RESISTANCE OF *PSEUDOMONAS AERUGINOSA* FROM CLINICAL AND ENVIRONMENTAL SOURCES TO HEAVY METALS IN HILLA CITY, IRAQ

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Abstract

The study included 300 samples collected from 150 clinical and 150 hospital environmental sources. Only 43 (14.3%) isolates belonged to *Ps. aeruginosa*. The isolates were tested for their susceptibility to 7 types of heavy metals (HM) namely Copper Sulfate, Silver Sulfate, Mercury chloride, Lead nitrate, Zinc sulfate, Cadmium sulfate, and Nickel sulfate. The screening test for ability of the isolates to resist HM was detected using lead nitrate in concentration of 400µg/ml. Results revealed that 37/43 were resistant to lead nitrate (400µg/ml). The MIC of 7 HM was detected by agar dilution and pouring method. Results revealed that most of the isolates were resistant to 7 HM in some of concentrations. The plasmid content was investigated for all 37 isolates of *Ps. aeruginosa* (34 clinical and 3 environmental). Results revealed that most isolates 32/37 harbored large (mega) plasmid. Biofilm production of *Ps. aeruginosa* isolates was investigated; results showed that 20/43 (47%) of isolates had biofilm. This study concluded that the increase of HM resistance was correlated with biofilm production for some HM used. The bacterial curing is proceeding for one isolate of *Ps. aeruginosa* (*Ps.*3). The results showed survived resistance to all HM used, which may be due to HM resistance trait was carried out on bacterial chromosome rather than plasmid.

Keywords: *Pseudomonas Aeruginosa, Resistance, Heavy Metals, Environmental.*

Introduction

Heavy metals, particularly silver and mercury, have a variety of applications in controlling microbial population (Kenneth and Jeffery, 2006). Silver salts alone or in combination with other drugs appear to have a significant potential as topical antimicrobial agent (Fox et al., 1977; De Gracia, 2001). Mercury in the form of less toxic organic compounds is being used as skin disinfectant (Gerald, 2007). Copper is considered as a safe agent to humans, as demonstrated by the widespread and prolonged use by women of copper intrauterine devices (Anonymous, 2002; O'Brien et al., 2008).

Pseudomonas aeruginosa is pathogen that causes a substantial portion of hospital infections. It is frequently multi drug resistant, which contributes to the high morbidity and mortality of patients in intensive care units (ICUs), burn units and surgery wards. A major reason for its prominence as a pathogen is its high intrinsic resistance to antibiotics and heavy metals (Fluit et al., 2000). This a ubiquitous, environmentally important microbe that may employ many resistance mechanisms against different heavy metals, such as the *mer* operon that reduces toxic Hg^{2+} to volatile Hg^{0}, which then diffuses out of the cell (Outten et al., 2000).

Pseudomonas aeruginosa is a prevalent hospital pathogen that is well known for its ability to form biofilms that are recalcitrant to many different antimicrobial treatments (Harrison et al., 2008).

It was found that biofilms were from 2 to 600 times more resistant to heavy metals stress than free-swimming cells. They also showed that biofilms are more resistant to heavy metals than either stationary-phase or logarithmically growing plankotonic cells (Teitzel and Parsek, 2003).

The aim of this study was to detect the prevalence of heavy metals resistant (HMR) *Ps. aeruginosa* isolates recovered from clinical and environmental samples and studying the correlation between biofilm production and HM resistance**.**

Materials and Methods

This study included 300 samples (150 Clinical and 150 Environmental samples). The clinical specimens were collected from patients suffering from burns, wounds, and otitis media, who attending Hilla teaching hospital; whereas hospital environmental samples included catheters, beds, bath rooms, fomites, wall of wards, and some types of disinfectants at the same hospital.

All samples were inoculated on MacConkey agar, Nutrient agar, and selective medium (cetrimide agar), then incubated at 37ºC for 24-48 hrs. *Ps. aeruginosa* were identified by routine diagnostic tests including cellular, cultural and biochemical characteristics.

All isolates were subjected to susceptibility testing by screening test using agar medium supplemented with ($PbNO_3$ 400µg/ml). The isolates were also tested for their susceptibility to 7 heavy metals (HM) represented by; Copper Sulfate, Silver Sulfate, Mercury chloride, Lead nitrate, Zinc sulfate, Cadmium sulfate, and Nickel sulfate. The minimum inhibitory concentration (MIC) of 7 HM was detected by agar dilution method (silver, zinc, cadmium, nickel) and pouring method (lead, copper, mercury), based on standard methods (Riley and Mee, 1982; Forbes, 1998).

The following concentrations of heavy metals were prepared:

1- 0.0001M, 0,001M, 0.01M for Cadmium and 0.0001M, 0.001M, 0.01M, 0.1M for Nickel, Zinc, and Silver.

2- (100, 200, 400, 800, 1600, 2400, 3200 µg/ml) for lead; (100, 200, 400, 800, 1600, 1750, 3200 µg/ml) for Copper; and (2.7, 5.4, 10.8, 21.6, 43.2, 54.3, 86.4 µg/ml) for Mercury.

Biofilm production, Plasmid profile, and Plasmid curing were studied as follows:

Biofilm formation was determined using tissue culture-treated, 96-well polystyrene plates, based on the methods of Christensen et al (1985) and Ziebuhr et al (1997).

Plasmid DNA extraction of gram negative bacteria was performed using Geneaid kit, the steps of the method was according to the manufacturing company (Geneaid kit, USA) and plasmid profile was carried out by electrophoresis (Sambrook and Rusell, 2001). Plasmid curing was carried out using Elevated Temperature method according to Kheder (2002).

Results and Discussion

Out of the 300 samples, only 43 (14.3%) isolates belonged to *Ps. aeruginosa*. 40 (26.6%) of these isolates belonged to clinical samples and 3 (2%) isolates belonged to the *Ps. aeruginosa*.

Heavy metals resistance and MIC of isolates:

All isolates were subjected to susceptibility testing by screening test using agar medium supplemented with $PbNO_3$ 400µg/ml. Results revealed that 37 isolates (85%) were resistant to lead nitrate, these isolates were distributed into 34 clinical and 3 environmental samples (Table 1).

Vaca Pacheco *et al*. (1995) used lead nitrate as a screening test for detection of heavy metals resistance in *P. aeruginosa*, and they found all their isolates were resistant to lead nitrate ($PbNO_3$) at a concentration of 400µg/ml.

Table (1) Numbers and percentage of clinical and environmental isolates of *Pseudomonas aeruginosa* detected by screening test:

Susceptibility to $PbNO_3$ (400µg/ml)	No. of isolates (%)		Total	(%)
	Clinical	Environmental		
Resistant	34(85%)	3(100%)	37	85%
Sensitive	6 (15%)	0	6	15%
Total	40(92.8%)	3(6.9%)	43	100%

Bacterial resistance to heavy metals (Table 2, 3) shows the MIC of *Ps. aeruginosa* to all studied heavy metals. In case of silver sulfate ($AgSO_4$), results showed that 34:37 isolates were resistant to $AgSO_4$ in concentration 0.0001M and the MIC of all isolates was 0.01M.

In case of zinc sulfate ($ZnSO_4$), four isolates were sensitive to $ZnSO_4$ in low concentration 0.0001M. The MIC of all isolates was 0.1M. In case of cadmium sulfate ($CdSO_4$), five isolates were sensitive to $CdSO_4$ in low concentration 0.0001M. The MIC values of all isolates were 0.1M. In case of nickel sulfate ($NiSO_4$), the results indicated the all of isolates were resistant to $CdSO_4$ in concentration 0.0001M and 0.001M. The MIC of all isolates was 0.01M (Table 2).

In case of copper sulfate ($CuSO_4$), results indicated that all isolates were resistant in concentrations of 100, and 200µg/ml and the MIC of most of the isolates was 1600 µg/ml. (Table 3).

In case of mercury chloride ($HgCl_2$), two isolates were sensitive to $HgCl_2$ in low concentration 2.7 µg/ml. The MIC of all isolates was 86.4µg/ml.

In case of lead nitrate ($PbNO_3$), results revealed that 30:37 isolates were resistant in

concentration of 2400 µg/ml and the MIC of all isolates was 3200 µg/ml.

Table (2): MIC values of *Pseudomonas aeruginosa* isolates to silver sulfate, zinc sulfate, cadmium sulfate, nickel sulfate in molar concentrations.

Isolates	MIC of Silver sulfate	MIC of Zinc sulfate	MIC of Cadmium sulfate	MIC of Nickel sulfate
*Ps.*1	0.01	0.1	0.001	0.01
*Ps.*2	0.01	0.1	0.001	0.01
*Ps.*3	0.01	0.1	0.01	0.01
*Ps.*4	0.01	0.1	0.001	0.01
*Ps.*5	0.01	0.1	0.001	0.01
*Ps.*6	0.01	0.1	0.01	0.01
*Ps.*8	0.01	0.01	0.01	0.01
*Ps.*9	0.01	0.1	0.01	0.01
*Ps.*12	0.01	0.1	0.001	0.01
*Ps.*13	0.01	0.1	0.001	0.01
*Ps.*14	0.01	0.1	0.001	0.01
*Ps.*15	0.01	0.1	0.01	0.01
*Ps.*16	0.001	0.01	0.001	0.01
*Ps.*17	0.001	0.1	0.0001	0.01
*Ps.*19	0.0001	0.0001	0.001	0.01
*Ps.*20	0.001	0.01	0.001	0.01
*Ps.*21	0.001	0.01	0.001	0.01
*Ps.*22	0.01	0.01	0.001	0.01
*Ps.*24	0.001	0.01	0.001	0.01
*Ps.*25	0.01	0.01	0.001	0.01
*Ps.*26	0.001	0.01	0.001	0.01
*Ps.*27	0.01	0.01	0.01	0.01
*Ps.*28	0.001	0.1	0.01	0.01
*Ps.*29	0.001	0.01	0.001	0.01
*Ps.*30	0.001	0.01	0.001	0.01
*Ps.*31	0.0001	0.0001	0.01	0.01
*Ps.*32	0.001	0.1	0.01	0.01
*Ps.*33	0.001	0.01	0.01	0.01
*Ps.*34	0.001	0.01	0.001	0.01
*Ps.*36	0.0001	0.0001	0.01	0.01
*Ps.*37	0.001	0.01	0.0001	0.01
*Ps.*38	0.001	0.01	0.01	0.01
*Ps.*39	0.001	0.01	0.001	0.01
*Ps.*40	0.01	0.01	0.0001	0.01
*Ps.*41	0.001	0.01	0.01	0.01
*Ps.*42	0.001	0.1	0.0001	0.01
*Ps.*43	0.001	0.0001	0.0001	0.01

The interpretation of these results may be due to the fact that *Ps. aeruginosa* has many mechanisms for heavy metals resistance; firstly, the accumulation of specific ions can be diminished, not by interference with uptake but by active extrusion of the heavy metals ion from the cells. This mechanism is specific only for *Pseudomonas* spp. Secondly; cations can be segregated into complex compound by thiol-containing molecules and then ejected from the cell. Thirdly, some metal ions may be reduced to a less toxic oxidative state by the complex enzymes and special oxidation mechanisms in the cells and finally, for many metals resistance and homoeostasis where is a combination of two or three of the mentioned basic mechanisms that is the case which *Ps. aeruginosa* success (Abdul-Sada, 2008).

Prasad *et al.* (2009) found that all isolates were sensitive to heavy metals (Cd^{2+}, Ag^{+}, Ar^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+}) at a concentration of 0.1M, and most of them were resistant to heavy metals at a concentration of 0.0001M. Singh *et al* (2010) found that the MIC values of Ni^{2+} were ranged from 80-250 µg/ml, Cd^{2+} was (80-210 µg/ml).

The results of this study are similar to those obtained by Prasad *et al* (2009) who found that all isolates of *P. aeruginosa* were resistant to silver nitrate at a concentration of 0.0001M and all isolates were sensitive to silver nitrate at concentrations of 0.01M and 0.1M. Dong *et al*.

(2001) found 100 µg/ml of sliver nitrate is effective to prevent *P. aeruginosa* ATCC 27853 production biofilm and inhibition of bacterial attachment.

In a local study, Abdul-Sada (2008) found that *P. aeruginosa* isolated from wastewater in Basrah, Iraq, were resistant to Zn_2O_3 and cadmium chloride at concentrations of 0.4M, and 0.1M, respectively, while Xiao-xi *et al*. (2009) found that *P. aeruginosa* isolate E_1 was resistant to Zn^{2+} and Cd^{2+} in concentrations of 16.5 mmol/L (0.0165M) and 18.5 mmol/L (0.018M) respectively. Nies (2003) interpreted that *P. aeruginosa* respond to excess Zn^{2+} and cd^{2+} by metal-inducible resistance mechanisms, Zn^{2+} and Cd^{2+} resistance in bacteria is mainly based on active efflux of metal ions to prevent toxic effects in the cell.

Regarding to mercury chloride, karbasizaed *et al* (2003) revealed that coliforms were tolerant to mercury chloride was in 54.3 µg/ml, while Prasad *et al* (2009) found that all isolates of *P. aeruginosa* were sensitive to mercury chloride in concentration 0.0001M, 0.001M , 0.01M and 0.1M. *Pseudomonas aeruginosa* were able to resist to mercury because it has *mer operon* that reduced toxic Hg^{2+} to volatile Hg^0, which then diffuses out of the cell.

According to lead nitrate, the results showed that all isolates were resistant to lead nitrate and the MIC values ranged from 800-3200 µg/ml (Table 3). These results are similar to that obtained by Karbasized *et al* (2003) who revealed the coliforms were tolerant to lead nitrate was in a MIC of 3200 µg/ml. Xiao-xi *et al*. (2009) found *P. aeruginosa* isolate E_1 was resistant to pb^{+2} in concentration 10.0 mmol/L (0.01M). Prasad *et al*. (2009) found that all isolates of *P. aeruginosa* were sensitive to lead nitrate at concentrations of 0.001M, 0.01M and 0.1M. Many authors found that *P. aeruginosa* were resistant pb^{2+} by the system localized in cad AC operon, cad A catalyzed the active efflux of Cd^{2+}, Zn^{2+}, and Pb^{2+}, also they found Pseudomonads have P-type ATPase that can resist Pb^{2+} (Nucifora *et al*., 1989).

Table (3): MIC values of *Pseudomonas aeruginosa* isolates to copper sulfate, mercury chloride, and lead nitrate in (µg/ml) concentrations.

Isolates	MIC of Copper sulfate	MIC of Mercury chloride	MIC of Lead nitrate
*Ps.*1	1600	2.7	3200
*Ps.*2	1750	54.3	3200
*Ps.*3	1750	86.4	3200
*Ps.*4	1600	86.4	3200
*Ps.*5	1750	43.2	3200
*Ps.*6	1750	54.3	3200
*Ps.*8	1750	86.4	3200
*Ps.*9	1750	86.4	3200
*Ps.*12	1600	54.3	3200
*Ps.*13	1750	43.2	3200
*Ps.*14	1750	86.4	800
*Ps.*15	1600	86.4	3200
*Ps.*16	1600	86.4	3200
*Ps.*17	1600	54.3	3200
*Ps.*19	1600	86.4	3200
*Ps.*20	3200	86.4	3200
*Ps.*21	1600	86.4	800
*Ps.*22	1600	2.7	2400
*Ps.*24	400	54.3	3200
*Ps.*25	1600	43.2	3200
*Ps.*26	1600	86.4	3200
*Ps.*27	1600	21.6	2400
*Ps.*28	800	43.2	2400
*Ps.*29	400	21.6	3200
*Ps.*30	1600	21.6	3200
*Ps.*31	1600	21.6	1600
*Ps.*32	1600	21.6	3200
*Ps.*33	1600	21.6	3200
*Ps.*34	1600	21.6	3200
*Ps.*36	1600	86.4	3200

*Ps.*37	800	21.6	3200
*Ps.*38	3200	21.6	3200
*Ps.*39	1600	43.2	١٦٠٠
*Ps.*40	1600	86.4	3200
*Ps.*41	1600	54.3	3200
*Ps.*42	1600	54.3	3200
*Ps.*43	1600	43.2	3200

Biofilm formation:

The biofilm formation by *Ps. aeruginosa* isolates was investigated. The results showed that 20/43 (47%) of isolates had biofilm (Table 4). Results also showed that all environmental isolates (3 isolates) and 17/40 (42.5%) of clinical isolates were biofilm producers. The relationship between biofilm production and heavy metal resistance (HMR) was studied. It was found that the HMR of *Ps. aeruginosa* isolates is not correlated with production of the biofilm. From these results, this study concluded that the increase of HM resistance was correlated with biofilm production for some (but not all) HM used.

Table (4): Biofilm production by *Pseudomonas aeruginosa* isolates recovered from clinical and environment samples

Isolate No.	A 492 (≥ 0.17)*	Biofilm production
Ps. 1	0.11	-
Ps. 2	0.20	+
Ps. 3	0.23	+
Ps. 4	0.17	+
Ps. 5	0.09	-
Ps. 6	0.11	-
Ps. 7	0.22	+
Ps.8	0.12	-
Ps. 9	0.22	+
Ps. 10	0.21	+
Ps.11	0.14	-
Ps. 12	0.11	-
Ps. 13	0.14	-
Ps. 14	0.19	+
Ps. 15	0.10	-
Ps. 16	0.15	-
Ps. 17	0.24	+
Ps. 18	0.14	-
Ps.19	0.11	-
Ps.20	0.20	+
Ps.21	0.16	-

No. of isolates	**A 492 (≥ 0.17)***	**Biofilm production**
Ps.22	0.09	-
Ps.23	0.09	-
Ps.24	0.17	+
Ps.25	0.20	+
Ps.26	0.26	+
Ps. 27	0.11	-
Ps.28	0.30	+
Ps.29	0.11	-
Ps.30	0.11	-
Ps. 31	0.16	-
Ps. 32	0.11	-
Ps. 33	0.12	-
Ps.34	0.20	+
Ps.35	0.14	-
Ps. 36	0.16	-
Ps. 37	0.29	+
Ps.38	0.24	+
Ps. 39	0.20	+
Ps.40	0.25	+
Ps.41	0.24	+
Ps.42	0.12	-
Ps.43	0.83	+

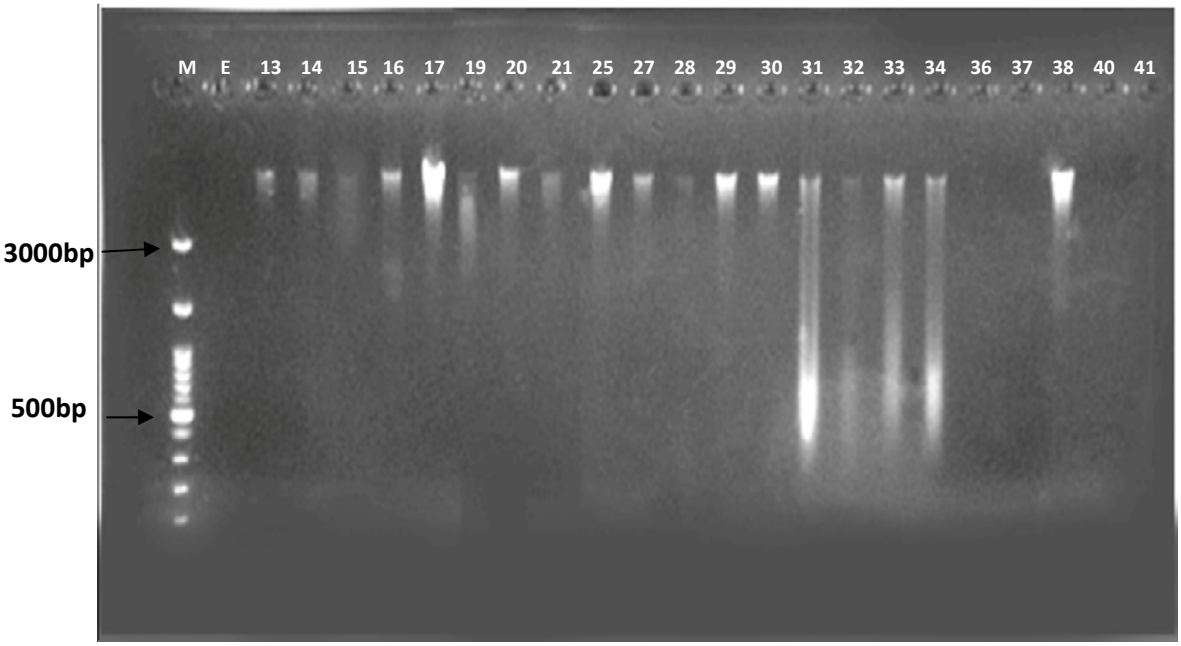
* The number between brackets indicates the standard value of biofilm production by ELIZA technique.

Plasmid profile and Curing of bacterial plasmids:

The plasmid content was investigated for all 37 isolates of *Ps. aeruginosa* (34 clinical and 3 environmental) (Figure 1). Results revealed that most isolates 32:37 harbored large (mega) plasmid with large (huge) molecular weight that couldn't be detected using 3000 bp size marker (ladder).

Many researchers worldwide reported that HMR in *P. aeruginosa* is carried on large (mega) plasmids. Raja and Selvam (2009) revealed isolate *P. aeruginosa* exhibited resistance to heavy metals such as cadmium, chromium, nickel and lead, due to the presence of plasmid DNA, which was designated as pBC15. The size of this plasmid DNA was approximately 23 kb, and they suggested that nickel and ampicillin resistance gene was conferred by plasmid DNA. Nikbin *et al* (2007) revealed that *P. aeruginosa* isolated from hospital in Tahran, Iran has plasmid with molecular weight 100 kbp. However in this study a large size marker (40000bp) was not available at the period of the study, so only 3000bp marker was used and all of the plasmid bands (in the gel) were out of ladder.

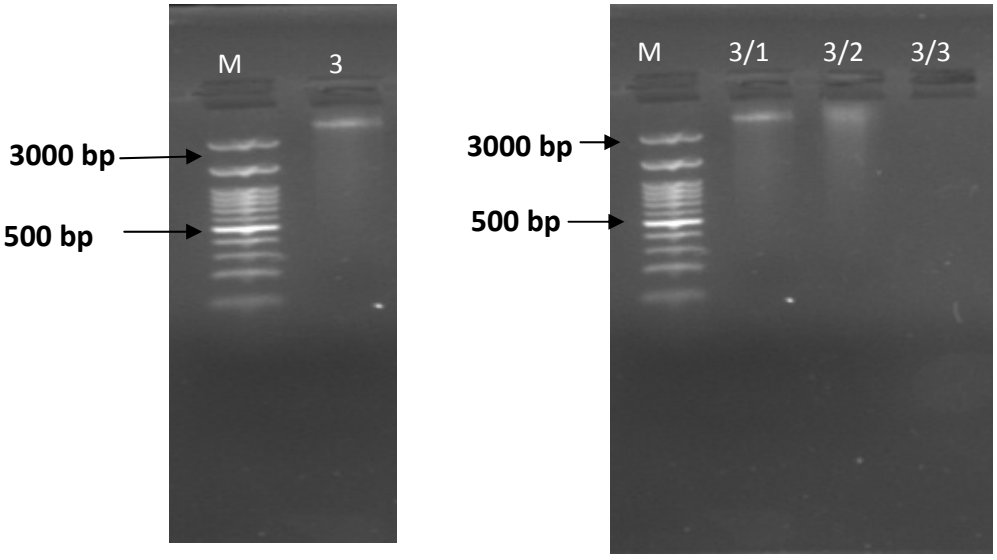
Figure (1): Gel electrophoresis of plasmid DNA content of *Ps. aeruginosa* isolates after (1:30) hr. at (60) voltage.



Lane (M): DNA molecular size marker (3000-bp ladder).
Lane (E): Show negative control (*E. coli standard strain MM294).*
Lanes: (P13), (P14), (P15), (P16), (P17), (P19), (P20), (P21), (P25), (P27), (P28), (P29), (P30), (P31), (P32), (P33), (P34), (P36), (P37), and (P38) shows clinical isolates.
Lanes :(P40), (P41) Show environmental isolates.

The bacterial curing was concluded for one isolate *Ps. aeruginosa* (*Ps.*3) (Figure 2). The results showed survived resistance to all HM. This result indicates that the HM resistance trait was carried on chromosome rather than plasmid. This could be due to that the plasmid is not cured out because it is really difficult to cure large mega plasmids. Many isolates of *P. aeruginosa* have no plasmid content and still show heavy metals resistance that lead to think that gene responsible for these resistances found on the chromosome (Raja and Selvam, 2009).

Figure (2): Gel electrophoresis of plasmid DNA content of *Ps. aeruginosa* isolate before and after curing after (1:30) hr. at(60) voltage.



Before curing **After curing**

Lane (M): DNA molecular size marker (3000-bp ladder).
Lane ($P_{3/1)}$: shows clinical isolate (first dilution).
Lanes ($P_{3/2}$): shows clinical isolate (second dilution).
Lanes ($P_{3/3}$): shows clinical isolate (third and last dilution).

References:

Abdul-Sada, H. K. (2008). A resistance study of *Pseudomonas aeruginosa* to Heavy Metals. Med. J. Babylon. 5 (1): 16-23.

Anonymous. (2002). Copper IUDs, infection and infertility. Drug Ther**.** Bull., 40: 67-69.

Christensen, G.D., Simpson, W.A., Younger, J.A., Baddour, L.M., Barrett, F.F., Melton, D.M. (1985). Adherence of coagulase negative *Staphyloccoci* to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol., 22:996-1006.

De Gracia, C. G. (2001). An open study comparing topical silver sulfadiazine and topical silver sulfadiazine-cerium nitrate in treatment of moderate and severe burns. Burns, 27:67-74.

Dong, Y.H., Wang, L.H., Xu, J.L., Zhang, H.B., Zhang, X.F., and Zhang, L.H. (2001). Quenching quorum-sensing-dependent bacterial infection by an n-acyl homoserine lactonase. Nature, 411:813–817.

Fluit, A. C., Verhoef, J. and Schmitz, F. J. (2000). The European sentray participants. Antimicrobial resistance in European isolates of *Ps. aeruginosa*. Eur. J. Clin. Microbiol. Infect. Dis. 19: 370-374.

Forbes, A. B. (1998). *Enterobacteriacaea*. Baily and Scotts diagnostic Microbiology. Baltimor, Mosby, pp: 509-526.

Fox, C.L. J.R., Monafo, W.W. J.R., Ayvazian, V.T.T., Skinner, A.M., Modaks, Stanford, J. (1977). Topical Chemotherapy for burn using cerium salts and silver sulfadiazine. Surg. Gynecol. Obstect., 144: 668- 672.

Gerald, E. (2007). Antisepsis, Disinfection and sterilization, types action and resistance. Treatment of skin and wound infections, 1st ed, Blackwell publishing, 156.

Harrison, J. J., Turner, R. J., and Joo, D. A. (2008). Copper and quaternary ammonium cations exert synergistic bactericidal and antibiofilm activity against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother., 52: 2870-2881.

Karbasizaed, V., Badami, N., and Emtiazi, G. (2003). Antimicrobial, heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in a hospital in Isfahan, Iran. Afr. J. Biotechnol., 2(10): 379-383.

Kenneth, A.A., and Jeffery, TSH. (2006). Manual of dermatologic therapeutics: with essential of

diagnosis. 7th ed, Lippincott Williams and Wilkins pp.314.

Kheder, A. K. 2002. Studies on antibiotic resistance by plasmid of *Pseudomonas aeruginosa*. Ph.D thesis. College of Education. University of Salahaddin, Iraq.

Nies, D. H., (2003). Efflux mediated heavy metal resistance in prokaryotes. FEMS Microbiol. Rev., 27 (2-3): 313-339.

Nikbin, V. S., Abd-Ali A., Feizabadi M. M., and Gharavi S. (2007). Indian J. Med. Res., 126: 146-151.

Nucifora, G., Chu L., Misra T. K., and Silver S. (1989). Cadmium resistance from *Staphylococcus aureus* plasmid pI258 cadA gene results from a cadmium-efflux ATPase. Proc. Natl. Acad. Sci. USA, 86:3544-3548.

O'Brien, P.A., Kulier, R., Helmerhorst, F.M., Usher-Patel, M., d'Arcangues, C. (2008). Copper-containing, framed intrauterine devices for contraception: a systematic review of randomized controlled trials. Contraception, 77: 318-327.

Outten, F. W., Outten, C. E., and O'Halloran, T. V. (2000). Metalloregulatory systems at the interface between bacterial metal homeostasis and resistance, pp: 145-157. In G. Stoz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, D.C.

Prasad, V. S., Mamatha, B., and Shivananda, P. G. (2009). Action of heavy metals on *Pseudomonas aeruginosa* strains isolated from infection wound. J. Chin. Clin. Med., 4 (3):132-135.

Raja, C. E., Selvam, G. S. (2009). Plasmid profile and curing analysis of *Pseudomonas aeruginosa* as metal resistant. Int. J. Environ. Sci. Tech., 6 (2): 259-266.

Riley, T.V. and Mee, B. J. (1982). Susceptibility of *Bacteroids* species to heavy metals Antimicrob. Agents Chemother., 22:889-892.

Sambrook, J. and Rusell, D. W. (2001). Molecular cloning. A laboratory manual. Third ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press, N.Y.8 (15):31-45.

Singh, V., Chauhan, P.K., Kanta, R., Dhewa, T., and Kumar, V. (2010). Isolation and characterization of *Pseudomonas* resistant to heavy metals contaminations, Int. J. Pharmaceutical Sci., 3(2): 164-167.

Teitzel, G. M. and Parsek, M. R. (2003). Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. Appl. Environ. Microbiol., 69: 2313-2320.

Vaca Pacheco, S., Raul, M. and Carlos, C. (1995). Inorganic-ion resistance by bacteria isolated from a Mexico City freeway. Antonie van leeuwenhoek, 67:333-337.

Xiao-xi, Z., Jian-xin, T., Xue-duan, L., and Pei, J. (2009). Isolation, identification and characterization of cadmium-resistant *Pseudomonas aeruginosa* strain E1. J. Cent. South Univ. Technol., 16: 0416-0421.

Ziebuhr, W., Heilmann, C., Gotz, F., Meyer, P., Wilms, K., Straube, E. and Hacker, J. (1997). Detection of the intercellular adhesion gene cluster (ica) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. Infect. Immun., 65: 890–896.

مقاومة الزوائف الزنجارية المعزولة من مصادر سريرية وبيئية للمعادن الثقيلة في مدينة الحلة، العراق

الملخص

تضمنت هذه الدراسة جمع ٣٠٠ عينة، ١٥٠ سريرية و ١٥٠ جمعت من بيئة المستشفى لغرض عزل بكتريا الزوائف الزنجارية. تم عزل ٤٣ (١٤,٣%) عزلة من الزوائف الزنجارية. تم إجراء مسح أولي للكشف عن العزلات المقاومة للمعادن باستخدام نترات الرصاص بتركيز (400 µg/ml) وأظهرت النتائج أن 37:43 من العزلات كانت مقاومة لهذا التركيز. تم الكشف عن حساسية هذه العزلات المقاومة لعدد من المعادن الثقيلة (كبريتات الفضة، كبريتات الزنك، كبريتات الكادميوم، كبريتات النيكل، كبريتات النحاس، كلوريدات الرئبق، ونترات الرصاص) من خلال تحديد التركيز المثبط الأدنى لهذه المعادن وبطريقتي التخفيف بالأكار وطريقة صب الأطباق وأظهرت النتائج ان معظم العزلات كانت مقاومة لهذه المعادن في بعض التراكيز. تم الكشف عن المحتوى البلازميدي للعزلات المقاومة للمعادن الثقيلة وأظهرت النتائج أن معظم العزلات ٣٢:٣٧ أحتوت على بلازميد عملاق. تم التحري عن انتاج الغشاء الحيوي للعزلات ووجد ان (٤٧%) من العزلات منتجة له. وأظهرت النتائج ان زيادة المقاومة للمعادن الثقيلة كان مرتبطا مع قابلية بعض العزلات على انتاج الغشاء الحيوي. أظهرت نتائج تحييد البلازميد البكتيري للعزلات بقاء المقاومة للمعادن الثقيلة بعد التحييد مما يشير الى ان صفة مقاومة المعادن الثقيلة محمولة على الكروموسوم وليست على البلازميد البكتيري.