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Detection of genetic mutations by insertion sequence IS*256* in *Staphylococcus aureus* under the stress of Vancomycin and Ciprofloxacin

A Thesis

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1. Introduction

Staphycoccus aureus is the most dangerous of the many common staphylococcal bacteria. This gram positive, sphere shaped (coccal) bacteria often causes skin infections but can cause pneumonia, endocarditis, osteomyelitis, bacteremia (Zycinska *et al.*, 2008).

Increasing trend of antimicrobial resistance in bacteria that causes infectious diseases is a global problem, although resistance significantly varies between geographical regions. The common bacterial pathogens can be resistant to all known antimicrobial agents (Prestinaci *et al*., 2015). Infections caused by resistant *S. aureus* can be difficult to treat, resulting in a greater risk of death. Especially Orthopaedic infections, such as *S. aureus*. Most clinical cases of orthopaedic surgeries have shown that patients infected with antibiotic resistant bacteria, such as methicillin resistant *S. aureus* (MRSA), are associated with increased morbidity and mortality (Walsh, 2016).

The date of multidrug-resistant *S.aureus* (MDR-SA) have been isolated from both adults and children around the word (Thapaliya *et al*., 2018), they are resistant to, methicillin, extended-spectrum beta-lactamase-producing bacteria, streptomycin, gentamycin, tetracycline and vancomycin (Schaumburg *et al*., 2016). The emergence of antibiotic resistance among bacterial pathogens is a major problem in treatment of infectious disease in both community and in healthcare settings throughout the world (Kleinert *et al.*, 2017).

Many virulent factors of *S. aureus* increase its susceptibility of antibiotics. The virulent factors is including adherence to host proteins do promotes attachment to blood clots and traumatized tissue (Thomer ,2016), and *S. aureus* is avoidance of host defenses by many defense mechanism such as capsule, teichoic acids, Protein A, fibronectin and

collagen banding protein (Tankeshwar, 2019). *S. aureus* is known of its capability to produce extra cellular toxins include haemolysis, leukcidin, enterotoxin, exfoliative and TSST-1 (Bukowski *et al*.,2010).

Stafhylococcus aureus has enzymes to help opsonization and phagocytosis to infection varies tissues include coagulase, staphylokinase, hyaluron -idase and deoxyribonuclease (Berends *et al*., 2010).

Methicillin resistant *S.aureus* (MRSA), vancomycin –resistant (VRSA) and multidrug-resistant have the ability to acquire mobile genitic elements and transposition of IS*256* has been investigated in *S.aureus*, where to play an important role in biofilm formation and antibiotic resistance (Gregorio *et al*., 2016).

IS*256* has been detected in the genome of several clinical isolates of *S. aureus* in multiple copies, alone or flanking both ends of the amino - glycoside resistance transposon Tn*4001*. Tn*4001* is composed of 1.9 Kb central region flanked 1.3 Kb (1324 bp) (Moffatt *et al*., 2011) inverted repeats, the IRs flanking the resistance determinant of Tn*4001* have been designated IS*256*. Transposon can transposes by a copy and paste mechanism (Vandecraen *et a l*., 2017).

Gene inactivation is Possible affects of IS transposition in the function and expression of a target gene. Many cases have been described illustrating the modulation of resistance, virulence and metabolic activities by IS-mediated gene inactivation (Vandecraen *et al*., 2017).

According to what mentioned above, this study was aimed to detection of genetic mutations by insertion sequence IS*256* in Staphylococcus aureus under the stress of Vancomycin and Ciprofloxacin antibiotics. This was achieved according to the following steps:

1. Collection of specimens from clinical sources (boil, wounds, nasal caviety, urine, vagina, blood, operation tip drainage, soft tissue, joint fluid and sputum).
2. Identification of *S. aureus* according to their morphological characteristic and biochemical tests.
3. Investigating the antibiotic resistance of bacterial isolates against different antibiotics from different groups, and detection of the minimum inhibitory concentration MIC and the minimum bactericidal concentration MBC for vancomycin and ciprofloxacin antibiotics.
4. Detection of IS*256* in bacterial isolates.
5. Choosing bacterial isolate which contained IS*256* and multi drug resistant and high minimum inhibitory concentration to Vancomycin and Ciprofloxacin.
6. Investigating the genetic mutation and survival fraction IS*256* in *S. aureus* under the influence of stress Vancomycin and Ciprofloxacin antibiotics on penicillin, Ampicillin, Azethromycin, Trimethoprim, Erythromycin, Rifampin, Gentamycin and Lincomycin.

Summary

A total of 109 clinical specimens were collected from burns, nose, urine, joint fluid, vagina, blood, sputum and tip drainage and soft tissues from surgical operations for patients attends Baghdad teaching hospital and the division of laboratories/ private nursing hospital in Baghdad Governorate for the period from July/2019 to September/2019. From these samples a total of 109 bacterial isolates were obtained after culturing on McConkey agar and Blood agar plates. After screening these bacterial isolates, it was found 47 of them were Gram positive and 62 Gram negative bacteria. Twenty-two of G^{+}ve bacteria were identified as *Staphylococcus aureus* according to their ability to cause β-heamolysis, monnitol fermentation, they were positive for catalase, lisithinase, urease, while they were negative for oxidase. Identification of these isolates was confirmed by using Viteck-2.

Susceptibility of *S.aureus* isolates to different antibiotics was determined. Results showed these isolates have different resistant profile for different antibiotics, where all the isolates (100%) were resistant to penicillin and methicillin, 77% were resistant to ampicillin, 53% were resistant to erythromycin, 45% to azithromycin, trimethoprim and chloramphenicol, 36% were resistant to tetracycline, 23% to ciprofloxacin, doxycillin and lincomycin, 18% to refampin and 4% for vancomycin.

Genomic DNA was extracted from all *S.aureus* isolates with high purity and concentration, then insertion sequence *256* (IS*256*) was detected by using specific primers. Results showed that IS*256* was detected in 18 isolates, and the prevalence of this transposable element in bacterial isolates from clinical samples was 100% in UTI isolates, nose, wounds, tip drainage and soft tissues isolates, 66% in respiratory isolates, 33% in joint