Study and identify the genetic factors that causing resistance against a group of antibiotics in *Pseudomonas aeruginosa* isolated from clinical cases in Iraq

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ABSTRACT

290samples at different areas of the human body were collected from patients (clinical cases) of Diwaniyah Teaching Hospital , the samples distributed as 123 male and 167 female. It was isolated group of bacteria included: *Pseudomonas aeruginosa*, *Pseudomonas fluorescence*, *Proteus mirabilis*, *Proteus vulgaris*, *Escherichia coli*, *Klebsella pneumonia*,*Staphylococcus* sp., *Enterococcus* sp. and *Streptococcus* sp.it was isolated 278 isolation of bacterial included: Pseudomonas aeruginosa, Pseudomonas fluorescence, Proteus mirabilis, Proteus vulgaris, Escherichia coli, Klebsella pneumonia, Staphylococcus sp. , Enterococcus sp. and Streptococcus sp.

It was found that the females bacterial were highest, amounting to 159 (57.58%) compared to males, which reached 119 (41.03%) also the isolation of *P. aeruginosa* reached to 19 isolated by (6.551%) distributed to 9 (3.103%) female and 10 (3.448%) males respectively.

The sensitivity of bacterial isolates are used as a test to six antibiotics in health institutions and considered resistance varies to antibiotics, isolates of *P. aeruginosa* have given a high resistance to antibiotics Trimethoprim, Nitrufuration and Tetracycline as it was (100%, 100%, 87.5%) Respectively also it showed very weak resistance to the antibiotic Amoxicillin and Ciproflxacin reached to (12.5%, 15.7%), respectively.

The detected genes that encode to the antibiotics resistance in *P. aeruginosa* isolates is*nfsA* gene, *tetA* gene and *tri* gene.

Keywords: *P. aeruginosa,* antibiotics resistance, resistancegenes

ITRODUCTION

Pseudomonas aeruginosa is one of enterobacteriaceae causing a severe infections ,it characterized by a high resistance to enormous range of antibiotics,disinfctants,antiseptic and some physical conditions such as: drought and heat, the resistance *of P. aeruginosa* returned to effective metabolic mechanisms such as: producing of enzymes which break antibiotic molecule and make it inactive [1]this species infects the patients in hospitals who are exposed to non-antiseptic air and polluted by ADIS ,this species can also invade and still in the damaged tissues and causing bacteremia [2].

This bacteria has many virulence factors which is assist to infect the host body and overcoming the body defenses, the virulence factors are: Pills which diffuse on cell surfaces enable them to adhesion and colonization on the surfaces of the epithelial tissues of the host body[3].Capsules have slim layer contain from glycoprotein material this layer providing protection for bacterial cells ,this layer also forming biofilm[4]these bacteria are capable of producing three types of protease enzymes: Esterase , Lipase and phospholipase ,these enzymes give this bacteria the ability to invade the tissue by separating layer of fat between dermis and epidermis[5]these bacteria capable of producing gelatiniase enzymes ,this enzyme gives this bacteria the ability of hydrolyzed gelatin into amino acids as nutrients[6]. *P. aeruginosa* have another enzymes which convert antibiotics molecules into active less molecules, these enzymes encoded by genes located on chromosomes or on extrachromosomal genetic factors[7]also the acetylase enzyme works to acetylation OH groups antibiotics molecules and this characteristic of species [8].

The high resistance of *P.aeruginosa* because of lack of permeability of the cell membrane to many antibiotics giving it resistance to antibiotics that belong to same group[9].Lipopolysaccharide (LPS) plays an important role in the permeability of membranes of bacteria and it is responsible for antibiotics resistance in gram negative bacteria for wide range of antibiotics and any change in (LPS) affects the permeability of the cell membrane[10].

Nitrofuration resistance showed because of nitroductases enzymes activity these enzymes encoded by *nfsA* ,*nfsB*genes[11].Tetracycline used against gram negative bacteria because of this antibiotic is wide range drug , tetracycline target is bacterial ribosome and inhibit the protein synthesis[12] the most important proteins in ribosomal subunit 30s which have a high ability to bind with tetracyclinesare: 3s, 7s, 14s and 19s but tetracyclines bind directly with 7s protein[13].

There are some genes had been located in *P.aeruginosa*isolates such as: *tetA. tetB*and *tetC* these genes responsible for bacterial resistance against tetracyclinegrug[14].

The major mechanism for bacterial resistance against trimethoprim antibiotic is extra producing of (DHFR) enzymes these enzymes has been encoded by genes located on extrachromosomal genetic factors and this mechanism mainly observed in enterobacteriaceae[15].

MTERIALAND METHODS

SAMPLESCOLLECTION

290 different clinical samples collectedfrom patients included: wounds, ear, sputum, lactation and feces were distributed among 123 samples from males and 167 samples from females patients fromDiwaniyahHospital for the period from September 1\ 2015 until January 31\2016.

BACTERIAL ISOLATIONANDIDENTIFICATION

Specimens cultivated on enrichment and differential media the bacteria has been identified according to culture and phenotypic characteristics, microscopic tests and biochemical characteristics according to procedure which listed in [16].

ANTIBIOTICS SENSIVITY TEST

Six types of antibiotics have been tested against p. aeruginosa the antibiotics are: Amoxicillin, Amikacin, Gentamycin, Trimethoprim, Nitrufuration, Tetracycillin according to procedure which listed in[17].

POLYMERASECHAINREACTION

The polymerase chain reaction has been done to investigate about antibiotics resistance genes existing which responsible for *P.aeruginosa* isolates resistance against the antibiotics according to procedure which listed in [18]. The primers have been designed by NCB1-Gebank Database web site and Primer3plus program .The primers have been prepared by the Korean BioneerCompany (Table1).

Table1. Primers that used in this study and their sequences .

Primer	Sequence		Amplicon
nfsA gene	F	GGGTATATATCGGCGGCCTG	245bp
	R	ATTATTGCTGCCACGGGTGA	
tetA gene	F	GCCGATATCACTGATGGCGA	323bp
	R	ACCTGTCCGACAAGTTGCAT	
Tri gene	F	TGTGATTGTGTCTGGTGGTGG	300bp
	R	TAGTGCATCTAACGCCTGGC	

RESULTSAND DISCUTIONS

19 *P. aeruginosa*isolates have been identified according to phenotypic characteristics and chemical tests .The results have been ensured by Api20E identifying system kit.

The isolates disrupted into 9 (6.55%) isolates from males and 10 (3.44%) isolates from females (Table2).

Table2. The number and ratio of bacterial species and the source of isolates.

Isolated bacteria	Isolates of bacterial species		Male source isolates		Female source isolates	
	No.	%	No.	%	No.	%
Ps. aeruginosa	19	6.551	9	3.103	10	3.448
Ps. fluorescence	28	9.655	11	3.793	17	5.862
Pr. meriabilbs	24	8.275	10	3.448	14	4.827
Pr. vulgaris	28	9.655	15	5.172	13	4.482
E. coli	61	21.034	23	7.9318	38	13.103
Staphylococcus sp.	51	17.586	18	6.206	33	11.379
Enterococcus sp.	17	5.862	6	2.068	11	3.793
Kl. peneumonia	12	4.137	7	2.413	5	1.724
Strptococcus sp.	38	9.655	20	6.896	18	6.206

The results have been explained that the bacterial resistance against Amikacinand Amoxicillin antibiotics in *P. aeruginosa* isolates arereached to 4.166%,this result conformity of [19],and

against Gentamycin and Nitrufurationare reached to 8.33% and 100% this result agree with [20],the resistance against Tetracyclineand Trimethoprim are reached to 87.5% and 100% and this result conformity with [21,22].

The results of PCR have been explained that the genes which responsible for antibiotics resistance are exist in *P. aeruginosa* isolates as following:

The *nfsA* gene is responsible for resistance against Nitrufuration, this gene has been detected in all isolates(the resisted isolates), the bundle 245bp has been showed in the electrophoresis,this prove that all isolates have this gene (Figure1).

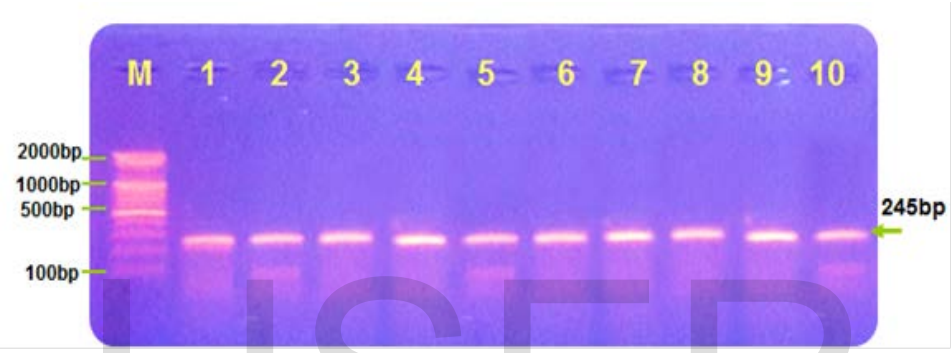

Figure1.Agarose gel Electrophoresis that contain PCR results .M represent Marker (100-2000bp) ,the pits (1-10) represent the result of some positive isolates for 245bp bundle.

The existing of this gene in *P. aeruginosa* isolates is conformity of [23].This gene encodes for Nitroductases these enzymes divided into two groupsfirst group which reduces nitro compounds with oxygen (Oxygen-investiveNitroductases) and another group which reduce nitro compounds without oxygen [24].There are several types of *nfs* genes such as: *nfsA* and *nfsB*, *nfsA* described as the major oxygen-investivenitroductase which requires NADPH and shows low activity with NADH,*nfsB* described as the minor oxygen-investivenitroductase [23].

The *tetA* gene is responsible for resistance against Tetracycline,this gene has been detected in 17 isolates (the resisted isolates) .The bundle 323bp has been showed in the electrophoresis, this prove that these isolates have this gene (Figure2).

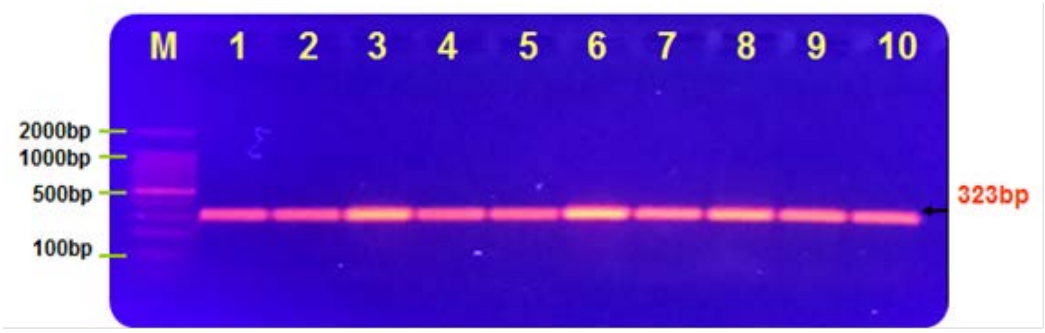

Figure2.Agarose gel Electrophoresis that contain PCR results .M represent Marker (100-2000bp) ,the pits (1-10) represent the result of some positive isolates for 323bp bundle.

The *tetA* gene encodes to efflux proteins that eject the antibiotic molecules outside the bacterial cell that makes the constriction of antibiotic inside the bacterial cell lower so that makes the cell resistance ,*tetA*,*tetC*,*tetD* genes encode to efflux proteins which give the resistance character against tetracyclines but not against minocyciline, so the*tetB* gene gives the resistance character against both of tetracyclines and minocyciline[25].

The *tri* gene is responsible for resistance against Trimethoprim, this gene has been detected in all isolates (the resisted isolates), the bundle 300bp has been showed in the electrophoresis,this prove that these isolates have this gene (Figure3).

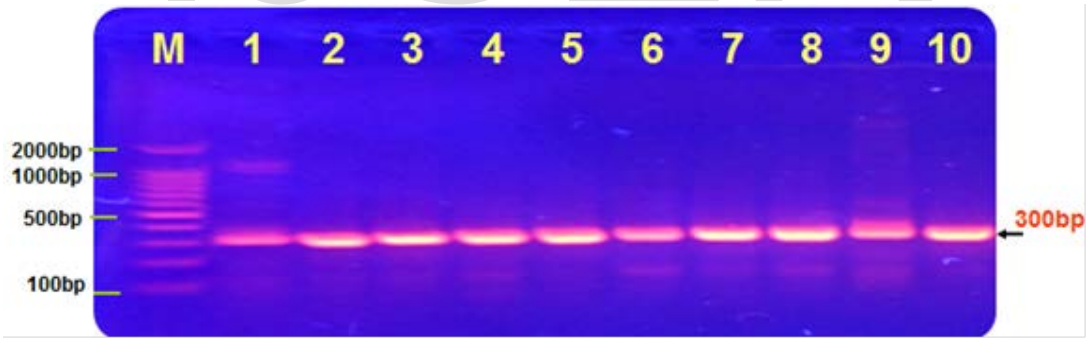

Figure3.Agarose gel Electrophoresis that contain PCR results .M represent Marker (100-2000bp), the pits (1-10) represent the result of some positive isolates for 300bp bundle.

Trimethoprim inhibits selectivelythe bacterialdihydrofolate-erductase (DHFR) that leads to obstruct reducing the dihydrofolate totetrohydrofolate so that theTrimethoprim used in place of Trimethoprim-sulfamethoxazole to treat UTIs infections [26]. There are several mechanisms to resist trimethoprim have been detected but the major mechanism is the extra producing of DHFR

enzyme which encoded by genetic factors such as plasmids and transposons this mechanism is common in gram negative bacteria [27].

We can conclude that *P. aeruginosa*isolates existing is low 6.55% and this presence high in females isolates if we compare with males isolates.As this isolates have multi-resistance against a group of antibiotics this resistance is encoded by resistance genes. Our commandment is the studies should be aboutanother clinical case and investigate about the capability of bacteria on make bacteremia and invade the brain and important organs and detect the genetic reason behind the multi-resistance against the new antibiotics.

REFERENCES

1- **Fraimow, H. S. and Abrutyn, E. (1995). "Pathogens resistant to antimicrobial agents, Epidemiology, molecular mechanisms and clinical management", Infect Dis Clin North Am., Vol. 9, pp. 497-530.Rozalski, A.; Sdorczyk, Z. and Kotelko, K. (1997).** Potential virulence factors of Proteus bacilli. Microbiol. Mol. Bio. Rev. 61: 65-89.

2- **Davey, M. E., and G. O. O'Toole. (2000).** Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Revs. 64(4):847-67.

3- **Brooks, G. F.;Butel, J. S. and Morse, S. A. (2001).***Pseudomonas*,*Acinetobacters*, and uncommon gram – negative bacteria .In :Jawet'z , Melnick and Adelberg,s Medical microbiology. 22nd ed . Lange medical books / McGraw – Hill. Medical publishing division .

4- **Todar, K. (2005).** Todar's Online Textbook of Bacteriology .University of Wisconsin-Madison Department of Bacteriology.

5- **Horn, M.P.; Zuercher, A.W.; Imboden, M. A.; Rudolf, M. P.; Lazar, H.; Wu, H.; Hoiby, N.; Fas, S. C. and Lang, A.B. (2010).** Preclinical In Vitro and In Vivo Characterization of the Fully Human Monoclonal IgM Antibody KBPA101 Specific for Pseudomonas aeruginosa Serotype IATS-O11.Antimicrob. Agents Chemother.,54(6):2338-2344 .

6- **Andrejko, M. ;Zdybicka-Barabes, A. ; Janczarek, M. and Cytrynska, M. (2013).** Three Pseudomonas aeruginosa strains with different protease profiles. on-line at: www.actabp.pl. 60(1): 83-90.

7- **Lepper , Y. C. ; Ahn , B. J. and Jin , J. S. (2002).** Molecular characterization of Pseudomonas aeruginosa isolate resistant all antimicrobial agents. But susceptible to colistin , in Daegu . Korea. J. Microbiol. 7:1969-1971.

8- **Westwood, S.; Tommassen, J. and Jaeger, K.E. (2005).** A novel lipolytic enzyme located in the outer membrane of Pseudomonas aeruginosa. J. Bacteriol. 181(22): 6977-6986 .

9- **Horn, M.P.; Zuercher, A.W.; Imboden, M. A.; Rudolf, M. P.; Lazar, H.; Wu, H.; Hoiby, N.; Fas, S. C. and Lang, A.B. (2010).** Preclinical In Vitro and In Vivo Characterization of the Fully Human Monoclonal IgM Antibody KBPA101 Specific for Pseudomonas aeruginosa Serotype IATS-O11.Antimicrob. Agents Chemother.,54(6):2338-2344 .

10- **Ernst, R. K.; Adams, K. N. et al. (2006).** The Pseudomonas aeruginosa lipid Adeacylase: Selection for expression and loss within the cystic fibrosis airway. J. Bacteriol., 188: 191-201.

11- **Zenno, S., T. Kobori, M. Tanokura, and K. Saigo. (1999).** Purification and characterization of NfrA1, a Bacillus subtilis nitro/flavinreductase capable interacting with the bacterial luciferase. Biosci. Biotechnol. Biochem. 62:1978–1987.

12- **Levy, S.B. (1992).** The Antibiotic Paradox: How Miracle Drugs are Destroying the Miracle. Plenum Press, NewYork.

13- **Buck, M.A. and Cooperman, B.C. (1990).**Singl e protein omission reconstitution studies of tetracycline binding to the 30 S subunit of Escherichia coli ribosomes. Biochemistry 29, 5374-5379.

14- **Jones,C.S.,Osborne,D.J.andStanley,J.(1992).**Enterobacterial tetracycline resistance in relation to plasmid incompatibility.Mol. Cell. Probes 6, 313-317.

15- **Heikkila¨, E., O. Renkonen, R. Sunila, P. Uurasma, and P. Huovinen. (1990).**The emergence and mechanisms of trimethoprim resistance in Escherichia coli isolated from outpatients in Finland. J. Antimicrob. Chemother. 25:275–283.

16- **Mcfaddin, J.F. (2004).** Biochemical test for identification of medical bacteria, 2nd ed. Waverly Press., Baltimore.

17- **Goldstein, F.W., Kitzis, M.D. and Acar, J.F. (1994).**N,Ndimethylglycyl-amido derivative of minocycline and 6-demethyl-6-desoxytetracycline, two new glycylcyclines highly effective

against tetracycline-resistant Gram-positive cocci. Antimicrob. Agents Chemother. 38, 2218-2220.

18- **Sloan, J., McMurry, L.M., Lyras, D., Levy, S.B. and Rood,J.I. (1994).** The ClostridiumperfringensTet P determinant comprises two overlapping genes: tetA(P), which mediates active tetracycline efflux, and tetB(P), hich is related to the ribosomal protection family of tetracycline-resistance determinants. Mol. Microbiol. 11,403-415.

19- **Raka, L.; Mulliqi,-Osmani, G.; Berisha, L.; Begolli, L.; Omeragiq, S.; Parsons, L.; Salfinger, M.; Jaka, A.; Kurti, A.andJakupi, X.(2004).** Etiology and susceptibility of urinary tract isolates in Kosova. Int. J.Antimicrob. Agents, 23(1): 2-5.

20- **Bendough , Z . ; Reddy, A . ; Simmons , W. and Sesrosiers , M . (2006).** Biofilm formation by some enterobacteriaceae associated with an unfavorable evolution after surgery. J. med .microbial .33 : 371 -922.

21- **Fluit. A.C.; Visser, M.R.and Schmitz, F.J. (2001).** Molecular detection of antimicrobial resistance. Clin. Microbiol. Rev., 41(4):836-871 .

22- **Kipnis , E.** ; Sawa, T. ; and Wiener-**Kronish , J.(2006).**Targeting mechanisms of Pseudomonas aeruginosapathogenesis. Médecine et maladies infectieuses .36 :78–91 .

23- **Jorgensen, M. A., M. A. Trend, S. L. Hazell, and G. L. Mendz. (2001).** Potential involvement of several nitroreductases in metronidazole resistance in Helicobacter pylori. Arch. Biochem. Biophys. 392:180–191.

24- **Petersen. (2005).** Initiation of protein synthesis in bacteria. Microbiol. Mol.Biol. Rev. 69:101–123.

25- **Benveniste, R. and Davies, J. (1973).** Aminoglycoside antibiotic-inactivation enzymes in actinomycetes similar to those present in clinical isolates of antibiotic resistant bacteria.Proc. Natl. Acad. Sci, USA 172, 3628-3632.Biofilm Produced by some Uropathogens Isolated from Patients.

26- **Cotton,L. A.** ; Graham, R. J. and. Lee, R. J.(2009). The Role of Alginate in P. aeruginosa PAO1 Biofilm Structural Resistance to Gentamicin and Ciprofloxacin. Journal of Experimental Microbiology and Immunology(JEMI). 13:58-62 .

27- **Engel, J. N. (2003).** Molecular pathogenesis of acute Pseudomonas aeruginosa infections. Severe Infections Caused by Pseudomonas aeruginosa. A. R. Hauser and J. Rello. Dordrecht, Kluwer Academic Publsihers, 201-229 .

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