Determination of Inducible Clindamycin Resistance and Correlation with Vitek2 Inducible Clindamycin Resistance Test in *Staphylococcus aureus* Isolated from Clinical Samples

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ABSTRACT

Objective: To compare the performance of Vitek2 with the gold standard D test in terms of inducible clindamycin resistance (ICR) detection.

Study Design: Descriptive study.

Place and Duration of the Study: Indus Hospital and Health Network Karachi, Pakistan, from November 2021 to April 2022.

Methodology: Standard operating procedures of the laboratory were followed for processing of clinical samples. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were included. The isolates from the same patient within two-week time period were excluded. Clinical laboratory standards institute guidelines were followed for performing and interpreting D test. The results of the D test were compared with Vitek2 results for ICR.

Results: A total of 313 isolates were MRSA, of which 93 isolates tested positive for ICR on both the D test and Vitek2. Nine isolates were positive for ICR on Vitek2 and negative on the Kirby-Bauer disk diffusion method. One isolate tested positive on the disk method and negative on Vitek2.

Conclusion: Vitek 2 appeared to give false positive results. Reporting false susceptibility of clindamycin can cause therapeutic failure which can markedly affect the patient's outcome. This discordance needs to be investigated further with a large sample size and stringent observation of D-test results to pick laboratory error.

Key Words: *Methicillin-resistant Staphylococcus aureus, Inducible clindamycin resistance, D zone, Vitek2.*

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INTRODUCTION

A significant number of infections in hospitals and general population are caused by *Staphylococcus (S.) aureus*.[1] The treatment options for treating methicillin-resistant *Staphylococcus aureus* (MRSA) are limited due to rising antimicrobial resistance (AMR).[2] Upto 80% of nosocomial *S. aureus* infections worldwide are caused by MRSA, which increases mortality, morbidity, hospital stays and expenses. According to the World Health Organization (WHO), patients with MRSA infections had a 64% higher mortality rate than other infections. MRSA can spread from one person to another through personal contact.[3] Rising resistance in *Staphylococcus aureus* is challenging due to limited treatment options.[4]

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Clindamycin, a macrolide lincosamide streptogramin-B (MLS-B) is an important antibiotic. This is an appropriate option for treating skin, bone and joint infections. It has low cost, few side effects, and good absorption both orally and parenterally.[5,6] The 50S ribosomal subunit is inhibited by all antibiotics in the MLS-B group, preventing protein synthesis.[7,8] Clindamycin resistance can be constitutive and inducible. When inducers like erythromycin are present, the inducible resistance manifests. It happens because the expression of clindamycin resistance is induced by the erythromycin ribosome methylase (*erm*) gene.[8,9]

Different resistance mechanisms for ICR have been identified in *S. aureus*. Active efflux pumps are encoded by *msr* gene while drug inactivation is caused by *lun* gene. Another important mechanism is by erm genes *ermA, ermB, ermC,* and *ermF*. These *erm* genes cause alteration of ribosomal binding by methylation of 23S rRNA gene. The primary *erm* genes involved are *ermA* and *ermC*.[9] When there is resistance to both erythromycin and clindamycin, which is caused by the constant presence of methylase in the isolate is called Constitutive (cMLSB) phenotype. Inducible clindamycin-resistant isolates

appear resistant to erythromycin only in presence of erythromycin. In this case there is flattening towards erythromycin giving an impression of D inhibitory zone, giving the impression that they are mistakenly susceptible to clindamycin (an inducible MLSB phenotype). The isolates that exhibit resistance to erythromycin and sensitivity to clindamycin have MS phenotype.[10] Failure to identify inducible clindamycin resistance could result in improper usage of medicine and unsuccessful clinical treatments.[11] Erythromycin clindamycin disc approximation test (D test) is advised by the Clinical Laboratory Standards Institute (CLSI) to identify inducible clindamycin resistance. This study was conducted to compare the effectiveness of Vitek2 and CLSI gold standard D test for identifying inducible clindamycin resistance in MRSA isolates from various clinical samples.

METHODOLOGY

This was a descriptive cross-sectional study conducted in the Microbiology laboratory of Indus Hospital and Health Network, Karachi from November 2021 to April 2022. The clinical samples included blood, respiratory, pus, tissues, and wound swabs from individuals of all ages and genders, including outpatients, inpatients, and those from emergency departments. Exclusion criteria were duplicate MRSA isolates within two weeks, Methicillin susceptible *Staphylococcus aureus* (MSSA) isolates and urine samples. The sample size was calculated from prevalence of MRSA found in the Pakistani population (36%).[12] The confidence interval was set at 95% and precision at 6%. The sample size was calculated online by using open EPI. The sample size calculated was 246, but this study was conducted on 313 isolates. The study was approved by the ethical review committee of Indus Hospital and Health Network, Karachi. The institutional review board number was obtained [Reference code: IHHN_IRB_2021_09_007].

Staphylococcus aureus isolates growing on 5% Sheep blood agar (SBA) were identified by colony morphology, Gram stain, and biochemical tests including catalase test, coagulase test, and DNASE test. Cefoxitin (30 µg) discs were used to identify MRSA isolates. Cefoxitin inhibition zone size ≤21 mm was regarded as the threshold for methicillin resistance, and ≥22 mm indicated methicillin susceptibility.[13] CLSI guidelines were followed for performance of D test. Mueller Hinton agar plates were used for inoculation. The erythromycin discs (15µg) and clindamycin discs (2µg) were spaced 15 to 26 mm apart. Plates were incubated for 24 hours at 37°C in ambient air. ICR was interpreted as flattening of zone adjacent to erythromycin disc. The MS phenotype was detected when there was no resistance to clindamycin (D test was negative). The isolates that showed flattening towards erythromycin (D test positive) were found to be resistant to erythromycin and sensitive to clindamycin. These isolates have ICR phenotype called iMLSB phenotype. Isolates resistant to both were interpreted as constitutive clindamycin resistance (cMLSB). Isolates sensitive to both were interpreted as susceptible phenotype.[14]

Bacterial suspension equal to 0.5 McFarland turbidity standard was prepared. Vitek2 ASP card for Gram-positive bacteria was inoculated and incubated according to the manufacturer's instructions. ASP-GP71 card was used to detect the ICR with two wells. The well with combination of 0.25µg/ml of clindamycin and 0.5 µg/ml of erythromycin was used to detect ICR. After recommended incubation, the results were noted. ATCC strains were used for quality of biochemical tests and Vitek2.

Statistical analysis was performed on SPSS 26.0 version. Sensitivity, specificity, positive predictive value, and negative predictive value, were calculated. Percentages were calculated for types of samples and phenotypes found in MRSA isolates.

RESULTS

MRSA isolated were 313, and both D zone and Vitek2 ICR test were positive for 93 isolates. Nine isolates were positive only on Vitek2 and one was positive only on the D test. The sensitivity of Vitek2 ICR was 98.93% whereas specificity was 95.90%. Positive predictive value was low (91.2%) whereas the negative predictive value was high (99.2%) as shown in Table I. The percentage of susceptible isolates to both erythromycin and clindamycin was 32.6%, cMLSB were 8.6%, MS phenotype D negative were 28.4%, and iMLSB were 29.7%. The percentage of different phenotypes was tested by D test is shown in Table II. Samples from which MRSA was isolated included pus and wound cultures 64.53% (n=202), respiratory samples 15.33% (n=48), blood 11.2% (n=35), tissues 3.8% (n=11), and others 6.3% (n=20).

Table I: Comparative performance of Vitek2 with D test in MRSA isolates in terms of inducible clindamycin resistance (No=313).

Vitek2 ICR	D test status		Total
	Positive	Negative	
Positive	93	09	102
Negative	01	210	211
Sensitivity	98.93%		
Specificity	95.90%		
Positive predictive value	91.2%		
Negative predictive value	99.5%		

Table II: Phenotypic susceptibility pattern of clindamycin and erythromycin in MRSA by D-test.

Phenotype	N (313)	Percentage (%)
ER-S, CL-S	102	32.6
ER-R, CL-R (cMLS-B)	27	8.6
ER-S, CL-R	02	0.6
ER-R, CL-S (D -ve) (MS)	89	28.4
ER-R, CL-S (D +ve) (iMLS-B)	93	29.7

ER (Erythromycin), CL (Clindamycin), R (Resistant), S (Sensitive), cMLS-B (constitutive macrolide linco-samide streptogramin B), iMLS-B (inducible macrolide lincosamide streptogramin B), D –ve D test negative, D +ve D test positive.

DISCUSSION

MRSA is a common pathogen causing infections in community. Rising antimicrobial resistance is a global threat responsible for increasing morbidity and mortality. Therefore, antibiotics with better bioavailability, easy route of administration, and reduced adverse effects are needed. Clindamycin has favourable pharmacokinetics. Appropriate laboratory testing

for ICR is mandatory to prevent therapeutic failure. The Vitek2 is a commercial system used for identification and antimicrobial susceptibility testing including ICR in *S. aureus*. The present study showed that 32.6% of isolates with clindamycin sensitivity and erythromycin resistance belong to inducible phenotype which can be reported as false susceptible if tested alone.

Vitek2's sensitivity was 98.93%, specificity was 95.90%, positive predictive value (PPV) was 91.2% and negative predictive value (NPV) was 99.2% according to the current study. Almost similar sensitivities of 99% were reported by Griffith *et al*.[15] Lower sensitivities of 91.1% were reported by Buchan *et al*.,[16] 93% by Lavalle *et al*.[17] and 95% by Gardiner *et al*.[18] Very low sensitivity of 36% was reported by Tazi *et al*.,[19] they examined ICR in group B streptococcal isolates.[20] The present study reported failure of detection of ICR by Vitek2 in one isolate. Bobenchik *et al*.[21] reported failure of detection of ICR by Vitek2 in 6 staphylococcal isolates which was confirmed by CLSI D test. Hassan *et al*.[20] reported failure of detection of ICR in one isolate out of six positive ICR isolates on Vitek2 with specificity calculated as 94% which is in concordance with this study's specificity of Vitek2 that was 95.90%. This result was discordant with most other studies which reported 100% specificity of the Vitek2 with no false positive results.

In another study, Vitek2 failed to detect ICR in 2 isolates which were confirmed by D test. The sensitivity, specificity, positive predictive value, and negative predictive value were calculated as 85.7%, 100%, 100%, and 84.6%, respectively.[22] Gardiner reported concordance between positive D zone and ICR but negative ICR on Vitek2 showed positive D zone test similar to this study.[18] Another study showed high concordance between Vitek2 and D zone with sensitivity of 98.8%, specificity of 98.2%, and agreement was 98.6%.[23] Jethwani *et al.* also showed concordant results for two negative ICR isolates which were positive on D zone.[24]

This study included only MRSA for ICR and D test and it is a single-centre study so results could not be generalised. Variables related to performing D test by Kirby-Bauer disc diffusion method can affect the outcome. Results of nine isolates positive on Vitek2 ICR but negative on D test were not verified by repeating D test because the isolates were not saved in the repository. Molecular test to evaluate genes responsible for inducible resistance was not performed due to limited resources.

CONCLUSION

Vitek 2 appeared to give false positive results. This discordance needs to be investigated further with a large sample size and stringent observation of D-test results to pick laboratory error because mistake in placing erythromycin and clindamycin discs at recommended distance can lead to such discordance. Reporting false susceptibility of clindamycin can cause therapeutic failure which can markedly affect the patient's outcome.

ETHICAL APPROVAL:
This study was conducted after receiving approval by the Institutional Review Board of Indus Hospital and Health Network, Karachi (Reference code: IHHN_IRB_2021_09_007).

PATIENTS' CONSENT:
Informed, written consent was taken from each participant enrolled in the study.

COMPETING INTEREST:
The authors declared no completing interests.

AUTHORS' CONTRIBUTION:
AZ: Performed the study, wrote the manuscript.
NK: Designed the study, critical proofing, and analysis of results.
FA: Supervised the research, critical proofing.
QZ: Did literature research, data collection.
All authors read and approved the study for publication.

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