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Original Article

Prevalence of Multi Drug Resistant *Pseudomonas Aeruginosa*

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[SF] Conception and design, Collection and assembly of data, [FA] Analysis and interpretation of the data, Statistical expertise, [MM]Final approval and guarantor of the article

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A B S T R A C T

Background: The increasing prevalence of multiple drug-resistant (MDR) bacterial infections is a serious concern for global health. *Pseudomonas aeruginosa*, a common pathogen, has been linked to various nosocomial infections associated.

Objective: The current study is designed to find out the prevalence of multidrug resistant *Pseudomonas aeruginosa*.

Methodology: Clinical isolates of P. aeruginosa obtained from 3 different hospitals in Islamabad as well as Rawalpindi, Pakistan over 2016 and 2017. In this study, various secretions, including ascetic fluid, aspiration fluid, bronchial secretion, bronchial washing, and abscess, were collected from patients belonging to different age groups. Samples were processed to isolate bacterial colonies that were identified through biochemical characterization. Isolated strains of *Pseudomonas aeruginosa* were evaluated through antibiotic susceptibility testing by Kirby-Bauer disc diffusion method.

Results: A total number of 52 samples were processed, followed by the isolation and identification of *Pseudomonas aeruginosa*. The maximum number of samples were collected from patients belonging to 61-70 years old age group, and female patients (55.76%) were higher in frequency than male patients (44.23%). The majority of *Pseudomonas aeruginosa* isolates displayed resistance against Amikacin, Tobramycin, and Piperacillin.

Conclusion: The prevalence of MDR *P. aeruginosa* is a serious healthcare problem. Available antibiotics are not useful against them. Therefore, there is an urgent need of new and improved drugs with minimized cost and toxic effects that can maximize the healthcare benefits.

Keywords: P. aeruginosa- MDR and ESBLs -Typing of P. aeruginosa. evaluators.

Introduction

The increasing prevalence of multiple drug-resistant (MDR) bacterial infections is a serious concern for global health.[1] Excessive use and misuse of antibiotics by human beings along with bacterial natural mechanisms to overcome drug resistance are the major causes for the emergence of bacterial drug resistance.[2] The species of Pseudomonas were the 2nd most frequent bacteria (14%) recovered in duration of six-month statewide clinical surveillance investigation in Ghana.[3] Pseudomonas aeruginosa, a common pathogen, has been linked to various nosocomial infections associated. It also causes pseudomonasis that can adversely affect poultry animals such as chickens, geese, ducks, as well as ostriches, while the infection present in eggs destroys embryos [4]. P. aeruginosa is innately resistant to several antimicrobial drugs, posing a significant challenge to doctors throughout therapy. Different MDR strains have been discovered in a variety of environmental niches across several nations.[5]

Resistance to multiple antibiotics, as well as cross-resistance across drugs, may lead to MDR P. aeruginosa. Several recent findings have attracted attention to the development and transmission of novel sequence types (STs) as well as clones of MDR P. aeruginosa, which pose a significant pandemic risk in hospitals across the world.[6] This problem was discovered largely in cystic fibrosis patients, where prolonged illness with P. aeruginosa contributes to the formation of resistance to numerous antibiotics. These MDR P. aeruginosa strains can be passed from patient to patient, resulting in epidemics among patients with cystic fibrosis who attend the same hospital.[7] P. aeruginosa (MDR) found

uncommon in patient of cystic fibrosis. Four-year research in a Boston hospital identified 22 incidences of MDR P. aeruginosa (a frequency of 5.5 cases/10,000 patients admitted per year) linked to resistance development during therapy.[8]

Resistance against Carbapenem in P. aeruginosa is developing internationally, however surveillance to determine the causes of such resistance is poor in the developing countries. A study conducted used whole-genome sequencing to identify the genotypic causes of b-lactam resistance in 142 P. aeruginosa clinical isolates obtained from 3 hospitals in Islamabad as well as Rawalpindi, Pakistan over 2016 and 2017. Antimicrobial susceptibility testing was performed on isolates using Kirby-Bauer disc diffusion, as well as their genomes were accumulated using Illumina technique. Resistance to b-lactams was substantial, with 46% of isolates tolerant to resistant to cefepime, 48% resistant to ceftolozane-tazobactam, piperacillin-tazobactam, 42% and 65% reduced susceptibility against carbapenem.[9]

Aeruginosa was found in just 9.6 percent of clinical, environmental, as well as poultry waste samples from Ghana's Ashanti Region. However, there has been a significant increase in the frequency of P. aeruginosa MDR strains in environmental and clinical samples.[4] The estimated incidence in common clinical samples of P. aeruginosa (MDR) from varies greatly across the MENA area, with Egypt having the greatest prevalence (75.6%) and Morocco having the lowest (0%), with 7.3% in Saudi Arabia and 8.1% from Qatar having small prevalence. The Levant nations (Palestine, Iraq, Jordan and Israel) were equally varied, with Jordan (52.5%), Lebanon (64.5%) Israel (30%), and Palestine (47.6%) exhibiting higher levels of opposition than Iraq (12.4%). This might be due to a regional practice of unrestricted antibiotic prescription or no uniform standards. It is important to note that it is critical to evaluate presented data with caution in order to prevent biased selection since it may represent various study techniques or source regions.[10]

Methodology

Clinical isolates of P. aeruginosa obtained from 3 different hospitals of Islamabad as well as Rawalpindi, Pakistan over 2016-2017. Samples were aseptically collected from 52 patients of different age groups. The nature of the sample varied for different patients. Samples included different secretions like ascetic fluid, aspiration fluid, bronchial secretion, bronchial washing, and abscess. An appropriate number of samples were collected from adults and infants into sterile bottles covered with cap that were properly labeled.

Samples were processed immediately within one hour of collection. A small amount of secretion was taken and cultured on specific isolation media. Agar plates were prepared aseptically to isolate clinical pathogens. Samples were aseptically inoculated on blood agar plates, and MacConkey agar plates. After overnight incubation, the isolated colonies were obtained, which were then further processed.

Identification of isolated bacterial colonies was based on Gram staining, colony morphology, and different biochemical tests including, Catalase, Oxidase, Sulfide, indole, citrate, urea, TSI reaction and lactose by following the protocols.[11]

The antibiotic susceptibility of different bacterial isolates was evaluated using disc diffusion method. A range of different antibiotic discs was used including Amikacin, Ciprofloxacin, Tobramycin, Levofloxacin, and many others. Aseptically plates of Muller Hinton Agar were prepared and applied antibiotics and bacterial cultures. After an overnight incubation, plates were examined for the appearance of zones of inhibition.[12]

Results

A total of 52 specimens, both from male and female patients of different age groups, were collected, including ascetic fluid, aspiration fluid, bronchial secretion, bronchial washing, and abscess. As given in Table I, the frequency of female patients was higher than male patients.

Table I: Frequency of male and female patients.

Patient Gender	Number	Percentage
Male	23	44.23 %
Female	29	55.76 %
Total	52	100 %

A different number of samples were obtained from different age groups, while the maximum number of samples were collected from patients belonging to 61-70 years old age group with a frequency of 23.07% as presented in Figure 1.

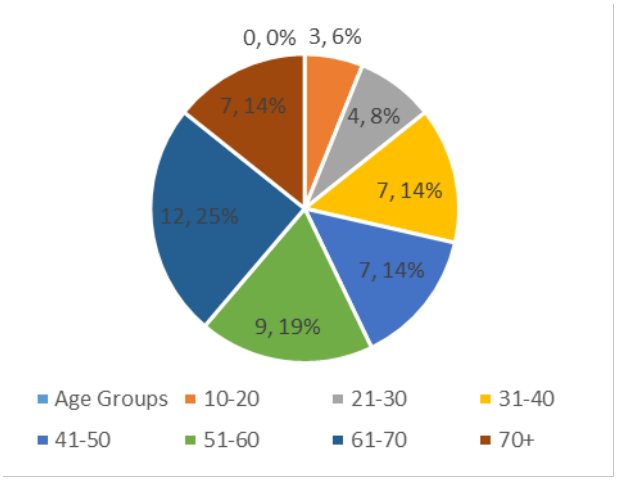

Figure 1. Frequency of patients from different age groups.

The frequency of different secretions collected also varied widely, with bronchial washings being the most frequently collected specimen (53.84%) while bronchial fluid was the least frequent one (3.84%), indicated by data presented in Table II.

Table II: Sources of origin of different bacterial isolates.

Specimens	Number of Patients	Percentage
Abscess	5	9.61 %
Ascitic fluid	11	21.15 %
Aspiration fluid	6	11.53 %
Bronchial fluid	2	3.84 %
Bronchial washing	28	53.84 %
Total	52	100 %

Aeruginosa was isolated from all of the collected specimens. The presence of P. aeruginosa was confirmed through different biochemical tests. The results of biochemical testing confirmed the presence of P. aeruginosa by negative lactose as well as sulphide test, whereas positive result was shown by all the other biochemical tests performed.

The disc diffusion method was utilized to evaluate the antibiotic senitivity pattern of bacterial isolates. The results demonstrated that both sensitive and resistant patterns were shown by strains in response to the antibiotics as given in Figure 2. Among total isolated P. aeruginosa, 20.43% displayed multi drug resistance, while 12.25% were sensitive to the tested antibiotics.

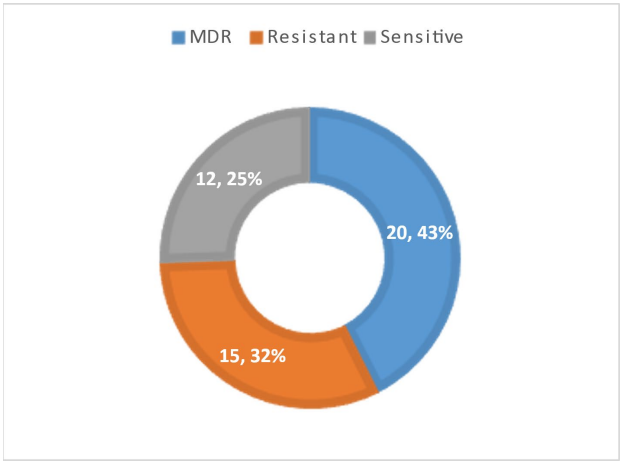

Figure 2. Drug Resistance Pattern among clinical isolates

The detailed antibiotic susceptibility pattern of P. aeruginosa isolates was determined as given in Figure 3.

Discussion

Diseases caused by MDR P. aeruginosa are increasing throughout the world, and they pose a major health concern that also increases the economic burden on the health system.[13] The present study revealed the antibiotics susceptibility pattern of different strains of P. aeruginosa isolated from different secretions. In this study, a total of 52 patients, including both males and female from different age groups were selected to collect different secretion specimens for the isolation of Pseudomonas.

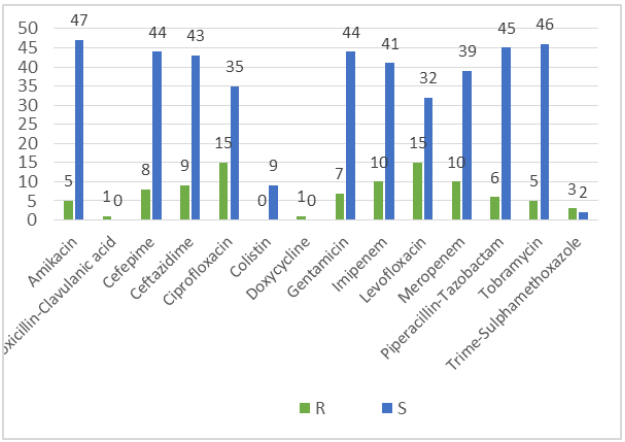

Figure 3. Antibiotic Susceptibility Pattern of P. aeruginosa against Different Antibiotics

The identification of the isolates was done on the basis of different biochemical tests by following the identification scheme. The results confirmed the presence of Pseudomonas. Different biochemical tests were run to properly identify the isolated strains. Among all the biochemical test sulphide test and lactose test showed negative results an all the samples,

while rest of the tests including catalase, oxidase, indole, citrate, urea, and TSI showed positive results. These biochemical tests, along with gram staining and colony morphology, confirmed the presence of P. aeruginosa.[14]

Antibiotic susceptibility was also checked to evaluate the antibiotic resistance in bacterial strains. The results concluded that 47 samples out of 52 were sensitive to amikacin while only 5 were resistant. Only one sample was resistant to Amoxicilin-Clavulanic acid. 8 strains were resistant to Cefepime, 9 were resistant to Ceftazidime, 15 were resistant to Ciprofloxacin and Levofloxacin, 1 was resistant to Doxycycline, 7 were resistant to Gentamicin, 10 were resistant to Imipenem and Meropenem, 6 had reduced ueptibility to Piperacillin-Tazobactam, 5 were resistant to Tobramycin, and 3 were resistant to Trime-Sulphamethoxazole. Most strains showed resistance against Ciprofloxacin and levofloxacin as shown in Figure 3. In one of the studies, 90% of Pseudomonas aeruginosa isolates were reported as resistant to Cotrimoxazole and 81.8% showed resistance against Tetracycline.[15] However, in another study, Pseudomonas displayed 98.2% resistance against amoxicillin.[16]

Conclusion

The prevalence of MDR *P. aeruginosa* is a serious healthcare problem. Available antibiotics are not useful against them therefore there is an urgent need of new and improved drugs with minimized cost and toxic effects that can maximize the healthcare benefits. The improper use of antibiotics should be stopped so that the spread of MDR bacterial strains can be minimized.

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