Antibiotic Resistance of Uropathogenic Pseudomonas Aeruginosa

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Abstract

Virulent and resistant strains Pseudomonas aeruginosa is one of the most important causes of UTIs in women. The present study was carried to investigate the frequency of virulence factors in the multi-drug resistant strains of P. aeruginosa isolated from women hospitalized due to the UTIs. One hundred urine samples were collected from women patients suffered from UTIs. Samples were cultured and those that were P. aeruginosa positive were analyzed for the presence of putative virulence genes. Sixty two of 100 samples (62/100%) were positive for P. aeruginosa. Monthly, sex and age-dependent prevalence were seen for P. aeruginosa. Bacterial strains had the highest levels of resistance against penicillin (26.6%), imipenem (26.6%) and Trimethoprim (26.6%). Of 62 P. aeruginosa isolates, 25 strains were resistant to more than 10 antibiotics (40.3%). Our findings should raise awareness about antibiotic resistance in hospitalized women with UTIs. Clinicians should exercise caution in prescribing antibiotics, especially in cases of UTIs.

Keywords: Pseudomonas aeruginosa, urinary tract infections, antibiotic disc, women.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections diseases in human (Mittal, R. et al., 2009) UTIs account for more than 8 million referrals to hospitals, 1.5 million hospitalization, and 300,000 severe clinical syndromes in the United States annually. UTI is an important cause of mortality and morbidity in pediatrics (Shaikh, N. et al.2008).

Pseudomonas aeruginosa is the third most common pathogen associated with hospital acquired UTIs. It is a non-fermentative, aerobic, Gram-negative rod shaped bacterium ((Fu XH et al., 2013)). P. aeruginosa is responsible for 9% of the cases of UTIs all-around the world (Sobczyk, D.et al., 2006). Its high ability to cause UTIs is related to certain virulence factors. Virulent strains of P. aeruginosa cause more severe clinical diseases which are mainly difficult to treatment with routine antibiotics. Treatment of UTIs caused by this bacterium is often started empirically, and therapy is based on information determined from the antimicrobial resistance pattern (Lister, P.D.et al., 2009). However, a large proportion of uncontrolled antibiotic usage has subsidized to the development of resistance in P. aeruginosa strains. P. aeruginosa exhibits the highest rates of resistance to the fluoroquinolones, with resistance to ciprofloxacin and levofloxacin ranging from 20 to 35%. Higher levels of antibiotic resistance in the P. aeruginosa isolates of UTIs have been reported previously (Narten M. et al., 2012).

Pseudomonas aeruginosa is a common gram-
negative rod-shaped bacterium that can cause disease in plants and animals, including humans. It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, thus has colonized many natural and artificial environments (Euzéby, J.P. 1997; Palleroni, N. J. 2010). It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonization occur in critical body organs, such as the lungs, the urinary tract, (Balch, A. and Smith, R. 1994). Species of considerable medical importance, P. aeruginosa is a prototypical "multidrug resistant (MDR) pathogen" recognized for its ubiquity, by its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses especially nosocomial infections such as ventilator-associated pneumonia and various sepsis syndromes. The organism is considered opportunistic except in immune compromised individuals, but the organism does produce a range of clinically important infections in the immune competent and/or in situations where no pre-existing vulnerability is required e.g. hot tub folliculitis. In all infections produced by P. aeruginosa, treatment is dually complicated by the organism's resistance profile, which may lead to treatment failure and/or expose patients to untoward adverse effects from advanced antibiotic drug regimens. This dilemma is a central clinical problem in the field of antimicrobial resistance in immune compromised individuals,(Stefani, S.et.al.,2017).

Pseudomonas aeruginosa resistance to antipseudomonal drugs has increased in several regions of the world (Sader, H. S. et al., 2012). and the Centers for Disease Control and Prevention (CDC),with the aim of enhancing the comparability of data and of promoting better comprehension of the problem of highly drug-resistant bacteria, recently created standardized international definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, and PDR was defined as non-susceptibility to all agents in all antimicrobial categories ((Magiorakos, A. P. et al., 2012)).

Urinary tract infection (UTI) is a common problem diagnosed and treated in urgent care medicine practice. A 2010 report indicated that 3.1% of urgent care visits were for UTIs. (24).An estimated eight million episodes of UTI occur in the US each year (Weinick, R.M. et al., 2010), with one out of three women requiring treatment for UTI before age 24 (David, R.D.et al., 2005).Urinalysis and urine gram stain and culture may assist with diagnosis, but add to the cost of care and are not always necessary. UTIs can affect the lower urinary tract (cystitis) or upper tract (pyelonephritis). Similar to other acute infections, initial antibiotic treatment for cystitis is empiric. A variety of antibiotics is available for treating UTIs, but changing antibiotic sensitivities make appropriate empiric treatment a moving target over time. A recently published guideline (Gupta, K. et al., 2010),by the Infectious Diseases Society of America and European Society for Microbiology and Infectious Diseases provides evidence-based recommendations for treating pre-menopausal, non-pregnant females with uncomplicated UTI. UTIs which occur in men, pregnant women, and patients with immunosuppression or urinary tract abnormalities, such as congenital malformations, urinary calculi, recent urologic instrumentation, indwelling catheters, neurogenic bladder, and kidney transplant, are considered complicated and require more complex decision-making than will be reviewed in this update. Because the majority of UTIs are uncomplicated, however, this review should have broad application.

Antibiotic resistance of P. aeruginosa

One of the most worrisome characteristics of P. aeruginosa is its low antibiotic susceptibility, which is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (e.g., Opr f , mexAB, mexXY, etc.) and the low permeability of the bacterial cellular envelopes(Poole, K., 2004).In addition to this intrinsic resistance, P. aeruginosa easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants. Development of multidrug resistance by P. aeruginosa isolates requires several different genetic events, including acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes. Hyper mutation favors the selection of mutation-
driven antibiotic resistance in *P. aeruginosa* strains producing chronic infections, whereas the clustering of several different antibiotic resistance genes in integrons favors the concerted acquisition of antibiotic resistance determinants. Some recent studies have shown phenotypic resistance associated to biofilm formation or to the emergence of small-colony variants may be important in the response of *P. aeruginosa* populations to antibiotics treatment. (Cornelis, P., 2008).

Mutations in DNA grease are commonly associated with antibiotic resistance in *P. aeruginosa* has. These mutations, when combined with others, confer high resistance without hindering survival. Additionally, genes involved in cyclic-di-GMP (also called cyclic diguanylate and c-di-GMP) signaling may contribute to resistance. When grown in vitro conditions designed to mimic a cystic fibrosis patient's lungs, these genes mutate repeatedly.

**The aim of this work was to:**

1-Identify the role of *Pseudomonas aeruginosa* as a cause of UTI among female patients in Beni Suef university hospital.

2-Study the antimicrobial susceptibility of the isolated *Pseudomonas aeruginosa* and study the genetic background of its antimicrobial resistance.

**Patients, material and methods:**

Samples and *Pseudomonas aeruginosa* isolation, was conducted during the period from January / 2014 to May/ 2015 on 100 patients, from the urology outpatient clinics in Beni-Suef university hospital, faculty of Medicine, Beni-Suef University, they were suffering from recurrent urinary tract infection, a total of 100 urine samples were collected from women patients suffered from UTIs. All patients of are females age range (from 19-45) years old. Urine samples were collected from the midstream using the suprapubic aspiration (SPA) .The urine samples were transferred to bacteriology laboratory within half an hour. Those samples were subjected to bacteriological examination. Urine samples were inoculated on to blood, MacConkey (Merck, Germany) and Nutrient agar (Merck, Germany) and incubated at 37°C for 18 - 24 h; colonies that produce pyocyanin, pyoverdin and pyorubin pigments were transferred to nutrient agar and subculture more than one time to obtain pure cultures. The isolates were identified using conventional biochemical tests such as oxidase test, motility test, citrate utilization test, catalase test, urease production test, gelatinase liquefaction, nitrate reduction test, triple sugar iron agar test, alkaline protease production, Indole test, oxidative-fermentative test, hemolysin production and lecithinase production. The results of the bacteriological. Antimicrobial susceptibility test pattern of antimicrobial resistance was studied using the simple disk diffusion technique. The Mueller–Hinton agar (Merck, Germany) medium was used for this purpose. Antibiotic resistance of *P. aeruginosa* strains against 22 commonly used antibiotics in the cases of UTIs was determined using the instruction of Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute., 2012).

**Susceptibility of *P. aeruginosa* strains were tested against ampicillin (10 μg/disk), gentamicin (10 μg/disk), amikacin (30 μg/disk), imipenem (30 μg/disk), Mezlocillin (30 μg/disk), Pipercillin (30 μg/disk), Cefotaxime (30μg/disk), ciprofloxacin (5 μg/disk), Norfloxacin (30μg/disk), Cotrimoxazole (30 μg/disk), Meropenem (10μg/disk), Ceftazidime (30 μg/disk), Tobramycin (10 μg/disk), Cefipime (30 μg/disk), Tazobactum (10μg/disk), Levofoxacin (5 μg/disk), Cefoperazone (30μg/disk), Ceftazidime (30 μg/disk), Ofloxacin (5μg/disk), Vancomycin (5 μg/disk), polymyxin B (300U/disk) and Aztreonam (30 μg/disk) antibiotic agents (Oxoid, UK). All of the inoculated plates were aerobically incubated at 37 °C for 18-24 h in an aerobic atmosphere. Results were interpreted based on the instruction provided by CLSI (2012). In all reactions, the *P. aeruginosa* (ATCC 10145) was used as quality control organisms.

**All participants were subjected to:**

- Detailed history: the duration of recurrent UTI, operations, instrumentation, history of diabetes mellitus, if under any treatment, especially antibiotics, its duration, dose and type. Participants who were on antibiotics were asked to come one week after the last dose.

- Mid stream voided urine samples were collected during active phase of infection under aseptic conditions in sterile containers then transported to the bacteriological laboratory microbiology department faculty of medicine Beni- Suef University, within half an hour.
Urine samples were studied bacteriologically as follows:

1. Quantitative viable bacterial counts.
2. Culture for isolation and identification of different organisms.

**Results:**

One hundred female patients suffering from recurrent UTIs were enrolled in the present study.

**Demographic data:**

The ages were ranges from 19-45 years old with the following distribution: 33 patients were from 19-30 years, 10 from 31-40 years and 57 from 41-49 years, with the mean age 42 years with standard deviation (SD) of ± 4.532 Table (1).

**Table (1): Age distribution among the studied patients**

<table>
<thead>
<tr>
<th>The age group</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 -30 years old</td>
<td>33</td>
<td>%33</td>
</tr>
<tr>
<td>31- 40 years old</td>
<td>10</td>
<td>10%</td>
</tr>
<tr>
<td>41 – 49 years old</td>
<td>57</td>
<td>57%</td>
</tr>
<tr>
<td>Total number</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Table 2.** Total distribution of *Pseudomonas aeruginosa* in the urine samples

<table>
<thead>
<tr>
<th>Age</th>
<th>No of sample collected</th>
<th>No. positive results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 -30 years old</td>
<td>33</td>
<td>17(51.5%)</td>
</tr>
<tr>
<td>31- 40 years old</td>
<td>10</td>
<td>6(60%)</td>
</tr>
<tr>
<td>41 – 49 years old</td>
<td>57</td>
<td>39(68.4%)</td>
</tr>
<tr>
<td>Total number</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Regarding predisposing factors and type of the recurrent urinary tract infections; it was found that 74 /100 (74%) of UTI patients were diagnosed after operation, cesarean section or diagnostic urological methods, while the remaining 26/100 (26%) of UTI patients had no history of hospital admission or any surgical manipulation. Moreover, 45/100 (45%) were diagnosed as having upper urinary tract infection (pyelonephritis) and 55/100 (55%) were suffering from lower urinary tract infection (cystitis).

**The results of the microbiological examination of the tested urine samples:**

The results of quantitative culture of the studied urine samples showed the presence of significant bacteriuria in most case (81%).

**Table (3): Resistance percentage of *P. aeruginosa* to various antimicrobial agents (Total population = 15)**

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Resistant</th>
<th>Moderate</th>
<th>Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.</td>
<td>%</td>
<td>NO.</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>14</td>
<td>93.3%</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13</td>
<td>86.6%</td>
<td>1</td>
</tr>
<tr>
<td>Amikacin</td>
<td>7</td>
<td>46.6%</td>
<td>5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>2</td>
<td>13.3%</td>
<td>5</td>
</tr>
<tr>
<td>Metzlocillin</td>
<td>4</td>
<td>26.6 %</td>
<td>6</td>
</tr>
<tr>
<td>Piperacilin</td>
<td>5</td>
<td>33.3%</td>
<td>4</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>4</td>
<td>26.6%</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>12</td>
<td>80%</td>
<td>1</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>7</td>
<td>46.6%</td>
<td>4</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>6</td>
<td>40%</td>
<td>4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4</td>
<td>26.6%</td>
<td>5</td>
</tr>
<tr>
<td>Cettazidine</td>
<td>5</td>
<td>33.3%</td>
<td>4</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>3</td>
<td>20%</td>
<td>5</td>
</tr>
<tr>
<td>Cefipime</td>
<td>3</td>
<td>20%</td>
<td>4</td>
</tr>
<tr>
<td>Tazobactum</td>
<td>3</td>
<td>20%</td>
<td>5</td>
</tr>
<tr>
<td>Levofloxacillin</td>
<td>4</td>
<td>26.6%</td>
<td>4</td>
</tr>
<tr>
<td>Vancomycine</td>
<td>5</td>
<td>33.3%</td>
<td>4</td>
</tr>
</tbody>
</table>

Data of Table (3). Clearly demonstrated that *P. aeruginosa* diversely react with different tested antibiotics. Our study had the highly susceptible this group comprises, Imipenem, Meropenem, Tobramycin, Tazobactum, Levofloxacillin, Cefipime and Vancomycine while resistance to
ampicillin (93.33%), gentamicin (96.6%) and ciprofloxacin (80%) were high.

Discussion:

Our work has identified the high prevalence of resistant and virulent strains of *P. aeruginosa* in the urine samples of women patients suffering from UTIs. Totally, 62% of women were infected with *P. aeruginosa*. As far as we know, this is the highest prevalence report of *P. aeruginosa* in the urine samples of women suffered from UTIs. Possible explanations for the high prevalence of *P. aeruginosa* in this study is the low levels of health care in hospitals, excessive application of urine catheter, lack of sanitary conditions in hospitals, improper use of effective drugs and occurrence of antibiotic resistance in *P. aeruginosa*. High prevalence of *P. aeruginosa* has been reported previously due to the inadequate disinfection procedures in a urology unit.

Another important finding of our investigation relates to the distributions of antibiotic resistance pattern in *P. aeruginosa* strains. Totally, our study had the highly susceptible this group comprises, Imipenem, Meropenem, Tobramycin, Tazobactam, Levofloxacillin, Cefipime and Vancomycine while resistance to ampicillin (93.33%), gentamicin (96.6%) and ciprofloxacin (80%) were high. All have shown a high distribution of antibiotic resistance against ampicillin, gentamicin, ciprofloxacin, and Amikacin. High efficacy of Imipenem, Tobramycin, Cefipime, Piperacillin, Tazobactum and Cefoperazone for the treatment of the cases of UTIs caused by *P. aeruginosa* strains.

Onguru *et al.*, (2008) reported that the *P. aeruginosa* strains of various clinical sources were resistant to imipenem (44.1%) which was entirely high. They showed that imipenem resistant strains were also resistant to amikacin (70%), gentamicin (85%), tobramycin (87%), Cefipime (81%), Piperacillin (61%) and ciprofloxacin (77%). The results of our study showed that considerable numbers of isolates were resistant to more than one antibiotic agent. Similar investigations have been reported previously. We identified a large number of antibiotic resistances in the *P. aeruginosa* strains isolated from women patients in urology outpatient clinics in Beni-Suef university hospital, faculty of Medicine, Beni-Suef University. Our data indicate that resistance against ampicillin and gentamicin, were the most commonly detected characteristics of the *P. aeruginosa* strains isolated from women patients with UTIs. Hence, judicious use of antibiotics is required by clinicians. Several different epidemiological studies indicate that *Pseudomonas aeruginosa* is a nosocomial pathogen - nosocomial infections are infections resulting from treatment in a hospital or a healthcare service unit; infections are considered nosocomial if they first appear within 48 hours or more after hospital admission or within 30 days after discharge. There is also evidence suggesting that antibiotic resistance is increasing in recent years in *Pseudomonas aeruginosa*. According to the Center for Disease Control and Prevention (CDC), the overall incidence of *Pseudomonas aeruginosa* infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10 percent of all hospital-acquired infections. *Pseudomonas aeruginosa* causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns, in cancer and in AIDS patients who are immune-suppressed.

*Pseudomonas aeruginosa* infection represents a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is almost 50 percent. *Pseudomonas aeruginosa* like other Gram-negative bacteria is difficult to treat with existing antibiotics, but may in addition develop resistance after unsuccessful treatment. Thus, *Pseudomonas aeruginosa* infections are an increasing threat to the community.

Conclusion:

Our study had the highly susceptible this group comprises, Imipenem, Meropenem, Tobramycin, Tazobactum, Levofloxacillin, Cefipime and Vancomycine while resistance to ampicillin (93.33%), gentamicin (96.6%) and ciprofloxacin (80%) were high. All have shown a high distribution of antibiotic resistance against ampicillin, gentamicin, ciprofloxacin, and amikacin. High efficacy of imipenem, tobramycin, Cefipime, Piperacillin, Tazobactum and cefoperazone for the treatment of the cases of UTIs caused by *P. aeruginosa* strains.
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لمرضى التهاب المسالك البولية بنسبة 100 %، الزائفة الزنجارية هي النوع الثالث من الكائنات الحية التي تم عزلها من عينات البول لمرضى التهاب المسالك البولية بنسبة 47 %، وأثبتت الدراسة أن البكتريا أكثر حساسية للمجموعة الآلية (Imipenem، Tobramycin، Tazobactum، Levofloxacin، Cefipime and Vancomycin، Ampicillin (gentamicin and ciprofloxacin)) أكثر مقاومة للفئة الآلية.