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DETECTION OF ANTISEPTIC RESISTANCE GENES AMONG CLINICAL ISOLATES OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

Thesis

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SUMMARY

Methicillin-resistant Staphylococcus aureus (MRSA) has emerged worldwide as a nosocomial pathogen of major importance, and the incidence of infections caused by MRSA continues to increase. As compared to methicillin-sensitive Staphylococcus aureus strains, MRSA infection has been associated with higher mortality and morbidity rates, longer hospital stays, and higher hospital charges.

Biocides are critical components of intervention strategies used in clinical medicine for preventing the dissemination of nosocomial infections. However, MRSA isolates with decreased biocide susceptibilities have been isolated from clinical samples, and MRSA isolates carrying antiseptic-resistance gene(s) have been prevalent worldwide. This has raised concerns that, as for antibiotics, intensive exposure of hospital pathogens to biocides may result in the emergence of resistance to these agents.

The aim of the present work was to study the susceptibility of methicillin-resistant isolates of *Staph. aureus*, obtained from different clinical samples, to commonly used biocides and to determine the prevalence of the biocide resistance genes, *qacA/B* and *smr*, among these isolates.

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LIST OF ABBREVIATIONS

CA-MRSA	Community-acquired methicillin resistant Staphylococcus aureus
CAPD	Continuous ambulatory peritoneal dialysis
CDC	Centers for Disease Control and Prevention
CoNS	Coagulase negative Staphylococci
CRAs	Chlorine-releasing agents
E. coli	Escherichia coli
EMRSA	Epidemic methicillin resistant Staphylococcus aureus
GISA	Glycopeptide-intermediate *Staph.aureus*
H_2O_2	Hydrogen peroxide
HA-MRSA	Hospital-Acquired methicillin resistant Staphylococcus aureus
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCWs	Healthcare workers
hGISA	Hetero-resistant Glycopeptide-intermediate *Staph. aureus*
HIV	Human immunodeficiency virus
HOCl	Hypochlorous acid
ICU	Intensive care unit
LPS	Lipopolysaccharides
M.tuberculosis	Mycobacterium tuberculosis
marA	Multiple antibiotic resistance activators
marR	Multiple antibiotic resistance repressor
MDR	Multidrug resistance
MFS	Major facilitator superfamily
MICs	Minimum inhibitory concentrations
MRSA	Methicillin resistant Staphylococcus aureus
MSSA	methicillin sensitive Staphylococcus aureus

NaDCC	Sodium dichloroisocyanurate
OCl^-	Hypochlorite ion
OmpF	Outer membrane porin proteins
OPA	Ortho-phthalaldehyde
P. aeruginosa	Pseudomonas *aeruginosa*
PAA	Peracetic acid
PBPs	Penicillin binding proteins
PCMX	Chloroxynol
PCR	Polymerase Chain Reaction
PMF	Proton motive force
PVL	Panton-Valentine leukocidin
PVP-I_2	polyvinylpyrolidone-iodine
QACs	Quaternary Ammonium Compounds
SASPs	Small acid-soluble spore proteins
SCC	Stapylococcal Cassette Chromosome
smr	Staphylococcal multidrug resistance
Staph. aureus	Staphylococcus aureus
STEL	Short-term exposure limit
TCC	Triclocarban
TSB	Trypticase soy broth
TSS	Toxic shock syndrome

INTRODUCTION

Antiseptics and disinfectants are used extensively in hospitals and other healthcare settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections ***(McDonnell and Russell, 1999)***. However, the widespread use of antiseptic and disinfectant products has prompted some speculation on the development of microbial resistance, in particular cross resistance to antibiotics ***(Noguchi et al., 2005)*.**

Some antiseptics and disinfectants were found, on the basis of minimum inhibitory concentrations (MICs), to be less inhibitory to *Staph. aureus* strains that contain a plasmid carrying genes encoding resistance to the aminoglycoside antibiotic gentamicin. These biocidal agents include chlorhexidine, diamidines, and quaternary ammonium compounds (QACs), together with ethidium bromide and acridines ***(Russell, 1997)***.

Mycock, (1985) reported that methicillin resistant *Staph. aureus* (MRSA) strains had a remarkable increase in tolerance (at least 5,000-fold) to povidone-iodine. However, there was no decrease in susceptibility of antibiotic-resistant strains to phenolics (phenol, cresol, and chlorocresol) or to the preservatives known as parabens ***(McDonnell and Russell, 1999)*.**

Moreover, interesting studies by ***Reverdy et al. (1993)*** demonstrated a relationship between increased *Staph. aureus* MICs to the β-lactam oxacillin and four antiseptics (chlorhexidine, benzalkonium chloride, hexamine, and acriflavine). A gene encoding multidrug resistance was not found in susceptible strains but was present in 70% of *Staph. aureus* strains for which the MICs of all four of these antiseptics were increased and in 45% of the remaining strains resistant to at least one of these antiseptics ***(Behr et al., 1994)*.**

Increased tolerance to disinfecting agents can be caused by energy-dependent efflux pumps located in the cell membrane ***(Noguchi et al., 1999; Kuroda et al., 2001)***. The genes encoding multidrug exporter proteins among Staphylococci can be divided into two families on the basis of DNA homology and phenotypic properties ***(Paulsen et al., 1996)*.** Members of the qacA-qacB family confer a resistance phenotype broader than that conferred by members of the smr family ***(Leelaporn et al., 1994)***. The *qacA* and *qacB* genes are closely related and differ at the nucleotide level by seven nucleotides. A single amino acid alteration (Ala in *qacB* to Asp in *qacA*, codon 323) is probably responsible for the differences in phenotypic expression, which gives a broader resistance phenotype in isolates harboring *qacA* ***(Narui et al., 2007)***.

AIM OF THE WORK

The aim of the present work is to study the susceptibility of methicillin-resistant isolates of *Staph. aureus*, obtained from different clinical samples, to commonly used biocides (antiseptic and disinfectant agents) and to determine the prevalence of the biocide resistance genes, *qacA/B* and *qaC*, among these isolates.

STAPHYLOCOCCUS AUREUS, AN OVERVIEW

Staphylococci are gram-positive, catalase-positive cocci that are placed together with Stomatococcus and Planococcus in the family Micrococcaceae. They range from 0.5 to 1.5μm in diameter, and occur in irregular grape-like clusters, tetrades and short chains. They are non motile, non-spore forming, typically unencapsulated, do not form gas from carbohydrates and are facultative anaerobes ***(Konneman et al., 2006)***.

Staphylococci are widespread in nature, their major habitat being the skin and mucus membrane of mammals and birds. In humans, sites of Staphylococcus (Staph.) aureus colonization include the anterior nares, intertriggious skin folds like axilla and groin. Other sites include the perineum, rectum and throat ***(Bannerman and Peacock, 2007)*.**

Staphylococcus aureus strains possess a large number of cell associated and extracellular factors, some of which contribute to the ability of the organism to overcome the body's defenses and invade, survive in and colonize the tissues. Though the role of each individual factor is not fully understood, it is likely that they are responsible for the establishment of infection, enabling the organism to bind to connective tissue, opposing destruction by bactericidal activities of humoral factors such as

complement and overcoming uptake and intracellular killing by phagocytes ***(Humphreys, 2004)*.** Staphylococcus aureus, almost uniquely among common pathogens, also has an astonishing history of changing clinical manifestations and epidemiologic behavior ***(McDonald, 2006)***.

Staphylococcus aureus is a major cause of both healthcare- and community-acquired infections. It is perhaps the single most common cause of healthcare-associated infections throughout the world ***(Diep et al., 2004)***. Moreover, it is a frequent cause of community-acquired bacteremia and is associated with significant mortality and morbidity ***(Shorr et al., 2006)***.

Infections caused by *Staph. aureus* range from minor skin disorders such as wound infections, furuncles, carbuncles, and bullous impetigo, through locally invasive diseases such as cellulitis, osteomyelitis, sinusitis, and pneumonia, to major life-threatening septicemia and meningitis ***(Fowler and Olsen, 2003)***. It is also a frequent cause of medical device-related infections such as intravascular line sepsis and prosthetic joint infections ***(Gardam, 2000)***. Although minor skin infections may resolve naturally without antibiotic intervention, once *Staph. aureus* invades deeper structures it often spreads hematogenously to other organ systems, leading to metastatic infection. Endocarditis and septicemia have significant