RESEARCH ARTICLE

Rapid Methods for Detection of Methicillin-resistant *Staphylococcus aureus* in Wasit Province, Iraq

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Received: 3rd July, 2021; Revised: 27th July, 2021; Accepted: 17th August, 2021; Available Online: 25th September, 2021

ABSTRACT

Background: Currently, the most important clinical challenge is *Staphylococcus aureus*, especially methicillin-resistant Staphylococcus aureus (MRSA), it has emerged as a nosocomial pathogen in the community and hospitals. The study was performed in the laboratory of microbiology, college of medicine, to investigate the effectiveness of molecular assay in detecting MRSA compared with congenital cefoxitin disk diffusion with antibiotic susceptibility pattern in Wasit Province, Iraq

Methods: one hundred and twelve clinical specimens were cultured to isolate the MRSA.

Results: Among 53 isolated from *S. aureus*, 42 (79.27%) isolates were developed an inhibition zone ≤ of 21 mm that indicates MRSA by cefoxitin disk diffusion method, and 50 (94.33%) isolates were positive for the *mecA* gene by polymerase chain reaction (PCR) method. MRSA was highly resistant to commonly used antibiotics such as amoxicillin, ciprofloxacin, and gentamycin.

Conclusion: Wounds infection are the most common sites for MRSA isolates followed by urine and blood; cefoxitin disk diffusion testing is not reliable for detecting MRSA, molecular assays such as polymerase chain reaction should be applied as a surrogate for disk diffusion testing. The results of this study indicated molecular assay is simple and valuable tools for the identification of MRSA in patients and carrier individuals. MRSA strains were highly resistant to different antibiotics used in this study.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Cefoxitin, MecA gene, Polymerase chain reaction.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.45

How to cite this article: Raheema RH. Rapid Methods for Detection of Methicillin-resistant *Staphylococcus aureus* in Wasit Province, Iraq. International Journal of Drug Delivery Technology. 2021;11(3):917-919.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a well-recognized public health problem in the world.[1] Its initially described in 1960s, emerged in the last decade as a cause of nosocomial infections responsible for potentially fatal diseases including osteomyelitis, necrotizing fasciitis, severe sepsis, endocarditis, life-threatening pneumonia and toxic shock syndrome.[2] It is the most microorganism in the skin infection with the ability to cause an assortment in hospital-acquired and community acquired. These infections are increasing and their treatment is becoming harder.[3]

S. aureus is the most isolated bacteria among both community-acquired and nosocomial infections. The most harmful factors delivered are various proteins and cytotoxins like exfoliative toxins,staphylokinase, hemolysins, leukocidins, nucleases, lipases, coagulase, and collagen and hyaluronidase.[4] MRSA has the propensity to form biofilms and might lead to significantly increased morbidity and mortality in the patients.[5] Community-associated MRSA was firstly reported in some high-risk individuals such as intravenous drug addicts, people in nursing homes, chronically ill people; nevertheless, MRSA are nowadays isolated even from healthy children.[6] The purpose of this study was to evaluate the effectiveness of molecular assay in detecting of MRSA compared with the conventional cefoxitin disk diffusion method.

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 were cultured on mannitol salt agar, and blood agar, then incubated for 24 hours at 37°C. Isolated bacteria were detected according to morphological, biochemical tests, and analytical profile index Staph

Detection of MRSA by Cefoxitin Disk Diffusion Method

Cefoxitin disk diffusion test was performed on all isolates of *S. aureus* by 30 µg disks, test inoculum 0.5 McFarland

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standards, suspension and lawn culture was 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.[7]

DNA Extraction

DNA extraction of MRSA isolates was carried out kit of gene aid Genomic DNA extraction, purity (1.7–2), and concentration was between 50–360 ng/µL by Nanodrop.

Detection of MecA gene by Polymerase Chain Reaction

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

Primers for amplification of mec Awas 5′G GG AT CA TA GCG TCA TT ATTC-3′and5′ AACGATTGTGACAC GATAGCC-3′. Thermo cycling was conducted in thermal cycle as follows: 5 minutes at 95°C; followed by 32 cycles of 95°C for 1-minute, 51°C for 30 seconds and 72°C for 1-minutes with a final step at 72°C for 5 minutes. Amplicon (527 bp of mecA) was detected in 1.5% agarose gel electrophoresis (70 volts for 1.5 hours) and visualized by staining with 2 µL 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 the Kirby Bauer method on Muller Hinton agar (Table 1). Results explicated via previous studies.[7]

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, only 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.*

The prevalence of *S. aureus* diversified among collected specimens relying on the source and type of clinical specimens, 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 wounds followed by midstream urine infection 11 (20.75%), then blood 10 (18.86%) and ultimately burn 5 (9.43%), Figure 1

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

Detection of *MRSA*

Accurate and rapid identification of MRSA strainsare essential to limit the spread of bacteria in patient care and infection control nursing programs. Cefoxitin is a better inducer for *mecA* regulatory system than other beta-lactams,[8] Recently, the[7] recommended the use of cefoxitin for the detection of MRSA. In this study, the cefoxitin sensitivity test was carried out for all *S. aureus* isolates, and results revealed that out of 53 *S. aureus* isolates, 42 (79.24%) developed an inhibition zone ≤ 21 mm that indicates the isolates were MRSA[9] showed cefoxitin is the best marker for mecA-mediated methicillin-resistant. This result is in agreement with[10] showed that 80% of *S. aureus* were identified as MRSA. Also,[11] reported in a study accomplished in an Al-Sulaimania city that MRSA covered 68% of all *S. aureus* isolates. A study performed by[12,13] reported the prevalence of MRSA in Iran was 16–35% in healthcare workers. In another study in Jordan[14] reported that's the rate was 10.1% in healthcare workers,[15] also showed that 73% in Saudi Arabia healthcare workers

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

This type of finding has earlier been notified by.[4,16] In a study conducted by.[17] it was reported that the conventional disk diffusion method usually offers (false negative) results with low sensitivity, especially in heterogeneous resistance strains. Polymerase chain reaction amplification of mecA gene was applied as" gold standard" for identification of MRSA(18; 19)

A suggestive study of polymerase chain reaction for mecA revelation gene is accurate, rapid with 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.[4].

Antibiotic Susceptibility Testing of MRSA

The antibiotic susceptibility test results for isolated MRSA indicated different antibiotic profiles as shown in Figure 3. MRSA isolates were resistant to many antibiotics applied routinely for this bacterium. Recently, many MRSA isolates were multidrug-resistant than MRSA isolates.

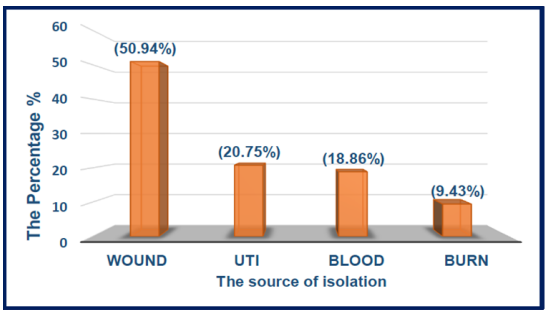

Figure 1: Distribution of *S. aureus* according to the source of isolation

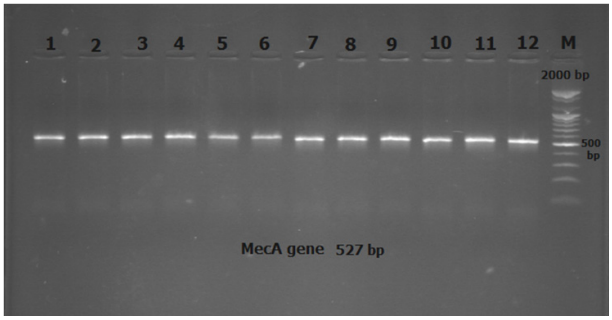

Figure 2: Gel electrophoresis of amplified Mec A gene for MRSA, the product size 527(bp). Lane (M): DNA ladder (100-2000bp),Lanes1-12

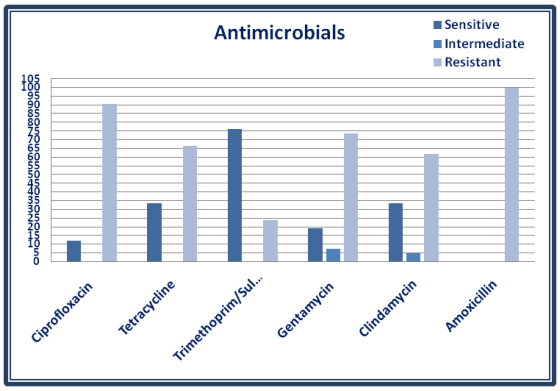

Figure 3: Antibiotics tested against MRSA isolates

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

CONCLUSION

The prevalence of MRSA varies depend on the type of clinical samples. It has been shown that wound infection had the highest (50.94%) proportion of MRSA isolates, cefoxitin disk diffusion testing is not reliable for detecting MRSA, 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 MRSA in patients and carrier individuals. MRSA isolates were multidrug-resistant.

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