

Short Communication

Detection of inducible clindamycin resistance by an automated system in a tertiary care hospital

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Clindamycin is commonly used in treatment of erythromycin resistant *Staphylococcus aureus* causing skin and soft tissue infections. *In vitro* routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to 'erm' genes resulting in treatment failure thus necessitating the need to detect such resistance by a rapid method. In the era of automation, vitek-2 system provides a panel for detection of inducible clindamycin resistance with conjunction of other antimicrobial susceptibility testing. The present study evaluated the performance of a vitek-2 card for detection of inducible clindamycin resistance in a tertiary care hospital. Non-duplicate clinical isolates of hundred *S. aureus* were obtained from various clinical samples. Antimicrobial susceptibility testing was carried out, including detection of clindamycin resistance and methicillin resistance pattern by vitek-2 identification and antimicrobial susceptibility testing (ID/AST) system by using AST-GP67 card. The results were compared to those of D-test as per CLSI guidelines on erythromycin resistant isolates. EPI INFO software, Licensed by CDC Atlanta was used for analysis of data. The sensitivity and specificity for the vitek-2 card was 95.4 and 100%, respectively in comparison to disk approximation test (D-test). The performance of vitek-2 card was 100% specific and rapid for detection of inducible clindamycin resistance with other antimicrobial susceptibility results.

Key words: Automated system, constitutive macrolide-lincosamide-streptogramin B (MLS_B) phenotype, inducible MLS_B phenotype, methicillin resistance *Staphylococcus aureus* (MRSA), msrA gene (MS phenotype).

INTRODUCTION

Emergence of methicillin resistance in *Staphylococcus aureus* (MRSA) has left us with very few therapeutic alternatives available to treat staphylococcal infections. The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due its excellent pharmacokinetic properties (Fiebelkorn et al., 2003).

The development of resistance in *Staphylococcus* species to Macrolide, lincosamide and streptogramin B has limited the use of these antibiotics. Macrolide resistance may be due to enzymes encoded by a variety of erm genes-MLS_B phenotype and may be constitutive (cMLS_B phenotype) or inducible (iMLS_B phenotype).

Another mechanism is active efflux pump encoded by the *mrs A* gene (MS phenotype). The MS and iMLS_B phenotypes are indistinguishable by using standard susceptibility test methods, but can be distinguished by erythromycin-clindamycin disk approximation test (D-test), automated vitek-2 system and demonstration of resistance genes by molecular methods (Steward et al., 2005; Pal et al., 2010).

The aim of this study was to determine

1. The rate of inducible clindamycin resistance in both methicillin-resistant and susceptible strains of *S. aureus* in our hospital as data describing iMLS_B prevalence among *S. aureus* isolates in this region is unknown.
2. To know the sensitivity and specificity of automated vitek-2 system in detecting inducible clindamycin resistance within 8 to 16 h with other antimicrobial susceptibility results in comparison to D-test which will

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Table 1. Distribution of isolates.

S/N	Phenotype	MRSA (%)	MSSA (%)	Total (%)
1	ER-S,CL-S	0	14	14
2	ER-R, CL-R (cMLS _B)	8	4	12
3	ER-R,CL-S,D ⁻ (MS)	6	25	31
4	ER-R,CL-S, D + (iMLS _B)	28	15	43
5	Total	42	58	100

ER, erythromycin; CL, clindamycin; cMLS_B, constitutive resistance to clindamycin; iMLS_B, inducible clindamycin resistance; D⁻, negative D-test; D+, positive D-test; MS, MS phenotype.

take 48 h as per CLSI guidelines.

MATERIALS AND METHODS

One hundred isolates of *S. aureus* were recovered from blood, pleural fluid, pus, sputum, tracheal aspirate, and other specimens received in the Department of Microbiology over a period of three and half months from December 2010 to mid March, 2011.

All the *S. aureus* were identified by conventional microbiological methods including colony morphology, gram stain, catalase, and slide coagulase and tube coagulase test.

All the *S. aureus* were subjected to antimicrobial susceptibility testing including detection of inducible clindamycin resistance and cefoxitin screening test by vitek-2 identification and antimicrobial susceptibility testing (ID/AST) system by using AST-GP67 card, which incorporated to the Advanced Expert System (AES), software which validates and interprets susceptibility test results and detects antibiotic resistance mechanisms. The CLSI susceptibility breakpoints were used.

The Vitek 2 AST-GP67 card (bioMérieux, Marcy l'Etoile, France) was used according to the manufacturer's recommendations. Briefly, three to five colonies of an 18 to 24 h-old culture of *S. aureus* were inoculated in a 0.45% NaCl solution and adjusted to a concentration equivalent to a 0.5 to 0.63 McFarland standard. The solution was then loaded with the card in the Vitek 2 system. The incubation period was determined by the Vitek 2 system. Two wells are used to detect inducible clindamycin resistance in the Vitek 2 card: One with 0.5 mg of clindamycin/liter and another one with a combination of 0.25 and 0.5 mg of clindamycin and erythromycin/liter, respectively. Both the instrument and the Advanced Expert System (AES) results were considered.

Disk approximation testing (D-test) was performed for each isolate according to Clinical and Laboratory Standards Institute (CLSI) method. A 0.5 McFarland suspension was prepared in normal saline for each isolate and inoculated on Mueller-Hinton agar plate. Clindamycin (CLI)-2 µg and erythromycin (ER)-15 µg disks (HIMEDIA, Mumbai) were placed 15 mm apart edge to edge manually. Plates were incubated at 35°C for 24 h and zone diameters were recorded. Induction test categories were interpreted given as:

1. MS phenotype - Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤ 13 mm) while sensitive to clindamycin (zone size ≥ 21 mm) and giving circular zone of inhibition around clindamycin was labeled as having this phenotype.
2. Inducible MLS_B phenotype - Staphylococcal isolates showing resistance to erythromycin (zone size ≤ 13 mm) while being sensitive to clindamycin (zone size ≥ 21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards

erythromycin disc were labeled as having this phenotype.

3. Constitutive MLS_B phenotype - this phenotype was labeled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤ 13 mm) and clindamycin (zone size ≤ 14 mm) with circular shape of zone of inhibition if any around clindamycin.

Statistical analysis

Statistical analysis was performed using EPI INFO software; Licensed by CDC Atlanta was used for analysis of data.

RESULTS

One hundred Staphylococcal isolates included in the present study were tested for antimicrobial susceptibility testing including detection of inducible clindamycin resistance and cefoxitin screening test by vitek-2 identification and susceptibility testing (ID/AST) system by using AST-GP67 card. Of the 100 *S. aureus* isolates, 86% were erythromycin resistant. These isolates when subjected to D test showed that 12% isolates were resistant to both erythromycin and clindamycin indicating constitutive MLS_B phenotype; 74% isolates showed clindamycin sensitivity. Out of these, 43% showed positive D test indicating inducible MLS_B phenotype while 31% gave negative D test indicating MS phenotype (Table 1). The overall percentage resistance for all three phenotypes was as follows:

Inducible clindamycin resistance = 43%
 Constitutive clindamycin resistance = 12%
 MS Phenotype = 31%

Percentage of inducible clindamycin resistance is higher in MRSA as compared to methicillin susceptible *S. aureus* (MSSA) (p value is 0.0119 which is highly significant, by z-test).

There was no difference between results obtained from the instrument and the advanced expert system (AES). Inducible clindamycin resistance was not detected in two strains that were D-test positive. The specificity was 100% (Table 2).

Table 2. The performance of the vitek-2 card.

Vitek-2 result	No. of isolates with:		Total
	D-test positive	D-test negative	
Positive	41	0	41
Negative	2	57	59
Total	43	57	100

Sensitivity = 95.4%; Specificity = 100%; Negative predictive value (NPV) = 96.6%; Positive predictive value (PPV) = 100%. When these 2 strains were retested, they were still negative with the vitek-2 card for the second time. Again, the results were similar when considering MSSA and MRSA, separately.

DISCUSSION

In the context of increasing prevalence of community-acquired MRSA, alternative drugs to treat skin and soft tissue Infections are needed. Clindamycin appears to be an interesting option because of the availability of an oral formulation, good bioavailability, and distribution in skin and abscesses. (Daurel et al., 2008) Resistance to clindamycin is highly variable in different patient populations (Moore et al., 2008; Schreckenberger et al., 2004) and, if this drug is to be used, rapid susceptibility testing for inducible clindamycin resistance must be available. In order to choose an appropriate method for each laboratory and patient populations, the performance has to be evaluated.

The vitek-2 system was reported to be 98% sensitive in detecting inducible clindamycin resistance in a study (Nakasone et al., 2007) that tested 62 strains of *Staphylococcus* spp. In two other studies, the sensitivity for inducible clindamycin resistance detection was 99% (Griffith et al., 2007; Sharp et al., 2009). In a study (Pal et al., 2010) sensitivity and specificity of vitek-2 system is 93 and 100%, respectively. In the present study, the Vitek 2 card failed to detect inducible clindamycin resistance in 2 strains (negative predictive value of 96.6%). Both of these isolates were from clinical specimens of different patients and were found in the different wards of the hospital. There were no false positive results for inducible clindamycin resistance with the vitek-2 card. So, positive results can be reported without further confirmation with D-test. Our findings indicate that the vitek-2 card would allow 96.6% of isolates to be correctly reported as resistant concomitantly with other antimicrobial susceptibility results. Laboratories that want to reach 100% sensitivity would still have to test erythromycin resistant/clindamycin susceptible isolates that showed negative results for inducible clindamycin resistance with the vitek-2 card with the D-test.

The use of vitek-2 system in routine Laboratory will enable us in guiding the clinicians regarding judicious use of clindamycin in skin and soft tissue infections as clindamycin is not a suitable drug for positive inducible clindamycin resistance (ICR) isolates while it can

definitely prove to be a drug of choice in case negative ICR isolates. Vitek-2 system also provides other therapeutic options of antibiotics along with ICR result.

As per our knowledge, this is the first study in India comparing two methods of detection of inducible clindamycin resistance. We raise the concern about the two strains of *S. aureus* in which the iMLS_B were not detected with vitek-2 card.

We conclude that it is important for laboratories to be aware of the local prevalence of iMLS_B isolates. On the basis of their data, they can choose which technique to use for detection of inducible clindamycin resistance in routine depending on their local epidemiology and availability of automated vitek-2 system in their institution. This prevalence of iMLS_B may change over time with the emergence of strains with different sensitivity patterns, so periodic surveys should be performed if testing is not routine.

REFERENCES

- Clinical and Laboratory Standards Institute (2007). Performance standards for antimicrobial susceptibility testing; Seventeenth informational supplement. Clinical Laboratory Standards Institute, 27(1).
- Daurel C, Huet C, Dhalluin A, Bes M, Etienne J, Leclercq R (2008). Differences in potential for selection of clindamycin-resistant mutants between inducible *erm* (A) and *erm*(C) *Staphylococcus aureus* genes. *J. Clin. Microbiol.*, 46: 546–550.
- Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH (2003). Practical disc diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase negative *Staphylococci*. *J. Clin. Microbiol.*, 41: 4740-4744.
- Griffith R, Messina-Powell S, Creely D, Dante M, Ullery M, Ledebor N, Shortridge S (2007). A new vancomycin test and inducible clindamycin resistance test for gram positive organisms with the Vitek 2 systems, abstr. C-014. Abstr. 107th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC.
- Moore ZS, Jerris RC, Hilinski JA (2008). High prevalence of inducible clindamycin resistance among *Staphylococcus aureus* isolates from patients with cystic fibrosis. *J. Cystic Fibrosis*, 7: 206–209.
- Nakasone I, Kinjo T, Yamane N, Kisanuki K, Shiohira CM (2007). Laboratory based evaluation of the colorimetric VITEK-2 Compact system for species identification and of the Advanced Expert System for detection of antimicrobial resistances: VITEK-2 Compact system identification and antimicrobial susceptibility testing. *Diagn. Microbiol. Infect. Dis.*, 58: 191–198.
- Pal N, Sharma B, Sharma R, Vyas L (2010). Detection of inducible clindamycin resistance among *Staphylococcal* isolates from different clinical specimens in western India. *J. Postgrad. Med.*, 56: 182-185.
- Schreckenberger PC, Ilendo E, Ristow KL (2004). Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. *J. Clin. Microbiol.*, 42: 2777–2779.
- Sharp S, Weber K, Bennett K (2009). Detection of inducible clindamycin resistance, *mecA*-mediated oxacillin resistance, and vancomycin resistance with the Vitek 2 GP67 card as compared to the D-test, and cefoxitin and vancomycin disk diffusion testing in clinically significant isolates of *Staphylococcus aureus*, abstr. C-007. Abstr. 109th Gen. Meet. Am. Soc. Microbiol. 2009. American Society for Microbiology, Washington, DC.
- Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L (2005). Testing for induction of clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus*. *J. Clin. Microbiol.*, 43: 1716-1721.