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Identification of MRSA (methicillin resistant *Staphylococcus aureus*) by *MECA* gene

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ABSTRACT: Staphylococcus aureus is a bacterium that may be found in the skin and upper respiratory system. Although S.aureus is a normal flora, it has evolved into an opportunistic pathogen that causes numerous infections that are difficult to cure due to the presence of virulence genes. The goal of this work is to identify MRSA in bacterial isolates using the mecA gene. In the current investigation, 213 samples were gathered from various clinical sources from patients hospitalized to various hospitals in Baghdad. The isolates were identified as S.aureus using normal bacteriological methods and verified using a molecular approach based on the nuc gene. Due to the many species of staphylococci that were expressed for varying resistance levels, the conventional approach was the best way for finding Methicillin resistant gene in S. aureus (mecA). Finally, mecAgene was found to be present in 76.67 % of bacterial isolates.

Keywords: **Antibiotic, Resistance, virulence gene**

1. INTRODUCTION

Staphylococcus aureus is gram positive bacteria that grow in the form of clusters, these bacteria are cocci, nonmotile, non-spore forming, aerobic or facultative anaerobic and highlighting a complex nutritional requirement for development, characterized by resistance to heat and tolerance to high concentration of salt, The Latin word aureus refers to colonies golden color that developed on solid media as a result of carotenoid production [1][2][3][4]. S.aureus a frequent pathogen for both human and animals that is considered as the causative agent for a wide variety of infections, which differ generally in symptoms and signs as well as the severity of the infection[5].The range of S.aureus infections includes skin , wound to deep tissue, pneumonia, septic arthritis, endocarditis, septicemia and nosocomial infection besides food poisoning, toxic shock syndrome and scalded skin syndrome. Presently, lower than 90% of S.aureus strains resist most penicillin derivatives and ordinary antibiotics like's tetracycline, fluoroquinolones, macrolides, aminoglycosides and chloramphenicol [6][7]. Efflux pumps have been identified for S.aureus encoded by chromosome or plasmids. The efflux pump lmrS, norC, mepA encoded by chromosome [8][9][10]. Vali et al reported that over expression of mepA convery a pattern of resistance to fluoroquinolones, tetracyclines, disinfectant, and dyes[11].

2. MATERIAL AND METHODS

2.1 Sample Collections

213 samples were obtained from patients hospitalized to Baghdad hospitals with a variety of clinical conditions (wounds, burns, ear infections, UTI, oral and nasal infections, etc). different hospitals and clinic from Baghdad city). Biochemical tests such as Gram stain, catalase test, coagulase test, and urease test were utilized to identify the clinical samples [12].

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2.2 Molecular Study DNA extraction:

. Table 1. Primers that used in this study

Primers	Sequence (5'……3')	PCR product bp	Reference
nuc_F	GCGATTGATGGTGATACGGTT	276	[13]
nuc-R	AGCCAAGCCTTGACGAACTAAAGC		
mecA-F	GTGAAGATATACCAAGTGATT	147	[13]
mec A-R	ATGCGCTATAGATTGAAAGGAT		

The manufacturer's instructions for Geneaid's Reagent Genomic DNA Kit were followed to isolate chromosomal DNA (Tiawan). Amplification of the S.aureus nuc gene and the mecA gene The nuc and mecA genes were amplified by PCR using the proper primers (table 1). The Go Taq®Green Master Mix (Promega, USA), 1 l of each primer (10 pmol), 7.5 l of DNase RNase free water, and 3 l of purified DNA template were used in a 25-l reaction with these primers.
A total of 35 cycles of denaturation at 95°C for 5 minutes, followed by 45-second annealing at nuc 54°C and mecA 60°C, 55-second extension at 72°C, and 7-minute final extension at 72°C were used to amplify the nuc and mecA genes. It was done by gel electrophoresis utilizing 1.5 percent agarose gel and ethidium bromide gel documentation system (Thermo Fisher Scientific, USA). A 1500 bp DNA Ladder (Bioland -USA) was used to establish the appropriate amplicon size.

3. STATISYICAL ANALYSIS

Statistical analysis was done by using (SPSS) (version 25) [14].

4. RESULT AND DISCUSSION

Out of 213 samples, routine bacteriological tests identified 120 as S.aureus. Nuc gene molecular detection was also used on the isolates to confirm their bacterial genus and species, as shown in (Fig.2). S.aureus isolates acquired locally are mostly multidrug resistant, meaning they are resistant to more than two antimicrobial agents[15]. In susceptibility testing, 76.67 percent of MRSA isolates were resistant to -lactam antibiotics [16][17][18]. 80 percent of S. aureus were MRSA, which is consistent with [19]. MRSA was found in 68% of all S. aureus isolates, according to research published in [20]. This study's S.aureus isolates had the greatest resistance to -lactam antibiotics, which is in line with[20findings]'s of strong resistance to mecillinam 76.5 percent, cefoxitin, ceftriaxone, and meropenem 66.12 percent.

4.1 Molecular detection of nuc gene in *S . aureus* Isolates

All 213 staphylococcus spp. DNA samples were PCR amplified using the specific primers (TABLE -1) to identify each staphylococcus species. According to the findings, all of the 120 isolates tested positive.

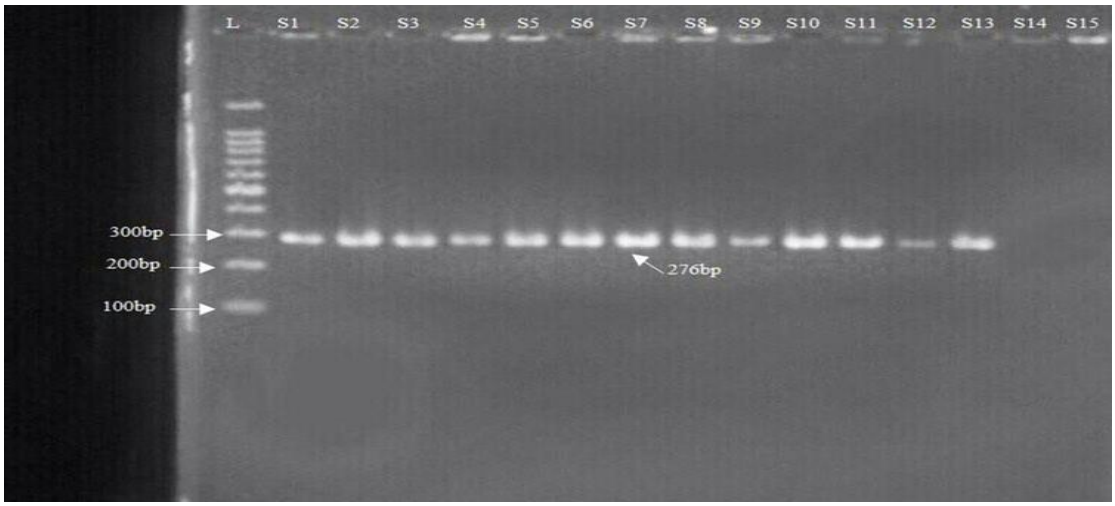

Figure 1: Gel electrophoresis of amplified nuc gene (276 bp), from *S.aureus* using conventional PCR. Agarose 1.5%, 70 V/cm for 1 hrs. and 20 min, stained with ethidium bromide dye and visualized on a UV

transilluminator. Lane L: 1500 bp DNA ladder. Lane 1-13: Amplicons 16srRNA gene for S.aureus (isolates from S1 to S 13).S14 represent positive control S15 represent negative control.

Using a total of 120 S.aureus DNA samples, a particular primer set was used to identify the chromosomal resistance mecA gene. After electrophoresis on a 2% agarose gel, 76.67% of the mecA gene generated a distinct band with a molecular size of 147 bp fig 2.

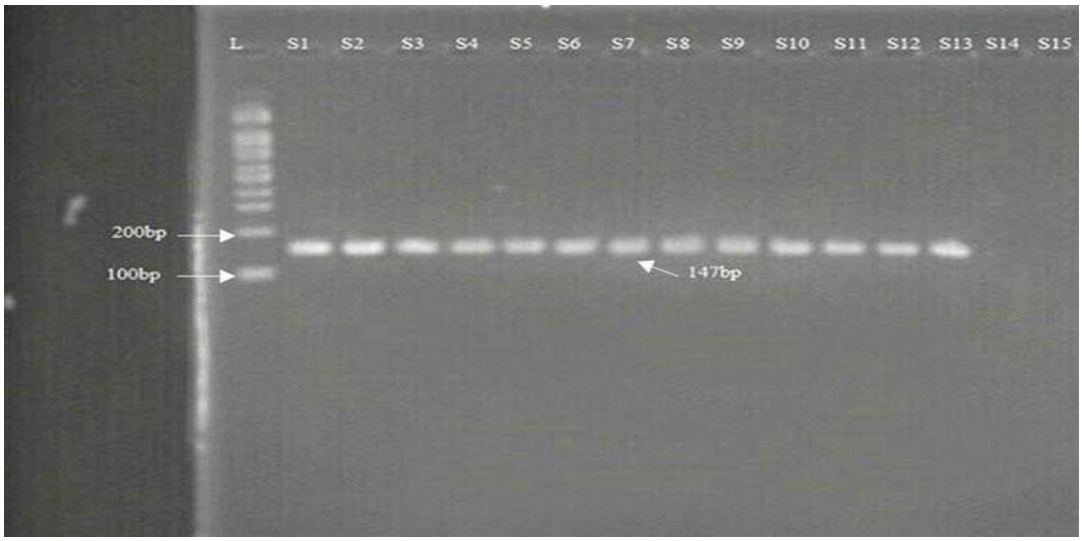

Figure 2: Gel electrophoresis for PCR amplification product of *mec*A gene (1.5% agarose, 70 voltage for 90 min), Lane L: 1500 bp DNA ladder. Lane S1-S13 represent the positive results of *S.aureus* isolates (147bp)Lane S14: Negative control.

Rasheed et al. (2020) found that 98.7 percent of MRSA had the mecA gene, which is consistent with this finding. The mecA gene was found in 39/78 percent of Staphylococcus aureus isolates, according to Alsadi et al. (2020). Potential resistance to -lactamases may be determined by the presence of mec A gene, which can be used as a marker to identify MRSA as shown in table 2.

Table2: The percentage of mecA gene in β- lactam resistance.

Antibiotics resistannce (R)	*mec* A positive	*mec*A negative	Total
ME	92(76.70)	0(0.0)	92(76.70)
CTX	92(76.70)	28(23.30)	120(100.00)
MEM	92(91.10)	9(8.90)	101(84.20)
MEC	92(87.60)	13(12.40)	105(87.50)
FOX	92(92.90)	7(7.10)	99(82.50)
CRO	92(91.10)	9(8.90)	101(84.20)

The result of correlation for antibiotic susceptibility with mecA gene in isolates in table 3, appearance most antibiotic susceptibility of isolates there are statistically significant of correlation with mecA gene(present or absent), except Ofloxacin and Trimethoprim there are the correlation no statistically significant.

Table3: The percentage of mecA gene in β- lactam and non in β- lactam antibiotics

Antibiotics	MRSA	MSSA	Total	*P value*

β-Lactams	ME	R	7 (50%)	7 (50%)	14 (100%)	-
		S	0 (0%)	0 (0%)	0 (0%)	
	CTX	R	7 (50%)	7 (50%)	14 (100%)	-
		S	0 (0%)	0 (0%)	0 (0%)	
	MEM	R	7 (50%)	2 (14.3%)	9 (64.3%)	0.005**
		S	0 (0%)	5 (35.7%)	5 (35.7%)	
	MEC	R	7 (50%)	3 (21.4%)	10 (71.4%)	0.018*
		S	0 (0%)	4 (28.6%)	4 (28.6%)	
	FOX	R	7 (50%)	2 (14.3%)	9 (64.3%)	0.005**
		S	0 (0%)	5 (35.7%)	5 (35.7%)	
	CRO	R	7 (50%)	2 (14.3%)	9 (64.3%)	0.005**
		S	0 (0%)	5 (35.7%)	5 (35.7%)	
Non–β-Lactams	Cip	R	7 (50%)	0 (0%)	7 (50%)	<0.0001**
		S	0 (0%)	7 (50%)	7 (50%)	
	E	R	7 (50%)	1 (7.1%)	8 (57.1%)	0.001**
		S	0 (0%)	6 (42.9%)	6 (42.9%)	
	ATH	R	7 (50%)	0 (0%)	7 (50%)	<0.0001**
		S	0 (0%)	7 (50%)	7 (50%)	
	OFX	R	1 (7.1%)	0 (0%)	1 (7.1%)	0.299 NS
		S	6 (42.9%)	7 (50%)	13 (92.9%)	
	NOR	R	7 (50%)	0 (0%)	7 (50%)	<0.0001**
		S	0 (0%)	7 (50%)	7 (50%)	
	TM	R	1 (7.1%)	0 (0%)	1 (7.1%)	0.299 NS
		S	6 (42.9%)	7 (50%)	13 (92.9%)	
	LEV	R	7 (50%)	0 (0%)	7 (50%)	<0.0001**
		S	0 (0%)	7 (50%)	7 (50%)	
	TS	R	0 (0%)	0 (0%)	0 (0%)	-
		S	7 (50%)	7 (50%)	14 (100%)	
	AK	R	0 (0%)	0 (0%)	0 (0%)	-
		S	7 (50%)	7 (50%)	14 (100%)	
	CO	R	7 (50%)	7 (50%)	14 (100%)	-
		S	0 (0%)	0 (0%)	0 (0%)	

Data presented as Chi-square independence test. NS=Non-significant, * the correlation is significant at the P < 0.05 level (Significant), ** the correlation is significant at the P < 0.01 level (Highly Significant). - No statistics are computed because data is a constant. R: resistance, S: sensitive. Ciprofloxacin(CIP), Colistin(CO), Levofloxacin(LEV), Meropenem(MEM), Mecillinam(MEC), Cefoxitin(FOX), Trimethoprim(TM), Methicillin(ME), Cefatoxime(CTX), Ceftriaxone(CRO),Ofloxacin(OFX),Amikacin(AK),Norafloxacin(NOR),Azithromycin(ATH),Erythromycin(E),Trimethoprim- Sulfamethoxazole (TS).

The result showed that 92 isolates were carriers of mec A gene and were resistant to all antibiotics that belong to β-lactam group that used in this study. Studies conducted by Hafez etal., (2009); Meshref and Omer (2011); Al zubi et al.,(2004) showed high prevalence of mecA gene among their *S.aureus* isolates and represent potential reservoir for the spread of this gene. Exogenous copies of the mec genes (mecA, mecB, mecC, and mecD) are found in all MRSA, and this confers resistance to -lactam antibiotics [21]. Some more mechanisms of resistance need to be investigated further.

But in non β-lactam antibiotics, the isolates were highly sensitivity although it was carriers *mec*A gene, it may be carrier of another gene responsible for resistance to this group of antibiotics or other mechanism cause resistance need further studies.

5. CONCLUSION

Finally, our data suggest that MRSA isolates have a high resistance to β-lactam drugs. To minimize an increase in the number of drug-resistant organisms in Baghdad's hospitals, it is recommended that antimicrobial use and infection control be optimized.

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