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## Pattern of Resistance to *Pseudomonas* infection in the North of Iraq: Emphasis on the Potential Role of a Combination Antibiogram

Nadia A. Al-Derzi, MD, MSc\*

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### ABSTRACT:

**Background:** The wide misuse of broad spectrum antibiotics causes increase in the ratio of antibiotic resistance in Iraq. Data are limited on the prevalence, pattern of resistance, and factors associated with resistant organisms.

**Objective:** The aim of this study was to determine the isolation prevalence and antibiotic resistance ratio of *P. aeruginosa* species isolated in the North of Iraq and to determine the optimum effect of a combination antibiogram and its potential role in empiric treatment.

**Patients and Methods:** The study samples were collected from the two cities Mosul and Duhok. Mosul samples were taken from inpatient specimens sent for culture and antibiotic sensitivity in Al-Salam teaching hospital for a three-year-period (2003-2005). Duhok samples were obtained mainly from outpatient specimens sent to the laboratory of one of the two main private hospitals in the city for another three-year-period (2007-2009). *P. aeruginosa* was identified by conventional methods and API 20 E (Biomérieux). Their antibiotic susceptibility was tested using the Kirby-Bauer plate diffusion method. Detection of combination effects was performed on Duhok samples by *in vitro* testing of different antimicrobial combination patterns commonly used in routine empirical practice. For this purpose combinations were chosen of the top four individual drugs demonstrating the highest susceptibilities by the standard antibiogram.

**Results:** Out of 8038 and 1878 clinical specimens submitted for culture in Mosul and Duhok, respectively, 180 and 21 clinically significant isolates of *P. aeruginosa* were isolated, resulting in a prevalence of 5.2% and 1.6%, respectively. The most common isolates were from pus, followed by urine specimens and ear discharges. The isolates in males were twice that of females. The pattern of resistance revealed that amikacin had the highest sensitivity (89.7%) followed by imipenem, tobramycin and piperacillin (85.6%, 84.1 %, and 82.1%, respectively). Cefotaxime showed the lowest sensitivity rate (66.4%) followed by ceftazidim, carbinicillin, ciprofloxacin and gentamycin (70.1%, 74.6%, 76.1%, and 79.1%, respectively). The study revealed that the optimum combination therapy with the highest sensitivity rate were the combination of amikacin with either piperacillin or imipenem (95.2%) and piperacillin with tobramycin (95.2%).

**Conclusions and Recommendations** A relatively high resistance rate to first line anti-pseudomonal drugs was observed, which should lead to continuous evaluation of hospital and community resistance pattern, the use of optimum combination therapy should be considered in the rational use of anti-pseudomonas drugs.

**Keywords:** *Pseudomonas aeruginosa*, Resistance pattern, Prevalence

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### Introduction:

Infections due to antibiotic-resistant bacteria are associated with increased morbidity and mortality due to the administration of inadequate empirical antibiotic therapy. This is mainly encountered among infections by gram-negative bacilli, especially *P. aeruginosa* with increases in adverse patient outcomes.<sup>1,2</sup>

*Pseudomonas* species are ubiquitous organisms which occur in water, soil, and decaying organic matter. They tend to be prevalent in hospitals where an exchange can occur between patients and environmental habitats. Normal individuals are generally resistant to infection but immunocompromised patients, particularly those treated with antibiotics to which the *pseudomonas* is resistant, are susceptible. Such treatment promotes infection with resistant strains of *pseudomonas*.<sup>3,4</sup>

Increasing antibiotic resistance contributes to difficulties with choosing antibiotics for these infections. Prominent examples include carbapenem resistance in *P. aeruginosa*, and now even resistance to antibiotics used as "salvage" therapy, such as tigecycline and the polymyxins. The mechanisms of this resistance are often complex but include production of multiple  $\beta$ -lactamase types, outer-membrane impermeability, up-regulated efflux pumps, and target-site mutation.<sup>5</sup>

In Iraq there is increasing concern regarding antimicrobial resistance. Data are limited on the prevalence, pattern of resistance and risk factors associated with resistant organisms. *P. aeruginosa* is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen.

Drug combinations have been used to increase the susceptibility of the microbe where combinations with the broadest coverage were consistently composed of an aminoglycoside and a  $\beta$ -lactam.<sup>6</sup> Bhat et al., for example, showed that adding amikacin to carbapenem had significantly improved the adequacy of the initial empirical antibiotic. It is important to point out that combination therapy for *P. aeruginosa* infection does not prevent the emergence of resistance.<sup>7,8,9</sup>

While the rate of antibiotic resistance is worsening, the antibiotic available for use against gram-negative bacilli is barely increasing. On a daily basis, clinicians are forced to choose an initial empirical therapy before identification of the bacteria and before susceptibility test results are available. Habitual use of the same antibiotic regimen for all patients may lead to increased resistance and/or increased rates of inadequate coverage.<sup>10,11</sup>

Increasing antibiotic resistance to *P. aeruginosa* is contributing to difficulties with choosing antibiotics for these infections. Close liaison with clinical microbiologists may allow clinical use of preliminary identification data. Anti-pseudomonal therapy must be ensured if *P. aeruginosa* is provisionally identified. An important role of the microbiology laboratory is to alert prescribers to the possibility of the presence of an organism not routinely covered by standard anti-pseudomonal therapy.

The aim of this study was to estimate the isolation prevalence and antibiotic resistance ratio of *P. aeruginosa* species isolated in the North of Iraq and to determine the potential role of a combination Antibioqram in the empirical therapy of severe pseudomonas infection.

### Patients and Methods:

The study was conducted in Mosul and Duhok, by prospective follow up of cultures. Mosul is the center of Nineveh governorate, which has a population of about 3 million and is situated 400km north of Baghdad. Duhok is the center of Duhok governorate with a population of about 1 million, about 65km North of Mosul in the Kurdistan Region of Iraq.<sup>12, 13</sup>

The study included all samples that were submitted to the microbiology laboratory for culture and antibiotic susceptibility examination. The samples were collected from abscesses, urine, and wound specimens from the two cities Mosul and Duhok.

Mosul samples were obtained from inpatients who were admitted to Al-Salam teaching hospital, which were collected for a three-year-period (2003-2005). This hospital is one of the two major teaching hospitals in Mosul serving mainly the west bank of Mosul city. A total of 8038 samples were sent to the microbiology laboratory.

Duhok samples were mainly collected from outpatients and sent to the laboratory of one of the two main private hospitals in the city for another three-year-period (2007-2009). A total of 1878 samples were sent to that laboratory.

*P. aeruginosa* was identified by conventional methods using the following criteria of the Clinical and Laboratory Standards Institute (CLSI; formerly, the NCCLS): recognizable colony structure and colony morphology gram stain, oxidase positive reaction, typical smell, and development of pyocyanin pigments.<sup>14</sup> The commercial identification systems Api 20E (Biomérieux API Products Ltd.) was used for other oxidase-positive gram-negative rods that did not have typical characteristics. Their antibiotic susceptibility was tested using the Kirby-Bauer plate diffusion method; .The antimicrobial susceptibility discs used were from (Oxoid Ltd.); susceptibility to the following agents was tested and reported: amikacin, ceftazidime, cefotaxim,

gentamicin, imipenem, carbenicillin, piperacillin, ciprofloxacin, and tobramycin. Susceptibility data were interpreted according to CLSI guidelines.<sup>15</sup>

To compare a combination antibiogram with a standard antibiogram, different clinically relevant antibiotic combinations used in routine empirical practice with a  $\beta$ -lactam-aminoglycoside were chosen. From the top individual drugs that demonstrated the highest susceptibilities tested by the standard antibiogram against 21 *P. aeruginosa* strains isolated in Dohuk to determine their optimum combined efficacy.

Qualitative measurement of drug interaction was performed by using the disk diffusion method. This technique utilizes the same inoculums and Mueller-Hinton agar as routine Bauer-Kirby susceptibility testing. Disks impregnated with individual antimicrobial agents are placed at a distance equal to the sum of the zone radii of inhibition of drugs when tested separately in the standard antibiogram. After overnight incubation, the interface of zone of inhibition was examined, and any inhibition of growth due to the combined effects of both antimicrobial agents were observed. and the number and percentage of isolates susceptible to to at least 1 of the 2 agents was recorded at different combinations<sup>16,17</sup>.

### Results:

Table 1 shows that out of 8038 clinical specimens submitted for culture during the three-year-period at Al-Salam Hospital in Mosul, 3429 were clinically significant isolates (42.7%) of which 180 were *P. aeruginosa*, resulting in a prevalence of 5.2%. In Duhok, out of the total 1878 specimens, 1292 were clinically significant isolates (68.8%) of which 21 were *P. aeruginosa*, resulting in a prevalence of 1.6%. In both settings, the *P. aeruginosa* isolates were much more frequent in males than females.

Figure 1 reveals that the most common *P. aeruginosa* isolates come from purulent specimens collected from skin wounds and burns, followed by isolates from urine and ear discharge specimens. The majority of cases were heavily colonized pure isolates. Only few showed mixed infections.

The overall pattern of sensitivity revealed that amikacin had the highest sensitivity (89.6%), followed by imipenem, tobramycin and piperacillin (85.6%, 84.1%, and 82.1% respectively).

A lower susceptibility pattern was noted with gentamicin (79.1%), ciprofloxacin (76.1%), carbenicillin (74.6%), ceftazidime (70.1), and cefotaxime (66.2%) (Table 2).

Table 3 reveals that the sensitivity pattern of combined therapy varied with different drug combinations. When combined with aminoglycosides, the best  $\beta$ -lactam antibiotics were imipenem and piperacillin; the least effective was

cefotaxime (except when combined with tobramycin).

The optimal aminoglycoside in combination therapy with  $\beta$ -lactams was amikacin, followed by tobramycin and gentamicin. Finally, table 3 shows that the highest sensitivity rate was found in the

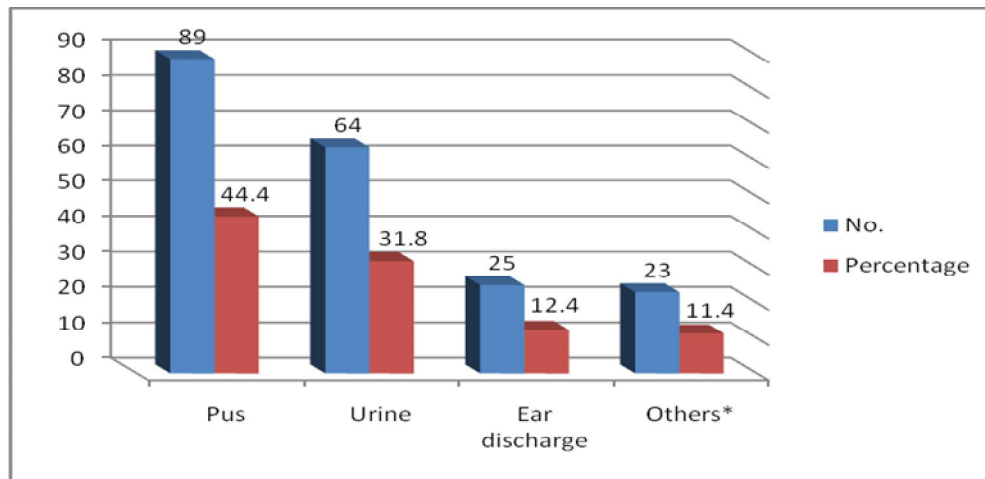
combination of amikacin with imipenem or piperacillin (95.2%) or piperacillin with tobramycin (95.2)%. The lowest combination effect was detected when cefotaxime was combined with amikacin or gentamicin (71.4)%.

| Place                               | Male | Female | Total         |
|-------------------------------------|------|--------|---------------|
| <b>Mosul</b>                        |      |        |               |
| Total samples                       | 2733 | 5305   | 8038          |
| Total positive isolates             | 1338 | 2091   | 3429 (42.7%)* |
| Total positive <i>P. aeruginosa</i> | 114  | 66     | 180 (5.2%)**  |
| <b>Duhok</b>                        |      |        |               |
| Total samples                       | 601  | 1277   | 1878          |
| Total positive isolates             | 491  | 801    | 1292 (68.8%)* |
| Total positive <i>P. aeruginosa</i> | 15   | 6      | 21 (1.6%)**   |

\*Out of the total samples  
\*\*Out of the total positive isolates

| Place         | Amikacin | Carbenicillin | Cefotaxime | Ceftazidime | Ciprofloxacin | Gentamicin | Imipenem | Piperacillin | Tobramycin |
|---------------|----------|---------------|------------|-------------|---------------|------------|----------|--------------|------------|
| Mosul (N=180) | 89.4     | 74.4          | 66.1       | 71.1        | 76.7          | 78.9       | 85.6     | 81.7         | 84.4       |
| Duhok (N=21)  | 90.5     | 76.2          | 66.7       | 61.9        | 71.4          | 81.0       | 85.7     | 85.7         | 81.0       |
| Total (N=201) | 89.6     | 74.6          | 66.2       | 70.1        | 76.1          | 79.1       | 85.6     | 82.1         | 84.1       |

|                 |            | $\beta$ -Lactams |            |             |          |              |
|-----------------|------------|------------------|------------|-------------|----------|--------------|
|                 |            | Carbenicillin    | Cefotaxime | Ceftazidime | Imipenem | Piperacillin |
| Aminoglycosides | Amikacin   | 81.0             | 71.4       | 90.5        | 95.2     | 95.2         |
|                 | Gentamicin | 81.0             | 71.4       | 85.6        | 81.0     | 85.7         |
|                 | Tobramycin | 76.2             | 90.5       | 88.0        | 85.7     | 95.2         |



\* include bronchial wash, nasal wash, sputum and other unidentified body fluids

**Figure 1:** Distribution of *P. aeruginosa* isolated by type of specimen

### Discussion:

Despite improvements in healthcare facilities and the introduction of a wide variety of antimicrobial agents, *P. aeruginosa* remains a major cause of nosocomial infection worldwide.<sup>3,4,17,18</sup>

Life-threatening infections caused by *P. aeruginosa* continue to be hospital infections. Hospital antibiograms are essential in monitoring drug resistance rates. However, antibiograms have several limitations, such as their inability to track the emergence of resistance during therapy.<sup>19</sup>

The study found that the isolation rate of *P. aeruginosa* was 4 times more frequent in Mosul than Duhok, which was expected as Mosul samples were collected from inpatients while most Duhok samples were obtained from outpatients. Studies found that the distribution of isolates is significantly correlated with the type of hospital from where the sample is obtained (general hospital vs. teaching hospital vs. specialized hospital).

Three to sixteen percent of isolates are reported to be due to nosocomial infection with the highest rates of *P. aeruginosa* isolates among those samples obtained in intensive care units.<sup>1,7,18</sup>

About one third of *P. aeruginosa* isolates stem from urine samples. This is similar to the findings of other studies where urine isolates constitute a considerable number of total isolates.<sup>20</sup> The rates of *P. aeruginosa* isolates were higher in males than in females. Similar results have been reported in other studies and might be due to the fact that males are more prone to occupational accidents with subsequent infections than females.<sup>21</sup>

Generally, aminoglycosides showed lower resistance than  $\beta$ -lactams. The latter exhibited sensitivity rates ranging from 66.4% to 76.1%, except for imipenem and piperacillin where higher rates were observed. Other studies found as well that aminoglycosides are potent anti-pseudomonal drugs. However, aminoglycoside-resistance has been reported virtually all over the world, particularly in Europe and Latin America.<sup>6</sup> Accordingly, imipenem

has been universally recommended as the drug of choice for *P. aeruginosa* infections, which has improved the coverage to 82%.<sup>7</sup>

In the study of Mizuta et al., *P. aeruginosa* isolates were most often susceptible to ceftazidime (87% of isolates), amikacin (84%), and tobramycin (84%). These findings are similar to those of this study with the exception of ceftazidime, which showed much lower susceptibility.<sup>8</sup>

Similarly, another study found that the lowest frequencies and appearance of resistance of spontaneous resistance mutations were found with cefepime and imipenem.<sup>22</sup> This study showed no substantial difference in the resistance pattern between inpatients in Mosul and outpatients in Duhok. However, the small number of isolates in Duhok might have affected the frequency distribution of the resistance antibiogram.

The highest sensitivity of *P. aeruginosa* was found in amikacin, imipenem, tobramycin and piperacillin. This might be due to the less frequent use of those drugs in the general practice because of the relatively high cost and the unsustained availability in hospitals and local markets.

On the other hand, the lowest sensitivity rates were detected in the third generation cephalosporins where about one third of *P. aeruginosa* isolates showed resistance to those drugs. This might be due to the high popularity and availability of those drugs as first-line empirical treatment. Ciprofloxacin was effective only in about three quarters of *P. aeruginosa* infections. This might be due to the widespread prescribing of fluoroquinolones in empirical therapy for pseudomonas infections, which may be associated with delays in administering effective therapy resulting in adverse outcomes. Similar results were noted in other studies.<sup>21</sup>

The combination efficacy was tested in aminoglycosides combined with  $\beta$ -lactams. Other studies have also recommend such

combinations.<sup>6,7,11,20</sup> Fluoroquinolones were not tested in the in those combinations.

The combination antibiogram allowed modest fine-tuning of choices for dual antibiotic therapy.

Assuming that clinicians use the standard antibiogram to guide their choice of combination antimicrobial therapy, they would most likely select the two antimicrobials most susceptible to *P. aeruginosa*.

Thus, amikacin-imipenem, amikacin-piperacillin, followed by tobramycin-imipenem and tobramycin-piperacillin would be the combinations chosen by clinicians.

Accordingly, this study found that amikacin combined with imipenem or piperacillin and tobramycin combined with piperacillin showed the greatest effect on *P. aeruginosa* isolates (95.2% susceptibility each).

However, the combination antibiogram demonstrates that tobramycin combined with imipenem did not follow that same rule, but resulted in a lower percentage of susceptible *P. aeruginosa* (85.7% vs. 95.2%).

Thus, when choosing dual therapy for *P. aeruginosa*, it should be based on combination antibiograms rather than on the choice of the two most susceptible drugs based on their individual antibiograms as that may lead to suboptimal coverage.

The study found that antibiotic selections based on the two antibiograms did not differ substantially. Similar results were noted by Mizuta et al.<sup>8</sup> This might be due to relatively lower resistance rates for aminoglycosids observed in comparison to studies conducted in developed countries.

The best *in vitro* combination, however, was for amikacin when combined with imipenem or piperacillin and for tobramycin with piperacillin. Similar results have been reported Bhat et al. who showed that the combination of cefepime with amikacin would be a more adequate initial empiric antibiotic therapy than the combination of cefepime with a fluoroquinolone.

They also noted that adding amikacin to carbapenem as initial empiric antibiotics would increase the coverage to 95%.<sup>7</sup> Nevertheless, the use of combinations of fluoroquinolones with  $\beta$ -lactams or amikacin was found to reduce the risk for *in vitro* selection of resistant *P. aeruginosa*.<sup>9</sup>

Similarly, another recent study on 183 patients with *P. aeruginosa* ventilator-associated pneumonia showed that the use of empirical combination therapy reduced microbiological failure significantly and improved their survival.<sup>23</sup> The rationale of adding aminoglycosides to  $\beta$ -lactams against *P. aeruginosa* to prevent emergence of resistance needs further examination.<sup>20</sup>

It is important to point out that combination therapy for *P. aeruginosa* infection does not prevent

the emergence of resistance Its purpose is to maximize the "coverage" of the infecting organism.<sup>9</sup>

In conclusion the sensitivity rates of first line anti-pseudomonal drugs were relatively low. Hospital antibiograms should be used to help guide empiric antimicrobial treatment. The antibiogram can serve as a valuable tool in guiding antimicrobial therapy, but other patient factors, such as previous infection history and antibiotic use need to be considered as well. Optimum dual combination therapy should be considered in the rational use of empiric anti-pseudomonal therapy.

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\* Lecturer, Department of Microbiology, Duhok College of Medicine, Duhok, Iraq  
Email: alshefaalabs2000@yahoo.co.uk  
Mobile +964-750-4161406