Article

Antimicrobial susceptibility of *Staphylococcus pseudintermedius* colonizing healthy dogs in Saskatoon, Canada

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Abstract — This study reports antimicrobial susceptibility of *Staphylococcus pseudintermedius* carried by healthy dogs in Saskatoon, and describes changes in antimicrobial resistance since a 2008 study. One hundred healthy dogs presenting to the wellness service at the Western College of Veterinary Medicine were screened for *S. pseudintermedius* by culturing rectal and pharyngeal swabs. *Staphylococcus pseudintermedius* was identified biochemically and antimicrobial minimum inhibitory concentrations were determined by broth micro-dilution. Methicillin resistance was confirmed by polymerase chain reaction (PCR) and sequencing of the *mec*A gene. Of 221 *S. pseudintermedius* isolates from 78 dogs, 7 were methicillin resistant. No resistance to the fluoroquinolones, nitrofurantoin, tigecycline, vancomycin, quinupristin-dalfopristin, linezolid, or daptomycin was identified. Of the 78 positive dogs, isolates resistant to penicillin were found in 78%, to ampicillin in 61% and to tetracycline in 26%; resistance to oxacillin, erythromycin, clindamycin, trimethoprim + sulfamethoxazole, chloramphenicol, and gentamicin was found in < 10% of dogs. Compared to the 2008 study, the frequency of resistance to all drugs increased, and the frequency of colonization with pan-susceptible isolates decreased from 46% to 30%.

Résumé — Susceptibilité antimicrobienne de *Staphylococcus pseudintermedius* colonisant des chiens en santé à Saskatoon, au Canada. Cette étude présente un rapport sur la susceptibilité de *Staphylococcus pseudintermedius* chez des chiens porteurs en santé à Saskatoon et décrit les changements de la résistance antimicrobienne depuis une étude réalisée en 2008. On a réalisé un dépistage auprès de 100 chiens en santé présentés au service de bien-être du Western College of Veterinary Medicine pour *S. pseudintermedius* en réalisant une culture d'écouvillons rectaux et pharyngés. *Staphylococcus pseudintermedius* a été identifié par des tests biochimiques et les concentrations minimales inhibitrices d'antimicrobiens ont été déterminées par micro-dilution en bouillon. La résistance à la méthicilline a été confirmée par ACP et le séquençage du gène *mec*A. Parmi les 221 isolats de *S. pseudintermedius* provenant de 78 chiens, 7 étaient résistants à la méthicilline. Aucune résistance aux fluoroquinolones, à la nitrofurantoine, à la tigecycline, à la vancomycine, à la quinupristine-dalfopristine, au linézolide ou à la daptomycine n'a été identifiée. Parmi les 78 chiens positifs, des isolats résistants à la pénicilline ont été trouvés chez 78 %, à l'ampicilline chez 61 % et à la tétracycline chez 26 %; la résistance à l'oxacilline, à l'érythromycine, à la clindamycine, au triméthoprime + sulfaméthoxazole, au chloramphenicol et à la gentamicine a été trouvée chez < 10 % des chiens. Comparativement à l'étude de 2008, la fréquence de la résistance à tous les médicaments a augmenté et la fréquence de la colonisation par des isolats sensibles a chuté de 46 % à 30 %.

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Introduction

Staphylococcus pseudintermedius (recognized as distinct from *S. intermedius* in 2005) colonizes the skin and mucosal surfaces of up to 90% of healthy dogs (1–3). Clinically, *S. pseudintermedius* is the most common cause of pyoderma and otitis externa, the second most common cause of urinary tract infections, and is frequently implicated in nosocomial infections in dogs (4,5). The ubiquity of canine *S. pseudintermedius* infections in the community and the frequency of empiric treatment by veterinarians highlight the importance of antimicrobial resistance surveillance to inform evidence-based empiric therapeutic selection.

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The emergence of antimicrobial resistance is a great challenge to antimicrobial therapy for animals and humans. The propensity of staphylococci to adapt to the selection pressure of antimicrobial use has been recognized since the first description of penicillin resistant *S. aureus* in the 1940s (6). Resistance to penicillin among staphylococci, including companion animal *S. pseudintermedius* isolates, is most commonly due to the production of staphylococcal beta-lactamase, conferred by the *bla*Z gene (7,8). *Staphylococcus pseudintermedius* has historically remained remarkably susceptible to antimicrobials, but since 2006 there has been a dramatic worldwide increase in the frequency of methicillin resistance (4,9). Methicillin resistance, which is rapidly emerging among *S. pseudintermedius* in dogs and common among *S. aureus* in humans is a serious threat to the efficacy of the most frequently used antibiotics, the beta-lactams (10–12). Methicillin resistance conferred by the *mec*A and *mec*C genes results in the production of altered cell wall proteins with a low affinity for beta-lactam drugs; leading to resistance to all beta-lactam antimicrobials currently licensed for use in veterinary medicine including the penicillins, cephalosporins, and carbapenems (13). Because methicillin resistance is not the product of beta-lactamase production, addition of beta-lactamase inhibitors such as clavulanic acid does not restore susceptibility. Furthermore, methicillin resistance in *S. pseudintermedius* is often associated with multidrug resistance, further limiting the treatment options available to veterinarians (4,9).

In the late 2000's there was an explosive increase in the incidence of MRSP associated with 2 lineages of *S. pseudintermedius,* sequence type (ST) 71 in Europe and ST68 in North America (11,14). Among healthy dogs in North America and Europe 0 to 4.5% have been found to carry MRSP, while up to 66% of clinical *S. pseudintermedius* isolates have been reported to be methicillin resistant (4,15–19). In Saskatoon, *S. pseudintermedius* carried by healthy dogs and those causing infections have historically been remarkably susceptible; a 2008 study failed to identify any animals carrying MRSP (2,5). Since 2009, reports of canine infections with MRSP in Saskatoon including urinary tract infections and necrotizing fasciitis suggest the emergence of resistance in this region (20,21). The objective of this study was to determine the antimicrobial susceptibility profiles of *S. pseudintermedius* colonizing healthy dogs in Saskatoon, and identify changes in the frequency of resistance since the 2008 investigation.

Materials and methods

Sample collection

Between June and September 2014, 100 clinically healthy dogs presenting to the wellness service of the Veterinary Medical Centre at the Western College of Veterinary Medicine were investigated (Table 1). Pharyngeal and rectal samples were collected using sterile swabs with Stuart transport media (Becton Dickinson, Sparks, Maryland, USA) as previously described (2). Pharyngeal samples were collected by gently rolling a sterile swab across the pharynx for 1 to 3 s, and rectal swabs were collected by gently inserting a second swab 3 cm into the dog's rectum and rotating for 1 to 3 s. All samples were processed within 4 h of collection. This study was approved by the University of Saskatchewan animal research ethics board (protocol #20130135).

Table 1. Characteristics of sampled dogs ($n = 100$)

Age	2.5 mo to 12 y (median = 3 y)
Gender[a]	
Intact male	18
Neutered male	33
Intact female	14
Neutered female	32
History of antimicrobial use, past 6 months	
Yes	9
No	91

[a] Information on gender was not recorded for 1 dog.

Culture and susceptibility testing

All swabs were plated on CHROMagar Staph aureus (CHROMagar, Paris, France), and Mueller-Hinton agar + 4 μg/mL oxacillin. Plates were then incubated overnight at 35°C and up to 5 *S. pseudintermedius*-like colonies (mauve color) were sub-cultured to Columbia agar with 5% sheep blood (Becton, Dickinson). Isolates were identified based on colony morphology (small, creamy grey to white, round colonies with a smooth margin and double zone of hemolysis on blood agar) and biochemically using the catalase test, and tube coagulase test with rabbit plasma, the production of acetoin and hyaluronidase, and the fermentation of mannitol, maltose, and trehalose (2,22). Since the carriage of genetically diverse *S. pseudintermedius* strains by individual dogs has been recognized, 3 isolates per animal were saved for future testing to increase the likelihood of detecting resistant organisms (23). Bacteria were stored at −80°C in trypticase soy broth + 15% glycerol. For dogs carrying *S. pseudintermedius* at both sites, 2 pharyngeal and 1 rectal isolate were saved.

Antimicrobial minimum inhibitory concentrations were determined by broth microdilution using the GAPLL1F Sensititre panel (Thermo Fisher Scientific, Oakwood Village, Ohio, USA). Tests were conducted according to the Clinical and Laboratory Standards Institute (CLSI) and manufacturer's guidelines (24). A panel of drugs including: penicillin (PEN), ampicillin (AMP), oxacillin (OXA) with 2% NaCl, erythromycin (ERY), clindamycin (CLI), tetracycline (TET), tigecycline (TGC), trimethoprim + sulfamethoxazole (SXT), ciprofloxacin (CIP), levofloxacin (LEV), moxifloxacin (MOX), gentamicin (GEN), chloramphenicol (CHL), rifampin (RIF), nitrofurantoin (NIT), vancomycin (VAN), linezolid (LZD), daptomycin (DAP) and quinupristin + dalfopristin (QDA) was used. For quality control *S. aureus* ACTCC 29213 and *Enterococcus faecalis* ACTCC 29212 were used (25). Antimicrobial MICs were used to categorized isolates as susceptible or resistant using CLSI breakpoints for all drugs except tigecycline and daptomycin for which the EUCAST interpretive criteria were used (25–27). Isolates were considered to be MRSP when resistant to oxacillin (MIC ≥ 0.5 μg/mL); genotypic resistance was confirmed by PCR and sequencing of the *mec*A and *mec*C genes using previously described primers (28). Isolates resistant

Table 2. MIC distribution of isolates and the percentage of animals colonized with resistant isolates in 2008 and 2014

Drug (μg/mL)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	% Resistant Isolates 2014 (*n* = 221)	% Animals 2014 (*n* = 78)	% Animals 2008 (*n* = 153)
PEN		75	6	8	15	12	6	11	42	46				63.3	73.0	39.9
AMP			82	32	26	37	26	11	3	4				48.4	61.5	9.8
OXA				213	2	1			5					3.6	9.0	0
ERY				134	73	3			11					5.0	9.0	3.3
CLI					214	1		6						2.7	5.1	2.6
TET							174	1			46			20.8	25.6	23.5
TGC		70	142	9										0	0	0
SXT					210	3		1	7					3.6	3.8	0
CIP						221								0	0	0
LEV				214	4	1	2							0	0	0
MOX				221										0	0	0
GEN							218	1	1		1			0.5	1.3	0
CHL								16	175	28	2			0.9	2.6	0
NIT											221			0	0	0
RIF					220	1								0	0	0
VAN				3	204	14								0	0	0
LZD						138	81	2						0	0	0
DAP					221									0	0	0
QDA					219	2								0	0	0

Antimicrobial minimum inhibitory concentration (MIC) distribution for *S. pseudintermedius* isolates (*n* = 221) for penicillin (PEN), ampicillin (AMP), oxacillin (OXA) with 2% NaCl, erythromycin (ERY), clindamycin (CLI), tetracycline (TET), tigecycline (TGC), trimethoprim + sulfamethoxazole (SXT), ciprofloxacin (CIP), levofloxacin (LEV), moxifloxacin (MOX), gentamicin (GEN), chloramphenicol (CHL), rifampin (RIF), nitrofurantoin (NIT), vancomycin (VAN), linezolid (LZD), daptomycin (DAP) and quinupristin + dalfopristin (QDA). Cells corresponding to concentrations tested are outlined in black while the resistance breakpoint is shaded. The number of isolates inhibited at each concentration is noted in each cell.

to erythromycin and susceptible to clindamycin were tested for inducible clindamycin resistance using the D-test as described by the CLSI (26).

Results

Of the 100 dogs tested, *S. pseudintermedius* was isolated from 78. A total of 221 isolates were collected, including single isolates from 5 dogs, 2 isolates from 3 dogs, and 3 isolates from 70 dogs. For dogs in which < 3 isolates were initially identified, all isolates were saved. No *S. pseudintermedius* was isolated from Müeller-Hinton agar with 4 μg/mL oxacillin; all isolates were recovered from CHROMagar *Staph aureus*. Antimicrobial susceptibility testing revealed phenotypic diversity among multiple isolates from individual dogs. Of the 78 positive animals, isolates with varying susceptibility profiles were grown from 30, while phenotypically homogeneous isolates were grown from 48. Consequently, the frequency of resistance among the overall isolate collection was lower than the percentage of animals carrying isolates expressing any particular resistance phenotype; for example, if 1 of 3 isolates carried by a dog was resistant to tetracycline, that dog was considered to carry tetracycline-resistant isolates (Table 2). No resistance to ciprofloxacin, levofloxacin, moxifloxacin, nitrofurantoin, rifampin, tigecycline, vancomycin, quinupristin + dalfopristin, linezolid or daptomycin was identified. The most common resistance profile was penicillin + ampicillin resistance (*n* = 70; 31.7%) followed by pan-susceptibility (*n* = 67; 30.3%) (Table 3). Methicillin resistant isolates (*n* = 8) were identified in 7 dogs carrying *S. pseudintermedius.* Resistance to trimethoprim + sulfamethoxazole, chloramphenicol, and gentamicin was less common (Table 3). All oxacillin resistant isolates posessed the *mec*A gene, while *mec*C was not identified. None of the 5 erythromycin resistant, clindamycin susceptible isolates were inducibly clindamycin resistant.

Fifteen multidrug resistant isolates (MDR; resistance to 3 or more drugs classes) were identified, all were methicillin susceptible *S. pseudintermedius* (MSSP) (Table 3). Notably, 1 isolate was resistant to PEN, AMP, TET, ERY, CLI, CHL and GEN.

Discussion

Compared to the previous resistance surveillance study targeting *S. pseudintermedius* from healthy dogs presenting to the wellness service at our institution in Saskatoon in 2008, a higher frequency of resistance to specific antimicrobials, and resistance to more drugs including methicillin was identified. Furthermore, only 30% of the dogs carried pan-susceptible isolates compared to 46% in 2008 (2). Differences in sample collection between the present investigation and that done in 2008 (the inclusion

Table 3. Summary of resistance profiles of *S. pseudintermedius* isolates ($n = 221$)

Resistance profile	Number of isolates
Pan-susceptible	67
PEN + AMP	70
PEN	27
TET	13
PEN + AMP + TET	13
PEN + AMP + OXA	6
PEN + AMP + SXT + TET	6
PEN + TET	4
PEN + AMP + TET + ERY + CLI	4
PEN + AMP + TET + ERY	3
PEN + AMP + SXT	2
PEN + AMP + OXA + TET	1
PEN + AMP + OXA + ERY	1
TET + ERY	1
PEN + ERY + CLI	1
PEN + AMP + TET + CHL	1
PEN + AMP + ERY + CLI + CHL + GEN	1

Resistance profiles of *S. pseudintermedius* isolates (left column), and number of isolates with each profile (right column). Penicillin (PEN), ampicillin (AMP), oxacillin (OXA) with 2% NaCl, erythromycin (ERY), clindamycin (CLI), tetracycline (TET), trimethoprim + sulfamethoxazole (SXT), gentamicin (GEN), chloramphenicol (CHL).

of a single isolate per dog in 2008 *versus* 3 presently, and the inclusion of nasal swabs in the 2008 investigation) preclude statistical comparisons between studies. However, the higher frequency of resistance including MRSP in 2014 is consistent with local clinical observations suggesting the emergence of resistance and with global MRSP trends. The frequency of carriage of healthy dogs with MRSP (7%) was higher than previously described elsewhere in North America or Europe ($\leq$ 4.5%) perhaps reflecting the continued emergence of MRSP following previous studies (4,18). This frequency was lower than that reported in Asia, where up to 45% colonization has been reported in Thailand, Japan and Hong Kong (29–31). The inclusion of Müeller-Hinton agar with 4 μg/mL oxacillin did not improve our ability to recover MRSP despite the identification of 5 isolates with oxacillin MICs of $>$ 4 μg/mL.

Risk factors for dogs to be infected with or carry MRSP have not been adequately characterized. There is conflicting evidence describing an association between infection with MRSP *versus* MSSP and previous antimicrobial administration (32,33). Hospitalization and surgical procedures have also been positively associated with MRSP colonization (33,34). In the present investigation, none of the 7 MRSP positive animals had been treated with antimicrobials in the previous 6 mo, and all were clinically healthy suggesting community acquisition of the MRSP. Further study is required to define risk factors associated with MRSP in dogs.

A total of 15 (6.8%) isolates from 7 dogs (9% of colonized dogs) were MDR, higher than in 2008 where only 1 dog carried MDR *S. pseudintermedius* (2). In contrast to the literature, MDR was more frequently identified among MSSP than MRSP (35). The most common resistance profile among MRSP, including 6 of 8 isolates, was simply beta-lactam resistance. MDR among MRSP is a serious threat to the ability of veterinarians to treat their patients. In 2009, a community associated urinary tract infection caused by MRSP resistant to the beta-lactams, macrolides, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole, chloramphenicol and rifampin was reported in an otherwise healthy, neutered male Pug dog (20). Elsewhere, MRSP resistant to all antimicrobials licensed for use in companion animals have been described, highlighting the critical role of culture and susceptibility testing to guide therapy (4,11,35). Differences in clinical outcome for human patients infected with methicillin resistant *versus* susceptible staphylococci have not been observed, although the typically superficial nature of staphylococcal infections (pyoderma and otitis) may mask differences which have been seen in invasive MRSA vs. MSSA infections in people (33,36). More studies are needed to define risk factors associated with MRSP infection so that appropriate empiric treatments can be applied pending laboratory guided therapy.

Although ill-defined, antimicrobial resistant *S. pseudintermedius* is also a public health risk; human infections have been reported (37). Because *S. pseudintermedius* is not part of the normal microbiota of humans, carriage has been reported to be sporadic, colonization or infection with this organism is likely zoonotic (38,39). Presumptive transmission of *S. pseudintermedius* from dogs to humans working closely with them (veterinary staff) and pet owners has been reported; 3.9% to 13% of those humans have been found to carry this organism (31,38,40). The frequency of human *S. pseudintermedius* infections may be under-appreciated due to its morphological and biochemical similarity to *S. aureus* leading to misidentification in diagnostic labs. The introduction of highly discriminatory identification methods such as MALDI-TOF which readily differentiate *S. pseudintermedius* and the closely related *S. intermedius* and *S. delphini* from *S. aureus* is helping to identify this previously under-recognized zoonosis (41).

Antimicrobial resistance appears to be emerging among *S. pseudintermedius* colonizing healthy dogs in Saskatoon. Although we presume that infections with MRSP are encountered with increasing frequency in our region, these data are not available. Culture and susceptibility testing should be encouraged to aid in the identification of MRSP in veterinary patients and to guide antimicrobial therapy. Methicillin resistance should be suspected when empiric beta-lactam therapy fails to cure *S. pseudintermedius* infections or for isolates demonstrated to be resistant to potentiated penicillins such as amoxicillin + clavulanic acid. Further studies to describe the susceptibility of clinical isolates in this region would be complementary to this investigation.

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