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Prevalence of MDR and XDR Pseudomonas aeruginosa and *Acinetobacter baumannii* isolated in clinical samples from Children Hospital

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ABSTRACT

Background: Acinetobacter baumannii and Pseudomonas aeruginosa are two major causative agents of nosocomial infections leading to the increase in morbidity and mortality rate globally, due to being MDR and XDR.
Objective: The aim of this study is to check the prevalence of MDR and XDR Acinetobacter baumannii and Pseudomonas aeruginosa phenotypes in hospital based samples.
Methods: Isolation of bacteria from various clinical samples is done using MacCokney agar and then identified using biochemicals. MDR and XDR strains were determined by antibiotic susceptibility testing (Kirby bauer disc diffusion assay).
Results: Out of 3248 clinical samples, Acinetobacter baumannii and Pseudomonas aeruginosa strains were detected in 309(9.51%) samples. Antibiotic susceptibility testing indicated that (16.50%) and (15.53%) of the P. aeruginosa and (74.75%) and (73.13%) of the Acinetobacter baumannii isolates were screened as the MDR and XDR strains. In wound samples, behavioral intensive care unit (BICU) and restoration ward, the frequency of MDR isolates was, 222 (71.8%), 187 (60.5%) and 63 (20.4%). The frequency of XDR isolates in burns was 30 (9.70%), while in BICU was 187 (59.54%), and restoration 58(18.77%).
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

In recent years, hospital-acquired infections (HAIs) and AMR have become one of the most prominent reason of morbidity and mortality[1.2.3]. Due to the increasing rates of antimicrobial resistant Gram-negative infections, selection of therapeutic options against MDR and XDR Acinetobacter baumannii and Pseudomonas is restricted[4]. In Europe, recent published studies estimates that 400,000 patients gets infected with resistant bacterial healthcare-acquired infections each year[5.6.7]. Mortality rates of Pseudomonas aeruginosa is 18 to 61% [8,9]. MDR *Acinetobacter baumannii* is lactose non-fermentor Gram-negative bacilli, most common nosocomial infections[8]. Limited treatment options for MDR and XDR bacterium leads to significant

infection control problems[10, 11]. Rates of MDR—P. aeruginosa are increasing in USA(14% in 2002), and Italy (4.1% in 2010) [4]. Moreover, in the U.S, MDR *Pseudomonas aeruginosa* account for 13–19% of HAIs each year. Mortality rates between 2007 and 2009 was estimated 46.1%)[12,13]. MDR *Pseudomonas aeruginosa* isolates in South America and Malaysia were estimated as 8.2% and 6.9%[14]. Mortality rate of MDR *Acinetobacter baumannii* in bacteremia was 21.2%[15]. Mortality rate in general wards was 5%, 54% in intensive care units (ICUs) is associated with *Acinetobacter baumannii*[11, 16]. Disinfection is the main approach to prevent nosocomial infections, otherwise, AMR is the major problem and may lead to high treatment cost, longer duration of hospitalization, and high mortality rate[17–19]. The present study was conducted to evaluate the prevalence and antibiotic susceptibility patterns of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Methods

Samples collection and culture: In this cross-sectional study, a total of 3248 clinical samples including wound, urine, sputum, blood, feces, and trachea were collected from Feb 2023 to July 2023 from a children hospital lahore. Samples were cultured on MacConkey agar.

Identification of Bacterial Isolates: Isolates were identified based on biochemical tests including oxidase, catalase, motility, citrate, Indol production, Methyl red, Voges–Proskauer, and presence of lysine decarboxylase, and arginine dehydrogenase enzymes were performed. After confirmation of isolates, disc diffusion assay was performed based on CLSI guidelines[23]. Antibiotics used were amikacin (30 µg), ceftazidime (30 µg), cephalexin (30 µg), ciprofoxacin (5 µg), imipenem (10 µg), meropenem (10 µg), gentamycin (10µg), tobramycin (10 µg), and cotrimoxazole[25]. ZOI is measured in mm and compared with CLSI to declare it sensitive, intermediate, or resistant. For Statistical analysis, data is entered to SPSS version 26, Chi square test($\chi 2$ test) applied and a P value < 0.05 is significant.

Results

309 (9.51%) *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from clinical-samples were collected. 234 (75.7%) and 75 (24.3%) were identified respectively as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* using biochemical profile. Out of 309, 246 (79.6%) were isolated from wound, 29 (9.4%) urine, blood 24 (7.8%), and sputum 10 (3.2%). AST indicated that 48 (15.53%) of the *Pseudomonas aeruginosa* isolates were imipenem resistant and 23 (7.44%) were susceptible. 228 (97.4%) *Acinetobacter baumannii* were imipenem resistant while 5 (2.1%) were sensitive. 47 (62.7%) of P. aeruginosa and 228(97.4%) of *A. baumannii* were ciprofloxacin resistant. Detailed data is listed. According to AST, 51(16.50%) *Pseudomonas aeruginosa* and 231 (74.75%) of *Acinetobacter baumannii* strains were categorized as MDR. 226 (73.13%) *Acinetobacter baumannii* and 48 (15.53%) *Pseudomonas aeruginosa* strains were categorized as XDR.

Table 1 Antibiotic susceptibility patterns of isolated P. aeruginosa and A. baumannii strains

AST Pattern	S	R	I
Amikacin	36	39	0
ceftazidime	30	44	1
cephalexin	5	70	---
ciprofloxacin	27	47	1
cotrimoxazole	16	59	---
Gentamycin	35	39	1
Imepenem	23	48	4
meropenem	24	45	6
Tobramycin	23	47	5

Frequency of MDR isolates from wound samples was higher, 222 (71.8%). MDR strains from BICU were 187 (60.5%) and restoration ward were 63 (20.4%) that is more isolated than other hospital wards.Frequency of XDR isolates in BICU was 184 (59.54%), and restoration ward 58 (18.77%), while that of burn department was 30 (9.70%). Considering the relationship between sex and age with the resistance to various antibiotics, only meropenem and aminoglycoside resistant strains were statistically significant (P value < 0.05).

Table 1 Antibiotic susceptibility patterns of isolated P. aeruginosa and A. baumannii strains

AST Pattern	S	R	I
Amikacin	14	208	12
ceftazidime	8	226	---
cephalexin	8	296	---
ciprofloxacin	6	228	---
cotrimoxazole	4	224	6
Gentamycin	---	234	---
Imepenem	5	228	1
meropenem	5	226	3
Tobramycin	5	227	2

Discussion

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[25]. Various studies have confirmed MDR *P. aeruginosa* isolates from burn hospitals, and ciprofoxacin (93.7%), imipenem (79.2%), and amikacin (82%) resistance strains were observed. 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%, ciprofoxacin 65%, and amikacin 90%. In this study, ciprofoxacin 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[27]. A study conducted by Nasimmoghadas et al., findings, *Pseudomonas aeruginosa* isolates were resistant to all tested antibiotics, except for ceftazidime (32%), and polymixin B (2%). 94% and 85% of isolates were categorized as MDR and XDR[24]. In my research, there are 16.50% MDR and 15.53% XDR *Pseudomonas aeruginosa* isolates (n=309). Preze et al., in 2019 indicated that the prevalence of the XDR and MDR isolates was 88.9%.

A study conducted by Saleem et al., reported that imipenem, amikacin, and ciprofloxacin *Pseudomonas aeruginosa* isolates were 30.2%, 37.2%, and 17.4%. Prevalence of MDR and XDR *Pseudomonas Aeroginosa* was 36.3% and 18.1%[30]. Resistance of imipenem and ciprofoxacin in my research were double of the Saleem findings, and frequency of XDR and MDR isolates in our results were higher than the mentioned study too. There is a difference between this study and Saleem et al study findings. Overuse of antibiotics is the major reason for this variation. Resistance to antibiotics (particularly for carbapenems) in Acinetobacter baumannii has reached alarming levels worldwide[10]. In this study, A. baumannii resistance pattern is tobramycin (97%), ceftazidime(96.6%), ciprofoxacin(97.4%), and imipenem(97%). According to Hatami's findings, tobramycin and ceftazidime A. baumannii isolates were 70%, while amikacin and imipenem resistant strains were 50% and 80%[32]. According to my findings, MDR isolates were 89.32%, and XDR were 91.90%. Resistance of A. baumannii to meropenem and imipenem has increased due to improper

infection control strategies in hospitals, and has become a major concern worldwide. According to the study Brazil in Brazil (2014), resistance of *A. baumannii* to carbapenem was 80.7%[33]. A cross sectional study in Brazil in 2017 conducted by Rossi and her colleagues reported that, in carbapenem resistant *A. baumannii*, a variation of 30% to 70% was observed between 2010 and 2014[34]. A study by Romanin in 2019 showed that carbapenem resistant A. baumannii isolates were 92.2%, while prevalence of XDR *A. baumannii* strains was 78.6%[35]. This study also demonstrated that 97% of Acinetobacter baumannii isolates were resistant to carbapenem (high resistant pattern). 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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