Antibiogram, Plasmid Pattern, and Biofilm Formation in Clinical Isolates of *P. aeruginosa*

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Abstract

In this study eight hundred sample of (wound, burn, cystic fibrosis) were collected from patients admitted to Emergency hospital, Hawler teaching hospital, Pediatric hospital and private laboratory in Erbil during the period of February 2011 to September 2011. One hundred isolates of *Pseudomonas aeruginosa* were identified by using cultural, morphological characteristics, biochemical test and Api 20NE system in addition to vitek machine. Results of pigments production revealed the ability of *P. aeruginosa* to produce various pigments including blue / green, yellow/ green , and brown/ blue . The susceptibility of *P. aeruginosa* isolates to different antibiotics was examined. Impenim was the most effective antimicrobial agents against *P. aerugionsa* isolates and most of isolates showed high resistance degree to doxycycline 100%, Tetracycline 100%, Vancomycin 100%, Rifampcin 96%, Ampicillin 95%, Chloramphenicol 94%, Trimethoprim 83%, Amoxacillin 81%, Streptomycin 76%, Ceftriaxone 73%, Amikacin 67%, Cefotaxime 62%, Gentamycin 54%, and Ciprofloxacillin 40%. Due to the resistance of the isolates to these antibiotics they were classified into twenty five groups that showed the sensitivity variation in their resistance to these antibiotics. All *P. aerugionsa* isolates were screened for their ability to produce *B- lactamase* & Extended spectrum Beta lactamase out of 69 of *P. aeruginosa* isolates 69% were found to produce β-lactamase using rapid iodometric method , while 48% of the isolates were found to produce ESBL. The plasmid profile for nine *P. aerugimosa* isolates which showed the highest antibiotic resistance were conducted and the results revealed that all isolates except p6, and p8 contain one band with molecular weight more than 10kb using Agarose gel electrophoresis technique. The ability of *P. aerugionsa* to produce biofilm were studied , and the results showed that An among 97% of the isolates 71% were strongly biofilm producer, 20% were moderately biofilm producer, and 6% were weakly produce biofilm while 3% were non biofilm producer

Key words: ***P. aeruginosa*** **, antibiotic resistance , plasmid, biofilm**

Introduction

Pseudomonas aeruginosa is the most important human pathogen of its genus and draws on an impressive array of mechanisms to infect almost any external site or organ (Williams *et al.*, 2000). Most community infections are mild and superficial, but in hospital patients, infections are more frequent, more severe and more varied. In fact, it is one of the most common Gram-negative bacteria found in hospital-acquired infections, representing a major cause of pneumonia, urinary tract, surgical wound, burn wound and bloodstream infections (Van Delden ,2004).Chronic pulmonary infection with *P. aerugionsa* are the major cause of morbidity and mortality in cystic fibrosis patients (Lyczak *et al.*, 2002).

Pseudomonas aeruginosa has an innate ability to produce specific fluorescent pigments , genetically encoded by two operons for the production of metabolites such as pyocyanin(blue-green) pyoverdin or fluorescein (greenish-yellow), pyomelanin (red-brown) and pyorubin (red). Various media are required to encourage different pigment production by pseudomonads. On chemically defined media, the pigments may exhibit a wide variety of colours depending on the carbon source used for growth (Mavrodi *et al.*, 2001).

The worldwide emergence of multi-resistant bacterial strain is a growing concern, especially in hospital infections caused by *Pseudomonas aeruginosa*. Among nosocomial bacterial infections, those caused *P. aeruginosa* are associated with highest mortality rate, and are difficult to eradicate from infected tissues or blood because these microorganisms are virulent and have a limited susceptibility to antimicrobials (Kettner *et al.* 1995). Besides its innate resistance, acquired additional resistance due to plasmids is also a problem. Resistance commonly involves plasmid-mediated production of drug-modifying enzymes especially β-lactamase and extended spectrum of β-lactamase.The widespread occurrence of resistance appears to reflect the dissemination of R plasmids or resistance plasmids encoding these enzymes among bacterial species that prosper in the hospital environment. R plasmids are small extra-chromosomal genetic elements, which code for resistance to antibiotics, usually to several antibiotics. These may be transferable to bacteria belonging to the same or to different species through conjugation, converting the recipients to resistance (Begum,2009).

Pseudomonas aeruginosa life includes stages where the cells are associated and form a biofilm on a surface (e.g. Costerton *et al* .,1999). The formation of these surface communities and their inherent resistance to antimicrobial agents are the cause of many persistent and chronic infections (Costerton *et al.*, 1999). Microscopic analysis has indicated that biofilm formation occurs in a sequential process of (i) transport of microbes to a surface;

(ii) initial attachment; (iii) formation of microcolonies; and (iv) biofilm maturation (Sauer *et al* ., 2002). An important consequence of biofilm is its marked antimicrobial resistance . Some studies indicates that *P. aeruginosa* within biofilms are metabolically less active and grow more slowly than cells at the biofilm periphery (owing to limited access to nutrients and oxygen), which may contribute to increasing biofilm tolerance to antimicrobials since antimicrobials often target metabolically active cell (Davies *et al.*, 2009). This study aimed to establish the antibiotic susceptibility patterns , pigment production and the plasmid nature of multiple antibiotic resistances in *P. aeruginosa* isolates with the final objective of finding the ability of *P. aeruginosa* to form biofilm for the different isolates.

Materials and Methods

Patients and specimens collection

One hundred isolates of *Pseudomonas aeruginosa* were identified among 800 samples were collected from different clinical specimens (burn , wound and cystic fibrosis), from Emergency hospital and Hawler teaching hospital in Erbil city, Paedraitic Hospital and private laboratory during the periods of (February 2011 to September 2011) . The samples were identified by performing morphological , cultural , biochemical tests , API 20 NE system, and vitek machine.

Antibiotic resistance profile of *P. aeruginosa*:

To study the effect of different antimicrobials on *P.aeruginosa,* isolates Mueller-Hinton agar was used as growth media, after sterilization and cooling at 45 ºC, final concentration of antibiotics as explained in table (1) were added to media and poured into sterile Petri-dishes. After solidification, the plates were inoculated by streaking method with *P.aeruginosa* then incubated at 37 ºC for 24 hours. The results were recorded next day (Jean *et al.*,2002).

Colour production by *P.aeruginosa* isolates

Bacterial samples of *P.aeruginosa* were inoculated on *Pseudomonas* Isolation Agar F, a medium that stimulates the production of fluorescein, *Pseudomonas* Isolation Agar P, a medium that stimulates the production of pyocyanin and also on cetrimide agar, a medium that stimulates the production of pyocyanin by streaking method and incubated at 37 ºC for 18-24 hours for the detection of pigment production.

β- lactamase test

Used for detection the ability of isolated bacteria to production β-lactamase enzyme , prepared by addition of 0.1 ml of Penicillin solution(6000 ml) to small test tube and then aloopful of young bacteria was added and left 30 minutes at room temperature , then a few drop of 0.1% starch , finally gram iodine reagent was added as

drops till color was changed, the appearance of white color directly indicate of *P . aeruginosa* to produce β- lactamase (Lennet *et al.*, 1991).

Detection of extended spectrum B- lactamases (ESBLs) in *P . aeruginosa*

Extended Spectrum B-lactamases (ESBLs) are plasmid- mediated enzymes produce by a number of gram negative bacteria . A double disc diffusion test was performed with amoxicillin- clavulanic acid surrounded by aztreonam and third generation cephalosporin disc cefotaxime and ceftazidime (DeFreitas *et al.*, 2003)

A bacterial suspension was prepared with the turbidity adjusted to a 0.5 McFarland standard.Muller Hinton agar plate were streaked with the suspension according to NCCLS guidelines.An amoxicillin (20 mg)-calvulanic acid 10 mg AMC disc was placed at the center of the plate.Individual disc containing 30 mg of CAZ ceftazidine , 30 mg CTX cefotaxime and 30 mg ATM aztreonam were placed on to the plates in a distance of 1 cm edge from an amoxicillin-clavulanic acid disk.

Isolation of plasmid DNA content from bacterial isolates under study for plasmid profile

Quantum Prep plasmid miniprep kit (fermentes)

The steps of plasmid DNA isolation are the following:

1. An overnight culture was transferred to a micro test tube centrifuged at 8000 round per minute (rpm) for (30 second). Supernatant was removed. Two hundred and fifty µl of re-suspension solution vortexed until the cell pellet is completely re-suspended. Two hundred and fifty µl lyses solution was added and mixed by inverting 10 times.tree hundred µl neutralization solution was added. Mixed by inverting 10 times then centrifuged for 5mint. A spin column inserted into a 2 ml wash tube. The supernatant to transferred to a spin column, centrifuged at 8ooo rpm for 1 mint to pull fluid though the column. The spin column was removed, the filtrate discarded, and the column replaced. 500 µl wash buffer added, centrifuge 2 minutes followed by a 2 minutes spin to remove all wash buffers. The spin column removed to a clean 1.5 ml tube, 100 µl sterilized H2O was added. Centrifuged for one minute.

Agarose electrophoresis technique (Woodford and jhonson, 2006)

Preparation of 1% agarose gel:

The gel (1%) was prepared by dissolving 1 gm of agarose powder in 100 ml of 0.5 X TBE (Tris base ethidium bromide) buffer, boiled until all agarose was dissolved and left to cool at 50 °C, 8 µl of ethidium bromide was added, the gel was poured in to the glass plate that contained appropriate comb, the gel was left to solidify and the comb was removed gently, the gel was soaked in a gel tank containing TBE buffer should cover the surface of the gel.

Sample loading

Ten µl of plasmid DNA samples were mixed with 5 µl of loading buffer, and the mixture was slowly loaded in to the wells on the gel, also a molecular weight marker was loaded as control.

Running the electrophoresis

The electrophoresis apparatus was joined to power supply, turned on and the samples electrophoresed at 10 volt/cm for 1 hour.The gel was visualized by UV-transilluminator, and then photographed.

Biofilm detection method (Hinsa and O'Toole,2006)

A loopful of bacterial culture of isolates to be screened was inoculated into separate test tubes containing 3ml of brain heart infusion broth (BHIB). Two hundred µl of each bacterial suspension was transferred to the microtiter plate wells and were covered and then incubated at 37°C for 24 hours.After incubation, the contents of the each wells were aspirated 3 times with 250µl of PBS.The remaining bacteria were fixed with 200 µl of 99% methanol per well and plate was emptied and left to dry for 15min.Then the wells of plates were stained for 5min with 0.2ml of 2% crystal violet (Hi-media-Mumbai). Excess stain was removed by gentle pipetting. Final wash was given 3 times with PBS (170 µl).The plates were air dried, the dye bound to the adherent cells was resolubilized with 160 µl of 33%(v/v) glacial acetic acid per well. Optical density (OD) of each wells were measured at 595nm by using Bio-rad model 550 microtiter plate reader.All strains were classified into the following categories, non adherent(-), weakly adherent(+), moderately adherent(++) or strongly adherent(+++), based upon the OD of bacterial biofilms.

Results

Collection of *P. aeruginosa* isolates

All bacterial isolates were subjected to a series of confirming tests to ensure that these isolates recovered belong to *P. aerugionsa* species. these bacterial cells from smear preparation are gram negative rods, motile, non-spore forming, arranged in single or short chain . The colonies on solid media were small, rough or smooth with flat edges and elevated appearance, but some have mucoid appearance, biochemical tests were negative to indol , positive to oxidase , and positive to citrate and presumptively are *P. aeruginosa* , which in accordance with previous observation (Buchanan and Gibbson, 1974; Altas *et al.*, 1995). Inaddition, API 20NE system was conducted which is specific for *P. aeruginosa* and the synonym number obtained was (0354555) which indicate that all isolates were *P. aerugionsa* as in figure (1). Also all isolates were identified using vitek system for identification of bacteria and the results revealed that all recovered isolates belong to *P .aeruginosa* species.

Colour production by *P.aeruginosa* isolates

The isolates were grown on a variety of media to investigate pigment production. The colours produced by the isolates when they were grown on selective media at 37°C were noted. All isolates were grown on *Pseudomonas* Isolation Agar F, a medium that stimulates the production of fluorescein, *Pseudomonas* Isolation Agar P, a medium that stimulates the production of pyocyanin and also on cetrimide agar, a medium that stimulates the production of pyocyanin. These pigments are typical of *Pseudomonas aeruginosa*.. The isolates produced yellow/green, indicative of fluorescein production, blue/green, indicative of the pyocyanin production, and brown / blue, indicative of pyomelanin production (table -2-).

Antibiotic sensitivity profile of *Pseudomonas aeruginosa* isolates:-

One hundred *P. aeruginosa* isolates were screened for their resistance to (15) widely used antibiotics in medicine which are (Amikacin, Amoxicillin, Ampicillin, Cefotaxime, Cefotrixone, Ciprofloxacin, Chloramphenicol ,Doxycyclin, Gentamcin , Impiene, Rifampicin , Streptomcin, Tetracyclin, Trimethprim, Vancomcin). Table (3) illustrates that all isolates vary in their response to the use of antimicrobial agents and the highest resistance percentage (%100) was to Doxcyclin, Tetracyclin, Vancomcin. For ciprofloxacillin 40%, for Gentamycin 54%, for Cefotaxime 62%, for Amikacin 67%, Ceftriaxone 73%, Streptomycin 76%, Amoxicillin 81%, Trimethoprim 83%, Chloramphenicol 94%, Ampicillin 95%, for Rifambicin 96% while the lowest percentage (20%) was to Imipiene. The isolated bacteria were grouped according to their resistance to the antimicrobials under study as in table (4) as antibiogram related to antimicrobial resistance and the resistant of each grouped to all antibiotics were ranged between 94 % for group No.1 to 60 % for group No25.

β - lactamase production in *P .aeruginosa*

Rapid iodometric method performed for detection of the ability of *P. aerugionsa* isolates to produce β–lactamase .that is responsible for their resistance to beta lactam antibiotics like penicillin and carbapenem . Among 100 isolates of P. aerugionsa (69%) 69 of these isolates were β-lactamase producer which indicated by the change from violet to colorless within 5 minutes (figure -2-) .

ESBL production in *P. aeruginosa*

One hundred isolates of *P. aeruginosa* were screened for ESBL production by double disk diffusion method . Of these 48 isolates were found to be ESBL positive , whereas 52 were ESBL negative. The results represented in figure (2).

Molecular Aspects

Plasmid profile in *P. aerugionsa*

Figure (3) shows the plasmid profile for nine isolates designated as p1, p2, p3, p4, P5, p6, p7, p8 and p9. The isolates that revealed the highest resistance toward antibiotics had been chosen for plasmid profile. Isolation of plasmid using agarose gel electrophoresis and observation under UV trans illuminator showed one band for all *P. aeruginosa* isolates with molecular weight more than 10 kbp except isolate No. 6,8 that lack any plasmids.

Biofilm formation

Table (5) and figure (4) shows the biofilm formation degree by *P. aerugionsa* isolates . Among 97% of biofilm producers, 71% of isolates were strongly adherent (biofilm producer) , 20% (20) were moderately adherent , 6% (6) were weakly adherent while 3% (3) were non adherent (produce no biofilm).

Discussion

Sample collection

The results showed that burn isolates were the most frequent forming 20% from 200 samples then cystic fibrosis 16%, while the wound was 9% , these samples were not taken regularly, but depend on the patients who admitted to these hospitals. Our results agreed with Hamasalih (2008) who collected fifty isolates 25% from 200 burn samples in Sulamaniya hospitalized patient. Kheder (2002) obtained 28% from burn, 20%for wound and 7.3% from urine. Lambert (2002) declared that the percent of *P. aeruginosa* from patient with cystic fibrosis was 15% .

Pigment production by *P. aeruginosa*

Pigment production is a contributory phenotypic characteristic in the classification of *P. aeruginosa* (Buchanan and Gibbson, 2001). Growth medium has the biggest effect on the development of pigment. Various media are required to encourage different pigment production by pseudomonads. The pigments exerts a proinflammatory effect on phagocytes, impairs the normal function of the human nasal cilia and inhibits the proliferation of human epidermis and lymphocytes. This is probably due to inhibition of electron transport. It have been reported to have antifungal properties that give *P. aeruginosa* an obvious selective advantage to these organisms in their natural environment (Kerr *et al*., 1999). It also induces rapid apoptosis of human neutrophils (Allen *et al*., 2005), directly oxidises glutathione and decreases its levels in airway epithelial cells (O'Malley *et al*., 2004).

Antibiotic resistance patter in *P. aeruginosa* isolates

It is note worthying to mention that the spreading of resistant *P. aeruginosa* in the last few decades is increadibly increased which limits the choise of the therapeutic option for the treatment of infections caused by this micro organism. . Now *P. aeruginosa* isolates across countries are increasingly resistant to greater number of anti-microbiol agents. Olayinka *et al.*,(2009) reported that 20% of *P. aeruginosa* isolated from clinical sample obtained from the surgical units Ahmadu Bello university teaching hospital in Nigeria were sensitive to impenim which is in a good agreement with our results . Also our results showed that all the isolates were highly resistant to Doxycyclin and tetracycline. These results agreed with those reported by Hamasalih (2008) who found that the resistant to Doxycyclin and tetracycline were 100% and 98% respectively. Othman (2010) pointed that more than 50 isolates of *p. aeruginosa* among different clinical specimen resist 98% to Amkacine 96% to Cephotaxime, 80% to Rifampicin, 70% to Ampicillin, 70% to Augment and 60% to Dooxycyclin respectively. Resistance mediated by *P. aeruginosa* can be attributed both to an inducible, chromosomally mediated beta lactamases that can render broad–spectrum cephalosporin inactive, and to a plasmid–mediated beta- lactamases that can lead to resistance to several penicillin and older cephalosporin (Barid ,1996).The mechanisms of bacterial resistance to aminoglycoside antibiotics in clinical isolates is usually controlled by enzymatic inactivation of the antibiotic, since nine different enzymes that catalyze the phosphorylation, acetylation, coradenylylation of aminoglycosides have now been identified in bacteria(Pollack 2000). The evolution of multi-resistant *P. aeruginosa* and its mechanisms of antibiotic resistance mechanisms include reduced cell permeability, efflux pumps, changes in the target enzymes and inactivation of the antibiotics (Lambert ,2002 ; Matsuo *et al.*, 2004) .

Detection of *B-lactamase* production

In the present study 69% of isolates have *B-lactamase* and this result agreed with Haleem (2011) who found that 66.66 % of *P. aeruginosa* isolated from different clinical cases have *β- lactamase* enzyme , also agreed with Manoharan *et al.*, (2010) who discovered that of (84.4%) the isolates have this enzyme. This method depends on the detection of penicilloic or cephalospoic acid, resulted from breakdown of amide bond in β-lactam ring for each of Penicillins or Cephalosporins (Itah and Essien, 2005, Sykes *et al.*, 1976). Iodine reacts with starch to form dark blue complex, which stays without changes in the absence of *β-lactamase* enzymes. In the case of *β-lactamase* producing bacteria, the resulting penicilloic or cephalospoic acid will reduce iodine into iodide; consequently, decolorization of starchiodine complex occurs (changing the color directly to white) if an isolates a *β-lactamase* producer (Sykes *et al.*, 1976). Strict antibiotic policies and

measure to limit the indiscriminative use of cephalosporins and carbapenems in the hospital environment should be undertaken to minimize the emergence of this multiple *β-lactamase* producing pathogen whose spread would leave no other option to treat Gram-negative nosocomial infections.

Detection of ESBL in clinical isolates of *P. aeruginosa.*

Our study reported (48 %) ESBL production among *P. aeruginosa* isolates. ESBL mediated resistance in *P.aeruginosa* as reported by Amutha *et al.,* (2008) was (25%) . In addition Hussein (2010) revealed that (37%) of *P. aeruginosa* were ESBL producer from burn patient. This study indicates a prevalence of *P. aeruginosa* producing ESBL which are very important as these strains may often cause out breaks in the pediatric population and causes increased morbidity and mortality in patients underlying diseases or limit therapeutic options due to the high degree of multidrug resistance. The highest rate of ESBL producing bacteria may be due to patients have predisposing factors such as the length of hospital stay, severity of illness , compromised immunity and urinary catheterization (Mirsalehian *et al.,* 2008) .

Plasmid pattern of *P. aerugionsa* isolates

Plasmid analysis of the multi- resistant isolates showed that 7 isolates of *P. aerugionsa* harboured plasmids which had similar plasmid band pattern with molecular weight more than 10 kb in size. Similar finding have been reported by Daini and Chinyere (2012) who found that *P. aeruginosa* isolated from clinical specimen harbored plasmid with molecular weight 19 kb. On the other hand our results matched with Othman (2011) and Hamasalih (2010) who studied molecular genetic of *P. aeruginosa* demonstrated plasmid profile of studied bacteria by agaraose gel electrophoresis and they obtained plasmid with molecular weight more than 10kb. A variety of plasmids have been isolated from *Pseudomonas aeruginosa* species such as R-factor plasmids, which inactivate certain antibiotics and S-factor plasmids capable of initiating chromosomal gene transfer (Chakrabarty, 1976). Plasmids in *Pseudomonas* species are also known to code for heavy metal resistance and degradation of organic compounds (Chakrabarty, 1976).

Biofolm formation by *P. aeruginosa*

Results revealed that 71% of the *P. aeruginosa* isolates were strongly biofilm producer 20% were moderately adherent , 6% were weakly adherent while 3% were non adherent. In study conducted by Delissalde *et al.,*(2004) to assess the capability of *P. aeruginosa* to produce biofilm using 162 clinical isolates. Only 14% of these isolates produce biofilm after an 8-hrs incubation and 8% after 24-hrs. On the other hand Kalaivani (2011) showed that 93%

of *P. aeuroginosa* isolated from pneumonia, urinary tract infection and bacteremia were biofilm producer among them 75% were strongly adherent 8% were moderately adherent, 11% were weakly adherent and 7% of the isolates didn't produce any amount of adherent which in good agreement with our results .In a study by Nagaveni *et al*, (2010) they found that 42% *of P . aeruginosa* were high biofilm producers, 33% were moderately and 25% were weak biofilm producers. High biofilm producers were more in our study which may be due to the increase number of multidrug resistant strains in the present study.

Luyan Ma *et al*., (2006) also proposed that there was strong correlation between biofilm formation and development of antibiotic resistance. One explanation for biofilms being generally refractory to antimicrobial chemotherapy is the presence of a highly resistant sub-population of cells called persisters in biofilm. Intriguingly, "late" isolates of *P. aeruginosa* in cystic fibrosis produce increased levels of drug-tolerant persister cells, which may be the primary "mechanism" for surviving chemotherapy and so, may explain the general recalcitrance of *P. aeruginosa* infections in cystic fibrosis (Mulcahy *et al.,* 2010) . Finally, the population of cells within a biofilm is heterogeneous, containing fast- and slow-growing cells, some resistant through expression of inactivating enzymes and efflux pumps, others conspicuously not expressing such systems. The overall resistance is therefore dependent upon an interaction between the entire population of cells and therapy needs to be directed against a multicellular community (Lambert 2002).

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Table (1): Antibiotics that used in this study (Jean *et al.*, 2002)

Solvent	Final (Working) Concentration μg/ml	Stock Solution mg / ml	Symbol	Antibiotic's Names
S.D.W.	25	25	Amc	Amikacin
S.D.W.			Am	Amoxacillin
S.D.W.	50	50	Amp	Ampicillin
S.D.W	30	10	Cef	Cefotaxime
S.D.W.			Cft	Ceftriaxion
D.W. or Ethanol	5	10	Cip	Ciprofluxacillin
Ethanol 100%	30	34	Cm	Chloroamphenicol
S.D.W.	30	10	Do	Doxycyclin
S.D.W	10	10	Gm	Gentamycin
S.D.W.			Imp	Imipinem
Methanol + 5drops of 10N NaOH	5	34	Rif	Rifampicin
S.D.W	25	20	Sm	Streptomycin
Ethanol 100%.	15	25	Te	Tetracycline
S.D.W.	20	10	Tri	Trimetheprim
S.D.W.	30	30	Van	Vancomycine

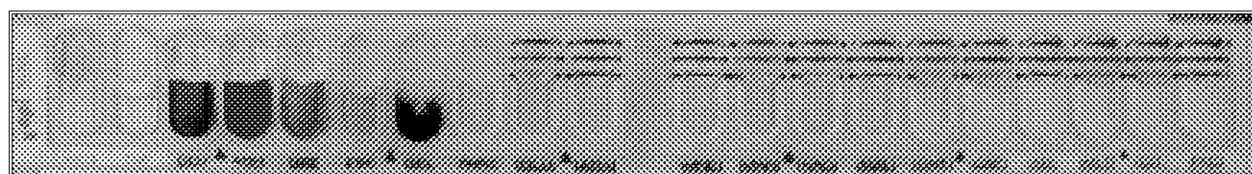

Figure (1): Strip of API 20 NE system

Table (2): The pigment production by *P .aeruginosa* isolates

		Pseudomonas Isolation No.			**Pseudomonas Isolation No.**
	PIA			**PIA**	
mucoid	Yellow \ Green	P51	mucoid	Blue/Green	P1
mucoid	Yellow\ Green	P52	Non mucoid	Blue/Green	P2
Non mucoid	Blue/Green	P53	Non mucoid	Blue/Green	P3
Non mucoid	Blue/Green	P54	Non mucoid	Blue/Green	P4
Non mucoid	Blue/Green	P55	Non mucoid	Blue/Green	P5
Non mucoid	Blue/Green	P56	mucoid	Blue/Green	P6
mucoid	Blue/Green	P57	mucoid	None	P7
mucoid	Blue/Green	P58	mucoid	Blue/Green	P8
Non mucoid	Yellow\ Green	P59	Non mucoid	Blue/Green	P9
mucoid	Yellow\ Green	P60	Non mucoid	Blue/Green	P10
Non mucoid	Brown/ Blue	P61	Non mucoid	Blue/Green	P11
Non mucoid	Brown / Blue	P62	mucoid	Blue/Green	P12
mucoid	Yellow\ Green	P63	mucoid	Blue/Green	P13
Non mucoid	Yellow\ Green	P64	Non mucoid	None	P14
Non mucoid	Blue/Green	P65	Non mucoid	Blue/Green	P15
Non mucoid	Blue/Green	P66	Non mucoid	Blue/Green	P16
Non mucoid	Blue/Green	P67	mucoid	Blue/Green	P17
mucoid	Blue/Green	P68	Non mucoid	Yellow \ Green	P18
mucoid	Blue/Green	P69	Non mucoid	Brown / Blue	P19
Non mucoid	Blue/Green	P70	Non mucoid	Yellow\ Green	P20
Non mucoid	Yellow\ Green	P71	Non mucoid	Yellow\Green	P21
Non mucoid	Yellow\ Green	P72	mucoid	None	P22
Non mucoid	Brown/ Blue	P73	mucoid	Blue/Green	P23
Non mucoid	Yellow\ Green	P74	Non mucoid	Blue/Green	P24
Non mucoid	Yellow\ Green	P75	Non mucoid	Blue/Green	P25
mucoid	Blue/Green	P76	Non mucoid	Blue/Green	P26
mucoid	Blue/Green	P77	Non mucoid	Blue/Green	P27
mucoid	Blue/Green	P78	Non mucoid	Blue/Green	P28
Non mucoid	Blue/Green	P79	mucoid	Blue/Green	P29
mucoid	Blue/Green	P80	mucoid	Blue/Green	P30
mucoid	Blue/Green	P81	Non mucoid	Blue/Green	P31
Non mucoid	Blue/Green	P82	Non mucoid	Blue/Green	P32
Non mucoid	Blue/Green	P83	mucoid	Blue/Green	P33
Non mucoid	Blue/Green	P84	mucoid	None	P34
Non mucoid	Yellow \ Green	P85	Non mucoid	Yellow\ Green	P35
Non mucoid	Yellow\ Green	P86	Non mucoid	Yellow\ Green	P36
Non mucoid	Yellow\ Green	P87	Non mucoid	Brown / Blue	P37
Non mucoid	Brown / Blue	P88	Non mucoid	Yellow\ Green	P38
mucoid	Blue/Green	P89	mucoid	Blue/Green	P39
mucoid	Yellow \ Green	P90	mucoid	Blue/Green	P40
mucoid	Blue/Green	P91	Non mucoid	Blue/Green	P41
Non mucoid	Blue/Green	P92	Non mucoid	Blue/Green	P42
Non mucoid	Blue/Green	P93	mucoid	Blue/Green	P43
mucoid	Blue/Green	P94	mucoid	Blue/Green	P44
mucoid	Blue/Green	P95	Non mucoid	Blue/Green	P45
Non mucoid	Blue/Green	P96	Non mucoid	Blue/Green	P46
Non mucoid	Blue/Green	P97	mucoid	Yellow\ Green	P47
Non mucoid	Yellow\ Green	P98	mucoid	Yellow\ Green	P48
mucoid	Yellow\ Green	P99	Non mucoid	Brown / Blue	P49
Non mucoid	Yellow\ Green	P100	Non mucoid	Yellow\Green	P50

Table (3): Resistance of *P. aeruginosa* to antibiotics

% of Resistant	No. of Isolates	Antibiotics
67%	67	**Amc***
81%	81	**Am**
95%	95	**Amp**
62%	62	**Cef**
73%	73	**Cft**
40%	40	**Cip**
94%	94	**Cm**
100%	100	**Do**
54%	54	**Gm**
20%	20	**Imp**
96%	96	**Rif**
76%	76	**Sm**
100%	100	**Te**
83%	83	**Tri**
100%	100	**Van**

*abbreviation are given in table 1

Table (4): Antibiogram groups of *P. aeruginosa* isolates and percent of resistance to antibiotics

Antibiotics															% of Resistances	No. of isolates	No. of Antibiogram
Van	Tri	Te	Sm	Rif	Imp	Gm	*Do	Cm	Cip	Cft	Cef	Amp	Am	Amc			
R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	94	1	1
R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	94	3	2
R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	87	4	3
R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	87	4	4
R	R	R	R	R	R	S	R	R	R	R	R	R	S	R	87	2	5
R	R	R	R	R	S	R	R	R	S	R	R	R	R	R	87	5	6
R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	87	3	7
R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	87	1	8
R	R	R	R	R	R	S	R	R	S	R	R	R	R	S	80	4	9
R	R	R	R	R	S	S	R	R	S	R	R	R	R	R	80	7	10
R	R	R	R	R	S	R	R	R	R	R	S	R	S	R	80	3	11
R	R	R	R	R	S	S	R	R	R	R	S	R	R	R	80	5	12
R	R	R	S	R	S	R	R	R	S	R	R	R	R	R	80	4	13
R	R	R	R	R	S	S	R	R	R	S	R	R	R	R	80	5	14
R	R	R	R	R	S	S	R	R	S	R	S	R	R	R	74	4	15
R	R	R	S	R	S	R	R	R	S	R	R	R	S	R	74	2	16
R	S	R	R	R	S	R	R	R	S	R	R	R	S	R	74	6	17
R	R	R	R	R	S	R	R	S	S	R	R	R	R	S	74	6	18
R	R	R	S	S	R	R	R	R	S	S	R	R	R	R	74	4	19
R	S	R	R	R	S	S	R	R	R	S	R	R	R	S	67	8	20
R	S	R	S	R	S	R	R	R	S	R	S	R	R	R	67	3	21
R	R	R	S	R	S	S	R	R	R	R	R	R	S	S	67	2	22
R	R	R	S	R	R	S	R	R	R	R	S	R	S	S	67	4	23
R	R	R	S	R	S	R	R	R	S	S	S	S	R	R	60	5	24
R	R	R	R	R	S	S	R	R	S	S	S	R	R	S	60	5	25
S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		DH5α	

*abbreviation are given in table 1

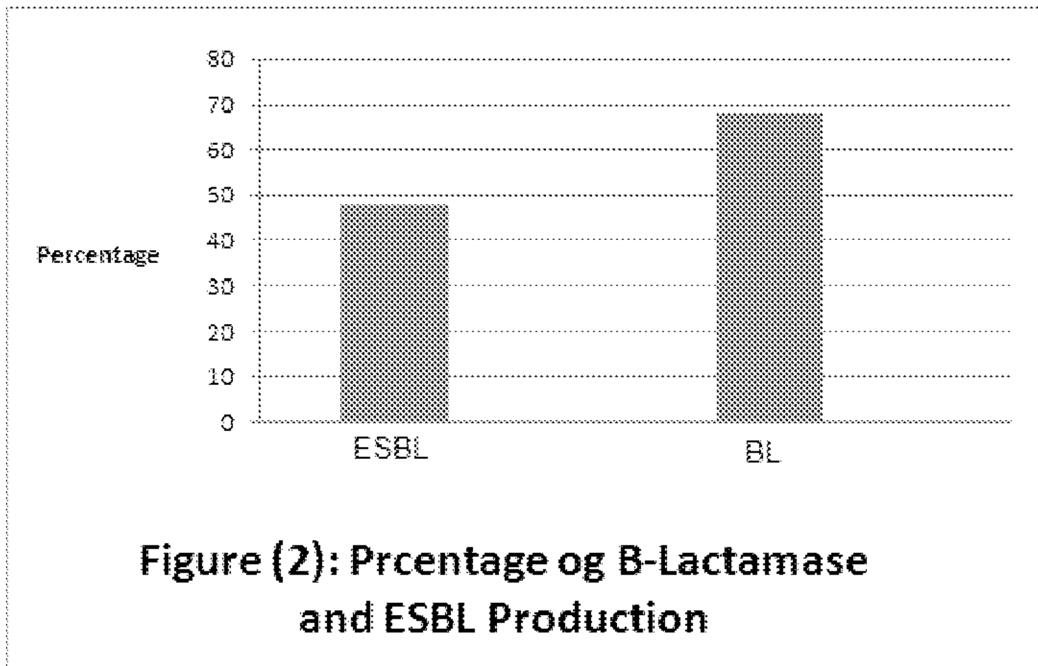

Figure (2): Prcentage og B-Lactamase and ESBL Production

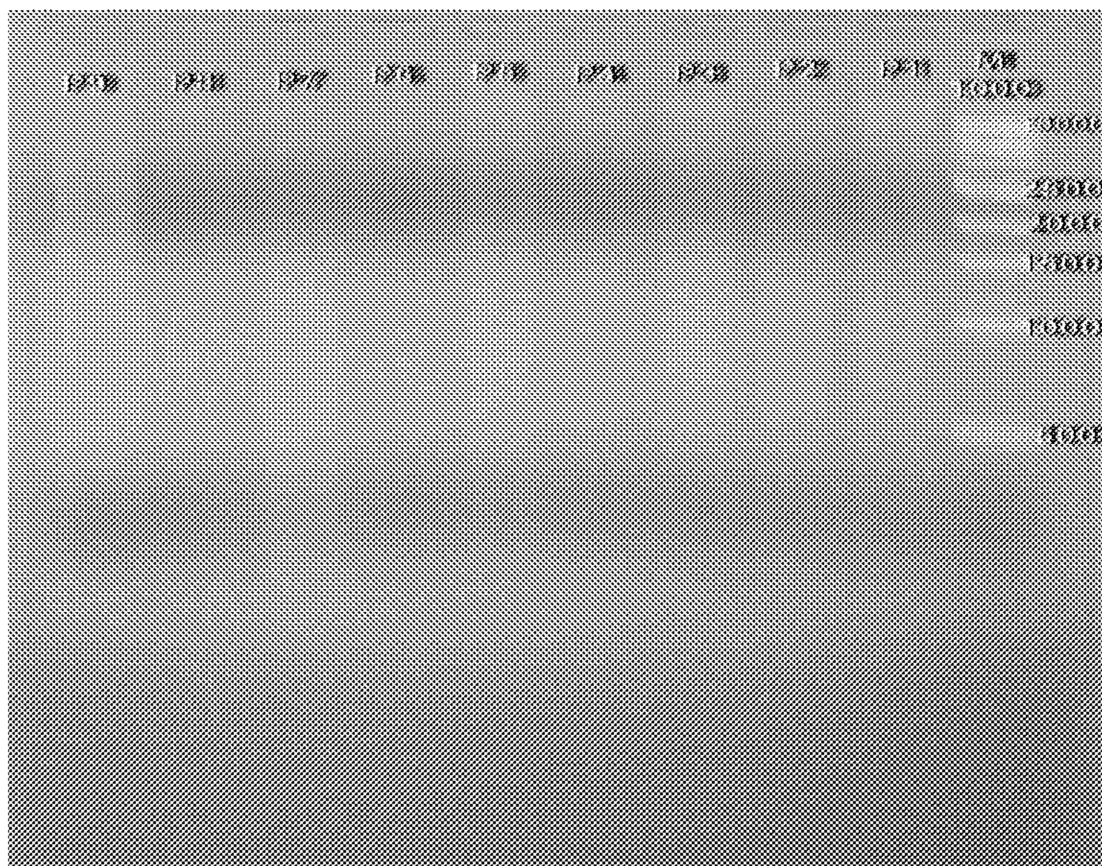

Figure (3): Plasmid profile in *P. aerugionsa*

Table (5): Percentage of Biofilm formation in *P. aeruginosa*

Bacterial Isolates		Biofilm Degree	Biofilm Formation (Absorbance at 600nm)
%	No.		
3%	3	No Biofilm	0.04-0.059
6%	6	Weak	0.06-0.099
20%	20	Moderate	0.1-0.2
71%	71	Strong	> 0.2
100%	100	Total	

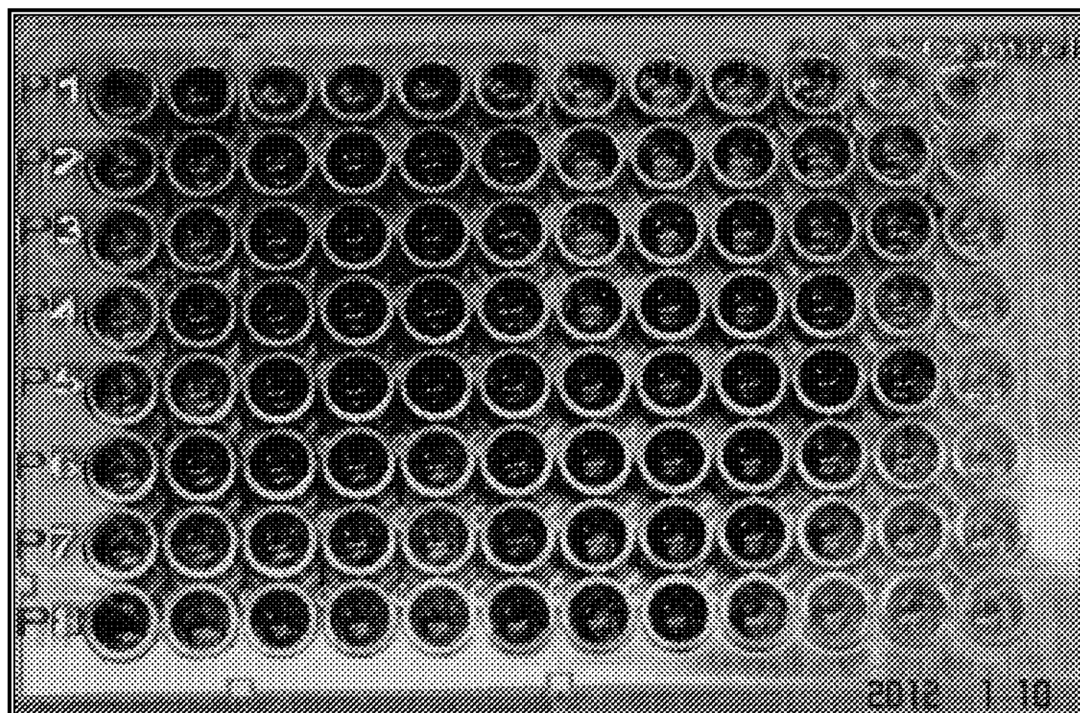

Figure (4): The biofilm formation by *P. aeruginosa* isolates

الخلاصة

المجاميع ضد المضادات الحيوية ، والمحتوى البلازميدي للعزلات الطبية لبكتريا *P. aeruginosa*

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في هذه الدراسة تم جمع 800 عينة من (الجروح والحروق، والتليف الكيسي) من المرضى وافدين الى مستشفى الطوارئ، هوليرالتعليمي، مستشفى الأطفال والمختبرات الخاصة في أربيل خلال الفترة من شباط 2011 إلى ايلول 2011. تم تشخيص مئة عزلة من بكتريا *P. aeruginosa* باستخدام الصّفات المزرعية، المورفولوجية، اختبار الكيمياء الحيوية و نظام API NE20 بالإضافة إلى تقنية VITEK.اظهرت نتائج انتاج الاصباغ قدرة بكتريا *P. aeruginosa* على انتاج اصباغ مختلفة تضمنت ازرق مخضر ، اصفر مخضر و قهوائي مزرق .تم فحص حساسية العزلات *P. aeruginosa* للمضادات الحيوية المختلفة. كان Imipenem من بين الأكثر المضادات الحيوية فعالية ضد عزلات *P. aeruginosa* وأظهرت معظم العزلات درجة مقاومة عالية ل doxycycline ‎100٪، Tetracycline ‎100٪، Vancomycin ‎100٪، Rifampcin ‎96٪، Ampicillin ‎95٪، Chloramphenicol ‎94٪ ، Trimethoprim ‎83٪، Amoxacillin ‎81٪، Streptomycin ‎76٪، Ceftriaxone ‎73٪، Amikacin ‎67٪، Cefotaxime ‎62٪، Gentamycin ‎54٪، و Ciprofloxacillin ‎54٪. وأيضا إستناداً الى مقاومة العزلات لهذه المضادات صنفت العزلات الى خمس و عشرون مجموعة التي أظهرت التباين حساسية في مقاومتهم لهذه المضادات الحيوية. تم فحص جميع العزلات *P. aeruginosa* لقدرتها على إنتاج بيتالاكتاميز و بيتالاكتاميز واسعة الطيف ، اظهرت النتائج ان من بين 100 عزلة *P. aeruginosa* تم العثور على 69٪ منتجة لبيتالاكتاميز باستخدام طريقة اليودومترية السريعة، في حين تم العثور على 48٪ من عزلات المنتجة لESBL. أظهرت المحتوى البلازميدي لتسعة العزلات *P. aeruginosa* التي أظهرت أعلى المقاومة للمضادات الحيوية أن جميع العزلات باستثناء P6 و P8تحتوي على حزمة واحدة مع الوزن الجزيئي أكثر من (KB10) باستخدام تقنية الاغاروز الكهربائي الهلامي. نتائج التقرير تشير إلى انتشار البلازميدات بين عزلات *P. aeruginosa* التي قد تحمل الجينات المقاومة ضد طيف واسع من المضادات الحيوية المستخدمة سريريا. تمت دراسة قدرة *P. aeruginosa* لإنتاج البيوفيلم، وأظهرت النتائج أن من بين 97٪ من العزلات ،71٪ انتجت بيوفيلم بقوة، و 20٪ كانت معتدلة الانتاج للبيوفيلم، و 6٪ ضعيفة انتاج للبيوفيلم بينما 3٪ لم ينتج البيوفيلم.