University of Thi-Qar Journal Of Science (UTsci)
Website: jsci.utq.edu.iq **Email: jsci@utq.edu.iq**
Volume 7, Number 1, June 2019

Potential effect of antimicrobial agents against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains from patients with skin infections

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Abstract

The present study is designed to investigate *S. aureus* and *Pseudomonas aeruginosa* bacteria isolated from wound and burn patients in Thi-qar province and detection of the optimal antibiotic. All *S. aureus* isolates were tested for their susceptibility to thirteen antibiotics, all *S. aureus* isolates 100% were resistant to ß-lactam antibiotics to both Ampicillin, penicillin, 91.5% to both cefoxitin, oxacillin, 89.1% to both Vancomycin, ciprofloxacin , iodine and ethanol has reduced 100% the growth of *S. aureus* and Tobramycin , 78.3% to tetracycline ,67.5% Netilmicin, 64.8% Clindamycin, 59.4% Erythromycin, 45.9% to both Amikacin , Ciprofloxacin and 29.7%Gentamicin while *Pseudomonas aeruginosa* isolates were tested for their susceptibility to twelve antibiotics, all isolates 100% were resistant to both Trimethoprim , Amikacin, Piperacillin, Ticarcillin, Aztreonam and Gentamicin ,95.2% cefotaxime , 85.7%Colistin sulphate, 80.9% to both Netilmicin, Ceftazidime, 76.1% Ciprofloxacin and 28.5% Imipenem. It concluded from the present study that all isolates of *S. aureus* were resistant to B-lactam antibiotics and Ciprofloxacin must be the best antibiotic that used against this microorganism.

Keywords: *S. aureus* , *pseudomonas aeruginosa*, Identification, antibiotic susceptibility.

Introduction:-

The skin is the largest organ in the body and the first line of defense against invading pathogens such as viruses, fungi, parasites and bacteria , the skin serves as a physical barrier to prevent entry of bacteria into deeper layers of tissue and or dissemination to internal organ systems, keratinocytes form this important physical barrier(Kobayashi *et al* .,2015). Considering the potential of different types of gram positive and gram negative bacteria it becomes important to clinically suspect of skin infections (Ghafur *et al.*,2017). Burn injury is one of the most common types of trauma that requires urgent medical attention(Church *et al* ., 2006).

Normal protective defense mechanisms of the skin are lost after a burn injury, resulting in rapid colonization of the wound surface, Initially, gram-positive organisms derived from skin commensals colonize the wound bed, followed later by gram-negative organisms and yeasts(Kennedy *et al* ., 2010). *Staphylococcus* species and *Pseudomonas aeruginosa* are two of the most frequently isolated microorganisms from burn wounds around the World (Altoparlak *et al* ., 2004). Wound sepsis is considered the most frequent cause of mortality and morbidity in such patients (Asati and Chaudhary, 2017).The World Health Organization(W.H.O) released a list of priority bacterial resistant pathogens, naming methicillin-resistant *S. aureus*, as a high priority target for further research and treatment development (W.H.O, 2017). Methicillin-resistant *Staphylococcus aureus*(MRSA) continues to be a significant human pathogen. For many years it has been a common cause of nosocomial infections, and variants capable of causing infections in the community (community-acquired Methicillin-resistant *Staphylococcus aureus* CA-MARSA) are an emerging and serious public-health issue (Chambers, 2001). More ever several studies have shown that the patient's own flora serves as the source of the infecting bacteria in about one-half of cases of surgical infections (Su and Cao , 2017 ; Fischetti , 2000). Burns are one of the most common and devastating forms of trauma, they induce a state of immunosuppression that predisposes burn victims to infectious complications (Church *et al*., 2006). The latter is the primary cause of morbidity and mortality in burn victims (Wibbenmeyer *et al*., 2006) . Colonization with *S. aureus* is an important risk factor for subsequent *S. aureus* infection (Wertheim *et al*., 2004; von Eiff *et al*., 2001) . Microbial resistance is

old as the advent of antimicrobial agents, antimicrobial resistance is the reduction in the susceptibility of pathogenic microorganism to one or more of the chemotherapeutic agents administered in clinical medicine (Elufisan *et al*., 2011). The pathogenesis of *Pseudomonas aeruginosa* infections is multifactorial, as suggested by the number and wide array of virulence determinants possessed by the bacterium (Barbieri , 2000).

Pseudomonas aeruginosa was reported to be the most commonly isolated Gram-negative bacteria for burning patients and the fifth most commonly isolated Gram-negative bacteria for nosocomial UTI in Najaf and Al-Diwaniya, Iraq (Fayroz-Ali, 2012; Al-Mayahi, 2013; Alshra, 2013). In addition to this intrinsic resistance, *Pseudomonas aeruginosa* frequently develops acquired resistance either by mutation in chromosomally-encoded genes or by the horizontal gene transfers of antibiotic resistance determinants (Takeda *et al*., 2007).

The present study related to the screening and characterization of two types of pathogenic bacteria and biofilm formation in patients with skin infections use antimicrobial agents in order to fight against *Pseudomonas aeruginosa* and *S. aureus*.

Materials and Methods:-

Samples collection:-

Samples were collected from 131 patients from both sexes with different ages, who suffered from skin infection; wound and burns.

Patients taking care and medications in AL Hussein Teaching hospital in Nasiriya City, during the period from August to December 2017. Swabs were collected from patients by disposable transport media. A formula for all patients was prepared, and included: date, age, sex, residence and health history . The swab was immediately inserted into a tube containing amies transport media, then transported to laboratory.

Isolation of *S. aureus* and *Pseudomonas aeruginosa*:-

The collected specimens were inoculated on types from culture media which included blood agar, mannitol salt agar, MacConkey agar and cetrimide agar base which considered as predominant enrich media, selective and differential media for the isolation, purification and identification of many types from bacteria. The plates were incubated at 37˚C for 24 hours then a single pure isolated colony was transferred to brain heart infusion agar slant for the preservation and to carry out other biochemical tests that confirmed the identification of isolates.

Identification of *S. aureus* and *Pseudomonas aeruginosa*:-

S. aureus and *Pseudomonas aeruginosa* were identified depending on the morphological features (colony size, shape, color ,hemolysis , translucency, edge, elevation and texture) on culture media and biochemical tests (MacFaddin, 2000). The isolates were stained by Gram stain to detect their response to stain, shapes and their arrangement (Benson, 2002).

The biochemical tests for *S. aureus* and *Pseudomonas aeruginosa*:-

A. **Coagulase test**
B. **Catalase test**
C. **Novobiocin test**
D. **Api Staph system :-** The Api Staph is the identification system for *Staphylococcus* and *Micrococcus*. This test is applied according to the company instructions.
E. **Oxidase Test**
F. **Kliglers Iron agar test**
G. **API 20 E System :-** The API-20E kit was used for confirmation the identification of *Pseudomonas aeruginosa* .the API 20 E strips consisted of 20 micro tubes containing dehydrated substrates. These micro tubes have been inoculated with bacterial suspension that reconstitutes the media. During incubation the bacterial metabolic activity in the medium caused change of its colors ,which might be spontaneous or revealed by the addition of reagents. The procedure adopted was following the manufacturer's instructions.

Antibiotics susceptibility test:-

The Kirby-Bauer method was used for establisher test (Kirby and Bauer, 1966) and the antibiotic used for *S.aureus* and *Pseudomonas aeruginosa* were Ampicillin, penicillin, cefoxitin, oxacillin, Vancomycin, Tobramycin , tetracycline , Netilmicin, Clindamycin, Erythromycin, Amikacin , Ciprofloxacin and Gentamicin for *S.aureus* while *Pseudomonas aeruginosa* isolates were twelve antibiotics, Trimethoprim , Amikacin, Piperacillin, Ticarcillin, Aztreonam ,Gentamicin , cefotaxime ,

Colistin sulphate, Netilmicin, Ceftazidime, Ciprofloxacin and Imipenem. Preparation of Mueller-Hinton plates It preparation according the manufacturer's instructions and Preparation of inoculum (Turbidity standard).To prepare the inoculums, colonies from overnight culture of tested isolates was transferred to a tube of 5 ml of normal saline to obtain culture with 1.5 × 108 CFU/ml by adjusting to McFarland standard tube No. 0.5. After incubation, the diameters of the inhibition zone were observed and measured in millimeters (mm). The diameter of inhibition zone for individual antimicrobial agent was translated in terms of sensitive (S), intermediate (I) and resistant (R) categories by comparison with the standard provided by the manufacturer (CLSI 2016).

Results:-

Isolation and characterization of *S. aureus* and *Pseudomonas aeruginosa*:-

A total of 131 sample from both sexes with different ages, who suffered from skin infection; wound and burns have been collected and tested during period from from August to December 2017 only (37) samples are given growth *S. aureus* with 28% and (21) sample are given growth *Pseudomonas aeruginosa* with 16% in figure (1).

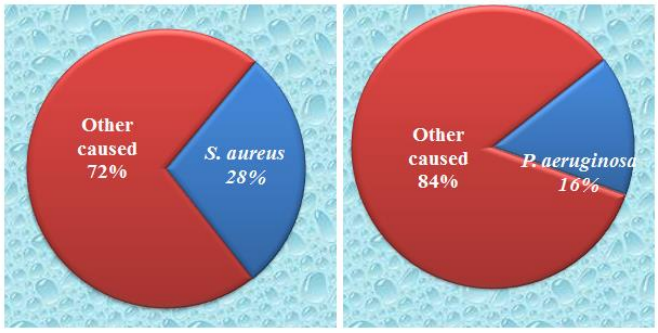


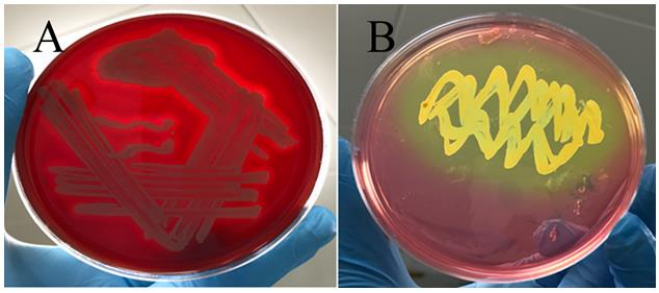
Figure (1) :- the occurrence of *S. aureus* and *Pseudomonas aeruginosa* isolated from 131 patient of skin infections

Colony Morphology:-

The results showed the morphology characteristics of *S. aureus* which grow on different media as in table (1)& figure (2).

Table (1):-Culture Characteristics of *S. aureus*

NO.	Culture Media	Morphology of colonies
1-	Blood Agar	Small , round , smooth ,golden yellow ,raised, glistening , hemolysis
2-	Mannitol Salt Agar	Yellow colonies

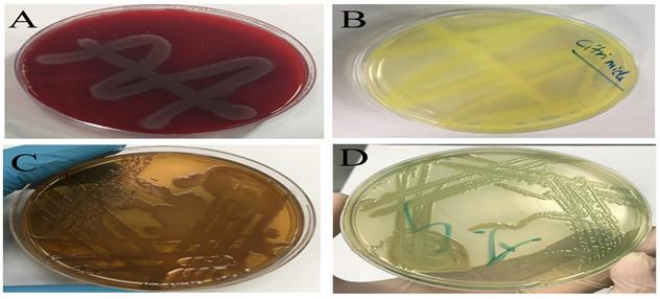


Figure(2) :- *S. aureus* on the blood agar (A) and on the mannitol salt agar (B).

While *Pseudomonas aeruginosa* which grow on different media as in table (2)& figure (4)

Table (2) :- Culture Characteristics of *Pseudomonas aeruginosa*

NO.	Culture Media	Morphology of colonies
1-	Blood Agar	large flat colonies, pale colonies, beta-haemolysis, a grape like oder
2-	MacConkey Agar	Sticky , convex pale color
3-	Cetrimide Agar Base	produce green- blue pigments
4-	Nutrient agar	produce green- blue pigments



Figure(3) :- The growth of Pseudomonas aeruginosa on blood agar (A), Cetrimide Agar Base (B) , MacConkey Agar (C) and Nutrient agar (D)

Microscopic examination:-

Microscopic examination was applied to all 37 isolates after having stained by Gram stain to detect their response to stain, the cells appeared as Gram positive cocci mostly arranged in grape like irregular clusters as well as all 21 isolates after having stained by Gram stain to detect their response to stain, the cells appeared as Gram negative rods occur as single or in pairs in figure (4).

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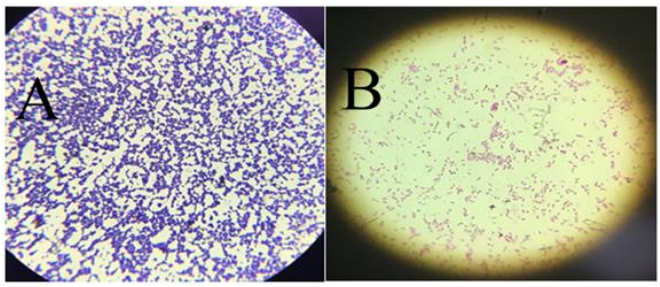


Figure (4):- Microscopic examination Gram positive cocci(*S.aureus*) A and Gram negative rods(*Pseudomonas aeruginosa)* B

Conventional Biochemical results:-

In the initial stage of identification, conventional biochemical tests were conducted, to identify the presumptive colonies Coagulase , Catalase test , Mannitol salt agar and Novobiocin for *S. aureus* as well as Oxidase test, Catalase test and Kligler Iron Agar(KI) for *Pseudomonas aeruginosa* as showen in table (3) (4); figure (5).

Table (3):- showed the result of (KIA).

NO.	Slant /Bottom	Symbol	Colour
1-	Alkaline/ Alkaline	K/K	Red/Red
2-	Alkaline/Acid	K/A	Red/yellow
3-	Acid/Acid	A/A	Yellow/Yellow
4-	H2S	-	Black precipitation
5-	Gas	-	Bubbles

Result of (KIA) of *Pseudomonas aeruginosa* :-

- Alkaline /Alkaline
- (negative H2S)
- Without gas

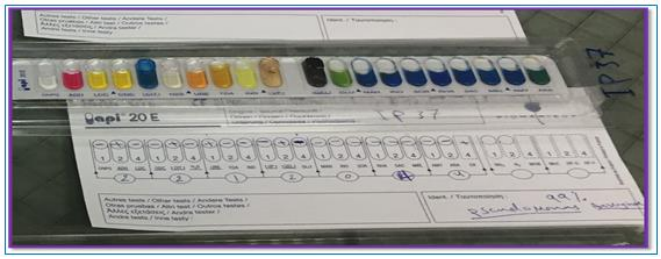
Table (4):- result of biochemical test to *S. aureus* and *Pseudomonas aeruginosa.*

NO.	Biochemical test	Result	Bacteria
1-	Catalase	+ve	*S. aureus*
2-	Coagulase	+ve	*S. aureus*
3-	Novobiocin	S	*S. aureus*
4-	KIA	K/K	*Pseudomonas aeruginosa*
5-	Catalase	+ve	*Pseudomonas aeruginosa*
6-	Oxidase	+ve	*Pseudomonas aeruginosa*

The result of API-20 Staph test has revealed that only 37 isolates from 131 sample were identified as *S. aureus* in figure (6).

Figure(6) :- Calculate the numerical profile in Api 20 system with Code 6736151 the test positive, *S. aureus*

The result of API-20E test has revealed that only 21 isolates from 131 sample were identified as *Pseudomonas aeruginosa* as in figure(7).



Figure(7) :- Calculate the numerical profile in Api 20NE,with code 2212044 the test positive, *P. aeruginosa*

Antibiotic susceptibility profile of *S. aureus* and *Pseudomonas aeruginosa* isolates:-

Thirteen kinds of antibiotics were used in the antimicrobial sceptibility test for(37) of *S. auruas* isolates that were resistant or sensitive or intermiadet to commonly used antibiotic are shown in figure (8).

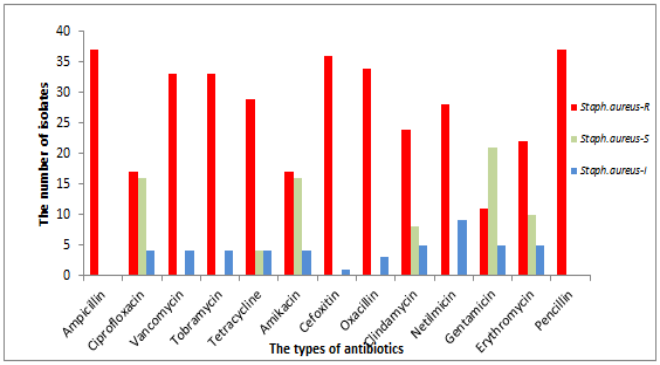


Figure (8):- Antibiotic susceptibility resistant pattern of *S. aureus*

The results indicated that the highest ratio of antibiotic resistance was 100% of isolates of *S. aureus* were both Ampicillin, pencillin and Less resistance to antibiotics gentamicin 29.7% as shown figure (9).

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The results also indicated that the antibiotic resistant between male and females showed no significant difference between males and females in antibiotics susceptibility as in figure (10).

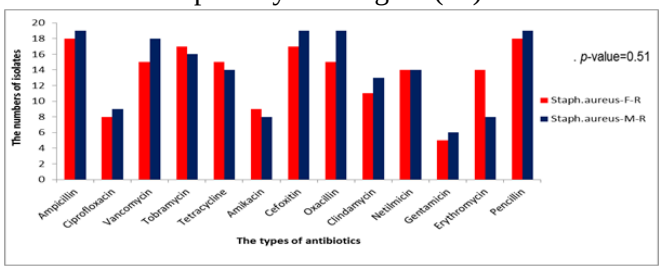

Figure (9):- Antibiotic susceptibility resistant pattern of *S. aureus*

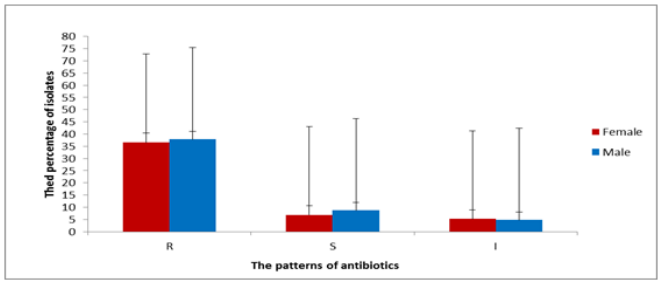

Figure (10):- The percentage of *S. aureus* strains isolated from female and male patients

There are different types of antibiotics were used as antimicrobial agents in the sceptibility test for (21)isolated of *pseudomonas aeruginosa* isolates that were resistant or sensitive or intermidate to commonly used antibiotic are shown in figure (11). The antibiotics susceptibility test showed that *Pseudomonas aeruginosa* resistance to the most antibiotics such as ciprofloxacin, Amikacin. Cefotaxim but sensitive to imipenem. The results also indicated that the antibiotic resistant between male and females as in figure (12) and showed no significant difference between males and females in antibiotics susceptibility as in figure (13).

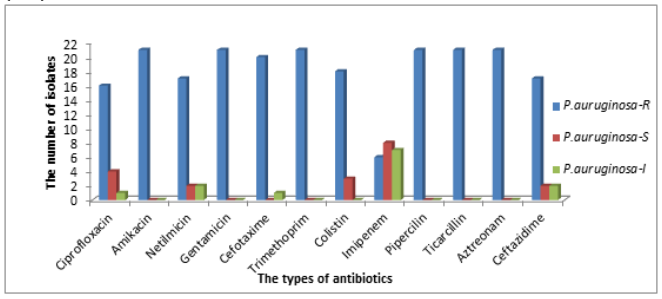

Figure (11):- Antibiotic susceptibility pattern of *p. aeruginosa*

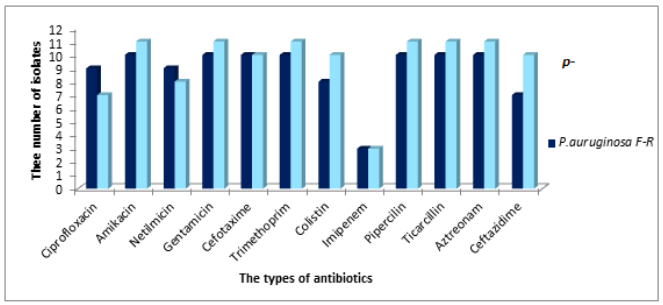

Figure (12):- Antibiotic susceptibility resistant pattern of *p. aeruginosa*

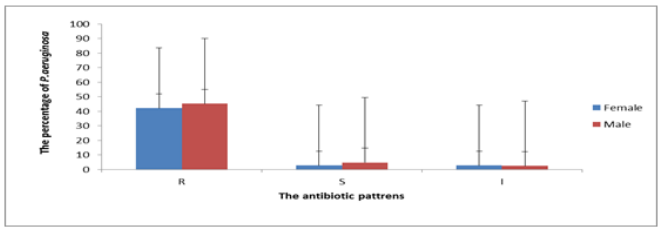

Figure (13):- The percentage of *p. aeruginosa* strains isolated from female and male patients with skin infections

Discussion:-

The results of the current study show all isolates of *S. aureus* is identified according to many characters such as microscopic examination, morphology characteristic on culture media like blood agar and detection the fermentation of mannitol sugar on mannitol salt agar, as revealed on Table (3-1) and Fig.(3-3). Further identification of a *S. aureus* is based on biochemical tests and API-20 Staph; and all isolates give positive results for all tests, these mothodes used by (Al- aawadi, 2014) to diagnosis this bacteria.

The results of the current study show that the occurrence of *S. aureus* is 37/131 (28.2 %), as shown in Fig. (3-1). The recent results are disagree with results of study Abid, (2015) who document that 45% of isolates identified as *S. aureus* that isolated from 200 sample collected from burn patients. The results of this study are analogous to other studies, like the results of Alwash and Saleh, (2013), described a *S. aureus* incidence in burns with a percentages of 33.3%. while the current percentage is slightly low when compared with results of study performed locally .

Alghalibi *et al.*, 2011 reffer to *S. aureus* may be considered as the most important isolated microorganism amongst the burn patients with burn wound infection. (Warner *et al*., 2009) noted it is one

of the greatest causes of nosocomial infection in burn patients.

Our results record that *S. aureus* followed by *Pseudomonas aeruginosa* these results disagree with Shahid and Malik,(2005) whom recorded that *Pseudomonas aeruginosa* caused skin infection , followed by *S. aureus* and other Gram negative bacteria such as *Klebsillea* spp can also cause infection in burn patients.

S. aureus is one of common species recovered from wound infection of patients after surgery and act as one of an important microorganisms which causes acute and chronic wound infection and also causes skin infections.

The identification of *Pseudomonas aeruginosa* is based on microscopic examination, colony morphology on culture media such as: blood agar, Nutrient agar, MacConkey agar and Cetrimide agar. And further identification by using the biochemical test and API 20NE.

The results of isolation and identification of *Pseudomonas aeruginosa* is 21/131 isolates (16.03 %), as revealed in Fig. (3-2). In addition to be associated with leg ulcers,*Pseudomonas aeruginosa* is one of the major causes of mortality and morbidity of burn victims, cystic fibrosis patients, and immunocompromised patients such as those suffering from AIDS (Tohidpour *et al*., 2009).

The recent data agree with results of Coetzee *et al.*, (2013) whom recorded that 14.5% of isolates were positive for *Pseudomonas aeruginosa*.

In our study, we investigated the antibiotic susceptibility on the both of *S. aureus* and *Pseudomonas aeruginosa* infected skin infection. The result show most of these types of bacteria resistance to different classes of antibiotics. The results also indicate that the antibiotic highest ratio of resistance 100% of isolates of *S. aureus* were both Ampicillin, pencillin and Less resistance to antibiotics gentamicin 29.7%.

Methicillin-resistant *S. auerus* (MRSA) strains are having a major impact worldwide, and due to their resistance to all β-lactams, Degaim, (2016) whom show that the occurrence of *S. aureus* which resistant to methicillin antibiotic (MRSA) is (67.5%). The detection of MRSA is an important mater for patients care and appropriate utilization of infection control resources (Al-Ruaily and Khalil, 2011).

Previous studies have also reported that because of increasing antibiotic resistance and the ability of pathogenic bacteria to resistant to many antibiotics and rapidly acquires additional drug resistance-conferring genetic information (Oli *et al*., 2017). In this regard, we investigat the relationship between the antibiotics susceptibility of these types of bacteria in males and females, several studies have been performed to evaluate the risk factors for resistance attributable mortality (Avitia-Domínguez *et al*. 2014; Bassetti *et al*. 2018; El Zowalaty and Gyetvai 2016). Bacteria exhibit multi-drug resistance (MDR) mechanisms to antibiotics including decreased permeability, expression of efflux systems, production of antibiotic inactivating enzymes and target modifications.(Dogonchia *et al.*, 2018; Robak *et al*., 2018). The antibiotics susceptibility test of the results performed that *Pseudomonas aeruginosa* resistance to the most antibiotics such as ciprofloxacin, Amikacin. Cefotaxim but sensitive to imipenem. The development of resistance has several risk factors linked to the severity of the infection and that resistance itself is associated with increased mortality (Dogonchia *et al*., 2018) . The resistance to the carbapenems in *Pseudomonas aeruginosa* is often caused by impermeability through alteration or loss of the porin , increased expression of an efflux pump (Lee and Ko *et al*., 2012).

Conclusions:-

From the present study it concluded the following:

1- *S. aureus* and *Pseudomonas aeruginosa* are an important causes of wound and burn infections.

2- The both pathogenic bacteria: *S. aureus* and *Pseudomonas aeruginosa* harbored some virulence factors that must be related for increasing the ability of those bacterial species to cause different diseases in burns and wounds.

3- All isolates of *S. aureus* were resistant to B-lactam antibiotics and Ciprofloxacin.

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