ปัจจัยที่มีผลต่อการคงอยู่ของเชื้อในกลุ่มเมธิซิลิน รีซิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค บนผิวหนังสุนัข และ ในโรงพยาบาลสัตว์

นางสาวพรรณพิชญา ฟุ้งวิทยา

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
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THE FACTORS EFFECT TO EXISTENCE OF METHICILLIN-RESISTANT COAGULASE POSITIVE STAPHYLOCOCCI (MRCoPS) ON DOG SKIN AND AN ANIMAL HOSPITAL

Miss Punpichaya Fungwithaya

จุฬาลงกรณ์มหาวิทยาลัย
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A Dissertation Submitted in Partial Fulfillment of the Requirements
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Department of Veterinary Pathology
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Thesis Title	THE FACTORS EFFECT TO EXISTENCE OF METHICILLIN-RESISTANT COAGULASE POSITIVE STAPHYLOCOCCI (MRCoPS) ON DOG SKIN AND AN ANIMAL HOSPITAL
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พรรณพิชญา ฟุ้งวิทยา : ปัจจัยที่มีผลต่อการคงอยู่ของเชื้อในกลุ่มเมธิซิลิน รีซิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค บนผิวหนังสุนัข และ ในโรงพยาบาลสัตว์ (THE FACTORS EFFECT TO EXISTENCE OF METHICILLIN-RESISTANT COAGULASE POSITIVE STAPHYLOCOCCI (MRCoPS) ON DOG SKIN AND AN ANIMAL HOSPITAL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. น.สพ. ดร. ณุวีร์ ประภัสระกูล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. นพ. ดร. ชาญวิทย์ ตรีพุทธรัตน์, 168 หน้า.

เชื้อในกลุ่มสตาฟฟิลโลคอคไคทที่สร้างเอนไซม์โคแอกกูเลส เป็นเชื้อแบคทีเรียก่อโรคที่พบมากในโรงพยาบาลสัตว์ เชื้อในกลุ่มเมธิซิลิน รีซิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค เป็นเชื้อดื้อยาที่มีความสำคัญในการก่อโรคจากสัตว์สู่คนที่มีวิชาชีพหรือพฤติกรรมที่เกี่ยวข้องกับสุนัข ในปัจจุบันมีการให้ความสำคัญกับการกระจายและอุบัติการณ์ที่เพิ่มขึ้นของเชื้อในกลุ่มเมธิซิลิน รีซิสแตน สตาฟฟิลโลคอคไคที่สร้างเอนไซม์โคแอกกูเลส ในโรงพยาบาลสัตว์ ในการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษา 1.) เพื่อตรวจหาการเพิ่มขึ้นของเชื้อเชื้อในกลุ่มเมธิซิลิน รีซิสแตน สตาฟฟิลโลคอคไคที่สร้างเอนไซม์โคแอกกูเลส ที่เพิ่มขึ้นหลังการรักษาด้วยยา เซฟฟาแลคซิน โมโนไฮเดรท 2.) เพื่อประเมินการกระจายของเชื้อในกลุ่มเมธิซิลิน รีซิสแตน สตาฟฟิลโลคอคไคที่สร้างเอนไซม์โคแอกกูเลส ในโรงพยาบาลสัตว์เพื่อการเรียนการสอน และ 3.) เพื่อประเมินประสิทธิภาพในการฆ่าเชื้อแบคทีเรียของยาฆ่าเชื้อ โพวิโดน ไอโอดีน และ คลอเฮกซิดีน กลูโคเนต ใน ไอโซโพพานอล ต่อเชื้อในกลุ่มเมธิซิลิน รีซิสแตน สตาฟฟิลโลคอคไคที่สร้างเอนไซม์โคแอกกูเลส ในห้องปฏิบัติการเชื้อเมธิซิลิน รีซิสแตน สตาฟฟิลโลคอคไคที่สร้างเอนไซม์โคแอกกูเลส สามารถแยกได้จากสุนัขทุกตัวที่ได้รับยาเซฟฟาแลคซิน โมโนไฮเดรท ในสัปดาห์แรก (n=38) และพบการคงอยู่ของเชื้อดื้อยาเมธิซิลิน รีซิสแตน สตาฟฟิลโลคัส ซูสอินเตอร์มิดิเดียส ต่ออีกมากกว่า 6 เดือนภายหลังจากหยุดการรักษา (n=10) เชื้อเชื้อ เมธิซิลิน รีซิสแตน สตาฟฟิลโลคัส ซูสอินเตอร์มิดิเดียส เป็นเชื้อที่มีการระบาดในโรงพยาบาลในวงกว้าง ซึ่งพบพื้นของคลินิกโรคผิวหนังมากที่สุด รองลงมาคือคลินิกสูติกรรม และยังพบในอุปกรณ์ทางการแพทย์ที่ได้รับการสัมผัสบ่อย ๆ เช่น โต๊ะตรวจ และเครื่องช่วยหายใจ เวลาและความเข้มข้นที่เหมาะสมของโพวิโดนไอโอดีนและคลอเฮกซิดีน กลูโคเนตในไอโซโพพานอล ที่สามารถทำลายเชื้อแบคทีเรีย คือ 0.1% ที่เวลา 45 วินาที และ 0.5% ที่เวลา 15 วินาที ตามลำดับ จากการศึกษานี้ปัจจัยที่ทำให้มีการระบาดของเชื้อดื้อยา เมธิซิลิน รีซิสแตน สตาฟฟิลโลคอคไคที่สร้างเอนไซม์โคแอกกูเลส ในโรงพยาบาลสัตว์คือการคงอยู่ของเชื้อดื้อยาภายหลังการรักษาโรคผิวหนังอักเสบ และความไม่เหมาะสมของการจัดการในการฆ่าเชื้อในสิ่งแวดล้อมและอุปกรณ์ การเลือกชนิดของยาฆ่าเชื้อและมีการใช้อย่างถูกต้องจะช่วยลดคววามเสี่ยงจากการระบาดของเชื้อดื้อยาได้

ภาควิชา พยาธิวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา พยาธิชีววิทยาทางสัตวแพทย์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5375956031 : MAJOR VETERINARY PATHOBIOLOGY
KEYWORDS: COAGULASE-POSITIVE STAPHYLOCOCCI / METHICILLIN-RESISTANT COAGULASE-POSITIVE STAPHYLOCOCCI / DISTRIBUTION / POVIDONE-IODINE / CHLORHEXIDINE GLUCONATE IN ISOPROPANOL

PUNPICHAYA FUNGWITHAYA: THE FACTORS EFFECT TO EXISTENCE OF METHICILLIN-RESISTANT COAGULASE POSITIVE STAPHYLOCOCCI (MRCoPS) ON DOG SKIN AND AN ANIMAL HOSPITAL. ADVISOR: ASSOC. PROF. DR. NUVEE PRAPASARAKUL, D.V.M., Ph.D., CO-ADVISOR: ASSOC. PROF. DR. CHANWIT TRIBUDDHARAT, M.D.,Ph.D., 168 pp.

Coagulase-positive staphylococci (CoPS) is common pathogenic bacteria distributing in veterinary hospitals. Methicillin-resistant coagulase-positive staphylococci (MRCoPS) is the important resistant trait and can cause zoonotic infection in human associated with dogs. In recently years, the distribution and contamination of MRCoPS in veterinary hospitals have been increasingly concerned. In this study, we proposed 1.) to determine the increasing of MRCoPS following the routine oral treatment by cephalexin monohydrate 2.) to investigate the distribution of MRCoPS in a veterinary school hospital and 3.) to determine the bactericidal efficacy of povidone-iodine (PI) and chlorhexidine gluconate in isopropanol (CGI) against MRCoPS, *in vitro.* MRCoPS were isolated from all dogs treated with cephalexin (n=38) at the first week administration and methicillin-resistant *S. pseudintermedius* (MRSP) had persisted until at least 6 months after drug-off date (n=10). MRCoPS were the most frequent at dermatological clinic followed by gynecological clinic especially on surfaces of floor and high touch sites such as examination table and rebreathing circuit. The optimal time and concentration of PI and CGI for bactericidal effect were 0.1% for 45s and 0.5% for 15s, respectively. In this study, the factors associated the distribution of MRCoPS in veterinary hospital were the consequence of dermatitis treatment causing persistence of bacterial resistance and certain inappropriate hygienic management in equipment and environment. To select the right antiseptic at optimal time and concentration could reduce risk of bacterial resistant outbreak in veterinary hospital.

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Department:	Veterinary Pathology	Student's Signature	.....
Field of Study:	Veterinary Pathobiology	Advisor's Signature	.....
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List of Abbreviations

mA	=	Milliampere
ATCC	=	American type culture collection
bp	=	Base pair
°C	=	Degree Celsius
CG	=	Chlorhexidine gluconate
CGI	=	Chlorhexidine gluconate in isopropanol
CLSI	=	Clinical laboratory standard institute
Cn	=	Gentamicin
CoPS	=	Coagulase-positive staphylococci
CU-ACUC	=	Chulalongkorn university animal care and use committee
Da	=	Clindamycin
DNA	=	Deoxyribonucleic acid
Do	=	Doxycycline
E	=	Erythromycin
EDTA	=	Ethylene diamine tetra acetic acid
Enr	=	Enrofloxacin
h	=	Hour
HCl	=	Hydrochloric acid
L	=	Liter
mg	=	Milligram

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List of Abbreviations (Continue)

MIC	=	Minimal inhibitory concentration
min	=	Minute
ml	=	Milliliter
mM	=	Millimolar
MRCoPS	=	Methicillin-resistant coagulase-positive staphylococci
MSCoPS	=	Methicillin-susceptible coagulase-positive staphylococci
MRSA	=	Methicillin-resistant *Staphylococcus aureus*
MSSA	=	Methicillin-susceptible *Staphylococcus aureus*
MRSP	=	Methicillin-resistant *Staphylococcus pseudintermedius*
MSSP	=	Methicillin-susceptible *Staphylococcus pseudintermedius*
MRSSc	=	Methicillin-resistant *Staphylococcus schleiferi* subsp. *coagulans*
MSSSc	=	Methicillin-susceptible *Staphylococcus schleiferi* subsp. *coagulans*
ND	=	Non-detectable
NT	=	Non-typeable
PI		Povidone-iodine
SIG	=	*Staphylococcus intermedius* group
Sxt	=	Co-trimoxazole
TSA	=	Tryptic soy agar

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List of Abbreviations (Continue)

U	=	Unit
VP	=	Voges-Proskauer

CHAPTER 1

THE RELATION BETWEEN ALL MANUSCRIPTS IN THE THESIS

The factor effect to existent of MRCoPS on dog skin and an animal hospital composed of antibiotic treatment, cleaning management and antiseptics. The first factor effect to existent of MRCoPS were revealed on “Association between cephalexin administration and the emergence of methicillin-resistant coagulase-positive staphylococci (MRCoPS) in dogs” and “Nasal carriage of methicillin-resistant *S. pseudintermedius* in dogs treated with cephalexin monohydrate”, respectively. The study showed MRCoPS and MRSP had increased at 1st week after treatment and could prolong persistent on dog skin up to 6-12 months after treatment. The second effect to existent of MRCoPS were revealed on “Prevalence and Molecular typing of Methicillin-Resistant *Staphylococcus pseudintermedius* (MRSP) in a Veterinary Teaching Hospital in Thailand” and “Distribution of methicillin-resistant coagulase-positive staphylococci (MRCoPS) in surgical unit and cystotomy operation sites at a veterinary teaching hospital, Thailand”, respectively. With unsuitable cleaning management, this study showed the high distribution and contamination of MRCoPS in veterinary teaching hospital in Thailand. The third effect to existent of MRCoPS were revealed on “Bactericidal effects of povidone-iodine and chlorhexidine gluconate against coagulase-positive staphylococci”. This study showed appropriate time and concentration can eliminate MRCoPS on dog skins.

All of manuscripts in partial fulfillment of the requirements for the degree of Doctor of Philosophy program in Veterinary Pathobiology.

IMPORTANCE AND RATIONALE

Coagulase-positive staphylococci (CoPS) are common resident and transient bacteria on animal and human (Chanchaithong and Prapasarakul, 2011). In general, these pathogens pose pyoderma, otitis external and surgery site infections (SSIs) in veterinary and human hospital (May et al., 2005; Bergstrom et al., 2012a). *Staphylococcus pseudintermedius* (*S. pseudintermedius*), *S. aureus* and *S. schleiferi* subsp. *coagulans* are the three major members of CoPS in domestic pets (Chanchaithong et al., 2014). In veterinary practitioner, *S. pseudintermedius* are the major population on dog skins (Beck et al., 2012). These opportunistic bacteria play a role in skin infection in dogs (Weese and van Duijkeren, 2010). Additionally, these pathogens have been reported to be zoonotic bacteria in humans (Riegel et al., 2011). *S. aureus* pose nosocomial infection in human and veterinary hospitals (Hsueh et al., 2004; Bergstrom et al., 2012b). Even if *S. aureus* have lower reports than *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* in veterinary hospitals (Sasaki et al., 2007a; Chanchaithong et al., 2014), these pathogens are important to be zoonotic bacteria in human and animal (Weese and van Duijkeren, 2010). Methicillin-resistant coagulase-positive staphylococci (MRCoPS) are CoPS that carried *mecA* gene on their

chromosome. In recent years, this resistant-trait have been increasingly concerned in human hospitals (Hsueh et al., 2004).

Cephalexin monohydrate is antibiotic drugs that is recommended for canine dermatitis treatment (Hillier et al., 2014). With antibiotic treatment, sensitive-trait was eliminated, while resistant-trait was selected (Andersson and Hughes, 2010). Then, MRCoPS are commonly discovered on patients associated with antibiotic treatment. Methicillin-resistant *S. aureus* (MRSA) are zoonotic bacteria in human (Leonard and Markey, 2008). In previous reports, these pathogens could be discovered from human, pets and environmental surfaces in households and hospitals (Bergstrom et al., 2012b; Davis et al., 2012). MRSA colonize on nasal cavities of humans and animals (Weese and van Duijkeren, 2010). Additionally, MRSA-positive pets can act as carrier (van Duijkeren et al., 2004; Rutland et al., 2009) and transferred to humans leading to re-current infection (Ferreira et al., 2011). Methicillin-resistant *S. pseudintermedius* (MRSP) are discovered on dog with underlined skin disease (Beck et al., 2012). Even if MRSP have low reported to be a zoonotic pathogen, MRSP ST71 were known as zoonotic infection leading to life-threatening agents in human (Stegmann et al., 2010). In veterinary practice, these pathogens were yielded from many surfaces such as weight scales, cages and treatment tables (Sasaki et al., 2007a; van Duijkeren et al., 2011). Additionally, MRSP could be discovered on human associated with dogs such as veterinarians and owners (Chanchaithong et al., 2014). Methicillin-resistant *S. schleiferi* subsp. *coagulans* (MRSSc) have been low reported in human and veterinary hospitals.

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However, MRSSc were reported to be minor population in the veterinary hospitals in Thailand (Chanchaithong et al., 2014). Hence, the distribution of MRSSc have been concerned as same as MRSP and MRSA.

In veterinary hospitals, the remaining of MRCoPS play role of recurrent infection especially operative patients (Bergstrom et al., 2012b). The monitoring and controlling of MRCoPS distribution must be emphasized in every hospitals. MRCoPS have been reported on many surfaces in veterinary hospitals such as doors, floors, mobile phones and keyboards (Bender et al., 2012; Bergstrom et al., 2012b). To limit the distribution, chlorhexidine gluconate in isopropanol (CGI) and povidone-iodine (PVP-I) which basically antiseptic in animals were the encrypted keys. However, the appropriate time and concentration of CGI and PI to *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* have not been reported.

LITERATURE REVIEW

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COAGULASE-POSITIVE STAPHYLOCOCCI (CoPS)

Staphylococci are Gram-positive cocci bacteria isolated from animal and human (Davis et al., 2012). CoPS is staphylococci associated with enzyme coagulase. These bacterium are known as a cause of nosocomial infection, pyoderma and post-surgical wound infection (Leonard and Markey, 2008). The seven members of CoPS composed of *S. aureus*, *S. delphini*, *S. hyicus, S. intermedius*, *S. lutrae*, *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* (Sasaki et al., 2007b), but only *S. aureus*, *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* play role as normal

flora on domestic animal (Bannoehr and Guardabassi, 2012; Beck et al., 2012; Chanchaithong et al., 2014). *S. pseudintermedius* are the major resident on dog skins (Bannoehr and Guardabassi, 2012). The carriage sites of these pathogens are nasal cavities (31%), oral mucosa (57%), perineum (52%) and groin (23%) (Bannoehr and Guardabassi, 2012). In recent years, the distribution of *S. pseudintermedius* have been increasingly reported in veterinary hospitals (Sasaki et al., 2007a; van Duijkeren et al., 2011). However, zoonosis infection causing of *S. pseudintermedius* have lower than *S. aureus* (Stegmann et al., 2010). *S. aureus* are recognized as zoonosis bacteria in human and animal (Rutland et al., 2009). In general, these microorganism act as opportunistic pathogen in humans, pigs and horses (Leonard and Markey, 2008) but have a low reported on the small animal such as dogs and cats (Chanchaithong et al., 2014). In recent years, *S. schleiferi* subsp. *coagulans* have been increasingly reported in veterinary practitioners (Riegel et al., 2011). These bacteria were minor population of the dog skin and posed otitis externa in dogs (May et al., 2005; Beck et al., 2012).

METHICILLLIN-RESISTANT COAGULASE-POSITIVE STAPHYLOCOCCI (MRCoPS)

MRCoPS have been discovered from human patient since 1961 (Jevons, 1961). After these pathogens were discovered, they were concerned as nosocomial problem in human and veterinary hospitals (Jevons, 1961; Leonard and Markey, 2008). To identify MRCoPS, the present of *mecA* gene on staphylococci chromosome must be confirmed (Strommenger et al., 2003). This gene is expressed to penicillin-binding

protein 2a (PBP2a) which has low-affinity to bind with beta-lactam ring in beta-lactam drugs (**Figure 1.1**). Hence, MRCoPS is not eliminated by beta-lactam antibiotic (Foster, 2004).

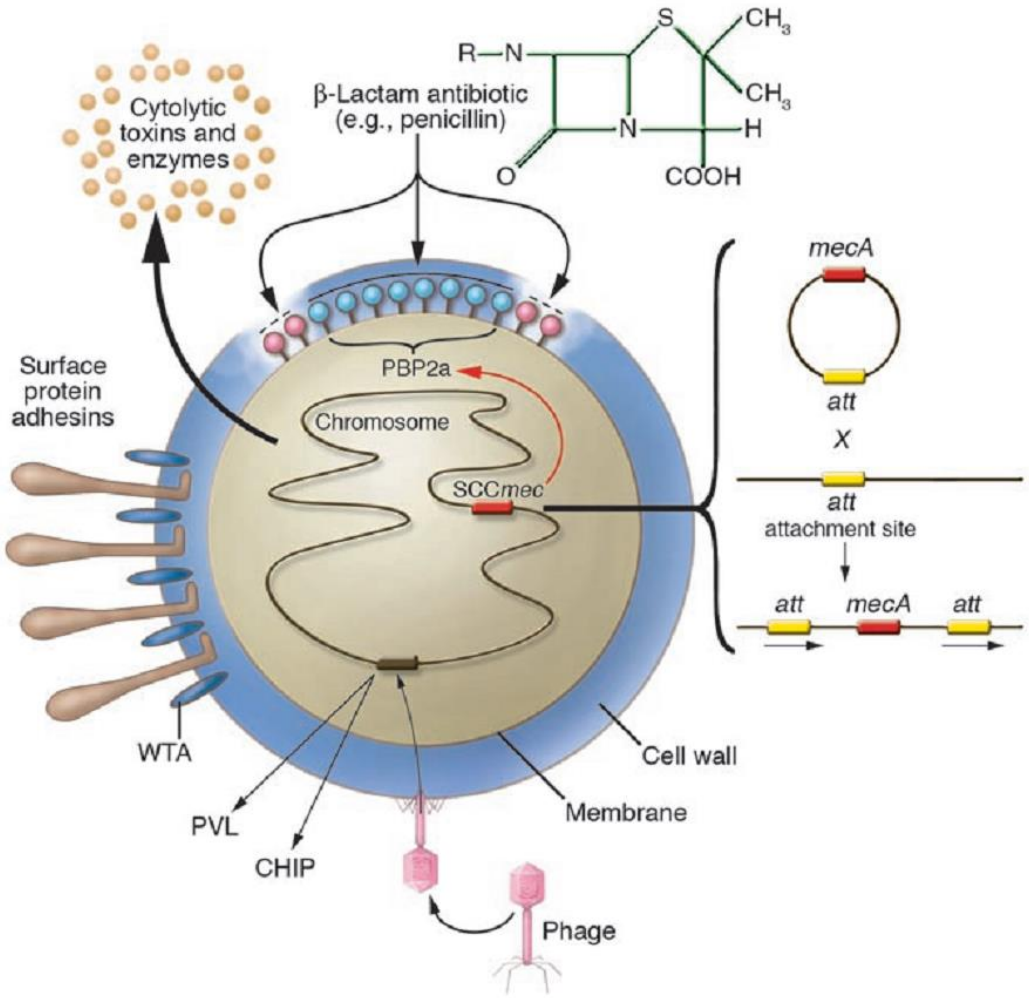

Figure 1.1 the structure of methicillin-resistant staphylococci (Foster, 2004)
WTA = wall teichoic acid, PLV = Panton-Valentine leucocidine and CHIP = gene on phage

The *mecA* gene is contained in "Staphylococcal Cassette Chromosome *mec*, SCC*mec*" which is the large mobile genetic elements (Ito et al., 2001; Foster, 2004). This mobile genetic element, which frequently acts as multi-drug resistance (MDR), is

able to interchange intra- and interspecies of staphylococci (Ender et al., 2004; Lloyd, 2012). To date, SCC*mec* is classified into XII classes (Wu et al., 2015) associated with pseudo-SCC*mec* elements (Perreten et al., 2013; Monecke et al., 2015). In veterinary practitioner, the first MRSA were discovered on mastitis cows in 1972 (Devriese et al., 1972). The prevalence of MRSA on dog skins was very low (less than 2%) (Hanselman et al., 2005). The prevalence of MRSP and MRSSc was very high (70-90%) in veterinary hospitals and dog skins (May et al., 2005; Sasaki et al., 2007a).

ANTIBIOTIC PRESSURE AND FITNESS COST

In general, bacteria survive and proliferate in the suitable environmental condition. Under antibiotic treatment (i.e. beta-lactam antibiotic, aminoglycoside and fluoroquinolone), most population of antibiotic-susceptible bacteria are inhibited or killed, while a resistant subset of organism survives (**Figure 1.2**). Finally, the population, which are selected under antibiotic pressure, proliferate and become predominant replacing susceptible-trait (Mulvey and Simor, 2009).

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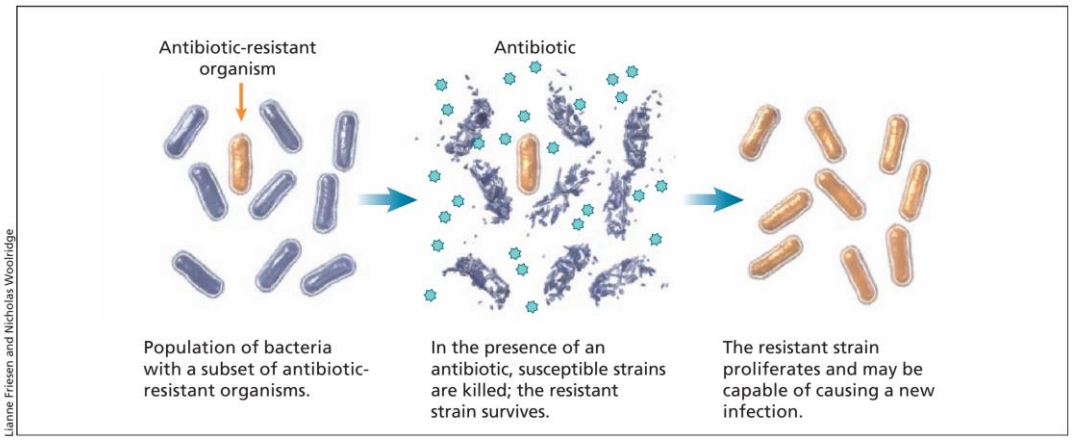

Figure 1.2 Effect of selective antibiotic pressure in bacteria (Mulvey and Simor, 2009).

The alterations in the microbiota are composed of four main factors; i) the spectrum of the agent, ii) dosage and duration of treatment, iii) route of administration and iv) the pharmacokinetic and pharmacodynamic properties of the agent (Jernberg et al., 2010). After drug-off, the ecology balance of normal flora bacteria are started (**Figure 1.3**). In Figure 1.3, it showed the population of bacteria in colon at pre-treatment, during treatment and drug-off. The increase in resistant bacteria are discovered after antibiotic treatment, while some of susceptible bacteria become resistant bacteria for survive in antibiotic pressure. After drug-off, the susceptible-trait that protected from antibiotic exposure in the mucin layer or in grooves between the villi are re-colonization in colon. In veterinary practices, MRSP were emphasized for remaining on the dog skin after drug-off. The previous reports revealed that some dogs could carry MRSP on their dog skins more than 14-16 months after drug-off (Beck et al., 2012; Windahl et al., 2012). However, no reports revealed the re-colonization of methicillin-susceptible *S. pseudintermedius* (MSSP).

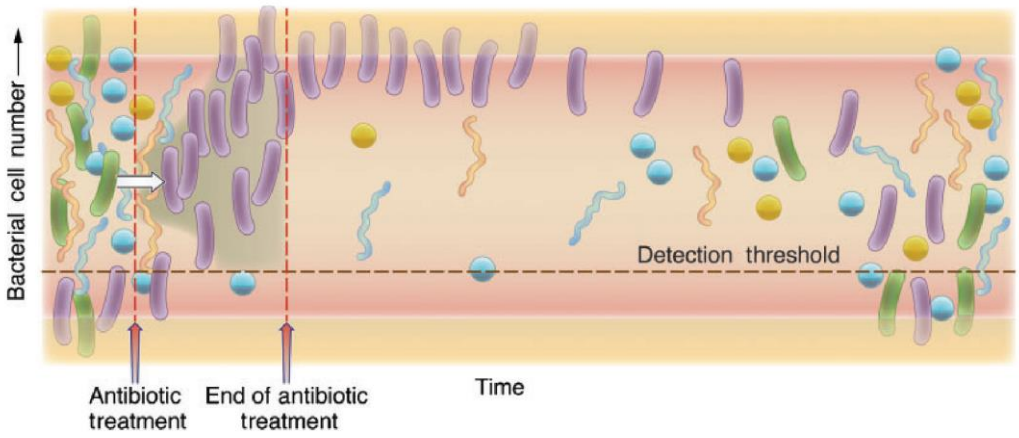

Figure 1.3 Representation of the impact of antibiotic administration on the bacterial community of the colon.

Purple rods = resistant bacteria; Green rods = susceptible bacteria; White arrow = transfer or mutation events; Yellow shading = mucin layer

THE DISTRIBUTION OF MRCoPS IN HOSPITAL

Staphylococci can persist on variable temperatures, pH, humidity, and sunlight exposure. In dry environment, staphylococci can survive over six months (Wagenvoort et al., 2000). The remaining of staphylococci have been reported on the veterinary hospitals and households especially human and animal contact surfaces (Weese, 2010; Davis et al., 2012; Hamilton et al., 2012). In veterinary hospital, examination tables, weight scales and floors are the major sources of MRCoPS (Aksoy et al., 2010; Hamilton et al., 2012). MRSP are the major population of MRCoPS in the veterinary environments. In the previous study, the clonality relationship between MRSP on dog and environment have been reported (Bergstrom et al., 2012a). The remaining bacteria posed re-current infection in veterinary hospital (Bergstrom et al., 2012b). Human is

the other important sources of MRCoPS in the veterinary hospitals (Weese and van Duijkeren, 2010). Both of MRSA and MRSP were discovered on veterinarians and owners' nasal cavities (Chanchaithong et al., 2014). Hence, MRCoPS in human associated with animal are emphasized to be a cause of the transmission of MRCoPS to animal patients (Bergstrom et al., 2012b; Chanchaithong et al., 2014).

POVIDONE-IODINE (PVP-I)

Polyvinyl pyrrolidone (PVP) and iodine are important sources of povidone-iodine (PVP-I) (Eel and Sebille, 1961; McDonnell and Russell, 1999). In the water, PVP-I is described into eight reactions (**Table 1.1**).

Table 1.1 Iodine-containing species in aqueous iodine solutions: Reactions and equilibria (Rackur, 1985)

I_2	$\leftrightarrow$	$I^+ + I^-$	$K = 9.9 \times 10^{-9}$
$I_2 + H_2O$	$\leftrightarrow$	$H2OI^+ + I^-$	$K = 1.2 \times 10^{-11}$
$I_2 + H_2O$	$\leftrightarrow$	$HOI + H^+$	$K = 3 \times 10^{-18}$
HOI	$\leftrightarrow$	$I^+ + OH^-$	$K = 3 \times 10^{-10}$
HOI	$\leftrightarrow$	$H^+ + IO^-$	$K = 4 \times 10^{-13}$
$I_2 + HOI$	$\leftrightarrow$	I_2HOI	$K = 2.7 \times 10^{-7}$
$I_2 + I$	$\leftrightarrow$	I_3^-	$K = 7.14 \times 10^{-2}$
3HOI	$\leftrightarrow$	$3H^+ + 2I^- + IO_3^-$	$K = 2.5 \times 10^{-11}$

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On theses, iodide (I^-), iodine (I_2) and triiodide (HOI) are important ion for bactericidal effect (Eel and Sebille, 1961; Rackur, 1985) (**Figure 1.4**) by rapidly penetrate into cell wall of bacteria and disrupt the proteins and nucleic acid structures resulting in cell death (McDonnell and Russell, 1999). In Figure 4, the iodine ion was increasing at 0.01 – 1% concentration. This is known as “dilution phenomenon”.

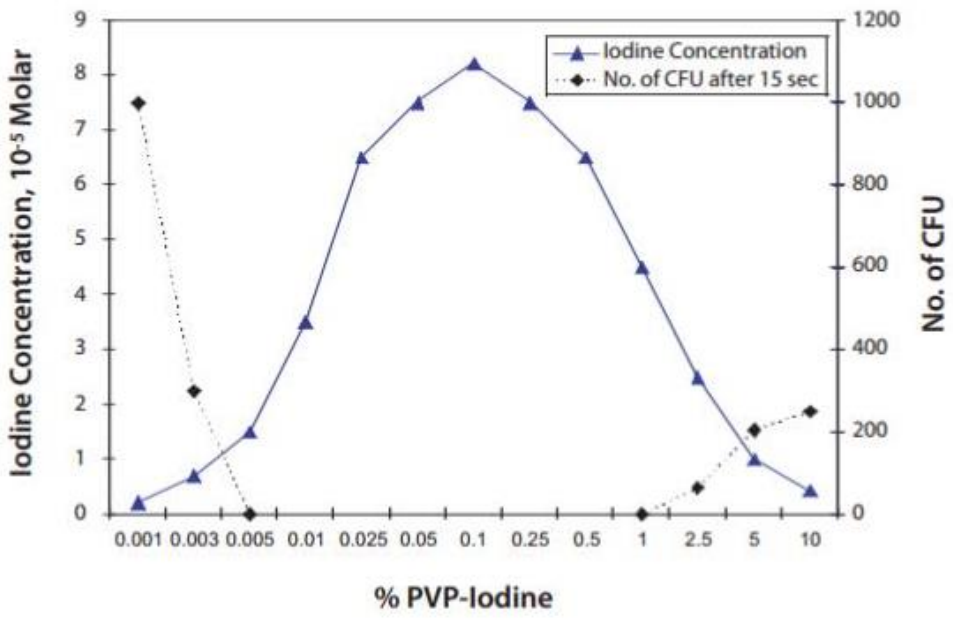

Figure 1.4 Correlation of the concentration of uncomplexed iodine with microbial reduction after 15 seconds for various concentrations PVP-I (Rackur, 1985)

This antimicrobial agents can kill bacteria and virus. Additionally, the prolonged contact time of PVP-I could terminate spore. In previous study, the bactericidal effect of PVP-I to *S. aureus* at a 1:100 dilution within 15s (Heiner et al., 2010). Up to date, the appropriate time and concentration of PVP-I to *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* have not been shown.

CHLORHEXIDINE GLUCINATE

Chlorhexidine gluconate composed of divalent, gluconate, acetate and hydrochloride (McDonnell and Russell, 1999). Due to binding with negatively charged of phospholipids on bacterial cell wall, this antiseptic can break down the cell wall causing cell death (**Figure 1.5**). This agent can kill bacteria, fungi and enveloped viruses. In previous study, mixing with 60% alcohol was increasing the bactericidal activity of chlorhexidine gluconate (Sakuragi et al., 1995) while, sole chlorhexidine gluconate eliminated *S. aureus* slowly (Sakuragi et al., 1995; Murayama et al., 2013). Even if *S. pseudintermedius* were killed at the 1 μg/mL, but not appropriate time had been reported (Murayama et al., 2013).

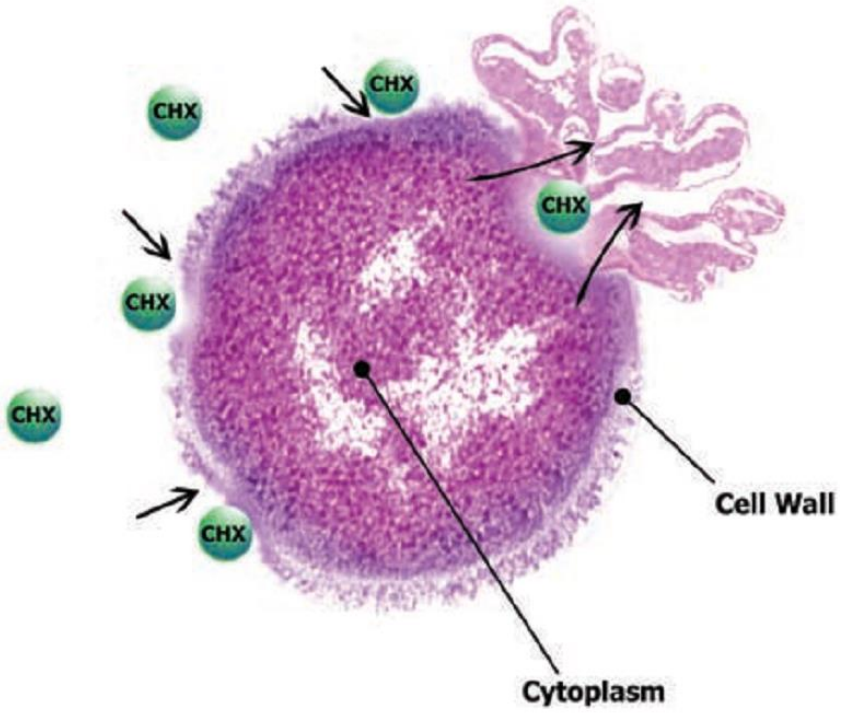

Figure 1.5 The mechanism of chlorhexidine molecules to and damage the surface of bacteria

HYPOTHESES

- The population of MRCoPS causing dermatitis is increased following the routine oral treatment by cephalexin monohydrate.
- There is the high distribution of MRCoPS in the veterinary school hospital.
- The decontaminating agents in appropriate exposure time and concentration can eliminate MRCoPS, *in vitro*.

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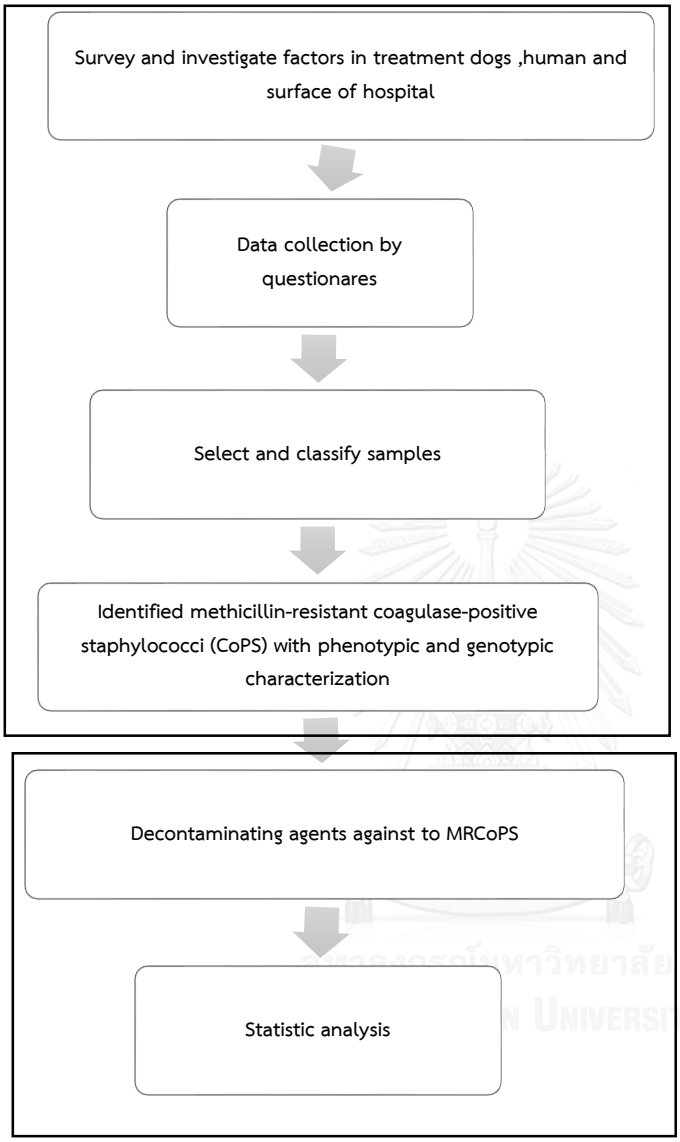
CONCEPTUAL FRAMEWORK



ADVANTAGES OF THE STUDY

1. Awareness of MRCoPS occurs on dog before and after treatment.
2. Provide the confirmation of MRCoPS distribution in the veterinary teaching hospital.

Provide optimal concentration and exposure time of antiseptics of PI and CGI against MRCoPS used in veterinary practice.

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CHAPTER 2

2.1 ASSOCIATION BETWEEN CEPHALEXIN ADMINISTRATION AND THE EMERGENCE OF METHICILLIN-RESISTANT COAGULASE-POSITIVE STAPHYLOCOCCI (MRCoPS) IN DOGS

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ABSTRACT

The potential transmission of MRCoPS between dogs and human has been noted as of potential public health concern. The current study aimed to determine the emergence of MRCoPS in dogs after oral administration of cephalexin. Skin swabs from 38 dogs without a history of antibiotic exposure were collected before drug administration (pre-treatment dogs) and during drug administration within one month (treatment dogs). A total of 196 CoPS were isolated from the nose, perineum and skin lesion. Fewer MRCoPS were isolated from pre-treatment dogs (7.89%) than from the treatment dogs ($P < 0.001$). MRSSc were only recovered from treatment dogs whereas MRSP were found in both groups. Overall, a high incidence of MRSP was found since the first week after administration. The nose and perineum were confirmed as the most common site of carriage of MRCoPS rather than skin lesions. In conclusion, the oral cephalexin administration was associated with the emergence of MRCoPS on dog skin within one week, a potential source of contamination to humans.

INTRODUCTION

S. pseudintermedius and *S. schleiferi* subsp. *coagulans* are the main CoPS found on canine skin, whereas, unlike the situation in humans, *S. aureus* is rarely found (Chanchaithong and Prapasarakul, 2011). Both microorganisms are part of the resident skin microbiota and as opportunist pathogens, depending upon factors such as the

host's immune status. The use of antibiotic treatment for skin infections is likely to encourage the emergence of resistant strains, which then may be a source of recurrent infection or increased risk of zoonotic bacterial transmission to owners and veterinarians.

Acquisition or expression of the methicillin-resistance trait is a potential bacterial adaptation following antibiotic treatment, and is characterized by the presence of the *mecA* gene and/or oxacillin disk screening test (Andersson et al., 1998). Most of methicillin-resistance trait also act as multidrug resistance to agents such as clindamycin, enrofloxacin, sulfamethoxazole/trimethoprim, gentamicin and tetracycline (Chanchaithong et al., 2014; Siak et al., 2014). MRCoPS, including MRSA, MRSP and MRSSc, have been reported in dogs and in associated people (Chanchaithong et al., 2014). Thus, these bacteria were emphasized to be zoonotic infection in veterinary and human hospital (Weese et al., 2012; Chanchaithong et al., 2014).

Cephalexin administration has been recommended as the primary choice of empirical therapy for routine treatment canine dermatitis (Hillier et al., 2014). Antimicrobial resistance can develop naturally following antibiotic exposure, and the persistence of antibiotic resistance depends on the genetic fitness of the wild type or impaired fitness of the mutant (Horvath et al., 2012). The high incidence rates of MRCoPS found in dogs might vary depending on management, especially the time of antibiotic administration (Lehner et al., 2014). An increase of MRCoPS strains in micro-environmental niches is a possible result of treatment, and this has potential public

health significance. Additionally, the timing of the onset of MRSP emergence after antibiotic treatment still needs to be clarified. This requires further specific investigation into the timing of MRCoPS emergence and the duration of antimicrobial use. This study was designed to determine the emergence of MRCoPS in dogs after oral cephalexin administration.

MATERIALS AND METHODS

Population

Thirty-eight dogs from households were recruited on a voluntary basis by the Dermatological Unit at a veterinary teaching hospital in Bangkok during 2012–2013. This study was approved by Institutional Animal Care and Use Committee (IACUC), with permit number 113/56. Male and female dogs ranging in age from 8 months to 2 years and of different breeds were presented. Two sample collections were carried out from the same dog depending on the cooperation of the animal owners. Prior to treatment a total of 38 dogs with superficial pyoderma were assigned as pre-treatment dogs. All dog samples were not treated with any antibiotic within 2 years. Subsequently, cephalexin monohydrate at a dose of 22-30 mg/kg were orally administered to all 38 dogs, twice per day for 4-8 weeks or until the patient had full skin recovery without any additional antibiotic or topical therapy. All dogs were followed up and categorized into subgroups representing 1, 2, 3 and 4 weeks of drug-exposure times. In each subgroup, one dog was sampled for two times at pre-treatment and during treatments

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depending on client convenience. Clinical sign of the dogs were observed for two months. The antibiotic treatment was determined and administered under the authority of the hospital's veterinary dermatologists. Dogs were excluded from the trial if they received other antibiotics during the observation period.

Bacterial collection

Sterile cotton swabs were used for sample collection from nares, perineum and/or affected lesions. Swabs were inserted at least 0.5 cm depth into the distal nares and approximately 1.0 cm around the peri-anal area. The affected tissue was either pyoderma or erythematous dermatitis. The swabs were stored in modified Stuart's transport medium (Difco, Paris, France) in an ice-box (Eriksen et al., 1994) and were cultured within 18 hours of collection.

Isolation and identification of CoPS and MRCoPS

Swabs were inoculated into 2 ml of enrichment broth containing 10 g/L tryptone (Difco, Paris, France), 75 g/L sodium chloride (Carlo erba, Rodano, Italy), 10 g/L mannitol (Flukka, Texas, USA) and 2.5 g/L yeast extract (Difco, Paris, France). Aliquots of 100 µL of sample suspension were inoculated onto nutrient agar (Merck, Darmstadt, Germany) supplemented with 5% sheep blood (blood agar) and on mannitol salt agar (MSA) (Difco, Paris, France) containing 0.5 µg/mL oxacillin (Sigma-Alrich, Missouri, USA) and then incubated at 37°C for 24 h and at 35°C for 48 h, respectively (Bemis et al., 2009). Up to three staphylococci-like colonies were collected from blood agar. Staphylococci were primarily confirmed by being gram-positive cocci

with glucose fermentation and catalase production, but negative in motility and oxidase production tests. For species identification, coagulase-positive staphylococci were identified based on their biochemical properties (Chanchaithong and Prapasarakul, 2011).

A multiplex PCR (M-PCR) with *nuc* amplification was performed for speciation of CoPS(Sasaki et al., 2010). The DNA was extracted using a Wizard Genomic® DNA purification kit (Wizard; Promega, Wisconsin, USA), and the M-PCR used a qPCR master mix (GoTaq®; Promega, Wisconsin, USA). The PCR products were detected by 1.5% agarose gel electrophoresis with ethidium bromide and were observed under a UV illuminator (Viber Lourmatt, Torcy, France). *S. aureus* ATCC 25923, *S. pseudintermedius* CVMC0108, *S. intermedius* CVMP 0309, *S. delphini* CVMP 0109 and *S. schleiferi* subsp. *coagulans* CVMC 0208 were used as the internal controls (Chanchaithong and Prapasarakul, 2011).

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MRCoPS identification

To screen MRCoPS, all CoPS were tested by standard disk diffusion method with oxacillin (1 mg). The protocol was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013). *S. aureus* ATCC 25923 was used as the standard control. Briefly, 0.5 McFarland units of bacterial suspension were spread on Mueller-Hinton agar (Difco, Paris, France) and the oxacillin disks (Oxoid, Hampshire, England) were placed on the agar surface. After incubation at 35°C for 24 h, the diameter of the zone of inhibition was measured and interpreted according to

CLSI criteria (CLSI, 2013). The *mecA* gene was detected in all isolates according to the approved protocol (Strommenger et al., 2003). MRSA strain NCTC 10422 and *S. aureus* ATCC 25923 were used as positive and negative controls, respectively.

Statistical analysis

Statistic 17 for Microsoft Windows (SPSS Inc.; Chicago, IL, USA) was used for all analyses. CoPS recovery rates between species was described by analysis of variance (ANOVA) and multiple comparisons. The different recovery rates between methicillin-resistant (MR) and methicillin-sensitive (MS) strains were analysed using the paired t-test. Values of $P<0.05$ were defined as being statistically significant. Reliability analysis between the existence of *mecA* gene and oxacillin resistant phenotype was performed by Cronbach's alpha coefficient (α). The criteria of reliability analysis is 1.) high reliability ($\alpha \geq 0.70$), 2.) fair reliability ($0.70 > \alpha > 0.30$) and 3.) low reliability ($\alpha \leq 0.30$).

RESULTS

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All 38 dogs were classified according to their initial condition and history of antibiotic treatment. They had superficial pyoderma with crusting and erythema. By two months from the onset therapy all dogs had recovered from the skin lesions. The population of MRCoPS and MSCoPS derived from each group are summarized in **Table 2.1.1**.

Table 2.1.1 Comparison between the recovery rates of MRCoPS and MSCoPS in the nasal cavity, perineum, and lesion sites in each group

Dog groups (n= dog numbers)	**Periods** (n= dog numbers)	**Total of dogs with**		*P-value*	**Sites**	**MRCoPS at site**
		***MRCoPS**	***MSCoPS**			
Pre-treatment (38)		3/38	38/38	*<0.001*	Nasal Perineum Skin lesion	1/38 2/38 0/38
Treatment (38)	1 week (11)	11/11	7/11	*<0.001*	Nasal Perineum Skin lesion	31/38 31/38 11/38
	2 weeks (7)	7/7	2/7			
	3 weeks (10)	10/10	3/10			
	4 weeks (10)	10/10	0/10			

*MRCoPS = methicillin-resistant coagulase-positive staphylococci; MSCoPS = methicillin-sensitive coagulase positive staphylococci

In pre-treatment dogs, MRCoPS were detected in the nares or perineum of 3 of the 38 animals (7.89%). In contrast, MRCoPS were isolated from either the nares or perineum of 31/38 (81.57%) of the treatment dogs, but only 11 of 38 (28.9%) were isolated from affected skin (paired *t*-test, *P <0.001*). MSCoPS was also detected in low numbers of treatment dogs (12 of 38; 31.57%) (paired *t*-test, *P = 0.003*). Coagulase-positive staphylococci species were identified by biochemical and genetic characterizations as *S. aureus*, *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans*; their frequencies and distribution are shown in **Table 2.1.2**.The correlation of *mecA* positive genotype and disk screening phenotype is shown in **Table 2.1.3**. The results

of *mecA* positive genotype in MRSSc did not correlate with the results of oxacillin screening method (α = 0.235). In this study, only one MRSP isolates was recovered from the nares of a pre-treatment dog. MRSP were commonly isolated from the treatment dogs, with the number of MSSP isolates being 4 time less than the MRSP isolates (*P<0.001*). Co-existence of resistant and susceptible strains was observed at all collection sites in the treatment dogs. Overall, 29 MRSSc isolates were recovered from the treatment dogs, but susceptible strains were found in both groups.


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Table 2.1.2 Frequencies and distributions of MRCoPS and MSCoPS belonging to three canine staphylococcal species at the sampling sites

Group (n= CoPS numbers)	**Sites**	***MRSP**	***MSSP**	*P-value*	***MRSSc**	***MSSSc**	**P-value**	***MRSA**	***MSSA**	**Total**
Pre-treatment (87)	Nares		38[†]			4		1	2	45
	Perineum	2	25	*<0.0001*		4				31
	Lesion		2							2
	Subtotal 1	2	65	*<0.0001*		8		1	2	78
Treatment (118)	Nares	28	5	*<0.0001*	12	1	<0.0001			46
	Perineum	30	7	*0.065*	13	2	0.001			52
	Lesion	11	4	*<0.0001*	4	1	0.83			20
	Subtotal 2	69	16	*<0.0001*	29	4	<0.0001			118
Subtotal 1+2= Total		71	81		29	12		1	2	196

*MRSP = methicillin-resistant *S. pseudintermedius*, MSSP = methicillin-sensitive *S. pseudintermedius*, MRSSc = methicillin-resistant *S. schleiferi* subsp. *coagulans*, MSSSc = methicillin-sensitive *S. schleiferi* subsp. *coagulans*, MRSA = methicillin-resistant *S. aureus*, MSSA = methicillin-sensitive *S. aureus*

Blank means no isolate.

[†]MRSP and MSSP in the nasal cavities of the control and treatment groups were significantly different (multiple comparisons, $P< 0.00$)

P-value determines significant difference between MRSP and MSSP at each carriage site and organs by paired t-test.

Table2.1.3 Time relapsing associated a possible selective pressure of MRCoPS on dog skin and agreement between *mecA* positive genotype and disk screening phenotype

Samples		Number of dogs with positive MRSP						Number of dogs with positive MRSSc					
		mecA positive	*OXA-R	*OXA-S	*CEP-R	*CEP-S	*Co-resistant	*mecA* positive	*OXA-R	*OXA-S	*CEP-R	*CEP-S	*Co-resistant
Pre-treatment		2	1	1		2							
	w1	11	11	0	7	4	7	3		3		3	
	w2	7	6	1	6	1	6	5	2	3		5	
Treatment	**w3**	10	10		5	5	5	2	1	1		2	
	≥4 week	10	9	1	9	1	9	8	3	5		8	
Total		40[a]	37[a]	3	27	13	27	18[b]	6[b]	12		18	

MRSP and MRSSc in this table were identified from *mecA* positive isolates that confirmed by PCR detection.

*OXA-R = oxacillin resistance; OXA-S = oxacillin sensitive including intermediate; CEP-R = cephalexin resistant; CEP-S = cephalexin sensitive including cephalexin intermediate; w = week of treatment; Co-resistant = resist to both cephalexin and oxacillin

Blank means 0 dogs

[a]α = 0.71

[b]α = 0.235

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DISCUSSION

In previous study, MRCoPS could be isolated from dog skins within one years after treatment (Beck et al., 2012). Then, the criteria of sample collection in this study could reduce remaining MRCoPS on their skin in pre-treatment dogs. This may explain why pre-treatment dogs had a very low incidence of resistant strains than was less than previously reported elsewhere (Beck et al., 2012).

CoPS were confirmed as being commensal on the skin of all tested dogs. *S. schleiferi* subsp. *coagulans* and *S. aureus* are moderate and minor components of the skin microbiota, respectively (Chanchaithong and Prapasarakul, 2011). All pre-treatment dogs contained MSCoPS at all collection sites, and co-colonization with MRCoPS and MSCoPS was confirmed. The existence of MRCoPS might reflect an irreversible acquisition of mutant strains in dogs exposed to an antibiotic for over a year (Craven and Neidle, 2007). In this study, one of the MRCoPS in a pre-treatment dog was MRSA, which is a common pathogen of human. This bacterium might be transferred from nasal cavities or skin of dog owners who was close contact with their dogs (Rutland et al., 2009). The nares and perineum have been deduced to represent a higher risk of transmissible contamination to clients than skin lesions (Walther et al., 2012). The very low recovery rate of MRSA might indicate that transmission from dogs to clients is not primarily a phenomenon of zoonosis, but vice versa (Rutland et al., 2009).

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In general, carriage sites (nares, oral cavity and perianal area) have been shown to be an important source of staphylococcal contamination to other hosts (Chanchaithong and Prapasarakul, 2011; Beck et al., 2012). This study revealed consistent evidence of MRSP from the nares and perineum, but it was less common in lesions. Hence, wound sites were not identified as a good screening area for MRSP in this study. The source of transmission might originated from environment and transferred to dogs during routine veterinary treatment. However, the very low recovery rate of MRSA might be that dog skin was not suitable for colonization of this pathogen (Rutland et al., 2009; Beck et al., 2012). In this study, *S. schleiferi* subsp. *coagulans* was recovered as well as *S. pseudintermedius* and *S. aureus,* nevertheless, the number of dogs which carried *S. schleiferi* subsp. *coagulans* was less than that of *S. pseudintermedius* under both conditions, with or without cephalexin administration. However, the emergence of MRSSc was potentially related only to the period of drug administration.

The criteria of MRSP oxacillin breakpoint were applied for MRSSc interpretation in this study (CLSI, 2013). However, the result of oxacillin disk screening did not correlate with *mecA*-positive results in MRSSc. This might possible that the criteria of MRSP did not suitable for screening MRSSc as well as MRSP. Hence, the MRSSc detection should be decided by *mecA* gene.

The influence of cephalexin treatment on the MRCoPS population were described in this study. MRCoPS were discovered on all dogs after first week of

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treatment. Then, the proportion of MRCoPS and MSCoPS were increase from 1st to 4th week of treatment. Hence, this might be linked with antibiotic stress theory (Andersson et al., 1998). With respect to this theory, the result showed that all MSCoPS completely disappeared within 4 week (100%) and this correlated with the previous reports (Beck et al., 2012). However, the increasing of MRCoPS population must be concerned in the veterinary treatment and the hospital management. The distribution of MRCoPS might be originate from treatment dogs. Therefore, control of this microorganism should be intensive cleaning management and sanitation in veterinary hospital.

In conclusion, CoPS comprising *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* were common at nasal cavities, perineum and lesion of dog patients. The co-colonization with resistant and sensitive strains was evident on pre-treatment and treatment dog skin, but the increase in MRCoPS were shown after antimicrobial administration. The emergence of MRSP might suggest an immediate onset of clonal selection with possible transmission.

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2.2 NASAL CARRIAGE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN DOGS TREATED WITH CEPHALEXIN MONOHYDRATE

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ABSTRACT

This study aimed to investigate the nasal carriage of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in dogs treated with oral cephalexin monohydrate. Ten dogs with superficial pyoderma were monitored longitudinally for MRSP carriage for up to 1 year after treatment, including typing of strains and determining antibiograms. Methicillin-susceptible *S. pseudintermedius* (MSSP) were recovered prior to treatment in all dogs and could be isolated after 12 months in 1 dog. MRSP was detected within 1 week of treatment in all dogs, and 3 clones represented by ST45, ST112 and ST181 were consistently present for up to 12 months after treatment. Susceptibility tests showed that all MRSP isolates were resistant to at least 7 common antimicrobials. Oral cephalexin monohydrate treatment selected for strains of multi-resistant MRSP, which were still present after 1 year.

INTRODUCTION

Staphylococcus pseudintermedius is a commensal bacterium on canine mucosa and skin that also can cause canine dermatitis. In rare cases it can opportunistically infect humans and contribute to detrimental outcomes such as septicemia, sinusitis and dog bite wound infection(Chuang et al., 2010; Stegmann et al., 2010; Wang et al., 2013). Systemic cephalexin administration is the primary choice of empirical therapy for canine superficial pyoderma (Hillier et al., 2014); however, the use of antibiotics may encourage an increased frequency of resistant strains, resulting

in recurrent infection or increased risk of bacterial zoonotic transmission to owners and veterinarians (Walther et al., 2008).

Antimicrobial resistant strains can be selected following exposure to antimicrobials. According to the selective pressure concept, antibiotic resistant strains may persist depending on the relative genetic fitness of resident susceptible and resistant strains(Horvath et al., 2012). Methicillin-resistant *S. pseudintermedius* (MRSP), can be increasingly detected after routine antibiotic treatment for canine dermatitis, and these also express resistance to other beta-lactam drugs, namely, penicillins and cephalosporins (Siak et al., 2014). Previously, emergence of MRSP from dogs with a history of treatment for dermatitis was observed in a longitudinal study and was shown to become the source of contamination for in-contact animals and the environment within the same household (Laarhoven et al., 2011). MRSP has been reported to persist on dog's skin for more than 6 month after antibiotic administration, and increased detection of MRSP during treatment seems to be common (Beck et al., 2012). However, the changes in the *S. pseudintermedius* population and the duration of persistence of MRSP strains on dog skin following antimicrobial treatment still needs to be determined in index dogs. This study aimed to determine changes in the *S. pseudintermedius* population in dogs after treatment with cephalexin and to evaluate the persistence of the resistant population in a longitudinal study.

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MATERIALS AND METHODS

Animals and treatment

This study was approved by the Chulalongkorn University Institutional Animal Care and Use Committee (permit number 113/56). Owner's permission was obtained through a consent form. Between 2011 and 2013, 10 dogs from different households were recruited on a voluntary basis by the Dermatological Unit at the University's Small Animal Teaching Hospital. Inclusion criteria for the dogs were generalized superficial pyoderma indicated by the presence of primary and secondary lesions including erythema, papules, pustules or epidermal collarets, and having no previous treatment with any drugs. All subjects were treated with oral cephalexin monohydrate at a dose of 22 to 30 mg/kg body weight (BW) q12h for 2 month with some topical therapy in most cases (Table 1). The dose and duration of treatment were prescribed by the veterinary dermatologist.

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Bacterial collection

Each dog was sampled at 3 times: 1) prior to treatment with antibiotics (Pre-treatment group), 2) 1 week after the start of treatment (Treatment group), and 3) 6-12 month after the onset of treatment (Follow-up group). Dog 9 was sampled at both 6 and 12 months post-treatment. Up to 4 samples from the same dog were collected over the duration of the study, depending on the cooperation of the animal owners.

Isolation and identification of *S. pseudintermedius*

Samples were collected using sterile cotton swabs inserted at least 0.5 cm into the left rostral nares of the dogs. The tip of the cotton swab was added to 1 mL of 0.85% normal saline in a microcentrifuge tube, then vigorously mixed and kept at 4°C for no longer than 2 h before it was cultured for bacteria. Ten-fold serial dilutions were prepared as described in the ISO6888-1 guideline (ISO6888-1, 1999), and 100 µL of each dilution was plated onto mannitol salt agar (MSA; Difco, Paris, France), and onto MSA containing 0.5 µg/mL oxacillin (Sigma-Aldrich, St. Louis, Missouri, USA) (MSA-O) (Chanchaithong et al., 2014). The plates were incubated at 37°C for 24 h and at 35°C for 48 h, respectively. Colonies of staphylococci that were pink, round, convex, smooth and 0.1 to 0.3 mm in diameter were counted on 2 plates per dilution series containing approximately 20 to 200 colonies and the average number was used to calculate the colony forming units (CFU)/swab.

At the highest serial dilution plate with visible growth of bacterial colonies, three suspected staphylococcal colonies were selected from MSA-O plates for species identification. In the case of no bacterial growth on MSA-O, pink colonies were collected from MSA without oxacillin. *Staphylococcus pseudintermedius* from either MSA-O or MSA was identified by routine primary biochemical tests, the tube coagulase test and secondary biochemical properties, with confirmation by amplification of the *nuc* gene by PCR (Sasaki et al., 2010; Chanchaithong and Prapasarakul, 2011). After identification, non-staphylococci and coagulase negative staphylococci were excluded

from the experiment. *Staphylococcus. aureus* ATCC (American Type Culture Collection) 25923^{T}, *S. pseudintermedius* CVMC [Chulalongkorn University Veterinary Microbiology (CUVM), canine strain) 0108, *S. intermedius* CVMP (CUVM pigeon strain) 0309, *Staphylococcus delphini* CVMP 0109 and *Staphylococcus schleiferi* subsp. *coagulans* CVMC 0208 were used as control strains. One *S. pseudintermedius* isolate per dog per time of collection, comprising 10 isolates from prior to treatment, 10 isolates from the first week and 11 isolates from follow-up dogs, were used for susceptibility testing and molecular typing.

Susceptibility testing and MRSP detection

All *S. pseudintermedius* isolates were assessed for susceptibility against 8 antimicrobials by the disk diffusion method including 1 µg oxacillin (OXA), 200 µg mupirocin (MUP), 15 µg erythromycin (ERY), 2 µg clindamycin (CLI), 30 µg doxycycline (DOX), 10 µg gentamicin (GEN), 5 µg enrofloxacin (ENR) and 25 µg sulfamethoxazole/trimethoprim (SXT). The protocol was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, Vet01-A4 (CLSI, 2013). *S. aureus* ATCC 25923^{T} was used as the standard control. Isolates were confirmed as MRSP by oxacillin resistance (Bemis et al., 2009) and possession of the *mecA* gene (Strommenger et al., 2003).

Molecular typing

SCC*mec* of MRSP isolates were classified by the presence of the *mec* complex class and the type *ccr* complex by multiplex PCR (Kondo et al., 2007). DNA fingerprints

were obtained for strain typing using *Cfr*9I-pulsed-field gel electrophoresis (PFGE) with the CHEF-DRIII apparatus (Bio-Rad, Hercules, California, USA), with a voltage of 6 V/cm and a switch time 0.5 to 15 s for 18 h and 20 to 25 s for 5 h (Soedarmanto et al., 2011). A The *ba*I-digested chromosome of *Salmonella* Braenderup H9812 was used as a standard marker for normalization, and a dendrogram was constructed using Gene Directory software (Syngene, Frederick, Maryland, USA) with UPGMA and setting at 1.0% position tolerance. A PFGE group was defined as clustering with an 80% similarity in pattern. Multilocus sequence typing (MLST) was performed to determine the sequence type (ST) of MRSP strains by amplification and sequencing of 7 housekeeping genes (*ack*, *cpn60*, *fdh*, *pta*, *purA*, *sar* and tuf), and analysis with the PubMLST database (http://pubmlst.org/spseudintermedius/) (Solyman et al., 2013).

Statistical analysis

Statistics 17 for Microsoft Windows (SPSS Inc.; Chicago, Illinois, USA) was used for all analyses. Category comparison for number of colonies cultured among groups (Pre-treatment, Treatment, and Follow-up) was done using the Kruskal-Wallis test. Differences between the numbers of colonies cultured at each observation were analysed using the Wilcoxon signed-ranks test. Values of $P < 0.05$ were statistically significant.

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RESULTS

All selected dogs were presumptively diagnosed with generalized superficial pyoderma. After 2 month of administration of cephalexin, all dogs had normal skin without the need for additional antibiotic or steroid therapies throughout the time of observation. All dogs had *S. pseudintermedius* isolated at each sampling time (**Tables 2.2.1 and 2.2.2**.). On MSA, the numbers of CFU of staphylococcus-like colonies between the 3 groups were significantly different (P = 0.007). Furthermore, the CFU for the dogs at follow-up were significantly greater than for the pre-treatment (P = 0.005) and treatment (P = 0.013) samples (**Table 2.2.1**). Only MSSP was isolated from all dogs prior to treatment, and dogs 9 and 10 also had MSSP isolated at 12 month post-treatment. Twelve MSSP isolates, including 10 from all dogs prior to treatment and 2 from Dog 9 and Dog 10 at 12 month post-treatment, were included for the PFGE fingerprint analysis. A total of 19 MRSP were selected from all dogs at the 1st week after treatment and the follow-up period (6 to 12 month after treatment) (**Table 2.2.2**). All MRSP isolates were characterized by SCC*mec* typing, MLST and DNA fingerprint analysis. A dendrogram from DNA fingerprint analysis of 31 *S. pseudintermedius* isolates illustrated with other characteristics and time of isolation is presented as a Supplementary Figure (available from the author). Isolates from the same dog having an identical PFGE pattern, sequence type (ST), SCC*mec* type and antibiogram are shown as one representative pattern.

Table 2.2.1 Age, sex, breed and treatment for the 10 dogs and the number of Staphylococcus-like colonies recovered at each collection time.

Dog	Age	Sex	Breed	Other treatments[a]	Log CFU/swab)					
					Pre-treatment		Treatment		Follow-up	
					MSA[c,e]	MSA+Oxa[d]	MSA[e]	MSA+Oxa	MSA[e,f]	MSA+Oxa
1	2 y	M	Beagle	2% Chlorhexidine	2.54	ND	2.31	1.31	3.05	2.44
2	8 mo	F	Mixed	Herbal cream[b]	2.84	ND	2.7	1.52	2.95	2.65
3	9 mo	F	Mixed	Herbal cream	2.48	ND	2.56	1.64	3.64	3.65
4	1 y	M	German shepherd	2% Chlorhexidine	2.75	ND	2.82	0.9	2.87	2.88
5	1.5 y	F	Mixed	2% Chlorhexidine	2.35	ND	2.45	1.22	2.65	2.12
6	10 mo	F	English cocker spaniel	None	2.56	ND	2.46	1.32	2.95	2.44
7	1 y	M	Pug	None	2.25	ND	2.56	1.64	2.65	2.77
8	1.5 y	M	Beagle	2.5% Benzyl peroxide	2.29	ND	2.54	1.02	2.4	2.55
9	9 mo	M	Mixed	Herbal cream	2.36	ND	2.51	1.54	2.96	ND
10	1 y	M	Mixed	2.5% Benzyl peroxide	2.9	ND	2.89	1.33	3.54	ND

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[a]Other treatments apart from oral cephalexin. [b]Local herbal product mainly containing custard apple seeds and other Thai herbal ingredients recommended for localized dermatitis. [c]Mannitol salt agar. [d]Mannitol salt agar contained 0.5 μg/ml oxacillin. [e]Numbers of *Staphylococcus*-like colonies between the 3 groups were significantly different (Kruskal-Wallis test; $P = 0.007$). [f]Numbers of *Staphylococcus*-like colonies in the follow-up group were greater than in the pre-treatment and treatment groups (Wilcoxon Signed Ranks test; $P = 0.005$ and $P = 0.013$). ND = not detectable. M – male, F – female, CFU – colony-forming units. Chlorhexidine and benzoyl peroxidase were shampoos.

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Table 2.2.2 Genotypic and antibiogram profiles of coagulase-positive staphylococci (CoPS) serially isolated from 10 dogs during the period prior to treatment (0 month) until a maximum of 12 month after treatment.

Dog	CoPS	PFGE type	ST	Antibiogram	SCC*mec*	Times of occurrence (months)				
						0	1[a]	6	8	12
1	MSSP	A			Neg	■				
	MRSP	F	45	OXA- ERY-CLI-GEN-DOX-SXT	NT		■	■		
2	MSSP	A			Neg	■				
	MRSP	F	45	OXA- ERY-CLI-GEN-DOX-SXT	NT		■		■	
3	MSSP	B			Neg	■				
	MRSP	F	45	OXA- ERY-CLI-GEN-DOX-SXT	NT		■	■		
4	MSSP	B			Neg	■				
	MRSP	C	112	OXA- ENR-ERY-CLI-GEN-DOX-SXT	A1		■		■	
5	MSSP	D			Neg	■				
	MRSP	C	112	OXA- ENR-ERY-CLI-GEN-DOX-SXT	A1		■		■	
6	MSSP	E			Neg	■				
	MRSP	H	181	OXA-ENR-ERY-CLI-GEN-DOX-SXT	V		■			■
7	MSSP	G			Neg	■				
	MRSP	H	181	OXA-ENR-ERY-CLI-GEN-DOX-SXT	V		■		■	
8	MSSP	I			Neg	■				
	MRSP	C	112	OXA- ENR-ERY-CLI-GEN-DOX-SXT	A1		■			■
9	MSSP	G			Neg	■				■
	MRSP	C	112	OXA- ENR-ERY-CLI-GEN-DOX-SXT	A1		■	■		
10	MSSP	J			Neg	■				■
	MRSP	F	45	OXA- -ERY-CLI-GEN-DOX-SXT	NT		■			

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MSSP = methicillin-sensitive *S. pseudintermedius*, MRSP = methicillin-resistant *S. pseudintermedius*, PFGE = pulsed-field gel electrophoresis, ST = sequence type in multilocus sequence typing, NT = non-typable, Neg = negative

1[a]: the samples were collected on the 7[th] day after onset of treatment

A grey block indicates the presence of the clones at the time of sampling. All dogs had CoPS at each sampling time. Three samples were obtained from each dog, except for dog 9 samples were obtained.

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By PFGE typing, 12 MSSP isolates clustered into 9 groups and 19 MRSP isolates clustered into 3 groups based on the 80% similarity cut-off. Typing by MLST identified 3 STs of MRSP including ST 45, ST 112 and ST 181. MRSP ST 181 contained SCC*mec* V (MRSP ST 181-V), and ST 112 carried non-typable SCC*mec* with a class A *mec* complex and type 1 *ccr* complex (MRSP ST 112-A1). Multiplex PCR could not identify the SCC*mec* type of MRSP ST 45 (MRSP ST 45-ND). Antibiograms of MRSP are presented in the Supplementary Figure (available from the author) and Table 2.

DISCUSSION

In previous studies, risk factors associated with increased detection of MRSP included frequent visits to veterinary clinics, prolonged hospital stays, and having a breeding bitch in the same household - but the effects of administration of antimicrobials have not been consistent (Lehner et al., 2014; Gronthal et al., 2015; Kjellman et al., 2015). Thus, this longitudinal study assessed the dynamic population change of *S. pseudintermedius* between pre-treatment and drug-use, as well as the persistence of MRSP post-treatment. Samples were taken from the nose, as this site is known to be an important source of staphylococcal carriage and contamination for other hosts (Beck et al., 2012). The careful selection of animals in the study may explain why untreated dogs had no resistant strains detected, which differed from previous reports(Beck et al., 2012; Kjellman et al., 2015). In our study, *S.*

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pseudintermedius could be a microbial marker for selection of antimicrobial resistant strains.

The use of MSA allowed growth of all staphylococci with pink colonies that could be used to differentiate them from other genera (Chanchaithong and Prapasarakul, 2011). MSA-O agar was used to screen the staphylococci with the methicillin resistance trait, thus the bacterial number tentatively represented the MRSP number (Bemis et al., 2009). The increased number of colonies of staphylococci on MSA found in the follow-up samples compared to pre-treatment might have arisen from co-colonization with MRSP and pre-existing MSSP strains. Adaptation mechanisms of bacterial strains in their ecological niche in the canine nose following antibiotic treatment have not been investigated. In all treated dogs, MSSP appeared to be replaced by MRSP as the dominant coagulase-positive *Staphylococcus* by the first week after treatment. Hence cephalexin treatment rapidly drove an increase in MRSP, consistent with the selective pressure theory for staphylococcal populations (Paul et al., 2011). This result confirms that selection of MRSP during treatment occurs frequently in the nasal environment (Beck et al., 2012). Moreover our follow-up demonstrated maintenance of a high level of persistence for 6 to 12 months in this longitudinal study, which was strongly concordant with the results of a previous cross sectional study (Beck et al., 2012).

PFGE typing is an approved genetic classification tool, and gave results highly consistent with the MLST results. The findings confirmed that clones of MSSP were

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genetically different from MRSP. The MLST and PFGE analysis confirmed that the persistent MRSP in follow-up dogs was the same clone in all cases (Laarhoven et al., 2011). The frequency of specific MRSP clones in an individual could be explained by a selective pressure phenomenon exerted by pre-existing resistant strains during antimicrobial exposure.

Isolates ST45-ND, ST112-A1 and ST181-V were shown to be multi-resistant to at least 5 additional antimicrobial classes. SCC*mec* of MRSP ST45 was not specifically identified in this study, but ΨSCC*mec*$_{57395}$ is commonly associated with this ST in Thailand and Israel(Paul et al., 2011; Chanchaithong et al., 2014). Additionally, ST45, ST112 and ST181 were previously reported as clones shared between dogs and owners (Chanchaithong et al., 2014). Our study showed that MRSP could be detected in healthy convalescent dogs, and that MLST and SCC*mec* typing were useful to study the molecular epidemiology of the infection.

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In conclusion, we demonstrated that oral cephalexin treatment of 10 dogs with pyoderma was associated with selection of MRSP clones with multidrug resistance. We observed a rapid onset of selective pressure and maintenance of MRSP for up to 12 months after treatment.

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2.3 PREVALENCE AND MOLECULAR TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* (MRSP) IN A VETERINARY TEACHNIG HOSPITAL IN THAILAND

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ABSTRACT

This study was to determine the prevalence of MRSP from environment to investigate the source of methicillin-resistant *S. pseudintermedius* (MRSP) distribution, within the Veterinary Teaching Hospital, veterinary staffs and dogs, and to characterize their species, antimicrobial susceptibility and clone types of MRSP. A total of 224 samples were collected from 188 environmental surfaces in 8 parts of hospital (28 samples per unit and 10 samples per hallways), 22 nasal carriage of veterinary staffs and 14 wound sites of dogs. The bacteria were counted on the selective media and identified using biochemical profiles and molecular analysis. Overall, high distribution of staphylococci was found on floor and medical instruments at the average of 41.10 CFU/swab. MRSP were performed on 4 floors, 3 tables, 2 syringe plates, 1 keyboard, 1 cotton and 2 knot doors. MRSP containing untypable SCC*mec* cassette presented multidrug resistance to doxycycline, gentamicin, erythromycin and clindamycin. By pulsed-field gel electrophoresis (PFGE) analysis, 6 of 10 PFGE types were found at dermatological unit that was identical to 3 types within gynecological unit. The clone type belonged to a veterinarian was different from the others, whereas type B MRSP from a dog was identical to that in Dermatological unit. Based on the results, there was a high distribution of CoPS circulating in the hospital with high number of colonization. Molecular typing indicated variance of MRSP representing the direction

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of distribution within the area study. Our insight is strongly helpful for strategic planning in hygienic recommendation in veterinary hospitals.

INTRODUCTION

Staphylococcus (S.) pseudintermedius has been majorly recognized as members of canine coagulase-positive Staphylococci (CoPS) causing dermatitis and septicemia (Frank et al., 2009; Morris et al., 2012) and is transmissible to owner and human patients (van Duijkeren et al., 2004). Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) commonly presents multidrug resistance to certain particular antibiotic groups such as β-lactam, macrolide, lincosamide, fluoroquinolone and aminoglycoside (Young et al., 2014). Recently, increase of MRSP distribution in veterinary hospital become the high risk of surgery site infections (SSIs) and septicemia in animal patients and also being threat of organization development and standardization (Dancer, 2004).

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The distribution of MRSP in the veterinary hospital poses nosocomial infection (Bergstrom et al., 2012a; Gronthal et al., 2015). Environmental surfaces, veterinary staffs and animal patients play role in MRSP circulation (Bergstrom et al., 2012a; Davis et al., 2012; Gronthal et al., 2015). The surveillance of MRSP is emphasized to predict the distribution of these pathogens in hospitals (van Duijkeren et al., 2011). In veterinary hospital, MRSP were discovered on medical instruments such as weight scales, examination tables and stethoscopes (Hamilton et al., 2012). The remaining MRSP in

environment were a potential cause for outbreak in veterinary hospital (Gronthal et al., 2015). Previously, outbreak caused by MRSP ST71 were concerned in a Finnish Veterinary Teaching Hospital (Gronthal et al., 2015).

Veterinarians were the other important carrier who shared MRSP to environmental and animal (Sasaki et al., 2007). However, the factor effect of MRSP distribution was independent in each hospital (Youn et al., 2011; Gronthal et al., 2015). To control an outbreak and spread of MRSP, the hygienic strategy and hospital accreditation must be applied in veterinary hospitals (van Duijkeren et al., 2011; Bergstrom et al., 2012b) including hand hygiene, protective gear and disinfectant (Gronthal et al., 2015).

In Thailand, sharing of MRSP among dogs, dog owners and veterinarians were confirmed in our teaching hospital (Chanchaithong et al., 2014). However, there has not been reported the information of MRSP distribution in the hospital environment. This study aimed to determine the prevalence of MRSP in the Veterinary Teaching Hospital in Thailand and analyze the clone relation in the units of investigation.

MATERIALS AND METHODS

Study design and ethic

A cross sectional study was performed on the Small Animal Teaching Hospital of the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. The

samples had been collected during November 2014. Dog and veterinary staff sampling protocols and consent forms were approved from the Institutional Animal Care and Use Committee (IACUC) (113/56) and the Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (137/57), respectively.

The hospital setting

The Veterinary Teaching Hospital (VTH), Faculty of Veterinary Science, Chulalongkorn University was the main veterinary hospitals in Bangkok, Thailand where approximately140,000 patients visited in each year. The hospital composed with 4 divisions; Emergency and Intensive Care Unit, General Medicine, Surgical Unit, Gynecological Unit and Special Units (Cardiology, Kidney & Urine care, Diabetes, Dermatological, Cancer, Feline disease, and Neurological).

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In 2014, approximately 389 pet patients daily visited to VTH divided as 96 at general medicine, 30 at gynecological unit, 27 at dermatological unit and 8 at surgical unit. The routine of cleaning management in this VTH composed of 1.) The floors are cleaned with 2.5% quaternary ammonium compound (UMONIUM38®, Laboratoire Huckert's International, Thailand) between 15.30-16.00 PM 2.) Examination tables, stethoscopes, syringe plates, waiting branch, drug cabinet, keyboard and knot door are cleaned with 0.5% quaternary ammonium compound (UMONIUM38®, Laboratoire Huckert's International, Thailand) when the items were not in use. 3.) Cotton for

wound dressing did not changed unit they were empty. and 4.) The disinfectant water in forceps jars which is 1% povidone iodine (Betadine® solution, Pathumthani, Thailand) is changed every day in all unit.

Populations and sample collections

Environmental samples

A total of 188 samples were retrieved from environmental surfaces in 8 parts of hospital; Division of general medicine, vaccination unit, gynecology, dermatology, surgery, post-surgery care and hall way (lower and upper). At the sample collection day, medicine, vaccination and dermatological unit located on new building of VTH while gynecology, surgery, post-surgery care and hall ways was parts of old building. The criteria of sample collections were described in **Table 2.3.1**. The criteria of room composing of 1.) Having at least 8 pet patients visited per day and 2.) Cleaning room at least 1 time per day. Most of samples were collected from the central examination room which at least 5 patients visited per day. One environmental sample were collected sample for two times in the same day; before clinics opened at 7.30 – 8.00 AM and after clinics cleaned at 15.30 – 16.00 PM at 22 May 2014. In one room, samples were collected from 5 parts of floors (Buttner et al., 2001) the central examination room. In case of medical instruments, if items in that room have more than one, the high frequency use item was chosen for sampling (Hoet et al., 2011). The data associated hospital management were collected by questionnaires. All surfaces were

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collected from sterile cotton swab. Before swabbing, cotton was dipped into 2 mL of peptone saline dilution (PSD; peptone 1 g, sodium chloride 8.5 g in 1000 mL of distill water). The moisten swab were rolled on the surface, cut the cotton part into the same tube and contained on ice until cultured.

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Table 2.3.1 The criteria of sample collection on floors and medical instruments

Environmental samples	**Type**	**Sample/room/time**	**Criteria (area per swab)**
Floor	Floor	5	3x3 cm2 from five parts; right up, right down, middle, left up and left down in the main examination room
	Cotton	1	One gram of bandaging wound cotton in the main examination room
	Stethoscope	1	0.1x0.1 cm2 of animal contact surface of stethoscopes in the main examination room
	Syringe plate	1	0.1x0.1 cm2 of syringe plate in the in the main examination room
Medical instruments	Disinfectant water[1]	1	1 mL of disinfectant water in forceps jar in the main examination room
	Examination table	1	10x10 cm2 on the surfaces of examination table in the main examination room
	Waiting branch	1	10x10 cm2 waiting branch in front of the main examination rooms
	Drug cabinet	1	10x10 cm2 of drug cabinet in Division or unit
	Keyboard	1	10x10 cm2 of keyboard in the main examination room
	Knot door	1	0.1x0.1 cm2 of knot door on the front door in the main examination

[1]negative control

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Veterinary staff samples

Veterinary staff samples composed of 1 veterinary nurse per unit and 16 veterinarians who had worked at the hospital for 2 years and rotated for veterinary practices in every unit. They were asked to provide a sterile cotton swabs into their nasal cavities (Chanchaithong et al., 2014) and their histories by questionnaires. Routinely, veterinarians and the other staffs wear the protective mask during working hours. The sterile cotton swabs were dipped into 2 mL of PSD in a sterile test tube (No. 9820, Cole-Parmer®, Thailand) before sampling (Chanchaithong et al., 2014). After sampling, the cotton part was cut into the same tube, contained on ice and cultured within 2 h.

Dog samples

A total of 14 samples were collected from nasal cavities of 14 dogs in surgery, post-surgery care and dermatological unit on the same day of human and environmental samplings. The outpatient dogs with wound infection or dermatitis were chosen from each clinical unit underneath authorization of veterinarian and permission of owner. The moist, sterile cotton swabs were dipped in 2 mL of PSD into a sterile test tube (Chanchaithong et al., 2014). The swab was rolled on the wound sites of dogs. The cotton part was cut into the same tube, contained on ice and cultured within 2 h.

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Bacteria enumeration, species identification and MRSP identification

In this study, samples were cultured within 2 h with enrichment prior for accuracy in the enumeration. The samples were vigorously shaken before ten-fold serial dilution were prepared as described in the ISO6888-1 guideline (ISO6888-1, 1999). A total of 0.1 mL of suspension was spread on Baird-Parker agars (Difco™, France) without and with 0.5 μg/mL of oxacillin (screening plate) for staphylococci selection and enumeration and was repeated this process at 3 times. After incubation at 35℃ for 48 h, black colonies of Staphylococci-like were counted. Three presumptive staphylococci colonies were purified on tryptic soy agar (TSA; Difco™, France) and confirmed with coagulase test. All CoPS were identified by primary and secondary biochemical tests and multiplex-PCR (M-PCR) for (Sasaki et al., 2010; Chanchaithong and Prapasarakul, 2011). *S. aureus* ATCC 25923, *S. pseudintermedius* CVMC 0108, *S. schleiferi* subsp. *coagulans* CVMC 0208 (canine origin), *S. intermedius* CVMP 0309 and *S. delphini* CVMP 0109 were used as control strains.

All CoPS were screened with oxacillin disk diffusion method. Oxacillin and cefoxitin breakpoints were confirmed methicillin-resistant coagulase-positive staphylococci (MRCoPS) definition by CLSI recommendation (CLSI, 2013). All oxacillin resistant isolates were defined as MRCoPS and confirmed by *mecA* gene detection (Strommenger et al., 2003). *S. aureus* ATCC 25923 and methicillin-resistant *S. aureus* (MRSA) N315 were used for the internal control strains.

MRSP antibiogram, SCC*mec* typing and clone typing

Thirty-four MRSP isolates from the screening plates (Baird-Parker agars with 0.5 µg/mL of oxacillin) were further determined antibiograms, SCC*mec* type and pulse-field gel electrophoresis (PFGE) patterns. They were determined by disk diffusion method (CLSI, 2013). The panel of antimicrobials were included clindamycin (DA, 2 µg), doxycycline (DO, 30 µg), gentamicin (CN, 10 µg), erythromycin (E, 15 µg), mupirocin (MUP, 200 µg) and sulfamethoxazole/trimethoprim (SXT, 25 µg). *S. aureus* ATCC 25923 was used as the control strain. The susceptibility level was interpreted as CLSI recommendation (2013).

The conserved fragments of the *mec* gene complex and *ccr* gene complex were detected by multiplex PCR to identify SCC*mec* type (Kondo et al., 2007). In order to determine clonal relation among MRSP, PFGE were performed as recommended (Chanchaithong et al., 2014). Briefly, bacterial DNA was plugged into Seakem® agarose (Bio-Rad, USA) and cut with *Crf9I* enzyme. DNA fragments were separated on a CHEFIII at 6 V/cm, 14ºC, 120º in a 1% Pulsed-field grade agarose gel with switching was 5-15 sec for 18 h and 15-60 sec for 5 h. Gels were stained with ethidium bromide, destained in water and digitally captured under UV light (Murchan et al., 2003). *Salmonella* serotype Braenderup H9812 was used as leader. The GeneDirectory program (Syngene, USA) associated with dice coefficient (1.5) were used for dendrogram construction (UPGMA and 1.0% position tolerance).

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Data analysis

Statistic 22 for Microsoft Windows (SPSS Inc.; Chicago, IL, USA) was the program for statistical analysis. Criteria of staphylococci population on hand touch sites and floor surfaces were interpreted by Dancer's criteria (2004); aerobic bacteria; ≤ 2.5 CFU/cm^2 = scanty growth; 2.5–12 CFU/cm^2 = light growth; 12–40 CFU/cm^2 = moderate growth; and 40–100 CFU/cm^2 = heavy growth (Dancer, 2004). The prevalence of CoPS, MRCoPS, MRSP, MRSSc and MRSA were described by percentile. The variable of colonies in each room were analyzed by Post Hoc test. The risk of MRCoPS in each room were described by odd ratios while the population of MRCoPS in Division of medicine were used as references.

RESULTS

Distribution of CoPS in the Small Animal Teaching Hospital

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The number of staphylococci on the floors were ranged from 0 – 200 CFU/cm^2 (average 61.09 CFU/cm^2 on BPA and 44.95 CFU/cm^2 on BPA-O). Dermatological unit showed the highest CoPS number (200.22 CFU/cm^2) while that of surgery unit was the lowest (0.22 CFU/cm^2) followed by down stair hallway (1.56-9.11 CFU/cm^2) (**Table 2.3.2**). The number of staphylococci among medical instruments was highly detected in post-surgical care unit (844.44 CFU/swab). Additionally, the number of colonies in the room where cleaned by broom without mopping and antiseptic was significantly higher than the others ($P < 0.05$, Post Hoc test).

In this experiment, CoPS strains retrieved from 60 of 224 samples; 26 parts of floors, 19 items of medical instruments, 14 dogs and 1 veterinarian. The population of CoPS isolates were performed highly on the floor, dog patients and examination table at 31, 14 and 9, respectively. CoPS was detected from only one veterinarian and no CoPS was detected from disinfectant water in forceps jars and drug cabinets.

Questionnaires

The veterinarians' questionnaires were collected from 16 veterinarians in surgery, dermatological, gynecology and post-surgery care unit. Eight of 16 veterinarians revealed that they used amikacin, amoxicillin/clavulanic acid, cefazolin, cephalexin, doxycycline, enrofloxacin, marbofloxacin, metronidazole and penicillin-streptomycin for treating pet patients. Veterinary assistants showed individual cleaning management in each unit and did not follow routine management. In all unit, examination tables were cleaned with UMONIUM38® immediately when the items were not in use.

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Table 2.3.2 Prevalence and bacterial count of coagulase positive staphylococci (CoPS) and methicillin resistant coagulase positive staphylococci (MRCoPS) in 8 parts of the veterinary hospital.

Places	Main antibiotic used	Routine Cleaning management (antiseptic/equipment/time)	Average of colonies on the floor					Average of colonies on the medical instruments[a]				
			BPA	BPA+O	CoPS positive surfaces (%)	MRCoPS positive surfaces (%)	Odd ratio of MRCoPS positive surfaces	BPA	BPA+O	CoPS positive surfaces (%)	MRCoPS positive surfaces (%)	Odd ratio of MRCoPS positive surfaces
			(CFU/cm^2)	(CFU/cm^2)				(CFU/swab)	(CFU/swab)			
Gynaecology	Enrofloxacin	2.5% quaternary ammonium compound/Mob/1	88.65	96.81	5/10 (50%)	5/10 (50%)	4	453.33	191.11	3/18 (16.67%)	2/18 (11.11%)	2.15
Medicine	Amoxicillin/clavulanic acid	2.5% quaternary ammonium compound/Mob/1	41.33	27.33	4/10 (40%)	2/10 (20%)	Reference	700	540.56	3/18 (16.67%)	1/18 (5.56%)	Reference
Post- surgery care	Enrofloxacin	2.5% quaternary ammonium compound/Mob/2	85.77	14.8	3/10 (30%)	3/10 (30%)	1.71	844.44	431.11	5/18 (27.78%)	3/18 (16.67%)	3.45
Surgery	Enrofloxacin	2.5% quaternary ammonium compound/Mob/1	0.22					572.22	338.33	3/18 (16.67%)	2/18 (11.11%)	2.15
Dermatological	Amoxicillin/clavulanic acid, Cephalexin and Metronidazole	No antiseptic/Broom/1	200.22[b]	122.44	7/10 (70%)	6/10 (60%)	6	499.44	299.44	5/18 (27.78%)	4/18 (22.22%)	4.91
Vaccine		2.5% quaternary ammonium compound/Mob/1	61.89	7.67	3/10 (30%)			221.67	34.44			
Lower floor		Unknown/Mob/1	9.11	0.67	2/10 (20%)							
Upper Floor		Unknown/Mob/1	1.56		2/10 (20%)							

[a]Medical instrument = cotton, stethoscope, disinfectant water, syringe plate, examination table, waiting branch, drug cabinet, keyboard and knot door

[b]Colonies (CFU/cm2) in dermatological unit are higher than the other rooms significantly ($P < 0.05$, Post Hoc test).

Grey box = not detectable

Prevalence of MRCoPS in the Small Animal Teaching Hospital

MRCoPS were found on 5 hospital units; 16 floors, 12 items, 14 dogs and 1 veterinarian. MRSP retrieved from 4 floors, 3 tables, 2 syringe plates, 1 keyboard, 1 cotton and 2 knot doors. Distribution of methicillin-susceptible coagulase-positive staphylococci (MSCoPS) and MRCoPS on floor and medical instruments are presented in **Table 2.3.3**. MRCoPS were detected on floor of 4 units; gynecology, medicine, post-surgery care and dermatological unit. On these, MRSP and methicillin-resistant *S. schleiferi* subsp. *coagulans* (MRSSc) were found on the floor at medicine and dermatological units. Among medical instruments, MRSP was found at examination tables (3/6) and in cotton for wound dressing at gynecology unit. In veterinary staff, one veterinarian carried both MRSP and MSSA on the nasal cavities.

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Table 2.3.3 MSCoPS and MRCoPS on floor and medical instruments

Sources (average colonies from BPA+OX)	Floor (33.71 CFU/cm^2)	Examination table (481.67 CFU/swab)	Syringe plate (252.5 CFU/swab)	Keyboard (402.5 CFU/swab)	Cotton (208 CFU/swab)	Knot door (272.5 CFU/swab)	Stethoscope (0 CFU/swab)	Waiting bench (1050 CFU/swab)
Gynecology	△	△▲			△○			
Medicine	△▲○		△▲	●				
Post- surgery care	△	△		△▲		△●		
Surgery		▲		○				○
Dermatological	△▲○ ●	△○●	△			△○	●	
Vaccine	▲✸							
Lower floor	●							
Upper Floor	▲●							

Grey = non detectable

▲ = methicillin-susceptible *S. pseudintermedius* (MSSP)

△ = methicillin-resistant *S. pseudintermedius* (MRSP)

● = methicillin-susceptible *S. schleiferi* subsp. *coagulans* (MSSSc)

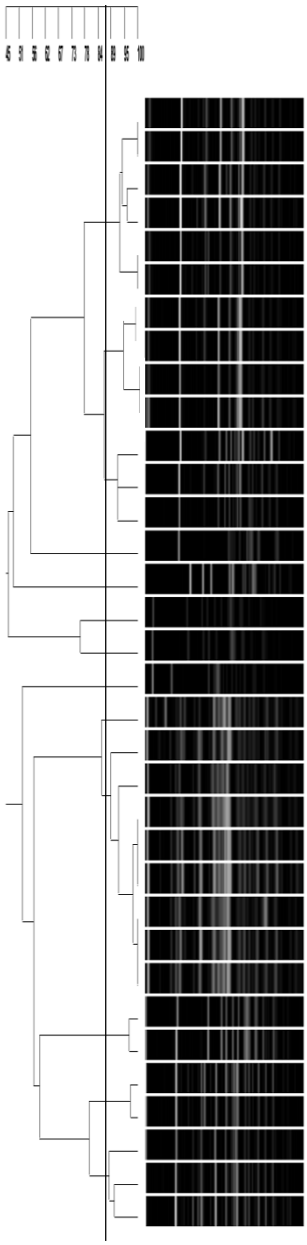
○ = methicillin-resistant *S. schleiferi* subsp. *coagulans* (MRSSc)

✸ = methicillin-susceptible *S. aureus* (MSSA)

Antimicrobial profiles, SCC*mec* type and PFGE types

At least one MRSP strain from the one positive place was further determined their antibiogram and molecular typing. A total of 34 MRSP were classified into five antibiogram patterns. All MRSP resisted to erythromycin and clindamycin. MRSP were resistant to doxycycline (Do), gentamicin (Cn), sulfamethoxazole/trimethoprim (Sxt) and mupirocin (Mup) at the percentage of 88.23, 85.29, 14.79 and 2.94, respectively. The most popular pattern was Cn-Do-E-Da (24/34, 70.59%) that belonged to untypeable SCC*mec* group (Figure 2.3.1). Antibiogram of human MRSP was SxT-Cn-Do-E-Da. Only one mupirocin-resistant MRSP was performed on dog patients in post-surgical care unit.

This study discovered two groups of SCC*mec*; untypeable (29/34, 85.29%) and V type (5/34, 14.70%). By pulsed-field gel electrophoresis (PFGE) analysis, 6 of 10 PFGE types (A, B, E, F, I and J) were found at dermatological unit that was identical to 3 types within gynecology unit (type A, I and J). The clone type belonged to a veterinarian was different from the others, whereas type B MRSP from dogs was identical to that in dermatological unit.



Samples	Sources	Places	Types	SCC*mec*	Antibiogram
G1CO2	cotton	Gynaecology	A	-	CnDoEDa
G1CO3	cotton	Gynaecology	A	-	CnDoEDa
SW2PO1	plates	Dermatological	A	-	CnDoEDa
SW2TO1	table	Dermatological	A	-	CnDoEDa
G1TO1	table	Gynaecology	A	-	CnDoEDa
G1TO2	table	Gynaecology	A	-	CnDoEDa
SW2F5O1	floor	Dermatological	B	-	CnDoEDa
SW2F5O2	floor	Dermatological	B	-	CnDoEDa
SW2F1O1	floor	Dermatological	B	-	CnDoEDa
SW2F1O2	floor	Dermatological	B	-	CnDoEDa
D10	dog	Surgery	B	-	CnDoEDa
D11	dog	Surgery	B	-	CnDoEDa
D12	dog	Surgery	B	-	CnDoEDa
D13	dog	Post care	C	V	SxtMupCnEDa
D14	dog	Surgery	D	V	DoEDa
SW1F201	floor	Dermatological	E	V	DoEDa
SW1F4O3	floor	Dermatological	F	V	SxtDoEDa
Vet staff	human	Hospital	G	V	SxtCnDoEDa
D1	dog	Dermatological	H	-	CnDoEDa
D2	dog	Dermatological	H	-	CnDoEDa
D3	dog	Dermatological	H	-	CnEDa
D4	dog	Dermatological	H	-	CnEDa
D5	dog	Dermatological	H	-	CnEDa
D6	dog	Dermatological	H	-	CnDoEDa
D7	dog	Dermatological	H	-	CnDoEDa
D8	dog	Dermatological	H	-	CnDoEDa
D9	dog	Dermatological	H	-	CnDoEDa
G1F2O1	floor	Gynaecology	I	-	SxtDoEDa
SW2F4O3	floor	Dermatological	I	-	SxtDoEDa
G1F4O2	floor	Gynaecology	J	-	CnDoEDa
G1TO3	table	Gynaecology	J	-	CnDoEDa
SW2F4O1	floor	Dermatological	J	-	CnDoEDa
G1F4O3	floor	Gynaecology	J	-	CnDoEDa
SW1F4O1	floor	Dermatological	J	-	CnDoEDa

Figure 2.3.1 Pattern of pulsed-field gel electrophoresis (PFGE), SCC*mec* type and antibiograms of methicillin-resistant *S. pseudintermedius* (MRSP) from many sources in the Small Animal Teaching Hospital.

DISCUSSION

CoPS and MRCoPS were tentatively enumerated and isolated from medical instruments and floors in each hospital unit. Our results demonstrated the high prevalence of MRCoPS including MRSP and MRSSc at floor and equipment in each unit. We could identify the possible source of MRSP leading to preventive and control in the veterinary teaching hospital. This cross sectional study was collected samples from environmental items comprising hand-touch sites, medical devices and materials, floor surfaces, veterinary staffs and dog patients in a veterinary teaching hospital where locates in the central area of Bangkok with high customer flow rate (approximately 389 cases/day). The hospital can take a high risk of MRCoPS distribution as a good model for study. Interestingly, the highest CoPS and MRCoPS were found at dermatological unit especially on the floor and in dog patients.

By questionnaire, the veterinarians routinely prescribed cephalosporin, metronidazole, tetracycline, fluoroquinolone and aminoglycoside to dog patients. Cephalexin monohydrate at 22-30 mg/kg twice a day for consecutively 4-8 weeks for treatment of canine dermatitis is mostly chosen as empirical use (Beco et al., 2013) and may immediately enhance survival of methicillin-resistant clones on skin lesion and carriage sites (Fungwithaya et al., 2016). Undoubtedly, we found such high population together with the variable of molecular types and antibiograms.

A high contamination of CoPS in equipment and surrounding is a major threat in veterinary hospitals (Aksoy et al., 2010) resulting nosocomial infection, risk of zoonotic distribution and uncertified standard accreditation. At the collection date, six of eight area (75%) were interpreted as the heavy microbial growth on plate (40-100 CFU/cm^2) especially dermatological unit base on the criteria of hygienic criteria from human hospital (Dancer, 2004). The microbial surveillance in hospitals was very useful for making a proper hygienic management and strategic plan. The goal of bacterial number per a hand touch site in human hospital was setup at to less than < 2.5 CFU/cm^2 (Dancer, 2004) but there have not been agreement documented for veterinary use (Aksoy et al., 2010).

Floor is the most frequent area for bacterial contamination. Use of proper disinfectant and cleaning protocol could restrict distribution of pathogen in hospital and reduced prevalence of nosocomial infection (Mullineaux and Jones, 2007; Portner and Johnson, 2010). However, our result was an importance evidence for revising a bacterial decontamination of the future hospital strategic program. Generally, cleaning with mops were recommended in veterinary hospitals (Portner and Johnson, 2010). Mopping with disinfectant (i.e. sodium hypochlorite, 2% phenolic solution and 0.5% chlorhexidine) is recommended for routine cleaning protocol in veterinary hospital (Portner and Johnson, 2010). In mopping method, the disinfectant have to contact with the surface of floor for 20 min and repeat the mop again. Additionally, we

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recommended to change mop bucket twice daily: at the beginning of the day and the final mopping of the day.

On the other hand, we concerned about MRCoPS contamination in veterinary instruments that would also indicate a risk of transmission to pet patients. After routine cleaning on the examination, MRCoPS was found at the great number in 4 units. In this hospital, examination tables were cleaned immediately when the items were not in use. Even if the cleaning patterns were the same, the remaining of MRSP were performed on the examination table of gynecology, post-surgery care and dermatological unit. Hence, we suggested that the remaining of MRSP on some examination tables might cause of cleaning practices and time-killed of antiseptic. On these, we recommended to revise the cleaning management following Portner and Johnson (2010). To control the contamintion on examination table, hand hygiene and appropriate disinfectant were recommended. With respect to hand hygiene, WHO recommended strictly hand hygiene with alcohol and 0.5% chlorhexidine (Ling and How, 2012). Additionally, the disinfectant for decontamination on *table surfaces* composed of ethanol, iodine iodophore, peroxygen compound, chlorhexidine, phenolic solution and sodium hypochlorite. With respect to low contact time (5 min), peroxygen compound (i.e. Virkon®) were suitable to clean the examination table.

One veterinarian carried MRSP presenting multidrug resistance on his nasal cavities. Human contained MRSP in nasal carriage is uncommon but closed associated

with pet owner and veterinary staff (Chanchaithong et al., 2014). The prevalence of human carrier was ranging from 1-5% but the highest prevalence (8%) was reported in Thailand (Sasaki et al., 2007; Chanchaithong et al., 2014). However, it was tough to conclude that dogs and high-touch sites in veterinary hospital was the potential source of transmission since the human strains (G type and SCC*mec* V) was not linked to those of dogs and environmental sources. The exposure time and host status may be a significant factor for explanation of MRSP transmission (Sakuragi et al., 1995; Laarhoven et al., 2011). However, a higher number from pair between human and animal strains should be recruited and analyzed in the further study.

PGFE typing is an approved genetic classification among intraspecies variation, and gave results highly consistent with the multi locus sequencing (MLST) results (Solyman et al., 2013). SCC*mec* typing is to initially describe detail of genes associated methicillin resistant trait. A total of 34 MRSP strains derived from 34 different sources, places and time showed the variety of clone type (A-J) distribution within the hospital. The strains from syringe plates, cotton, tables and floors were closely related but apart from those of human and dog origins. Human strain showed a distinguish electrophoretic type whereas only one dog shared PGFE B type with floor origin was confirmed. It is thought that PGFE type of MRSP may associate with isolation sources. The virulent factors and difference of their pathogenicity are needed to be warrant in further study. Most of strains were untypable SCC*mec* by the previous

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recommendation. Previously, we identified the untypable cassette as ΨSCCmec57395 that showed multidrug resistance and commonly associated with the untypable strains in Thailand. The frequency of specific MRSP clones in an individual could be explained by a selective pressure phenomenon exerted by pre-existing resistant strains during antimicrobial exposure.

In veterinary hospitals, pet patients are mostly administrated by antimicrobials and raising of resistant commensal microbes on skin becomes the consequence phenomenon. A high prevalence of CoPS circulating in the veterinary hospital was confirmed by isolation and identification. The clinical unit with prolong use of antimicrobial administration to patients had the highest risk of MRSP existence and may be the potential source of hospital distribution. The monitoring of MRCoPS number in positive area gave a highly concern on revision of the hygienic management.

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2.4 DISTRIBUTION OF METHICILLIN-RESISTANT COAGULASE-POSITIVE STAPHYLOCOCCI (MRCoPS) IN SURGICAL UNIT AND CYSTOTOMY OPERATION SITES AT A VETERINARY TEACHING HOSPITAL, THAILAND

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ABSTRACT

This study aim to investigate the distribution of methicillin-resistant coagulase-positive staphylococci (MRCoPS) from veterinary staff, hand-touch sites and surgical tissue during cystotomy operations in pet patients and to analyze their genetic relations and antimicrobial resistant profiles. Human and environmental samples were obtained from 12 surgeons and veterinary assistants at their nasal carriage and 29 hand-touch sites of instruments in operative unit areas for bacterial isolation and enumeration. Swabbed samples were triply collected from 29 dog and 3 cat patients at the site of incision; at incision area; at peritoneum during operating and at peritoneum before suture. MRCoPS were defined by *mecA* gene detection and characterized by antibiograms profile, SCC*mec* type and pulsed-field gel electrophoresis. Twenty-four staphylococci were isolated from 1 veterinary assistant, 12 operating room floor areas and hand-touch sites, 3 dogs and 1 cat. Methicillin-resistant *S. pseudintermedius* (MRSP) was found on an electric clipper and rebreathing circuits in the operating room. Three dog patients were positive for MRSP during surgery, and one methicillin-resistant *S. aureus* (MRSA) was detected in the cat patient. All MRCoPS were resistant to doxycycline, erythromycin, clindamycin and enrofloxacin, but there were no patients that developed surgical site infections. By genotypic patterns, the clones obtained from the environment and human sources were different from animal clones. With intensive hygienic management, there is a variety of MRCoPS

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clone existence within the surgical unit and during surgery. Finding MRCoPS cannot be a predictable marker for surgical site infections.

INTRODUCTION

Staphylococcus (S.) aureus, *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* are the major members of coagulase-positive staphylococci (CoPS) in dogs with and without dermatitis (Chanchaithong et al., 2014). These bacteria are believed to be an important cause of canine pyoderma, surgery site infections (SSIs) and otitis externa in pet patients (Igmi et al., 1990; Weese, 2008), escpecially the methicillin-resistant coagulase-positive staphylococci (MRCoPS) group. MRCoPS is among CoPS isolates containing *mecA* gene, which plays a significant role in nosocomial infection in human and veterinary hospitals (Coombs et al., 2009; Weese and van Duijkeren, 2010), with evidence of pet to client transmission (Morris et al., 2012).

In recent years, the high prevalence of MRCoPS in veterinary hospitals has been increasingly reported (Coombs et al., 2009; Weese and van Duijkeren, 2010; Bergstrom et al., 2012b), but a consensal linkage between MRCoPS and related clinical impact is still unclear. In bacterial ecology, MRCoPS is located on skin and nasal carriages of pet carriers and veterinary staff as well as contaminated hand-touch sites such as keyboards, weight scales and floors in animal hospitals (Bender et al., 2012; Hamilton et al., 2012). More recently, the relation between MRCoPS in the environment and recurrent infection at surgery units and wards has been suspected in a horse hospital

(Bergstrom et al., 2012b). Thus, a high distribution of MRCoPS may be seen as a risk of SSIs for post-operative animals in veterinary hospitals (Bergstrom et al., 2012a; Bergstrom et al., 2012b; Davis et al., 2012; Turk et al., 2014).

Veterinary surgeons certainly encounter SSIs during post-operative periods. Therefore, systemic surveillance would be very helpful to define the source of contamination. Furthermore, an association between the existence of MRCoPS on surgical tissue and SSIs has not revealed a clear connection. Thus, this study aimed to explore the occurrence of MRCoPS in the environment of a surgical unit, veterinary staff and surgical patients from cystotomy to post-operation. All MRCoPS were analyzed for their molecular genetic relation and antimicrobial resistant profiles.

MATERIALS AND METHODS

Hospital background

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Bacterial samples were collected from the Small Animal Teaching Hospital of the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand where they annually treat approximately140,000 patients per year. About 250 surgical cases were routinely handled by the surgical unit. In this unit, the floors were cleaned with 2.5% quaternary ammonium compound (UMONIUM38®, Laboratoire Huckert's International, Thailand) between 6.30 to 7.00 AM, while electric dog clippers, hematocrit centrifuges, the operating theater and surgical cabinets were cleaned with 0.5% quaternary ammonium compound (UMONIUM38®, Laboratoire Huckert's

International, Thailand) when the unit was not in use. Scrub suits, endotracheal tubes and scissors were routinely autoclaved daily. In this study, the surgical unit was chosen since the incident of SSIs had been observed in cystotomy patients before this study was started.

Sample collections

Hand-touch sites in surgical unit

The samples obtained from 29 areas were singly collected from environments including floors and all hand-touch sites within the surgical unit from 7.00 to 8.00 AM after routine cleaning. The surgical unit consisted of three subunit rooms: 1) the operating theater for abdominal surgery, including cystotomy; 2) the Preparation room for hair shaving and pre-anesthetic medication and 3) the Central room for surgeons to washing and dress for surgery (**Figure 2.4.1**). Swab samples from the floors in the surgery (3x4 m^2/room) and preparation room (3x4 m^2/room) were obtained using 3x3 cm^2/swabs from 3 parts/room: left, middle and right, whereas the 3x3 cm^2/swabs from the central room (6x8 m^2) were collected from 6 parts/room: left up, left down, middle up, middle down, right up and right down. The sample swabbing was obtained from hand-touch sites of different equipment such as electric dog clippers, rebreathing circuits, tables, a hematocrit centrifuge, scrub suits, endotracheal tubes, pipes, scissors, lights, operating theater, air filters, surgery cabinets and an electrocardiography unit,

either as individual from hand touch sites or pooled samples from floors, as previously described (Hoet et al., 2011). The samples were kept on ice for less than 2 h before culture processing. The medical chemicals and antiseptics, including lidocaine spray, xylocaine jelly, povidone-iodine, alcohol bottom, normal saline and cotton with alcohol, were also collected. Each swab was the separately grown and counted. The colony numbers in each part were then analyzed by determining the average number/area.

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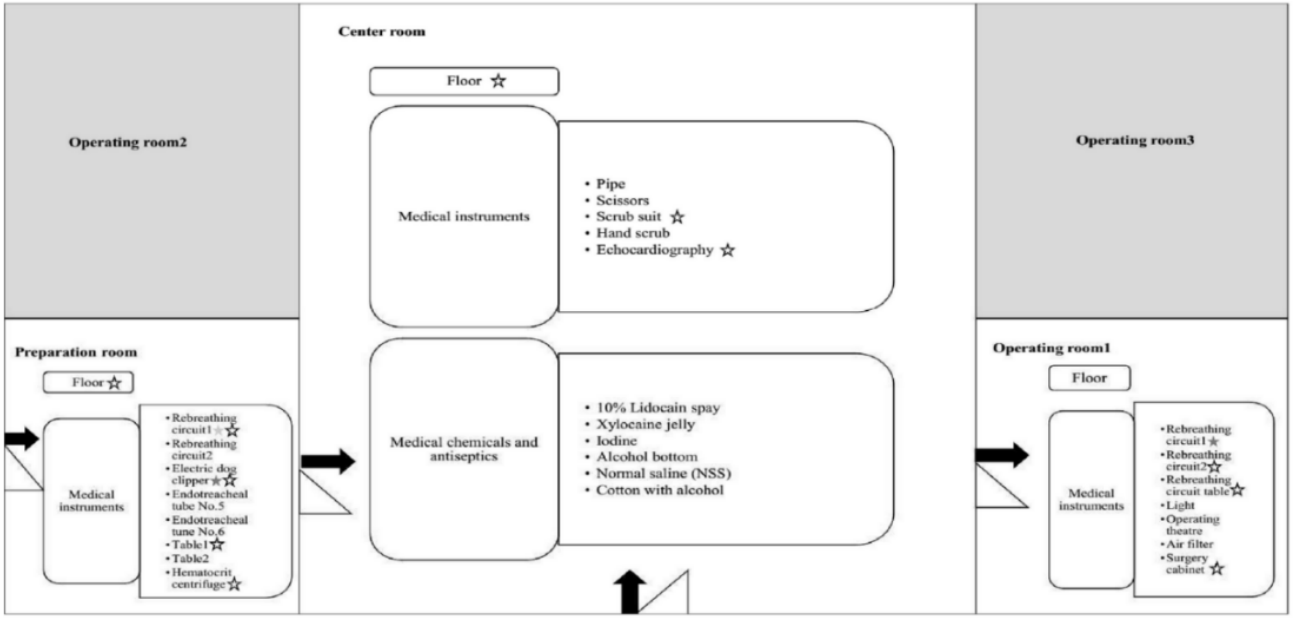

Figure 2.4.1 Presence of staphylococci divided by area, hand-touch sites according to floor plan of surgery unit in the veterinary teaching hospital.

Star markers represent subjects contaminated with staphylococci.

★ Methicillin-susceptible coagulase-positive Staphylococci (MSCoPS)

★ Methicillin-resistant coagulase-positive Staphylococci (MRCoPS)

☆ Coagulase-negative Staphylococci

Veterinarians and Nurses in surgical unit

Twelve nasal carriage samples were obtained from healthy veterinarians and veterinary assistants in the surgery unit after receiving approval by the Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (081/54). All staff worked at the surgical unit for at least 5 years (40 h/week of work-hour). Staff histories, age, gender, previous illnesses, prior antimicrobial uses and occupations, were recorded. The nasal swab was soaked with sterile peptone dilution saline (PDS) (0.09% NaCl, 0.1% peptone) before swabbing. Then, the swab was inserted at least 0.5 cm into nasal cavities and stored in transport tubes containing 1 ml of PDS (Chanchaithong et al., 2014). The samples were kept on ice less than 2 h before culture processing.

Pet patients in surgical unit

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Samples were collected from 32 surgical patients treated with cystotomy (29 dogs and 3 cats) aged from 3 months to 11 years during 2012-2013. Sampling protocols and consent forms were approved by the Institutional Animal Care and Use Committee (IACUC) (113/56). Pre/post-operative antimicrobial therapies and previous illness histories were recorded thoroughly under veterinarian authorization. Urinalysis, bacterial culture and antimicrobial susceptibility were performed prior to the operation for follow-up treatment decision.

After induction of anesthesia by 2% xylazine HCl (Rompun® vial, Bayer, Istanbul), the surgical area of the animal patients was shaved by an electric clipper in the preparation room. After that it was well scrubbed with 4% chlorhexidine gluconate scrub (Ecolab, Thailand) for 5 min and wiped with 95% ethyl alcohol. This process was conducted at least 2 times until all skin debris was noticeably removed. Before the patient was moved to the operating room, the antimicrobial based compound to test for antimicrobial susceptibility was intramuscularly injected.

In the operating room, the surgery site was triply cleaned with 10% povidone-iodine (LF Asia, Thailand) for 1 min and wiped with 95% ethyl alcohol before initial incision. The sample was firstly collected using sterile cotton swab (T1) at a 1x1 cm^2 site at the incision line. Within 5 min after entering the abdomen, a second sampling was swabbed from the peritoneum 0.5 cm under the incision line (T2). After the cystotomy procedure, approximately 200 mL of 37^{o}C sterile normal saline was used for abdominal lavage 4 to 5 times. The third collection (T3) was obtained at the same site as T2. The skin incised line was stitched with monofilament sutures at both the inner and outer layers of the skin. The entire procedure was completed within less than 2 h in all cases. The pets were then taken home for convalescence. At day 7-10 post-operation, the sutures were removed from the uncomplicated operation wound at the surgical unit. If SSI was presented, the fourth collection (T4) was performed at the infected site.

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Staphylococcal and MRCoPS identification

All swabbed samples were cultured within 2 h after collection. A total of 100 µL sample suspensions from floors, instruments and disinfectant agents were placed and incubated on Baird-Parker agar at 37°C for 48 h for enumeration (Dancer et al., 2008) and mannitol salt agars containing 0.5 µg/mL of oxacillin (MSA-O) at 35°C for 48 h for isolation of methicillin-resistant *S. pseudintermedius* (Sasaki et al., 2007). After the bacteria were enumerated, at least three staphylococcus-like colonies were picked for identification.

The staphylococci were identified by biochemical tests (Chanchaithong and Prapasarakul, 2011). Species identification was confirmed by multiplex-PCR (M-PCR) (Sasaki et al., 2010). The control strains were *S. aureus* ATCC 25923, *S. pseudintermedius* CVMC 0108, *S. schleiferi* subsp. *coagulans* CVMC 0208 (canine origin), *S. intermedius* CVMP 0309 and *S. delphini* CVMP (Chanchaithong and Prapasarakul, 2011).

To define MRCoPS, the suspected colonies grown on MSA-O were confirmed by oxacillin disk diffusion following the Clinical Laboratory Standardization Institute procedure (CLSI, 2013) and their possessing *mecA* gene (Strommenger et al., 2003). *S. aureus* ATCC 25923 and methicillin resistant *S. aureus* (MRSA) N315 were used as negative and positive control of *mecA* gene, respectively.

Antibiograms

Antimicrobial susceptibility of MRCoPS to 11 antimicrobials composed of amoxicillin/clavulanic acid (20 µg/10 µg), cefazolin (30 µg), cefoxitin (30 µg), clindamycin (2 µg), doxycycline (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (30 µg), imipenem (5 µg), mupirocin (5 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg) were determined by a disk diffusion method (CLSI, 2013).

Molecular typing

SCC*mec* types of methicillin resistant *S. pseudintermedius* (MRSP) were identified by a multiplex PCR for detection of the conserved fragments of the *mec* gene complex and *ccr* gene complex (Kondo et al., 2007). Pulsed-field gel electrophoresis (PFGE) illustrated the DNA fingerprint pattern of *S. pseudintermedius* using the *Cfr*9I restriction enzyme. DNA separation was achieved using 6 V/cm of voltage with a switch time of 0.5-5 s for 18 h and 20-25 s for 5 h using a CHEF-DRIII apparatus (Bio-rad, Hercules, USA) (Soedarmanto et al., 2011). Genetic relatedness of the strains was analyzed by dendrogram construction using UPGMA in the GeneDirectory program (Syngene, USA) and setting 1.0% position tolerance. DNA markers for gel normalization was *Xba*I-digested chromosomal DNA of *Salmonella* Braenderup H9812. PFGE clusters were grouped by more than 80% similarity of patterns.

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Data analysis

In this study, descriptive analysis was described by the IBM SPSS Statistics Desktop version 22.0 (SPSS Inc.; Chicago, IL, USA). The populations of CoPS and MRCoPS were described by percentile. The criteria for bacterial growth on hand-touch sites and floors were applied from Dancer (2008); < 2.5 CFU/mL = -, 2.5 – 12 CFU/mL = +; 12 – 40 CFU/mL = ++ and ≥ 40 CFU/mL = +++. GeneDirectory® software associated with dice coefficient (1.5) analyzed PFGE patterns.

RESULTS

Hand-touch sites and humans

Staphylococci were isolated from 12 hand touch sites consisted of 11/29 coagulase negative staphylococci (CoNS) and 3/29 CoPS in all rooms. All rebreathing circuits were positive for staphylococci, including MRSP in the operating room. In operating room 1 and the preparation room either co-existence of CoPS and CoNS or single existence on the rebreathing circuit and electric clippers were found (**Table 2.4.1 and Figure 2.4.1**). Two of 3 CoPS were MRSP with a high number of colonization. Regarding staff in the surgical unit, there was no detectable MRSP in their nasal carriage, but MRSA was found on one veterinarian.

Table 2.4.1 Occurrence and number score of staphylococci detection within three rooms of the surgical unit.

Samples	Sources	Places	CFU/ml	Coagulase test		MRSP	MSSP
				CoPS	CoNS		
Operative room1	Floors		-				
	Medical Instruments	Rebreathing circuit1	+++	+		+	
		Rebreathing circuit2	+		+		
		Rebreathing circuit table	+		+		
		Light1	-				
		Operating theater	-				
		Air filter	-				
		Surgery cabinet	+++		+		
Preparation room	Floors		+		+		
	Medical Instruments	Endotracheal tube No.6	-				
		Endotracheal tube No.5	-				
		Electric dog clipper	+++	+	+	+	
		Rebreathing circuit1	++	+	+		+
		Rebreathing circuit2	++				
		Table1	+		+		
		Table2	++		+		
		Hematocrit centrifuge	+++		+		
Center room	Floors		+		+		
	Medical Instruments	Pipe	-				
		Scissors	-				
		Hand scrub	-				
		Scrub suit	++		+		
		Normal saline	-				
		Echocardiography	-				
	Medical Chemicals and Antiseptics	10% lidocain spay	-				
		Xylocaine jelly®	-				
		Alcohol	-				
		Iodine	-				
		Cotton with alcohol	-				

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CFU = colonies forming unit; CoPS = coagulase-positive Staphylococci; CoNS = coagulase-negative Staphylococci; MRSP = methicillin-resistant *S. pseudintermedius*; MSSP = methicillin-susceptible *S. pseudintermedius*

*Bacterial umber score: < 2.5 CFU/ml= -, 2.5 – 12 CFU/ml = +; 12 – 40 CFU/ml = ++ and ≥ 40 CFU/ml = +++

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Pet patients

The 29 dogs and 3 cats at average age 6.78 ± 2.3 years had follow-up examinations for MRCoPS detection during their cystotomy operation. Their histories based on urine culture and antibiotic uses are presented in Table 8. Seven of 32 pets (9 observations) were found to have staphylococci in at least one observation comprising 4 MSSP and 4 MRSP on dogs at T2 and T3 and one MRSA on a cat at T1. The bacterial number appeared on the primary agar ranging from 0-7 CFU/swab. There was no staphylococci detection on dog patients at T1. Six of 7 cases were treated with enrofloxacin during or after surgery. Only Dog 8 and Cat 13 were positive to MRCoPS for at least 2 observations. There was no SSI presented during post-operative care (T4).

Antibiograms, SCC*mec* type and MRSP clone relation

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The antibiogram patterns and PFGE typing of *S. pseudintermedius* isolated from surgical patients and hand-touch sites are shown in **Table 2.4.2**. PFGE types: G (D15T3, D8T2 and D8T3) and I (D19T2), belonging to MRSP were detected on dog patients at T2and T3 of cystotomy, whereas B and C types were found on veterinary equipment. PGFE types A and D belonging to MSSP were detected on the tables, scrub suits and floor, but E, F and H types were found on surgical tissue. Feline MRSA was resistant to oxacillin, cefoxitin, erythromycin and clindamycin, while all MRSP were resistant to

amoxicillin/clavulanic acid, cefazolin, cefoxitin, doxycycline, erythromycin, clindamycin and enrofloxacin. MSSP (A, D, E and H) were resistant to only erythromycin and clindamycin, and one MSSP (F) was also resistant to enrofloxacin. By SCC*mec* typing, only D19T2 could be classified as V type, whereas the others were presented on an untypable cassette. Four identical PFGE types were distinguished, including G type from Dog 8 and Dog 15, I type from Dog 19, B type from the electric clippers, C type from the rebreathing circuit and D type from Dog 19.


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Table 2.4.2 Staphylococcal detection and identification in surgical patients and their urine culture and antimicrobial histories

Pet No.	Sp.	Positive sample			Urine culture	Pre-operating antimicrobial history (OP)	Pre-operative medicine (IM)	Post-operating antimicrobial history (OP)
		T1	T2	T3				
Dog6	D		MSSP		CoNS	Enrofloxacin	Enrofloxacin	Enrofloxacin
Dog8	D		MRSP	MRSP	CoPS	Enrofloxacin	Enrofloxacin	Enrofloxacin
Dog11	D		MSSP		*Corynebacterium* spp.	Amoxicillin/clavulanic acid	Cefazolin	Cephalexin
Cat13	C	MRSA		MSSP	ND	Enrofloxacin	Enrofloxacin	Enrofloxacin
Dog 15	D			MRSP	CoPS, Pseudomonas *spp.*	Enrofloxacin, Doxycycline	Enrofloxacin	Enrofloxacin
Dog 18	D			MSSP	CoPS, Proteous *spp.*	Enrofloxacin, Amoxicillin/clavulanic acid	Enrofloxacin	Enrofloxacin
Dog 19	D		MRSP		CoPS, Corynebacterium *spp.*	Marbofloxacin, Metronidazole	Enrofloxacin	Enrofloxacin

Sp. = Species; C= cat; D = dogs; MRSP = Methicillin-resistant *S. pseudintermedius*; MRSSc = Methicillin-resistant *S. schleiferi* subsp. *coagulans*; MRSA = Methicillin-resistant *S. aureus*; MSSP = Methicillin-sensitive *S. pseudintermedius*, CoNS = Coagulase-negative staphylococci; SSIs = Surgery site infection; ND = not detected

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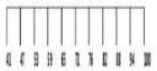
Table 2.4.3 PFGE patterns, SCC*mec* types and antibiograms of *S. pseudintermedius* derived from patient and environmental sources.



Isolate Code	Source	Type	PFGE groups	SCC*mec* type	Antibiograms
ET1	Table	MSSP	A	-	EDa
ET2	Table	MSSP	A	-	EDa
EE	Electric clipper	MRSP	B	NT	KZAmcCnDoEnrEDa
EC	Rebreathing circuit	MRSP	C	NT	KZAmcCnDoEnrEDa
D11T2	Dog	MSSP	D	-	EDa
D18T3	Dog	MSSP	E	-	EDa
C13T3	Cat	MSSP	F	-	EnrEDa
D15T3	Dog	MRSP	G	NT	KZAmcCnDoEnrEDa
D8T2	Dog	MRSP	G	NT	KZAmcCnDoEnrEDa
D8T3	Dog	MRSP	G	NT	KZAmcCnDoEnrEDa
D6T2	Dog	MSSP	H	-	EDa
D19T2	Dog	MRSP	I	V	DoEnrEDa

Kz = cefazolin, Do = doxycycline, E = erythromycin, Da = clindamycin, Mup = mupirocin, Sxt = co-trimoxazole, Cn = gentamicin, Amc = Amoxicillin/clavulanic acid, Enr = enrofloxacin,

DISCUSSION

The results revealed the determination of MRCoPS distribution within surgical unit among veterinary staff, environment and animal patients during cystotomy but there was no SSI concern. The surgical unit in the veterinary teaching hospital was used as the model of observation based on consistency of good hygienic management, antibiotic protocol, high frequency of daily operations and restriction zone from outsiders. For these reasons, this surgery was a very good model for monitoring bacterial distribution in a hospital unit with very low confounders. In this study, occurrences of staphylococci in the surgical unit and on equipment fixed within each room such as rebreathing circuits, surgical cabinets, electronic clippers, and centrifuge as well as animal patients and veterinary staff were persistent, whereas those on the scrub suits might have been contaminated by the reservoir user. To detect the bacteria from disinfectant at routine preparation within the forceps jar and all chemicals associated with patients, the result ensured that all agents were free from bacteria and not a source of bacterial distribution. Interestingly, the higher CFU number presented on hand touch sites over those on the floor could suggest improved hygienic manipulation and strategy in the animal hospital. CoNS was more commonly found in this study, which implies that low pathogenicity staphylococci might be more viable in both equipment and animal hosts (De Visscher et al., 2014).

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Sample collection and cultures were carried out by an aseptic technique to ensure that most of the cases were free from staphylococci. Surprisingly, MSSP, MRSP and MRSA still remained on the incision site and abdominal operative area, even though all patients underwent standard aseptic preparation (Zubrod et al., 2004). Moreover, the result from urine cultures was not consistent enough to detect staphylococci during surgery. It is speculated that the bacterial contamination might pass from areas adjacent to the incision site. However, the existence of staphylococci contamination on the surgical site was not the only factor associated SSI, which might also result from underlining patient factors such as immunological defect and household management (Bannoehr and Guardabassi, 2012).

DNA analysis showed a variety of MRSP and MSSP clones around the unit. This confirmed that the animal clones found in surgical tissue were distinguished from environmental clones. Thus, the most possible staphylococcal contamination to patients during surgical procedure was from their own or other patient sources. The PFGE type G strain, with identical antibiogram and SCC*mec* type was found in abundance in this study. Nevertheless, this could not be considered a representative outbreak strain unless the experiment was consecutively observed long term with relation to SSIs (Bergstrom et al., 2012a). Using an antibiogram, enrofloxacin resistant MRSP was detected at the surgical site, but there was no SSI observation; therefore, this finding confirms that low CoPS or MRCoPS contamination during surgery was

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insignificant in inducing SSIs (Schmid-Hempel and Frank, 2007). On the other hand, MRSA was found on one veterinarian and cat patient. However, clonal relation of MRSA was not performed since the cat had not been exposed to the positive veterinarian. Therefore, it would have been difficult to anticipate a relation in transmission.

In a previous report, the risk factors of SSIs were related to pet health, pre-operative conditions, operating room environment, duration of operation, surgical instrument management, surgical attire and post-operative factors (Weese, 2008). One incident of recurring infection derived from environmental surfaces was confirmed in a Swedish veterinary hospital (Bergstrom et al., 2012a). However, most risk factors in the surgical unit were controllable, while this was not the case during convalescent, as all patients convalesced in their home. Another study showed that SSI might be caused by household contamination or wound management by clients (Davis et al., 2012). Therefore, pet owners are likely to be the key person in hygiene management this case.

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In conclusion, the existence of staphylococci, MSSP and MRCoPS, at hand-touch sites can be detectable in a surgical unit of a veterinary hospital. There was no relative transmission between the environmental and animal patients detected, and MRSP during surgery was not enough to induce SSIs.

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2.5 BACTERICIDAL EFFECTS OF POVIDONE-IODINE AND CHLORHEXIDINE GLUCONATE AGAINST COAGULASE-POSITIVE STAPHYLOCOCCI

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ABSTRACT

We aimed to evaluate an *in vitro* bactericidal efficacy of the routine antiseptics using in veterinary hospital; povidone-iodine (PI) and chlorhexidine gluconate in isopropanol (CGI) to canine CoPS. Twenty CoPS were divided to 5 methicillin-resistant *Staphylococcus* (*S.*) *pseudintermedius* (MRSP) and 5 isolates of each *S. pseudintermedius*, *S. aureus* and *S. schleiferi* subsp. *coagulans* defined as methicillin-susceptible CoPS (MSCoPS). The bactericidal efficacy was determined by broth microdilution according to European Standard EN 1656:2000 at the concentration of 0.1, 1 and 10% PI and 0.5, 1 and 2% CGI for 15s, 30s, 45s, 1 min, 3 min and 5 min, respectively. There was no difference in susceptibility values between strains. Regarding the fastest bactericidal effects, all tested CoPS were killed with 0.1% PI within 45s, whereas 0.5% CGI could kill within only 15s.

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INTRODUCTION

Coagulase-positive Staphylococci (CoPS) is gram positive cocci bacteria comprising the three members of CoPS on dog skin; *Staphylococcus (S.) pseudintermedius*, *S. aureus* and *S. schleiferi* subsp. coagulans. Methicillin-resistant coagulase positive staphylococci (MRCoPS), poses a prolong infection in canine allergic dermatitis that may be a source of spreader in the hospital and animal household (Windahl et al., 2012). For public health concern, these bacteria have been reported

as the cause of secondary infection in immunocompromised and operated human patients (Bergstrom et al., 2012). To reduce an impact of MRCoPS dissemination in veterinary hospital, use of antiseptic is the empirical tool for decontamination and wound management.

Povidone-iodine (PI) and chlorhexidine gluconate (CG) are the common antiseptics in veterinary and human hospitals. The antimicrobial agent of PI composed of three ion including iodine (I_2), hypoiodous acid (HOI) and iodide (I^-) (Rackur, 1985) that yielded from iodine reaction in aqueous solution. In veterinary use, PI is used for pre-surgical skin preparation (Osuna et al., 1990) and treatment of infectious wound (Sanchez et al., 1988). However, the knowledge of the exposing time and concentrations to kill *S. pseudintermedius*, *S. aureus* and *S. schleiferi* subsp. coagulans that isolated from dog is unrevealed.

Chlorhexidine gluconate is a chemical compound against bacteria, fungi and enveloped virus. By bactericidal mechanism, the positively-charged ion of CG attached with negatively charged site of cell wall leading to cell death (McDonnell and Russell, 1999). Practically, mixing with alcohol solution can increase the efficacy of bactericidal activity of CG (Sakuragi et al., 1995). However, the bactericidal effect of chlorhexidine gluconate in isopropanol (CGI) to common CoPS on the dog skins had not been reported. Then, time exposures and concentrations of each disinfectant compound are the important parameters resulting difference of bactericidal effects to each

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microorganism. In this study, we aimed to evaluate, *in vitro,* bactericidal efficacy of PI and CGI to canine CoPS member in different concentrations and exposing times.

MATERIAL AND METHODS

Antiseptics

PI and CGI were routinely used at the Veterinary Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand.

Betadine® solution (Reg. No. 1A 675/31, IDS manufacturing LTD., Pathumthani, Thailand) contained with 10% of PI (Mundidone®, Netherlands) in aqueous solution. Betadine was diluted to 1% and 0.1% by ultrapure water. All solutions (10, 1 and 0.1%) were freshly prepared before the determination.

Q-Bac2A® (Reg. No. 1-1-03-02-13-00463, Pose Health Care Limited, Bangkok, Thailand) composed of 2% CGI. CGI was prepared at 2%, 1% and 0.5% diluting by ultrapure water.

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Bacteria isolation and identification

A total of 20 isolates dividing 5 methicillin-resistant *S. pseudintermedius* (MRSP) and 15 MSCoPS; 5 samples of each *S. pseudintermedius*, *S. aureus* and *S. schleiferi* subsp. *coagulans* were derived from a previous study of Chanchaithong and Prapasarakul (2011). All bacteria were identified by the approved biochemical analysis

(Chanchaithong and Prapasarakul, 2011) and confirmed by multiplex PCR (Sasaki et al., 2010). The methicillin-resistant trait was characterized by oxacillin disk diffusion test (CLSI, 2013) and *mecA* identification (Strommenger et al., 2003) whereas the susceptible strains defined by these negative results. The bacteria were prepared by cultivation on tryptic soy agar (TSA) contained with 5% sheep blood at 37ºC for overnight.

Bactericidal determination

The broth microdilution was modified from the European Standard EN 1656:2000 (2000) and Banovic et al (2013). Briefly, the bacterial concentration was adjusted at 0.5 McFarland using a densitometer (DEN-1B McFarland Densitometer, Grant-bio®, Cambridgeshire, UK). The stock solutions of bacteria were finally adjusted to approximately 10^6 CFU/ml and confirmed the population followed by ISO standard enumeration (ISO6888-1, 1999). One hundred microliters of bacterial suspension were mixed with 100 µl of chemical agent dilution in 96-well sterile plate (Nunclon™ Delta Surface, Thermo Scientific, Jiangsu, China)(Banovic et al., 2013). After exposure at 15s, 30s 45s, 60s, 180s and 300s, the reactions between antiseptic and bacteria were stopped by mixing with 20 µl of the suspension with 180 µl of D/E neutralizer (BBL®, French) for 5 min. Thereafter, 10 µl of the suspension were placed on 5% sheep blood and incubated at 37ºC for 24 h (BSEN, 2000). *S. aureus* ATCC 6538 was used as the strain control. The procedure was repeated at least two times.

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Statistical analysis

All statistical analysis were calculated by IBM SPSS Statistics Desktop version 22.0 (SPSS Inc.; Chicago, IL, USA). The difference of time-kill between MRSP and MSCoPS were analyzed with Chi-square statistic. The minimum bactericidal concentration (MBC) was described as the lowest concentration and minimal time that could kill all bacteria (BSEN, 2000).

RESULTS AND DISCUSSION

D/E neutralizer and ultrapure water did not affect to bacterial growth. The bactericidal efficacy of PI to canine staphylococci is presented in **Figure 2.5.1**. At 10% PI, *S. schleiferi* subsp. coagulans and *S. pseudintermedius* were terminated within 180s while *S. aureus* and MRSP were determined to kill within 60s. By controversy, 10% PI is recommended as the most stable form of PI preparation due to their slow releasing property of polyvinylpyrrolidone (PVP) for at least 18 hours (Kunkle et al., 2015).

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At 0.1 and 1% concentration, PI were determined to kill both MRCoPS and MSCoPS within 45s. The results could be explained by the dilution phenomenon (Rackur, 1985). The dilution phenomenon is known as low concentration but high bactericidal activity. In PI, 0.01 -1 % concentration released the highest concentration of iodine that was a main antimicrobial substrate (Rackur, 1985) and immediately reacted to bacterial cell membrane. However, this aqueous dilution was unstable

(Mueller et al., 2012), which could not prolong bactericidal activity on applied site. In additional, use of 0.1 and 1% PI were recommended in treatment of mouthwash (Higashitsutsumi et al., 1993), eye infection (Pinto et al., 2015) and pre-surgery preparation (Ferguson et al., 2003) in human and wound dressing in dogs (Sanchez et al., 1988). For all tested concentrations of PI, 3 mins exposure time was recommended for aseptic preparation such as a pre-surgical site (Reichman et al., 2009). The notable side effect of PI is the inhibition of human skin fibroblast growth (Balin and Pratt, 2002) and skin irritation in dogs (Osuna et al., 1990). However, the reports of 0.1 to 10% PI side effect were very low in animal (Mueller et al., 2012).

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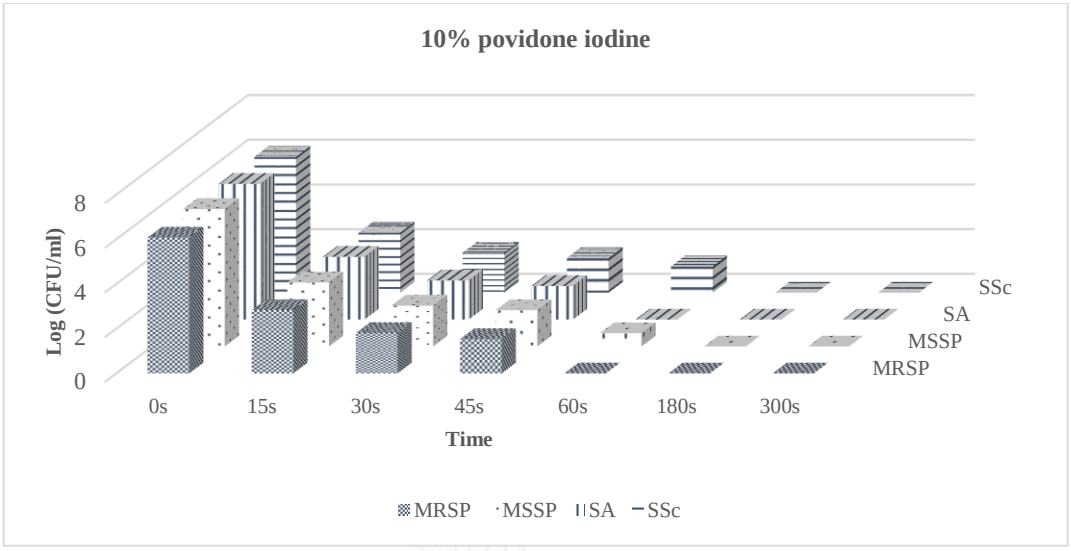
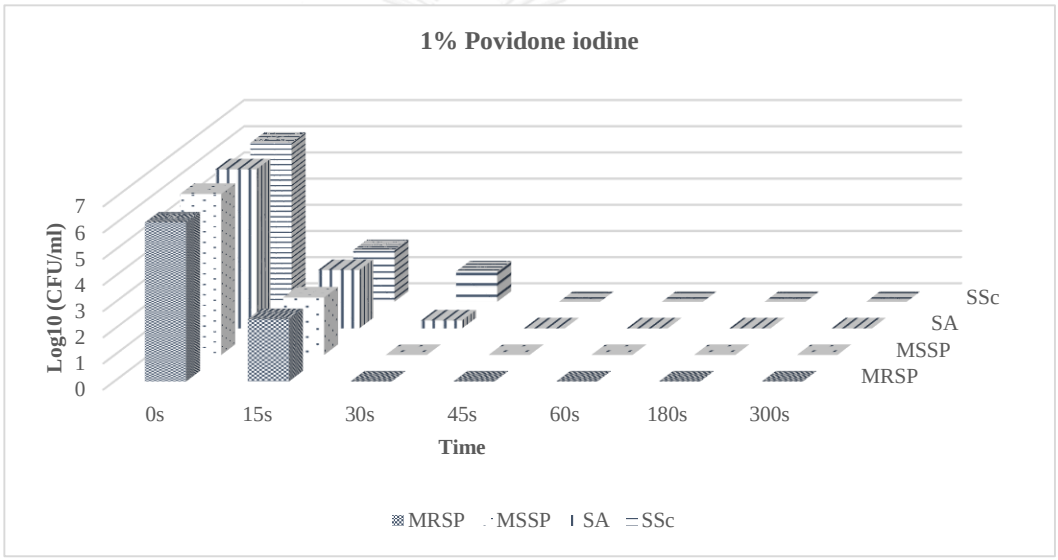
a)


10% povidone iodine
Log (CFU/ml)
0
2
4
6
8
0s
15s
30s
45s
60s
180s
300s
Time
SSc
SA
MSSP
MRSP
MRSP
MSSP
SA
SSc

b.)


1% Povidone iodine
Log10 (CFU/ml)
0
1
2
3
4
5
6
7
0s
15s
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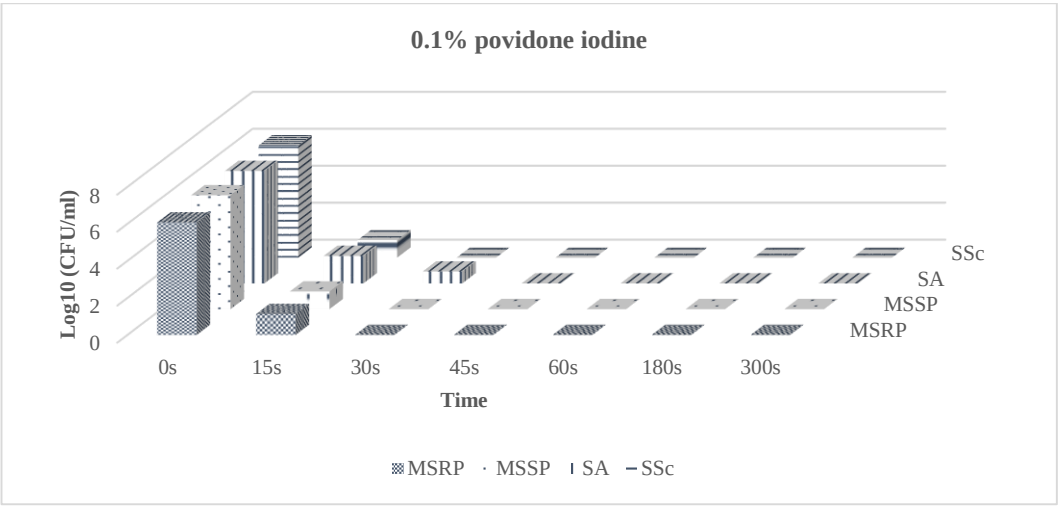
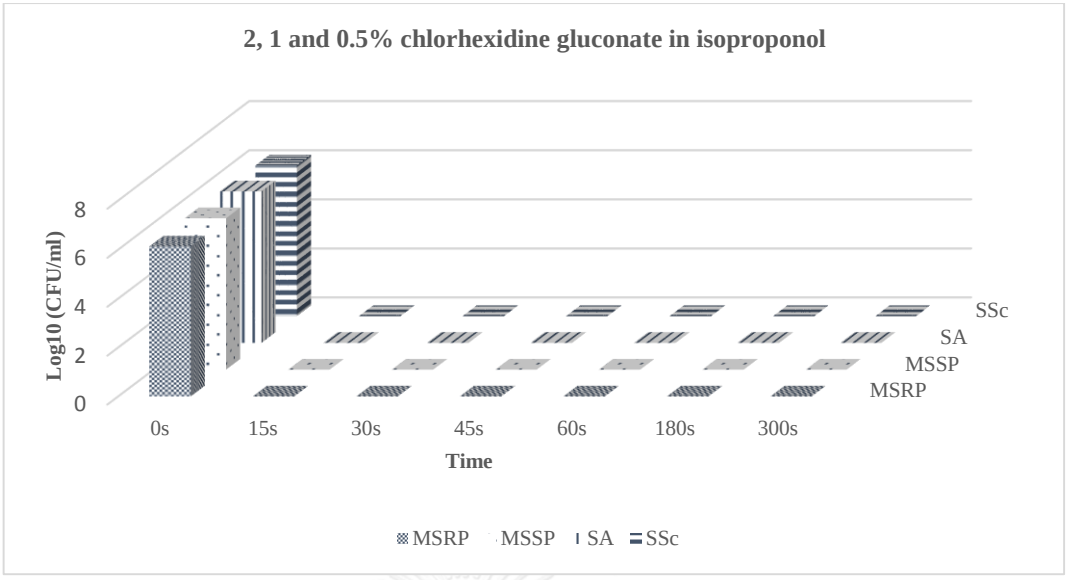
60s
180s
300s
Time
SSc
SA
MSSP
MRSP
MRSP
MSSP
SA
SSc

c.)


0.1% povidone iodine
Log10 (CFU/ml)
0
2
4
6
8
0s
15s
30s
45s
60s
180s
300s
Time
SSc
SA
MSSP
MSRP
MSRP
MSSP
SA
SSc



d.)

Figure 2.5.1 Exposing time and concentration of PI to methicillin-resistant *S. pseudintermedius* (MRSP), methicillin-susceptible *S. pseudintermedius* (MSSP) and *S. schleiferi* subsp. *coagulans* (SSc) and *S. aureus* (SA); a) 10% povidone-iodine, b) 1% PI, c) 0.1% povidone-iodine and d.) 0.5-2% chlorhexidine gluconate in isopropanol.

In this study, the concentrations of 0.5 – 2.0 % CGI could kill all MRSP and MSCoPS within 15s. There was no difference between methicillin-susceptible and methicillin-resistant trait. In the previous study, CG without alcohol eliminated staphylococci over 5 min (Banovic et al., 2013). Due to mixed with alcohol, this product could eliminate much faster. In veterinary practices, 0.5% CG is the active ingredient in antiseptic shampoo in dogs (Kwochka and Kowalski, 1991) and also is easy to prepare for hygienic use in household and hospitals (Mueller et al., 2012). In veterinary clinic, amount 2% chlorhexidine could lead to an ototoxic effect in cats (Igarashi and Oka,

1988). Also, the important side effect of CGI is breaking of red blood cell (Gabler et al., 1987). Therefore, CGI was not recommended for treatment of open wound with a lot of capillary injury. However, there have not been report of the animal adverse effect at 0.5-1% chlorhexidine.

In summary, we recommended the time-kill and concentration-kill to CoPS eradication of the two common antiseptics used in veterinary hospitals. With respect to the result of this study, we suggest 10% povidone-iodine, should expose to CoPS for at least 3 min, whereas 1:10 and 1:100 dilutions from its original concentration showed the higher efficacy by shortening to 45s of time-kill. On the other hand, at least 0.5% of CGI had the bactericidal activity within 15s.

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CHAPTER 3

General Discussion and conclusion

Methicillin-resistant is an important resistant trait of Staphylococci that are commonly associated with underlying skin conditions and dogs with antimicrobial exposure (Weese and van Duijkeren, 2010). This study gathered the animal and environmental factors concerned the incidence of MRCoPS existence in veterinary teaching hospital including MRSP, MRSSc and MRSA. The results from veterinary and dog sites revealed that the routine administration for canine dermatitis by cephalexin monohydrate could select the resistant strain and could occupy within nasal carriage as the micro-environmental niches instead of MSCoPS population at the first week after antimicrobial exposure. MRSP was the most common staphylococci distributing in dogs and veterinary hospital environment. The longitudinal observation presented dynamic changes of *S. pseudintermedius* population from prior to the end of treatment. MRSP could persist on nasal mucosa more than 12 months after treatment that also presented in previous studies (Laarhoven et al., 2011; Beck et al., 2012). It is strongly confirmed that selective pressure phenomenon was the cause of MRCoPS raising and the followed up dogs firstly demonstrated clone persistence for over 6 months. Thus, the treated animals become as the source of MRCoPS distribution in hospital and household (Davis et al., 2012; Bergstrom et al., 2012a).

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MRCoPS has been believed as a common cause of postoperative infection or surgical site infection (SSI). Investigation of MRCoPS distribution in hospital can provide epidemiologic data related to prevention and hygienic management in the hospital unit. Monitoring of bacterial contamination in surgical unit and operation rooms is a crucial action for observation for SSI cause . By using the routine animal preparation prior to operation, there was no SSIs developing during observation. However, MRSP and MRSA could be detected within operation room and pot-operative care unit and even at operative tissue. The clonal differences between animal and equipment origin might indicate strain pathogenicity and an irrelativeness of SSI incidence. Regarding to risk analysis, floors and examination tables are taken into the significant risk site of MRCoPS in the veterinary teaching hospital. Their surfaces can be a major MRCoPS source reflecting underestimate bacterial survival ability and under standard of the hospital hygienic strategy. Disinfectant with chemical agents (i.e. alcohol, biguanide, quaternary ammonium compound, inorganic solution and peroxygen compound) is recommended for routine decontamination on surface of veterinary hospitals with optimal contact time at 20 minutes (Portner and Johnson., 2010). Hand washing and gloving together with appropriate disinfectant are very helpful for controlof MRCoPS on highly-touched surfaces (Portner and Johnson., 2010; Ling and How, 2012). The decontamination by disinfectant on highly-touched surfaces are routinely recommended composing ethanol, iodine iodophore, peroxygen compound, chlorhexidine, phenolic solution and sodium hypochlorite. However, peroxygen

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compound is the most trustable agent to clean *highly-touched surfaces*. To reduce MRCoPS contamination on the dog skin, PI and CGI are commonly used as the antiseptic of choice in veterinary practice. To our knowledge, this is the first report of the endpoints of time-kill and concentration-kill of PI against canine CoPS in veterinary practice. The study proposed a suitable guideline for wound management and preoperative preparation protocol by using 10% povidone iodine for at least 3 minute exposure and wipe the incision line by 0.5% CGI for at least 15 second. Use of 10-100 time PI dilution will be very useful in the process of wound dressing that could enhance the bactericidal efficacy at infective site. At 0.1% concentration, PI releases the highest concentration of iodine that was a main antimicrobial substrate (Rackur, 1985) and immediately reacts to bacterial cell membrane. However, this aqueous dilution is unstable (Mueller et al., 2012), which and bactericidal effect is not prolonged during application. In veterinary practices, use of 0.1% and 1% PI are recommended for mount washing (Higashitsutsumi et al., 1993), eye dropping (Pinto et al., 2015) and pre-surgical preparation (Ferguson et al., 2003) in human, and wound dressing in dogs (Sanchez et al., 1988). At 10% dilution, PI preparation due to their slow releasing property of polyvinylpyrrolidone (PVP) for at least 18 hours (Kunkle et al., 2015). With respected to this result, it is important to remember that bactericidal effect depends on time and concentration parameters. However, even high bactericidal efficacy of CGI was found within a little moment, the use of CGI is not recommended for open wound

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with bleeding complication. Because CGI might delay wound healing by injuring capillary vessels and is not recommended for treatment or open wound.

In conclusion, *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* was the common staphylococci species colonizing on nasal mucosa, perineum and infecting skin lesions of canine patients. Co-colonization between resistant and sensitive strains was commonly found on dog skin pre-treatment and during the treatment and the MRCoPS was increasingly isolated after oral cephalexin treatment and the MRSP clone with multidrug resistant characteristics maintained until over 6 months.. This study also pointed out the risk source of MRCoPS in the hospital surrounding and equipment and also in the process during operation. Linkage between MRCoPS detection and hygienic management protocol could be used for reset as the guideline of MRCoPS and other bacterial distribution in veterinary hospital and at dog household under one health approach concept.

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Limitation of the study

1. Samples were collected from environment, veterinary staffs and dog patients only one time.
2. Bacteria in urinalysis of cystotomy patients could not follow-up.
3. The bactericidal effect of povidone-iodine and chlorhexidine gluconate in isopropanol were not tested, *in vivo*.

Suggestion from the study

The result of this study related to the factors effect to existent of MRCoPS on animal skin and animal hospital. The study suggested the hygienic policy on table 3.1 .

Table 3.1 The hygienic policy of veterinary hospital

	Veterinarian, veterinary staffs and human associated pet in veterinary hospital
	Hygiene management
1	Remove jewelry before treatment
2	Hand should be washed with at least 0.5% chlorhexidine gluconate in isopropanol before and after contact with animals.
3	Gloves should be removed promptly after use and hand should be washed after taking off gloves.
4	Mask should be worn in working time.
	Antibiotic usage
1	Antibiotic drugs should be used under recommendation doses.
2	Drug sensitivity test should be done before antibiotic treatment.
3	If it possible, antibiotic treatment should be replaced by suitable antiseptic in case of dermatitis treatment.
4	Multi-drug resistant bacteria should be emphasized in veterinary hospital by monitoring every years.
	Hospital cleaning managements
	Floor
1	The mop head should be cleaned with 100 ppm sodium hypochlorite for 20 mins at least 2 time per day.

2	Change the disinfectant solution between rooms, or dirty floor
3	Mop heads should be washed by the washing machine (>40° C) daily.
	Medical instruments
1	Medical instruments should be cleaned with quaternary ammonium compounds for 10 mins every time after use.

Decontamination on animal skin

1	A dilution of 0.1-1% povidone-iodine are recommended to wound cleaning with at least 45s of contact time.
2	In skin preparation for surgery, the recommended antiseptics are 10% povidone-iodine at least 3 mins and 0.5% chlorhexidine gluconate in isopropanol at least 15s.
3	The awareness of povidone-iodine 1.) 0.1-1% povidone-iodine can cause of toxic to fibroblast growth. 2.) 10% povidone-iodine can kill bacteria, slowly.
4	The awareness of chlorhexidine gluconate 1.) 2% chlorhexidine gluconate can cause of ototoxic in cat. 2.) Chlorhexidine gluconate can break cell wall of red blood cell.

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APPENDIX

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Preparation of media, solutions and buffers

Media and biochemical tests

Salt to tolerance

Peptone	10	g
Beef extract	3	g
Sodium chloride	70	g
Distilled water	1000	mL

Potassium Hydroxide (40%)

Potassium Hydroxide	40	g
Sterile De-ionized Water	100	mL

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Voges-Proskauer (VP) Reagents

Peptone	5	g
Glucose	5	g
K_2HPO_4	5	g
Distilled water	1000	mL

Oxidation-fermentation medium

Glucose	10	g
Peptone	2	g
Sodium chloride	5	g
K_2HPO_4	0.3	g
Agar	3	g
Bromothymol purple	0.04	g
Distilled water	1000	mL

Purple base medium

Proteus peptone No.3	10	g
Beef extract	1	g
NaCl	5	g
Bromothymol purple	0.04	g
Distilled water	1000	mL

*add 10 ml of 20% sugar in 200 ml of this media before use

Mannitol fermentation

Proteus peptone No.3	10	g
Beef extract	1	g

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NaCl	5	g
Bromothymol purple	0.04	g
agar	10	g
Distilled water	1000	mL

*add 10 ml of 20% mannitol sugar in 200 ml of this media before use

0.85% normal saline solution

NaCl	8.5	g
Distilled water	1000	mL

Peptone Dilution Saline (PDS)

Peptone	1	g
NaCl	9	g
Distilled water	1000	mL

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Stock media

Proteus peptone No.3	10	g
Beef extract	5	g
Yeast extract	3	g
NaCl	5	g

K_2HPO_4	0.8	g
Agar	10	g
Distilled water	1000	mL

Urea medium

Peptone	1	g
NaCl	5	g
K_2HPO_4	2	g
Agar	20	g
0.2% Phenol red	6	mL
Distilled water	1000	mL

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Buffer and solution for PFGE

6 N HCl

HCl	50.4	mL
Ultrapure water	49.6	mL

10 N NaOH

NaOH	200	g
Sterile ultrapure water	diluted to 1000	mL

5 M NaCl

NaCl	146.25	g
Sterile ultrapure water	diluted to 1000	mL

1 M Tris-HCl, pH 8.0

Tris base	60.55	g
Sterile ultrapure water	diluted to 500	mL

*adjust pH with 6 N HCl

0.5 M EDTA, pH 8.0

Na_2EDTA	93.05	g
Sterile ultrapure water	diluted to 500	mL

*adjust pH with 10 N NaOH

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1.8% SeaKem Gold agarose (for Staphylococci plugs)

SeaKem Gold agarose	0.45	mg
TE buffer	25	mL

1% SeaKem Gold agarose (for Salmonella plugs)

SeaKem Gold agarose	0.25	mg

TE buffer	25	mL

1 mg/ml lysostaphin stock solution

lysostaphin	5	mg
1 mM sodium acetate	5	mL

10 mg/ml lysozyme stock solution

lysozyme	100	mg
Sterile ultrapure water	10	mL

20 mg/ml Proteinase K stock solution

Proteinase K	100	mg
Sterile ultrapure water	5	mL

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Cell suspension buffer

1 M Tris, pH 8	10	mL
0.5 M EDTA, pH 8	20	mL
Sterile ultrapure water	70	mL

TE buffer

1 M Tris, pH 8	10	mL
0.5 M EDTA,pH 8	2	mL
Sterile ultrapure water	diluted to 1000	mL

EC lysis buffer

1 M Tris, pH 8	5.4	mL
5 M NaCl	180	mL
0.5 M EDTA, pH 8	180	mL
Brij-58	4.5	g
Sodium deoxycholate	1.8	g
Sodium lauryl sarcosine	4.5	g
Sterile ultrapure water	70	mL

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

10x Tris-Borate EDTA buffer

Tris base	108	g
Boric acid	55	g
Sterile ultrapure water	diluted to 1000	mL

Ethidium Bromide for gel staining

10 mg/ml of EtBr	10	mL
distilled water	100	mL

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ใบยินยอมให้ใช้สัตว์ทดลองเพื่อโครงการวิจัยและการเรียนการสอนทางสัตวแพทย์

ข้าพเจ้า (นาย/นาง/นางสาว).....ที่อยู่.....ซอย.....
ถนน.....ตำบล.....อำเภอ.....จังหวัด.....
รหัสไปษณีย์..... ได้อนุญาตให้สัตวแพทย์หญิง พรรณพิชญา ฟุ้งวิทยา เลขที่ใบอนุญาต 01-6149/2550 เป็นหัวหน้าหรือเจ้าของโครงการใช้ตัวอย่างเชื้อจากทางช่องจมูกด้านหน้า ง่ามขาและแผลที่ผิวหนังจากสุนัขชื่อ.....เพื่อศึกษาปัจจัยที่มีผลต่อการคงอยู่การคงอยู่ของเชื้อ เมธิซิลิน รีสิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค บนผิวหนังสุนัข และในโรงพยาบาลสัตว์เล็ก

ลงนาม.....
(.....)
เจ้าของสัตว์
ลงนาม.....
(.....)
สัตวแพทย์
ลงนาม.....
(.....)
นายพ
วันที่.....ศ.พ.....เดือน.....

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

หนังสือแสดงความยินยอมเข้าร่วมการวิจัย (สำหรับสัตวแพทย์)

ทำที่.....

วันที่.....เดือน.....พ.ศ..... .

เลขที่ ประชากรตัวอย่างหรือผู้มีส่วนร่วมในการวิจัย.....

ข้าพเจ้า ซึ่งได้ลงนามท้ายหนังสือนี้ ขอแสดงความยินยอมเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย ปัจจัยที่มีผลต่อการคงอยู่ของเชื้อในกลุ่มเมธิซิลิน รีสิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไคใน โรงพยาบาลสัตว์เล็ก

ชื่อผู้วิจัย นางสาว พรรณพิชญา ฟุ้งวิทยา

ที่อยู่ที่ติดต่อ ภาควิชาจุลชีววิทยาทางสัตวแพทย์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ 02 218 9582

ข้าพเจ้า ได้รับทราบรายละเอียดเกี่ยวกับที่มาและวัตถุประสงค์ในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยง/อันตราย และประโยชน์ซึ่งจะเกิดขึ้นจากการวิจัยเรื่องนี้ โดยได้อ่านรายละเอียดในเอกสารชี้แจงผู้เข้าร่วมการวิจัยโดยตลอด และได้รับคำอธิบายจากผู้วิจัย จนเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้าจึงสมัครใจเข้าร่วมในโครงการวิจัยนี้ ตามที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย โดยข้าพเจ้ายินยอม ตอบแบบสอบถามเกี่ยวกับการในยาต้านจุลชีพในโรงพยาบาลสัตว์เล็ก จุฬาลงกรณ์ มหาวิทยาลัย *ทั้งนี้จะมีการสัมภาษณ์เป็นเวลา 15 นาที และ*เก็บตัวอย่างด้วยสำลีพันปลายไม้(cotton bud) *เป็นเวลา 5 นาที* ข้อมูลเกี่ยวกับแบบสอบถามและตัวอย่างที่เก็บได้จะถูกเก็บเป็นความลับ

ข้าพเจ้ามีสิทธิถอนตัวออกจากการวิจัยเมื่อใดก็ได้ตามความประสงค์ โดยไม่ต้องแจ้งเหตุผล ซึ่งการถอนตัวออกจากการวิจัยนั้น จะไม่มีผลกระทบในทางใดๆ ต่อข้าพเจ้าทั้งสิ้น ซึ่งรวมถึงตำแหน่งในปัจจุบันของข้าพเจ้า

ข้าพเจ้าได้รับคำรับรองว่า ผู้วิจัยจะปฏิบัติต่อข้าพเจ้าตามข้อมูลที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และข้อมูลใดๆ ที่เกี่ยวข้องกับข้าพเจ้า ผู้วิจัยจะเก็บรักษาเป็นความลับ โดยจะนำเสนอข้อมูลการวิจัยเป็นภาพรวมเท่านั้น ไม่มีข้อมูลใดในการรายงานที่จะนำไปสู่การระบุตัวข้าพเจ้า

หากข้าพเจ้าไม่ได้รับการปฏิบัติตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าสามารถร้องเรียนได้ที่ คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ จุฬาลงกรณ์มหาวิทยาลัย ชั้ 1น ชอย 2 อาคารสถาบัน 4 10330 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 62 จุฬาลงกรณ์

โทรศัพท์ 8147-2218-0, 0-2218-8141 โทรสาร 8147-2218-0E-mail: eccu@chula.ac.th

ข้าพเจ้าได้ลงลายมือชื่อไว้เป็นสำคัญต่อหน้าพยาน ทั้งนี้ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงผู้เข้าร่วมการวิจัย และสำเนาหนังสือแสดงความยินยอมไว้แล้ว

ลงชื่อ.....
นางสาว พรรณพิชญา ฟุ้งวิทยา
ผู้วิจัยหลัก

ลงชื่อ.....
(.....)
ผู้มีส่วนร่วมในการวิจัย

ลงชื่อ.....
(.....)
พยาน

แบบสอบถาม : ปัจจัยที่มีผลต่อการคงอยู่ของเชื้อในกลุ่มเมธิซิลิน รีสิสแตน

โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค ในผิวหนังสัตว์และโรงพยาบาลสัตว์เล็ก

หลักสูตรพยาธิวิทยาทางสัตวแพทย์ ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

สถานที่เก็บข้อมูล .....วันที่.....

วัตถุประสงค์ : แบบสอบถามนี้จัดทำขึ้นเพื่อรวบรวมข้อมูลเกี่ยวกับการจัดการความสะอาดในบริเวณห้องต่างๆของโรงพยาบาลสัตว์เล็ก จุฬาลงกรณ์ มหาวิทยาลัย ซึ่งอาจเป็นปัจจัยหนึ่งที่ทำให้เกิดการคงอยู่ของเชื้อดื้อยา ในกลุ่มเมธิซิลิน รีสิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค บนผิวหนังสัตว์และในโรงพยาบาลสัตว์ โดยแบบสอบถามนี้ เป็นส่วนหนึ่งของงานวิจัยของนักศึกษาระดับปริญญาเอก ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์ มหาวิทยาลัย และจะนำข้อมูลไปใช้ประโยชน์ในการศึกษาเท่านั้น ทั้งนี้ขออนุญาตใช้เวลาในการสัมภาษณ์เป็นเวลา 15 นาที

ส่วนที่ 1 ข้อมูลทั่วไป (สัตวแพทย์(

คำชี้แจง : กรุณาทำเครื่องหมาย √ ลงในช่องว่าง ☐ หน้าคำตอบที่ท่านต้องการ

1.1. เพศ : ☐ ชาย ☐ หญิง

1.2. อายุ : ☐ ต่ำกว่า 20 ปี ☐ 21-30 ปี ☐ 31-40 ปี
☐ 41-50 ปี ☐ 51-60 ปี ☐ 61 ปีขึ้นไป

1.3. แผนกที่ปฏิบัติงาน : ☐ ห้องอายุศาสตร์ ☐ ห้องสูติกรรม ☐ ห้องฉุกเฉิน ☐ ห้องวัคซีน
☐ ห้องผ่าตัด ☐ ห้องโรคผิวหนัง

1.4. อายุงาน (ปี): ☐ < 1 ☐ 1 - 2 ☐ 3-5 ☐ 6-10 ☐ 10 ปีขึ้นไป

1.5. เวลาการทำงานภายในหนึ่งสัปดาห์ ☐ <40 hr./week ☐ 40 hr./week ☐ >40 hr./week

ส่วนที่ 2 ความคิดเห็นเกี่ยวกับการใช้ยาต้านจุลชีพในชีวิตประจำวัน

2.1. ท่านเคยใช้ยาต้านจุลชีพสำหรับตัวท่านเองในชีวิตประจำวันหรือไม่ ☐ เคย ระบุ_____ ☐ ไม่เคย

2.2. ท่านมีสุนัขเลี้ยงไว้ในบริเวณบ้านหรือไม่ ☐ มี ☐ ไม่มี (ข้ามไปข้อ 2.5)

2.3. ความใกล้ชิดของท่านกับสุนัขเลี้ยงของท่าน ☐ มาก ☐ ปานกลาง ☐ น้อย

2.4. สุนัขเลี้ยงของท่านเคยได้รับยาปฏิชีวนะหรือไม่ ☐ เคย ระบุ_____ ☐ ไม่เคย

2.5. จำนวนสุนัขที่ท่านทำการรักษาในแต่ละวัน
☐ 1 case/ต่อวัน ☐ 2-5 case/ต่อวัน ☐ 5-10 case/ต่อวัน ☐ ≥10 case/ต่อวัน

2.6 ท่านเคยใช้ยาต้านจุลชีพเพื่อการรักษาสุนัขหรือไม่ ☐ เคย ระบุ_____ ☐ ไม่เคย

ขอขอบพระคุณในความร่วมมือมา ณ โอกาสนี้เป็นอย่างสูง
สพ.ญ. พรรณพิชญา ฟุ้งวิทยา นิสิตผู้ดำเนินงานวิจัย

หนังสือแสดงความยินยอมเข้าร่วมการวิจัย (สำหรับผู้ช่วยสัตวแพทย์(

ทำที่.....

วันที่.....เดือน.....พ.ศ.....

เลขที่ ประชากรตัวอย่างหรือผู้มีส่วนร่วมในการวิจัย.....

ข้าพเจ้า ซึ่งได้ลงนามท้ายหนังสือนี้ ขอแสดงความยินยอมเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย ปัจจัยที่มีผลต่อการคงอยู่ของเชื้อในกลุ่มเมธิซิลิน รีสิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไคใน โรงพยาบาลสัตว์เล็ก

ชื่อผู้วิจัย นางสาว พรรณพิชญา ฟุ้งวิทยา

ที่อยู่ที่ติดต่อ ภาควิชาจุลชีววิทยาทางสัตวแพทย์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ 02 218 9582

ข้าพเจ้า ได้รับทราบรายละเอียดเกี่ยวกับที่มาและวัตถุประสงค์ในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยง/อันตราย และประโยชน์ซึ่งจะเกิดขึ้นจากการวิจัยเรื่องนี้ โดยได้อ่านรายละเอียดในเอกสารชี้แจงผู้เข้าร่วมการวิจัยโดยตลอด และได้รับคำอธิบายจากผู้วิจัย จนเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้าจึงสมัครใจเข้าร่วมในโครงการวิจัยนี้ ตามที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย โดยข้าพเจ้ายินยอม ตอบแบบสอบถามเกี่ยวกับการจัดการความสะอาดในบริเวณห้องต่างๆของโรงพยาบาลสัตว์เล็ก จุฬาลงกรณ์ มหาวิทยาลัย ทั้งนี้จะมีการสัมภาษณ์เป็นเวลา 15 นาทีและเก็บตัวอย่างด้วยสำลีพันปลายไม้(cotton bud) *เป็นเวลา 5 นาที* ข้อมูลเกี่ยวกับแบบสอบถามและตัวอย่างที่เก็บได้จะถูกเก็บเป็นความลับ

ข้าพเจ้ามีสิทธิถอนตัวออกจากการวิจัยเมื่อใดก็ได้ตามความประสงค์ โดยไม่ต้องแจ้งเหตุผล ซึ่งการถอนตัวออกจากการวิจัยนั้น จะไม่มีผลกระทบในทางใดๆ ต่อข้าพเจ้าทั้งสิ้น ซึ่งรวมถึงตำแหน่งในปัจจุบันของข้าพเจ้า

ข้าพเจ้าได้รับคำรับรองว่า ผู้วิจัยจะปฏิบัติต่อข้าพเจ้าตามข้อมูลที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และข้อมูลใดๆ ที่เกี่ยวข้องกับข้าพเจ้า ผู้วิจัยจะเก็บรักษาเป็นความลับ โดยจะนำเสนอข้อมูลการวิจัยเป็นภาพรวมเท่านั้น ไม่มีข้อมูลใดในการรายงานที่จะนำไปสู่การระบุตัวข้าพเจ้า

หากข้าพเจ้าไม่ได้รับการปฏิบัติตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าสามารถร้องเรียนได้ที่ คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ ซอย 2 อาคารสถาบัน 4 จุฬาลงกรณ์มหาวิทยาลัย ชั้น 1 10330 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 62 จุฬาลงกรณ์

โทรศัพท์ 8147-2218-0, 0-2218-8141 โทรสาร 8147-2218-0E-mail: eccu@chula.ac.th

ข้าพเจ้าได้ลงลายมือชื่อไว้เป็นสำคัญต่อหน้าพยาน ทั้งนี้ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงผู้เข้าร่วมการวิจัย และสำเนาหนังสือแสดงความยินยอมไว้แล้ว

ลงชื่อ ลงชื่อ.....

..... (.....)

นางสาว พรรณพิชญา ฟุ้งวิทยา
ผู้วิจัยหลัก

ผู้มีส่วนร่วมในการวิจัย

ลงชื่อ.....

(.....)

พยาน

แบบสอบถาม : ปัจจัยที่มีผลต่อการคงอยู่ของเชื้อในกลุ่มเมธิซิลิน รีสิสแตน
โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไคบนผิวหนังสัตว์และในโรงพยาบาลสัตว์เล็ก
หลักสูตรพยาธิวิทยาทางสัตวแพทย์ภาควิชาจุลชีววิทยาคณะสัตวแพทยศาสตร์จุฬาลงกรณ์มหาวิทยาลัย
สถานที่เก็บข้อมูล.....วันที่ .....

วัตถุประสงค์ :แบบสอบถามนี้จัดทำขึ้นเพื่อรวบรวมข้อมูลเกี่ยวกับการจัดการความสะอาดในบริเวณห้องต่างๆของโรงพยาบาลสัตว์เล็ก จุฬาลงกรณ์ มหาวิทยาลัย ซึ่งอาจเป็นปัจจัยหนึ่งที่ทำให้เกิดการคงอยู่ของเชื้อดื้อยา ในกลุ่มเมธิซิลิน รีสิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค บนผิวหนังสุนัขและในโรงพยาบาลสัตว์ โดยแบบสอบถามนี้ เป็นส่วนหนึ่งของงานวิจัยของนักศึกษาระดับปริญญาเอก ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์ มหาวิทยาลัย และจะนำข้อมูลไปใช้ประโยชน์ในการศึกษาเท่านั้น

ส่วนที่ 1 ข้อมูลทั่วไป (สำหรับผู้ช่วยสัตวแพทย์(

คำชี้แจง:กรุณาทำเครื่องหมาย √ ลงในช่องว่าง☐หน้าคำตอบที่ท่านต้องการ

1.6. เพศ : ☐ ชาย ☐ หญิง

1.7. อายุ : ☐ ต่ำกว่า 20 ปี ☐ 21-30 ปี ☐ 31-40 ปี
☐ 41-50 ปี ☐ 51-60 ปี ☐ 61 ปีขึ้นไป

1.8. วุฒิการศึกษา : ☐ มัธยมศึกษาหรือต่ำกว่า ☐ ประกาศนียบัตรวิชาชีพ
☐ ปริญญาตรี ☐ สูงกว่าปริญญาตรี

1.9. แผนกที่ปฏิบัติงาน: ☐ห้องอายุศาสตร์ ☐ห้องสูติกรรม ☐ห้องฉุกเฉิน ☐ห้องวัคซีน
☐ห้องผ่าตัด ☐ห้องโรคผิวหนัง

1.10. อายุงาน (ปี): ☐< 1 ☐ 1 - 2 ☐3-5 ☐6-10 ☐10 ปีขึ้นไป

ส่วนที่ ความคิดเห็นเกี่ยวกับการทำความสะอาดในชีวิตประจำวัน2

คำชี้แจง : กรุณาทำเครื่องหมาย √ ลงในช่องว่าง☐ตามความคิดเห็นของท่านว่า"เห็นด้วย" หรือ "ไม่เห็นด้วย"

คำถาม	เห็นด้วย	ไม่เห็นด้วย
2.1. การทำความสะอาดพื้นห้องตรวจด้วยน้ำยาฆ่าเชื้อแบบถูพื้น ควรทำอย่างน้อยหนึ่งถึงสองครั้งต่อวัน	☐	☐
2.2. การเพิ่มความเข้มข้นขึ้น 2 เท่าของน้ำยาฆ่าเชื้อแบบถูพื้นควรจะกระทำในพื้นที่ๆมีความเคร่งครัดเรื่องความสะอาด อย่างเช่น ห้องศัลยกรรม	☐	☐
2.3. ควรเอาสิ่งสกปรกออกจากไม้ถูพื้นให้หมดก่อนที่จะทำความสะอาดครั้งต่อไป	☐	☐
2.4. การใช้หัวผ้าถูพื้นซ้ำ ควรนำผ้าถูพื้นแช่ผงซักฟอกที่อัตราส่วน 1000 ppm (1:50) ในน้ำร้อนที่อุณหภูมิ เกิน 160 องศาเซลเซียสเป็นระยะเวลา 10 นาที เพื่อฆ่าเชื้อหลังใช้งาน หรือก่อนเริ่มงานทำความสะอาดพื้น	☐	☐
2.5. ควรเติม(ในกรณีที่วันนั้นไม่มีสัตว์ที่มีโรคติดเชื้อร้ายแรงเข้ามารับการรักษา) หรือเปลี่ยนถ่าย(ในกรณีที่มีสัตว์ที่มีโรคติดเชื้อร้ายแรงเข้ามารับการรักษา) น้ำยาฆ่าเชื้อ ที่ใช้สำหรับอุปกรณ์ทุกวัน	☐	☐
2.6. ในบริเวณที่คนและสัตว์ป่วยมากกว่า 10 คนและ/หรือ 10 ตัวขึ้นไป การทำความสะอาดพื้นควรทำอย่างน้อย สองครั้งต่อวัน	☐	☐
2.7. ควรทำความสะอาดผนังห้องที่ใช้ทำการบำบัดโรคที่มีการสัมผัสกับสัตว์ป่วย	☐	☐
2.8. ควรมีการฆ่าเชื้ออุปกรณ์ทั้งเล็กและใหญ่ทุกชนิดที่ทำได้อย่างน้อยทุกสัปดาห์	☐	☐
2.9. ในการฆ่าเชื้อกับอุปกรณ์ทำความสะอาดต่างๆก่อนที่จะรับสัตว์ใช้ใหม่ควรแช่แอลกอฮอล์มาตรฐาน 70% เป็นเวลา 10-15 นาที	☐	☐

ส่วนที่ 3ข้อมูลการทำความสะอาดพื้น

คำชี้แจง : กรุณาทำเครื่องหมาย √ ลงในช่องว่าง ☐ ตามกิจกรรมทำความสะอาดของท่านตามจริง

3.1. จำนวนครั้งในการทำความสะอาดพื้น : ☐ ครั้งต่อวัน 1 ☐ ครั้งต่อวัน 2 ☐ มากกว่า 2 ครั้งต่อวัน

3.2. ช่วงเวลาทำความสะอาดพื้น : ☐ เช้า ☐ เย็น ☐ เช้าและเย็น

3.3. อุปกรณ์ทำความสะอาดพื้น : ☐ ไม้กวาด☐ ไม้ถูพื้น ☐ ทั้งสองอย่าง

3.4. การทำความสะอาดไม้กวาด : ☐ เปลี่ยนใหม่ .....(นานเท่าใด) ☐ ไม่เคยทำความสะอาด
☐ อื่นๆ ..... (ระบุ)

3.5. การทำความสะอาดไม้ถูพื้น : ☐ ซักด้วยน้ำเปล่า ☐ ซักด้วยน้ำยาฆ่าเชื้อ
☐ ไม่เคยทำความสะอาด

*<u>ข้อ 3.6-3.10 สำหรับผู้ทำความสะอาดด้วยไม้ถูพื้น</u>

3.6. ชนิดของน้ำยาถูพื้น : ☐ น้ำยาถูพื้นที่ใช้ตามบ้าน ☐ น้ำยาถูพื้นเฉพาะโรงพยาบาล
☐ อื่นๆ ☐ ไม่ทราบชนิด

3.7. ระยะเวลาที่น้ำยาถูพื้นสัมผัสกับพื้น : ☐ < 10 นาที ☐ 11-15 นาที ☐ มากกว่า 15 นาที

3.8. จำนวนการซักผ้าถูพื้นต่อการทำความสะอาดหนึ่งครั้ง : ☐ครั้ง 1 ☐ ครั้ง 2
☐ มากกว่า 2 ครั้ง

3.9. คุณมีการทำความสะอาดมากขึ้น ในวันที่มีสุนัขที่มารับการรักษามากกว่าปกติ : ☐ ใช่ ☐ ไม่ใช่

3.10. ระยะเวลาที่ใช้ในการทำความสะอาดต่อครั้ง : ☐ < 15 นาที ☐ 15-30 นาที ☐ มากกว่า 30 นาที

ส่วนที่ 4ข้อมูลการทำความสะอาดอุปกรณ์ในห้อง

คำชี้แจง : กรุณาทำเครื่องหมาย √ ลงในช่องว่าง☐ตามกิจกรรมทำความสะอาดของท่านตามจริง

4.1. จำนวนครั้งในการทำความสะอาดอุปกรณ์ต่างๆที่สัมผัสกับตัวสัตว์หลังใช้งาน :

☐ 1 ครั้ง ☐ ≥ 1 ครั้ง ☐ ไม่เคยทำความสะอาด

4.2. จำนวนครั้งที่เปลี่ยนน้ำยาทำความสะอาดอุปกรณ์:

☐ 1 วัน ☐ ≥ 1 วัน ☐ ไม่เคยเปลี่ยน

4.3. จำนวนครั้งที่เช็ดโต๊ะรักษาสัตว์ป่วย (หรือไข้):

☐ 1 ครั้ง ☐ ≥ 1 ครั้ง ☐ ไม่เคยทำความสะอาด

4.4. น้ำยาทำความสะอาดที่ใช้เช็ดโต๊ะรักษาสัตว์ป่วย (หรือไข้): ☐ใช้ ได้แก่.....☐ ไม่ใช้

4.5. ระยะเวลาที่น้ำยาทำความสะอาดสัมผัสกับโต๊ะรักษาสัตว์ป่วย (หรือไข้):

☐ 30 วินาที ☐ 0.31 - 5 นาที ☐ >5 นาที

4.6. การทำความสะอาดคอมพิวเตอร์ประจำห้องตรวจ:

☐ 1 ครั้งต่อวัน ☐ 2 ครั้งต่อวัน ☐ อื่นๆ ระบุ..... ☐ ไม่เคย

4.7. การทำความสะอาดเก้าอี้เก้าอี้ที่เจ้าของสัตว์ป่วยนั่งรอรับการรักษา:

☐ 1 ครั้งต่อวัน ☐ 2 ครั้งต่อวัน ☐ อื่นๆ ระบุ..... ☐ ไม่เคย

4.8. สำลีที่ใช้ในห้องตรวจ :

☐ เปิดใหม่ทุกวัน ☐ ใช้จนหมดค่อยเปิดใหม่ ☐ อื่นๆ ระบุ.....

4.9. การทำความสะอาดตู้เก็บยา:

☐ 1 ครั้งต่อวัน ☐ 2 ครั้งต่อวัน ☐ อื่นๆ ระบุ..... ☐ ไม่เคย

4.10. การทำความสะอาดถาดรองอุปกรณ์:

☐ 1 ครั้งต่อวัน ☐ 2 ครั้งต่อวัน ☐ อื่นๆ ระบุ..... ☐ ไม่เคย

4.11. การทำความสะอาด Stethoscope:

☐ 1 ครั้งต่อวัน ☐ 2 ครั้งต่อวัน ☐ อื่นๆ ระบุ..... ☐ ไม่เคย

4.12. การทำความสะอาดลูกบิดประตู:

☐ 1 ครั้งต่อวัน ☐ 2 ครั้งต่อวัน ☐ อื่นๆ ระบุ..... ☐ ไม่เคย

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

ขอขอบพระคุณในความร่วมมือมา ณ โอกาสนี้เป็นอย่างสูง

สพ พรรณพิชญา ฟุ้งวิทยานิสิตผู้ดำเนินงานวิจัย .ญ.

หนังสือแสดงความยินยอมเข้าร่วมการวิจัย (สำหรับผู้ทำความสะอาด(

ทำที่.....

วันที่.....เดือน.....พ.ศ.....

เลขที่ ประชากรตัวอย่างหรือผู้มีส่วนร่วมในการวิจัย.....

ข้าพเจ้า ซึ่งได้ลงนามท้ายหนังสือนี้ ขอแสดงความยินยอมเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย ปัจจัยที่มีผลต่อการคงอยู่ของเชื้อในกลุ่มเมธิซิลิน รีสิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไคในโรงพยาบาลสัตว์เล็ก

ชื่อผู้วิจัย นางสาว พรรณพิชญา ฟุ้งวิทยา

ที่อยู่ที่ติดต่อ ภาควิชาจุลชีววิทยาทางสัตวแพทย์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ 02 218 9582

ข้าพเจ้า ได้รับทราบรายละเอียดเกี่ยวกับที่มาและวัตถุประสงค์ในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยงอันตราย และประโยชน์ซึ่งจะเกิดขึ้นจากการวิจัยเรื่องนี้ โดยได้อ่านรายละเอียดในเอกสารชี้แจงผู้เข้าร่วมการ/วิจัยโดยตลอด และได้รับคำอธิบายจากผู้วิจัย จนเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้าจึงสมัครใจเข้าร่วมในโครงการวิจัยนี้ ตามที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย โดยข้าพเจ้ายินยอม ตอบแบบสอบถามเกี่ยวกับการจัดการความสะอาดในบริเวณห้องต่างๆของโรงพยาบาลสัตว์เล็ก จุฬาลงกรณ์ มหาวิทยาลัย ทั้งนี้จะมีการสัมภาษณ์เป็นเวลา 15 นาที ข้อมูลเกี่ยวกับแบบสอบถามและตัวอย่างที่เก็บได้จะถูกเก็บเป็นความลับ

ข้าพเจ้ามีสิทธิถอนตัวออกจากการวิจัยเมื่อใดก็ได้ตามความประสงค์ โดยไม่ต้องแจ้งเหตุผล ซึ่งการถอนตัวออกจากการวิจัยนั้น จะไม่มีผลกระทบในทางใดๆ ต่อข้าพเจ้าทั้งสิ้น ซึ่งรวมถึงตำแหน่งในปัจจุบันของข้าพเจ้า

ข้าพเจ้าได้รับคำรับรองว่า ผู้วิจัยจะปฏิบัติต่อข้าพเจ้าตามข้อมูลที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และข้อมูลใดๆ ที่เกี่ยวข้องกับข้าพเจ้า ผู้วิจัยจะเก็บรักษาเป็นความลับ โดยจะนำเสนอข้อมูลการวิจัยเป็นภาพรวมเท่านั้น ไม่มีข้อมูลใดในการรายงานที่จะนำไปสู่การระบุตัวข้าพเจ้า

หากข้าพเจ้าไม่ได้รับการปฏิบัติตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าสามารถร้องเรียนได้ที่ ซอย 2 อาคารสถาบัน 4 จุฬาลงกรณ์มหาวิทยาลัย ชั้น 1 คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 10330 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 62 จุฬาลงกรณ์
โทรศัพท์ 8147-2218-0, 0-2218-8141 โทรสาร 8147-2218-0E-mail: eccu@chula.ac.th

ข้าพเจ้าได้ลงลายมือชื่อไว้เป็นสำคัญต่อหน้าพยาน ทั้งนี้ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงผู้เข้าร่วมการวิจัย และสำเนาหนังสือแสดงความยินยอมไว้แล้ว

ลงชื่อ.....
นางสาว พรรณพิชญา ฟุ้งวิทยา
ผู้วิจัยหลัก

ลงชื่อ.....
(.....)
ผู้มีส่วนร่วมในการวิจัย

ลงชื่อ.....
(.....)
พยาน

แบบสอบถาม : ปัจจัยที่มีผลต่อการคงอยู่ของเชื้อในกลุ่มเมธิซิลิน รีสิสแตน
โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค ในผิวหนังสัตว์และโรงพยาบาลสัตว์เล็ก
หลักสูตรพยาธิวิทยาทางสัตวแพทย์ ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
สถานที่เก็บข้อมูล.....วันที่ .....

วัตถุประสงค์ :แบบสอบถามนี้จัดทำขึ้นเพื่อรวบรวมข้อมูลเกี่ยวกับการจัดการความสะอาดในบริเวณห้องต่างๆของโรงพยาบาลสัตว์เล็ก จุฬาลงกรณ์ มหาวิทยาลัย ซึ่งอาจเป็นปัจจัยหนึ่งที่ทำให้เกิดการคงอยู่ของเชื้อดื้อยา ในกลุ่มเมธิซิลิน รีสิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค บนผิวหนังสัตว์และในโรงพยาบาลสัตว์ โดยแบบสอบถามนี้ เป็นส่วนหนึ่งของงานวิจัยของนักศึกษาระดับปริญญาเอก ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์ มหาวิทยาลัย และจะนำข้อมูลไปใช้ประโยชน์ในการศึกษาเท่านั้น
ทั้งนี้ขออนุญาตใช้เวลาในการสัมภาษณ์เป็นเวลา 15 นาที

ส่วนที่ 1 ข้อมูลทั่วไป (สำหรับผู้จัดการทำความสะอาด(

คำชี้แจง : กรุณาทำเครื่องหมาย √ ลงในช่องว่าง ☐ หน้าคำตอบที่ท่านต้องการ

1.1 เพศ : ☐ ชาย ☐ หญิง

1.2 อายุ : ☐ ต่ำกว่า 20 ปี ☐ 21-30 ปี ☐ 31-40 ปี
☐ 41-50 ปี ☐ 51-60 ปี ☐ 61 ปีขึ้นไป

1.3 วุฒิการศึกษา : ☐ มัธยมศึกษาหรือต่ำกว่า ☐ ประกาศนียบัตรวิชาชีพ
☐ ปริญญาตรี ☐ สูงกว่าปริญญาตรี

1.4 อายุงาน (ปี) : ☐ < 1 ☐ 1 - 2 ☐ 3-5 ☐ 6-10 ☐ 10 ปีขึ้นไป

ส่วนที่ 2 ความคิดเห็นเกี่ยวกับการทำความสะอาดในชีวิตประจำวัน

คำชี้แจง : กรุณาทำเครื่องหมาย √ ลงในช่องว่าง ☐ ตามความคิดเห็นของท่านว่า "เห็นด้วย" หรือ "ไม่เห็นด้วย"

คำถาม	เห็นด้วย	ไม่เห็นด้วย
2.1 การทำความสะอาดพื้นห้องตรวจด้วยน้ำยาฆ่าเชื้อแบบถูพื้น ควรทำอย่างน้อยหนึ่งถึงสองครั้งต่อวัน	☐	☐
2.2 การเพิ่มความเข้มข้นขึ้น 2 เท่าของน้ำยาฆ่าเชื้อแบบถูพื้น ควรจะกระทำในพื้นที่ๆ มีความเคร่งครัดเรื่องความสะอาด อย่างเช่น ห้องศัลยกรรม	☐	☐
2.3 ควรเอาสิ่งสกปรกออกจากไม้ถูพื้นให้หมดก่อนที่จะทำความสะอาดครั้งต่อไป	☐	☐
2.4 การใช้หัวผ้าถูพื้นซ้ำ ควรนำผ้าถูพื้นแช่ผงซักฟอกที่อัตราส่วน 1000 ppm (1:50) ในน้ำร้อนที่อุณหภูมิ เกิน 160 องศาเซลเซียส เป็นระยะเวลา 10 นาที เพื่อฆ่าเชื้อหลังใช้งาน หรือก่อนเริ่มงานทำความสะอาดพื้น	☐	☐
2.5 ในบริเวณที่คนและสัตว์ป่วยมากกว่า 10 คนและ/หรือ 10 ตัวขึ้นไป การทำความสะอาดพื้นควรทำอย่างน้อย สองครั้งต่อวัน	☐	☐
2.6 ในการฆ่าเชื้อกับอุปกรณ์ทำความสะอาดต่างๆ ก่อนที่จะรับสัตว์ไข้ใหม่ ควรแช่แอลกอฮอล์มาตรฐาน 70% เป็นเวลา 10-15 นาที	☐	☐

ส่วนที่ 3 ข้อมูลการทำความสะอาดพื้น

คำชี้แจง : กรุณาทำเครื่องหมาย √ ลงในช่องว่าง ☐ ตามกิจกรรมทำความสะอาดของท่านตามจริง

.1 จำนวนครั้งในการทำความสะอาดพื้น : ☐ 1 ครั้งต่อวัน ☐ 2 ครั้งต่อวัน ☐ มากกว่า 2 ครั้งต่อวัน

.2 ช่วงเวลาทำความสะอาดพื้น : ☐ เช้า ☐ เย็น ☐ เช้าและเย็น

.3 อุปกรณ์ทำความสะอาดพื้น : ☐ ไม้กวาด ☐ ไม้ถูพื้น ☐ ทั้งสองอย่าง

.4 การทำความสะอาดไม้กวาด : ☐ เปลี่ยนใหม่ (นานเท่าใด)..... ☐ ไม่เคยทำความสะอาด ☐ อื่นๆ (ระบุ) .....

.5 การทำความสะอาดไม้ถูพื้น : ☐ ซักด้วยน้ำเปล่า ☐ ซักด้วยน้ำยาฆ่าเชื้อ ☐ ไม่เคยทำความสะอาด

*<u>ข้อ 3.6-3.10 สำหรับผู้ทำความสะอาดด้วยไม้ถูพื้น</u>

.6 ชนิดของน้ำยาถูพื้น : ☐ น้ำยาถูพื้นที่ใช้ตามบ้าน ☐ น้ำยาถูพื้นเฉพาะโรงพยาบาล ☐ อื่นๆ ☐ ไม่ทราบชนิด

.7 ระยะเวลาที่น้ำยาถูพื้นสัมผัสกับพื้น : ☐ < 10 นาที ☐ 11-15 นาที ☐ มากกว่า 15 นาที

.8 จำนวนการซักผ้าถูพื้นต่อการทำความสะอาดหนึ่งครั้ง : ☐ 1 ครั้ง ☐ 2 ครั้ง ☐ มากกว่า 2 ครั้ง

.9 คุณมีการทำความสะอาดมากขึ้น ในวันที่มีสุนัขที่มารับการรักษามากกว่าปกติ : ☐ ใช่ ☐ ไม่ใช่

.10 ระยะเวลาที่ใช้ในการทำความสะอาดต่อครั้ง : ☐ < 15 นาที ☐ 15-30 นาที ☐ มากกว่า 30 นาที

ขอขอบพระคุณในความร่วมมือมา ณ โอกาสนี้เป็นอย่างสูง

สพพรรณพิชญา ฟุ้งวิทยา .ญ. นิสิตผู้ดำเนินงานวิจัย

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

การคำนวณความน่าเชื่อถือของแบบสอบถาม

แบบสอบถามเรื่องการทำความสะอาด (cleaning management)

แบบสอบถามในส่วนที่ 2 ความคิดเห็นเกี่ยวกับการทำความสะอาดในชีวิตประจำวัน ดัดแปลงมาจาก routine cleaning and disinfectant protocol ซึ่งเขียนโดยสัตวแพทย์ Joshua A. Portner และ Justine A. Johnson เนื้อหาดัดแปลงมาจากวารสาร Guidelines for reducing pathogens in veterinary hospitals: disinfectant selection, cleaning protocols, and hand hygiene ที่ถูกตีพิมพ์เมื่อ 2010 ซึ่งจะใช้เป็นแนวทางการทำความสะอาดของโรงพยาบาลต่อไป ในส่วนนี้ผู้วิจัยมีวัตถุประสงค์เพื่อทดสอบความรู้ของผู้ทำความสะอาดและผู้ช่วยสัตวแพทย์ซึ่งคัดเฉพาะข้อที่ควรจะรู้ในการทำความสะอาดมาทดสอบ โดยใช้คำถาม “เห็นด้วย” หรือ “ไม่เห็นด้วย” เพื่อหลีกเลี่ยง bias คำถามที่เลือกมาทดสอบความรู้ของผู้ช่วยสัตวแพทย์มี 9 ข้อและผู้จัดการทำความสะอาดมี 6 ข้อ ในข้อนี้มีการตั้งสมมุติฐานว่า ความรู้ในการทำความสะอาดที่ถูกต้องอาจจะมีผลต่อการทำความสะอาดของผู้ปฏิบัติ

ในผู้ช่วยสัตวแพทย์ เกณฑ์การให้คะแนนคือ 1.) เห็นด้วย 9-5 คะแนน = ความรู้ดี 2.) เห็นด้วย 4-0 คะแนน = ไม่มีความรู้

ในผู้ทำความสะอาด เกณฑ์การให้คะแนนคือ 1.) เห็นด้วย 6-4 คะแนน = ความรู้ดี 2.) เห็นด้วย 3-0 คะแนน = ไม่มีความรู้

แบบสอบถามในส่วนที่ 3 และ 4 พัฒนามาจากผลการเก็บเชื้อในสถานที่ต่างๆ โดยเฉพาะอย่างยิ่งบนพื้น ที่มีเป็นตำแหน่งที่มีการเจอเชื้อมากอย่างนัยสำคัญ คำถามของการทำความสะอาดบนพื้นห้องจึงจำเป็นต้องละเอียดมากกว่าตำแหน่งอุปกรณ์ที่เคลื่อนได้และเคลื่อนที่ไม่ได้ และเมื่อได้สอบถามอย่างคราวๆพบว่ามีความแตกต่างกันในแต่ละห้องค่อนข้างมาก จึงจำเป็นต้องลงลึกถึงปัจจัยอย่างละเอียด

แบบสอบถามเรื่องการใช้ยาต้านจุลชีพ (antimicrobial use**)**

พัฒนามาจากแบบสอบถามของ อ.น.สพ. ภัทรรัฐ จันทร์ฉายทอง ที่ได้ผ่าน Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University เลขที่ 081/54

Reliability and validity

เครื่องมือที่ใช้ทดสอบใช้ reliability และ validity โดยมีผู้ทรงคุณวุฒิคือ รศ.ดร.น.สพ .ณุวีร์ ประภัสระกูล, นางวารี นิยมธรรม (นักวิทยาศาสตร์ผู้ชำนาญการพิเศษ (และผศ.ดร.น.สพ.ชาญวิทย์ ตรีพุทธรัตน์

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

แบบสอบถามเรื่องการทำความสะอาด รวมทั้งผู้ทำความสะอาดและผู้ช่วยสัตวแพทย์

ผลจากการพิจารณาโดยผู้เชี่ยวชาญ เพื่อหาค่า IOC

ประเด็นที่ต้องการ	ข้อคำถาม	ระดับความสอดคล้อง			รวม	IOC
		สอดคล้อง	ไม่แน่ใจ	ไม่สอดคล้อง		
ส่วนที่ 2						
1.) ทดสอบความรู้ในการทำความสะอาดพื้น	2.1	3	0	0	3	1
	2.2	2	1	0	2	0.67
	2.3	3	0	0	3	1
	2.4	2	1	0	2	0.67
	2.6	2	1	0	2	0.67
2.) ทดสอบความรู้เกี่ยวกับการเปลี่ยนน้ำยาฆ่าเชื้อสำหรับอุปกรณ์	2.5	3	0	0	3	1
3.) ทดสอบความรู้เกี่ยวกับการทำความสะอาดผนังที่มีแนวโน้มจะสกปรก	2.7	3	0	0	3	1
4.) ทดสอบความรู้ในการทำความสะอาดอุปกรณ์	2.8	3	0	0	3	1
	2.9	2	1	0	2	0.67
ส่วนที่ 3						
1.) วิธีและความถี่ในการทำความสะอาด	3.1	3	0	0	3	1
	3.2	3	0	0	3	1
	3.3	3	0	0	3	1
	3.4	3	0	0	3	1
	3.5	2	1	0	2	0.67
2.) วิธีการทำความสะอาดด้วยน้ำยาถูพื้น	3.6	2	1	0	2	0.67
	3.7	2	1	0	2	0.67
	3.8	2	1	0	2	0.67
	3.9	3	0	0	3	1
	3.10	3	0	0	3	1
ส่วนที่ 4						
1.) ความถี่ในการทำความสะอาดอุปกรณ์ต่างๆ	4.1	3	0	0	3	1
	4.2	3	0	0	3	1
	4.3	3	0	0	3	1
	4.4	3	0	0	3	1
	4.5	2	1	0	2	0.67
	4.6	2	1	0	2	0.67

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4.7	2	1	0	2	0.67
4.8	2	1	0	2	0.67
4.9	2	1	0	2	0.67
4.10	2	1	0	2	0.67
4.11	2	1	0	2	0.67
4.12	3	0	0	3	1

1.) ผลจากค่าลองทดสอบแบบสอบถาม จากผู้ทดสอบที่เป็นผู้ที่คุ้นเคยกับการทำความสะอาดในโรงพยาบาลสัตว์แต่ไม่ใช่กลุ่มตัวอย่าง จำนวน 6 คน

ส่วนที่ 2 ให้ค่า Cronbach's Alpha = 0.78

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
q2.2	5	1.6	0.866	0.611
q2.4	5.17	1.767	0.777	0.654
q2.5	5.33	3.067	-0.093	0.899
q2.6	5.33	2.267	0.542	0.745
q2.9	5.17	1.767	0.777	0.654

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ส่วนที่ 3 (ข้อ 3.1-3.5) ให้ค่า Cronbach's Alpha = - 0.89

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
q3.4	1.83	0.167	-0.316	.
q3.5	1.67	0.267	-0.316	.

ส่วนที่ 3 (ข้อ 3.6-3.10) ให้ค่า Cronbach's Alpha = -0.278

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
q3.6	6.33	1.867	-0.189	1.48E-16
q3.7	7.5	1.1	0.342	-1.374[a]
q3.8	7.17	1.767	0.033	-.453[a]

q3.9	7.5	2.3	-0.12	-.193[a]
q3.10	7.5	3.1	-0.518	0.201

ส่วนที่ 4 ให้ค่า Cronbach's Alpha = 0.7

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
q4.2	17.17	26.967	0.362	0.679
q4.3	17.33	33.467	-0.621	0.749
q4.5	17.33	29.867	0.149	0.703
q4.6	16.5	18.7	0.716	0.586
q4.7	16.83	28.567	0.168	0.704
q4.8	16.5	27.5	0.422	0.676
q4.9	15.33	23.867	0.479	0.654
q4.10	16.83	29.367	0.024	0.73
q4.11	16.67	22.667	0.495	0.649
q4.12	16	16	0.913	0.516

คำชี้แจงที่ 1

เนื่องจากแบบทดสอบการทำความสะอาดของผู้ที่ทำความสะอาดและผู้ช่วยสัตวแพทย์มีความคล้ายคลึงกัน โดยแบบสอบถามของผู้ทำความสะอาดนั้นจะถูกตัดให้เหลือเพียงแค่ส่วนที่เกี่ยวข้องกับการทำความสะอาดพื้นทางเดินเท่านั้น จึงขออนุญาตใช้ค่า validity และ reliability ร่วมกันค่ะ

คำชี้แจงที่ 2

ในส่วนที่ 3 ของแบบสอบถามการทำความสะอาดของผู้ช่วยสัตวแพทย์และผู้ทำความสะอาด ให้ ค่า reliability ที่เป็นค่าติดลบ ซึ่งคำถามในส่วนนี้มีความสำคัญในการหาปัจจัยในการคงอยู่ของเชื้อบนพื้นในแต่ละสถานที่ ถึงแม้ค่าที่ได้จะติดลบ แต่ก็อยากจะขออนุญาตเก็บคำถามเอาไว้เพื่อหาคำตอบที่แท้จริงจากการทำงานจริงในห้องต่างๆค่ะ

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แบบสอบถามเรื่องการใช้ยาต้านจุลชีพ) สัตวแพทย์)

1.) ผลจากการพิจารณาโดยผู้เชี่ยวชาญ เพื่อหาค่า IOC

ประเด็นที่ต้องการ	ข้อคำถาม	ระดับความสอดคล้อง				
		สอดคล้อง	ไม่แน่ใจ	ไม่สอดคล้อง	รวม	IOC
ส่วนที่ 2						
1.) การใช้ยาปฏิชีวนะสำหรับตัวเอง	2.1	3	0	0	3	1
2.) การใช้ยาปฏิชีวนะกับสัตว์เลี้ยงของตัวเอง	2.2	3	0	0	3	1
	2.3	3	0	0	3	1
	2.4	3	0	0	3	1
3.) การใช้ยาปฏิชีวนะกับสัตว์ป่วย	2.5	2	1	0	2	0.67
	2.6	3	0	0	3	1

2.) ผลจากค่าลองทดสอบแบบสอบถาม จากผู้ทดสอบที่เป็นสัตวแพทย์ (ที่ไม่ใช่กลุ่มตัวอย่าง) จำนวน 6 คน

ส่วนที่ 2 (เฉพาะข้อ 2.3 และ 2.4 เพราะข้ออื่นตอบเหมือนกัน) ให้ค่า Cronbach's Alpha = 0.713

	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Squared Multiple Correlation
Q2.3	.67	.267	.843	.711
Q2.4	1.50	1.900	.843	.711

VITA

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EDUCATION

MS Chulalongkorn University, Department of Veterinary Pharmacology, Faculty of Veterinary Science, 2006-2009

Thesis: "Species and antimicrobial susceptibility of methicillin-resistant coagulase-positive Staphylococci spp. (MRCPS) isolated from dogs in Thailand"

Advisor: Associate Professor Supatra Srichirat

BS Mahidol University, Faculty of Veterinary Science, 2000-2006

Research: "Prevalence of PBFD and Avian polyoma in Psittacines in the central part of Thailand by multiplex PCR"

Advisor: Miss Roschong Boonyarittichaikij, DVM

EXPERIMENT

1. Dog trainer of Good Dog Club at Mahidol University, 2004-2006.
2. Organizer of "ประกวดสุนัขนิสัยดี", 2004-2006.
3. Lecturer at faculty of Veterinary Science, Western University, Oct 2009 – Apr 2010.
4. Part-Time Clinician at Markpol Clinic, 2010-2013.
5. MALDI-TOF MS training at Free University of Berlin, 27 Feb - 27 May 2015.

CONFERENCE AND POSTER PRESENTATIONS

1. Fungwitaya P, Naenna P, Pongpech P and Srichirat S. "Antimicrobial Susceptibility of Methicillin-Resistant Coagulase-Positive Staphylococci Isolated from Dogs in Thailand." VPAT Regional Veterinary Congress 26-29 April 2009 at Bangkok, Thailand
2. Fungwitaya P, Chanchaithong P, Muaungkong P, Bumpenpol P, Kaewparuehaschi M, Chongthaleong A, Tribudharat C and Prapasarakul N. "Appearance of MRCoPS on dog skin following oral cefalexin monohydrate administration." The 38th International Conference on Veterinary Science (The 38th ICVS) 16-18 January 2013 at Bangkok, Thailand
3. Fungwitaya P, Chongthaleong A, Tribudharat C and Prapasarakul N. "Prevalence of coagulase-positive staphylococci (CoPS) on veterinarians, surfaces and cotton ball at small animal hospital, faculty of Veterinary Science, Chulalongkorn University during 2010-2011." The 38th International Conference on Veterinary Science (The 38th ICVS) 16-18 January 2013 at Bangkok, Thailand
4. Fungwithaya P,Chanchaithong P, Chongthaleong A, Tribuddharat C, Phumthanakorn N, and Prapasarakul N. "Prolongation of methicillin resistant Staphylococcus pseudintermedius (MRSP) following cefalexin monohydrate administration." The 1st ASM Conference on Experimental Microbial Evolution 19-22 July 2014 at Washington DC, USA.
5. Fungwithaya Punpichaya and Prapasarakul Nuvee.. "Approximately time and concentration of povidone iodine to kill Staphylococcus pseudintermedius" The first grand progress presentation 12 Deccember 2014 at Chulalongkorn University, Bangkok, Thailand.
6. Fungwithaya P. and N. Prapasarakul. "Risk Assessment of Coagulase Positive Staphylococci (CoPS) on Materials and Equipment Surfaces in a Veterinary Teaching Hospital, Thailand" CUVC 2014 13th, Bangkok, Thailand.
7. Fungwithaya P. and N. Prapasarakul. "Time to Kill Evaluation of Silver-Nano Agents to Canine Coagulase Positive Staphylococci (CoPS)" CUVC 2014 13th, Bangkok, Thailand.
8. Fungwithaya Punpichaya, Phumthanakorn Nathita, Chanchaithong Pattrarat, Chongthaleong Anan, Tribuddharat Chanwit, Brikshavana Pasakorn and Prapasarakul. Nuvee "Distribution of methicillin-resistant Staphylococcus pseudintermedius (MRSP) in a small animal hospital" 40th WSAVA 15-18 May 2015, Bangkok, Thailand.

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