INTRODUCTION

Staphylococci are type of bacteria that usually associated with infected indwelling medical devices, chronic infections and serious consequences which had a huge impact on clinical medicine. The most isolated species in these types of infections are *Staphylococcus epidermidis* and *Staphylococcus aureus* ***(Donlan and Costerton, 2002).***

Staphylococcus aureus had b.een associated with high mortality rate bacteremia in the pre-antibiotic era which reached up to 80%. However the introduction of penicillin in the early 1940s had dramatically improved the outcome of *staphylococcal* infection. But at the beginning of 1942, penicillin-resistant *staphylococci* had emerged, first in hospitals and subsequently in the community ***(Franklin, 2003)***.

Despite of the low rates of penicillin susceptible *Staphylococcus aureus*, from 5 to 20% **.** Penicillin remains the antibiotic of choice for penicillin-susceptible *Staphylococcus aureus* ***(Nissen et al., 2013).***

Two mechanisms confer penicillin resistance in staphylococci. First, the most important is production of β-lactamase, which inactivates penicillin by hydrolysis of its beta-lactam ring. The second is primarily associated with

human isolates and confers resistance due to a penicillin-binding protein, PBP2a, encoded by mecA ***(Niemeyer et al., 1996).***

More than 90% of staphylococcal isolates now produce penicillinase, regardless of the clinical setting. The gene for β-lactamase is part of a transposable element located on a large plasmid, often with additional antimicrobial resistance genes (e.g., gentamicin and erythromycin). Spread of penicillin resistance primarily occurs by spread of resistant strains ***(Nissen et al., 2013).***

Staphylococcal resistance to penicillin is mediated by *blaZ* gene, the gene that encodes β-lactamase. This predominantly extracellular enzyme, synthesized when staphylococci are exposed to β-lactam antibiotics, hydrolyzes the β-lactam ring, rendering the β-lactam inactive. *blaZ* gene is under the control of two adjacent regulatory genes, the antirepressor *blaR1gene* and the repressor *blaI* gene. Studies have demonstrated that the signaling pathway responsible for β-lactamase synthesis requires sequential cleavage of the regulatory proteins BlaR1 and BlaI. Following exposure to β-lactams the BlaR1which is a transmembrane sensor-transducer, cleaves itself ***(Zhang et al., 2001)***.

A number of phenotypic methods for the detection of β-lactamase production in *Staphylococcus* species have been investigated and compared to detection of the *blaZ gene* by PCR. All phenotypic methods had a sensitivity of less than 72%. Various PCR methods have been described for the detection of *blaZ gene* in *Staphylococcus species*. Clinical Laboratory Standards Institute (CLSI) recommend that *blaZ gene* detection should be considered for Penicillin Sensitive Staphylococcus Aureus (PSSA) isolates from cases of serious infection requiring penicillin therapy ***(Pereira et al., 2014).***

The advantages of the real-time PCR over conventional PCR include a faster turnaround time, less specimen handling with subsequent reduced workload and risk of specimen contamination, lower cost, and equivalent sensitivity (100%) and specificity (100%). These advantages would facilitate more routine testing of the *blaZ* gene ***(Nissen et al., 2013).***

Aim of the Work

The present study aims to identify prevalence of Staphylococcus Aureus (*S.aureus)* resistance to penicillin and to evaluate the nitrocefin reaction in detecting β-lactamase production in (*S. aureus)* isolates. This would be compared to detection of blaZ- *gene* presence by real time PCR in *S. aureus* isolates obtained from various clinical samples.

Overview on Staphylococcus Aureus

Staphylococcus aureus (*S.aureus)* is a facultative anaerobic gram-positive coccal bacteria. *S. aureus* looks like a cluster of grape. *S. aureus* multiply in asexual manner by binary fission. The two newly formed cells still attached to one another. This is why the cells are seen in clusters ***(Li and Nikaido, 2009).***

Virulence factors of Staphylococcus aureus:

Staphylococcus aureus has various types of virulence factors including genetic, structural and biochemical factors, the majority of clinical significant diseases caused by S. aureus depend on a combination between these factors:

1- Cell wall virulence factors:

a- Capsule:

Staphylococcus aureus has a very thin capsule that help in evading phagocytosis ***(Harvey, 2013).***

b- Protien A :

Protein A is an IgG-binding protein which related to staphylococcal peptidoglycan bridges. It has the ability to bind to Fc region of an antibody.So it has anti opsonin effect ***(Campeotto et al., 2014).***

c- Fibronectin binding protein:

Fibronectin binding protein (FnBP) is an important component of Staphylococcus aureus that help the bacteria to attach to mucosal surface and tissue matrix ***(Harvey, 2013).***

d- Clumping factor:

Is called coagulase which Plasma can enhance Fibronectin binding protein (FnBP) to clump the organisms on tissue ***(Harvey, 2013).***

2- Cytolytic exotoxins:

Hemolysin such as (A, b, gamma toxins) can lysis red blood cells membranes and mamalina cells, exotoxin a is the most studied type ***(Bryan et al., 2013).***

3- Panton-Valentine leukocidin:

An important toxin produced by MRSA it lyses the membrane of PMNS, strains which produce this type of toxins is considered more virulent than non-producers ***(Deacon et al., 2016).***

4- Superantigen exotoxin:

Virulence of this kind of toxins derived from its ability to stimulate T-lymphocytes enhancing it to produce large amount of cytokines such as interleukin 2 (IL-2) and

tumor necrosis factor alpha (TNF a). superantigen exotoxins include many types such as ***(Adam et al., 2013)***:

a- Enterotoxins:

S. aureus can secret an enterotoxin that is responsible for causing self-limiting gastroenteritis which characterized by diarrhea and vomiting which last for six hours and recovery in 24 hours. Symptoms also usually come with nausea, vomiting, diarrhea, and abdominal pain**.**

Enterotoxins are more heat stable than S.aureus so infected food would not always contain the bacteria itself but its enterotoxin products ***(Jarraud et al., 2001)***.

b- Toxic shock syndrome toxin (TSST-1):

This type of toxin has similar structure to enterotoxins, however it does not cause gastroenteritis but it cause a classical toxic shock syndrome with Signs and symptoms include erythematous rash, fever, multiple organ failure, and hypotension along with skin desquamation ***(Katarina et al., 2014).***

c- Exofoliatin toxin (ET):

Exfoliative toxin (ET) toxins are responsible for producing scalded-skin syndrome which associated with epidermal staphylococcal infection in infants and young

children. It might spread as epidemics in hospitals and nurseries ***(Harvey, 2013).***

5- Enzymes:

Staphylococcus aureus secrets various types of enzymes such as:

- Coagulase enzyme which function in plasma clot formation to prevent phagocytosis and reduce blood supply to infection site.
- Hyaluronidase enzyme: This enzyme helps the spread of infection by breaking down hyaluronic acid and weakening the connection within tissue.
- DNAse (deoxyribonuclease): which digests the DNA.
- Lipase enzyme: which breakdown lipids.
- Staphylokinas enzyme: help spread of infection by dissolving fibrin.
- Beta-lactamase enzyme: for drug resistance especially penecillins.

(Costa et al., 2013).

6- Staphylococcal pigments

Staphylococcus aureus produces many types of pigments such as *staphyloxanthin* (a golden coloured

carotenoid pigment) which is a golden dye that gives yellowish colonies in blood agar.

This pigment is considered a virulence factor, because it help the bacteria to escape killing by the reactive oxygen compounds on which the host immune system depends on to eliminate the pathogens ***(Clauditz et al., 2006).***

Some Mutations of *S. aureus* cause them not to produce staphyloxanthin causing a reduced ability to survive when introduced with an oxidizing chemical, such as hydrogen peroxide. These mutant colonies are easily eliminated when exposed to human granulocytes, while colonies which produce the pigmented colonies survive.

Drugs designed to inhibit the production of staphyloxanthin may weaken the bacterium and renew its susceptibility to antibiotics ***(Liu et al., 2005).***

Table (1): Virulence factors of S. Aureus**.**

Virulence factor	Activity
Cell wall polymers 1. Peptidoglycan	▪ Inhibits response to inflammation; endotoxin like activity
2. Teichoic acid	▪ Phage adsorption function.
Cell surface proteins 1. Protein A	▪ Reaction along with Fc region of IgG
2. Clumping factor	▪ Fibrinogen binding
3. Fibronectin-binding protein	▪ Fibronectin binding
Exoproteins ***A. Toxins*** 1) α- toxin, β- toxin, γ-toxin, δ-toxin, Panton - Valantine, and leucocidin	▪ Impairment of membrane permeability; cytotoxic effect on phagocytic and tissue cells
2) Epidermolytic toxin (Exfoliatin A and Exfoliatin B)	▪ Staphylococcal scalded skin syndrome
3) Enterotoxin	▪ Staphylococcal food poisoning
B. Enzyme 1. Toxic shock syndrome toxin 2. Coagulase 3. Hyaluronidase 4. Staphylokinase 5. Lipase	▪ Induce multisystem effects ▪ Converts fibrinogen to fibrin ▪ Degrades hyaluronic acid; spreading effect ▪ Degrades fibrin; spreading effect ▪ Degrades lipid; act on substances present on surface of the skin particularly fats and oil secreted by sebaceous glands
6. Phospholipases: Phosphatidylinositol -specific phospholipase C	▪ Degrade phospholipids; is associated with strains recovered from patients with adult respiratory distress syndrome and disseminated intravascular coagulation
7. Deoxyribonuclease	▪ Degrades DNA
8. Catalase	▪ Is a protective enzyme released by almost all staphylococcal species. It catalyzes conversion of hydrogen peroxide found during metabolism or released after phagocytosis into water and oxygen
9. Beta lactamase	▪ It render S.aureus resistant to penicillin and ampicillin. ▪ Cause proteolysis

Table (1): Continued

Virulence factor	Activity
10. Proteases	▪ Staphylococcal food poisoning ▪ Induce multisystem effects ▪ Converts fibrinogen to fibrin ▪ Degrades hyaluronic acid; spreading effect ▪ Degrades fibrin; spreading effect ▪ Degrades lipid; act on substances present on surface of the skin particularly fats and oil secreted by sebaceous glands ▪ Degrade phospholipids; is associated with strains recovered from patients with adult respiratory distress syndrome and disseminated intravascular coagulation ▪ Degrades DNA ▪ Is a protective enzyme released by almost all staphylococcal species. It catalyzes conversion of hydrogen peroxide found during metabolism or released after phagocytosis into water and oxygen ▪ It render S.aureus resistant to penicillin and ampicillin ▪ Cause proteolysis ▪ Staphylococcal scalded skin syndrome

(Quoted from Humphreys, 2004).

Diseases caused by S. Aureus

S. aureus causes many disease according to way of entry into human body, it can produce tissue infection, localized abscesses when it involved in a skin prick, another portal of infection is respiratory root which can cause pneumonia, introducing *S. aures* by blood has much more dangerous consequences such as endocarditis, osteomyelitis and meningitis ***(Harvey, 2013).***

S. aureus infection is a major cause of morbidity and mortality among humans and animals. It may infect soft tissue, skin, bone, respiratory, and endovascular disorders ***(Harvey, 2013).***

A. Localized skin infections:

S. aureus most commonly cause superficial, small abscesses which involve different areas of skin such as hair follicules (folliculitis), eye lash (stye) or it may be carried to subcutenous by an object causing boils (furuncles) or larger lesion (carbuncle). Impetigo another form of skin infection common in children which include superficial spread of *s.aureus* causing crusty skin ***(Thomer et al., 2016).***

B. Deep localized infections:

S. aureus can invade deeply in body either from superficial lesions or mechanical introduced by trauma or foreign body. For example, acute osteomyelitis or septic arthritis are medical emergencies that require surgical drainage and antibiotics ***(Thomer et al 2016).***

C. Acute endocarditis:

Injection abuse, poor antisepsis in superficial skin surgeries and elderly are most common risk factors for to s.aureus endocarditis.

Signs and symptoms of acute endocarditis includes; fever, malasia hypotension, changing heart murmurs and septic embolic signs such as osler nodules and Roth spots.

S. aureus acute endocarditis can affect normal and prosthetic valves. The rate of endocarditis has elevated and accounts for 22.9% to 34% of cases. S. aureus is the highest pathogen that cause mortality in nosocomial endocarditis with mortality rate 40-56% of elderly and immune deficient patients ***(Georgescu et al., 2016).***

D. Sepsis

S. aureus sepsis is a life threatening condition of untreated patients. Risk factors for sepsis include Immune compromised patients such as elderly patients who

undergoing Invasive procedures. The presentation of staphylococcal sepsis symptoms are most commonly fever, hypotension, tachycardia, and tachypnea. Usually symptoms arise from releasing TNF a, IL-1, and IL-6 from immune cells. Severe cases can lead to multiorgan failure, DIC and death ***(Harvey, 2013).***

E. Pnumonia:

S. aureus can cause a caveating, necrotizing pneumonia and death especially in immunocompromised patients. It is associated with high mortality rate if associated with influenza or if it came as a secondary infection ***(McDanel et al., 2016).***

F. Nosocomial infections:

S. aureus is one of the most common bacteria that causes hospital-acquired infections such as wound infections, catheter and medical devices contamination causing bacteremia which progress to septicemia ***(Harvey, 2013).***

G. Toxicosis:

1- Toxic shock syndrome (TSS):

It is a life threatening condition cause by *S.aureus* with signs and symptoms including (fever, sunburn like

rash, diarrhea, vomiting, multi organ failure). In 1970 infected tampons caused TSS for menstruating women. These tampons stimulated the production of TSST into circulation without introducing the *S.aureus* ***(Katarina et al., 2014).***

2- Staphylococcus gastroenteritis :

Ingestion of food (especially salty such as egg salad or creamy pasta) contaminated by *S.aures*, cause it to produce heat stable enterotoxin causing nausea vomiting and diarrhea in less than 6 hours ***(Puah et al., 2016)***.

3- Scaled skin syndrome:

S. aureus exofoliative toxin causes appearance of superficial bullae due to destruction of interacellar adhesion molecules in stratum granulosum causing epithelial desquamation. Following acute exfoliation, erythematous cellulitis occurs. MRSA suspected it should be treated urgently with Vancomycin or according to antibiotic sensitivity tests ***(Mishra et al., 2016).***