Genotypic, Phenotypic Identification and Antibiogram Assay of Mrsa Isolated from Healthy Individuals in Wasit Province

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Abstract

Staphylococcus aureus is a Gram-positive, facultative anaerobic, non-spore-forming, coagulase -positive bacterium. the Methicillin resistance is mostly mediated by the presence of mecA gene acquisition of mecA gene leads to altered penicillin binding protein (PBP), called PBP2a, with low binding affinity to methicillin. A total of 52 *S. aureus* strains were analysed. Antibiogram assay for 52 healthy carriers of MRSA isolates by using Vitek 2 system to examine 16 different antibiotics.Methicillin resistance was confirmed by presence of the mecA gene by PCR. Of 52 *S. aureus* samples, 52 isolates of them were MRSA confirmed by targeting mecA gene using PCR. Aims of this study detect methicillin resistant *Staphylococcus aureus* from nose of healthy individuals in Wasit province and confirming that by PCR to detect mecA Gene.

Key words: *Staphylococcus aureus, MRSA, Antibiogram , healthy individuals , mecA gene.*

Introduction

Staphylococci are gram positive bacteria , the cells of *S aureus* about 1 μm in diameter arranged in irregular single small spherical (cocci), clusters, grow most rapidly at 37°C ,*Staphylococci* are relatively resistant to drying, heat (they withstand 50°C for 30 minutes), and 9% sodium chloride , their PH growth ranges 7-7.5 [(1)]. Approximately 30% of healthy individuals carry *S. aureus* in their anterior nares, is also considered to be the most pathogenic of the *staphylococci* and has been associated with a wide range of infections such as skin and soft tissue infections [(2)]. The problem is compounded by the fact that many hospital strains of *S. aureus* are resistant to most of the commonly used antimicrobial agents, especially methicillin, these strains known as methicillin-resistant *S. aureus* [(3)]. Resistance of MRSA is caused by possession of the mecA gene, which codes for a penicillin-binding protein [(4)] that binds the drug less well ,this alternative protein confers resistance to all beta -lactam antibiotics [(5,6)]. Nasal *S. aureus* carriage plays a key role in the pathogenesis of infection by representing the source and the independent risk factor for subsequent infections [(7,8)].

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Methods

Sample Collection

A 231 sample were obtained from nasal of healthy carrier, who attended from Wasit hospitals during the period from November-2018 to March-2019.

Isolation and Identification of S. aureus

Isolation of S. Aureus : The samples& specimens were cultured directly on blood agar medium and incubated at 37Cº for 24 hours to study characteristic of growing colonies and capability of blood hemolysis,for the primary identification of S. aureus. After that it was transported to Mannitol-salt agar as a selective medium for these bacteria and incubated at 37°C for 24 hours [(9)].

Primary Identification of S. aureus

A. Colonial Morphology: its identification was depended on the morphological properties (Colony size, color , edge , texture and hemolysis on blood agar

. The single selected colonies culture on mannitol salt agar plates ,Growth of yellow colonies as a result of fermentation of mannitol [10].

B. Microscopic Examination **:** Single purefied of bacterial colony used to stained with gram stain for the primary identification [11].

C. Biochemical Tests : positive result for Catalase [12], Oxidase [13], Haemolysis [14], Coagulase [11].

Confirmation of Biochemical Test by API Staph System

The API Staph System, produced by bio-Merieux is a reliable method for identifying 23 species of staphylococci and it was used to confirm the biochemical identification of the isolates [3,4].

Identification of Methicillin Resistance *Staphylococcus aureus*(MRSA) by PCR

1. Phenotypically Detection of MRSA

Detection of MRSA by cefoxitin disc diffusion method: All the isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc of cefoxitin . A 0.5 McFarland standard suspension of the isolate was made and the culture was done on MHA plate. Plates were incubated at 37ºC for 18 hr. An inhibition zone diameter of ≤ 19 mm was reported as Methicillin-resistant isolate while ≥20 mm of zone diameter inhibite was considered as methicillin- sensitive isolate [15,16].

confirmation of detected of MRSA by vitek2 system: The AST-P592 card used for *staphylococci* contained , Beta-Lactamase, Cefoxitin screen, oxacillin

A. Inoculum Preparation : Suspensions were set up by emulsifying bacterial isolates in 0.45% saline to what might be compared to a 0.5 McFarland turbidity standard [17].

B. Sensitivity Test by Vitek2: prepared Suspensions as previously was used for VITEK 2 system. AST-P592 Card Used for Contained *Staphylococcus*, Beta-Lactamase Screen, Cefoxitin. The antibiotic groups of the VITEK 2 system in this study are described in table (1) [18].

Table (1) antibiotics included in vitek card and their abbreviation:

Antibiotics	Abbreviations
Erythromycin	E
Piperacillin	PRL
Moxifloxacin	MFX
Aztreonam	ATM
Ceftazidim	CAZ
Rifampicin	RP
Penicillin G	PG
Clindamycin	CD
Ciprofloxacin	CIP
Gentamycin	CN
Chloramphenicol	C
Imipenem	IPM
Cefoxitin	FOX
Oxacillin	OX
Sulphamthoxazol/ Trimethoprim	SXT
Tetracycline	TE
Vancomycin	VA
Azithromycin	ATH
Trimethoprim	TM

Genotyping Detection of MRSA Isolate

Extraction of bacterial genome : Extraction of genomic DNA was preformed by using FavorPrep Blood/ Cultured cells Genomic DNA extraction Mini kit and protocol was followed as per manufacturer's instructions.

Detection of DNA product by agarose gel electrophoresis : DNA samples were electrophoresed by horizontal agarose gel electrophoresis according to [19].

Polymerase Chain Reaction Protocols : Methicillin resistant strains were determined by the presence of the mecA gene by monoplex PCR as described previously. MRSA isolates were tested for the presence of the 533-bp PCR product of the mecA gene using the following primers: forward 5'-TGGCTA TCG TGT CAC AAT CG-3'and reverse 5'-CTGGAA CTT GTT GAG CAG AG-3'.

A. Monoplex PCR Mixture: The DNA extract of MRSA isolates were subjected to *mecA* gene by monoplex PCR. The protocols used depending on manufacturer's instruction [20].

B. Detection of DNA Product by Agarose Gel Electrophoresis: DNA samples were electrophoresed by horizontal agarose gel electrophoresis as previously reported according to [19].

Results and Discussion

Isolation and Identification of *Staphylococcus aureus*

Cultural Characteristics for Isolation and Identification of *S. Aureus*

Identification was performed by streaking all specimens onto blood agar (BA) which were then incubated at 37˚C for 24hours. the colonies on BA plates showed up enormous, round, velvety/buff shaded settlements, delivering total away from of β-hemolysis . results were agreed with [22]and WHO 2003, the bacterial isolates were purified by culture isolates on the selective media of *S. aureus* which was the mannitol salt agar . the colonies appeared as small, circular smooth and yellow-golden color colonies, these results were agreed with [21].

Microscopical Characteristic and Biochemical Tests

S. aureus was isolated and identified depending on the cultural and microscopical properties as long biochemical test Catalase test is useful to distinguish staphylococci from enterococci The formation of bubbles indicates a positive test [22]. coagulase tests is performed to identify the ability of *S. aureus* isolates to coagulate plasma Positive result indicated by clot formation within 4 hr Where clotting did not occur the tubes were incubated at room temperature for an additional 18hr agreed with [10,23].

Number of *S. aureus* and MRSA isolate

A total of 231 specimens were collected from healthy people, 52 were identified as MRSA by disc diffusion method and all of them contains mecA. This study show a high prevalence of MRSA carriage in healthy people that indicating high risk of spreading infections as shown in table (2). this mismatches with [24] and [25]. There are no studies that are completely match these results.

Table (2): Percentage of antibiotic resistance for healthy carriers MRSA

Types of specimens	Total NO.	No. negative	S.aureus	No.(%)of MRSA
Nasal swab	231	179(77.4%)	52(22.5%)	52(100.0)

Identification of MRSA

All the isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc of cefoxitin, Methicillin resistant *S. aureus* (MRSA) emerged, resistance is caused by possession of the mecA gene which codes for a penicillin-binding protein that binds the drug less well, this alternative protein confers resistance to all beta -lactam antibiotics [5,6]. The confirmation of detected of MRSA by vitek2 system [18]. Of the 52 *S. aureus* isolates in Wasit province , 52 were identified as MRSA by disc diffusion method and all of them contains mecA ,this mismatch with [19]. No studies that are completely match these results.

Antibiotic Susceptibility Test of MRSA

The susceptibility test was done for MRSA isolates. Table (3) shows percentages of antibiotic resistance for 52 healthy carriers MRSA isolates by using Vitek 2 system to examine 16 different antibiotics. the present study, MRSA showed the highest resistance for each two group as 100% for both oxacillin and cifoxitine and Vancomycin group with present 98% .this study were similar to [26], Whereas, the remaining classes of antibiotics tested came with a different resistance rate as showen in table (3).

Table (3): Percentage of antibiotic resistance for healthy carriers MRSA isolate

Group of study / Antimicrobials	isolates from Healthy Carriers %	Mean + Std. Deviation
Linezolid	100	A 100 + 0.000
Ciprofloxacin	94	B 95.00 + 1.41421
Gentamicin	93	B 93.500 + 0.70711
Tigecycline	92	C 91.00 + 1.41421
Trimethoprim/Sulfamethoxazole	90	C 91.00 + 1.41421
Moxifloxacin	88	C 89.00 +1.41421
Imipenem	80	D 80.00+ 0.000
Clindamycin	72	E 70.500 +2.12132
Teicoplanin	50	H 48.00 + 2.82843
Erythromycin	41	I 42.00 +1.41421
Rifampicin	29	J 29.500 + 0.70711
Tetracycline	24	K 25.00 + 1.41421
Vancomycin	2	L 2.500 + 0.70711
Benzylpenicillin	0	L 0.0000 + 0.00000
Oxacillin	0	L 0.0000 + 0.00000
Cifoxitine	0	L 0.0000 + 0.00000

Table (4) represents the percentages of antibiotic sensitivity of MRSA isolates isolated from healthy carriers ts groups by using Vitek 2 system to examine 16 different antibiotics with the using of minimum inhibitory concentration (MIC) [15]. In the light of this results, MRSA showed that the highest mean + the standard deviation of sensitivity to

100 + antibiotics were 0.000 for class oxazolidinones (linezolid) followed by 95.00 + 1.41421and 93.500 + 0.70711 for class fluoroquinilones (ciprofloxacin) and aminoglycosides (gentamicin), respectively.

Table (4) The percentages of sensitivity of MRSA isolates isolated from healthy carriers

No.	Antimicrobial Group	No.(%) Healthy Carrier
1	Benzylpenicillin	100
2	Oxacillin	100
3	Imipenem	20
4	Vancomycin	98
5	Teicoplanin	50
6	Ciprofloxacin	6
7	Moxifloxacin	12
8	Erythromycin	59
9	Clindamycin	28
10	Linezolid	0
11	Gentamicin	7
12	Tetracycline	76
13	Tigecycline	8
14	Rifampicin	71
15	Trimethoprim/Sulfamethoxazole	10
16	Cifoxitine	100

Molecular Identification of MRSA Isolates

Several methods have been described that utilize polymerase chain reaction (PCR) for the detection of MRSA directly from clinical specimens (27) or in combination with broth enrichment. The feasibility of the PCR methodology for the identification of *S. aureus* strains and for the detection of antibiotic resistance genes has already been shown [28].

DNA extraction from MRSA

DNA was extracted by using several processed demodulation of the company Geneaid and took time from 24 to 72 hr.

PCR Detection of *mecA* gene

Monoplex polymerase Chain Reaction was used in the present study for the detection of *mec*A gene. *mec*A gene has been detected among all tested *S. aureus* isolates that considered phenotypically as MRSA by using the disk diffusion method and Vitek 2 system and subjected to PCR to detect the presence of *mecA* gene, all of them gave positive results using PCR . So , the correlation between phenotypic and genotypic method was 100% .These result agreement with the results of [29]. PCR product appeared as a DNA band with an about (533) bp in size as shown in figure (1) for healthy carriers. These results were agreement with previously published results by [30] and inconsistent with [29] and [31] whom detected a (310bp) DNA fragment in all tested *S. aureus* isolates.

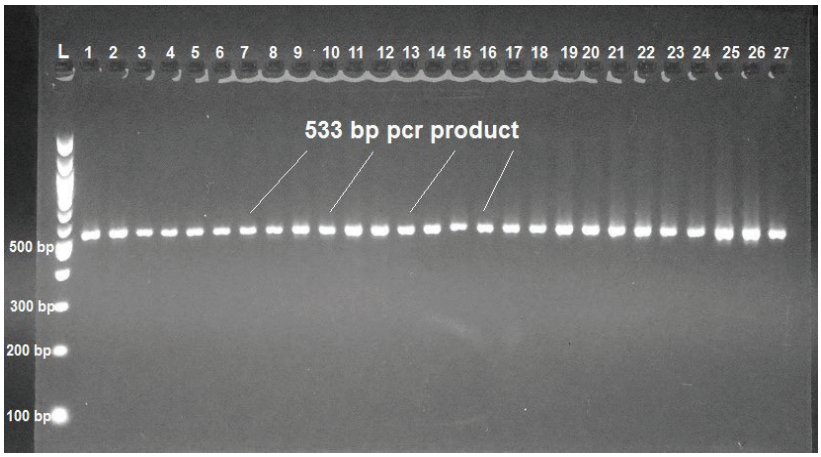

Figure (1): Agarose gel electrophoresis of PCR amplification products from carriers group of *S. aureus ,mecA* **gene (2% agarose, TBE buffer (0.5X), 100 volt and 50 AM for 1 hour.). L:The DNA molecular weight marker (100 bp ladder)**

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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