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Sayran Hamad Haji
College of Pharmacy

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Abstract

Pseudomonas aeruginosa has become a major cause of life-threatening nosocomial infections that can rapidly develop resistance to multiple antimicrobial agents, mostly due to its ability to produce extended-spectrum β-lactamase (ESBL),AmpC and metallo-β-lactamase(MBL).Biofilm formation is another important factor contributes to the chronicity of infections as they reduce susceptibility to antimicrobial agents. This study was aimed to find prevalence of ESBL and AmpC β-lactamases production and biofilm formation in burn isolates of P. aeruginosa. Further we find out the potential to form biofilm in relation to antibiotic resistance and β-lactamase productions.A total of 90 isolates of P. aeruginosa were isolated from burn samples .We applied Vitek-2 automated system at Rozhawa hospital as a panel of antimicrobial agents. Microtiter plate assay was chosen to detect the biofilm formation. Combined disk diffusion method and AmpC disc test were followed for phenotypic detection of ESBL and AmpC production, respectively. Of the 90 P. aeruginosa burn isolates, 31.1% of isolates formed biofilms ,the majority of which (71.4%) were moderate biofilms. ESBL production 58 (64.4%) was found to be the predominant resistance mechanism followed by AmpC β-lactamase production 46(51.1%). Production of biofilm were higher in the ESBL,AmpC and ESBL+AmpC β-lactamases producers (34.4%,32.6%and37.9%) respectively,compared with the ESBL,AmpC and ESBL+AmpC β-lactamases non-producers (25%,31.8%and 27.8%) respectively.A significant association was found between the degree of biofilm formation and betalactamase production in P. aeruginosa.($P<0.05$) The biofilm producing P.aeruginosa were significantly ($P<0.01$) more resistant compared to biofilm non producers. Biofilm production was strongly correlated to antibiotic resistance .

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Prevalence of ESBL and AmpC β-Lactamase Production and Biofilm Formation in Burn Isolates of *Pseudomonas aeruginosa* From Rozhawa Hospital in Erbil City

Sayran Hamad Haji

Hawler Medical University/College of Pharmacy

ABSTRACT

Pseudomonas aeruginosa has become a major cause of life-threatening nosocomial infections that can rapidly develop resistance to multiple antimicrobial agents, mostly due to its ability to produce extended-spectrum β-lactamase (ESBL),AmpC and metallo-β-lactamase(MBL).Biofilm formation is another important factor contributes to the chronicity of infections as they reduce susceptibility to antimicrobial agents. This study was aimed to find prevalence of ESBL and AmpC β-lactamases production and biofilm formation in burn isolates of P. aeruginosa. Further we find out the potential to form biofilm in relation to antibiotic resistance and β-lactamase productions.A total of 90 isolates of P. aeruginosa were isolated from burn samples .We applied Vitek-2 automated system at Rozhawa hospital as a panel of antimicrobial agents. Microtiter plate assay was chosen to detect the biofilm formation. Combined disk diffusion method and AmpC disc test were followed for phenotypic detection of ESBL and AmpC production, respectively. Of the 90 P. aeruginosa burn isolates, 31.1% of isolates formed biofilms ,the majority of which (71.4%) were moderate biofilms. ESBL production 58 (64.4%) was found to be the predominant resistance mechanism followed by AmpC β-lactamase production 46(51.1%). Production of biofilm were higher in the ESBL,AmpC and ESBL+AmpC β-lactamases producers (34.4%,32.6%and37.9%) respectively,compared with the ESBL,AmpC and ESBL+AmpC β-lactamases non-producers (25%,31.8%and 27.8%) respectively.A significant association was found between the degree of biofilm formation and betalactamase production in P. aeruginosa.(P<0.05) The biofilm producing P.aeruginosa were significantly (P<0.01) more resistant compared to biofilm non producers. Biofilm production was strongly correlated to antibiotic resistance .

Keywords: ***Pseudomonas aeruginosa, burn, ESBL,Ampc, biofilm,antibiotic resistance***

1.INTRODUCTION

Pseudomonas aeruginosa(P. aeruginosa) is an increasingly predominant Gram-negative opportunistic pathogen in nosocomial infections that can rapidly develop resistance to different

types of broad-spectrum antibiotics and its potential to form biofilms(Vahdani *etal*.,2012).*P. aeruginosa* is frequently causing various acute and chronic opportunistic infections such as bacteremia in hospitalized patients(Heydari and Eftekhar,2015).They can frequently complicated in infections of immunocompromised patients with severe underlying diseases including burn sufferers, cystic fibrosis and cancer .*P.aeruginosa*as an important life-threatening nosocomial pathogen plays a prominent role in serious infections in burn wounds, it has been implicated in high morbidity and mortality in burn patients(Vahdani *etal*.,2012;Neopane *etal*.,2017).Despite of the treatment for burns has been developed, infectionremains the main causes of mortality and represents a major threat to critically ill patients especially burn patients(Dou *etal*.,2017).About 45% of mortality in burn patients is due to infections(Kaur and Wankhede,2013).Infections caused by *P. aeruginosa*are difficult to treat as the majority of isolates exhibit high level of innate resistance to many antibiotics and disinfectants including anti-Pseudomonal antimicrobial classes (Tavajjohi and Moniri,2011).Further, acquired drug resistance mostly in hospital area among *P. aeruginosa* strains is reported (Neopane *etal*.,2017)with the ability to propagate on medical devices including colonization of ventilators, sinks, drains, disinfectant solutions, and catheters on which these organisms grow as a biofilm, subsequently it can be transmitted rapidly among burn patients in hospital settings(Shaikh *etal*.,2015).In *P. aeruginosa*, mechanisms of resistance to various antimicrobials may be due to outer membrane impermeability, target site modification,porinmutations,biofilmformation,and multidrug efflux pumps(Lathamani and Kotigadde,2016).In addition to low susceptibility of *P. aeruginosa* to antimicrobial agent emergence of new resistance mechanisms have been reported, among these, the important roles of various β-lactamases such as ESBL,AmpC,Klebsiella Pneumoniae Carbapenemase (KPC) and MBLs (Heydari and Eftekhar,2015; Akhter,2015).Genes for all these enzymes are often encoded by mobile genes carried on plasmids, facilitating rapid transmition among microorganisms (Sreeshma *etal*.,2013).ESBLs are responsible to hydrolyzing a broad range of β-lactams which include penicillins, cephalosporins and aztreonam (except for cephamycins or carbapenems)(Rafiee *etal*.,2014).AmpC β-lactamases are clinically significant because they confer resistance to a wide variety of beta-lactam antibiotics like penicillins, first, third generation cephalosporins (e.g.,ceftriaxone, cefotaxime, and ceftazidime), cephamycins (e.g., cefoxitin and cefotetan)and frequently aztreonam.In contrast to ESBLs they can be degrade cephamycins and are not affected by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam but they are inhibited by cloxacillin and phenylboronic acid (Moxon and Paulus,2016).In *P. aeruginosa*, resistance to the extended-spectrum cephalosporins such as ceftazidime mostly results from over expression of the naturally occurring AmpC β-lactamases (Rafiee *etal*.,2014).Coexistence of multiple β-lactamases in clinical isolates of *P. aeruginosa* is common, causing resistance to almost all β-lactam antibiotics and consequently decreased therapeutic options(Norouzi *etal*.,2010; Lathamani and Kotigadde,2016).Resistance to carbapenems is of great concern as these are considered to be antibiotics of last resort to combat infections by MDR bacteria, particularly in intensive care units and burn wards(Długaszewska *etal*.,2016).More studies found that biofilm formation can enhance antibiotic resistant in *P. aeruginosa*(Neopane *etal*.,2017).Biofilm formation is one of a common strategy for bacterial survival in hard environmental conditions.Biofilms have a significant impact in causing fatal infections, and are estimated to be contributed with 65% of nosocomial infections(El-Khashaab

etal.,2016).Bacterial biofilms is a major cause of recurrent or chronic infections and may impair cutaneous wound healing and reduce topical antibacterial efficiency in treating infected skin wounds(Fazeli *etal*.,2012; El-Khashaab *etal*.,2016).Biofilm production is well established for a number of *P.aeruginosa* infections on different host tissues as well as different medical devices(Norouzi *etal*.,2010;Kaur and Wankhede,2013).Biofilm is a complex communities of microorganisms embedded in a self-produced extracellular polymeric substance(EPS) consisting of polysaccharide, protein and DNA(Kaur and Wankhede,2013).Which make them to adherent to inert or living surfaces in order to resisting phagocytosis and other activity of defense mechanism of the host.Bacterial biofilms cause chronic infections because they show increased tolerance to antibiotic therapy and the action of disinfectants(Manchanda and Singh,2003).Resistance of biofilm against component of host immune system as well as their capacity to acquire resistance to different antibiotics appears to be risk factor for persistent infections and makes infections due to *P. aeruginosa* difficult to eradicate (Kumar *etal*.,2012).The present study was undertaken to find prevalence of ESBL and AmpC β-lactamases production in burn isolates of *P. aeruginosa* and to know number of isolates forming biofilm. Further we have evaluated the correlation between biofilm formation and β-lactamase production among the isolates along with we studied the associtation of biofilm producer with antibiotic resistant pattern of the isolates.

2.MATERIALS AND MWTHODS

2.1. Specimen collection

The present study was conducted at Roshawa Hospital in Erbil City,Iraqi Kurdistan Region. From June to October 2017, a total of 90 consecutive non-repeat clinical isolates of *P. aeruginosa* were obtained from hospitalized burned patients .The isolates were identified by conventional biochemical methods and Vitek 2 system at Rozhawa hospital. All isolates were studied for biofilm formation and its association to antimicrobial resistance pattern.These isolates were further tested for ESBL and AmpC production. ATCC *P. aeruginosa* 27853 strain was used as quality control reference strain for all experiments .This project was approved at the first site by the Scientific and Research Ethics Committee at College of Pharmacy/ Hawler Medical University.

2.2. Bacterial identification

Vitek-2 automated system (bioMérieux,USA)was used for diagnosis to the species level. The isolated bacteria were stored in trypticas soy broth (TSB) with 40% glycerol at -70°C until used (AL-Marjani and Khadam,2016).

2.3. Antimicrobial susceptibility

A panel of antimicrobial agents (ampicillin, cefepim, cefotaxime, cefoxitine, ceftazidime, cefuroxime, gentamycin, imipenem,meropenem, norfloxacin, ciprofloxacin, tobramycin, and colistin) were determined by Vitek-2 automated system (Khosravi and Mihani,2008).

2.4.Phenotypic detection of ESBL production

ESBL activity among the isolates was screened out by using the combined disk diffusion method recognized by Clinical and Laboratory Standards Institute (CLSI)(CLSI,2011). A 0.5 McFarland

of test culture was swabbed on Mueller Hinton Agar plates. The disks of cefotaxime (30µg), cefotaxime/clavulanic acid (20/10µg), ceftazidime (30µg) and ceftazidime/clavulanic acid (20/10µg) were placed at a distance of 20 mm (centre to centre) on the Muller Hinton agar plates containing the inoculum. The plates were incubated for 24 hrs at 37^0C, ESBL production was inferred if the inhibition zone increased by 5 mm towards the cefotaxime plus clavulanic acid disc or ceftazidime plus clavulanic acid disc when compared to the ceftazidime and cefotaxime disc alone (Norouzi *etal*.,2010).

2.5.Phenotypic detection of AmpC beta lactamases production

Cefoxitin disks (30 µg) were used for detection of AmpC producing isolates, as described by Parveen*et al*.(2010) and Madhumati *et al*.(2015). Isolates exhibiting zone diameters less than 18 mm were considered positive for AmpC screening and were subjected to phenotypic AmpC confirmatory tests using AmpC disc test (Patwary *etal*.,2010).The test is based on use of tris-EDTA to permeabilize a bacterial cell and release of β-lactamases in to the external environment.The AmpC discs were prepared in-house by applying 20 µl of a 1:1 mixture of normal saline and 100xTris-EDTA to each sterile filter paper disks, which were allowed to dry, and storing them at 2 to 8°C. The surface of a Mueller-Hinton agar plate (LAB M Limited, UK) was inoculated with a lawn of 0.5 McFarland suspension of cefoxitin susceptible *E.coli* ATCC 25922. A 30µg cefoxitin disc was placed on the inoculated MHA plate.An AmpC disc was inoculated with several colonies of the test organisms was placed besides the cefoxitin disc almost touching it, with the inoculated disk face in contact with the agar surface.After overnight incubation at 37ºC, the plates were examined for either an indention or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin.

2.6.Biofilm formation Assay

microtiter plate assay was chosen to detect thebiofilm formation, aspreviously reported (Heydari and Eftekhar,2015;AL-Marjani and Khadam,2016).All isolates were grown overnight in tubes containing 5 mL trypticase soy broth (TSB)(Merck, Germany) at 37°C. The growth were then suspended in to fresh medium in 1:100 dilution. 200 µL of diluted cultures were inoculated into 96 Well polystyrene microtitre plates (Costar/USA) and incubated at 37°C for 24 hours without shaking.The edge of the plate was covered with parafilm to avoid evaporationduring incubation.The broth was then removed by washing the wells three times with 200 µL of Phosphate Buffer Saline pH 7.2 then were exposed to air-dry.The wells were then stained with 200 µL of 0.1% crystal violet for 30 minutes in room temperature.The plates were washed with distilled water to remove the unbounded dye, allowed to dry .The adhered stain was solubilized by addition 200µl of 95% ethanol.The optical density (OD) of the dissolved crystal violet was measured at 630nm using ELISA reader.Biofilm formation was considered negative at ODs below 0.12, weakly positive at ODs 0.12-0.24 and strong positive at ODs > 0.24. Each test was repeated on three different days and the results were reported as the mean of the obtained values.

Statistical Analysis

GraphPad Prism(version5;GraphPadSoftware,SanDiego,CA).Chi-square test was used for analysis of categorical data. A P-value of < 0.05 was considered statistically significant.

3. RESULTS

Of the 90 isolates of *P. aeruginosa* isolated from burn samples, biofilm formation was detected by microtitre plate method in 28 isolates (31.1%) and 62(68.8%) were negative for biofilm formation. ESBL production was observed in 58 isolates (64.4%) and AmpC β-lactamase production was observed in 46 isolates (51.1%). 29 isolates (32.2%) co-produced ESBL and AmpC β-lactamases.Table1

Table1.Prevalence of biofilm formation and β-Lactamase production in burn isolates of Pseudomonas aeruginosa (n=90)

β-Lactamase production	POSITIVE	NEGATIVE
ESBL	58 (64.4%)	32 (35.5%)
AmpC	46 (51.1%)	44 (48.8%)
Both ESBL & AmpC	29(32.2%)	61 (67.7%)
Biofilms	28 (31.1%)	62 (68.9%)

Production of ESBL and AmpC β-lactamases by biofilm and non-biofilm producers *P. aeruginosa* isolates is presented in Table 2. Among 28(31.1%) biofilm producing isolates, 20(34.4 %) were ESBL producers,15(32.6%) produced AmpC, and 11(37.9%) were positive for ESBL+ AmpC β-lactamases production. Whereas production of biofilm in the ESBL, AmpC and ESBL+AmpC β-lactamases non-producers occurred in 25%,31.8% and 27.8% isolates, respectively.As observed, the ability of biofilm formation was found to be high in β-lactamase producing isolates than that of β-lactamase non-producing isolates.Association was found to exist between biofilm formation and β-lactamase productions in *P. aeruginosa* burn isolates.

Table 2. Correlation between ESBL and AmpC production and biofilm formation among burn isolates of Pseudomonas aeruginosa (n=90)

	ESBL		AmpC*β*-lactamase		ESBL + AmpC*β*-lactamase	
	producers (no. and %)	non-producers (no. and %)	producers (no. and %)	non-producers (no.and%)	producers (no. and %)	non-producers (no. and %)
Biofilm producers N=28	20(34.4%)	8(25%)	15(32.6%)	14(31.8%)	11(37.9)	17(27.8%)
Biofilm non-producers N=62	38(65.5%)	24(75%)	31(67.3%)	30(68.1%)	18(62 %)	44(72.1%)
Total isolates	58	32	46	44	29	61

Our study displayed that, out of a total of 90 *P. aeruginosa* isolates, 28(31.1%) formed biofilms.8(28.5%) of the isolates were strongly adherent, 20(71.4%) moderately adherent and 62(68.8%) weakly adherent.The results show significantly (P<0.05) higher percentages of β-lactamase (ESBL and AmpC) productions in strong biofilm producing isolates compred to β-lactamase (ESBL and AmpC) productions in moderate and weak or non biofilm producing isolates.Of the 58 ESBL producers and 46 AmpC β-lactamase producers, 75% and 62.5% were strong biofilm producers, respectively, whereas 66% and 50% were weak or non biofilm producers. Among 29 ESBL & AmpC β-lactamase producers, the majority of isolates were formed moderate biofilms, 45%.The degree of biofilm formation by *P. aeruginosa* in relation to β-lactamase(s) production has been shown in Figure 1.

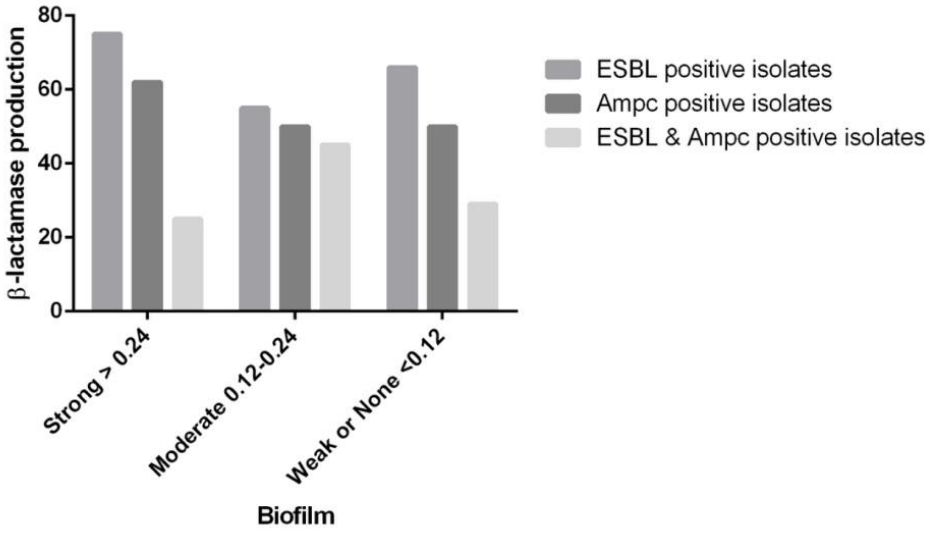

Figure 1.The association between the degree of biofilm formation and β-lactamase production among burn isolates of Pseudomonas aeruginosa

Out of 13 antibiotics tested, all ninety burn isolates were found to be 100% sensitive to colistin. They revealed a high resistance rate for ampicillin, cefotaxime and cefuroxime (100%). Biofilm producer among *P. aeruginosa* displayed the highest resistance to cefoxitin 27(96%) followed by cefepim and norfloxacin 26(93%), meropenem 22(79%), imipenem and ceftazidime 21(75%), gentamicin 20(71%),tobramycin18(64%),ciprofloxacin17(61%).The non biofilm producers after colistin showed lowest resistance to meropenem 25(40%) followed by imipenem33(53%), ciprofloxacin 35(56%),gentamicin 36(58%),ceftazidime 39(63%)and tobramycin40(65%). The antibiotic resistance pattern of *P. aeruginosa* was found significantly (Pvalue < 0.001) higher in biofilm producers than in biofilm non-producers(Figure 2).

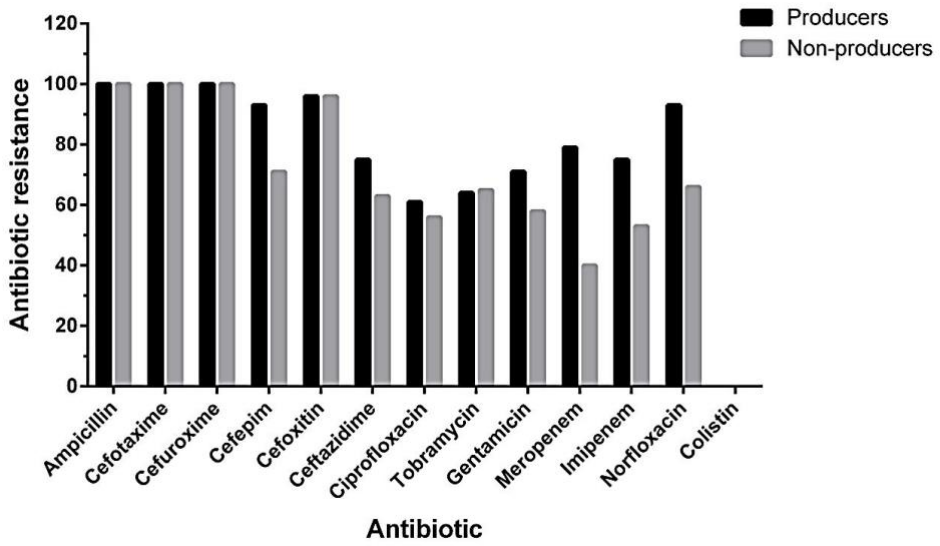

Figure 2. Antibiotic resistance pattern of P. aeruginosa among biofilm producers and non-producers

4. DISCUSSION

P. aeruginosa is a well-known colonizer of burn wound (de Almeida Silva *etal.*,2017). Burn patients may have a skin deficiency, moist and warm wound environments, and are therefore more prone to infection (Dou *etal.*,2017).Biofilm formations by *P. aeruginosa* are difficult to treat with antimicrobial agents as they reduce susceptibility to antimicrobial agents, and consequently decreased therapeutic options(de Almeida Silva *etal.*,2017).Among more than 800 different known β-lactamases in Gram-negative bacteria, at least 120 were detected in *P. aeruginosa*, among which, ESBLs ,AmpC and MBLs are clinically significant. Detection of ESBLs and AmpC β-lactamases are of particular concern (Rafiee *etal.*,2014).This study revealed, out of 90 *P. aeruginosa* burn isolates, only 31.1% of isolates formed biofilms Table1, the majority of which (71.4%) were moderate biofilms. In a recent study, 76.4% of *P. aeruginosa* burn isolates were shown to form moderate to strong biofilms(Neopane *etal.*,2017).In a study, 43.5% of *P. aeruginosa* burn isolates were shown to form biofilms(Heydari and Eftekhar,2015).Whereas another study conducted by El-Khashaab *et al.*(2016) showed 25.7% *P. aeruginosa* were biofilm producer.Among various mechanisms of resistance, ESBL enzyme was found to be more effective.In our study, ESBL production was seen in 64.4 % (58/90) of *P. aeruginosa* isolates. Incidence of ESBL in different studies were 39.2% (Rafiee *etal.*,2014), 40% (Neopane *etal.*,2017) and 45.83% (Qureshi and Bhatnagar,2016). However, a study by Chika *etal.*(2017) phenotypically revealed that (86.4%) of *P. aeruginosa* were ESBL positive. In our study, we have observed a little high prevalence of AmpC β-lactamase production occurred in (51.1%) (46/90) of the isolates. This finding corresponds to the findings of Hentzer *etal.*(2001) who observed 50% *P.aeruginosa* to be AmpC producers in their study. Another study conducted at Iran reported higher results(68.6%)(Rafiee *etal.*,2014).Sometimes ESBL is co-produced with AmpC and MBL β-lactamase in bacterial pathogens may cause a major therapeutic failure if remain undetected (Neopane *etal.*,2017).We have also observed the coproduction of ESBL and AmpC β-lactamases which was detected in 29(32.2%) of the isolates.This is in agreement with the results of Akhter(2015) who found

(30.4%) isolates were positive for both ESBL and AmpC in Gram negative bacilli including *P. aeruginosa*. But our result was higher compared to the other studies worldwide (25.5-26%) (Kumar,2012; Sreeshma *etal*.,2013;Neopane *etal*.,2017).Presence of two different kinds of β-lactamase producing *P. aeruginosa* including ESBL and AmpC β-lactamases and the correlation of these enzymes with biofilm formation has been shown in a number of studies (Rafiee *etal*.,2014;Heydari and Eftekhar,2015; Neopane *etal*.,2017). In current study, Biofilm formations were higher in the ESBL,AmpC and ESBL+AmpC β-lactamases producers(34.4%,32.6%and37.9%,respectively) compared to the ESBL,AmpC and ESBL+AmpC β-lactamases non-producer ones (25%,31.8% and 27.8%, respectively). In Table 2 we detected biofilm formation correlated with β-lactamase productions. These results were comparable with what have been found in published literature (Kalaivani *etal*.,2013;Qureshi and Bhatnagar,2016;Neopane *etal*.,2017).*P. aeruginosa* display an elevated level of antibiotic resistance mechanisms among them production of different types of β-lactamases primarily ESBL, AmpC and MBLs also contribute to the survival of bacteria in biofilms(Emami and Eftekhar,2015;Neopane *etal*.,2017).Figure 1 represents the degree of biofilm formation in relation with β-lactamase production among our isolates. A significant association was found between the degree of biofilm formation and β-lactamase production. Similar results have been shown for *P. aeruginosa* burn isolates, where the presence of multiple-β-lactamase was associated with formation of strong biofilms(Heydari and Eftekhar,2015). In another study, a highly significant correlation was found between the degree of biofilm formation and ESBL production in *P. aeruginosa* (Neopane *etal*.,2017). Although another study exhibited that ESBL (but not MBL or AmpC) inhibited biofilm production by impairing the twitching motility which plays an important role in manifesting as perturbation of structures involved in bacterial adhesion that are required to induce biofilm formation(Gallant *etal*.,2005).It is evident from Figure 2; there was a high frequency (>70%) of resistance against all the frequently used antimicrobial agents,except for colistin.This is presumably due to the fact that clinicians and other health care providers may be unaware ofthe problem of ESBL production by Gram negative bacilli resulting in inappropriate medication. According to the current results among carbapenems, imipenem and meropenem resistance was observed to be 54(60%) and 47(52%) respectively, which is an "alarming sign", since carbepenems were the antibiotics of choice for treating *P. aeruginosa* infections. In recent years, extensive use of carbapenems to treat AmpC and ESBL producing *P. aeruginosa*, has raised the incidence of bacterial resistance markedly due to production of MBLs (Rafiee *etal*.,2014). A study by Qurish and Bhatnagar(2016) also showed 45.83% of *P. aeruginosa*, were resistant to imipenem and 54.16% to meropenem. Furthermore, another reports from Iraq by AL-Marjani and Khadam(2016) confirmed that all their isolates were sensitive to colistin. This study also highlights the existence of association between antibiotic resistance and biofilm formation, exposed that the biofilm producing *P.aeruginosa* were significantly more resistant compared to biofilm non producers ($p<0.001$) (Figure 2). This incidence is possibly attributed to gene transfer mechanisms within the biofilm environments, which is often acquired by transfer of genetic information from one organism to another as well as delayed diffusion of antibiotics inside the bacterial cell (Sahal and Bilkay,2015).

5.CONCLOSION

ESBL is shown to be predominant mechanism for development of resistance in the present study.

Alarmingly, β-lactamase and biofilm production in our isolates confer a high level of resistance to almost all the commercially used antibiotics including carbapenems.This indicates that a significant correlation exists between biofilm formation and antibiotic resistance as well as association was found to exist between biofilm formation and β-lactamase productions. More importantly, β-lactamase (ESBL and AmpC) phenotype was associated with strong biofilm formations in *P. aeruginosa* burn isolates . Early detection of biofilm is essential.

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بوختة

بلاوبوونةوةى دروستكردنى ESBL ,AmpC β-lactamse لةطةل بةرهةمهيَنانى ثةردةى زيندةطى لةلايةن *Pseudomonas aeruginosa* جياكراوة لة برينى سوتاو دا لةنةخؤشخانةى رؤذئاوا لة هةوليَر

pseudomonas aeruginosa بؤتةهؤى هؤكاريَكى ترسناك و سةرةكى لة تووشبون بةو نةخؤشيانةى كة هةرةشةن لة سةر ذياني مرؤظايةتى ضونكة لةتواناى د ا هةية بةخيرَايي بةرطرى دروست بكات بةرامبةر دذة زيندةطى بةكتريا.ئةمةش بةشيَوةيةكى دةطةريتةوة بؤ تواناى بةكترياكة لة بةرهةمهيَنانى Metallo-β-lactamse(MBL) (ESBL),AmpC.سةرةكى شةبةنطيَك لة ثيَكهيَنانى ثةردةى زيندةطى هؤكاريَكى طرنطى ترة كة بةشدار دةبيَت لة طةشةسةندنى نةخؤشى ضونكة دةبيَتةهؤى كةمكردنةوةى ضالاكيةكانى دذ ة زيندةطى بةكتريا.ئامانج لةم ليَكؤلينةوة يةبريتى يةلة زانينى مةود اى بلاَو بونةوةى دروستكرد نى *P.aeruginosa* لة برينى سوتاندا سةرباري ئةوةش تواناى دؤزينةوةى دروست بوونى ثةردةى زيندةطى و ثةيوةندى لةنمونةى

ثةردةكةلةطةل بةرطرى بؤ دذة زيندةطى وبةرهةمهيَنانى β-lactamase لة و بةكترياية د ا .بة جؤريَك كة لة 90جياكراوة لة بةكتريا كة لة برينى سوتان.سيستةمى ظايتك-2 ى ئؤتوماتيكى لةسةرجىَ بةجيَكرا بؤ دؤزينةوةى كاريطةرى دذة زيندةطى بةكتريا

لة نةخؤشخانةى فرياكةوتنى رؤذئاوا.هةروةك تاقيكردنةوةى تةختةى مايكروتايتر هةلَبذيَردرا و د ياري كرا بؤ دؤزينةوةى دروست كردنى ثةردةى زيندةطى.

هةردوو Combined disk diffusion method and AmpC disc بةكارهاتووة بؤ دؤزينةوةى روكةشى ريَطاى

ESBL,AmpCيةك لةدواى يةك .

لة 90نمونةى جياكراوةى بةكترياكة ئةوا (32.2%) ثةردةى زيندةطيان دروست كردووة كة زؤربةيان برى 71.4% بريتى بوون لة ثةردةى زيندةطى ناوةندى . ESBL (66.4%) 58 ميكانيزميكى بةرطرى باو بوو لة دواى ئةو بةرهةمهيَنانى

46 (51.1%) AmpC β-lactamase بةرهةم هاتووة .

بةجؤريَك كة بةرهةمهيَنانى ثةردةى زيندةطى بةرزترة لة بةرهةم هاتوةكانى

وAmpC ,ESBL)(34.4%,32.6%و37.9%) يةك بةدواى يةك بة بةراورد لةطةلَ بةرهةم نةهاتوةكان ESBL+AmpC)

لة ثةردةى زيندةطى (ESBL,AmpCوESBL+AmpC) (25%,31.8%,27.8%) يةك بةدواى يةك .

. ثةردةى زيندةطى بةرهةم هيَن بةرطريان بؤ دذة زيندةطي بةكتريا كان زؤر تربوو .وةئةوةى دؤزرايةوة بريتى بوو لة هةبوونى ثةيوةندى لةنيوان ثةردةى زيندةطى بةرهةم هاتوولةطةل بةرهةمهيَنى بيتالاكتةمةيز .

ثةيوةنديةكي توند وتؤل هةية لة نيوان بةرهةمهيَنانى ثةردةى زيندةطى لةطةل بةرطرى دذة بةكتريا زيندةكيةكان.

انتشار تكوين الانزيماتESBL, AmpC β-lactamase و الاغشية الحيوية في عزلات البكتريا *Pseudomonas aeruginosa* المعزولة من مرضى الحروق فى مستشفى روزئاوا فى اربيل

لقد اصبحت *Pseudomonas aeruginosa* سببا رئيسيا وخطرا للعدوى التي تهدد حياة الانسان والتي يمكن ان تطوربسرعة و مقاومة للعديد من العوامل المضادة للجراثيم,ويرجع ذلك في الغالب الى قدرتها على انتاج طيف ممتد من انزيما ت β-lactamase(ESBL),AmpC وMetallo-β-lactamse(MBL) فأن تشكيل الاغشية الحيوية هو عامل أخرومهم حيث يساهم في ازدهار العدوى لأنها تقلل من التعرض للعوامل المضادة للجراثيم.هدفت هذه الدراسة الى معرفة مدى انتشارESBL β-lactamase و AmpC ضمن عزلات (*P.aeruginosa*) المسببة للحروق علاوةعلى ذلك يمكننا اكتشاف امكانية تكوين الاغشية الحيوية فيما يتعلق بمقاومة المضادات الحيوية ومنتجات (β-lactamase). بحيث تم عزل 90 عزلة من*(P.aeruginosa)*من عينات الحروق.وطبقنا نظام فيتك-2 الالي لكشف عن العوامل المضادة للجراثيم في مستشفى الغرب في مدينة اربيل.تم أختيار أختبار لوحة مايكروتايتر للكشف عن تشكل بايوفلم.اتبعت طريقة نشر القرص المشترك واختبار القرص للكشف عن المظاهر (ESBLوAmpC) على التوالي.

في90 عزلة *P.aeruginosa* شكلت 32.2% من العزلات الاغشية الحيويةوكانت نسبة الاغلبية71.4% عبارة عن الاغشية الحيوية المعتدلة .انتاج ESBL (66.4%) 58 هو الية المقاومة السائدة يليه انتاج (AmpC) 46 (51.1%) .كان انتاج الاغشية الحيوية أعلى في المنتجين ل AmpC, ESBL و (ESBL+AmpC β-lactamases) (34.4%,32.6%و37.9%) على التوالي. مقارنة بغيرالمنتجين ل(ESBL+AmpC β-lactamasesوAmpC, ESBL) (25%,31.8%,27.8%) على التوالي.

تم العثور على ارتباط معنوى بين درجة انتاج الاغشية مع (B-lactamse) في (*P.aeruginos)* . الاغشية الحيوية لها علاقة قوية بمقاومة العزلات للمضادات الحيوية.