Original Article

Association between Cephalexin Administration and Emergence of Methicillin-resistant Coagulase-positive Staphylococci (MRCoPS) in Dogs

Punpichaya Fungwithaya[1] Pattrarat Chanchaithong[1] Nathita Phumthanakorn[1]

Passana Muaungkong[1] Pitikarn Bumpenpol[1] Mutchamon Kaewparuehaschai[1]

Chanwit Tribudharat[2] Nuvee Prapasarakul[1*]

Abstract

The potential transmission of methicillin-resistant coagulase-positive staphylococci (MRCoPS) between dogs and human has been noted as of potential public health concern. The current study aimed to determine the emergence of methicillin-resistant coagulase-positive staphylococci (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 lesions. Fewer MRCoPS were isolated from the pre-treatment dogs (7.89%) than from the treatment dogs (p<0.001). Methicillin-resistant *Staphylococcus* (*S.*) *schleiferi* subsp. *coagulans* (MRSSc) were only recovered from the treatment dogs, whereas methicillin-resistant *S. pseudintermedius* (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 the skin lesions. In conclusion, the oral cephalexin administration was associated with the emergence of MRCoPS on dog skin, a potential source of contamination to humans.

Keywords: cephalexin monohydrate, dog, methicillin-resistant, methicillin-resistant coagulase positive staphylococci, *Staphylococcus pseudintermedius*

[1]*Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand*
***Correspondence**: Nuvee.P@chula.ac.th*

Introduction

Staphylococcus pseudintermedius (S. pseudintermedius) and *S. schleiferi* subsp. *coagulans* are the main coagulase-positive staphylococci (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 opportunist pathogens, depending on 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 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 methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *S. pseudintermedius* (MRSP) and methicillin-resistant *S. schleiferi* subsp. *coagulans* (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 hospitals (Weese et al., 2012; Chanchaithong et al., 2014).

Cephalexin administration has been recommended as the primary choice of empirical therapy for routine treatment of 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 in 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 methicillin-resistant coagulase-positive staphylococci (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 the Chulalongkorn University 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 had not been 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 treatment depending on client convenience. Clinical signs of the dogs were observed for two months. 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 in 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: The 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). DNA was extracted using a Wizard Genomic® DNA purification kit (Wizard; Promega, Wisconsin, USA), and a qPCR master mix (GoTaq®; Promega, Wisconsin, USA) was used for the M-PCR. 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 internal controls (Chanchaithong and Prapasarakul, 2011).

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). *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 were described by analysis of variance (ANOVA) and multiple comparisons. 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 was 1.) high reliability ($\alpha \geq 0.70$), 2.) fair reliability ($0.70 > \alpha > 0.30$) and 3.) low reliability ($\alpha \leq 0.30$).

Results

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 of therapy all dogs recovered from the skin lesions. The population of MRCoPS and MSCoPS derived from each group are summarized in Table 1. In the 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%) treatment dogs, but only 11 of 38 (28.9%) were isolated from the 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. The correlation of *mecA* positive genotype and disk screening phenotype is shown in Table 3. The results of *mecA* positive genotype in MRSSc did not correlate with the results of oxacillin screening method ($\alpha = 0.235$). In this study, only one MRSP isolate 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 times 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.

Table 1 Comparison between recovery rates of MRCoPS and MSCoPS in 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 (49)		3/38	38/38	*<0.001*	Nasal	1/38
					Perineum	2/38
					Skin lesion	0/38
Treatment (38)	1 week (11)	11/11	7/11	*<0.001*	Nasal	31/38
	2 weeks (7)	7/7	2/7		Perineum	31/38
	3 weeks (10)	10/10	3/10		Skin lesion	11/38
	4 weeks (10)	10/10	0/10			

*MRCoPS = methicillin-resistant coagulase-positive Staphylococci, MSCoPS = methicillin-sensitive coagulase positive Staphylococci

Discussion

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

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 originate from the environment and transfer 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 (Routh 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.

Table 2 Frequencies and distributions of MRCoPS and MSCoPS belonging to three canine staphylococcal species at 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 *Staphylococcus 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.

Table 3 Association between time relapsing and 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							
Treatment	**w1**	11	11	0	7	4	7	3		3	3		
	w2	7	6	1	6	1	6	5	2	3	5		
	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 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 dog.

[a]$\alpha = 0.71$

[b]$\alpha = 0.235$

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 be that the criteria of MRSP were 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 was described in this study. MRCoPS were discovered on all dogs after the first week of treatment. Then, the proportion of MRCoPS and MSCoPS increased from the 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 weeks (100%) and this correlates with previous reports (Beck et al., 2012). However, the increase in MRCoPS population must be concerned in veterinary treatment and hospital management. The distribution of MRCoPS might originate from treatment dogs. Therefore, control of this microorganism should be intensive cleaning management and sanitation in veterinary hospitals.

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 was shown after antimicrobial administration. The emergence of MRSP might suggest an immediate onset of clonal selection with possible transmission.

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บทคัดย่อ

การปรากฏของเชื้อ Methicillin-resistant Coagulase-positive Staphylococci (MRCoPS) ในสุนัขภายหลังจากได้รับเซฟฟาแลคซิน

พรรณพิชญา ฟุ้งวิทยา[1] ภัทรรัฐ จันทร์ฉายทอง[1] นทิตา ภูมิธนากรณ์[1] พาสนา ม่วงคง[1] ปิติกาญจน์ บำเพ็ญผล[1] มัชฌมณ แก้วพฤหัสชัย[1] ชาญวิทย์ ตรีพุทธรัตน์[2] ณุวีร์ ประภัสระกูล[1*]

มีรายงานการส่งผ่านของเชื้อดื้อยาชนิด methicillin-resistant coagulase positive staphylococci (MRCoPS) ระหว่างสุนัขกับมนุษย์ ซึ่งเป็นประเด็นสำคัญในทางสาธารณสุข การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาการปรากฏของเชื้อ MRCoPS ในสุนัขระหว่างการให้ยาเซฟฟาแลคซิน โมโนไฮเดรทแบบกิน เก็บตัวอย่างเชื้อบนผิวหนังสุนัขจำนวน ๓๘ ตัว สุนัขทุกตัวไม่มีประวัติการได้รับยาปฏิชีวนะ ก่อนการรักษา (กลุ่มก่อนได้รับการรักษา) และหลังการรักษา (กลุ่มระหว่างรักษา) พบเชื้อ MRCoPS จำนวน ๑๙๖ ตัวอย่างจากช่องจมูก ขาหนีบ และรอยโรคบนผิวหนัง ในกลุ่มก่อนได้รับการรักษาพบเชื้อดื้อยาเมธิซิลินจำนวนน้อยมากบนผิวหนังสุนัข (7.89%) เมื่อเปรียบเทียบกับสุนัขกลุ่มการรักษา (p<0.001) พบเชื้อ methicillin-resistant *Staphylococcus (S.) schleiferi subsp. coagulans* (MRSSc) ในระหว่างการรักษาเท่านั้น ในขณะที่พบเชื้อ methicillin-resistant *S. pseudintermedius* (MRSP) จากสุนัขทั้งสองกลุ่ม ในภาพรวมเชื้อ MRSP เป็นเชื้อหลักที่พบตั้งแต่สัปดาห์แรกภายหลังการรักษา ที่บริเวณจมูกและขาหนีบเป็นตำแหน่งที่พบเชื้อ MRCoPS ได้มากกว่าที่บริเวณรอยโรคที่ผิวหนัง จากการศึกษาสรุปได้ว่าการรักษาด้วยยาเซฟฟาแลคซินมีความเกี่ยวข้องกับการปรากฏของเชื้อดื้อยาชนิด MRCoPS บนผิวหนังของสุนัข ซึ่งอาจเป็นแหล่งของการปนเปื้อนไปสู่มนุษย์

คำสำคัญ: เซฟฟาแลคซิน โมโนไฮเดรท สุนัข เมธิซิลิน รีสิสแตน เมธิซิลิน รีสิสแตน โคแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค สตาฟฟิลโลคอคคัส ซูอินเตอร์มิเดียส

[1]ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330

[2]ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ศิริราชพยาบาล กรุงเทพฯ เขตบางกอกน้อย กรุงเทพฯ 10700

****ผู้รับผิดชอบบทความ** E-mail: Nuvee.P@chula.ac.th*