# **Relation between biofilm formation and resistance to antibacterial agents of pseudomonas aeruginosa isolated from different sources**

**ELZahraa Radwan, Emad.M. Al-Ebshahy, Samy A. Khalil and Helmy A.Torky**

Microbiology Department, Faculty of Veterinary Medicine, Alexandria University

**Abstract**

*Pseudomonas aeruginosa* *(P. aeruginosa* ) is an opportunistic pathogen that is a leading cause of morbidity and mortality in immune-compromised individuals. Moreover, it continues to be a major problem in veterinary practice. Overcoming of p. aeruginosa has become so difficult due to its noticed capacity to resist antibiotics. This capacity is owing to its intrinsic and acquired resistance mechanism to resist most antibiotics. Additionally, adaptive antibiotic resistance of *P. aeruginosa* is recently characterized mechanism, which includes biofilm- mediated resistance and formation of multidrug presister cells and this is responsible for relapse of infections.

The study is carried on 160 samples (110 human samples (50 pus, 60 urine), and 50 animal samples (mastitis milk)). The samples were directly transferred to laboratory where they prepared for bacteriological examination for isolation of *P.aeruginosa* and determination of antibiotic sensitivity test.

Result of this study showed that 11 human samples ( 6 urine , 5 pus) and 4 mastitis milk samples were positive for *P. aeruginosa* . By testing positive samples for antibiotic sensitivity test, the result showed the higher sensitivity observed for colistin (100%) for all samples. Meanwhile, all samples show resistance for (SXT, ,MFX, TGC, ETP, FOX ,AUG ,CTX) . The rest of antibiotics (AK, TOB, CN, CIP, LEV, IMP, MEM, ETP, PTZ, CRO, CAZ, SCF, CPZ) were variable in sensitivity between samples.

Thus, this work is aimed to highlight on antibiotic resistance of *P. aeruginosa* in both human and animal.

**Key words:** *Pseudomonas aeruginosa*, human, animal, Biofilm, Antibiotics Sensitivity

**1 .Introduction**

*Pseudomonas aeruginosa* is a Gram-negative, aerobic rod shaped bacterium, which

belongs to the bacterial family *Pseudomonadaceae. P. aeruginosa* is wide spread in

nature. Many be reside in the water, plants, soil, and the predisposing environment,

like hospital locations( **Jones et al., 2008**).

The pathogen is a free- living organism in diverse planktonic form environment.Itis characterized by its high genetic plasticity and potential for adapting to various environments. This pathogen also can form biofilm and is responsible for 10–20% of infections in hospitals.

*P. aeruginosa* is a most common nosocomial in the human pathogen in immuno-

compromised patients like cancer and burns ( **Campana S*,* et al.,2014)**. this

bacteria can cause some diseases include pneumonia, endocarditis **( Baltch, A. L.etal .,1994**) and inflammation in the urinary tract (**Bayer, A. S et al., 2005**) , there are some organs may be affected by this bacteria like central nervous system, eyes, ears, skin, wounds, and musculoskeletal system and causing cystic fibrosis, burns, and

immune deficiency.

In animals Serious *P. aeruginosa* infections, both acute and chronic, are often nosocomial and associated with compromised host defenses; however, this opportunistic pathogen is more and more recognized as the cause of disease in both livestock and companion animals, including otitis and urinary

tract infections in dogs and cats, mastitis in dairy cows and goats, hemorrhagic

pneumonia in mink and foxes, and endometritis in horses **(Haenni et al., 2015).**

As global problem, the rate of multidrug-resistance (MDR) strains has

resulted in the medical therapy against *P. aeruginosa* be complicate ( **Dogonchi et** **al ., 2018** ), in addition, the ability of *P. aeruginosa* to produce biofilm is thought to be a main factor involved in chronic infections. Biofilms are complex of microbial cells embedded in an extracellular matrix composed of proteins, extracellular DNA, and exopolysaccharides, providing a protective life-style for bacteria and are extremely challenging and costly to treat by antimicrobial compound.

**The present work was designed to highlight on** 1) Isolation and identification of

*Pseudomonas aeruginosa* from human (wounds and UTI ) and from animal (mastitis

Infection ). 2) Susceptibility of the isolated *P. aeruginosa* to antimicrobial agents.

**2. Materials and methods**

**2.1. samples :-**

A total of 160 samples (50 pus samples, 60 urine samples of human) collected from

different hospitals of Alexandria and (50 mastitis milk samples of animal) collected

from Behira, Gharbia governments farms and from animal health research

institute(AHRI) in cairo . The samples were collected, labeled and transported with a

minimum of delay in ice box to the laboratory of the Department of Microbiology,

Faculty of Veterinary Medicine, Alexandria University**.**

**2.2.Isolation and identification of the bacteria:**

The samples were incubated at 37°C for one to one and half hour. Then a loopful

from each sample was streaked on to MacConkey’s agar media, Cetrimide agar media and nutrient agar then incubated aerobically at 37°C for 24-48 hrs. Suspected colonies were picked up and then purified on nutrient agar and MacConkey’s agar. Suspected colonies were picked up and streaked on solid plate media. The purified colonies were sub cultured by streaking on nutrient agar slope and subsequently inoculated in to semisolid agar medium. Which used for preservation as a stock culture for further identification **(Elmanama** **et al., 2019)**.

The identification by using traditional bacteriological methods according to **( lusis and soltys et al., 1971**) ; morphology by macroscopic appearance and special grapes or tortilla odder and by its characteristic diffuse coloured pigment , and by microscopic examination according to( **Joklik et al., 1984)** using grams stain, and by

vitec-2 compact system and different biochemical tests according to (**Qunin et al., 2002**) as oxidase, catalase, citrate , indole tests.

**2.3. Antimicrobial susceptibility testing:**

Antimicrobial discs used in human and veterinary therapy like tazobactam/piperacillin, ceftazidime, cefepime, aztreonam, meropenem, imipenem, tobramycin, amikacin, gentamicin, and ciprofloxacin were assessed in each laboratory according to their routine testing methods. by using **kirby Disk diffusion for antibacterial sensitivity**

Each bacterial isolate was inoculated into nutrient broth. About 150 μL of the broth

was inoculated on the surface of the Muller-Hinton plate and spread thoroughly by a

sterile glass rod to be sure of an even distribution of the inoculum. After replacing the

plate lid, It was allowed to remain undistributed on a leveled surface for 3-5 minutes

to allow the adsorption of the moisture then the disks were applied. The selected

antibiotics disks were placed on the inoculated plate and pressed firmly in to agar to

endure complete contact with the agar. The plates were inverted and incubated at

37ºC for 24 hours. After incubation, the degree of sensitivity was determined by

measuring the easily visible and clear zone of inhibition growth produced by diffusion

of the antibacterial agent from the disks in to the surrounding medium. The results

were interpreted according to the diameter of inhibition zone produced around each antimicrobial disk was measured and interpreted using the Clinical Laboratory Standardization Institute (CLSI) zone diameter interpretative standards (**Wayne, 2008) .**

**3. RESULTS AND DICUSSION**

**3.1. Table (1): Results of isolation of *Pseudomonas* species from human and animal samples:-**

|  |  |  |  |
| --- | --- | --- | --- |
| **Negative** | **Positive** | **Number** | **sample type** |
| **45** | **5** | **50** | **human pus** |
| **54** | **6** | **60** | **human urine** |
| **46** | **4** | **50** | **animal mastitis milk** |

* 1. **Results of antibacterial sensitivity of *P. aeruginosa*** The *Pseudomonas spp*. isolated from various sources (Pus, Urine and mastitis milk) were tested for antimicrobial susceptibility against antibiotic used.
     1. **Human pus**:-

Our result cleared that, the antibiotic sensitivity test for the bacteria isolated from pus showed a significant differences among different antibiotics used. The higher sensitivity observed for the antibiotics of COL while, the sensitivity for (AK, CN, TOB, CIP, IMP, MEM and CAZ) showed a moderate sensitivity level as its level reached to (60 %). While, the lower sensitivity observed in LEV, CPM, PTZ and SCF as its sensitivity reached to 40 % and in case of CPZ it reached to (20 %). resistance observed in case of SXT, TGC, ETP, FOX, CRO, AUG and CTX, as its sensitivity reached to (0 %).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antibiotic** | **N** | **Sensitive** | **Moderate sensitivity** | **Resistant** | **Sensitivity %** |
| **AK** | **5** | **3** | **0** | **2** | **60** |
| **CN** | **5** | **3** | **0** | **2** | **60** |
| **TOB** | **5** | **3** | **0** | **2** | **60** |
| **SXT** | **5** | **0** | **0** | **5** | **0** |
| **CIP** | **5** | **3** | **0** | **2** | **60** |
| **LEV** | **5** | **2** | **0** | **3** | **40** |
| **MFX** | **5** | **2** | **0** | **3** | **40** |
| **COL** | **5** | **5** | **0** | **0** | **100** |
| **TGC** | **5** | **0** | **0** | **5** | **0** |
| **IMP** | **5** | **3** | **0** | **2** | **60** |
| **MEM** | **5** | **3** | **0** | **2** | **60** |
| **ETP** | **5** | **0** | **0** | **5** | **0** |
| **FOX** | **5** | **0** | **0** | **5** | **0** |
| **CPM** | **5** | **2** | **1** | **2** | **40** |
| **PTZ** | **5** | **2** | **0** | **3** | **40** |
| **CRO** | **5** | **0** | **0** | **5** | **0** |
| **CAZ** | **5** | **3** | **0** | **3** | **60** |
| **AUG** | **5** | **0** | **0** | **5** | **0** |
| **CTX** | **5** | **0** | **0** | **5** | **0** |
| **SCF** | **5** | **2** | **1** | **2** | **40** |
| **CPZ** | **5** | **1** | **1** | **3** | **20** |

**Table (2): Antibiotic sensitivity test for the samples of human pus3.2.2 Human Urine:**

Our result cleared that, the antibiotic sensitivity test for the bacteria isolated from pus showed a significant differences among different antibiotics used.

The higher sensitivity observed for the antibiotics of COL (100 %) while, the sensitivity for SCF and CIP reached to 66.67%. While, the sensitivity of AK, TOB, IMP, PTZ, MEM and CAZ) showed a moderate sensitivity level as its level reached to (50 %). While, the lower sensitivity observed in Lev, CPM as its sensitivity reached to 16.76 %. Resistance observed in case of SXT, TGC, ETP, FOX, CRO, AUG, CTX and CPZ, as its sensitivity reached to (0 %).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antibiotic** | **N** | **Sensitive** | **Moderate sensitivity** | **Resistant** | **Sensitivity %** |
| **AK** | **6** | **3** | **0** | **3** | **50** |
| **CN** | **6** | **1** | **0** | **5** | **16.67** |
| **TOB** | **6** | **3** | **0** | **3** | **50** |
| **SXT** | **6** | **0** | **0** | **6** | **00** |
| **CIP** | **6** | **4** | **0** | **2** | **66.67** |
| **LEV** | **6** | **1** | **0** | **5** | **16.67** |
| **MFX** | **6** | **0** | **1** | **5** | **00** |
| **COL** | **6** | **6** | **0** | **0** | **100** |
| **TGC** | **6** | **0** | **0** | **6** | **00** |
| **IMP** | **6** | **3** | **0** | **3** | **50** |
| **MEM** | **6** | **3** | **0** | **3** | **50** |
| **ETP** | **6** | **0** | **0** | **6** | **00** |
| **FOX** | **6** | **0** | **0** | **6** | **00** |
| **CPM** | **6** | **1** | **0** | **5** | **16.67** |
| **PTZ** | **6** | **3** | **0** | **3** | **50** |
| **CRO** | **6** | **0** | **0** | **6** | **00** |
| **CAZ** | **6** | **3** | **0** | **3** | **50** |
| **AUG** | **6** | **0** | **0** | **6** | **00** |
| **CTX** | **6** | **0** | **0** | **6** | **00** |
| **SCF** | **6** | **4** | **0** | **2** | **66.67** |
| **CPZ** | **6** | **0** | **0** | **6** | **00** |

**Table (3): Antibiotic sensitivity test for the samples of human**

**3.2.3 Animal samples (mastitis milk):**

Our result cleared that, the antibiotic sensitivity test for the bacteria isolated from animal mastitis milk showed a significant differences among different antibiotics used.

The higher sensitivity observed for the antibiotics of COL, AK, TOB (100 %)

While, the sensitivity of CIP showed a moderate sensitivity level as its level reached to (50 %). While, the lower sensitivity observed in IMP, CPM , PTZ, CAZ and SCF as its sensitivity reached to (25 %). resistance observed in case of SXT, LEV, MFX, TGC, ETP, FOX, CRO, AUG ,CPZ and CTX, as its sensitivity reached to (0 %)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antibiotic** | **N** | **Sensitive** | **Moderate sensitivity** | **Resistant** | **Sensitivity %** |
| **AK** | **4** | **4** | **0** | **0** | **100** |
| **CN** | **4** | **4** | **0** | **0** | **100** |
| **TOB** | **4** | **4** | **0** | **0** | **100** |
| **SXT** | **4** | **0** | **0** | **4** | **00** |
| **CIP** | **4** | **2** | **0** | **2** | **50** |
| **LEV** | **4** | **0** | **0** | **4** | **00** |
| **MFX** | **4** | **0** | **0** | **4** | **00** |
| **COL** | **4** | **4** | **0** | **0** | **100** |
| **TGC** | **4** | **0** | **0** | **4** | **00** |
| **IMP** | **4** | **1** | **0** | **3** | **25** |
| **MEM** | **4** | **1** | **0** | **3** | **25** |
| **ETP** | **4** | **0** | **0** | **4** | **00** |
| **FOX** | **4** | **0** | **0** | **4** | **00** |
| **CPM** | **4** | **1** | **0** | **3** | **25** |
| **PTZ** | **4** | **1** | **0** | **3** | **25** |
| **CRO** | **4** | **0** | **0** | **4** | **00** |
| **CAZ** | **4** | **1** | **0** | **3** | **25** |
| **AUG** | **4** | **0** | **0** | **4** | **00** |
| **CTX** | **4** | **0** | **0** | **4** | **00** |
| **SCF** | **4** | **1** | **0** | **3** | **25** |
| **CPZ** | **4** | **0** | **0** | **4** | **00** |

**Table (4): Antibiotic sensitivity test for the samples of animal mastitis milk**

*Pseudomonas aeruginosa* is an opportunistic pathogen that frequently causes severe systemic infections However, it may cause wide spectrum of infection in urinary, respiratory , gastrointestinal tract, eyes and other sites , and it contributes to high morbidity and mortality rates among infected hosts ( **Hotack and Majt et al., 1997**) .

In veterinary medicine, *P. aeruginosa* is not a widespread pathogen but is responsible for a variety of difficult to treat infections *P .aeruginosa* has a remarkable ability to acquire and harbor diverse resistance determinants **(Hirsch EB et al., 2010**).

Due to its intrinsic and acquired antimicrobial resistance, only limited classes of antibiotics are effective for the treatment of *P. aeruginosa* infection, so outbreaks due to MDR *P. aeruginosa* infection in hospitals despite efficient infection control policies, occurs worldwide ( **Azar Dokht Khosravi et al, 2016**).

**In this study our results on the Antibiotic sensitivity test cleared that, in human pus samples** the higher sensitivity observed for the antibiotics of COL while, the sensitivity for (AK, CN, TOB, CIP, IMP, MEM and CAZ) showed a moderate sensitivity level as its level reached to (60 %). While, the lower sensitivity observed in Lev, CPM, PTZ, SCF and SCF as its sensitivity reached to 40 % and in case of CPZ it reached to (20 %). No sensitivity observed in case of SXT, TGC, ETP, FOX, CRO, AUG and CTX, as its sensitivity reached to (0 %). Meanwhile, our results on the

**Human** **urine samples cleared that,** the antibiotic sensitivity test for the bacteria isolated from pus showed a higher sensitivity observed for the antibiotics of

COL (100 %) while, the sensitivity for SCF and CIP reached to 66.67%. while, the sensitivity of AK, TOB, IMP, PTZ, MEM and CAZ) showed a moderate sensitivity level as its level reached to (50 %). While, the lower sensitivity observed in LEV, CPM as its sensitivity reached to 16.76 %. And there is no sensitivity observed in case of SXT, TGC, ETP, FOX, CRO, AUG, CTX and CPZ, as its sensitivity reached to (0 %).

**Our results on mastitis milk samples revealed that,** The higher sensitivity observed for the antibiotics of COL, AK , TOB (100 %) While, the sensitivity of CIP showed a moderate sensitivity level as its level reached to (50 %) While, the lower sensitivity observed in IMP, CPM ,PTZ, CAZ and SCF as its sensitivity reached to (25 %).resistance observed in case of SXT, LEV, MFX, TGC, ETP, FOX, CRO, AUG ,CPZ and CTX, as its sensitivity reached to (0 %)

This results agreed with those of ( **[Javiya](https://www.ncbi.nlm.nih.gov/pubmed/?term=Javiya%20VA%5BAuthor%5D&cauthor=true&cauthor_uid=20040963) et al., 2008)** where they reported that, the highest number of Pseudomonas infections was found in urine, followed by pus and sputum. *Pseudomonas* species demonstrated marked resistance against monotherapy of penicillins, cephalosporins, fluoroquinolones, tetracyclines and macrolides. Only combination drugs like Ticarcillin + Clavulanic acid, Piperacillin + Tazobactum, Cefoperazone + Sulbactum, Cefotaxime + Sulbactum, Ceftriaxome + Sulbactum and monotherapy of amikacin showed higher sensitivity to Pseudomonas infections; however, the maximum sensitivity was shown by the Carbapenems.

Among the aminoglycosides, amikacin has the highest sensitivity against P. aeruginosa, which is in corroboration with an earlier report published from India. **(Smitha et al., 2005).** Amikacin was designed as a poor substrate for the enzymes that bring about inactivation by phosphorylation, adenylation or acetylation, but some organisms have developed enzymes that inactivate this agent as well. Amikacin seems to be a promising therapy for *Pseudomonal* infection. Hence, its use should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains. **(Smitha et al., 2005).**

The problem of increasing resistance to P. aeruginosa has limited the use of other classes of antibiotics like the fluoroquinolones, tetracyclines, macrolides and chloramphenicol. **(Chambers et al., 2006).**

our results on mastitis millk agreed with ( **Amel E. Ghazy et al., 2015)** as she foundresistance of all isolates of *P.aeruginosa* to sulphamethoxazole (SXT ), and is supported by the high degree of resistance to this antibacterial agent reported by ( **Reali and roastai et al., 1994**) . Also, (**Amal et al., 2002**) found all isolates of *Pseudomonas aeruginosa* to be resistant to (SXT) and chloramphenicol. Concerning the antimicrobial sensitivity pattern of *P. aeruginosa*, it was shown that the organism was refractory to most chemotherapeutic agent (**Brooks et al., 1995**).

*P. aeruginosa* has a great intrinsic resistance to antibiotics arising from the combination of unusually restricted outer membrane permeability and secondary resistance mechanisms such as energy –dependent multidrug efflux and chromosomally encoded periplasmic beta-lactamase given this high level of natural resistance and mutational resistance to most classes of antibiotics

can readily arise **(Hancock and Speert et al., 2000**).

**4. CONCLUSION**

We conclude that *P.aeruginosa* is really a disaster that threats our life either for human and animal. So, we should limit our misuse of antibiotics.

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