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ISOLATION AND CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA* FROM BABYLON PROVINCE

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ABSTRACT : A total samples were collected during the period from April, 2017 to June, 2017. It included the isolation and identification of 20 *Pseudomonas aeruginosa* isolates from 80 samples collected from different clinical and environmental sources. The distributions of these isolates were 14(70%) from 40 burn samples, 4(20%) from 25 samples of urinary tract infections, 2(10%) from 15 samples of operative rooms. The isolates were identified according to cultural properties, microscopic, biochemical test and confirmed by VITEK 2 system compact.

Key words : *P. aeruginosa*, antibiotic susceptibility.

INTRODUCTION

Pseudomonas is one of the most diverse genera known, found in diverse environmental niches, such as soil, water, animals and plants. *Pseudomonas aeruginosa* differs from other species of this genus in being pathogenic to animals (Aghamollaei *et al*, 2015). This Gram-negative bacterium is rod shaped and an opportunistic pathogen, causing various types of infection (*e.g.*, skin, eyes, ears, respiratory tract, urinary tract, gut-derived sepsis, soft tissue infections, bone and joint infections). Immunocompromised patients and patients on immunosuppressive treatments, such as patients suffering from cystic fibrosis, burn wounds, AIDS and cancer, are the most frequently infected by this organism. *Pseudomonas aeruginosa* strains are naturally resistant to several antibacterial drugs (Pereira and Cardoso, 2014). In addition, some clinical isolates have a hypermutator phenotype, facilitating the rapid evolution of resistance to drugs to which they were initially sensitive. *P. aeruginosa* strains can also acquire resistance from mobile genetic elements, such as plasmids (Peng *et al*, 2014). Most *P. aeruginosa* strains are still susceptible to colistin, a polymyxin antibiotic, but increasing numbers of reports of pan-resistant strains are emerging (Bleves *et al*, 2010; Heiniger *et al*, 2010).

Due to its nominal nutritional requirement *P. areuginosa* has theabilitytosurvivein soil, plant surfaces, waste water, moist environment, surface water, or even on inert materials (Rakesh *et al*, 2012). Using life support equipments, cosmetics, dilute antiseptics even through the washing liquids and soaps, it can be transmitted person to person. However, *P.aeruginosa* is mostly acquired from environment and spread person-to-person rarely (Berrouane *et al*, 2000).

*P. areuginosa*is highly ubiquitous in water system and capable to acquire antibiotic resistance due to its low outer membrane permeability and extensive efflux pump system. The overall prevalence of antibiotic resistant *P. aeruginosa* is increasing, with up to 10% of global isolates found to be multi-drug resistance. It is recognized as the second leading cause of gram negative nosocomial infection and a major treatment challenge for *Pseudomonas aeruginosa* (Moazami-Goudarzi and Eftekhar, 2013).

The Emergence of multidrug-resistant (MDR) strains in burns units, particularly in economically under developed and developing countries, has become a major problem in the control of infections (Japoni *et al*, 2009). Multidrug-resistance, caused by a variety of resistance mechanisms, leaves few alternatives for treatment of some patients. Carbapenems are the selective drugs for treatment of MDR isolates (Lister *et al*, 2009). The carbapenems; (imipenem & meropenem) represent important therapeutic options for serious infections caused by *P. aeruginosa*. This pathogen may become resistant to the carbapenems by modulating and combin-ing different intrinsic mechanisms such as down regula-tion or loss of porins (Picao *et al*, 2012).

Pseudomonas aeruginosa has been described as a 'phenomenon of bacterial resistance' so the present study

aim to isolate and identify *Pseudomonas aeruginosa* in Al-Hilla Hospitals.

MATERIALS AND METHODS

Samples collection

Bacterial Isolates : In this study, 20 isolates of *P. aeruginosa* were isolated from 80 clinical (for both sexes of different ages who referred to AL-hilla Teaching Hospital, during the period from April, 2017 to June, 2017) and hospital environmental sources. These included 40 swabs from burn patients, 25 samples from patients suffering from urinary tract infections (UTI) for both sexes of different ages and 15 swab from operation rooms (Surgical operative beds, surgical instruments, tables, sink, walls and floor). All swabs and specimens were transported to the laboratory of Hilla Teaching hospital without delay. The samples were immediately inoculated in MacConkey agar, blood agar and nutrient agar and incubated for overnight at 37°C aerobically (Forbes *et al*, 2007).

Preparation of the culture media

Culture media have been prepared according to the information, which reported on the containers and sterilized by the autoclave (121°C for 15 minutes under 1.5 bar) and to ensure these media were not contaminated by put them in the incubated at 37°C for 24 hours. After that there were prepared according to manufucture companies, which reported on the containers including: put them in the refrigerator. The important culture media in the diagnosis have been prepared at the following method:

A. Blood agar base
B. MacConkey agar
C. Mueller Hinton agar
D. Nutrient agar
E. Nutrient broth

Identification of *P. aeruginosa* isolates

Colonial morphology

The grown colonies on the nutrient agar and Muller-Hinton agar characterized by producing diffusible pigments and sweet-grape odor were selected for further diagnostic tests for *P. aeruginosa* (MacFadden, 2000).

Cultural characteristics include; colonial morphology (smooth mucoid), grape odor, diffusible pigments on Muller-Hinton agar (bluish green or yellowish green) (Collee *et al*, 1996).

Nutrient agar : The growing colonies were identified according to pigments and odor production (grape like odor), blood agar the growing colonies of most isolates were identified according to type of hemolysis, swarming movement and metallic sheen iridescence appearance. And MacConkey agar, the growing colonies were identified according to their ability to ferment lactose (Macfaddin, 2004).

Microscopic examination

Microscopic examination includes gram–stain reaction (negative), the examination of shape (rods), arrangement of cells with each other, motility and capsule presence (Isenberg, 2004).

Growth at 42°C

P. aeruginosa has a characteristic ability to grow at 42°C in successive subculture. Tubes containing nutrient broth were inoculated with selected colonies and incubated at 42°C for 24 hours. The ability to grow was indicated for the positive result.

Identification of *P. aeruginosa* Isolates via VITEK2

VITEK 2 is the next generation of the gold standard in microbial identification and represents advanced colorimetric technology.

Procedure

All the following steps were done according to the manufacturer's instructions (Biomerieux) (Fritsche *et al*, 2011) :

1. Three ml of normal saline was placed in plane test tube and inoculated with a lope full of isolated colony.
2. The test tube was inserted into a Dens Check machine for standardization of colony to McFarland standard solution (1.5×10^8 cell/ml)
3. The standardized inoculum was placed in the cassette and a sample identification number entered into the computer software via barcode.
4. The VITEK 2 card type was then read from barcode placed on the card during manufacture and the card was thus connected to the sample ID.
5. The cassette was placed in the filler module, when the cards were filled, transferred the cassette to the reader/ incubator module.

All subsequent steps were handled by the instrument; the instrument controls the incubation temperature. The optical reading of the cards were continually monitors and transfers test data to the computer for analysis. When the test cycle is completed, the system automatically ejected the cards into a waste container.

Maintenance Media

This medium contained nutrient broth 85% and glycerol 15%; then was distributed in 5ml. amount in sterile tubes and autoclaved. This medium was used to preserve the bacterial isolates at -20^{0}C for several months (Forbes *et al*, 2007).

Antibiotic susceptibility testing

P. aeruginosa isolates were tested for their sensitivity to 18 commonly used antibiotics by disk diffusion method (Perilla *et al*, 2010). Results were recorded by measuring the inhibition zone (in millimeters) and interpreted according to Clinical and Laboratory Standards Institute documents (CLSI, 2014).

The susceptibility of *P. aeruginosa* isolates were determined by disk diffusion method as follows:

The Kirby–Bauer standardized disk method

The method according to procedure of Kirby *et al* (1966) was carried out as follows:

- Mueller–Hinton medium was employed for this test. The medium was cooled to 45–50°C and with a sterile wire loop, the 4–5 pure colonies were transferred to a tube containing 5ml of BHI broth. The broth was incubated at 37°C until its turbidity standard. This usually required at least 4–6 hours incubation. The cells density was compared with McFarland standard tube No. 0.5.
- Within 15 minutes of adjusting the density of the inoculum, sterile cotton swab on a wooden applicator stick was dipped into the standardized bacterial suspension. The swab was then used to streak the dried surface of a Mueller–Hinton plate in 3 different directions to obtain an even distribution of the inoculums. The plates were allowed to remain on a flat and level surface undisturbed for 3 to 5 minutes to allow absorption of excess moisture.
- With a sterile forceps, the selected disks were placed on the surface of medium and pressed firmly but gently into the agar with sterile forceps. Within 15 minutes the inoculated plates were incubated at 37°C for 18 hours in an inverted position.
- After incubation the diameters of the complete inhibition zones were noted measured by using a ruler. The end point, measured to the nearest millimeter was compared with zones of inhibition determined by CLSI (2012) and to decide the susceptibility of bacteria to antimicrobial agent, whether being resistant or sensitive.

RESULTS AND DISCUSSION

Isolation and identification of *P. aeruginosa*

Twenty isolate of *P. aeruginosa* isolated from 80 clinical and hospital environmental samples, these included 40 swab from burned patients, 25 sample from patients suffering from urinary tract infections (UTI) for both genders of different age groups, and 15 swab from operative rooms.

Morphological and cultural characterization tests showed 20 (25%) isolates were identified as *P. aeruginosa*, 14(70%) isolates from burns, 4(20%) isolates from UTI infections and the last 2 (10%) isolates from operative rooms. Table 1 showed number and percentage of bacterial isolates among clinical samples in Hilla city.

The high percent of *Pseudomonas* isolation was observed in females 12/40 (66.7%), while in male it was 6/25 (33.3%) shown in the Table 2.

Identification of *P. aeruginosa* isolates usually based on cultural properties, the presence of characteristics pigments, and growth at 42°C and finally, confirming the identification by VITEK2-Compact system, all the isolates were cultured on nutrient agar and blood agar. The characteristic feature on nutrient agar *P. aeruginosa* colonies were smooth, translucent large low convex 2-4 mm in diameter with irregular spreading edge. In addition, most the isolates produced the diffusible pigments on nutrient agar, such as bluish–green (pyocyanine) especially in burns and UTI isolates. On blood agar, most isolates produce β-hemolysis. All isolates were Gram negative rods. All isolates showed ability to grow at 42^{o}C.

P. aeruginosa is ubiquitous microorganism widely distributed in soil, water and on living hosts and it is one of the most important factors of nosocomial infection, which threatens the lives of many patients annually (Loveday *et al*, 2014).

These results showed that burns are the most accessible site by *P. aeruginosa* (14, 70%). The high frequency of *P. aeruginosa* may be explained by the fact that it is regarded as an opportunistic pathogen (Ranjan *et al*, 2010). This result was expected with high prevalence of *P. aeruginosa* in community may be related to the increasing numbers of the immune compromised patients in our population due to different diseases and contaminations of the environment in hospital and in country special patients with long stay in hospital. This agree with several studies conducted by Saleh (2012), Peng *et al* (2014).

In return to the site of infection, *P. aeruginosa* in UTI less than burns (4, 20%). These recurrent infections

Table 1 : Number and percentage of bacterial isolates among clinical samples in Hilla city.

Clinical sample type	No. of sample	No. of *P. aeruginosa* isolates
Burn	40	14 (70%)
UTI	25	4 (20%)
Operative room	15	2 (10%)
Total	80	20 (100%)

Table 2 : Frequency of *Pseudomonas* isolates in clinical samples according to gender.

Gender	No. of sample	Positive results	%
Male	25	6	33.3%
Female	40	12	66.7%
Total	65	18	100%

Table 3 : Biochemical tests for identification of *P. aeruginosa* isolates.

Test	Result
Gram-stain	-
Oxidase test	+
Catalase test	+
Pigments production	+
Hemolysis (β-hemolysis)	+
Indole test	-
Methyl-red	-
Voges-Proskauer	-
Simmon's citrate	+
Urease production	-
H_2S production	-
Kligler's iron agar	K/K

are mainly due to the accumulation of mixed biofilms on the artificial surface of the catheter or other implant. A biofilm on an indwelling urinary catheter consists of adherent microorganism, their extracellular products and host components deposited on the catheter. Biofilm on urinary catheters results in persistent infections that are resistant to antimicrobial therapy (Mahmoud *et al*, 2013).

The results showed that the distribution rate of *P. aeruginosa* in operation rooms was 2 (10%). This can be attributed to it's high adaptability to extreme environmental conditions and hence tolerates and survives in such conditions (Pirnray *et al*, 2003). Hospital environment has identified *Pseudomonas* spp, the majority of moist sites of hospital. The important and the most environmental sources of *Pseudomonas* spp. are humid places, many factors that predispose hospitals contamination, such as crowded condition in the hospital with patients, visitors and medical staff, in addition to contamination of objects, devices, materials, water and food with micro-flora, whereas the widespread of antibiotic for the treatment of bacterial infections increased the emergence of multi-drug resistant strains of bacteria. The role of contamination of the environment with *Pseudomonas* in hospital has been the subject of several studies that agree with our study (Pal *et al*, 2010; Atlanta, 2014).

In Table 2 shows the high percent of *Pseudomonas* isolation was observed in females 12/40 (33.3%), while in male it was 6/25 (66.7%) this may be to the presence of small amount of *P. aeruginosa* as part of normal flora of the gut, therefore fecal contamination is the reason for President in the incidence of these infections, also *P. aeruginosa* is one of the main reasons for the formation of urinary tract infections in females (Akinloye *et al*, 2006; Fayroz-Ali, 2012).

Identification of *P. aeruginosa* isolates showed that most isolates produced pigments diffuses into medium on nutrient agar called pyocyanin and most isolates produce β-hemolysis and other do not produce hemolysis. Most of *P. aeruginosa* isolates able to producing pigments on nutrient and MacConky agar such as pyocyanin (blue-green), pyoverdin (yellow-green) and pyomelanin (brown)pigment (Sundararaj *et al*, 2004; Mowat *et al*, 2010).

Some biochemical tests were carried out and the results compared with standard result documented by Collee *et al* (1996), MacFadden (2000).

All isolates were Gram negative rods and positive for oxidase, catalase and positive to motility test. All isolates able to grow at 42°C (Ryan and Ray, 2004) mentioned that the combination of characteristic oxidase positive colonies, pyocyanin production and the ability to grow at 42°C is sufficient to distinguish *P. aeruginosa* from other *Pseudomonas* species. The ability of *P. aeruginosa* to growth at 42°C consider the best method to identification the bacterium from other and used by Otajevwo (2013).

Antibiotic susceptibility profile for *P. aeruginosa* isolates

Twenty isolate of *P. aeruginosa* were tested for their antibiotics susceptibility, toward 18 antibiotics using Kirby-Bauer disc diffusion methods. Screening for antibiotic susceptibility was done by agar streaking methods on Muller-Hinton agar. The antimicrobial potency of selected antibiotics against the *Pseudomonas* is summarized in Table 3.

The present study, revealed a remarkable elevation *P. aeruginosa* resistance to antibiotics that were used in this study, In general especially toward the beta-lactam antibiotics, represented by penicillins such as piperacillin, ticarcilline and carbenicllline since the level of resistance

Fig. 1 : *P. aeruginosa* on nutrient agar after 24 hr. at 37°C.

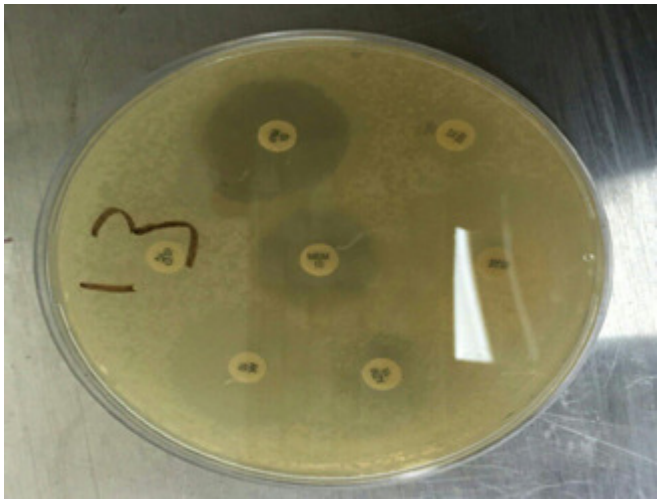

Fig. 2 : Antibiotic susceptibility of *P. aeruginosa*on Muller Hinton agar after 24 hr. at 37°C.

recorded for this antibiotics 70%, 92.5% and 87.5%, respectively and for the cephalosporin III represented by ceftazidime, cefotaxime, and ceftriaxone, since the percentage of resistance recorded about 90%, 85% for cefotaxime and ceftriaxone, respectively. The resistance rates to other antibiotics represented by cephalexin, cefepime, cefixime, cefoxitin were 90%, 77.5%, 85% for each cefixime and cefoxitin, respectively.

The results also showed that the resistance to aztreonam is 80%, while the resistance to amoxi-clav and nalidixic acid were 87.5%, 80%, respectively.

The current study explained that *P. aeruginosa* isolates were resistant to other antibiotics represented by amikacin, ciprofloxacin, gentamicin accounted for 27.5%, 42.5% and 32.5%, respectively. The results showed that all *P. aeruginosa*isolates were sensitive to imipenem and meropenem and 38(95%) isolates sensitive for meropenem, all isolates from urine were sensitive to imipenem, meropenem, amikacin and ciprofloxacin.

Twenty isolate of *P. aeruginosa* were tested for their antibiotics susceptibility, toward 18 antibiotics using Kirby-Bauer disc diffusion methods. Screening for antibiotic susceptibility was done by agar screen methods on Muller-Hinton agar. The antimicrobial potency of selected antibiotics against the *P. aeruginosa*is summarized in Table 3. Prolonged antimicrobial use as treatment for pseudomonic infections is seemed to be risk factor for infection with *P. aeruginosa* especially multidrugs–resistant *P. aeruginosa* (MDRPA) (Obritsch *et al*, 2005). Obtained results from this study shown in Table 4 reveal a remarkable elevation in pseudomonal resistance to the beta-lactam antibiotics represented by penicillins such as ticarcilline, piperacillin and carbenicllline since the level of resistance accounted for this antibiotics 92.5%, 90% and 87.5%, respectively. These results were agreed with studies reported by (Golshani and Sharifzadeh, 2013).

The levels of resistance to the third generation cephalosporins including ceftazidime, cefotaxime and ceftriaxone are 90%, 85% (each cefotaxime and ceftriaxone).This rate of resistance nearly similarwith studies (Golshani and Sharifzadeh, 2013).

The resistance to the penicillins and cephalosporins has become an important issue in most hospitals in which resistance rate have reached greater levels. Resistance mediated by *P. aeruginosa* can be attributed both to an inducible, chromosomally mediated beta-lactamases that can render broad–spectrum cephalosporins inactive and to a plasmid–mediated beta- lactamases that can lead to resistance to several penicillins and older cephalosporins (Kaye *et al*, 2000; Giamarellou and Antoniadou, 2001).

The resistance level to other antibiotics represented by cephalexin, cefepime, cefixime, cefoxitin were 90%, 77.5%, 85% for each cefixime and cefoxitin, respectively *P. aeruginosa* resistance to this antibiotics can be conferred by the outer membrane, which provides an effective intrinsic barrier to accessing the targets are located either in the cell wall or cytoplasmic membrane or within the cytoplasm and modifications in outer membrane permeability via alterations in porin protein channels represent a component of many resistance mechanisms. In addition, inactivating enzymes released from the inner membrane can function more efficiently within the confines of the periplasmic space. The mechanisms by which intracellular concentrations of drugs are limited include decreased permeability through the outer membrane, decreased uptake through the cytoplasmic membrane and active efflux back out across the cytoplasmic membrane (Henwood *et al*, 2001; Po-Ren *et al*, 2002).

Results of the present study showed the level of resistance to aztreonam, nalidixic acid were 80% for

Table 3 : Antibiotic susceptibility of *P. aeruginosa*.

Antibiotic disk	Resistance according to site of infection			Total resistance of bacterial isolates
	Burns (N=14)	Urine (N=4)	Operative rooms (N=2)	
Piperacillin	12(55%)	3(75%)	2(100%)*	17(70%)
Ticarcilline	13(95.4%)	3(75%)	2(100%)*	18(92.5%)
Carbenicllline	13(95.4%)	3(75%)	1(80%)	17(87.5%)
Cefotaxime	13(95.4%)	3(62.5%)	1(80%)	17(85%)
Ceftriaxone	12(91%)	3(75%)	1(80%)	16(85%)
Ceftazidime	14(91%)	3(75%)	2(100%)*	19(90%)
Aztreonam	13(86%)	2(62.5%)	1(80%)	16(80%)
Cefoxitin	13(91%)	3(75%)	1(80%)	17(85%)
Cephalexin	13(91%)	3(75%)	2(100%)*	18(90%)
Cefixime	13(95.4%)	3(62.5%)	1(80%)	17(85%)
Cefepime	12(91%)	3(75%)	1(62.5%)	16(77.5%)
Augmentin	13(91%)	2(62.5%)	2(100%)*	17(87.5%)
Nalidixic acid	11(86%)	3(62.5%)	1(80%)	15(80%)
Amikacin	4(31.8%)	0(0%)**	1(40%)	11(27.5%)
Gentamicin	5(45.5%)	1(25%)	1(62.5%)	7(42.5%)
Ciprofloxacin	3(36%)	0(0%)**	5(62.5%)	8(32.5%)
Imipenem	0(0%)**	0(0%)**	0(0%)**	0(0%)**
Meropenem	0(0%)**	0(0%)**	0(0%)	0(0%)

*: all the isolates were resistant for this antibiotic.**: all the isolates were sensitive for this antibiotic.

each.These resistances, mainly, are an important indicator for the presence of ESBLs. ESBL-producing Gram-negative rods are undoubtedly one of the most important etiological agents of many severe and life-threatening nosocomial infections (Kang *et al*, 2005). The genes encoding ESBLs are usually localized on large, transferable plasmids that can easily become widespread in Gram negative bacilli (Franiczek *et al*, 2007), while the resistance level to amoxi-clav is 87.5%.This is likely to be due to the heavy selection pressure from overuse of this antibiotic and seem to be losing the battle (Ryan and Ray, 2004).

Present study explained that *P. aeruginosa* isolates were intermediate resistance to other antibiotics represented by amikacin, ciprofloxacin and gentamicin accounted for 27.5%, 42.5% and 32.5% respectively. This results were nearly accordant with results of AL-Muhannak (2010), Mahmoud *et al* (2013), Ghamgosha *et al* (2014). This resistance against fluoroquinolones in this study may reflect significant antibiotic pressure in the environment rather than co-carriage of this resistance gene on plasmids. Quinolone resistant is usually caused by various chromosomal mutations that alter the target enzymes, such as DNA gyrase and topoisomerase IV, or activate efflux systems (Jeong *et al*, 2005). Plasmid-mediated quinolone-resistant has only recently discovered. The plasmid genes responsible for quinolone-resistance, termed *qnr* (Shigemura *et al*, 2008). The reason behind these high results of resistance is that the intrinsic antibiotic resistance of *P. aeruginosa* may be due to the expression of chromosomally encoded efflux pumps (Li and Nikaido, 2004).

The results showed that all *P. aeruginosa* isolates were sensitive to imipenem and meropenem and 20(100%) isolates sensitive for meropenem, all isolates from urine were sensitive to imipenem, meropenem, amikacin and ciprofloxacin. This results were agreement with studies of Harran (2012), Ghamgosha *et al* (2014). It appears that imipenem are the drug of choice for serious infection with broad spectrum *â*-lactamase producing organisms as has been recommended earlier (Pongpech *et al*, 2008; Farajzadeh *et al*, 2014).

REFERENCES

Aghamollaei H, Moghaddam M M, Kooshki H, Heiat M, Mirnejad R and Barzi N S (2015) Detection of *Pseudomonas aeruginosa* by a triplex polymerase chain reaction assay base donlasI/Randgyr B genes. *J. Infect. Public Health* **8**, 314–322.

Akinloye O, Ogbolu D O, Akinloye O M and Terry A O A (2006) Asymptomatic bacteriuria of pregnancy in Ibadan, Nigeria: a reassessment. *Br. J.*

AL-Muhannak F H N (2010) Spread of some Extended-Spectrum Beta-Lactamases in Clinical Isolates of Gram-Negative in Najaf. *M.Sc. Thesis*. College of Medicine, Kufa University.

Atlanta G A (2014) *Pseudomonas aeruginosa* in healthcare settings.: Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences.

Berrouane F Y, McNutt L, Buschellman B J, Rhomberg P R, Sanford M D, Hollis R J, Pfaller M A and Herwaldt L A (2000) Outbreak of Severe *Pseudomonas aeruginosa* Infection Caused by Contaminated Drains in a Whirlpool Bathtubs. *Clinical Infectious Diseases* **31**, 1331-1337.

Bleves S, Viarre V, Salacha R, Michel G P F, Filloux A and Voulhoux R (2010) Protein secretion systems in *Pseudomonas aeruginosa*: A wealth of pathogenic weapons. *Int. J. Med. Microbiol.* **300**, 534–543.

Collee J G, Fraser A G, Marmion B P and Simmons A (1996) Mackie and Mecartney. Practical Medical Microbiology. 14th ed., USA. Pp: 413-424.

Farajzadeh S, Rostami S, Jolodar A, Tabatabaiefar M, Khorvash F and Saki A (2014) Detection of metallo-beta lactamases among carbapenem resistant.

Fayroz-Ali J M H (2012) Detection of Quinolone Resistance Genes in *Escherichia coli* Isolated from Patients with Significant Bacteriuria in Najaf Province. *Ph.D. Thesis*. Sc. University of Babylon, Iraq.

Franiczek R, Dolna I, Krzy¿anowska B, Szufnarowski K and Krochmal B (2007) Conjugative Transfer of Multiresistance Plasmids from ESBL-

positive Escherichia coli and Klebsiella spp. Clinical Isolates to *Escherichia coli* Strain K12 C600. *Adv Clin Exp Med*. **16**(2), 239–247.

Fritsche T R, Sader H S, Toleman M A, Walsh T R and Jones R N (2005) Emerging metallo-β-lactamase-mediated resistances: a summary report from the worldwide SENTRY antimicrobial surveillance program. *Clinical Infectious Diseases* **41**(Supplement-4), S276-S278.

Ghamgosha M, Shahreki Z, Kafilzadeh F and Bameri Z (2014) Metallo-Beta- Lactamase Genes Vim-1, Spm-1 and Imp-1 in *Pseudomonas aeruginosa* Isolated From Zahedan Hospitals. *Int J Infect*. **1**(1).

Giamarellou H and Antoniadou A (2001) Antipseudomonal Antibiotics. *Medical Clinics of North America* **85**(1), 354-360.

Golshani Z and Sharifzadeh A (2013) Prevalence of blaOxa10 Type Betalactmase Gene in Carbapenemase Producing *Pseudomonas aeruginosa* Strains Isolated From Patients in Isfahan. *J J Microbiol*. **6**(5).

Henwood C J, Livermore D M, James D and Warner M (2001) Antimicrobial Chemotherapy Disc Susceptibility Test. *J-Antimicrob-Chemother* **47**(6), 789-799.

Isenberg H D (2004) Clinical Microbiology Procedures Handbook, 2nd ed.

Japoni A, Alborzi A, Kalani M, Nasiri J, Hayati H and Farshad S (2009) Susceptibility patterns and cross resistance of antibiotics against *Pseudomonas aeruginosa* isolated from burn patients in the South of Iran. *Burns* **32**, 343-347.

Jeong J Y, Yoon H J, Kim E S, Lee Y, Choi S H, Kim N J, Woo J H and Kim Y S (2005) Detection of qnr in Clinical Isolates of *Escherichia coli* from Korea. *Antimicrob Agents Chemother* **49**, 2522-2524.

Kang H Y, Jeong Y S and Oh J Y (2005) Characterization of antimicrobial resistance and class 1 integrons found in Escherichia coli isolates from humans and animals in Korea. *J Antimicrob Chemother* **55**(5), 639-644.

Kaye K S, Fraimow H S and Abrutgn E (2000) Antibacterial Therapy. *Infections Disease clinics of North America* **14** (2), 451-460.

Li X Z and Nikaido H (2004) Efflux-mediated drug resistance in bacteria. *Drugs* **64**, 159-204.

Lister P D, Wolter D J and Hanson N D (2009) Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. *Clin. Microbiol Rev.* **22**(4), 582-610.

Loveday H P, Wilson J A, Kerr K, Pitchers R, Walker J T and Browne J (2014) Association between healthcare water systems and *Pseudomonas aeruginosa* infections: a rapid systematic review. *J. Hosp. Infect*. **86**, 7–15.

Mac Fadden J (2000) Biochemical tests for Identification of Medical Bacteria 3rded. The Williams & Wilkins Co., USA.PP: 689 - 691.

MacFaddin J F (2004) *Biochemical tests for Identification of Medical Bacteria*. **4**th ed., Waverly press, Inc., Baltimore, U.S.A.

Mahmoud A B, Zahran W A, Hindawi G R, Labib A and Galal R (2013) Prevalence of Multidrug-Resistant *Pseudomonas aeruginosa* in Patients with Nosocomial Infections at a University Hospital in Egypt, with Special Referenceto Typing Methods. *Journal of Virology & Microbiology* 10-13.

Moazami-Goudarzi S and Eftekhar F (2013) Assessment of Carbapenem Susceptibility and Multidrug-Resistance in *Pseudomonas aeruginosa* Burn Isolates in Tehran. *Jundishapur Journal of Microbiology* **6**, 162-165.

Obritsch M D, Fish D N, MacLaren R and Jung R (2005) Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: epidemiology and treatment options. *Pharmacotherapy* **25**(10), 1353-1364.

Otajevwo F D (2013) Urinary Tract Infection among Symptomatic Outpatients Visiting a Tertiary Hospital Based in Midwestern Nigeria. *Glob. J. of Heal. Sci*. **5**(2), 187-199.

Pal R B, Rodrigues M and Datta S (2010) Role of Pseudomonas in nosocomial infections and biological characterization of local strains. *J Biosci Tech*. **1**(4), 170-179.

Peng Y, Bi J, Shi J, Li Y, Ye X and Chen X (2014) Multidrugresistant Pseudomonas aeruginosa infections pose growin health care-associated infection control in the hospitals of Southern China: a case- control surveillance study. *Am. J. Infect. Control* **42**, 1308–1311.

Pereira S G and Cardoso O (2014) Mobile genetic elements of *Pseudomonas aeruginosa* isolates from hydrotherapy facility and respiratory infections. *Clin. Microbiol. Infect*. **20**, 203–206.

Perilla M J, Ajello G, Boop C, Elliott J, Facklam R, Popovic T and Wells J (2010) Manual for laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in developing World. CDC, Atlanta, Georgia, USA, 2010.

Picao R C, Carrara-Marroni F E, Gales A C, Emerson Jose Venancio E J, Xavier D E, Tognim M C and Pelayo J S (2012) Metallo-â-lactamase-production in meropenem-susceptible *Pseudomonas aeruginosa* isolates: risk for silent spread. *MemInstOswaldo Cruz, Rio de Janeiro* **107**(6), 747-751.

Pongpech P, Naenna P, Taipobsakul Y, Tribuddharat C and Srifuengfung S (2008) Prevalence of Extended spectrum beta lactamase and class 1 Integron integrase gene INTI1 in *Escherichia coli* from Thai patients and health Southeast Asian. *J TROP MED Public Health* **39**(3), 425-433.

Ranjan K P, Ranjan N, Bansal S K and Arora D R (2010) Prevalence of *Pseudomonas aeruginosa* in post-operative wound infection in a referral hospital in Haryana, India. *J. Lab. Physicians* **2**, 74-77.

Rakesh M R, Govind N L, Kalpesh M, Rosy P, Kanu P and Vegad M M (2012) Antibioitc Resistance Pattern in *Pseudomonas aeruginosa* Species Isolated at a Tertiary Care Hospital, Ahmadabad. *National Journal of Medical Research* **2**, 156-159.

Ryan K J and Ray C G (2004) Sherris medical microbiology an introduction to infectious diseases. The McGraw-Hill Companies, 4th ed. PP: 385-393.

Ryan K J and Ray C G (2004) *Sherries medical microbiology*. 4th ed. McGraw-Hill-New York.: 5-9.

Saleh B H, Al-Jumaily E F and Hussain S M (2012) Production and purification of exotoxin a extracted from social strains of *Pseudomonas aeruginosa* in iraq. *DAMA International* **1**(1), 15-23.

Shan-Chwen C, Shen W H, Chin Y, Wei-Chuan H and Kwen T (2002) Antimicrobial drug resistance in Pathogens causing nosocomial infections at a university Hospital in Taiwan. *Medline J*. **8**(1), 1981–1999.

Shigemura K, Arakawa S, Miura T, Nakano Y, Tanaka K and Fujisawa M (2008) Significance of floroquinolone-resistant E. coli in urinary tract infections. *Jpn J Infect Dis*. **61**, 226-228.

Sundararaj T, Anthoniraj S, Kannan N and Muthukaruppan S M (2004) Microbiology. TamilNadu text book corporation, Government of Tamil Nadu. 1st ed. PP.129-147.

Washington D C, ASM Forbes B A, Sahm D F and Weissfeld A S (2007) *Bailey and Scott's Diagnostic Microbiology*. **12**th ed., pp. 1031, Mosby-Elsevier.