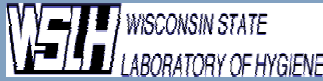


# Large Sample Comparison of Cefoxitin and Oxacillin Disk Diffusion Methods to Detect *mecA*-mediated Resistance in *Staphylococcus aureus*

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## REVISED ABSTRACT

**Background:** In January 2007 the Clinical and Laboratories Standards Institute (CLSI) modified the recommended resistant and susceptible breakpoints for the 30 µg cefoxitin disk test to detect *mecA*-mediated resistance in *Staphylococcus aureus* from ≤19 mm and ≥20 mm to ≤21 mm and ≥22 mm, respectively. The objective of this study was to compare the performance of the cefoxitin disk at the new breakpoints with that of the 1 µg oxacillin disk using a large sample of recent *S. aureus* isolates.

**Methods:** Non-duplicate *S. aureus* isolates were consecutively collected by 53 participating Wisconsin clinical laboratories between August and September of 2007 and submitted to the Wisconsin State Laboratory of Hygiene (WSLH). Species identification was confirmed at WSLH by coagulase slide test and subsequent tube test and biochemicals. Susceptibility to oxacillin was evaluated by the disk diffusion method using 30 µg cefoxitin and 1 µg oxacillin disks according to current CLSI guidelines. Oxacillin disk error rates were determined by comparison to the cefoxitin disk result as the CLSI preferred method. All discrepancies were resolved using the PBP2a latex agglutination method for detection of the *mecA* gene product.

**Results:** A total of 1611 isolates were evaluated. Of the 813 isolates that tested oxacillin susceptible using the cefoxitin disk, 791, 0, and 22 were interpreted as susceptible, intermediate and resistant to oxacillin, respectively, using the oxacillin disk method. Of the 798 isolates that tested resistant to oxacillin using the cefoxitin disk, 797, 1, and 0 were interpreted as resistant, intermediate and susceptible to oxacillin, respectively, using the oxacillin disk method. Cefoxitin disk sensitivity and specificity as compared to oxacillin disk was 97.3% and 100%, respectively. After discrepant analysis the cefoxitin disk sensitivity increased to 99.9%. Not considered in the above analysis, was 1 isolate that tested intermediate to oxacillin and resistant to cefoxitin. This isolate was negative for PBP2a by latex agglutination.

**Conclusion:** The results of this large sample comparison validate the new CLSI cefoxitin disk test breakpoints for the detection of *mecA*-mediated resistance in *S. aureus*.

## BACKGROUND

Accurate detection of methicillin resistance in *S. aureus* is of the utmost importance to ensure the correct treatment for the affected patient. The *mecA* gene confers resistance to methicillin in *S. aureus*. The Clinical Laboratory Standards Institute (CLSI) recommends usage of cefoxitin instead of oxacillin when using the disk diffusion method to determine resistance against methicillin for *S. aureus* (1). Cefoxitin results are easier to interpret, and thus, more sensitive for the detection of *mecA*-mediated resistance than oxacillin (2, 3).

The recommended resistant and susceptible breakpoints for the 30 µg cefoxitin disk test, used to detect *mecA*-mediated resistance in *S. aureus*, were changed in January 2007 by the CLSI from ≤ 19mm and ≥ 20mm to ≤ 21mm and ≥ 22mm, respectively. This study sampled a large number of recent *S. aureus* isolates to compare the performance of the 30 µg cefoxitin disk test at the new breakpoints to the 1 µg oxacillin disk test.

## METHODS

**Organisms.** A total of 1611 isolates of both methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA) were collected from 53 Wisconsin clinical laboratories between the months of August and September of 2007. Participating laboratories were asked to submit their first 20 consecutive isolates each of MRSA and of MSSA, excluding duplicate isolates. Isolates were submitted to the Wisconsin State Laboratory of Hygiene (WSLH) for reference testing. The isolation sites of the *S. aureus* isolates collected were as follows: 1159 skin/soft tissue (71.9%), 136 urine (8.4%), 130 respiratory (8.1%), 65 bloodstream (4.0%), 79 other (4.9%), and 42 not given (2.6%).

**Identification of isolates.** *Staphylococcus aureus* isolates were originally identified by the Wisconsin clinical laboratory that submitted the isolate to the WSLH. Identification was then confirmed by colony morphology and a slide coagulase test. A tube coagulase test was performed in the event that the slide coagulase was negative. Coagulase negative isolates were then identified by biochemicals and excluded from this analysis.

**Antimicrobial Susceptibility Testing.** Susceptibility to antimicrobial agents was evaluated by the CLSI reference disk diffusion method (1) on Mueller Hinton Agar (Remel, Lenexa, KS). *MecA*-mediated resistance detection was evaluated by disk diffusion methods for both 1 µg oxacillin and 30 µg cefoxitin impregnated disks (Remel) and results were compared. Discrepant isolates were resolved using a PBP2a latex agglutination kit (Denka Seiken Co. LTD., Tokyo, Japan).

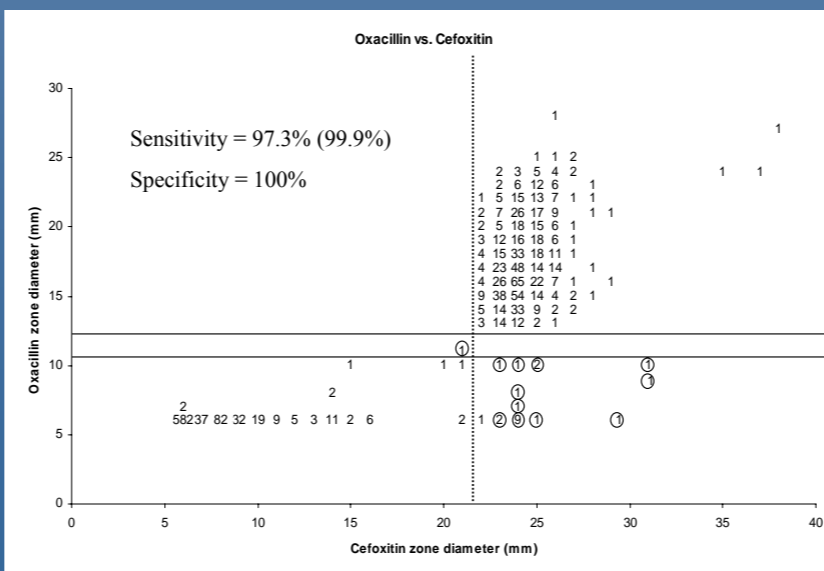
## RESULTS

- The cefoxitin disk demonstrated an initial sensitivity and specificity of 97.3% and 100%, respectively, compared to the oxacillin disk.
- Through discrepant analysis, 21 of the 22 isolates that tested resistant to oxacillin and susceptible to cefoxitin were found to be negative by PBP2a latex agglutination. An additional isolate that tested intermediate to oxacillin and resistant to cefoxitin was also found to be negative for PBP2a.
- Absence of the PBP2a protein is indicative of non-*mecA*-mediated methicillin resistance. Therefore, in this collection a 1.4% rate (22/1611) of non-*mecA*-mediated MRSA was detected.
- Following discrepant analysis and exclusion of the non-*mecA*-mediated MRSA isolates, the cefoxitin disk test sensitivity increased to 99.9% and the specificity remained at 100%.
- Cefoxitin incorrectly identified only one isolate as susceptible (zone diameter = 22mm) that was oxacillin resistant and PBP2a positive.

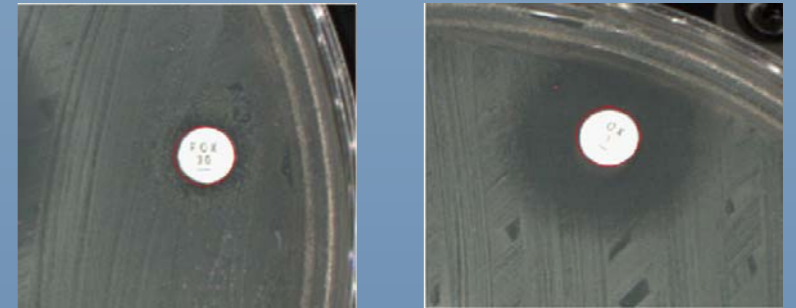
**Table 1.** Number of oxacillin (1 µg) and cefoxitin (30 µg) disk diffusion test results by CLSI interpretive criteria obtained from 1611 *Staphylococcus aureus* isolates.

Cefoxitin (30 µg)	Number of Isolates			Total
	Oxacillin (1 µg)			
	S	I	R	
S	791	0	22	813
R	0	1	797	798
Total	791	1	819	1611

**Figure 1.** Correlation of oxacillin (1 µg) and cefoxitin (30 µg) disk test zone diameters obtained from 1611 *Staphylococcus aureus* isolates. Circled isolates indicate PBP2a negative isolate(s) at that position. The unbroken lines represent the current oxacillin CLSI interpretive criteria for *S. aureus*; the broken line represents the new (January 2007) cefoxitin CLSI interpretive criteria for *S. aureus*.



**Figure 2.** Cefoxitin and oxacillin disk diffusion results for MRSA isolate # 1382 as an example of zone readability. On the left, the zone diameter of the 30 µg cefoxitin disk is clearly 6mm (read using reflected light). On the right, the zone diameter of the 1 µg oxacillin disk is 6mm by reading the haze up to the disk using transmitted light, but can easily be misinterpreted as susceptible.



## DISCUSSION

- Using a large sample of *S. aureus* isolates, this study affirms the newly modified CLSI susceptibility breakpoint of ≥ 22 mm for cefoxitin disk diffusion (1). Four isolates testing resistant to oxacillin from this collection would have otherwise been misinterpreted as susceptible using the previous cefoxitin susceptible breakpoint of ≥ 19 mm.
- Another new addition to the CLSI M100S-17 is a warning of the limitation of cefoxitin as a substitute test for oxacillin, as cefoxitin will only detect MRSA with a *mecA*-mediated resistance mechanism. However, non-*mecA*-mediated MRSA are a rare occurrence, as evidenced in this study by a non-*mecA*-mediated resistant rate of 1.4%.
- Even with this limitation, cefoxitin disk diffusion results can be much easier to read than oxacillin due to sometimes hazy results which are commonly misinterpreted as oxacillin susceptible (Figure 2). This rate of false susceptibility has been noted as high as 4.4% in some studies (4, WSLH publication pending), well above the CLSI recommended acceptable limit of ≤ 1.5%.

## CONCLUSION

- This large sample study validates the new CLSI breakpoints for the cefoxitin disk test as a surrogate for the oxacillin disk test to detect *mecA*-mediated resistance in *S. aureus*.

## REFERENCES

1. CLSI. 2007. Performance Standards for Antimicrobial Susceptibility Testing; 17th Informational Supplement. CLSI Document M100-S17. Wayne, PA.
2. Swenson, J. M., Tenover, F. C., and the Cefoxitin Disk Study Group. 2005. Results of Disk Diffusion Testing with Cefoxitin Correlate with Presence of *mecA* in *Staphylococcus* spp. J. Clin. Microbiol. 43:3818-3823.
3. Witte, W., Pasemann, B., and Cuny, C. 2007. Detection of Low-Level Oxacillin Resistance in *mecA*-positive *Staphylococcus aureus*. Clinical Microbiology and Infection. 13:408-412.
4. Potumarthy, S., Fritsche, T., Jones, R. Evaluation of alternative disk diffusion methods for detecting *mecA*-mediated oxacillin resistance in an international collection of staphylococci: Validation report from the SENTRY antimicrobial surveillance program. Diag. Microbiol. and Infect. Dis., 51: 57 – 62.

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