to the situation in hospitals, little attention has been given to prevention of infection caused by MRSA in nursing homes. There is a paucity of large-scale longitudinal studies monitoring the occurrence of MRSA in the residential homes. In addition, the prevalence of the elderly in the community harbouring MRSA has received little attention. It is likely that the prevalence of MRSA within nursing homes is increasing as a result of the increased prevalence of MRSA within hospitals, which may have been compounded by the considerable

movement of patients from long-stay hospitals to community-based nursing homes.

QAC genes conferring low-level resistance to cationic biocides have been widely studied in clinical isolates. However, to date, there are no reports regarding presence of QAC genes staphylococci from the elderly. The frequency of QAC genes in Staphylococci from the elderly is likely to differ considerably from the general population. There may also be differences between isolates form elderly living in residence homes and those living in the community.

The study aimed to determine the prevalence of QAC gene carriage in isolates of staphylococci from the elderly residents of residential homes in Hong Kong and to compare these with isolates from elderly living in their own homes and with those of the general population. The prevalence of MRSA carriage in the elderly was also investigated.

6.2 Materials and Methods

6.2.1 Study Design

In Hong Kong, the whole range of residential long-term care facilities are collectively termed "residential care homes for the elderly" (RCHEs). The Hong Kong Council of Social Service classifies RCHEs into 4 categories: (1) hostels for elderly persons, (2) homes for the aged, (3) care-and-attention homes providing continuum of care (CAPCCs), and (4) nursing homes. A hostel for elderly persons provides only residence without skilled nursing care. In homes for the aged some medical care is

provided, but these facilities are mainly residential with some domestic support. In contrast, CAPCCs cater to elderly patients with mild-to-moderate disabilities who require feeding assistance and skilled nursing care. In addition to services provided by the government, there are large numbers of private RCHEs in Hong Kong, which are not categorized may have residents of several categories. A large number of elderly subjects living in their own homes attend daycare centres run by both the Government and charitable organizations. There is no medical care provided at these centres but attendees may receive talks and information about health care topics.

A cross-sectional study of prevalence of *S. aureus* carrying QAC genes from the elderly was performed. The elderly were recruited from four RCHEs and a DC. The DC was provided by the Hong Kong Social Welfare department. The elderly were aged ≥ 60 years. In each RCHE, all residents were initially approached by the in-house healthcare assistants. A sample size of 202 elderly persons was estimated based on a *S. aureus* carriage rate of 20% and assumed 5% carriage rate of QAC genes in *S. aureus* and CNS within 3% error with 95% confidence level according to the formula $N=\mu a^2\pi (1- \pi)/\delta^2$.

6.2.2 Confidentiality and Ethical Considerations

The study was approved by the Human Subjects Ethics Sub-Committee of The Hong Kong Polytechnic University. In the recruitment phase, invitation letters that explained the study were sent to the RCHEs. Residents who consented to

participate were given an information sheet that explained the study, and a consent form. The consent form is shown in Appendix (III and IV).

6.2.3 *S. aureus* and CNS Isolation and Identification

A sterile cotton-tipped swab which was pre-moistened with sterile saline was inserted approximately 1-2cm into one nostril and rotated against the nasal mucosa before carefully being withdrawn. A second swab was used for each subject to sample the oropharynx. All swabs were placed into transport medium and transferred to the laboratory within four hours. They were held at 4°C until culture was performed within eight hours of collection.

Swabs were placed into 5% salt Brain Heart Infusion broth (Oxoid, Basingstoke, UK). After 24h of incubation, broths were subcultured onto *S. aureus* selective agar (SA Select, Biorad, Marnes-la-Coquette, France) and incubated for 24h. All organisms with staphylococcal morphology were identified by Gram stain and catalase test, and confirmed by 16S *r*RNA genes (Martineau et. al., 2001). Tube coagulase test negative strains were reported as CNS. *S. aureus* was confirmed by a positive Staphaurex Plus test (Murex Biotech, Dartford, UK) and presence of *fem*A gene (Mehrotra et. al., 2000). *S. epidermidis* was differentiated from other CNS by PCR (Martineau et.al. 2001). The detailed procedure is ad described in Chapter 3.

6.2.4 Identification and Genotyping of MRSA

S. aureus isolates were subcultured onto MRSA selective agar. If strains grew on this agar, then identification of methicillin-resistance by *mec*A gene detection was carried out and SCC*mec* types were determined. The detailed methodology was as shown in Chapter 3.

6.2.5 Antibiotic Susceptibility Testing

Susceptibility to a range of antibiotics was determined by disc diffusion for all *S. aureus i*solated from the elderly, following CLSI guidelines (CLSI 2009), except for fusidic acid, when British Society for Antimicrobial Chemotherapy guidelines were used (Andrews et al., 2001). The detailed procedure is as described in Chapter 3.

6.2.6 Detection of QAC Genes

The detection of QAC genes was performed as described in Chapter 3.

6.2.7 Statistical Analysis

Statistical analyses were performed using SPSS system for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Association of categorical variables was determined by Chi-square statistic or Fisher's exact test. Mann-Whitney U-test was used to compare the MBC and MIC results. Carriage rates in the elderly were compared with those of the general population.

6.3 Results

6.3.1 Demographic Data

A total of 335 elderly were recruited into the study, 222 (66.3%) from four RCHEs and 113 (33.7%) elderly from the daycare centre (DC) (Table 6.1). The four RCHEs were included in the study: ALCE, YLE, HSQE, and HFE. ALCE was categorized as a home for the aged. The residents of the remaining three RCHEs had various levels of ability to perform activities of daily living, some requiring feeding assistance and skilled nursing care. YLE and HSQE were private RCHEs. ALCE and HFE were government subsidized.

6.3.2 *S. aureus* and MRSA Carriage Rate of the Elderly from RCHEs and DC

The 416 and 239 isolates from RCHEs and DC residents respectively consisting of 285 CNS and 131 *S. aureus* from the RCHEs and 200 CNS and 39 *S. aureus* from the DC (Table 6.1). Some subjects yielded more than one colony type of *S. aureus* or CNS. A maximum of two types were included for any subject.

The carriage rates of *S. aureus* and MRSA in the elderly from the RCHEs were 39.6% (88/222) and 28.4% (63/222), respectively. The rates varied widely between the elderly homes: *S. aureus* carriage ranged from 20-63.9%; and MRSA from 7.5-58.3% (Table 6.1).

The carriage rates of *S. aureus* and MRSA in the elderly from DC were considerably lower at 24.8% (28/113) and 2.65% (3/113) (Table 6.1). Significant differences between carriage rates in attendants of the DC and residents of RCHEs were found for *S. aureus* (p=0.007, OR 2.0, 95% CI: 1.2-3.3) and MRSA (p<0.001, OR 14.6, 95% CI: 4.5-47.7) (Table 6.2).

However, these differences only applied to the average carriage rates in the elderly homes. There was no significant difference in the *S. aureus* and MRSA carriage rates between residents of ALCE, which had the lowest carriage rates, and those of the DC attendees (p=0.54 for *S. aureus*, p=0.175 for MRSA) (Table 6.2.).

6.3.3 Comparison of *S. aureus* and MRSA Carriage Rates from the Elderly and the General Population

There was significant difference in both *S. aureus* and MRSA carriage rates between the elderly (116/335, 34.6%) and the general population: *S. aureus*: (186/775, 24.0%). *(*p<0.001, OR 1.7, 95% CI: 1.3-2.2); MRSA: (66/335, 19.7 %), (4/775, 0.62%). (p<0.001, OR 47.3, 95% CI: 17.1-131.0) (Table 6.3).

However, stratification of the elderly results revealed that although there was significant difference in *S. aureus* carriage rate between the RCHEs (88/222, 39.6%) and the general population (186/775, 24%) (p<0.001, OR 2.1, 95% CI: 1.5-2.9) there was no significant difference in *S. aureus* carriage rate between that from the DC

(24.8%) and the general population (24.0%) (p=0.857) (Table 6.3).

With respect to MRSA carriage, there were significant differences between both groups of the elderly and the general population. Between RCHEs (28.4%) and the general population: (p<0.001, OR 76.4, 95% CI: 27.4-212.8); between DC (2.65%) and the general population (0.52%) (p=0.016, OR 5.3, 95% CI: 1.2-23.8) (Table 6.3).

6.3.4 MRSA from the Elderly in Hong Kong

The distribution of SCC*mec* types of the MRSA from the elderly are listed in Table 6.4. SCC*mec* types IV and V were predominant representing 64.9% of the isolates. Only 25.5% MRSA consisted of typical HA-MRSA types (p<0.05). The remaining 9.6 % strains were not typeable (Table 6.4).

6.3.5 MRCNS in the Elderly and the General Population

Of the CNS isolates from the DC, 39.0% (78/200) were *mec*A positive. In contrast, 58.2% (166/285) RCHEs CNS were *mec*A positive. This difference in proportion of methicillin resistant strains was significant (p<0.001, OR 2.2 95% CI: 1.5-3.2). There was also a difference between elderly isolates and those of the general population (11%) (p<0.001, OR 11.3, 95% CI: 4.4-28.9). The rates of methicillin resistance varied between RCHE with *mec*A present in 56.7% (51/90) CNS strains from ALCE, 68.9% (31/45) from YLE, 60.3% (38/63) in HSQE, and 52.9% (46/87) from HFE, though this variation was much less than that observed for MRSA.

6.3.6 Susceptibility of *S. aureus* from the Elderly

There were high levels of resistance to ciprofloxacin (38.5 %), tetracycline (27.2 %), fusidic acid (10.1 %), gentamicin (10 %), and erythromycin (21.9 %) with lower levels observed to chloramphenicol (7.1 %), trimethoprim–sulphamethoxazole (2.4%), clindamycin (8.9%), and linezolid (1.8 %). No strain was resistant to vancomycin (Table 6.5).

These were significant differences in resistance rates between methicillin sensitive and resistant strains for all antibiotics other than fusidic acid (Table 6.5). Comparison of resistance patterns of HA-MRSA and CA-MRSA revealed that there were significant differences for some agents including tetracycline, gentamicin and erythromycin (Table6.6).

The susceptibilities for the general population are also listed in Table 6.7 for comparison. Strains isolated from the elderly were more resistant to oxacillin, ciprofloxacin, gentamicin, fusidic acid, tetracycline and chloramphenicol compared with isolates from the general population (p<0.05). For other antibiotics, although the resistance frequencies of strains from the elderly were a little higher than that from the general population, the differences did not reach significance.

The susceptibilities for *S. aureus* from RCHEs and DC are listed in Table 6.7 Strains isolated from the RCHEs were more resistant to oxacillin, ciprofloxacin, clindamycin,

and fusidic acid compared with the isolates from the DC ($p<0.05$), and the resistance frequencies of strains for other antibiotics from the RCHEs were generally a little higher than those from the general population, but did not reach significance.

Multi-resistance, defined as resistance to three or more classes of antibiotics, was observed for 29.8% (28/94) MRSA isolates. Of these, 54.2% (13/24) HA-MRSA isolates and 50% (4/8) untypeable MRSA were multi-resistant, compared to only 17.7% (11/62) CA-MRSA (Table 6.6).

6.3.7 Presence of QAC Genes in CNS and *S. aureus*

The prevalence of *qac*A/B and *qac*J in CNS was significantly higher than in *S. aureus*. ($p=0.006$, OR 2.1, 95% CI: 1.2-3.6 for *qac*A/B; $p=0.003$, OR 3.7, 95% CI: 1.5-9.0 for *qac*J). However, there was no significant difference in the prevalence of other QAC genes (*smr*, *qac*G, *qac*H) ($p>0.05$) (Table 6.8).

6.3.8 Comparison of QAC Gene in the Elderly and the General Population

***qac* A/B**

Of the strains from the DC, 13.4% (32/239) strains were *qac*A/B gene positive, including 5.1% (2/39) *S. aureus* and 15% (30/200) CNS. Compared to this, 19.7% (82/416) of RCHEs isolates were *qac*A/B positive, including 23.2% (66/285) CNS and

12.1% (16/131) *S. aureus*. Analysis revealed isolates from the RCHES overall carried significantly more *qac*A/B gene than those from DC (*p*=0.04, OR 1.6, 95% CI: 1.0-2.5). The difference in *qac*A/B carriage for CNS was significant (*p*=0.026, OR 1.7 95% CI : 1.1-2.7), but not for *S. aureus* (*p*=0.207).

The *qac*A/B rate varied between the elderly homes (range 8.3-19.6% for *S. aureus*, 16.7-34.8% for CNS). This did not reach significance for *S. aureus*, but CNS rates of *qac*A/B in HFE were significantly different from those of ALCE and HSQE (ALCE *p*=0.007, OR 2.6, 95% CI: 1.3-5.3; HSQE *p*=0.021 OR 2.5 95% CI:1.1-5.5) (Table 6.8).

smr gene was present in 10.8% (45/416) of isolates from the RCHEs consisting of 9.1% (26/285) CNS and 14.5% (19/131) *S. aureus*. The *smr* gene was not detected in DC isolates. As with *qac*A/B, *smr* positive rates varied between RCHEs, with 0% *S. aureus* and 1.1% (1/90) CNS in ALCE, 9.3% (4/43) *S. aureus* and 6.7% (3/45) CNS in YLE, 6.7% (2/30) *S. aureus* and 15.9% (10/63) CNS in HSQE, 14.9% (13/87) *S. aureus* and 26.1% (12/46) CNS in HFE. There were significant differences in *smr* positive rates of *S. aureus* and CNS between different RCHES (*p*=0.02 for *S. aureus*, *p*=0.003 for CNS) (Table 6.8).

***qac*G, *qac*H and *qac*J**

Only two isolates with *qac*G gene were identified. Both were from CNS, one from DC (0.42%) and one from an RCHE (0.24%). *qac*H was not detected. Similar to *qac*G, *qac*J was only isolated from one CNS from a DC attendee (0.5%), but 4.6% (19/416)

RCHEs isolates were *qac*J positive, making a total of 20 *qac*J positive isolates in the elderly. (*p*=0.003, OR 11.4, 95% CI: 1.5-85.6).

Of the 19 *qac*J positive isolates from the RCHES, 2.82% (8/285) CNS and 8.40% (11/131) *S. aureus* harboured the *qac*J gene. *qac*J gene was present in 16.7% (2/12) *S. aureus* and 6.7% (6/90) CNS in ALCE, and 19.6% (9/46) *S. aureus* and 2.3% (2/87) CNS in HFE, but was not detected in other RCHES isolates (Table 6.8). Rates of all QAC genes were compared with those of the general population and were found to be higher for RCHE residents for all genes.

6.3.9 Association between the *mec*A and QAC Genes in *S. aureus* and CNS from the Elderly

Overall, methicillin resistant isolates were more likely to carry *qac*A/B gene than methicillin sensitive isolates (*p*<0.001, OR 2.6 95% CI: 1.7-4.0) (Table 6.9), with both MRSA having more *qac*A/B gene than MSSA (*p*<0.001, OR 16.6 95% CI: 2.1-127.6), and MRCNS than MSCNS (*p*=0.001, OR 2.2, 95% CI: 1.4-3.5).

Similarly, the methicillin resistant strains carried more *smr* than the methicillin sensitive (*p*<0.001, OR 4.7 95% CI: 2.4-9.2) (Table 6.9), with statistically higher positivity in both MRSA (17.0%, 16/94) (*p*=0.002, OR 7.6, 95% CI: 1.7-34.2), and MRCNS more than MSCNS (*p*<0.001, OR 4.0, 95% CI: 1.9-8.6). MR strains carried more *qac*J gene than the MS one (*p*=0.033, OR 2.9, 95% CI: 1.0-8.1) (Table 6.9).

6.3.10 Antibiotic Resistance and QAC Gene Carriage

Resistance to ciprofloxacin, gentamicin, tetracycline and erythromycin was significantly associated with presence of QAC genes (Table 6.10).

6.4 Discussion

6.4.1 *S. aureus* and MRSA Prevalence in the Elderly in Hong Kong

To our knowledge, this is the first report comparing the prevalence of MRSA colonization in the elderly subjects recruited from nursing homes and DC in Hong Kong. The mean carriage rate of *S. aureus* in the elderly from the RCHEs was 39.6% but this varied widely from 20 to 63.9% between the homes. The carriage rates of *S. aureus* and MRSA in the elderly from the DC were considerably lower at 24.8% and 2.65% than those of the RCHEs. The mean rate in the RCHEs was significantly higher than for the general population in Hong Kong. *S. aureus* colonization increases with aging and the mortality rate of *S. aureus* infection is markedly increased in elderly persons (Reunes et al., 2011).

In a recent study of MRSA colonization of elderly subjects in 13 RCHEs in Hong Kong, 2.8% were found to carry MRSA (Ho et al., 2007b). No MRSA was detected in two facilities. The rate of MRSA carriage in the other 11 facilities ranged from 1.9% to 4.2%. Ho et al. (2008b) reported a somewhat higher MRSA colonization rate of 5.1% from 1563 residents in 487 RCHEs in Hong Kong.

In our investigation, the carriage rate of MRSA was 28.4%, far exceeding that of the general public in Hong Kong. This MRSA carriage rate was obviously much higher than the previous reports for Hong Kong (Ho et al., 2007b; 2008b). This may indicate that the MRSA colonization rate in the elderly in RCHEs had increased greatly over a relatively short period of time. However, MRSA carriage rate differed greatly between RCHEs elderly homes varying from 7.5 to 58.3%, among which, the lowest MRSA colonization rate was from ALCE. In their 2008b study, the researchers only collected 3-4 samples at each RHCE and failed to detect MRSA in 85% of homes (Ho et al, 2008b). Although the residents to be sampled were meant to be selected by randomization, such small samples from each home may lead to bias if the selected subject was unsuitable for sampling due to their state of health or refusal to participate.

In Asian countries, MRSA has been reported to reach high levels in hospital settings, with 40%-75% of *S. aureus* isolates resistant to methicillin (Chongtrakool et al., 2006). A recent report of a four year study (2006-2009) in the UK showed 9-22% of residents from 65 care homes carried MRSA, mostly HA- MRSA (SCC*mec* IV) (Horner et al., 2013). However, a report from the emergency department of a French teaching hospital showed that the prevalence of MRSA carriage at the time of admission of patients from nursing homes was 31.9% (Eveillard et al., 2006). A study in Singapore reported 41% MRSA colonization in hospital patients admitted from nursing homes and 6.0% for non-nursing home residents [RR: 6.89; 95% CI 5.74-8.26] (Verrall et al., 2013). Additionally, a high frequency of MRSA was detected in

environmental samples from a new building after nursing home residents had moved in, providing strong evidence that nursing home residents act as reservoirs of MRSA (Ludden et al., 2013).

Outbreaks in hospitals may sometimes be attributed to MRSA acquired in nursing homes (Bradley, 1997; Kerttula et al., 2005). Although MRSA infections in the care home setting are infrequent, colonized residents have an increased risk of developing an infection. In order to avoid large outbreaks in Hong Kong, It is important to urgently implement strategies to control the transmission of MRSA in RCHEs, as well as to monitor MRSA colonization of the elderly admitted to the hospital.

RCHEs are an important part of the health care system for the old age group. Infection is an important cause of morbidity and mortality in the institutionalized elderly. The elderly homes in Hong Kong are fairly crowded, and such conditions have been associated with MRSA transmission and higher rates of MRSA carriage (Beam and Buckley, 2006). In our investigation, the distance between beds was less than two metres. The limited room for each elderly person may greatly increase the risk for MRSA spread.

Ho et al. (2007b) reported that residents had an average living space of 16 m^2 per person, which is substantially higher than the 5.5 m^2 per person or greater that was adopted by the Hong Kong Housing Authority as a standard. However, the 16m^2

also takes into account the space taken up by communal areas but many of the more frail occupants spend the majority of their time in the shared bedrooms. Ho et al. (2008b) surveyed only subsidized RCHEs managed by the Hong Kong Council of Social Service, and their results might not be applicable to private RCHEs, where the facilities frequently are not as well-appointed as those run by the Government. Three of the homes we visited were privately run and average space was considerably less with four elderly persons in 12 m^2 cubic area. This difference could possibly lead to underestimation of the prevalence of MRSA if samples are only collected in the less crowded facilities.

In this study, we also surveyed the elderly attending a DC. These subjects live in their own homes and gather in the DC irregularly. The carriage rates of *S. aureus* and MRSA in the elderly from DC were considerable lower at 24.8% and 2.65%. This strongly supports the evidence that living in elderly home increases the risk for MRSA colonization in comparison to living in their own home. The *S. aureus* carriage rate in elderly from the DC did not differ from the general population, but these elderly subjects did carry more MRSA. Possibly gathering in the DC would increase the MRSA transmission between the elderly especially if some had recently been hospitalized. However, our current study could not provide more evidence to support this and further investigation should be carried out.

The antimicrobial susceptibility profile of *S. aureus* in Hong Kong, especially for MRSA colonizing residents in care homes is largely unknown, because no national

system for tracking trends in antibiotic resistance in nursing homes exists. The data herein revealed that the majority of the MRSA isolates in the elderly possessed SCC*mec* type IV and V, which is similar to the result showed reported by Ho et al (2008b). This is in contrast with the clinical isolates in which SCC*mec* type III, predominated. All the MRSA isolates from the elderly attending the DC were SCC*mec* type IV or V. These results indicate that CA-MRSA has disseminated widely in both the elderly homes and the community in Hong Kong.

In recent years, sharp increases in infections caused by antibiotic-resistant bacteria appear to be occurring in US nursing homes as well as hospitals (Crnich et al., 2007; Viray et al., 2005). In this study, there were high levels of resistance in *S. aureus* to ciprofloxacin, tetracycline, and erythromycin. Linezolid resistance in staphylococci was still rare, but should be closely monitored.

MRSA susceptibility patterns evolve continuously. Ho et al., (2008b) reported somewhat higher resistance rates for MRSA isolates from RCHEs than those in our study, with 86.3% for ciprofloxacin, 65% for erythromycin, 66.3% for tetracycline, and 42.5% for gentamicin. This may also reflect geographical variation in MRSA between different RCHEs.

In our study, 29.8% MRSA isolates were multi-resistant. CA-MRSA is reported to be more likely to be sensitive to a wider range of these antibiotics than HA-MRSA and the data in this study was consistent with this definition. Compared to the clinical

MRSA isolates described in Chapter 5 in which there was no difference in the antibiotic profiles of HA-MRSA and CA-MRSA, the CA-MRSA from the elderly differed from HA-MRSA in the frequency of resistance to many antibiotics. It indicates that although the CA-MRSA in the hospitals in Hong Kong have evolved to become more resistant to antibiotics, CA-MRSA isolated from elderly in nursing home and DC have remained closer to the initial definition of CA-MRSA.

In this study, 12.5% HA- MRSA and 11.3% CA-MRSA were resistant to clindamycin, a little lower to reports elsewhere (Dryden et al., 2010) and than clinical isolates described in Chapter 5. It was also found that the *S. aureus* strains from the elderly were more resistant to antibiotics than strains from the general population ($p<0.05$) and that isolates from RHCEs were more resistant than those from the DC. This reflects that elderly are prone to carry more antibiotic resistant determinants owing to their poor immunities or/and frequent attendance at the hospital. Although strains isolated from the elderly attending DC are currently not multi- resistant, monitoring of their antibiotic profiles should be considered.

6.4.2 QAC Genes in Staphylococci isolates form the Elderly in Hong Kong

Although *S. aureus* including MRSA have been used as indicator organisms for assessment of surface hygiene, there have been no reports related to QAC genes in isolates from the elderly. Previous research has focused on the prevalence of QAC genes in *S. aureus* and MRSA clinical isolates (Smith et al 2008; Wang et al., 2008a;

Sheng et al., 2009).

This study showed a significant difference in the prevalences of *qac*A/B, *smr*, and *qacJ* in *S. aureus* and CNS isolates from the elderly in the RCHEs and the general public. It showed that the elderly were more likely to be colonized with *S. aureus* and that their strains were more likely to harbor QAC genes than the general public. The RCHEs may act as a reservoir of QAC genes. Consequently, the elderly, acting as an interface between hospitals, long-term care facilities, and the community, may be important in cross-transmission of antiseptic resistant *Staphylococci* between hospitals, RCHEs, and the community.

Additionally, our study also indicates that the elderly in the RCHEs harboured more QAC genes in both *S. aureus* and CNS isolates than the elderly attending a DC. However, rates of staphylococci with QAC genes in DCs may increase due to transfer of strains from the hospital to the DC attendants accompanied by increasing usage of home biocides. It is necessary to monitor rates of the QAC gene positive strains and their levels of resistance in the long term.

There have been few reports related to *qac*G/H/J genes in isolates of *S. aureus*. In our clinical study, 6.7% *S. aureus* and 10% MRSA were *qac*J positive and no *qac*G, *qac*H positive strains were detected. In the study of hospital surfaces (Chapter 4), *qac*G and *qac*J gene were each only present in two isolates. In the elderly isolates, only two carried *qac*G gene, but carriage of *qac*J was higher with 2.82% CNS and

8.40% *S. aureus* positive isolates.

In the environment of the elderly home, residual concentrations of biocides may play an important role in the transfer of QAC genes. Attempts to improve environmental hygiene in RCHEs may result in higher rates of QAC genes if this process is not carried out correctly. Many of the staff in RCHEs are poorly trained and may need additional education in the correct use of antiseptic agents.

6.4.3 Conclusion

The data shows that MRSA has spread from the hospital environment to other healthcare institutions in Hong Kong, which must now be regarded as important reservoirs for MRSA transmission.

It also showed that the guidelines for the MRSA control may not be well implemented in RCHEs, especially in some of the private ones. The hygiene in the RCHEs should be monitored. Our results demonstrate that RCHE residents are at high risk for colonization by MRSA carrying QAC genes. The high prevalence of MRSA colonization in nursing homes underscores the importance of good adherence to standard infection control precautions even in the absence of a diagnosed infection.

Appropriate interventions are urgently needed in RCHEs to reduce endemic MRSA

colonization. Further studies on interventions to reduce MRSA in RCHEs should be performed to determine their effectiveness.

In this study the carriage rates of *S. aureus* and MRSA in elderly home varied widely. Different conditions have been observed in investigated residential homes, with in-patients having differing amounts of individual space and differences in standards of hygiene. The patients also differed between locations with some (ALCE) being mainly mobile and able to leave the premises for exercise and recreation to others in which a considerable proportion of residents were bed-bound. Additionally, two of the residential homes were subsidized by the government and the other two were private ones. These factors are likely to have contributed to the variation observed in this study. Only one daycare center was included. Therefore, more daycare center should be sampled in a future study.

In this study, only four RCHEs were sampled, and further studies should include more RCHEs to better determine the profile associated with the high prevalence of MRSA and *S. aureus* carrying QAC genes.

Table 6.1 *S. aureus* and MRSA carriage in the elderly from RCHEs and DC

RCHEs	N (%)	Total isolates		*S. aureus* carriage rate (%)	MRSA carriage rate (%)
		S. aureus N (%)	CNS N (%)		
ALCE	40 (18.1)	12 (9.2)	90 (31.6)	20 (8/40)	7.5 (3/40)
YLE	36 (16.2)	43 (32.8)	45 (15.8)	63.9 (23/36)	58.3 (21/36)
HSQE	46 (20.7)	30 (22.9)	63 (22.1)	45.6 (21/46)	28.3 (13/36)
HFE	100 (45.1)	46 (35.1)	87 (30.5)	35 (35/100)	26 (26/100)
Overall	222 (100)	131(100)	285 (100)	39.6 (88/222)	28.4 (63/222)
DC	113 (100)	39 (100)	200 (100)	24.8 (28/113)	2.65 (3/113)
Total	345 (100)	170 (100)	485 (100)	34.6 (116/335)	19.7 (66/335)

Table 6.2 Comparison of *S. aureus* and MRSA carriage rates of the elderly between from RCHEs and the day care centre

Species	RCHEs	Positive N (%)	Negative N (%)	*p* value [a]	OR	95% CI
S. aureus	ALCE	8 (20)	32 (80)	0.54	-	-
	YLE	23 (63.9)	13 (36.1)	**<0.001***	**5.4**	**2.4-12.0**
	HSQE	21(45.6)	21 (54.4)	**0.001***	**3.3**	**1.6-6.8**
	HFE	35 (35)	65 (65)	0.103	-	-
	DC	28 (24.8)	85 (75.2)	-	-	-
MRSA	ALCE	3 (7.5)	37 (92.5)	0.175	-	-
	YLE	21 (58.3)	15 (41.7)	**<0.001***	**55**	**14.6-208.1**
	HSQE	13 (28.3)	23 (71.7)	**<0.001***	**14.4**	**3.9-53.8**
	HFE	26 (26)	74 (74)	**<0.001***	**12.9**	**3.8-44.1**
	DC	3 (2.65)	110 (97.35)	-	-	-

a: compared to the DC; *$p<0.05$

Table 6.3 Comparison of *S. aureus* and MRSA carriage rates between the elderly and the general population

	Group	Positive N (%)	Negative N (%)	*p* value [a]	OR	95% CI
S. aureus	DC	28 (24.8)	85 (75.2)	0.857	-	-
	RCHEs	88 (39.6)	134 (60.4)	**0.001***	**2.1**	**1.5 - 2.9**
	Overall elderly	116 (34.6)	219 (65.4)	**<0.001***	**1.7**	**1.3 -2.2**
	General population	186 (24.0)	589 (76.0)	-	-	-
MRSA	DC	3 (2.65)	110 (97.35)	**0.016***	**5.3**	**1.2 -23.8**
	RCHEs	63 (28.4)	159 (71.6)	**0.001***	**76.4**	**27.4 - 212.8**
	Overall elderly	66 (19.7)	269 (80.3)	**<0.001***	**47.3**	**17.1-131.0**
	General population	4 (0.52)	771 (99.38)	-	-	-

a: p value calculated when compared to the general population; *$p<0.05$

Table 6.4 SCC*mec* types of MRSA from the elderly in Hong Kong

Sources	Untypable N (%)	I N (%)	II N (%)	III N (%)	IV N (%)	V N (%)	Total N (%)
ALCE	-	-	5 (100)	-	-	-	5 (100)
YLE	-	-	-	-	25 (63.1)	14 (35.9)	39 (100)
HSQE	1 (7.1)	-	-	1 (7.1)	3 (21.3)	9 (63.9)	14 (100)
HFE	8 (26.7)	3 (10)	2 (6.7)	13 (43.3)	4 (13.3)	-	30 (100)
Over all RCHEs	9 (10.2)	3 (3.4)	7 (8.0)	14 (15.9)	32 (36.4)	23 (26.1)	88 (100)
DC	-	-	-	-	4 (66.7)	2 (33.3)	6 (100)
Total	9 (9.6)	3 (3.2)	7 (7.4)	14 (14.9)	36 (38.3)	25 (26.6)	94 (100)

Table 6.5 Susceptibility of *S. aureus* from the elderly

Antibiotics	*S. aureus* (n=170) Resistant N (%)	MRSA (n=94) Resistant N (%)	MSSA (n=76) Resistant N (%)	Difference between MRSA and MSSA (*p* value) [a]
Oxacillin	94 (55.3)	94 (100)	0 (0)	-
Ciprofloxacin	66 (38.8)	63 (67)	3 (3.9)	**<0.001***
Trimethoprim–sulphamethoxazole	4 (2.4)	3 (3.2)	1 (1.3)	0.629
Gentamicin	17 (10)	11 (11.7)	6 (7.9)	0.452
Clindamycin	15 (8.9)	11 (11.7)	4 (5.3)	0.179
Tetracycline	47 (27.6)	35 (37.2)	12 (15.3)	**0.002***
Fusidic acid	17 (10.1)	8 (8.5)	9 (11.8)	0.608
Erythromycin	37 (21.9)	24 (25.5)	13 (17.1)	0.197
Chloramphenicol	12 (7.1)	6 (6.4)	6 (7.9)	0.769
Linezolid	3 (1.8)	2 (2.1)	1 (1.3)	1.0
Imipenem	11 (6.5)	9 (9.6)	2 (2.6)	0.114

* Difference is statistically significant; [a] Statistical analysis: Chi-Square tests or Fisher's exact test

Table 6.6 Comparison of antibiotic susceptibility between HA-MRSA and CA-MRSA

Antibiotic	N (%) of MRSA resistance to antibiotics (n=86)		
	HA-MRSA (n=24)	CA-MRSA (n=62)	Difference between HA-MRSA and CA-MRSA (p value)[a]
	Resistant	Resistant	
Ciprofloxacin	14 (58.3)	44 (71.0)	0.309
Trimethoprim–sulphamethoxazole	0 (0)	3 (4.8)	0.557
Gentamicin	6 (25)	3 (4.8)	**0.013***
Clindamycin	3 (12.5)	7 (11.3)	1
Tetracycline	17 (70.8)	11 (17.7)	**<0.001***
Fusidic acid	3 (12.5)	4 (6.5)	0.393
Erythromycin	11 (45.8)	7 (11.3)	**0.001***
Chloramphenicol	2 (8.3)	4 (6.5)	0.67
Linezolid	1 (4.2)	1 (1.6)	0.483

[a] Statistical analysis: Chi-Square tests or Fisher's exact test

Table 6.7 Comparison of general population and elderly groups for antibiotic resistance among *S. aureus* isolates

Antibiotics	R/S	Group		Difference between elderly and GP (*p* value)	Group		Difference between EH and DC (*p* value)
		Elderly N (%) (n=170)	GP N (%) (n=186)		EH N (%) (n=131)	DC N (%) (n=39)	
Oxacillin	R	94 (55.3)	3 (3.8)	**<0.001***	88 (67.2)	6 (15.4)	**<0.001***
	S	76 (44.7)	179 (96.2)		57 (32.8)	33 (84.6)	
Ciprofloxacin	R	65 (38.5)	4 (2.2)	**<0.001***	63（48.1）	3（7.7）	**<0.001***
	S	105 (61.5)	182 (97.8)		68 (54.9)	36 (92.3)	
Trimethoprim–sulphamethoxazole	R	4 (2.4)	0 (0)	0.051	4（3.1）	0（0）	0.575
	S	164(97.6)	186(100)		127 (96.7)	39 (100)	
Gentamicin	R	17 (10)	8 (4.3)	**0.036***	16（12.2）	1（2.6）	0.125
	S	153 (90)	178 (95.7)		115 (87.6)	38 (97.4)	
Clindamycin	R	15 (8.9)	10 (5.4)	0.204	15（11.5）	0（0）	**0.024***
	S	155 (91.1)	176 (94.6)		116 (88.5)	39 (100)	
Tetracycline	R	46 (27.2)	31 (16.7)	**0012***	41（31.3）	6（15.4）	0.066
	S	124(72.8)	155(83.3)		90 (68.7)	33 (84.6)	
Fusidic acid	R	17(10.1)	2(1.1)	**<0.001***	17（13）	0（0）	**0.014***
	S	153 (89.1)	184 (98.9)		114 (87)	39 (100)	
Erythromycin	R	37 (21.9)	50 (26.9)	0.262	27 (20.6)	10 (25.6)	0.512
	S	163 (78.1)	136 (73.1)		104 (79.4)	29 (74.4)	
Chloramphenicol	R	12 (7.1)	14 (7.5)	0.865	8(6.1)	4 (10.3)	0.474
	S	158 (92.9)	172 (92.5)		123 (93.9)	35 (89.7)	
Linezolid	R	51 (30)	16 (8.6)	0.056	3 (2.3)	0 (0)	1.0
	S	119 (70)	170 (91.4)		128 (97.7)	39 (100)	
Imipenem	R	3 (1.8)	11 (5.9)	**<0.001***	10 (7.6)	1 (2.6)	0.46
	S	167 (98.2)	175 (94.1)		121 (92.4)	38 (97.4)	

R, antibiotic resistance; S, antibiotic sensitive; * Difference is statistically significant ($p<0.05$);

GP, general population; EH, elderly home; DC, daycare center

Table 6.8 Comparison of QAC gene prevalence in *S. aureus* and CNS from the elderly and the general population

QAC gene	Source	Positive N (%)	*p* value*[a]	OR [a]	95% CI [a]	*p* value*[b]	OR [b]	95% CI [b]
*qac*A/B	RCHES	82 (19.7)	**<0.001***	**2.4**	**1.7 -3.5**	**0.04***	**1.6**	**1.0 - 2.5**
	DC	32 (13.4)	0.729	-	-	-	-	-
	General population	48 (12.4)	-	-	-	-	-	-
smr	RCHEs	45 (10.8)	**0.012***	**1.9**	**1.1 - 3.1**	**<0.05***	-	-
	DC	0 (0)	0.0068	0.93	0.91-0.96	-	-	-
	General population	27 (7.0)	-	-	-	-	-	-
*qac*G	RCHEs	1 (0.24)	0.153	-	-	**<0.05***	-	-
	DC	1 (0.42)	0.399	-	-	-	-	-
	General population	4 (1.03)	-	-	-	-	-	-
*qac*J	RCHEs	19 (4.6)	**0.001***	**6.1**	**1.8-20.8**	**0.003***	**11.4**	**1.5-85.6**
	DC	1 (0.42)	0.585	-	-	-	-	-
	General population	3 (0.7)	-	-	-	-	-	-

* Difference is statistically significant ($p<0.05$); a: compared to general population; b: compared to DC.

Table 6.9 Association between the *mec*A gene and *qac* gene in *S. aureus* and CNS from the elderly

QAC genes	Species	Positive N (%)	Negative N (%)	*p* value [a]	OR	95% CI
qacA/B	MR	80 (23.7)	258 (76.3)	**<0.001***	**2.6**	**1.7-4.0**
	MS	34 (10.7)	283 (89.3)			
smr	MR	49 (14.5)	289 (85.5)	**<0.001***	**4.7**	**2.4-9.2**
	MS	11 (3.5)	306 (96.5)			
qacG	MR	1 (0.3)	227 (99.7)	>0.05	-	-
	MS	1 (0.3)	316 (99.7)			
qacJ	MR	15 (4.4)	323 (95.6)	**0.033***	**2.9**	**1.0-8.1**
	MS	5 (1.6)	312 (98.4)			

* Difference is statistically significant ($p<0.05$); MR: methicillin resistant, MS: methicillin sensitive; a, MR compare to MS

Table 6.10 Association between antibiotic resistance and QAC genes in *S. aureus* from the elderly

Antibiotics	R/S	Number of *S. aureus* isolates with QAC gene (%)		*p* value
		Positive	Negative	
Ciprofloxacin	R	23 (57.5)	42 (32.6)	**0.005***
	S	17 (42.5)	87 (67.4)	
Trimethoprim–sulphamethoxazole	R	2 (5)	2 (1.6)	0.210
	S	38 (95)	127 (98.4)	
Gentamicin	R	10 (25)	6 (4.7)	**<0.001***
	S	30 (75)	123 (95.3)	
Clindamycin	R	5 (12.5)	10 (7.8)	0.356
	S	35 (87.5)	119 (92.3)	
Tetracycline	R	21 (52.5)	25 (19.4)	**<0.001***
	S	19 (47.5)	104 (80.6)	
Fusidic acid	R	6 (15)	11 (8.5)	0.234
	S	34 (85)	118 (91.5)	
Erythromycin	R	18 (45)	19 (14.7)	**<0.001***
	S	22 (55)	110 (85.3)	
Quinupristin/Dalfopristin	R	10(25)	41 (31.8)	0.414
	S	30(75)	88 (68.2)	
Chloramphenicol	R	5 (12.5)	7 (5.4)	0.128
	S	35 (87.5)	122 (94.6)	
linezolid	R	2 (5)	1 (0.78)	0.077
	S	38 ((5)	128 (99.22)	
Imipenem	R	4 (10)	6 (4.7)	0.210
	S	36 (90)	123 (95.3)	

*Difference is statistically significant (p<0.05) R: Resistant; S: Sensitive

CHAPTER 7

DEVELOPMENT AND EVALUATION OF THE SPIRAL GRADIENT ENDPOINT TECHNIQUE FOR DETERMINATION OF MICs OF ANTISEPTICS

7.1 Introduction

Spiral gradient endpoint (SGE) technique is a gradient method for MIC determination which has been previously used for antibiotic susceptibility determination (Paton et al., 1990; Doddangoudar et al., 2010; Pong et al., 2010). SGE was developed some years ago and has been shown to be an accurate, simple, low cost method. Its use has been evaluated for antibiotic susceptibility testing of a number of organisms including those with non-standard nutritional or atmospheric requirements (Wallace & Corkill, 1989; Hill & Schalkowsky, 1990; Doddangoudar et al., 2010; Pong et al., 2010). In addition to the flexibility of medium and incubation conditions, the use of a gradient allows a more accurate MIC determination especially at higher concentrations. Gradient methods provide the ability to see small increments in susceptibilities and resistance which can be missed by dilution methods, which suffer from large increments as concentration increases. It is very tedious to introduce intermediate dilutions to overcome this problem.

SGE uses a spiral plater to deposit a known concentration of an antimicrobial agent onto the surface of an agar plate, producing a concentration gradient in a precise spiral as a way of increasing the dilution of the compound from near the centre of the plate to the periphery (Spiral Biotech Inc, Norwood, MA, USA). Use of the SGE method has several advantages compared to other MIC methods, including decreased labour and materials, accurate mechanical dilution, and good correlation with reference methods (Wallace & Corkill, 1989; Hill & Schalkowsky, 1990).

However, owing to the introduction of alternative gradient methods especially E-test, SGE has not been widely applied. Recently, SGE has been shown to be suitable for determination of MICs for fastidious organisms and detection of vancomycin resistance as well as for susceptibility testing of Shiga toxin–producing *Escherichia coli* (Pong et al, 2010; Doddangoudar et al., 2010; Khatiwada et al., 2012). For such applications, SGE offers a simple, rapid and cost-effective alternative method for MIC determination (Doddangoudar et al., 2010; Khatiwada et al., 2012).

In the case of antibiotics, the use of E-test offers a convenient gradient method for routine MIC determination. However for other compounds, such as biocides, E-tests are not available. Preparation of dilutions for such substances can be tedious especially if a large number of isolates need to be tested. The SGE technique only requires one or two plates to cover a wide range of concentrations. Each plate can be used to determine the MIC of three strains in duplicate. As these plates are

prepared individually, the technique can be developed and evaluated for a wide range of substances.

The aim of this study was to optimize the SGE method for determination of MIC of antiseptics for *S. aureus* and to evaluate it in comparison with standard methods.

7.2 Materials and Methods

This study comprised of two stages. In the first part the method was optimized and the accuracy and reproducibility were assessed using a selection of strains. In the second stage the MIC values determined for these strains and the same strains following induction by the presence of antiseptics (as described in Chapter 8) and displaying higher MICs were compared to those determined by agar dilution.

7.2.1 Strains and Media

7.2.1.1 Strains

Reproducibility and accuracy phase: For intra-batch and inter batch reproducibility the following strains were included: a control strain of *S. aureus* (ACTT25923) which is negative for QAC genes and should not display elevated tolerance to antiseptics thereby acting as a baseline; four clinical isolates of *S. aureus* negative for QAC genes; five clinical *S. aureu*s isolates positive for *qac*A; and five clinical *S. aureus* isolates positive for *smr*. *qac*A positive strains were chosen rather than *qac*A/B

because *qac*A, unlike *qac*B, usually mediates resistance to CHG. *smr* usually mediates resistance to BC but not CHG.

Evaluation Phase: For determination of Inter-observer reliability and comparison of SGE with AD: The same strains as described above were included together with their induced derivatives from the induction study described in Chapter 8. The induced forms of the 14 clinical isolates had been exposed to antiseptics for two weeks. The strains used were renamed so as to match their use in the induction study. These pre and post-induction strains were designated a1-a5: *qac*A positive strains; c1-c5: *smr* positive strains; n1 (ATCC 25923) and n2-n5: clinical strains without QAC genes. The name was followed by the suffix p0 for pre-induction and p2 for after induction for 12 days.

Strains carrying the *qac*A gene were differentiated from clinical isolates of *S. aureus* shown positive for *qac*A/B by PCR by sequencing the amplified gene product. Those isolates with a product matching the published sequence (Genebank) for *qac*A were chosen for the study. For all MIC determinations, bacterial suspensions were prepared from overnight cultures in brain heart infusion (BHI) broth adjusted to a turbidity equivalent to that of a 0.5 McFarland standard.

7.2.1.2 Storage of Isolates

Isolates were sub-cultured onto BHI agar plates and incubated overnight at 37°C.

Single colonies were inoculated into BHI broth in an Eppendorf tube and incubated overnight. After addition of 20% glycerol to each tube, the isolates were stored at -80 ^{o}C.

7.2.1.3 Preparation of Stock Solutions

Chlorhexidine gluconate (CHG) and Benzalkonium Chloride (BC) (Sigma-Aldrich, St Louis, MO, USA) were used to prepare solutions for the reference agar dilution test (AD), and SGE technique. The antiseptics were diluted with milli-Q water and the solutions sterilized by filtration (Opticap XL 10 Capsule Filterk, Millipore). The formula below was used to calculate the weight of the antiseptic in the stock solution:

$$1000 \times V \times \frac{C}{P} = Wt$$

P=potency given by the manufacturer.

V=Volume in ml required

C=Final concentration (multiples of 1000)

Wt= Weight of antiseptic in mg

The antiseptic concentrations required for SGE to produce the exponential concentration gradients (1–8 mg/L and 8–64 mg/L) were calculated using SGE software (Spiral Biotech). Stock solutions of 1072 mg/L (low range) and 8576 mg/L (high range) were prepared and aliquots stored at -80 ^{o}C. The working solutions

were was freshly prepared each time and used within 24 hours. The weight of the antiseptics for stock solutions of 1000 mg/L for AD were calculated using the equation above and the concentration range for AD was 1 to 128 mg/L. The stock concentrations should include the range of MICs expected and MIC breakpoint concentrations as well as the known MICs of control strains.

7.2.1.4 MIC Determination by Spiral Gradient Endpoint Method

SGE uses a spiral plater to deposit biocides on the agar surface in a decreasing concentration gradient, providing an antimicrobial concentration gradient on the agar to determine MIC. Mueller Hinton agar (MHA) (Oxoid, Basingstoke, UK) was prepared following the instructions of the manufacturer. Agar plates of exactly 20 ml were prepared and dispensed using the Mediaclave and Technomate line (INTEGRA Biosciences Holdings, Switzerland). It is important that plates are poured on a completely level surface.

Using a spiral plater (Auto plate 4000, Spiral Biotech) 50 µL of the stock solution was deposited onto a 10 cm agar plate containing 20 mL agar in a spiral pattern to produce an exponential concentration gradient. The antiseptic solution (BC or CHG) was applied to a distance of 13 mm from the centre of the MHA, using the exponential disposition mode. The plates were air dried for one hour at room temperature under a laminar flow hood. After one hour, suspensions of three strains equivalent to a 0.5 McFarland turbidity standard were swabbed radially in

duplicate on each plate across the spiral using a template as a guideline, thereby exposing the organisms to the full concentration gradient. The swab with culture must not be streaked in the opposite direction as this could lead to carry over of antiseptic from high concentration to low concentration. The conditions used had been optimized previously by Pong et al. (2010).

Following inoculation, the plates were incubated overnight at 37 ^{o}C. The plates were then examined and the distance from the centre of the plate to the commencement of growth was measured. The radius at which all growth ends is termed a ‘‘trailing endpoint concentration’’ (TEC). This represents the concentration at the maximum extent of growth of outlying colonies beyond the end of confluent growth and represents the MIC of reduced susceptibility subpopulation if this is present. The point at which a heavy, confluent line of growth became less dense (a marked change from confluent growth) is termed as ‘‘endpoint concentration’’ (EC). EC is representative of the MIC of the majority of the population and should be equivalent to the concentration determined by the standard dilution method. The radial measurements were entered into a software program provided by the manufacturer, using the molecular weights and diffusion characteristics of the antimicrobial agent, to calculate the corresponding concentrations at the TEC and EC. The MIC was determined from the low-range plate unless the endpoint was beyond 8 mg/L.

7.2.1.5 MIC Determination by Agar Dilution Method

The MICs were determined by the use of the Clinical Laboratory Standards Institute (2009) recommended method for AD. A stock solution of 1000mg for AD was calculated using equation in section 7.2.1.2 and the concentration range for reference tests was 1 to 128 mg/L in $\log_2$ dilution concentration increments. A calculated volume of stock solution was added to sterile MHA media at 50-60 ^{0}C to give a volume of 20 mL, then poured into 10 cm Petri dishes to achieve a series of concentrations for standard agar dilution.

0.5 McFarland bacterial suspensions were dispensed onto the MHA plates containing CHG or BC prepared as described above using a multi-point inoculator (Mast Co Ltd, Bootle, UK). The plates were incubated at 37 ^{o}C for 24 h. The test was performed in duplicate. The MIC determinations were based on the presence of growth of bacteria on the screening plates. MICs were determined as the lowest concentration inhibiting growth.

7.2.2 Reproducibility Study of SGE

Five *S. aureus* isolates carrying *qac*A, five with s*mr* gene, and five control strains (1 ACTT 25923, and four *S. aureus* without QAC genes), were used for the intra-batch and inter-batch reproducibility study. In order to assess the validity and reproducibility of SGE, an intra-batch reproducibility study was performed on a

single occasion using 36 replicates of single isolates of selected strains. SGE tests were performed on six plates, and the test was repeated six times on each plate.

An inter-batch reproducibility study was performed on six separate occasions by SGE using single isolates of selected strains. Each time, SGE was repeated six times on one plate. The mean MIC was calculated. A control strain with known MIC was included in the test for each batch of determination.

To observe the inter-observer reproducibility, the results of the fifteen strains used in the accuracy study were interpreted by three observers (one experienced and two inexperienced in using SGE method.

7.2.3 Evaluation of SGE

MICs were determined for the original 15 strains and the 15 induced variants after 14 days CHG exposure (for CHG) and 15 variants before and after BC exposure (for BC). SGE technique was performed as described above. MIC for each strain was performed in duplicate. Both the endpoint concentration (EC) and the trailing endpoint concentration (TEC) were determined. MICs by SGE were compared with those determined by AD. To allow comparison between a categorical and gradient method, SGE results were rounded up to the nearest AD dilution.

7.2.4 Statistical Analysis

Mean, Standard Deviation (SD) and Coefficient of Variance (CV) were determined for the three parameters of the reproducibility study. In order to determine the degree of agreement between AD and SGE, statistical analysis was performed on the results as described below and agreement between the two methods was assessed by preparation of a Bland and Altman plot.

To determine the degree of agreement between AD and SGE tests, the distribution of MIC results among the strains was examined. Regression analysis was carried out and Pearson correlation coefficient was calculated to measure the overall association between the log_2 dilution MIC results of the SGE method and the AD method.

To determine whether there was a trend for the SGE method to produce significantly higher or lower results than the AD method, Wilcoxon signed rank test was performed on the difference of log_2 MIC of the SGE test results compared with AD test. MIC results within log_2 dilution were assumed to be equivalent for this test.

7.3 Results

7.3.1 Reproducibility Study

CHG: The results of intra-batch, inter-batch and inter-observer reproducibility are shown in Tables 7.1, 7.2, 7.3, and 7.4. For interbatch variability the SDs for

individual strains ranged from 0 – 1.63 and the CV from 0 – 0.387 with means of 0.41 and 0.15 respectively. The results were similar for Intrabatch variability with a mean SD of 0.20 (range of 0 – 1.63) and mean CV of 0.07 (range 0 – 0.35). Inter-observer variation yielded a mean SD of 0.71 (range 0 – 4.62) and CV of 0.09 (range 0 – 0.43). The disagreements in readings were limited to one strain and involved only one log_2 dilution. This level of agreement is within the limits for dilution methods.

BC: The results of intra-batch, inter-batch and inter-observer reproducibility are shown in Tables 7.5, 7.6, 7.7, and 7.8. For interbatch variability the SDs for individual strains ranged from 0 – 1.63 and the CV from 0 – 0.35 with means of 0.27 and 0.038 respectively. The results were similar for Intrabatch variability with a mean SD of 0.27 (range of 0 – 1.63) and mean CV of 0.06 (range 0 – 0.35). Inter-observer variation yielded a mean SD of 0.62 (range 0 – 9.24) and CV of 0.04 (range 0 – 0.43). The disagreements in readings were limited to four strains and involved only one log_2 dilution. This level of agreement is within the limits for dilution methods.

7.3.2 Evaluation of SGE for Determination of MICs

CHG: Comparison of MICs obtained by SGE showed that 93.3% of test strains matched the MIC obtained by AD. There was 100% agreement for results of both methods for all strains except for *qac*A positive strains before induction, for which

there was 60% agreement. Thus overall there were only 6.7% minor errors (within 1 $\log_2$ dilution) in determination of the MIC of CHG by SGE method. The discrepancy represented a difference of only one dilution (Table 7.9 and 7.10).

Wilcoxon signed-rank test showed that there was no significant trend for higher or lower MIC values if SGE method was used (p=0.82).

Regression analysis and Pearson correlation coefficients were used to measure the overall association between the SGE method and AD. R^2 was found to be 0.99. The regression coefficient of SGE MIC was 0.995 (SE = 0.018, p<0.001), showing that SGE MIC is statistically associated with AD MIC and is likely to be a predictor of the true MIC. The regression equation could be written as expected dilution MIC=1.02 + 0.995 SGE MIC.

The Bland and Altman plot (Fig 7.1) showed good agreement of MIC obtained by both AD and SGE as mean of difference was 0.13 and SD of difference (S) was 0.51, with only one point outline the d $\pm$ 2S lines.

BC: Comparison of MICs obtained by SGE showed that 87% of test strains matched the MIC obtained by AD. There was 100% agreement for results of both methods for all strains except for 80% agreement for control strains before induction, 80% agreement for *qac*A positive strains after induction and 60% agreement for *smr* positive strain before induction. Thus overall there were 13% errors (within 1 $\log_2$

dilution) in determination of the MIC of BC by SGE method. The discrepancy represented a difference of only one dilution (Table 7.11 and 7.12).

Wilcoxon signed-rank test showed that there was no significant trend for higher or lower MIC values if SGE method was used (p = 0.68).

Regression analysis and Pearson correlation coefficients were used to measure the overall association between the SGE method and AD. R^2 was found to be 0.87. The regression coefficient of SGE MIC was 0.995 (SE = 0.113, p<0.001), showing that SGE MIC is statistically associated with AD MIC and is likely to be a predictor of the true MIC. The regression equation could be written as expected dilution MIC=1.047 + 0.87 SGE MIC.

The Bland and Altman plot (Fig 7.2) showed good agreement of MIC obtained by both AD and SGE as mean of difference for 0.87 and SD of difference for 3.15, without points outlining the d $\pm$ 2S lines.

7.4 Discussion

Biocides are widely used in the both hospitals and the community as an intervention for reducing the spread of infection. There is increasing concern that increased use of biocides may contribute to the emergence and/or selection of resistant pathogens. Compared to our understanding of antibiotic resistance, bacterial resistance against different types of biocides has received less attention.

The MICs of bacteria to biocides may be determined by agar or broth dilution methods, which continue to be the gold standards for MIC determination (CLSI, 2009). Susceptibility to antiseptics is commonly tested using phenotypic and MIC- or MBC-based methods. However, the results of phenotypic susceptibility testing are strongly affected by the methodology adapted and differences between techniques used makes inter-study comparisons difficult. In addition, there are no internationally consistent breakpoint values for biocide susceptibility testing. Thus, the method to determine MIC of antiseptics has not been standardized (Horner et al., 2012a).

Both broth and agar dilution methods are technically demanding, time consuming, labour intensive, and expensive. They both have the disadvantage of large increments at higher concentrations with high standard allowable error. Broth dilution endpoints can be difficult to determine and lead to ambiguous results. Therefore a rapid, accurate and cost-effective determination tool is urgently needed (Reller et al., 2009). This study evaluated the SGE method, to determine if it could provide accurate and reliable results. For antibiotics, precise estimation of MIC can be obtained by E-test, but this methodology is not available for antiseptic agents. A previous evaluation study of SGE for non-fastidious organisms reported good correlation (0.86–0.96) and percentage agreement (46%–88% within one dilution) for MIC determined by SGE compared with broth dilution (Paton et al., 1990). Pong et al. (2010) in an evaluation of SGE for fastidious organisms, showed SGE MICs were in 95–100% agreement within one dilution and had good susceptibility

category agreement with broth dilution between tests for all organisms and antibiotics compared, with excellent correlation with the reference method (0.908–0.930). Similarly, Doddangoudar et al. (2010) showed low SD and CV values and good reproducibility and accuracy for vancomycin MIC determination for *S. aureus.*

In the current study, the results showed that the SGE technique is a precise and accurate method for MIC measurement of *S. aureus* to antiseptics. Comparison of the results of SGE tests with the gold standard AD showed that the association between the two methods was excellent, with good Pearson correlation coefficient. There was no tendency for SGE to produce higher or lower MICs than the reference method. Reproducibility of the SGE MIC was in excellent agreement with AD as demonstrated by the Bland and Altman plot. All the results met the requirement of the Food and Drug Administration (FDA) and the International Organization for Standardization for Evaluation of the Performance of Antimicrobial Susceptibility Test Devices for Susceptibility Test Systems (US FDA, 2003; International Organization for Standardization 2007).

Our study indicated that the SGE provides a suitable alternative method for rapid and accurate detection of strains with reduced biocide susceptibility with good correlation of MICs with those determined by AD method.

SGE is easy to perform in the routine laboratory, and although SGE methods need

an initial investment for purchasing a spiral plater for laboratories, this cost could be recovered in short period because of the cost-effective advantage of SGE compared with other methods. SGE costs six Hong Kong Dollars per plate and three isolates can be tested on one plate. The SGE method only requires antiseptic stock solution and the test could be carried out at any time. SGE is a suitable alternative to determine antiseptic MICs, which allows for more surveillance of antiseptic MICs to monitor both overall resistance and MIC increments. In addition to the flexibility of medium and incubation conditions, the use of a gradient allows a more accurate MIC determination especially at higher concentrations. Gradient methods increase the capability to see small increments in susceptibilities and resistance, in comparsion to dilution methods which suffer from large increments as concentration increases and cannot allow observation of small er changes in susceptibility.

In summary, the development of a standardized method for the detection of reduced susceptibility and/or resistance to in-use concentrations of antiseptics is urgently needed. Our study showed SGE method offers an accurate, reliable, economical and time-effective method for determination of *S. aureus* susceptibility to CHG and BC as an alternative to AD. It is necessary to carry out further large scale of evaluations for SGE and to evaluate the method for other kinds of bacteria and different biocides.

Table 7.1 Intra-Batch reproducibility for CHG MIC (mg/L) by SGE

Control strain	R1	R2	R3	R4	R5	R6	Mean	SD	CV
1 (ATCC)	2	2	2	2	2	2	2	0	0
2	2	2	2	2	2	2	2	0	0
3	2	2	2	2	2	2	2	0	0
4	2	2	2	2	2	2	2	0	0
5	2	2	2	2	2	2	2	0	0
qacA									
1	4	4	4	4	4	4	4	0	0
2	4	4	4	4	4	4	4	0	0
3	4	4	4	4	4	4	4	0	0
4	4	4	4	4	4	8	4.67	1.63	0.35
5	4	4	4	4	4	4	4	0	0
smr									
1	2	2	2	2	4	2	2.33	0.82	0.35
2	2	2	2	2	2	2	2	0	0
3	2	2	2	4	2	2	2.33	0.82	0.35
4	2	2	2	2	2	2	2	0	0
5	2	2	2	2	2	2	2	0	0
Total Mean SD=0.20; Total Mean CV=0.07									

R: replicates

Table 7.2 Inter-batch variation for determination of MIC (mg/L) by SGE for CHG

Control strain	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Mean	SD	CV
1(ATCC)	2	2	2	2	2	2	2	0	0
2	2	2	2	2	2	2	2	0	0
3	2	2	2	2	2	2	2	0	0
4	2	2	2	2	2	2	2	0	0
5	2	2	2	2	2	2	2	0	0
qacA									
1	4	4	4	4	4	4	4	0	0
2	4	4	4	4	4	4	4	0	0
3	4	4	4	4	4	4	4	0	0
4	4	4	4	4	4	8	4.67	1.63	0.35
5	4	4	4	4	4	4	4	0	0
smr									
1	2	2	2	2	2	2	2.67	1.03	0.387
2	2	2	2	2	2	2	2.33	0.82	0.35
3	2	2	2	4	2	2	2.33	0.82	0.35
4	2	2	2	2	2	4	2.33	0.82	0.35
5	2	2	2	4	2	4	2.67	1.03	0.387
Total Mean SD=0.41; Total Mean CV=0.15									

Table 7.3 Inter-Observer Data for CHG (mg/L)

Strain	Inexperienced observer		Experienced observer	Inter		
	1	2	1	Mean	SD	CV
a1-p0	4	4	4	4	0	0
a2-p0	4	4	4	4	0	0
a3-p0	2	4	4	3.33	1.15	0.35
a4-p0	4	4	4	4	0	0
a5-p0	4	4	4	4	0	0
a1-p2	16	8	16	13.33	4.62	0.35
a2-p2	16	16	16	16	0	0
a3-p2	16	16	16	16	0	0
a4-p2	16	16	16	16	0	0
a5-p2	8	16	16	13.33	4.62	0.35
c1-p0	2	2	2	2	0	0
c2-p0	2	2	2	2	0	0
c3-p0	2	2	2	2	0	0
c4-p0	2	2	2	2	0	0
c5-p0	8	8	8	8	0	0
c1-p2	8	4	8	6.67	2.31	0.35
c2 p2	8	8	8	8	0	0
c3-p2	8	8	8	8	0	0
c4-p2	8	8	8	8	0	0
c5-p2	8	8	8	8	0	0
n1-p0	1	2	2	1.67	0.56	0.33
n2-p0	2	2	2	1	0	0
n3-p0	2	4	2	2.67	1.15	0.43
n4-p0	2	2	2	2	0	0
n5-p0	2	2	2	2	0	0
n1-p2	8	8	8	8	0	0
n2-p2	8	16	8	10.67	4.62	0.43
n3-p2	8	8	8	8	0	0
n4-p2	4	8	8	6.67	2.31	0.35
n5-p2	8	8	8	8	0	0

a1-a5: only *qac*A positive strains; c1-c5: only *smr* positive strains; n1:ATCC 25923 n2-n5: clinical strain without *qac* gene; p0 = parent non-induced strain; p2 = after 12 days induction

Table 7.4 Summary of reproducibility data for determination of MICs of *S. aureus* to chlorhexidine by SGE method

Reproducibility	SD range	Mean SD	CV range (%)	Mean CV (%)
Intra-batch	0 - 1.63	0.20	0 - 35	7
Inter-batch	0 - 1.63	0.41	0 - 38.7	15
Inter-observer	0 - 4.62	0.71	0 - 43	9

Table 7.5 Intra-Batch reproducibility for BC MIC (mg/L) by SGE

Control strain	R1	R2	R3	R4	R5	R6	Mean	SD	CV
1(ATCC)	2	2	2	4	2	2	2.33	0.82	0.35
2	4	4	4	4	4	4	4	0	0
3	4	4	4	4	4	4	4	0	0
4	4	4	4	8	4	4	4.67	1.63	0.35
5	4	4	4	4	4	4	4	0	0
qacA									
1	8	8	8	8	8	8	8	0	0
2	8	8	8	8	8	8	8	0	0
3	8	8	8	8	8	8	8	0	0
4	8	8	8	8	8	8	8	0	0
5	8	8	8	8	8	8	8	0	0
smr									
1	8	8	8	8	8	8	8	0	0
2	8	8	8	8	8	8	8	0	0
3	8	4	8	8	8	8	7.33	1.63	0.22
4	8	8	8	8	8	8	8	0	0
5	8	8	8	8	8	8	8	0	0
Total Mean SD=0.27; Total Mean CV=0.06									

R: replicates

Table 7.6 Inter-batch variation for determination of MIC (mg/L) by SGE for BC

Control strain	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Mean	SD	CV
1(ATCC)	2	2	2	2	4	2	2.33	0.82	0.35
2	4	4	4	4	4	4	4	0	0
3	4	4	4	4	4	4	4	0	0
4	4	4	4	4	4	4	4	0	0
5	4	4	4	4	4	4	4	0	0
qacA									
1	8	8	8	8	8	8	8	0	0
2	8	8	8	8	8	8	8	0	0
3	8	8	8	8	8	4	7.33	1.63	0.22
4	8	8	8	8	8	8	8	0	0
5	8	8	8	8	8	8	8	0	0
smr									
1	8	8	8	8	8	8	8	0	0
2	8	8	8	8	8	8	8	0	0
3	8	8	8	8	8	8	8	0	0
4	8	8	4	8	8	8	7.33	1.63	0.22
5	8	8	8	8	8	8	8	0	0
Total Mean SD=0.27; Total Mean CV=0.038									

Table 7.7 Inter-Observer Data for BC (mg/L)

Strain	Inexperienced observer		Experienced observer	Inter		
	1	2	1	Mean	SD	CV
a1-p0	8	8	8	8	0	0
a2-p0	8	8	8	8	0	0
a3-p0	8	8	8	8	0	0
a4-p0	8	8	8	8	0	0
a5-p0	8	8	8	8	0	0
a1-p2	16	16	16	16	0	0
a2-p2	16	32	16	21.33	9.24	0.43
a3-p2	16	16	16	16	0	0
a4-p2	16	16	16	16	0	0
a5-p2	32	32	32	32	0	0
c1-p0	8	8	8	8	0	0
c2-p0	8	8	8	8	0	0
c3-p0	8	8	8	8	0	0
c4-p0	8	16	8	10.67	4.62	0.43
c5-p0	8	8	8	8	0	0
c1-p2	16	16	16	16	0	0
c2-p2	16	16	16	16	0	0
c3-p2	16	16	16	16	0	0
c4-p2	16	16	16	16	0	0
c5-p2	16	16	16	16	0	0
n1-p0	2	2	2	2	0	0
n2-p0	4	4	4	4	0	0
n3-p0	4	4	4	4	0	0
n4-p0	4	4	4	4	0	0
n5-p0	4	4	4	4	0	0
n1-p2	16	8	8	10.67	4.62	0.43
n2-p2	8	8	8	8	0	0
n3-p2	8	8	8	8	0	0
n4-p2	8	8	8	8	0	0
n5-p2	16	16	16	16	0	0

a1-a5: only *qac*A positive strains; c1-c5: only *smr* positive strains n1:ATCC25923; n2-n5: clinical strain without *qac* gene; p0 = parent non-induced strain; p2 = after 12 days induction

Table 7.8 Summary of reproducibility data for determination of MICs of *S. aureus* to BC by SGE method

Reproducibility	SD range	Mean SD	CV range (%)	Mean CV (%)
Intra-batch	0-1.63	0.27	0-35	6
Inter-batch	0-1.63	0.27	0-35	3.8
Inter-observer	0-9.24	0.62	0-43	4

Table 7.9 Determination of CHG MIC (mg/L) by AD and SGE

Strain	SGE	AD
a1-p0	4	2
a2-p0	4	2
a3-p0	4	4
a4-p0	4	4
a5-p0	4	4
a1-p2	16	16
a2-p2	16	16
a3-p2	16	16
a4-p2	16	16
a5-p2	16	16
c1-p0	2	2
c2-p0	2	2
c3-p0	2	2
c4-p0	2	2
c5-p0	2	2
c1-p2	8	8
c2-p2	8	8
c3-p2	8	8
c4-p2	8	8
c5-p2	8	8
n1-p0	2	2
n2-p0	2	2
n3-p0	2	2
n4-p0	2	2
n5-p0	2	2
n1-p2	8	8
n2-p2	8	8
n3-p2	8	8
n4-p2	8	8
n5-p2	8	8

a1-a5: only *qac*A positive strains; c1-c5: only *smr* positive strains

n1: ATCC25923; n2-n5: clinical strain without QAC gene;

p0 – before induction; p2 – after induction

Mean of Difference (d) = 0.13; SD of Difference (S) = 0.51

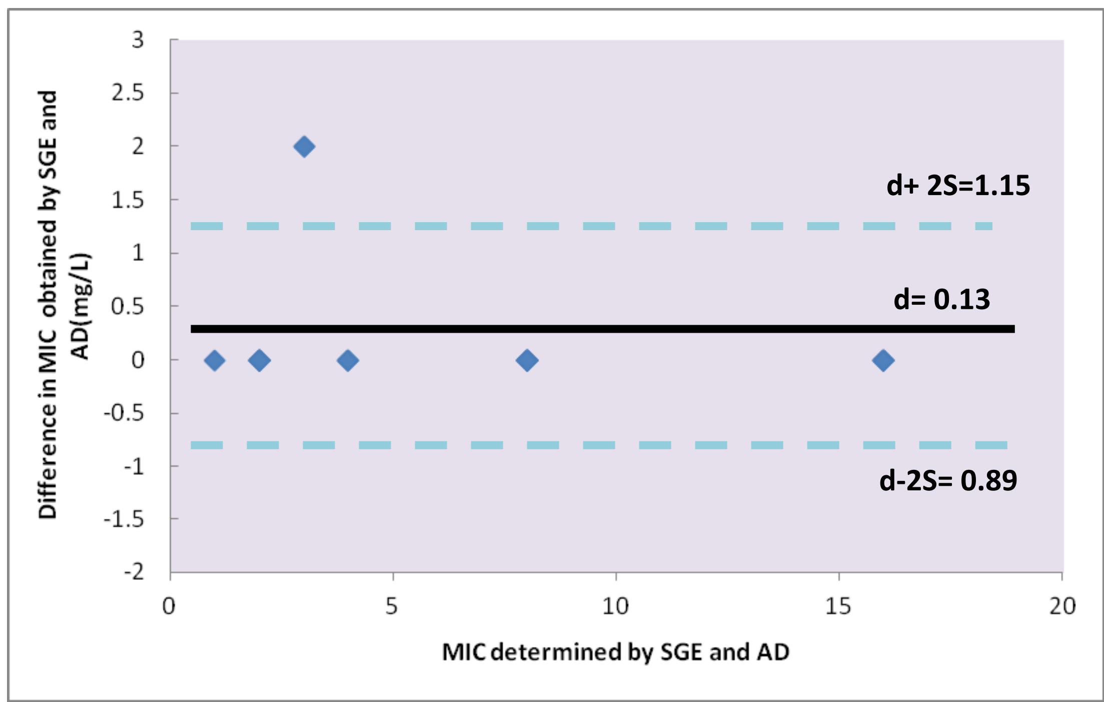


Fig7.1 Bland and Altman Plot of evaluation of MIC Values of SGE with respect to AD for CHG

Each point shown on the plot may represent several determinations (all 30 isolates are included).

Table 7.10 Comparison of MICs for CHG and agreement between sensitivity categories obtained by SGE and AD on MH agar

Dilution test sensitivity category	Difference in MIC for CHG(SGE MIC vs. AD MIC) (% agreement within)						
	>-2	-2	-1	0	1	2	Number within 1 dilution
Control strains				5 (100)			5 (100)
control strains induced by CHG				5 (100)			5 (100)
*qac*A strain				3 (60)	2 (40)		5 (100)
*qac*A strain induced by CHG				5 (100)			5 (100)
smr strain				5 (100)			5 (100)
smr strain induced by CHG				5 (100)			5 (100)
Total				28 (93.3)	2 (6.7)		30 (100)

Table 7.11 Determination of BC MIC (mg/L) by AD and SGE

Strain	SGE	AD
a1-p0	8	8
a2-p0	8	8
a3-p0	8	8
a4-p0	8	8
a5-p0	8	8
a1-p2	16	16
a2-p2	16	16
a3-p2	16	16
a4-p2	16	16
a5-p2	32	16
c1-p0	8	4
c2-p0	8	4
c3-p0	8	8
c4-p0	8	8
c5-p0	8	8
c1-p2	16	16
c2-p2	16	16
c3-p2	16	16
c4-p2	16	16
c5-p2	16	16
n1-p0	2	2
n2-p0	4	4
n3-p0	4	4
n4-p0	4	2
n5-p0	4	4
n1-p2	8	8
n2-p2	8	8
n3-p2	8	8
n4-p2	8	8
n5-p2	16	16

a1-a5: only *qac*A positive strains; c1-c5: only *smr* positive strains;

n1: ATCC25923; n2-n5: clinical strain without QAC gene;

p0 – before induction; p2 – after induction

Mean of Difference (d) = 0.87; SD of Difference (S) = 3.15

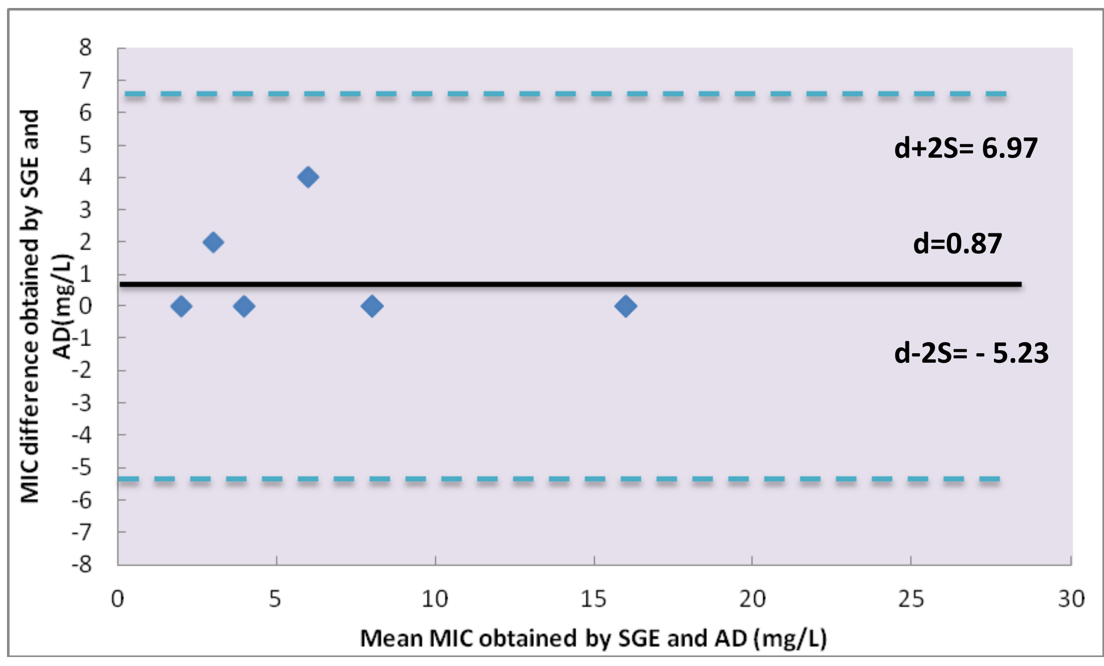


Fig 7.2 Bland and Altman Plot of evaluation of MIC Values of SGE with respect to AD for BC

Each point shown on the plot may represent several determinations (all 30 isolates are included).

Table 7.12 Comparison of MICs for BC and agreement between sensitivity categories obtained by SGE and AD on MH agar

Dilution test sensitivity category	Difference in MIC for BC(SGE MIC vs. AD MIC) (% agreement within)						
	>-2	-2	-1	0	1	2	Number within 1 dilution
Control strains				5 (100)			5 (100)
control strains induced by BC				4 (80)	1 (20)		5 (100)
*qac*A strain				5 (100)			5 (100)
*qac*A strain induced by BC				4 (80)	1 (20)		5 (100)
smr strain				3 (60)	2 (40)		5 (100)
smr strain induced by BC				5 (100)			5 (100)
Total				26 (87)	4 (13)		30 (100)

CHAPTER 8

EFFECT OF PROLONGED EXPOSURE TO SUB-INHIBITORY CONCENTRATIONS OF ANTISEPTICS ON MINIMUM INHIBITORY CONCENTRATIONS OF *S. AUREUS* STRAINS

8.1 Introduction

It has been widely suggested that exposure to biocides may increase tolerance of bacteria to these agents. There is concern that increased use of biocides contributes to the emergence and/or selection of pathogens. Residues of biocides could provide low level exposure, leading to reduced susceptibilities to biocides (Suller & Russell., 1999; Smith et al., 2008; Vali et al., 2008).

In many bacteria, efflux pumps expressed at basal levels, confer low-level protection and enable the bacteria to survive exposure to sub-clinical levels of antimicrobial agents. The wide spectrum of substrates recognized by efflux systems has prompted concern that exposure of a bacterium to one substrate could select for over-expression of an efflux system and consequent resistance to other substrates. It is possible that exposure of a bacterium to a biocide could result in

selection of an efflux mutant which has reduced susceptibility to biocides and antibiotics (Suller et al 1999; Smith et al., 2008; Vali et al., 2008; Wang et al., 2008).

While numerous authors have postulated the relationship between low level antiseptic exposure and the development of antiseptic resistance, there is little evidence to support this theory in the literature. Most studies on the induction of antibiotic resistance have involved effects of antiseptic exposure of Gram negative organisms, especially *Pseudomonas aeruginosa* (Abdel-Malek et al, 2002; Fraud et al, 2008). It was shown that exposure to pyrithione biocides led to decreased susceptibility of *P. aeruginosa* to these agents and this resistance was irreversible in the absence of the agent (Abdel-Malek et al, 2002). There have been a number of studies involving staphylococcal isolates from the teats of dairy cattle that had been treated with antiseptics to prevent mastitis (Hogan & Smith, 1995; Behiry et al, 2012). In a recent study by Behiry et al, (2012), teat isolates were exposed to low concentrations of two antiseptics, nonoxinal-9 iodine complex and CHG. Although increased resistance to nonoxinal-9 were observed in *S. aureus* isolates passaged ten times in low concentrations of this agent, a similar result was only observed for one of the ten isolates exposed to CHG. This suggested that repeated exposure to sub-lethal levels of CHG did not lead to reduced susceptibility of these strains. This seems to contradict the hypothesis of low level biocide exposure leading to more resistance to these agents.

The aim of this study was to determine if exposure to sub-inhibitory levels of

antiseptic leads to an increase in MICs and MBCs of exposed strains of *S. aureus.* MICs were performed using SGE as this allows smaller increments in MICs to be observed which could be missed if a conventional dilution MIC method was employed.

8.2 Materials and Methods

8.2.2 Strains

Twenty clinical *S. aureus* isolates were randomly selected from the QAC gene positive isolates from a local district hospital used in Chapter 4. The 20 strains included five QAC gene negative strains (N1: *S. aureu*s ATCC 25923; N2-N5), twelve clinical *S. aureus* isolates carrying *qac*A (A1-A12) and five clinical *S. aureus* isolates with *smr* (C1-C5). The initial CHG MICs and BC MICs were determined by SGE methods as described in Chapter 7.

8.2.3 Preparation of Antiseptic Stock Solutions

Stock solutions of CHG and BC (Sigma-Aldrich, St Louis, MO, USA) were prepared as described in Chapter 7.

8.2.4 Induction Study

In order to determine if continued selection pressure from presence of CHG or BC would lead to further increases in CHG or BC MICs, respectively, all isolates were incubated in BHI broths supplemented with a CHG or BC concentration equivalent to 50% of the initial MIC at 37 ^{o}C for 48 h. The strains were transferred to fresh

media every 48 h and MIC determination was performed by SGE every 6 days. If the MIC increased, the CHG/BC concentration of the next passage was increased to the newly determined 50% MIC. This process was carried out for a period of 24 days.

8.2.5 Determination of the Stability of Elevated MICs

The stability study was carried out for the strains exposed to CHG or BC. The stability of MICs was determined by further incubation of the induced strains in antiseptic free broth at 37 ^{o}C. Strains were transferred to fresh medium at 48 h intervals. The MICs were determined by SGE after two weeks.

8.3 Results

CHG: Before induction, all Group A isolate had a CHG MIC of 4 mg/L, except A12 with initial MIC 2 mg/L. All Group C and Group N isolates had an initial CHG MIC of 2 mg/L. Over the period of CHG exposure, the MICs of A1- A12 strains rapidly increased reaching 32 mg/L by day 21 and remained unchanged until day 24. With two exceptions, the MICs of all Group C and N isolates reached a maximum CHG MIC of 16 mg/L. One isolate from each group (C1 and N4) reached a CHG MIC of 32 mg/L (Table 8.1). During the stability phase of the study, it was observed that by the end of the two week period all isolates had reverted to their initial MIC. Patterns of development and loss of non-susceptibility were observed and appeared to be similar for all isolates (Table 8.1 & Figure 8.1).

BC: all *qac*A positive isolates except A12 and all *smr* positive isolates had initial MIC

to BC of 8 mg/L, while A12 and all control isolates (C) initial MIC was 4 mg/L except one control (ATCC) was 2 mg/L. Following induction, the MIC of all *qac*A and *smr* positive isolates reached 32 mg/L while control strains increased more slowly reaching a maximum of 16 mg/L. Following passage in BC-free broth, all isolates reverted to their pre-induction MIC levels within 12 days (Table 8.2 & Figure 8.2).

8.4 Discussion

In this study, the changes in MICs to antiseptics of *S. aureus* under selection pressure with CHG and BC were estimated using SGE, as this method of MIC determination can detect smaller changes. Using SGE allows the presence of any more resistance sub-populations of isolates to be observed, as occurs in resistance to vancomycin (Doddangoudar et al, 2010). However, there was no evidence of such sub-populations. *qac*A is commonly associated with reduced susceptibility to CHG and BC in staphylococci while *smr* gene confers resistance to certain biocides including BC but not CHG.

In staphylococci, reduced susceptibility to CHG is defined as an MIC ≥4 mg/L by Alam et al. (2003a), Wang et al. (2008) and Sheng et al.(2009). BC resistant or reduced suscepitiblity strains is defined as MIC >2 mg/L (Anthosisen et al., 2002; Sidhu et al., 2001; 2002) Sidhu et al. (2002) considered isolates with MIC between 3-8 mg/L to be BC resistant and BC sensitive strains to have an MIC<=2 mg/L. Nakaminami et al. (2010) reported that *qac*A and *qac*B variants in *S. aureus* strains RND1, which derived from RN4220 without *nor*A gene, showed different

susceptibility to BC, such as MIC of QacBIII variants to BC was 8 mg/L, while the mutants E320A with 4 mg/L.

In this study, the initial MICs of 11 of the 12 *qac*A positive parent strains (Day 0) were higher than for *smr* positive and QAC gene negative isolates. This supports the evidence that *qac*A mediates reduced susceptibility to CHG, as has been demonstrated by induction of *qac*A by the presence of CHG which was quantified by a luciferase assay (Smith et al, 2008). Both CHG and BC, as substrates, were found to induce the expression of the genes encoding the QacA efflux pumps in *S. aureus* (Smith et al, 2008). Expression in isolates with *qac* genes had significantly ($p < 0.0001$) higher MBCs for biocides containing QACs and CHG.

It is possible that the remaining *qac*A positive isolate with a lower MIC was not expressing phenotypic resistance to CHG. An altered QacA protein coded for by some *qac*A positive isolates has been described (Smith et al, 2008). This protein has an amino acid substitution resulting in an altered biocide profile. Nevertheless, all twelve isolates showed rapid increase in MIC following induction.

All *smr* positive and QAC gene negative controls gave initial CHG MICs of 2 mg/L. In the studies reported in Chapters 3 & 4, MIC determination using broth dilution method, found several strains carrying *smr* gene with MIC > 2 mg/L. This may be due to difficulties in interpretation of the broth dilution, but it is possible that these raised MICs in *smr* gene positive isolates may be due to the presence of base-pair

mutations that cause a change in substrate specificity or there may be another undetected mechanism of resistance present. Over-expression of *nor*A may produce a similar resistance phenotype and other researchers have reported *smr* positive CNS isolates exhibiting CHG MICs of 4 mg/L (Leelaporn et al., 1994).

All *qac*A positive isolates achieved an MIC 32 mg/L over 24 days of exposure to sub - inhibitory levels of CHG, reaching their maximum MICs within the induction period but rapidly reverting to susceptibility in the absence of CHG. Other workers have failed to observe an increase in resistance to CHG (Suller & Russell, 1999) or resistance increase in only some isolates (Behiry et al., 2012). The rapid loss of resistance in the absence of CHG was also noted for most strains in other studies (Behiry et al., 2012).

It was interesting to note that strains carrying *smr* and control strains also developed higher tolerance to CHG reaching MICs of 16 mg/L. This may represent presence of other unidentified efflux pumps in these strains which may be stimulated by the presence of CHG. All isolates returned to the initial MIC within two weeks in the absence of CHG after two weeks.

The development of reduced susceptibility in QAC negative strains may also indicate that these strains had acquired the QAC-resistant phenotype unrelated to changes in genes for efflux pumps. This phenotype could reflect some sort of general stress such as decreased cell wall permeability as has been described in

Mycobacteria (Svetlíková et al., 2009) or in vancomycin intermediately resistant staphylococci (Gould, 2010) or by means of a global resistance response (Grkovic et al., 2002; Hecker et al., 2007). The rapid return to susceptibility level may be attributed to the fitness cost required to maintain the thick cell wall associated with a reduced susceptibility. In addition, biocide resistance genes are part of tightly regulated virulence systems, and the wider effects of global regulatory systems as well as consistent genetic targets associated with cell wall biosynthesis and degradation may be involved in reduced susceptibility (DeMarco et al., 2007; Huet et al., 2008).

Results for BC induction were quite similar to those for CHG. Both *qac*A and *smr* positive isolates were induced to considerably higher MIC levels than the control strains. It was expected that both gene positive types would be tolerant to BC. Interestingly, A12 had a lower initial MIC to BC than other *qac*A positive isolates as was observed for CHG, strengthening the suspicion that gene expression is altered in this isolate. Once again MICs of control strains increase during the induction phase, indicating that other factors than the QAC genes may allow for increased tolerance. Following removal of the antiseptic selection pressure, all isolates reverted to their initial MIC values, showing again that stable antiseptic phenotypes are rare.

While this and other studies have clearly shown that it is possible for *S. aureus,* especially those carrying QAC genes, to develop biocide non-susceptibility,

formation of stable phenotype would appear to be rare. None of our isolates or those reported in most other studies retained resistance (Suller & Russell, 1999; 2000; Vali et al, 2008; Smith et al, 2008). One stable isolate was reported in isolates from dairy cattle (Behiry et al., 2012). It is possible that stable phenotypes may only develop after prolonged exposure such as regular use of an antiseptic as in dairy farming. There is a need to further investigate the factors associated with development of stable phenotypes.

In this study, the maximum MICs against CHG were 32 mg/L. However, in practice, much higher concentrations of disinfections are used (40,000 mg/L in 4% aqueous CHG solution) (Paul et al., 1988) and the increased tolerance seen in the induced isolates remains far below in-use levels. If biocides are used at concentrations recommended for use in the hospital environment, none of the clinical isolates would be able to withstand their presence. Although, information about the clinical impact of reduced susceptibility to CHG remains limited, recently studies have suggested that staphylococci exhibiting reduced susceptibility to CHG and/or the presence of CHG efflux-mediated resistance genes may be associated with clinical failure of the decolonization/treatment of staphylococci (Batra et al., 2010; Lee et al., 2011; Horner et al., 2013). Larger scales of screening studies are needed for further investigation considering the effect of residual concentrations of biocides encountered in the healthcare environment.

Although, findings from this study help to increase the understanding of biocide

non-susceptibility, establishment of long term monitoring of biocide non-susceptibility in clinical settings, further research into the relationship between the carriage of QAC and antiseptic residues, and phenotypic reduced CHG susceptibility are urgently needed.

Table 8.1 CHG MICs (mg/L) of *S. aureus* clinical isolates following induction and reversion

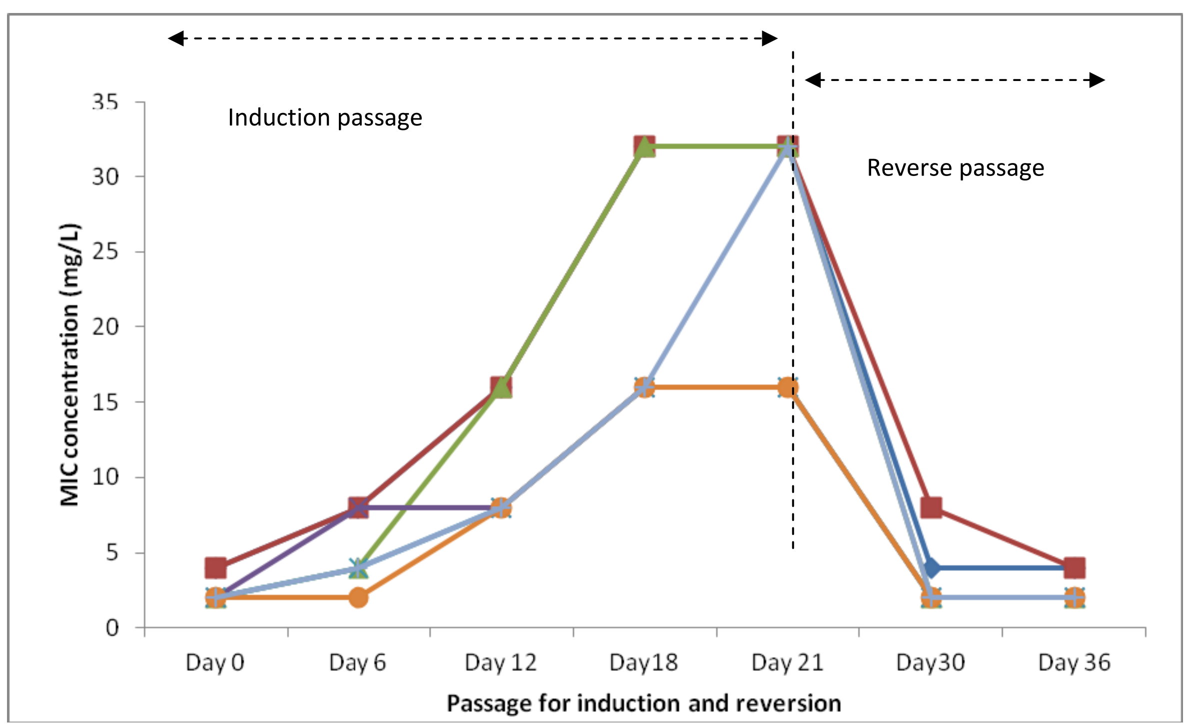
NO.	MICs in Induction passage (mg/L)					MICs in reverse passage (mg/L)	
	Day 0	Day 6	Day 12	Day18	Day 24	Day30	Day 36
A1	4	8	16	32	32	4	4
A2	4	8	16	32	32	4	4
A3	4	8	16	32	32	4	4
A4	4	8	16	32	32	4	4
A5	4	8	16	32	32	8	4
A6	4	8	16	32	32	4	4
A7	4	8	16	32	32	4	4
A8	4	8	16	32	32	4	4
A9	4	8	16	32	32	4	4
A10	4	8	16	32	32	4	4
A11	4	8	16	32	32	4	4
A12	2	4	16	32	32	2	2
C1	2	4	8	16	32	2	2
C2	2	4	8	16	16	2	2
C3	2	4	8	16	16	2	2
C4	2	8	8	16	16	2	2
C5	2	4	8	16	16	2	2
N1	2	2	8	16	16	2	2
N2	2	4	8	16	16	2	2
N3	2	4	8	16	16	2	2
N4	2	4	8	16	32	2	2
N5	2	4	8	16	16	2	2

A = *qac*A positive; C = *smr* positive; N = control strain (negative for *qac*A-J)

Table 8.2 MICs to BC of *S. aureus* clinical isolates following induction and reversion

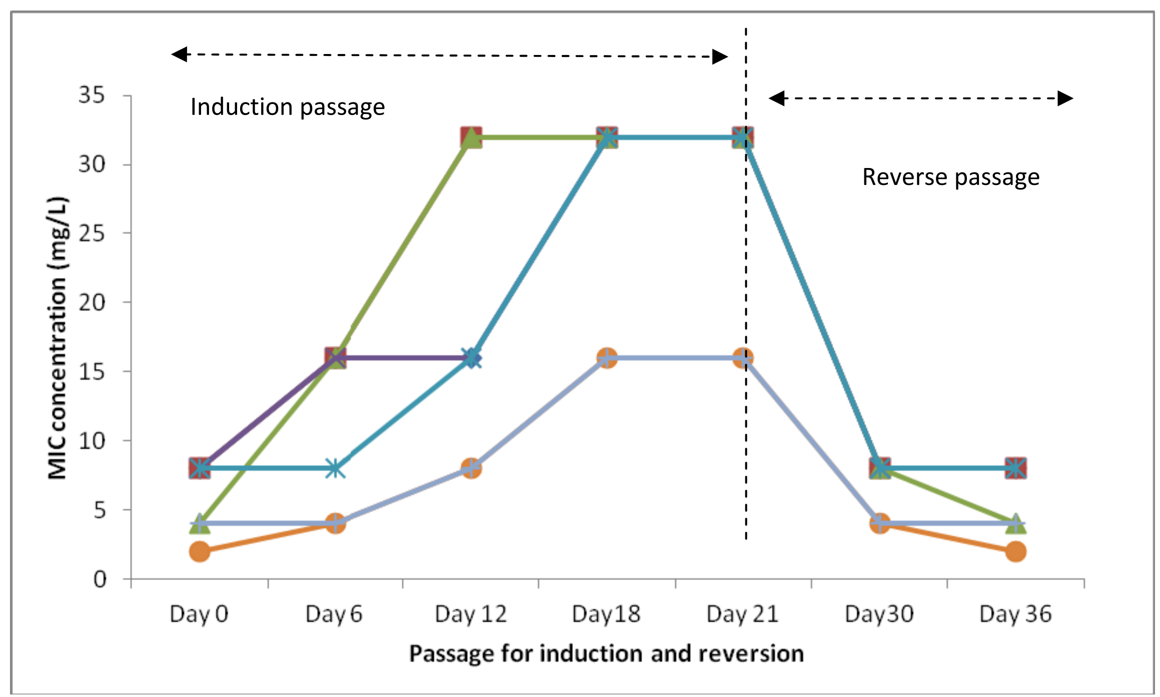
NO.	MICs in Induction passage (mg/L)					MICs in reverse passage (mg/L)	
	Day 0	Day 6	Day 12	Day18	Day 24	Day30	Day 36
A1	8	16	16	32	32	8	8
A2	8	16	16	32	32	8	8
A3	8	16	16	32	32	8	8
A4	8	16	16	32	32	8	8
A5	8	16	32	32	32	8	8
A6	8	16	16	32	32	8	8
A7	8	16	16	32	32	8	8
A8	8	16	16	32	32	8	8
A9	8	16	16	32	32	8	8
A10	8	16	16	32	32	8	8
A11	8	16	16	32	32	8	8
A12	4	16	32	32	32	8	4
C1	8	16	16	32	32	8	8
C2	8	16	16	32	32	8	8
C3	8	8	16	32	32	8	8
C4	8	8	16	32	32	8	8
C5	8	8	16	32	32	8	8
N1	2	4	8	16	16	4	2
N2	4	4	8	16	16	4	4
N3	4	4	8	16	16	4	4
N4	4	4	8	16	16	4	4
N5	4	4	8	16	16	4	4

A = *qac*A positive; C = *smr* positive; N = control strain (negative for *qac*A-J)



The diagram shows the development of increased CHG resistance and its rapid loss in four representative strains.

Fig 8.1 Development and loss of non-susceptibility to CHG in *S. aureus* exposed to increasing concentrations of CHG



The diagram shows the development of increased BC resistance and its rapid loss in four representative strains.

Fig 8.2. Development and loss of non-susceptibility to BC in *S. aureus* exposed to increasing concentrations of BC

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1 Introduction

The overall purpose of this research was to provide significant and original contributions to the knowledge base of QAC genotypes in *S. aureus* and CNS in selected populations and environments in Hong Kong, providing a framework for effective detection, antimicrobial selection in treatment of *S. aureus* and MRSA carrying QAC genes, as well as to improve clinical outcomes and infection control. This chapter summarizes the conclusion of the results of this research while providing relevant recommendations. According to the findings from this study, recommendations are identified and further, the potential for utilization and healthcare applications involved are also indicated. In addition, the limitations of this research work are identified and suggestions for further work are included.

9.2 Major Findings and Recommendations

This section reviews the main issues addressed in this research and summarizes the conclusions and recommendations.

9.2.1 QAC Gene Prevalence In Hong Kong

Widespread use of biocides may impose selective pressure and contribute to the emergence of bacteria with decreased antiseptic susceptibility. The use of biocides may contribute to the emergence of cross-resistance and co-resistance between widely used biocides and antibiotics (Koljalg et al., 2002; Walsh et al., 2003; Buffet-Bataillon et al., 2012). In this study, a series of cross-sectional studies were performed to determine the prevalence of *Staphylococcus* spp. carrying QAC genes in Hong Kong, and investigate risk factors for carriage of QAC genes in staphylococci.

The first prevalence study, with a control group consisting of the general population, concluded that nurses, especially those who had recent contact with MRSA positive patients, were a high risk population for carriage of QAC genes in staphylococci. Previous research focused on the prevalence in *S. aureus* and MRSA clinical isolates (Mayer et al., 2001; Noguchi et al., 2006; Smith et al., 2008; Wang et al., 2008a; Vali et al., 2008; Sheng et al., 2009; Longtin et al. 2011; Ho et al 2012a,b; Karki et al.,2012; Lepainteur et al., 2013) and few have determined QAC gene rates in carriage isolates, especially CNS isolates (Leelaporn et al., 1994; Anthonisen et al., 2002; Sidhu et al., 2002; Lepainteur et al. 2013) or colonizing strains from healthy subjects.

This study of carriage in nurses also suggested that the hospital environment may exert selective pressure for carriage of these strains, leading to further studies

investigating the prevalence of QAC genes in staphylococci in the hospital environment as well as in the community.

With this in mind, the second study involving investigation of the dissemination of staphylococcal isolates carrying QAC genes contaminating items in both public and hospital environments was performed. This study revealed that staphylococcal species harbouring QAC genes were widespread on ATMs and on several surfaces investigated at the selected hospital. ATM machines were chosen as a marker to represent the public environment, and it was concluded that such items in the environment could act as a common source in the community for dissemination of strains positive for QAC genes. To date, there were few reports about QAC gene prevalence in the community. Studies of *qac*G, H and J have revealed that these were mainly associated with food (Bjorland et al., 2005; Smith et al., 2008; Akinkunmi & Lamikanra, 2011; Ho et al., 2012b).

In the hospital environment, cultures obtained from items such as blood pressure cuffs revealed a high rate of *qac*A/B positive isolates, substantiating the evidence that the hospital environment can provide a survival advantage for QAC gene positive strains, as well as serve as a source for their dissemination. To date, there are few reports about QAC gene prevalence in *Staphylococcus* spp. isolated from the hospital environment.

In addition to revealing high rates of carriage of QAC genes, the results of both the

nurses' carriage study and the environmental contamination study showed there was a widespread prevalence of MRSA in Hong Kong hospitals. Analysis revealed that a high percentage of those strains containing QAC genes simultaneously harboured *mec*A. This association between presence of *mec*A and QAC genes especially *qac*A/B has been reported in several other studies (Smith et. al. 2008; Vali et. al., 2008). It has been suggested that selective pressure of low level antiseptic concentrations could favour the survival of MRSA in strains with both forms of resistance.

The third study investigated clinical isolates of *S. aureus* to determine the prevalence of QAC genes in local isolates from both BSI and SSI and compare these with reports elsewhere. The study confirmed that a high percentage (41.5%) of MRSA isolates in Hong Kong harboured *qac*A/B. This indicated that the *qac*A/B gene plasmid had been horizontally transmitted between the different MRSA clones and was wide spread in the Hong Kong clinical isolates. While this carriage rate appeared high, similar or even higher rates have been reported in MRSA isolates elsewhere, including Japan , China and Europe, where rates approaching 60% have been observed (Noguchi et al., 2005; Wang et al. 2008a; Vali et al., 2008), and reached 80% in Brazil (Miyazaki et al., 2007). Longtin et al. (2011) reported the MRSA from Canadian intensive care unites carried *qac*A/B (2%) and *smr* (7%) gene, respectively. Ho et al. (2012a) found that 45.2% MRSA isolated from the chlorhexidine-impregnated catheter related bloodstream infections harbored the *qac*A/B gene.

In the final cross-sectional study, the results indicated that the elderly appeared to be more likely to be colonized with *S. aureus* and that their strains are more likely to harbour QAC genes than those of the general public. However, there was a significant difference in both colonization rates and frequency of QAC gene presence between the elderly who were resident in nursing homes and those in the community, with nursing home residents being at higher risk. These care home residents were also more likely to be colonized with MRSA as has been shown in numerous studies (Chongtrakool et al., 2006; Eveillard et al., 2008; Ho et al., 2008b; Pfingsten-Würzburg et al., 2011). However, the presence of QAC genes has not previously been reported in carriage strains of the institutionalized elderly. Their colonization by MRSA carrying QAC genes suggests co-selection of these strains in these residents, allowing elderly care homes to act as a reservoir of QAC genes.

In all of these studies, the prevalence of the more recently described QAC genes, *qac*G, H, and J, was investigated. In all sets of isolates, carriage of these genes in both *S. aureus* and CNS remained low. These genes were first identified from food and animal sources (Bjorland et. al., 2005; Smith et.al. 2008) and appear not to have become widespread in human colonizing strains. Of those isolates positive for these genes, *qac*J was present more frequently in CA-MRSA than HA-MRSA, and may be spreading in the community. Although the *qac*J gene originated from animals and their surrounding environment, it now appears to be transferring into human isolates possibly via food. A similar scenario may apply to *qac*G which was identified in all groups of staphylococcal isolates except clinical isolates. Close monitoring of

the spread of these genes is necessary, especially as co-carriage of these genes with the well-recognised *qac*A/B and *smr* leads to elevated antiseptic MICs.

9.2.2 Relationship between Antibiotic Resistance and QAC genes in Staphylococci

There has been considerable discussion as to whether the presence of genes that encode for efflux-mediated resistance to CHG selects for antibiotic resistance genes (Berg et al., 1998; Sidhu et al., 2001b; 2002; Perez-Roth et al., 2010).

In each of our observations, there appeared to be strong associations between resistance to non-beta lactam antibiotics and the presence of QAC genes. Using the nurses' study as an example, a significant association was noted between isolates carrying the *qac*A/B and resistance to clindamycin, tetracycline, ciprofloxacin, and trimethoprim/sulphamethoxazole. As mentioned above, the association with methicillin resistance, described in other studies (Smith et al. 2008; Vali et al., 2008), was also seen in our isolates, but it was observed that the QAC genes were more common in multi-resistant MRSA strains.

Overall, our results suggest that co-selection of MRSA with QAC genes under selection pressure which may contribute to survival of MRSA. Use of antiseptics may be selecting for other antibiotic resistant strains and assisting their survival in the environment, especially for health care settings.

It is possible that there is also co-selection for other virulence genes. In the ATM study, enterotoxin SEB and QAC genes co-existed in some isolates, but there was no association between QAC genes and genes for other enterotoxins. It is possible there was co-selection for these genes, but it may just reflect selection for disinfectant resistant strains at the site.

9.2.3 Reduced Susceptibility to Biocides

Our study showed that strains harbouring QAC genes had significantly higher MICs than control and QAC negative strains. This elevation of the MIC was enhanced in isolates having two QAC genes, notably the presence of *qac*G with either *qac*A/B or *smr.* Such increase in MICs has been observed by other researchers (Suller & Russell, 1999; Smith et al., 2008; Vali et al., 2008, Ienardo et al., 2013). It is often remarked, that although there is an increase in the MIC due to the presence of the QAC genes, these increases may not be clinically significant as the elevated MICs are still well below the in-use values for the disinfectants most frequently tested, CHG and BC, and that therefore even QAC gene positive strains would be killed in the presence of in-use concentrations. However, actual concentrations in the environment may be considerably lower due to dilution effects caused by use of cloths, infrequent rinsing out of cloths and poor compliance with the correct use of disinfectants (Rutala & Weber, 2002; Karki & Cheng et al., 2012). Exposure to lower concentrations could allow for survival of strains with even a moderately increased MIC. In addition, some related agents are used at much lower concentrations such as QAC-like disinfectants in contact lens cleaning solutions (Penna et al., 2001). In

these situations survival of QAC gene positive strains may be of more significance.

Determination of an accurate MIC of antiseptic agents poses certain problems. There is no standardized methodology and researchers have largely adapted dilution methods used for antibiotics which may not be entirely suitable for antiseptics. In this study, a method previously used to determine concentration effects by means of gradient technique, SGE, was evaluated for determination of MICs. It appeared that SGE may offer an accurate, reliable, economical, and time-effective method for determination of *S. aureus* susceptibility to antiseptics as an alternative to dilution methods. However, further studies are needed in order to evaluate SGE for other bacterial species, different media, and different biocides in order to determine the scope of applicability of SGE for antiseptic MIC determination.

Our study of the effects of prolonged biocide exposure demonstrated that strains with increased tolerance to biocides could be selected for by repetitive passage in sub-inhibitory concentrations of biocides. This study helps to broaden the understanding of biocide non-susceptibility. An important feature reported by this and other studies investigating the effects of exposure to sub-inhibitory concentrations, is that removal of the stimulus almost always results in the reversion of the strains to MICs at the pre-exposure level. This at least demonstrates that strains that do develop enhanced resistance are not able to maintain this resistance in the absence of the stimulating antiseptic. This further

reinforces the importance for correct use of disinfectants following well-designed protocols.

9.2.4 MRSA Prevalence in Hong Kong

Although not a primary objective, this study also provided a current picture of MRSA colonization in specified populations in Hong Kong. It was somewhat surprising to find that the majority of the nurse MRSA isolates harboured SCC*mec* types associated with CA-MRSA as exposure to the hospital environment would lead to an expectation of mainly SCC*mec* III. The predominant SCC*mec* type seen in the clinical isolates remained SCC*mec* type III, although the prevalence of CA-MRSA was increasing. A similar predominance of CA-MRSA types was observed in the isolates from the elderly, the hospital surfaces, and ATMs. It is possible that CA-MRSA may survive better on surfaces than HA-MRSA allowing a higher chance to be transmitted to a site for colonization or infection. Nevertheless, the prevalence of MRSA in the general population and public environment still remains low. The rate in the general population seems to have changed little from a decade ago (O'Donoghue & Boost, 2004). Although the colonization rate was somewhat higher in nurses, this is still lower than those reported elsewhere (Wang et al., 2004).

9.3 Limitations and Scope for Further Studies

This study was able to successfully accomplish all the objectives described in Chapter 2, but mainly due to limited resources, did suffer from some limitations.

The prevalence rate of QAC genes among *S. aureus* and CNS colonizing nurses as well as from general population was determined and the results demonstrated that nurses may act as an important reservoir of QAC gene positive *Staphylococcus* spp. However, this study was somewhat limited as demographic information for the general population was not available. Therefore, information for antiseptic use from the general population was unknown although it is very likely that the nurses are exposed to more disinfectants than the general public in their daily life. Another limitation is that other information related to nurses is not included in this investigation such as working time and schedules, and working department which might lead to missing some risk factors for presence of QAC genes in staphylococci.

Nurses can act as a “lynchpin” between hospitals, long-term care facilities, nursing homes, and the community and act as reservoirs (Eveillard et al., 2004; Verrall et al. 2013). High rates of MRSA and MRCNS especially MRSE in healthcare workers were detected. CA-MRSA type was the predominant type in the MRSA isolates from nurses. Therefore, this study recommends long term monitoring of methicillin resistant *Staphylococcus* spp. and tracking the molecular type changes in MRSA and MRCNS.

This study also determined *Staphylococcus* spp. harbouring QAC genes in both the public and hospital environments, showed widespread dissemination of such strains. However, this study was limited to strains on the surfaces from one selected hospital and ATMs as the only site as a representative for the community

environment. A further large scale study including more sites in the community and sampling of a range of hospitals is needed to investigate the prevalence of the *Staphylococcus* spp. harbouring QAC genes in various environments. Further studies to evaluate the effectiveness of interventions to reduce contamination are needed using several potential inanimate objects as markers of contamination. Due to worries about transmission of infectious agents during disease outbreaks, the practice of regular wiping of surfaces sometimes incorporating use of a disinfecting agent is clearly meant to reduce levels of contamination. However, the presence of low levels of disinfectant may select for strains harbouring QAC genes allowing persistence in the environment. The effects of such practices should be evaluated though it is recognized that due to large numbers of variables including surface type, cleaning agent used, type of cloth, time between cleanings etc, this may present considerable difficulties.

Investigation of clinical isolates revealed that the main MRSA types in the investigated hospital was still SCC*mec* type III, although the prevalence of CA-MRSA was increasing and the main MRSA types colonizing nurses were CA-MRSA. This suffered the limitation of data being derived from only one hospital in Hong Kong, which may not be generalizable to the whole of Hong Kong. Studies at other hospitals are needed and change of predominance of HA-MRSA to CA-MRSA carefully monitored. Differences in survival in the presence of disinfectant between HA- and CA-MRSA should be investigated.

In conclusion, surveillance of clinical isolates with QAC genes is necessary for evidence of increasing tolerance or resistance that might lead to a reconsideration of CHG use as the recommended hand and patient hygiene agent in hospitals. Presence of *qac*A/B genes in certain MRSA clones associated with higher resistance to CHG and various antiseptic agents as well as having increased antibiotic resistance, might limit the choice of drugs for treating MRSA infections, and presents difficulties in MRSA infection control. Well designed surveillance systems for biocide resistance and antibiotic resistance are required to continuously monitor the situation in clinical isolates.

There are several limitations for the study related to the elderly. The antiseptic usage information was not included in the study, so any relationships of antibiotic exposure with presence of QAC gene carriage could not be identified. Appropriate interventions are urgently needed for care home residents to reduce endemic MRSA colonization in care homes. Interventions in cleaning, hygiene and patient density should be considered as well as training in particular techniques such as enteral feeding which has been shown to reduce colonization (Warren et al., 2006; Ho et al., 2012). The effects of such interventions need to be carefully evaluated. However, this can be difficult as many care homes in Hong Kong are privately run and are unwilling to take part in research. Nevertheless the situation in care homes does need urgent attention as these homes are serving as reservoirs of MRSA and possibly QAC genes for the community.

A small number of isolates carrying *qac*G, H and J were identified in our study, and these genes have been only reported at low prevalence in most studies of human isolates. However, their presence in food animals at considerably higher levels may lead to their spread into the community (Bjorland et. al., 2005; Smith et.al. 2008; Wong et al., 2013). Further monitoring of these genes in food and carriage strains of food handlers may help to determine if these genes are indeed transferring from animal to the human population.

Recently, there has been increased interest in antiseptic resistance, with reports of high levels of *qac*A/B in clinical isolates from Malaysia (Shamsudin et al., 2012), Canada (Longtin et al., 2011), United States (McGann 2012 et al., 2012) and Australia (Ho et al., 2012b). Other researchers have examined the presence of these genes in *Enterococcus,* leading to the first detection of *qac*A/B in *Enterococcus* isolates from blood and stool from hospitalized humans, from faeces of farm animals, and from food (Bischoff et al., 2012). A novel QAC gene, *qac*Z, was also reported recently in *Enterococcus* with widespread prevalence in clinical isolates and dairy products (Braga et al., 2011). In addition to studies on Gram positive cocci, other studies have included the investigation of association between biocide resistance and presence of *qac*ΔE and *qac*E genes in *Klebsiella pneumoniae* clinical isolates (Abuzaid et al., 2012) and between *qac*EΔ1 genes in *P. aeruginosa* (Romão et al., 2011). It has been reported that reduced susceptibility to a biocide at its in-use concentration*,* may be independent of the presence of the *qac*EΔ1 gene in *P. aeruginosa* (Ortega Morente et al., 2013). Recently, Farkas et al. (2013) detected

that urban aquatic environments were contaminated by bacteria in biofilms all containing *qac*EΔ1 gene. Ciusa et al. (2012) found a novel resistance mechanism to triclosan mediated by an sh-fab I allele mutation, and demonstrated potential selective pressure for reduced biocide susceptibility in clinical *S. aureus* isolates. Therefore, continued investigation is needed, not only in Staphylococcal species, but in other micro-organisms and biocides, as well as into the relationship with other kinds of QAC genes and reduced susceptibility.

Currently, many questions relating to the transmission of resistance associated with QAC genes and mobile genetic elements leading to cross-resistance and/or co-resistance with antibiotics remain unanswered. Our study has provided evidence for the hypothesis that multiple antibiotic resistances are associated with the reduced susceptibility to biocides in staphylococci isolates. However, a recent study by Coelho et al. (2013), using a decision tree algorithm to evaluate the relationship between antibiotic resistance and reduced biocide susceptibility in *Staphylococcus*, whilst recognizing the strong association between these resistances, could not conclude whether antibiotic resistance selected for biocide resistance or vice versa (Coelho et al., 2013). Further work is needed to clarify this situation.

Other researchers support the concept of co-selection of antibiotic resistance in the presence of QAC genes and this hypothesis was strengthened by recent reports suggesting reduced susceptibility to CHG in MRSA may have a clinical impact. Batra et al. (2010) determined that MRSA strains harbouring the *qac*A/B genes with an

elevated MBC to CHG spread more rapidly after use of a CHG-based surface antiseptic protocol in an ICU. Akinkunmi & Lamikanra (2011) reported that MRSA isolated from facecal samples of children in the community, were less susceptible to antiseptics than MSSA and had 2-3 times greater MICs to antiseptics. In a nested case-control study performed by Lee et al. (2011), the presence of genotypic CHG resistance (*qac*A/B genes) coupled with mupirocin resistance was shown to significantly increase the risk of persistent MRSA carriage after decolonization therapy, independently predicting failure of MRSA decolonization of patients (Lee et al., 2011). In another study by Lee et al. (2013), 65% mupirocin-resistant clinical isolates of MRSA carried *qac*A/B and 71% the *smr* gene. However, McDanel et al. (2013) only detected two isolates from nursing home residents carrying *qac*A with low level mupirocin resistance. Recently, Lepainteur et al. (2013) reported the mupirocin resistance and reduced antiseptic susceptibility among CNS, 59% (30/51) of which were found carrying *qac*A/B gene, 41.2% with reduced susceptibility to at least one antiseptic including BC and CHG, and 61% resistant to mupirocin.

Skovgaard et al. (2013), by means of comparing *S. epidermidis* isolated from nurses between to that from blood isolates from the 1960's, found absence of *qac*A/B genes in *S. epidermidis* isolates obtained in the 1960s, which implied that long-term use of biocides like chlorhexidine or related compounds may select for the presence of *qac*A/B genes. Lenonardo et al. (2013) detected the presence of QAC gene positive *S. aureus* among 1602 clinical isolates showed the increased MIC to BC and CHG, but no to MBC.

These studies indicated that staphylococci exhibiting reduced susceptibility to CHG and/or the presence of CHG efflux-mediated resistance genes may be associated with clinical failure of the decolonization/treatment of staphylococci.

Otter et al. (2013) evaluated the carriage of QAC resistance genes and CHG susceptibility in MRSA before and after introduction of an institutional MRSA control programme incorporating CHG-based decolonization (Otter et al., 2013). The results suggested presence of *qac*A might confer a selective advantage in response to CHG-based decolonization in the dominant endemic MRSA clone (CC22), but not other (CC30) MRSA clones. Infection control practice may lead to a change of MRSA epidemiology with increasing global dominance of CC22 and ST239 clones harbouring increased virulence determinants. In Hong Kong, as elsewhere in SE Asia, the dominant MRSA clone is ST239 and this may help explain the high rates of *qac*A/B positivity detected in isolates from this region. In contrast, Sangal et al. (2012) reported that long-term CHG bathing was not associated with any detectable loss of efficacy or increase in resistance in MRSA or with any increase in infection with other organisms.

Therefore, further studies investigating the relationship between the carriage of QAC genes and phenotypic reduced CHG susceptibility are needed. The clinical relevance of low-level mupirocin resistance and QAC genes also needs to be addressed. A correlation between the occurrence of reduced CHG susceptibility and decolonization failure does not necessarily indicate causality, as there are other

possible confounding factors. Investigation of a correlation and causality between increased CHG use should be performed with particular attention to the types of use and an increased prevalence of reduced susceptibility to CHG.

While this study did investigate and characterize MRSA in the populations chosen (nurses, general population, the elderly, clinical isolates) and the environment (public environment and hospital surfaces) in Hong Kong, owing to the limited time and resources, some of these studies, such as the elderly study, included fairly small numbers of subjects, and while having adequate power to investigate presence of QAC genes in staphylococci in general, lacked sufficient sample size to fully investigate MRSA. Expanded studies are needed, especially for the elderly as conditions between the elderly homes vary widely.

MRSA control has become a priority in healthcare facilities, and eradication of carriage will be beneficial for the individual and other patients at risk of MRSA contamination. However, any intervention using antimicrobial agents can become a potential threat increasing risk of emergence of resistance. This has been observed in the emergence of mupirocin resistance following the implementation of decontamination programmes (Wisniewska et al., 2002; Rossney & O'Connell, 2008; Liu et al., 2010; Desroches et al., 2013; Abbasi-Montazeri et al., 2013). In healthcare facilities, large amounts of chlorhexidine have been used for the decolonization of patients carrying MRSA to effectively reduce the risk to acquire hospital infection (Climo et al., 2013). A recent study by Huang et al., (2013), showed that using

chlorhexidinde in routine ICU practice, universal decolonization (i.e., no screening, and decolonization of all patients was more effective than targeted decolonization (i.e., screening, isolation, and decolonization of MRSA carriers) or screening and isolation in reducing rates of MRSA clinical isolates and bloodstream infection. Despite the effectiveness of universal decolonization, decolonization of all patients with chlorhexidine could bring about over use of antiseptics and biocide residue lead to the selection of pressure. Further studies are needed to investigate the effects of such infection control strategies.

Improper disinfectant use, especially in healthcare settings could select for *Staphylococcus* spp. carrying QAC genes as such strains can survive in the presence of biocide residues in the environment, increasing the risk of infection control failure.

According to our observations, continued investigations are needed not only in the laboratory and in healthcare settings, but in all areas of biocide usage, and should include a range of micro-organisms and biocides. It is suggested that rigid adherence to the correct cleaning procedure is of vital importance for effective infection control and institutional hygiene. Widespread use of decolonization therapies should be coupled with procedures to monitor for emergence of resistance. Appropriate and prudent biocide use is paramount to prevent the emergence of bacterial resistance and cross-resistance. Alternative agents or practices are required in settings where resistance has caused MRSA control

measures to be ineffective.

In summary, this is the first study that has examined the QAC gene prevalence in staphylococcal isolates in Hong Kong from a wide variety of sources. The study aimed to determine if the health care environment increased risk of strains carrying these QAC genes. The increased proportion of QAC gene positivity in *mec*A positive isolates suggests co-selection of these genes, contributing to survival of MRSA in community and health care facilities, including hospitals and nursing homes. Use of antiseptics may be selecting for antibiotic-resistant strains and assisting their survival in the environment. In order to carry out further studies on increasing biocide resistance, a simple, flexible test for determining the MIC of a biocide or biocide mix is needed. This study evaluated SGE for determination of MICs of antiseptics and showed that SGE offers a precise and accurate method for biocide MIC measurement. The induction study demonstrated that biocide exposure could indeed select for strains with increased tolerance.

APPENDIX

Appendix I Consent Form for the Nurses

THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學

香港 九龍 紅磡
Hung Hom Kowloon Hong Kong

Code number:__________________

Consent Form

Carriage and contamination of surfaces in the hospital and community with antiseptic resistant *Staphylococcus aureus*

This study aims to determine the proportion of antiseptic resistant Staphylococcus aureus isolates carried by nurses and the general public in Hong Kong. It will also examine environmental and hospital surfaces for antiseptic resistant S.aureus. If you agree to participate ini the study, you will be asked to provide a nose swab on one occasion only and to complete a very brief questionnaire providing details of your most recent hospital placement preferred antiseptic hand scrubs, recent hospitalization etc.

I ....(name) agree to participate in the study entitled "Carriage and contaminiation of surfaces in the hospital and community with antiseptic resistant *Staphylococcus aureus*"

I have read and understood the information presented to me. I understand that must provide a nose swab and complete a short questionnaire. I understand I am free to leave the study at any time without penalty.

I have had an opportunity to ask questions about the study and these have been answered to my satisfaction.

I understand that the results of this study may be published, but my own results will remain confidential and I will not be identified personally in any published work.

Signature.....................................
(or mark)

Date..

Appendix II Questionnaire for the Nurses

香港 九龍 紅磡
Hung Hom Kowloon Hong Kong

Please help to complete the questionnaire for additional information:

1. Age ____________________
2. Sex ____________________
3. Last hospital attended: ____________________
4. Underlying conditions and chronic illness: NO ☐ Please tick

If Yes, please list e.g.Eczema, Asthma, Diabetes, Hypertension, Thyrotoxicosis, Chronic renal faileure)

5. History of antibiotics use in past 6 months:

Yes □ NO □

If YES, please list types/or name/s if known*

____

6. Have you in contact with known MRSA patient/s in the last 3 months:

   Yes □ NO □

7. Which antiseptics did you commonly use in the last 3 months in between patients for disinfection? **You may select the same answer in more than one question**

A Always (>75%) B. Very often (50%-74%) C. Sometimes (25%-49%) D. Rarely & Never (<25%)

1. Alcohol hand rub: ____________________
2. Hisbiscrub(Chlorohexinde): ____________________
3. Soap: ____________________
4. Povidone Iodine: ____________________

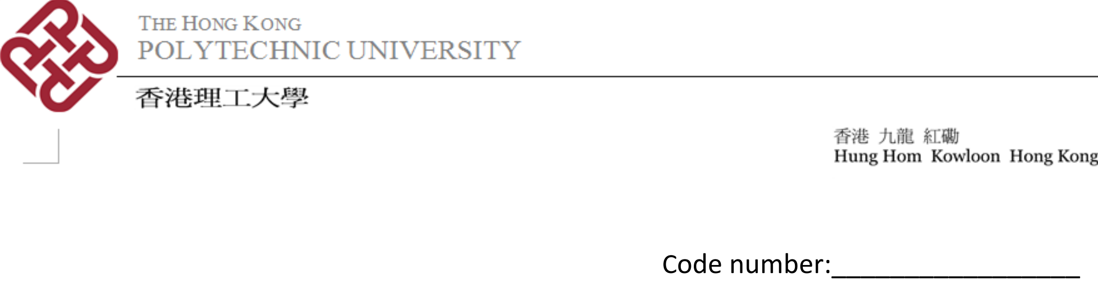
-END-

Common antibiotics prescribed by GP:

Amoxycillin, Augmentin, Ampicillin; Bactrim/Septrin,Azithromycin;Tetracyclines: Doxycylcine;

Fluroquinolones: Ofloxacin, Levofloxacine; Cephalosporins: Zinnaat(Cefuroxime)

Appendix IIIa Consent Form for the Elderly in the Nursing Home (English Version)



Code number:_________________

Consent Form
(For Elderly Persons in Residential Home)

Prevalence of carriage and characterisation of strains of methicillin resistant *Staphylococcus aureus* (MRSA) in residents of nursing homes and day care centres for the elderly in Hong Kong

This study aims to determine the amount of MRSA that elderly people in Hong Kong carry in their nose. If you agree to participate in the study, you would be asked to allow us take a nose sample using a swab of cotton wool two times over a six week period, and to provide some personal details such as age, length of time you are living in the nursing home etc. You are free to leave the study at any time with no penalty.

I ....(name) agree to participate in the study of carriage of methicillin resistant *Staphylococcus aureus* in elderly persons in Hong Kong.

I have read and understood the information presented to me.

I have had an opportunity to ask questions about the study and these have been answered to my satisfaction.

I understand that I must provide a nose swab two times times over a six week period and complete a questionnaire.

I understand that the results of this study may be published, but my own results will remain confidential and I will not be identified personally in any published work.

Signature.....................................
(or mark)

Date..

Appendix IIIb Consent Form for the Elderly in the Nursing Home (Chinese Version)

香港 九龍 紅磡
Hung Hom Kowloon Hong Kong

同意書

(適用於安老院中居住的長者)

研究在安老院中居住的長者及日間護理中心之長者的耐甲氧西林金黄色葡萄球菌(MRSA)帶菌情況以及對不同種類耐甲氧西林金黄色葡萄球菌的特徵

此項研究目的是為了測定耐甲氧西林金黄色葡萄球菌（MRSA）寄生於香港長者鼻腔中的比例。如果您同意參與這項研究，我們會於六星期內分別以藥棉拭子對您的鼻腔進行取樣兩次。同時，您需要提供一些個人資料，如年齡、您在安老院的居住時期等。閣下有權於任何時候退出是次研究而毋須負任何責任。

本人__________(姓名) 同意參與有關在香港長者耐甲氧西林金黄色葡萄球菌（MRSA）的帶菌趨勢之研究。

上述研究的有關資料已經被解釋得十分清楚，本人已經看過並且明白所有內容。.

本人清楚我有權對是次研究發出任何提問，直至滿意為止。

本人明白我必須於六星期內分別提供兩次鼻腔樣本以及回答一份問卷。.

本人了解是次研究結果有機會被公開發表，惟我的個人資料將被保密而個人身份將不被洩露。.

參加者簽署：......................................
(或指紋)

日期：..
..

Appendix IVa Consent Form for the Elderly in the Daycare Center (English Version)

香港 九龍 紅磡
Hung Hom Kowloon Hong Kong

Code number:__________________

Consent Form
(For Elderly Persons in Day Care Centres)

Prevalence of carriage and characterisation of strains of methicillin resistant *Staphylococcus aureus* (MRSA) in residents of nursing homes and day care centres for the elderly in Hong Kong

This study aims to determine the amount of MRSA that elderly people in Hong Kong carry in their nose. If you agree to participate in the study, you would be asked to allow us take a nose sample using a swab of cotton wool two times over a six week period, and to provide some personal details such as age, length of time you have been attending a day care centre etc. You are free to leave the study at any time with no penalty.

I ....(name) agree to participate in the study of carriage of methicillin resistant *Staphylococcus aureus* in elderly persons in Hong Kong.

I have read and understood the information presented to me.

I have had an opportunity to ask questions about the study and these have been answered to my satisfaction.

I understand that I must provide a nose swab twice over a six week period and complete a questionnaire.

I understand that the results of this study may be published, but my own results will remain confidential and I will not be identified personally in any published work.

Signature.....................................
(or mark)

Date...

Appendix IVb Consent Form for the Elderly in the Daycare Center (Chinese Version)

香港 九龍 紅磡
Hung Hom Kowloon Hong Kong

同意書

(適用於日間護理中心活動的長者)

研究在安老院中居住的長者及日間護理中心之長者的耐甲氧西林金黄色葡萄球菌(MRSA)帶菌情況以及對不同種類耐甲氧西林金黄色葡萄球菌的特徵

此項研究目的是為了測定耐甲氧西林金黄色葡萄球菌（MRSA）寄生於香港長者鼻腔中的比例。如果您同意參與這項研究，我們會於六星期內分別以藥棉拭子對您的鼻腔進行取樣兩次。同時，您需要提供一些個人資料，如年齡、您在安老院的居住時期等。閣下有權於任何時候退出是次研究而毋須負任何責任。

本人____________(姓名) 同意參與有關在香港長者耐甲氧西林金黄色葡萄球菌（MRSA）的帶菌趨勢之研究。

上述研究的有關資料已經被解釋得十分清楚, 本人已經看過並且明白所有內容。.

本人清楚我有權對是次研究發出任何提問，直至滿意為止。

本人明白我必須於六星期內分別提供兩次鼻腔樣本以及回答一份問卷。.

本人了解是次研究結果有機會被公開發表，惟我的個人資料將被保密而個人身份將不被洩露。.

參加者簽署：......................................
(或指紋)

日期：...

Appendix V Ethics Approval

PART IV: DECLARATION by Investigators

Note. Certain trial information will be passed to a Central Database for risk management purpose and to assist HA's finance controller in sourcing insurance coverage for clinical trial activities.

Scientific Title of Study: *(MUST appear on every page of the declaration)*	Epidemiology and molecular characteristics of antiseptic resistance genes in Staphylococcus aureus (S.aureus) and Coagulase negative Staphylococci (CNS) in Hong Kong

1. I/We declare that the information supplied is to the best of our knowledge and accurate.
2. I/We agree to uphold the protection of research subjects' rights and safety through adherence to local laws, Declaration of Helsinki, institutional policies and ICH-GCP.
3. I/We understand that approval by the Cluster REC is subject to regular renewal according to local policy.
4. I/We agree to report to the REC(KC/KE)
   - any planned change(s) to the study, and further agree not to implement any change(s) without receiving prior approval, except to eliminate immediate hazard to research subjects or when the change(s) involve only logistical or administrative issues.
   - any fatal events in applying site within 48 hours while pending investigation, and any serious adverse events in applying site (with an extended report) preferably within 7 days but not later than 15 days (from the day it was made known to me/us).
   - any new information on the project that adversely influences the risk/benefit ratio.
   - progress report(s) (as requested by the Cluster REC) and a final report (after completion of study).
5. I/We agree to keep all study documents for a period of at least three years after study closure.
6. I/We agree to maintain adequate records and to make them available for audit / inspection.
7. I/We agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

	Title and Name	Signature	Date
Principal investigator	phD student, ZHANG MI NG		04 / 01 / 2010
Co-investigators			/ /
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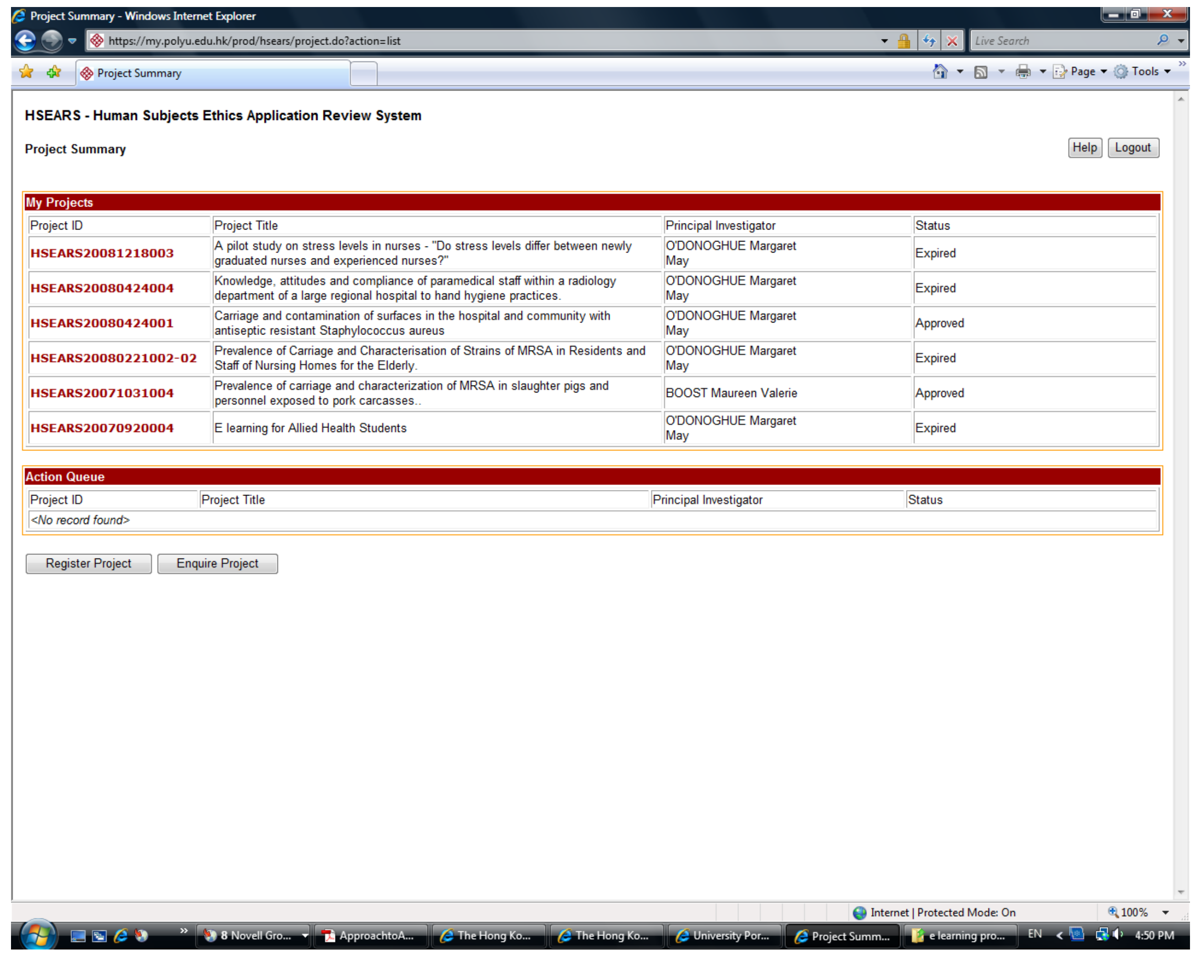
For student project			
Academic supervisor	Associate Prof, Maureen BOOST		01 / 03 / 2010
Site supervisor			/ /

Appendix V Ethics Approval



HSEARS - Human Subjects Ethics Application Review System

Project Summary

My Projects

Project ID	Project Title	Principal Investigator	Status
HSEARS20081218003	A pilot study on stress levels in nurses - "Do stress levels differ between newly graduated nurses and experienced nurses?"	O'DONOGHUE Margaret May	Expired
HSEARS20080424004	Knowledge, attitudes and compliance of paramedical staff within a radiology department of a large regional hospital to hand hygiene practices.	O'DONOGHUE Margaret May	Expired
HSEARS20080424001	Carriage and contamination of surfaces in the hospital and community with antiseptic resistant Staphylococcus aureus	O'DONOGHUE Margaret May	Approved
HSEARS20080221002-02	Prevalence of Carriage and Characterisation of Strains of MRSA in Residents and Staff of Nursing Homes for the Elderly.	O'DONOGHUE Margaret May	Expired
HSEARS20071031004	Prevalence of carriage and characterization of MRSA in slaughter pigs and personnel exposed to pork carcasses..	BOOST Maureen Valerie	Approved
HSEARS20070920004	E learning for Allied Health Students	O'DONOGHUE Margaret May	Expired

Action Queue

Project ID	Project Title	Principal Investigator	Status
<No record found>			

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