UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION AND GENETIC PROFILING OF ANTIBIOTIC RESISTANCE AMONGST Staphylococcus aureus AND METHICILLIN-RESISTANT Staphylococcus aureus ISOLATES FROM SELANGOR, MALAYSIA

ASINAMAI ATHLIAMAI BITRUS

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By

ASINAMAI ATHLIAMAI BITRUS

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

April 2017

DEDICATION

This thesis is dedicated to:

My beloved parents**,**

Mr. & Mrs. Bitrus Asinamai Bituku

My beloved siblings

Mrs. Blessing Simon Udurbu, Engr. Andrew Bitrus Asinamai, Mrs. Munagkur Philibus Gonya, Engr. Ijudigal Bitrus Emmanuel and Engr. Abraham Bitrus Bituku

Who always pray, supported and encourage me to do my best

And finally,

My nephews and nieces

Gideon Simon, Godiya Simon and Jasku Simon, Samuel and Hephzibah Andrew

Kishak and Princess

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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By

ASINAMAI ATHLIAMAI BITRUS

April 2017

Chairman : Associate Professor Zunita Zakaria, PhD
Faculty : Veterinary Medicine

Staphylococcus aureus is a good model to illustrate the importance of gene transfer events in the global spread and dissemination of resistant clones worldwide. A total of 29 isolates (8 humans, 11 horses, 4 dogs, 4 chickens, 1 cat, and 1 from environmental surface) were used in this study. The isolates were reconfirmed as *S. aureus* using phenotypic and genotypic methods. Antibiotic susceptibility test was carried out using disk diffusion method. While screening of 10 selected virulence gene was carried out PCR. Mix liquid culture plating using graded doses of antibiotics was carried out to determine the *in vitro* transfer of methicillin resistant determinants *mec*A. PCR detection of SCC*mec* types and characterization of the universal attachment of SCC*mec* (*orf*X) and the SCC*mec* insertion site was carried out by PCR and Sanger's sequencing and Molecular typing of the isolates was carried out using multilocus sequence typing (MLST) technique.

The result of this study showed that, all isolates were *nuc* gene positive and (10/29) 34 % of the isolates were positive for *mec*A. While three (3/29) 10.34% isolates lack expression of *mec*A. There was a general reduction in the pattern of resistance to antibiotics previously tested. Reduced susceptibility was observed in two (2) isolates SDG2 and SH4 which were seen to be resistant to (8/13) 62% and (7/13) 54% of the antibiotics tested respectively. Resistance to amoxicillin, cefoxitin and oxacillin was also observed in (8/14) 57%, (9/14) 64% and (12/14) 86% of the MRSA isolates respectively. Four (4/14) 29% of the isolates were phenotypically resistant to vancomycin, doxycycline and amoxicillin-clauvulanic acid, while phenotypic resistance to tetracycline and erythromycin was also seen in (6/14) 43% and (5/14) 36% of the isolates respectively.

Profiling of the virulence gene showed that a total of 20/29 (68.9%) and 14/29 (48%) of the isolates were positive for staphylococcus exotoxin-like toxin 1 (*set1*) and lipase encoding gene (*geh*). Also, twelve 11/29 (37%), and 7/29 (24.1%) of the all the isolates were positive for beta hemolysin (*hlβ*) and V8 protease (*SspA*) respectively. The isolates were also positive for exfoliative A (*etA*) 2/29 (6.9%), alpha hemolysin (*hlα*) 2/29 (6.9%) and staphylococcus enterotoxin u (*Seu*) 4/29 (13.8%). Additionally, a total of 5/29 (17.2%) and 3/29 (10.34%) of the isolates were positive for phage-borne Panton valentine leukocidine (PVL) and exfoliative toxin B. While only 1/29 (3.4%) of the isolates was positive for gene coding for toxic shock syndrome toxin-1 (*tst*). The result of the antibiogram revealed a reduced susceptibility and multidrug resistance in four isolates (and susceptibility to all antibiotics observed in two isolates.

The findings of the *in vitro* transfer of *mec*A gene using mix liquid culture plating on Luria-Bertini (LB) agar each separately containing antibiotics 100 µg/mL, 50 µg/mL and 30 µg/mL of erythromycin and cefpodoxime for selection of donor cells, tigecycline and levofloxacin for selection of recipient transconjugants showed that transfer of resistance determinants occurred between MRSA to MSSA.

Screening of SCC*mec* types showed that majority of the MRSA isolates carried SCC*mec* typing III 7/11(63%). While only 3/11 (27%) and 1/11 (9.0%) of the isolates were positive for SCC*mec* type II and IVe. PCR amplification of the universal insertion site of the SCC*mec* (*orf*X) and attachment site showed that all the isolates 16/16(100%) were positive for the *orf*X gene while 7/16(43.8%) were positive for the *att*B. In addition, phylogenetic tree analysis using Maximum Likelihood method showed that the isolates demonstrate a significant level of diversity.

Multilocus sequence typing (MLST) analysis revealed two types of sequence types (STs) ST1 and ST177.The findings of this study showed the effect of prolonged storage temperatures on resistance gene, the carriage of virulence genes and demonstrate the transfer of methicillin resistance genes. Additionally, SCC*mec* types were screened, its insertion site characterised and finally, the clonal types in a collection of *S. aureus* isolates was determined. Hence, providing baseline data on the global spread of resistance and virulence genes as well as transmission of MRSA from humans to animals.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENCIRIAN DAN PROFIL GENETIK KERINTANGAN ANTIBIOTIK DI KALANGAN *Staphylococcus aureus* DAN *Staphylococcus aureus* RINTANG METISILIN (MRSA) DARI SELANGOR, MALAYSIA

Oleh

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Staphylococcus aureus merupakan model yang baik untuk menggambarkan kepentingan acara pemindahan gen dalam penyebaran klon yang mempunyai rintangan secara global dan meluas di seluruh dunia. *Staphylococcus aureus* yang rintang metisilin (MRSA) merupakan patogen yang amat penting kerana diakui mempunyai impak ke atas kesihatan manusia disebabkan oleh potensinya yang zoonotik dan balikan zoonotik. Sebagai contoh, jangkitan yang disebabkan MRSA telah mendapat perhatian khusus kerana pilihan rawatan yang terhad. Kajian ini bertujuan untuk menentukan mekanisme perolehan rintangan antibiotik dalam pencilan *S. aureus* dan *S. aureus* rintang metisilin (MRSA) yang didapati daripada haiwan dan manusia.

Sejumlah 29 pencilan (8 manusia, 11 kuda, 4 anjing, 4 ayam, 1 kucing, dan 1 dari permukaan alam sekitar) telah digunakan dalam kajian ini. Pencilan disahkan sebagai *S. aureus* menggunakan morfologi koloni, pewarnaan Gram untuk morfologi sel, media terpilih, ujian biokimia, dan amplifikasi PCR gen 'thermo-nuclease' *S. aureus* dan rintangan metisilin penentu *mec*A. Ujian kerentanan terhadap antibiotik dijalankan dengan menggunakan kaedah resapan disk. Cerakinan tindak balas rantai polymerase (PCR) terhadap 10 gen virulen terpilih telah dijalankan menggunakan primer dan kitaran yang sesuai. Di samping itu, pengkulturan teknik 'cecair kultur campuran' menggunakan dos antibiotik berbeza telah dijalankan untuk menentukan pemindahan *in vitro* gen kerintangan *mec*A. Pengesanan PCR terhadap jenis SCC*mec* dan penjujukan lekatan universal SCC*mec* (*orf*X) dan tapak sisipan SCC*mec* telah dijalankan, manakala pencirian molekul pencilan dijalankan dengan menggunakan pencirian jujukan multilokcus (MLST).

Hasil kajian ini menunjukkan bahawa semua pencilan adalah gen *nuc* positif dan (10/29) 34% daripada pencilan adalah positif untuk *mec*A. Manakala tiga (3/29) 10.34% pencilan *mec*A positif pada pencilan dan identifikasi awal kini didapati adalah *mec*A negatif. Terdapat penurunan dalam corak kerintangan terhadap antibiotik yang diuji sebelum ini.Pengurangan kerentanan diperhatikan dalam dua (2) pencilan SDG2 dan SH4 yang masing-masing dilihat menjadi rintang terhadap (8/13) 62% dan (7/13) 54% daripada antibiotik yang diuji. Kerintangan terhadap amoksilin, sefoksitin dan oksasilin masing-masing juga diperhatikan dalam (8/14) 57%, (9/14) 64% dan (12/14) 86% daripada pencilan MRSA. Empat (4/14) 29% daripada pencilan adalah rintang vankomisin, doksisiklin dan asid amoksisilin-klavulanik secara fenotip, manakala kerintangan fenotip untuk tetrasiklin dan eritromisin masing-masing juga dilihat dalam (6/14) 43% dan (5/14) 36% pencilan.

Profil gen virulen menunjukkan sejumlah 20/29 (68.9%) dan 14/29 (48%) daripada pencilan adalah positif untuk staphylococcus exotosin bersamaan toksin 1 (*set1*) dan pengekodan gen lipase (*geh*). Selain itu, dua belas 11/29 (37%), dan 7/29 (24.1%) daripada semua pencilan masing-masing adalah positif untuk beta hemolisin (*hlβ*) dan protease V8 (*SspA*). Pencilan juga positif exfoliative A (*etA*) 2/29 (6.9%), hemolisin alfa (hlα) 2/29 (6.9%) dan staphylococcus enterotoxin u (*Seu*) 4/29 (13.8%). Di samping itu, sebanyak 5/29 (17.2%) dan 3/29 (10.34%) daripada pencilan adalah positif untuk faj bawaan Panton valentine leukocidine (PVL) dan toksin exfoliative B yang sering dikaitkan dengan jangkitan MRSA di kalangan masyarakat. Manakala hanya 1/29 (3.4%) daripada pencilan adalah positif untuk gen pengekodan untuk sindrom kejutan toksik toksin-1 (*tst*).

Hasil antibiogram menunjukkan penurunan kerentanan dan rintangan terhadap pelbagai antimikrobial dalam empat pencilan (SEQ5, SEQ7, SEQ10 dan SEQ11) dan kerentanan terhadap semua antibiotik dilihat dalam dua pencilan (SEQ 6 dan SCH4). Sebanyak 9/16 (56.2%) dan 3/16 (18.75%) daripada pencilan S. aureus masing-masing rintang terhadap oksasilin dan sefoksitin. Di samping itu, 6/16 (37.5%) daripada pencilan adalah rintang terhadap amoksisilin, tetrasiklin dan vankomisin dari segi fenotip. Begitu juga, 5/16 (31.25%) daripada pencilan adalah rintang terhadap streptomisin dan linzolide. Seterusnya, frekuensi kerintangan kepada neomisin dan tilmikosin adalah 9/16 (56.2%) dan 8/16 (50%), manakala hanya 3/16 (20%) daripada pencilan adalah rintang terhadap doksisiklin. Begitu juga, 4/16 (25%) daripada pencilan yang digunakan dalam kajian ini adalah rintang terhadap rifampisin, mupirosin dan eritromisin.

Hasil pemindahan *in-vitro* gen *mec*A menggunakan pemplatan cecair kultur campuran ke atas agar Luria-Bertini (LB) yang mengandungi 100 µg / mL, 50 µg / mL dan 30 µg / mL antibiotik eritromisin dan cefpodoksim setiap satu secara berasingan untuk pemilihan sel penderma, tigesiklin dan levoflosaksin untuk pemilihan transkonjugan penerima, mendedahkan dua jenis transkonjugan; transkonjugan penderma yang tidak rintang terhadap eritromisin, cefpodoksim, oksasilin, cefoksitin, amoksisilin dan neomisin. Transkonjugan penerima adalah rintang terhadap levoflosaksin, tigesiklin dan neomisin. Keputusan yang sama diperolehi apabila kultur campuran masing-masing diplatkan ke atas agar LB yang mengandungi 50 µg / mL dan 30 µg / mL.

Namun, tidak ada pertumbuhan apabila SEQ5 dan SEQ1 telah diplatkan ke atas agar LB yang mengandungi 100 µg / mL eritromisin dan tigesiklin tetapi, pertumbuhan diperhatikan apabila kultur yang sama telah diplatkan ke atas agar LB yang mengandungi 50 µg / mL dan 30 µg / mL eritromisin dan tigesiklin. Tetapi, kesemua transkonjugan adalah *mec*A negatif.

Amplifikasi PCR gen rintang terhadap metisilin *mec*A selepas pemplatan cecair campuran ke atas agar yang mengandungi 100 µg / mL mendedahkan bahawa 75% (9) daripada sel-sel transkonjugan penderma dan 58.3% (7) transkonjugan penerima adalah positif untuk *mec*A. Walau bagaimanapun, apabila kultur diplatkan ke atas agar yang masing-masing mengandungi 50 µg / mL dan 30 µg / mL, 61.5% (8) kedua-dua penderma dan penerima transkonjugan adalah positif untuk *mec*A manakala hanya 46.2% (7) dan 41.75% (5) kedua-dua penderma dan penerima transkonjugan adalah *mec*A positif.

Penyaringan jenis SCC*mec* menunjukkan majoriti pencilan MRSA membawa SCC*mec* penjenisan III 7/11 (63%). Manakala hanya 3/11 (27%) dan 1/11 (9.0%) daripada pencilan adalah positif untuk SCC*mec* penjenisan II dan Ive. Pencilan yang membawa SCC*mec* penjenisan II dan III secara universal diiktiraf sebagai MRSA yang diperolehi hospital dan bersaiz lebih besar daripada SCC*mec* jenis IV dan V yang dikaitkan dengan MRSA yang diperolehi masyarakat. Amplifikasi PCR akan tapak sisipan SCC*mec* (*orf*X) dan tapak lekatan menunjukkan bahawa semua pencilan 16/16 (100%) adalah positif bagi gen orfX manakala 7/16 (43.8%) adalah positif bagi *att*B.

Di samping itu, analisis filogenetik menggunakan kaedah kebolehjadian maksimum menunjukkan bahawa pencilan mempunyai tahap keertian yang pelbagai. Pencilan UPM 03, 04, 05, 06 dan 08 yang telah dipencilkan daripada manusia, anjing dan kuda didapati berada di kelompok berbeza daripada pencilan UPM 02, 07 dan 01 yang telah diisolasi daripada manusia dan ayam. Walau bagaimanapun, pencilan UPM 01 dan 07 berkait rapat daripada pencilan UPM 02 walaupun ia telah diisolasi daripada spesies yang berbeza.

Analisis penjenisan jujukan multilocus (MLST) menunjukkan bahawa, analisis eBURST mengumpul sembilan pencilan kepada dua jenis jujukan (ST) ST1 dan ST177. Jujukan jenis ST 1 milik klon kompleks CC5 manakala ST177 adalah unsur tunggal yang melekat pada dua jujukan jenis lain ST3100 dan ST 350. Jujukan (ST3100) telah diperolehi daripada basuhan trakea kuda di UK, manakala ST350 diisolasi daripada tangki susu lembu di USA. Satu-satunya perbezaan antara ST3100 dan ST177 adalah profil alel untuk jujukan jenis 3100 *gmk*, *tpi* dan *yqi*L adalah 7,70 dan manakala untuk ST177 adalah 47, 61 dan 70 dengan perubahan hanya dalam bilangan alel tpi dan yqiL . Begitu juga, ST 350 masing-masing berbeza daripada ST177 pada *glp*F, *tpi* dan *yqi*L. Kesimpulannya, hasil kajian ini melaporkan isolasi pertama ST177 di seluruh Asia. Dengan itu, mengesahkan kegunaan MLST sebagai teknik penjenisan molekul yang kukuh dan cekap. Penemuan dalam penyelidikan ini menunjukkan kesan penyimpanan dalam suhu rendah terlampau kepada gen kerintangan, pembawaan gen virulen dan menunjukkan pemindahan gen rintang

metisilin. Di samping itu, penjenisan SCC*mec* telah dijalankan, tapak sisipan telah dicirikan dan jenis klon terhadap sekumpulan pencilan *S. aureus* telah dijalankan. Hasil kajian juga menyediakan data asas bagi merekabentuk strategi yang berkesan yang dapat mengekang penyebaran MRSA secara global.

Kata kunci: Pemindahan gen, metisilin, penjenisan jujukan multilocus, *Staphylococcus aureus,* Staphylococcal kaset kromosom mec, rintangan, virulen

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I certify that a Thesis Examination Committee has met on 10 April 2017 to conduct the final examination of Asinamai Athliamai Bitrus on his thesis entitled "Characterisation and Genetic Profiling of Antibiotic Resistance Amongst *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* Isolates from Selangor, Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS AND SYMBOLS

AMC	Amoxicillin-Clauvulanic acid
AML	Amoxicillin
ATCC	American Type Culture Collection
*att*B	Bacterial attachment site
agr	Accessory gene regulator
BA	Blood agar
bp	Base pairs
CC	Clonal complex
ccr	Cassette chromosome recombinase
CLSI	Clinical Laboratory Standard Institute
°C	Degree Celsius
DNA	Deoxyribonucleic acid
E	Erythromycin
EDTA	Ethylene diamine tetra acetic
*et*A	Exfoliative toxin A
*et*B	Exfoliative toxin B
*arc*C	Carbamate kinase
*aro*E	Shikimate dehydrogenase
*glp*F	Glycerol Kinase
gmk	Guanylate Kinase
pta	Phosphate acetyltransferase
tpi	Triose phosphate isomerase
*yqi*L	Acetyl coenzymes A acetyltransferase

FOX	Cefoxitin
MLST	Multilocus sequence typing
Mup	Mupirocin
PFGE	Pulse Field Gel Electrophoresis
DLST	Double Locus Sequence typing
DO	Doxycycline
G	Gram(s)
geh	Lipase encoding gene
H	Hour(s)
hlα	Alpha hemolysin
hlβ	Beta hemolysin
Kb	Kilo base
LB	Luria Bertani agar
LZD	Linezolid
mL	Milliliter
mg	Milligram
mec	Methicillin resistance determinants
Min	Minute(s)
MDR	Multidrug resistance
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MSA	Mannitol salt agar
MLST	Multilocus Sequence typing
mPCR	Multiplex Polymerase Chain Reaction
N	Neomycin

NaCl	Sodium chloride
Nuc	Thermostable nuclease
NCCLS	National Committee for Clinical Laboratory Standards
SCC	Staphylococcal cassette chromosome
SCH	Staphylococcus chicken
SDG	Staphylococcus dog
SCT	Staphylococcus cat
SEQ	Staphylococcus equine
SH	Staphylococcus human
SspA	V8 protease
TSA	Tryptone soy agar
*orf*X	Open reading frame of unknown origin
ORSAB	Oxacillin resistance screening agar base
PFGE	Pulsed Field Gel Electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PVL	Panton Valentine Leukocidine
RD	Rifampin
VA	Vancomycin
S	Second(s)
S	Streptomycin
set-1	Staphylococcus exotoxin-like toxin
Seu	Staphylococcal enterotoxin like toxin
tst-1	Toxic shock syndrome toxin
CFU	Colony forming units

Spp.	Species
TAE	Tris-acetate-EDTA
TBE	Tris-borate EDTA
UV	Ultraviolet
UPM	Universiti Putra Malaysia
V	Volt
Taq	*Thermophilus aquaticus*
TGC	Tigecycline
TE	Tetracycline
WHO	World Health Organization
µL	Micro litre
µg	Micro Gram
µM	Micro molar
ST	Sequence type
NA	Nutrients Agar

CHAPTER 1

INTRODUCTION

Staphylococcus aureus is a ubiquitous, versatile and highly adaptive pathogen that colonizes the skin and mucous membrane of the anterior nares, gastrointestinal tracts, perineum, the genitourinary tracts and pharynx (den Heijer *et al.*, 2013). It is the causative agent of a wide range of infections in humans and animals with a significant impact on public health (Luzzago *et al*., 2014). Host specialization, ability to acquire and loss resistance and virulence determinants as well as its potential of being a zoonosis poses a significant public health implication (Holden *et al*., 2004; Saleha and Zunita, 2010; Luzzago *et al.*, 2014).

Clinically, *S. aureus* is the most pathogenic member of the genus staphylococci and the etiologic agent of a wide variety of diseases that ranges from superficial skin abscess, food poisoning and life threatening disease such bacteremia, necrotic pneumonia in children and endocarditis (Shaw *et al*., 2004). In animals, it causes mastitis in cow, botryomycosis in horses, and dermatitis in dogs, septicemia and arthritis in poultry (Zunita *et al*., 2008; Luzzago *et al*., 2014). The severity of the disease is made due to the production of a number of putative virulence factors and possession of antibiotic resistance determinants such as *mecA, VanA* and staphylococcal exotoxins which helps in the initiation of disease process, immune evasion and host tissue destruction (Holden *et al*., 2004; Shaw *et al*., 2004). *Staphylococcus aureus* is one of the most studied members of the coagulase positive staphylococci due to its ability to develop resistance to almost all classes of antibiotics and produce a repertoire of virulence factors (Noto, 2008). This unique feature led to the emergence of highly resistant strains and difficulty in the treatment of *S. aureus* infection.

Antibiotics resistance development in *S. aureus* was first reported in the mid-1940s when a strain of *S. aureus* developed resistance against penicillin by the production of a hydrolyzing enzyme called penicillinase (Basset *et al*., 2011). Since then, *S. aureus* strains resistant to penicillin were widely isolated in cases of bacteremia in the UK and United States. Initially those resistant strains were only isolated from patients and health care personnel where it derives the name nosocomial associated penicillin resistant *S. aureus*. However, resistant strains without apparent identifiable risk factors associated with the hospital strains were later isolated among individuals in the community (Chuang and Huang, 2013). This led to a scenario where increased resistance to penicillin were observed from the late 1940s until the early 1960s when a semi-synthetic homologue of penicillin called methicillin was introduced into the clinics as a strategic drug of choice for the treatment of *S. aureus* infection (Jevon, 1961). However, resistance development to methicillin in *S. aureus* was reported within a year of its introduction as a strategic drug of choice for the treatment of *S. aureus*

Methicillin resistant *S. aureus* (MRSA) arises as a result of the acquisition of a genomic island carrying methicillin resistance determinant, *mec*A. Ever since its discovery in the early 1960s in the UK, methicillin resistant *S. aureus* have gain global notoriety as the most common cause of human, community and livestock infections. Thus, leading to a reduction in the therapeutic value of many critically important antibiotics and prolonging the length of hospital admission (Purrello *et al*., 2011). Over the past decades, MRSA has evolved, and this could probably be due to clonal expansion of previously existing clones and from the conversion of methicillin susceptible *S. aureus* (MSSA) to MRSA. This is a sequel to the acquisition of a methicillin resistance determinants coding for an alternative penicillin binding protein with reduced or less susceptibility to all classes of beta lactams antibiotics (Noto *et al*., 2008).

Methicillin resistant determinant *mec*A is located on a large 25-65kb mobile cassette chromosomes called SCC*mec* which facilitates the horizontal transfer of resistance determinants in and out of the bacteria (Chambers, 1997). In addition, it was observed that the acquisition of *mec*A seems to have occurred independently in a number of *S. aureus* strains, with some clonal lineages having the propensity to colonize specific species, and may be adapted to either humans or animals. Other lineages have less host-specificity, and can infect a wide variety of species (Center for Food Security and Public Health, University of Iowa, 2011). Moreover, transfer and worldwide dissemination of antibiotic resistance determinants among clinically important bacteria and their mobile genetic element have long been observed to have occurred between bacteria of the same and different clusters (Khan *et al*., 2000; Wielders *et al*., 2001; Sabet *et al*., 2014). Some studies have also demonstrated the role of horizontal gene transfer in rapid acquisition and dissemination of antibiotics resistance determinants in *S. aureus* (Khan *et al*., 2000; Barlow, 2009; Sabet *et al*., 2014). The report of Huddleston, (2014) and Lindsay, (2014) further gives credit to this findings where they reported the role of horizontal gene transfer events in ensuring wide genetic variability as well as successful adaptation between bacteria through high transfer frequency of resistance determinants.

The evolutionary origin as well as detailed mechanism of transfer of *mec*A is not fully understood (Barlow, 2009; Hanssen *et al*; 2004). However, studies on *Staphylococcus sciuri* and *Staphylococcus hominis* have revealed the presence of methicillin resistant determinant *mec*A with 88% similarity in sequence of amino acid and 80 % DNA sequence identity to the *mec*A gene of MRSA (Wu *et al*., 1998). In addition, transfer of methicillin resistance have been observed to have occurred both *in vitro* and *in vivo* from *Staphylococcus epidermidis* to *S. aureus* indicating the role of coagulase negative Staphylococci serving as reservoirs of *mec*A (Forbes and Schaber, 1983 ;Khan *et al*., 2000). Furthermore, it has been observed that the most common pathway of gene transfer events in *S. aureus* is generalized transduction, however transformation and conjugative plasmid transfer have been observed to have occurred too (Lacey, 1975 ; Khan *et al*., 2000; Huddleston, 2014; Lindsay, 2014). Similarly, only i*n vivo* conjugative plasmid transfer has been reported to be of significant importance (Khan *et al*., 2000). Conjugative transfer of resistance determinants in *S. aureus* is known to be mediated by conjugative plasmids; however transfer of

resistance determinants in the absence of conjugative plasmids have been reported to have occurred (Forbes and Schaberg,

Most studies on transfer of antibiotic resistance in human *S. aureus* strains have indicated coagulase negative staphylococci (CoNS) as reservoirs of resistant determinants (Forbes and Schaberg 1983; Wu *et al*., 1998; Khan *et al*., 2000). Similarly, studies on antibiotic resistance transfer between human and animal isolates was reported to occur, indicating the importance of resistance transfer in the dissemination and successful adaptation of methicillin resistant S. *aureus* (Khan *et al*., 2000; Sabet *et al*., 2014).

The rapid spread of resistance between bacteria has been one of the factors limiting the production of new antibiotics in order to curb the increasing impact of antibiotics resistance on healthcare cost (Barlow, 2009). The increase in the emergence of highly pathogenic strains of methicillin resistant *S. aureus* and its impact on public health cannot be over emphasizing. There is paucity of information with respect to the profiling of resistance and virulence genes of *S. aureus* and MRSA. Thus, this study was designed to characterize the antibiotics resistance and virulence gene profiles of *S. aureus* and MRSA isolated from animals and humans. It is hypothesized that the carriage rate of antibiotic resistance and virulence gene profiles of *S. aureus* and MRSA is facilitated by gene acquisition.

The objectives of this study were;

1. to determine the persistence of antibiotic resistance in MRSA.
2. to establish the antimicrobial and virulence profile of MRSA and MSSA isolated from animals and humans.
3. to investigate the *in vitro* transfer of antibiotic resistance from MRSA to MSSA.
4. to screen for Staphylococcal cassette chromosome *mec* (SCC*mec*) and to characterized the attachment site (*att*B) of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin susceptible *Staphylococcus aureus* (MSSA) isolate
5. Multilocus Sequence Typing (MLST) of *Staphylococcus aureus* isolated from horse wounds

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