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In Vitro Antimicrobial Susceptibility of *Staphylococcus pseudintermedius* Isolates of Human and Animal Origin

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MIC results for 115 *Staphylococcus intermedius* group isolates are presented. Of these, 33% were methicillin resistant, among which 51.4% were susceptible to doxycycline, 29.7% to clindamycin, and 21.6% to trimethoprim-sulfamethoxazole. All of the isolates were susceptible to ceftaroline, daptomycin, linezolid, nitrofurantoin, quinupristin-dalfopristin, rifampin, tigecycline, and vancomycin. Of all the isolates, 82.6%, 67.8%, and 23.5% were susceptible to ciprofloxacin, erythromycin, and penicillin, respectively. No isolates harbored *mupA* or *qacA/B* genes, which suggested a lack of resistance to mupirocin or chlorhexidine.

The *Staphylococcus intermedius* group (SIG) is comprised of *Staphylococcus intermedius*, *Staphylococcus pseudintermedius*, and *Staphylococcus delphini*. These Gram-positive cocci (except for *S. intermedius*) are positive for tube coagulase and negative for slide coagulase and may be misidentified as *Staphylococcus aureus* by clinical laboratories that test human specimens (1). A colonizer of the nares and anal mucosa of cats and dogs, *S. pseudintermedius* is increasingly being recognized in human diagnostic specimens (2). This may be due in part to improved diagnostic technologies, such as matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), now being used in many clinical laboratories. *S. pseudintermedius* has been documented to cause invasive infections, including brain abscesses, endocarditis, and bacteremia, in humans (3). Methicillin resistance among *S. pseudintermedius* isolated from dogs is increasing (4), with rates of up to 47% in some regions of the world (5). This resistance is predominantly due to the dissemination of the ST71 clonal lineage in Europe and the ST68 clonal lineage in North America (4). Methicillin-resistant isolates often display resistance to other classes of antimicrobials used in veterinary medicine, including aminoglycosides, fluoroquinolones, lincosamides, macrolides, and tetracyclines, and to chloramphenicol and trimethoprim-sulfamethoxazole (SXT) (6). However, limited susceptibility data are available for *S. pseudintermedius* with antimicrobials used for humans. We recently conducted a study to evaluate oxacillin and cefoxitin disk and MIC results as predictors of methicillin resistance (encoded by *mecA*) in a collection of 115 SIG isolates from human and veterinary specimens associated with clinical infections. This study documented that cefoxitin testing, which is recommended by the Clinical and Laboratories Standards Institute (CLSI) to predict methicillin resistance for other species of staphylococci, is a poor predictor of *mecA* in SIG, whereas both oxacillin disk and MIC tests accurately detect *mecA*-mediated oxacillin resistance in these isolates (7). As a result of our study, CLSI published *S. pseudintermedius*-specific oxacillin breakpoints in the 26th edition of the M100S standard (8). In the present study, we document the results of antimicrobial susceptibility testing (AST) for this collection of 115 SIG isolates, including 111 isolates of *S. pseudintermedius* (45 from human, 56 from canine, 7 from feline, 2 from avian, and 1 from porcine sources) and 4 isolates of *S. delphini* (3 from equine and 1 from avian sources).

Bacterial isolates were described in our previous article (7). AST was performed according to the CLSI reference broth microdilution (BMD) MIC method (8), using panels prepared in-house with cation-adjusted Mueller-Hinton broth (MHB). MHB was supplemented with 50 mg/liter $CaCl_2$ for daptomycin testing and 2% NaCl for oxacillin testing (9). Fifteen antimicrobial agents were tested (Table 1). BMD tests were read after 16 to 20 h of incubation at 35°C in ambient air for all of the antimicrobials except oxacillin and vancomycin, where the final reading was done after 24 h of incubation. MIC results were interpreted according to the *Staphylococcus* spp. breakpoints listed in CLSI M100S, 26th edition, including use of the new oxacillin *S. pseudintermedius* breakpoints and ceftaroline and vancomycin breakpoints for *S. aureus* (8). Because there are no CLSI tigecycline breakpoints, the Food and Drug Administration (FDA) breakpoint for *S. aureus* was used. All isolates with penicillin-susceptible MICs (≤0.12 μg/ml) were tested by penicillin disk diffusion using the standard CLSI method and examined for β-lactamase production using a BBL Cefinase disk (BD, Sparks, MD). In addition to taking zone measurements, the zone edges were evaluated for sharp versus fuzzy borders around the penicillin disks. β-Lactamase testing was performed using growth taken from the zone margin surrounding a penicillin disk test on BBL Mueller-Hinton agar (MHA) (BD) after 16 to 18 h of incubation. *mecA* PCR and

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TABLE 1 MIC values of 15 antimicrobial agents for *Staphylococcus intermedius* group ($n = 115$) when tested by the CLSI reference broth microdilution MIC method in cation-adjusted Mueller-Hinton broth

Antimicrobial agent[a]	Number of isolates at MIC (μg/ml) of[b]:										Percent of isolates susceptible				
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	Human	Animal	*mecA*$^-$	*mecA*$^+$	All
Ceftaroline			115[c]								100	100	100	100	100
Ciprofloxacin				94[c]	1			20[d]			91.1	77.1	97.4	51.4	82.6
Clindamycin				78[c]		1			36[d]		80.0	60.0	85.9	29.7	67.8
Daptomycin			115[c]								100	100	100	100	100
Doxycycline					69		20	23	3[d]		84.4	72.9	89.7	51.4	77.4
Erythromycin				78[c]					37[d]		80.0	60.0	85.9	29.7	67.8
Linezolid			1[c]		63	50	1				100	100	100	100	100
Nitrofurantoin									114	1	100	100	100	100	100
Oxacillin			77[c]		3[e]	6	2	1	2	24[d]	91.1	51.4	98.7	0	66.9
Penicillin	28	5	3	1	2		76[d]				26.6[f]	21.4[f]	50	0	23.5[f]
QDA				115[c]							100	100	100	100	100
Rifampin				115[c]							100	100	100	100	100
SXT				54[c]	25	1	4	31[d]			84.4	60.0	92.3	21.6	69.6
Tigecycline			115[c]								100	100	100	100	100
Vancomycin			14[c]		100	1					100	100	100	100	100

[a] QDA, quinupristin-dalfopristin; SXT, trimethoprim-sulfamethoxazole.
[b] MIC values in the unshaded part of the table fall in the susceptible interpretive category; those in the shaded parts of the table are in the intermediate and/or resistant category.
[c] MIC less than or equal to value in column header.
[d] Value greater than or equal to value in column header.
[e] Includes 1 isolate that was *mecA negative*.
[f] Includes 5 human isolates and 1 animal isolate that had penicillin-susceptible MICs but were β-lactamase positive.

SCC*mec* typing were performed as described in our previous article (7). Mupirocin resistance was determined by PCR for the *mupA* gene, and chlorhexidine resistance was determined by PCR for the *qacA/B* gene, as described elsewhere (10).

MIC results obtained for the 115 isolates are shown in Table 1. Thirty-seven isolates (32.2%), 4 of human origin and 33 of veterinary origin, harbored the *mecA* gene. Using CLSI M100S *Staphylococcus* spp. interpretive criteria, 33 (42.3%) of the 78 *mecA*-negative isolates had penicillin-susceptible MICs of ≤0.12 μg/ml (Table 1). For 27 of 33 isolates, MICs were ≤0.06 μg/ml, penicillin zone measurements were susceptible at ≥29 mm, and induced nitrocefin tests were negative. Six (18.2%) of 33 isolates, 5 human and 1 animal isolate, yielded a positive induced nitrocefin test, indicating the presence of a β-lactamase. Six isolates demonstrated penicillin zones of ≤28 mm (resistant), and all had sharp zone edges. Five of these isolates had penicillin MICs of 0.12 μg/ml, and 1 isolate had a penicillin MIC of ≤0.03 μg/ml. Repeat testing in two laboratories confirmed the results. When the nitrocefin tests were performed using uninduced colonies (i.e., not from a penicillin zone margin), variable results were obtained, with 0 to 4 of the 6 isolates yielding a positive result in different laboratories on different days, when testing colonies grown on blood agar plates or on MHA. As such, a test for β-lactamase production should be performed for all penicillin-susceptible *S. pseudintermedius* isolates, as done for other *Staphylococcus* spp. Whether a penicillin zone edge test is sufficient for this purpose or an induced nitrocefin-based test is needed remains to be determined. However, in our limited analysis, the penicillin zone edge test was 100% concordant with the nitrocefin results obtained when testing induced colonies. All isolates were susceptible to ceftaroline, the cephalosporin with high-affinity binding to PBP 2a expressed by *mecA*.

With regard to the non- β-lactam agents, significant differences were noted in the percentage of methicillin-resistant isolates susceptible to doxycycline, SXT, and clindamycin, compared to what has been documented with contemporary isolates of *S. aureus* (11). This constellation of multidrug resistance is consistent with the multidrug-resistant (MDR) *S. pseudintermedius* clones ST68 and ST71, which harbor mutations within *gyrA* and *grlA* (conferring resistance to fluoroquinolones), as well as a Tn*5404*-like transposon element that harbors the *dfrG* (sulfamethoxazole resistance) and *ermB* (clindamycin and erythromycin resistance) genes (4). Interestingly, differences were noted in our collection based on the SCC*mec* type. Isolates with SCC*mec* V were more commonly resistant to erythromycin and clindamycin (10/11 isolates, 90.9%), SXT (10/11 isolates, 90.9%), doxycycline (8/11 isolates, 72.7%), and ciprofloxacin (9/11 isolates, 81.8%) than those with SCC*mec* types IV or III. For SCC*mec* type IV, 4 of 8 (50.0%), 8 of 8 (100%), 1 of 8 (12.5%), and 0 of 8 (0.0%) isolates were resistant to these antimicrobials, respectively. For isolates with SCC*mec* type III, 4 of 9 (44.4%), 2 of 9 (22.2%), 4 of 9 (44.4%), and 0 of 9 (0.0%) were resistant. Isolates of the MDR North American ST68 lineage harbor SCC*mec* V, similar to the more resistant isolates in our collection (4).

Doxycycline susceptibility was 89.7% among *mecA*-negative isolates and only 51.4% among *mecA*-positive isolates (Table 1). This was in striking contrast to doxycycline susceptibility rates among human isolates of methicillin-resistant *S. aureus* (MRSA), which were 96% among a collection of >4,000 isolates recovered from human diagnostic specimens in 2010 (12). Doxycycline susceptibility rates were similarly high among methicillin-resistant coagulase-negative *Staphylococci* (CoNS), at 94.1% in one study of 1,473 isolates (13). Our data are consistent with previous studies that documented 31% to 38% doxycycline susceptibility among methicillin-resistant *S. pseudintermedius* (MRSP) isolates from canine sources (14, 15). No difference was found in susceptibility to doxycycline between human ($n = 5$, 40.0% susceptible) and

veterinary ($n = 32$, 53.1% susceptible) MRSP isolates in the present study.

Of note, canine-specific breakpoints for doxycycline have been proposed to accommodate the pharmacokinetics of doxycycline doses used for dogs. The canine breakpoints are ≤0.125 μg/ml (susceptible), 0.25 μg/ml (intermediate), and ≥0.5 μg/ml (resistant) (16). The lowest concentration of doxycycline tested in our study was 1 μg/ml, so we cannot estimate the effect that these breakpoints would have on our collection of isolates. However, 35% of *mecA*-positive and 10.2% of *mecA*-negative isolates had MICs of 2 to 4 μg/ml, which are considered resistant by the canine breakpoints but susceptible by the human breakpoints. Resistance to the tetracyclines is mediated through acquisition of tetracycline resistance genes (*tet* genes), four of which have been identified among *S. pseudintermedius* isolates: *tet*(M) and *tet*(O), which mediate ribosomal protection, and *tet*(K) and *tet*(L), which encode efflux pumps. The most commonly occurring of these genes are *tet*(M) and *tet*(K) in *S. pseudintermedius* (16, 17). Isolates that harbor none of these genes typically have MICs of ≤0.125 μg/ml to doxycycline, whereas acquisition of the *tet*(M) gene can be associated with MICs that are elevated but below the susceptible breakpoint of 4 μg/ml given in CLSI document M100S, 26th edition. Clinically, it is unclear whether isolates that are susceptible by the CLSI M100S breakpoint and harbor a *tet* gene are associated with treatment failures, but these isolates would be considered resistant by the proposed veterinary breakpoint (16). The EUCAST susceptible breakpoint for doxycycline is ≤1 μg/ml for human isolates of *Staphylococcus* spp. (www.eucast.org), and when applying this breakpoint, only 18.1% of methicillin-resistant and 79.5% of methicillin-susceptible isolates in our study would be considered doxycycline susceptible. Regardless, the *tet* genes are carried on Tn*5801* and Tn*916* elements (6), the same as found in human and veterinary isolates of tetracycline-resistant *S. aureus* (18). The Tn*916 tet*(M) gene was found in all isolates of the clonal complex (CC) 398 of *S. aureus*, suggesting this element was integrated into the genome of the clone early and disseminated vertically. This may be the case for the ST71 and ST68 clonal lineages of *S. pseudintermedius* and may account for the common occurrence of doxycycline resistance in these isolates. Doxycycline resistance may also be selected for through the common use of this agent for the treatment of pyoderma in small-animal veterinary medicine.

SXT susceptibility was only 21.6% among *mecA*-positive isolates. In contrast, human isolates of MRSA are typically susceptible to this agent; in 2013, 98.0% of isolates in a collection of >9,000 MRSA isolates were susceptible to SXT (19). SXT susceptibility is lower among CoNS. In the same study conducted in 2013, 52.7% of 2,268 methicillin-resistant CoNS isolates were susceptible to SXT (19).

All isolates in this study that were resistant to erythromycin were also resistant to clindamycin, and susceptibility rates for both agents were only 29.7% among MRSP isolates (Table 1). Consequently, no inducible clindamycin resistance was observed, although an inducible *erm* gene was previously documented in *S. pseudintermedius* (20).

We documented 51.4% ciprofloxacin susceptibility in MRSP isolates, which was similar to observations in MRSA and MR CoNS isolates (19). However, this susceptibility rate was significantly higher than rates documented in some studies of veterinary SIG isolates, where susceptibility rates as low as 2.7% were reported using the same susceptible breakpoint of 1 μg/ml (21). A single point mutation in topoisomerase II or IV genes confers fluoroquinolone resistance in *S. pseudintermedius* (22).

All isolates were susceptible to ceftaroline, daptomycin, linezolid, nitrofurantoin, quinupristin-dalfopristin, rifampin, tigecycline, and vancomycin. There are currently no vancomycin breakpoints for SIG, as CLSI only publishes *S. aureus* and CoNS breakpoints for this antimicrobial agent. However, unlike for CoNS, where the modal MIC for vancomycin is 2.0 μg/ml, we found the vancomycin MIC mode to be 1.0 μg/ml, similar to that documented for *S. aureus*. As such, it may be reasonable for clinical laboratories to interpret vancomycin MICs by using the more conservative *S. aureus* susceptible breakpoints of ≤2.0 μg/ml when SIG is encountered, compared to the ≤4-μg/ml breakpoint for CoNS in CLSI M100S or for *Staphylococcus* spp. in the CLSI VET01 standards. Similar to other studies of SIG (23), we did not find any cases of high-level mupirocin resistance among the isolates in this collection, nor did we detect the presence of the *qacA/B* gene in any isolates, suggesting the absence of chlorhexidine resistance in these isolates.

In summary, we present *in vitro* susceptibility results for a large collection of SIG clinical isolates tested by the CLSI reference BMD MIC method. Laboratories should carefully review susceptibility results for all coagulase-positive staphylococci and consider using additional identification procedures, such as MALDI-TOF MS or an automated instrument, for isolates that are doxycycline and/or SXT resistant, a phenotype common to *S. pseudintermedius* but unusual for *S. aureus*. This is important, as correct identification of these isolates is critical to accurate testing of SIG with oxacillin to detect methicillin resistance. Clinicians should be cognizant of the dramatic difference in SXT, clindamycin, and doxycycline susceptibility between SIG and *S. aureus*, as these agents are commonly prescribed as empirical therapy for MRSA in wound and skin structure infections. While susceptibility to these antimicrobials was higher in human than in animal isolates overall (Table 1), this was likely due to the significantly higher proportion of *mecA*-positive isolates in the veterinary collection, a bias of our data set. A second limitation of the present study was the inclusion of only 4 *S. delphini* and no *S. intermedius* isolates; further data will determine if susceptibility rates differ significantly between these isolates and *S. pseudintermedius*. It is worth noting, however, that *S. intermedius* is rarely isolated in veterinary or human clinical laboratories but rather is a constituent of the normal nares flora of the wild pigeon (24).

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