Original Article

In Vitro Activity Of Colistin And Other Agents Against Multidrug Resistant Pseudomonas Aeurginosa

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Abstract

Objective
The current research sought to evaluate the in vitro activity of Colistin and other agents against multi-drug resistant Pseudomonas aeruginosa isolates**.**

Methodology
This in vitro cross-sectional research was carried out October and December 2018 after receiving ethical approval. Pus, wound swabs, blood, tracheal aspirates, and urine samples were regularly collected and obtained from in-patient and out-patient clinics. All samples were given to the department of microbiology at Ziauddin University Hospital's North Nazimabad campus for culture and sensitivity testing. All samples were analyzed in accordance with the CLSI Guidelines 2018.

Results
Of the 276 Pseudomonas aeruginosa isolates, the majority were from pus (34.2%). MDR Pseudomonas aeruginosa were present in 152 (55%). Female isolates were more resistant than male isolates Antibiotic susceptibility tests revealed that 24% and 20% of P. aeruginosa isolates, respectively, were susceptible to ceftazidime and Imipenem. While Colistin was effective against all P. aeruginosa isolates (p- 0.001).

Conclusion
Colistin outperformed Imipenem and Ceftazidime against MDR P. Aeruginosa isolates. Colistin has thus emerged as an effective and significant substitute against MDR isolates of P. Aeruginosa, it could be used when no other options are available or the disease is severely detrimental.

Keywords: Pseudomonas aeruginosa, Colistin, Ceftazadine, Imipenem, Antibacterial resistance

INTRODUCTION

The development of antibacterial drugs represents a turning point in modern science (1). Antibacterial drugs are the most commonly used and effective agents in medicine for treating a wide variety of diseases caused by bacterial infections (2). Every day, antibiotic agents save millions of lives all over the world. However, there is a major global threat: the rise of antibacterial resistance (1). The increased use, and repeatedly misuse, of antibacterial drugs has resulted in the occurrence of bacteria that no longer respond to therapeutic interventions (3).

Pseudomonas aeruginosa has remained one of the most concerning pathogenic organisms associated with antibiotic resistance. P. aeruginosa causes the blood infections, respiratory infections, skin conditions, urinary tract infections, and surgical site infections (4, 5). The occurrence of significant innate and acquired resistance factors in this pathogen frequently leads in poor outcome measures. Pseudomonas aeruginosa has been categorized as an ESKAPE organism and is associated with a broad range of acquired infection (6).

Multi - drug resistant bacteria are those that are resistant to at least one antibiotic in three or more different categories. The rise in multi-drug resistance Pseudomonas aeruginosa (MDRPA) bacterial infection in past few years has hampered the choice of a suitable antibiotic therapy, resulting in a spike in rates of mortality and morbidity in patients with such an infection (7).

Resistance to commonly used antimicrobials, such as antipseudomonal beta-lactams or quinolones, has resulted in the adoption of alternative medicines (8, 9).

Consequently, there are limited treatment options available due to the development of these pathogenic organisms' resistance processes, primarily as a result of the indiscriminate use of antibiotics, inability to follow therapeutic regimens, variability in dosage administered, and accessibility of over-the-counter medications (10).

Recently, due to the shortage of conventional antibiotics with activity against Gram-negative bacteria, Colistin has been reevaluated to treat a variety of infections caused by MDR strains. Colistin resistance is incredibly low; however, much work remains to be done before it can be used in clinical settings (11). As a result, the purpose of this study was to assess the in vitro activity of Colistin and other agents against multi-drug resistant isolates of P. aeruginosa.

MATERIALS AND METHODS

It was a quasi-experimental study performed in a laboratory setting. The study was carried out at Microbiology lab of Ziauddin Hospital, North Campus, and Karachi. The study was approved by the ethics committee of the University. Between October and December 2018, 276 pus, ear swab, blood, sputum, tracheal aspiration and urine specimens were processed for culture and sensitivity testing in accordance with established guidelines.

Samples were cultured with normal culture media on MacConkey agar (Oxoid) and Blood agar (Oxoid). The plates were kept at 37° Celsius for 24 hours. All gram negative, catalase, and oxidase positive colonies were identified to the species level using standard microbiological procedures.

Antibiotic susceptibility was determined using Kirby Bauer's disc diffusion method. Using this method, a bacterial inoculum surface was created on a 150 mm Mueller Hinton Agar plate (Oxoid UK). Colistin, Imipenem and Ceftazidime antibiotic discs were placed on an agar plate. Before determining the results, the plates were incubated at 35°C for 16-24 hours. CLSI (2018) recommended that the zones of growth inhibition around each antibiotic disc be quantified and labelled as sensitive or resistant (12).

Determination of MIC

The MICs of the strains for both antimicrobial were determined using 96-well microtitre plates and the broth microdilution method. The bacterial inoculum was prepared in the amount of 0.5 McFarland (approximately 1.5 x 108 CFU/ml). To achieve the final inoculum (approx. 1.5 x 106 CFU/ml), the inoculum was diluted 1:100 by cation-adjusted MuellerHinton broth (CA-MHB), and 10 ul of this was added to 100 µl of CA-MHB(13, 14). The plates were incubated for 18 hours at 35±2°C. After incubation, the presence or absence of turbidity in wells was noted and interpreted using CLSI supplement 2013 MIC criteria.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 21 was used to analyse the data. For numerical variables, descriptive analyses were expressed as Mean with Standard Deviation. For categorical variables, frequencies and percentages were determined. The Chi square test was used to evaluate the relationship between drug sensitivity and resistance patterns, with a p value less than 0.05 considered significant.

RESULTS

On the basis of identification method, two hundred and seventy six strains of P. Aeruginosa were isolated from 1900 specimen. Out of which 55% were MDR P. Aeruginosa and 45% were only P. Aeruginosa. MDR P. Aeruginosa frequency was slight more in females 54% as compared to male 46%. P-value of 0.52 was statistically non-significant.

Table-I: Total samples of Pseudomonas Aeruginosa

Total Sample	**MDR**	**Non- MDR**	**p-value**
276	152 (55%)	124 (45%)	0.52
Male 133 (46%)	70 (46%)	63 (51%)	
Female 143 (54%)	82 (54%)	61 (49%)	

As shown in **Table-II**, the majority of the isolates (34.2%) were obtained from pus, followed by tracheal aspiration (20.6%), urine (18.6%), and ear swab (2.1%). The P - value was 0.024, indicating statistical significance.

Table-II: Frequency of MDR Pseudomonas Aerigonsa in specimen.

Source	**MDR** **152 (55%)**	**Non- MDR** **124 (45%)**	**p-value**
Urine	28 (18.6%)	38 (30.6%)	0.024
Blood	16 (10.5%)	13 (10.5%)	
Ear Swab	3 (2.1%)	6 (4.8%)	
Pus	52 (34.2%)	19 (15.3%)	

Tracheal asp	31 (20.6%)	26 (20.9%)	
Sputum	22 (14.4%)	22 (17.7%)	

As demonstrated in **Table-III**, the majority of MDR P.Aerigonsa strains were isolated from the outpatient department, followed by surgical ward, ICU, medicine ward and Gynecology ward.

Table-III: Percentage of MDR isolates in different Departments.

Department	**MDR** **152**	**Non- MDR** **124**	**p-value**
Gynecology ward	5 (3%)	0 (0%)	0.29
Medicine ward	18 (11.8%)	12 (10.1%)	
Surgery ward	41 (27.1%)	47 (38%)	
ICU	25 (16.5%)	27 (21.5%)	
Outpatient Department	63 (41.2%)	38 (30.4%)	

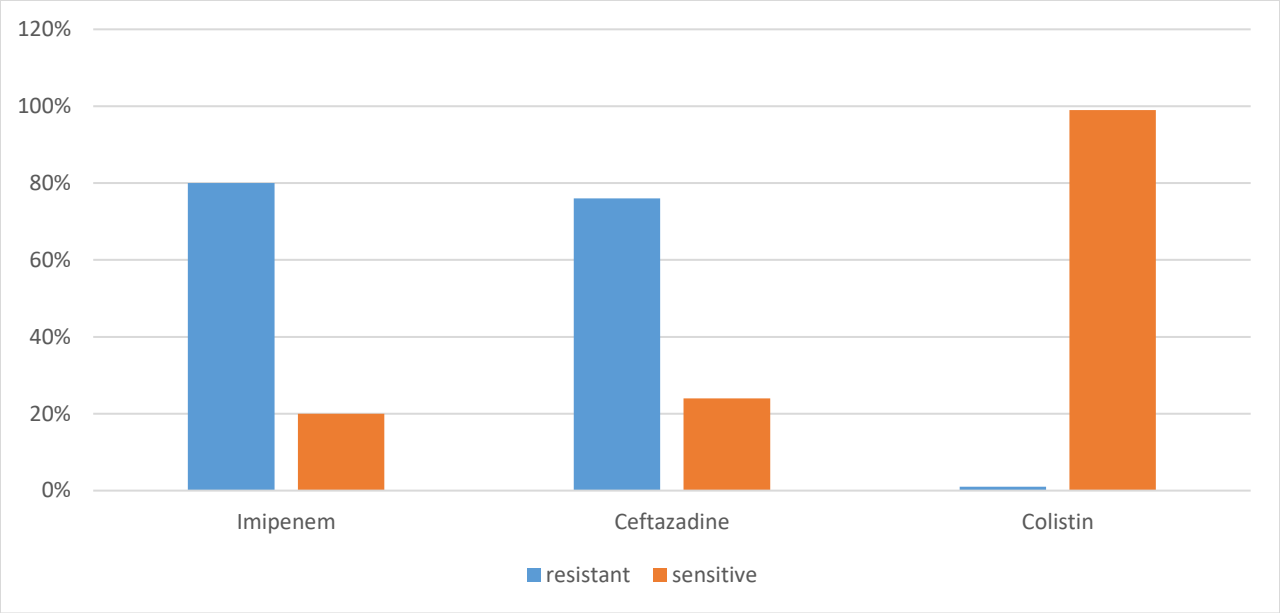

Figure 1: Antibiotic susceptibility of MDR P. Aeruginosa

Imipenem (80%) and Ceftazidine (76%) showed more resistant pattern against MDR Pseudomonas Aerigonsa than Colistin which showed only 1% resistant pattern as shown in **Figure 1**.

Table-IV: Sensitive pattern of Drugs against MDR Pseudomonas Aeruginosa

Drug	Sensitive	Resistant	p-value
Imipenem	20%	80%	0.0001
Ceftazadine	24%	76%	
Colistin	99%	1%	

The sensitive pattern of the three drugs differed significantly (p-0.0001) against MDR Pseudomonas Aeruginosa, with Colistin being the most susceptible agent. **(Table-IV)**

DISCUSSION

Multidrug-resistant P. aeruginosa is a serious global problem that affects many people. In an era of emerging resistant strains, particularly among MDR bacteria, there is renewed interest in the use of new antimicrobial options (15).

In the present study, Multi-Drug Resistant strains of P. aeruginosa were discovered to be more widespread in the female population (54%), compared to 46% in the male population. The findings are comparable to those of a study performed in Nepal, which found that MDR P. aeruginosa strains were (64%) higher in females than (31%) relatively lower in males(16).

Previously, clinical research findings evaluated the efficacy of various antimicrobial treatments in patients with MDR bacteria infectious diseases (17). The most effective antibiotics for treating Pseudomonas aeruginosa are beta lactams (Imipenem, meropenem, and doripenem). The ineffectiveness of beta-lactams is caused by the actions of beta-

lactamases, which break the amide bond of the -lactam ring, impeding infection treatment and leading to poor clinical outcomes (18).

A study by D'Souza et al. found that the majority of the Pseudomonas Aeruginosa isolates were resistant to Ceftazidime (19). Hence, Colistin might be an efficient solution, as a last resort for the treatment of multi-drug-resistant Pseudomonas aeruginosa, therefore, its antimicrobial activity should be tested on an international and domestic scale to ensure its useful application (20, 21).

In the current study, multi-drug resistant P. aeruginosa isolates obtained from inpatient and outpatient were susceptible to 2µg/ml Colistin. These findings are consistent with the findings of the CANWARD research, which found that the MIC values of 76 MDR Pseudomonas Aeruginosa toward Colistin were ≤ 2 µg/ml (22). In another research by Hsueh et al. in Taiwan found that Colistin at a concentration of 2 µg/ml suppressed 90% of isolates that were susceptible to Colistin (23).

In addition Memer et al. executed a comparable study to determine the role of Colistin in the treatment of multidrug resistant Pseudomonas and discovered that Colistin is more efficacious than beta lactam drugs (24).

In a Saudi Arabian study, the susceptibility of 33 MDR P. aeruginosa isolates to Colistin and carbapenems was determined using the E test and the CLSI breakpoint recommendation. Their findings, however, demonstrates that Colistin had significant activity against 93.9% of the strains, a result that is validated by our data, most likely because Saudi Arabia express the same activity to Pakistan in terms of living (25).

Tam et al. (2010) discovered that Colistin was effective against 95% of MDR-PA isolates with 1.5 and 2 µg/ml. One out of the 19 strains isolated in this research, 5% was Colistin resistant with 3 µg/ml (26).

The antimicrobial sensitivity screening of P. aeruginosa isolates from tracheal secretions conducted in a research by Yayan also demonstrated that Colistin was the only antibacterial agent that displayed no resistance over the years, despite the reality that the number of P. aeruginosa isolates evaluated for Colistin was quite low. Little recent evidence on the susceptibility of Gram-negative bacteria to Colistin are accessible, in part because Colistin clinical isolates remains difficult and in part because Colistin use is not widely spread.

CONCLUSION

In conclusion, Colistin demonstrated encouraging in vitro experiments against MDR strains in our geographical area. These findings point to Colistin-based treatment for patients with MDR infections. However, further research on a larger collection of drug-resistant strains is required to back up these findings in the near future.

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Informed consent: Informed consent was obtained

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