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Original Article

Antibiotic Sensitivity Pattern of Carbapenem-Resistant Pseudomonas Aeruginosa

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ABSTRACT

Objective: To determine the sensitivity of Carbapenem-resistant *Pseudomonas aeruginosa* (CARP) with the panel of antibiotics according to Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines.

Study Design: Cross-sectional study.

Duration and Place of Study: Armed Forces Institute of Pathology, Rawalpindi from Pakistan, Apr to Sep 2020.

Methodology: All samples received at the Microbiology Department during the study period were included. Various samples such as blood, pus, sputum, Endobronchial washing (EBW), non-directed bronchial lavage (NBL), tissue and urine were processed in the laboratory. Samples were inoculated on appropriate culture media, and bacteria were identified according to their colony morphology, Gram staining characteristics and biochemical tests. Antibiotic susceptibility testing was performed using the Kirby Bauer Disk Diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines. However, for susceptibility to Colistin, the agar dilution method was performed.

Results: In our study, fifty-five Carbapenem-resistant *Pseudomonas aeruginosa* were isolated. Out of these fifty-five isolates, thirteen (23.6%) were sensitive to Tazocin, seven (12.7%) were sensitive to Ceftazidime, fourteen (25.4%) were sensitive to Gentamicin, 7(12.7%) were sensitive to Cefepime, (12.7%) to Aztreonam, 10(18.2%) were sensitive to Amikacin, 7(12.7%) to Ciprofloxacin, 6(11%) to Levofloxacin and forty-two (76.4%) were sensitive to Colistin.

Conclusion: The susceptibility of Carbapenem-resistant *Pseudomonas aeruginosa* to all the antibiotics tested in this study was low. Isolates displayed the highest susceptibility to Colistin.

Keywords: Antibiotic susceptibility profile, Carbapenems, Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), Disk diffusion technique.

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INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen causing infections associated with high morbidity and mortality. It implicates various infections such as wound infections, soft tissue infections, respiratory tract infections, urinary tract infections, endocarditis, pneumonia, eye infections, ear infections, bacteremia and sepsis, especially in immunocompromised and burn patients.[1,2] Carbapenems (Imipenem, Meropenem, Doripenem) are very effective in treating infections caused by *Pseudomonas aeruginosa*.[3] There are already fewer treatment options available for treating infections caused by *Pseudomonas aeruginosa* due to its intrinsic resistance to various antimicrobials. A high percentage of hospital-acquired infections are caused by multidrug-resistant *Pseudomonas aeruginosa*.[4] Carbapenems are used as the drug of choice against many multidrug-resistant bacterial pathogens, but now infections caused by Carbapenem-resistant pathogens are rising.[5-7] In Pakistan, the first case of Carbapenem-resistant *Pseudomonas aeruginosa* was reported in December 2003.[8] However, Carbapenemase-producing *Pseudomonas aeruginosa* was first reported in the United States in 2001.9 Infections caused by Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) are major threats to public health as they are difficult to treat. As a result, patients show poor prognosis. Different studies suggest that the risk of bacteremia caused by Carbapenem-resistant *Pseudomonas aeruginosa* is increased due to various risk factors such as a nasogastric tube, mechanical ventilation and prior use of antibiotics.[10]

The objective of this study was to determine the complete antibiotic susceptibility profile and limited therapeutic options available to treat infections caused by Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA).

METHODOLOGY

The cross-sectional was conducted from April to September 2020, at the Microbiology Department, Armed Forces Institute of Pathology (AFIP) Rawalpindi Pakistan The samples were received from Combined Military Hospital, Pak Emirates Military Hospital, Government, and Private Sector Hospitals of Rawalpindi and Islamabad seeking microbiological services

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from our lab. Non-probability consecutive sampling technique was used to collect the samples. Ethical approval was taken from Institutional Review Board (MP-MIC19-3/READ-IRB/21/118).

Inclusion Criteria: All the samples that yielded growth of Gram-negative bacteria during the study period at the Department of Microbiology were included in the study.

Exclusion Criteria: Repeat samples from the same patient were excluded. Samples that yielded growth of Gram-positive bacteria were also excluded.

Various samples such as blood, pus, sputum, non-directed bronchial lavage (NBL), Endobronchial washings (EBW), tissue, fluid and urine were processed in the laboratory. These samples were inoculated on appropriate culture media. Before inoculation, media was examined for expiration date and contamination. Media were inoculated in the order of least selective media first to selective media. Blood Agar, Chocolate Agar and MacConkey Agar were used for routine aerobic culture. Blood agar and Cystine Lactose Electrolyte Deficient (CLED) agar were used for urine cultures. The culture plates were incubated for 18-24 hours at 35°C±2°C.

0in ambient air. The bacteria were initially identified according to their growth characteristics on culture med=ia plates, such as colonial morphology, odour and pigment production etc. Then, a Gram staining procedure was performed from the colony grown on the culture plates to identify Gram-negative bacilli. Other rapid identification procedures and biochemical tests like motility, catalase, and oxidase tests were performed.[11] Motile, Gram-negative bacilli that were oxidase positive were presumptively identified as *Pseudomonas aeruginosa* and considered as the 'Test isolates'. To confirm the identification of the test isolates up to the genus and species level, definitive biochemical testing was done using API 10S and API 20NE (Biomerieux). The test isolates were further dealt with by performing antibiotic susceptibility testing by the Kirby-Bauer Disk Diffusion technique on Mueller Hinton Agar according to the CLSI 2020 guidelines.[12] Initially, a colony suspension equivalent to 0.5 McFarland was made and inoculated on Mueller Hinton Agar. The same colony suspension was also used to subculture the organism on BAP and MAC to achieve and validate the pure growth of the organism and to ensure contamination-free growth on the MHA. Antibiotic disks were applied at a distance of at least 24 mm from centre to centre so that a 90 mm plate accommodated six disks. Following antibiotics were tested; Piperacillin-Tazobactam (100/10µg), Ceftazi dime (30µg), Gentamicin (10µg), Cefepime (30µg), Aztreonam (30µg), Imipenem (10µg), Meropenem (10µg), Amikacin (30µg), ciprofloxacin (5µg), Levofloxacin (5µg). Colistin susceptibility testing for *Pseudomonas aeruginosa* was performed according to the agar dilution method. A total of 2 Petri plates of 90mm, each having 20 ml of MHA with a final Colistin concentration of 2µg/ml and four µg/ml, was prepared to test at least ten isolates at a time. A colony suspension equivalent to 0.5 McFarland was made. This colony suspension was then diluted 1:10 in saline and inoculated using a 10µL loop onto a Colistin agar plate. Similarly, using the original bacterial suspension, subculture on BAP was done as a purity check. Lastly, the Colistin agar plate and the purity plate were incubated at 33-35oC in ambient air for 16-20 hours. After incubation, Colistin plates were carefully observed under transmitted light. Even one colony was considered to grow. For Quality Control *Pseudomonas aeruginosa* ATCC 27853 was used. The plates and the API were then incubated at 35 C ± 2 C in ambient air for 16-18 hours. After the completion of the incubation period, API web was used to confirm the identification of *Pseudomonas aeruginosa* isolates according to the biochemical reactions shown by the isolates on the API 10s and API 20s.

Isolates showing resistance or intermediate susceptibility to either or both the Carbapenems were considered Carbapenem-resistant *Pseudomonas aeruginosa*, and the susceptibility pattern of the rest of the antibiotics in the panel was also noted. The petri dish was held a few inches against a black background illuminated with reflected light. The diameter of the zone of complete inhibition was noted with the unaided eye in mm.

Statistical Package for Social Sciences (SPSS) version 26.0 was used for the data analysis. Quantitative variables were summarized as Mean±SD and qualitative variables were summarized as frequency and percentages.

RESULTS

Samples from fifty-five patients which yielded growth of Carbapenem-resistant *Pseudomonas aeruginosa* were included. Out of fifty-five patients, 43 (78.1%) were males and 12(21.9%) were females. Mostly the samples were received from patients admitted to either ICUs or various other wards fifty-one (92.7%), whereas only four (7.2%) patients were from the Outpatient Department.

Samples that yielded Carbapenem-resistant *Pseudomonas aeruginosa* were blood (5,9%), fluid (1,1.8%), Non-directed Bronchial Lavage (7,12.7%), Endobronchial washings (1,1.8%), pus (18,32.7%) and sputum (1, 1.8%) (Figure-1).

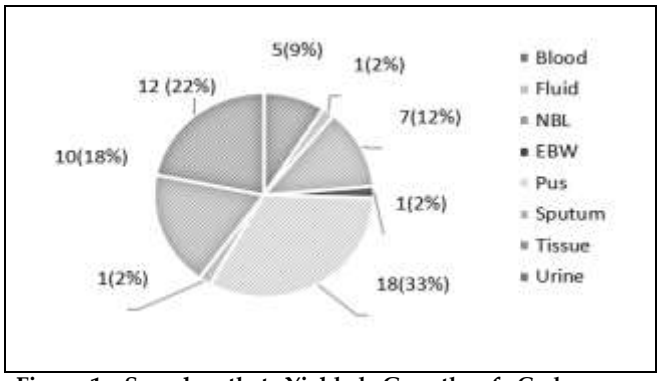

Figure-1: Samples that Yielded Growth of Carbapenem-Resistant Pseudomonas aeruginosa (n=55)

Out of fifty-five Carbapenem-resistant *Pseudomonas aeruginosa,*thirteen (23.6%) were sensitive to piperacillin-tazobactam, seven (12.7%) were sensitive to Ceftazidime, fourteen (25.4%) were sensitive to Gentamicin, seven (12.7%) were sensitive to Cefepime, seven (12.7%) to Aztreonam, ten (18.2%) were sensitive to Amikacin, seven (12.7%) to Ciprofloxacin, six (11%) to Levofloxacin and forty-two (76.4%) were sensitive to Colistin (Figure-2).

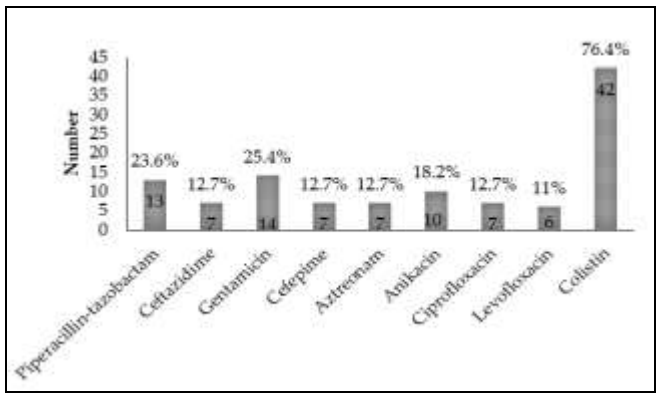

Figure-2: Comprehensive Antimicrobial susceptibility Pattern of Carbapenem Resistant Pseudomonas aeruginosa (n=55)

Out of 55 *Pseudomonas aeruginosa* isolates, 100% were resistant to Imipenem. The isolates resistant to the tested Carbapenems (Imipenem and Meropenem) were 52(94.5%). In the same number of isolates,52 (94.5%) were also resistant to Meropenem. Only 3(5.5%) isolates were sensitive to Meropenem but resistant to Imipenem (Figure-3).

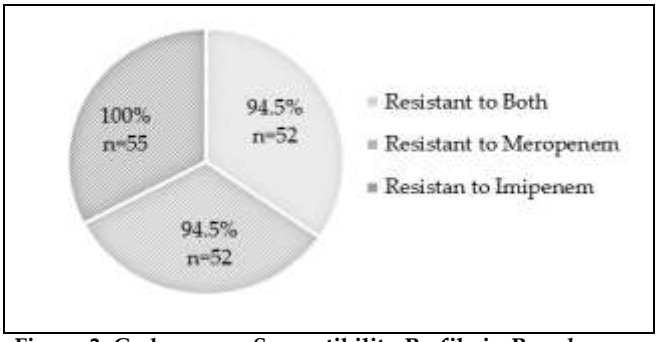

Figure-3: Carbapenem Susceptibility Profile in Pseudomonas Aeruginosa (n=55)

Few isolates (1,1.8%) were pan-drug resistant. Multidrug resistance was observed in 13(23.7%) isolates. Then, there were 29(52.8%) extensively drug-resistant isolates. Among XDR isolates, susceptibility to two classes of antibiotics (Amikacin and Colistin) was shown by 1(1.8%) isolates. Similarly, another 1 (1.8%) showed susceptibility to Gentamicin and Colistin. Isolates showing sensitivity to piperacillintazobactam and Colistin were 2(3.7%). Isolates that showed sensitivity to quinolones and Colistin were 1(1.8%).

DISCUSSION

According to European Center for Disease Control (ECDC) and the Center for Disease Control and Prevention (CDC), an MDR isolate shows acquired non-susceptibility to at least one agent in three or more antimicrobial categories, and XDR isolate remains susceptible to only one or two categories. A PDR pathogen is non-susceptible to all agents from all antimicrobial categories.[13,14]

In our study, the antibiotic of choice for treating Carbapenem-resistant *Pseudomonas aeruginosa* remains uncertain, as isolates showed maximum susceptibility towards Colistin which was 76%. Colistin should be considered an antibiotic of last resort for treating multidrug-resistant infections as it has limited clinical efficacy and should be co-administered with another active antimicrobial. It is preferable to use other antimicrobials, but susceptibility to all other antibiotics effective against *Pseudomonas aeruginosa* isolates was not more than 30%. In our study, sensitivity to Amikacin, piperacillin-tazobactam, Aztreonam, Ciprofloxacin, Cefepime, Ceftazidime and Colistin was low as compared to the study conducted at Sir Aurobindo Medical College Indore by Sharan *et al.,* where the sensitivity for Colistin was reported to be 100%, and sensitivity of isolates to Amikacin was found to be 80%.[15] The study conducted at the University of Pittsburgh medical centre by Buehrle *et al.* showed that

the pathogens showed 68% susceptibility to Cefepime and 57% to piperacillin-tazobactam.[16] The study conducted at a Brazilian tertiary hospital by Dias *et al.* reported sensitivity for Gentamicin to be 80%, Piperacillin Tazobactam 56%, and Amikacin 36%, but sensitivity to Aztreonam and Ciprofloxacin was 9.8% which is quite similar to our study.[17]

It is time to explore novel treatment options for multi and extensively-drug-resistant *Pseudomonas aeruginosa.* In our study, 23.7% of isolates were multidrug-resistant, and 52.8% were extensively drug-resistant. Different studies were conducted on the effectiveness of Ceftolozane-Tazobactam, and it was concluded that it is effective against multidrug-resistant *Pseudomonas aeruginosa* especially non-Carbapenemase-producing isolates.[18,19] According to a study a third-generation cephalosporin (Ceftazidime) along with a non-β-lactam-β-lactamase inhibitor (avibactam) is found to be effective against drug-resistant *Pseudomonas aeruginosa* isolates causing UTIs, Intra-abdominal infections and other hospital-acquired infections.[20]

At the same time, we should follow the hospital antibiotic stewardship programs and restrict the use of Carbapenems against Gram-negative rods as it is reported that hospitals that restrict the use of Carbapenems or use few Carbapenems have a very low incidence of Carbapenem-resistant *Pseudomonas aeruginosa.*

CONCLUSION

The susceptibility of Carbapenem-resistant *Pseudomonas aeruginosa* to other recommended antibiotics is very low, and the treatment of patients depends on individual pathogen-directed susceptibility. In order to know the best available antibiotic to be administered as empirical treatment for *Pseudomonas aeruginosa* isolates in our part of the world, continuous surveillance of antibiotic susceptibility is required.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

FA & IAM: Conception, study design, drafting the manuscript, approval of the final version to be published.

HW & HZ: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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