Research Article

# Determination of Multidrug Resistance and Extended Drug Resistance Pattern of Pseudomonas aeruginosa in Clinical Isolates of Tertiary Care Hospital Lahore

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## Abstract

**Background:** Pseudomonas aeruginosa (PA) is a major public health community-acquired threat around the globe due to the growing rate of antimicrobial resistance. It is one of the most common trending causes of nosocomial infections.

**Objective:** The current study aimed to check the rate of multidrug-resistant (MDR) and extended drug-resistant (XDR) PA and to analyze the invitro activity of different antimicrobial agents against clinically isolated bacterial samples. Molecular Detection and amplification of L lipoprotein (OprL gene) were also done to determine the frequency and species of different strains of Pseudomonas aeruginosa (PA2192, C3719, PA01, PA14 and PACS2).

**Method:** This cross-sectional study was conducted at at the Department of Pathology, King Edward Medical University/ Mayo Hospital Lahore. Total 140 patients were included in the study. Biochemical characterization, molecular identification, antimicrobial susceptibility profiling and DNA sequencing of the desired gene were done to confirm different strains' identification.

**Results:** MDR and XDR Pseudomonas aeruginosa infection are more common among males 81(57.5%) and high among the 31-45 age group, i.e., 55 (39.3%). After antibiotic susceptibility testing, 60% of strains were found to be MDR PA, while 40% were categorized as XDR PA. Doripenem showed the highest sensitivity, 93 (66.4%) among all carbapenems. Polymyxin B showed the highest activity against Pseudomonas aeruginosa multi and extensively drug-resistant strains, i.e., 108 (77.1%).

**Conclusion:** The present study suggests that doripenem can be the only active agent for combating infections, and the Carbapenem drug appears effective against highly resistant strains of Pseudomonas aeruginosa.

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## Introduction

Antimicrobial resistance has become one of the emerging challenges globally, and infections due to multidrug-resistant (MDR)and extended drug-resistant (XDR) organisms are recognized as major threats to the healthcare community because these are difficult to treat. Pseudomonas aeruginosa, an opportunistic human pathogen, is among the GN rods (Gram-negative); majorly cause of healthcare-associated infections such as urinary tract infections, bloodstream infections, pneumonia, and surgically acquired infections. Pseudomonas

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aeruginosa antibiotic resistance is increasing nowadays all over the world.[1,2] It is the third leading cause of gram-negative infections in the United States, infecting 4% of 24,179 adult cases with nosocomial bloodstream infections.[2] In Pakistan, due to the highest frequency among all MDR organisms isolated in clinical investigation, epidemiological surveillance for susceptibility of Pseudomonas aeruginosa is essential to select the best empirical antibiotics. Local data is scanty for multi-drug resistant (MDR) and extended drug resistant (XDR) for Pseudomonas aeruginosa. Anti-Pseudomonas aeruginosa antimicrobial drug classes include penicillin with β-lactamase inhibitors (Ticarcillin-clavulanic acid and Piperacillin-tazobactam); Carbapenems[3] (imipenem, doripenem and meropenem); Aminoglycoside[4] (amikacin, gentamicin, tobramycin and netilmicin); Fluoroquinolones[5] (ciprofloxacin and levofloxacin); Polymyxins (Colistin and polymyxin B); Monobactams (aztreonam); Phosphonic acids (fosfomycin).[6] The nonspecific usage of antimicrobials and increased rate in invasive procedures, along with the expansion of intrinsic in addition to acquired resistance among P. aeruginosa strains, resulted in the progression of the emergence of diverse multidrug-resistant P. aeruginosa epidemics in medical settings.[7] The threat s to carriers admitted in acquiring MDR pathogens correlates to carriers admitted in a similar ward but also to personalized risk factors, i.e., patient features and hospital proceedings such as the use of invasive devices in addition to antibiotic intake.[8] Accurate, rapid identification of different Pseudomonas aeruginosa species and various antimicrobial drugs against PA is a critical constituent to treat MDR/XDR infections. Detection of PA with the conventional qualitative method requires at least 3 or 4 days due to 48 hours of incubation time for pure culture, which is used for biochemical characterization. One of the major drawbacks of the conventional method is that it is easily misunderstood with other types of gram negatives bacilli. So, using molecular detection techniques (polymerase chain reaction) can improve more precise Detection of Pseudomonas strains. L lipoprotein (OprL)is an outer membrane protein of PA that is critical in antibiotic resistance. So, this protein is an important factor for identifying Pseudomonas strains in clinical isolates by molecular characterization method.[9,10] Knowledge about epidemiology and possible risk elements for acquiring MDR/XDR-PA infection is vital for an appropriate and early assortment of appropriate antibiotics. So, this study was designed to investigate the rate and molecular characterization of MDR and XDR Pseudomonas aeruginosa among hospitalized or immunosuppressed patients.

## Methods

Sampling for a comparative cross-sectional study was done using a non-probability purposive sampling technique from Tertiary Care Hospitals in Lahore and managed at the Department of Pathology, King Edward Medical University, Lahore. Each sample was obtained from a single individual. Out of 140 samples selected for this study, 53.6 % samples were cultured from pus, 20.7% from sputum,9.3% from urine, 5% from endotracheal tube, 2.9% from pleural fluid and blood, 2.1% from Foley tip, 1.4% from chest tube, 0.7% bronchial washing, HVS and tissue fluid. Microbiological identifications were proceeded by shape and size of colonies which were determined by using Centrimide agar, gram staining and biochemical characterization such as oxidase, urease, triple sugar iron agar and citrate utilization test was done for structural conformation.[11]

Antibiotic sensitivity testing was performed to find out the sensitivity profile of bacteria by using the Kirby-Bauer Disk Diffusion method.[12] The antimicrobial susceptibility profiling was done by using sixteen antimicrobial agents from seven antimicrobial classes (carbapenems, cephalosporin, aminoglycosides, fluoroquinolones, penicillin, B-lactamase inhibitors, monobactams, polymyxins) were determined. Different antibiotics were used which included gentamicin (CN), amikacin (AK), netilmicin (NET), tobramycin (TOB), ceftazidime (CAZ), cefepime (FEP), levofloxacin (LVX), ciprofloxacin (CIP), piperacillin-tazobactam (TZP), ticarcillin-clavulanic acid (TIM), imipenem (IPM), meropenem (MEM), doripenem (DOR), aztreonam (ATM), colistin (CST), polymixin B (PMB).

Genomic DNA isolated from all strains was amplified using a polymerase chain reaction. Two Selective OprL gene primers (5'ATGGAAATGCTGAAATTCGGC 3' and 5' CTTCTTCAGCTCGACGCGACG 3') were used and result observed on 1% agarose gel under UV trans illuminator. For sequencing of 25 OprL, amplified gene samples were sent to Macrogen Korea. Sequences were obtained and then analyzed online with BLAST.

## Results

This study was conducted in the Microbiology department at King Edward Medical College University from June 2019 to December 2019. During this period, 140 samples of Pseudomonas aeruginosa were collected from the same number of patients and processed at the Microbiology lab of the Department of Pathology, King Edward Medical University, Lahore. All Molecular Biological work was done at the Advance Diagnostic lab, Punjab AIDS Control Program, Lahore. The MDR and XDR infection rateThe MDR and XDR infection rate is high in male patients, i.e., 81 (57.5%) and low in females, 59(42.1%). The infection rate was high among the 31-45 age group, i.e., 39.3%, followed by 46-60 years of age (27.1%). Microbiological identification of Pseudomonas aeruginosa was done based on their biochemical properties. Among all pseudomonas, only Pseudomonas aeruginosa can grow on cetrimide agar. Gram staining showed gram-negative rods on microscopy and positive citrate and oxidase test results. 60% of Pseudomonas aeruginosa strains fitted the criteria of being MDR PA, while 40% of strains were classified as XDR PA based on drug susceptibility profiling.[13,14] From all antibiotics tested for their activity against MDR strains, in total, 84 subjects showed the following results such as in aminoglycosides class amikacin 58(69.0%) showed the highest sensitivity and highest resistances showed by tobramycin 38(45.3%). In the carbapenem class, the highest sensitivity showed by doripenem 71(84.5%), and the high resistance showed by imipenem 18(21.5%). Cephalosporins found the highest sensitivity in cefepime 59(70.2%) and the highest resistance in ceftazidime 55(53.6%). In fluoroquinolones, the highest sensitivity was shown by levofloxacin 55(65.5%), and ciprofloxacin 40(47.6%) showed the highest resistance. In Pencillin-β-lactamase inhibitors highest sensitivity was found in Piperacillin-tazobactam (TZP) 66 (78.5%), and the highest resistance was found in ticarcillin-clavulanic acid (TIM)30(35.7%). Aztreonam (ATM) showed high resistance 46(54.8%) in monobactam. In polymyxins, Colistin (CST) showed the highest resistance 33(39.3%), and polymixin B (PMB) showed the highest sensitivity 68(80.9%). Among all classes doripenem showed highest activity 71(84.5%) followed by polymyxin B 68(80.9%), meropenem 67 (79.7%), imipenem and piperacillin tazobactam 66 (78.5%). Aztreonam showed the least activity, 38(45.2%) among all, as shown in Table 1. Drug sensitivity of XDR strains (total patients 56) was checked with all selected antibiotics, and in the aminoglycosides class, amikacin 20(35.7%) showed the highest sensitivity and highest resistances showed by tobramycin 51(91.1%). In the carbapenem class, the highest sensitivity showed by doripenem 22(39.3%), and high resistance showed by meropenem (MEM) 47(78.6%). Cephalosporins found the highest sensitivity in cefepime 4(7.1%) and the highest resistance in ceftazidime 56(100%). In fluoroquinolones, the highest sensitivity was shown by levofloxacin 5(8.9%), and the highest resistance showed by ciprofloxacin 52(92.9%). In Pencillin-β-lactamase inhibitors highest sensitivity was found in Piperacillin-tazobactam (TZP) 9(16.1%), and the highest resistance was found in ticarcillin-clavulanic acid (TIM) 53(94.7%). Aztreonam (ATM) showed resistance 50(89.3%) in monobactam. In polymyxins, Colistin (CST) showed the highest resistance, 30(53.6%), and polymixin B (PMB) showed the highest sensitivity, 40 (67.8%). All antibiotics tested for XDR showed that polymyxin B and colistin Colistin are more sensitive among all antibiotics. Ceftazidime didn't show any activity against XDR strains. Activities of different antibiotics are shown in Table 2. By -cross-comparison between MDR and XDR antibiotic profiling, it is interpreted that outcomes were the same in both cases. All highly resistant or sensitive for MDR, and sensitive for MDR were also given the same results in the case of XDR. For precise and fast identification of species of PA OprL gene was amplified using a thermocycler, and the size of the OprL gene was found to be about 504bp, as shown in Figure 1. Sequences of the OprL gene were obtained and then analyzed online with BLAST; the results are shown in Table 3.

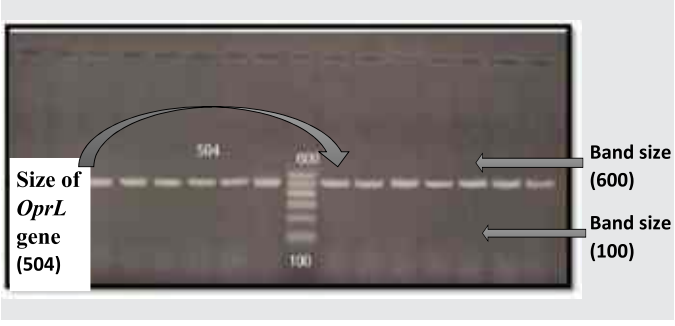


**Figure 1:** *PCR products of OprL gene*

**Table 1:** *Drug sensitivity of MDR strains*

| Drug class | Drug name | Sensitivity | Resistance |
|---|---|---|---|
| Aminoglyco-sides | Gentamicin | 55(65.5%) | 29(34.5%) |
| | Tobramycin | 46(54.7%) | 38(45.3%) |
| | Amikacin | 58(69.0%) | 26(31.0%) |
| | Netilmicin | 50(70.2%) | 34(29.8%) |
| Carbapenem | Imipenem | 66(78.5%) | 18(21.5%) |
| | Meropenem | 67(79.7%) | 17(20.3%) |
| | Doripenem | 71(84.5%) | 13(15.5%) |
| Cephalosporins | Ceftazidime | 39(46.4%) | 55(53.6%) |
| | Cefipime | 59(70.2%) | 25(29.8%) |
| Fluoroqinolones | Ciprofloxacin | 44(52.4%) | 40(47.6%) |
| | Levofloxacin | 55(65.5%) | 29(34.5%) |
| Penicillin-β-lactamase inhibitors | Ticarcillin-clavulanic acid (TIM) | 54(64.3%) | 30(35.7%) |
| | Piperacillin-tazobactam (TZP) | 66(78.5%) | 18(21.5%) |
| Monobactam | Aztreonam (ATM) | 38(45.2%) | 46(54.8%) |
| Polymyxins | Colistin (CST) | 51(60.7%) | 33(39.3%) |
| Drug class | Polymixin B (PMB) | 68(80.9%) | 16(19.1%) |

**Table 2:** *Drug sensitivity of XDR strains*

| Drug class | Drug name | Sensitivity | Resistance |
|---|---|---|---|
| **Aminoglycosides** | Gentamicin (CN) | 8(14.3%) | 48(85.7%) |
| | Tobramycin (TOB) | 5(8.9%) | 51(91.1%) |
| | Amikacin (AK) | 20(35.7%) | 36(64.3%) |
| | Netilmicin (NET) | 7(12.5%) | 49(87.5%) |
| **Carbapenem** | Imipenem (IPM) | 13(23.2%) | 43(76.8%) |
| | Meropenem (MEM) | 12(21.4%) | 47(78.6%) |
| | Doripenem (DOR) | 22(39.3%) | 34(60.7%) |
| **Cephalosporins** | Ceftazidime (CAZ) | 00(0%) | 56(100%) |
| | Cefipime (FEP) | 4(7.1%) | 52(92.9%) |
| **Fluoroqinolones** | Ciprofloxacin (CIP) | 4(7.1%) | 52(92.9%) |
| | Levofloxacin (LVX) | 5(8.9%) | 51(91.1%) |
| **Penicillin-β-lactamase inhibitors** | Ticarcillin-clavulanic acid (TIM) | 3(5.3%) | 53(94.7%) |
| | Piperacillin-tazobactam (TZP) | 9(16.1%) | 47(83.9%) |
| **Monobactam** | Aztreonam (ATM) | 6(10.7%) | 50(89.3%) |
| **Polymyxins** | Colistin (CST), | 26 (46.4%) | 30(53.6%) |
| | Polymixin B (PMB) | 40 (67.8%) | 16(32.2%) |

**Table 3:** *BLAST analysis of sequenced OprL gene*

| Sequence number | BLAST Results | Accession number |
|---|---|---|
| **1** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **2** | Pseudomonas aeruginosa strain CR1 | submitted |
| **3** | Pseudomonas aeruginosa strain PPF1 | submitted |
| **4** | Pseudomonas aeruginosa strain PPF1 | submitted |
| **5** | Pseudomonas aeruginosa strain CR1 | submitted |
| **6** | Pseudomonas aeruginosa strain SP2230 | submitted |
| **7** | Pseudomonas aeruginosa strain CR1 | submitted |
| **8** | Pseudomonas aeruginosa strain CR1 | submitted |
| **9** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **10** | Pseudomonas aeruginosa strain PPF1 | submitted |
| **11** | Pseudomonas aeruginosa strain SP2230 | submitted |
| **12** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **13** | Pseudomonas aeruginosa strain PA01 | submitted |
| **14** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **15** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **16** | Pseudomonas aeruginosa strain SP2230 | submitted |
| **17** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **18** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **19** | Pseudomonas aeruginosa strain PPF1 | submitted |
| **20** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **21** | Pseudomonas aeruginosa strain SP2230 | submitted |
| **22** | Pseudomonas aeruginosa strain PA -3 | submitted |
| **23** | Pseudomonas aeruginosa strain SP2230 | submitted |
| **24** | Pseudomonas aeruginosa strain CR1 | submitted |

## Discussion

In recent years, a growing population of multidrug-resistant bacteria (MDR) threatens to move us into the "post-antibiotic era" of bacterial infectious diseases. Pseudomonas aeruginosa is responsible for morbidity, mortality and healthcare costs and is also responsible for hospital-associated infections (HAIs). Infections due to Pseudomonas aeruginosa are quite difficult to treat. This study was conducted in Lahore Metropolitan, one of Pakistan's highly populated cities, one of Pakistan's highly populated cities. Pseudomonas aeruginosa, the global threat of antibiotic resistance, was selected for this study. In the present study, male patients outnumbered female patients, i.e., 57.9% male, while 42.1% of females were found to be affected by either MDR or XDR P. aeruginosa. Increased prevalence of male patients than female patients is evident from the present study. It can be clarified by the statement that in our province, adult males have greater exposure to outdoor surroundings due to their mobility than females.[15] The maximum infection rate was found in people aged between 31 and 45 (39.3%), followed by patients aged between 46 and 60 (27.1%). Most MDR/XDR P. aeruginosa was isolated from pus samples (53.3%). The results are consistent with the study conducted by Khan et al. in which maximum MDR P. aeroginusa strains were isolated from pus (33.3%). Antibiotic susceptibility

testing was also checked via 'The Kirby Bauer Disc Diffusion method'.[16,17] In the present research, 40% of P. aeruginosa infections displayed an XDR phenotype which was far more than reported by Palavutitotai et al., i.e., 22%. Different strains of Pseudomonas aeruginosa have generally low susceptibility to various antibiotics than Gram-negative bacteria of another genus.[18] This organism is intrinsically resilient to most antibiotics. The phenomenon is reported as intrinsic resistance.[19] Effective antimicrobial therapy is severely restricted to treating HAI (Hospital-acquired infections) caused by XDR (extensively drug-resistant) and multidrug-resistant (MDR) P. aeruginosa and now becoming a therapeutic challenge. The current research shows that P. aeruginosa was less resistant to the carbapenem group of antibiotics like imipenem, meropenem (43.6%), and doripenem (33.6%). In a study by Anupurba et al., resistance to ceftazidime was 46%, and ciprofloxacin was 42%,[20,21] much lower than the present study. This might be attributed to the environmental state of a particular region, genetic circumstantial of the organism or extensive use of antimicrobials among patients. In our study, levofloxacin (75.7%) showed the greatest resistance among all MDR and XDR cases. In research on multidrug-resistant Pseudomonas aeruginosa, Khan et al. reported the highest resistance against the Cephalosporin group of antibiotics. Among carbapenems, doripenem was found to be more sensitive among all MDR/XDR patients, i.e., 93 (66.4%). But overall, carbapenem resistance was high, and the results were similar to a study conducted by Palavutitotai et al., in which 40% of P. aeruginosa were reported to exhibit carbapenem resistance. National Healthcare Safety Network reported that HAI presented a lower CRPA frequency than among 4,669 P. aeruginosa isolates collected from 4,515 hospitals in the U.S.A. between 2011 and 2014, with 7% to 28% resistance seen.[22] Present results are also consistent with the outcomes of research performed in Thailand, in which a high incidence of CRPA, i.e. (72%) amongst 261 isolates of multidrug-resistant P. aeruginosa, was reported.[23] It is notifiable that there is considerable variation in the prevalence of drug-resistant P. aeruginosa among studies, clearly dependent on the study population and the isolation site. The present study indicated that 67.8% of XDR-PA remained sensitive to polymyxin B, followed by colistin (46.4%). In combination, colistin and aminoglycosides probably enhance renal toxicity, predominantly in critically ill personnel. Although preceding in vitro studies have revealed a synergistic activity of doripenem, imipenem,[24] amikacin or ceftazidime with Colistin against MDR or XDR P. aeruginosa.[24,25] The amplification and results of the molecular analysis reveal that the OprL gene was present in all isolates, and amplicon size was found to be 504bp which is closely related to the size reported by Abdullahi et al.[26] Peptidoglycan-associated lipoprotein OprLis the particular of Pseudomonas aeruginosa hence considered as a diagnostic marker. Billard-Pomare et al. showed that qPCR of the OprL gene has high specificity, and it is more suitable than culture to show bacterial colonization.[27] Xu et al. (2004) demonstrated that conventional PCR based on the amplification of the OprL (outer-membrane lipoprotein) and exoA (exotoxin A) gene allows the early Detection of new colonization. There is. Still, various sub-strains, such as PPF1, CR1 etc., but BLAST results of the OprL gene revealed that most strains in our population belong to PA3 and CR1 sub-strains.[28,29]

## Conclusion

A high resistance rate of Pseudomonas aeruginosa infections to carbapenem drugs makes establishing a suitable empirical therapy challenging. The present study suggests that doripenem can be the only active agent for combating infections caused by MDR/ XDR-PA. Another approach to treat the infections caused by MDR/XDR pathogens is to combine several antibiotics i.e., polymyxin B or colistin, with doripenem to gain a synergistic effect and decrease the probable toxicities associated with each drug. Several strains prevail in communities like PA 2192, but results revealed that RA-3 and CR-1 majorly exist in our population. By using more effective treatment approaches, and drugs, gaining the expedition for novel antibiotics, emphasizing the communication route of XDR, MDR and focusing on healthier usage and administration of the current antimicrobial armamentarium, we can control the resistance and mortality rate.

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