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Detection of the Toxin Associated Genes of Methicillin-Resistant *Staphylococcus Aureus* Using a Multiplex PCR Assay in Wasit General Hospitals

Rana Hussein Raheema* and Ban Hamid Qaddoori
Medical Microbiology, Faculty of Medicine, Wasit University, Iraq.

Corresponding Author: Rana Hussein Raheema
Email: rraheema@uowasit.edu.iq

ABSTRACT

Staphylococcus aureus, especially MRSA, has spread to all parts of the world and is becoming a significant concern in public health as one of the most common causes of nosocomial infections. This study aimed to assess the prevalence of MRSA toxin genes. The study was conducted in the medical microbiology laboratory of medicine college, Wasit University, Iraq, from January 2017 - June 2019. Fifty-seven isolates were isolated from blood culture, wound infections, urine culture, and nasal swabs, which were identified using microbiological and biochemical tests; antibiotic susceptibility was estimated by the clinical laboratory standards institute. Then the isolates were tested for Molecular screening to detect toxin genes. *S. aureus* was found in 33 (57.89%) isolates. MRSA was detected in 29 (87.8%) *S. aureus* isolated by cefoxitin, the results showed high susceptibility to imipenem (94%), ciprofloxacin (91%), clindamycin (91%) and nitrofurantoin (91%). In comparison, high resistance was observed with penicillin (94%). Molecular screening for *eta*, etb and *tsst-1* genes, revealed positive amplification with 41.3%, 27.5 %, and 31 %, respectively. Fem A and luk-PV genes were detected in 44.8% and 10.3% of the isolates, respectively. There is a high prevalence of MRSA among different clinical isolates, which harbors toxin genes.

Keywords: *Staphylococcus aureus*, Methicillin resistance Staphylococcal, toxins

Correspondence:
Rana Hussein Raheema
Medical Microbiology, Faculty of Medicine, Wasit University, Iraq.
Email: rraheema@uowasit.edu.iq

INTRODUCTION

Over recent years, the incidence of nosocomial infection has been growing. It involves the emergence of new strains of antibiotic Resistance pathogens (Sahetal and al. 2014, Allegranzi *et al.* 2011) as significant public health concerns due to the existence of an increased number of hospitalized immunocompromised patients. A hospital can have the most common forms of NIs: infection with the blood supply, urinary tract infections, and infections at operating sites. Respiratory, pneumonia, meningitis, and other soft tissue (Endalafer *et al.* 2011). (Raka *et al.*,2006).

S. aureus is a common agent for potentially lethal infections of the bloodstream, in particular, because of its remarkable ability to colonize numerous areas (including medical equipment) and spread through various environments. The average frequency of *S. aureus*-induced bloodstream infections in developed countries is around 80 to 190 cases/100,000 people annually (Talan *et al.*, 2011; Arias *et al.*,2017). All isolates of *S. aureus* produce enzymes and a range of more than 30 different extracellular proteins, most of which play a direct role in pathogenesis (Ladhani *et al.*,1999). *Staphylococcus aureus* is the most isolated bacterium among both community-acquired and nosocomial infections. The majority of harmfulness factors delivered are various proteins and cytotoxins like staphylokinase, exfoliative toxins, leukocidins, hemolysins, hyaluronidase, coagulase, lipases, collagenase, and nucleases. (Raheema & Abed., 2019). Toxic S. aureus can generate a range of enzymes that support its virulence and spread through the host, such as coagulase, hyaluronidase, deoxyribonuclease, and lipase. Other variations of extracellular protein toxins, including enterotoxins, TSST -1, exfoliated (ET) toxins, hemolysins, and coagulases, increase body pathogenicity and invader hosts, have also been reported. Also, (Kong et coll., 2016).

These protein toxins have been more commonly observed for MRSA than in non-MRSA (Liu *et al.* 2010), both of which have been reported as a cause of toxic shock and scalded human host skin syndrome (TSST -1) and exfoliative toxins (ETA and ETB) (Johnson *et al.*, 1991). One of the most significant virulence factors in *S. aureus* is the panton-valentine Leukocidin (PVL). (Shrestha *et al.*, 2014). An important virulence factor produced by many methicillin-resistant *S. aureus* (MRSA) strains is the Panton-Valentine Leukocidin (PVL). This bicomponent pore-forming cytolytic toxin targets cell membranes of leukocytes (McClure *et al.* 2006), which is associated with skin and soft tissue infections (SSTI), necrotizing pneumonia and epidemiologically linked to community-associated MRSA (CA-MRSA) (Chambers 2005, DeLeo *et al.* 2009). FemA gene encodes proteins that influence the level of methicillin resistance of *S. aureus*. (Kobayashi *et al.*,1994). This study aimed to investigate resistance trends, methicillin prevalence, and toxin genes in S. aureus from isolated clinical samples.

METHODOLOGY

This research was conducted in the medical microbiology laboratory of medicine college, Wasit University, from January 2017 - June 2019. Fifty-seven isolates were collected from blood culture, wound infections, urine culture, and nasal swabs. cultured on blood agar, mannitol salt agar, and all isolates were identified as *S. aureus* by morphological characteristics, Gram-staining, and biochemical tests. (Raheema & Abed., 2019). Antibiotics tested against *S. aureus* include Vancomycin (30 µg), penicillin G (10 units), tobramycin (10 µg), nitrofurantoin (300 µg), levofloxacin (5 µg), imipenem (10 µg), gentamycin (10 µg), erythromycin (15 µg), chloramphenicol (30 µg) clindamycin (2 µg), cefoxitin (30 µg), ciprofloxacin (5µg) then plates were incubated at 37 °C for 24 h. The inhibition region diameter has been calculated using a ruler and interpreted according to CLSI

(2018) criteria. All *S. aureus* genomic DNA has been extracted using DNA kit (intron kit, Korea) and PCR amplified with the use of TSST, ETA and ETB, fem A, and luk PV primers. Multiplex PCR has been performed in toxin genes (tsst-1, eta, ETB) as defined by (Mehrotra *et al*, 2000). The amplification reactions consisted of Initial denaturation at 95 °C for 3 min, denaturation 94 °C for 30 s, annealing 40 °C for 30 s, extension 72 °C for 30 s, and final extension 72 °C for 7 min. PCR analysis has been performed fem A as (Kobayashi *et al*.) described and, luk – PV analyzes have been performed according to Jarraud *et al*., 2002 description.

Table 1: Oligonucleotide primers used in this study

Primer	Oligonucleotide sequence, 5' to 3'	(bp)	References
FemA	Fem-Al AGACAAATAGGAGTAATGAT Fem-A2 AAATCTAACACTGAGTGATA	509	Kobayashi *etal*.,1994
Luk-PV	F: TTCACTATTTGTAAAAGTGTCAGACCCACT R: TACTAATGAATTTTTTTATCGTAAGCCCTT	180	Jarraud *etal*.,2002
TSST	F: ATGGCAGCATCAGCTTGATA R: TTTCCAATAACCACCCGTTT	350	Johnson etal.,1991
ETA	F: CTAGTGCATTTGTTATTCAA R: TGCATTGACACCATAGTACT	119	Johnson etal.,1991
ETB	F: ACGGCTATATACATTCAATT R: TCCATCGATAATATACCTAA	200	Johnson etal.,1991

Statistical Analysis

Statistical data analysis using SAS (Statistical Framework-version 9.1) was carried out. Using the Chi-square test, the percentages were compared. The significant level was set at $P < 0.05$.

RESULTS AND DISCUSSION

According to Bergey's Manual of Systematic Bacteriology, in 33 (57.89%) of 57 patients collected, aureus was isolated (William *et al*., 2009). Staining with Gram-stain exhibited spherical cocci, and the predominant shape was grape-like clusters, Results of biochemical tests disclosed positive results for catalase and coagulase. As well as other characters mentioned by (Raheema and Abed, 2019) and (Raheema 2016), the primary diagnosis findings for these 33 aureus isolates were characterized by selective and differential culture media.

Urology 15 (45.45%), 12 wound infections (36.36%), follows by four blood cultures (12.12%), and two nasal swabs (6.06%) (Figure 1) is the most significant number of isolates. The clinical S. aureus infection percentage obtained in other research was 21.87% (Suo'd, 2005), 30.1% (Albaldawi,2005) inducing human inflammation, and isolated from several infection sites (Todar, 2008).

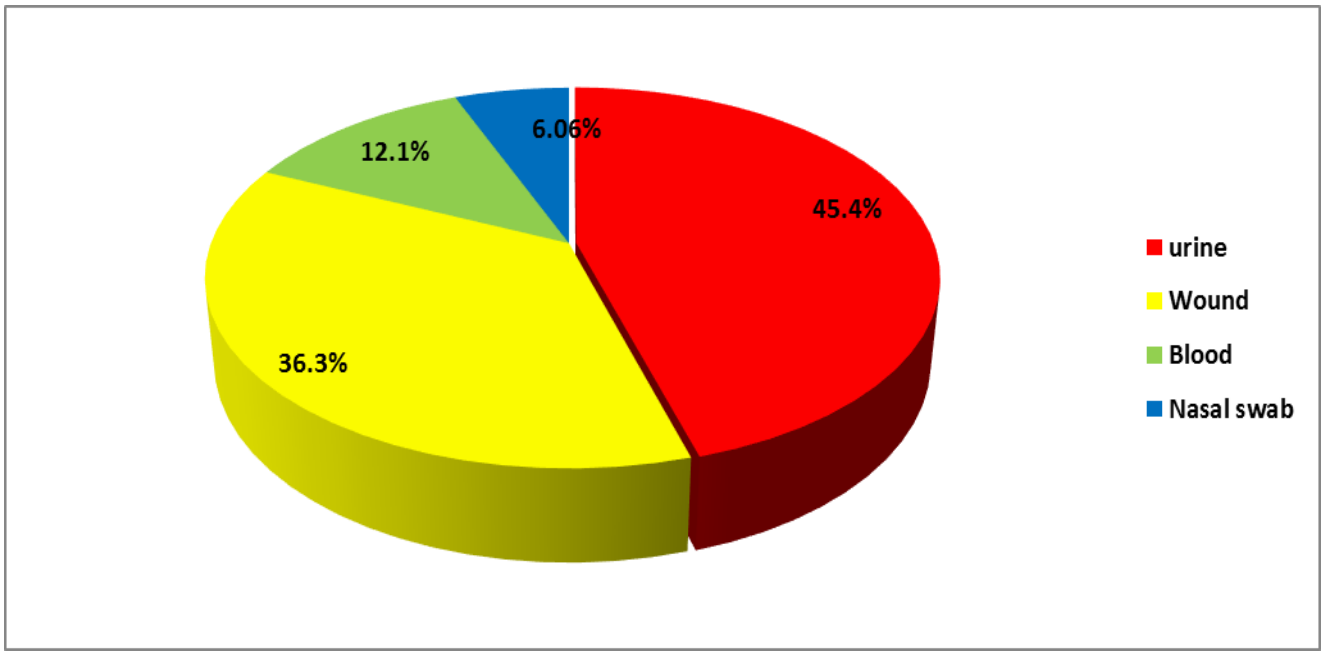

Figure 1: Distribution of clinical samples

Figure 2 reported antibiotic susceptibility test findings, with Staphylococcus aureus isolates displaying elevated susceptibility to imipenem (94%), ciprofloxacin (91%), clindamycin (91%), and nitrofurantoin (91%). Strong resistance (94 percent) was observed with penicillin.

The second generation of penicillins has been developed to combat antibiotics' resistance, including methicillin: Oxacillin, a Pencillinase enzyme-resistant synthetic semi-synthetic penicillin (Al-Taai *et al*., 2017). In 29 (87.8%) S, MRSA was identified. The overall prevalence of MRSA was 27.8 percent in aureus isolated by cefoxitin. Ciprofloxacin and Vancomycin were the most compelling antagonists against isolated clinical MRSA isolates from wounds and burns. This is consistent with the result (Shekarabi *et al*., 2017), where thirty-six percent of isolates reported complete vancomycin resistance (VRSA).

The absence of Vancomycin resistance is due to the lack of vanA and vanB genes (Zhang *et al*., 2004). Al-Hossainy (2007) showed that S. VRSA accounted for 20%. From aureus. The findings did not align with Al-Geobory's (2011) finding that the vancomycin resistance rate was 2.27 percent, where VRSA isolates among S.aureus was 4 out of 50 (8%) (Mohammed, 2011).

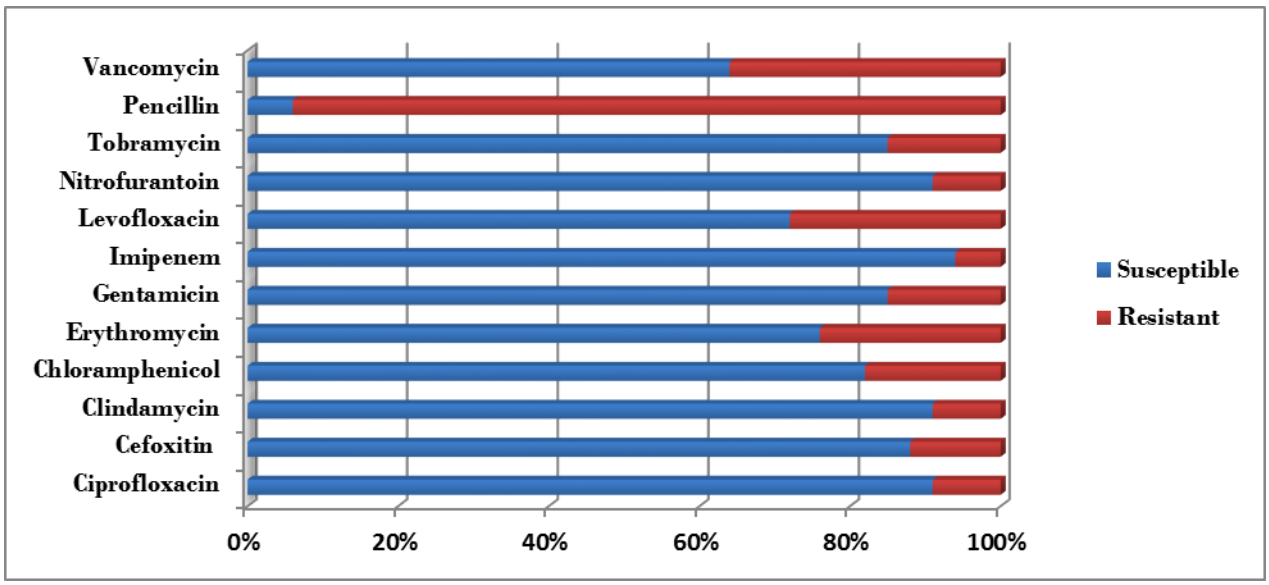

Figure 2: Antimicrobial susceptibility profile

The discrepancies in the degree of susceptibility to some antibiotics and the antibiotic resistance mechanism may be due to reduced cell wall permeability, chromosomal, and plasmid-mediated β-lactamase development is the crucial methicillin resistance mechanism. (Katzif *et al*., 2005), biofilm production also helped generate highly resistant organisms to destroy (Otto.,2006; Raygada and Levine, 2009).

Figure 3 shows the results of molecular detection of *eta*, ETB and *tsst-1* genes with 12 (41.3%), 8 (27.5 %) and 9 (31 %) respectively. Exfoliating concentrations of exfoliating genes were comparable to previous cases (eta, 6%, and etb 4%) (Ezeamagu *et al*. 2018). In this research, the low rate of positive exfoliative toxin-producing isolates is consistent with previous studies (Megevand *et al*., 2010; Sila *et al*., 2009). Unlike early study (Tokajian *et al*., 2011) documented a high prevalence of tsst-1 among the MSSA strains.

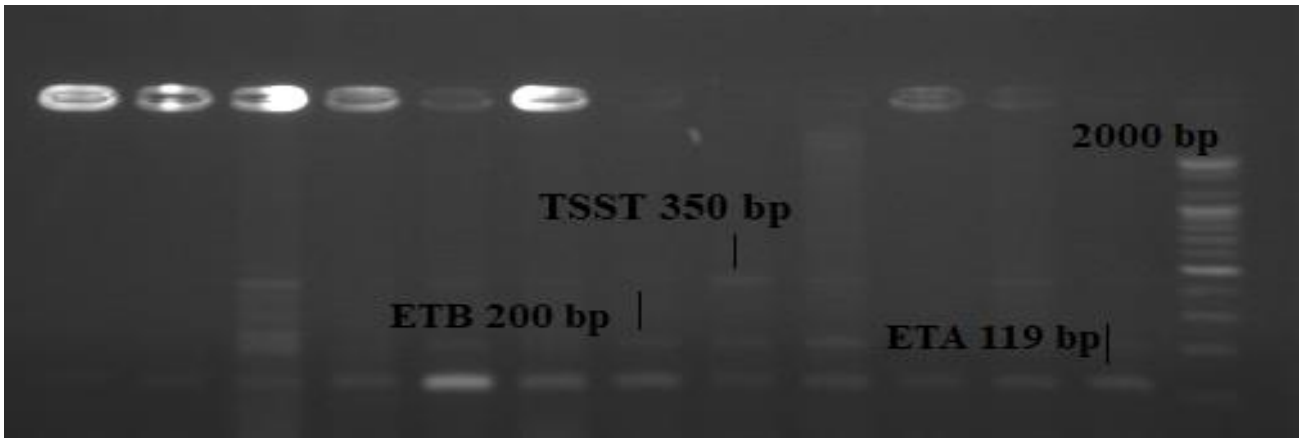

Figure 3: Gel electrophoresis of amplified (*eta*, *etb* and *tsst-1*) genes of *S.aureus* using multiplex PCR . DNA ladder(100-2000bp).

Of the 29 isolates used in the study, 13 (44.8%) of isolates are made from the gene FemA, as shown in figure 4. FemA factor is central to the encoding of proteins in methicillin-resistance genes that affect the methicillin resistance levels involved in forming the cell wall. The absence of the gene FemA is related to reducing the glycerin content in peptidoglycan (Stranden,1997).

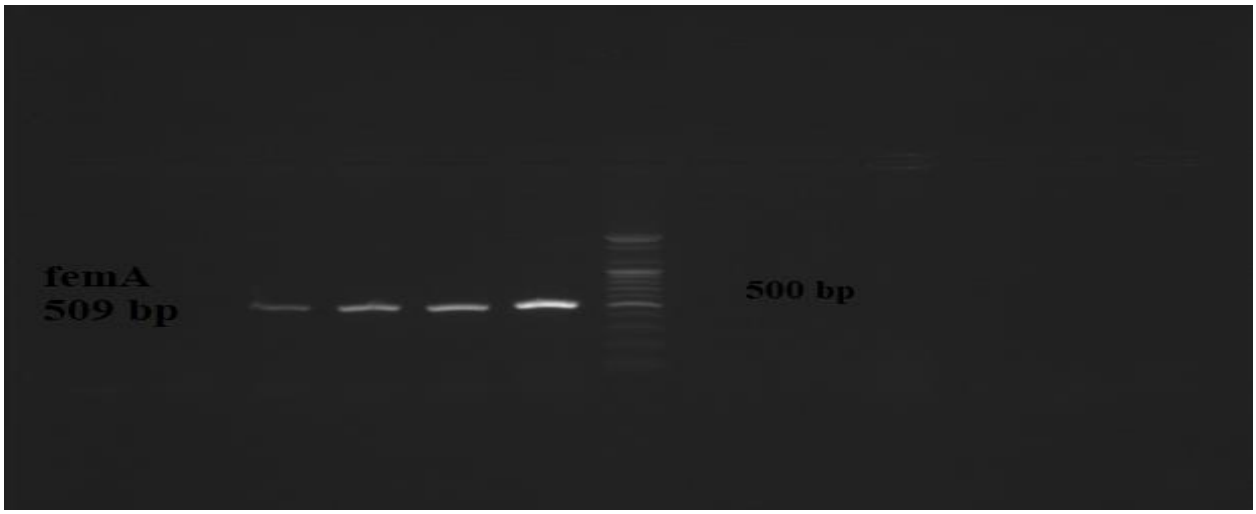

Figure 4: Gel electrophoresis of amplified (*FemA*) gene of *S.aureus* using conventional PCR. DNA ladder (100-2000bp).

The detection results of the luk-pv gene coded for Leukocidin showed good genetic results (figure 5). MRSA strains have a luk-pv gene of 3 (10.3%) – that's in line with Kareem's discovery (2016), which was revealed in Baghdad at 6.55% to MRSA pvl-harbor isolates. Methicillin-Resistant S developed from panton-valentine Leukocidin (luk-pv). Heart and soft tissue infections (Kadhim *et al.* .2020) are the causes of severe pathogenic pathologies that endanger health. Responsive to methicillin. Aureus may have greater secretive capacity than MRSA (Varshney *et al.* 2010) for toxins such as leucocidin Panton-Valentine. It may be attributed to the lack of SCCmec's carriage elements that MSSA isolates have less genetic fitness pressure. Virulence and antibiotic resistance are significant determinants of the types of infections caused by *Staphylococcus aureus* (Jiménez *et al.*,2011).

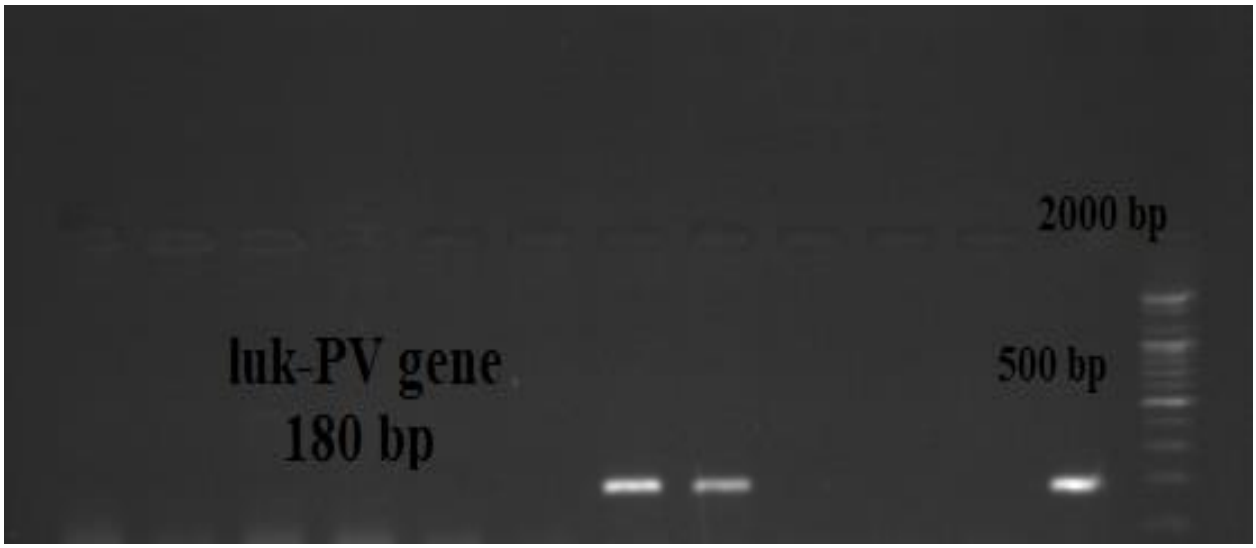

Figure 5: Figure 3: Gel electrophoresis of amplified (*luk-pv*) gene of *S.aureus* using conventional PCR . DNA ladder (100-2000bp).

CONCLUSION

MRSA prevalence in clinical isolates is high. Moreover, MRSA's toxin genes display an interplay and co-operation of resisting genes within the strain, so efficient monitoring of MRSA by sensitizing the use of antibiotics is essential and developing a drug resistance surveillance system. The results display that MRSSA contains toxin Genes.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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