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Detection of some virulence factors and antibiotic resistance of Staphylococcus aureus isolated from clinical cases

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Abstract*---Staphylococcus aureus* is a gram-positive bacteria that cause a wide variety of clinical diseases, appears as grape like clusters, non-sporing, nonmotile and usually non capsulated bacteria. The current study was carried out from September to December 2021 , in which 61 *S. aureus* isolated from 150 clinical specimens. It is resistant to most β-lactam antibiotics due to an alteration in the penicillin-binding protein (PBP2a) that is encoded by the *mec* genes ,during that can be classified as Methicillin-resistant *S. aureus* (MRSA) that resistant to a wide range of β-lactam antimicrobial agent due to the presence of the *mecA* gene and methicillin-sensitive *S. aureus* (MSSA) that usually resistant penicillin due to β-lactamases. antibiotic susceptibility of all 61 *S. aureus* isolates showed resistant to Amoxicillin/clavulanic and Cefepime (100%) followed by Cefotaxime(94%) , Azithromycin (80%) and Erythromycin (60.6%) while the Vancomycin, Ceftriaxone, Amikacin, Gentamicin and Imipenem antibiotic gives high sensitivity (100%) followed by Meropenem and Levofloxacin(80%). *S. aureus* contains many genes which encode the most important toxin like the *tst* gene which encode toxic shock syndrome toxin (TSST-1) and *luks* gene which encode Panton Valentine leukocidin (PVL) toxin. We looked at the prevalence and screening of this genes which are considered one of the most important factors of virulence in *S. aureus*. We performed polymerase chain reaction for *mecA*, *tst* and *luks* gene for *S. aureus* clinical isolates . the *mecA* gene appear in all *S. aureus* isolates(100%) while the *luks* gene appeared in (20%) and the *tst* gene in (10%) of the isolates .

***Keywords*---**MRSA, MSSA, β- lactam antibiotics.

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Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive, round-shaped facultatively anaerobic bacterium. It is an opportunistic infection that colonizes the skin and upper respiratory tract. (Horn *et al* ., 2018). *S. aureus* is hemolytic, forming a transparent hemolytic ring around the colonies on blood agar plates (Gonzalez-Perez *et al.*, 2019 ; Sato *et al.*, 2019). While *S. aureus* has a commensal relationship with humans, it will adapt to a pathogenic lifestyle on entering host tissues(through injury or medical intervention) (Reddy *et al.*, 2017) . Methicillin is a β-lactam antibiotic was introduced as an effective agent against *S. aureus* in 1959. However resistance was reported within a year(Gordon and Lowy, 2008). The widespread use of Methicillin in the late 1960 led to the emergence of Methicillin resistant *S. aureus* (MRSA). The acquisition and expression of the *mecA* gene, which codes for a modified penicillin binding protein (PBP) known as PBP2a, is required for methicillin resistance in *S. aureus* (Monecke *et al.*, 2011). *S. aureus* produces toxins that may cause such diverse manifestations as gastroenteritis, toxic shock syndrome and scalded skin syndrome. A large selection of *S. aureus* virulence factors includes enzymes i.e, coagulase, nucleases and catalases that help it to evasion from immune system and many exotoxins such as enterotoxins and toxic shock syndrome toxin (TSST)(Adalbert *et al* .,2021). Presence of virulence factors appears to vary among *S. aureus* strains due to loss and acquisition of genes (Shittu *et al* ., 2007). Because this methodology identifies the strains of *S. aureus* bearing the genes of toxins, the introduction of the PCR method may assist in providing the necessary information during *S. aureus* outbreaks for proper infection treatment (Sultan and Al Meani , 2019).

Materials and Methods

Samples collection

A total of 150 clinical specimens collected from September to December of 2021 from different sources in Al-Najaf province,(Al-Sadr Medical City, Al-Furat Al-Awsat and Burn Center) . Clinical specimens included burns , wounds , urine and abscesses. Burn, wound and abscesses specimens were collected using sterile cotton swabs , while the urine collecting by sterile plastic bottle . Swab was taken swab from infection area and then transport the swab by transport media to the lab . All The collected Specimens were transported by sterile transport swabs and inoculated using direct method of inoculation on culture of selective media namely Blood and Mannitol salt agar then inoculated at 37°C for 18-24 (Cheesbrough,2010).

Diagnosis of *S. aureus* isolates

Isolates were recognized and diagnosis based upon the characters of cell and colony morphology toward growing bacteria on media as well as some essential of biochemical tests agreeing with (MacFaddin , 2000) . Suspected bacterial isolates which were Gram – positive , coagulase positive, negative to oxidase , as well as non - motile , were further identified by biochemical tests. The final identification was performed using the automated VITEK-2 compact system using ID-GP cards. Detection the susceptibility of *S. aureus* to group of antibiotics by Kirby - Bauer method and the results interpreted according to (CLSI, 2020).

S. aureus important gene identification

Polymerase Chain Reaction (PCR) was performed in monoplex patterns in order to amplify different fragments of genes under study for detecting *S. aureus* virulence factors genes. The following primer used in this study:

Primer Type		(Primer Sequence (5'-3'))	amplicon size (bp)	Reference
mecA	F	TAGAAATGACTGACGTCCG	154 bp	(Santos *et al.*,1999)
	R	TTGCGATCAATGTTACCGTAG		
tst	F	AAG CCC TTT GTT GCT TGC G	445bp	(Becker *et al.*,1998)
	R	ATC GAA CTT TGG CCC ATACTT T		
luks	F	CAGGAGGTAATGGTTCATTT	151 bp	(Al-Talib *et al.*,2009)
	R	ATGTCCAGACATTTTACCTAA		

2 µL of forward gene primer, 2 µL of Reverse gene primer, 4µL of free nuclease water, 5 µL of DNA, and 12 µL of master mix , thin walled PCR tube were included in each 25 µL of PCR reaction mixture for PCR. The following were the thermal cycler settings:

PCR gene	Temperature (c) / Time					Cycle Number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
mecA	95/2 min	95/30 sec.	55/30 sec.	72/10 sec.	72/5 min.	30
tst	95/2 min.	95/30 sec.	56/30 sec.	72/50 sec.	72/5 min.	30
luks	95/2 min.	95/30 sec.	52/30 sec.	72/20 sec.	72/5 min.	30

Results and Discussion

S. aureus were isolated from different clinical specimens using specific media such as Mannitol Salt Agar and CHROM™ agar .

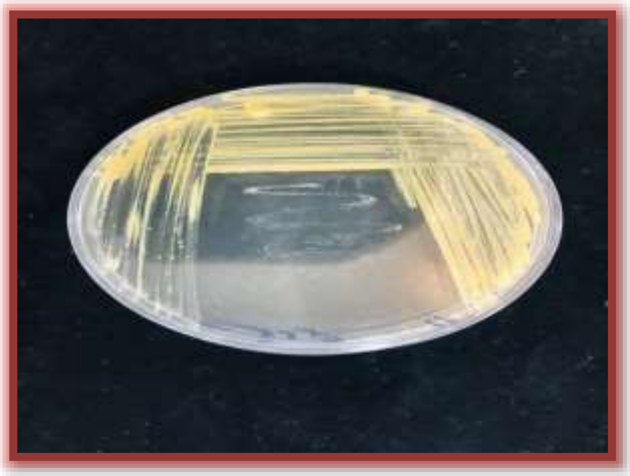

Figure (1): growth of *S. aureus* on Chrom ™ agar orientation medium

Antimicrobial susceptibility test of clinical *S. aureus* isolate using disc diffusion method

Antimicrobial susceptibility testing was performed on all of the detected *S. aureus* isolates, (CLSI , 2020) was used to read the findings of this test , which included 14 antibiotic kinds. The results in a Table (1)

Table (1) : Determination the Sensitivity of the clinical *S. aureus* to Antibiotics

Antibacterial agent	Sensitive (%)	Resistance(%)
Amoxicillin/clavulanic(AMC)	0	61(100%)
Cefepime (FEP)	0	61(100%)
Vancomycin (VA)	61(100%)	0
Ceftriaxone (CTR)	61(100%)	0
Trimthoprim (TMP)	36(59%)	25(41%)
Meropenem (MEM)	49(80%)	12(20%)
Azithromycin (AZM)	12 (20%)	49(80%)
Levofloxacin(LEV)	49(80%)	12(20%)
Cefotaxime (CTX)	4 (6%)	57(94%)
Amikacin (AK)	61(100%)	0
Erythromycin (E)	24 (39.4%)	37(60.6%)
Gentamicin (CN)	61(100%)	0
Imipenem(IMP)	61(100%)	0
Ceftazidime (CAZ)	36(59%)	25(41%)

Molecular Detection of Bacterial Isolates

Detection of *mecA* in clinical *S. aureus* isolates

A total of 10 *S. aureus* isolates were molecularly examined for the presence of *mecA* genes after being acquired from diverse clinical sources , fig (2) illustrates the frequency of genes in the isolates under research as percentages: 10/10 (100%) from *S. aureus* isolates.

Figure (2) : Electrophoresis diagram of Monoplex PCR generated products for extracted DNA of 10 *S. aureus* isolates ,utilizing Primer *mecA* gene with Product (154bp). For 1.5 hours , the electrophoresis was run at 70 volts. lane (L), DNA Molecular Size Marker (100 bp ladder) . Gene *mecA* produces positive results in all Lanes.

This findings of *S. aureus* isolates show that 100% of them had the *mecA* gene and this result is comparable to that of (Yurtsever *et al* .,2020) they observed that the *mecA* gene is present in all of the isolates(100%) however this result is not comptible to that of (Alhamadani *et al* ., 2020) and (Zarei Koosha *et al* ., 2016) , who showed that the *mecA* gene ratio reached(80%), (87.3%) of *S. aureus* isolates included the *mecA* genes. the *mecA* mediates Methicillin resistance in *S. aureus* . Methicillin resistance has occurred in *S. aureus* by mutation of a penicillin-binding protein , a chromosome - encoded protein . This type of resistance is transferred between *S. aureus* by bacteriophages (Lakhundi *et al* ., 2018).

Molecular detection of *tst* genes

Polymerase chain reaction technique of the 10 *S. aureus* isolates revealed that *tst* were gave positive result for only one isolate (10%), (Fig. 3).The current result agree with (Mir *et al* ., 2019) who showed the frequency of *tst* was (10.7%), this result is in agreement with many reports that met on the little appearance of this gene such as (Wang *et al* ., 2017) and (Ghasemzadeh-Moghaddam *et al* ., 2011) they showed the frequency of gene (18.0%),(6.3%), while these results did not agree with the study conducted by (Zhao *et al* ., 2019) who found 208 of the identified *S. aureus* strains were *tst* – positive .

The toxic shock syndrome toxin-1 (TSST-1) encoded by *tst* gene , is a significant member of superantigens (Sags) and may lead to staphylococcal toxic shock syndrome (TSS) in a susceptible host (Spaulding *et al* ., 2013).

Figure (3-) : Ethidium bromide – stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* isolates using primer *tst* _ gene with product445bp . The electrophoresis was performed at 70 volt for 1.5 hr . lane (L) , DNA molecular size marker (1500 bp ladder). Only Lane (8) show positive result.

Molecular detection of *luks* genes

The presence of the *luks* gene (151pb) was determined by polymerase chain reaction amplification among *S. aureus* isolates from diverse clinical sources . This finding indicated that only (20%) of clinical isolates produced a positive result prevalence of *luks* among *S. aureus* isolates, figure (4).

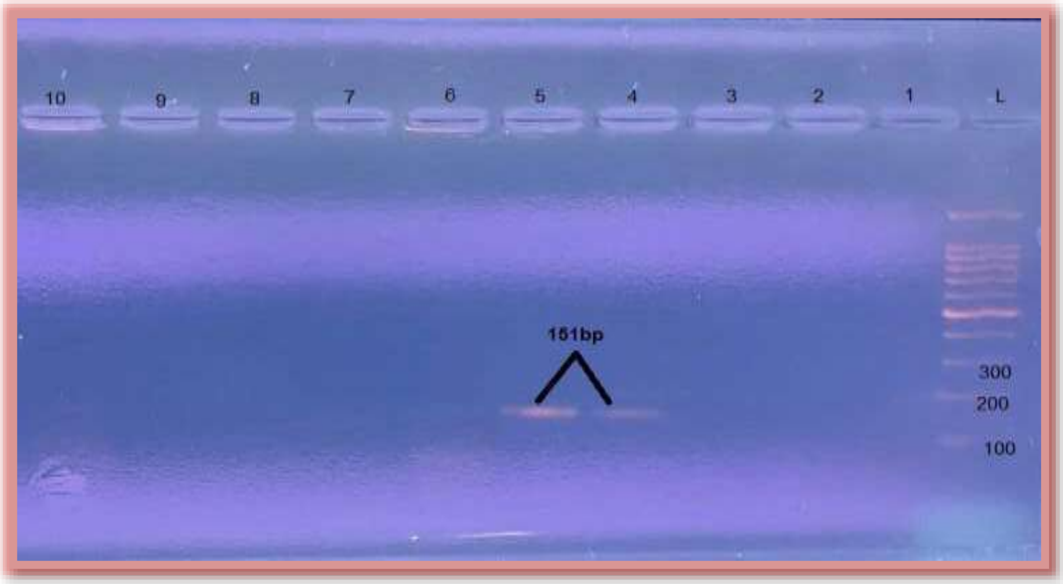

Figure (4) : Ethidium bromide – stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* isolates using primer *luks* _ gene with product 151 bp . The electrophoresis was performed at 70 volt for 1.5 hr. Lane (L) , DNA molecular size marker (1500 bp ladder). Lanes (4,5) show positive results.

The results showed that (20%) of *S. aureus* isolates contained *luks* gens , the same results appeared in (Katsarou *et al* ., 2020) and (Neamah *et al* ., 2019) they found (21.5%),(27.7%) *S. aureus* isolates carried *luks*. *luks* gene encoding Panton – Valentine leukocidin (PVL) , it is a powerful cytotoxin that is made up of two chromatographically distinct protein components , *LukS-PV* (slow) and *LukF-PV* (fast) . The active toxin promotes neutrophil lysis by generating a hole ontheir membrane , which is linked to dermonecrosis (Jauneikaite *et al* .,2020).

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