International Validation of Alternative Disk Diffusion Methods for Detecting *mec*A-Mediated Oxacillin (OX) Resistance (R) in Staphylococci: Report from the SENTRY Antimicrobial Surveillance Program

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ABSTRACT

Background: The phenotypic detection of OX (*mec*A mediated) R in *S. aureus* (SA) using standardized dilution or disk diffusion (DD) methods remains suboptimal. Attempts to enhance DD testing accuracy have been proposed by the NCCLS. We report the comparison of OX, cefoxitin (FOX) and ceftizoxime (CTIZ) DD test results to those of the OX broth microdilution (BMD) test.
Methods: The collection consisted of 203 SA and 101 coagulase-negative staphylococci (CoNS) originating from 49 sites in 16 countries of the SENTRY Antimicrobial Surveillance Program. OX susceptibilities (S) were determined using the NCCLS BMD method and compared with results of standardized DD testing using OX, FOX and CTIZ disks. Organisms were also tested for the *mec*A gene product (PBP2a).
Results: Inter-method error rates are listed in the Table:

			Occurrences by OX MIC category (% error):	
Organism (no.)	Disk	Category	S	R
SA (203)	OX	S	99	8(3.9)
		I	1(0.5)	2(1.0)
		R	0	93
	FOX	S	99	1(0.5)
		R	1(0.5)	102
	CTIZ	S	98	0
		I	0	1(0.5)
		R	2(1.0)	102
CoNS (101)	OX	S	61	0
		R	3(3.0)	37
	FOX	S	61	1(1.0)
		R	3(3.0)	36
	CTIZ	S	64	2(2.0)
		I	0	1(1.0)
		R	0	34

For SA, very major (Vm; 4.4%) and minor (1.5%) errors were detected for OX disks; no errors were detected for FOX and 1.0% major and 0.5% minor errors for CTIZ. Among CoNS, OX and FOX produced 4.0 and 3.0% Ma errors, respectively. CTIZ produced only 1.0% Vm and minor errors each. Modification of the interpretive zone breakpoints for CTIZ (≥ 25 mm = S, ≤ 24 mm = R) reduced the error to 0.0%.
Conclusions: Use of surrogate markers (FOX and CTIZ) for DD proved superior to OX in detecting OX R among staphylococci. Modification of the CTIZ breakpoint produced complete inter-method concordance at an affordable cost for routine clinical practice.

INTRODUCTION

Oxacillin resistance in both community and hospital acquired strains of staphylococci has emerged as a growing resistance threat. Oxacillin resistant *Staphylococcus aureus* (ORSA) which was first recognized in the United Kingdom in 1961, has now become the leading cause of nosocomial infections worldwide. BSI pathogens from the SENTRY program indexed by geographic region over the five year period from 1997 - 2001 showed increasing ORSA rates: North America (22.4 to 38.7%); Europe (22.1 to 30.4%); and Latin America (29.2 to 36.0%).

While oxacillin resistance in staphylococci has been recognized for nearly half a century, a simple and reliable phenotypic method to detect resistance has remained elusive. Detection of oxacillin resistance by phenotypic methods relies on modification of culture conditions to improve expression, and thus detection of resistance. This year NCCLS has recommended the use of the cefoxitin disk as a surrogate marker for the detection of oxacillin resistance in staphylococcal isolates. Zones of ≤ 19 mm and ≤ 24 mm imply resistance for *S. aureus* and coagulase-negative staphylococci, respectively, whereas ≥ 20 mm and ≥ 25 mm imply susceptibility.

To validate this recommendation we tested a contemporary international collection of 304 clinically significant staphylococcal isolates (203 *S. aureus* and 101 coagulase-negative staphylococci [CoNS]) submitted to the SENTRY Program, by comparing the results of oxacillin, cefoxitin and ceftizoxime disk diffusion methods to those obtained by the oxacillin broth microdilution method and using the PBP2a latex agglutination test to resolve discrepancies.

MATERIALS AND METHODS

Strain collection. An international collection of 304 staphylococci; including 203 *S. aureus* and 101 CoNS, obtained from 49 sites in the 16 countries of the SENTRY Antimicrobial Surveillance Program (2003) were tested. 194 (95.6%) of *S. aureus* were blood isolates and 9 (4.4%) were from documented skin and soft tissue infections. 98 (97.0%) of CoNS were blood isolates and 3 (3.0%) were from documented skin and soft tissue infections.

Susceptibility test methods. Oxacillin MIC values were determined by the NCCLS standardized broth microdilution method. Oxacillin susceptibility testing by disk diffusion was performed using oxacillin (1-µg), and cefoxitin (30-µg) disks [NCCLS, 2004]. Disk diffusion testing by ceftizoxime (30-µg) disk was performed to simultaneously evaluate its value in predicting oxacillin resistance. The current NCCLS [2004] criteria for susceptibility testing of staphylococcal isolates to ceftizoxime were used (i.e. ≤14 mm resistant, 15-19 mm intermediate and ≥ 20 mm sensitive).

Data analysis. A two-part analysis was performed: 1) results of the disk diffusion tests (i.e., oxacillin, cefoxitin and ceftizoxime) were compared with those obtained from the oxacillin broth microdilution method; and 2) inter-disk diffusion method error rates were also calculated by comparing the oxacillin disk and ceftizoxime disk results to those of the cefoxitin disk. Discrepancies between methods were resolved using the PBP2a latex agglutination test.

RESULTS

- Inter-method error rates of the three ß-lactam disks (oxacillin, cefoxitin and ceftizoxime) for predicting oxacillin resistance in staphylococcal isolates as measured by the NCCLS broth microdilution method are shown in **Table 1**.
- For *S. aureus* isolates, the oxacillin disk test had 3.9% major and 1.5% minor errors; the cefoxitin disk had 0.5% very major and 0.5% major errors. The ceftizoxime disk had 1.0% major and 0.5% minor errors (**Figure 1**).
- The corrected error rates for discrepant results following testing with the PBP2a latex test for the three disks were: oxacillin remained unchanged, cefoxitin error rate was reduced to 0.0%, and ceftizoxime displayed 0.5% major and 0.5% minor errors.
- For CoNS, the oxacillin disk had 3.0% major errors; cefoxitin disk had 1.0% very major and 3.0% major errors; and the ceftizoxime disk had 2.0% very major and 1.0% minor errors.
- The corrected error rates for discrepant CoNS results following testing with the PBP2a latex test for the three disks were: the oxacillin error remained unchanged, cefoxitin had 3.0% major and ceftizoxime 1.0% very major and 1.0% minor errors.
- The corrected disk diffusion error rates for oxacillin and ceftizoxime disks for predicting oxacillin resistance in staphylococcal isolates when compared to the NCCLS criteria for the cefoxitin disk test are shown in **Table 2**.
- For *S. aureus*, no errors were detected when testing with cefoxitin disks. Oxacillin disk testing had 4.4% major and 1.5% minor errors; ceftizoxime disk testing had 1.0% major and 0.5% minor errors (**Figure 2**).
- Use of a single breakpoint for ceftizoxime for *S. aureus* (≥ 20 mm = susceptible and ≤ 19 mm = resistant) reduced the error rate to 1.0% major (no false-susceptible).
- For CoNS, the cefoxitin and oxacillin disk tests had major error rates of 3.0% and 4.0%, respectively; ceftizoxime had 1.0% very major and 1.0% minor errors (**Figure 3**).
- Modification of the interpretative breakpoints for ceftizoxime for CoNS to ≥ 25 mm (susceptible) and ≤ 24 mm (resistant) reduced the error rate to 0.0%.

Table 1. Intermethod error rates of three ß-lactam disk tests (oxacillin, cefoxitin, ceftizoxime) for predicting staphylococcal oxacillin resistance as measured by the NCCLS broth microdilution method.

			Occurrences by oxacillin MIC category (% error):[a]	
Organism (no. tested)	Disk tested	Category[a]	Susceptible	Resistant
S. aureus (203)	Oxacillin	Susceptible	99	8(3.9)
		Intermediate	1(0.5)	2(1.0)
		Resistant	0	93
	Cefoxitin	Susceptible	99	1(0.5)[b]
		Resistant	1(0.5)[b]	102
	Ceftizoxime	Susceptible	98	0
		Intermediate	0	1(0.5)
		Resistant	2(1.0)[c]	102
CoNS (101)	Oxacillin	Susceptible	61	0
		Resistant	3(3.0)	37
	Cefoxitin	Susceptible	61	1(1.0)[b]
		Resistant	3(3.0)	36
	Ceftizoxime	Susceptible	64	2(2.0)[c]
		Intermediate	0	1(1.0)
		Resistant	0	34

a. NCCLS [2004] categorization criteria.
b. Errors were eliminated when the disk results were compared to the *mec*A gene product detection results.
c. One error was resolved by comparison to the *mec*A test.

Table 2. Error rates for the disk diffusion methods for detecting *mec*A-mediated oxacillin resistance among international isolates of staphylococci (304 strains).

		Error rates by type:[a]		
Tested organisms (no. tested)	Disk tested	Very major	Major	Minor
S. aureus (203)	Oxacillin	4.4	0.0	1.5
	Cefoxitin	0.0	0.0	-[b]
	Ceftizoxime	0.0	1.0[c]	0.5[c]
CoNS (101)	Oxacillin	0.0	4.0	-[b]
	Cefoxitin	0.0	3.0	-[b]
	Ceftizoxime	1.0[c]	0.0	1.0[c]

a. Results compared to proposed NCCLS M100-S14 [2004] tables for each tested compound or existing criteria for oxacillin and ceftizoxime.
b. - = no criteria are published for the intermediate category.
c. Modification of the breakpoint zone diameter for ceftizoxime for CoNS would reduce the errors to zero (≥ 25 mm = susceptible and ≤ 24 mm = resistant). A single breakpoint for ceftizoxime for *S. aureus* (susceptible at ≥ 20 mm and resistant at ≤ 19 mm) would reduce error to 1.0% compared to *mec*A tests. Overall performance accuracy versus all 304 staphylococci was 99.3%.

Figure 1: Scattergram comparing results of the reference oxacillin MIC determined by the NCCLS broth microdilution method and zone diameters around the 30-µg ceftizoxime disk (203 *S. aureus* strains). P = single result from a *mec*A-positive strain and N = a result for a *mec*A-negative organism.

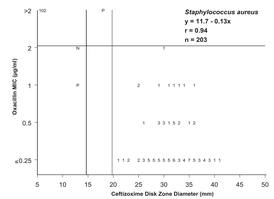


Figure 2: Scattergram comparing the zones of inhibition for cefoxitin (30-µg) and ceftizoxime (30-µg) disks when testing 203 *S. aureus* strains. P = single result from a *mec*A-positive strain and N = a result for a *mec*A-negative organism.

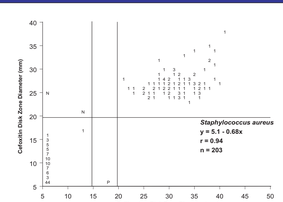
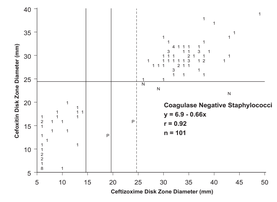


Figure 3: Scattergram comparing zone diameter results for the cefoxitin and ceftizoxime disk tests against 101 strains of coagulase-negative staphylococci. Vertical broken line indicates the modified interpretive breakpoint that produces no errors when using the ceftizoxime disk test to predict the presence of *mec*A. P = single result from a *mec*A-positive strain and N = a result for a *mec*A-negative organism.



CONCLUSIONS

- Our findings confirm the superiority of the cefoxitin disk as a surrogate marker to detect oxacillin resistance in an international collection of *S. aureus* and CoNS, with > 95% being bloodstream isolates.
- The overall accuracy of the four tests in the 304 staphylococcal isolates processed was as follows: modified ceftizoxime disk (99.3%) > oxacillin MIC test = cefoxitin disk test (99.0%) > current ceftizoxime disk (98.4%) > oxacillin disk (94.7%).
- A minor modification of ceftizoxime interpretive breakpoints produced complete inter-method concordance with the reference method, and all staphylococcal accuracy exceeding that of 30-µg cefoxitin disk (99.3% vs. 99.0%).
- Cefoxitin and ceftizoxime disks provide an easy, familiar and affordable method for the clinical laboratories to rapidly and accurately identify oxacillin resistance in staphylococcal isolates.

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