Study of Biofilm Formation and Antibiotic Resistant Profile in Multidrug Resistant and Extensive Drug Resistance Pseudomonas Aeruginosa Isolated from Burn Wound Infections in Southwest Iran

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Research Article

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Abstract

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen that has remained on the 'top 10' common hospital 'superbugs' worldwide for more than a decade. Study of biofilm formation and antibiotic resistant profile in multidrug resistant and extensive drug resistance *P. aeruginosa* isolated from burn wound infections in southwest Iran

Methods and Results: This study, which was performed in 110 *P. aeruginosa* isolates culture-positive reports. Assessment of biofilm formation via microtitre plate and Congo red agar. Overall, 110 clinical *P. aeruginosa* isolates were confirmed from wound burn infections. The maximum resistance rate among *P. aeruginosa* isolates to antibiotics tested was as follow Piperacillin, ceftazidime, and minimum resistance rate among *P. aeruginosa* isolates to antibiotics tested such as ticarcillin-clavulanic acid. The isolates were then evaluating the MICs by using the E-test. only 7 isolates were confirmed as colistin-resistant. Colistin reference MICs for the The prevalence of MDR *P. aeruginosa* was 38% and XDR- *P. aeruginosa* was 22% respectively. One of *P. aeruginosa* isolates were PDR. In microtitre plate assay,76% of the isolates have ability for biofilm, formation, 40% were categorized as strong biofilm-formers; 32% were moderate; 21% were weak biofilm formers and 43% could not form any detectable biofilm.

Conclusion: in our study development of resistance by *P. aeruginosa* to many antimicrobial agents is a great challenge in controlling its infections. Therefore, the transmission of these isolates to patients leads to higher resistance. Therefore, the necessary hygiene measurements should be taken for the prevention of transferring the *P. aeruginosa* isolates to hospitalized patients.

Introduction

Burn patients are one of the most serious patients that doctors can see in their careers. These patients are in a dangerous period of various organ damage, in the acute phase; infection is the main cause of death. More than 75% of burn deaths in patients with severe burns are related to sepsis, infectious complications, and respiratory trauma. (1) Around 180,000 deaths occur annually due to burns, and the majority of these deaths occur in low- or middle-income developing countries. (2) The mortality rate reported by different studies in Iran varies from 1.4/100,000 to 9.7/100,000 and regardless of study population types, case-fatalities have been variably reported in a range of 2–98%. (3)

Damage to the skin barrier, larger burns, immunosuppression caused by burns, and prolonged hospital stay are important risk factors for infection. The type and size of the wound and the type and number of microorganisms. (4) *Pseudomonas aeruginosa* as one of the main causes of burn infections is responsible for an extensive range of serious infections (5). *P. aeruginosa* is a conditional pathogenic microorganism and has been among the top ten most common hospital "super bacteria" in the world for more than a decade. Therapeutic alternatives are nonetheless seriously constrained because of unfold of antimicrobial resistant lines; thus, *P. aeruginosa* contamination stays a life-threatening complication (6). The up-law of innate resistance mechanisms along with overexpression of efflux pumps and the purchase of overseas genetic determinants along with plasmids are crucial traits for the survival of *P. aeruginosa* at some stage in environmental pressures along with clinic environments. (7) The developing worldwide emergence of *P. aeruginosa* lines immune to all β-lactam sellers highlights the cap potential of this microorganism to evolve hastily to selective environmental stresses. (8) Multi-drug resistance of *P. aeruginosa* is diagnosed to be the various maximum hard antibiotic-resistant gram-poor bacilli to manipulate and treat. (9) Serious *P. aeruginosa* infections, each acute and chronic, are regularly nosocomial and related to compromised host defenses; Once an opportunistic pathogen like *P. aeruginosa* enters the host, its capacity to motive contamination has additionally been correlated with its tendency to shape biofilms. (10) Biofilm formation is a procedure wherein microorganisms irreversibly connect to and develop on a floor and bring extracellular polymers that facilitate attachment and matrix formation, ensuing in an alteration with inside the phenotype of the organisms for increase price and gene transcription (11). The aim of this study is Study of biofilm formation and antibiotic resistant profile in multidrug resistant and extensive drug resistance *P. aeruginosa* isolated from burn wound infections in southwest Iran.

Materials And Methods

Ethics declaration

The study was approved by the Research Ethics Committee (REC) of the Ahvaz Jundishapur University of Medical Sciences (No: IR. AJUMS.RE.1399.669) Iran. As the study was an observational one and did not involve any new intervention, it was conducted by the Declaration of Helsinki of 1975.

Specimens collection and Microbiological procedures

This study, which was performed in Taleghani Burn Center University Hospital, which is the major burn center in Ahvaz, Iran, from May 2019 to November 2020. One hundred and ten *P. aeruginosa* strains (replica isolates aren't included) culture-advantageous reviews consisting of wound 345 sufferers have been included. Patients who had an extended or admitted medical institution live because of burning infections with a hospitalization length of equal, extra than forty-eight hours have been studied. Patients have been excluded with any records of cancer, way of life-negative, immunocompromised diseases, and the usage of antibiotic tablets on the time of admission. Before present process wound excisions, the injuries of hospitalized sufferers have been controlled with topical 1% (Silver sulfadiazine) SSD cream with cumbersome dressings and modified each 24 hours. Samples have been accumulated from burn wounds via way of means of way of life swabs below aseptic techniques. According to the American Burn Association, wound colonization is described because the presence of a low awareness of microorganism at the floor without invasion or systemic manifestations. When there are greater than 10 five bacterial isolates of tissue way of life with inside the wound, we name it a wound infection. When greater than 10 five bacterial isolates of tissue way of life with inside the burn wound reasons the formation of pus and separation of the eschar, lack of graft with the involvement of tissue, or the presence of systemic sepsis, then it's far referred to as invasive infection (12). The samples were transferred to the laboratory immediately to the Department of Microbiology of Ahvaz Jundishapur
University of Medical Sciences. \textit{P. aeruginosa} isolates were confirmed by conventional and biochemical tests such as: gram stain, triple sugar iron (TSI), MRVP, oxidation fermentation (OF) test, citrate test, and pigment production in Mueller Hinton Agar (Biolife, Italia) and growth at 42°C. (13) The gram negative rod shape, MRVP negative, oxidative positive, citrate positive, triple sugar iron (TSI) agar reaction of alkaline over no change, growth at 42°C and production of bright-blue to blue green diffusible pigment on Mueller-Hinton agar.

**Confirmation of \textit{P. aeruginosa} -specific PCR assay**

The boiling technique turned into used to extract genomic DNA from \textit{Paeruginosa} isolates. A few bacterial colonies of \textit{Paeruginosa} lines grown in a single day on nutrient agar (Merck, Germany) have been suspended in micro tubes containing 500 µl of Tris-EDTA buffer, then the micro tubes have been located in Incubock micro tube incubators (Denville Scientific, USA) for five min at 95°C, after which centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant turned into used because the DNA template with inside the PCR assays. The DNA amount and fine have been assessed the usage of Nano Drop Spectrophotometer

**Data analysis**

C for 24 h aerobically. (18) red dye was prepared separately and added into the autoclaved BHI medium. The plates were incubated at 37°C.

**Overall, 110 clinical \textit{P. aeruginosa} isolates were confirmed from wound burn infections. All \textit{P. aeruginosa} isolates detected in biochemical tests were also confirmed by the molecular method. The mean age of the patients in the study was 35.32 years and (SD) was 11.74 years. (Table 1).**
Antibiotic resistance in *P. aeruginosa* isolates

The maximum resistance rate among *P. aeruginosa* isolates to antibiotics tested was as follow: piperacillin 68\% (n = 75), ceftazidime 59\% (n = 65), meropenem 56\% (n = 63), gentamycin 56\% (n = 63), gatifloxacin 56\% (n = 63) and minimum resistance rate among *P. aeruginosa* isolates to antibiotics tested such as ticarcillin-clavulanic acid 22\% (n = 25), and ceftolozane-tazobactam 16\% (n = 6) (Table 2). These isolates were then evaluated by using the E-test. only 7 isolates were confirmed as colistin-resistant. Colistin reference MICs for the 110 *P. aeruginosa* bacteria were from 6 to 128 mg/L.

The incidence of MDR and XDR in *P. aeruginosa* isolates

The incidence of MDR *P. aeruginosa* was 38\% (n = 42), and XDR- *P. aeruginosa* was 22\% (n = 25) respectively. One of *P. aeruginosa* isolates were PDR. The phenotypic antibiotic resistance pattern, prevalence, and diversity of the 110 *P. aeruginosa* isolates from the wound are shown in Table 1. The table reveals, that 74 different combination patterns were ranging from thirteen antibiotics in each combination. (Table 3) Out of the 110 *P. aeruginosa* isolates, one isolate was resistant to the 7 antibiotics representing thirteen classes.

The evolution of biofilm production in *P. aeruginosa* isolates

The biofilm manufacturing evaluation through MTP and Congo pink agar methods. In the CRA method, 66(67\%) isolates have been taken into consideration as generating biofilm and produced black colonies while 44 (50\%) isolates produced pink colonies. In MTP differentiated isolates into sturdy, mild, susceptible, and no biofilm-forming in step with the OD values at 570 nm for (as effective control) and TSB (as poor control) have been 0.525 ± 0.062 and 0.055 ± 0.009, respectively. The OD570 values for the medical lines ranged from 0.125 ± 0.056 to 1.745 ± 0.054.Again, 76\% (n = 84) of the isolates have capacity for biofilm, formation, 40\% (n = 45) have been classified as sturdy biofilm-formers; 32\% (n = 19) have been mild; 21\% (n = 20) have been susceptible biofilm formers and 43\% (n = 26) couldn't shape any detectable biofilm. In the forty-five sturdy biofilm-manufacturer, 76\% (n = 9) isolates have been XDR and 76\% (n = 18) isolates have been MDR and one remoted turned into PDR. Among the 76\% (n = 19) mild biofilm formers, 76\% (n = 9) isolates have been XDR and 76\% (n = 6) isolates have been MDR. Also, of the 20 susceptible biofilm-formers, 76\% (n = 3) isolates have been XDR and 76\% (n = 8) isolates have been MDR (Table 2). According to results, antibiotic resistance in *P. aeruginosa* non-biofilm manufacturer is better than *P. aeruginosa* isolates biofilm manufacturer (Fig. 1 and table4). The statistical evaluation of the no counting among biofilm-forming capacity and antibiotic resistance (P = 0.781). However, the statistical evaluation of the connection among biofilm-manufacturer capacity and XDR (P = 0.042).

Discussion

*P. aeruginosa* is one of the main etiological agents of wound infections. In our study, the prevalence of *P. aeruginosa* in burn wound was 31\%. In previous studies, in our region *P. aeruginosa* in burn wound were found to be 44\% to 69.9\%. (19,20) The rate of *P. aeruginosa* vary between different in our region, which may be due to the performance of various infection control procedures. today, drug resistance is a massive developing trouble in treating infectious illnesses. The effects of us examine confirmed that 38\% of *P. aeruginosa* isolates had been MDR. The excessive incidence of MDR *P. aeruginosa* isolates turned into additionally suggested from a few research in Iran and different countries. (21,22) The multidrug antibiotic resistance can lower the efficacy of the not unusual place antibiotics used with inside the scientific putting specially with inside the infections. During the current decades, the carbapenems have encouraged because the first-line antibiotics for the remedy of *P. aeruginosa* infections. However, unfortunately, growing resistance to carbapenems has been suggested global amongst *P. aeruginosa* isolates. (23) In us examine, the antibiotic susceptibility takes a look at effects confirmed that almost all of those isolates had been proof against meropenem (57\%), imipenem (47\%) and doripenem (67\%). In settlement with ours, the excessive quotes of carbapenem-resistant and doripenem *P. aeruginosa* isolates had been suggested from different research. (24–26) Also, our effects confirmed that the antibiotic resistance quotes to gentamycin, amikacin, tobramycin, piperacillin, aztreonam, imipenem, meropenem, ceftazidime, gatifloxacin, and norfloxacin amongst MDR *P. aeruginosa* isolates had been extra than 50\%. In much like our work, Perez et al, Del Barrio et al additionally, suggested the excessive incidence of the resistance to those antibiotic dealers amongst *P. aeruginosa* isolates. (27,28) Colistin are the most effective remedy alternatives for infections as a result of appreciably drug resistant (XDR) or MDR A. *P. aeruginosa* However, this antibiotic has a few unwanted facet effects, along with nephrotoxicity and neurotoxicity. (29) The antibiotic susceptibility takes a look at effects confirmed that almost all of *P. aeruginosa* isolates had been liable to colistin which can be in settlement with different reviews acquired from preceding research in Iran and different countries. (30–32) Hence, those effects endorse that colistin remains the only antibiotic dealers in opposition to MDR *P. aeruginosa* isolates. The biofilm matrix can extensively defend microorganism from each the immune device cells and antibiotic dealers. Biofilm formation of *P. aeruginosa* ends in lack of antibacterial susceptibility and the usage of extra concentrations of antibiotics with inside the remedy of infections as a result of such isolates. In us examine, maximum *P. aeruginosa* isolates had the cappotential of biofilm manufacturing however with unique capacities. As stated above, we located a big inverse dating among the capability of biofilm formation and resistance to all antibiotic dealers (p = 0.781), i.e. the biofilm density in touchy traces turned into extra than biofilm density in resistant traces. In constant with us examine, a few researchers additionally proven that the touchy traces tended to supply more potent biofilms than the resistant traces while a few others confirmed that MDR traces had extra functionality for the biofilm manufacturing than touchy traces. (33,34) Biofilm-forming microorganism are embedded in a matrix and gather houses that render them fairly tolerant to antibiotics, UV light, chemical biocides, host immune response, and different outside stresses. Biofilm can defend microorganisms from harsh environmental situations along with hot temperature and pH, excessive salinity and pressure, negative nutrients, antibiotics, etc., with the aid of using appearing as a barrier. Structural barriers, at the side of chronic cells inside biofilm, play a decisive position in antibiotic resistance. As reviews indicate, biofilm-associated infections are tough for remedy and could now no longer be cured easily. Consequently, the prescription of antibiotics will now no longer remedy or take away biofilm-associated contamination because of their antibiotic tolerance and genetic mutation. Biofilm is now taken into consideration to be a number one reason of continual contamination, and antibiotic-resistant microorganism are popular in biofilm form. Currently, it's miles believed that over 80\% of continual infectious illnesses are as a result of biofilm, and it’s miles recognized that
traditional antibiotic medicinal drugs are insufficient at removing those biofilm-mediated infections. (35) In end in our examine improvement of resistance with the aid of using *P. aeruginosa* to many antimicrobial dealers is a first rate undertaking in controlling its infections. Therefore, the transmission of those isolates to sufferers ends in better resistance. In total, >50% of *P. aeruginosa* scientific isolates had been manufacturers of sturdy biofilm. Therefore, the vital hygiene measurements ought to be taken for the prevention of moving the *P. aeruginosa* isolates to hospitalized sufferers.

**Declarations**

**Funding**

Not applicable

**Consent for publication**

Not applicable

**Competing interests**

The authors report no conflicts of interest in this work.

**Availability of data and materials**

Any additional information can be obtained from the corresponding author on request.

**Code availability**

Not applicable

**Authors’ contributions**

The concept and the design of the study were developed by Aram asareh zadegan dezfuli. The methodology was designed by Arshid Yousef-Avarvand. Data collection and the experimental works were carried out by Aram asareh zadegan dezfuli. The formal analyses and interpretation of data were carried out by Arshid Yousef-Avarvand. The original draft was prepared by Arshid Yousef-Avarvand. All the authors have read and approved the final manuscript for submission. The Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, financially support this project.

**Ethics approval**

The study was approved by the Research Ethics Committee (REC) of the Ahvaz Jundishapur University of Medical Sciences (No: IR. AJUMS.RE .1399.669) Ahvaz, Iran.

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Tables

Table 1- Patients and results of biofilm formation, antibiotic resistance and *P. aeruginosa* isolates
| ID | G  | Duration of hospitalization | MTP   | Congo red | ARPs                                                                 | Co Mi |
|----|----|----------------------------|-------|-----------|----------------------------------------------------------------------|-------|
| 1  | -29| First 48 hrs               | Moderate | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP G, AMK, LVX, NOR, OFX, GAT | 3     |
| 2  | -33| Third week                 | Strong  | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP G, AMK, LVX, NOR, OFX, GAT | 2.5   |
| 3  | -39| Last of first week         | Strong  | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP E, GN, T, AMK, LVX, NOR, OFX, GAT | (R)   |
| 4  | -27| Fourth week                | Strong  | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP CIP, G, AMK, LVX, NOR, OFX, GAT | 1     |
| 5  | -27| First 48 hrs               | Moderate | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP CIP, G, AMK, LVX, NOR, OFX, GAT | 1     |
| 6  | -28| Fourth week                | Strong  | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP G, AMK, LVX, NOR, OFX, GAT | 3     |
| 7  | -45| Second week                | Moderate | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP CIP, E, C/T, T, GN, AMK, LVX, NOR, OFX, GAT | 2.5   |
| 8  | -35| First 48 hrs               | Strong  | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP CZA, C/T, G, AMK, LVX, NOR, OFX, GAT | 2.5   |
| 9  | -33| Fourth week                | Weak    | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP G, AMK, LVX, NOR, OFX, GAT | 1     |
| 10 | -42| Second week                | Strong  | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP CIP, C/T, GN, AMK, LVX, NOR, OFX, GAT | 0.7   |
| 11 | -47| Last of first week         | Moderate | -         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, C/T, TZPPIP T, C/T, GN, AMK, LVX, NOR, OFX, GAT | 1     |
| 12 | -43| Fourth week                | Strong  | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, PIPTZPCIP CZA, C/T, AMK, LVX, NOR, OFX, GAT | 2.5   |
| 13 | -22| First 48 hrs               | Strong  | +         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, PIPTZPCIP CZA, C/T, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| 14 | -28| Third week                 | Moderate | -         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, T, TZPPIP CIP, C/T, GN, AMK, LVX, NOR, OFX, GAT | 3     |
| 15 | -32| Last of first week         | -       | -         | NET, TOB, MEM, DOR, ATM, CAZ, FEPTIM, TZPPIP CZA, GN, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| 16 | -36| Third week                 | Strong  | +         | NET, TOB, MEM, IPM, O, DOR, ATM, CAZ, FEPTIM, T, TZPPIP CIP CZA, GN, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| 17 | -21| Fourth week                | -       | -         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP C, GN, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| 18 | -51| First 48 hrs               | Moderate | -         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP CIP C, GN, FOX, AMK, LVX, NOR, OFX, GAT | 3     |
| 19 | -53| Fourth week                | Strong  | +         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, PIPTZPCIT CZA, C/T, GN, AMK, CIPLVX, NOR, OFX, GAT | 2.5   |
| 20 | -36| Last of first week         | Weak    | -         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP CIP, GN, AMK, LVX, NOR, OFX, GAT | 0.7   |
| 21 | -37| Third week                 | Weak    | -         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP C, RGGN, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| 22 | -40| Second week                | Moderate | +         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP E, GN, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| 23 | -34| Fourth week                | -       | -         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP E, GN, AMK, CIPLVX, NOR, OFX, GAT | 3     |
| 24 | -22| First 48 hrs               | Moderate | +         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP C, CD, GN, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| 25 | -26| Third week                 | Moderate | -         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, CZA, TZPPIP E, GN, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| 26 | -39| Second week                | Strong  | +         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP CIP, GN, AMK, LVX, NOR, OFX, GAT | 0.7   |
| 27 | -43| First 48 hrs               | Strong  | +         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP CIP, AMK, LVX, NOR, OFX, GAT | 3     |
| 28 | -27| Fourth week                | Strong  | +         | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPTIM, CZA, TZPPIP G, FOX, AMK, CIPLVX, NOR, OFX, GAT | 0.7   |
| Week          | Action       | Duration | Effect | Medications                                                                 |
|--------------|--------------|----------|--------|----------------------------------------------------------------------------|
| Third week   | Weak         | 29-41 M  | -      | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT |
| First 48 hrs | Moderate     | 30-25 F  | -      | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT |
| Second week  | Strong       | 31-36 M  | -      | NET, TOB, MEM, IPM, DOR, ATM, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT |
| Fourth week  | Strong       | 32-33 M  | -      | NET, TOB, MEM, IPM, ATM, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT     |
| First 48 hrs | Moderate     | 33-30 M  | -      | TOB, MEM, ATM, CAZ, PIP, GN, AMK, LVX, NOR, OFX, GAT                       |
| Fourth week  | Weak         | 34-30 M  | -      | TOB, MEM, IPM, AT, CAZ, PIP, GN, AMK, LVX, NOR, OFX, GAT                  |
| Second week  | Weak         | 35-28 F  | -      | TOB, MEM, IPM, AT, CAZ, PIP, GN, AMK, LVX, NOR, OFX, GAT                  |
| First 48 hrs | Weak         | 36-39 F  | -      | TOB, MEM, IPM, AT, CAZ, PIP, GN, AMK, LVX, NOR, OFX, GAT                  |
| Last of first week | Strong | 37-47 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Fourth week  | -            | 38-46 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| First 48 hrs | -            | 39-45 F  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Last of first week | Strong | 40-35 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Fourth week  | -            | 41-42 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| First 48 hrs | Strong       | 42-39 F  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Fourth week  | -            | 43-37 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Last of first week | Strong | 44-16 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| First 48 hrs | Moderate     | 45-23 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Last of first week | Moderate | 46-26 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Second week  | -            | 47-45 F  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| First 48 hrs | Moderate     | 48-67 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Third week   | Strong       | 49-54 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Last of first week | Strong | 50-33 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Third week   | -            | 51-65 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| Last of first week | Strong | 52-43 M  | -      | TOB, MEM, IPM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT       |
| First 48 hrs | Strong       | 53-55 F  | -      | MEM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT                 |
| Fourth week  | Strong       | 54-76 F  | -      | MEM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT                 |
| Third week   | Weak         | 55-43 F  | -      | MEM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT                 |
| Second week  | Strong       | 56-12 M  | -      | MEM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT                 |
| Last of first week | Weak | 57-34 M  | -      | MEM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT                 |
| Last of first week | -      | 58-35 M  | -      | MEM, AT, CAZ, FEPT, TZP, PIP, GN, AMK, LVX, NOR, OFX, GAT                 |
| M     | Week               | Reaction | Associated Molecules                  |
|-------|--------------------|----------|---------------------------------------|
| 59    | Fourth week        | -        | MEM, ATM, CAZ, PIP, GN, GAT            |
| 60    | Second week        | Moderate | MEM, ATM, CAZ, PIP, GN, GAT            |
| 61    | Last of first week | -        | ATM, CAZ, PIP, GN, GAT                |
| 62    | First 48 hrs       | Strong   | ATM, CAZ, PIP, GN, GAT                |
| 63    | Fourth week        | Weak     | IPM, ATM, PIP, GN, GAT                |
| 64    | Last of first week | Strong   | IPM, ATM, PIP, GN, GAT                |
| 65    | Last of first week | -        | IPM, ATM, PIRGAT                      |
| 66    | Fourth week        | Strong   | IPM, ATM, PIRGAT                      |
| 67    | First 48 hrs       | Weak     | IPM, ATM, PIP                         |
| 68    | Fourth week        | Weak     | IPM, ATM, PIP                         |
| 69    | Last of first week | -        | IPM, ATM, PIP                         |
| 70    | First 48 hrs       | -        | IPM, ATM, PIP                         |
| 71    | Last of first week | Strong   | PIP                                   |
| 72    | Fourth week        | -        | PIP                                   |
| 73    | First 48 hrs       | Strong   | -                                     |
| 74    | Fourth week        | Weak     | -                                     |
| 75    | Last of first week | -        | -                                     |
| 76    | Fourth week        | Strong   | -                                     |
| 77    | Last of first week | Weak     | -                                     |
| 78    | Last of first week | -        | -                                     |
| 79    | Fourth week        | Strong   | -                                     |
| 80    | First 48 hrs       | Moderate | -                                     |
| 81    | Last of first week | -        | -                                     |
| 82    | Fourth week        | Strong   | -                                     |
| 83    | First 48 hrs       | Moderate | -                                     |
| 84    | First 48 hrs       | Strong   | -                                     |
| 85    | First 48 hrs       | -        | -                                     |
| 86    | Last of first week | Weak     | -                                     |
| 87    | First 48 hrs       | Moderate | -                                     |
|    |   |                                             |        |    |   |        |
|----|---|--------------------------------------------|--------|----|---|--------|
| 88 | 17 | Fourth week                                | -      | -  | - | -      |
| 89 | 65 | First 48 hrs                               |        |    | + | -      |
| 90 | 43 | Fourth week                                | Strong | +  | - | -      |
| 91 | 22 | Fourth week                                | Strong | +  | - | -      |
| 92 | 23 | Last of first week                         | Moderate + | - | - | -      |
| 93 | 43 | Last of first week                         | Strong | +  | - | -      |
| 94 | 34 | First 48 hrs                               | Strong | +  | - | -      |
| 95 | 43 | Fourth week                                | -      | -  | - | -      |
| 96 | 22 | Fourth week                                | -      | -  | - | -      |
| 97 | 33 | Fourth week                                | -      | -  | - | -      |
| 98 | 43 | First 48 hrs                               | Strong | +  | - | -      |
| 99 | 43 | Last of first week                         | -      | -  | - | -      |
| 100| 22 | Fourth week                                | Weak   | +  | - | -      |
| 101| 13 | First 48 hrs                               | Weak   | +  | - | -      |
| 102| 23 | Fourth week                                | Strong | +  | - | -      |
| 103| 34 | Last of first week                         | Weak   | +  | - | -      |
| 104| 22 | Fourth week                                | Strong | +  | - | -      |
| 105| 44 | First 48 hrs                               | Strong | +  | - | -      |
| 106| 33 | Fourth week                                | Weak   | +  | - | -      |
| 107| 33 | First 48 hrs                               | Strong | +  | - | -      |
| 108| 34 | Fourth week                                | Weak   | +  | - | -      |
| 109| 23 | Fourth week                                | Weak   | +  | - | -      |
| 110| 43 | First 48 hrs                               | Strong | +  | - | -      |

Table 2 - Results of antimicrobial resistance tests by disk diffusion method
| Antimicrobial category | Antimicrobial agent | resistant | susceptible |
|------------------------|---------------------|-----------|-------------|
| PENICILLINS            | Piperacillin         | (68%)75   | (31%)35     |
| B-LACTAM COMBINATION AGENTS | Piperacillin-tazobactam | (44%)49   | (55%)61     |
|                        | Ceftazidime-avibactam | (26%)29   | (73%)81     |
|                        | Ceftolozane-tazobactam | (14%)16   | (85%)94     |
|                        | Ticarcillin-clavulanate | (22%)25   | (77%)85     |
| CEPHEMS                | Cefepime            | (29%)32   | (70%)78     |
|                        | Ceftazidime         | (59%)65   | (40%)45     |
| MONOBACTAMS            | Aztreonam           | (66%)73   | (36%)37     |
| CARBAPENEMS            | Doripenem           | (65%)33   | (65%)77     |
|                        | Imipenem            | (47%)52   | (52%)58     |
|                        | Meropenem           | (57%)63   | (47%)52     |
| AMINOGLYCOSIDES         | Gentamycin          | (60%)67   | (39%)43     |
|                        | Tobramycin          | (62%)55   | (62%)55     |
|                        | Amikacin            | (49%)54   | (62%)56     |
|                        | Netilmicin          | (35%)39   | (64%)71     |
| FLUOROQUINOLONES        | Ciprofloxacin       | (53%)59   | (46%)51     |
|                        | Levofoxacin         | (55%)61   | (44%)49     |
|                        | Norfloxacin         | (53%)59   | (46%)51     |
|                        | Ofloxacin           | (39%)44   | (58%)66     |
|                        | Gatifloxacin        | (62%)69   | (37%)41     |

piperacillin (PIP), piperacillin-Tazobactam (TZP), ceftazidime-Avibactam (CZA), ceftolozane-tazobactam (C/T), ticarcillin-clavulanic acid (TIM), cefepime (FEP), ceftazidime (CAZ), aztreonam (ATM), doripenem (DOR), imipenem (IPM), meropenem (MEM), tobramycin (TOB), gentamicin (GN), amikacin (AMK), netilmicin (NET), ciprofloxacin (CIP), levofloxacin (LVX), norfloxacin (NOR), ofloxacin (OFX), gatifloxacin (GAT)

Table 3. Antibiotic resistance patterns among *P. aeruginosa* isolates
Table 4: The relation between biofilm formation and the antibiotic susceptibility patterns

| Rate of biofilm |  n  | susceptible | MDR | XDR |
|-----------------|-----|-------------|-----|-----|
| Strong          | -   | 1           | 18  | 10  |
| Moderate        | -   | 1           | 5   | 9   |
| Weak            | -   | 3           | 8   | 3   |
| Nonbiofilm      | 26  | 12          | 11  | 3   |
| Total           | 26  | 17          | 42  | 25  |

Figures
Figure 1

The frequency of antibiotic resistance in biofilm producer and non-biofilm producer P. aeruginosa.