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Antimicrobial Susceptibility Patterns of Clinical Isolates of *Pseudomonas aeruginosa* Isolates from Patients of Respiratory Tract Infections in a Tertiary Care Hospital, Karachi

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Abstract

Objectives: The current study was aimed to investigate pathogen burden and antimicrobial susceptibility of conventional use antibiotics against MDR *P. aeruginosa.*

Design: *In vitro* Preclinical study.

Patients and Methods: Sputum samples were collected by using non-probability sampling techniques from in-door and out-door patients; a total of 560 sputum samples were collected. Standard and specific microbiological methods were used to identify the clinical isolates. Antibiotic susceptibility pattern was determined by using the Kirby Bauer Disc-diffusion method. Clinical and Laboratory Standards Institute (CLSI) guidelines 2019 were used to process the samples.

Results: About 155 samples were positive for *P. aeruginosa*, out of which 95 were positive for MDR *P. aeruginosa*. Analysis of demographic data showed 39 (n=41%) female and 56 (n=59%) male patients. Majority cases were obtained from 41-60 years of age group, with p- value<0.02. Antibiogram showed resistance pattern of MDR against empirical and conventionally used antibiotics. The highest sensitivity was recorded against Ceftolozane/Tazobactamthat is 94% and the highest resistance recorded against Amikacin 48%, followed by Ceftazidime 34%.

Conclusion: Ceftolozane/Tazobactam could be an alternative treatment option for MDR *P. aeruginosa* infections.

Keywords: *P. aeruginosa* infections; Antibiogram; Antimicrobial susceptibility; Ceftolozane/Tazobactam; Nosocomial infections

Introduction

Antimicrobials are the salutary tool for treating a list of bacterial infections. Millions of lives have been saved because of antibiotic usage that is important progress in the field of medical sciences. Resistance development against antibiotics or other anti-infective agents is an important threat globally [1,2] Therapeutic choices are limited due to persistent antimicrobial resistance presents significant morbidity and mortality rates in human. According to the World Health Organization (WHO), antibiotic resistance has now become a major clinical concern and global public health problem in this era [3,4]. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a versatile bacterium causing opportunistic and nosocomial infections. Center for Disease Control and Prevention (CDC) reported that infections caused by *P. aeruginosa* are often hospital-acquired and associated with patients with immunological disorders such as cystic fibrosis and severe burns [5,6].

Multidrug Resistance (MDR) antibiotics by definition can be stated as "resistance to at least three or more than three antibiotics groups" [5]. Bacteria acquire this resistance through the production of efflux pumps, the formation of biofilm and production of Extended Beta-Lactamase Enzyme (ESBL), AmpC Beta Lactamases (AmpC), and mutational change in protein OprD, all these mechanisms are proposed for the development of resistance in *P. aeruginosa* against antimicrobials. Furthermore, in developing countries, the irrational prescription of antimicrobials without performing diagnostic susceptibility tests by physicians has increased the production

of resistant strains. The threat of growing resistance by MDR strains is increasing morbidity or mortality rates and imposes grave concern to human health care costs [7,8]. Over the last few years' particular attention has been given in the development of new agents that target gram-positive organisms while persistent lacunae are lagging in the development of antimicrobials to cure the infections from gram-negative organisms. Decreasing the efficacy of antimicrobial agents due to the increase in resistance, treating *P. aeruginosa* is a hallmark for clinicians [9]. Therefore, the current study aimed to evaluate the susceptibility of 5th generation antibiotic (Ceftolozane/Tazobactam) in treating respiratory tract infections caused by *P. aeruginosa*. To the best of our knowledge, no data is available in Pakistan regarding this as the antibiogram of the drugs varies from place to place.

Methods

An *in-vitro* preclinical study was conducted in the hospital of Karachi; samples were recruited by using consecutive sampling techniques from January to September 2019. The study got approval from the ethical review committee of the associated center. About 560 samples of sputum were collected from the patients with respiratory tract infections who either visited the out-patient department or admitted to the hospital ward. There was no discrimination in the selection of gender and age of the patients. Samples showing double growth or contamination on agar plates were excluded from the study. Those patients who were reported a positive growth of *Pseudomonas aeruginosa* on culture test included in the study. The minimum sample size was ninety-five (n=95), calculated via sealed envelope software. Labeled sterile containers were used for the collection of samples of sputum. MacConkey's agar was used for inoculated and samples were incubated at 37°C for 24-48 hours. For detecting the organisms multiple biochemical tests including colony morphology Gram staining, positive oxidase reaction, production of pyocyanin on Mueller-Hinton agar (Oxoid UK), citrate utilization and growth at 42°C, were used by following standard protocol

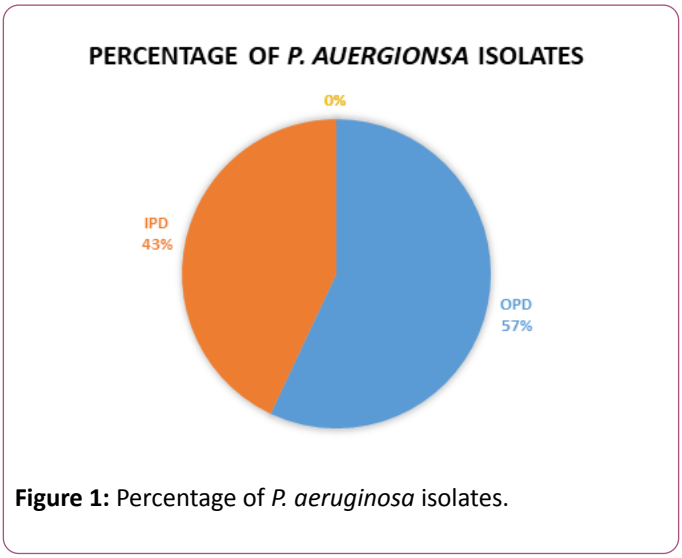

Figure 1: Percentage of *P. aeruginosa* isolates.

Incubation conditions for plates were 16-24 h at 35°C before analyzation of results; " commercially prepared fixed concentration paper antibiotic discs" were placed on an agar plate. Growth inhibition zones around each of the antibiotic discs were demarcated in accordance with CLSI guidelines (2018) and labeling was performed as sensitive or resistant. Antibiotic discs i.e. Imipenem (10 mcg), Amikacin (30 mcg), Ceftazidime (30 mcg), Ciprofloxacin (5 mcg), Ceftolozane/Tazobactam (30/10 mcg), Azithromycin (30 mcg), Levofloxacin (10 mcg) were taken for identification of susceptibility pattern (**Figure 1**).

Data were analyzed by using SPSS version 21. Numerical data were presented in mean with standard deviation. Associations between sensitivity and resistance patterns of drugs were analyzed by using the chi-square test while p value ≤ 0.05 was considered as significant. Antibiotics susceptibility pattern of MDR *P. aeruginosa* with different drugs was presented in the form of bar-charts while pie-charts were used to illustrate the departmental Percentage of MDR *P. aeruginosa* positive isolates (**Figures 2 and 3**).

Results and Discussion

Out of 560 sputum cultures, 155 (27%) showed positive growth of *P. aeruginosa* and 95 (61%) were MDR positive. Out of 95 samples, 56 (59%) specimens were males and 39 (41%) were females. The mean age was 42.29 ± 21.72, age range starting from 1 month up to 80 years, with most of the isolates recovered from 41-60 years of age i.e. 47% (**Table 1**).

Table 1: Demographics individualities of patients.

Age	**MDR** *P. aeruginosa*	*P. aeruginosa*	**p-value**
1 month-20 years	8 (8.4%)	18 (11.6%)	
21-40 years	26 (27%)	41 (26%)	**<0.02**
41-60 years	45 (47%)	74 (47%)	
61-80 years	16 (16%)	22 (14%)	
Gender distribution	**MDR** *P. aeruginosa*	*P. aeruginosa*	
Females	39 (41%)	58 (37%)	**<0.03**
Male	56 (59%)	97 (62.5%)	
Total	N 95	N 155	

Antimicrobial susceptibly showed the highest sensitivity toward Ceftolozane/Tazobactam 94% followed by Azithromycin 89%, Imipenem 86%, Levofloxacin 78%, and Ciprofloxacin 76%. The highest resistance recorded against Amikacin 48% followed by Ceftazidime 34%.

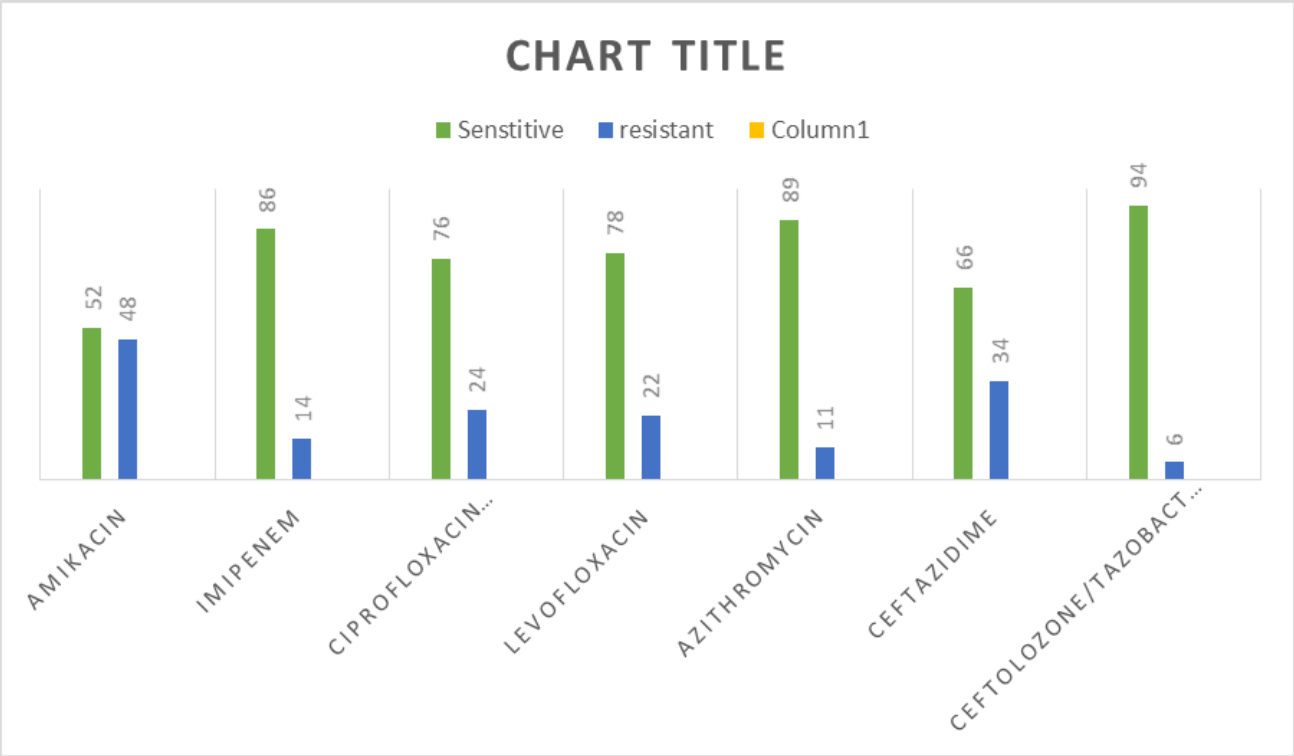

Figure 2: Susceptibility pattern of antibiotics.

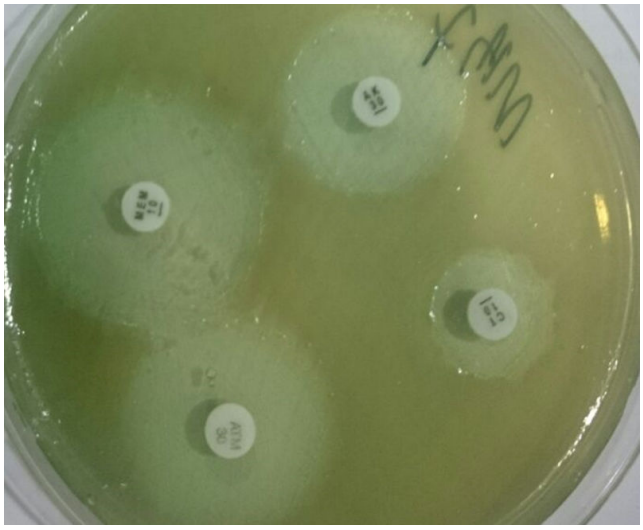

Figure 3: Zone diameters of the antibiotic disc by disc diffusion method.

Discussion

P. aeruginosa notorious infections have been marked with the highest priority for surveillance on the basis of parameters such as incidence, prevalence, mortality and morbidity rates, and chronicity of the disease. Available regimens for inhibition and treating the life-threatening infections due to clinical isolates of *P. aeruginosa* have demonstrated resistance to multiple antibiotics that have led to a few therapeutic antibacterial drugs as an option for treatment [10,11]. Due to its peculiar structure and the large genome, it is included in the sixth ESKAPE organism that is a major reason behind nosocomial infections in the US and therapeutic threats around the globe because of their ability to become resistant to almost all available antibiotics [12,13]. The frequency of *P. aeruginosa* was 23% of total sputum cultures in our study. Studies from different regions of Pakistan have shown a higher frequency of *P. aeruginosa* from sputum isolates [14-16]. Similar alarming results are also reported in middle east countries [17,18]. A high proportion of males were seen in our study and results were parallel with other published studies [19,20]. Antibiogram of antibiotics showed the best drug against *P. aeruginosa* Isceftolozane/Tazobactam which is (94%) sensitive to MDR isolates. A similar set of data in the United States showed the highest MIC (Minimal Inhibitory Concentration) and sensitivity of Ceftolozane/Tazobactam for *P. aeruginosa* isolates [21]. Ceftolozone/Tazobactam showed potent action against gram-negative organisms such as *P. aeruginosa* including its resistant strains and ESBL (Extended Spectrum Beta-Lactamase) producing *enterobacteriaceae*, *E. coli* and *K. pneumonia* [22]. Carbapenems considered being last resort therapy against gram-negative microbes. High sensitivity recorded against Imipenem 86% as compared to studies done in Pakistan and India [23,24]. This might be because of the selective usage of carbapenems in the current setting due to the narrow therapeutic index. Fluoroquinolones are widely used drugs for treating respiratory tract infections but because of irrational use of these drugs resistance has been observed in our study and also similar observations are

available around the globe [25]. The current study revealed relatively high resistance of Amikacin i.e. 48. This high resistance rate of Amikacin was also reported from other studies [26,27]. The findings of the current study are parallel with the findings of Shortridge et al. who also have suggested Ceftolozane/Tazobactam is a potent drug in terms of pseudomonal infections [28]. Conventional agents and empirical therapy for treating the respiratory tract infection is becoming hallmark because of the increase in resistance rate [29,30].

Conclusion

Ceftolozane/Tazobactam is an effective drug of choice in treating respiratory tract infections, especially when it is started soon after the occurrence of infection. Antimicrobial stewardship and multidrug-resistant surveillance programs are needed to prevent treatment failure and to reduce antibiotic selection pressure.

Limitation and Future Recommendations

Samples were recruited from one center of Karachi. We strongly recommend conducting a study on a larger scale in order to explain the exact resistance pattern in the Pakistani population. There is also an increasing need for awareness programs to control the irrational usage of antibiotics.

Conflict of Interest

None

Funding

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