An Investigation into the association of plasmid-borne *qacAB* and antimicrobial resistance in Meticillin-Resistant *Staphylococcus aureus*

Azra Khan MSc BSc (Hons) CSci FIBMS

September 2013

A thesis submitted in partial fulfilment of the
Degree of Professional Doctorate in Biomedical Science,
School of Pharmacy and Biomedical Sciences

University of Portsmouth
Faculty of Science

Abstract

Meticillin-resistant *Staphylococcus aureus* (MRSA) is globally recognised as a major causative organism of hospital acquired infection (HAI) and continues to present many challenges for infection prevention and control. Once established within hospitals and healthcare centers, the control of spread of MRSA and therapy is difficult due to resistance to otherwise effective antimicrobials. Government initiatives in the United Kingdom (UK) have led to considerable investments in improving infection control practices, with emphasis on improving hand hygiene compliance of healthcare professionals and hospital environmental cleanliness to control the spread and limit the source of MRSA and other HAIs. This has resulted in the subsequent increase in disinfectant and antiseptic usage containing, quaternary ammonium compounds (QACs), cationic biocides such as chlorhexidine and the bisphenol ether, triclosan, for decontamination of surfaces and disinfection of skin. Thus, there is serious concern that as with antibiotic resistance, continual and intensive exposure of MRSA (and other hospital pathogens) to biocides, may result in the emergence of resistance to these agents with further detrimental consequences and substantial burden for prevention, treatment and control of hospital infections.

MRSA carry a number of plasmid-borne *qac* genes, predominantly *qacA*, *qacB* and *smr* that encode resistance to commonly used antiseptics and disinfectants in hospitals, nursing homes and other healthcare establishments. The proteins encoded by *qacA* and *qacB* mediate efflux via active transport; QacA multidrug exporter mediates resistance to monovalent, divalent cationic and lipophilic antimicrobial compounds, whilst the closely related export protein QacB mediates lower levels of resistance to divalent cations.

In this research a "snapshot" study of hospital strains of MRSA stored at the Hospital Infection Research Laboratory (HIRL), City Hospital, Birmingham, was carried out to determine the prevalence and distribution of *qacAB* in these isolates and determine a possible association between presence of these genes and biocide resistance. The intercalating dye, ethidium bromide (EtBr) is a substrate for many *S. aureus* multi-drug resistant (MDR) efflux pumps and was used in the present study as a marker for detection of efflux pump activity. Previous studies have reported that MRSA strains with an MIC of $\geq$ 64 mg/L to EtBr have *qacAB*, however, the present study used a lower baseline value of $\geq$ 32 mg/L resistance to EtBr to capture any isolates with low MICs that may have *qacAB* and may be missed.

Initially 3,400 MRSA strains collected between October 2002 and October 2006 were screened to identify and select isolates with $\geq$ 32 mg/L resistance to EtBr. A second MRSA collection stored at the Antimicrobial Chemotherapy Laboratory, City Hospital, Birmingham, comprised 63 isolates that showed MICs of $\geq$ 64mg/L, were also included in the study. At this stage the study set (Set A) comprised 112 isolates with varying MIC to EtBr ranging from $\geq$ 32 mg/L to 256 mg/L. At a later date an additional 400 strains were screened from the same stored collection to include strains with lower MICs, i.e. < 32 mg/L. Thus a total of 336 isolates with varying levels of resistance to EtBr were studied.

PCR was carried out on all 336 isolates for detection of *qacAB, smr, qacG, qacH* and *qacJ* to determine the presence and prevalence of the genes. Set A isolates positive for *qacAB* were further investigated to differentiate between *qacA* and *qacB*. Restriction

digestion using the restriction enzyme *Rsa*1 was carried out on PCR products followed by PCR using specific primers for detection of the two genes. Urease activity and neomycin sensitivity were used as a means of basic characterization applied to all the study isolates. A select number of samples negative for *qacA* and *qacB* were typed using *spa* typing.

Transfer studies involving, conjugation, plate mating and transformation on selected strains were carried out to attempt transfer of *qacAB* using the marker EtBr from a strain of MRSA with an MIC of $\geq$ 256 mg/L to EtBr and *qacAB* positive to a strain with < 32mg/L MIC to EtBr and lacking *qacAB*. Unfortunately, conjugation experiments were not successful in this study. Plasmid curing experiments were also carried out to demonstrate loss of plasmid through continual passaging onto selective plates.

A variety of antiseptics and disinfectants are used in hospitals for prevention of HAIs. The present study was limited to carrying out minimum bactericidal concentration (MBC) determinations and MIC of four commonly used hospital biocides against randomly selected strains. The strains reflected ranges of MICs to EtBr and presence or absence of *qacAB*. These experiments, determined the efficacy of the biocides tested, to effectively destroy MRSA on skin and environment when used in healthcare settings.

The results suggest that in the majority of strains showing high MICs to EtBr i.e. $\geq$ 64 mg/L, *qacAB* is present and thus, the mechanism of resistance to biocides may be attributed to an efflux protein pump encoded by these genes. Following restriction digestion of *qacAB* positive strains, with the restriction enzyme *Rsa1*, 81 of the 112 *qacAB* positive strains tested positive for *qacA*, i.e. 90% and 9 (11%) for *qacB*. The predominant prevalence of the *qacA* gene indicates that most of these strains are likely to be resistant to organic cationic biocides and intercalating dyes such as EtBr and acriflavine. However, the results of the MIC and MBC determinations carried out on a selection of biocides commonly used in the healthcare environment implies that the four biocides tested are likely to be 99.9% effective at killing the majority of isolates in this study set. However, five isolates demonstrated MBCs to chlorhexidine of > 32 mg/L. Chlorhexidine is a compound that is widely used in hand hygiene and surgical antisepsis products, and the results suggest that solutions containing this compound would be ineffective in removing MRSA from the hands of healthcare workers and skin sites if used.

Molecular *spa* typing of selected samples negative for *qacAB* revealed that Endemic-MRSA (EMRSA) type 15 was the most frequent *spa* type identified in this study, followed by EMRSA-16 and EMRSA-1. Three strains identified jointly as EMRSA-3 and EMRSA-1. One strain identified as the Berlin clone

With regards to the challenges presented to infection prevention and control, MRSA has the potential to develop increased tolerance to biocides commonly used in the hospital environment, due to expression of efflux pumps, although currently there is little evidence of this. Further research is required to understand and learn of the various mechanisms of resistance, supported by adherence to control of infection strategies for prevention and spread of infections in healthcare facilities.

List of Contents

List of Tables

List of Figures

List of Abbreviations

ABC	Adenosine Triphosphate-Binding Cassette
ATP	Adenosine Triphosphate
BAC	Bacitracin
BHIA	Brain Heart Infusion Agar
BHIB	Brain Heart Infusion Broth
BMS	Biomedical Scientist
BSAC	British Society of Antimicrobial Resistance
BSc (Hons)	Bachelor of Science (Honours)
BZC	Benzalkonium chloride
CA	Community Acquired
$CaCl_2$	Calcium Chloride
CA-MRSA	Community-Acquired Meticillin-Resistant *Staphylococcus aureus*
CCC DNA	Covalently Closed Circles Deoxyribonucleic acid
CDR	Communicable Disease Report
Cip	Ciprofloxacillin
CoNS	Coagulase Negative Staphylococci
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DoH	Department of Health
dNTP(s)	Deoxyribonucleic triphosphate(s)
EARSS	European Antimicrobial Surveillance System

EARSS-NET	European Antimicrobial Surveillance Network
EMRSA	Epidemic Meticillin-Resistant *Staphylococcus aureus*
Ery	Erythromycin
E. coli	*Escherichia coli*
EtBr	Ethidium Bromide
Fus	Fusidic acid
Gm	Gentamicin
HAI(s)	Healthcare Associated Infection(s)
HA-MRSA	Hospital Acquired Meticillin-Resistant *Staphylococcus aureus*
HCW	Healthcare Worker
HDCPM	Hexadecylpyridinium chloride monohydrate
HIRL	Hospital Infection Research Laboratory
HIS	Healthcare Infection Society
ICNA	Infection Control Nurses Association
ICU(s)	Intensive Care Unit(s)
IPC	Infection Prevention and Control
IPS	Infection Prevention Society
K	Kanamycin
$MgCl_2$	Magnesium Chloride
MATE	Multi-drug and Toxic Compound Extrusion
Mb	Megabase
MBC	Minimum Bactericidal Concentration
MCT	Mixed Culture Technique

MFS	Major Facilitator Superfamily
$MgCl_2$	Magnesium chloride
MGE	Mobile Genetic Element
MIC	Minimum Inhibitory Concentration
MLST	Multi-Locus Sequence Type
MR-MRSA	Multi-drug-Resistant *Staphylococcus aureus*
MRSA	Meticillin-Resistant *Staphylococcus aureus*
MSSA	Meticillin-Sensitive *Staphylococcus aureus*
mM	Micro moles
MSc	Master of Science
NHS	National Health Service
OC DNA	Open Circles Deoxyribonucleic acid
PBP2a	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
PEG	Poly Ethylene Glycol
PMF	Proton Motive Force
pmol	Pico mole
PVL	Panton-Valentine Leucocidin
QAC(s)	Quaternary Ammonium Compound(s)
RC	Rolling Circle
Rif	Rifampicin
S. aureus	*Staphylococcus aureus*
SCC	Staphylococcal Cassette Chromosome

SMR	Small Multi-drug Resistance
ST	Sequence Type
Taq-polymerase	*T*hermos-*ac*quaticus polymerase (enzyme)
TBE buffer	Tris/borate/EDTA/buffer
Tet	Tetracycline
TSB	Tryptone Soya Broth
UK	United Kingdom
µL	Micro litre
UV	Ultra violet
VRSA	Vancomycin-Resistant *Staphylococcus aureus*
v/v	Volume to Volume
WHO	World Health Organisation
w/v	Weight per volume

Acknowledgements

I would like to express my sincere gratitude and appreciation to all those who have assisted me, advised me, supported and encouraged me throughout the Professional Doctorate course. First and foremost, I sincerely thank Professor Graham Mills for all his help and support given to me over the years, and particularly so through the last hurdle of writing up. I am extremely appreciative and indebted to him for all his efforts for my success.

I am most thankful to Dr Adam Fraise (Consultant Microbiologist/Director HIRL) and Christina Bradley (Laboratory Manager) for allowing me the opportunity to undertake the Professional Doctorate, and to Dr Nigel Brenwald (Senior Biomedical Scientist, Antimicrobial Chemotherapy Department, City Hospital), for his initial supervision of the research and for his advice and support. I shall always remember his words "research is 90% failure and 10% success".

I am eternally grateful to Professor Barry Cookson (Director of The Laboratory of HealthCare Associated Infection, Colindale), for his invaluable guidance and advice and for taking over the role of an additional supervisor/advisor at the later stages of my research. I would not have been at the stage I am without his continual, unconditional, kind help and support which has been crucial to completion of the research and the thesis.

I am extremely indebted to Professor Elizabeth Wellington (School of Life Sciences, University of Warwick) for allowing me to continue with the practical research in her department. Without her support I would have had to abandon my studies. I have appreciated all her help and advice and co-supervision of my studies with Dr William Gaze (School of Life Sciences, University of Warwick). I would not have achieved progress without their continual guidance, help and encouragement of which I am immensely grateful. I would like to extend my thanks to Mr Andrew Mead (School of Life Sciences, University of Warwick) for his statistical expertise and help.

I am also extremely grateful to Dr Alan Cockayne (Centre for Biomolecular Science, Queens Medical Centre, Nottingham) for his much appreciated advice, assistance and kind

arrangements to carry out transfer studies in his department with the use of specialist equipment and materials.

I am most appreciative to Dr Mark Sutton and Dr Matthew Wand (HPA-Porton Down), for allowing me to continue with some of my later experimental work in their laboratory and for all their supervision and advice.

I would like to thank all those who have entered my life, on a professional and/or personal basis, whether fleetingly or permanently, and whom have inspired me, mentored me, encouraged me, guided me, given me their support and motivation to complete my studies. My deepest gratitude and appreciation for the support and friendship shown to me by my dear friends and colleagues, Kulvir Chana, Rajinder Pnaiser, Becky Walker and Margo Spears and my fellow Professional Doctorate class members, Dr Hugh Hurst, Dr David Ricketts and Mr Allen Roberts, for helping me to keep focused and positive through the difficult times in my life.

Last, but not least I would like to express my heartfelt appreciation to all my family for their love and support of my determination to achieve the Professional Doctorate. We have gone through some devastating times recently, but the support of my loved ones and thoughts of my family have kept me motivated to complete.

Dedication

I dedicate this thesis to the memory of my beloved father, who always encouraged all his children to embrace education and knowledge as a very precious “gift”, to strive to achieve excellence in our studies and to confront all life’s challenges with courage and perseverance. He instilled in us the merits of education through, example, encouragement and advice. He himself held high personal and professional ethics and he was very much admired and respected for those qualities by his family, friends, colleagues and students. “My dearest papa, my aspiration to achieve this highest qualification came from you and was for you, to keep your memory alive and for you to be proud of me. I hope you are”.

I also dedicate this to my darling mother and I thank her for all her prayers, support and love throughout my studies and in all aspects of my life.

Declaration

I declare that whilst studying for the Doctorate in Biomedical Science at the University of Portsmouth, I have not been registered for any other award at another University. The work undertaken for this degree has not been submitted elsewhere for any other award. The work contained within this submission is my own work and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due acknowledgment has been made in the text.

Azra Khan
September 2013