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Marked Dominance of methicillin Resistant *Staphylococcus aureus* among Iraqi Patients

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection in human beings and animals stands out as one of the leading pathogens causing nosocomial and community infections. Likewise, slightly increasing drug resistance in MRSA has narrowed the treatment choices. This work focuses on estimating the prevalence of MRSA in Baghdad, Iraq. A total of 130 specimens were collected from patients visiting various hospitals in Baghdad, Iraq. The present results revealed that 50 (92.6%) isolates were identified as *Staphylococcus aureus*. Noticeably, *mecA* gene was detected in 44 (88%) isolates. Hence, the light must be shed on this marked prevalence of methicillin resistant *S. aureus*.

Keywords: *MRSA, Staphylococcus aureus, mecA, Baghdad.*

Introduction

Staphylococcus aureus is very hardy pathogen for human and it is responsible for a wide variety of infections. Its pathogenesis is a complex process due to the release of different range of secreted and surface-associated virulence factors [(1)].

Strains of *S. aureus* harbouring mecA gene will resist all beta lactam antibiotics. Nevertheless, just after the presenting methicillin as a medicine to treat penicillin-resistant *S. aureus* strains in 1961, methicillin resistant *S. aureus* (MRSA) strains were reported, [(2)]. Thereupon, MRSA has emerged as a boundless challenge in human medicine, particularly as a nosocomial pathogen, nonetheless, this type of *S. aureus* has developed resistance against nearly all common antibiotics; rendering its treatment into a problematic concern. Moreover, since the 1990s, MRSA is considered as a worrisome problem in hospitalized patients or those who recently had intensive surgery; henceforth MRSA strains are known as community-acquired or community-associated MRSA [(3)].

During the past decades, MRSA has spread throughout the world and has become highly endemic in many geographical areas. Due to the changing pattern of antibiotic resistance in S. aureus and the prevalence of multidrug resistance (MDR) in MRSA, some investigators have suggested that the resistance patterns should be evaluated periodically and antibiotic therapy should be guided by susceptibility testing [(4)].

The prevalence of MRSA underlies a serious risk and potentiates a problematic concern that lead to the emergence of strains with enhanced virulence [(5)]. Upon that, the present work was undertaken to investigate the prevalence of MRSA amongst patients attending Baghdad hospitals.

Materials and Methods

Ethical statement

All participants agreed to provide the investigator with the specimens. Informed consent according to the Declaration of Helsinki was obtained from all participants.

Staphylococcus aureus isolation and identification

One hundred and thirty specimens included mid-stream urine, burn swabs, wound swabs, and blood were collected from hospitalized patients referring Al-Yarmouk teaching Hospital and Baghdad Medical City in Baghdad, Iraq.

All these specimens were cultured onto plates of Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hr. Colonies that appeared from primary cultures were re-inoculated by sub-culturing on BHI agar, then re-cultured onto MSA and incubated at 37°C for 24 hr to obtain purified bacterial isolates. Thereafter, discrete colonies were cultured onto blood agar for haemolysis behaviour detection. Oxidase, catalase, and coagulase were assayed as well.

Detection of methicillin resistance by cefoxitin disk method

All the isolates were subjected to cefoxitin disk (30 µg) diffusion assay. A 0.5 McFarland standard compatible suspension of the isolate was prepared and lawn culture was spread on Muller-Hinton agar plate. Plates were incubated at 37ºC for 18 hr. and zone diameters were measured. An inhibition zone diameter of ≤ 21 mm was reported as Methicillin-resistance [6].

Detection of ***16SrRNA*** and ***mecA***

Extraction of Bacterial DNA

Genomic DNA was extracted using Presto™ Mini gDNA Bacteria (Geneaid, Thailand). Upon the procedure itemized by the manufacturing company, DNA was extracted from overnight cultures of the carefully chosen staphylococcal isolates. Purified DNA concentration was measured using Biodrop (Biodrop, Canada).

Gene amplification protocol

To confirm the identification of *S. aureus* isolates, conventional PCR technique was carried out to amplify fragments of *16SrRNA* (108bp) genes. Two microliters of each primer Sa442-1 (5′-AATCTTTGTCGGTACACGATATTCTTCACG-3′) and Sa442-2 (5′-CGTAATGAGATTTCAGTAGATAATACA ACA-3′). *mecA* gene as a determinant of methicillin resistance was detected with primers MecA1 (5′-GTAGAAATGACTGAACGTCCGATAA-3′) and MecA2 (5′-CCAATTCCACATTGT TTCGGTCTAA-3′). Different concentrations of DNA (depending on DNA yield) extracted from each *S. aureus* isolate and deionized D.W. were added to PCR premix tubes in order to reach 20 µl as a final volume. The PCR conditions were as follows: 2 µl of template DNA in a 20 µl final reaction volume containing 0.2 µM for the primers with the thermocycling conditions (Bio-Rad T100, USA) set at 94°C for 3 min, followed by 35 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 15s for *16SrRNA* [7] and 94°C for 10 min, followed by 10 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 75 s and 25 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 75 s for *mecA* [8]. PCR products were visualized using 2% agarose gel stained with diamond nucleic acid dye (Promega, USA).

Results

Staphylococcus aureus isolation and identification

One hundred and thirty different clinical specimens collected from patients attending hospitals in Baghdad were streaked on MSA. Fifty-four isolates appeared as round yellow colonies and positive for beta haemolysis, catalase, oxidase, and coagulase; therefore, primarily identified as *S. aureus*. What's more, out of these 54 isolates, 50 (92.5%) developed methicillin resistance by cefoxitin disk diffusion method (CDD).

DNA extraction and preparation

After DNA extraction by Presto™ Mini gDNA Bacteria Kit, DNA concentration was between 22 and 81 ng/ml; whereas, purity was about 1.89- 1.92. A ratio of 1.8 -2.0 is generally accepted as "pure" for DNA [9]. Gel electrophoresis was done to confirm the integrity of extracted DNA.

Detection of ***16SrRNA*** and ***mecA*** genes by polymerase chain reaction

In this study, PCR technique was applied to confirm the presence of *16SrRNA* and *mecA* genes. The existence of genes was detected by presence of single band at a given molecular weight. (viz. 108 bp and 310 bp for *16SrRNA* and *mecA*, respectively) of the DNA marker.

The current results revealed that *16SrRNA* was located in 50 (92.6%) out of 54 biochemically *S. aureus* isolates. Correspondingly, four isolates were identified using traditional methods as *S. aureus*, they did not have this gene. Furthermore, of these 50 isolates, *mecA* gene was detected in 44 (88%) isolates. Of interest, 47 (94%) isolates were detected as MRSA by CCD, all of which carried *16SrRNA*.

Discussion

The increasing incidence of multi-drug resistant *S. aureus* strains, particularly, MRSA is a serious problematic issue in therapeutic strategies and simultaneously considered as a threat to both the clinical settings and community.

Karmakar et al. [10] mentioned that among 165 samples, 100 strains (60.60%) were isolated from a selective MSA media and then these isolates were identified as *S. aureus* by different biochemical tests. Gram staining, catalase, coagulase, and thermonuclease were important phenotypic identifying markers of *S. aureus*. They found that 100%, 92%, and 84% isolates were positive for catalase, coagulase, and heat-stable nuclease, respectively. The results of present study agreed with a study done by Rusenova and Rusenov [11] that total of 156 isolates suspicious for *S. aureus* were detected by a conventional biochemical method. The majority of *S. aureus* strains gave typical biochemical reactions with the exception of 30 (19.2%) and 25 (16%) that were VP negative and weak positive in fermenting mannitol respectively. Twelve strains were found to be non-haemolytic (7.7%). However, precise detection of *S. aureus* was done by combination of conventional and molecular methods. Ibraheem and Al-Mathkhury [12] reached similar findings as they highlighted the inaccuracy of traditional methods for the identification of *S. aureus*. Same authors recommend that all the traditional identification should be confirmed through molecular methods, in order to avoid false-positive results.

The *mecA* gene synthesizes penicillin binding protein (PBP2a) and it is the cause of methicillin resistance in MRSA. This protein able to reduced affinity for βlactam antibiotics**. This gene resides on the staphylococcal cassette chromosome (SCC). Staphylococcal cassette chromosome is a** large genetic mobile element which varies in size and genetic composition among the strains of MRSA (10). To treat staphylococcal infections, various classes of antibiotics including beta-lactams, glycopeptides, lipopeptide, oxazolidones, aminoglycosides, macrolides, and fluoroquinolones [13].

The present study agreed with a local study performed by Al-Dahbi and Al-Mathkhury [14] as they mentioned that the incidence of MRSA among *S. aureus* was 94.3%. However, it compatible with another local studies [12, 15, 16] demonstrated that most isolates of *S. aureus* developed methicillin resistance.

To investigate the distribution of methicillin resistance staphylococci among the patients, Muhammad and Al-Mathkhury (17) performed the antibiotic sensitivity test to 137 *Staphylococcus* isolates using CCD. The results revealed that 68% of *S. aureus* isolates developed methicillin resistance.

Interestingly, the present work revealed that MRSA detected by CDD outnumbered the PCR technique. The reason of such difference could be due to presence of other genes responsible for the methicillin resistance other than *mecA*.

Owing to unnecessary and unrestrained use of antibiotics, the **bacterial species developed multidrug resistance; hence narrowing the therapeutic choices for the treatment [18]. MRSA originated from nosocomial infections highpoints this species as a potential pathogen; which have the capacity to cope with different antibiotics [19].**

Conclusion

MRSA isolates in Iraq are increasing with time, an issue need to be highlighted.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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