Automated Vitek-2 System versus D Test in Detection of Inducible Clindamycin Resistance Staphylococcus aureus

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ABSTRACT

Background: Macrolide, lincosamide and streptogramin type B (MLS) antibiotics are important in the treatment of Staphylococcus aureus infections and existence of isolates with ability to resist against MLSB antibiotics is worrisome. 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: Evaluation the utility of automated vitek-2 system in detection of inducible clindamycin resistance S. aureus compared to D zone test and detection the prevalence of erm A and erm C genes among isolated strains. Method: 25 clinical staphylococcus aureus isolates (Erythromycin intermediate and resistant, Clindamycin susceptible) 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 25 isolates, ICR was detected by Vitek-2 in 11 isolates (44%). Two of the isolates were not detected by Vitek-2 but confirmed by D-test. sensitivity, specificity, positive and negative predictive values were calculated as 85.7%, 100%, 100% and 84.6% respectively. erm C and A genes were detected in (40%) and (24%) of the studied isolates respectively. Both C and A genes were detected in (12%). 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 Staph aureus (MRSA) were known as the most important pathogens that were frequently isolated, and caused serious and life threatening clinical infections such as nosocomial and community-acquired infections. Vancomycin and teicoplanin are commonly used to treat the infections with MRSA, however, recently isolation of S. aureus with decrease susceptibility or resistance to glycopeptides caused encourage of physicians to prescribe of other alternative treatments such as Macrolide - Lincosamide - Streptogramin (MLS). 

Clindamycin is often utilized for the treatment of skin, soft tissue, bone and joint infections due to S. aureus because of its high oral bioavailability and good tissue penetration. It is less costly with fewer side effects than alternative anti-staphylococcal agents such as vancomycin, linezolid and daptomycin, and may provide additional benefit by inhibiting toxin production due to its mechanism of action (inhibition of protein synthesis by binding to the 50S subunit of the bacterial ribosome).
In this context, clindamycin is considered a good option, because of its action against biofilm formation and bacterial adherence, a high level of bone and joint penetration, and a good tolerance. Also, clindamycin induces bacterial resistance only slowly. (3) Resistance to MLS antibiotics among staphylococci can be occurred by various mechanisms, including: an active efflux pump encoded by msrA gene (cause resistance to macrolids and type B streptogramins, and not to clindamycin, Enzymatic inactivation of antibiotic and ribosomal target modification that is the major mechanism of resistance and affects macrolides, lincosamides, and type B streptogramins (MLS resistance). In staphylococci, the four genes, ermA, ermB, ermC and ermF, are frequently involved in resistance to MLS. (1)

Three MLSB phenotypes are known in S. aureus, a constitutive resistant phenotype (cMLSB), a clindamycin-susceptible phenotype in vitro with inducible resistance in vivo (iMLSB), and a clindamycin-susceptible and macrolide-streptogramin B-resistant phenotype (MSB). (4)

Inducible resistance is observed when the inactive mRNA produced by the production of methylases becomes active in the presence of an inducer, while active methylase mRNA is produced in strains where constitutive expression is seen. The strains carrying the inducible erm gene are resistant to the inducer and remain susceptible to non-inducer macrolides and lincosamides. (5)

A simple laboratory test (as titled D-zone test) can differentiate between staphylococci that have inducible erm genes-mediated resistance and those which have efflux pump-mediated resistance (6).

When an organism expressing iMLSB resistance is tested according to Clinical and Laboratory Standards Institute (CLSI) methods with a 15-mg erythromycin disk placed close to a 2- mg clindamycin disk, the zone of inhibition around the clindamycin disk is flattened to form a “D” shape (positive D-test), whereas in the MS phenotype, the clindamycin zone remains circular. (7).

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 (CLSI) reference methods of broth micro-dilution (BMD) and disk diffusion (DD) (8).

The automated system Vitek 2 (bioM'érieux, Marcy l'E' toile, France) now offers a panel that detects inducible clindamycin resistance directly(9).

The aim of this work is evaluation the utility of automated vitek-2 system in detection of inducible clindamycin resistance S. aureus compared to D zone test and detection the prevalence of erm A and erm C genes among isolated strains.

**Methodology**

This work was carried out at Microbiology and Immunology Department, Benha Faculty of Medicine from May2017 to November 2017.

The study was carried out on 25 S. aureus isolates (Erythromycin intermediate and resistant. Clindamycin susceptible) from 60 different clinical samples (abscess, burns, wounds and respiratory secretions). These patients were attending wards of intensive care unit (ICU), chest, and general surgery departments of Benha University Hospitals.

**Antibiotic susceptibility and antibiogram:**

Antibiotic susceptibility was performed for all isolates by using a sterile swab, the plates of Mueller Hinton agar were inoculated after dipping the swab in the bacterial suspension equal to 0.5
McFarland. Using sterile forceps, the antibiotic discs were placed in the center of the M.H agar plates and pressed gently to ensure good contact. The discs were Cefoxitin (FOX=30µ g), Amoxicillin (AX=25µ g), Vancomycin (VA=30µ g), Ceftriaxone (CAZ=30µ g), Ofloxacin (OFX=5µ g), Cefotaxime (CTX=30µ g), Erythromycin (E=15µ g) and Clindamycin (DA=2µ g).

**Detection of inducible MLSB resistance (D test):**

A 0.5 McFarland equivalent suspension of organisms was inoculated onto a Mueller–Hinton agar (MHA). Clin (2 µ g) and Ery (15 µ g) discs were placed 15-21 mm edge to edge on the MHA. Plates were analyzed after 24 h of incubation at 35°C. When the Ery zone is ≤22 mm and the Clin zone is 21 mm with a D-shaped zone around the CLin, the organism is positive for inducible resistance (D-test positive). (10)

**Antibiotic susceptibility by Vitek-2 system:**

The AST card for VITEK-2 Systems is an automated test methodology based on the MIC technique reported by MacLowry and Marsh and Gerlach. The organism suspension to be tested must be diluted to a standardized concentration in 0.45% saline before being used to rehydrate the antimicrobial medium within the card. The card was then filled, sealed, and placed into the instrument incubator/reader VITEK-2 system. The instrument monitored the growth of each well in the card over a defined period of time (up to 18 hours for bacteria). At the completion of the incubation cycle, MIC values were determined for each antimicrobial contained on the card. (11) The card that used in this study was AST-GP67 (BioMerieux, France).

**Genotypic identification of ICR S.aureus:**

**DNA extraction:**

Extraction was done using Zymo Research (ZR) Fungal/Bacterial DNA MiniPrep™ Catalog No. D6005 (U. S. A). The procedure was carried out according to manufacturer's instructions.

**DNA amplification:**

Four primers were used to perform a multiplex PCR protocol, to detect both ermA and ermC. The primers were designed according to (12). The sequence of the first pair targeting ermA gene was: 5’GTICAAGAACC AATCAATAAC GAG3’ and 5’GGATCAGGAA AAGGACATTT TAC3’ amplifying a 421 bp DNA fragment. The sequence of the second pair targeting ermC: 5’GCTAATATTG TTAAATCCTG CAATTCC3’ and 5’GGATCAGGAA AAGGACATTT TAC3’ amplifying a 572 bp DNA fragment.

PCR was carried out in 50 µl volume reaction mixtures containing 1µl of each primer, 5 µl of crude template DNA, Water, nuclease-free 16 µl and 25 µl DreamTaq Green PCR Master Mix. The Denaturation, the annealing and the Extension temperature were 95°C, 55°C and 72°C respectively for ermA and ermC. The number of cycles for them was 25-40 cycles. Final extension done on 72°C for 5-15 min for one cycle. PCR products were separated by gel electrophoresis on 1.5 % agarose gel containing 2.5µ g/ml ethidium bromide.

**Results**

The study was carried out on 25 S. aureus isolates (Erythromycin intermediate and resistant, Clindamycin susceptible) by both Disc diffusion method and Vitek-2 system. MRSA was detected in 18 (72%) of S. aureus isolates by Cefoxitin screening by both methods.

ICR was detected in 13 (52%) and 11 (44%) of S. aureus isolates by D test (Fig: I) and Vitek 2 system respectively. Automated Vitek 2 system was unable to detect 2 positive ICR isolates done by the D test. PPV and NPV of the Vitek 2 system was 100% and 84.6% respectively. The sensitivity and specificity of the test
were 85.7% and 100% respectively. (table I)
All ICR isolates were MRSA by both disc diffusion method and Vitek 2 system.
About distribution of ICR S. aureus in different clinical samples no statistical significant differences were detected regards ICR in various clinical samples by D test and Vitek-2 system. (P = 1, 0.48 ) respectively. (table II)
By multiplex PCR showed that *erm* C and A genes were detected in (40%) and (24%) of the studied isolates respectively. Both C and A genes were detected in (12%). (table III, Fig: II)

Table (I) Prevalence of ICR among S. aureus by D test and Vitek 2 system:

<table>
<thead>
<tr>
<th></th>
<th>D-test +ve (no.=13/25=52.0%)</th>
<th>D-test –ve (no.=12/25=48.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitek-2 test +ve</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>(11/25= 44.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitek-2 test -ve</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>(14/25= 56.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>85.7</td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>PPV (%)</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>NPV (%)</td>
<td>84.6</td>
<td></td>
</tr>
</tbody>
</table>

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

Table (II) Distribution of ICR S. aureus in different clinical samples:

<table>
<thead>
<tr>
<th></th>
<th>All=S25</th>
<th>Sputum (no.=9)</th>
<th>BAL (no=3)</th>
<th>Abscess (no=8)</th>
<th>Burn (no=5)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D test</td>
<td></td>
<td>No.</td>
<td>No.</td>
<td>No.</td>
<td>No.</td>
<td>1.00</td>
</tr>
<tr>
<td>Vitek 2</td>
<td></td>
<td>5,55.56</td>
<td>1,33.33</td>
<td>4,50.0</td>
<td>3,60.0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*Fisher Exact Test

Table (III) Distribution of *erm* A gene and *erm* C gene among the studied isolates:

<table>
<thead>
<tr>
<th></th>
<th>erm A only</th>
<th>erm C only</th>
<th>erm A+C</th>
<th>No erm A+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of strains</td>
<td>6</td>
<td>10</td>
<td>12.0</td>
<td>6</td>
</tr>
<tr>
<td>24.0</td>
<td>40.0</td>
<td>24.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION
Clindamycin, an antimicrobial belonging to the MLSB family, is frequently used for treatment of skin and soft tissue infections caused by S. aureus. However, the widespread use of the MLSB family of antimicrobials has led to the emergence of resistance. Isolates with ICR are found to be resistant to erythromycin but susceptible to clindamycin when these discs are not placed adjacent to each other during antimicrobial sensitivity testing. (13).
Conversely, labeling all EryR staphylococci as ClinR or not will likely prevent the use of Clin in treating infections that would likely respond to Clin therapy (14).
A series of the VITEK systems (bioMerieux, Marcy l’Etoile, France) has been a fully automated instrument that provides species identification and antimicrobial susceptibility testing for a variety of clinical isolates, and are presently used in many clinical microbiology laboratories worldwide. (15; 16).

The VITEK Advanced Expert System (AES) has been created to analyze the AST results using the well-established knowledge base of approximately 100 species and 20000 ranges of MIC to detect more than 2300 phenotypic antimicrobial resistances. (17)

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 85.7% and the specificity was 100%. The PPV and NPV were 100% and 84.6% respectively. The automated system failed to detect 2 isolates as ICR positive while confirmed by D test.

Similar sensitivities were reported by Lavallee et al., (2010), Nimmo et al., (2011), Jethwani et al. (2011), Buchan et al., (2012), Gardiner et al., (2012), reported a 93%, 92.5%, 95.4%, 91.1%, 95% respectively.

The specificity and PPV of Vitek-2 test in this study were (100%), these findings were confirmed by many studies. Nakasone et al., (2007) (17), Lavalle e et al. (2010) (9) and Jethwani et al. (2011) (10) who reported the same results of us as specificity of the test with no false positive results. They recommended that positive vithek 2 results should be reported without confirmation by D test.

A possible reason for the false negatives of the ICR test is insufficient incubation time in the Vitek-2 for induction to occur. The card is typically incubated for 4–10 hours, with the time variability being dependent on the inoculum and organism growth characteristics. If a slower growing organism is inoculated at the lower end of the recommended range of 0.5–0.63 MacFarlane, insufficient incubation time may contribute to a false negative result. (2)

The prevalence of ICR among MRSA, MSSA in this study illustrated that all ICR positive were MRSA. This results coincided with Renushri, et al., (2011) (11) who reported that more MRSA isolates showed higher MLSBi phenotype (27.8%) compared to MSSA isolates (5.8%).

In contrast to our results Buchan et al., (2012) (18) showed that from 43 ICR positive S. aureus isolates MRSA was 15 isolates while MSSA was 28 isolates.

In the present study frequency of ICR in clinical samples were 60%, 55.56% , 50% and 33% from burn, sputum, abscess and BAL respectively. These results showed a high similarity with Taie D. (2013) (21) who reported the most frequent positive (D Test) from Blood samples 4(4.4%), followed by Pus samples 2(2.2%), while the least frequent were from sputum samples 1(1.1%).

As regard the association between phenotype and genotype for the studied S. aureus isolates, the distribution of erm C gene and erm A gene were detected in (40%) and (24%) of the studied isolates respectively. Both C and A genes were detected in (12%). None C and A genes were detect in 24%.

Our findings agreed with Pereira et al., (2016) (22) who reported that in the 44 isolates subjected to PCR, the erm C gene was detected in higher frequency than the erm A gene17 (38.6%) and 4 (9.1%) respectively. The presence of both gene was detected in only one (2.3%) of the isolates.

However Hosseini et al., (2016) (23) found 10 MRSA isolates having inducible phenotype among which 5 isolates harbored erm A and 3 other isolates only consisted erm C
A possible explanation for the predominance of erm C positive MRSA isolates in our study was cleared by Spiliopoulou et al. (2004) who reported that their isolates were belonged to two different MRSA clones even though they were isolated in different hospitals. The prevalence of erm C positive isolates was most likely due to the selection and dissemination of these two multi-resistant MRSA clones.

CONCLUSIONS

This study highlights the crucial role of antibiotic susceptibility testing. As clindamycin is one of the most commonly used antibiotics for MRSA isolates. High prevalence of ICR among MRSA isolates limits the therapeutic options for MRSA to the antibiotics like linezolid and vancomycin.

The D test is a simple, reliable and inexpensive test to perform along with routine susceptibility testing which delineates the iMLS B and the cMLS B resistance.

Vitek-2 system is a potentially reliable method for antimicrobial testing including ICR.

Vitek-2 system combined with AES will greatly contribute to laboratory function in the field of clinical microbiology.

The predominance of erm C as the genetic determinant for the expression of resistance to MLS antibiotics among the total S. aureus population.

On the other hand, increasing of (ermA + ermC) genes in the MRSA emphasizes on the accurate use of these antibiotics to prevent any treatment failure.

REFERENCES


