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RESEARCH ARTICLE

Prevalence of Tst Genes in Methicillin-resistant *Staphylococcus aureus* (MRSA)

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) are common and important causes of nosocomial infections worldwide in hospitals and health care facilities where people are less immune, MRSA frequently spread in hospitals by patients that conceder as a major reservoir, healthcare workers (HCWs), environmental surfaces, and occasionally through the air, MRSA has many virulence factors that play an important role in bacterial spread and severity including's a superantigen that encoded by *Tst* gene Toxic shock syndrome-1 (*TSS-1*) Secondary inflammatory complications include invasive forms of bacterial diseases Such as inflammation of the lungs, lung abscesses, urinary tract infections, food poisoning, osteoarthritis, Endocarditis, meningitis, arthritis, toxic shock syndrome, septicemia and death.The study showed a clear outbreak of methicillin-resistant staphylococcus bacteria (MRSA), 39/ 46(85%), *Tst* gene was absent in most isolates, only 6/39 (15.4%) harboring *Tst* gene.

Keywords: Methicillin Resistant Staphylococcus aureus (MRSA), Tst gene

1 | INTRODUCTION

Staph aureus is one of the most common pathogenic bacteria, as its pathology is dependent on a group of virulence factors that affect the host and cause the disease. (Hoseini Alfatemi et al. 2014)It is also one of the "ESKAPE" organisms, can cause many serious infections so it is considered a serious and growing threat worldwide that can affect various groups and can cause serious nosocomial infections. (Liang et al. 2019)

Staph aureus can avoids further clearance by the immunity through the expression of surface-linked proteins and polysaccharide capsules that prevent opsono-phagocytic killing. *Staphylococcus* protein A (SpA) is a membrane-bound protein that binds with Fc region in IgG, thereby avoiding recognition

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of macrophages (Walton 2013) The success of *Staph aureus* a pathogen is explained by it ability to express:

(1) Its ability to invade and inflammate. This includes a number of mechanisms including colonization, synthesis of extracellular structure of molecules that facilitate adherence and help them avoid host defenses.

(2) Its ability to produce toxins. (Zhu 2010) and(Haghkhah 2003)

Neutrophils are the decisive defense of the body in controlling the colonization and spread of *Staph aureus* despite the behavior of Staphylococcus for many evasion strategies.One of these strategies is the induction of neutrophil cell death, which causes inflammations and tissue damage and increased disease severity. (Yang et al. 2019) In addition to evading strategies, *Staph aureus* produces many virulence factors, which include enzymes and toxins, in addition to its ability to produce septic shock by activating and interacting with the immune system and coagulation (Rigby and DeLeo 2012) and (Gordon and Lowy 2008) . *Staph aureus* produces a wide variety of exotoxins, among the numerous toxins of including enterotoxins, the enterotoxins super antigens have already been assigned to the pyrogenic toxin super antigen family based on their biological activity and structural similarity, toxic shock toxin-1 (TSST-1) that induces super antigenic activity, and exfoliative toxins (ETs), these toxins are responsible for specific acute clinical syndromes such as toxic shock syndromes (TSS) , food poisoning due to staphylococcus enterotoxins and staphylococcal scarlet fever (a mild form of TSS), all these toxins share in their structural and biological properties, and this indicates that they are derived from a common ancestor. (Zhu 2010) and (Thomas et al. 2006) . Another class of genetic characteristics of staphylococci is a super-antigen that encoded by *Tst* gene, that carried on mobile genetic elements (MGE) named (*SaPIs*.), nearly 15 kb genomic regions that significantly denote a number of virulence genes, (*SaPIs*) linked to specific *Staph aureus* genetic families, known as lineages (Sharma et al. 2018) and (Shien 2014) Toxic shock syndrome-1 (*TSS-1*) Secondary inflammatory complications include invasive forms of bacterial diseases Such as inflammation of the lungs, lung abscesses, urinary tract infections, food poisoning, osteoarthritis, Endocarditis, meningitis, arthritis, toxic shock syndrome, septicemia, Death (Bocskay 2016)

2 | MATERIALS AND METHODS

Sampling

A total of 484 samples collected from patients (Skin swab, Nasal swab and Wound swab), Health Care Workers (Skin swab and Nasal swab), hospital words (Orthopedic and Surgical words) and Operation Theater (various places of Operation Theater before and after sterilization) . Samples were collected in the period Between November 2018 and August 2019, from two locations, Al-Basrah Teaching Hospital and Al-Saddr Teaching Hospital. Each swab was transferred in to enrichment medium (brain heart infusion broth (BHIB)) for 2-4 hour at 37^{o}C. (Nicholas P. Vitko and Anthony R. Richardson 2014)

Culturing and identification

Staphylococcus grows easily on most routine media at aerobic or micro-aerophilic conditions. It was quickly grows at (37°C), and the ideal temperature in which the pigment is formed is 20-25°C *Staph aureus* usually forms grey to golden yellow colonies due to carotenoids, Produces β-haemolysis on horse, sheep or human blood agar plates (Suzuki et al. 2012) and (Gillet et al. 2002) The bacterial morphology was observed microscopically as Gram-positive cocci arranged in grape-like irregular clusters (Gillet et al. 2002) All *Staph aureus* strains produce coagulase enzyme. *Staph aureus* are catalase positive and oxidase negative (Suzuki et al. 2012) *Staph aureus* express a clumping factor (fibrinogen affinity factor) (Reddy, Srirama, and Dirisala 2017) *Staphylococcus* can grow in a medium with a high salt concentration, so they can grow easily in MSA. The acidity of the medium changes as the bacteria ferments mannitol and turn phenol red pH-indicator; *Staph aureus* changes color of MSA from the alkaline (red)to the acidic(yellow), while the rest of the *Staphylococcus* will grow without changing the color of the medium. (Gillet et al. 2002)

Detection of MRSA

- **Cefoxitin disc diffusion,** Significant method to detect MRSA, by testing MRSA resistance to the cefoxitin disc, culture was done on MHA plate , incubation temperatures at 35-36 0C and times of 18-24 hour strains of Staph aureus having zone of inhibition less than 19mm defined as MRSA.
- **PCR Methods,** Strains of Staph aureus harboring MecA gene defined as MRSA

Genomic DNA extraction

As the instruction of manufacturer (promega company) Polymerase Chain Reaction (PCR) according to the manufacturer's instructions, the DNA were detected by gel electrophoresis, the samples were loaded in 0.8% agarose gel 1×TBE (54 g Tris-base, 0.5M EDTA, 1-l distilled water, pH=8 and diluted with 400 ml of distilled water) and electrophoresed at 60 V for 30 min.

Polymerase chain reaction technique

PCR is a very effective method to amplify a particular DNA as many copies of a specific DNA (Bartlett 2003), all MRSA isolates were assayed for the presence of the *Tst* gene by PCR using previously described primers, for PCR used diluted forward and reverse primers to reach (100 pmol/μl) concentration as stock solution, distilled water was used as the negative control.

TABLE 1: PCR mastermix Volum:

PCR mix		Volum
Promega Green Master Mix		12.5µL
DNA template		2.5µL
Primer	Forward Primer	1.5 µL
	Reverse Primer	1.5 µL
nuclease free water		7 µL
Total		25 µL

TABLE 2: OligonucleotideSequences and Amplicon Size of Each Gene Used in This Study

Gene	Oligonucleotide Sequence (5'-3')	Amplicon Size, bp	Reference
MecA		147	Funaki, *et al.*,2019
MECA-1	GTGAAGATATACCAAGTGATT		
MECA-2	ATGCGCTATAGATTGAAAGGAT		
Tst		326	Rossato, *et al.*, 2018
GTSSTR-1	ACCCCTGTTCCCTTATCATC		
GTSSTR-2	TTTTCAGTATTTGTAACGCC		

TABLE 3: Thermal Cycler ProgramsUsed in This Study

Gene	Temperature (°C)/Time					Cycle No.
	Initial denaturation	Cycling condition			Final extension	
		denaturation	annealing	extension		
MecA	94/5 min	95/30 sec	50/45 sec	72/1 min	72/7 min	30
Tst	96/2 min	94/30 sec	55/1 min	72/1min	72/7 min	30

Statistical analysis

Statistical analysis was done using SPSS (Statistical Package for Social Sience) program V. 20, Exper-imental data were presented in terms of observed

numbers and percentage frequencies, and then analyzed by using Chi-square ($\chi 2$) test to determine the relationship between the variables, P value $\leq$ 0.05 was considered statistically significant.

3 | RESULTS

Identification of bacterial isolates

Samples were collected in the period between November 2018 and August 2019 from two locations, Al-Basrah Teaching Hospital and Al-Sadder Teaching Hospital. Out of 485 samples only 46 (9.48 %) were identified as coagulase positive staphylococci, as shown in table (3-1) PCR product was electrophoresed in 1.5 % agarose gel, Stained with ethidium bromide, , 7 μL of PCR products and promega DNA ladder (50-1000bp) carefully loaded

in the wells and electric current was matched (65 volt for 45 h). The gel was then observed under a UV light and compare with ladder (50-1000bp).

Detection of MRSA isolate

Cefoxitin resistance staph aureus isolate harboring mecA gene (MRSA) was detected in 39from 46(85%) S. aureus isolates. 10(90%) isolates of MRSA were from wound samples, 6 (75%) from patient skin swab and 5 (83%) from patient noses, 4(80%) from hospital wards, 4(80%) from health care workers hands, 7(100%) from health care workers noses and 3 (75%) from hospitals theaters samples.

df=6, P Value =0.851, $\square^2$ =2.654

MecA gene detactions

TABLE 4: Number of Staph aureus isolates

Type of specimens	*Staph aureus*				Total *Staph aureus* isolate	
	MRSA		MSSA			
	No.	%	No.	%	No.	%
Hospital wards	4	80	1	20	5	100
Hospital theaters before sterilization	3	75	1	25	4	100
H.C.Ws hands	4	80	1	20	5	100
H.C.Ws nasal swab	7	100	0	0	7	100
Wound swab	10	90	1	10	11	100
Patient skin swab	6	75	2	25	8	100
Patient nasal swab	5	83	1	17	6	100
Total	39	85	7	15	46	100

Staphylococcus MecA gene presence in all MRSA isolates, to detect *Staph aureus* isolates with *MecA* gene, it was subjected to PCR technique, *MecA* gene band detected at 147bp region.

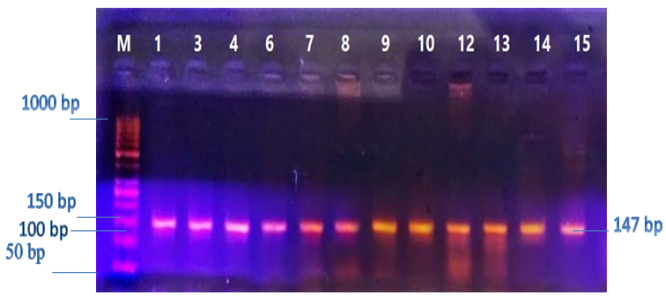

FIGURE 1: Figure-1:Amplified MecA gene (147bp) of Staph aureus isolates in PCR techniqueAgarose gel (1.5%), (95voltage for 45minutes)

M: DNA Ladder (50-1000bp). All MRSA was positive for *MecA* gene

***Tst* gene detection**

Tst gene detected only in 15.4% of MRSA isolate.

After amplification by PCR technique. Genes were detected by Gel electrophoresis of amplified PCR products of Tst genes (326bp) of *Staph aureus* isolates in PCR technique.

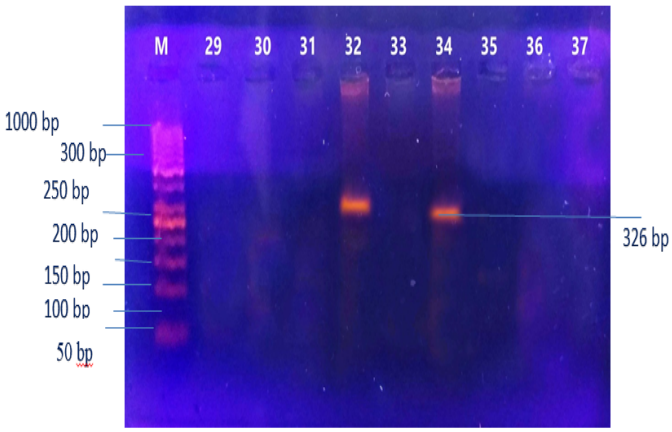

FIGURE 2: Amplified PCR products of Tst gene (326bp) of Staph aureus isolatesin PCR technique. Agarose gel (1.5%), (95voltage for 45minutes), M: DNA Ladder(50-1000bp), 32, 34 samples harboring

Sequencing for *Tst* gene

One sample was subjected to sequencing isolated from patient nose (10), as showen in the Figure 3 , with alignment bellow Figure 4.

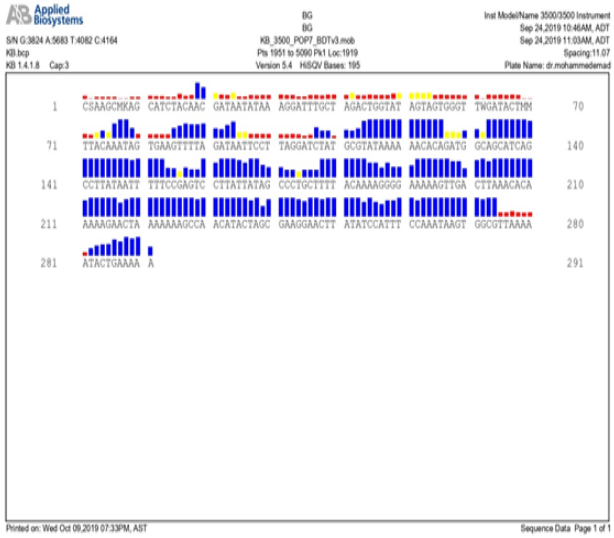

FIGURE 3: DNA Sequence for Tst gene

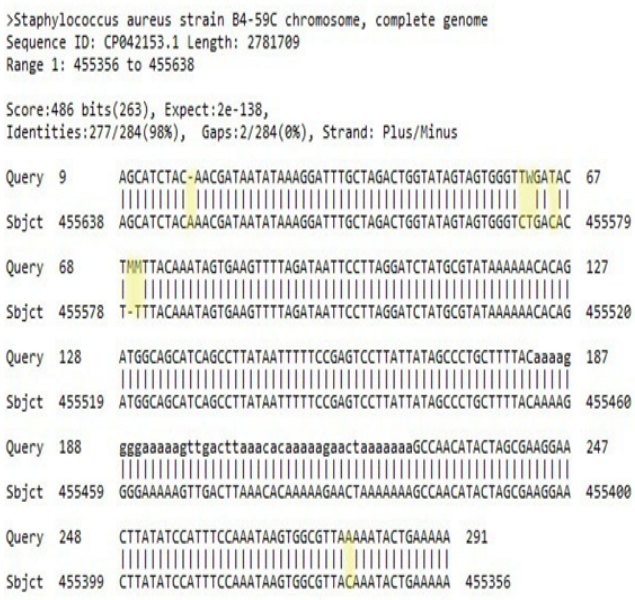

FIGURE 4: Alignment of Tst gene.

Query isolate (our isolate) begin from (9-291) bp when compared with subject isolate (Stander isolate) begin from (455638-455356) bp, where the compat-ibility occur between the two isolates for identifica-tion Query isolate, identities was 98%, There was a mismatch at seven places.

Source of isolates	MRSA genes	
	MecA	*Tst*
Hospital wards	4 (100%)	1 (25%)
Operating Theatres	3 (100%)	0
H.C.Ws hand	4 (100%)	2 (50%)
H.C.Ws nasal swab	7 (100%)	1 (14.3%)
Wound swab	10 (100%)	1 (10%)
Patients skin swab	6 (100%)	0
Patients nasal swab	5 (100%)	1 (20%)
Total of positive result	39 (100%)	6 (15.4%)
Negative result	0	33 (84.6%)
df	-	6
P value	-	0.433
$?^2$	-	5.913

TABLE 5: Prevalence of various genes ofMRSA isolated from different specimens.

4 | DISCUSSION

The study showed that these bacteria isolated from the hospital environment (operating rooms and patients' rooms) and hands and noses of workers and patients may be causes wound infection, There is increasing concern about MRSA contamination and infections in the hospital words meanly in post-operative wound, in our study isolation showed high prevalence range of MRSA strains 85% (39/46) of the total *staph aureus* isolated from various samples, higher rate of MRSA isolations from H.C.W nasal swabs 100% (7/7), followed by wound swabs 90.9% (10/11), Nasal swab from patient 83% (5/6), H.C.Ws hand swabs and hospital words 80% (4/5) for each, lowest rate were recorded for the patient Skin swab (6/8) and operative rooms (3/4) 75% each. The finding about high prevelance of MRSA is not surprising and is also in line with several studies carried out in Iraq. (Al-azawi et al. 2016)(Al-dahbi and Al-mathkhury 2013) and (Al-Maliki 2009). The ratio of MRSA was relatively low in a study conducted in Kurdistan region of Iraq, in 2015 where the MRSA prevalence was 53% (Hussein et al. 2015) In another study in Iran was 69% (Jahanshahi, Zeighami, and Haghi 2018) while in a study conducted in India, the percentage was much lower 16.6% (Goud et al. 2011) MRSA prevalence 51.4% at the Korean hospital from the *Staph aureus* collected from blood and nasal colonizers (Peck et al. 2009) In general MRSA was highly prevalent in Asian countries (Hussain et al. 2019) In the German study there was a decrease in MRSA rate (Schubert, Kämpf, Wahl, et al., 2019b) In Turkey 2017, high rates of *staph aureus* high resiste to penicillin and ampicillin (Yılmaz and Aslantaş 2017) A study in Isfahan, Iran, in 2018 showed a nasal carriage of MRSA 51.9% among patient and 16% among health workers (Moshtagheian et al. 2018) MRSA prevalence in wounds was high, and this is consistent with previous studies, 76.9% (Khanal and Jha 2010); 44% (Tyagi, Kapil, and Singh 2008); 60.1% (Orrett and Land 2006); 34.8% (Hafeez, Chughtai, and Aslam 2004) The presence of MRSA in wounds delays healing (Solomkin 2001). In other study the average of MRSA rate for Wound Infections After Cardiothoracic Surgery was 54% in a three-year period

from 2007 to 2010 (Walsh, Greene, and Kirshner 2011) During the present study, MRSA isolate from HCWs noses was (19.4%) Which corresponds to previous studies (Caceres 2011) (Shittu et al. 2011) and (Munoz et al. 2008); 12.7% (Shibabaw, Abebe, and Mihret 2013) 12% in (Ibarra et al. 2008); 14.3% (Radhakrishna et al. 2013), MRSA rate was low in other studies; only 5.3% in Iran study (Askarian et al. 2009); and it was 0% in Kenya (Omuse, Kariuki, and Revathi 2012) With increasing of MRSA colonization rate, there is greater risk in developing drug-resistant wound infections. Therefore, it is necessary to avoid infection as much as possible. Previous reserchs have shown a large infections rate due to cross-contamination by hands of health staff (Rotter and Koller 1991) All MRSA isolate in our study harboring MecA gene. This result agreed with many other studies that showed all MRSA isolate harboring *MecA* gene (Yang et al. 2020) (Mussa and Al-mathkhury 2018) (Karmakar, Dua, and Ghosh 2016)(Al-Charrakh, Al-Hassnawi, and Al-Khafaji 2015); (Dağı et al. 2015) Other studies considered that Methicillin resistance can be happen in MecA absence, MRSA could have other mechanism(s) for resistance; e.g., altered target site or may be reduced drug accumulation. MecA gene absence may also due to a technical error upon detection. (Mahdi et al. 2016) (Carpaij et al. 2011) and (Wielders et al. 2002) The TSST coded by Tst gene (Dinges, Orwin, and Schlievert 2000) The *Tst* gene was detected in (15.4%) MRSA isolates in our study, The percentage was close to these studies (Ezeamagu et al. 2018) and (Hoseini Alfatemi et al. 2014) (14%,11.6%) respectively.In other studies, the *Tst* gene ratio was slightly higher, *Tst* gene was detected in (26.31%) (Costa et al. 2018) (27.9%) (Megevand,*et,al,*2010), Other studies recorded highly prevalent *Tst* gene (72.2%) in MRSA isolates from blood (Peck et al. 2009) Whereas in another study, *Tst* genes were non-detected (Motallebi et al. 2019)

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