In Vitro Antimicrobial Susceptibility of Staphylococcus pseudointermedius Isolates of Human and Animal Origin


Background: Methicillin-resistant Staphylococcus pseudointermedius (MRSP) poses a significant challenge for the treatment of infections. In this study, we evaluated the in vitro susceptibility of 115 S. pseudointermedius isolates to a range of antimicrobial agents.

Methods: Clinical isolates were collected from human and veterinary specimens associated with clinical infections. Antibiotic susceptibility was determined using a broth microdilution (BMD) method, and oxacillin disc diffusion with confirmation of disc inhibition using a BBL Cefinase disk. Methicillin resistance was confirmed by PCR amplification of the mecA gene. The Staphylococcus aureus 2928 strain was used as a positive control.

Results: Of the 115 isolates, 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 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.

Conclusion: The susceptibility patterns of MRSP isolates from human and animal sources suggest the need for careful antibiotic stewardship to prevent the emergence of resistance.
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

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Number of isolates at MIC (µg/ml) (a)</th>
<th>Percent of isolates susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Ceftaroline</td>
<td>115°</td>
<td>94°</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>115°</td>
<td>78°</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>115°</td>
<td>78°</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>1°</td>
<td>1°</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>1°</td>
<td>1°</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1°</td>
<td>1°</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1°</td>
<td>1°</td>
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<tr>
<td>Nitrofurantoin</td>
<td>1°</td>
<td>1°</td>
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<tr>
<td>Oxacillin</td>
<td>1°</td>
<td>1°</td>
</tr>
<tr>
<td>Penicillin</td>
<td>28°</td>
<td>5°</td>
</tr>
<tr>
<td>QDA</td>
<td>115°</td>
<td>1°</td>
</tr>
<tr>
<td>Rifampin</td>
<td>115°</td>
<td>1°</td>
</tr>
<tr>
<td>SXT</td>
<td>115°</td>
<td>1°</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>115°</td>
<td>1°</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1°</td>
<td>1°</td>
</tr>
</tbody>
</table>

(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*me*c typing was 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 Tn5404-like transposon element that harbors the *dfgR* (sulfamethoxazole resistance) and *ernB* (clindamycin and erythromycin resistance) genes (4). Interestingly, differences were noted in our collection based on the SCC*me*c type. Isolates with SCC*me*c 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*me*c types IV or III. For SCC*me*c 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*me*c 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*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 $\leq 0.125 \mu g/ml$ (susceptible), $0.25 \mu g/ml$ (intermediate), and $\geq 0.5 \mu g/ml$ (resistant) (16). The lowest concentration of doxycycline tested in our study was 1 $\mu g/ml$, 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 $\mu g/ml$, 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 $\leq 0.125 \mu g/ml$ to doxycycline, whereas acquisition of the $tet(M)$ gene can be associated with MICs that are elevated but below the susceptible breakpoint of 4 $\mu g/ml$ 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 $\leq 1 \mu g/ml$ 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 Tn5801 and Tn916 elements (6), the same as found in human and veterinary isolates of tetracycline-resistant S. aureus (18). The Tn916 $tet(M)$ 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 $\mu g/ml$ (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, linzolid, 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 $\mu g/ml$, we found the vancomycin MIC mode to be 1.0 $\mu g/ml$, 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 $\geq 2.0 \mu g/ml$ when SIG is encountered, compared to the $\leq 4\mu g/ml$ 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. intermedii isolates; further data will determine if susceptibility rates differ significantly between these isolates and S. pseudintermedius. It is worth noting, however, that S. intermedii is rarely isolated in veterinary or human clinical laboratories but rather is a constituent of the normal nares flora of the wild pigeon (24).

REFERENCES


