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CHARACTERIZATION OF Staphylococcus aureus ISOLATED FROM SMALL RUMINANTS AND FARM WORKERS IN ALBATINAH SOUTH, THE SULTANATE OF OMAN

SALIM SULAIMAN RASHID ALMAKHLADI

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By

SALIM SULAIMAN RASHID ALMAKHLADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

This thesis is specially dedicated to:

the soul of my father: Sulaiman Rashid Almakhladi

(May Allah have mercy on him)

And

my beloved mother,

My beloved wife,

and my children;

Sulaiman Almakhladi

Anafal Almakhladi

Mohammad Almakhladi

Abrar Almakhladi

Hebba Almakhladi

Abdulhamed Almakhladi

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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

Staphylococcus aureus has long been recognised as an important pathogen causing nosocomial, community and livestock-associated infections worldwide. The prevalence and incidence rates of *S. aureus* and methicillin resistant *S. aureus* (MRSA) infections are on the rise worldwide but differ in the rates from country to another. Similarly, in the Sultanate of Oman there is a significant increase in the incidence of MRSA infection at major hospitals. While there are few numbers of research on the organism in human, there is no information on *S. aureus* and MRSA carriage among livestock and livestock workers in the country to date. This study is designed to investigate the prevalence of nasal carriage of *S. aureus* and MRSA in goat, sheep, and farm workers and subsequently the isolates were characterized using phenotypic and genotypic methods. Antibiotic susceptibility test (AST) was carried out using the minimum inhibitory concentration (MIC). Molecular identification was performed for the presence of the *nuc* gene and *mec*A gene, while molecular typing was done for six *S. aureus* isolates using multilocus sequencing typing (MSLT). The sampling frame for sample collection from all the different Wilayats (Districts) of Al Batinah South Governorate in the Sultanate of Oman was calculated and designed according to the Agricultural Census, Ministry of Agriculture and Fisheries, Sultanate of Oman. A total of 876 nasal swab samples were collected from Barka (n=341), Rustaq (n=209), Musanah (n=190), Nakhal (n=60), Wadi Al Maawil (n=49), Al and Alawaby (n=27). The samples were from goat (n=413), sheep (n=408) and farm workers (n=55). Upon identification and confirmation of the bacteria using PCR detection of *nuc* and *mecA* gene, 18 samples (2.05%) were positive for *S. aureus* which included five isolates (1.21%) from goats, 6 from sheep (1.47%) and 7 from farm workers (12.73%). In total,

18/876 (2.06%) of the livestock and farm workers were positive for *S. aureus*. In goats, 5/413 (1.21%) of the samples were positive for *S. aureus*. In sheep, 6/408 (1.47%) of the samples were positive for *S. aureus*. In farm workers, 7/55 (12.73%) of the samples were positive for *S. aureus*, and 1/7 (14.29%) of them had MRSA. Only one isolate was positive for *mec*A gene confirming the presence of only one MRSA in the tested samples. Minimum Inhibitory Concentration results showed that the isolates were mostly susceptible to the majority of the tested antibiotics which comprised of; benzylpenicillin, oxacillin, gentamicin, tobramycin, levofloxacin, moxifloxacin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, tetracycline, tigecycline, fosfomycin, nitrofurantoin, fusidic acid, mupirocin, rifampicin, trimethoprim/sulfamethoxazole. It was found that 10/18 (55.56%) were resistant to benzylpenicillin, 7/18 (38.89%) were resistant to erythromycin, 5/18 (27.78%) were resistant to clindamycin, 1/18 (5.65%) were resistant to oxacillin and tetracycline and 3/18 (16.67%) were intermediately resistant to levofloxacin. Multilocus sequencing typing (MLST) performed on six isolates revealed from different sequence types (STs); ST1290, ST522, ST2884 and ST6. This study reported that the prevalence of nasal carriage of *S. aureus* in goat, sheep and farm workers is low in Albatinah South. The prevalence of MRSA was also very low. Neither sheep nor goat had MRSA. This study provides the fundamental information on the current status of *S. aureus* and MRSA in the small ruminants and humans having close contact with the animals. It also indicates the need for preventive measures to maintains the low prevalence of *S. aureus* and MRSA in small ruminant and to control the spread of *S. aureus* in the livestock, in Oman.

Keywords: Antibiotic, AST, MLST, MRSA, *Staphylococcus aureus,* Sultanate of Oman

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENCIRIAN *Staphylococcus aureus* DIPENCILKAN DARIPADA RUMINAN KECIL DAN PEKERJA LADANG DI ALBATINAH SELATAN, KESULTANAN OMAN

Oleh

SALIM SULAIMAN RASHID ALMAKHLADI

Mei 2018

Pengerusi : Profesor Madya Zunita Zakaria, PhD
Fakulti : Perubatan Veterinar

Staphylococcus aureus telah lama dikenali sebagai patogen penting yang menyebabkan jangkitan berkaitan nosokomial, komuniti dan ternakan di seluruh dunia. Kadar prevalens dan insiden *S. aureus* dan *S. aureus* tahan metisillin (MRSA) berbeza dari negara ke negara yang lain dan pada umumnya ia meningkat. Begitu juga, di Kesultanan Oman, terdapat peningkatan yang ketara dalam kejadian jangkitan MRSA di hospital-hospital besar. Walaupun terdapat beberapa penyelidikan mengenai organisma bawaan manusia, tidak ada maklumat sama sekali mengenai pembawa *S. aureus* dan MRSA di kalangan pekerja ternakan dan ternakan di negara ini sehingga kini. Kajian ini direka untuk menyiasat prevalens *S. aureus* dan MRSA pada hidung kambing, biri-biri dan pekerja ladang dan seterusnya isolat akan dicirikan menggunakan kaedah fenotip dan genotip. Ujian kerentanan antibiotik (AST) dijalankan dengan menggunakan kepekatan perencat minimum (MIC). Pengenalpastian molekul dilakukan untuk kehadiran gen *nuc* dan gen *mec*A. Sementara itu, analisis molekular dilakukan untuk semua isolat *S. aureus* dengan menggunakan analisis penjujukan multilokus (MSLT). Kerangka pensampelan untuk pengambilan sampel dari semua Wilayats (Daerah) Al-Batinah Selatan Governorate di Sultanate Oman dihitung dan direka mengikut Banci Pertanian, Kementerian Pertanian dan Perikanan, Kesultanan Oman. Sebanyak 876 sampel swab hidung dikumpulkan dari Barka (n = 341), Nakhal (n = 60), wadi Al Maawil (n = 49), Musanah (n = 190), Al Rustaq (n = 209) 27) dan Alawaby (n = 27). Sampel terdiri daripada kambing (n = 413), biri-biri (n = 408) dan pekerja ladang (n = 55). Setelah pengenalpastian dan pengesahan bakteria menggunakan PCR untuk mengesan gen *nuc* dan *mec*A, 18 sampel (2.05%) didapati positif kepada *S. aure*us yang mana termasuk lima isolat daripada

kambing (1.21%), enam daripada bebiri (1.47%) dan tujuh daripada pekerja ladang (12.73%). Secara keseluruhan, 18/876 (2.06%) ternakan dan pekerja ladang adalah positif kepada *S. aureus*. Pada kambing, 5/413 (1.21%) sampel adalah positif kepada *S. aureus*. Pada biri-biri 6/408 (1.47%) sampel adalah positif untuk *S. aureus*. Pada pekerja ladang, 7/55 (12.73%) daripada sampel positif kepada *S. aureus,* dan 1/7 (14.29%) daripadanya mempunyai MRSA. Dalam sampel MRSA yang diuji, hanya satu isolat yang disahkan positif kepada gen *mec*A. Kepekatan perencat minimum (MIC) menunjukkan bahawa isolat tersebut rentan kepada kebanyakan antibiotik yang diuji termasuk benzilpenisillin, oksasilin, gentamisin, tobramisin, levofloksasin, moksifloxacin, erithromisin, clindamisin, linesolid, teikoplanin, vankomisin, tetrasikline, tigesikline, fosfomisin, nutrafurantoin, fusidik acid, mupirosin, rifampisin, trimetoprim/sulfametozole. Didapati bahawa 10/18 (55.56%) isolat adalah rintang terhadap benzilpenisillin, 7/18 (38.89%) rintang terhadap eritromisin, 5/18 (27.78%) rintang terhadap clindamisin1/18 (5.65%) rintang terhadap oksasilin dan tetrasikline manakala 3/18 (16.67%) adalah dipertengahan resintan terhadap levofloksasin. Analisis penjujukan multilokus (MLST) yang telah dilakukan terhadap enam isolat menunjukkan bahawa terdapat empat jenis jujukan yang berbeza (STs): ST1290, ST522, ST2884 dan ST6. Kajian ini menunjukkan bahawa prevalens *S. aureus* pada hidung kambing, biri-biri dan pekerja ladang adalah rendah di Albatinah Selatan. Prevalens MRSA juga sangat rendah. Tiada terdapat MRSA pada biri-biri atau kambing. Kajian ini memberikan maklumat asas mengenai status semasa *S. aureus* dan MRSA pada ruminan kecil dan manusia yang mempunyai hubungan rapat dengan haiwan ini juga menunjukkan keperluan untuk langkah pencegahan untuk memelihara kelaziman rendah *S. aureus* dan MRSA dalam ruminan kecil dan untuk mengawal tahap masa depan *S. aureus* yang tersebar di ternakan. di Oman.

Kata kunci: antibiotik, AST, Kesultanan Oman, MLST, MRSA, *Staphylococcus aureus*

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I certify that a Thesis Examination Committee has met on 11 May 2018 to conduct the final examination of Salim Sulaiman Rashid Almakhladi on his thesis entitled "Characterization of *Staphylococcus aureus* Isolated from Small Ruminants and Farm Workers in Albatinah South, the Sultanate of Oman" 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 Master of Veterinary Science.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science.The members of the Supervisory Committee were as follows:

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Name of Member of Supervisory Committee: Professor Dr. Mohamed Ariff Omar

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Siti Khairani-Bejo

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ABR	Antibacterial resistance
AGISAR	Advisory Group on Integrated Surveillance of Antimicrobial Resistance
agr	Accessory gene regulator
AHL	Animal Health Laboratory
AHRC	Animal Health Research Centre
AMC	Amoxicillin-Clavulanic acid
AML	Amoxicillin
AMR	Antimicrobial resistance
*arc*C	Carbamate kinase
*aro*E	Shikimate dehydrogenase
Asian	Network for Surveillance of Resistant Pathogens
AST	Antibacterial susceptibility testing
ATCC	American Type Culture Collection
*att*B	Bacterial attachment site
BA	Blood agar
bp	Base pairs
BPW	Buffered Peptone Water
CAESAR	Central Asian and Eastern European Surveillance of Antimicrobial Resistance
CA-MRSA	community-acquired MRSA
CC	Clonal complex
ccr	Cassette chromosome recombinase
CDC	US Centres for Disease Control and Prevention
CFU	Colony forming units
CI	confidence interval
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CLSI	Clinical Laboratory Standard Institute
DALY	disability-adjusted life years DR-TB drug-resistant TB
DLST	Double Locus Sequence typing
DNA	Deoxyribonucleic acid
DO	Doxycycline
DST	Drug susceptibility testing
E	Erythromycin
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EDTA	Ethylene diamine tetra acetic
EFSA	European Food Safety Authority
EMB	Eosin Methylene Blue
ESBL	extended spectrum beta-lactamase
ESCMID	European Society of Clinical Microbiology and Infectious Diseases EQA external quality assessment
*et*A	Exfoliative toxin A

*et*B	Exfoliative toxin B
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
flaA	Flagellin A gene
FOX	Cefoxitin
FWD-Net	Foodborne and Waterborne Diseases and Zoonoses Network
G	Gram(s)
GASP	Gonococcal Antimicrobial Surveillance Program
GCC	Gulf Cooperation Council
geh	Lipase encoding gene
GFN	Global Foodborne Infections Network
GISP	Gonococcal Isolate Surveillance Project
*glp*F	Glycerol Kinase
glyA	Serine hydroxyl methyl transferase gene
gmk	Guanylate Kinase
GRASP	Gonococcal Resistance to Antimicrobials Surveillance Program
h	Hour(s) MRSA MRSA
HA- MRSA	Hospital-associated methicillin-resistant *Staphylococcus aureus*
HCA-MRSA	Health care-associated community methicillin-resistant *Staphylococcus aureus*
hlα	Alpha haemolysin
hlβ	Beta haemolysin
ICU	Intensive care unit
IDSR	Integrated Disease Surveillance and Response
Kb	Kilo bas
kOH	Potassium hydroxide
KSA	Kingdom of Saudi Arabia
LA-MRSA	Livestock associated methicillin-resistant *Staphylococcus aureus*
LB	Luria Bertani agar
LOS	length of stay
LZD	Linezolid
MD	mean difference
MDR	Multidrug resistance
mec	Methicillin resistance determinants
mg	Milligram(s)
MIC	Minimum Inhibitory Concentration
Min	Minute(s)
ml	Millilitre
mg	Milligram
MLST	Multilocus Sequence Typing
mPCR	Multiplex Polymerase Chain Reaction
MRSA	methicillin-resistant *Staphylococcus aureus*
MR-VP	Methyl Red- Voges-Proskauer

MSA	Mannitol salt agar
MSSA	Methicillin-susceptible *Staphylococcus aureus*
Mup	Mupirocin
N	Neomycin
NA	Nutrients Agar
NaCl	Sodium chloride
NCCLS	National Committee for Clinical Laboratory Standards
NICD	National Institute for Communicable Diseases
NRL	National Reference laboratory
NS	Non-susceptible
nuc	Thermostable nuclease
C°	Degree Celsius
OIE	World Organization for Animal Health
*orf*X	Open reading frame of unknown origin
ORSAB	Oxacillin resistance screening agar base
P	Probability
PBP	penicillin-binding protein
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
PICO	population, intervention, comparison, outcome
pta	Phosphate acetyltransferase
PVL	Panton Valentine Leukocidine
RD	Rifampin
ReLAVRA	Latin American Antimicrobial Resistance Surveillance Network
RNA	Ribonucleic acid
RR	Relative risk
rRNA	Ribosomal RNA s Second(s)
S	Second(s)
S	Streptomycin
SA	*Staphylococcus aureus*
SCC	Staphylococcal cassette chromosome
SCH	*Staphylococcus* chicken
SCT	*Staphylococcus* cat
SDG	*Staphylococcus* dog
SEQ	*Staphylococcus* equine
set-1	*Staphylococcus* exotoxin-like toxin
Seu	Staphylococcal enterotoxin like toxin
SH	*Staphylococcus* human
SIM	Sulfide-Indole-Motility medium
Spp.	Species
SspA	V8 protease
ST	Sequence type
TAE	Tris-acetate-EDTA
Taq	*Thermophilus aquaticus*
TBE	Tris-borate EDTA
TE	Tetracycline

TGC	Tigecycline
tpi	Triose phosphate isomerase
TSA	Tryptone soy agar
tst-1	Toxic shock syndrome toxin
UAE	United Arab Emirates
UPM	Universiti Putra Malaysia
UV	Ultraviolet
V	Volt
VA	Vancomycin
WHO	World Health Organization
*yqi*L	Acetyl coenzymes A acetyltransferase
χ2	Chi-square Tests
µg	Micro Gram
µL	Micro litre
µM	Micro molar

CHAPTER 1

INTRODUCTION

Staphylococcus aureus is a normal flora of humans and many species of animals (Graveland et al., 2011), and it has long been associated with livestock (Fluit, 2012). In healthy humans, three patterns of *S. aureus* carriage can be distinguished; about 20% of humans are persistent carriers, 60% are intermittent carriers, and approximately 20% almost never carry *S. aureus* (Williams, 1963). The ecological niche of *S. aureus* is the anterior nares. In addition, carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of infection (Williams, 1963). Therefore, it is vital to institute measures that will help prevent the onset of *Staphylococcus* infections (Kluytmans et al., 1997).

Staphylococcus aureus belongs to the family *Staphylococcus*, which is a member of the *Micrococcus* family (Lowy, 1998). It is a gram-positive coccus (1µm) in diameter, non-motile, non-spore forming facultative anaerobe, catalase and coagulase positive and stable in the environment (Quinn et al., 2011). *Staphylococcus aureus* is an opportunistic, high adaptive and an important pathogen that can colonize the nares of farm animals and other animals (Weese & van Duijkeren, 2010). It is often found in the nose of healthy individuals as a commensal bacteria and also on the skin, mucous membranes and glands (Plata et al., 2009).

Staphylococcus aureus is a host specialization bacteria, causing severe animal diseases, such as urinary tract infection, suppurative disease, mastitis, and arthritis (Lee, 2003), and it has the ability to acquire and lose resistance and virulence determinants as well as its potential of being zoonotic. It has been reported in cattle, pets, and chickens and has significant public health implication (Holden et al., 2004; Luzzago et al., 2014). *Staphylococcus intermedius, S. hyicus, S. aureus* are among the most important pathogens in genus *Staphylococcus* (Quinn et al., 2011). *Staphylococcus aureus* is the most pathogenic specie due to acquisition of *mec*A gene which encodes an additional altered low-affinity penicillin-binding protein (PBP2a) (Hackbarth & Chambers, 1989).

Nasal carriage of *S. aureus* is a risk factor for subsequent infection. It is able to cause infections in both humans and a large number of animal species with a large diversity of both benign and lethal infections, because of a wide range of virulence factors that include various toxins known as staphylococcal enterotoxins (SEs) and enzymes (Bal & Gould, 2005), leading to high cost on health care and livestock production.

In human, the pathogen is commonly carried on the skin or in the nasal cavity and can be found in throat, intestine and pubic region of the human body with a significant impact on health (Luzzago et al., 2014). In animals, mastitis caused by *S. aureus* is the major problem in dairy milk production (Fluit, 2012).

The most common strain of *Staphylococcus species*. is one of the most common cause of community-acquired and *S. aureus* nosocomial infections throughout the world (Muthukrishnan et al., 2011). *Staphylococcus aureus* is considered as an important pathogenic bacterium worldwide causing a broad spectrum of diseases such as systemic infections, skin infections, soft-tissue infection and toxic shock syndrome (Oluseun, 2012).

Drug-resistant infections occur when pathogens resist the effects of antimicrobials that render the drugs ineffective (World Bank, 2016). There are many strains of *S. aureus* and the important one which is resistant to antimicrobial drugs is Methicillin-Resistant *S. aureus* (MRSA). MRSA evolved from methicillin susceptible *S. aureus* that is resistant to a family of antibiotics related to penicillin that includes methicillin and oxacillin and other antibiotics caused by the *mec*A gene via acquisition of mobile genetic elements called staphylococcal cassette chromosome *mec* (SCC*mec*), where *mec*A gene is situated on it. This mobile genetic element has two essential components, the ccr gene complex (*ccr*) and the *mec* gene complex (*mec*) (Chongtrakool et al., 2006; Deurenberg et al., 2007b). MRSA have become a leading cause of morbidity and mortality worldwide (Lowy, 1998). Report on multidrug resistant (MDR) organism including *S. aureus* from UAE documented high prevalence in human hospitals (Shibl et al., 2012, Sonnevend et al., 2012). In recent years, emergent multidrug-resistant strains of *S. aureus* (MDRSA) have made treatment of *S. aureus* infections more protracted, more burdensome, and less successful. Food animal production facilities (farms and slaughterhouses) have been identified as a source of human exposure to antibiotic-resistant *S. aureus*, including MRSA and MDRSA. The practices of confinement and administration of antibiotics to animals including non-therapeutically for growth promotion are commonly used in industrial food animal production and provide a reservoir for the selection of novel, antibiotic-resistant bacteria that can be exchanged between animals and humans.

To date, there is no information on *S. aureus* and MRSA carriage among livestock and livestock workers in the Sultanate of Oman. However, there is possible risk of animals to become the source for MRSA in humans.

It was hypothesized that there was a high prevalence of MRSA in the animals and humans in close contact with the animals and there were diverse strains of MRSA in the Sultanate of Oman.

The objectives of this study were:

1- To determine the prevalence of nasal carriage of *S. aureus* and MRSA among livestock (sheep and goat) and the farm workers.
2- To determine antibiogram of the *S. aureus* isolates.
3- To determine the molecular characteristics of *S. aureus* and MRSA using Multi Locus Sequencing Typing (MLST).

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