MOLECULAR CHARACTERISATION OF METHICILLIN-RESISTANT *Staphylococcus aureus* AND *Staphylococcus pseudintermedius* IN CATS, DOGS AND PET OWNERS IN A VETERINARY HOSPITAL

By

AFSHAR MOHAMMAD FARZAD

Thesis Submitted to 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 dedicated to my parents, my brother and sisters
with love, respect and a bunch of memories
Indeed, we belong to Allah and indeed to Him we will return. So, which of the favours of your Lord would you deny? Ar-Rahman [Verse:16].

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 : Professor Zunita Zakaria, PhD
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The genus staphylococcus includes opportunistic pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), which are of public health importance. This study aimed to investigate the presence of MRSA and MRSP bacteria in dogs, cats, and their owners in University Veterinary Hospital, UPM and understand the possible zoonotic transmission of these pathogens. Samples were collected from 150 dogs, 100 cats and 100 pet owners that visited the University Veterinary Hospital, Faculty Veterinary Medicine, UPM, and dogs and cats from animal pounds. The obtained bacterial cultures were phenotypically and genotypically identified using selective agar, a series of biochemical tests and Polymerase Chain Reaction (PCR) for species and methicillin resistance confirmation.

Staphylococcus pseudintermedius and *Staphylococcus aureus* were present in 17 (4.85%), and seven (2%) samples, respectively. One of these isolates (2%) was identified as MRSA, one (1.3%) isolate from pet dogs, one (2%) from pet cats and one (1%) from pet owners were confirmed to be MRSP. Antimicrobial susceptibility tests were performed using the standard disk diffusion method. Two (50%) isolates (one MRSA and one MRSP) showed multidrug resistance, while the other two MRSP isolates showed resistance against one and two antimicrobial agents.

Multilocus sequence typing was performed by amplifying seven housekeeping genes, Sanger's sequencing and using PubMLST for sequence type assignment. ST789 was assigned for *S. aureus* (76_C_M). *S. pseudintermedius* (65_C_F) isolated from a cat was assigned as ST 2296, which is related to clonal complex 45 and the other *S. pseudintermedius* (18_W_M) isolated from a pet owner (ST 2297) is a corresponding sequence type of 2296. A singleton was one of *S. pseudintermedius* (ST 2298) (88_D_F) isolates from a dog.

Staphylococcal protein A (spa) typing for both MRSA and MRSP isolates was performed and typed. Only one MRSA isolate was typable as spa type t091, and the rest of the isolates were not typable. Staphylococcal Cassette Chromosome *mec* (SCC*mec* typing) typing was performed using multiplex PCR. The sole MRSA identified as SCC*mec* type V and MRSP isolates were type II and VII.

The risk factors associated with the spread of staphylococci were investigated using a questionnaire distributed to 125 pet owners. Having close contact with animals like allowing them to lick face and having other animals had a significant association with carriage of staphylococci in this study. However, due to a small number of isolates, other factors were not significantly associated with carriage of our target organisms; therefore, there is a need to further study the risk factors that are associated with carriage of staphylococci the future. In brief, both MRSA and MRSP that were detected in the current study were multidrug resistant and molecularly related to other Southeast Asian countries. The findings from this study have brought new insights into the current status of antimicrobial resistance and molecular characteristics of both *S. aureus* and *S. pseudintermedius* isolated from dogs and cats in Malaysia.

Keywords: Antimicrobial resistance; antimicrobial susceptibility testing; companion animals; public health

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

PENCIRIAN MOLEKULAR *Staphylococcus aureus* RINTANG-METHISILIN DAN *Staphylococcus pseudintermedius* PADA KUCING, ANJING DAN PEMILIK HAIWAN PELIHARAAN DI HOSPITAL VETERINAR

Oleh

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Genus staphylococcus merangkumi patogen oportunistik seperti *Staphylococcus aureus* rintang metisilin (MRSA) dan *Staphylococcus pseudintermedius* rintang metisilin (MRSP) merupakan mikroorganisma-mikroorganisma penting dalam kesihatan awam. Kajian ini bertujuan untuk mengkaji kewujudan bakteria MRSA dan MRSP pada anjing, kucing dan pemiliknya di Hospital Veterinar Universiti, UPM serta memahami kemungkinan penularan zoonotik patogen-patogen ini. Sampel telah dikumpulkan daripada 150 ekor anjing, 100 ekor kucing dan 100 orang pemilik haiwan yang telah datang ke Hospital Veterinar Universiti, Fakulti Perubatan Veterinar, UPM, serta anjing dan kucing dari pusat kurungan haiwan. Kultur bakteria yang diperoleh telah dikenal pasti secara fenotip dan genotip menggunakan agar terpilih, beberapa siri ujian biokimia dan Tindak Balas Berantai Polimerase (PCR) bagi tujuan pengesahan spesies dan kerintangan terhadap methisilin.

Staphylococcus pseudintermedius dan *Staphylococcus aureus* masing-masing terdapat dalam 17 (4.85%) dan tujuh (2%) sampel. Salah satu daripada pencilan tersebut (2%) dikenal pasti sebagai MRSA, satu (1.3%) pencilan daripada anjing peliharaan, satu (2%) daripada kucing peliharaan dan satu (1%) daripada pemilik haiwan peliharaan disahkan sebagai MRSP. Ujian kerentanan antimikrob telah dilaksanakan dengan menggunakan kaedah piawaian resapan cakera. Dua (50%) pencilan (satu MRSA dan satu MRSP) menunjukkan ketahanan terhadap pelbagai agen antimikrob, manakala dua lagi pencilan MRSP menunjukkan ketahanan terhadap satu dan dua agen antimikrob.

Pentipan jujukan multilokus dilaksanakan dengan menguatkan tujuh gen penyelenggara, penjujukan Sanger dan PubMLST bagi tujuan penjujukan jenis umpukan. ST789 telah diumpukkan adalah berkaitan dengan *S. aureus* (76_C_M). *S. pseudintermedius* (65_C_F) yang telah dipencil daripada kucing telah diumpukkan sebagai ST 2296, di

mana ia dikaitkan dengan kompleks klon 45 dan *S. pseudintermedius* (18_W_M) yang dipencil daripada pemilik haiwan (ST 2297) adalah jujukan jenis 2296. Satu *singleton* diperoleh daripada satu *S. pseudintermedius* (ST 2298) (88_D_F) yang dipencil daripada anjing.

Pentipan staphylococcus protein A (spa) untuk kedua-dua pencilan MRSA dan MRSP telah dilaksanakan. Hanya satu MRSA dikategorikan sebagai jenis spa t091 manakala selainnya tidak dapat dikategorikan. Staphylococcal Cassette Chromosome *mec* (penjenisan SCC*mec*) dijalankan dengan menggunakan multipleks PCR. MRSA tunggal yang dikenal pasti sebagai pencilan SCC*mec* jenis V dan MRSP ialah jenis II dan VII.

Faktor-faktor risiko yang berkaitan dengan penularan staphylococci telah dikaji dengan menggunakan soal selidik yang telah diedarkan kepada 125 orang pemilik haiwan peliharaan. Mempunyai hubungan rapat dengan haiwan seperti membenarkan mereka menjilat muka dan mempunyai haiwan lain merupakan perkaitan yang signifikan dengan pembawaan staphylococci dalam kajian ini. Walau bagaimanapun, disebabkan hanya terdapat pencilan yang kecil, faktor-faktor lain tidak dikaitkan secara signifikan dengan pembawaan organisma yang disasar, oleh itu, terdapat keperluan untuk mengkaji lebih lanjut faktor-faktor risiko yang mempunyai kaitan dengan pembawaan staphylococci pada masa hadapan. Secara ringkas, kedua-dua MRSA dan MRSP yang dikesan dalam kajian ini adalah rintang terhadap pelbagai agen antimikrob dan mempunyai kaitan molekular dengan negara-negara Asia Tenggara yang lain. Penemuan daripada kajian ini telah membawa kepada pandangan baharu mengenai status terkini rintangan antimikrob dan ciri-ciri molekular kedua-dua *S. aureus* dan *S. pseudintermedius* yang dipencil daripada anjing-anjing dan kucing-kucing di Malaysia.

Kata kunci: Ketahanan antimikrob; ujian kerentanan antimikrob; haiwan pendamping; kesihatan awam

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This thesis was submitted to the Senate of 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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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

ack	Acetate kinase
yqiL	Acetyl coenzymes A acetyltransferase
purA	Adenylosuccinate synthetase
ATCC	American Type Culture Collection
AMR	Antimicrobial resistance
bp	Base pair
arc	Carbamate kinase
catA	Catechol dioxygenase A
C	Chloramphenicol
da	Clindamycin
CLSI	Clinical Laboratory and Standard Institute
CC	Clonal complex
CoPS	Coagulase-positive staphylococci
CFU	Colony forming unit
CA-MRSA	Community-associated MRSA
CI	Confidence interval
°C	Degree of Celsius
DNase	Deoxyribonuclease
DBKL	Dewan Bandaraya Kuala Lumpur
DLV	Double locus variant
Do	Doxycycline
eBURST	Electronic Based upon related sequence type
enr	Enrofloxacin

E	Erythromycin
erm	Erythromycin ribosomal methylase
JKEUPM	Ethics committee for research involving human subjects
Tris-EDTA	Tris-Ethylenediamine Tetraacetic Acid
Fdh	Formate dehydrogenase
CN	Gentamicin
goeBURST	Global optimized electronic Based Upon Related Sequence Types
glpF	Glycerol Kinase
g	Gravity
gmk	Guanylate Kinase
GyrA	Gyrase A
HCWs	Healthcare workers
cpn60	Heat shock protein
HA-MRSA	Hospital-associated MRSA
HIV	Human immunodeficiency virus
HA	Hyaluronic acid
hysA	Hyaluronidase enzyme A
HCL	Hydrochloric acid
IACUC	Institutional Animal Care and Use Committee
ICU	Intensive care unit
IL	Interleukin
IU	International Unit
kg	Kilo gram
Luk-1	Leukotoxin-1

lnu	Lincosamide nucleotidyltransferase
L	Litre
LA-MRSA	Livestock-associated MRSA
MPSJ	Majlis Perbandaran Subang Jaya
mec	Methicillin resistance determinants
MRSP	Methicillin-resistant *S. pseudintermedius*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MSSP	Methicillin-susceptible *Staphylococcus pseudintermedius*
MIC	Minimum inhibitory concentration
µM	Micro molar
mg	Milli gram
mL	Milli litre
MGE	Mobile genetic elements
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRS	Methicillin-resistant staphylococci
MLST	Multilocus sequence typing
MUSCLE	Multiple Sequence Comparison by Log- Expectation
NETs	Neutrophil extracellular traps
N	Normal
OR	Odds ratio
OPNG	O-nitrophenyl-beta-D-galactopyranoside
ORSAB	Oxacillin-resistant staphylococcal agar base
PVL	Panton-Valentine leukocidin
PBP2a	Penicillin-binding protein

PSMs	Phenol-soluble modulins
pta	Phosphate acetyltransferase
PCR	Polymerase chain reaction
pH	Power of hydrogen
p	Probability
ProT	Prothrombin T
PFGE	Pulsed-field gel electrophoresis
RD	Rifampicin
RpoB	RNA polymerase
SI	*S. intermedius*
SSSS	Staphylococcal Scalded skin syndrome disease
ST	Sequence type
exi	Serine protease
aroE	Shikimate dehydrogenase
SLV	Single locus variant
SSTIs	Skin and soft tissue infections
NaCl	Sodium Chloride
sar	Sodium sulfate symporter
spa	Staphylococcal protein a
S.D	Standard Deviation
SCCmec	Staphylococcal cassette chromosome mec
SE	Staphylococcal enterotoxins
SFP	Staphylococcal food poisoning
SA	*Staphylococcus aureus*

SIG	*Staphylococcus intermedius* group
SP	*Staphylococcus pseudintermedius*
SPSS	Statistical Package for the Social Sciences
Tet M	Tetracycline resistance gene M
nucA	Thermostable nuclease
TSST	Toxic shock syndrome toxin
tuf	Translation elongation factor
SXT	Trimethoprim-Sulphamethoxazole
tpi	Triose phosphate isomerase
TBE	Tris borate ethylene diamine tetra acetic acid
TNF	Tumor necrosis factor
UPM	Universiti Putra Malaysia
UV	Ultra violet
UVH	University Veterinary Hospital
WGS	Whole-genome sequencing
Wbp	Wilbrand factor-binding protein
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Staphylococcus aureus and *Staphylococcus pseudintermedius* are widespread skin and mucous membrane colonisers and may cause opportunistic infections in humans and animals, particularly mammals. *S. aureus* is the most prevalent coagulase-positive staphylococci (CoPS) found in humans, with about 25% of healthy people colonised on a long-term basis(Gómez-sanz et al., 2013). Nevertheless, *S. pseudintermedius* is the most common CoPS found in healthy dogs and cats, though *S. aureus* can also be found in these animals, especially those that live with their owners (20%). Furthermore, the existence of SP in humans who come in contact with these animals should not be overlooked, especially given the possibility of *S. pseudintermedius* misidentification with *S. aureus* or *Staphylococcus. intermedius* (SI). MRSA is detected in a small percentage of healthy canines (0–4%). In this context, it is thought that MRSA in household pet animals arose as a result of MRSA in humans based on typing data and clonal relatedness investigations. Observations on MRSA and methicillin-resistant *S. pseudintermedius* (MRSP) in dogs and cats suggest that these resistant bacteria are becoming pathogens in these animals. Alternatively, since MRSP's incidence in humans appears to be extremely low, a definite zoonotic origin is likely to occur (Gómez-sanz et al., 2013).

Methicillin-resistant bacteria are classified according to their resistance to the antibiotic methicillin which is useful in infection control and surveillance. The existence of MRSA (Methicillin-resistant *S. aureus*) is a significant burden on the public health care system, and precise molecular typing is critical for infection management and MRSA surveillance. SCC*mec* (staphylococcal cassette chromosome *mec*) in MRSA is important because it contains the *mec*A or *mec*C gene and allows staphylococci to adapt to varied situations, such as hospitals, the community, and animals. The chromosomal background, identified by the multilocus sequence type (ST) or clonal complex (CC), and the kind of staphylococcal cassette chromosome *mec* (SCC*mec*) element are both included in the current standard MRSA nomenclature (indicated by Roman numerals I to XIII) (Kaya et al., 2018).

Molecular typing techniques are essential tools for identifying and tracking the primary spreading clones and lineages of MRSA and MSSA (Methicillin-susceptible *Staphylococcus aureus*). Because *S. aureus* will continue to evolve, it is important to watch the changing epidemiology of *S. aureus* using PFGE, MLST, microarray, whole-genome sequencing (WGS), SCC*mec*, and spa typing (staphylococcal protein a) approach in the future. Multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), spa typing, and SCC*mec* typing are the most widely utilized techniques for typing *S. aureus* nowadays (Kumar et al., 2021).

As MRSA became more common among community members, it was perhaps unavoidable that domestic animals, particularly domestic pets, would be exposed to the bacteria. MRSA's appearance in pets has the potential to have serious consequences for both animal and human health. Most animals that contact MRSA are unaffected, as indicated by MRSA colonisation in clinically normal animals; however, opportunistic infections can arise. The most prevalent infections include wound infections, surgical site infections, pyoderma, otitis, and urinary tract infections, however opportunistic infections at numerous other body sites can also occur (Weese & Duijkeren, 2010).

Staphylococcus pseudintermedius, like methicillin-resistant *S. aureus*, can develop resistance to a variety of antimicrobial treatments. MRSP resistance to erythromycin, clindamycin, trimethoprim-sulfamethoxazole, gentamicin, and levofloxacin was discovered in 17 of 57 (30%) dogs at a veterinary clinic in Japan in 2007, with the majority of the canines having received antimicrobial drugs during the previous six months (Sasaki et al., 2007). All of these findings highlight the necessity of managing the presence of SA and SP in the home, with a focus on the risk of human-to-animal bacterial transfer and vice versa (Gómez-sanz et al., 2013).

There have been several reports on the prevalence of MRSA in dogs, cats, environment, horses, and stray cats in Malaysia, and their molecular typing has been investigated (Aklilu et al., 2012; Bitrus et al., 2017; Ghani et al., 2010; Kanagarajah et al., 2017). *Staphylococcus*. *pseudintermedius* and MRSP have also been addressed in pets, abandoned dogs, and cats (Mohamed et al., 2017, 2020). However, the studies on MRSA are limited to veterinary personnel and animals. To the best of our knowledge, a few studies addressed pet owners as a potential carrier of MRSA (Chai et al., 2021, 2022), and there are no studies on pet owners carrying MRSP. In addition, there is no molecular information on the *S. pseudintermedius* that were isolated from those studies. Therefore, it is timely to update the information regarding these two prominent bacteria. Therefore, this study was designed to identify *S. aureus* and *S. pseudintermedius* from the pet and stray dog and cats and owners at the University Veterinary Hospital (UVH), UPM. Isolates were then characterised, and the risk factors associated with the spread of *S. aureus* and *S. pseudintermedius* were determined. The alternative hypotheses were as following:

1. MRSA and MRSP are present in the apparently healthy dogs and cats that visited the University Veterinary Hospital, UPM
2. More than half of the *S. aureus* and *S. pseudintermedius* isolates in the present study are multidrug resistant
3. The methicillin-resistant *S. aureus* and *S. pseudintermedius* strains in dogs and cats that visited the UVH are molecularly related to isolates from other Southeast Asia countries.
4. There is a significant association between the risk factors included in the questionnaire and the carriage of *S. aureus* and *S. pseudintermedius* in pet dogs, pet cats and their owners.

Finally, the specific objectives of this study were to:

1. Isolate and identify MRSA and MRSP from pet dogs and cats and their owners in University Veterinary Hospital and from cats and dogs at animal pounds.
2. Determine the antibiotic-resistant profiles of MRSA and MRSP.
3. Characterise MRSA and MRSP using multilocus sequence typing, staphylococcal protein a typing, and SCC*mec* typing.
4. Determine the risk factors associated with the spread of MRSA and MRSP.

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