Detection of *mecA*-mediated Methicillin Resistance in *Staphylococcus pseudintermedius*

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Clinical Problem

- *Staphylococcus pseudintermedius* colonizes oropharynx and anal mucosa of cats/dogs
- Human infections reported
- Often mis-identified in clinical laboratories as *Staphylococcus aureus* (coagulase positive)
- As laboratories implement MALDI-TOF for identification of bacteria, increase in *S. pseudintermedius* isolations

VET01-S2 (2013)

Antimicrobial Agent	Disk Content	Zone Diameter (mm) S	Zone Diameter (mm) I	Zone Diameter (mm) R	MIC Breakpoint (µg/mL) S	MIC Breakpoint (µg/mL) I	MIC Breakpoint (µg/mL) R	Comments
β-Lactams/Penicillins								
Ampicillin								
Enterobacteriaceae	10 µg	≥17	14–16	≤13	≤8	16	≥32	(6) Ampicillin is used to test for susceptibility to amoxicillin and hetacillin.
Staphylococcus spp.	10 µg	≥29	–	≤28	≤0.25	–	≥0.5	
Streptococci								
β-hemolytic group	10 µg	≥24	–	–	≤0.25	–	–	
viridans group		–	–	–	≤0.25	0.5–4	≥8	
Listeria spp.	–	–	–	–	≤2	–	–	(7) For some organism/antimicrobial combinations, the absence of resistant strains precludes defining any results categories other than "susceptible." For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed.
Enterococci	10 µg	≥17	–	≤16	≤8	–	≥16	(8) For enterococci: Strains producing β-lactamase are best detected by a chromogenic cephalosporin β-lactamase test.
Oxacillin								(9) Oxacillin is used to test for susceptibility to methicillin, nafcillin, and cloxacillin. *S. aureus* interpretive criteria should be used for strains of *S. aureus* and not for other coagulase-positive staphylococci isolated from veterinary sources such as *S. pseudintermedius*.
Staphylococcus aureus and *S. lugdunensis*		–	–	–	≤2 (oxacillin)	–	≥4 (oxacillin)	(10) Oxacillin disk testing is not reliable for *S. aureus* and *S. lugdunensis*. For disk testing, see cefoxitin and comment (11) for reporting oxacillin when using cefoxitin as a surrogate test.
	30 µg cefoxitin	≥ 22	–	≤21	≤ 4 (cefoxitin)	–	≥ 8 (cefoxitin)	(11) Cefoxitin is used as a surrogate for oxacillin; report oxacillin susceptible or resistant based on the cefoxitin result.
CoNS except *S. lugdunensis*	30 µg cefoxitin	≥ 25	–	≤24	–	–	–	(12) Oxacillin interpretive criteria may overcall resistance for some CoNS from bovine mastitis because some strains for which the oxacillin MICs are 0.5 to 1 µg/mL lack *mecA*. Testing for *mecA* or for PBP 2a is recommended for strains for which the oxacillin MICs are 0.5 to 1 µg/mL before reporting complete β-lactam resistance.[4]
					≤0.25 (oxacillin)	–	≥0.5 (oxacillin)	(13) MIC distributions of canine isolates support these breakpoints for use in canine skin and soft tissue infection; however, efficacy data and PK-PD targets were unavailable.
Staphylococcus pseudintermedius	1 µg oxacillin	≥18	–	≤17	≤0.25	–	≥0.5	(14) Cefoxitin breakpoints are not predictive of *mecA*-mediated resistance in *S. pseudintermedius* isolated from animals.

VET01-S2 (2013)

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Streptococci								
β								
vi								
L								or some organism/antimicrobial combinations, the absence of resistant strains udes defining any results categories other than "susceptible." For strains ing results suggestive of a "nonsusceptible" category, organism identification ntimicrobial susceptibility test results should be confirmed.
E								or enterococci: Strains producing β-lactamase are best detected by a chromogenic alosporin β-lactamase test.
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Staph *lugdunensis*		–	–	–	≤2 (oxacillin)	–	≥4 (oxacillin)	(10) Oxacillin disk testing is not reliable for *S. aureus* and *S. lugdunensis*. For disk testing, see cefoxitin and comment (11) for reporting oxacillin when using itin as a surrogate test.
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(9) "Oxacillin is used to test for susceptibility to methicillin, nafcillin and cloxacillin. S. aureus interpretive criteria should be used for strains of S. aureus and not for other coagulase-negative staphylococci isolated from veterinary sources such as S. pseudintermedius"

(14)"Cefoxitin breakpoints are not predictive of mecA-mediated resistance in S. pseudintermedius isolated from animals"

J Vet Diagn Invest 21:53–58 (2009)

Bemis et al. J Vet Diagn Invest. 21:53-58 (2009)

Evaluation of susceptibility test breakpoints used to predict *mecA*-mediated resistance in *Staphylococcus pseudintermedius* isolated from dogs

David A. Bemis,[1] Rebekah D. Jones, Linda A. Frank, Stephen A. Kania

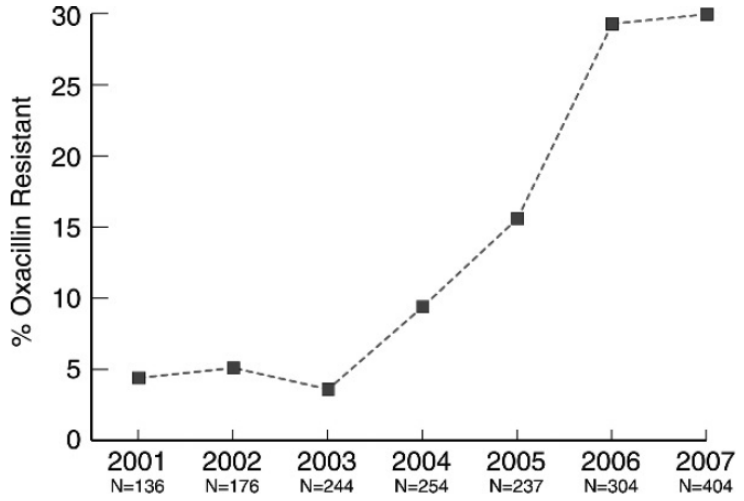


Figure 2. Frequencies of oxacillin resistance among *Staphylococcus pseudintermedius* isolates from dogs from 2001 to 2007. Disk diffusion method; resistant: ≤17 mm, susceptible: ≥18 mm.

Application of CLSI M100 Oxacillin and Cefoxitin Breakpoints to *S. pseudintermedius*

Table 1. Disk diffusion breakpoints (current and former) for oxacillin and cefoxitin with summary of intermethod error rates.*

Antimicrobial agent (no. of tests/no. of resistant strains†)	Current CLSI (2008) breakpoints					Former CLSI (2004–2008) breakpoints				
	Criterion (mm)		Error rate (%)			Criterion (mm)		Error rate (%)		
	Susceptible	Resistant	Vm	Ma	Mi	Susceptible	Resistant	Vm	Ma	Mi
Oxacillin (436/230)	≥13	≤10	14.9	0	5.9	≥18	≤17	0.9	0.6	NA
Cefoxitin (292/88)	≥22	≤21	29.1	0	NA	≥25	≤24	14.7	0	NA

* Cefoxitin breakpoints were those recommended by the Clinical and Laboratory Standards Institute (CLSI) for use with *Staphylococcus aureus* (current) and coagulase-negative staphylococci (former). There are no CLSI-recommended cefoxitin breakpoints specified for use with *S. pseudintermedius*. Vm = very major (false-susceptible) error; Ma = major (false-resistant) error; Mi = minor errors (discords involving an intermediate interpretation); NA = not applicable (no intermediate interpretation).

† Resistance detected by *mecA* polymerase chain reaction.

2008, test and interpret like *S. aureus*

2004-2008, test and interpret like CoNS

Note: M100-S25 cefoxitin zones same as above; oxacillin disk test no longer recommended

Conclusions:

- Cefoxitin disk test underestimates *mecA*-mediated resistance in *S. pseudintermedius*

Bemis et al. J Vet Diagn Invest. 21:53-58 (2009)

J Vet Diagn Invest 21:684–688 (2009)

Evaluation of Clinical Laboratory Standards Institute interpretive criteria for methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs

Jennifer R. Schissler,[1] Andrew Hillier, Joshua B. Daniels, Lynette K. Cole, Wondwossen A. Gebreyes

30 *mecA* positive canine isolates from 2007-2008

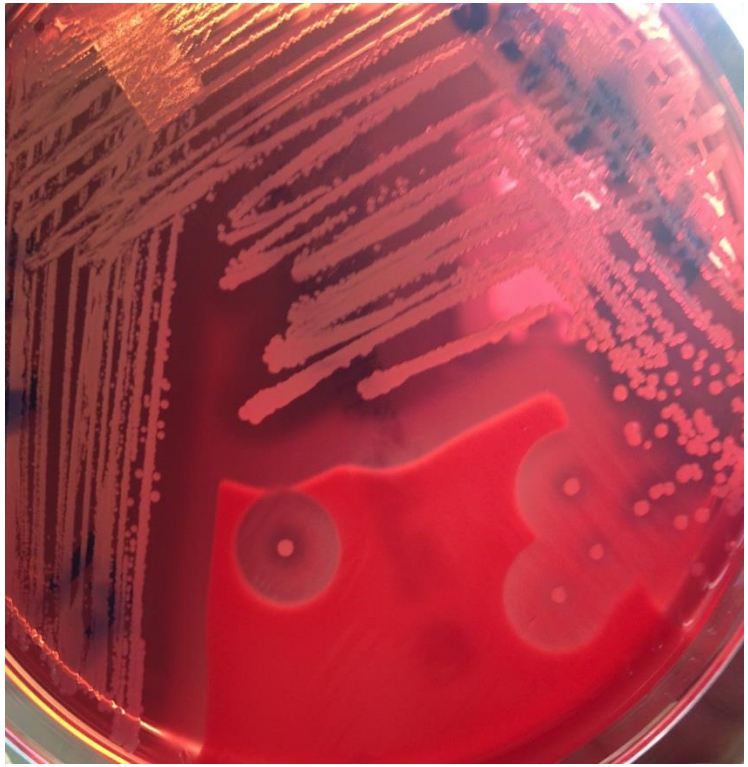
The Ohio State University Veterinary Teaching Hospital

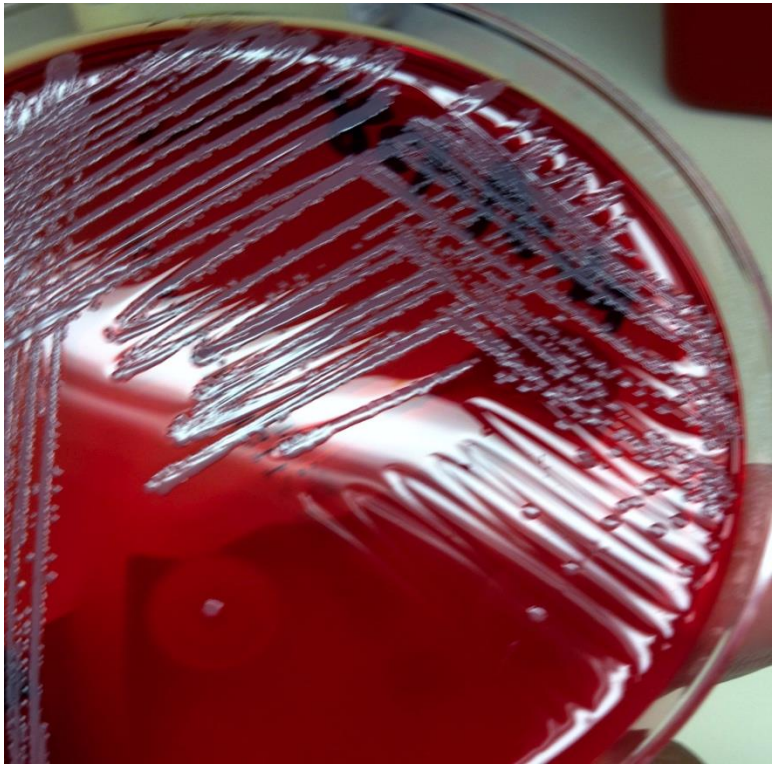
CLSI M100 Breakpoint Criteria Applied		No. (%) Resistant
Organism	R Breakpoint	
S. aureus / lugdunensis	oxacillin ≥4 µg/mL	22 (73.3%)
CoNS	oxacillin ≥0.5 µg/mL	29 (97%)
S. aureus / lugdunensis	cefoxitin ≤21 mm	2 (6.7%)
CoNS	cefoxitin ≤24 mm	13 (43.3%)

Example Case (UCLA)

- A 3 year old male with a history of renal and liver transplant and newly diagnosed hepatoblastoma s/p 6 cycles of chemotherapy
- Admitted for gastrostomy tube site cellulitis
- Blood cultures were drawn and patient started on IV clindamycin.
- Blood cultures grow Gram positive cocci in clusters
  - **Tube coagulase from blood broth is positive → reported as *S. aureus***
  - Subculture yields:
    - Off-white colonies with double-zone beta hemolysis
    - **Slide coagulase negative, tube coagulase positive (rabbit plasma)**
    - MALDI TOF (Vitek MS) → *S. pseudintermedius* (99%)
    - Susceptible to: oxacillin, clindamycin, erythromycin, ciprofloxacin daptomycin, linezolid, vancomycin
    - Resistant to: penicillin, trimethoprim/sulfamethoxazole
- History of frequent encounters with dogs, although none in family home
- Patient does well on clindamycin, discharged home

Typical colony morphology on BAP following 24 hr ambient air incubation @35°C





Staphylococcus pseudintermedius

S. pseudintermedius (UCLA 2015)

Patient		Collection Date	Specimen	MIC (µg/ml)*	
No.	Age/Sex			Oxacillin	Cefoxitin
1	41 YO M	1-8	Skin, right temple	≤0.25	≤4
2	3 YO M	2-12	Blood	≤0.25	≤4
3	52 YO M	2-17, 3-5	Sphenoid sinus	1	≤4
4	43 YO M	3-5, 3-24	Sphenoid sinus	1	≤4
5	23 YO F	3-4	Abscess, left foot	≤0.25	≤4
6	86 YO M	3-10	Right maxillary sinus	>16	≤4
7	55 YO F	3-13	Nasal vestibule cellulitis	≤0.25	≤4

*CLSI reference broth microdilution method, in-house prepared panels

Current Breakpoints

Breakpoints	Oxacillin				Cefoxitin			
	Disk (mm)*		MIC (µg/ml)		Disk (mm)		MIC (µg/ml)	
	S	R	S	R	S	R	S	R
CLSI M100-S25 *S. aureus / S. lugdunensis*	-	-	≤2	≥4	≥22	≤21	≤4	≥8
CLSI M100-S25 Coagulase-negative *Staphylococcus*	-	-	≤0.25	≥0.5	≥25	≤24	-	-
CLSI VET01-S2 *S. pseudintermedius*	≥18	≤17	≤0.25	≥0.5	-	-	-	-
EUCAST *S. pseudintermedius*	-	-	-	-	≥35	<35	-	-

*Oxacillin disk breakpoints (S, I, R):
M100-S22 - ≥13, 11-12, ≤10 (S. aureus / S. lugdunensis); eliminated 2013
M100-S18 - ≥18, ≤17 (CoNS); eliminated 2009

Study

- **Purpose:**

  Evaluate oxacillin and cefoxitin tests (disk and MIC) for detection of methicillin resistance due to *mecA* in *S. pseudintermedius* human and veterinary isolates
- **Participating institutions:**
  - UCLA
  - Emory University School of Medicine /Children's Healthcare of Atlanta (CHOA)
  - Texas A & M
  - Washington University (WUST)
  - Children's Hospital of Los Angeles (CHLA)
  - Also received isolates from RM Alden Research Laboratory (RMARL)

Isolates Included in Study (n=114)

Institute	Quantity	Source	Specimen source	mecA+	SCCmec	Rep-PCR*
UCLA	11	Human	Blood, ethmoid sinus drainage, wound, ear, skin, abscess	3	IV, V	A, D, F
RMARL	8	Human	Blood, dog bites, cat bites, wounds	0	n/a	F only
WUST	3	Human	Wounds	0	n/a	F only
CHOA	23	Human	Blood, wound, endotracheal aspirate, skin	1	V	A, D, E, F
Texas A&M	69	Canine, feline, equine, and porcine	Pyoderma	33	III, IV, V	A, B, C, D, E, F

N=45 Human Isolates; N=69 Veterinary Isolates

- 37 with *mecA*
- 77 negative for *mecA* and *mecC*

Study Design

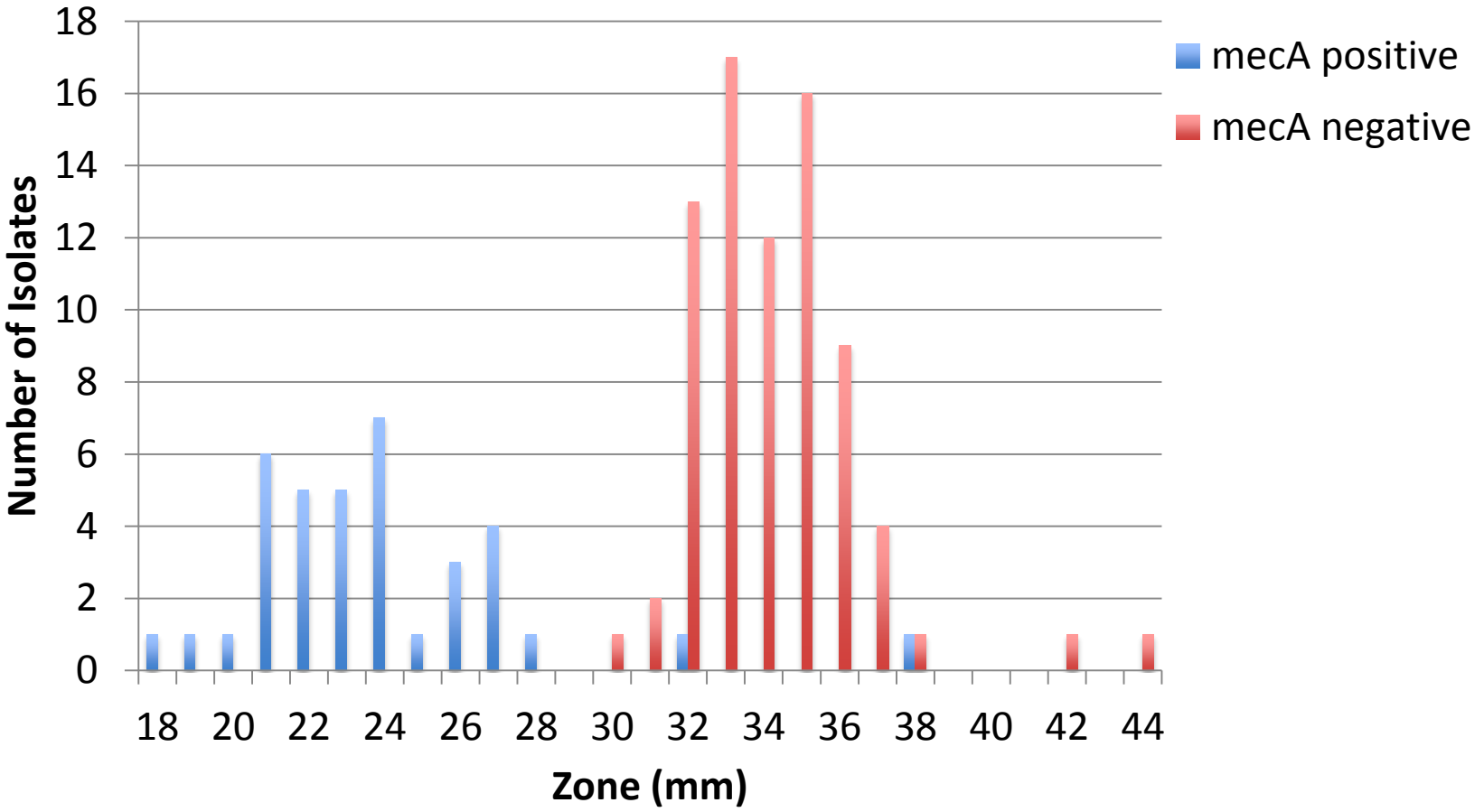
Tests Performed	Lab	Notes
mecA and *mecC* PCR *sccmec* typing, rep-PCR	WASH U	
Disk Diffusion*	UCLA	BD MHA
BMD*	UCLA	Panels made at UCLA (Difco MHB) OX –0.25-16 µg/ml (CAMHB + 2% NaCl) CX – 4 µg/ml
MicroScan	CHOA	
BD Phoenix	CHLA	
Vitek2	UCLA	
Alere PBP2a	UCLA	Induced with CX and uninduced

*CLSI reference method

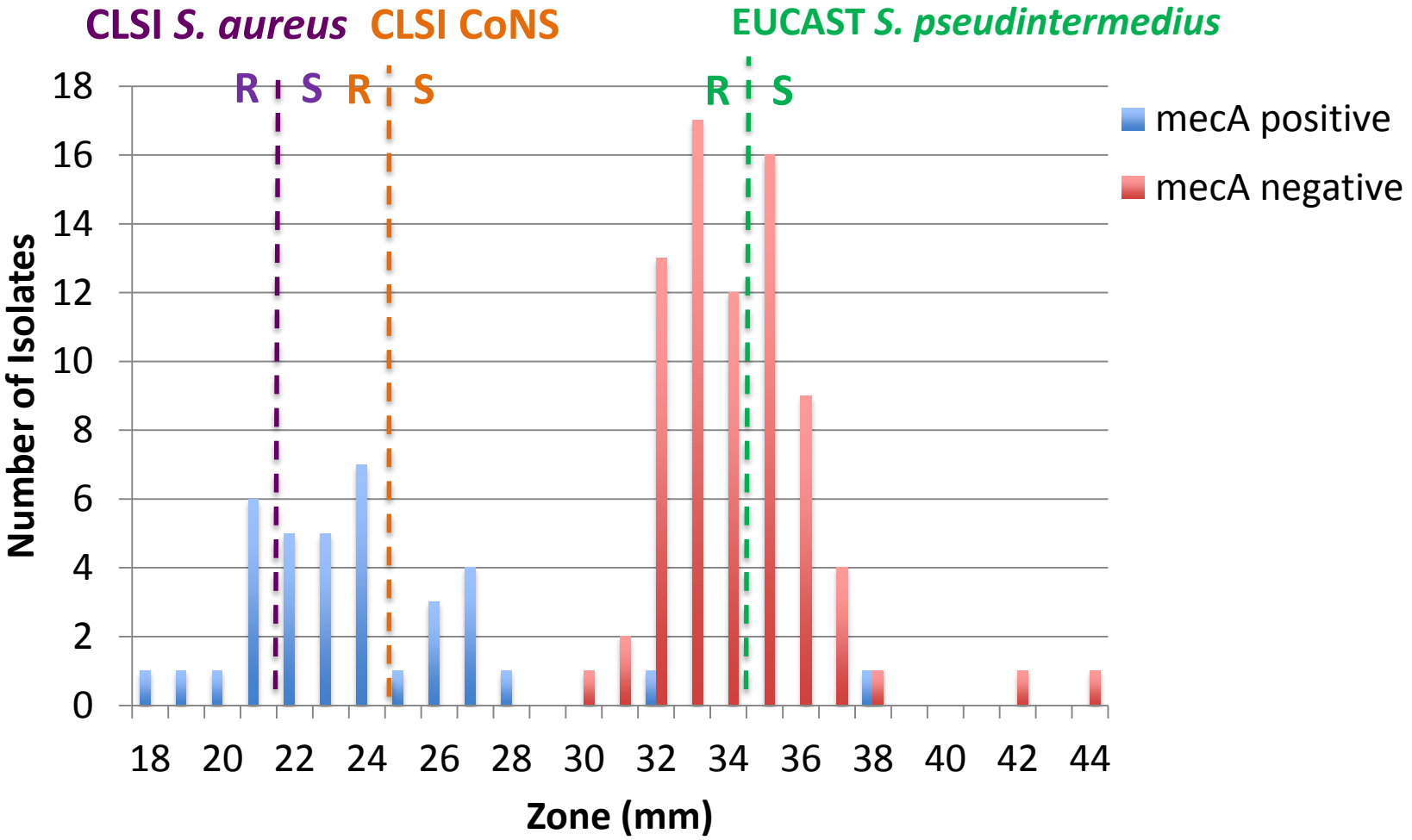
Data Analysis

- Oxacillin and/or cefoxitin zone diameters and MICs interpreted using breakpoints listed in:
  - M100-S25 *S. aureus* / *S. lugdunensis*
  - M100-S25 Coagulase-negative *Staphylococcus*
  - VET01-S2 *S. pseudintermedius*
  - EUCAST *S. pseudintermedius*
- Results compared to *mecA* PCR to define “R” vs. “S” (categorical agreement)
- All errors (VME and ME) retested for *mecA* and retested by disk diffusion and MIC

Cefoxitin Zone Diameter Distribution (n=114)



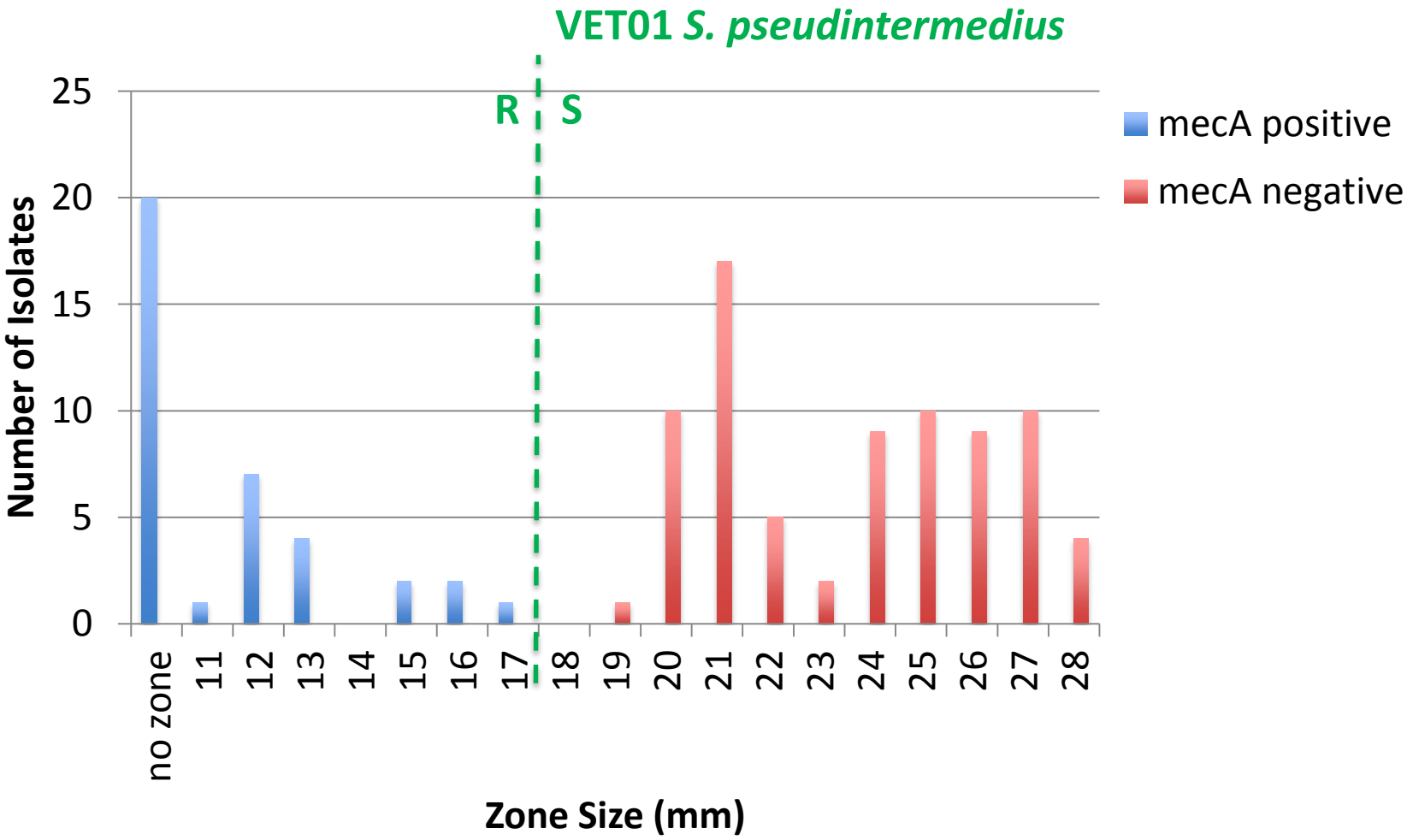
Cefoxitin Zone Diameter Distribution (n=114)



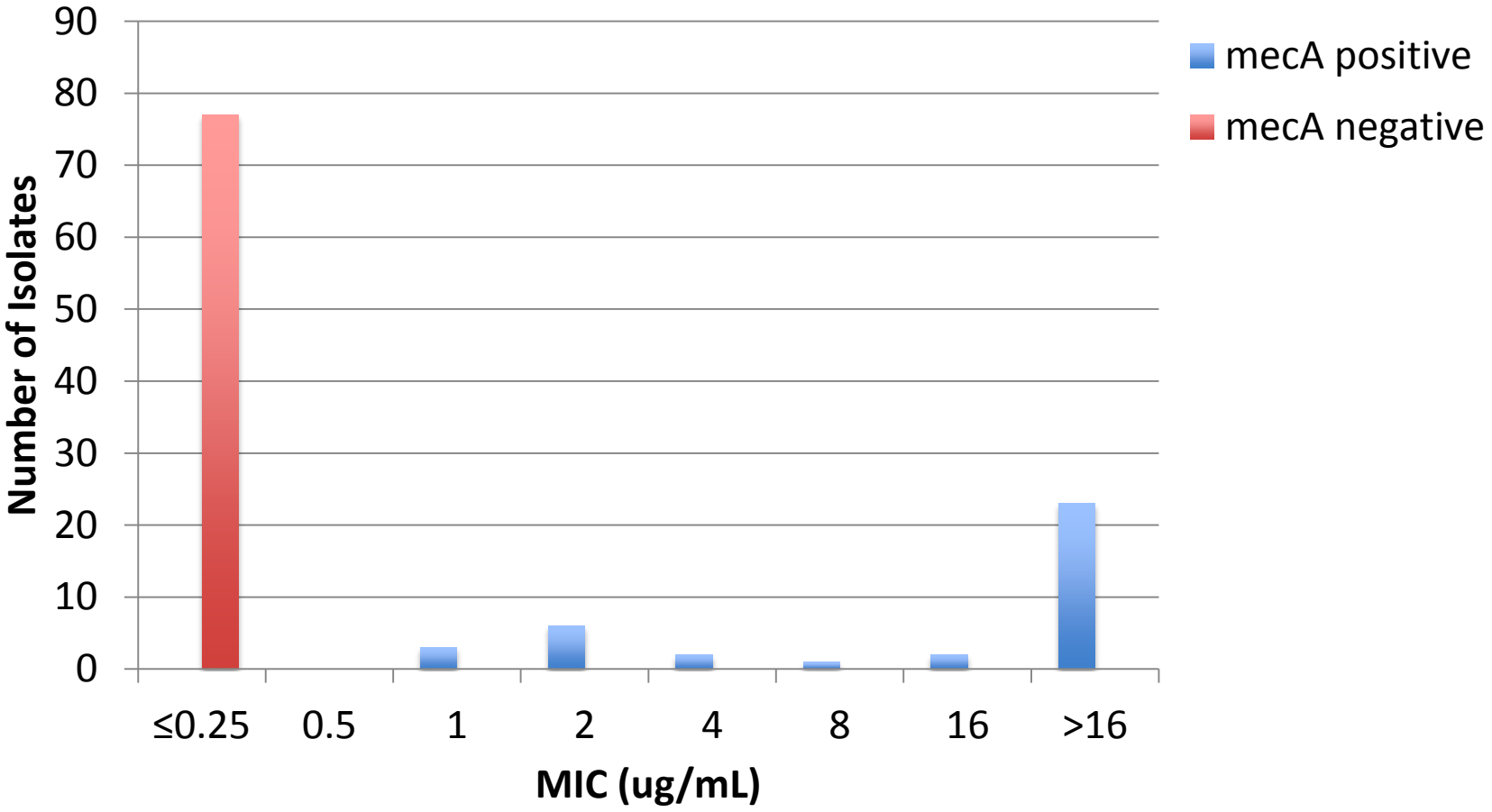
Performance of Cefoxitin tests (n=114)

Breakpoint	CA	VME (%)	ME (%)
CLSI M100-S25 Disk *S. aureus/S. lugdunensis*	76%	28/37 (76%)	0/77 (0%)
CLSI M100-S25 Disk Coagulase-negative *Staphylococcus*	90%	11/37 (30%)	0/77 (0%)
EUCAST Disk *S. pseudintermedius*	58%	1/37 (3%)	48/77 (62%)
CLSI M100-S25 MIC *S. aureus/S. lugdunensis* MIC	71%	33/37 (89%)	0/77 (0%)

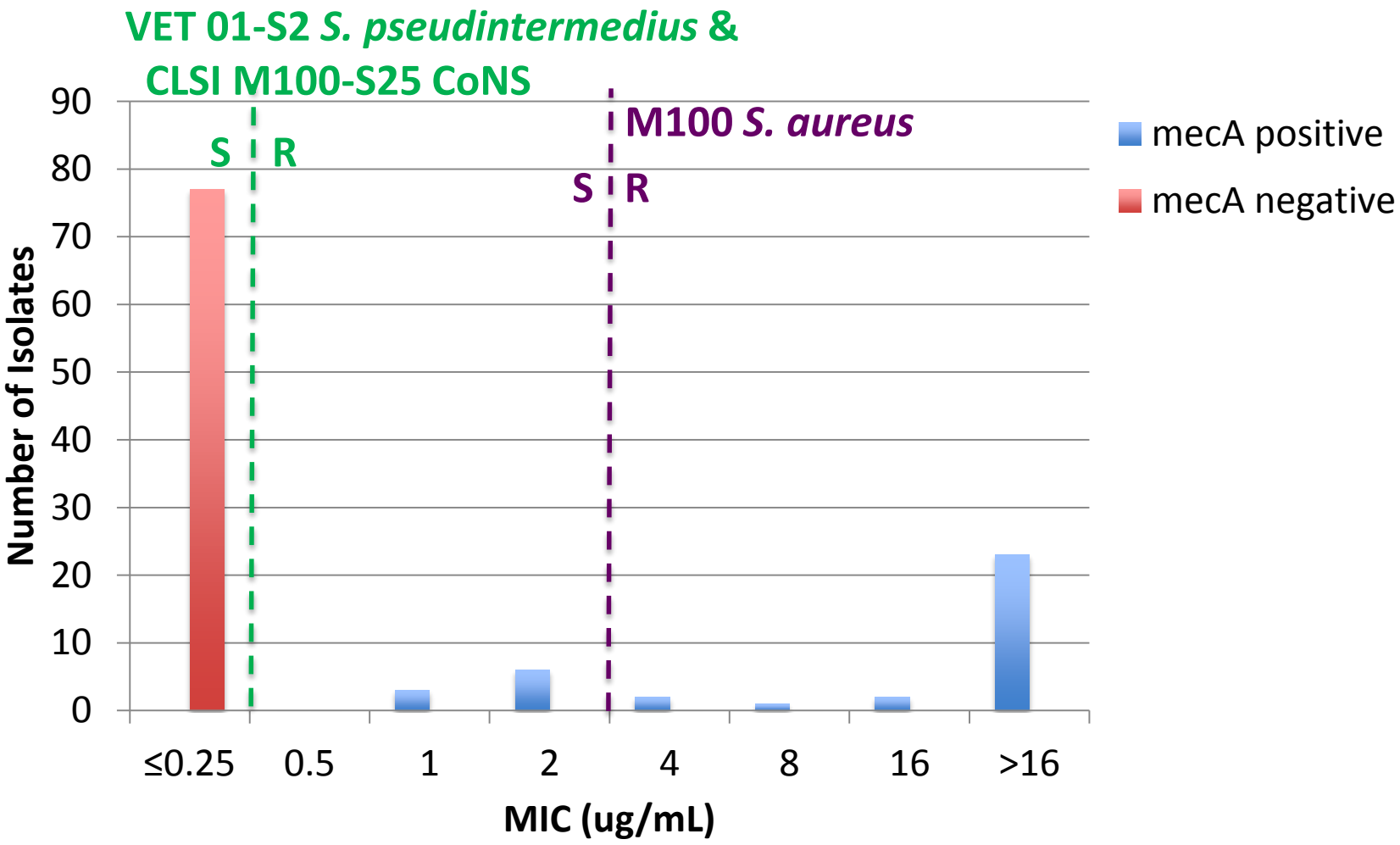
Oxacillin Zone Diameter Distribution (n=114)



Oxacillin MIC Distribution (n=114)



Oxacillin MIC Distribution (n=114)



Performance of Oxacillin MIC tests (n=114)

Same BPs

Breakpoint	CA	VME (%)	ME (%)
CLSI M100-S25 MIC *S. aureus / S. lugdunensis*	93%	8/37 (22%)	0/77 (0%)
CLSI M100-S25 MIC Coagulase-negative *Staphylococcus*	99%	0/37 (0%)	0/77 (0%)
CLSI VET 01-S2 MIC *S. pseudintermedius*	99%	0/37 (0%)	0/77 (0%)
VET01-S2 Disk	99%	0/37 (0%)	0/77 (0%)

Performance of Composite Cefoxitin and Oxacillin MIC (n=114)

M100-S25 *S. aureus* / *S. lugdunensis* breakpoints

mecA	No.	Number (%)		
		Oxacillin-R MIC ≥4 µg/ml	Cefoxitin-R MIC ≥8 µg/ml	Oxacillin–R and /or Cefoxitin-R
positive	37	29 (78%)	9 (24%)	30 (82%)
negative	77	0 (0%)	0 (0%)	0 (0%)

Conclusions

- Oxacillin interpreted by M100-S25 CoNS / VET01-S2 *S. pseudintermedius* MIC breakpoint reliably categorizes *mecA* positive and *mecA* negative isolates. Categoric agreement (n=114) in this study was 100%

- Neither cefoxitin single well MIC (4 µg/mL) nor cefoxitin disk diffusion tests using either *S. aureus / S. lugdunensis* or CoNS breakpoints reliably separate *mecA* positive from *mecA* negative isolates of *S. pseudintermedius.*

- Recommendation: Modify M100 Table 2C to indicate *S. pseudintermedius* should be tested by oxacillin MIC and interpreted by CoNS breakpoints.

Options – M100 Table 2C

Option 1:
Add oxacillin MIC breakpoints for *S. pseudintermedius*

Option 2:
Add oxacillin MIC breakpoints and oxacillin disk diffusion breakpoints for *S. pseudintermedius*; reinstate oxacillin disk diffusion test for *S. pseudintermedius* (use breakpoints listed in current Vet01-S2 which are the same as previous M100 CoNS breakpoints)

For either option, add comment “Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius.*”

Options – M100 Table 2C

Option 1 (without oxacillin disk diffusion BPs)
Option 2 (with oxacillin disk diffusion BPs).

Table 2C. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	I	R	MIC Interpretive Criteria (µg/mL) S	I	R	Comments
PENICILLINASE-STABLE PENICILLINS (Continued)									
A	Oxacillin For *S. aureus* and *S. lugdunensis*.		–	–	–	≤ 2 (oxacillin)	–	≥ 4 (oxacillin)	For use with *S. aureus* and *S. lugdunensis*. (11) Oxacillin disk testing is not reliable. See cefoxitin and comment (4) for reporting oxacillin when testing cefoxitin as a surrogate agent.
		30 µg cefoxitin (surrogate test for oxacillin)	≥ 22	–	≤21	≤ 4 (cefoxitin)	–	≥ 8 (cefoxitin)	(12) Cefoxitin is tested as a surrogate for oxacillin; report oxacillin susceptible or resistant based on the cefoxitin result. See comments (4), (7), and (10).
A	Oxacillin For CoNS except *S. lugdunensis*	–	–	–	–	≤0.25 (oxacillin)	–	≥0.5 (oxacillin)	For use with CoNS except *S. lugdunensis*. (13) Oxacillin MIC interpretive criteria may overcall resistance for some CoNS, because some non–*S. epidermidis* strains for which the oxacillin MICs are 0.5–2 µg/mL lack *mecA*. For serious infections with CoNS other than *S. epidermidis*, testing for *mecA* or for PBP 2a or with cefoxitin disk diffusion may be appropriate for strains for which the oxacillin MICs are 0.5–2 µg/mL.
		30 µg cefoxitin (surrogate test for oxacillin)	≥25	–	≤24	–	–	–	See comments (4), (7), (10), and (12).
A	Oxacillin For *S. pseudintermedius*	1 µg oxacillin	≥ 18	–	≤ 17	≤ 0.25	–	≥ 0.5	(14) Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA* mediated resistance in *S. pseudintermedius*.

FOR INFORMATION ONLY

Performance of Alere PBP2a (n=114)

mecA	No.	Number Positive (%)	
		Not induced	Induced w/ Cefoxitin disk
positive	37	33 (89%)	37 (100%)
negative	77	0 (0%)	0 (0%)

PBP2a testing was performed according to the manufacturer's instructions using colonies from a BAP incubated 24 hours, or from the edge of the cefoxitin disk diffusion zone on MHA

Performance of Automated AST Instruments – Oxacillin (n=114)[1]

System / Panel (Concentration range)	Performance Using CLSI M100-S25 S. aureus/S.lugdunensis or CoNS Oxacillin Breakpoints					
	S. aureus/S. lugdunensis R ≥4 µg/mL			CoNS R ≥0.5 µg/mL		
	CA	VME	ME	CA	VME	ME
BD Phoenix / PMIC-8 (0.25, 0.5, 1, 2)	92%	9 (24%)	0 (0 %)	97%	2 (5%)	0 (0%)
bioMerieux Vitek2 / AST-GP71 (0.5, 1, 2)	93%	8* (22%)	0 (0 %)	99%	0 (0 %)	0 (0%)
Beckman Coulter MicroScan / Pos MIC 29 (0.25, 0.5, 1, 2)	96%	5 (14%)	0 (0 %)	99%	0 (0 %)	0 (0%)

[1] RUO for all systems

*One isolate has growth failure (x2) in the AST-GP71 panel