Prevalence of Multidrug-resistant, Extensively Drug-resistant, and Pandrug-resistant *Pseudomonas Aeruginosa* in Different Clinical Samples in Tertiary Care Center

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Abstract
Antibiotic resistance is one of the alarming issues, affecting human health. *P. aeruginosa* is a prototype of “multidrug-resistant pathogen” and is recognized for its ubiquitous distribution, advanced antibiotic resistance mechanisms, and nosocomial infections. The organism is classified into various phenotypes based on the drug resistance pattern, namely, drug-resistant (DR), multi-DR (MDR), extensively DR (XDR), and pan-DR (PDR).

Methods: This cross-sectional study was conducted in Microbiology Department of Agartala Govt Medical College under GBP hospital, from 1st January 2016 to 31st December 2016. Clinical specimens were collected from different clinical departments of GBP hospital. Clinical isolates were identified by standard and specific microbiological methods. The antibiotic susceptibility pattern was determined by Kirby Bauer Disc diffusion method. Clinical and Laboratory Standards Institute (CLSI) guidelines were used to determine the results.

Results: The frequency of MDR *P. aeruginosa* isolated from different clinical specimens was found to be 43.43%. Antibiotic resistance studies revealed that 50.51% of *P. aeruginosa* isolates were DR, and 6.06% were XDR phenotype. None of the strains showed PDR phenotype. Amikacin was found to be the most effective antibiotic, followed by Carbapenem group of drugs.

Conclusion: Our data revealed a high prevalence of DR phenotypes of *P. aeruginosa* in the total isolate of *P. aeruginosa*. There is an urgent need to resolve the issue by taking some preventive measures. Combined efforts of health care professionals and researchers are required to educate people about the proper use of antibiotics and other infection control measures.

Keywords: Drug resistance, multidrug-resistant phenotype, Extensively Drug-resistant, and Pandrug-resistant of *Pseudomonas aeruginosa*.

Introduction
Antibiotic resistance is one of the alarming issues, affecting human health. There are various factors responsible for the emergence of resistance such as, misuse and overuse of antibiotics, patient related factors, inappropriate prescriptions by the physicians, self medications especially young adults, use of broad spectrum antibiotics and...
synergistic combinations, unnecessary promotions by pharmaceutical industry, untrained staff in microbiological testing laboratories, lack of awareness with the new guidelines recommended for antimicrobial testing etc.\(^1\)

Pseudomonads are diverse group of established and emerging pathogen and are major agents of nosocomial and community acquired infections, widely distributed in the hospital environment where they are particularly difficult to eradicate.\(^2\)

\textit{P. aeruginosa} is notorious for being intrinsically resistant to many structurally unrelated antimicrobial agents by exhibiting low permeability of its outer membrane, the constitutive expression of various efflux pumps and the naturally occurring chromosomal AmpC \(\beta\)-lactamase, and it can acquire additional resistant gene form other organisms via plasmids, transposons, bacteriophages, and also by biofilm production.\(^3,4\)

\textit{P. aeruginosa} is divided into different phenotypes based on the drug resistance patterns of the organism.\(^5\) Multidrug-resistant (MDR) phenotype is defined as \textit{P. aeruginosa}, which is resistant to more than one antimicrobial agent in three or more antimicrobial categories. A similar resistance to more than one antimicrobial agent in \(<3\) antimicrobial categories is defined as drug-resistant (DR) \textit{P. aeruginosa}. Extensively DR (XDR) phenotype is defined as \textit{P. aeruginosa}, which is resistant to more than one antimicrobial agent in all the antimicrobial categories, except in two or less. Pan-DR (PDR) phenotype is defined as a bacterium which is resistant to all antimicrobial agents in all antimicrobial categories.\(^6\)

MDR, XDR, and PDR phenotypes elaborate inactivating enzymes, such as extended-spectrum beta-lactamases (ESBL) and metallo-\(\beta\)-lactamases (MBL), that make beta-lactams and carbapenems ineffective.\(^7\) ESBL-producing \textit{P. aeruginosa} was initially detected in Europe in the mid-1980s, and MBL-producing \textit{P. aeruginosa} was first reported from Japan in 1991. They have rapidly spread over different parts of the world since then.\(^8\)

Literature is sparse regarding the incidence of different phenotypes of \textit{P. aeruginosa} from our country as we could retrieve only two articles in the PubMed with the relevant (\textit{P. aeruginosa}, India, phenotype, incidence) keywords.\(^9,10\) Hence, we conducted this study to find the incidence of MDR, XDR, and PDR phenotypes of \textit{P. aeruginosa} in the tertiary care hospital of Agartala.

**Materials and Methods**

The study was carried out in Microbiology department in Agartala Government Medical College and Govind Ballap Pant Hospital at Agartala, Tripura during period of January 2016 to December 2016. A total of 5562 routine clinical specimens were collected from different samples and process using conventional microbiological methods.

Samples were cultured on blood agar and Mac Conkeys Agar and CLED (urine) and plates were incubated overnight at 37º C. \textit{P. aeruginosa} was identified on the basis of colony morphology, Gram staining and biochemical tests including Catalase, Oxidase, Indole, Motility, Citrate, Urea, TSI reaction.

The antibiotic sensitivity test was performed by Kirby Bauer disc diffusion technique with commercially available discs (Hi-Media) on Muller Hinton Agar using Amikacin (30mcg), Netilmicin (30mcg), Ciprofloxacin (5mcg), Ofloxacin (5mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefotaxime (30mcg), Piperacillin (10mcg), Piperacillin Tazobactam (100/10mcg), Amoxyclav 20/10 (30mcg), Cefoperazone-Sulbactam (75/15mcg), Imipenem (10mcg), Nitrofurantoin (300mcg for urinary isolates). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Pseudomonas aeruginosa. ATCC 27853 strain was used for quality control in the study.

In our work, MDR \textit{P. aeruginosawas} detected as a bacterium which was resistant to three or more anti-Pseudomononal antimicrobial classes.
(carbapenems, fluoroquinolones, penicillins /cephalosporins and aminoglycosides). A similar resistance to more than one antimicrobial agent in <3 antimicrobial categories is defined as drug-resistant (DR) \( P. \) \( aeruginosa \). Extensively DR (XDR) phenotype is defined as \( P. \) \( aeruginosa \), which is resistant to more than one antimicrobial agent in all the antimicrobial categories, except in two or less. Pan-DR (PDR) which is resistant to all antimicrobial agents in all antimicrobial categories.

Statistical analysis was performed by SPSS version 17. Frequency of MDR \( P. \) \( aeruginosa \) and percentage of resistant antibiotics were calculated.

Results

\( P. \) \( aeruginosa \) were isolated from clinical samples. Out of 151 isolates, 43 were found to be MDR \( P. \) \( aeruginosa \) \( \text{ATCC} \) 27853 was used as positive control.

![Fig no.1. Prevalence of \textit{Pseudomonas aeruginosa} drug-resistant strains](image)

| Table no: 1. Prevalence of \textit{Pseudomonas aeruginosa} drug-resistant strains |
|------------------|-----------------|----------|-----------------|-----------------|-------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| SN | Pus | Sputum | Wound swab | Urine | Blood | ENT | EYE SWAB | TOTAL |
|-----|-----|--------|------------|-------|-------|-----|----------|--------|
| DR | 16  | 7      | 2          | 18    | 2     | 4   | 1        | 50 (50.51%) |
| MDR| 12  | 13     | 2          | 12    | 1     | 3   | X        | 43(43.43%)    |
| XDR| X   | 4      | 1          | 1     | X     | X   | X        | 6(6.06%)      |
| PDR| X   | X      | X          | X     | X     | X   | X        | X                |

The incidence rate of MDR in \( P. \) \( aeruginosa \) was highest in the age group of patients between 21 to 40 years. Urine (12 out of 43) and pus (12 out of 43) samples accounted for the majority of the MDR isolates. Our data showed that 50.51% strains were DR, 43.43% were MDR, and 6.06% were of XDR phenotype. None of the strains showed PDR phenotype.

| Table no: 2. Age distribution of MDR \( P \) \( aeruginosa \): |
|-----------------|-----------------|-----------------|-----------------|
| SN | AGE | MALE | FEMALE | TATOL |
|-----|-----|------|--------|-------|
| 1   | 0-20| 5    | 3      | 8     |
| 2   | 21-40| 10   | 5      | 15    |
| 3   | 41-60| 10   | 3      | 13    |
| 4   | >61 | 6    | 1      | 7     |
| 5   | 0>61| 31   | 12     | 43    |

**Discussion**

The percentage of MDR PA in India ranges from 11.36% reported by Siti Nur Atiquah Idris et al., \(^{11}\) to 91.6% reported by S.Panranjothi et al., \(^{12}\). In our study, 43.43% \( P. \) \( aeruginosa \) were found to be Multi drug resistant (MDR) which is comparable...
with above studies and result is similar with the study conducted by Senthamarai S., et al.\textsuperscript{13} where it was 41.35%.

Altered target sites, bacterial efflux pumps, enzyme production or inhibition, loss of membrane protein, etc are different mechanisms mediated by multidrug-resistance (MDR) P. aeruginosa.\textsuperscript{14} Our study showed the prevalence of drug resistance in 66.66% out of 151 of the isolates of \textit{P. aeruginosa}. No isolate was found to be PDR phenotype, thereby showing the efficacy of certain antibiotics against this organism.

Increasing resistance of beta-lactam in nosocomial \textit{P. aeruginosa} has become a serious threat particularly against third and fourth generation Cephalosporins, is of major concern. There are a lot of molecular mechanisms to develop resistance against these antibiotics; generation of extended-spectrum beta-lactamases (ESBL), by incorporation of bla genes in integrons and inability of porin genes to enhance their expression level and/or alteration of antibiotic target sites\textsuperscript{15}

Present study showed that \textit{P. aeruginosa} was found to be highly resistant against cephalosporin group of antibiotics. Study reported by Wang et al, explained the absolute resistance of Ampicillin, Cephazolin, Cefuroxime and Cefotaxime, which is in accordance with our results.\textsuperscript{16} Our study was also supported by Hamza et al, exhibited 100% resistance against Cefixime.\textsuperscript{17} While Jombo et al, reported 86% susceptibility of \textit{P. aeruginosa} against cefurixime.\textsuperscript{18}

Our study showed that the Carbapenem group of drugs are still effective against \textit{P. aeruginosa} infection than Piperacillin or combination of Piperacillin /Tezobactum where resistance rate was 72.85% and 66.00% which is comparable to a report showing it to be 73%.\textsuperscript{19} The current study demonstrated that 30% \textit{P. aeruginosa} were resistant against Carbapenem antibiotics (Imepenem, Meropenem), Rodríguez-Martínez JM et al,\textsuperscript{20} showed that 87% of strains of \textit{P. aeruginosa} were resistant against Imepenem\textsuperscript{21} Another study reported 100% resistance against Carbapenems\textsuperscript{22} it is very obvious that efficacy of this particular antibiotic is declining. Clonal spread contributes lesser importance in the statistics and epidemiology of infections caused by \textit{P. aeruginosa}, and the main mechanism associated with increased resistance to Imipenem was reduced expression of OprD (outer membrane protein) found in the isolates.\textsuperscript{21} Aminoglycosides is a significant member of broad spectrum antibiotics with a peculiar structure of an aminocyclitol ring. They are outstandingly active against aerobic and facultative aerobic Gram-negative bacteria. They mainly act by inhibiting protein synthesis and break cell membrane.\textsuperscript{23} Our study showed that Amikacin was the most effective drug against infection showing sensitivity of 74.84%. Amikacin was constructed as a weak candidate for the enzymes that are responsible to bring chemical modifications but some organisms have developed specific enzymes to inactivate Amikacin.\textsuperscript{24} One study declared 25.15% resistance against Aminoglycoside.

Fluroquinolone compounds are one of the important antimicrobial agents that have been used for variety of infections. New groups of Fluroquinolone are beneficial against Gram-negative and Gram-positive bacteria as far as older Fluroquinolones are concerned, they were effective against aerobic Gram-negative bacteria.\textsuperscript{21} Present study showed 29.80% sensitivity against Ciprofloxacin while 100% resistance against Ciprofloxacin was exhibited in one study.\textsuperscript{24} Similarly, 87.8% resistance was also claimed by another study.\textsuperscript{25} This study indicated Amikacin as an efficient treatment of choice against MDR \textit{P. aeruginosa} among all the tested antibiotics and Fouzia Khan et al\textsuperscript{26} showed the same result in their study.

Conclusion Our data revealed a high prevalence of DR phenotypes of \textit{P. aeruginosa} in the total isolate of \textit{P aeruginosa}. There is an urgent need to resolve the issue by taking some preventive measures. Combined efforts of health care professionals and researchers are required to
educate people about the proper use of antibiotics and other infection control measures.

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