



UNIVERSITI PUTRA MALAYSIA

***OCCURRENCE OF *Staphylococcus pseudintermedius* IN DOGS AND
CATS AND THEIR ANTIBIOTIC RESISTANCE PROFILES***

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By

MOHAMED ABDELRAHMAN MOHAMED

**Thesis submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfillment of the Requirements for the Degree of Master of
Veterinary Science**

November 2014

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DEDICATION

This thesis is dedicated to my parents and all members of my family who always supported me and encouraged me to do the best



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Veterinary Science

OCCURRENCE OF *Staphylococcus pseudintermedius* IN DOGS AND CATS AND THEIR ANTIBIOTIC RESISTANCE PROFILES

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November 2014

Chairman: Professor Saleha Abdul Aziz, PhD

Faculty : Veterinary Medicine

Staphylococcus pseudintermedius is an emerging coagulase positive staphylococci and has now become a threat to animal health due to multidrug resistance with very limited therapeutic options particularly in small animals and it requires urgent action to control its spread. The organism is now recognised as a significant pathogen in veterinary medicine causing skin, ear and wound infections. To date, there has been no studies done on *S.pseudintermedius* in dogs and cats in Malaysia. The aims of this study were to determine the presence of *S.pseudintermedius* in dogs and cats, to assess their antibiotic susceptibility and to detect *mecA* and *blaZ* genes in the isolates. Swab samples were taken aseptically from 200 apparently healthy dogs and cats; the sites swabbed were nasal cavity, buccal cavity, rectum and perineal skin. The animals consisted of pet dogs and cats that were brought to the Universiti Veterinary Hospital (Universiti Putra Malaysia) and stray dogs and cats that were kept in an animal shelter. All samples were cultured on selective media, presumptively identified by biochemical tests and *Staphylococcus spp* were confirmed by PCR assay. The isolates were tested for antibiotic resistance using standard disc diffusion method. The total prevalence of *S.pseudintermedius* in this study was 11.5% (23/200), of which 22% (11/50) in stray cats, 18% (9/50) stray dogs, 6% (3/50) pet dogs and 0% in pet cats animals were found positive to *S.pseudintermedius*. Results showed that a high number of stray animals were colonized with *S.pseudintermedius* compared to pet animals; this may be due to high animal density in the shelter, in contact with animals from many sources and the unknown pathogen carriage status of the majority of animals. This study also revealed that nasal cavity is the best site for isolation of the organism (5.5%), followed by buccal cavity (3.5%), skin (2.5%) and

rectum (2.5%) but the difference was statistically not significant. In the second part of the study, *S.pseudintermedius* were tested against 12 antibiotics. The isolates were 100% resistant to penicillin, erythromycin and tetracycline, while they showed 100% susceptible to oxacillin, amoxicillin-clavulanic acid, gentamicin, chloramphenicol, vancomycin, ciprofloxacin, enrofloxacin, cephalexin and rifampicin. The third part of the study was carried out to detect the presence of *mecA* and *blaZ* genes that are responsible for the resistance to methicillin and penicillin, respectively. The *blaZ* gene which codes for β -lactamases production was found in all of the isolates that were resistant to penicillin but not to methicillin. The resistance to three antibiotics showed the isolates as multidrug resistant and poses a significant risk for choosing drug of choice for treatment that may limit therapeutic options. Although the prevalence is low compared to other studies, the presence of *S.pseudintermedius* in dogs and cats could be a potential source of zoonotic infection and it has been reported in veterinary personnel and pet owners. Therefore, it is recommended that contact with infected animals should be reduced, hand washing should be performed after animal contact and risk of transmission from animal to animal should be reduced in veterinary clinics. Antibiotic resistance is a global public health concern and the presence of resistant genes remains an important topic of public health debate, therefore, common mechanisms of staphylococcal antibiotic resistant must be understood clearly and management of resistances should be practised such as prudent antimicrobial usage, including routine treatment options must be based on culture and susceptibility data when suitable to decrease the impact of this problem. This study has provided an overview of *S.pseudintermedius* in stray and pet dogs and cats in Selangor, Malaysia. Although the prevalence is quite low, however, those handling dogs and cats need to take extra precaution. For a better understanding of *S.pseudintermedius* concerning pathogenesis and transmission, more studies on the prevalence and characterization of *S.pseudintermedius* isolated pet animals, from veterinary staff and pet owners are needed.

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

KEHADIRAN *Staphylococcus pseudintermedius* PADA ANJING DAN KUCING SERTA PROFIL KERINTANGAN ANTIBIOTIK

Oleh

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Staphylococcus pseudintermedius adalah organism koagulas positif baru muncul dan kini telah menjadi ancaman kepada kesihatan haiwan berikutan kerintangan yang majemuk rawatan dan dapat menyebabkan yang terhad terutamanya pada haiwan kesayangan dan memerlukan tindakan segera bagi mengawal perebakannya. Organisma tersebut kini dikenalpasti sebagai patogen signifikan dalam perubatan veterinar yang mengakibatkan jangkitan kulit, telinga dan luka. Sehingga kini, tiada kajian *Staphylococcus pseudintermedius* pada anjing dan kucing dilakukan di Malaysia. Tujuan kajian ini adalah bagi menentukan kehadiran *S.pseudintermedius* pada anjing dan kucing, menilai kerentanan antibiotik dan mengesan gen *mecA* dan *blaZ* dalam isolat. Sampel calitan telah diambil secara aseptik daripada 200 ekor anjing dan kucing; kawasan yang dicalit adalah rongga nasal, rongga bukal, rektum dan kulit perineal. Haiwan terdiri daripada anjing dan kucing peliharaan yang telah dibawa ke Hospital Veterinar Universiti (Universiti Putra Malaysia) dan anjing dan kucing terbiar yang disimpan di tempat perlindungan haiwan. Kesemua sampel telah dikultur di media selektif, pengenalan pestian dibarat melalui ujian biokimia dan *Staphylococcus spp* telah disahkan melalui Kaedah PCR. Kerintangan antibiotik isolat telah diuji dengan menggunakan kaedah "standard disc diffusion". Jumlah *S.pseudintermedius* yang diasingkan dalam kajian ini adalah 11.5%, yang terdiri daripada 22% pada kucing liar, 18% anjing liar, 6% anjing peliharaan dan 0% kucing peliharaan yang didapati positif *S.pseudintermedius*. Hasil kajian ini telah mendapati bilangan haiwan liar yang dijangkiti *S.pseudintermedius* adalah lebih tinggi berbanding haiwan peliharaan, hal ini mungkin disebabkan ketumpatan haiwan yang tinggi dipusat perlindungan, entuhan dengan haiwan lain dari pelbagai sumber dan status patogen yang dibawa majoriti haiwan tidak diketahui. Kajian ini juga mendedahkan rongga nasal adalah kawasan yang terbaik bagi pengasingan organisma tersebut (5.5%), diikuti oleh rongga bukka (3.5%), kulit (2.5%) dan rektum

(2.5%) tetapi tiada perbezaan signifikan secara statistik. Pada bahagian kedua kajian ini, *S.pseudintermedius* telah diuji terhadap 12 antibiotik. Isolat didapati 100% rintang terhadap penisilin, eritromisin dan tetrasiklin, dan telah menunjukkan 100% sensitif terhadap oksasilin, amoksisilin-asid klavulanik, gentamisin, kloramfenikol, vankomisin, siproflosaksin, enroflosaksin, sefalaksin dan rifampisin. Bahagian ketiga kajian telah dijalankan bagi mengesan kehadiran gen *mecA* dan *blaZ* yang bertanggung jawab ke atas kerintangan terhadap metisillin dan penisilin. Gen *blaZ* yang mengkod penghasilan β -lactamases telah dikesan pada kesemua isolat yang rintang terhadap penisilin tetapi tidak pada metisillin. Kerintangan terhadap tiga antibiotik menunjukkan isolat adalah kerintang majemuk dan menimbulkan risiko signifikan dalam memilih ubat untuk rawatan yang boleh menghad pilihan terapeutik. Walaupun prevalen adalah rendah berbanding kajian lain, kehadiran *S.pseudintermedius* pada anjing dan kucing boleh menjadi sumber berpotensi jangkitan zoonotik dan ianya telah dilaporkan pada personel veterinar dan pemilik haiwan peliharaan. Oleh itu, adalah disyorkan sentuhan dengan haiwan terjangkit harus dikurangkan, cucian tangan perlu dilakukan selepas sentuhan haiwan dan risiko transmisi dari haiwan ke haiwan perlu dikurangkan di klinik veterinar. Kerintangan antibiotik merupakan kebimbangan kesihatan awam global dan kehadiran gen rintang menjadi topik penting perdebatan kesihatan awam. Oleh itu, mekanisma kerintangan antibiotik *Staphylococcus* perlu difahami dengan jelas dan pengurusan kerintangan perlu dipraktikkan seperti penggunaan antimikrobia dengan berhemah, termasuk rawatan perlu berdasarkan kepada data kultur dan kerentanan yang bersesuaian bagi mengurangkan impak masalah ini. Kajian ini telah memberi gambaran menyeluruh *S.pseudintermedius* terhadap anjing dan kucing liar dan peliharaan di Selangor, Malaysia. Untuk pemahaman yang lebih baik tentang pathogenesis dan transmisi *S.pseudintermedius*, kajian yang lebih terperinci terhadap prevalens dan pencirian *S.pseudintermedius* yang didapati daripada haiwan peliharaan, pegawai veterinar dan pemilik haiwan peliharaan diperlukan.

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I certify that a Thesis Examination Committee has met on 13 November 2014 to conduct the final examination of Mohamed Abdelrahman Mohamed on his thesis entitled "Occurrence of *Staphylococcus pseudintermedius* in Dogs and Cats and Their Antibiotic Resistance Profiles" 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 Science.

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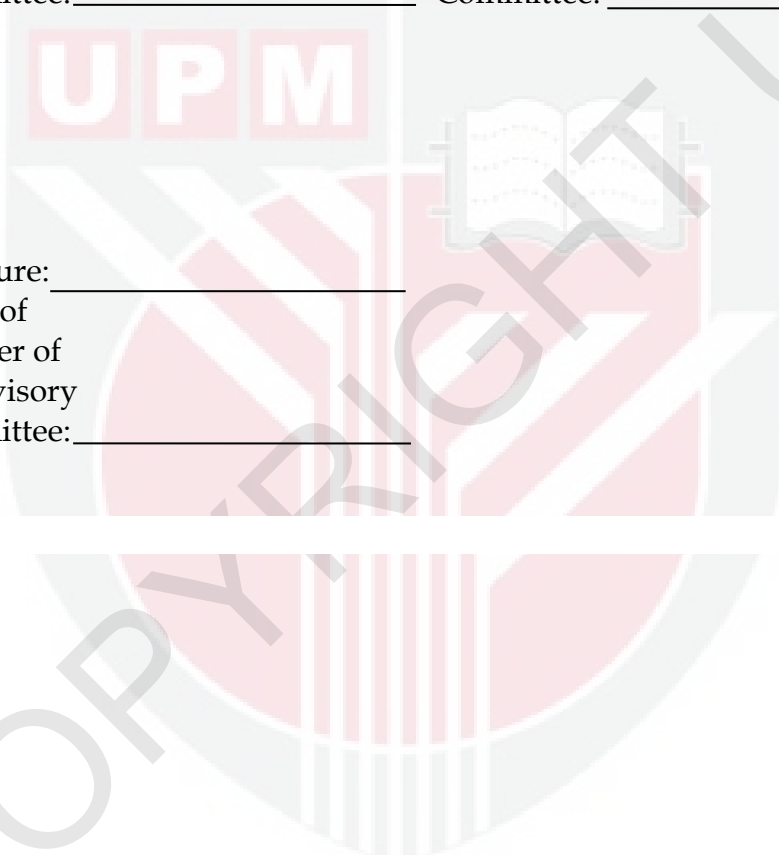


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LIST OF ABBREVIATIONS

AST	Antibiotic susceptibility test
blaZ	beta lactamase Z
balR1	beta lactamase regulatory protein R1
blaR2	beta lactamase regulatory protein R2
bp	Base pairs
CBA	Colombia blood agar
CPS	Coagulase positive staphylococci
CCUG	Culture Collection of the University of Goteborg
CLSI	Clinical and laboratory Standards Institute
CNS	Coagulase negative staphylococci
DNA	Deoxyribonucleic Acid
DBKL	Dewan Bandaraya Kuala Lumpur
mecA	gene coding for penicillin-binding protein 2a
Mg	Microgram
MHA	Muller-Hinton Agar
MIC	Minimum Inhibitory Concentration
ml	Micro liter
MRS	Methicillin resistant staphylococcus
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MRSP	Methicillin resistant <i>Staphylococcus pseudintermedius</i>
MSA	Manitol Salt Agar
ORSAB	Oxacillin Resistance Screening Agar Base
OSA	Oxacillin Salt Agar
PCR	Polymerase Chain Reaction
PBP	Penicillin-binding protein

SCC	Staphylococcal Chromosomal Cassette element
SPCA	Society for the Prevention of Cruelty to Animals
TBE	Tris borate-EDT buffer
TSB	Tryptone soya broth
UPM	Universiti Putra Malaysia
UVH	Universiti Veterinary Hospital



CHAPTER 1

INTRODUCTION

The *Staphylococcus* genus involves groups of organisms that are common commensals on the skin and mucous membranes of humans and a wide range of animal species. Although they are generally found in or on healthy individuals, they are also important causes of opportunistic infections. The clinical significance of *Staphylococcus* species is quite variable, with some being important causes of diseases and others minimally pathogenic. Among the important species of *Staphylococcus* in animals are: *Staphylococcus aureus*, *S. Staphylococcus intermedius* and *Staphylococcus pseudintermedius*.

Up to 2005, *S.intermedius* was considered the most common and clinically important canine *Staphylococcus* species; however it is now known that previous reports on *S.intermedius* in dogs and cats almost certainly were actually the closely related *S.pseudintermedius*. This is because a number of studies reported that *S.intermedius* is actually rare to non-existent in dogs and cats (Sasaki et al., 2007a; Devriese et al., 2005) . As of 2005, *S.pseudintermedius* was described and recognised as a new coagulase-positive *Staphylococcus* species based on 16S rRNA gene sequence analysis of isolates from a cat, a dog, a horse and a parrot (Devriese et al., 2005).

The organism was grouped and classified as a member of a genetically heterogeneous group of bacteria called *Staphylococcus intermedius* group (SIG) which consists of three related species: *S.intermedius*, *S.pseudintermedius* and *S.delphini* (Sasaki et al., 2007b). Since then, *S.pseudintermedius* is recognised as the species which colonizes and causes infections in pet dogs and cats more than the other staphylococcal species (Perreten et al., 2010). A number of animal body sites can be colonized by the organism, especially the nasal passages, oral cavity, skin, and perineal mucosa (Fazakerley et al., 2009; Hartmann et al., 2005).

The majority of pyoderma cases in dogs and a smaller percentage in cats are usually caused by *S. pseudintermedius*. The organism is considered as an opportunistic pathogen that can also cause a variety of other infections such as wound infections, surgical site infections, septic arthritis, osteomyelitis, urinary tract infections, endocarditis, liver abscess, peritonitis, and ocular infections (Zubeir et al., 2007; Cabassu et al., 2007; Holm et al., 2004; Cohn et al., 2003; Gerding et al., 1988). However, little is known about the pathogenesis of this organism (Fitzgerald, 2009), but like other staphylococci, *S.pseudintermedius* produces a number of enzymes such as (coagulase, protease, thermonuclease) and toxins such as (haemolysins, exfoliative toxins and enterotoxins) which are reported may be involved (Fitzgerald, 2009).

Human infection by *S.pseudintermedius* is uncommon and most of the reported infections were due to dog-bite wounds (Lee, 1994); also,

bacteremia (Vandenesch et al., 1995), pneumonia (Daly et al., 1999), ear infections (Tanner et al., 2000; Kikuchi et al., 2004), varicose leg ulcers (Atalay et al., 2005), infected suture lines (Lee, 1994), and brain abscess (Atalay et al., 2005) have been reported as caused by the organism .

The emergence of methicillin resistant *S.pseudintermedius* (MRSP) has been reported and similar to MRSA, MRSP is mediated by the *mecA* gene

(Moodley et al., 2009; Loeffler et al., 2007). Veterinarians and owners of dogs are considered at higher risk of MRSP infection (Weese and van Duijkeren, 2010). Antibiotic resistant staphylococci have been reported to increase alarmingly particularly methicillin resistant staphylococcal which have increased significantly in medical and veterinary health care arenas. Infections related with antibiotic resistant *Staphylococcus* species are increasingly encountered by veterinary practitioners. This presents substantial challenges for successful empiric therapy, limits antibiotic treatment options, and raises concerns of their zoonotic transmission. It is reported that the excessive use of antibiotic agents in pet animals and their close contact with humans lead to these animals as possible sources of antibiotic resistance spread to humans (Guardabassi et al., 2004).

MRSP are found to be resistant to many classes of antibiotic agents apart from resistant to β -lactams (Perreten et al., 2010) and occasionally to four or more classes to be multidrug-resistant strains. Today, MRSP is considered a serious emerging problem in small animal medicine and one that needs crucial action to control its spread (Gronlund , 2009). Due to limited treatment options, there is a challenge in veterinary medicine in the treatment of infections caused by multidrug resistant MRSP (Wettstein et al., 2008). In addition, veterinarians are under pressure not to use antibiotics that are important in human medicine and needs careful evaluation of extra-label drug use in veterinary medicine (Weese and van Duijkeren, 2010). There is great variation in the prevalence of MRSP according to different geographical locations.

In Malaysia, there is a lack of study on *S.pseudintermedius* and MRSP in cats and dogs. This study was conducted to fill this lack of information. At the same time, to study if there is any difference in infection rates between stray and pet animals.

Problem Statement (justification)

Staphylococcus pseudintermedius is common colonizer of the skin and mucosal surfaces of dogs and cats, but also important opportunistic pathogen that causes numerous infectious diseases. The organism has also been recognized as a zoonotic pathogen in the last few years.

For several decades, there was dramatic increase of antibiotic resistance worldwide particularly MRSA and recently MRSP due to several factors that contribute significantly to the current situation.

Hypothesis

1. There is high prevalence of *Staphylococcus pseudintermedius* in dogs and cats
2. *Staphylococcus pseudintermedius* isolates carry at least one resistant gene (*mecA* or *blaZ*).

Objectives

The objectives of this study were:

1. to determine the presence of *S.pseudintermedius* in dogs and cats.
2. to compare the infection rates in stray and pet animals and associated risk factors.
3. to detect the presence of resistant genes (*mecA* and *blaZ*) in the isolates.



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