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Original Article

Microbiology Section

A Longitudinal Study on Antimicrobial Resistance Dynamics in *Pseudomonas aeruginosa* at a Government Superspeciality Hospital

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ABSTRACT

Introduction: *Pseudomonas aeruginosa*, primarily an opportunistic hospital pathogen, has now become an increasing cause of infections in the community as well. It significantly contributes to the rising burden of Antimicrobial Resistance (AMR), making treatment challenging. In high-risk settings such as superspeciality hospitals, the impact of AMR is magnified, necessitating continuous surveillance of susceptibility patterns to guide empirical therapy and escalation/de-escalation strategies for definitive treatment as part of antimicrobial stewardship.

Aim: To analyse the Antimicrobial Susceptibility Testing (AST) patterns of *Pseudomonas aeruginosa* isolates at a government superspeciality hospital.

Materials and Methods: This longitudinal study analysed data from three years (January 2021 - January 2024) at the 250-bedded ESIC super-speciality government teaching hospital, Hyderabad , Telangana, India. Adhering to Clinical and Laboratory Standards Institute (CLSI) M-100 guidelines, samples were collected and processed on clinical requests; those showing growth were analysed using an automated identification and AST analyser. Among all the samples received in the microbiology laboratory, 517 samples with growth of *Pseudomonas aeruginosa* were included. Colistin resistance was confirmed using Colistin Broth Disc Elution (CBDE) and compared with automated analyser results to assess the difference between both methods. Suitable antibiotics with potential for treatment were evaluated based on CLSI M39 guidelines. Demographic parameters such as age groups (paediatric and adult), gender distribution and sample types (urine, blood, respiratory specimens and exudates) were considered to evaluate resistance patterns. Descriptive statistics were used and presented in terms of percentages.

Results: In the current study, among the 517 isolates of *Pseudomonas aeruginosa*, 440 (85%) were from adults and 77 (15%) from paediatric population. *Pseudomonas aeruginosa* showed varying susceptibility rates to different antibiotics, ranging from 193 (43.8%) to 276 (62.7%) in adults and 32 (41.5%) to 38 (49.3%) in paediatric population. Multidrug Resistant (MDR) isolates totalled 170 (32.8%), with 123 (43.2%), 6 (12.5%), 20 (22.1%) and 21 (22.5%) among the received urine, blood, respiratory and exudate samples, respectively. MDR *Pseudomonas aeruginosa* rates increased from 2021 to 2023, reaching 68 (43.6%). Colistin susceptibility rates were relatively better, with 371 (84.3%) in adults and 70 (90.9%) in the paediatric population. Colistin susceptibility ranged from 38 (79%) to 83 (93%) across samples. A discrepancy of 76 (14.7%) was observed between the CBDE and automated methods for detecting colistin resistance.

Conclusion: This study highlights the increase in MDR *Pseudomonas aeruginosa*, with higher resistant rates observed among paediatric population. While colistin remains effective, its resistance requires ongoing monitoring, necessitating the need for alternative strategies, enhanced infection control measures and antimicrobial stewardship to effectively combat the growing threat of AMR in *Pseudomonas aeruginosa*.

Keywords: Age groups, Antimicrobial stewardship, Colistin, Gram-negative bacterial infection, Multidrug resistance

INTRODUCTION

Pseudomonas aeruginosa, one of the World Health Organisation (WHO) priority pathogens, once known for being an opportunistic hospital-acquired pathogen, has since evolved to cause a range of infections in the community as well. It is distinct from the wide array of pathogenic organisms due to its highly adaptable genome, which enables it to thrive in various environments [1,2]. *Pseudomonas aeruginosa* makes considerable contributions to the growing burden of AMR with its arsenal of intrinsic and acquired resistance mechanisms, exhibiting innate resistance to many antibiotics and further developing resistance through genetic mutations and horizontal gene transfer. This multifaceted issue is driven by factors including the overuse of antibiotics, selective pressure in clinical settings and the inherent ability to acquire and disseminate resistance mechanisms. The spectrum of resistance mechanisms exhibited by *Pseudomonas aeruginosa* further complicates treatment efforts [1-3].

There has been a rise in MDR *Pseudomonas aeruginosa* isolates in recent years. Conventional anti-pseudomonal beta-lactam antibiotics, used for the empirical treatment of such isolates, are now being rendered ineffective, posing a significant hurdle to treatment [4]. Understanding the dynamics of resistance in *Pseudomonas aeruginosa* requires longitudinal studies that track changes in resistance profiles over time. Such studies provide valuable insights into the evolving resistance patterns and the factors driving these changes. By examining temporal trends, future resistance developments can be predicted and the effectiveness of existing treatment regimens can be assessed [4,5].

The use of colistin has re-emerged, mainly for treating infections caused by MDR Gram-negative pathogens; however, the emergence of colistin resistance has been observed in recent years [5,6]. Effective management of *Pseudomonas aeruginosa* infections necessitates a comprehensive approach, including careful antibiotic use, the development of novel therapeutic agents and strict implementation and adherence to infection control practices.

Understanding resistance dynamics can help guide the optimisation of empirical treatment regimens and the use of combination therapies to counteract resistance [4-6].

Present study aimed to analyse the AST patterns of *Pseudomonas aeruginosa* isolates, with the primary objective of assessing the differences in AST patterns between adult and paediatric populations. The secondary objectives are to determine the burden of MDR isolates, evaluate the temporal trends and assess the difference in colistin susceptibility between the automated AST analyser and CBDE. The current study site is an exclusively superspeciality hospital, a referral centre for all other ESIC dispensaries and hospitals in the state, comprising a patient population that often has multiple co-morbidities and prior exposure to antibiotics. Thus, the study site offers unique insights into the antibiotic susceptibility dynamics of *Pseudomonas aeruginosa* in such settings and the differences between adult and paediatric populations.

MATERIALS AND METHODS

The current longitudinal study analysed data from three years (January 2021-January 2024) at the ESIC superspeciality government teaching hospital in Hyderabad, encompassing surgical departments (Paediatric Surgery, Cardio-Thoracic and Vascular Surgery, Neurosurgery and Urology) and medical departments (Anaesthesia, Cardiology, Neurology, and Nephrology). Ethical approval (ESICMC/SNR/IEC-F641/09-2024) has been obtained for the study.

Inclusion criteria: All samples, including urine, blood, exudates, sterile body fluids and others from patients received for bacterial culture in the microbiology lab that grew *Pseudomonas aeruginosa*, from all age groups and genders were included in the study.

Exclusion criteria: Repeat samples from the same patient, contaminants, surveillance samples and samples with growth of organisms other than *Pseudomonas aeruginosa* were excluded from the study.

Sample size: All samples meeting the inclusion criteria during the study period were included, in accordance with CLSI M-39 guidelines, which recommend testing at least 30 isolates to obtain statistically reasonable susceptibility rates [7].

Study Procedure

Samples were collected and processed upon request from clinicians. Samples showing growth were subjected to analysis by an automated identification and antibiotic susceptibility test analyser (Vitek-2) following CLSI M100 recommendations. Colistin resistance was confirmed using CBDE and compared with automated analyser results. Suitable antibiotics for treatment were evaluated based on CLSI M39 guidelines. MDR was defined as nonsusceptibility to ≥1 agent in ≥3 antimicrobial categories [8]. For making a choice for empirical therapy, antibiotics with a susceptibility of >80% (green) were considered reasonable options, while those with <60% (red) were not and those between 60-80% (yellow) may be considered in particular circumstances, such as Out-Patient Department (OPD) settings or for stable patients [7,9].

STATISTICAL ANALYSIS

Data were entered into Microsoft Excel and categories were presented in terms of percentages. Descriptive statistics were used to analyse the data. Cohen's Kappa value was employed to measure agreement between the two methods of testing colistin susceptibility (CBDE and Vitek) and was interpreted as follows [10,11]:

0-0.2 None

0.21-0.39 Minimal

0.4-0.59 Weak

0.6-0.79 Moderate

0.8-0.9 Strong

>0.9 Almost perfect

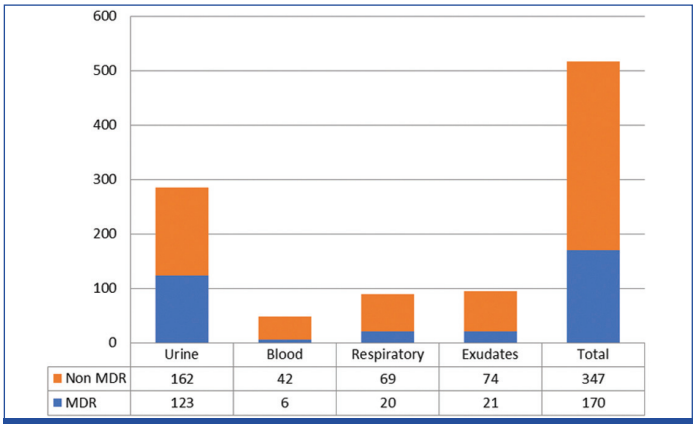
RESULTS

In the present study, among the 517 isolates of *Pseudomonas aeruginosa*, 440 (85%) were from adults and 77 (15%) were from the paediatric population. Varying susceptibility rates to different antibiotics were observed among the adult and paediatric populations, with colistin showing the highest susceptibility rates of 371 (84.3%) and 70 (90.9%), respectively, followed by amikacin at 276 (62.7%) and 38 (49.3%). Both populations exhibited susceptibility rates of less than 50% for carbapenems. Overall, paediatric isolates had lower susceptibility compared to adults [Table/Fig-1].

Antibiotics		Adult	Paediatric
BL-BLIs	Piperacillin/ tazobactam	205 (46.5)	33 (42.8)
	Cefoperazone/ Sulbatam	238 (54.0)	34 (44.1)
3rd generation cephalosporins	Ceftazidime	241 (54.7)	34 (44.1)
4th generation cephalosporins	Cefepime	236 (53.6)	35 (45.4)
Aminoglycosides	Amikacin	276 (62.7)	38 (49.3)
Fluoroquinalones	Ciprofloxacin	193 (43.8)	36 (46.7)
	Levofloxacin	206 (46.8)	35 (45.4)
Carbapenems	Imipenem	221 (50.2)	32 (41.5)
	Meropenem	219 (49.7)	34 (44.1)
Polymyxins	Colistin	371 (84.3)	70 (90.9)

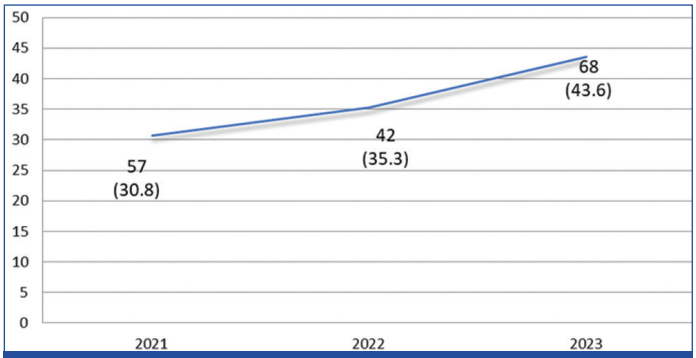
[Table/Fig-1]: Percentage susceptibility of *Pseudomonas aeruginosa* to various antibiotics among adult and paediatric populations during the study period given as n (%).

Among the isolates from urine, blood, lower respiratory and exudate samples, 123 (43.2%), 6 (12.5%), 21 (22.5%), and 20 (22.1%) were classified as MDR [Table/Fig-2].



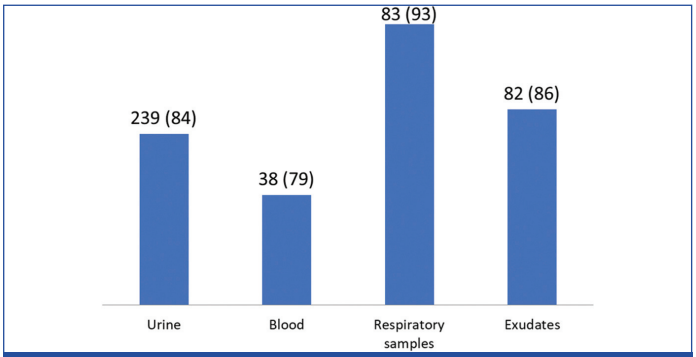
[Table/Fig-2]: MDR and Non-MDR *Pseudomonas aeruginosa* isolates across various samples given as 'n'.

The isolation of MDR *Pseudomonas aeruginosa* has shown an upward trend, reaching 68 (43.6%) in 2023, suggesting a rising burden of resistant infections, which has direct implications for treatment strategies as it further limits the antibiotic options available [Table/Fig-3].



[Table/Fig-3]: Percentage of MDR *Pseudomonas aeruginosa* from 2021-2023 given as n (%).

Colistin susceptibility rates varied across samples, ranging from 38 (79%) in blood to 83 (93%) in respiratory samples [Table/Fig-4].



[Table/Fig-4]: Colistin susceptibility among various samples from January 2021- January 2024 given as n (%).
U: Urine; B: Blood; R: Respiratory; E: Exudates

There was a discrepancy of 76 (14.7%) between the automated and CBDE methods for the detection of colistin resistance, with a Kappa value of 0.44 suggesting moderate agreement between the two methods [Table/Fig-5].

		VITEK		Total	Kappa value
		Intermediate (%)	Resistant (%)		
CBDE	Intermediate (%)	397 (76.8)	44 (8.5)	441 (85.3)	0.44
	Resistant (%)	33 (6.4)	43 (8.3)	76 (14.7)	
Total		430 (83.2)	87 (16.8)	517	

[Table/Fig-5]: Colistin AST by VITEK and CBDE from January 2021- January 2024 given as n (%).

DISCUSSION

The present study showed a higher prevalence of *Pseudomonas aeruginosa* in adults (85%) compared to paediatric patients (15%), which was similar to the findings of several studies conducted in various locations, such as Chennai (94.6% vs. 5.4%), Kolkata (67.26% vs. 32.74%), and Ethiopia (56.2% vs. 43.8%). Despite these overall trends, the prevalence rates varied between studies among paediatric and adult patients [12-14]. This highlights the necessity of localised research to identify the factors responsible for such differences and to understand geographic and environmental influences on infection patterns.

A high level of antibiotic resistance was observed in the present study, with most antibiotics showing less than 60% susceptibility in both adult and paediatric patients. However, colistin demonstrated relatively higher susceptibility in adults (84.3%) and paediatric patients (90.9%). Similar findings were observed among adults in other studies; however, several others have reported some variations in paediatric populations, with some antibiotics showing slightly higher susceptibility rates [15-18]. This emphasises the concerning scenario which necessitates the use of last resort restricted antibiotics like colistin or newer beta-lactam/beta-lactam inhibitor combinations to treat *Pseudomonas aeruginosa* infections.

Studies from Kerala and central India conducted in 2023 found higher rates of MDR *Pseudomonas aeruginosa* at 74.1% and 51.7%, respectively, in comparison to the present study. In contrast, a similar rate of 40.9% was reported by the study conducted by Patidar M and Dhingra N in Indore [19-21]. The burden of MDR *Pseudomonas aeruginosa* isolates was noted to vary across samples in studies from Indore, Kerala and Central India, with MDR isolates predominantly found in pus and other exudate samples. This contrasts with the present study's findings, where the majority of MDR *Pseudomonas aeruginosa* was isolated from urine samples [19-21], underscoring the importance of sample-specific prescription of antibiotics for *Pseudomonas aeruginosa*.

However, a rising trend of MDR *Pseudomonas aeruginosa* has been reported by studies conducted in Nepal (8.1% in 2014 to 4.8% in 2016) and Salem (64% in 2008 to 71% in 2010), revealing a worsening antibiotic resistance problem [18,22]. This could be attributed to existing issues such as over-prescription of antibiotics and weak public health policies contributing to insufficient infection control and antibiotic stewardship prevalent in resource-limited settings. In contrast, decreasing trends were noted in a study from Qatar, which could be due to effective antibiotic stewardship and robust infection control practices typically followed in such developed countries [22,23].

Colistin susceptibility in the present study was high across all sample types, with the highest in respiratory samples (93%), followed by exudates (86%), and the lowest in blood samples (79%). The study from Indore by Patidar M and Dhingra N also showed high susceptibility to colistin among respiratory samples, followed by pus samples, which aligns with the findings of the present study; however, in contrast, the lowest susceptibility was observed in urine samples [19]. Both Vitek (83.2% intermediate, 16.8% resistant) and CBDE (85.3% intermediate, 14.7% resistant) testing methods yielded high colistin susceptibility rates in the present study. These findings are similar to those reported by Rout B et al., which also found comparable susceptibility and resistance rates using the same testing methods [24]. The discrepancy between the results of these methods suggests that CBDE should be relied upon for colistin susceptibility testing of *Pseudomonas aeruginosa*.

Furthermore, the statistical analysis (Cohen's Kappa value) demonstrated a moderate agreement between both methods, indicating that VITEK may produce false-resistant or false-intermediate results. This suggests that VITEK results should be interpreted with caution, especially in critical cases such as bloodstream infections or patients in the ICU, where precise AST results are essential. Clinicians should consider confirmatory testing with CBDE. Hence, continuous tracking of resistance patterns helps healthcare facilities to better adjust treatment protocols according to the changing landscape of AMR in various clinical settings [22]. The findings of the present study therefore provide a valuable baseline for monitoring resistance trends in *Pseudomonas aeruginosa* over time and pave the way for future research.

The results of this study also shed light on the clinical implications for treatment strategies regarding *Pseudomonas* infections. The rising trend of MDR isolates necessitates looking beyond conventional antibiotics and exploring new, innovative therapeutic options. Besides the already available newer beta-lactam combinations, bacteriophage therapy, phage-derived endolysins and natural bioactive compounds are a few such innovations that could hold potential as alternative treatment options, especially for difficult-to-treat infections. Interrupting resistance mechanisms and biofilm formation could be aided by measures such as nanoparticles and quorum sensing inhibitors, while vaccines and monoclonal antibodies could be potentially used for long-term preventive and therapeutic benefits [1,3].

Monotherapy has its limitations, which can be overcome by using combination regimens that should be guided by real-time antibiograms to optimise both empirical and definitive treatment options [1,3]. Colonisation leading to subsequent infections in high-risk patients is another area of concern that may require preventive measures such as microbiome modification and decolonisation strategies [3]. Polymyxins, which have been reintroduced as last-resort options, have also faced emerging resistance, necessitating continuous surveillance and the development of safer and more effective alternatives [1-5,22].

Limitations

The current study had certain limitations, as phenotypic and molecular methods were not utilised for confirmation and detection of the type of carbapenem resistance among the *Pseudomonas aeruginosa* isolates. Moreover, the colistin susceptibility results from CBDE and the automated method were not compared with the reference standard Broth Microdilution (BMD).

CONCLUSION(S)

This study highlights a notable increase in MDR *Pseudomonas aeruginosa* over time. While higher resistance rates were observed among paediatric population in comparison to adults for most antibiotics, colistin resistance was found to be higher in adults. These findings emphasise the need for alternative or combination treatments to effectively manage MDR *Pseudomonas aeruginosa* infections. This underscores the necessity for a multi-faceted approach involving infection prevention and control practices, antimicrobial stewardship, the development of new treatment strategies, continuous surveillance, and the implementation of effective global strategies to combat the growing threat of AMR in *Pseudomonas aeruginosa*.

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