



Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolated from urine samples of urinary tract infection patients in Nagpur region of Maharashtra state

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Abstract:

Pseudomonas aeruginosa is often encountered in urinary tract infection (UTI) worldwide and has shown varied antibiotic susceptibility patterns. This study was therefore designed to ascertain the antibiotic susceptibility patterns of the organism in tertiary care hospital of Nagpur region. Data on antimicrobial susceptibility of *P. aeruginosa* generated from urine samples collected from indoor and outdoor patients for the period of one year (October 2014 to October 2015). Samples were collected, stored and processed using standard laboratory procedures. Many different bacteria are isolated from the different 648 urine samples out of which 245 (44%) *pseudomonas aeruginosa* samples were confirmed. 147 (60%) species of *pseudomonas* were isolated from females and 97 (40%) were isolated from male patients. Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Bauer disc diffusion method) according to CLSI guidelines. The isolated cultures were sensitive to amikacin (82.6%), aztreonam (88%), piperacillin-tazobactam (78.2%), nitrofurantoin (82.1%) and imipenem (98.9%). The sensitivity to ampicillin (49%), cefuroxime (62%), ceftriaxone (61%), norfloxacin (57%), ciprofloxacin (59%), kanamycin (63%), tobramycin (76%), Gentamycin (51%), neomycin (55%), ciprofloxacin (59%), cefuroxime (62%), levofloxacin (65%), Ceftriaxone (61%), ceftazidime (64%), ampicillin (39%), Cefuroxime (70%), Cefoxitin (68%). In conclusion, the rate of antibiotics resistance against *P. aeruginosa* is extremely high. Prudent and more justifiable reasons for antibiotics consumption both for prophylactic and therapeutic use against UTI should be critically weighed against the side effect of resistance development. Furthermore, antimicrobial susceptibility testing should be performed as a basic laboratory procedure among hospitals and clinics so as to aid in the choice of antibiotics prescriptions in health centre.

Introduction:

Pseudomonas aeruginosa is one of the important bacterial pathogens isolated from various samples. Despite advances in medical and surgical care and introduction of wide variety of antimicrobial agents against having anti-pseudomonal activities, life threatening infection caused by *Ps. aeruginosa* continues to cause complications in hospital acquired infections. Several different epidemiological studies indicate that antibiotic resistance is increasing in clinical isolates. *Pseudomonas aeruginosa* is a bacterium that is often encountered in urinary tract infection (UTI) worldwide and has shown varied antibiotic susceptibility patterns. This study was therefore designed to ascertain the antibiotic susceptibility patterns of the organism in tertiary care hospital of Nagpur region. Data on antimicrobial susceptibility of *P. aeruginosa* generated from urine samples collected from indoor and outdoor patients in the hospital in microbiology department of present hospital for the period of one year (October 2014 to October 2015). Samples were collected, stored and processed using standard laboratory procedures. Among the most common infectious diseases urinary tract infections UTIs are common encountered disease in developing countries. UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site: bladder (cystitis), kidney (pyelonephritis) or urine and also can be asymptomatic. Many different bacteria are isolated from the different 648 urine samples out of which 245 *pseudomonas aeruginosa* samples

were confirmed. 147 species of *pseudomonas* were isolated from females and 97 were isolated from male patients.

Pseudomonas aeruginosa has emerged as a major cause of infection in the last few decades. It is an increasingly prevalent opportunistic pathogen and is the fourth most frequently isolated nosocomial pathogen rate was 19.69%.⁽²³⁾ *P. aeruginosa* were more sensitive to combination drugs like piperacillin+tazobactam (93.5%) and cefoperazone+sulbactam (92.3%) followed by imipenem (88.2%), meropenem (87.1%). Sensitivity to amikacin, tobramycin, gentamicin and ceftazidime ranges from 35% to 55%. Highest resistance rate was seen for amoxicillin followed by doxycycline. From our study, we concluded that *P. aeruginosa* is one of the most common nosocomial pathogen.

It is sensitive to combination drugs like piperacillin+tazobactam and cefoperazone+sulbactam. It is also sensitive to carbapenems like imipenem, meropenem and aminoglycosides like amikacin, tobramycin, gentamicin, and cephalosporins like ceftazidime. Rational use of these drugs is necessary to prevent further spread of antimicrobial resistance among *P. aeruginosa* strains and also emergence of multi drug resistance. Hence this study was designed to ascertain the antibiotic susceptibility patterns of the organism in tertiary care hospital of Nagpur region.

Material and Method: Materials and methods: The present study was conducted during October 2014 to October 2015. The samples were collected and stored and then processed using the

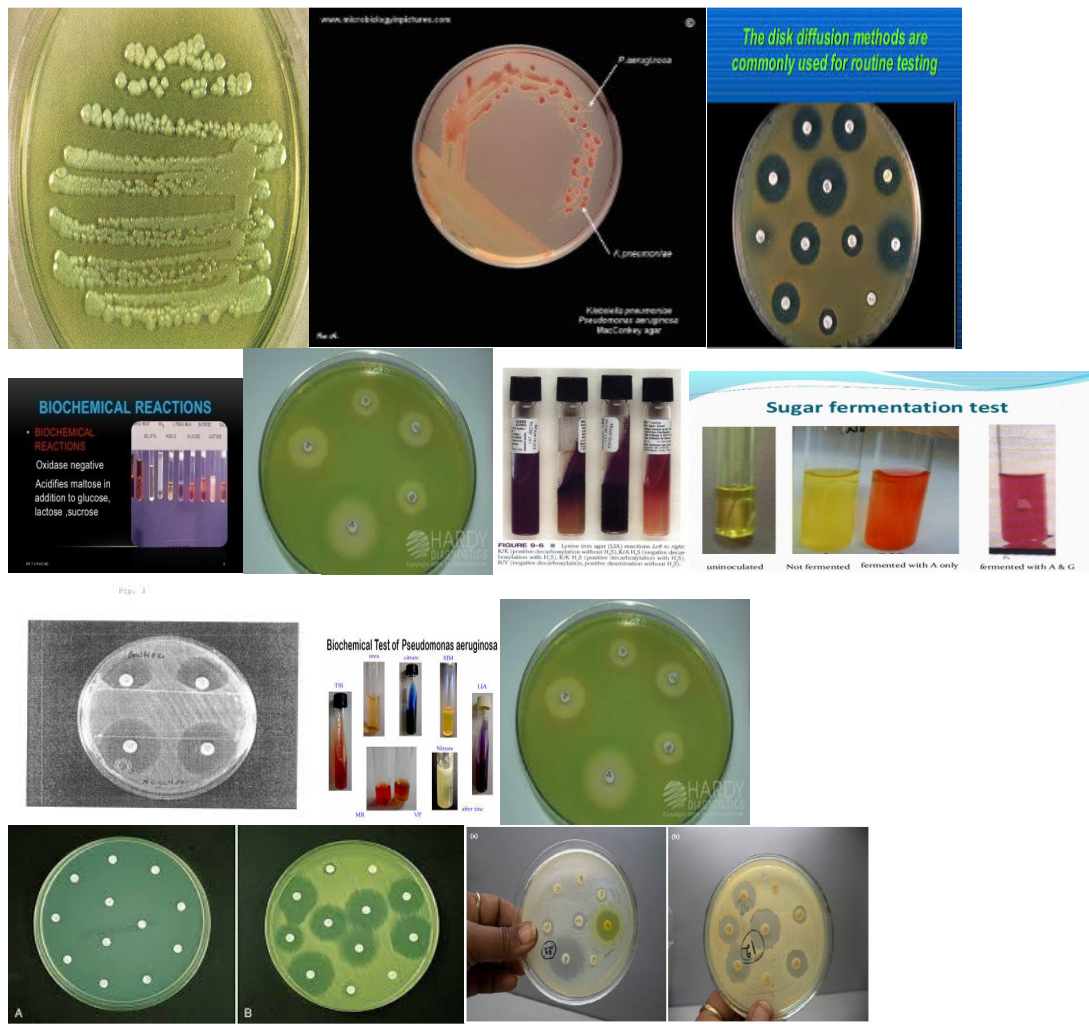
standard procedure for isolation and identification of the *pseudomonas species*. A total of 245 *pseudomonas aeruginosa* strains are isolated from 648 urine samples from indoor and outdoor patients. The study was carried out in microbiology department of tertiary care hospital of Nagpur region. Out of 648 samples 547 sample showed growth of bacteria (84%), 245 (44%) were *Pseudomonas aeruginosa*. 53 (10%) samples show no growth and 51 (9%) samples showed mixed growth.

The samples were selected on the basis of their growth on routine MacConkey medium which showed lactose non-fermenting pale colonies which were oxidase test positive and on nutrient agar pigmented and non-pigmented colonies with oxidase positive. Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Bauer disc diffusion method) according to CLSI guidelines. Antimicrobial disc

for susceptibility test were procured from Hi-Media Mumbai. Muller Hinton agar was used application of antibiotic discs to the inoculated agar plates: Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Bauer disc diffusion method) according to CLSI guidelines. The following antibiotics were tested by disc diffusion

method. Amikacin (30µg), Ciprofloxacin (30µg), Norfloxacin (30µg), Levofloxacin (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Cefuroxime (30µg), Cefoxitin (30µg), Kanamycin (30µg), Tobramycin (30µg), Gentamycin (30µg), Neomycin (30µg), Augmentin (30µg), Aztreonam (30µg), Piperacillin (30µg), Nitrofurantoin (30µg), Nalidixic acid (30µg), Tazobactam (30µg), Imepemem (10µg), Meropenem (10µg), Ticarcillin (30µg) were used for antibiotic susceptibility test.

Fig:1 Pseudomonas colonies in Nutrient Agar Fig :2 Pseudomonas colonies on Mac conkey agar
 Fig:3 Antibiotic sensitivity against antibiotic Fig :4 Biochemical reactions
 Fig:5 Triple sugar iron slant Fig:6 Sugar Fermentation reaction Reactions
 Fig :7 Antibiotic sensitivity test Fig:8 IMViV on Muller Hinton Agar



Observation Table:

Sr.no	Name of the antibiotic	No of female patients showing susceptibility in %	No of male patients showing susceptibility in %	Total % showing susceptibility
1	Amikacin	82%	80%	84%
2	Ciprofloxacin	60%	55%	43%
3	Norfloxacin	64%	60%	57%
4	Levofloxacin	66%	61%	66%
5	Ceftriaxone	68%	67%	63%
6	Ceftazidime	65%	62%	65%
7	Cefuroxime	58%	49%	75%
8	Cefoxitin	78%	71%	68%
9	Kanamycin	54%	49%	57%
10	Tobramycin	58%	55%	65%
11	Gentamycin	60%	51%	49%
12	Neomycin	53%	49%	45%
13	Augmentin	69%	68%	75%
14	Amoxicillin	53%	45%	33%
15	Ampicillin	34%	41%	35%
16	Aztreonam	79%	71%	68%
17	Piperacillin	86%	75%	66%
18	Nitrofurantion	69%	63%	73%
19	Nalidixic acid	76%	67%	69%
20	Tazobactam	92%	93%	83%
21	Imepenem	97%	90%	89%
22	Meropene m	84%	81%	87%
23	Ticarcillin	89%	82%	78%
24	piperacillin	86%	86%	75%

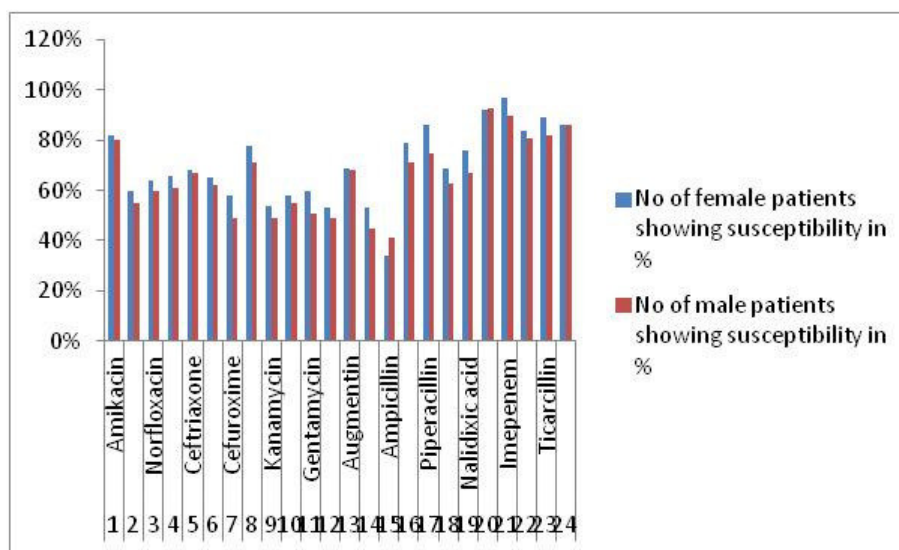


Figure : 9 Bar Diagram showing the male , female ratio for antibiotic susceptibility test.

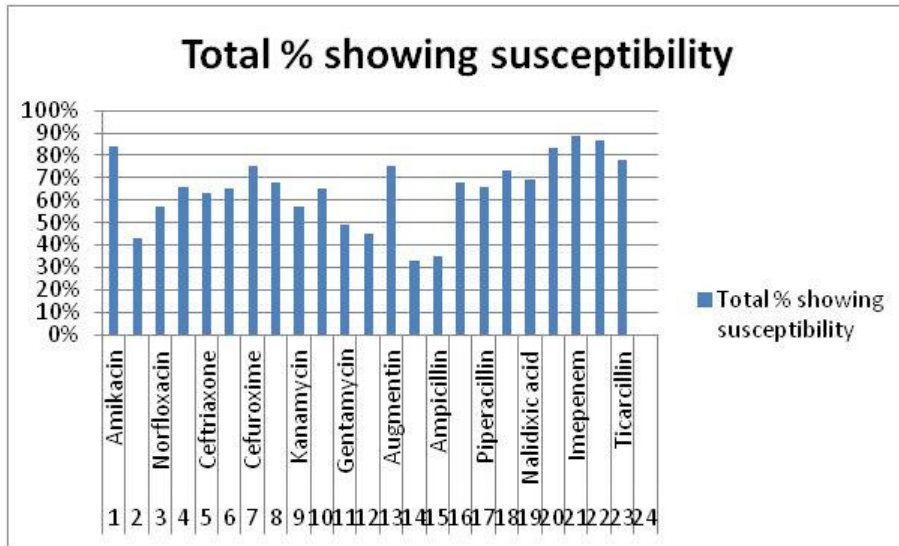


Figure :10 BarDiagram showing total antibiotic susceptibility values for given no of sample

Results:

In the present study we have used near about 20 different generation antibiotics along with beta lactam antibiotics which are given in some of the cases.

A total of 648 urine samples were received for culture and sensitivity during the study period. Among these, 547 samples (84%) yielded significant growth 57 samples (10.1%) showed no growth and 53 samples (10%) showed mixed growth. From various organisms isolated from urine culture only *Pseudomonas aeruginosa*, was selected for the study and all were uropathogens. After obtaining the pure strains of *pseudomonas aeruginosa*, the strains were subjected to biochemical identification tests to identify *Pseudomonas spp.* For this purpose samples were inoculated in Triple Sugar Iron media (TSI), Citrate media, Peptone water, Urease media and kept in an incubator for 18 hrs at 37°C. Next day the results were noted on TSI, Citrate media and Urease media. Part of growth on Peptone water was subjected to Indole test with Kovac’s Reagent and part for motility test by Hanging drop’ method. A strain of *Pseudomonas* in the TSI medium showed alkaline slant, no reaction in butt. It showed negative reaction for indole test, negative urease test and positive citrate test. Glucose is utilized oxidatively, forming acid only.

After confirmation the isolates were further subjected to antibiotic sensitivity test. These isolated were found to be sensitive to amikacin (84.6%), aztreonam (79%), piperacillin-tazobactam (78.2%), nitrofurantoin (73.1%) and imipenem (98.9%). The sensitivity to ampicillin (41%), cefturoxime (75%), ceftriaxone (63%), norfloxacin (57%), ciprofloxacin (59%), kanamycin (57%), tobramycin

(67%), gentamycin (53%), neomycin (55%), cefturoxime (72%), levofloxacin (66%), ceftazidime (64%), amoxicillin (39%), ceftiofloxacin (68%).

During the analysis it was observed that empirical therapy was started in 50 cases. In 32 cases ceftriaxone was used, in 20 ciprofloxacin, in seven cases norfloxacin and in the remaining few ampicillin, amoxicillin, cephalexin and doxycycline were used in the study.

Discussion:

All the isolates of *P. aeruginosa* (100%) in the present study were resistant to number of antibiotics, ampicillin, amoxicillin, neomycin, kanamycin, ciprofloxacin while the highest sensitivity rate of 92% was recorded with Tazobactam, 97% with imipenem. Antibiotics like meropenem, ticarcillin, piperacillin, nalidixic acid shows the sensitivity in the range of 80% to 90%. The antibiotics ceftazidime, tobramycin, augmentin, levofloxacin, norfloxacin, nitrofurantoin all shows the antibiotic susceptibility in the range of 55% to 75%.

These findings compare favorably with that of: Goniugur *et al* (2003) in Turkey on 249 isolates of *P. aeruginosa* in a teaching hospital where 100% resistance to ampicillin and penicillin was recorded; Sule *et al*, (2002) in Sagamu, Nigeria who reported a sensitivity of *P. aeruginosa* to aminoglycosides in the range of 61.8%- 75%, fluoroquinolones, 82.8%-89.2% and ampicillin, and tetracycline, 1.7%- 46.8%; Taiwo *et al*, (2002) in Ilorin who reported *P. aeruginosa* to be sensitive in the range 70%- 94% to ofloxacin and ciprofloxacin, and 55%- 90% to gentamicin, ceftriaxone, azithromycin, and ampicillin; and Olayinka, *et al*, (2004), in Zaria who reported a 27.8% of the 92 isolates of *P. aeruginosa* to be of Ceftazidime + gentamicin + pefloxacin + ofloxacin resistance pattern. Also in Pakistan (Ogundipeju, & Nwobu, 2004), *P. aeruginosa* was similarly reported to be 75%, 30%, and 10% susceptible to

gentamicin, streptomycin and tetracycline respectively; while in Japan, Ogiwara, *et al* (1999) as well reported a high resistance (over 90%) of the organisms to tetracycline, nalidixic acid, nitrofurantoin and ampicillin. Similar susceptibility patterns of *P. aeruginosa* were also reported in Saudi Arabia and Kuwait (Rotimi, *et al*, 1998), Canada and Brazil (Pfaller, *et al*, 1998), and South Korea (Cane, & Walsh, 1999). Findings from this study also compares well with that from Egypt which showed a resistance of about 95% *P. aeruginosa* isolates against ampicillin (Elkholy, *et al*, 2003). This high resistance of *P. aeruginosa* is believed to be as a result of the ability of the organism to undergo mutation and acquire resistant genes at a faster rate compared to *Enterobacteriaceae* (Woods, *et al*, 1986).

Administration of antibiotics as a prophylactic measure against UTI should always be analyzed critically and the benefits seen to be well above the side effects of which resistance is one of them, before choosing such a management option (Struelens, 1998). Prolonged or permanent urethral catheterizations are notable scenarios often encountered and their benefits need to be periodically reviewed. Also, health personnel should be aware of the prevailing antimicrobial activity pattern of, at least, the locally available antibiotics against *P. aeruginosa* so as to make correct or near correct prescriptions in the absence of a comprehensive antimicrobial susceptibility report (Swedish-Norwegian Consensus Group, 1998). This would help reduce the external stimuli from inappropriate drug prescriptions towards the development and acquisition of resistance genes by bacteria (Ayliffe, 1996).

In view of the current rate of antimicrobial resistance of *Pseudomonas* as found in the present study, antimicrobial susceptibility testing by Microbiology laboratories of hospitals and clinics should be made a routine practice in such health centers. Also the procurement of reagents and materials for susceptibility testing along with the requisite personnel should be considered a basic laboratory requirement in order to boost this conventional practice. In conclusion, this study has shown that, the rate of antibiotics resistance against *P. aeruginosa* is extremely high. Prudent and more justifiable reasons for antibiotics consumption both for prophylactic and therapeutic use against UTI should be critically weighed against the side effect of resistance development.

Furthermore, antimicrobial susceptibility testing should be performed as a basic laboratory procedure among hospitals and clinics so as to aid in the choice of antibiotics prescriptions.

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