Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* in Regional Tertiary Care Hospitals of Saudi Arabia

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**Abstract:**

**Objectives:** To determine antimicrobial resistance pattern of *P.aeruginosa* in regional tertiary care hospitals of Saudi Arabia.

**Method and materials:** A total of 121 clinical isolates of *P.aeruginosa* from eight different hospitals were studied. The clinical data of the patients was collected using a predesigned questionnaire. All isolates were identified as *P.aeruginosa* using standard bacteriological methods and API 20E kits. Antibiotic susceptibility testing was performed by disk diffusion method recommended by Clinical and Laboratory Standards Institute.

**Results:** Majority of *P.aeruginosa* isolates were from patients suffering from respiratory tract infections (43.8%), followed by wound infections (21.5%). The overall drug resistance data of 121 *P.aeruginosa* strains showed low to moderate resistance to all drugs tested (4.9% to 30.6%). The highest resistance was exhibited by meropenem (30.6%) followed by ticarcillin (22.3%), imipenem (19%), and piperacillin (17.3%). Piperacillin-tazobactam showed lowest resistance (4.9%). Irrespective of site of infection, *P.aeruginosa* strains showed high resistance to meropenem followed by ticarcillin and imipenem. About 13.2% strains were multidrug resistant.

**Conclusion:** This study showed low to moderate rate of drug resistance in *P.aeruginosa*. Resistance rate <10% to amikacin, cefepime and piperacillin-tazobactam is encouraging for the treatment of pseudomonal infection. Continuous monitoring of antimicrobial susceptibilities at each hospital is important to help in deciding the most adequate therapy for *P.aeruginosa* infection and to know the developing resistance pattern.

**I. Introduction:**

Members of genus *Pseudomonas* are Gram-negative, aerobic, rod-shaped bacteria with widespread occurrence in nature, especially in damp areas. The clinically important species of *Pseudomonas* are *P.aeruginosa*, *P.putida*, *P.fluorescens*, *P.stutzeri*, *P.alcaligenes*, *P.pseudoalcaligenes*, *P.putrefaciens*, and *P.mendocina*. However, the most important among them is *P.aeruginosa* as the total number of infections produced by other species is far lower than that produced by *P.aeruginosalanone*.

*Pseudomonas aeruginosa*, being an opportunistic human pathogen, is the leading cause of nosocomial infections, especially among patients who are admitted to intensive care units. It has been implicated in various nosocomial infections e.g., pneumonia, urinary tract infection, skin and soft tissue infections, in severe burns and wound infections and in infections in immuno-compromised individuals. Compared to *Enterobacteriaceae*, *P.aeruginosa* is relatively resistant to many antibiotics. The rate of antibiotic resistance to *P.aeruginosa* is increasing in many parts of the world, in particular to β-lactams, aminoglycosides, and fluoroquinolones.

Its general resistance is due to a combination of factors. It is intrinsically resistant to antimicrobial agents, due to low permeability of its cell wall. It has the genetic capacity to express a wide range of resistance mechanisms. It can become resistant through mutations in the chromosomal genes which regulate the resistance genes. It can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages. The resistance of *P. aeruginosa* is not the same in different countries. For example drug resistance to antipseudomonal drugs was highest in Turkey (70-93%); India (43-68%); Bangladesh (40-86%); Pakistan (35-40%) and in Iran (36-75%), but low in Singapore (10-23%); West Indies (2.6-12.3%) and Malaysia (6.7-23%).
In some countries variable rates with increasing trend in resistance were seen. In UK resistance to antipseudomonal drugs was 8% in 2001 which increased to 43% in 2003 and in Saudi Arabia, resistance rate raised from 10% in 1998 to 30% in 2004. Other studies from Saudi Arabia also showed a gradual increase in antimicrobial resistance against *P. aeruginosa* over a period of 5 years from 4% in 2001 to 20% in 2005 for antipseudomonal drugs. In another study from Riyadh high rates of drug resistance (36-54%) to antipseudomonal drugs was found. Similar results were reported in a study from Makkah where *P. aeruginosa* showed 29-51% resistance to antipseudomonal drugs. While an earlier study showed 50-90% resistance by *P. aeruginosa* to β-lactams, aminoglycosides, and fluoroquinolones.

In recent years, a considerable increase in the prevalence of multidrug resistance (MDR) in *P. aeruginosa* has been noticed, which is related to high morbidity and mortality. Regional variations were seen in multidrug resistance *P. aeruginosa* (MDRPA) from 0.6-32% according to geographic location and type of surveillance study. Studies from Saudi Arabia showed low as well as high rate of MDRPA. The study from Riyadh reported low rate of MDRP *P. aeruginosa* among resistant *P. aeruginosa* isolates (2% in 2004 and 3% in 2005). While the study from Makkah showed very high rate of MDRPA (50%).

*Pseudomonas aeruginosa* has emerged as a major cause of healthcare-associated infections. Its clinical significance has greatly increased due to its ability to rapidly develop resistance to major groups of antibiotics used for its treatment. It is important to know the changing pattern of antimicrobial susceptibility for adequate therapy of pseudomonal infection. Therefore, this study was undertaken to determine the antimicrobial resistance pattern of *P. aeruginosa* including magnitude of multidrug resistance in various tertiary care hospitals.

### II. Materials and Methods:

**Bacterial isolates:** In this study a total of 121 *P. aeruginosa* clinical isolates were collected from eight different hospitals during August – October 2013. The distribution of these isolates among participating hospitals is given in the bracket after the name of each hospital. The hospitals from Makkah were: Maternity and Children hospital (32), Al-Noor Specialist hospital (13); King Abdul Aziz hospital (7) and Her’a General hospital (3). The hospitals from Jeddah included in the study were: Saudi National Guard hospital (35); Maternity and Children hospital (22); King Fahad General Hospital (6) and King Fahad Armed Forces hospital (3).

**Patients’ data:** The clinical information about the site of infection from where *P. aeruginosa* strains were isolated was collected using a predesigned questionnaire.

**Identification of clinical isolates of *P. aeruginosa***: All clinical isolates were sub cultured on blood agar plates and incubated at 37°C for 16-24 hours. Following incubation, plates were examined for the growth and processed for the identification of the organism using standard bacteriological methods and API20E kit.

**Antibiotics susceptibility test:** Antimicrobial susceptibility testing of each *P. aeruginosa* isolate was performed using Kirby Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute. Antibiotic disks used in the susceptibility test were: amikacin (30µg), aztreonam (30µg), cefepime (30µg), ceftazidime (30µg), ciprofloxacin (5µg), gentamicin (10µg), imipenem (10µg), levofloxacin (5µg) meropenem (10µg), piperacillin (100µg), piperacillin–tazobactam (100/10µg), and ticarcillin (75µg). Using standard interpretive tables of CLSI, the test organism was classified as susceptible, intermediate or resistant to the panel of antimicrobials used.

**Data Analysis:** The data of all patients obtained through a predesigned questionnaire was entered and analyzed using Microsoft Excel2007.

### III. Results

**Clinical information of *P. aeruginosa* infected patients:** Distribution of *P. aeruginosa* according to site of infection revealed highest isolation rate from respiratory tract infections (43.8%). The next in order were surgical wound infection (21.5%), genital infection (14%), urinary tract infection (10.8%), and blood stream infection (3.3%). *P. aeruginosa* was isolated in equal number (2.5% each) from eye and ear infections (Fig.1).

**Drug resistance in *P. aeruginosa* isolates:** The antibiotic resistance pattern of 121 *P. aeruginosa* isolates from eight different hospitals of Makkah and Jeddah were studied for antibiotics commonly used for the treatment of pseudomonal infections. Both, overall drug resistance pattern and data of individual hospitals for *P. aeruginosa* isolates is presented here.

The overall drug resistance data of 121 *P. aeruginosa* strains showed low to moderate resistance to all drugs tested (4.9% to 30.6%) (Fig.2). Highest resistance was exhibited by meropenem (30.6%), followed by...
ticarcillin (22.3%), imipenem (19%), piperacillin (17.3%) Both, aztreonam and ciprofloxacin showed equal rate of resistance (16.5%, each). Drug resistance rate for other antibiotics were: levofloxacin (15.7%), ceftazidime (14%) and gentamicin (11.6%). Other antibiotics, which showed resistance <10% were: cefepime (8.3%), amikacin (7.4%). Piperacillin-tazobactam showed lowest resistance (4.9%).

The drug resistance pattern of clinical isolates of *P.aeruginosa* recovered from different clinical sites was compared (Table-2). It was found that all clinical isolates irrespective of site of infection showed high resistance to meropenem followed by ticarcillin and imipenem.

In this study *P.aeruginosa* clinical isolates from eight hospitals (four hospitals each from Makkah and Jeddah) were studied and their drug resistance pattern is presented in Table-3.
Antimicrobial Resistance Pattern Of Pseudomonas Aeruginosa In Regional Tertiary Care Hospitals...

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance Pattern (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime</td>
<td>8(15%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>13(24.5%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>14(26.4%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10(19%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>15(28.3%)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>13(24.5%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>22(41.5%)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>8(15%)</td>
</tr>
<tr>
<td>Piperacillin-</td>
<td>4(7.5%)</td>
</tr>
<tr>
<td>Tazobactam</td>
<td></td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>19(36%)</td>
</tr>
</tbody>
</table>

Table 3: Antibiotic resistance pattern in the hospitals of Jeddah and Makkah

<table>
<thead>
<tr>
<th>Name of the Hospital</th>
<th>Antibiotic Resistance Pattern in Participating Hospitals (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Amikacin</td>
</tr>
<tr>
<td>SNG-J(35)</td>
<td>8.6</td>
</tr>
<tr>
<td>MCH-J(22)</td>
<td>0</td>
</tr>
<tr>
<td>KFGH-J(6)</td>
<td>16.7</td>
</tr>
<tr>
<td>KFAFH-J(3)</td>
<td>0</td>
</tr>
<tr>
<td>MCH-M(32)</td>
<td>0</td>
</tr>
<tr>
<td>NSH-M(13)</td>
<td>30.7</td>
</tr>
<tr>
<td>KAH-M(7)</td>
<td>14.2</td>
</tr>
<tr>
<td>HGH-M(3)</td>
<td>0</td>
</tr>
</tbody>
</table>

Jeddah Hospitals: SNGH-J= Saudi National Guard Hospital; MCH-J= Maternity and Children; KFGH-J= King Fahad General Hospital; KFAFH-J= King Fahad Armed Forces Hospital

Makkah Hospitals: MCH-M= Maternity and Children; NSH-M= Al-Noor Specialist Hospital; KAH-M= King Abdul-Aziz Hospital; Hospital; Hirra General Hospital

Among all the hospitals, the highest drug resistance in P.aeruginosa isolates was found at King Fahad General Hospital, Jeddah (16.7- 66.7%). The lowest resistance rate was seen at Maternity and Children hospital, Jeddah (4.5- 22.7%) and Maternity and Children hospital, Makkah (6.2-21.9%).

In all the hospitals, whether from Makkah or Jeddah, P.aeruginosa isolates exhibited highest resistance to meropenem (21.97- 66.7%), except at King Fahad Armed Forces hospital, Jeddah where all the strains studied showed no resistance to any antibiotics tested. The highest resistance to meropenem was seen in the isolates from King Fahad General hospital, Jeddah (66.7%), followed by Al-Noor Specialist, hospital, Makkah (61.5%). The lowest resistance to meropenem was found at Maternity and Children hospital, Makkah (21.9%).

Ticarcillin was found to be the second in the order of resistance in almost all the hospitals (4.5- 22.8%).

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66.7%). Similar pattern as that of meropenem was seen for ticarcillin at King Fahad General hospital, Jeddah, where it was found in the highest rate (66.7%), followed by Al-Noor Specialist, hospital, Makkah where resistance to ticarcillin was 46.1%. The lowest resistance to ticarcillin (4.5%) was seen at Maternity and Children hospital, Jeddah

No resistance was shown by *P. aeruginosa* to piperacillin-tazobactam from all the hospitals, except King Fahad General Hospital, Jeddah, Al-Noor Specialist hospital, Makkah, and Saudi National Guard hospital, Jeddah, where resistance rate to piperacillin-tazobactam was 33.3%, 15.4% and 5.8%, respectively.

The cumulative drug resistance data from Makkah hospitals (Fig. 3) showed highest resistance to meropenem (34.5%), followed by piperacillin and ticarcillin (25.4%, each), aztreonam (20%), levofloxacin, ciprofloxacin (18.1%, each), ceftazidime and gentamicin (16.3%, each) and imipenem (14.5%). The antibiotics which showed drug resistance <10% included: amikacin and Resistant Intermediate Susceptible cefepime (9%, each) and the lowest resistance rate exhibited by piperacillin-tazobactam (3.6%).
From the hospitals of Jeddah (Fig. 4), cumulative drug resistance also showed highest rate of resistance to meropenem (27.2%), followed by imipenem (22.7%), ticarcillin (19.6%), ciprofloxacin (15.1%), ceftazidime (14.5%), aztreonam and levofloxacin, (13.6%, each), piperacillin (10.6%). The antibiotics which showed drug resistance <10% included: gentamicin and cefepime (7.5%, each). Amikacin and piperacillin-tazobactam exhibited lowest rate of resistance (6%, each).

Multidrug resistance (MDR) was found to be 13.2% among the isolates studied. *P. aeruginosa* strains from the hospitals of Makkah and Jeddah showed MDR in 7.4% and 5.8% strains, respectively (Fig. 5).

Considering individual hospitals, maximum MDR rate was seen at Al- Noor Specialist Hospital, Makkah (46.1%), followed by Saudi National Guard hospital, Jeddah (17.1%). Other hospitals MDR rates were: 16.6% from King Fahad General Hospital, Jeddah, 14.2% from King Abdul Aziz hospital, Makkah and 6.2% from Maternity and Children hospital, Makkah. No MDR strain was found at Maternity and Children hospital Jeddah, King Fahad Armed Forces hospital, Jeddah and Hirra General Hospital, Makkah (Fig. 6).

**Figure 5: Multidrug resistance among *P. aeruginosa* isolates.**

**Figure 6: Distribution of multidrug resistant isolates of *P. aeruginosa* in various hospitals of Makkah and Jeddah (n=16)**

**Makkah Hospitals:** NSH-M= Al-Noor Specialist Hospital; KAH-M= King Abdul-Aziz Hospital; MCH-M= Maternity and Children Hospital

**Jeddah Hospitals:** SNGH-J= Saudi National Guard Hospital; KFGH-J= King Fahad General Hospital

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Pseudomonas aeruginosa has emerged as an important pathogen and found implicated in variety of nosocomial infections, in particular respiratory tract infections. In our study P. aeruginosa was responsible for 43% of respiratory tract infections. Similarly, 41.2% and 42.7% cases of respiratory tract infection from Malaysia and 42.7% and 52% cases from Makkah, Saudi Arabia were due to P. aeruginosa. P. aeruginosais also a common isolate from surgical wounds. Studies from Italy, Turkey and Saudi Arabia found P. aeruginosa as a causative agent in 25.2%, 22.2% and 26% of the surgical wound infections, respectively. Our results coincide with these studies as 20.6% of surgical wound infections were due to P. aeruginosa.

In our study, P. aeruginosa was responsible for 14% of female genital infections. Similar results found in a study from Makkah in which P. aeruginosa was responsible for 14.7% cases of female genital infections. However, another study from Makkah reported low rate (3%) of P. aeruginosa genital infection. Variable rates of urinary tract infections due to P. aeruginosa were reported from different parts of the world e.g., from USA (8%); Nigeria (4.6%); Saudi Arabia (8.9%), and (12%). Our findings are in agreement with these studies where 10.8% of urinary tract infections were caused by P. aeruginosa.

Pseudomonas aeruginosa is one of the major causative agents of burn wound infections. General prevalence studies conducted does not represent its actual rate for burn wound infections because less number of burn cases was brought to general hospitals. Our study falls in this category and where only 0.8% cases of burn wound infection were due to P. aeruginosa. Whereas, studies conducted in special burn treatment centers gives actual rate of infection caused by P. aeruginosain burn cases. The studies from Tehran and Italy reported P. aeruginosa in 57% and 59% cases of burn infection, respectively.

Determining antibiotic resistance pattern of bacterial agents may assist in the appropriate selection of antibiotic for treatment. In the present study P. aeruginosa isolates showed low to moderate antibiotic resistance pattern (4.9% to 30.6%). These findings are in agreement with the studies conducted in Saudi Arabia, Malaysia, Singapore, West Indies, and UK. Whereas, high drug resistance rate (>50% to 98%) for P. aeruginosa was reported from Turkey, Bangladesh, Iran, and as well as from Saudi Arabia.

In our study, highest drug resistance for P. aeruginosa isolates was found with meropenem (30.6%). This rate is comparable with the rates reported in recent studies from Makkah (36.4%) and Riyadh (38.3%). However, a low rate of resistance to meropenem was reported from Riyadh (5%) and Al-Khobar (18%).

The resistance to ticarcillin was 22.3% in our study which is less compared to report earlier from Makkah (56.3%). A very high rate of resistance to ticarcillin (93%) was reported in a study from Turkey. This variation in resistance rate may be correlated with the inappropriate use of relevant antibiotics for the treatment.

The resistance to imipenem in this study was 19%. Similar rates were found in studies from Al-Khobar (20%) and Makkah (25.3%). The low rates of resistance were also reported from other parts of Saudi Arabia: Riyadh (9%), Dhahran (5.8%). However, in a study from Riyadh high rate of imipenem resistance (38.6%) was also reported. The variation in the resistance rates of P. aeruginosa may be related to antibiotic prescribing habits at these settings.

In this study, the resistance rate of P. aeruginosa to piperacillin was moderate (17.3%). Compared to our study, a high rate of resistance to piperacillin was reported in a study from Riyadh (54%), and two studies from Makkah (49.4%) and (47%). However, one study from Dhahran showed low rate of resistance to piperacillin (4-11%). The differences in the resistance rates are probably related to differences in antibiotic use in these settings.

The encouraging finding of our study is the lowest rate of resistance shown by piperacillin-tazobactam (4.9%), which is one of the mainstay for the treatment of pseudomonal infections. Similar results were shown for piperacillin-tazobactam resistance in two studies from Malaysia (9.4%), (12%), and one study from Singapore (11.7%). However, other studies conducted in different parts of Saudi Arabia showed much higher rate of resistance to this antibiotic combination; Riyadh (50.3%), and Makkah (41.2%) and (33.5%).

Ceftazidime resistance in this study and a study from Riyadh was same (i.e., 14%). However, a much higher rate of resistance for ceftazidime was reported in earlier studies from Makkah (51.3%), (52.7%), and other parts of Saudi Arabia: Al-Khobar (53%) and Riyadh (45.1%). Thus, the differences in the resistance rates usually correlate with the prescribing practices of each hospital and the selective pressure of certain antibiotics.
Resistance of *P. aeruginosa* to ciprofloxacin is becoming a rising problem in many parts of the world. In our study, the resistance rate to ciprofloxacin was 16.5%. Much higher rate was reported from Saudi Arabia and other parts of the world. From Saudi Arabia resistance to ciprofloxacin was found to be (42.8%) and (50.9%) from Makkah, and 35% from Al-Khobar. Similar rates were also reported from Iran (58%), India (49%) and Turkey 48.9%. The difference in the rate of ciprofloxacin resistance is usually related to the intensity of the use of fluoroquinolones.

In our study, although, overall drug resistance data compared to individual hospitals data is giving variable rate of drug resistance for each hospital, meropenem and ticarcillin exhibited high resistance in all hospitals. The low rates of resistance to amikacin, cefepime and piperacillin-tazobactam are encouraging as these can be used appropriately in these hospitals.

The resistance rate of *P. aeruginosa* to different antimicrobials isolated from different sites varied greatly. In a study from Bangladesh, organisms isolated from surgical wound infection were more resistant to azithromycin (100%), ceftazidime (86.8%), and ciprofloxacin (75.5%). However, isolates from respiratory infection were 100% resistant to ciprofloxacin, ceftazidime, and amikacin. In contrast, in a study from Saudi Arabia, piperacillin has shown high resistance among the isolates of respiratory, urinary and wound infections. The similar pattern was seen in our study where meropenem has shown high resistance among *P. aeruginosa* strains isolated from respiratory, surgical, genital and urinary tract infections. This phenomenon that all clinical isolates of *P. aeruginosa* irrespective of site of infection has shown high resistance to one drug i.e., piperacillin in an earlier study from Saudi Arabia and meropenem in our study may be attributed to antibiotic prescribing polices of these settings; however, it needs further evaluation.

The rate of MDR-*P. aeruginosa* is increasing in many parts of the world and poses a serious therapeutic problem. In this study, the rate of MDR- *P. aeruginosa* was 13.2%, which is high. Similar results were reported from Malaysia where MDR-*P. aeruginosa* was found in 19.6% strains. Whereas, in contrast studies from Saudi Arabia, reported 1-2% MDR from Dhahran and 2-3% MDR-*P. aeruginosa* from Riyadh. Continuous monitoring of drug resistance pattern at healthcare facilities will be of help to know the trend of MDR among *P. aeruginosa* in Saudi Arabia.

In conclusion, this study found low to moderate rate of drug resistance among *P. aeruginosa* in various hospitals of Makkah and Jeddah. Low resistance rate (<10%) to amikacin, cefepime and piperacillin-tazobactam is encouraging. This study also reports high MDR (13.2%) in *P. aeruginosa* strains.

Since, drug resistance pattern of *P. aeruginosa* is keep on changing, therefore such type of study needs to conducted at each institute at regular intervals which will help in selection of appropriate antimicrobial therapy for the treatment of *P. aeruginosa* infection. Further, prudent use of antibiotics for the treatment should be included in the antibiotic policy of the hospital so that multiple drug resistant *P. aeruginosa* strains may not develop in short term.

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