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ORIGINAL ARTICLE

Performance of Vitek-2 System for Detection of Inducible Clindamycin Resistance among Clinical Isolates of Staphylococci in Comparison to the D-Test

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Key words:

Inducible clindamycin resistance,
Vitek-2,
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ABSTRACT

Background: *Clindamycin resistance among clinically significant staphylococcal isolates is now increasing. Routine testing of staphylococcal isolates for inducible clindamycin resistance (ICR) is advocated by the Clinical Laboratory Standards Institute (CLSI). Automated system Vitek 2 offers a panel that detects inducible clindamycin resistance directly. it is easy and more cost-effective than the more labor-intensive CLSI reference methods.* ***Objectives:*** *The aim of this study was to assess the performance of Vitek-2 system for ICR detection against the reference D-test, as well as to detect the presence of erm-A and erm-C genes among these isolates.* ***Methodology:*** *22 clinical staphylococcal isolates (15 Staph aureus and 7 coagulase negative Staph (CoNS) were examined for ICR both by D- test and Vitek-2 system. multiplex PCR was performed for the isolates to detect ermA and ermC genes.* ***Results:*** *Out of the 22 isolates, ICR was detected by Vitek-2 in 6 isolates (27.7%). five of the 6 isolates were confirmed by D-test. sensitivity, specificity, positive and negative predictive values were calculated as 100%, 94.1%, 83.33% and 100% respectively. ermC gene was detected in 20/22 isolates, 5 isolates were positive for both genes.* ***Conclusion:*** *Vitek-2 is considered a potentially reliable test for detection of ICR, further studies are recommended on large number of isolates.*

INTRODUCTION

Methicillin resistant Staphylococci *(MRS)* with decreased susceptibility or resistance to glycopeptides have been isolated[1]. Alternative treatments such as macrolides (e.g. erythromycin), lincosamides (clindamycin), and streptogramin B (quinupristin-dalfopristin) antimicrobial agents (collectively known as MLSB agents) have been used to treat *staphylococcal* infections[2].

There are two primary mechanisms that provide resistance to macrolide antibiotics. Among Staphylococci, the gene *msr* A encodes efflux pump which is a primary mechanism of defense and quite common in some geographical areas. The second mechanism includes modification of drug binding sites on the ribosomes that also enhances resistance to macrolides[3]. These two mechanisms promote resistance to macrolides, lincosamides and streptogramins B group of antibiotics and t*erm*ed as MLSB resistance.

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An *erm* gene (usually *erm* A or *erm* C) encodes methylation of 23S rRNA- binding site, which is shared commonly by these three drug classes[4].

As regards *erm* genes they are mainly borne by plasmids and transposons which are capable of being self-transferable. Twenty one classes of *erm* genes and as many corresponding *erm* proteins gets differentiated by this current nomenclature system. *erm* A, *erm* B, *erm* C, and *erm* F are the four major classes that are seen in pathogenic microorganisms The *erm* A and *erm* C det*erm*inants are predominant in Staphylococci[5]. The *erm* A genes are mainly spread in methicillin resistant strains which are borne by transposons, and *erm* C genes are frequently responsible for erythromycin resistance in methicillin-susceptible strains that are plasmids borne, Whereas *erm* B class genes are mainly restricted to Streptococci and Enterococci, and the *erm* F class genes to Bacteroides species and other anaerobic bacteria[6].

The expression of MLSB resistance can be inducible (iMLSB) or constitutive (cMLSB)[7]. Staphylococcal isolates with constitutive resistance in vitro demonstrate resistance to both erythromycin and clindamycin whereas isolates that harbor inducible resistance are resistant to erythromycin but appear susceptible to clindamycin (iMLSB). Inducible clindamycin resistance is caused by a ribosomal

methylase encoded by *erm* genes[8]. The inducible phenotype is expressed only in the presence of macrolides, but not lincosamides[9].

The Clinical and Laboratory Standards Institute (CLSI) recommends testing of erythromycin-resistant and clindamycin susceptible isolates of Staphylococcus spp. by either D-zone test or broth microdilution to detect inducible clindamycin resistance[10]. However, previous reports demonstrated that the inducible resistance (MLSBi) cannot be routinely detected by standard broth- or agar-based susceptibility test methods[11,12]. It is important to distinguish the MLSBi strains from macrolide-resistant strains that contain the gene *msr*(A), encoding an efflux pump that affects only macrolides, not clindamycin[13].

Fernandes and his colleagues[14] described performing the automated system Vitek 2 which offers a panel that detects inducible clindamycin resistance directly. Commercial automated systems for identification and susceptibility testing of bacteria are used in most clinical microbiology laboratories in the United States. Due to their ease of use and cost-effectiveness they are often the preferred methods over the more labor-intensive Clinical and Laboratory Standards Institute (CLSI) reference methods of broth microdilution (BMD) and disk diffusion (DD)[15].

AIM OF THE WORK

The aim of this study was to evaluate the performance of Vitek-2 system for detection of inducible clindamycin resistance among a group of Staphylococci isolated from different clinical samples, in comparison to D-test as well as to detect the presence of erm-A and erm-C genes among these isolates.

METHDOLOGY

Bacterial isolates:

A total number of 22 clinical isolates of *Staphylococci* [15 Staphylococcus aureus and 7 coagulase negative Staphylococci (CoNS) obtained from different samples from the Department of Medical Microbiology; Ain shams University, in the period between January 2015 to June 2015 were included in the study. Isolates were collected from different clinical specimens. All the isolates of Staphylococcus spp. included in the study were resistant (zone ≤13 mm) or intermediate (zone 14-22 mm) to erythromycin but susceptible to clindamycin (zone ≥ 21mm) according to

Detection of inducible clindamycin resistance:

The D-zone test performed according to the CLSI guidelines[10]:

Three to five colonies of a 24-hour-old culture of Staphylococcus spp. were suspended in a 0.45% NaCl solution and adjusted to a concentration equivalent to a 0.5 McFarland standard. The solution after that was streaked on Muller Hinton agar plates (standard disk diffusion procedure). Erythromycin (15µg) and clindamycin (2µg) disks (Oxoid, United Kingdom) were placed, separated by 15mm. the plates were incubated overnight at 37°C.

Flattening of the zone of inhibition adjacent to the erythromycin disks (D zone) was considered a positive result. (Figure 1)

Fig. 1: A Muller Hinton agar plate showing positive D-test

The Vitek 2 AST-P580 card (bioMe´rieux, Marcy l'E´toile, France):

The procedure was performed according to the manufacturer's instructions. Briefly, three to five colonies of a 24-h-old culture of Staphylococcus spp. were inoculated in a 0.45% NaCl solution and adjusted to a concentration equivalent to a 0.5 McFarland standard. Suspensions were vortexed before ID cards inoculations. Cards were inoculated within 15 minutes. Cards were allowed to reach room temperature. Protective package liners were removed and cards were inspected for holes or cracks in the foil material.

The suspensions were then loaded with the card in the Vitek 2 system for further dilution and card filling. Two wells were used to detect inducible clindamycin resistance in the Vitek 2 card: one with 0.5 mg of clindamycin/liter and the other contains a combination of 0.25 and 0.5 mg of clindamycin and erythromycin/liter, respectively according to Lavallee et al[16].

Detection of ermA and ermC genes by multiplex PCR:

DNA extraction.

Extraction was done using QIAamp® DNA Blood Mini Kits (Catalog no. 51104, 51106) (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol with prior heating to 100°C for 10 minutes.

A 2-McFarland-standard bacterial suspension was prepared in saline, and bacterial DNA was extracted from 200µl ($1.2X10^8$ CFU) of the suspension. Purified DNA was eluted from the QIAamp Spin Column in a concentrated form in either Buffer AE. Elution buffer was applied to the column. The QIAamp Spin Column was incubated with the elution buffer at room temperature for 5 minutes before centrifugation to

increase the yield. Purified DNA was stored at –20°C in Buffer AE (10 mM Tris·Cl; 0.5 mM EDTA; pH 9.0).

DNA amplification.

Four primers were used to perform a multiplex PCR protocol, to detect both *ermA* and *ermC* . The primers were designed according to Lina et al[5]. The sequence of the first pair targeting *erm A gene* was: 5′GTTCAAGAAC AATCAATACA GAG3′ and 5′GGATCAGGAA AAGGACATTT TAC3′ amplifying a 421 bp DNA fragment. The sequence of the second pair targeting *ermC:* 5′GCTAATATTG TTTAAATCGT CAATTCC3′ and 5′GGATCAGGAA AAGGACATTT TAC3′ amplifying a 572 bp DNA fragment.

PCR was carried out in 50 µl volume reaction mixtures containing 0.5µl of each primer, 10 µl of crude template DNA and 25 µl Qiagen master mix. The annealing temperature was 55°C for *ermA* and *ermC*. PCR products were separated by gel electrophoresis on 2% agarose gel containing 0.5µg/ml ethidium bromide (Figure 2)

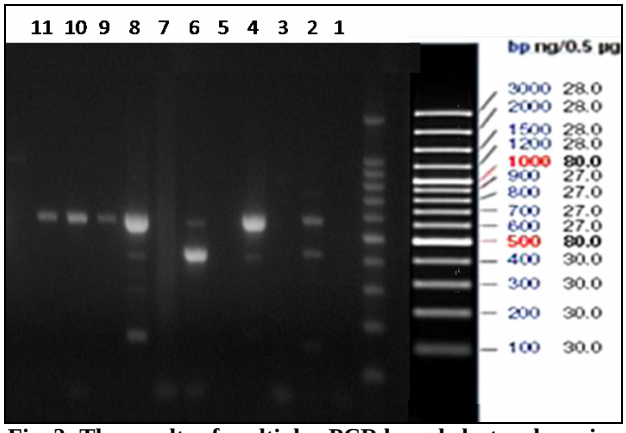

Fig. 2: The results of multiplex PCR by gel electrophoresis

RESULTS

The present study was conducted on 22 Staphylococcal clinical isolates from the Medical Microbiology and Immunology laboratory-Ain Shams University. All the isolates were erythromycin resistant and clindamycin sensitive by the Kirby Bauer disk diffusion method. 15 isolates were identified as *Staph aureus*, (of which 8 isolates were identified as MRSA using standard methods according to the CLSI guidelines). 7 isolates were coagulase negative Staph.

Out of the 22 isolates, 5 (22.7%) cases were positive for inducible clindamycin resistance (ICR) by the D test.

The results of Vitek-2 showed a 100% agreement with the results of conventional methods regarding identification of the tested strains as *Staph aureus* and *coagulase negative Staph*. In addition, regarding the results of antimicrobial resistance for erythromycin and clindamycin compared to the results of Kirby Bauer disk diffusion method (100% of isolates were erythromycin resistant and clindamycin sensitive by Vitek-2 test). (Table 1)

Regarding ICR detection by Vitek-2, 6 (27.7%) isolates were positive, one of them was not confirmed by D test. the sensitivity of Vitek-2 ICR was calculated as 100%, and a specificity of 94.1%. positive and negative predictive values were 83.33% and 100% respectively. (Table 2)

The results of multiplex PCR showed that 20(91%) of the tested isolates were positive for erm C gene. of which 5 isolates (22.7%) were positive for both genes, and 2 isolates (9%) were negative for both genes.

The Vitek-2 card used in this study performed testing of oxacillin and cefoxitin resistance for identification of mecA resistance (methicillin resistance). Among *Staph aureus* isolates, 13 isolates were oxacillin resistance, of which 8 isolates were confirmed by cefoxitin resistance. 6 out of 7 CoNS were oxacillin resistance by Vitek-2, none of them was confirmed by cefoxitin resistance testing.

The results of conventional identification of MRSA agreed with the vitek-2 test in 10/15 (66%) of *Staph aureus* isolates. the discordance rate was 5/15 (33%). (Table 3)

Table 1: The results of antimicrobial sensitivity by Vitek-2 test

	Staph aureus (N = 15)			***Coagulase negative Staph (N = 7)***		
	S	**I**	**R**	**S**	**I**	**R**
Erythromycin	-	1	14	-	-	7(100%)
Clindamycin	15(100%)	-	-	7(100%)	-	-
ICR	-	-	5 (33%)	-	-	1(14%)
Oxacillin	2(13%)	-	13(87%)	1(14%)	-	6(86%)
Vancomycin	10	-	5	7(100%)	-	-
Linzeloid	10	-	5	7(100%)	-	-
Trimethoprim	12(80%)	-	3(20%)	6(86%)	-	1(14%)
Rifampin	9(60%)	1(7%)	5(33%)	7(100%)	-	-
Mupirocin	12(80%)	-	3(20%)	7(100%)	-	-
Nitofurantine	15(100%)	-	-	7(100%)	-	-
Tigecycline	11(73%)	-	4(27%)	7(100%)	-	-
Levofloxacin	7(47%)	-	8(53%)	6(86%)	-	1(14%)
Tobramycin	5(33%)	1(7%)	9(60%)	6(86%)	-	1(14%)
Cefoxitin	7 (47%) -ve	-	8 (53%) +	-	-	7(100%) -ve

Table 2: The results of Vitek-2 compared to the results of D-test

	D-test +ve	***D-test –ve***
Vitek-2 test +ve	5	1
Vitek-2 test –ve	0	16
Sensitivity: 100%		
Specificity: 94.1%		
PPV: 83.33%		
NPV: 100%		

Table 3: Results of detection of mecA oxacillin/cefoxitin resistance among *Staphylococcal* isolates by Vitek-2 and standard methods

Oxacillin/cefoxitin sensitivity by Vitek-2	***Diagnosis of MRSA by standard methods***	
	MRSA (N= 8)	**MSSA (N= 7)**
+ve Vitek-2 test	8	5
-ve Vitek-2 test	-	2

DISCUSSION

Increasing prevalence of community-acquired MRS, 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 and good bioavailability[17]. Resistance to clindamycin is highly variable in different patient populations so rapid susceptibility testing for clindamycin resistance must be available[18].

Several previous studies assessed the performance of Vitek-2 system. Previous reports recommended that The ICR test is reliable in the presence of a positive result; however, there is a false negative rate of approximately one in four. This will lead to susceptibility reporting errors, with potentially serious clinical implications. A negative ICR should be confirmed by CLSI D-test before reporting clindamycin as susceptible where the organism is not susceptible to erythromycin[19].

In the present study, by comparing the automated Vitek-2 system for detection of ICR with the results of the D-test as a gold standard technique. The sensitivity of the Vitek-2 test was 100% and the specificity was 94.1%. The positive and negative predictive values were 83.33% and 100% respectively.

Similar sensitivities were reported, 98% sensitivity was reported in a study that tested 62 strains of Staphylococcus spp. [20]. Griffith et al[21] and Sharp et al[22] reported a sensitivity of 99% for inducible clindamycin resistance detection using Vitek-2 system.

Buchan et al[23], Lavallee et al[16] and Gardiner et al[19] reported a lower sensitivities of 91.1%, 93% and 95% respectively.

A very low sensitivity for Vitek-2 test (36%) was reported by Tazi et al[24], they examined ICR in a group of group B streptococcal isolates. also Bobenchik et al[15] reported failure of detection of ICR by Vitek-2 in 6/30 (20%) of resistant Staphylococcal isolates confirmed by CLSI standard methods. They considered Vitek-2 as a non-reliable test for ICR detection among the tested isolates.

In our study, considering the D-test as the gold standard technique, the specificity of Vitek-2 test was calculated as 94.1%. One out of the 6 isolates identified as ICR was not confirmed with the D-test. This result disagreed with the results of most previous studies who reported a 100% specificity of the test with no false positive results. They recommended that positive vitek-2 results should be reported without confirmation by D-test[15,16]. Buchan et al[23] tested 524 clinical Staphylococcal isolates; they reported a specificity of the test of 99.8%.

In the present study, ICR was detected by D-test in 5/22 (22.7%) of the tested isolates, this rate was lower than that reported by other studies. This percentage was very low when compared with the results of Gardiner et al[19], who detected ICR in 191/217 (88%) of Staphylococcal isolates. also, Lavallee et al[16] who detected ICR in 134 isolates out of 163 (82%) using D-test. However, similar to our results Bobenchik et al[15] reported that ICR could be detected in 30/134 (22.3%) of the tested Staphylococcal isolates.

The presence of erm genes (A or C) in 20/22 (91%) of the isolates in this study goes with erythromycin resistance. the two negative isolates were also negative for ICR by both the phenotypic methods used. Similarly, Huang et al[25] detected ermA and/or ermC in 97.7% of the *Staph aureus* strains with erythromycin resistance.

Interestingly, in the study by Lavallee et al[16], one strain of Staph aureus was D-zone test positive and positive for inducible clindamycin resistance by the agar dilution method and by the Vitek 2 card but was negative for *ermA* or *ermC*. This strain was tested in another laboratory for the presence of *ermB* by PCR and was negative for this gene. They referred this finding possibly to the presence a previously described *ermY* gene. In addition, mutations at the target sites of primers

for *ermA*, e*rmB*, and *ermC* could be responsible for this observation.

In this study, there was a 100% agreement between Vitek-2 test and the standard methods regarding antimicrobial sensitivity testing of erythromycin and clindamycin. Regarding MRSA detection, the results of Vitek-2 test agreed with the standard methods in 66% of Staph aureus isolates. We recommend detection of mec genes in the discordant cases as a gold standard to evaluate the sensitivity of the test.

CONCLUSIONS

Routine testing for inducible clindamycin resistance is important to identify those strains that remain susceptible to clindamycin despite macrolide resistance, in order to circumvent treatment failure.

The results of this study points to the Vitek-2 system as a potentially reliable method for bacterial identification and antimicrobial testing including inducible clindamycin resistance.

A limitation of this study was the small number of isolates tested.

Further studies are strongly recommended on large number of isolates and different types of microorganisms to evaluate the utility of the Vitek-2 automated system.

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