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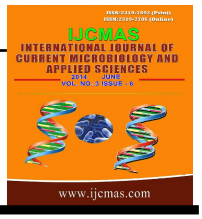


Original Research Article

Prevalence of genes encoding Exfoliatin toxin A and Panton-Valentine Leukocidin among Methicillin resistant *Staphylococcus aureus* in Baghdad

Raghad A. Abdulrazaq, Mohammed F. AL-Marjani* and Sussan H. Othman

Department of Biology, College of Science Al- Mustansiriya University, Baghdad, Iraq.
**Corresponding author*

Keywords

PVL toxin, Exfoliative toxin, *Staphylococcus aureus*, PCR

A B S T R A C T

The aim of this study was to determine the distribution of *pvl* and *eta* genes in Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are leading causes of hospital-acquired infections in Baghdad . A total of (100) MRSA isolates were recovered from hospitalized patients. From the screening profile of 100 MRSA isolates enrolled in this study, the percent of PVL-Positive and ETA-Positive *S. aureus* were represented by 27% and 13% of isolates respectively. The resistance patterns of MRSA isolates were determined, All isolates were resistant to cloxacillin , followed by cefoxitin (86 %) and cephalexin (51%) , 26% to lincomycin ,23% to trimethoprim and 22% to rifampicin. Results of Nitrogen bases sequencing for PCR product of 16 samples in this study revealed consistency reaching up to 99 % as compared nitrogen bases sequence of the *pvl* gene present in the *Staphylococcus aureus* strain in NCBI

Introduction

Staphylococcus aureus is an important human pathogen capable of causing diseases in the hospital and community settings . The increased incidence of multidrug-resistant *S. aureus* strains among nosocomial (or hospital-acquired [HA]) infections has added a challenging dimension to the *S. aureus* problem (Saïd-Salim *et al.*, 2005; McCarthy and Lindasy, 2012).

The pathogenicity of *Staphylococcus aureus* infections is related to various bacterial surface components (e.g., capsular polysaccharide and protein A), including those recognizing adhesive matrix molecules (e.g., clumping factor and fibronectin binding protein), and to extracellular proteins (e.g., coagulase, hemolysins, enterotoxins, toxic-shock syndrome [TSS] toxin, exfoliatins, and Panton-Valentine leukocidin [PVL] (Archer, 1998).

Panton-Valentine leukocidin (PVL) is a bi-component, pore-forming exotoxin produced by some strains of *Staphylococcus aureus*. Also termed a

synergohymenotropic toxin (i.e. acts on membranes through the synergistic activity of 2 non-associated secretory proteins, component S and component F) (Lina *et al.,* 1999).The epidemiological association of PVL-producing *S. aureus* strains from patients with necrotizing pneumonia suggested PVL was a major virulence factor (Gillet *et al.,* 2002).

Exfoliative toxins (also known as "epidermolytic" toxins) are particularly interesting virulence factors of *S. aureus*. These extremely specific serine proteases recognize and cleave desmosomal cadherins only in the superficial layers of the skin, which is directly responsible for the clinical manifestation. of staphylococcal scalded skin syndrome (SSSS) (Bukowski *et al.,* 2010).

There are two serological forms of staphylococcal ETs(ETA and ETB) which are responsible for human SSSS.The gene encoding ETA is situated in the bacterial chromosome, but the gene encoding ETB is located on the plasmid. ETA and ETB are composed of 242 and 246 amino acids, respectively and they have approximately40% amino acid similarity (Ladhani *et al.,* 1999). The aim of this study was to determine the frequency of *pvl* and *eta* genes in MRSA in cutaneous infections,and also to determine the nitrogen bases sequence of the *pvl* gene present in the *Staphylococcus aureus* in Baghdad.

Materials and Methods

Bacterial isolates

A total of 100 MRSA isolates were collected from cutaneous samples (abscess and wound) from patients who were admitted to Baghdad hospitals in 2013.These isolates were identified by conventional biochemical reactions according to the criteria established by (Forbes *et al.,* 2007).

The isolates were inoculated a CHROM agar MRSA plate. The results were read after 24 and 48 h of incubation at 35°C. The growth of colonies showing any pink or mauve coloration was considered to be positive (indicating MRSA).

Antimicrobial susceptibility test

Antimicrobial susceptibility of the isolates were tested by using Kirby-Bauer disk diffusion method following CLSI guidelines (CLSI,2009) , using commercially available 6mm discs (Bioanalyse /Turkey) The susceptibility of the isolates was determined against 12 antibacterial agents, They include: Clindamycin, rifampicin, tecoplanine, trimethoprime, cloxacillin, gentamicin, cephalexine, cefoxitine lincomycin, levofloxacin, Azithromycin and vancomycin, on Mueller Hinton agar Plate (Lab M Limited Topley House,United Kingdom), using overnight culture at a 0.5 McFarland standard followed by incubation at 35^{o}C for 16 to 18 h.

DNA Preparation and PCR

A PCR reactions with specific primers were performed to identify *pvl* and *eta* genes of each MRSA isolates (Table 1).DNA template was prepared as described by (Olsvik and Strockbin, 1993) (25µl) of PCR amplification mixture contained deionized sterile water,(12.5)µl Green Go *Taq* Master Mix pH (8) (Promega,USA) .

The thermocycling were as follows: Initial denaturation at 94°C for 10 min and 35

cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1min and extension at 72 C for 45 Sec. and a final extension was performed at 72°C for 10 minutes . All PCR products were analyzed by electrophoresis through 1% agarose gels.

DNA sequence analysis

The DNA fragments for sequencing were obtained by PCR amplification, the fragments of each PCR products were sequenced with the set of primers by Macrogen , USA). The program (BioEdit Pro.version: 7.0.0) was used for bioinformatic analysis of nucleotide sequences.

Results and Discussion

In this research 100 Methicillin resistant *S. aureus* isolates were collected from hospitalized patients in Baghdad . The resistance patterns of MRSA isolates to 12 antimicrobial agents are shown in Table 2.

All isolates were resistant to cloxacillin , followed by cefoxitin (86%) and cephalexin (51 %), 26% to lincomycin, 23% to trimethoprim and 22% to rifampicin .First recognized in 1960, methicillin-resistant *Staphylococcus aureus* (MRSA) was considered to be a medical oddity. Now, MRSA is the most common nosocomial bacterial pathogen isolated in many parts of the world (Grundmann *et al.,* 2006). Other resistance rates were: 18 % gentamicin, 17% vancomycin, while the minimum resistance were seen with teicoplanin (4%).

In 1997, first strain of *Staphylococcus aureus* reduced susceptibility to vancomycin was reported from Japan, after that ,two more cases were reported from united state . In 2002 ,the first clinical isolate of vancomycin –resistant *Staphylococcus aureus* was reported by workers from Brazil and Jordan (Ng *et al.*,2011).

A total of 100 isolates of MRSA were tested for the presence of the *pvl* and *eta* genes by PCR, 27% and 13 % were positive respectively (Fig 1) (Fig 2). Narita et al (2001) showed that the temperate phage φSLT infected only 3% of clinical PVL-negative *S. aureus* strains, leading to PVL production. Cabrera et al (2010) showed that 83% of *S. aureus* isolates carrying *pvl* genes. When isolates were categorized according to type of staphylococcal infection, the PVL genes were strongly associated with skin and soft tissue infections, such as abscesses, skin lesions, and boils (furuncles). By contrast, no statistically significant association was observed with impetigo, blisters, or SSS (Holmes *et al.,* 2005). PVL has been linked to specific human *S. aureus* infections such as primary skin and soft tissue disease and severe necrotizing pneumonia, where the mortality rate is about 75% (Gillet *et al.*,2002).

A higher occurrence of exfoliative toxins is associated with SSSS diagnosis, where *eta* is present at higher rates , but the prevalence shows geographical differences (in Japan, *etb* is more frequent) (Sauer *et al.,* 2008) .

Results of Nitrogen bases sequencing for PCR product of 16 samples in this study revealed consistency reaching up to 99 % as compared Nitrogen bases sequence of the *pvl* gene present in the *Staphylococcus aureus* strain in NCBI .

Table.1 Sequence of forward and reverse primers used for detecting *pvl* and *eta* genes among MRSA isolates.

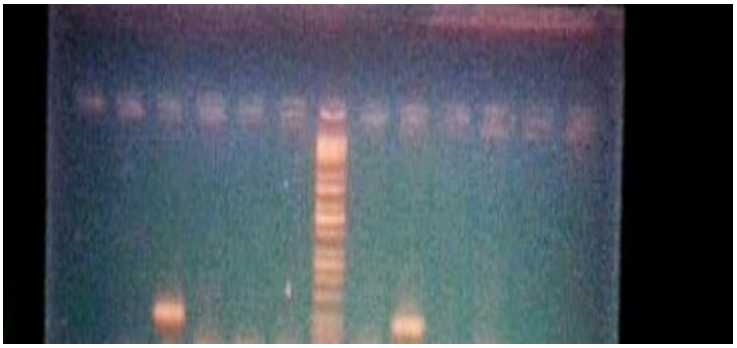
Primer type	**Primer sequence 5----3**	**Product size**	**reference**
Forward primer *pvl* Reverse primer *pvl*	'ATCATTAGGTAAAATGTCTGCACATGATCCA GCATCAASTGTATTGGATAGCCAAAAGC	433bp	Jarraud *et al.*,2002
Forward primer *eta* Reverse primer *eta*	ACTGTAGGAGCTAGTGCATTTGT TGGATACTTTTGTCTATCTTTTTCATCAAC	190 bp	Jarraud *et al.*,2002

Table.2 Susceptibility of the 100 isolates of methicillin-resistant *S.aureus* to 12 Antibiotics.

Antibiotic		**Resistant (%)**
Rifampicin	**RA**	**22**
Clindamycin	**DA**	**13**
Lincomycin	**L**	**26**
Levofloxacin	**LEV**	**13**
Trimethoprime	**TEM**	**23**
Cloxacillin	**CX**	**100**
Azithromycin	**AZM**	**16**
Cefoxitine	**CX**	**86**
Gentamicin	**CN**	**18**
Cephalexine	**CL**	**51**
Tecoplanine	**TEC**	**4**
Vancomycin	**VA**	**17**

Figure.1 Gel electrophoresis (1% agarose, 7 v/cm) of *pvl* (433 bp) .

Figure.2 Gel electrophoresis (1% agarose, 7 v/cm) of *eta* (190 bp)



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