Isolation and detection of pseudomonas aeruginosa from post-surgery operatives wound in Babylon provine hospital/ Iraq

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Abstract—A study was carried out to assess isolation and detection of*Pseudomonas aeruginosa* from post-surgery operatives wounds in Babylon Province Hospitals (General Al-Hashemeyah hospital and Al-Shomali General Hospital). **Methods:** 100 specimens were taken from post operative surgery wounds. The identity of isolates was confirmed by staining method (Gram stain) , culturing (Nutrient,Macconkey,Blood and TSA agars), biochemical tests (Catalase , Oxidase, Citrate, Methyl red, Voges-Proskauer,Indole and Lactose fermentation) and Antibiogramtests. **Results:** from 100 samples, 24 isolates of *Pseudomonas aeruginosa*were collected. The biochemical activities were positive in Catalase , Oxidase and Citrate and negative in Methyl red, Voges-Proskauer,Indole and Lactose fermentationwere fixed as features of it. Among the variety of antibiotics tested, the highests resistance were found with Ampicillin (75%) and Oxytetracycline (70.8%) followed by Amoxicillin (66.6%) and Tetracycline (62.5%), On the other hand, both the isolates were intermediately sensitive to Gentamicin (62.5%), Kanamycin (58.3%) and Erythromycin (54.1%).while the isolates were highly sensitive to Chloramphenicol (70%) and Ciprofloxacin (66.6%). **Conclusion:** *P. aeruginosa* one of the most important pathogenic bacteria that infect the post-operative wounds.and in some cases even with antibiotic therapy. All the human ages may undergo the infection with *P. aeruginosa* with different immunity conditions.Reccurent uses of the same antibiotics may be lead to bacterial resistance because the multi-antibiotics resistance mechanism of *P. aeruginosa*.Hyagenic managment include Sterilization and Disinfectant methods which established in some hospitals are not enough to prevent the infection of pathogenic bacterial as nasocomial infections.

Index Terms— *Pseudomonas aeruginosa*, nasocomial infections, ß-lactamase production.

1 INTRODUCTION

Pseudomonas aeruginosa is a gram negative, rod-shaped bacterium, cells measure 0.5 to 1.0 µm by 3 to 4 µm. It is an obligate aerobe and forms smooth round colonies with a fluorescent greenish color. It often produces the non-fluorescent bluish pigment pyocyanin, which diffuses into the agar. Other *Pseudomonas* species do not produce pyocyanin. Many strains of *P. aeruginosa* also produce the fluorescent pigment pyoverdin, which gives a greenish color to the agar, Some strains produce the dark red pigment pyorubin or the black pigment pyomelanin.(1)

P. aeruginosa has a relatively large genome that presumably promotessurvival in diverse habitats(2). In addition to inhabiting a wide variety of environmental positions, *P. aeruginosa* is also a multi-host pathogen, capable of infecting hosts as divergent as amoebae, plants, insects, flies, nematodes, and mice (3).

Extensive studies have shown that *P. aeruginosa* is armed with a large arsenal of virulence factors enabling it to breach the human innate immune system, to intoxicate host cells, and to modulate human adaptive immune mechanisms, serving the goal of establishing systemic infection or more localized, chronic colonization (4).

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A) Extracellular toxins

First, *P. aeruginosa* virulence factors include a variety of extracellular toxins (exotoxin A, phospholipase C, elastase) that could cause extensive damage to host tissues through their enzymatic activities. Obviously these factors play important roles in the acute infections (e.g., in burn patients, where massive tissue damage and septicemia are the most common manifestation)(5).

B) Flagellum, pilus and alginate

Another group of virulence factors are the attachment and motility organelles, including flagella (swimming), pili (twitching), and extropolysaccharide (alginate).

These factors give the bacterium the ability to attach to host cells and move around the host environment, facilitating colonization and immune avoidance. *P. aeruginosa* usually possesses a single polar flagellum, responsible for the primary function of bacterial motility .Interestingly, the flagellar cap protein has been shown to be involved in the adherence of *P. aeruginosa* to the human respiratory mucin, which is important for the initial colonization of the Cystic Fibrosis (CF) patient's airway (6). Pseudomonal pili also are polarly localized motility/adherence organelles, responsible for twitching motility , specific adherence to eukaryotic host cells, and nonspecific binding to other surfaces, where these pili could mediate close contact and bacterial colonization.One distinctive feature of *P. aeruginosa* pathogenicity is the existence of the mucoid phenotype, a condition where bacterial cells overproduce extracellular polysaccha-

ride alginate during colonization of the CF patient's lung. It has been suggested that alginate may enhance *P. aeruginosa* virulence/survival in several ways (7).

C) Type III secreted exotoxins

In recent years, *P. aeruginosa* has been shown to have the so-called type III secretion system (TTSS), These protein factors can be directly distributed to the host cells; and once transferred, can elicit various host responses, facilitating successful dissemination and infections.

D) Biofilm

P. aeruginosa has been shown to be able to form a biofilm in various environments. Biofilms have been known to have a rather complex structure with differentiated bacterial populations, and increased resistance against hostile environmental factors, including host immune mechanisms and therapeutic treatments such as antibiotics (8). Evidence indicates that *P. aeruginosa* forms a biofilm in (CF) lungs where the bacterium lives in an anaerobic environment, as opposed to the aerobic biofilm formed in laboratory conditions (9). *Pseudomonas aeruginosa* is a highly adaptable microorganism able to tolerate low oxygen conditions. It can survive with low levels of nutrients and grow in temperatures ranging from 4-42°C (10). These characteristics allow it to attach itself and survive on medical equipment and on other hospital surfaces,which favors the beginning of infections in immunocompromised patients(11).One likely reason that P. aeruginosa is a common nosocomial pathogen is because it is capable of thriving in a wide variety of environmental niches, including surfaces in hospital rooms, water, soil and plants(12).

Pseudomonas aeruginosa is an important cause of both communityacquired and hospital-acquired infections (13). Community-acquired infections include among others ulcerative keratitis (usually associated with contact lens use), otitis externa, skin and soft tissue infections (including diabetic foot infections). Hospitalized patients may be colonized with *P. aeruginosa* on admission or during hospital stay. Nosocomial infections include pneumonia, urinary tract infections, bloodstream infections, surgical site infections and infection of the skin after burn injuries. *P. aeruginosa* is the leading cause of respiratory tract infections with patients which are intubated during a hospital stay and has a high mortality of 40% to 50%.*P. aeruginosa* infections also occur in immunocompromised patients e. g. AIDS especially in patients who have a compromised phagocytic system (14). Wound infections due to *P.aeruginosa* are especially difficult in burn patients. A high percentage of the wound infections will lead to sepsis with significant mortality rates. *P. aeruginosa* infections of the eye usually follows minor trauma to the cornea.These infections are frequently associated with contact lens use.

Post-operative wound infection or surgical site infection is an important cause of health care associated infections among surgical patients. Over the last few decades, a steady increase in drug resistant *P. aeruginosa* strains has made antibiotic treatment more difficult (15). In part because no new antibiotics effective against *P. aeruginosa* are imminentlyavailable as treatment options, the pressing need for drugs to fight this pathogen has focused study on its virulence factors as potential drug targets, and more generally active a search for novel antiinfectives(16,17) . *P. aeruginosa* produces various mechanisms of resistance to antibiotics such as broad-spectrum b-lactamases, alteration of protein binders of penicillin (PBP), porin mutations, plasmid enzymatic modification, DNA-gyrase mutation and active expulsion pumps(18,19).

2 MATERIALS AND METHODS

2.1 specimens

One hundreds specimens were take as from patients in General Al-Hashemeyah hospital and Al-Shomali General Hospital. These specimens distributed according to the age and gender as in table (1) and according to the inpatients and outpatients as in table (2).

TABLE 1
DISTRIBUTION OF THE CASES ACCORDING TO AGE AND GENDER

Ages / Gender	1-10 years	11-20 years	21-30 years	31-40 years	41-50 years	51-60 years	61-70 years	Total
Male	7	6	5	2	6	2	0	28
Female	1	19	36	10	3	1	2	72
Total	8	25	41	12	9	3	2	100

TABLE 2
DISTRIBUTION OF THE CASES ACCORDING TO INPATIENTS AND OUTPATIENTS.

Ages	Inpatients		Outpatients	
	Male	Female	Male	Female
1-10 years	6	1	1	0
11-20 years	2	23	0	0
21-30 years	3	34	2	2
31-40 years	1	7	1	3
41-50 years	4	2	2	1
51-60 years	1	1	1	0
61-70 years	0	2	0	0
Total	17	70	7	6

2.2 identification of samples

The samples were streaked on nutrient agar plates and the plates were incubated at 37 oC for 24 hours, then the characteristic suspected single colonies were subjected to Gram's staining and then sub-cultured in MacConkey agar, blood agar and Tryptose Soya Agar (TSA agar) at 42oC. The pure isolates of Pseudomonas aeruginosa were transferred to 1% nutrient agar slant and stored in the refrigerator at 4 oC. P. aeruginosa was identified by biochemical tests (Catalase, Oxidase, Citrate, Methyl red, Voges-Proskauer, Indole and Sugar fermentation) (20).

2.3 Antiobiogram Testing

Antimicrobial susceptibilities were determined by Kirby-Bauer disk diffusion according to the CLSI recommendation (21). The antibiotic disks used were as follow: Ampicillin (AMP.)10 unit, Tetracycline (TET)30 µg, Oxytetracycline (OXY.)10 µg, Amoxicillin(AMX.)10 µg, Chloramphenicol (CHL) 30µg, Ciprofloxacin (CIP.) 5 µg, Gentamicin (GEN.)10 µg, Erythromycin (ERY.) 15 µg and Kanamycin (KAN.) 30 µg.

3 RESULTS

3.1 speciems

The Pseudomonas aeruginosa isolates were distributed as in table (3).

TABLE 3
DISTRIBUTION OF PSEUDOMONAS AERUGINOSA ISOLATES

Ages / Gender	1-10 years	11-20 years	21-30 years	31-40 years	41-50 years	51-60 years	61-70 years	Total
Male	2	1	1	0	2	1	1	8
Female	1	3	5	3	2	2	0	16
Total	3	4	6	3	4	3	1	24

3.2 Identifcation of the Bacteria

By culture, P. aeruginosa produces circular mucoid smooth colonies with emits sweat grape odor in nutrient agar. On TSA agar, the colonies were show circular shape of colonies with undulate margin with blue-green color.

All isolates produced β-hemolysis on blood agar and grew on MacConkey agar, but did not ferment lactose sugar. By gram staining, the morphology of isolated P. aeruginosa showed Gram-negative, pink colored, medium rod shaped appearance. Isolates of P. aeruginosa were found to be motile when examined using hanging drop slide under microscope. Results of biochemical test were positive in Catalase , Oxidase and Citrate and negative in Methyl red, Voges-Proskauer,Indole and Lactose fermentation.

3.3 Antibiogram Test

The response of P. aeruginosa isolates agaist the antibiotics were showed in table 4.

TABLE 4
ANTIBIOTIC SENSITIVITY PATTERN OF THE ISOLATES OF PSEUDOMONAS AERUGINOSA.

Antibiotics	Disc Concentration (µg /disc)	Bacterial Isolates		
		Resistant	Intermediate	Sensitive
Ampicillin	10 unit	18 (75%)	4 (16.7%)	2 (8.3%)
Tetracycline	30 µg	15 (62.5%)	6 (25%)	3 (12.5%)
Oxytetracycline	10 µg	17 (70.5%)	4 (16.7%)	3 (12.5%)
Amoxicillin	10 µg	16 (66.6%)	5 (20.8%)	3 (12.5%)
Chloramphenicol	30 µg	3 (12.5%)	4 (16.7%)	17 (70.5%)
Ciprofloxacin	5 µg	3 (12.5%)	5 (20.8%)	16 (66.6%)
Gentamicin	10 µg	2 (8.3%)	15 (62.5%)	7 (29.2%)
Erythromycin	15 µg	5 (20.8%)	13 (54.2%)	6 (25%)
Kanamycin	30 µg	3 (12.5%)	14 (58.3%)	7 (29.2)

4 DISCUSSION

By culture, *P. aeruginosa* produces circular mucoid smooth colonies with produces sweat grape odor in nutrient agar and the blue-green appearnace of the colonies on TSA agar because the productio of pyocyanin pigment, these finding granted with those ofLau *et al.,*(2004) (22). Also the producing of β-hemolysis on blood agar and growing on MacConkey agar with no lactose sugar fermentation. These characteristics colonies were similar with finding of Haleem *et al.,* (2011) (23).

The morphology of isolated *P. aeruginosa* with gram staining showed Gram-negative, pink colored rods with medium size appearance.These findings agreed with the findings reporteby Tripathi *et al.,* (2011) (24). The Pseudomonal isolates appeared motile with hanging drop method because it has pili that help it in movement, This finding similar to that which fixed by Quinn *et al.,* (2002) (25).

Results of biochemical tests granted with that which established byMeyer *et al* .,(2002) (26) .

Antibiogram test means, the test that is done in laboratories or in in-vitro condition to detect the sensitiveness of antibiotics against certain bacteria which is responsible for specific disease. On the other hand this test is done to measure the ability of antibiotics to prevent the growth of bacteria under in-vitro condition or in a suitable environment or outside the body. Being Gram-negative bacteria, most *Pseudomonas spp.* are naturally resistant to penicillin and the majority of related beta-lactam antibiotics, but a number are sensitive to chloramphenicol andciprofloxacin. Aminoglycosides such as gentamicin, and amikacin are other choices for therapy (27).

P. aeruginosa is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. One of its most worrying characteristics is its low antibiotic susceptibility. This ability to thrive in harsh conditions is a result of their hardy cell walls that contain porins. Their resistance to most antibiotics is attributed to efflux pumps, which pump out some antibiotics before they are able to act.*P. aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. One of its most worrying characteristics is its low antibiotic susceptibility (28). This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (e.g., *mexAB-oprM*, *mexXY*, etc.,). and the low permeability of the bacterial cellular envelopes. Besides intrinsic resistance, *P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer ofantibiotic resistance determinants. Some recent studies have shown phenotypic resistance associated to biofilm formation or to the emergence of small-colony-variants may be important in the response of *P. aeruginosa* populations to antibiotic treatment (29).

All isolates of *P. aeruginosa* were investigated for susceptibility and resistance patterns by disc diffusion method using 9 antibiotics. Among the variety of antibiotics tested, the highests resistance were found with Ampicillin (75%) and Oxytetracycline (70.8%) followed by Amoxicillin (66.6%) and Tetracycline (62.5%) (Table 4). These findings were more or less similar to other researchers Ferguson *et al.,* (2007) (30) where the authors concluded that 100% *P. aeruginosa* were resistant to Ampicillin, Amoxicillin and Tetracycline.

On the other hand, both the isolates were intermediately sensitive to Gentamicin (62.5%), Kanamycin (58.3%) and Erythromycin (54.1%). These results were nearly comparable with Tripathi *et al.,* 2011 where the authors found that all the clinical isolates of *P. aeruginosa* were sensitive to variety of antibiotics including gentamicin.

most isolates of *P. aeruginosa* were highly sensitive to Chloramphenicol (70%) and Ciprofloxacin (66.6%). These results of antibiotic sensitivity test were similar with Corona-Nakamura *et al.,* 2001(31) where the authors interpreted that *P. aeruginosa* absolutely sensitive to Ciprofloxacin.

7.3 Additional Formatting and Style Resources

Additional information on formatting and style issues can be obtained in the IJSER Style Guide, which is posted online at: http://www.ijser.org/. Click on the appropriate topic under the Special Sections link.

4 Conclusion

1. P. aeruginosa one of the most important pathogenic bacteria that infect the post-operative wounds.and in some cases even with antibiotic therapy.
2. All the human ages may undergo the infection with P. aeruginosa with different immunity conditions.
3. Reccurent uses of the same antibiotics may be lead to bacterial resistance because the multi-antibiotics resistance mechanism of P. aeruginosa.
4. Hyagenic managment include Sterilization and Disinfectant methods which established in the hospitals are not enough to prevent the infection of pathogenic bacterial as nasocomial infections.

Recommendations:

The excessive focusing on the molecular biology study specially that related to the resistant genes of the pathogenic bacteria like P. aeruginosa to reduce the antibiotics resistance.

Appropriate hygienic management should be establish in the hospitals to reduce the incidance of the nasocomial infections including post-operative infections.

References

[1] J.S. Bridle, "Probabilistic Interpretation of Feedforward Classification Network Outputs, with Relationships to Statistical Pattern Recognition," *Neurocomputing – Algorithms, Architectures and Applications,* F. Fogelman-Soulie and J. Herault, eds., NATO ASI Series F68, Berlin: Springer-Verlag, pp. 227-236, 1989. (Book style with paper title and editor)

[2] .Tatterson, L. E.; Poschet, J. F.; Firoved, A. ; Skidmore, J. and Deretic,V. **(**2001). CFTR and *Pseudomonas* infections in cystic fibrosis. Front Biosci. **6:**D890-7.

[3] Pukatzki, S.; Kessin, R. H.; Mekalanos, J. J. (2002). The human pathogen *Pseudomonas aeruginosa* utilizes conserved virulence pathways to infect the social amoebaDictyostelium discoideum. Proc. Natl. Acad. Sci. U. S. A. 99: 3159–3164.

[4] Apidianakis, Y.; Rahme, L. G. (2009) Drosophila melanogaster as a model host forstudying Pseudomonas aeruginosa infection. Nat Protoc 4: 1285–1294.

[5] Lau, G. W.; Goumnerov, B. C.; Walendziewicz, C. L.; Hewitson, J.; Xiao, W. et al.(2003). The Drosophila melanogaster toll pathway participates in resistance toinfection by the gram-negative human pathogen Pseudomonas aeruginosa. Infect.Immun. 71: 4059–4066.

[6] Bitter, W. (2003). Secretins of *Pseudomonas aeruginosa*: large holes in the outer membrane. Arch Microbiol. **179:**307-14. Epub 2003 Mar 28.

[7] Fleiszig, S. M.; Arora, S. K.; Van, R. and Ramphal,R. (2001). FlhA, a component of the flagellum assembly apparatus of *Pseudomonas aeruginosa*, plays a role in internalization by corneal epithelial cells. Infect Immun. **69:**4931-7.

[8] Mattick, J. S.(2002). Type IV pili and twitching motility. Annu Rev Microbiol. **56:**289-314.

[9] Mah, T. F.; Pitts, B. ; Pellock, B. G.; Walker, C.; Stewart, P. S. and O'Toole, G. A.**(**2003). A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature. **426:**306-10.

[10] Yoon, S. S.; Hennigan, R. F.; Hilliard, G. M.; Ochsner, U. A.; Parvatiyar, K.; Kamani, M. C.; Allen, H. L.; DeKievit, T. R.; Gardner, P. R.; Schwab, U.; Rowe, J. J.; Iglewski, B. H.; McDermott, T. R.; Mason, R. P.; Wozniak, D. J.; Hancock, R. E.; Parsek, M. R.; Noah, T. L.; Boucher, R. C. and Hassett.D. J. (2002). *Pseudomonas aeruginosa* anaerobic respiration in biofilms: relationships to cystic fibrosis pathogenesis. Dev Cell. **3:**593-603.

[11] Stover, C. K.; Pham, X. Q.; Erwin, A. L.; Mizoguchi, S. D.; Warrener, P. ;Hickey, M. J. et al. (2000). Complete genome sequence of *Pseudomonasaeruginosa* PAO1, an opportunistic pathogen. Nature406:959-964.

[12] Engel, J. and Balachandran, P. (2009). Role of *Pseudomonas ae-*

*ruginosa*type III effectors in disease. Curr Opin Microbiol.12:61-66.

[13] Lederberg, Joshua et al.(2000). *Pseudomonas*. Encyclopedia of Microbiology. Second Edition. Volume 3. San Diego,. p. 876-891.

[14] Driscoll, J. A.; Brody, S. L. and Kollef, M. H. (2007). The Epidemiology, Pathogenesis and Treatment of Pseudomonas aeruginosa Infections.*Drugs*. 67:351-368.

[15] Lang, A. B.; Horn, M. P.; Imboden, M. A. and Zuercher, A. W. (2004). Prophylaxis and therapy of Pseudomonas aeruginosa infection in cysticfibrosis and immunocompromised patients. *Vaccine*. 22:S44-S48.

[16] .Moore, N. M. and Flaws, M. L. (2011). Antimicrobial resistance mechanisms in *Pseudomonas aeruginosa*. Clin Lab Sci. 24: 47–51.

[17] Lesic, B.; Lepine, F.; Deziel, E.; Zhang, J. and Zhang, Q. (2007). Inhibitors of pathogen intercellular signals as selective anti-infective compounds. PLoS Pathog 3: 1229–1239.

[18] Sintim, H. O.; Smith, J. A.; Wang, J.; Nakayama, S. and Yan, L. (2010). Paradigm shift indiscovering next-generation anti-infective agents: targeting quorum sensing, cdi-GMP signaling and biofilm formation in bacteria with small molecules.Future Med Chem 2: 1005–1035.

[19] Garza-Ramos, U.; Silva-Sánchez, J. and Martínez-Romero, E. (2009). Genetics and genomics for the study of bacterial resistance. Salud Publica Mex.51(suppl 3):S439-S446.

[20] Tan, T. T. (2008). "Future" threat of gram-negative resistance in Singapore. Ann Acad Med Singapore.37:884-890.

[21] . MacFadden, J. F. (2000). Biochemical tests for Identification of Medical Bacteria3rd Ed.The Williams & Wilkins Co., USA: 689-691.

[22] Wikler, M. A. (2006). Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement: Clinical and Laboratory Standards Institute. 26(3):1–35.

[23] Lau, G.W.; Hassett, D.J.; Ran, H.; Kong, F. (2004). Trends in molecular medicine, 10 (12), 599-606.

[24] Haleem, H.; Kadhim, J; Ilham, T. and Banyan, A. (2011). Isolation of *Pseudomonas aeruginosa* from Clinical Cases and Environmental Samples, and Analysis of its Antibiotic Resistant Spectrum at Hilla Teaching Hospital. Med. J. Babylon. 8: 618-624.

[25] Tripathi, P.; Banerjee, G.; Saxena, S.; Gupta, S. M. and Ramteke, P. W. (2011). Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from patients of lower respiratory tract infection. African J. Microbiol. Res., 5(19): 2955-2959.

[26] Quinn, P. J.; Markey, B. K.;Carter, M. E.; Donnelly, W. J. and Leonard, F. C. (2002). Veterinary Microbiology and Microbial Disease. Iowa State University Press, Ames, USA. pp.124-126.

[27] Meyer, J. M.; Geoffroy, V. A. and Baida, A. (2002). Appl. Environ. Microbiol. 68 (6), 2745-2753.

[28] Ryan, K. J. and Ray, C.G. (2004). Sherris Medical Microbiology. 4th ed. McGraw Hill. ISBN. 8385-8529-9. 10.

[29] Van Eldere, J. (2003). "Multicentre surveillance of *Pseudomonas aeruginosa*susceptibility patterns in nosocomial infections". *J. Antimicrob. Chemother.* **51** (2): 347–352.

[30] Poole, K. (2004). "Efflux-mediated multiresistance in Gram-negative bacteria".*Clin. Microbiol. Infect.* **10** (1): 12–26.

[31] Ferguson, D.; Cahill, O. J. and Quilty, B. (2007). Phenotypic, molecular and antibiotic resistance profiling of nosocomial *Pseudomonas aeruginosa* strains isolated from two Ir. Hospitals. J. Med., 1(1): 201-210.

[32] Corona-Nakamura, A. L.; Miranda-Novales, M. G.; Leanos-Miranda, B.;*et al.,* (2001). Epidemiologic study of Pseudomonas aeruginosa in critical patients and reservoirs. Arch. Med. Res., 32: 238-242.