

Resistance Trends among *Pseudomonas aeruginosa* Isolates in a Tertiary Care Centre in South Gujarat

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Abstract

It is necessary to determine the susceptibility pattern of clinical isolates especially nosocomial one in the clinical settings for making strategy for effective empirical treatment & to reduce incidence of multidrug resistant bugs. Aim of this study was to detect the antimicrobial susceptibility pattern of *P. aeruginosa* isolates from clinical samples between January 2014 to December 2015, received at department of Microbiology, GMC, Surat. Clinical isolates were confirmed as *P. aeruginosa* by phenotypic methods/Vitek2 compact system as per availability. Genetic sequencing could not be performed due to unavailability. Antimicrobial susceptibility tests were performed by Kirby-Bauer disc diffusion method/Vitek2 compact system & Interpretation was done according Clinical and Laboratory Standards Institute (CLSI) of that year [1] [2]. Seven hundred fifty seven *P. aeruginosa* strains were studied during the study period. Most of the isolates were from surgery ward (62%), followed by orthopaedic ward (15%). 65% of the total isolates were from swab samples followed by urine (7%), pus, fluid (5%) & devices (4%). 60% isolates were resistant to Ceftazidime & for other drugs resistance pattern was as follows: Cefepime (52%), Levofloxacin (49%), Ticarcillin/clavulanic acid (49%), Meropenem & Gentamycin (44%), Ciprofloxacin (43%), Amikacin (41%), Tobramycin (39%), Netilmycin (36%), Piperacillin (32%), Aztreonam (31%), Piperacillin/tazobactam (26%), Imipenem (23%), Doripenem (12%) & Gatifloxacin (10%). As there is predominance of isolates from surgical ward in present study & resistance to carbapenem group of drugs was also found, indicating that most of the infection caused by *Pseudomonas aeruginosa* may be nosocomial.

Keywords

P. aeruginosa, Antimicrobial Sensitivity, Drug Resistance

1. Introduction

There is increase in multidrug resistance in clinical isolates in all the clinical settings these days. It is matter of concern as it can become a cause of threat to public health. There is need for making strategies that are efficient to prevent further increase of drug resistance in clinical isolates. Regular monitoring of antimicrobial susceptibility pattern of clinical isolates can greatly contribute in making preventive strategies for multidrug resistance. Prevalence of *P. aeruginosa* is 15% - 80% in chronic wounds [3]. *P. aeruginosa* is an organism that is present in many diverse environmental settings. It has ability to survive on minimal nutritional requirements and it can tolerate a variety of physical conditions. All these properties have allowed this organism to persist in both community and hospital settings. *P. aeruginosa* can be isolated from a variety of sources in hospital settings like respiratory therapy equipment, antiseptics, soap, sinks, mops, medicines, and physiotherapy and hydrotherapy pools [4]. *Pseudomonas aeruginosa* is an important cause of hospital acquired infection due to all these properties. *P. aeruginosa* & *P. maltophilia* are causative agents of 80% opportunistic infections caused by *Pseudomonas* [5]. It causes wide variety of infections. There is availability of various antimicrobial agents with anti-pseudomonas activity these days but *Pseudomonas aeruginosa* is still an important cause of morbidity & mortality in hospitalized patients. Therefore, this study has been carried out to study the current antimicrobial sensitivity pattern of *Pseudomonas aeruginosa* in patients attending New Civil Hospital, Surat.

2. Material & Method

It was a retrospective study done in Department of Microbiology, GMC, Surat. Antibiotic susceptibility pattern of *P. aeruginosa* isolated from the clinical samples of patients attending the New Civil Hospital, those were received in department of Microbiology, GMC, Surat during period of January 2014-December 2015 were analyzed. Repeat isolates from same sample of patient within 2 days of isolation of first isolate were not included in study (there is no standard currently available which tell about optimal frequency to do repeat antimicrobial susceptibility testing of repeat isolate from same sample when an organism is isolated from the same specimen source repeatedly but, testing every 2 to 5 days is thought sufficient) [6]. Species level identification was done by phenotypic methods (oxidase test, growth at 42°C, TSI test, arginine decarboxylation, OF sugar fermentation test) & Vitek 2 compact system as per availability. Genetic sequencing could not be done due to unavailability. Antimicrobial susceptibility was done by Kirbybauer technique (Figure 1)/Vitek 2 compact systems as per availability. Antimicrobial tested are Piperacillin (100 ug), Piperacillin/Tazobactam (100/10ug), Ticarcillin/Clavulanic acid (75/10ug), Ceftazidime (30 ug), Cefipime (30 ug), Aztreonam (30 ug), Doripenem (10 ug), Imipenem (10 ug), Meropenem (10 ug), Gentamycin (10 ug), Tobramycin (10 ug), Amikacin (30 ug), Netilmycin (30 ug), Ciprofloxacin (5 ug), Levofloxacin (5 ug) & Gatifloxacin (5 ug) according to CLSI guideline 2014 & 2015 [1] [2]. For Kirbybauer technique Mul-

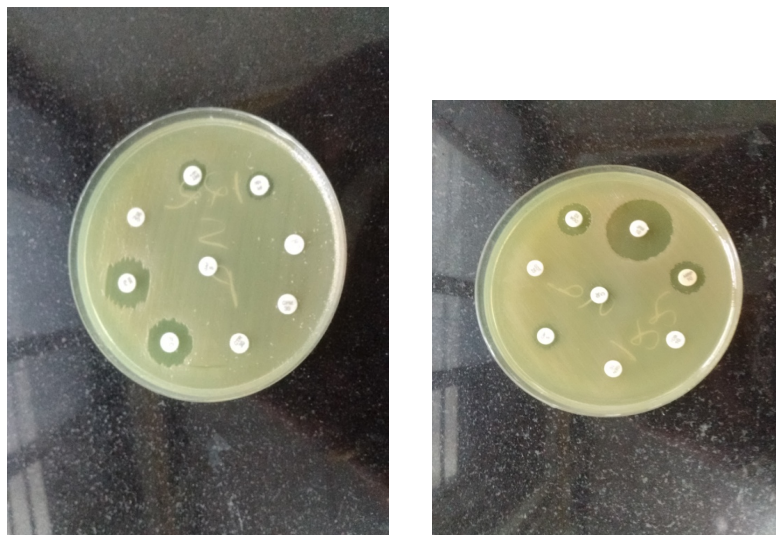


Figure 1. Showing antimicrobial susceptibility testing of *P. aeruginosa* by Kirby-bauer technique.

ler Hinton agar(HiMedia) medium having pH 7.2 to 7.4 at room temperature was used. Before doing antimicrobial susceptibility testing it was dried to remove droplet of moisture but taking care of not let it overdry. The antibiotic disks were maintained at -20°C until needed, according to the manufacturer's recommendations. Antibiotic disks were allowed to come at room temperature before use. They were checked for expiry date & quality control testing was done time to time for ensuring correct results. Antibiotic susceptibility testing was done as follows:

4 to 5 well-isolated colonies of the same morphological type from an agar plate were selected from 18 - 24 hour old culture. Top of each colony was touched with a wire loop and transferred to a tube containing 4 to 5 mL of peptone water. This suspension was incubated at 37°C for 2 hours. Turbidity of suspension was adjusted to 0.5 McFarland standard. A sterile non toxic swab was dipped into this suspension & then rotated & pressed several times on inside wall of test tube above the level of fluid to remove excess inoculums. Dried surface of Muller Hinton agar was inoculated with the swab three times at 60° from previous stroke. Plate was allowed to dry for about 5 minutes to remove the excess moisture. Antibiotic discs were placed appropriately on agar surface (minimum 24 mm distance between discs & 15 mm distance between disc & margin of plates). On one 120mm plate 6 antibiotic discs were placed. Once the disc was placed care was taken not to move the disk. Then plate was put in incubator at 37° in inverted position. Incubation was done for 16-18 hours aerobically. Zone of inhibition was measured to the nearest millimeter using scale. Results were interpreted according to CLSI guidelines 2015 & 2016 respectively. Results were entered in Excel sheet & analysis was performed.

3. Result

Pseudomonas aeruginosa comprised of 23 % (757 out of 3329) of all gram nega-

tive organism isolated from various clinical samples received in department of microbiology, GMC, Surat from January 2014-December 2015. Ward-wise analysis showed that most of the isolates were from surgery ward (62%), followed by orthopaedic ward (15%) (Figure 2). Sample-wise analysis showed that 65% of the total isolates were from swab samples followed by urine (7%), pus & fluid (5%), & devices (4%) (Figure 3). Most of the isolates were resistant to Ceftazidime (60%) & for other drugs resistance pattern was as follows: Cefepime (52%), Levofloxacin (49%), Ticarcillin/clavulanic acid (49%), Meropenem & Gentamycin (44%), Ciprofloxacin (43%), Amikacin (41%), Tobramycin (39%), Netilmicin (36%), Piperacillin (32%), Aztreonam (31%), Piperacillin/tazobactam (26%), Imipenem (23%), Doripenem (12%) & Gatifloxacin (10%) (Figure 4).

4. Discussion

Prevalence rate of *Pseudomonas aeruginosa* was 23 % (757 out of 3329) of all gram negative organism isolated from various clinical samples received in department of microbiology, GMC, Surat from January 2014-December 2015. Most of the isolates were from Surgery ward (62%), followed by Orthopaedic ward (15%). Sample-wise analysis showed that 65% of the total isolates were from swab samples followed by urine (7%), pus & fluid (5%), & devices (4%). These findings are similar with Muktikesh Das *et al.* who showed 67.6% isolation rate of *P. aeruginosa* from pus & swab samples. In present study isolation rate

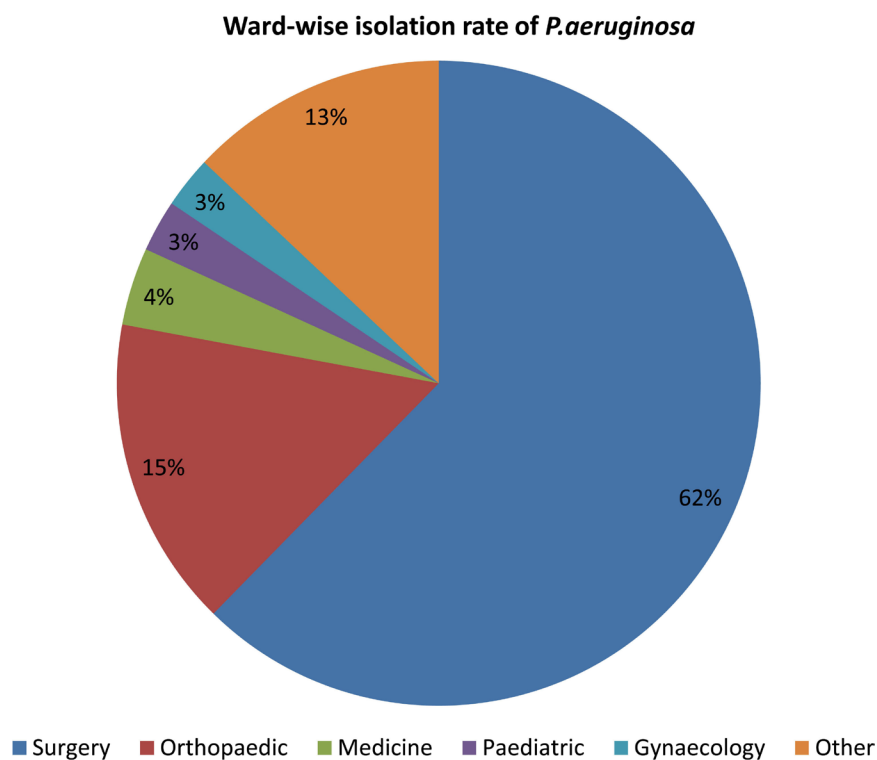


Figure 2. Ward-wise isolation rate of *P.aeruginosa* in present study. Showing ward-wise isolation rate of *P. aeruginosa*. Most of the isolates were from surgery ward (62%), followed by orthopaedic ward (15%), medicine (4%), Paediatric (3%), Gynaecology (3%) & other (13%).

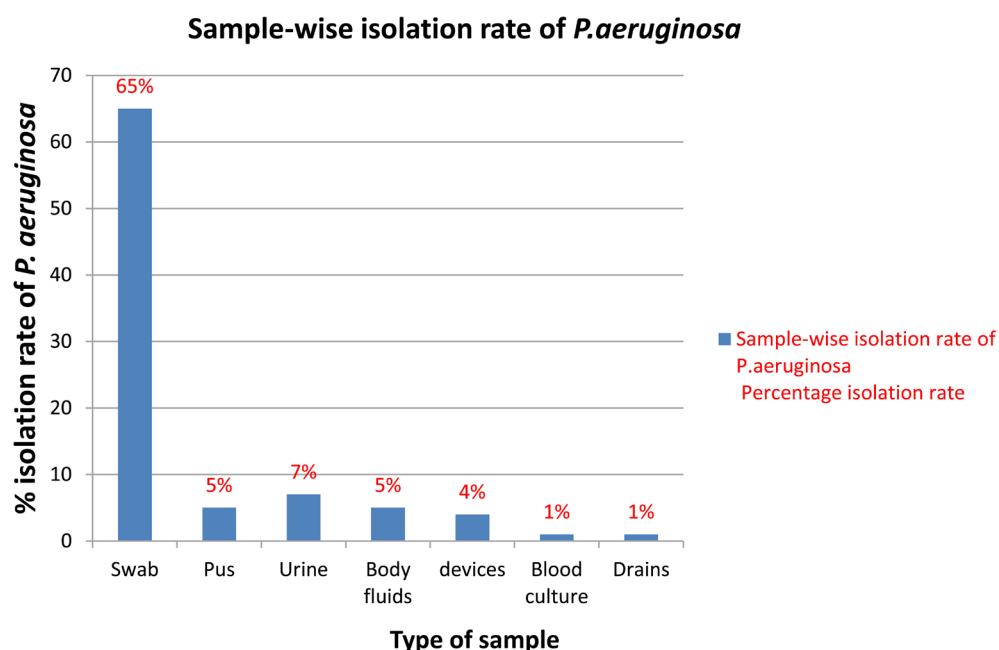
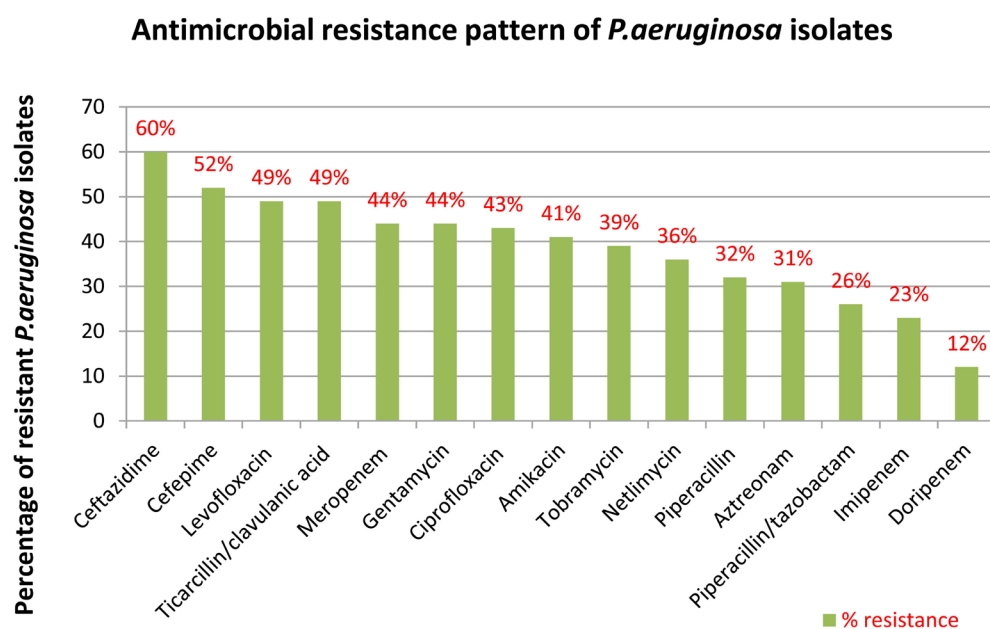


Figure 3. Sample-wise isolation rate of *P. aeruginosa* in present study. (Showing isolation rate of *P. aeruginosa* from various clinical samples in present study. Type of sample is plotted on X-axis & percentage isolation rate is plotted on Y-axis. Highest rate of isolation was from samples followed by urine (7%), pus & fluid (5%), & devices (4%), Blood culture & drains (1%).).



Antimicrobials used for analysis of resistance pattern of *P.aeruginosa*

Figure 4. Antimicrobial resistance pattern of *P. aeruginosa* isolates in present study. (Showing antimicrobial resistance pattern of *P. aeruginosa* isolates in present study. On X-axis antimicrobial tested in present study are plotted. On Y-axis percentage of resistant *P. aeruginosa* isolates to antimicrobials is plotted. 60% of the isolates were resistant to Ceftazidime & for other drugs resistance pattern was as follows: Cefepime (52%), Levofloxacin (49%), Ticarcillin/clavulanic acid (49%), Meropenem & Gentamycin (44%), Ciprofloxacin (43%), Amikacin (41%), Tobramycin (39%), Netilmycin (36%), Piperacillin (32%), Aztreonam (31%), Piperacillin/tazobactam (26%), Imipenem (23%), Doripenem (12%) & Gatifloxacin (10%).)

of *P. aeruginosa* from urine sample was 7%, which is less than previous studies carried out by Sheetal Sharma *et al.* (2016), Amandeep kaur *et al.* (27.1%), Sunal Lakum *et al.* (20%), Muktikesh Das *et al.* (15%) [7] [8] [9] [10]. These studies also showed high isolation rate of *P. aeruginosa* from pus sample. Most of the isolates in present study were resistant to Ceftazidime (60%). O. A. Igbalajobi *et al.* (73.8%), Amandeep Kaur *et al.* (62.8%), Sonal Lakum *et al.* (45.95%), Muktikesh Das *et al.* (77.7%) & Javed R. Al Zaidi *et al.* (100%) have shown similar high degree resistance of *P. aeruginosa* to ceftazidime [8] [9] [10] [11] [12]. For other drugs resistance pattern was as follows: Cefepime (52%), Levofloxacin (49%), Ticarcillin/clavulanic acid (49%), Meropenem & Gentamycin (44%), Ciprofloxacin (43%), Amikacin (41%), Tobramycin (39%), Netilmicin (36%), Piperacillin (32%), Aztreonam (31%), Piperacillin/tazobactam (26%), Imipenem (23%), Doripenem (12%) & Gatifloxacin (10%). Resistance to carbapenems was also present in this study & it was ranges from 12% - 44% for different drugs of this group. This high degree of resistance of *P. aeruginosa* for carbapenem group of drugs was also found in previous studies. Nandkuduru Premanandham *et al.* reported 55.56% resistance to doripenem & 25.8% for Imipenem, Amandeep Kaur *et al.* reported 33% resistance to meropenem & 17.8% resistance to Imipenem, Oman Basir Ahmed *et al.* reported 25% resistance to Imipenem & Sonal Lakum *et al.* reported 21.6% resistance to Imipenem for *P. aeruginosa* isolates [8] [9] [13] [14]. This increasing drug resistance to *P. aeruginosa* isolates is a matter of great concern for medical fraternity. As most of the isolates in present study were from surgical ward, hospital acquired or iatrogenic cause seems to play a main role. Increasing carbapenem resistance is also seen that arise a need to take further steps to do antibiotic susceptibility testing before giving empirical therapy & prevent abuse of antimicrobial therapy.

Limitations

- 1) Modified hodge test could not be done to confirm carbapenemase resistance.
- 2) There is need to do molecular study to identify resistant gene prevalent in setting.

5. Conclusion

Injudicious use of drugs is one of main contributors to emerging resistance of *P. aeruginosa* to antimicrobial therapy. It also contributes to appearance of multi-drug resistant strains of bacterium. Most of the isolates in present study were from surgical ward & isolated mainly in swab sample. Resistance to carbapenem group of drugs was also found. These findings indicate that most of the infection caused by *Pseudomonas aeruginosa* may be iatrogenic or hospital acquired.

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