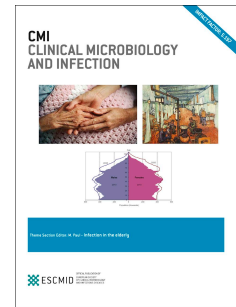


Accepted Manuscript

EUCAST disk diffusion Criteria for the Detection of *mecA*-Mediated β -lactam resistance in *Staphylococcus pseudintermedius*: oxacillin versus cefoxitin

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PII: S1198-743X(19)30215-0

DOI: <https://doi.org/10.1016/j.cmi.2019.05.002>

Reference: CMI 1663

To appear in: *Clinical Microbiology and Infection*

Received Date: 8 March 2018

Revised Date: 24 April 2019

Accepted Date: 7 May 2019

Please cite this article as: Skov R, Varga A, Matuschek E, Åhman J, Bemis D, Bengtsson B, Sunde M, Humphries R, Westblade L, Guardabassi L, Kahlmeter G, EUCAST disk diffusion Criteria for the Detection of *mecA*-Mediated β -lactam resistance in *Staphylococcus pseudintermedius*: oxacillin versus cefoxitin, *Clinical Microbiology and Infection*, <https://doi.org/10.1016/j.cmi.2019.05.002>.

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1 EUCAST Disk Diffusion Criteria for the Detection of *mecA*-Mediated β -Lactam
2 Resistance in *Staphylococcus pseudintermedius*: Oxacillin Versus Cefoxitin

3

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18

19 **Keywords:** *Staphylococcus pseudintermedius*, susceptibility testing, oxacillin, cefoxitin,

20 **methicillin resistance, MRSP**

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24

25 **Objectives:** Until recently, the European Committee on Antimicrobial Susceptibility Testing
26 (EUCAST) recommended the cefoxitin disk to screen for *mecA*-mediated betalactam resistance in
27 *Staphylococcus pseudintermedius*. A recent study indicated that cefoxitin was inferior to oxacillin
28 in this respect. We have re-evaluated cefoxitin and oxacillin disks for screening for methicillin
29 resistance in *S. pseudintermedius*. **Methods:** We included 224 animal and human *S.*
30 *pseudintermedius* isolates from Europe (n=108) and North America (n=116), of which 109 were
31 *mecA*-positive. Disk diffusion was performed per EUCAST recommendations using 30 µg cefoxitin
32 and 1 µg oxacillin disks from three manufacturers and Mueller-Hinton agar from two
33 manufacturers. **Results:** Cefoxitin inhibition zones ranged from 6-33 mm for *mecA*-positive *S.*
34 *pseudintermedius* (MRSP) and from 29-41 mm for *mecA*-negative *S. pseudintermedius* (MSSP). The
35 corresponding oxacillin zone intervals were 6-20 mm and 19 – 30 mm. For cefoxitin 16% (14.8%-
36 18.0%, 95% CI) of the isolates were in the area where positive and negative results overlapped. For
37 oxacillin the corresponding number was 2% (1.6%-2.9%). For oxacillin a breakpoint of S, ≥ 20 mm
38 and R, < 20 mm resulted in only 0.4% and 1.1% VME and ME rates respectively.

39 **Conclusions:** This investigation confirms that the 1 µg oxacillin disk predicts *mecA*-mediated
40 methicillin resistance in *S. pseudintermedius* better than the 30 µg cefoxitin disk. For a 1 µg
41 oxacillin disk we propose that 20 mm should be used as cut off for resistance i.e. isolates with a
42 zone diameter < 20 mm are resistant to all beta- lactam antibiotics except those with effect against
43 methicillin resistant staphylococci.

44

45 **Introduction**

46 *Staphylococcus pseudintermedius* is a coagulase-positive *Staphylococcus* species adapted
47 to *Canidae* and one of the most important bacterial pathogens in dogs but also causes
48 infections in humans including serious infections (1-4). The introduction of matrix-assisted
49 laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for bacterial

50 identification has shown that the incidence of *S. pseudintermedius* infections in humans is
51 probably underestimated due to mis-identification as *Staphylococcus aureus* (4-6).

52 Methicillin (β -lactam)-resistant *S. pseudintermedius* (MRSP) was first reported in 1999 in
53 North America (7) and in 2006 in Europe (8). Since then, five MRSP lineages (CC45, 68, 71,
54 112, 258) with specific traits regarding antimicrobial resistance, genetic diversity and
55 geographical distribution have spread globally (1, 9). Hitherto, according to our
56 knowledge, only *mecA*-based resistance have been reported in *S. pseudintermedius*.

57 Variable MRSP prevalence among clinical isolates (1-33%) has been reported by recent
58 studies from different geographical areas and study populations (2, 10-15). A study in the
59 United States (US) showed that the prevalence of methicillin resistance in canine clinical
60 isolates increased from <5% in 2001 to nearly 30% in 2007 . Some MRSP clones such as
61 sequence type (ST) 71 display resistance to virtually all antimicrobial agents licensed for
62 veterinary use, posing one of the most challenging problems so far encountered in the
63 antimicrobial management of veterinary infectious diseases. According to a recent review,
64 approximately two thirds of MRSP isolates submitted to the multilocus sequence typing
65 (MLST) database originate from skin samples associated with pyoderma, surgical site and
66 wound infections (1).

67 Cefoxitin is endorsed by both the European Committee on Antimicrobial Susceptibility
68 Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) as the
69 preferred agent for detecting methicillin-resistant *Staphylococcus aureus* (MRSA) and
70 methicillin-resistant coagulase-negative *Staphylococcus* (MRCONS) isolates by disk
71 diffusion (16-18). In contrast, there has been divergence between EUCAST and CLSI on the
72 antimicrobial agent to use for the detection of MRSP by disk diffusion. EUCAST has

73 advocated for the use of cefoxitin, whereas CLSI recommends oxacillin for detection of
74 MRSP (17, 18). Previous studies have shown that cefoxitin growth inhibition zone
75 diameter breakpoints recommended for detection of MRSA (susceptible, ≥ 22 mm;
76 resistant, < 22 mm) and MRCoNS (S, ≥ 25 mm; R, < 25 mm) are not reliable for MRSP (19). In
77 2012, based on a study of 1,146 *S. pseudintermedius* isolates originating from different
78 regions in the US, Bemis *et al.* proposed an epidemiological cut-off value for non-wildtype
79 of ≤ 30 mm to maximize sensitivity (97%) and specificity (92%) for predicting methicillin
80 resistance by cefoxitin disk diffusion (20). Our group further investigated 243 *S.*
81 *pseudintermedius* isolates to identify the most suitable cefoxitin breakpoint to distinguish
82 between MSSP and MRSP. The isolates were predominantly of European origin and the
83 results indicated a breakpoint of S, ≥ 35 mm and R, < 35 mm with only two (0.4%) major
84 errors (ME) and one (0.2%) very major error (VME) (unpublished own data). On the basis
85 of these data, these breakpoints were added to the EUCAST breakpoint table 4.0
86 published January 2014 (21). However, in a subsequent study Wu *et al.* showed that the
87 EUCAST breakpoint produced a significant number of major errors (ME) in a study using
88 115 human and veterinary "*Staphylococcus intermedius* group" isolates (111 *S.*
89 *pseudintermedius* and four *Staphylococcus delphini* isolates) from the US. The authors
90 concluded that cefoxitin disk diffusion is not reliable for MRSP detection and that
91 laboratories should perform oxacillin disk diffusion or broth-based minimum inhibitory
92 concentration tests (22). This was confirmed by Yarbrough *et al.* who found that none of
93 12 MRSP isolates were detected by cefoxitin disk diffusion whereas all 12 were detected
94 using oxacillin disk diffusion (4).

95 The current study was conducted to re-evaluate disk diffusion breakpoints using cefoxitin
96 (30 µg disk) and oxacillin (1 µg disk) disk diffusion to detect *mecA*-mediated β-lactam
97 resistance in *S. pseudintermedius* using disks from three manufacturers and Mueller-
98 Hinton agar (MHA) from two manufacturers. For the present evaluation, our strain
99 collection included strains from both Europe and North America to take the marked
100 differences in the distribution of clonal lineages existing between these two geographical
101 regions into account (1).

102

103 **Materials and Methods**

104 *Bacterial isolates*

105 A total of 224 clinical *S. pseudintermedius* isolates were tested, including 115 *mecA*-
106 negative (MSSP) isolates and 109 *mecA*-positive (MRSP) isolates. The isolates were
107 obtained from colleagues in Europe and North America representing a convenience
108 sampling and included the 111 *S. pseudintermedius* isolates described by Wu and
109 colleagues. Sixty-seven isolates from dogs and six from cats isolated between 2006 and
110 2011 were from a strain collection at the National Veterinary Institute in Sweden (SVA).
111 Forty-nine of these isolates were from different European countries, three from Canada
112 and two from the US (23). Forty canine isolates isolated between 2008 and 2011 were
113 from the Norwegian Veterinary Institute (NVI). The remaining 111 isolates described by
114 Wu *et al.* were obtained and included in this present study (the four *S. delphini* isolates
115 were not included) (22) to investigate if the difference between the data published by Wu
116 *et al.* and those obtained in our previous investigation were explained by differences
117 between isolates from Europe and isolates from North America. The isolates originated

118 from humans (n=45) and animals (n=66), including dogs, cats, birds and pigs. MLST data
119 were available for 76 of the 78 MRSP isolates from the SVA and NVI collections using the
120 MLST_5 scheme for 52 isolates (SVA) and the MLST_7 scheme for 24 isolates (NVI) (24,
121 25). A total of 18 different MLST types including world epidemic lineages such as ST68,
122 ST71 and ST258 were represented in the study. While no MLST data were available for the
123 isolates described by Wu and co-workers, repetitive-sequence PCR (rep-PCR)
124 demonstrated the collection was composed of six different rep-PCR clonal lineages
125 (designated A to F) (22). No correlation between rep-PCR clonal type and antimicrobial
126 susceptibility data was encountered, implying results were not due to a specific *S.*
127 *pseudintermedius* lineage. All isolates were identified in the laboratory at Växjö to the
128 species level with MALDI-TOF MS using the Microflex system with the MALDI Biotyper 3.1
129 software and MBT 6903 Library (Bruker Daltonics, Bremen, Germany) per the
130 manufacturer's instructions. *mec* status was determined by the contributing laboratories:
131 SVA (*mecA*) (26) and NVI (*mecA*) (27), or as described in Wu *et al.* (*mecA* and *mecC*) (22).
132 In case of discrepancy between the *mec* status and the phenotypic results obtained in this
133 study, the *mec* status were confirmed by a real-time PCR assay that tested for both *mecA*
134 and *mecC* (28). The study did not require patient consent or ethical approval since isolates
135 were not associated with any identifiable patient information.

136

137 *Antimicrobial susceptibility testing*

138 Disk diffusion was performed according to EUCAST recommendations (29) using 30 µg
139 cefoxitin and 1 µg oxacillin disks from Oxoid/ThermoFisher Scientific (Basingstoke, UK),
140 Mast Diagnostics (Bootle, UK) and Becton Dickinson (Heidelberg, Germany). All isolates

141 were tested in parallel from the same inoculum on in-house prepared MHA plates using
142 pre-formulated powder from ThermoFisher Scientific (Oxoid agar) and Becton Dickinson
143 (BBL agar), and commercial plates from Becton Dickinson (BBL agar). *Staphylococcus*
144 *aureus* ATCC® 29213 was used as quality control.

145

146 *Data analysis*

147 The ability of cefoxitin (30 µg) and oxacillin (1 µg) disks to predict the presence of *mecA*-
148 mediated β-lactam resistance in *S. pseudintermedius* was evaluated by 1) comparing the
149 degree of measurements placed in the interval where both *mecA*-negative and *mecA*-
150 positive isolates presented values (disregarding the measurements of the aberrant strain,
151 and 2) the number of major Errors (ME) and very major errors (VME) for the present
152 EUCAST breakpoint for cefoxitin (S, ≥35 mm and R, <35 mm) and for oxacillin using the
153 CLSI breakpoint (S, ≥18 mm and R, ≤17 mm) as well as an alternative breakpoint (S, ≥20
154 mm and R, <20 mm) based on the present study (total isolate set). ME and VME were
155 calculated based on the number of susceptible and the number of resistant tests,
156 respectively.

157 Analyses on performance were done disregarding the clearly aberrant *mecA*-negative
158 isolate (see results) for a) the total aggregated set of measurements: 2,007 data points
159 (223 isolates × 3 different disk manufacturers × 3 different MHAs), b) for isolates from
160 Europe vs isolates from North America and c) for each of the individual combinations of
161 MHAs and disk brands. Comparison of the distributions of zone diameters were
162 performed using the Mann-Whitney U test, p>0.05 were used as significance level

163

164 **Results**

165 The results for the cefoxitin 30 µg and oxacillin 1 µg disk screening tests are shown in
166 Table 1/Figure 1 and Table 2/Figure 2, respectively. One *mecA*- (and *mecC*) negative
167 isolate was clearly aberrant by oxacillin testing with an inhibition zone size between 14-16
168 mm for oxacillin and 28-29 mm for cefoxitin. This isolate was also clearly resistant in the
169 investigation by Wu *et al.* (22), the mechanism of resistance for this has not been
170 elucidated. Disregarding this isolate, the inhibition zone sizes of isolates from Europe and
171 North America spanned over similar ranges; *i.e.*, a maximum difference of 2 mm for both
172 cefoxitin and oxacillin except for *mecA*-positive isolates tested against cefoxitin where
173 isolates from Europe ranged from 6-33 mm versus 21-32 mm for isolates from North
174 America (Tables 1 and 2). Nevertheless, comparison of isolates from Europe and North
175 America for each of the four distributions; cefoxitin *mecA*-negative, cefoxitin *mecA*-
176 positive, oxacillin *mecA*-negative and oxacillin *mecA*-positive were significantly different
177 ($p < 0.0001$, $p < 0.01$, $p < 0.002$ and, $p < 0.0001$). Measurements from the individual disk and
178 MHA combinations only showed minor differences (*i.e.*, maximum difference in minimum
179 or maximum values of 1-2 mm [Tables 1 and 2]).

180 For the aggregated dataset for the 30 µg cefoxitin disks, 16% (14.8%-18.0%, 95% CI) of the
181 zone size measurements were in the region (29 -33 mm) where both *mecA*-negative and
182 *mecA*-positive isolates tested (Table 1). For the 1 µg oxacillin disks, only 2% (1.6%-2.9%,
183 95% CI) of the measurements were in the region (19-20 mm) where both *mecA*-negative
184 and *mecA*-positive isolates tested (Table 2). Furthermore, the vast majority of the *mecA*-
185 positive isolates displayed no zone of inhibition with the 1 µg oxacillin disk which provides

186 much better separation between the *mecA*-negative and the *mecA*-positive populations
187 compared to the 30 µg cefoxitin disk (Figure 1 and Figure 2).
188 For the 1 µg oxacillin disk the number of MEs and VMEs using both the CLSI breakpoint (S,
189 ≥18 mm and R, ≤17 mm) and the breakpoint suggested on the data in this publication (S,
190 ≥20 mm and R, <20 mm) are shown in Table 1 and 2 both for the total dataset as well as
191 for the individual datasets (excluding the aberrant *mecA*-negative isolate). The CLSI
192 breakpoint resulted in a total of nine *mecA*-positive isolates (six European and three North
193 American isolates, 40 data points) being reported as susceptible resulting in a VME rate of
194 4.1%, and one *mecA*-negative isolate (one North American isolate, 9 data points) would be
195 reported as resistant; *i.e.*, 0.9% ME. In contrast, changing the breakpoint to S, ≥20 mm and
196 R, <20 mm the corresponding VME and ME rates were 0.4% (one European isolate, 4 data
197 points) and 1.1% (2 North American isolates, 11 data points), respectively.

198

199 Discussion

200 Detection of *mecA*-based methicillin resistance using cefoxitin or oxacillin disks is in fact a
201 dichotomous screening test where the ideal substance has a cutoff that clearly
202 distinguishes between *mecA*-positive and *mecA*-negative isolates with no or very little
203 overlap. In this study, where *S. pseudintermedius* isolates from Europe and North America
204 were tested by using disks from three different manufacturers and MHA from two
205 different manufacturers, oxacillin was markedly better than cefoxitin in separating *mecA*-
206 negative from-positive isolates. By the 1 µg oxacillin disk, only 2% of the total number of
207 data points were in the interval where zone sizes for *mecA*-negative and *mecA*-positive
208 isolates overlapped (it was not possible to classify an isolate as either susceptible or

209 resistant) in comparison to 16% of the data points for the 30 µg cefoxitin disk diffusion.

210 Thus, our previous finding that cefoxitin disk diffusion can reliably differentiate between

211 *mecA*-negative and *mecA*-positive isolates of *S. pseudintermedius* has been modified

212 based upon our current data where a greater variety of strains, disks and media were

213 assayed. Furthermore, the oxacillin disk had the advantage that the majority of *mecA*-

214 positive isolates did not exhibit any zone of growth inhibition (they grew up to the edge of

215 the disk), permitting good separation of MSSP and MRSP.

216 Our data confirm the recommendation made by Wu *et al.* in favour of using oxacillin disk

217 diffusion for detection of methicillin resistance in *S. pseudintermedius* (22). However,

218 using the breakpoint suggested by Wu *et al.* (the breakpoint adopted by CLSI) nine (8%) of

219 the *mecA*-positive isolates, would be classified as false susceptible in comparison to one

220 isolate (0.9%) using a breakpoint of S, ≥20 mm and R, <20 mm. In a previous study, Bemis

221 *et al.* also found two PBP2a-positive isolates that displayed zone sizes greater than 17 mm

222 (18 mm and 23 mm) (19), (Bemis personal communication).

223 Interestingly six of the nine isolates were of European origin and none of the three North

224 American isolates were false susceptible in all tested variants, providing a possible

225 explanation for the difference found in this evaluation compared to the evaluation by Wu

226 *et al.* (22). Accordingly, for both cefoxitin and oxacillin the zone size distribution of isolates

227 from Europe were significantly different from the North American isolates possibly

228 reflecting differences in clonal distribution between Europe and North America.

229 The findings in this study stresses the need for testing isolates from different clonotypes

230 and to use disks and media from more manufacturers when setting breakpoints. Thus, for

231 the 1 µg oxacillin disks, we propose that 20 mm is a more appropriate breakpoint to

232 distinguish between *mecA*-negative (zone diameter ≥ 20 mm) and *mecA*-positive (zone
233 diameter < 20 mm) isolates. This new breakpoint should reduce the frequency of VME
234 (resistant isolates that test as susceptible) compared to the current CLSI breakpoint. The
235 breakpoints generated by this study are now accepted by the EUCAST (EUCAST breakpoint
236 table v 7.1, 2017 (30)).

237 The inclusion of media and disks from different manufacturers which is an integrated part
238 of EUCAST method development is a strength and demonstrates study originality since it
239 incorporates the unavoidable variation in materials between manufacturers. An important
240 limitation of the study is that the strain collection does not include isolates from Africa,
241 Asia, or Australia which potentially could affect the proposed breakpoints. We did not test
242 all isolates for *mecC*, however, isolates resistant for cefoxitin or oxacillin by disk diffusion,
243 but negative for *mecA* were tested for *mecC*. Nevertheless, we cannot exclude that among
244 the phenotypically susceptible isolates there were *mecC*-positive isolates, why our findings
245 only apply for *mecA*-mediated β -lactam resistance (as reflected in the title).

246

247 In conclusion, the present investigation confirms the findings from previous studies that
248 oxacillin is better than cefoxitin for detection of *mecA*-mediated β -lactam resistance in *S.*
249 *pseudintermedius*. As a result of this study, oxacillin is now recommended by CLSI and
250 EUCAST for detecting *mecA*-mediated β -lactam resistance in *S. pseudintermedius*. This
251 outcome contributes to optimize MRSP detection in both veterinary and human diagnostic
252 laboratories and has therefore important implications for antimicrobial treatment in both
253 populations.

254

255 **Acknowledgement:** we thank the “MRSP enthusiasts” consortium from a previous
256 publication (Perreten *et al.*, J Antimicrob Chemother. 2010, 65: 1145-54.) for contributing
257 to the strain collection at SVA.

258

259 **Funding:** this study was performed without external funding.

260

261 **Conflicts of Interest:** Drs. Skov, Varga, Matuschek, Åhman, Bemis, Bengtsson, Sunde,
262 Westblade, Guardabassi and Kahlmeter report nothing to disclose. Dr. Humphries reports
263 employment by Accelerate Diagnostics, Inc. and stocks with Accelerate Diagnostics.

264

265 Table 1. Cefoxitin 30 µg disk inhibition zone sizes (mm), Major errors (ME) and Very Major errors (VME) using a breakpoint for S, ≥34 mm and R, <35
 266 mm for *S. pseudintermedius* isolates (n=223*) obtained from Europe and North America for the total number measurements and for individual
 267 subgroups.

MHA Manufacturer	Disk Manufacturer	Number of measurements	Zone diameter, mm		Interval (mm) with measurements from both <i>mecA</i> negative and <i>mecA</i> positive isolates (% of total values)	Number (%) of ME (Breakpoint R<35 mm)	Number (%) VME (Breakpoint S≥34 mm)	
			<i>mecA</i> - positive	<i>mecA</i> - negative*				
All	All	2007 (223x3x3)	6-33	29-41	29-33 (16.3)**	376 (36.3%)	0 (0%) ²⁷¹	*
Europe	All	972 (108 x3x3)	6-33	31-40	31-33 (6.9%)	68 (18.4%)	0 (0%) ²⁷²	
North America	All	1035 (115 x3x3)	14-32	29-41	29-32 (12.2%)	308 (46.2%)	0 (0%) ²⁷³	D
BBL commercial	BD	223 (223x1x1)	10-31	29-40	29-31 (8.0%)	64 (55.7%)	0 (0%) ²⁷⁴	
	Mast	223 (223x1x1)	12-32	29-40	29-32 (9.8%)	59 (51.3%)	0 (0%) ²⁷⁵	at
BBL prepared in-house	Oxoid	223 (223x1x1)	9-32	30-40	30-32 (6.7%)	45 (39.1%)	0 (0%) ²⁷⁶	
	BD	223 (223x1x1)	9-32	31-40	31-32 (6.3%)	34 (29.6%)	0 (0%) ²⁷⁷	a
Oxoid prepared in-house	Mast	223 (223x1x1)	10-32	31-41	31-32 (5.8%)	26 (22.6%)	0 (0%) ²⁷⁸	
	Oxoid	223 (223x1x1)	6-33	31-41	31-33 (7.1%)	24 (20.9%)	0 (0%) ²⁷⁹	fo
Oxoid prepared in-house	BD	223 (223x1x1)	6-32	30-40	31-32 (6.3%)	51 (44.3%)	0 (0%) ²⁸⁰	
	Mast	223 (223x1x1)	6-33	30-40	30-33 (14.7%)	41 (35.7%)	0 (0%) ²⁸¹	r
	Oxoid	223 (223x1x1)	6-33	31-41	31-33 (9.4%)	32 (27.8%)	0 (0%) ²⁸²	

277 the aberrant *mecA/C*-negative strain with cefoxitin readings of 28-29 mm and oxacillin readings of 14-16 mm are omitted.

278 ** Percentage of measurements that overlap between the zone sizes for *mecA*-negative and *mecA*-positive isolates. The interval is greater for all

279 media/disks combined than for each individual media as the overlapping zones differ for the individual media/disks

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281 Table 2. Oxacillin 1 µg disk inhibition zone sizes (mm), Major errors (ME) and Very Major errors (VME) using breakpoint for S, ≥20 mm and R, <20 mm
 282 and S, ≥18 mm and R, ≤17 mm for *S. pseudintermedius* isolates (n=223*) obtained from Europe and North America for the total number measurements
 283 and for individual subgroups.

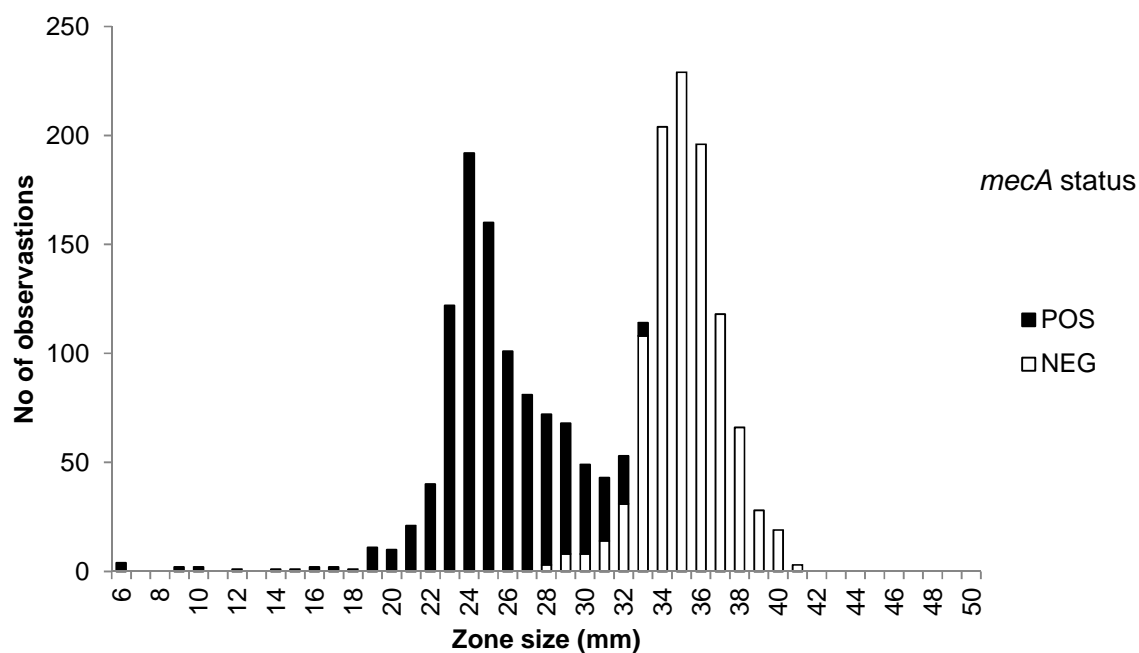
MH Agar Manufacturer	Disk Manufacturer	Number of measurements	Zone diameter, mm		Interval (mm) with measurements from both <i>mecA</i> negative and <i>mecA</i> positive isolates (Pct of total values)	Number (%) of ME (Breakpoint R≤17 mm)	Number (%) VME (Breakpoint S≥18 mm)	Number (%) of ME (Breakpoint R<20mm)	Number (%) VME (Breakpoint S≥20 mm)
			<i>mecA</i> - positive	<i>mecA</i> - negative*					
All	All	2007 (223x3x3)	6-20	19-30	19-20 (2.2%)**	9 (0.9%)	40 (4.1%)	11 (1.1%)	4 (0.4%)
Europe	All	972 (108 x3x3)	6-20	20-29	20 (0.9%)	0 (0.0%)	32 (4.9%)	0 (0.0%)	4 (0.6%)
North America	All	1035 (115 x3x3)	6-19	19-30	19 (0.2%)	9 (1.4%)	8 (2.4%)	11 (1.7%)	0 (0.0%)
BBL commercial	BD	223 (223x1x1)	6-19	19-28	19 (1.3%)	1 (0.9%)	4 (3.7%)	2 (1.8%)	0 (0.0%)
	Mast	223 (223x1x1)	6-18	19-28	-	1 (0.9%)	3 (2.8%)	2 (1.8%)	0 (0.0%)
BBL prepared in-house	Oxoid	223 (223x1x1)	6-20	20-29	20 (1.3%)	1 (0.9%)	4 (3.7%)	1 (0.9%)	1 (0.9%)
	BD	223 (223x1x1)	6-19	20-28	-	1 (0.9%)	5 (4.6%)	1 (0.9%)	0 (0.0%)
Oxoid prepared in-house	Mast	223 (223x1x1)	6-19	20-29	-	1 (0.9%)	5 (4.6%)	1 (0.9%)	0 (0.0%)
	Oxoid	223 (223x1x1)	6-19	20-28	-	1 (0.9%)	4 (3.7%)	1 (0.9%)	0 (0.0%)
Oxoid prepared in-house	BD	223 (223x1x1)	6-20	20-29	20 (0.9%)	1 (0.9%)	4 (3.7%)	1 (0.9%)	1 (0.9%)
	Mast	223 (223x1x1)	6-20	20-29	20 (1.8%)	1 (0.9%)	6 (5.5%)	1 (0.9%)	1 (0.9%)
	Oxoid	223 (223x1x1)	6-20	21-30	-	1 (0.9%)	5 (4.6%)	1 (0.9%)	1 (0.9%)

284 *Data for the aberrant *mecA/C*-negative strain with cefoxitin readings of 28-29 mm and oxacillin readings of 14-16 mm are omitted

285 ** Percentage of measurements that overlap between the zone sizes for *mecA*-negative and *mecA*-positive isolates. The interval is greater for all

286 media/disks combined than for each individual media as the overlapping zones differ for the individual media/disks

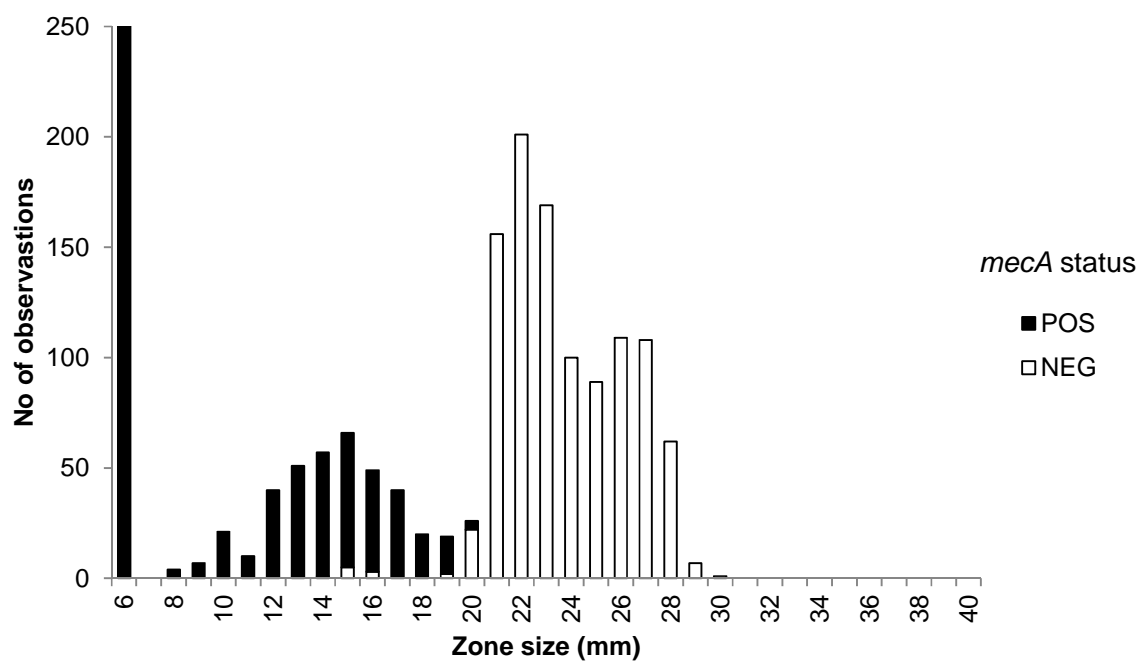
287 **Figure 1.** Cefoxitin 30 µg disk inhibition zone sizes versus *mecA* status for the 224 *S.*
288 *pseudintermedius* isolates from Europe and North America (2,016 data points, each isolate
289 tested using disk and media from three manufacturers [$3 \times 3 \times 224 = 2,016$]).



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291

292 **Figure 2.** Oxacillin 1 µg disk inhibition zone sizes versus *mecA* status for 224 *S.*
293 *pseudintermedius* isolates obtained from Europe and North America (2,016 data points,
294 each isolate tested using disk and media from three manufacturers [$3 \times 3 \times 224 = 2,016$]).



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Figure 1

