Antimicrobial activities against biofilm formed by bacteria isolated from patients with Burns in Al-Samawah city

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Abstract

The present study includes 145 samples collected from (100) patients, who suffered from burn wound infections, admitted to burn unit of the teaching medical Al-Hussein hospital in AL-Samawah City with different ages from both sexes. The period of collection were extended from December 2012 to May 2013. Out of 145 samples, About 315 bacterial isolates were found in 137 (94.5%) burn wound swabs and only 8 swabs (5.5%) were negative in bacterial growth. Results revealed the dominance of *Staphylococcus aureus* (42.3%) followed by *Staphylococcus epidermidis* (28.5%), *Pseudomonas aeruginosa* (22.6%), *Pseudomonas luteola* (20.4%), *Acinetobacterlwoffii* (16.79%), *Acinetobacterbaumannii*(14.6), *Raoultellaterrigena* (11.6%), *Klebsiellapneumoniae* (10.2%), *Aeromonashydrophila* (9.5%), *Proteus mirabilis* (8.75%), *Escherichia coli*(8.75%), *Providenciarettgeri* (8%), *Bacillus spp.* (8%), *Corynebacterium spp.*(7.3%) and *Proteus vulgaris* (6.67%) respectively. In this study, Antimicrobial susceptibility was carried out to the bacterial isolates against 19 antibiotics, the most of bacterial isolates were multi-drug resistance and other bacterial species were susceptible to the most antibiotics in this study. In multi-drug resistance bacteria, Imipenem was found to be the most effective drug against *Staphylococcus epidermidis*, *Pseudomonas luteola,Raoultellaterrigena, Aeromonashydrophila*and *Proteus mirabilis*, while meropenem was the most effective drug against both *Acinetobacterlwoffii*and *Acinetobacterbaumannii.* for *Pseudomonas aeruginosa,* Piperacillin/Tazobactam was the most effective drug and Linezolid was the most effective drug against *Staphylococcus aureus*. Whereas Ciprofolxacin was the most effective drug against *Klebsiellapneumoniae.* The results showed that The capacity of burn wound isolates to produce biofilms was high with a significant differences between bacterial species and between the different time periods ($P<0.05$). Also, The results revealed that 0.5 MIC of the most effective antibioticshad no significant effect on viable cells within biofilm matrix or on the total biofilm biomass of most tested bacteria, but there is significant effect of some antibiotics on biofilm biomass.

Keywords:
Burns, Bacterial Biofilm.

Physiology Classification Qp1 - 345

***The Research is apart on M.Sc. thesis in the case of the First researcher**

INTRODUCTION

(eschar) that provides a favourable niche for microbial colonization and proliferation [9]. Although biofilms are best known for their role in foreign device-related infections, recent studies have confirmed the importance of biofilms in the pathogenesis of burn wound infections [10]. More details, Biofilms are complex communities of surface attached aggregates of microorganisms embedded in a self-secreted extracellular polysaccharide matrix, or slime [11]. Once formed, biofilms act as efficient barriers against antimicrobial agents and the host immune system, resulting in persistent colonization and/or infection at the site of biofilm formation [12]. The emergence worldwide of antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections [13]. Recently, It has been observed that subinhibitory concentrations (sub MICs) of various antibiotics are able to modify the molecular architecture of the external surface of bacteria and some bacterial functions, such as the ability to adhere to the host cells or to the biomaterial surfaces, the ability of biofilm formation, the ability of bacterial invasion to host cells, the surface bacterial energy, the susceptibility to host defense mechanisms, motility etc., thus influencing bacterial virulence [14, 15, 16, 17].

Nosocomial infections are those infections that develop during hospitalization and are neither present nor incubating at the time of patient's admission. It represents a major problem in health care facilities, resulting in prolonged hospital stays, substantial morbidity and mortality, and excessive costs [1]. Nosocomial infections, even in this modern era of antibiotics, continue to remain an important and formidable consequence of hospitalization. It has been estimated that about 3.5% of patients leave the hospital after having acquired infections, depending on the case, hospital size and multiple other factors [2].Burn injuries by fire and hot liquids and contact with hot surfaces have been recognized as a significant and major public health problem [3]. Where Burns, are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality [4]. In patients with severe Burns, over more than 40 per cent of the total body surface area (TBSA), 75 per cent of all deaths are currently related to sepsis from burn wound infection or other infection complications and/or inhalation injury [5,6]. Although burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms [8]. Where The burn wound surface provides a protein-rich environment consisting of avascular necrotic tissue

MATERIAL AND METHODS

Samples Collection&Bacterial identification

laboratory of Al-Muthanna university for conduct the rest of tests. According to Eldere[19], Nosocomial infections were defined as infections diagnosed > 72 hr after the start of hospitalization, So only patients who are admitted for more than 3 days were included in this study.All swabs obtained were cultured directly on blood agar and MacConkey agar forisolation of aerobic bacteria and Identification of pathogenic bacteria was based on Microscopic and Morphological examination [20], biochemical tests[21,

145 burn wound swabs were taken from 100 burned patients, who presented invasive burn wound infection and admitted to burn unit of the teaching medical Al-Hussein hospital in AL-Samawah City from December 2012 to May 2013. burn wound swabs were collected from burn wounds after the removal of dressings and topical antimicrobial agents and cleansing of the wound surface with 70% alcohol [18]. the burn wound swabs were transported by the Amies medium to the college of medicin

Kit, Masta Staph Kit and VITEK 2 Compact System.

Antimicrobial susceptibility

Cefotaxime (CTX10), Chloramphenicol (C10), Ciprofloxacin(CIP10), Doxycycline (DO10), Erythromycin (E15), Gentamicin (CN 10), Imipenem (IPM10), Linezolid (LNZ30), Meropenem (MEM10), Oxacillin (OX10), Penicillin (P10), Piperacillin/tazobactam (TPZ100/10), Rifampin (RA5), Streptomycin (S10) andTicarcilin(TIC75).

isolate were measured by using the Broth Macrodilution Method recommended by [24].The sub-MIC that used in this study was ½ of the lowest MIC value for the most effective antimicrobial agent of each bacterial species.

effect on viable bacteria within the biofilm matrix and on total biofilm biomass**.**

adherent film; and 200 µl of the particular antibiotic (0.5 MIC of antimicrobial agent) dilution in tryptis soy broth was added. The plates were incubated at 37°C for 24 hr. After exposure to antibiotic 24 hr, the solutions were discarded and the wells were filled (200 µl) with PBS. And then The quantitative determination of biofilm formation was carried out by using Microtiter Plate Biofilm Formation Assay. This assay was repeated in absence of 0.5 MIC of antimicrobials (without exposure to antibiotic 24 hr) as a control.

eliminate the unbound bacteria, without disturbing the adherent film; and 200 µl of the particular antibiotic (0.5 MIC of antimicrobial agent) dilution in tryptis soy broth was added. The plates were incubated at 37°C for 24 hr. After incubation, the wells were Washed six times With PBS, each time using a multichannel pipettor to add 200 µl sterile PBS per well and then vigorously shaking out the liquid over a waste container to remove planktonic cells.Scissors were used to cut each individual well from the microtiter plate and added 200 µl PBS to each well.Each well (i.e., the actual plasticwell plus its contents) was added to a separate 13-ml tube containing 3.8 ml PBS (for a final liquid volume of 4 ml).

22] and the final identification was done by using diagnostic Kits such as API 20 E

In this test, the selection of antibiotics and its standard inhibition diameters was used as it recommended by [23]. The procedure of this test was performed according to MaccFaddin, (2000) by the Kirby-Bauer standardized method. The antibiotics tested for bacterial isolates were: Amikacin (AK10), Amoxicillin (AX25), Ampicillin (AM10), Azithromycin (AZM15),

Determination of MIC and sub-MIC of antimicrobials on planktonic cultures The Minimum inhibitory concentrations of the most effective antimicrobial agent for each bacterial

The effect of antimicrobials on pre-formed biofilms

The antimicrobial effect on pre-formed biofilm was determined by examining its

The effect of 0.5 MIC of antimicrobials on total biofilm biomass

This assay was used according to Tetz*et al.*[25] with some of modifications.Biofilm formation was carried out in a 96-well plate by using Microtiter Plate Biofilm Formation Assay [26, 27]. After 24, 48, 72 and 96 hr. of incubation at 37°C. The supernatant from each well was gently aspirated with a micropipette. Each well was washed three times withPhosphate Buffer Saline(PBS) under aseptic conditions to eliminate the unbound bacteria, without disturbing the

1- **The effect of 0.5 MIC of antimicrobials on viable bacteria within the biofilm matrix**.

The biofilm formed in the microtiter plate can also be quantitated by directly enumerating the viable bacteria within the biofilm matrix. This assay was performed according to Merritt *et al.*[28] with some of modifications in incubation time and in adding 0.5 MIC of antimicrobials.WhereBiofilm formation was carried out in a 96-well plate by using Microtiter Plate Biofilm Formation Assay [26, 27]. After 24, 48, 72 and 96 hr. of incubation at 37°C.The supernatant from each well wasgently aspirated with a micropipette. Each well was washed three times with PBS under aseptic conditions to

by plating on agar medium to enumerate bacteria that were attached to the microtiter well surface.All steps above was repeated, but without exposure to antibiotic 24 hr, as a control.

Statistical analysis

and analyzed by using statistical program social science (SPSS 20) and the results were expressed as Mean for three independent experiments [30].

Caped before and after addition.Sonicatormicrotip was Inserted and the contents of each tube were sonicated for 5 min at 40 KHz. viable counts were Performed according to Phelan [29] on the resulting suspensions

Completely Randomized Design (CRD) was used in the analysis of variance for data by using one way ANOVA test, Treatment means were compared using the least significant difference (LSD) at a 5% level of significance. Data were processed

RESULTS AND DISCUSSION

Isolation and Identification of Bacteria

About 315 bacterial isolates were found in 137 (94.5%) burn wound swabs, and only 8 swabs (5.5%) were negative in bacterial growth. *Staphylococcus aureus* was the commonest pathogen isolated (42.3%) followed by *Staphylococcus epidermidis* (28.5%), *Pseudomonas aeruginosa* (22.6%), *Pseudomonas luteola* (20.4%), *Acinetobacterlwoffii* (16.79%), *Acinetobacterbaumannii*(14.6), *Raoultellaterrigena* (11.6%), *Klebsiellapneumoniae* (10.2%), *Aeromonashydrophila* (9.5%), *Proteus mirabilis* (8.75%), *Escherichia coli*(8.75%), *Providenciarettgeri* (8%), *Bacillus spp.* (8%), *Corynebacterium spp.*(7.3%) and *Proteus vulgaris* (6.67%) respectively as shown in table (1). There is a significant difference between different bacterial genera ($p<0.05$), but does not have a significant difference between the species of the same bacteria ($p<0.05$).

Table (1). Distribution of bacteria isolated from burn wounds.

Staphylococcus aureus	**58**	**42.3**
Staphylococcus epidermidis	**39**	**28.5**
Pseudomonas aeruginosa	**31**	**22.6**
Pseudomonas luteola	**28**	**20.4**
Acinetobacterlwoffii	**23**	**16.79**
Acinetobacterbaumannii	**20**	**14.6**
Raoultellaterrigena	**16**	**11.6**
Klebsiellapneumoniae	**14**	**10.2**
Aeromonashydrophila	**13**	**9.5**
Proteus mirabilis	**12**	**8.75**
Escherichia coli	**12**	**8.75**
Providenciarettgeri	**11**	**8**
Bacillus spp.	**11**	**8**
Corynebacterium spp.	**10**	**7.3**
Proteus vulgaris	**9**	**6.67**
Pantoeasp 3	**8**	**5.8**
LSD value	**19.2**	

The culture positivity of this study (94.5%) in agreement with positive cultures obtained by Al-Azzawi and Al-Dulaimi[31], Bnain*et al.* [3], Al-saad*et al.*[32] and Al-kaabi[33] (80.16%, 84%, 89% and 80.59%), but this result in disagreement with other studies within Iraq such as Mohammed [34], Al-Asadi [35] and Alwan*et al.*[2011], who found that the culture positivity is (65.4%), (67%) and (64.3%) respectively. The high percentage of positive bacterial cultures of

Samawah city or even in Iraq, as *Raoultellaterrigena* (previously known as *Klebsiellaterrigena*), *Pseudomonas luteola* (previously known as *Chryseomonasluteola*) and *Pantoea sp3*, and This may be due to the large number of people who visits the patients in hospital, where most of those visitors are from rural areas. In addition to, they did not take the conditions of prevention during the visit. As well as, the contamination of tap water -which used in washing the patient every day- with new types of bacteria. However, The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent or long-term hospitalization, complicating illnesses, prior administration of antimicrobial agents, or the immunosuppressive effects of burn trauma. This evidence was consistent with previous observation mentioned by some workers. Initially, the immunologic response to severe burn injury is proinflammatory but later becomes predominately anti-inflammatory responses in an effort to maintain homeostasis and restore normal physiology; cytokines and cellular response mediate both of these phases [36].

Antimicrobial susceptibility

*Acinetobacterlwoffi*and *Acinetobacterbaumannii.* for *Pseudomonas aeruginosa,* Piperacillin/Tazobactam was the most effective drug and Linezolid was the most effective drug against *Staphylococcus aureus*. Whereas Ciprofolxacin was the most effective drug against *Klebsiellapneumoniae.* MIC and 0.5 MIC values of the most effective antibiotics (for each tested bacterium) were shown in table (2).

the skin swab may be attributed to the fact that the burn wound has a much higher incidence of infections compared with other forms of trauma because of extensive skin barrier disruption as well as alteration of cellular and humoral immune responses [37]. As described above, *Staphylococcus aureus*was the most common pathogen of burn wound infection of this study, as isolated in 42.3% of burn wound infection cases. These results bring into line with Komolafe*et al.* [38] 37.6%, Also This pathogen has been reported as a major cause of nosocomial infection in Europe [39], but Nasser *et al.*[40] and Bnain*et al.* [3] found that the most common pathogen of burn wound infection was *Pseudomonas aeruginosa* in percentage of (21.6%) and (66%). In addition, other researchers reported that the most predominate pathogen was *Acinetobacterbaumannii,* as Keen *et al.* [41](58%). Meanwhile,Prominence of *Staphylococcus aureus* in this study may be due to thatBurn units have become major reservoir for *S. aureus* that has the special characteristics for spreading quickly in a hospital environment.Also, the results of this study showed that some of bacterial species were first isolated from burn wound infection in AL-

The antimicrobial activities of 19 antibiotics were varied between bacterial isolates, where the most of bacterial isolates were multi-drug resistance and other bacterial species were susceptible to the most antibiotics in this study. In multi-drug resistance bacteria, Imipenem was found to be the most effective drug against *Staphylococcus epidermidis, Pseudomonas luteola,Raoultellaterrigena, Aeromonashydrophila*and *Proteus mirabilis*, while meropenem was the most effective drug against both

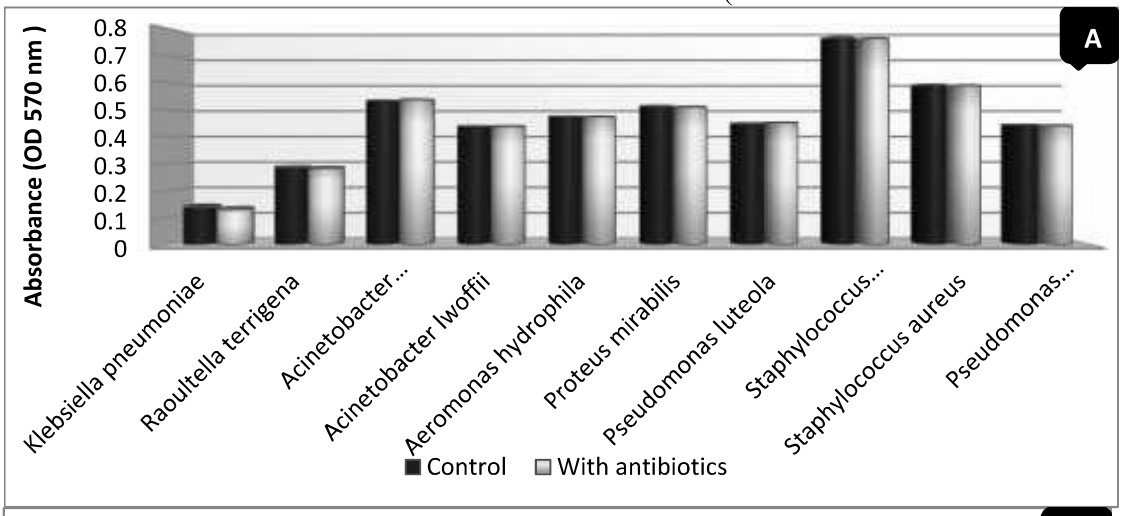
Table (2). Minimum inhibitory concentrations (MICs)and Sub-inhibitory concentration (0.5 MIC) values for bacterial isolates.

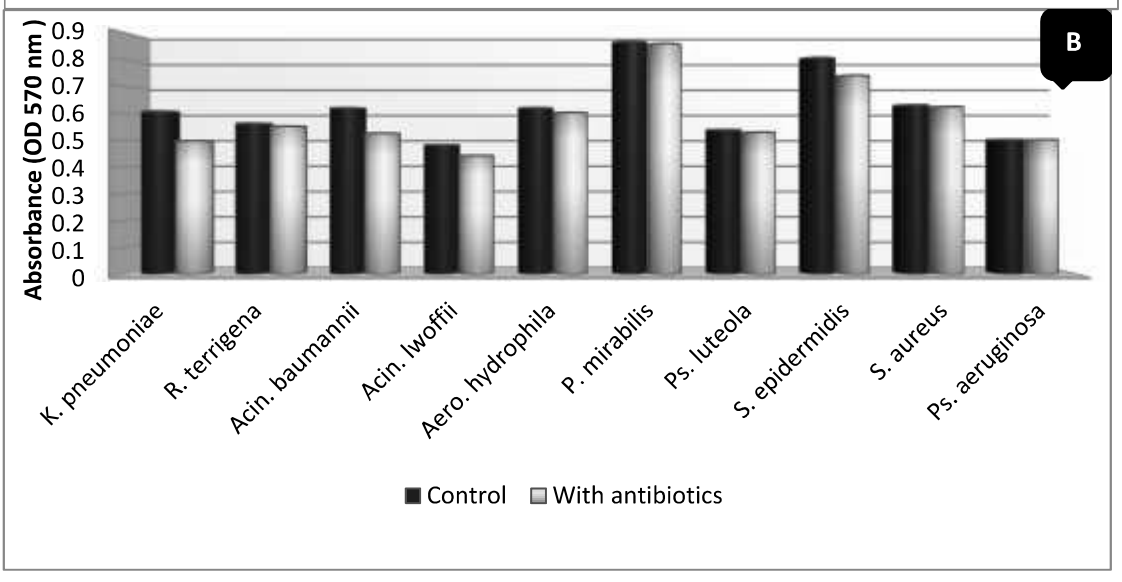
Microorganism	Antibiotics	MIC µl/ml	Sub-MIC (0.5 MIC)
Staphylococcusaureus	Linezolid	**4**	**2**
Staphylococcus epidermidis	Imipenem	**1**	**0.5**
Pseudomonas aeruginosa	Piperacillin/Tazobactam	**32**	**16**
Pseudomonas luteola	Imipenem	**64**	**32**
Acinetobacterlwoffii	Meropenem	**16**	**8**
Acinetobacterbaumannii	Meropenem	**16**	**8**
Raoultellaterrigena	Imipenem	**2**	**1**
Klebsiellapneumoniae	Ciprofolxacin	**16**	**8**
Aeromonashydrophila	Imipenem	**16**	**8**
Proteus mirabilis	Imipenem	**16**	**8**

antibiotic) at different biofilm ages (24-h-old biofilms, 48-h-old biofilms, 72-h-old biofilms and 96-h-old biofilms) are summarized in figure (1).

4.6.2.1 The effect of 0.5 MIC of antimicrobials on total biofilm biomass

Effect of 0.5 MIC of Imipenem, Meropenem, Piperacillin/Tazobactam, Ciprofolxacin and Linezolid on pre-formed biofilm biomass of bacterial isolates (each bacterium with their





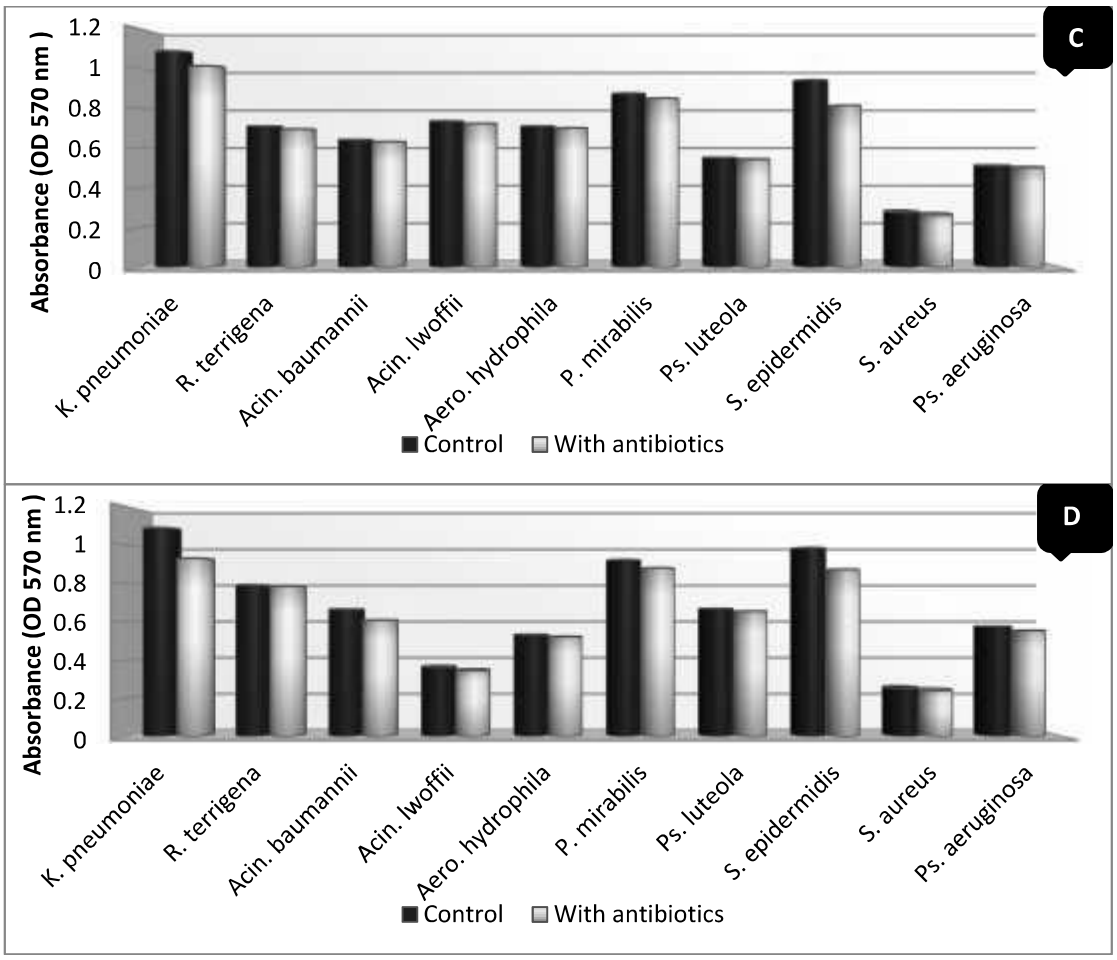


Figure (1). Effect of 0.5 MIC of antimicrobials on total biofilm biomass at 24-h-old biofilms

all isolates at the different biofilm biomass ages, except the 0.5 MIC of Ciprofloxacin and Imipenem were had a significanteffect on 48, 72, 96-h-old biofilm biomass of *Klebsiellapneumoniae* and *Staphylococcus epidermidis.* in addition, 0.5 MIC of Meropenem was also had a significanteffect only on 48-h-old biofilm biomass for both *Acinetobacterlwoffii* and*Acinetobacterbaumannii* ($p<0.05$).

The resistance of bacterial species to 0.5 MIC of antimicrobial agents when be in biofilm phase that observed in this study was also observed in other studies [42, 43, 44, 45]. These results may be due to that Biofilm formation almost always leads to a large increase in resistance to antimicrobial agents (up to 1000-fold decrease in susceptibility) in comparison with planktonic cultures grown in conventional liquid media [46]. The differences in biofilm resistance to antibiotics between bacterial species in this study may be attributed to that (A), 48-h-old biofilms (B), 72-h-old biofilms (C) and 96-h-old biofilms (D). Bars represent the mean of absorbance (optical density OD at 570nm) in presence/absence antibiotics (0.5 MIC) for each tested bacteria. Results are representative of three independent experiments and four replicates.

In the absence of 0.5 MIC of antimicrobials, the results of present study revealed that there are a significant differences between the different biofilm biomass ages (24, 48, 72 and 96-h-old biofilm) for each tested bacterium at ($p<0.05$). also, there is a significant difference between bacterial species for biofilm biomass formation in the same biofilm age ($p<0.05$). After these bacterial species in the different biofilm biomass ages were incubated in the presence of a sub-MICs (0.5 MIC) of antimicrobial agents for 24 h, The results of this study showed that 0.5 MIC of antimicrobials had no significant effect onbiofilm biomass of

Ciofu*et al.*[47] demonstrated that the resistance of *P. aeruginosa* biofilms to *Acinetobacterlwoffii* and*Acinetobacterbaumannii* in this study may be attributed to fact that these sub-MICs of Ciprofloxacin, Imipenem and Meropenem was effective in reduction of biofilm biomass but not effective in reduction of viable cells within biofilm matrix (As will explain in the subsequent test: Effect of 0.5 MIC of antimicrobials on viable bacteria within the biofilm matrix), where Merritt *et al.* [28] mentioned that there is one drawback of the crystal violet assay (Microtiter plate assay), this drawback is: crystal violet stains not only cells, but essentially any material adhering to the surface of the plate (e.g., matrix components); therefore, crystal violet staining may overestimate the number of adherent bacteria. As well as, Di Bonaventura *et al.* [50] states that "crystal violet staining assays measure total biofilm biomass but provide no information about cell viability". However, this means that these antimicrobials reduced the thickness of biofilm by decreasing the adhesion of some of matrix components to the surface of the plate well but was not effective on cell viability. These result were in agreement with other results obtained by Wasfi*et al.* [17], who reported that The sub-MICs ofceftriaxone and nitrofurantoin had no significant effect on viable cells, while they reduced the bioflm biomass of Proteus mirabilis.

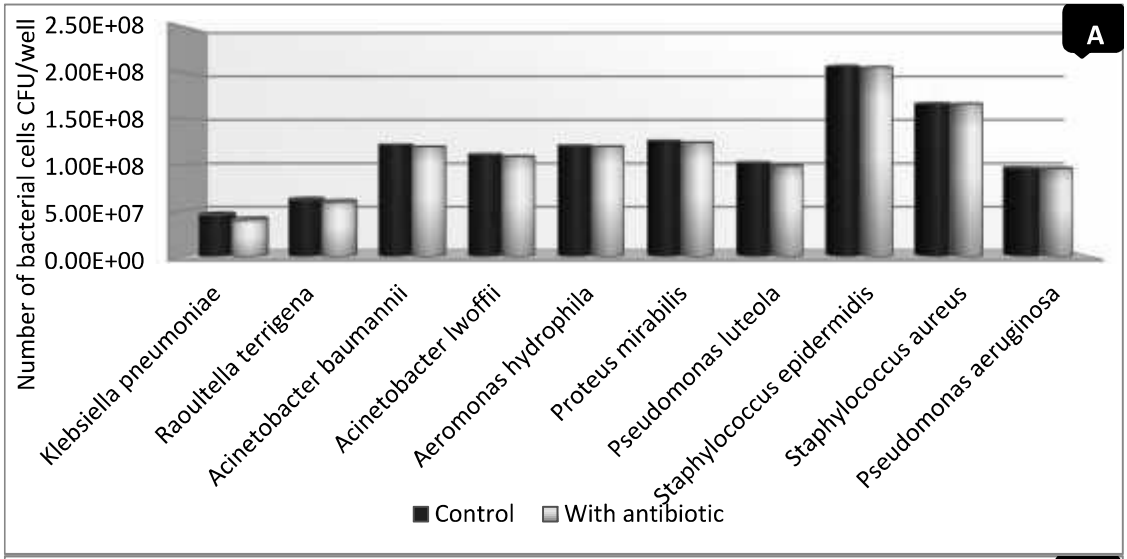
their antibiotic) at different biofilm ages (24-h-old biofilms, 48-h-old biofilms, 72-h-old biofilms and 96-h-old biofilms) are summarized in figure (2).

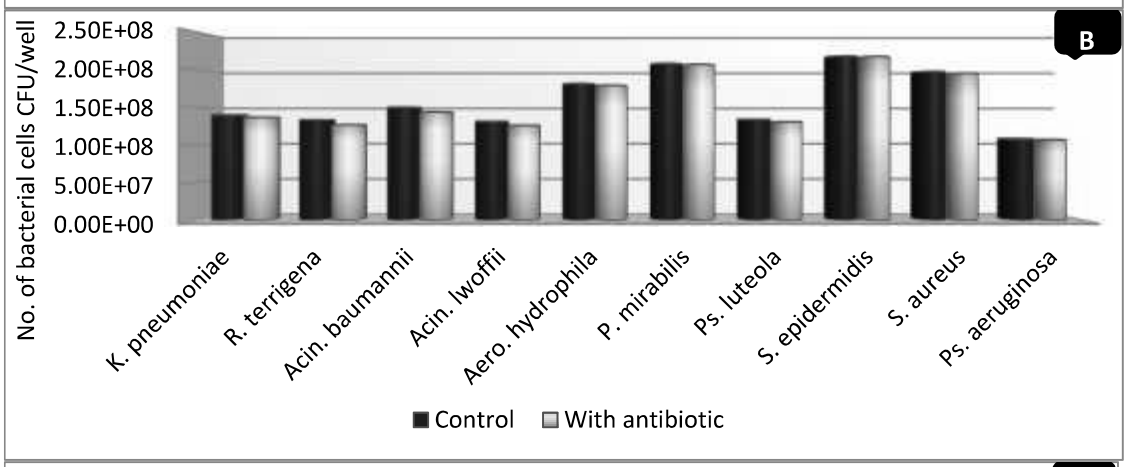
mechanisms of biofilm resistance to antibiotics varied between bacteria, where antimicrobial treatment is related to mucoidy. Mucoid biofilms were up to 1000 times more resistant to tobramycin than nonmucoid biofilms, in spite of similar planktonic MICs. Whereas Rachid*et al.* [48] and Kuehl *et al.*[49] reported that Some antimicrobials at subinhibitory concentrations, such as tetracycline, erythromycin and quinupristin-dalfopristin, vancomycin, and furanone also enhance coagulase-negative Staphylococci (such as *Staphylococcus epidermidis*) biofilm formation by stimulating *ica*expression or withunknown mechanisms. Moreover, Frank *et al.* [44] noted that sub-MIC concentrations of linezolid and ciprofloxacin, have been shown to induce *S. aureus* biofilm formation by increasing of expression of the *S. aureus* fibronectinbinding proteins (FnBPs). Also, Nucleo*et al.* [45] showed that sub-MIC imipenem induced biofilm formation *A. baumannii* by inducing expression of *A. baumannii*iron uptake genes and proteins. As described above, these examples of mechanisms of biofilm resistance to antibiotics justified The differences in biofilm biomass resistance to antibiotics between bacterial species in this study.

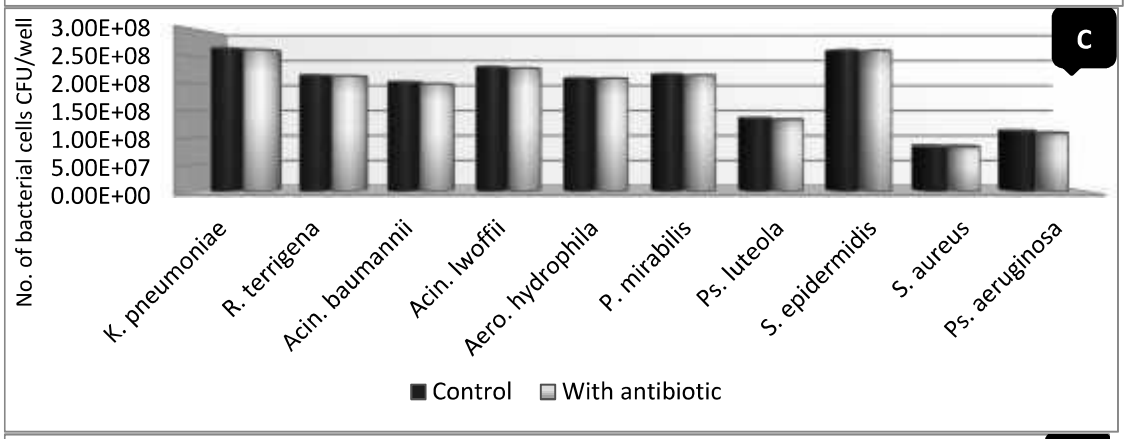
A significanteffects of 0.5 MIC of Ciprofloxacin and Imipenemon 48, 72, 96-h-old biofilm biomass of *Klebsiellapneumoniae* and *Staphylococcus epidermidis*,or 0.5 MIC of Meropenem on 48-h-old biofilm biomass for both

Effect of 0.5 MIC of antimicrobials on viable bacteria within the biofilm matrix.

Effect of 0.5 MIC of Imipenem, Meropenem, Piperacillin/Tazobactam, Ciprofolxacin and Linezolid on viable cells within pre-formed biofilm matrix of bacterial isolates (each bacterium with


A
Number of bacterial cells CFU/well
2.50E+08
2.00E+08
1.50E+08
1.00E+08
5.00E+07
0.00E+00
Klebsiella pneumoniae
Raoultella terrigena
Acinetobacter baumannii
Acinetobacter lwoffii
Aeromonas hydrophila
Proteus mirabilis
Pseudomonas luteola
Staphylococcus epidermidis
Staphylococcus aureus
Pseudomonas aeruginosa
Control
With antibiotic


B
No. of bacterial cells CFU/well
2.50E+08
2.00E+08
1.50E+08
1.00E+08
5.00E+07
0.00E+00
K. pneumoniae
R. terrigena
Acin. baumannii
Acin. lwoffii
Aero. hydrophila
P. mirabilis
Ps. luteola
S. epidermidis
S. aureus
Ps. aeruginosa
Control
With antibiotic


C
No. of bacterial cells CFU/well
3.00E+08
2.50E+08
2.00E+08
1.50E+08
1.00E+08
5.00E+07
0.00E+00
K. pneumoniae
R. terrigena
Acin. baumannii
Acin. lwoffii
Aero. hydrophila
P. mirabilis
Ps. luteola
S. epidermidis
S. aureus
Ps. aeruginosa
Control
With antibiotic

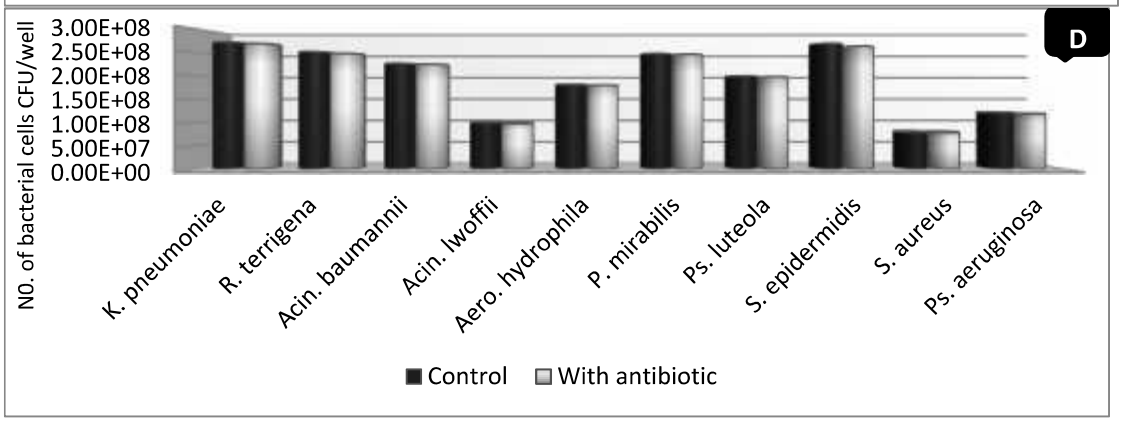

D
NO. of bacterial cells CFU/well
3.00E+08
2.50E+08
2.00E+08
1.50E+08
1.00E+08
5.00E+07
0.00E+00
K. pneumoniae
R. terrigena
Acin. baumannii
Acin. lwoffii
Aero. hydrophila
P. mirabilis
Ps. luteola
S. epidermidis
S. aureus
Ps. aeruginosa
Control
With antibiotic

Figure (2). Effect of 0.5 MIC of Imipenem, Meropenem, Piperacillin/Tazobactam, Ciprofolxacin and Linezolid on viable cells within pre-formed biofilm matrix of bacterial isolates (each bacterium with their antibiotic) at different biofilm ages 24-h-old biofilms (A), 48-h-old biofilms (B), 72-h-old biofilms (C) and 96-h-old biofilms (D).Bars represent the mean of the number of CFU per well in presence/absence antibiotics (0.5 MIC) for each tested bacteria. Results are representative of three independent experiments.

molecular size to penetrate the pore structure of extracellular polymer substance physical barrier; 2) electrostatic repulsion or sequestration of antimicrobials by surface polymers of biofilms, e.g. positively charged antimicrobials, aminoglycoside, binding to the negatively charged exopolymer; and 3) the presence of enzymatic substances in biofilm matrix specifically hydrolyzing the antimicrobial agents [54].

Moreover, other hypothesizes in biofilm resistance to sub-MICs, where Jefferson *et al.*[55] suggested that even though the matrix may not inhibit thepenetration of antibiotics, it may retard the rate of penetration enough to induce the expression of genes within the biofilm that mediates resistance. A second hypothesis to explain reduced biofilm susceptibility to antibiotics concernsthe metabolic state of microorganisms in a biofilm. Some ofthe cells located deep inside the biofilm structure experiencenutrient limitation and therefore exist in a slow-growingor starved state [56]. third hypothesis involvesgenetic adaptation to different conditions. the mutation frequencyof a biofilm-growing microorganism is significantlyhigher than that of its planktonic form; for *P. aeruginosa*,up to a 105-fold increase in mutability has been observed[57].

In toto, studying of Effect of 0.5 MIC of antimicrobials on viable bacteria within the biofilm matrix was more accuracy than studying of Effect of 0.5 MIC of antimicrobials on total biofilm biomass. Also, The majority of skin pathogenic and non-pathogenic biofilm research is performed *in vitro*. Although *in vitro* assays have several advantages, including lower cost, and the ability to control the number of bacteria, they do not take into consideration the effect of wound fluid, growth factors, proteases, and antimicrobial peptides [58]. Therefore, the laboratory animals must use in the prospective studies to help clarify the role of bacterial biofilms in burn wound infection and healing, also to explain biofilm resistance to antimicrobial agents.

In the absence of 0.5 MIC of antimicrobials, the results of present study showed that there are a significant differences in the number of viable cells of tested bacteria between the different biofilm ages (24, 48, 72 and 96-h-old biofilm) for each tested bacterium at ($p<0.05$). as well as, there are a significant differences in the number of viable cells between bacterial species in the same biofilm age ($p<0.05$).

After these bacterial species in the different biofilm ages were incubated in the presence of a sub-MICs (0.5 MIC) of antimicrobial agents for 24 h, The results of this study showed that 0.5 MIC of antimicrobials had no significant effect onviolable cells within biofilm matrix of all tested isolates at the different biofilm ages (24, 48, 72 and 96-h-old biofilm) ($p<0.05$). these results was in agreement with other studies [51,25,17], who noted that sub-MICs of some antimicrobials did not statistically significantly reduce the numbers of CFU of some bacterial species. Whereas, previous studies showed that some of sub-MICs of antimicrobial agent had a significant effect onviable cells within biofilm matrix for *Pseudomonas aeruginosa*[52] and *Staphylococcus aureus* [53]. The differences between this study and other studies may be due to that Changes in environmental conditions between studies can result in switch on/off of biofilm formation and different biofilm properties, such as their thickness and antimicrobial resistance characteristic.

However, Failure of antimicrobial agents to reach the biofilm-grown cells in this study and other studies might be due to: 1) difficulty of antimicrobials of large

REFERENCES

1- Tayh, G.A. 2011. Risk Factors and Antimicrobial Resistance of Pathogens Isolated from Burn Units at Local Hospitals in Gaza Strip, Palestine. MSC Thesis. Islamic University-Gaza, Biological Sciences.

2- Prakash, S.k. 2001.Nosocomial infection an overview. In: Maulana Azad Medical College, New Delhi. 6: 1-13.

3- Bnain A.A., Kahawish, Z.A., Kahawish, R.A., Kadom M., Jasim Z.M. 2008. Bacteriological study in burn wound infection. *Med. J. Baby.,* 5(2): 242-246.

4- Lionelli, G.T., Pickus, E.J., Beckum, O.K., , Decoursey, R.L., &Korentager, R.A. 2005. A three decade analysis of factors affecting burn mortality in the elderly. *Burns,* 31: 958-963.

5- Bang, R.L., Sharma, P.N., Sanyal, S.C. & Al Najjadah, I. 2002. Septicaemia after burn injury: a comparative study. *Burns,* 28: 746-51.

6- Taneja, N., Emmanuel, R., Chari, P.S. & Sharma, M. 2004. A prospective study of hospital-acquired infections in burn patients at a tertiary care referral centre in north India. *Burns*, 30: 665-9.

7- Weber, J.M., Sheridan, R.L., Pasternack, M.S. & Tompkins, R.G. 1997. Nosocomial infections in pediatric patients with Burns. *Am. J. Infect. Control.,* 25: 195-201.

9- Erol, S., Altoparlak, U., Akcay, M.N., Celebi, F. &Parlak, M. 2004. Changes of microbial flora and wound colonization in burned patients. *Burns,*. 30: 357-61.

10- Trafny, E.A. 1998. Susceptibility of adherent organisms from *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains isolated from burn wounds to antimicrobial agents. *Int J. Antimicrob. Agents*, 10: 223–228.

11- Stoodley, P., Sauer, K., Davies, D.G., &Costerton, J.W. 2002. Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.,* 56: 187–209.

12- Edwards, R., & Harding, K.G. 2004. Bacteria and wound healing. *Curr. Opin. Infect. Dis.,* 17: 91–96.

13- Sharma, M. &Taneja, N. 2007. *Burns,*, antimicrobial resistance & infection control. *Indian J. Med. Res.,* 126: 505-507.

14- Jabalameli, F., Malekzadeh, F. &Mirsalehianl, A. Effects of antibiotics on adhesion and invasion of *Proteus mirabilis*. *Acta. Med. Iranica*., 43(1): 55-59.

15- Vidya, K.C., Mallya P.S. & Rao, P.S. 2005. Inhibition of bacterial adhesion by subinhibitory concentrations of antibiotics. *Indian J. Med. Microbiol*.,23(2): 102-105.

16- Pour, N.K., Dusane, D. H., Dhakephalkar, P. K., Zamin, F. R., Zinjarde, S. S. &Chopade, B. A. 2011.Biofilm formation by Acinetobacterbaumannii strains isolated from urinary tract infection and urinary catheters. *FEMS Immunol. Med. Microbiol.*, 62: 328–338.

17- Wasfi, R., Abd El-Rahman, O.A., Mansour, L.E., Hanora, A.S., Hashem, A.M., Ashour, M.S. 2012. Antimicrobial activities against biofilm formed by *Proteus mirabilis* isolates from wound and urinary tract infections. *Indian J. Med. Microbiol.*, 30(1): 76-80.

18- Church, D., Elsayed, S., Reid, O., Winston, B., Lindsay, R. 2006. Burn Wound Infections. *Clin. Microbiol. Rev.*, 19 (2): 403-434.

19- Eldere, J. 2003. Multicentre surveillance of *Pseudomonas aeruginosa* susceptility patterns in nosocomial infections, *J. antimicrob. chem.,* 51(2): 347-352.

20- Leboffe, M.J., & Pierce, B.E. 2005. A Photographic Atlas For The Microbiology Laboratory. Morton Publishing Company, Colorado.

21- Collee, J. G., Fraser, A. G., Marmino, B. P. & Simons, A. 1996.Mackie and McCartney Practical Medical Microbiology.14th ed. The Churchill Livingstone, Inc. USA.

22- MaccFadin, J. K. 2000. Biochemical test for identification of medical bacteria. 3rd ed . Lippincott Williams and Winkins . AwolterKlumer Company . Philadelphia Baltimor .New York.

23- Clinical and Laboratory Standards Institute. (2012). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically;*

operation wounds in General Al-Hussein hospital of Karbala city. *Sci. J. Karb.,* 7(3): 35-41.

36- Alwan, M.J., Lafta, I.J. &Hamzah, A.M. 2011. Bacterial isolation from burn wound infections and studying their antimicrobial susceptibility. *Kuf. J. Vet. Med. Sci.,*2(1): 121-131.

37- Sanyal, S.C., Mokaddas, E.M., Gang, R.X. & Bang, R.L. 1998. Microbiology of septicemia in burn patients. *Ann. Burns fire disasters,* 11: 19-22.

38- Komolafe , O.O., James, J., Kalongolera, L. &Makoka, M. 2003. Bacteriology of Burns at the Queen Elizabeth Central Hospital, Blantyre, Malawi, *Burns,* 29: 235-238.

39- Wildemauee, C., Godard, C., Vershragen, G., Claeys, G., Duyck, C. &Beenhouwer, H. 2004. Ten years phage typing of Belgian clinical methicillin-resistant *S. aureus* isolates. *J. Hosp. Infect.*, 56: 16-18.

40- Nasser S., Mabrouk A. & Maher, A. 2003. Colonization of burn wounds in Ain Shams University Burn Unit. *Burns,* 29: 229–33.

41- Keen, E.F., Robinson, B.J., Hospenthal, D.R., Aldous, W.K., Wolf S.A., Chung K.K. & Murray C.K., 2009. Incidence and bacteriology of burn infections at a military burn center, *Burns,* 36(4): 461-468.

42- Bagge, N., Schuster, M., Hentzer, M., Ciofu, O., Givskov, M., Greenberg, E.P. &Høiby, N. 2004. *Pseudomonas aeruginosa* biofilms exposed to imipenem exhibitchanges in global gene expression and beta-lactamaseand alginate production. *Antimicrob. Agents Chemother.,* 48(4): 1175-1187.

43- Linares, J.F., Gustafsson, I., Baquero, F. & Martinez, J.L. 2006. Antibiotics as intermicrobialsignaling agents instead of weapons. *Proc. Natl. Acad. Sci. USA*, 103(51): 19484-19489.

44- Frank, K.L., Reichert, E.J., Piper, K.E. & Patel, R. 2007. In vitro effects of antimicrobial agents on planktonic and biofilm forms of *Staphylococcus lugdunensis* clinical isolates. *Antimicrob. Agents Chemother.*, 51(3): 888-895.

45- Nucleo, E., Steffanoni, L., Fugazza, G., Migliavacca, R., Giacobone, E., Navarra, A., Pagani, L. &Landini, P. 2009.

Approved Standard—Ninth Edition. CLSI document M07-A9. Wayne, PA: CLSI.

24- Clinical and Laboratory Standards Institute. (2013). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement.* CLSI document M100-S23. Wayne, PA: CLSI.

25- Tetz, G.V., Artemenko, N.K., &Tetz, V.V. 2009. Effect of DNase and Antibiotics on Biofilm Characteristics. *Antimicrob. Agents Chemother*, 53(3): 1204–1209.

26- Christensen, G.D., Simpson, W.A., Younger, J.J., Baddour, L.M., Barrett, F.F., Melton, D.M. &Beachey, E.H. 1985. Adherence of coagulase negative staphylococci to plastic culture plate a quantitative model for the adherence of staphylococci to medical divices. *J. Clin. Microbiol.,* 22: 996-1006.

27- O'Toole G. A. 2011. Microtiter dish biofilm formation assay. *J. Vis. Exp.*, 30(47): 2437-2438. doi: 10.3791/2437.

28- Merritt J.H., Kadouri D.E. & O'Toole G.A. 2011. Growing and Analyzing Static Biofilms. *Current Protocols in Cell Biology* . John Wiley & Sons, Inc. pp. 1B.1.1-1B.1.18.

29- Phelan, M.C. 2006. Techniques for mammalian cell tissue culture. *Curr. Protoc. Mol. Biol* 74:A.3F.1-A.3F.18.

30- Chanel, S.R.S.(2002). A handbook of biostatistics .S. Chand and company. India.

31- Al-Azzawi, S.A.N& Al-Dulaimi A.A.F. 2007. A study of some virulence factors in bacteria contaminated Burns. *J. Al Fath.*, 29: 357-368.

32- Al-saad, N.f. AL-Saeed, M.S. &Bnyan, I.A. 2012. Biofilm Formation by Bacterial Isolates from Burn Infected Patients. *Med. J. Baby.,* 9(3): 517-525

33- Al-kaabi, S.A. 2013. Bacterial Isolates and Their Antibiograms of Burn Wound Infections in Burns Specialist Hospital in Baghdad. *J. Bagh. Sci.,* 10(2): 331-340.

34- Mohammed, S.W. 2007. Isolation and Identification of Aerobic Pathogenic Bacteria from Burn Wound Infections. *J. Al-Nah. Univ.*, 10(2): 94-97.

35- Al-Asadi, K.A.H. 2009. A Study of the prevalence of bacteria in *Burns,* and

infection: Variations in virulence factors and virulence genes. *J. Hosp. Infect.*, 43:211-8.

52- El-Feky, M.A, El-Rehewy, M.S., Hassan, M.A, AboLella, H.A, Abd El-Baky, R.M. & Gad, G.F. 2009. Effect of ciprofloxacin and N-acetyl cystein on bacterial adherence and biofilm formation on uretral stent. *Pol. J. Microbiol*., 58:261-7.

53- Abd El-Aziz, A., El-Banna, T., Abo-Kamar, A., Ghazal, A., &AboZahra R. 2010. *In vitro* and *in vivo* Activity of some Antibiotics against Staphylococcal Biofilm and Planktonic Cells Isolated from Diabetic Foot Infections. *J. Am. Sci.,* 6(12): 760-770.

54- Qu, Y. 2010. Biofilm Formation and Antimicrobial Resistance of Coagulase-Negative Staphylococci Isolated From Neonatal Intensive Care Units. Thesis Ph.D. RMIT University.

55- Jefferson, K.K., Goldmann, D.A., & Pier, G.B. 2005. Use of confocal microscopy to analyze the rate of vancomycin penetration through *Staphylococcus aureus* biofilms. *Antimicrob. Agents Chemother.,* 49(6): 2467–2473.

56- Brown, M.R., Allison, D.G., & Gilbert, P. 1988. Resistance of bacterial biofilms to antibiotics: a growth-rate related effect?. *J. Antimicrob. Chemother.,* 22(6): 777–780.

57- Driffield, K., Miller, K., Bostock, J.M., O'neill, A.J., & Chopra, I. 2008. Increased mutability of *Pseudomonas aeruginosa* in biofilms. *J. Antimicrob. Chemother.,* 61(5): 1053–1056.

58- Davis, S.C., Ricotti, C., Cazzaniga, A., Welsh, E., Eaglstein, W.H., & Mertz, P.M. 2008. Microscopic and physiologic evidence for biofilm-associated wound colonization in vivo. *Wound. Rep. Reg.,* 16: 23-29.

Growth in glucose- based medium and exposure to subinhibitory concentrations of imipenem induce biofilm formation in a multidrugresistant clinical isolate of *Acinetobacterbaumannii*. *BMC Microbiol.*, 9(270): 1-14.

46- Taraszkiewicz, A., Fila, G., Grinholc, M., &Nakonieczna J. 2013. Innovative Strategies to Overcome Biofilm Resistance. *BioMed Res. Int.,* 2013:1-13, Article ID 150653. doi:10.1155/2013/150653.

47- Ciofu, O., L.F. Mandsberg, H.W. &Hoiby, N. 2012. Phenotypes selected during chronic lung infection in cystic fibrosis patients: implications for the treatment of *Pseudomonas aeruginosa* biofilm infections. *FEMS Immunol. & Med. Microbiol.,* 65(2): 215–225.

48- Rachid, S., Ohlsen, K., Witte, W., Hacker, J. &Ziebuhr, W. 2000. Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcusepidermidis*. *Antimicrob. Agents Chemother.*, 44(12): 3357-63.

49- Kuehl, R., Al-Bataineh, S., Gordon, O., Luginbuehl, R., Otto, M., Textor, M. &Landmann, R. 2009. Furanone at subinhibitory concentrations enhances staphylococcal biofilm formation by luxS repression. *Antimicrob. Agents Chemother.*, 53(10): 4159-4166.

50- Di Bonaventura, G., Spedicato, I., D'Antonio, D., Robuffo, I., & R. Piccolomini. 2004. Biofilm formation by *Stenotrophomonasmaltophilia*: modulation by quinolones, trimethoprim-sulfamethoxazole, and ceftazidime. *Antimicrob. Agents Chemother*, 48:151–160.

51- Rumbaugh, K.P., Griswold, J.A. &Hamood, A.N. 1999. *Pseudomonas aeruginosa* strains obtained from patients with tracheal, urinary tract and wound

فعاليات المضادات ضد الفلم الحيوي المتكون بواسطة بكتريا معزولة من مرضى الحروق في محافظة السماوة

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الخلاصة

تضمنت الدراسة الحالية 145 عينة جمعت من 100 مرضى, الذين يعانون من اخماج جروح الحروق, دخلو وحدة الحروق لمستشفى الحسين التعليمي في مدينة السماوة بأعمار مختلفة ولكلا الجنسين. أمتدت فترة الجمع من شهر كانون الاول 2012 الى شهر أيار 2013. من بين 145 عينة, وجدت حوالي 315 عزلة في 137(94.5%) مسحة حروق وفقط 8مسحات (5.5%) كانت سالبة للنمو البكتيري. اظهرت النتائج سيادة بكتريا *Staphylococcusaureus* (42.3%) تتبعها بكتريا *Staphylococcusepidermidis* (28.5%), *Pseudomonasaeruginosa* (22.6%), *Acinetobacterlwoffii* (20.4%), *Pseudomonas luteola* (16.79%), *Acinetobacterbaumannii* (14.6%), *Raoultella*terrigena (11.6%), *Klebsiella pneumonia* (10.2%), *Aeromonashydrophila* (9.5%), *Proteusmirabilis* (8.75%), *Escherichia coli* (8.75%), *Providenciarettgeri* (8%), *Bacillusspp.* (8%), *Corynebacterium spp.* (7.3%) و بكتريا *Proteusvulgaris* (6.67%) على التوالي. في هذه الدراسة, انجزت الحساسية المايكروبيةللعزلات البكتيرية ضد 19 مضاد حيوي, معظم العزلات البكتيرية كانت متعددت المقاومة لهذه المضادات و انواع بكتيرية اخرى كانت حساسة لمعظم المضادات الحيوية في هذه الدراسة. في البكتريا متعددة المقاومة للمضادات, وجد بأن مضاد Imipenem كان الدواء الاكثر فعالية ضد بكتريا *Staphylococcus epidermidis*, *Pseudomonas luteola*, *Raoultellaterrig ena*, *Aeromonashydrophila* و بكتريا *Proteus mirabilis*, بينما كان Meropenem المضاد الاكثر فعالية ضد كلا من *Acinetobacterlwoffii* و*Acinetobacterbaumannii* , كان المضادPiperacillin/Tazobactam الاكثر فعاليةلبكتريا *Pseudomonasaeruginosa* و مضاد Linezolid كان المضاد الاكثر فعالية ضد بكتريا *Staphylococcusaureus*, بينما مضاد Ciprofloxacin كان الاكثر فعالية ضد *Klebsiella pneumonia*. اظهرت النتائج بأن قابلية عزلات جرح الحرق لأنتاج الفلم الحيوي كانت عالية مع وجود فروقات معنوية بين الانواع البكتيرية و بين فترات الوقت المختلفة ($P<0.05$). كذلك, اظهرت النتائج بأن نصف التركيز المثبط الادنى (0.5 MIC) للمضادات الاكثر فعالية لا تمتلك تأثير معنوي على الخلايا الحية داخل حشوة الفلم الحيوي أو على الكتلة الكلية للفلم الحيوي لأغلب البكتريا المفحوصة, لكن هنالك اختلافات معنوية لبعض المضادات على كتلة الفلم الحيوي.

الكلمات المفتاحية

السطوح الحيوية البكتيرية، الحروق.