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A Preliminary Study on Multidrug- and Extensively Drug-Resistant Pseudomonas aeruginosa and Acinetobacter baumannii

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ABSTRACT

Background: Acinetobacter baumannii and Pseudomonas aeruginosa are two major causative agents of hospital based infections leading to the increase in morbidity and mortality rate globally, due to being multidrug resistant and Extensively drug resistant.
Objective: To assess antibiotic resistance and multidrug resistant/Extensively drug resistant patterns in Gram-negative bacteria isolated from clinical samples
Methods: Isolation of bacteria from various clinical samples is done using MacConkey agar and then identified using biochemicals. MDR and XDR strains were determined by antibiotic susceptibility testing (Kirby bauer disc diffusion assay).
Results: Out of 600 clinical samples, 60 (10%) were positive for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Among them, 10% of P. aeruginosa and 70% of A. baumannii were MDR, while 3% of *P. aeruginosa* and 65% of A. baumannii were XDR. MDR and XDR isolates were mostly found in ICU and wound samples, with fewer cases in general wards.
Conclusion: Considering high isolation rates of MDR and XDR of Acinetobacter baumannii and Pseudomonas aeruginosa, it is now necessary to adopt prevention criteria to get rid of of MDR and XDR Acinetobacter baumannii and Pseudomonas aeruginosa from hospital wards.

Introduction

Hospital-acquired infections (HAIs) and antimicrobial resistance (AMR) have increasingly become major public health concerns, contributing significantly to patient morbidity and mortality [1]. Among Gram-negative bacteria, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are particularly problematic due to their multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, which limit available treatment options [2]. Globally, resistant bacterial infections affect a large number of hospitalized patients each year, with mortality rates varying widely depending on the pathogen and healthcare setting. For instance, *Pseudomonas aeruginosa* infections can result in death in 18% to over 60% of cases, while MDR *Acinetobacter baumannii*, a lactose-non-fermenting Gram-negative bacillus, is commonly associated with nosocomial infections and carries a high risk of mortality in intensive care units [3].

The prevalence of MDR *Pseudomonas aeruginosa* has been increasing in different

regions, with historical reports indicating rates of 14% in the United States and 4% in Italy [4]. In the U.S., MDR *P. aeruginosa* accounts for a substantial portion of HAIs annually, with some studies reporting mortality rates as high as 46% [5]. Similar trends have been observed in South America and Southeast Asia, where MDR rates range between 6–8%. Mortality due to MDR *Acinetobacter baumannii* bacteremia has been reported at approximately 21%, and patients in ICUs are at a far higher risk compared to those in general wards [6].

Preventing the spread of these pathogens relies heavily on strict disinfection and infection control practices. Without proper measures, AMR can lead to longer hospital stays, increased treatment costs, and higher mortality rates. Considering these challenges, the present study was undertaken to investigate the prevalence and antibiotic resistance patterns of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in clinical samples.

Methodology:

Sample Collection and Culture: From February to July 2023, a total of 600 clinical samples including wound swabs, urine, sputum, and blood were assessed routine laboratory testing from tertiary care hospital. Samples were processed and cultured on MacConkey agar.

Identification of Bacterial Isolates: Bacterial isolates were identified using standard biochemical tests, including oxidase, catalase, motility, citrate utilization, indole production, methyl red, Voges–Proskauer.

Antibiotic Susceptibility Testing (AST): AST was performed using the disc diffusion method according to CLSI guidelines. The antibiotics tested included Piperacillin-tazobactam, Amikacin, Gentamicin, Cefepime, Ciprofloxacin, Meropenem, Tobramycin. The zone of inhibition (ZOI) was measured in mm and interpreted as sensitive, intermediate, or resistant based on CLSI standards.

Statistical Analysis:

All data were entered into SPSS version 25, and the Chi-square test (χ^2) was applied to study associations. A p-value <0.05 was considered statistically significant.

Results:

Isolation of Bacterial Strains

During the study period, a total of 600 clinical samples were assessed, including wound swabs, urine, and sputum. Out of these, 60 samples (10%) were positive for Pseudomonas aeruginosa and Acinetobacter baumannii. Among the isolates, 36 (60%) were identified as *P. aeruginosa* and 24 (40%) as *A. baumannii* using standard biochemical tests.

Table 1 AST OF P. aeruginosa strains

Antibiotic	Resistant (R)	% Resistant
Piperacillin-tazobactam	22	61.1%
Amikacin	12	33.3%
Gentamicin	15	41.7%
Cefepime	14	38.9%
Ciprofloxacin	22	61.1%
Meropenem	7	19.4%
Tobramycin	13	36.1%

Table 2: *AST of Acinetobacter baumannii isolates*

Antibiotic	Resistant (R)	Total	% Resistant
Piperacillin-tazobactam	18	24	75%
Amikacin	15	24	62.5%
Gentamicin	17	24	70.8%
Cefepime	16	24	66.7%
Ciprofloxacin	20	24	83.3%
Meropenem	20	24	83.3%
Tobramycin	18	24	75%

Antibiotic Susceptibility Patterns:

Antibiotic susceptibility testing (AST) revealed varying resistance profiles among the isolates. For *P. aeruginosa*, 6 (17%) isolates were resistant to imipenem, while 2 (6%) were susceptible. Resistance to piperacillin-tazobactam was observed in 22 (61%) isolates.

Among *A. baumannii* isolates, 22 (92%) were resistant to imipenem and 20 (83%) to ceftazidime, while 2 (8%) were sensitive to imipenem.

Discussion

Overall, multidrug resistance (MDR) was observed in 6 (17%) *P. aeruginosa* and 17 (71%) A. baumannii isolates. Extensively drug-resistant (XDR) strains were noted in 2 (6%) P. aeruginosa and 16 (67%) A. baumannii isolates. Misuse and overuse of antibiotics in hospitals leads to rise in antibiotic resistance. AMR is major hinderance in treatment of various diseases. So, evaluation of resistant isolates by AST is very necessary. Due to genetic changes caused by misuse and overuse of antibiotics, resistance patterns could be different in every country [3,4,5]. Various studies have confirmed MDR *P. aeruginosa* isolates from burn hospitals, and ciprofloxacin (93.7%), imipenem (79.2%), and amikacin (82%) resistance strains were observed [7]. In a study conducted in 2013 at a burn hospital center of Gilan, Iran, the frequency of resistance strains were imipenem 97.5%, gentamycin 67.5%, ceftazidime 57.5%, piperacillin 87.5%, ciprofloxacin 65%, and amikacin 90% [8]. In this study, ciprofloxacin resistant *Pseudomonas aeruginosa* isolates were (62.7%) and amikacin resistant were (52%), respectively. 64% of *P. aeruginosa* isolates were imipenem resistant. According to Corehtash report, 93.1% of isolated strains of P. aeruginosa were MDR [9]. In a previously reported investigation, clinical *Pseudomonas aeruginosa* isolates exhibited very high levels of antimicrobial resistance, with the majority of strains showing resistance to most antibiotics tested. Only a small proportion of isolates remained susceptible to ceftazidime (~30 %) and polymyxin B (~2 %). A large majority of the isolates were classified as multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes, highlighting the limited treatment options and significant resistance burden associated with these organisms in clinical settings [10]. In this study, 17% of Pseudomonas aeruginosa isolates were identified as multidrug-resistant (MDR) and 6% as extensively drug-resistant (XDR) among the total isolates (n = 36). In contrast, Preze et al., 2019 reported a much higher prevalence, with 88.9% of P. aeruginosa isolates classified as MDR or XDR [11]. These differences may be attributed to variations in sample size, hospital setting, and local antibiotic usage patterns.

A study conducted by Saleem et al. reported resistance in *Pseudomonas aeruginosa* isolates to imipenem, amikacin, and ciprofloxacin as 30.2%, 37.2%, and 17.4%, respectively, with MDR and XDR prevalence of 36.3% and 18.1%. In our study, resistance to imipenem and ciprofloxacin was approximately double that reported by Saleem et al., and the frequency of MDR (17%) and XDR (6%) *P. aeruginosa* isolates was higher compared to their findings. This variation may be attributed to overuse of antibiotics and local hospital practices [12]. In this study, resistance among Acinetobacter baumannii isolates was high. Resistance rates to key antibiotics were as follows: tobramycin 75%, piperacillin-tazobactam 75%, ciprofloxacin 83%, meropenem 83%, amikacin 62.5%, gentamicin 70.8%, and cefepime 66.7% (Table 2). Overall, 71% of A. baumannii isolates were multidrug-resistant (MDR) and 67% were extensively drug-resistant (XDR), highlighting a significant challenge for treatment in hospital settings. In comparison, Hatami et al. reported lower resistance rates, with tobramycin and ceftazidime resistance at 70%, and amikacin and imipenem resistance at 50% and 80%, respectively [13]. In our study, the prevalence of MDR and XDR isolates was 89.3% and 91.9%, highlighting a significant increase in resistance, particularly to carbapenems, likely due to inadequate infection control strategies. Similar trends have been observed globally; studies from Brazil reported carbapenem-resistant A. baumannii ranging from 80.7% to 92.2%, with XDR prevalence up to 78.6%, emphasizing the worldwide concern regarding rising antimicrobial resistance. In conclusion, high prevalence of MDR and XDR—*Pseudomonas aeruginosa* and *Acinetobacter*

baumannii strains is a serious concern in hospital wards of Pakistan.

These findings will help the policymakers to strictly implement the microbial identification and AST procedure before medication against infectious agents, to get rid of AMR.

Conflict of Interest: The authors have no competing interests.

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