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Diagnostic accuracy of phenotypic assays for determining antimicrobial resistance status in *Staphylococcus pseudintermedius* isolates from canine clinical cases

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ABSTRACT

This study evaluated the diagnostic test accuracy of disc diffusion relative to broth-microdilution for clinical *Staphylococcus pseudintermedius* isolated from dogs in Australia (n = 614). Accuracy of disc diffusion and broth-microdilution for oxacillin relative to *mecA* real-time PCR was also assessed. Each isolate had paired minimum inhibitory concentration and zone diameter values for ten antimicrobial agents. Data was dichotomised using Clinical and Laboratory Standards Institute susceptible and resistant clinical breakpoints. Test accuracy was reported using relative diagnostic sensitivity (RSe), specificity (RSp), likelihood ratio pairs, diagnostic odds ratio, and area-under-the receiver-operating characteristic (ROC AUC) analysis. Disc diffusion was found to have high test accuracy for most antimicrobials (ROC AUC range: 0.96 – 0.99) except rifampicin (ROC AUC = 0.80). The RSp of disc diffusion was high for all antimicrobials (range, 97.1%–100%). However, RSe was considerably variable (range, 35.7%–98.8%), particularly for amoxicillin-clavulanic acid (51.5%, 95% CI, 38.9%, 64.0%), cefoxitin (35.7%, 95% CI, 12.8%, 64.9%), and cephalothin (43.6%, 95% CI, 27.8%, 60.4%). When disc diffusion and broth-microdilution were compared to *mecA* real-time PCR, the overall accuracy of both assays was similar (ROC AUC, 0.99 respectively). However, the RSe for broth-microdilution (96.1%, 95% CI, 88.9%, 99.2%) was significantly higher than for disc diffusion (86.8%, 95% CI, 77.1%, 93.5%) (McNemars mid-*p* value 0.01). Overall, these findings demonstrate that for most antimicrobials, disc diffusion performed according to CLSI guidelines can be used to differentiate clinical *S. pseudintermedius* isolates that might otherwise be assessed by broth-microdilution, provided consideration is given to the performance estimates reported here.

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1. Introduction

The acquisition of resistance genes in clinically important bacterial pathogens of animals is of great concern. In particular, methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) poses a major challenge owing to extensive multidrug resistance, limited therapeutic options for infected hosts, and in rare cases, risk of sporadic zoonotic infection in people (van Duijkeren et al., 2011). For these reasons, accurate assessment of *S. pseudintermedius* susceptibility to a wide range of antimicrobials is required, not only as an objective basis for clinical therapy but also to monitor the occurrence of antimicrobial resistance in animal populations.

In most staphylococci, methicillin resistance is mediated by the mecA gene which encodes expression of the modified penicillin-binding protein, PBP2a (CLSI, 2015). Clinical and Laboratory Standards Institute (CLSI) guidelines specify the most reliable test for determination of methicillin resistance in *S. pseudintermedius* is detection of *mecA* by polymerase chain reaction (PCR) (CLSI, 2018). However, few veterinary laboratories routinely perform *mecA* PCR due to cost to owners. Typically, diffusion- or dilution-based phenotypic assays (using oxacillin as a surrogate for methicillin) are used to predict methicillin resistance, or more accurately, pan-beta-lactam resistance (CLSI, 2018). In most veterinary laboratories, diffusion-based assays such as disc diffusion are performed rather than dilution-based assays such as broth-microdilution (Hardefeldt et al., 2018). However, for most national surveillance programs, broth-microdilution is the preferred assay to generate antimicrobial susceptibility data in animals.

Comprehensive surveillance of antimicrobial resistance in animal pathogens such as *S. pseudintermedius* may be achieved if susceptibility data generated from disc diffusion can be acquired from those veterinary laboratories that routinely evaluate such pathogens. However, the acquisition of susceptibility data is only of value if the accuracy of disc diffusion is comparable to that of broth-microdilution. Additionally, factors associated with assay performance including consistent application of standardised protocols for identification of isolates, quality control, conduct of the test, antibiotic panels, and reading of zones must be widely adopted.

At present, there is limited understanding of the accuracy of disc diffusion when it is compared to broth-microdilution, especially in relation to animal pathogens. This poses a barrier to the use of disc diffusion susceptibility data in national surveillance programs. Standard statistical measures, such as relative diagnostic sensitivity and specificity, receiver-operating characteristic (ROC) analysis, area-under-the-curve (AUC), likelihood ratios, and diagnostic odds ratios (DOR) for a range of antimicrobial agents are essential to determine if disc diffusion and broth-microdilution generate comparable results. However, few studies have reported these estimates for disc diffusion in relation to *S. pseudintermedius*. Indeed, Bremis et al (2006, 2009) and Schissler et al (2009) are the only studies where diagnostic sensitivity and specificity of disc diffusion were reported although these studies limited analysis to oxacillin and cefoxitin alone. Other studies have also evaluated the appropriateness of oxacillin or cefoxitin interpretative criteria to predict methicillin resistance compared to the presence of the *mecA* gene (Bemis et al., 2012; Schmidt et al., 2014; Siak et al., 2014; Wu et al., 2016). However, analysis in these studies was mostly restricted to descriptive measures such as categorical agreement and error-rates, estimates which cannot be relied upon alone to adequately describe the accuracy of disc diffusion.

Therefore, the objective of this study was to evaluate the relative accuracy of disc diffusion compared to broth-microdilution to a range of antimicrobials for clinical *S. pseudintermedius* isolates derived from dogs. In addition, the accuracy of disc diffusion and broth-microdilution was compared to *mecA* real-time PCR for the prediction of methicillin resistance. An improved understanding of the accuracy of these assays will help determine if susceptibility data from *S. pseudintermedius* can be included in national surveillance.

2. Methods

2.1. Sample acquisition, characterisation, and antimicrobial susceptibility testing

S. pseudintermedius isolates were derived from a structured survey of antimicrobial resistance in veterinary pathogens between January 2013 and January 2014, involving all 22 veterinary diagnostic laboratories in Australia (Saputra et al., 2017). Coagulase-positive staphylococci isolates considered clinically relevant to the presenting condition (as determined by the diagnostic microbiologist) were sent to The University of Adelaide during the collection period. Species identification was confirmed by the University of Adelaide reference laboratory using the BD™ Bruker MALDI Biotyper. Isolates in the collection underwent broth-microdilution (minimum inhibitory concentration, MIC, μg/ml) and disc diffusion (zone diameter, mm) testing according to CLSI VET01-A4 protocols (CLSI, 2013b) at the University of Adelaide reference laboratory. *Staphylococcus aureus* ATCC 25923 and ATCC 29213 were used as quality control strains. The MIC results for the isolates were obtained from a previous study (Saputra et al., 2017). Disc diffusion testing was performed independently to when broth-microdilution testing occurred. Antibiotic discs were obtained from ThermoFischer Scientific (Australia). Antibiotics evaluated in this study are listed in Table 1. An antibiotic was included for evaluation if there were corresponding MIC and zone diameter measurements for isolates included in the study.

Table 1
Disc diffusion and broth-microdilution Clinical Laboratory Standards Institute (CLSI) interpretative criteria for canine clinical *Staphylococcus pseudintermedius* isolates (n = 614) evaluated in this study.

			Broth-microdilution (μg/ml)			Disc Diffusion (mm)	
Antimicrobial	Abbreviation	Disc content (ug)	MIC range	Susceptible breakpoint	Resistant breakpoint	Susceptible breakpoint	Resistant breakpoint
Amoxicillin-clavulanic acid[a]	AMC	20/10	0.06 – 32	≤4/2	≥8/4	≥20	≤19
Cefovecin[b]	CVN	30	0.06 – 64	≤0.5	≥2	≥24	≤20
Cefoxitin[b]	FOX	30	0.06 – 64	≤4	≥8	≥22	≤21
Cephalothin[a]	CEF	30	0.06 – 64	≤8	≥32	≥18	≤14
Chloramphenicol[b]	CHL	30	2.0 – 64	≤8	≥32	≥18	≤12
Ciprofloxacin[c]	CIP	5	0.03 – 8	≤1	≥4	≥21	≤15
Clindamycin[b]	CLI	2	0.03 – 32	≤0.5	≥4	≥21	≤14
Oxacillin[b]	OXA	1	0.03 – 64	≤0.25	≥0.5	≥18	≤17
Rifampicin[b]	RIF	5	0.004 – 4	≤1	≥4	≥20	≤16
Tetracycline[b]	TET	30	0.06 – 64	≤0.25	≥1	≥23	≤17

[a] CLSI VET01S2: Table 2B. Human Derived Zone Diameter Interpretation Standards and Minimum Inhibitory Concentration Breakpoints for Veterinary Pathogens.
[b] CLSI VET08: Table 2C. Zone Diameter and Minimum Inhibitory Concentration Breakpoints for *Staphylococcus* spp.
[c] CLSI M100-S25. Table 2C. Zone Diameter Interpretative Standards and MIC Breakpoints for *Staphylococcus* spp.

2.2. Screening for mecA

To assess *mecA* status, copies of the collection were sent to the Antimicrobial Resistance and Infectious Diseases Laboratory at Murdoch University. Isolates were cultured from frozen (−80 °C) stock culture according to CLSI protocols (CLSI, 2013b). DNA was extracted as described in Abraham et al. (2012) with minor modifications (Abraham et al., 2018). Presence of the *mecA* gene was determined by singleplex real-time probe-based PCR, as described previously (Costa et al., 2005). The probe (FAM-TTCCAGGAATGCAGAAAGACCAAAGCA-BHQ) and forward primer (5`-TGGTATGTGGAAGTTAGATTGGGAT-3`) used in this study were identified in a previous study (Nakagawa et al., 2005). The reverse primer was identified by performing a Basic Local Alignment Search Tool (BLAST) search on the online NCBI GenBank database. Based on sequence alignment, the reverse primer was designed manually (5`-CTATCTCATATGCTGTTCCTGTATTGGC-3`). MRSP isolates previously characterised by whole-genome sequencing for strain typing and detection of *mecA* genes by Worthing et al (2018a) were used as controls for comparison. Real-time PCR was performed in duplicate in 96-well plates using a 10 µL reaction mixture on QuantStudio™ 6 Flex Real-Time PCR system (Thermo Fisher Scientific, Australia). The reaction mixture comprised a final concentration of 0.4µM of each primer, 0.2µM probe, 5 µL TaqMan® Fast Advanced Master Mix, 2 µL nuclease-free water, and 2 µL DNA template.

3. Whole genome sequencing

Isolates with discordant oxacillin phenotypic and *mecA* real-time PCR results underwent whole genome-sequencing. DNA extractions were performed using a MagMax DNA multi-sample kit (ThermoFisher Scientific) according to the manufacturer's instructions, with the modification to omit the RNAse treatment step. Library preparation was performed with a Nextera XT kit with an increased tagmentation time of seven minutes. Sequencing was performed on an Illumina Nextseq 500 platform using a high-output V2 (2 × 150 cycles) reagent kit (O'Dea et al., 2018). Sequencing files were uploaded to the Center for Genomic Epidemiology (https://genomicepidemiology.org) and the ResFinder application used to check for the presence of acquired resistance genes.

3.1. Data analysis

Data used in this study comprised paired MIC and zone diameter values for 614 canine clinical *S. pseudintermedius* isolates for ten antibiotics.

For evaluation of test accuracy, MIC and zone diameter values were dichotomised using corresponding CLSI clinical interpretative criteria (Table 1). Where veterinary-specific *S. pseudintermedius* clinical breakpoints were unavailable, veterinary-specific *Staphylococcus* genus clinical breakpoints were used. Where veterinary-specific clinical breakpoints were unavailable or did not have corresponding MIC and zone diameter breakpoints, human breakpoints were used. For amoxicillin-clavulanic acid, we used the corresponding zone diameter and MIC breakpoints given by the last CLSI document containing them – CLSI VET01-S2, Table 2B (2013a). When the susceptible breakpoint was used to dichotomise MIC and zone diameter results, isolates in the 'intermediate' and 'resistant' range were collectively classified as 'non-susceptible'. When data were dichotomised using the resistant clinical breakpoint, isolates were classified as 'non-resistant' if their MIC or zone diameter value was within the 'intermediate' or 'susceptible' range. For real-time PCR, samples showing a sigmoidal curve with a cycle threshold (C_T) ≤ 40 were considered positive for the presence of *mecA* and negative if no sigmoidal curve was observed.

The accuracy of disc diffusion classification relative to broth-microdilution (the reference method) was evaluated by estimating relative diagnostic sensitivity (RSe) and specificity (RSp), likelihood ratios of positive (LR+) and negative (LR-) results and summarised using DOR and ROC AUC. For evaluation of the accuracy of disc diffusion and broth-microdilution to predict methicillin resistance in *S. pseudintermedius*, *mecA* real-time PCR was the reference test. ROC plots and AUC were estimated using non-parametric analysis since MIC data cannot be assumed to be normally distributed. Two-graph (TG) ROC plots were used to visualise the relative accuracy of disc diffusion to broth-microdilution across a range of cut-off values. Details on diagnostic sensitivity, specificity, LR pairs, DOR, and ROC analysis are given elsewhere (Glas et al., 2003; Greiner and Gardner, 2000).

Observed agreement was calculated as the proportion of isolates with the same interpretative classification by disc diffusion and broth-microdilution. Similarly, agreement between broth-microdilution, disc diffusion, and real-time PCR was calculated as the proportion of isolates with the same oxacillin interpretative classification and *mecA* status. McNemar's mid-*p* test was used to assess the extent of disagreement between two tests where a P value < 0.05 was considered significant (Fagerland et al., 2013).

Data were entered into MS Excel files and imported into Stata version 15.1 (Stata Corporation, College Station, TX) for all analysis. Data is available in supplementary information.

4. Results

4.1. Disc diffusion accuracy relative to broth-microdilution

RSp was consistently high across all antimicrobials (range, 97.1%–100%), whereas RSe estimates showed considerable variability (range, 35.7%–98.8%) when zone diameter and MIC values were dichotomised using susceptible breakpoints (Table 2). Similar results were recorded when resistant breakpoints were applied. Poor RSe estimates were recorded for amoxicillin-clavulanic acid (51.5%, 95% CI,

Table 2
Diagnostic performance estimates of disc diffusion relative to broth-microdilution for 614 canine *Staphylococcus pseudintermedius* isolates from clinical cases. Clinical and Laboratory Standards Institute (CLSI) susceptible and resistant breakpoints were used to dichotomise minimum inhibitory concentration (MIC) and zone diameter values. RSe, relative diagnostic sensitivity; RSp, relative diagnostic specificity; AUC, area-under-the-curve. Exact 95% confidence intervals are given.

	Susceptible Breakpoint Estimates			Resistant Breakpoint Estimates		
Antimicrobial	%RSe (95%CI)	%RSp (95%CI)	AUC (95%CI)	%RSe (95%CI)	%RSp (95%CI)	AUC
Amoxicillin-clavulanic acid	51.5 (38.9, 64.0)	99.8 (99.0, 100.0)	0.99 (0.97, 1.0)	51.5 (38.9, 64.0)	99.8 (90.0, 100.0)	0.99 (0.97, 1.0)
Cefovecin	72.3 (61.4, 81.6)	99.1 (97.8, 99.7)	0.95 (0.92, 0.98)	85.7 (74.6, 93.3)	98.6 (97.2, 99.4)	0.99 (0.98, 1.0)
Cefoxitin	35.7 (12.8, 64.9)	99.3 (98.3, 99.8)	0.96 (0.92, 1.0)	35.7 (12.8, 64.9)	99.3 (98.3, 99.8)	0.96 (0.92, 1.0)
Cephalothin	66.7 (51.1,80.0)	99.7 (98.7, 99.7)	0.99 (0.97, 1.0)	43.6 (27.8, 60.4)	99.8 (99.0, 100.0)	0.98 (0.97, 1.0)
Chloramphenicol	85.0 (70.2, 94.3)	100.0 (99.4, 100.0)	0.97 (0.93, 1.0)	94.4 (81.3, 99.3)	100.0 (99.4, 100.0)	0.99 (0.98, 1.0)
Ciprofloxacin	90.9 (80.1, 97.0)	99.8 (99.0, 100.0)	0.98 (0.96, 1.0)	88.2 (76.1, 95.6)	99.8 (99.0, 100.0)	0.98 (0.96, 1.0)
Clindamycin	98.8 (93.4, 100.0)	99.3 (98.1, 99.8)	0.99 (0.97, 1.0)	81.0 (70.6, 89.0)	99.8 (99.0, 100.0)	0.99 (0.97, 1.0)
Oxacillin	88.6 (79.5, 94.7)	100.0 (99.3, 100.0)	0.99 (0.98, 1.0)	88.6 (79.5, 94.7)	100.0 (99.3, 100.0)	0.99 (0.98, 1.0)
Rifampicin	80.0 (28.4, 99.5)	99.5 (98.6, 99.9)	0.80 (0.41, 1.0)	60.0 (14.7, 94.7)	99.7 (98.8, 100.0)	0.80 (0.41, 1.0)
Tetracycline	93.4 (87.9, 96.6)	97.1 (95.1, 98.4)	0.95 (0.93, 0.98)	94.1 (88.7, 97.4)	97.3(95.4, 98.6)	0.96 (0.93, 0.98)

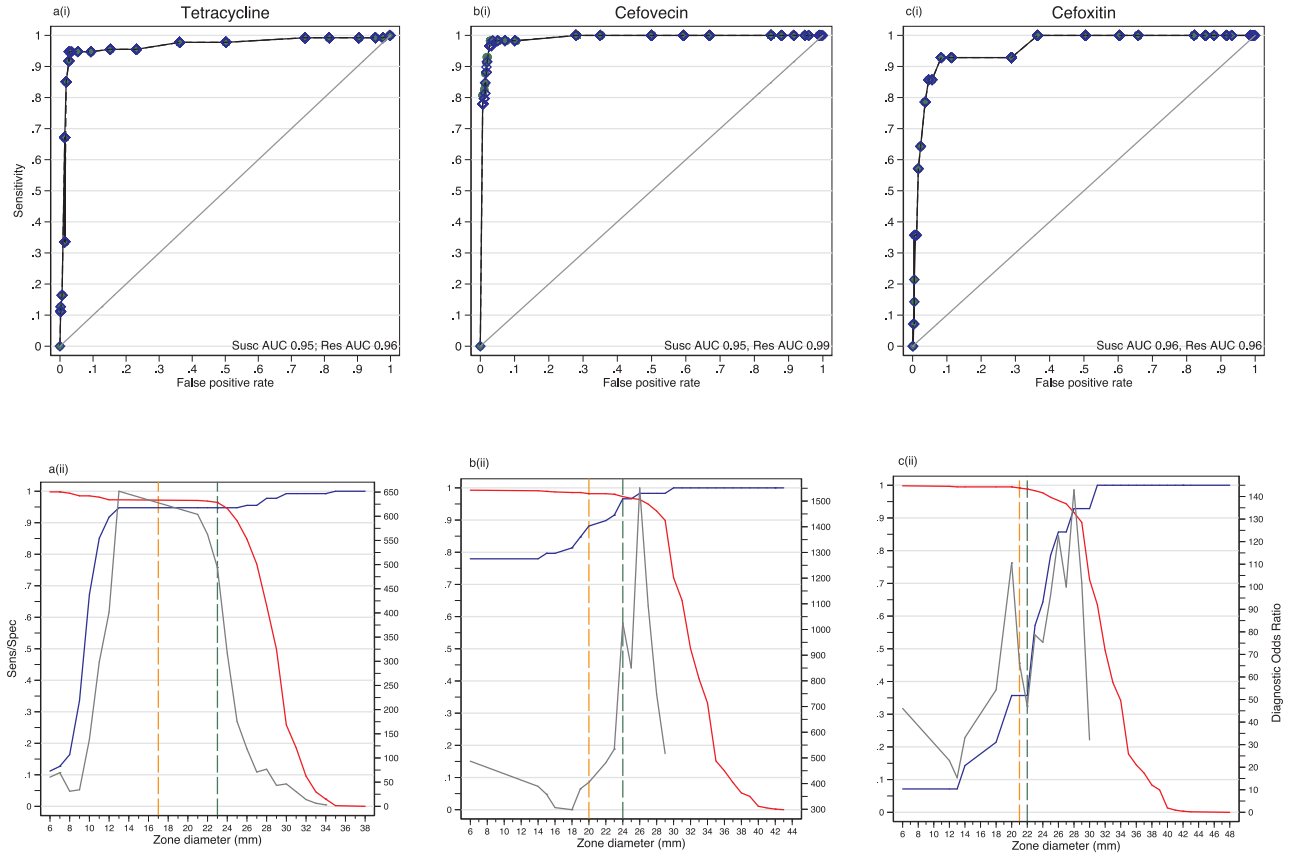

Fig. 1. Receiver-Operating Characteristic (ROC) analysis demonstrating overall performance of disc diffusion relative to broth-microdilution for three selected antimicrobials applied to canine *Staphylococcus pseudintermedius* isolates (n = 614) from clinical cases. ROC plots for tetracycline (a(i)), cefovecin (b(i)), and cefoxitin (c(i)). The green-circle curve is representative of the resistant clinical breakpoint and blue-diamond curve is representative of the susceptible clinical breakpoint for the minimum inhibitory concentration (MIC) as defined by Clinical and Laboratory Standards Institute (CLSI). Diagonal line represents an AUC of 0.5. Two-graph-ROC plots of relative sensitivity (Sens) and relative specificity (Spec) for tetracycline (a(ii)), cefovecin (b(ii)), and cefoxitin (c(ii)). Relative sensitivity (Sens, dash blue line), relative specificity (Spec, solid red line) diagnostic odds ratio (dash-dot grey line), CLIS susceptible breakpoint (green short-dash line) and resistant breakpoint (orange dash line) are plotted on each graph.

38.9%, 64.0%), cefoxitin (35.7%, 95% CI, 12.8%, 64.9%), and cephalothin (43.6%, 95% CI, 27.8%, 60.4%) irrespective of the breakpoint used to dichotomise MIC and zone diameter values.

The accuracy of disc diffusion relative to broth-microdilution, measured as the AUC, was greater than 0.96 for all antimicrobials, except rifampicin (AUC, 0.80) (Table 2). Overall test performance based on ROC analysis (ROC plots and TG-ROC) for three antimicrobials are shown in Fig. 1 (see supplementary materials for ROC plots for other antimicrobials). The ROC plots show minor differences in the accuracy of disc diffusion with the curves for all three antimicrobials approaching the top left-hand corner of the graph. However, the accuracy of disc diffusion varies considerably according to the TG-ROC plots for each antimicrobial evaluated. Disc diffusion is most accurate when the curves for RSe and RSp are close to one at the recommended breakpoints. For tetracycline, both the susceptible and resistant breakpoints correspond to near perfect RSe and RSp estimates, with the DOR indicating disc diffusion has high test discriminatory ability relative to broth-microdilution (Table 3). Drift between RSe and RSp estimates can be seen with cefovecin, with the DOR not at its highest discriminatory ability until sensitivity is over 95%. For cefoxitin, the performance of disc diffusion relative to broth-microdilution is poor when evaluated against both CLSI breakpoints. Here, disc diffusion maximises RSp at the expense of RSe at the recommended cefoxitin breakpoints.

Across all antimicrobials, there was strong diagnostic evidence from positive (resistant) disc diffusion results supporting the presence of resistance as classified by MIC (large LR^+, Table 3). The evidence provided by negative (susceptible) disc diffusion results (small LR^-) was strong using either breakpoint to determine susceptible status. For cefoxitin, disc diffusion was less than accurate at distinguishing susceptible isolates across both breakpoints (LR- = 0.65). The overall discriminatory ability of disc diffusion for all antimicrobials, as assessed by the DOR, was high (DOR > 82) (Table 3).

Observed agreement estimates were > 94.0% for all antimicrobials and breakpoints (Supplementary Tables 1 and 2). Across all antimicrobials and clinical breakpoints, negative percent agreement was > 97% (range 97.1%–99.8%). However, positive percent agreement was much more variable with low values (range, 43.5%–97.1%), particularly for cefoxitin (43.5%, 95% CI 23.2%, 65.5%) and amoxicillin-clavulanic acid (67.3%, 95% CI 57.3%, 76.3%), indicating disagreement between MIC and zone diameter values is associated with the clinical interpretation of resistant (or non-susceptibility) isolates. Antimicrobials with > 1% difference between proportion resistant by broth-microdilution and proportion resistant by disc diffusion recorded statistical significance (mid-*p* McNemar's < 0.05, Supplementary Tables 1 and 2). A higher number of antimicrobials recorded significant mid-*p* McNemar's estimate when the susceptible breakpoint dichotomised MIC and zone diameter values, including amoxicillin-clavulanic acid, cefovecin, cephalothin, and oxacillin. Prevalence-adjusted bias-adjusted Kappa estimates were > 0.9 for all antimicrobials.

The distribution of zone diameters for a selection of four antimicrobials can be appreciated in Fig. 2 (see supplementary materials for histograms of other antimicrobials). Estimates of relative diagnostic accuracy are maximal when there is a clear separation between

Table 3
Estimates of likelihood ratio pairs and diagnostic odds ratios of disc diffusion relative to broth-microdilution for 614 canine *Staphylococcus pseudintermedius* isolates from clinical cases. Clinical and Laboratory Standard Institute (CLSI) susceptible and resistant breakpoints were used to dichotomise minimum inhibitory concentration (MIC) and zone diameter values. LR^+, likelihood ratio of a positive test result; LR^-, likelihood ratio of a negative result, DOR, diagnostic odds ratio. Exact 95% confidence intervals given.

	Susceptible Breakpoint Estimates			Resistant Breakpoint Estimates		
Antimicrobial	LR^+ (95%CI)	LR^- (95%CI)	DOR (95%CI)	LR^+ (95%CI)	LR^- (95%CI)	DOR (95%CI)
Amoxicillin-clavulanic acid	282 (39, 2029)	0.49 (0.38, 0.62)	581 (97, ∞)	282 (9, 2029)	0.49 (0.38, 0.62)	581 (97, ∞)
Cefovecin	77 (32, 186)	0.28 (0.20, 0.40)	274 (103, 726)	59 (20, 118)	0.14 (0.08, 0.27)	407 (153, 108,748)
Cefoxitin	54 (16, 179)	0.65 (0.44, 0.96)	83 (20, 338)	54 (16, 179)	0.65 (0.44, 0.96)	83 (20, 338)
Cephalothin	190 (47, 768)	0.33 (0.22, 0.51)	576 (135, ∞)	251 (34, 1835)	0.57 (0.43, 0.74)	444 (71, ∞)
Chloramphenicol	∞ (117, ∞)	0.15 (0.07, 0.31)	∞ (1671, ∞)	∞ (136, ∞)	0.06 (0.01, 0.21)	∞ (13550, ∞)
Ciprofloxacin	508 (72, 3608)	0.09 (0.04, 0.21)	5580 (768, ∞)	497 (70, 530)	0.12 (0.06, 0.25)	4215 (601, ∞)
Clindamycin	131 (50, 349)	0.01 (0.0, 0.09)	10,692 (1404, ∞)	433 (61, 3080)	0.19 (0.12, 0.30)	2278 (368, ∞)
Oxacillin	∞ (114, ∞)	0.11 (0.06, 0.21)	∞ (1893, ∞)	∞ (114, ∞)	0.11 (0.06, 0.21)	∞ (1893, ∞)
Rifampicin	162 (48, 545)	0.20 (0.03, 1.0)	808 (86, ∞)	183 (39, 868)	0.40 (0.14, 1.0)	455 (57, 3892)
Tetracycline	32 (19, 53)	0.07 (0.04, 0.13)	470 (201, 1102)	35 (20, 59)	0.06 (0.03, 0.12)	569 (233, 1388)

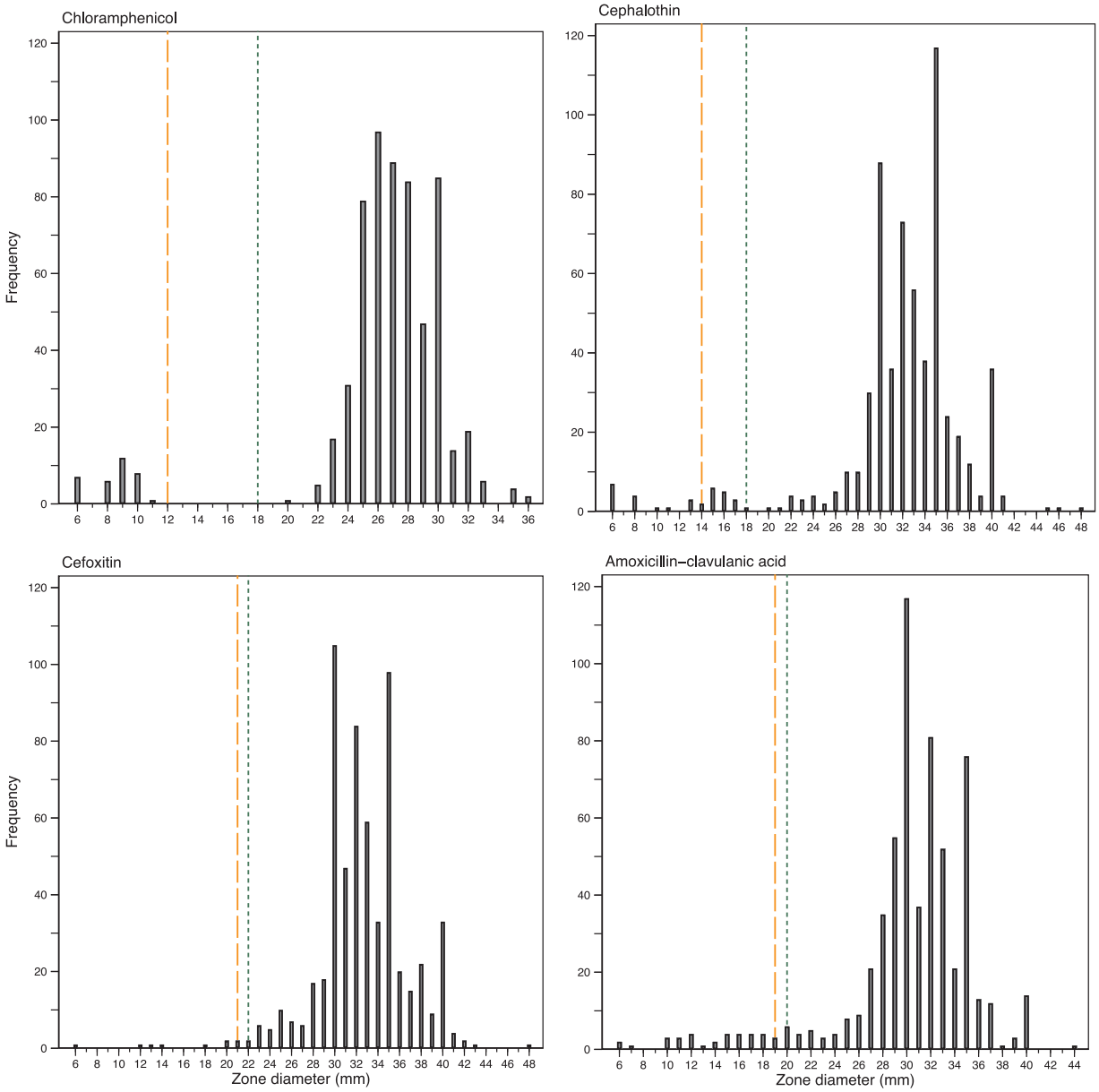

Fig. 2. Distribution of zone diameters for four antimicrobials in canine *Staphylococcus pseudintermedius* isolates (n = 614) from clinical cases. The Clinical and Laboratory Standards Institute (CLSI) susceptible breakpoint (green short-dash) and resistant breakpoint (orange long-dash) is plotted on each distribution.

Table 4
Diagnostic test performance of broth-microdilution and disc diffusion relative to *mecA* real-time PCR for 576 clinical canine *Staphylococcus pseudintermedius* isolates. Clinical and Laboratory Standards Institute (CLSI) susceptible and resistant breakpoints for oxacillin were used to dichotomise minimum inhibitory concentration (MIC) and zone diameter values. RSe, relative diagnostic sensitivity; RSp, relative diagnostic specificity; AUC, area under the curve; LR^+, likelihood ratio of a positive test result; LR^-, likelihood ratio of a negative result, DOR, diagnostic odds ratio. Exact 95% confidence intervals are given.

Test Performance estimates	Broth-microdilution (95% CI)	Disc Diffusion (95% CI)
RSe (%)	96.1 (88.9, 99.2)	86.8 (77.1, 93.5)
RSp (%)	99.6 (98.6, 100.0)	99.8 (98.9, 100.0)
AUC	0.99 (0.97, 1.00)	0.99 (0.96, 1.00)
LR +	240 (60.2, 958)	434 (61.2, 3082)
LR-	0.04 (0.01, 0.12)	0.13 (0.07, 0.24)
DOR	6059 (1059, 33,932)	3293 (511, ∞)
Oxacillin resistance (%)	13.0 (10.4, 16.1)	11.6 (9.1, 14.5)
Observed Agreement (%)	99.1 (98.0, 99.7)	98.1 (96.6, 99.0)
McNemars mid-*p* value	1.0	0.01

populations as demonstrated on the zone diameter histogram for chloramphenicol. However, performance estimates were imperfect when populations overlap and, or breakpoints were close together (*e.g.*, cefoxitin, cephalothin, amoxicillin-clavulanic acid, Fig. 2). Moreover, estimates of relative accuracy may be imprecise when the range of zone diameters is narrow (*i.e.*, mostly all susceptible), as can be seen for cefoxitin and rifampicin (supplementary materials).

4.2. Broth-microdilution and disc diffusion performance relative to mecA real-time PCR

For the evaluation of broth-microdilution and disc diffusion relative to *mecA* real-time PCR, 576 isolates were evaluated. In total, 13.2% (n = 76) of isolates were *mecA*-positive by real-time PCR, while 13.0% (n = 75) were oxacillin resistant by broth-microdilution and 11.6% (n = 67) by disc diffusion (Table 4). The relative diagnostic test accuracy of broth-microdilution and disc diffusion was high (AUC > 0.99). DOR estimates reflect the strong RSe and RSp estimates for both assays, however, disc diffusion's RSe was significantly lower than broth-microdilution (McNemars mid-*p* value < 0.01). There was a significant difference between the proportion of isolates identified as oxacillin-resistant by disc diffusion and by *mecA* real-time PCR (McNemars mid-*p* value < 0.01, Table 4). The overlap between oxacillin-susceptible isolates by disc diffusion and *mecA* positive status can be seen in Fig. 3. An overall comparison of the ROC analysis demonstrated negligible difference in the accuracy of broth-microdilution and disc diffusion. However, the robustness of the assays varies according to their corresponding TG-ROC plots (Fig. 3). For both assays, small movements in clinical breakpoints (and zone measurements for decreased susceptible isolates) will result in a noticeable change in RSe and RSp, especially so for disc diffusion.

4.3. mecA sequence analysis

Nine isolates identified as phenotypically susceptible to oxacillin by disc diffusion (9/9) or broth microdilution (3/9) and *mecA* positive on real-time PCR underwent whole-genome sequencing. Details of phenotypic and genotypic characteristics of the isolates are detailed in Supplementary Table 3. Six strain types were identified including two from the same sequence type, ST498. Two strains were from new sequence types. The nine isolates were confirmed *mecA* positive with 99.5%–100% identity and 100% full length coverage and all contained *blaZ* or *blaZ*-like elements.

5. Discussion

The main finding from this study is the high level of accuracy of disc diffusion relative to broth-microdilution for most antimicrobials evaluated in clinical *S. pseudintermedius* when performed according to CLSI guidelines. This finding holds regardless of the CLSI interpretative criteria used to evaluate the performance of disc diffusion. For clindamycin, an antibiotic commonly recommended as a first-line treatment for *S. pseudintermedius* infections in dogs (Hillier et al., 2014), the accuracy of disc diffusion was comparable to broth-microdilution. Similarly, disc diffusion was accurate for cefovecin; an antibiotic often recommended as a final treatment option. However, the accuracy of disc diffusion was unsatisfactory for amoxicillin-clavulanic acid and cephalothin, antimicrobials that are also frequently used to *S. pseudintermedius* infections. For these antimicrobials, disc diffusion has limitations when determining the phenotypic susceptibility of *S. pseudintermedius*.

These findings are of concern for amoxicillin-clavulanic acid which is widely used to treat skin infections in dogs as there are no published veterinary-specific zone diameter interpretative criteria for *S. pseudintermedius*, and CLSI removed zone diameter clinical breakpoints for all antistaphylococcal beta-lactams in 2012 (Dien Bard et al., 2014). Thus, the amoxicillin-clavulanic acid zone diameter breakpoints listed in older versions of CLSI document VET01 are likely to be unreliable. Considering this, and the findings from this study, veterinary laboratories that evaluate the susceptibility of clinical *S. pseudintermedius* isolates to amoxicillin-clavulanic acid using the disc diffusion assay are advised to discontinue the practice. Furthermore, veterinary laboratories are advised to limit disc diffusion testing of clinical *S. pseudintermedius* isolates to oxacillin and penicillin to infer the susceptibility for other beta-lactam antimicrobial agents, except for newer cephalosporins with anti-MRSA activity. These recommendations are consistent with CLSI (2018), and findings reported by Dien Bard et al. (2014) and Siak et al. (2014). This study also provides quantitative and graphical evidence to confirm the inadequacy of disc diffusion testing for determining the susceptibility of *S. pseudintermedius* to cefoxitin. Given the potential for a high number of misclassification errors, cefoxitin should not be included in the panel of antibiotics used to evaluate antimicrobial susceptibility of animal-derived *S. pseudintermedius*.

Broth-microdilution and disc diffusion were shown to be relatively comparable at predicting the presence of the *mecA* gene in clinical *S. pseudintermedius* isolates. For both assays, predicting of the absence of the *mecA* gene was high, while broth-microdilution out-performed disc diffusion when predicting the presence of the *mecA* gene. Other studies have also found phenotypic oxacillin resistance is a reliable predictor of the presence of the *mecA* gene in *S. pseudintermedius* (Bemis et al., 2009; Schissler et al., 2009; Worthing et al., 2018a; Wu et al., 2016). In this study, nine isolates were identified as oxacillin-susceptible by disc diffusion or broth-microdilution yet harboured the *mecA* gene. A similar observation has been reported in other studies (Eckholm et al., 2013; Feng et al., 2012; Griffeth et al., 2008; Kania et al., 2004; Kuwahara-Arai et al., 1996). It has been suggested that failure to express the PBP2a protein may be due to mutation, down-regulation or suppression of *mecA* gene expression (Kania et al., 2004; Kuwahara-Arai et al., 1996). The type of staphylococcal cassette chromosome that harbours the *mecA* gene (SCC*mec*) has also been shown to affect oxacillin MIC in MRSP isolates (Kasai et al., 2016; Worthing et al., 2018b). The collection of *mec*A-positive, oxacillin-susceptible isolates included isolates from ST498, ST539 and ST547. These sequence types were previously shown to harbour SCC*mec* types IVg and NA45, both of which have significantly lower oxacillin MIC values than other SCC*mec* types (Worthing et al., 2018b). Heterogeneous resistance, where there is existence of susceptible and resistant organisms within a single strain has also been proposed (Kania et al., 2004; Savini et al., 2013). Variations in salinity, temperature, pH, or the presence of beta-lactam during laboratory culture is also reported to have an effect on phenotype

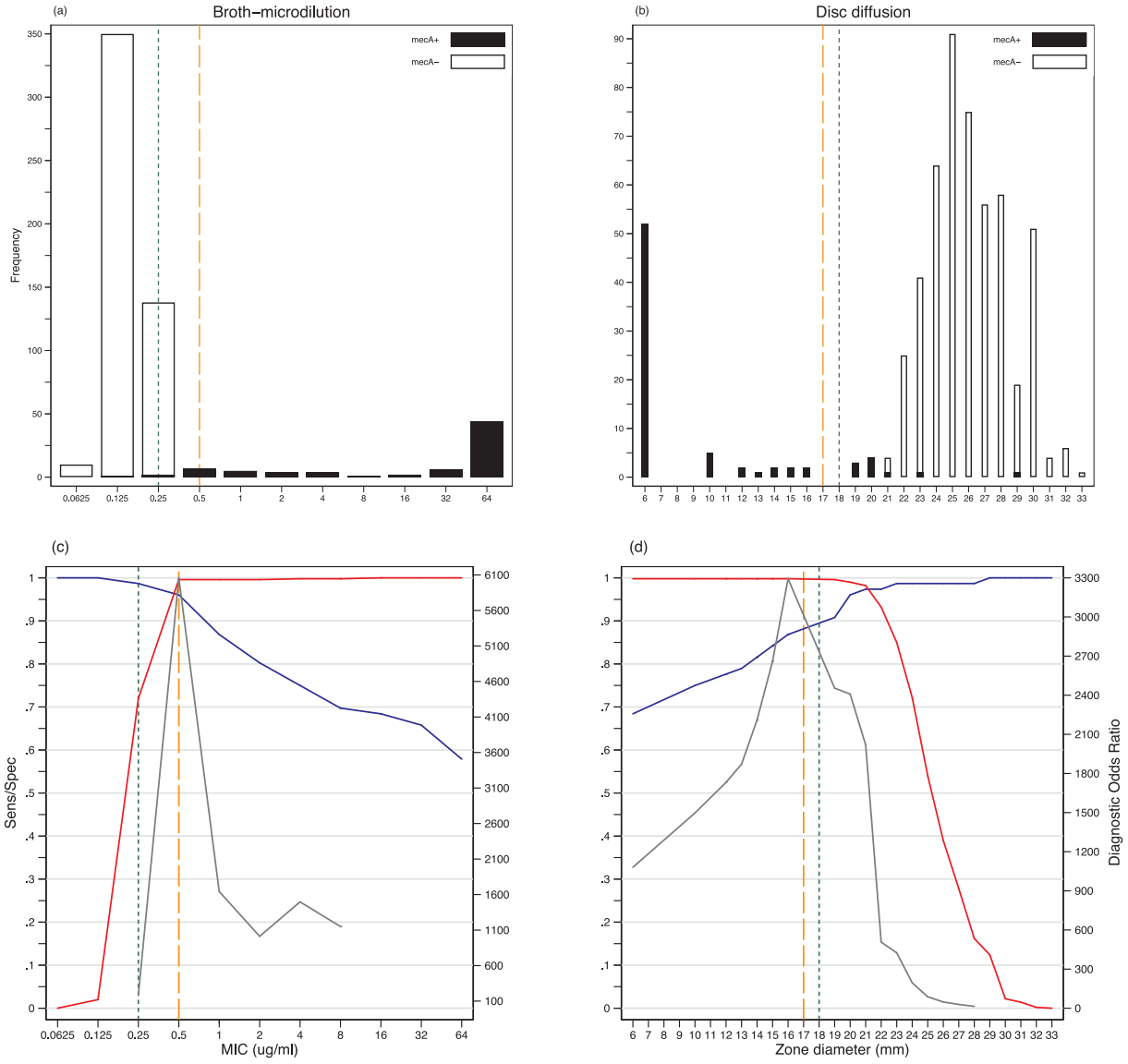

Fig. 3. Diagnostic test performance attributes for broth-microdilution and disc diffusion for oxacillin in canine *Staphylococcus pseudintermedius* isolates (n = 576) from clinical cases. (a) Minimum inhibitory concentration (MIC) values by broth-microdilution, and (b) zone diameter values by disc diffusion are compared to *mecA* real-time PCR status. (c) Two-graph Receiver-Operating Characteristic (TG-ROC) plots for broth-microdilution, and (d) disc diffusion, plot the relative sensitivity (Sens), relative specificity (Spec), and diagnostic odds ratio (DOR). Real-time *mecA* PCR is the reference test. Relative sensitivity (Sens, dash blue solid line), relative specificity (Spec, solid red line), diagnostic odds ratio (dash-dot grey line). Clinical and Laboratory Standards Institute (CLSI) susceptible (green short dash line) and resistant (orange long-dash line) breakpoints are plotted on all graphs.

expression (Griffeth et al., 2008). Additionally, the precision of measurements derived from the phenotypic assays for decreased susceptible isolates must also be considered. For example, six of the nine isolates identified as oxacillin-susceptible by disc diffusion were within 3 mm of the resistant clinical breakpoint, while five of the nine MICs were equal to the resistant clinical breakpoint. The detection of isolates with decreased susceptibility depends very much on the validity of the assay, the proficiency of technicians, and the breakpoint used to classify the isolates.

In parallel with an earlier study involving *Escherichia coli* (Badger et al., 2018), we report a broad range of measurements for disc diffusion applied to *S. pseudintermedius*. Quantitative estimates from large studies of these kind are generally lacking in veterinary literature for a broad range of diagnostic tests. Evidenced-based clinical decisions are better supported with robust estimates of test accuracy. Also, in surveillance settings, diagnostic sensitivity and specificity can be used in standard equations to adjust the apparent prevalence of disc diffusion, thereby allowing direct comparison with ('true') prevalence measured by broth-microdilution. ROC analysis is useful to determine test accuracy and assist in defining breakpoint values since it is independent of prevalence in a population. Depending on the purpose of the test and the clinical or epidemiological setting, either diagnostic sensitivity or specificity can be improved by altering the breakpoint used to define resistance.

While this study was comprised of a comprehensive collection of clinical *S. pseudintermedius* isolates submitted from all veterinary laboratories in Australia over one year, it cannot be considered a broad representation of all clinical cases presented to primary care veterinarians. Findings from this study may be biased towards the inclusion of resistant isolates since veterinarians are more likely to submit samples from cases that may have already failed initial treatment. Biases arising from selective inclusion of animals or isolates in studies are well recognised (Lash et al., 2014; Laupland et al., 2007). Ciprofloxacin was used as a representative of the fluoroquinolone class as it is commonly

used in national surveillance owning to its relevance to public health. However, there is a need to evaluate the performance of other fluoroquinolone class members specific to animal health, such as enrofloxacin and marbofloxacin, for clinical decision-making. Data for this study were generated in two reference laboratories (University of Adelaide, Murdoch University) and may not reflect the variation in results which may occur when multiple primary laboratories perform phenotypic assays. Moreover, since broth-microdilution is an imperfect reference test the performance estimates for disc diffusion reported here can never exceed those of broth-microdilution.

6. Conclusion

Overall, this study demonstrates that for most antimicrobials evaluated, disc diffusion can be used to differentiate veterinary *S. pseudintermedius* isolates that might otherwise be assessed by broth-microdilution when performed according to CLSI guidelines. However, disc diffusion performance was less favourable for amoxicillin-clavulanic acid, cephalothin, and cefoxitin. Therefore, veterinary laboratories are advised to use oxacillin or penicillin to infer phenotypic susceptibility of *S. pseudintermedius* to other beta-lactam antibiotics, except for newer anti-MRSA cephalosporins. We found disc diffusion and broth-microdilution approximated genotypic results from *mecA* real-time PCR when using oxacillin to predict methicillin resistance, and there was minimal difference in the performance estimates between both phenotypic assays relative to PCR. These findings demonstrate that disc diffusion susceptibility data from clinical *S. pseudintermedius* could be acquired for national surveillance provided consideration is given to the diagnostic test performance estimates reported here.

Declaration of interest statement

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Ethical approval

None required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.05.024.

References

Abraham, S., Chin, J., Brouwers, H.J., Zhang, R., Chapman, T.A., 2012. Molecular serogrouping of porcine enterotoxigenic Escherichia coli from Australia. J. Microbiol. Methods 88, 73–76.

Abraham, S., Kirkwood, R.N., Laird, T., Saputra, S., Mitchell, T., Singh, M., Linn, B., Abraham, R.J., Pang, S., Gordon, D.M., Trott, D.J., O'Dea, M., 2018. Dissemination and persistence of extended-spectrum cephalosporin-resistance encoding IncI1-blaCTXM-1 plasmid among Escherichia coli in pigs. ISME J. 12, 2352–2362.

Badger, S., Abraham, S., Saputra, S., Trott, D.J., Turnidge, J., Mitchell, T., Caraguel, C.G.B., Jordan, D., 2018. Relative performance of antimicrobial susceptibility assays on clinical Escherichia coli isolates from animals. Vet. Microbiol. 214, 56–64.

Bemis, D.A., Jones, R.D., Frank, L.A., Kania, S.A., 2009. Evaluation of susceptibility test breakpoints used to predict mecA-mediated resistance in Staphylococcus pseudintermedius isolated from dogs. J. Vet. Diagn. Investig. 21, 53–58.

Bemis, D.A., Jones, R.D., Videla, R., Kania, S.A., 2012. Evaluation of cefoxitin disk diffusion breakpoint for detection of methicillin resistance in Staphylococcus pseudintermedius isolates from dogs. J. Vet. Diagn. Investig. 24, 964–967.

CLSI, 2013a. Performance Standards for Antimicorbial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; (VET01-S2), 2nd ed. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI, 2013b. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals (VET01-A4), 4th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI, 2015. Performance standards for antimicrobial susceptibility testing; (M100-S25). 25th Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI, 2018. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals (VET08), 4th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

Costa, A.M., Kay, I., Palladino, S., 2005. Rapid detection of mecA and nuc genes in staphylococci by real-time multiplex polymerase chain reaction. Diagn. Microbiol. Infect. Dis. 51, 13–17.

Dien Bard, J., Hindler, J.A., Gold, H.S., Limbago, B., 2014. Rationale for eliminating Staphylococcus breakpoints for beta-lactam agents other than penicillin, oxacillin or cefoxitin, and ceftaroline. Clin. Infect. Dis. 58, 1287–1296.

Eckholm, N.G., Outerbridge, C.A., White, S.D., Sykes, J.E., 2013. Prevalence of and risk factors for isolation of meticillin-resistant Staphylococcus spp. From dogs with pyoderma in northern California. USA. Vet Dermatol 24 (154-161), e134.

Fagerland, M.W., Lydersen, S., Laake, P., 2013. The McNemar test for binary matched-pairs data: mid-p and asymptotic are better than exact conditional. BMC Med. Res. Methodol. 13, 91.

Feng, Y., Tian, W., Lin, D., Luo, Q., Zhou, Y., Yang, T., Deng, Y., Liu, Y.-H., Liu, J.-H., 2012. Prevalence and characterization of methicillin-resistant Staphylococcus pseudintermedius in pets from South China. Vet. Microbiol. 160, 517–524.

Glas, A.S., Lijmer, J.G., Prins, M.H., Bonsel, G.J., Bossuyt, P.M., 2003. The diagnostic odds ratio: a single indicator of test performance. J. Clin. Epidemiol. 56, 1129–1135.

Greiner, M., Gardner, I.A., 2000. Epidemiologic issues in the validation of veterinary diagnostic tests. Prev. Vet. Med. 45, 3–22.

Griffeth, G.C., Morris, D.O., Abraham, J.L., Shofer, F.S., Rankin, S.C., 2008. Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and Staphylococcus schleiferi in dogs with healthy and inflamed skin. Vet. Dermatol. 19, 142–149.

Hardefeldt, L.Y., Marenda, M., Crabb, H., Stevenson, M.A., Gilkerson, J.R., Billman-Jacobe, H., Browning, G.F., 2018. Antimicrobial susceptibility testing by Australian veterinary diagnostic laboratories. Aust. Vet. J. 96, 142–146.

Hillier, A., Lloyd, D.H., Weese, J.S., Blondeau, J.M., Boothe, D., Breitschwerdt, E., Guardabassi, L., Papich, M.G., Rankin, S., Turnidge, J.D., Sykes, J.E., 2014. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial guidelines working group of the international society for companion animal infectious diseases). Vet. Dermatol. 25 (163-175), e142–163.

Kania, S.A., Williamson, N.L., Frank, L.A., Wilkes, R.P., Jones, R.D., Bemis, D.A., 2004. Methicillin resistance of staphylococci isolated from the skin of dogs with pyoderma. Am. J. Vet. Res. 65, 1265–1268.

Kasai, T., Saegusa, S., Shirai, M., Murakami, M., Kato, Y., 2016. New categories designated as healthcare-associated and community-associated methicillin-resistant Staphylococcus pseudintermedius in dogs. Microbiol. Immunol. 60, 540–551.

Kuwahara-Arai, K., Kondo, N., Hori, S., Tateda-Suzuki, E., Hiramatsu, K., 1996. Suppression of methicillin resistance in a mecA-containing pre-methicillin-resistant Staphylococcus aureus strain is caused by the mecI-mediated repression of PBP 2′ production. Antimicrob. Agents Chemother. 40, 2680–2685.

Lash, T.L., Fox, M.P., MacLehose, R.F., Maldonado, G., McCandless, L.C., Greenland, S., 2014. Good practices for quantitative bias analysis. Int. J. Epidemiol. 43, 1969–1985.

Laupland, K.B., Ross, T., Pitout, J.D., Church, D.L., Gregson, D.B., 2007. Investigation of sources of potential bias in laboratory surveillance for anti-microbial resistance. Clin. Invest. Med. 30, E159–166.

Nakagawa, S., Taneike, I., Mimura, D., Iwakura, N., Nakayama, T., Emura, T., Kitatsuji, M., Fujimoto, A., Yamamoto, T., 2005. Gene sequences and specific detection for Panton-Valentine leukocidin. Biochem. Biophys. Res. Commun. 328, 995–1002.

O'Dea, M.A., Laird, T., Abraham, R., Jordan, D., Lugsomya, K., Fitt, L., Gottschalk, M., Truswell, A., Abraham, S., 2018. Examination of Australian Streptococcus suis isolates from clinically affected pigs in a global context and the genomic characterisation of ST1 as a predictor of virulence. Vet. Microbiol. 226, 31–40.

Saputra, S., Jordan, D., Worthing, K.A., Norris, J.M., Wong, H.S., Abraham, R., Trott, D.J., Abraham, S., 2017. Antimicrobial resistance in coagulase-positive staphylococci isolated from companion animals in Australia: a one year study. PLoS One 12, e0176379.

Savini, V., Di Giuseppe, N., Fazii, P., D'Amario, C., D'Antonio, D., Carretto, E., 2013. Staphylococcus pseudintermedius heterogeneously expresses the mecA gene. Vet. Microbiol. 165, 489–490.

Schissler, J.R., Hillier, A., Daniels, J.B., Cole, L.K., Gebreyes, W.A., 2009. Evaluation of Clinical Laboratory Standards Institute interpretive criteria for methicillin-resistant Staphylococcus pseudintermedius isolated from dogs. J. Vet. Diagn. Invest.: Off. Publ. Am. Assoc. Vet. Lab. Diagn., Inc 21, 684–688.

Schmidt, V.M., Williams, N.J., Pinchbeck, G., Corless, C.E., Shaw, S., McEwan, N., Dawson, S., Nuttall, T., 2014. Antimicrobial resistance and characterisation of staphylococci isolated from healthy Labrador retrievers in the United Kingdom. BMC Vet. Res. 10, 17.

Siak, M., Burrows, A.K., Coombs, G.W., Khazandi, M., Abraham, S., Norris, J.M., Weese, J.S., Trott, D.J., 2014. Characterization of meticillin-resistant and meticillin-

susceptible isolates of Staphylococcus pseudintermedius from cases of canine pyoderma in Australia. J. Med. Microbiol. 63, 1228–1233.
van Duijkeren, E., Catry, B., Greko, C., Moreno, M.A., Pomba, M.C., Pyorala, S., Ruzauskas, M., Sanders, P., Threlfall, E.J., Torren-Edo, J., Torneke, K., 2011. Review on methicillin-resistant Staphylococcus pseudintermedius. J. Antimicrob. Chemother. 66, 2705–2714.
Worthing, K.A., Abraham, S., Coombs, G.W., Pang, S., Saputra, S., Jordan, D., Trott, D.J., Norris, J.M., 2018a. Clonal diversity and geographic distribution of methicillin-resistant Staphylococcus pseudintermedius from Australian animals: discovery of novel sequence types. Vet. Microbiol. 213, 58–65.
Worthing, K.A., Schwendener, S., Perreten, V., Saputra, S., Coombs, G.W., Pang, S., Davies, M.R., Abraham, S., Trott, D.J., Norris, J.M., 2018b. Characterization of staphylococcal cassette chromosome mec elements from methicillin-resistant Staphylococcus pseudintermedius infections in australian animals. mSphere 3.
Wu, M.T., Burnham, C.A., Westblade, L.F., Dien Bard, J., Lawhon, S.D., Wallace, M.A., Stanley, T., Burd, E., Hindler, J., Humphries, R.M., 2016. Evaluation of Oxacillin and Cefoxitin Disk and MIC Breakpoints for Prediction of Methicillin Resistance in Human and Veterinary Isolates of Staphylococcus intermedius Group. J. Clin. Microbiol. 54, 535–542.