Int.j.pathol.2021;19(4): 172-177

Original Article

Susceptibility Profile of *Pseudomonas Aeruginosa* to Amikacin and Imipenim by Disc Diffusion and Minimum Inhibitory concentration Method

Mehveen Iqbal[1], Shahid Ali[2], Syeda Atiya Batool Gardezi[3] and Mahin Shams[4]

[1, 3, 4] Department of Pathology, United Medical and Dental College. [2]Department of Pharmacology Bolan University of Medical and Health Sciences

ABSTRACT

Background: *The resistant strains of Pseudomonas aeruginosa* become life threatening if not treated promptly. The objective of the study was to determine the susceptibility pattern of *Pseudomonas aeruginosa* to amikacin and imipenem by Disc diffusion and Minimum Inhibitory Concentration method.

Materials &Method: This cross sectional study was conducted from December 2019 to December 2020 at Creek general hospital and Zubaida medical center, Karachi. Samples of pus, blood and tracheal aspirates were collected. Samples were streaked on blood and Mac Conkey`s agar and further confirmed by API 20 NE system. Antimicrobial susceptibility of amikacin and imipenem was performed by Kirby- Bauer disk diffusion method and Minimum inhibitory concentration testing method. SPSS version 22.0 was used for statistical analysis

Results: A total of 200 samples were collected. Out of 200, 64 (32%) positive samples were further analyzed. 60.9 % (n=39) strains were isolated from pus. 64 positive samples 32% were tested for antimicrobial susceptibility of amikacin and imipenem by disc diffusion and minimum inhibitory concentration method. Out of 64, 82.8% (n=53) of isolates were observed resistant to amikacin by disc diffusion method followed by 62.5% (n=40) by minimum inhibitory concentration method. Intermediate sensitivity of amikacin was observed 28.12% (n=18) by minimum inhibitory concentration. 67.18% (n=43) isolates showed resistance to imipenem with minimum inhibitory concentration method as compared to 53.98% (n=34) resistant isolates when tested with disc diffusion method

Conclusions: Two testing methods should be used in-conjunction to determine susceptibility of antimicrobials to assess the correct burden of resistant isolates of *Pseudomonas aeruginosa*

Key words: *Pseudomonas aeruginosa,* antimicrobial susceptibility, imipenem, amikacin, Disc Diffusion, Minimum inhibitory concentration.

Introduction

Pseudomonas aeruginosa is an opportunistic bacterium causing multiple local and systemic infections ranging from nonthreatening to life threatening ones[1]. The diverse Gram-negative bacterium has been turn into one of the most common nosocomial pathogen connected to ample morbidity and mortality in recent years[2].

CORRESPONDENCE AUTHOR

Dr. Mehveen Iqbal

Assistant Professor (Microbiology)

United Medical and Dental College, Karachi

Email: mehveenfarhan2@gmail.com

In particular *Pseudomonas aeruginosa* has primary significance and have bleak projection in causing diseases like pneumonia and sepsis among those who are hospitalized and suffering with devastating conditions, burn patients, HIV positive, cystic fibrosis and others.[2, 3]

Among several factors, *the current surveillance of antimicrobial resistance is of major concern* that *makes these gram-negative bacilli infection even more hazardous. This is indicating towards a worrisome situation in the health care locale[1, 3]. Therefore it is necessary to monitor the resistant strains of Pseudomonas aeruginosa to observe susceptibility trends and to provide treatment guidelines for* physicians.

Pseudomonas aeruginosa has beta lactamase enzyme, efflux pump and low permeable membrane and number of transport mechanism that provide innate resistance to many antibiotics.[4] These antibiotics include penicillins and amino-penicillins, the macrolides such as erythromycin, the tetracyclines including doxycycline, the first and second generation Cephalosporins and the 3rd generation oral Cefixime. Not only this but resistance against aminoglycosides, the significantly effective element against *Pseudomonas aeruginosa,* is also frequently present nearly all over world[5]. Besides this the conversion of *Pseudomonas* into cocoid bacterium under the influence of antibiotic stress is another way of increasing drug resistanc.[6]Adding more to this, irrational use of antibiotics is another fundamental issue in emergence of resistant bacteria around the globe.[7]Hence multidrug resistance among *Pseudomonas* is worrisome and are deceptively the cause of higher morbidity, since these infections may not only lead to septicemia and other serious complications, but necessitate effective antibiotherapy.[8]In our country, nonexistence of infection Control training and extensive use of formal antibiotics have further amplified this difficulty. To avoid all these, testing of clinical isolates with standard methods is another fundamental aspect of consideration.

The dynamic and diagnostic role of clinical laboratories in testing clinical samples is undeniable. The methods of antimicrobial susceptibility testing are important as number of bacterial isolates become multi drug resistant. The different methods of antibiotic susceptibility testing not only postulates effective dosage of antibiotics but also frames sketch of empirical therapy of a patient for correct management. Disc diffusion methods (DDM) and Minimum inhibitory concentration (MIC) are among the methods of testing the effectiveness of antibiotics. The disc diffusion method is the qualitative, simple, economical and supple method of testing that allows easy interpretation and detection of various grouping of resistance[9]. whereas MIC is the quantitative, more expensive testing presents lowest levels of antibiotics that inhibit organism`s growth. MIC is of great significance in determining the susceptibility of an organism to selected antibiotics. Keeping the importance of *Pseudomonas aeruginosa*, beta lactam drug imipenem and protein inhibitor amikacin were taken into consideration. Since these are effective and frequently used drugs to stamp out *Pseudomonas* infections[10,11]. Therefore a research was planned to study the susceptibility pattern of amikacin and imipenem against *Pseudomonas aeruginosa* by DDM and MIC. The purpose of the project was to ascertain the resistance profiles of *Pseudomonas aeruginosa* circulating in our surroundings.

Materials & Methods

This cross sectional research project was carried out at Creek general hospital (United medical and dental college), Karachi and Zubaida medical centre, Karachi during the period of December 2019 to December 2020 . The research project was approved by United Medical and Dental College Ethical review committee (UMDC/Ethic/2019/27/2/247). Sample size was estimated by using WHO calculator with the prevalence of 15% and confidence interval of 95%[4, 8.]

After taking consent from patients, 200 sample of pus from skin wound, blood and tracheal aspirates were taken for isolation of organism. Brief clinical history of patient was taken. Samples were collected from hospitalized patients having wound, secondary pneumonia and those who were on ventilators. Those having urinary tract infections, taking anti-pseudomonal antibiotic and outdoor patients were not included in this study. Samples were cultured on blood agar, MacConkey's agar and incubated at 37°C. The organism growth was identified by their colonial morphology, gram staining reactions, and further confirmed on the basis of biochemical reactions reaction and using API 20 NE system[12]. The biochemical reagents and media used for the identification of *Pseudomonas aeruginosa* were Oxidase reagent, Triple sugar iron agar, Simmon`s citrate agar, Urea agar base, Sulphide indole motility medium[12]

Drug susceptibility was done according to Kirby Bauer disc diffusion method.[13] For this standardized inoculum was prepared, the organism was swabbed on the surface of a Mueller-Hinton agar plate. The Commercially available filter paper disks impregnated with amikacin (30µg) and imipenem (10µg) were used (Oxoid, UK). After overnight incubation at 37°C, the zone of inhibition around disk was measured in mm. The zone of diameter $\leq$ 15mm is considered as resistant , 16-21mm intermediate and $\geq$ 22mm sensitive for amikacin and that of imipenem was $\leq$ 16mm as resistant , 17-22 mm intermediate and $\geq$ 23mm sensitive The results were interpreted according to CLSI (clinical laboratory standard institute) guidelines[14]

All the isolated *Pseudomonas aeruginosa* were tested to the MIC for amikacin and imipenem by using commercially available Ezy MIC™ strip coated with

respective antibiotics. The concentration for amikacin tested ranged from 0.016-256 µg/ml and for imipenem 0.002-32 µg/ml. With the help of forceps, the strips were placed on Mueller Hinton agar using overnight incubation at 37 ^{0} C[15] with the MIC scale facing upwards (towards the opening of the plate) and the handle 'E' at the rim of the plate. The whole strip was ensured to be in complete contact with the agar surface. After 24 hours at 37 ^{0} C, MICs were read on the basis of intersection of elliptical zone of growth inhibition with MIC scale on the strip. For MIC break points CLSI[14] and Guidelines of EUCAST[16] testing was followed. AMK (Amikacin); Resistance >32mm intermediate > 16mm and < 32mm and sensitive ≤ 16mm. IPM (Imipenem); Resistance >8mm and <16mm, Intermediate > 4mm and <8mm, and Sensitive <4mm. Inoculum was prepared according to 0.5 Mc Farland index for both testing. *Pseudomonas aeruginosa* ATCC 27853 was used as control in this study.

Results were calculated in frequencies (%) by using SPSS 22.0 version. Cohen`s kappa statistical analysis was done to measure the concordance between the two testing methods. Kappa values were determined by performing the cross tab analysis. The percent concordance was calculated as follows [(a + d)/ (a + b + c + d)]*100, where *a* is the number of sensitive isolates by both tests, *b* is the number of sensitive isolates sensitive by MICs method and resistant by disk diffusion, *c* is the number of isolates resistant by MICs and sensitive by disk diffusion, and *d* is the number of isolates resistant by both tests[17]

Results

Total of 200 samples were included in this study. Percentages of different isolates were shown in Table No 1.

Table No-1: Percentages of Different Isolates

S.No	Name of isolates	Number of isolates	% of isolated strains
1	Pseudomonas aerugenosa	64	32%
2	Staphylococcus aureus	58	29%
3	Proteus spp	32	16%
4	Klebsiella spp	24	12%
5	E.coli	22	11%

Out of 200 samples, 64 samples showed positive growth for *Pseudomonas aureginosa* which were further analyzed. Among these 64 positive samples, 60.9% (n=39) of isolates were isolated from pus (skin wound) as shown in Table No 2.

Table-No 2: Number of Pseudomonas Aeruginosa Isolated from Different Samples

S.No	Source of strain	Number of isolates	% of isolated strains
1	Pus	39	60.9%
2	Blood	20	31%
3	Tracheal Aspirate	04	9%

Percentages of drug susceptibility of amikacin against isolated *Pseudomonas aeruginosa* by DDM and MIC method of testing is shown in Figure No 1 and 2.

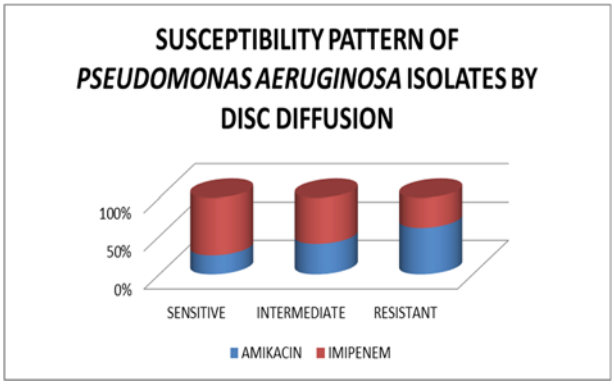

Figure No-1: Susceptibility Pattern of Isolated Pseudomonas Aeruginosa to Amikacin and Imipenem by Disc Diffusion Method

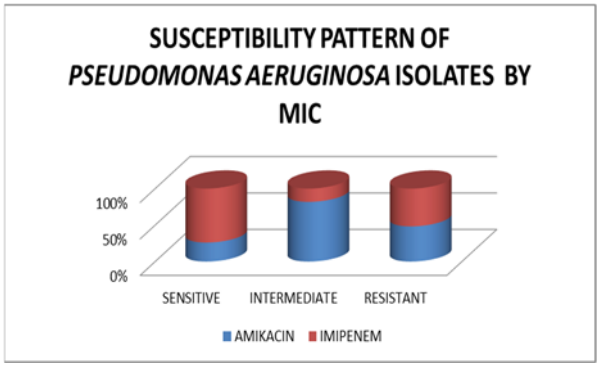

Figure No-2: Susceptibility Pattern of Isolated Pseudomonas Aeruginosa to Amikacin and Imipenem by Minimum inhibitory concentration Method

Comparison of DDM and MIC method used to determine susceptibility of amikacin, can be observed in Figure No 3 which revealed higher percentage of intermediate results 28.12% (n=18) by MIC method of testing.

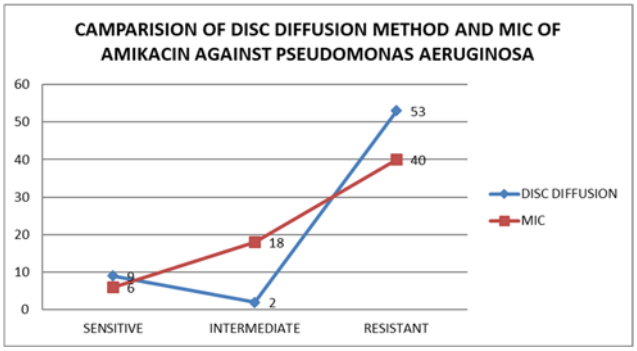

Figure No-3: Comparison of Disc Diffusion and Mic Method

Figure No 4 showed the comparison of isolate tested against imipenem by DDM and MIC methods of testing.

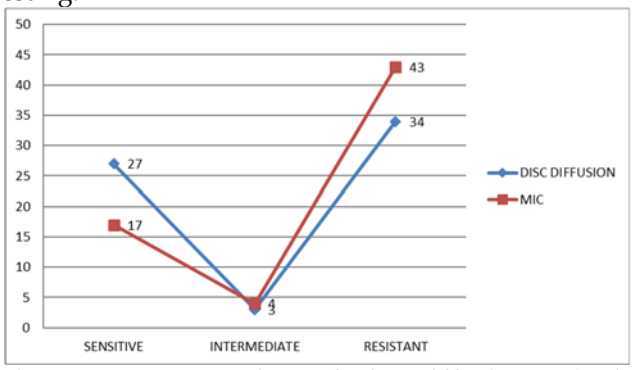

Figure No-4: Comparison of Disc Diffusion and Mic Method

Statistical analysis showed good measure of agreement between two tests as shown in Table No 3

Table No-3: Agreement Between Disc Diffusion Method and Mics Method

S.No	Name of antibiotics	Kappa value	% of agreement
1.	AMIKACIN	0.709	96
2	IMIPENEM	0.798	96

KEY: poor agreement = <0.20; fair agreement = 0.200.40; moderate agreement = 0.400.60; good agreement = 0.600.80; very good agreement = 0.801.00

Discussion

In present study dissimilarities between the susceptibility pattern of amikacin and imipenem against *Pseudomonas aeruginosa* can be witnessed by both MIC method and the DDM methods of testing. Being an opportunistic organism, resistant isolates of *Pseudomonas aeruginosa* are questionably one of the multifaceted to treat as they can cause life-threatening infections and serious complications.[8] The resistance among *Pseudomonas* species to number of antibiotics has intensified extensively and consequently required to be calculated accurately to be treated effectively.[8,10] . Interestingly in recent study the difference in susceptibility can be observed more in case of amikacin which yielded 28.12% intermediate sensitive isolates by MIC as compared to only 3% by DDM (3%). Changiz et al (2020) also documented similar results[18]. Moreover current study also recorded 13.7% amikacin sensitive strains of *Pseudomonas aeruginosa* by DDM in comparison with 9.38% by MIC. Our results are in close contact with Gill *et al* (2011)[19]. In contrast to a study conducted by Samad and co-researchers in 2017 demonstrated 82.14% in vitro sensitivity of amiakcin against multidrug resistant *Pseudomonas aeruginosa* [4] In a study by David P *et al*[20], amikacin showed 91% sensitivity. In addition 62% susceptibility for amikacin was observed by Paul and his co mates[21]. Difference in resistant pattern among *Pseudomonas aeruginosa* by both methods is alarming. This dissimilarity might be due to variation in pattern of prescribing amikacin and limited use of conventional antibiotics for Pseudomonal infections[12,20]. Therefore amikacin should be given for severe nosocomial infections[18]. Keith also pointed towards the geographical aspects in development of resistance[22].

In recent project 26.57% isolates were observed as sensitive to imipenem by MIC as compared to 42.8% by DDM. MIC being more sensitive and quantitative could be a reason for difference between two methods in current study. Lubna *et al* reported 19% sensitivity to imipenem by DDM[23]. In another study by Sidra and Habib (2020) reported 56%sensitivity to imipenem [24]. In a research conducted by David and his co-mates 59% sensitivity of imipenem was observed[20].In Europe, the CDC report (2016) revealed 33.9% of *Pseudomonas aeruginosa* were resistant to imipenem group by MIC[25]. Contradictory a research conducting in 2018 showed only 1 % resistance to imipenem[26]. As we know that the resistance to commonly used antibiotic for management of *Pseudomonas* infections are more common as compared with other bacteria, and antibiotic monotherapy is also related with treatment problems compared with combination regimens.[27, 28]. The use of imipenem should be limited to multidrug resistant pathogens to lower down the resistance because imipenem is still a good choice of drug for such patients. Furthermore, inappropriate use of empiric treatment, unreasonable practice of prescribing antibiotics particularly, self medication

and handiness of skills in terms of testing techniques could be a reason for resistance [24, 26].

The difference in susceptibility pattern by two methods is still questionable and directing towards emphasizes on proper antimicrobial testing and usage of exact dose of the drug. Faulty testing and dissimilarities in results may produce undesirable clinical outcomes else putting additional liability on health services.[28,29]In current study there was a disparity between the outcomes of sensitivity testing by DDM and MIC testing although statistical analysis showed a good Kappa measure of agreement between the two methods of testing. Disc diffusion method is cheaper, simple and easy to handle method[30] of testing whereas Minimum Inhibitory Concentration (MIC) is a rapid costly procedure used to govern the quantitative activity of antimicrobials. It is the "Gold Standard" for defining *in vitro* activity[10, 29]. In our setup most of the diagnostic laboratories do not perform the MIC susceptibility of clinical isolates. Our study also pointed towards the importance of MIC in order to regulate the minimum dose that would be clinically effective. It is desired that a standardized method of testing should be implicated for tracing of resistant strains else better impact on commonly used antibiotics, new advancement in this field and health policies. Well-timed and precisely performance of susceptibility testing marks a meaning full change in management of patient[27, 31]. Therefore keeping in mind the advantages and disadvantages of both DDM and MIC, MIC method of testing should be used paralleled to disc diffusion, and should be fortified, in conjunction with culture/sensitivity testing of clinical isolates in all surroundings, especially the hospital wards.

Unfortunately, there are few limitations of our research that should not be overlooked. The study was conducted on limited isolated samples which doesn't reflect the true demography of population. This project is lacking complete clinical history and follow-up of a patient. Samples of pus, blood and tracheal aspirates were considered only. In addition combination of antibiotics was not tested in recent project which might be very useful and effective in resistant cases.

Conclusion

In conclusion, antimicrobial susceptibility of *Pseudomonas aeruginosa* should be determined with advanced and better testing methodology along with routine testing methods. Correct documentation of resistant strains would be helpful in treatment of *Pseudomonas aeruginosa* infections as well in limiting the drug resistance.

Acknowledgement

Our earnest gratitude to the authorities of Zubaida medical centre and United Medical and Dental College.

CONFLICT OF INTEREST: No

FUNDING: No

References

1. Dawra R, Sharma R, Bachhiwal R et al. High incidence of multidrug resistant Pseudomonas aeruginosa isolated from infected burn wounds in a tertiary hospital. Int J Curr Microbiol Appl Sci. 2017;6(2):1134–1139.
2. Benie C, Dadie A, Guessennd N, et al. Characterization of virulence potential of Pseudomonas aeruginosa isolated from bovine meat, fresh fish, and smoked fish. Eur J Microbiol Immunol. 2017;7(1):55–64.
3. Brogan D.M., Mossialos E. A critical analysis of the review on antimicrobial resistance report and the infectious disease financing facility. Glob. Health. 2016;12(8):3-7.
4. Samad A, Ahmed T, Rahim A, Khalil A, Ali I. Antimicrobial susceptibility patterns of clinical isolates of Pseudomonas aeruginosa isolated from patients of respiratory tract infections in a Tertiary Care Hospital, Peshawar. Pak J Med Sci. 2017;33(3):670–674.
5. Pitt TL, Sparrow M, Warner M, and Stefanidou M.. . Survey of resistance of Pseudomonas aeruginosa from UK patients with cystic fibrosis to six commonly prescribed antimicrobial agents. Thorax.2017;58(27):794-796.
6. Khare A, Kothari S, Misra V. Incidence and sensitivity pattern of Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa in a tertiary care hospital. Int J Basic Clin Pharmacol. 2017; 6:(4);329-33.
7. Fernandez-Barat L, Ferrer M, De Rosa F, Gabarrus A, et al . Intensive care unit-acquired pneumonia due to Pseudomonas aeruginosa with and without multidrug resistance. J Infect. 2017;74(2):142–52.
8. Riu M, Chiarello P, Terradas R, et al. Cost attributable to nosocomial bacteremia. analysis according to microorganism and antimicrobial sensitivity in a university hospital in Barcelona. PLoS One. 2016;11(4):45-50
9. Sanchez MA, Sanchez Del Saz B, Loza E, et al : Evaluation of the OSIRIS video reader system for disk diffusion susceptibility test reading. Clin Microbiol Infect. 2001, 7: 352-357.
10. Nabil A, Ahmed A, KarimA. Evaluating Antibiotic Sensitivity Patterns of Pseudomonas in Relation to Specimen Type in Hospital. J Pak Med Assoc. 2019.69(2);168-172
11. Nathwani D, Raman G, Sulham K, et al. Clinical and economic consequences of hospital- acquired resistant and multidrugresistant Pseudomonas aeruginosa

infections: a systematic review and meta-analysis. Antimicrob Resist Infect Control, 2014; 3(1):32–47
12. Ewa M.k, Michal A.K, Grzegorz S, et al . Isolation and Characterization of Pseudomonas spp. Strains That Efficiently Decompose Sodium Dodecyl Sulfate Front. Microbiol., 07 2017:8 (4):1872
13. Jorgensen J, and Turnidge J. Susceptibility test methods: dilution and disk diffusion methods. 2007.1152–1172. In P.R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller(ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington,D.C
14. Document; Twenty-Fifth International Supplement Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing.M100-S26. Wayne, PA: CLSI; 2016.
15. Citron, D. M., Ostovari, M. I., Karlsson, A.,, et al. Evaluation of the E test for susceptibility testing of anaerobic bacteria. Journal of Clinical Microbiology, 1991;29(10), 2197-2203
16. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Ver. 6.0, 2016. EUCAST. 2016.
17. Siva G,Nor Azu and Ramelah M. Antimicrobial susceptibility of clinical isolates of Pseudomonas aeruginosa from a Malaysian Hospital.Malays J Med Sci. 2009; 16(2): 27–32
18. Changiz A and Mehrdad P. Comparison of two disk diffusion and E-test methods in determining antibiotic susceptibility of Pseudomonas aeruginosa strains in isolated burn infections of Ahvaz City. Bangl J Med Sci.2020;19 (4);646-651
19. Gill MM, Usman J, Kaleem F et al. Frequency and Antibiogram of Multi-drug Resistant Pseudomonas aeruginosa. J Coll Physicians Surg Pak. 2011;21(9):531–534.
20. David P N, Joseph L K, Hongbin C, Henan L. Defining the potency of amikacin against Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii derived from Chinese hospitals using CLSI and inhalationbased breakpoints.Infection and Drug Resistance. 2018;11: 783–790
21. Paul G H, Fluit A.C, Milatovic D,verhoef Jand Schmitz F.J. Antimicrobila susceptibility of imipenim resistant Pseudomonas aeuroginosa. Joural of antimicrobial chemotherapy, 2002; 50 293-307
22. Keith P. Aminoglycoside Resistance in Pseudomonas aeruginosa.Antimicrob Agents Chemother. 2005 ; 49(2): 479–487
23. Farooq L, Memon Z, Muhammad O et al. Frequency and antibiogram of multi-drug resistant pseudomonas aeruginosa in a Tertiary Care Hospital of Pakistan. Pak J Med Sci. 2019; 35(6): 1622–1626
24. Sidra S and Habib B. Resistance profile of genetically distinct clinical Pseudomonas aeruginosa isolates from public hospitals in central Pakistan. Journal of Infection and Public Health, 2020;13(4) : 598–605
25. European Centre for Disease Prevention and Control. Surveillance Report: Antimicrobial resistance surveillance in Europe 2016.
26. López C, Cabot G, Del B, et al. The Versatile Mutational Resistome of Pseudomonas aeruginosa. Front Microbiol. 2018;9(4):685
27. Arti N, Mridu A, Avinash S, et al . Assessment of Doripenem, Meropenem, and Imipenem against Respiratory Isolates of Pseudomonas aeroginosa in a Tertiary Care Hospital of North India.Indian J Crit Care Med. 2017; 21(10): 703–706
28. Barrasa-V, Aibar-R, PrietoA P, et al. Impact on morbidity, mortality, and length of stay of hospital-acquired infections by resistant microorganisms. Clin Infect Dis. 2017;65(4):644–52.
29. Lucasti C, Vasile L, Sandesc D, et al, Dose-Ranging study of relebactam with imipenem-cilastatin in subjects with complicated intra-abdominal infection. Antimicrob Agents Chemother. 2016;60(10):6234–43.
30. Ayukekbong J., Ntemgwa M., Atabe A.. The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrob. Resist. Infect. Control. 2017;6(47):2-8
31. Kassim A., Omuse G., Premji Z.,et al. Comparison of Clinical Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing guidelines for the interpretation of antibiotic susceptibility at a University teaching hospital in Nairobi, Kenya: A cross-sectional study. Ann. Clin. Microbiol. Antimicrob. 2016;15(3):21-29.

HISTORY	
Date received:	24-11-2021
Date sent for review:	15-01-2022
Date received reviewers comments:	09-02-2022
Date received revised manuscript:	11-02-2022
Date accepted:	05-03-2022

CONTRIBUTION OF AUTHORS	
Author	**Contribution**
Mehveen Iqbal	A, B, C
Shahid Ali	A, B, C
Syeda Atiya Batool	B, C
Mahin Shams	B, C

KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion