

Investigation of *Staphylococcus aureus* with Vitek-1 Erythromycin-Intermediate Susceptibilities: Comparison with Vitek-2 and Phoenix

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ABSTRACT

BACKGROUND: Most laboratories use automated susceptibility testing systems as their primary method to detect erythromycin resistance in *S. aureus*, and most default clindamycin as resistant when erythromycin resistance is detected. While inducible MLS resistance is common among erythromycin-resistant *S. aureus*, no studies have examined strains with intermediate susceptibility to erythromycin.

METHODS: In total, 69 isolates (63 cross-Canada, 6 U.S.) that were erythromycin-intermediate according to the Vitek-1 system were identified from a diverse collection of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA). Of these, 57 isolates (55 MRSA, 2 MSSA) were available for further testing: susceptibilities to erythromycin and clindamycin were determined by NCCLS broth microdilution and disk diffusion, and the inducible MLS phenotype was detected by placing the erythromycin and clindamycin disks 15 mm apart and measuring clindamycin resistance induction zones. Results were compared to those of Vitek-2 and Phoenix systems.

RESULTS: According to Vitek-1, 44/57 (77%) strains had erythromycin MIC of 1 µg/mL, 5/57 (9%) were 2 µg/mL and 8/57 (14%) were 4 µg/mL; all had clindamycin MIC of ≤ 0.5 µg/mL. All 57 were found to have erythromycin MIC ≥ 8 µg/mL by broth microdilution and 56/57 (98%) were erythromycin-resistant by disk diffusion (1 MRSA was erythromycin-intermediate at 16 mm). 54/57 (95%) had an inducible MLS phenotype. The Phoenix correctly identified 100% and the Vitek-2 identified 55/57 (96%) of strains as erythromycin-resistant (MIC ≥ 8 µg/mL). 2/57 strains had Vitek-2 erythromycin MIC of 1 and 4 µg/mL; both had the inducible MLS phenotype.

CONCLUSIONS: The erythromycin-intermediate *S. aureus* identified in this study represent anomalous results generated by the Vitek-1 system. As 95% of *S. aureus* exhibited the anticipated inducible MLS phenotype, it is recommended that all *S. aureus* identified with erythromycin-intermediate and clindamycin-susceptible profiles by the Vitek-1 system be interpreted and reported as resistant to both erythromycin and clindamycin.

BACKGROUND

Erythromycin and clindamycin belong to the macrolide, lincosamide and streptogramin (MLS) family of antibiotics. Expression of MLS resistance can be either constitutive or inducible. Previous studies have shown that inducible MLS resistance is common among erythromycin-resistant *S. aureus*. As the majority of MLS resistance in *S. aureus* is *erm*-mediated, erythromycin-resistant strains are automatically reported as clindamycin resistant. However no studies have looked at strains identified to have intermediate susceptibility to erythromycin.

Many microbiology laboratories today use automated systems to determine antibiotic susceptibilities in *S. aureus*. We identified *S. aureus* isolates that exhibited erythromycin-intermediate susceptibility according to the bioMerieux Vitek-1 system. These were subjected to further antibiotic susceptibility testing that included broth microdilution and disk diffusion. These results were also compared to those from the Vitek-2 and Becton Dickinson Phoenix systems.

METHODS

The *S. aureus* strains used in this study were selected from a diverse collection of MRSA and MSSA at the Microbiology Department at Mount Sinai Hospital that included various isolates from across Canada and the U.S. The isolates had been collected between 1997 and 2002 and stored frozen. A total of 69 *S. aureus* isolates that had been reported with erythromycin intermediate susceptibility were identified for this study. Of these, 57 were available for further testing.

Broth microdilution was done according to NCCLS guidelines. Organisms were screened for inducible MLS resistance with 2 µg clindamycin disks placed precisely 15 and 20 mm (edge-to-edge) from a 15 µg erythromycin disk on Mueller-Hinton plates. Blunting of the zone of inhibition around the clindamycin disk after 18 to 24 h incubation was interpreted as positive for inducible MLS resistance. GPS-105, AST-P526 and PMIC/ID-14 cards were used in the Vitek-1, Vitek-2 and Phoenix systems respectively as per the manufacturers' instructions.

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RESULTS

According to Vitek-1, of the 57 *S. aureus* strains, 44 (77%) had an erythromycin MIC of 1 µg/mL, 5 (9%) were 2 µg/mL and 8 (14%) were 4 µg/mL (Table 1). All of the isolates had clindamycin MIC ≤ 0.5 mg/mL on the Vitek-1 system and these had been interpreted as susceptible. When broth microdilution was performed, all strains were erythromycin-resistant (MIC ≥ 8 µg/mL) and clindamycin-susceptible (MIC ≤ 0.5 µg/mL).

Antimicrobial susceptibilities by disk diffusion showed 56 of the 57 strains (98%) were erythromycin-resistant while one isolate was erythromycin-intermediate with an inhibition zone diameter of 16 mm. The double disk method showed 54 of 57 (95%) to have the inducible MLS phenotype with the remaining 3 isolates truly susceptible to clindamycin. The results were equivalent

whether the clindamycin and erythromycin disks were 15 or 20 mm apart. However, blunting of the zone of resistance was more evident when disks were 15 mm apart with the mean reduction in radius at 7.6 ± 2.1 mm with disks 15 mm apart and a reduction of 5.5 ± 1.5 mm with disks 20 mm apart.

In comparison, the Phoenix system correctly identified all isolates as erythromycin-resistant (MIC ≥ 8 µg/mL) and Vitek-2 found 55 of 57 (96%) isolates to be erythromycin-resistant (MIC ≥ 8 µg/mL). The two remaining strains had Vitek-2 erythromycin MICs of 1 and 4 µg/mL. These two latter isolates had the inducible MLS phenotype. Both the Phoenix and Vitek-2 systems found all the strains to be clindamycin susceptible and neither had expert rules overriding the susceptibility results.

Table 1. *S. aureus* Erythromycin Susceptibilities According to Different Techniques

Vitek-1		Vitek-2		Phoenix		Broth Microdilution		Disk Diffusion
Ery MIC (µg/mL)	n	Ery MIC (µg/mL)	n	Ery MIC (µg/mL)	n	Ery MIC (µg/mL)	n	Inducible MLS Resistance (n)
1	44	1	1	8	44	8	44	44
		4	1					
		8	42					
2	5	8	5	8	5	8	5	5
4	8	8	5	8	8	8	8	5

CONCLUSIONS

The erythromycin-intermediate *S. aureus* identified in this study represent anomalous results generated by the Vitek-1 system. Of 57 isolates from across North America, all were found to be erythromycin resistant by the NCCLS reference method of broth microdilution. The Vitek-2 and Phoenix automated systems have improved detection of erythromycin resistance in *S. aureus*, identifying 96% and 100% of isolates as resistant respectively.

Double disk diffusion testing for the MLS resistance phenotype found that 95% of the strains were inducible. Given our high rates of inducible MLS resistance, it would be neither cost effective nor an acceptable alternative to routinely screen all Vitek-1 erythromycin-intermediate *S. aureus* by this method. More importantly, this would delay reporting of results. We recommend that all erythromycin-intermediate *S. aureus* identified by Vitek-1 be automatically interpreted as resistant. In addition, the clindamycin susceptibility in such strains should also be changed to resistant to reflect the predominance of inducible MLS phenotype. Similar problems may exist for antimicrobial susceptibility testing of other gram-positive organisms in the Vitek-1 system and we are currently addressing this issue.