

ABSTRACT

Objective: To validate antimicrobial susceptibility testing (AST) results for antimicrobial agents commonly tested against *Staphylococcus aureus* in Wisconsin hospital and clinical laboratories.
Study Design: Isolates of *S. aureus* were collected by 53 laboratories in Wisconsin during August and September of 2007 and submitted to the Wisconsin State Laboratory of Hygiene (WSLH) for validation testing using the disk diffusion method performed according to Clinical Laboratory Standards Institute (CLSI) guidelines. Participating laboratories were asked to submit 20 consecutive isolates each of MRSA and methicillin susceptible *S. aureus* (MSSA). Species identification was confirmed by coagulase slide test, and subsequent tube test and biochemicals. Inducible clindamycin resistance was evaluated by the D-test method as per CLSI guidelines.
Results: Over 1600 *S. aureus* isolates were tested. Only 16 (<1%) misidentifications were detected. Comparison of clinical and WSLH susceptibility interpretation data will determine very major, major and minor error rates in aggregate for all commonly tested antimicrobial agents.
Conclusions: This validation will help clinical laboratories in Wisconsin evaluate their antimicrobial susceptibility testing methods for MRSA and implement changes to improve their performance. This will result in better patient care and management for the people of Wisconsin. Future analyses will determine susceptibility patterns, and identify educational opportunities for the practical application of AST standards.

BACKGROUND

Of the 135 clinical laboratories in Wisconsin, 87 (64%) perform antimicrobial susceptibility testing (AST). Most Wisconsin clinical laboratories are small, rural laboratories with limited staffing. All clinical laboratories strive to adhere to the most recent CLSI (formerly NCCLS) recommendations (1), but AST complexity and staffing limitations can be challenging. This study recruited 53 of 87 (61%) AST performing laboratories to participate in an evaluation of AST performance using eleven antimicrobial agents commonly tested against *Staphylococcus aureus* as an AST quality indicator.

METHODS

Organisms. Isolates of *S. aureus* were collected by 53 laboratories in Wisconsin during August and September of 2007 and submitted to the Wisconsin State Laboratory of Hygiene (WSLH) for validation testing. Over 1600 isolates were collected during the two-month period and were categorized by isolation site as follows: 1159 skin/soft tissue (71.9%), 136 urine (8.4%), 130 respiratory (8.1%), 65 bloodstream (4.0%), 121 other (7.5%).

Antimicrobial Susceptibility Testing.

Clinical Laboratories: Clinical laboratories used automated systems: vitek (71%), microscan (21%), and phoenix (4%) as well as non automated: disk diffusion (4%) for AST.

Reference Laboratory (WSLH): Susceptibility to antimicrobial agents was evaluated, according to CLSI guidelines, by the disk diffusion method on Mueller Hinton Agar (Remel, Lenexa, KS) (1). The following 11 antibiotics were tested: oxacillin (1 µg), cefoxitin (30 µg), penicillin (10 IU), erythromycin (15 µg), clindamycin (2 µg), ciprofloxacin (5 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), rifampin (5 µg), gentamicin (10 µg), and vancomycin (30 µg) (Remel, Lenexa, KA).

D-Test. Erythromycin induced clindamycin resistance was detected by D-test method using disk diffusion on Mueller Hinton Agar. The detection of a D-shaped zone of complete clearance around the clindamycin disk was read as a positive result.

Table 1. Error rates calculation and acceptable range.

Error	Acceptable Rate ^a	Calculation	Meaning
Very Major	< 1.5%	# called S by clinic and R by ref. lab total # R according to ref. lab	% False Susceptible
Major	< 3%	# called R by clinic and S by ref. lab total # S according to ref. lab	% False Resistant
Minor	< 5%	# discrepant called I by either lab total # isolates tested	% False Intermediate

^a NCCLS. 2001. Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameter; Approved Guideline-2nd Edition.

RESULTS

- **Identification:** 1611/1627 (99%) were correctly identified as *S. aureus* by clinical laboratories. 9/16 non *S. aureus* isolates were identified as coagulase negative *Staphylococcus*.
- **% Agreement:** The percent agreement for every antimicrobial was above 96% except the quinolones. The results of ciprofloxacin and levofloxacin from clinical laboratories were compared to only ciprofloxacin from the reference laboratory leading to a higher number of minor errors.
- **Error Rates Out of the Acceptable Range:**
 - Minor:** quinolones (If levofloxacin is excluded, there are no out of range error rates)
 - Major:** rifampin
 - Very Major:** oxacillin, clindamycin, quinolones, rifampin¹, and tetracycline²
 - ¹Not statistically significant due to the low number of resistant isolates
 - ²Also all errors occurred with the Vitek system
- **D Test.** Analysis of 742 *S. aureus* isolates from clinical laboratories with submitted D Test results.
 - 96% Agreement (712/742)
 - 10 false sensitive isolates
 - 20 false resistant isolates

Table 2. % Agreement for *S. aureus* isolates disk diffusion testing comparing WI clinical laboratory data and reference laboratory (WSLH).

Antimicrobials	% Agreement	% Discrepant	No. tested
Cefoxitin	98.4	1.6	190
Oxacillin	96.6	3.4	1503
Clindamycin	97.1	2.9	1493
Erythromycin	96.2	3.8	1505
Gentamicin	99.8	0.2	1427
Penicillin	97.7	2.3	477
Quinolone	91.4	8.6	1482
Rifampin	99.6	0.4	1405
Tetracycline	99.3	0.7	1496
Trim-sulfa	99.4	0.6	1493
Vancomycin	100	0.0	1591
Total^a	97.7	2.3	14,062

^a Mean Values

Table 3. Error Rates for *S. aureus* isolates disk diffusion testing comparing WI clinical laboratory data and reference laboratory (WSLH).

Antimicrobials	% (No.) of errors				No. tested
	Very Major	Major	Minor	Total	
Cefoxitin	1.2 (1)	1.9 (2)	NA	3	190
Oxacillin	4.4 (34)	2.2 (16)	0.1 (1)	51	1503
Clindamycin	4.3 (10)	2.5 (33)	0.1 (1)	44	1493
Erythromycin	1.2 (11)	1.2 (7)	2.7 (40)	58	1505
Gentamicin	0	0.1 (2)	0.1 (1)	3	1427
Penicillin	0.5 (2)	16.1 (9)	NA	11	477
Quinolone	2.3 (13)	0.9 (8)	7.2 (107)	128	1482
Rifampin	16.7 (1)	0.2 (3)	0.1 (2)	6	1405
Tetracycline	5.6 (3)	0.3 (4)	0.3 (4)	11	1496
Trim-sulfa	0	0.5 (7)	0.1 (2)	9	1493
Vancomycin	0	0	NA	0	1591
Total^a	2.5 (75)	0.8 (91)	1.33 (158)	324	14,062
Acceptable Rate	< 1.5	< 3.0	< 5.0		

^a Mean Values

Figure 1. Map of 53 participating WI clinical laboratories

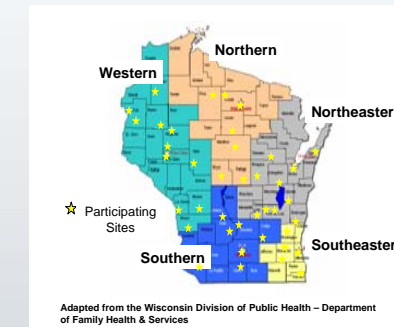


Figure 2. D test positive result showing clear D-shaped zone of clearance around the clindamycin disk.



DISCUSSION

- **Error Rates:**
 - According to the CLSI ciprofloxacin and levofloxacin have nearly complete cross-resistance and –susceptibility. When ciprofloxacin results from the clinical reference laboratories were compared, all errors were within the acceptable range. However, when results from the clinical laboratories using levofloxacin were included in the analysis there were increased error rates.
 - The very major error rate for rifampin was well out of the acceptable range due to the fact that there were only 6 resistant isolates in total. This error rate, therefore, is not statistically significant.
 - The greater than 90% agreement for all antimicrobials suggests that Wisconsin clinical laboratories are doing an excellent job with susceptibility testing.
 - The average very major error rate for all antimicrobials was outside of the acceptable range. This discrepancy appears to be due mostly to errors in interpreting oxacillin, clindamycin and quinolone results.
- **D Test:** Clindamycin susceptible and erythromycin resistant *S. aureus* isolates should be tested for inducible resistance by the D test method. The high number of major errors could be explained by an incorrect interpretation of a hazy D zone as a positive result.

CONCLUSION

- Identification to the species level was adequate. 99.0% of isolates referred for this study were correctly identified as *S. aureus*.
- All antimicrobial agents tested were in adequate agreement with reference laboratory results with an average of >97%, with the exception of the quinolones (91.4%).
- The overall major (<3.0%) and minor (<5.0%) testing error rates were acceptable, however the overall very major error rate (2.5%) was in the unacceptable range (>1.5%).
- Of the limited number of reported D test results, 712 of 742 (96.0%) were in adequate agreement with reference laboratory testing.

REFERENCES

1. CLSI. 2007. Performance Standards for Antimicrobial Susceptibility Testing; 17th Informational Supplement. CLSI Document M100-S17. Wayne, PA.
2. NCCLS. 2001. Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameter; Approved Guideline-2nd Edition. NCCLS Document M23-A2. Wayne, PA.

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