ORIGINAL ARTICLE

Identification of Inducible Clindamycin Resistance in *Staphylococcus aureus* using Automated Vitek-2 Compact System and D test

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ABSTRACT

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogen causing serious and life-threatening clinical infections. Detecting inducible resistance to clindamycin (ICR) in S. aureus is challenging with routine testing methods, potentially leading to treatment failure. Hence, use of both automated systems like VITEK-2 and conventional methods for detecting ICR in routine microbiology laboratories will be helpful in accurate diagnosis. **Aim and Objectives:** To identify S. aureus in clinical samples and assess the reliability of VITEK-2 for detecting inducible clindamycin resistance as compared to routine D-test. **Materials and methods:** A total of 80 isolates of S. aureus were identified from clinical samples by routine conventional microbiological methods and antibiotic susceptibility testing was performed using both automated VITEK-2 and conventional technique. **Results:** The sensitivity of the Vitek-2 test for detecting ICR was 91.18% and specificity was 100% as compared to D-test. The PPV and NPV were 100% and 94.23% respectively. However, 3 isolates which showed ICR by D-test could not be detected in VITEK-2 system. **Conclusion:** Use of both automated systems and routine conventional techniques together in detecting ICR in Staphylococcus aureus will accurately give the diagnosis and accelerate the treatment.

KEY WORDS: VITEK-2, D-test, Resistance, Staphylococcus aureus, Inducible clindamycin resistance (ICR).

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogen causing serious and life-threatening clinical infections[1].MRSA is grouped under HA MRSA (Healthcare associated methicillin resistant *Staphylococcus aureus*) and CA MRSA (Community acquired methicillin resistant *Staphylococcus aureus*). HA MRSA is acquired either during prolonged or frequent hospitalizations and CA MRSA usually affects healthy people owing to transmission of pathogen within community[1,2]. *In vitro* susceptibilities of MRSA strains, especially those from community-acquired infections to clindamycin (CLI), erythromycin (ERY), quinolone antibiotics, tetracyclines, and trimethoprim-sulfamethoxazole have frequently been reported[3,4].Macrolide-lincosamide- streptogramin B (MLS_B) family is most commonly used for the treatment of MRSA and Clindamycin is the frequent choice due to its excellent pharmacokinetic properties[5].

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Widespread use of MLS_B antibiotics has led to an increase in the number of Staphylococcal strains acquiring resistance to MLS_B antibiotics[6]. Macrolide resistance may be due to enzyme encoded by a variety of erm genes. MLS_B phenotypes in S. aureus is of three types, a constitutive resistant phenotype ($cMLS_B$), a clindamycin- susceptible phenotype in vitro with inducible resistance in vivo ($iMLS_B$) and a clindamycin-susceptible and macrolide-streptogramin B-resistant phenotype (MS phenotype)[7].

In case of inducible resistance, inactive mRNA produced by the production of methylases becomes active in the presence of an inducer. But in constitutive resistance active methylase mRNA is produced[1]. The MS and $iMLS_B$ phenotypes are indistinguishable by using standard susceptibility test methods, but it can be identified by erythromycin-clindamycin disk approximation test (D-test), Vitek-2 Compact automated system and demonstration of resistance genes by molecular methods[5].

Staphylococci strains having efflux pump-mediated resistance and inducible erm genes-mediated resistance, makes D-zone test easy to perform[8]. Automated antimicrobial susceptibility testing systems Vitek 2 (bioMe´rieux, Marcy l'Etoile, France) are widely used in clinical laboratories and provide results with shorter incubation times than disk diffusion testing[9].

The aim of this study is to identify S. aureus in clinical samples and assess the reliability of VITEK-2 for detecting inducible clindamycin resistance as compared to routine D-test in a tertiary care hospital.

Materials and methods

This prospective study was conducted from August 2022 to July 2023. A total of 80 isolates of S.aureus were identified from clinical samples like pus, wound swab, tracheal aspirate, blood and sterile fluid are tested. All the S.aureus isolates were identified by conventional microbiological methods including colony morphology, gram stain, catalase, slide and tube coagulase tests[6].

Antibiotic susceptibility and antibiogram

Antibiotic susceptibility testing was performed on all the 80 S. aureus isolates by Kirby Bauer's disc diffusion method on Muller Hinton agar (MHA) plates. Antibiotic discs used were Cefoxitin (30μg), Oxacillin (1μg), Erythromycin (15μg), Clindamycin (2μg), Pencillin (10U), Ciprofloxacin (5μg), Ceftazidime (30μg), Cefotaxime (30μg), Amoxacillin (25μg) as per CLSI guidelines. An inhibition zone of 21 mm or less around cefoxitin disc indicated MRSA[3].

Detection of inducible MLSB resistance (D test) was done by bacterial suspension equal to 0.5 McFarland. For this test, Erythromycin (15μg) and Clindamycin (2μg) discs were placed at 15mm distance edge to edge on MHA plate. Plates were analysed after overnight incubation at 37^0C, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance[3] (Figure 1). Three different phenotypes were isolated after testing and interpreted as follows:

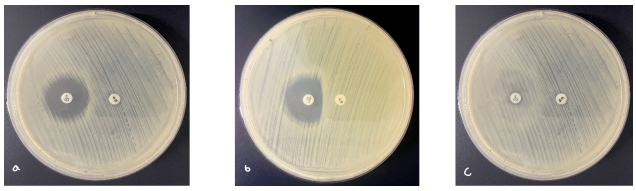

Figure 1: Disc diffusion test for inducible clindamycin resistance (a)Erythromycin resistant and clindamycin sensitive Staphylococcal isolate with circular zone of inhibition around clindamycin suggestive of MS phenotype. (b) Erythromycin resistant and clindamycin sensitive Staphylococcal isolate giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc suggestive of inducible MLSB phenotype. (c) Staphylococcal isolate resistant to both erythromycin and clindamycin suggestive of constitutive MLSB phenotype

I. MS Phenotype: - Staphylococcal isolates exhibiting resistance to erythromycin (zone size $\leq$ 13 mm) while sensitive to clindamycin (zone size $\geq$ 21mm) and giving circular zone of inhibition around clindamycin was labelled as having this phenotype.

II. Inducible MLS_B phenotype ($iMLS_B$): - Staphylococcal isolates showing resistance to erythromycin (zone size $\leq$ 13 mm) while being sensitive to clindamycin (zone size $\geq$ 21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype.

III. Constitutive MLS_B phenotype ($cMLS_B$): - This phenotype are the Staphylococcal isolates which shows resistance to both erythromycin (zone size $\leq$ 13 mm) and clindamycin (zone size $\leq$ 14 mm) with

circular shape of zone of inhibition if any around clindamycin.

Quality control (QC) of the erythromycin and clindamycin discs was performed with S. aureus ATCC 25923, according to the standard disc diffusion QC procedure. Additional QC was performed with separate in-house selected S. aureus strains that demonstrated positive and negative D-test reactions.

Antibiotic susceptibility by Vitek-2 Automated system

The AST card for VITEK-2 system is an automated test methodology based on the MIC technique reported by MacLowry and Marsh and Gerlach. The Vitek 2 AST-GP67 card (bioMe´rieux, Marcy l'Etoile, France) was used according to the manufacturer's recommendations. Briefly, three to five colonies of an 18 to 24 hr 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 standards. 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 were used to detect inducible clindamycin resistance in the Vitek 2 cards. At the end of the incubation cycle, MIC values were determined for each type of antimicrobial on the card[3,10].

The median time to final susceptibility reporting based on the Vitek 2 system was 7 h (range, 6 h 15 min to 12 h 30 min) and showed no difference between MSSA strains (median time, 6 h 45 min) and MRSA strains (median time, 7 h).

Results

All the eighty isolates of S.aureus were tested for susceptibility by routine disc diffusion method and Vitek 2 System. MRSA was identified in 33 (41.25%) S.aureus isolates by cefoxitin screening in both conventional and automated (VITEK-2) methods.

Of the 80 S.aureus isolates, 65 (81.25%) were erythromycin resistant and 70 (87.5%) were clindamycin resistant. All the isolates were tested for D test, out of which, 10 (12.5%) isolates were resistant to both erythromycin and clindamycin showing constitutive MLS_B ($cMLS_B$) phenotype, among which 18.18% were MRSA, as shown in following Table 1.

Positive D-test was seen in 31 (38.75%) isolates indicating inducible MLS_B ($iMLS_B$) phenotype, out of which 69.69% were MRSA, as depicted inTable 1.

Negative D test indicating MS phenotype was seen in 24 (30%) out if which 12.12% were MRSA, as shown in followingTable 1.

Automated (Vitek 2) system was unable to detect 3 positive $iMLS_B$isolates done by D test. PPV and NPV of the Vitek 2 system was 100% and 94.23% respectively. The sensitivity and specificity of the test were 91.18% and 100% respectively (Table 2).

Inducible clindamycin resistance was higher in Methicillin resistant S. aureus (MRSA) as compared to Methicillin susceptible *S. aureus* (MSSA).

Table 1: Distribution of isolates

Susceptibility pattern (Phenotype)	MRSA (%)	MSSA (%)	Total (%)
ERY-S, CL-S	0	15 (31.91)	15 (18.75)
ERY-R, CL-R ($cMLS_B$)	6 (18.18)	4 (8.51)	10 (12.5)
ERY-R, CL-S, D test positive ($iMLS_B$)	23 (69.69)	8 (17.02)	31 (38.75)
ERY-R, CL-S, D test negative (MS)	4 (12.12)	20 (42.55)	24 (30)
Total (n)	33	47	80

*ERY- Erythromycin; CL- Clindamycin; S- Sensitive, R- Resistant; $cMLS_B$- Constitutive resistance to clindamycin; $iMLS_B$- Inducible resistance to clindamycin; MS- MS phenotype.

Following Table 2 shows, prevalence of inducible clindamycin resistance by D test and Vitek-2 system.

Discussion

The increasing frequency of Staphylococcal infections among patients and changing patterns in antimicrobial resistance have led to renewed interest in the use of clindamycin therapy to treat such infections. Clindamycin, a member of the MLSB family, is commonly employed for treating skin and soft tissue infections due to its tolerability, availability in oral form, excellent tissue penetration, good bioavailability, and cost-effectiveness. Macrolide resistance may be constitutive or inducible in the presence of either a macrolide or a lincosamide inducer[11].

The VITEK systems from bioMerieux in Marcy l'Etoile, France, are fully automated instruments

Table 2: Prevalence of inducible clindamycin resistance by D test and Vitek-2 system

	No. of isolates with,		
	D test positive (no.= 34/80=42.5%)	D test negative (no.= 46/80=57.5%)	Total
Vitek- 2 test positive (31/80=38.75%)	c31	0	31
Vitek- 2 test negative (49/80=61.25%)	3	46	49
Total	34	46	80
Sensitivity (%)	91.18		
Specificity (%)	100.0		
PPV (%)	100.0		
NPV (%)	94.23		

*PPV: positive predictive value *NPV: negative predictive value.

widely employed in clinical microbiology laboratories globally. These systems play a crucial role in species identification and antimicrobial susceptibility testing for various clinical isolates. VITEK Advanced Expert System (AES) is designed to analyze antimicrobial susceptibility testing (AST) results by utilizing a well-established knowledge base encompassing around 100 species and 20,000 MIC ranges. This allows the system to identify over 2,300 phenotypic antimicrobial resistances[12].

The analysis revealed a higher prevalence of both inducible resistance and constitutive resistance in MRSA compared to MSSA, with rates of 69.69% and 18.18% for inducible resistance and 17.02% and 8.51% for constitutive resistance, respectively. These findings align with several earlier studies[13–16].

In the present study, we compare the reliability of automated (Vitek-2) system for detection of ICR with the results of the D-test. The sensitivity of the Vitek-2 test was 91.18% and specificity was 100%. The PPV and NPV were 100% and 94.23% respectively. The automated system failed to detect 3 isolates as ICR positive while confirmed by D test. These isolates were from clinical specimen of different patients and were found in the different wards of the hospital.

One potential explanation for the false negatives in the Inducible Clindamycin Resistance (ICR) test could be an insufficient incubation time in the Vitek-2 system for the induction process to take place. The card typically undergoes incubation for a duration of 4–10 hours, with the specific time dependent on factors such as the inoculum size and the growth characteristics of the organism. In cases where a slower-growing organism is inoculated at the lower end of the recommended range (0.5–0.63 MacFarland), an incomplete incubation time might contribute to a false negative result[17].

The VITEK system reported similar sensitivities by Griffith et al.[18], Pal et al.[19], Jethwani et al.[5], Lavallée et al.[9], Buchan et al.[20], Nimmo et al.[21], Gardiner et al.[17], Heba-Allah et al.[10], reported a 99%,93%, 95.4%, 93%, 91.1%, 92.5%, 95%, 85.7% respectively. The specificity and PPV of Vitek-2 test in this study were 100%. Numerous studies, including those conducted by Heba-Allah et al.[10], Nakasone et al.[12], Jethwani et al.[5], and Lavallée et al.[9], have validated our findings, highlighting the consistent specificity of the test without any occurrences of false positives. These researchers advocated for the reporting of positive VITEK 2 results without the necessity for confirmation through the D test.

Considering the limited range of antibiotics accessible for treating methicillin-resistant staphylococcal infections and the acknowledged constraints associated with vancomycin, it is advisable to contemplate clindamycin as a viable option for managing serious soft tissue infections caused by methicillin-resistant Staphylococci that exhibit sensitivity to clindamycin[14].

The use of Vitek-2 system in routine laboratory will enable microbiologists 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[5].

The Vitek 2 results indicated a consistent time to response for both MSSA and MRSA strains. Notably, the Vitek 2 system facilitated a rapid response within regular working hours ($\leq$8 hours) for 88.7% of S. aureus isolates. In contrast, the disk diffusion method necessitated a longer incubation period of 24 hours, following the guidelines recommended by the CLSI[3]. Implementing the D test in routine

laboratory procedures helps guide clinicians in making informed decisions about the appropriate use of clindamycin in the treatment of skin and soft tissue infections. Identifying D test positive isolates indicates that clindamycin may not be the most suitable drug for treatment. Conversely, D test negative isolates suggest that clindamycin can be considered as a preferred drug for effective treatment[22].

Conclusion

Our article suggests that use of both Automated systems and routine conventional techniques together in detecting resistance pattern of Staphylococcus aureus will accelerate the diagnosis and further assist the clinicians in tailoring antibiotic therapy based on the specific characteristics of the bacterial isolates, optimizing patient care.

Conflicts of Interest

Nil

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