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RAPID METHODS FOR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN WASIT PROVINCE, IRAQ

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Abstract

One of the most current critical clinical challenges is *Staphylococcus aureus* pathogen, especially the Methicillin-Resistant *Staphylococcus aureus* (MRSA). It has emerged as a nosocomial pathogen in both community and hospitals. This study was performed in the laboratory of microbiology, college of medicine, Wasit University to investigate the effectiveness of molecular assay in detecting MRSA compared to cefoxitin disk diffusion with antibiotic susceptibility pattern in specimens collected from patients lived in Wasit Province, Iraq. One hundred and twelve clinical specimens were cultured to isolate the MRSA. Total 53 Staphylococcus aureus (*S. aureus*) isolates, 42 (79.27%) were developed an inhibition zone with about ≤ 21 mm which indicates MRSA by cefoxitin disk diffusion method whereas 50 (94.33%) isolates were positive for the *mecA* gene by PCR. MRSA was highly resistant to commonly used antibiotics such as amoxicillin, ciprofloxacin and gentamycin. It can be concluding that the wounds infection are the most common sites for MRSA isolates followed by urine and blood. Cefoxitin disk diffusion testing is not reliable for detecting methicillin-resistant *Staphylococcus aureus* compared to molecular assays such as polymerase chain reaction when applied as a surrogate for disk diffusion testing. The current study validates the molecular assay is a simple and valuable tool for identification of Methicillin-resistant *Staphylococcus aureus* in patients and carrier individuals. MRSA strains were highly resistant to different antibiotics used in this study.

Key words: Methicillin-resistant *Staphylococcus aureus*, Cefoxitin, MecA gene, PCR.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are well-recognized as worldwide public health problem (Garoy *et al*., 2019). Although it is first described in the 1960s, it been emerged in the last decade as an important cause of nosocomial infections which is responsible for potentially fatal diseases including osteomyelitis, necrotizing fasciitis, severe sepsis, endocarditis, life-threatening pneumonia and toxic shock syndrome (Monecke *et al*., 2011). It's the most common microorganism to cause skin infection and has the ability to cause an assortment in hospital-acquired and community-acquired. The prevalence of these infections is increasing and the treatment is becoming more difficult (Oliveira *et al*., 2018).

S. aureus is the most isolated bacteria in both community-acquired and nosocomial infections. The majority of harmful factors delivered by this pathogen are proteins and cytotoxins like exfoliative toxins, staphylokinase, haemolysins, leukocidins, nucleases, lipases, coagulase, collagenase and hyaluronidase (Raheema and Abed, 2019). MRSA has the propensity to form biofilms and might significantly increase morbidity and mortality in the affected patients (Neopane *et al*., 2018). Community-associated MRSA was firstly reported in some high-risk individuals such as intravenous drug addicts, people in nursing homes and chronically ill people, nevertheless, MRSA are nowadays isolated even from healthy children (Tenover and Goering, 2009). The purpose of this study was to evaluate the effectiveness of molecular assay in detecting MRSA compared with the conventional cefoxitin disk diffusion method.

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Materials and methods

Sample collection

From July 2018 to January 2019, a total of one hundred and twelve clinical (wound swabs, burn swabs, midstream blood and urine) samples were sent to the medical microbiology laboratory for routine analysis. Different samples cultured on mannitol salt agar and blood agar then incubated for 24 hrs at 37°C. Isolated bacteria

were detected according to morphological, biochemical tests and analytical profile index of Staph.

Detection of MRSA by Cefoxitin Disk Diffusion method

Cefoxitin disk diffusion test was performed for all isolates of *S. aureus* by use 30 µg disks, test inoculum 0.5 McFarland standards, suspension and lawn culture were performed on Mueller-Hinton agar plate for 18hrs at 36ºC. The inhibition zone diameter was measured using a metric ruler. An inhibition zone diameter of ≤ 21 mm was reported as Methicillin-resistant and ≥ 22 mm was considered as methicillin-sensitive (CLSI , 2016).

DNA extraction

DNA extraction of MRSA isolates was *carried out using a* kit of generated Genomic DNA extraction, purity (1.7-2) and concentration were between 50-360 ng/µl by Nanodrop.

Detection of MecA gene by Polymerase chain reaction

Molecular technique was used for amplifying of MecA genes. The reaction was performed in a total volume 20µl of Pre Mix (Bioneer, South Korea) consisting of 1µl from each primer forward and reverse, 3µl of DNA and, the volume made up to 20 µl with free nucleases deionized water according to the instructions of manufacturing company.

Primers for amplification of mecA were 52 GGGATCATAGCGTCATT ATTC-32 and 52 AACGATTGTGACACGATAGCC-32 . Thermocycling was conducted in the thermal cycle as follows: 5 min at 95°C followed by 32 cycles of 95°C for 1 min, 51°C for 30 sec and 72°C for 1 min with a final step at 72°C for 5 min. Amplicon (527 bp of mecA) was detected in 1.5% agarose gel electrophoresis (70 volts for 1.5 h) and visualized by staining with 2µl red stain then documentation was performed by the gel documentation saving picture (Bio-Rad).

Antibiotic susceptibility test

All MRSA isolates were further screened for their susceptibility of various antibiotics via Kirby Bauer method on Muller Hinton agar table 1. Results explicated according to clinical and laboratory standards (CLSI, 2016).

Results and Discussion

Isolation and identification of *Staphylococcus aureus*

Out of one hundred and twelve specimens, including wound infection, burns, urinary tract infection and a blood infection, 53 isolates (47.32 %) were able to grow on mannitol salt agar and developed yellow colonies on this medium, thereby; they were conventionally confirmed as *S. aureus*.

Table 1: Antibiotics Disks used in this Study.

No.	Antibiotic	Concentration (µg)
1	Ciprofloxacin	5
2	Tetracycline	30
3	Trimethoprim/Sulfamethoxazole	1.25/23.75
4	Gentamycin	10
5	Clindamycin	2
6	Amoxicillin	10

The prevalence of *S. aureus* diversified among collected specimens relying on the source and type of clinical specimens. The highest percentage of *S. aureus* infections were observed in wound infection 27 (50.94%), this bacterium can be considered the major agents of nosocomial infections in wound followed by midstream urine infection 11 (20.75%), then blood 10 (18.86%) and ultimately burn 5 (9.43%) Fig. 1.

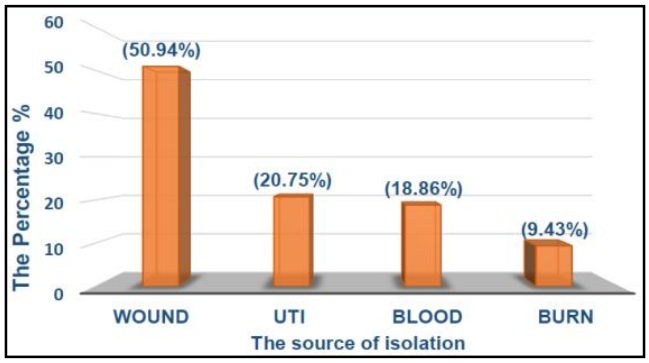

Fig. 1: The percentage of distribution of *S. aureus* according to the source of isolation.

Detection of *MRSA*

Accurate and rapid identification of *methicillin-resistant Staphylococcus aureus* strains is essential to limit the spread of bacteria through patient healthcare and infection control nursing programs. It has been demonstrated that Cefoxitin is a better inducer for *mecA* regulatory system than other beta-lactams (McKinney *et al*., 2001). Recent study strongly recommended using of cefoxitin for MRSA detection (CLSI, 2016).

In this study, the cefoxitin sensitivity test was carried out for all *S. aureus* isolates and results revealed that out of 53 *S. aureus* isolated, 42 (79.24%) developed an inhibition zone ≤ 21 mm that indicates the isolates were MRSA. Moreover, it has been shown that cefoxitin is the best marker for *mecA*-mediated methicillin-resistant (Bonjean *et al*., (2016).

Importantly, these observations are profoundly agreed with previous work (Karam and Al-Mathkhury, 2017)

showed that 80% of *S. aureus* were identified as MRSA. In addition, a study accomplished in an Al-Sulaimania city reported that MRSA covered 68% of all *S. aureus* isolates (Muhammad and Al-Mathkhury, 2014). A study performed by (Havaei 2014 ; Mirkarimi *et al*., 2016), reported the prevalence of MRSA in Iran was 16% - 35%in healthcare workers. In same line of thought, it has been shown that the rate was 10.1% in healthcare workers in Jordan (Aqel *et al*., 2015). Another research (Iyer *et al*., 2014), also showed that 73% in Saudi Arabia healthcare workers.

The results of the *Staphylococcus aureus* isolated from the 53 specimens tested in the second step of the study, 42 (79.24%) were detected as methicillin-resistant *staphylococcus aureus* via conventional disc diffusion. Detection of the mecA gene by using molecular techniques since 53 isolated of *S. aureus*. The results of PCR revealed 50 (94.33%) were positive for the mecA gene as shown in Fig. 2.

This type of finding has earlier been notified by (Raheema and Abed., 2019) and (Cekovska *et al*., 2005). In a study conducted by (Davoodi *et al*., 2012), it has been reported that conventional disk diffusion method usually offers (false negative) results with *low sensitivity*,

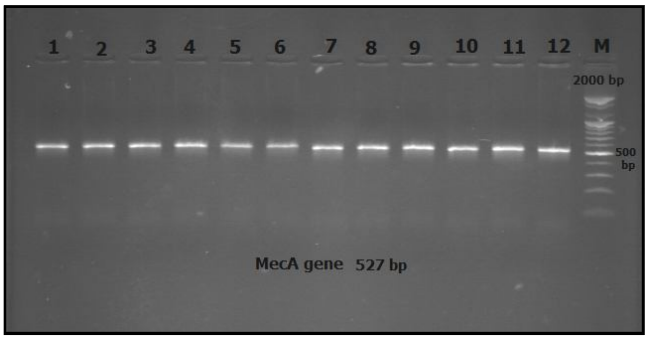

Fig. 2: Gel electrophoresis of amplified MecA gene for MRSA, the product size 527 (bp). Lane (M): DNA ladder (100-2000bp), Lanes1-12.

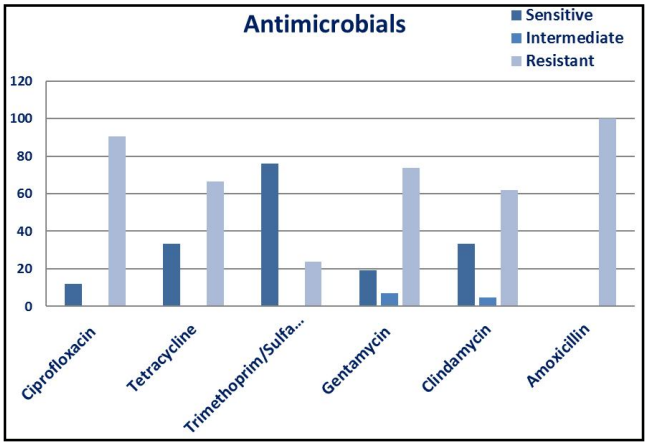

Fig. 3: Antibiotics tested against MRSA isolates.

especially in heterogeneous resistance strains. Polymerase chain reaction amplification of the mecA gene was applied as" gold standard" for identification of MRSA (Jonas *et al*., 2002, Pournajaf *et al*., 2014).

Furthermore, a suggestive study of a polymerase chain reaction for mecA revelation gene is accurate, rapid with the helpful diagnostic tool, especially where MRSA strains are endemic in rural hospitals, the competence of *S. aureus* to cause various diseases is attributed to various virulence genes (Raheema and Abed., 2019).

Antibiotic susceptibility testing of MRSA

Results of antibiotic susceptibility test for isolated MRSA indicated different antibiotic profiles as shown in Fig. 3. In fact, MRSA isolates were resistant to many antibiotics applied routinely for this bacterium. Recently, many *MRSA* isolates were multidrug-resistant than methicillin-sensitive *Staphylococcus aureus* isolates.

In our study, high resistance of MRSA isolates was observed against amoxicillin, ciprofloxacin and gentamycin.

Conclusion

Prevalence of methicillin-resistant *Staphylococcus aureus* varies depend on the type of clinical samples. It has been shown that wound infection had the highest (50.94%) proportion of MRSA isolates. Notably, cefoxitin disk diffusion testing is not reliable for detecting methicillin-resistant *Staphylococcus aureus*. Importantly, molecular assays such as polymerase chain reaction should be used as a replacement for Conventional method, it has also been indicated that molecular assay is simple and valuable tools for identification of Methicillin-resistant *Staphylococcus aureus* in patients and carrier individuals. Consistently, Methicillin-resistant *Staphylococcus aureus* isolates were multidrug-resistant.

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