LAB RESEARCH

Antibiogram of ESBL and MBL producing *Pseudomonas aeruginosa* among the population of Hazara division, KPK, Pakistan

Farooq Ali[1], Zeeshan Niaz[2], Pir Tariq Shah[3], Qismat Shakeela[4], Bibi Uzma[5], Shehzad Ahmed[6]

Abstract

Objective: To investigate the frequency rate and sensitivity pattern of extended-spectrum beta-lactamase and metallo-beta-lactamase producing *Pseudomonas aeruginosa* isolated from major hospitals.

Methods: The cross-sectional study was conducted in the Microbiology section of the Pathology Department of Ayub Medical College, Abbottabad, Pakistan, from September 2017 to April 2018, and comprised clinical samples collected from different medical wards of major hospitals in the study area. For the selective growth of *Pseudomonas aeruginosa*, Cetrimide agar was used, and different antibiotics were evaluated for the sensitivity pattern following Kirby-Bauer diffusion method. *Pseudomonas aeruginosa* producing extended-spectrum beta-lactamase and metallo-beta-lactamase were identified through double disk synergy test and imipenem-ethylenediaminetetraacetic acid tests respectively. Patient's demographic and medical history was noted on a proforma. Data was analysed using SPSS 22.0.

Results: Of the 242 samples screened, 46 (19%) were positive for *Pseudomonas aeruginosa*. These samples were highly sensitive to levofloxacin, amikacin, imipenem, meropenem and ciprofloxacin ($p<0.05$). Of the positive cases, 11 (23.91%) were detected for extended-spectrum beta-lactamase production, while 3 (6.52%) samples were detected for metallo-beta-lactamase production.

Conclusion: *Pseudomonas aeruginosa* samples were widely resistant to most antibiotics, but were sensitive for some antibiotics which may be recommended by physicians when treating *Pseudomonas aeruginosa* infection.

Keywords: ESBL, MBL, Susceptibility, DDST, Imipenem-EDTA test.

(JPMA 70: 1979; 2020) **DOI: https://doi.org/10.5455/JPMA.19089**

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a rod-shaped, motile, oxidase- and catalase-producing gram-negative bacterium, which is known for incendiary bacterial infection in humans and animals. This opportunistic pathogen is linked with diverse infections such as urinary tract infection (UTI), surgical and nosocomial infections.[1,2] As an opportunistic pathogen, it infects two-third of the hospitalised population which makes it the most aggressive bacterial pathogen. It is the leading gram-negative pathogen in health centres with about 60% mortality rate. Moreover, it complicates almost 90% cystic fibrosis deaths in the community.[3]

Gasserd discovered and isolated this gram-negative bacillus for the first time from the green pus in 1882. The unique style and diverse nutritional profile of *P. aeruginosa* permit it to colonise everywhere.[4] Routinely, this gram-negative rod survives in hospital environment and its reservoir for infection and colonisation cannot be traced.[5]

Wounds of burn patients are colonised by different strains of *P. aeruginosa*. The frequency of the infections in these patients ranges 22-73%.[6] Multi-drug resistance (MDR) is a big health challenge linked with *P. aeruginosa* and this pathogen resists a wide range of antibiotics. The susceptibility pattern of *P. aeruginosa* is limited to a number of antimicrobial agents because of its genetic resistance to a wide range of antibiotics,[7] and emerging resistance to the previously used sensitive antibiotics.[8] Resistance to antibiotics in case of hospital infections are associated with higher costs and adverse effects.[9] Extended spectrum beta lactamase (ESBL)-producers are the bacterial strains that hydrolyse and emerge resistant to ceftazidime, ceftriaxone, cefotaxime (3G cephalosporin).[10] Studies have reported the frequency rate of ESBL-producing pseudomonas species ranging 22-36%.[11,12]

A ventilator-based prevalence rate of pneumonia due to *P. aeruginosa* was reported as 15.6%, in which Asia contributed 16%, United States 13.5%, Latin America 13.8% and Europe 19.4%.[13] A study reported patient-to-patient transmission of *P. aeruginosa* in Vancouver, Canada, in 174 isolates; 157 genetic types were recognized as *P. aeruginosa*.[14]

The current study was planned to assess the occurrence rate, susceptibility pattern and the prevalence rate of ESBL- and metallo-beta-lactamase (MBL)-producing *P. aeruginosa* in indoor and outdoor patients at different hospitals.

[1-4,6]Department of Microbiology Hazara University Mansehra, KPK Pakistan;
[5]Department of Pathology, Ayub Medical College Abbottabad, KPK, Pakistan.
Correspondence: Shehzad Ahmed. e-mail: shehzadlughmani@gmail.com

Materials and Methods

The cross-sectional study was conducted in the Microbiology section of the Pathology Department of Ayub Medical College, Abbottabad, Pakistan, from September 2017 to April 2018, and comprised clinical samples collected from different medical wards of major hospitals in the study area. After approval from the institutional bioethical committee of Hazara University, Mansehra, Pakistan. A total of 242 different clinical samples were collected from the patients of different medical wards, like ear, nose, throat (ENT) the burns unit act of the hospitals, and the sample included ear swabs, pus, urine, wounds, body fluids, blood and sputum. Each patient was briefed about the purpose of the study and was also ensured for confidentiality of individual result along with his/her consent.

The samples were collected from the patients in the presence of a medical officer and were inoculated on suitable culture media, such as cystine–lactose–electrolyte-deficient (CLED) agar, blood agar, MacConkey agar, nutrient agar, and after overnight incubation at 37ºC, the culture media were examined for the colonies of gram-negative rod *P. aeruginosa*. Cetrimide agar was used for selective growth. The colonies were also identified based on staining character, growth pigmentation, colonial morphology and other biochemical tests for *P. aeruginosa* as per standard lab identification methods.[15,16]

For antibacterial susceptibility patterns, tryptic soy broth (CM129-Oxoid) was prepared and was poured 4-5ml into each capped tubes. These tubes after sterilisation by autoclaving were left overnight at 35ºC prior to inoculation of samples. Turbidity of inoculum was standardised to 0.5 McFarland index according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[17] Routinely used different antibiotics (commercially available, Oxoid) were used to determine the susceptibility pattern by following disc diffusion method.[18] Minimum inhibitory concentration (MIC) of each antibiotic to *P. aeruginosa* was found, and the break points were standardised in line with literature.[19]

For the detection of ESBL-producing *P. aeruginosa*, all bacterial isolates were screened as prescribed by a study.[20] All the stored bacterial isolates at -20ºC were refreshed on nutrient agar to find out ESBL via synergy disc diffusion method. ESBL-producing *P. aeruginosa* were identified using double disk synergy test (DDST). Three discs of third-generation (3G) cephalosporine, ceftriaxone (CRO), cefotaxime (CTX) and ceftazidime (CAZ) 30µg each were placed on Mueller Hinton Agar (MHA) at a distance of 25mm centre-centre from amoxicillin-clavulanic acid (AMC-20/10µg).[20] Enhancement in zone of inhibition of any in the 3G cephalosporine towards AMC indicated the presence of ESBL-producing *P. aeruginosa*.

MBL-producing *P. aeruginosa* were identified using imipenem-ethylenediaminetetraacetic acid (IMP-EDTA) method. Two IMP-10µg discs and 0.5M EDTA were used. A 0.5M EDTA was prepared by dissolving 186.1g of EDTA (disodium EDTA.$2H_2O$) in one litre of distilled water and potential of hydrogen (pH) was adjusted up to 8.0. IMP-resistant culture was inoculated and two 10µg IMP discs were placed on MHA medium and appropriate amount of half-Mole EDTA was added to one of them to obtain the desired concentration of 1000µg. The culture plates were examined for the synergistic effect of IMP and chelator agent EDTA after 16 hours of incubation at 37ºC.[21]

Data was analysed using SPSS 22.0. Two-way analysis of variance (ANOVA) and chi-square test were applied as appropriate. $P<0.05$ was considered significant.

Results

Of the 242 samples screened, 119(49%) came from males and 123 (51%) from females. The samples included ear swabs 114 (47%), blood 14(5.8%), body fluid 3 (1.2%), pus 54(22.3%), sputum 5 (2.1%), urine 49 (20.2%) and wound 3(1.2%). The mean age of each type of sample along gender lines were noted (Figure 1), and significance was checked in terms of both and gender age (Table 1). Overall, 46(19%) samples were positive for *P. aeruginosa*; 27 (58.7%) from males and 19 (41.3%) from females (Table 2). Also, 32 (69.57%) of the positive samples related to indoor patients and 14 (30.43%) to outdoor patients. Of the positive cases, 11 (23.91%) were detected for ESBL production, and 3 (6.52%) for MBL production (Figure 2). The highest number of positive cases of *P. aeruginosa* were identified in ear swab specimens, followed by pus and urine specimens, while no cases were identified positive in body fluid and sputum specimens (Figure 3).

Susceptibility patterns of *P. aeruginosa* to different classes of antibiotics were checked (Table 3) and the same was

Table-1: Tests of Between-Subjects Effects.

Source	df	F	*p*-value
Dependent Variable: Age of Patients			
Gender	1	3.291	0.071
Sample Type	6	3.936	0.001
Gender * Sample Type	5	2.491	0.032

Table-2: Gender wise frequency of *Pseudomonas aeruginosa*.

Gender	Total samples	Positive	Percent
Male	119	27	58.7
Female	123	19	41.3
Total	242	46	19

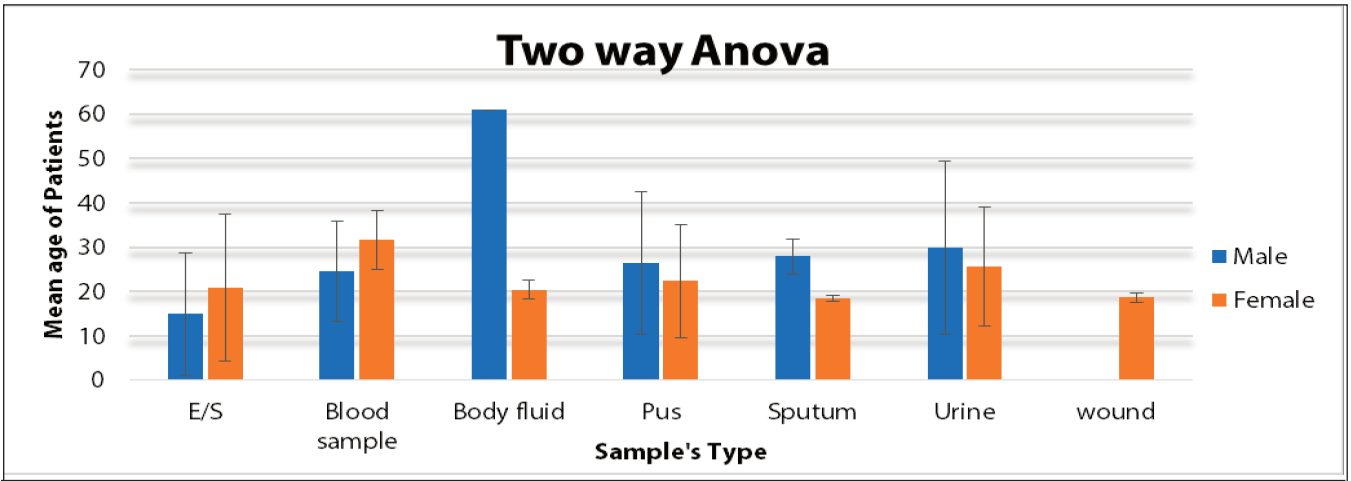

Figure-1: Statistics of factorial analysis of variance (ANOVA) among gender and age of patients and type of clinical sample.

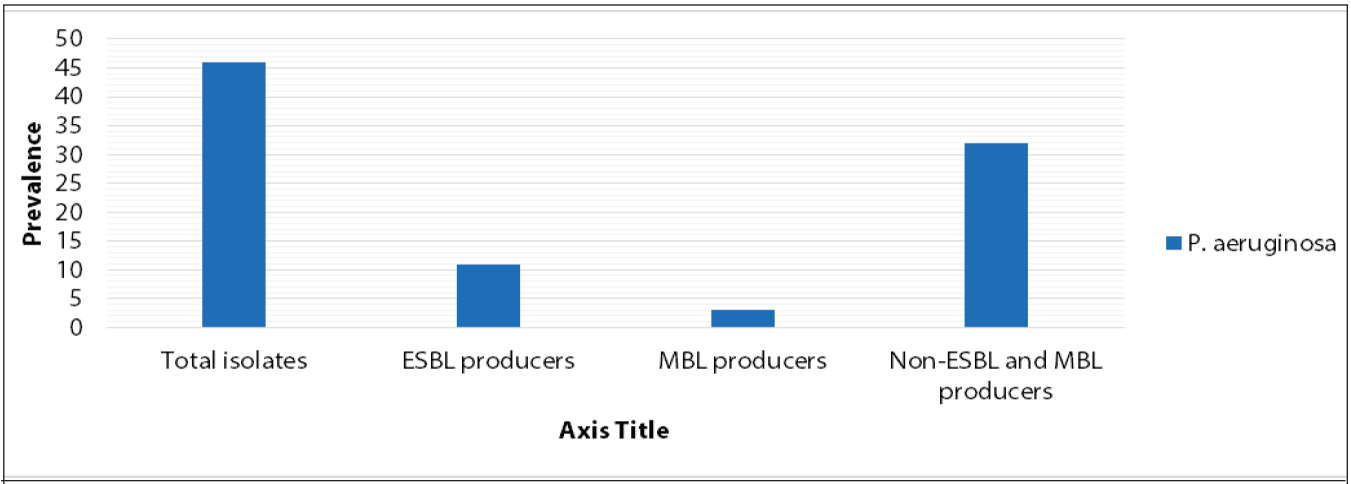

Figure-2: Frequency rate of ESBL- and MBL-producing *Pseudomonas aeruginosa* among clinically pathogenic isolates.

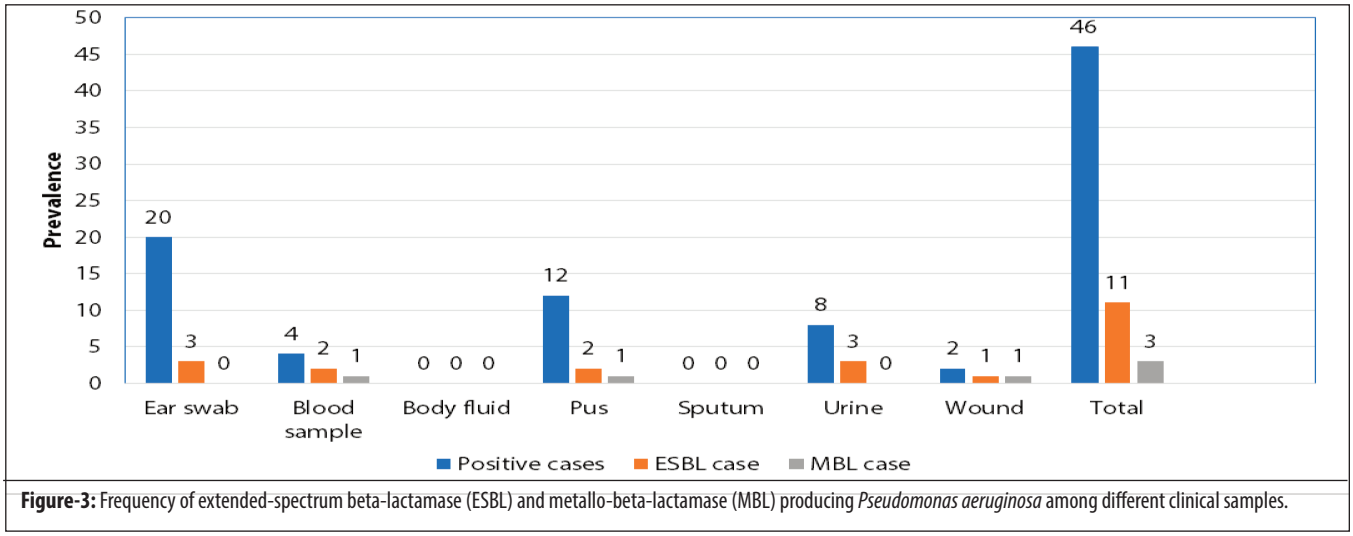

Figure-3: Frequency of extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* among different clinical samples.

done for ESBL- and MBL-producers (Tables 4-5) as well as in relation to indoor and outdoor patients (Table 6).

Table-3: Susceptibility pattern of *Pseudomonas aeruginosa* isolates to different antibiotics.

Antibiotics Name	Codes	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Levofloxacin	LEV	26 (56.52)	12 (26.09)	8 (17.39)
Amikacin	AK	18 (39.13)	18 (39.13)	10 (21.74)
Carbenicillin	CAR	11 (23.91)	21 (45.65)	14 (30.43)
Ceftazidime	CAZ	14 (30.43)	17 (36.96)	15 (32.61)
Ceftriaxone	CRO	6 (13.04)	3 (6.52)	37 (80.43)
Aztreonam	ATM	4 (8.7)	29 (63.04)	13 (28.26)
Cefotaxime	CTX	3 (6.52)	13 (28.26)	30 (65.22)
Tazobactam- piperacillin	TZP	11 (23.91)	24 (52.17)	11 (23.91)
Gentamycin	CN	9 (19.57)	8 (17.39)	26 (56.52)
Ciprofloxacin	CIP	20 (43.48)	8 (17.39)	18 (39.13)
Imipenem	IMP	30 (65.22)	12 (26.09)	4 (8.7)
Meropenem	MEM	20 (43.48)	20 (43.48)	6 (13.04)

Table-4: Susceptibility pattern of extended-spectrum beta-lactamase (ESBL)-producing *Pseudomonas aeruginosa* to various antibiotics.

Antibiotics Name	Codes	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Levofloxacin	LEV	8 (72.73)	2 (18.18)	1 (9.09)
Amikacin	AK	4 (36.36)	5 (45.45)	2 (18.18)
Carbenicillin	CAR	1 (9.09)	7 (63.64)	3 (27.27)
Ceftazidime	CAZ	2 (18.18)	3 (27.27)	6 (54.55)
Ceftriaxone	CRO	0	1 (9.09)	10 (90.91)
Aztreonam	ATM	0	4 (36.36)	7 (63.64)
Cefotaxime	CTX	0	0	11 (100)
Tazobactam- piperacillin	TZP	3 (27.27)	7 (63.64)	1 (9.09)
Gentamycin	CN	2 (18.180	8 (72.73)	1 (9.09)
Ciprofloxacin	CIP	7 (63.64)	3 (27.27)	1 (9.09)
Imipenem	1MP	8 (72.73)	3 (27.27)	0
Meropenem	MEM	7 (63.64)	4 (36.36)	0

Discussion

Bacterial species are commonly known for spreading various infections in both humans and animals. These species can survive in the environment of common healthcare facilities. Some of these, especially *P. aeruginosa* and *Enterobacter*, can survive even in toxic conditions. These properties make them able to become resistant to all the drugs available in an environment.[22]

The current study had more infected samples coming from the male population compared to females which is the opposite of earlier findings.[23] The positive rate of *P. aeruginosa* was high in pus, secretion and urine samples as compared to sputum, blood etc. This finding is in line with an earlier study.[24] In terms of susceptibility pattern in the present study, the clinical isolates of *P. aeruginosa* showed high susceptibility to imipenem, meropenem, ciprofloxacin, levofloxacin and amikacin. An earlier study highlighted the same susceptibility pattern for the *P. aeruginosa*.[25] But another study disagreed with the efficacy of ciprofloxacin for *P. aeruginosa*. It is known that bacterial susceptibility pattern is geographically different.[26]

Table-5: Susceptibility pattern of metallo-beta-lactamase (MBL)-producing *Pseudomonas aeruginosa* to various antibiotics.

Antibiotics Name	Codes	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Levofloxacin	LEV	3 (100)	0	0
Amikacin	AK	1 (33.33)	0	2 (66.67)
Carbenicillin	CAR	0	2 (66.67)	1 (33.33)
Ceftazidime	CAZ	3 (100)	0	0
Ceftriaxone	CRO	3 (100)	0	0
Aztreonam	ATM	1 (33.33)	1 (33.33)	1 (33.33)
Cefotaxime	CTX	1 (33.33)	1 (33.33)	1 (33.33)
Tazobactam- piperacillin	TZP	1 (33.33)	2 (66.67)	0
Gentamycin	CN	1 (33.33)	0	2 (66.67)
Ciprofloxacin	CIP	1 (33.33)	2 (66.67)	0
Imipenem	1MP	0	0	3 (100)
Meropenem	MEM	0	0	3 (100)

Table-6: Susceptibility pattern of *Pseudomonas aeruginosa* between indoor and outdoor patients.

Antibiotic name	Indoor patients (n=32)		Outdoor patients (n=14)		*p* value
	Sensitive	Resistant	Sensitive	Resistant	
	n (%)	n (%)	n (%)	n (%)	
LEV	20 (62.5)	12 (37.5)	6 (42.9)	8 (57.1)	< 0.216
AK	10 (31.3)	22 (68.9)	8 (57.1)	6 (42.9)	< 0.098
CAR	6 (18.8)	26 (81.3)	5 (35.7)	9 (64.3)	< 0.215
CAZ	12 (37.5)	20 (62.5)	2 (14.3)	12 (85.7)	< 0.115
CRO	4 (12.5)	28 (87.5)	2 (14.3)	12 (85.5)	< 0.869
ATM	1 (3.1)	31 (96.9)	3 (21.4)	11 (78.6)	< 0.043
CTX	1 (3.1)	31 (96.9)	2 (14.3)	12 (85.7)	< 0.158
TZP	5 (15.6)	27 (84.4)	6 (42.9)	8 (57.1)	< 0.046
CN	7 (21.9)	25 (78.1)	2 (14.3)	12 (85.7)	< 0.550
CIP	15 (46.9)	17 (53.1)	5 (35.7)	9 (64.3)	< 0.482
IMP	22 (68.8)	10 (31.2)	8 (57.1)	6 (42.9)	< 0.447
MEM	16 (50.0)	16 (50.0)	5 (35.7)	9 (64.3)	< 0.371

Commonly, cephalosporins are recommended in general practice for the treatment of infections caused by *P. aeruginosa*.[27,28] Ceftriaxone (13.04%) and ceftazidime (30.43%) were less effective against *P. aeruginosa* in the current study. Results for the 3G cephalosporin are affirmed by a study.[29] Susceptibility pattern of the cephalosporin reported in India had the same hypothesis for *P. aeruginosa*.[30]

In the current study, carbapenem had higher frequency rate of susceptibility against *P. aeruginosa* species. Imipenem exhibited 68.8% susceptibility rate to indoor and 57.1% to outdoor isolates, while meropenem showed 50% and 35.7% efficacy respectively. These results are supported by a study.[31] Aminoglycosides are recommended for the treatment of gram-negative bacteria,[32] and the current study also found that non β-lactams isolates showed good susceptibility to gentamycin (19.57%) and amikacin (39.13%). Our results are in accordance with an earlier study.[33] Other studies confirmed that amikacin was more effective against *P. aeruginosa*

infections in comparison with gentamycin.[30,34,35] From the results obtained in our study, it appears that gentamycin was widely prescribed in health practices and misused in the community without any consultation with physicians.

A total of 6.52% MBL-positive cases were detected in the current study among all the positive cases for *P. aeruginosa*. These were mostly sensitive to levofloxacin, cefotaxime and ceftazidime, while 100% resistant to imipenem and carbapenem. The findings are in line with a reported study.[36]

Also, ESBL-positive samples were 23.91% and exhibited high resistance to gentamycin, imipenem and meropenem while remaining sensitive to levofloxacin, ceftazidime and ceftriaxone. These results match earlier findings. [27,31,34] However, other antibiotics, such as amikacin, aztreonam and tazobactam / piperacillin have shown less resistance.[24]

The present study used two drugs-based tests for the detection of ESBL- and MBL-producing *P. aeruginosa* which is in agreement with earlier studies.[21,23]

The findings of the current study indicate that there were a few loopholes in hospital and medical setups, and improving these can significantly reduce or eradicate not only the spread, but also the emergence of MDR strains of *P. aeruginosa*. In order for that to happen, the overall hygienic conditions at hospitals need to be standardised, proper diagnosis and treatment regime must be adopted where antibiotic susceptibility must be detected before a prescription is handed out. The patients must be made aware of the pitfalls of irrational use of antibiotics with incomplete dosage.

Conclusion

P. aeruginosa samples were found to be widely resistant to most antibiotics, but were sensitive for some others which may be prescribed by physicians when treating *P. aeruginosa* infections.

Disclaimer: None.

Conflict of interests: None.

Source of Funding: None.

References

1. Kiska D, Gilligan *P. Pseudomonas*. Manual of clinical microbiology. Washington DC: ASM Press; 2003, pp 719-28.
2. Sekiguchi JI, Asagi T, Miyoshi-Akiyama T, Kasai A, Mizuguchi Y, Araake M, et al. Outbreaks of multidrug-resistant *Pseudomonas aeruginosa* in community hospitals in Japan. J Clin Microbiol 2007; 45: 979-89.
3. Fick R. *Pseudomonas aeruginosa*, the opportunist : pathogenesis and disease. London, Tokyo: CRC Press; 1993.
4. Favero M, Carson L, Bond W, Petersen N. *Pseudomonas aeruginosa*: growth in distilled water from hospitals. Science 1971; 173: 836-8.
5. Malani PN. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. JAMA 2010; 304: 2067-71.
6. Japoni A, Farshad S, Alborzi A. *Pseudomonas aeruginosa*: Burn infection, treatment and antibacterial resistance. Iranian Red Crescent Med J 2009; 11: 244-53.
7. Ruimy R, Genauzeau E, Barnabe C, Beaulieu A, Tibayrenc M, Andremont A. Genetic diversity of *Pseudomonas aeruginosa* strains isolated from ventilated patients with nosocomial pneumonia, cancer patients with bacteremia, and environmental water. Infection and Immunity 2001; 69: 584-8.
8. Akanji B, Ajele J, Onasanya A, Oyelakin O. Genetic fingerprinting of *Pseudomonas aeruginosa* involved in nosocomial infection as revealed by RAPD-PCR markers. Biotechnology 2011; 10: 70-7.
9. Afunwa RA, Odimegwu DC, Iroha RI, Esimone CO. Antimicrobial resistance status and prevalence rates of extended spectrum beta-lactamase producers isolated from a mixed human population. Bosn J Basic Med Sci 2011; 11: 91-6.
10. Peirano G, Pitout JD. Molecular epidemiology of Escherichia coli producing CTX-M β-lactamases: the worldwide emergence of clone ST131 O25: H4. Int J Antimicrob Agents 2010; 35: 316-21.
11. Bell JM, Chitsaz M, Turnidge JD, Barton M, Walters LJ, Jones RN. Prevalence and significance of a negative extended-spectrum β-lactamase (ESBL) confirmation test result after a positive ESBL screening test result for isolates of *Escherichia coli* and *Klebsiella pneumoniae*: results from the SENTRY Asia-Pacific surveillance program. J Clin Microbiol 2007; 45: 1478-82.
12. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan. Burns 2009; 35: 1020-5.
13. Kollef MH, Chastre J, Fagon JY, François B, Niederman MS, Rello J, et al. Global prospective epidemiologic and surveillance study of ventilator-associated pneumonia due to *Pseudomonas aeruginosa*. Crit Care Med 2014; 42: 2178-87.
14. Speert DP, Campbell ME, Henry DA, Milner R, Taha F, Gravelle A, et al. Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis in British Columbia, Canada. Am J Resp Crit Care Med 2002; 166: 988-93.
15. Cheesbrough M. District laboratory practice in tropical countries: Cambridge university press; 2006.
16. Cowan ST, Steel KJ. Cowan and Steel's manual for the identification of medical bacteria: Cambridge university press; 2004.
17. Weinstein MP, Patel JB, Bobenchik AM, Campaeu S, Cullen SK, Galas MF, et al. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute (CLSI). 29th ed. 2019.
18. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45: 493-6.
19. Patel JB, Weinstein MP, Eliopoulos GM, Jenkins SG, Lewis JS, Limbago B, et al. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute (CLSI). 27th ed. 2017.
20. Hawser S, Bouchillon S, Lascols C, Hackel M, Hoban D, Badal R, et al. Susceptibility of European Escherichia coli clinical isolates from intra-abdominal infections, extended-spectrum β-lactamase occurrence, resistance distribution, and molecular characterization of ertapenem-resistant isolates (SMART 2008–2009). Clin Microbiol Infect 2012; 18: 253-9.
21. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas spp*. and Acinetobacter spp. J Clin Microbiol 2002; 40: 3798-801.
22. Alp E, Güven M, Yıldız O, Aygen B, Voss A, Doganay M. Incidence, risk factors and mortality of nosocomial pneumonia in intensive care units: a prospective study. Ann Clin Microbiol Antimicrob 2004; 3: 17.
23. Ahmad M, Hassan M, Khalid A, Tariq I, Asad MHHB, Samad A, et al. Prevalence of Extended Spectrum β-Lactamase and Antimicrobial

Susceptibility Pattern of Clinical Isolates of Pseudomonas from Patients of Khyber Pakhtunkhwa, Pakistan. BioMed Res Int 2016; 2016: 6068429.

24. Bashir D, Thokar MA, Fomda BA, Bashir G, Zahoor D, Ahmad S, et al. Detection of metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* at a tertiary care hospital in Kashmir. Afr J Microbiol Res 2011; 5: 164-72.
25. Shahcheraghi F, Badmasti F, Feizabadi MM. Molecular characterization of class 1 integrons in MDR *Pseudomonas aeruginosa* isolated from clinical settings in Iran, Tehran. FEMS Immunol Med Microbiol 2010; 58: 421-5.
26. Messadi AA, Lamia T, Kamel B, Salima O, Monia M, Saida BR. Association between antibiotic use and changes in susceptibility patterns of *Pseudomonas aeruginosa* in an intensive care burn unit: a 5-year study, 2000–2004. Burns 2008; 34: 1098-102.
27. Cavallo J, Fabre R, Leblanc F, Nicolas-Chanoine M, Thabaut A. Antibiotic susceptibility and mechanisms of β-lactam resistance in 1310 strains of *Pseudomonas aeruginosa*: a French multicentre study (1996). J Antimicrobial Chemotherapy 2000; 46: 133-6.
28. Gales A, Jones R, Turnidge J, Rennie R, Ramphal R. Characterization of *Pseudomonas aeruginosa* isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the global SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis 2001; 32 : S146-S55.
29. Strateva T, Ouzounova-Raykova V, Markova B, Todorova A, Marteva-Proevska Y, Mitov I. Widespread detection of VEB-1-type extended-spectrum beta-lactamases among nosocomial ceftazidime-resistant *Pseudomonas aeruginosa* isolates in Sofia, Bulgaria. J Chemotherapy 2007; 19: 140-5.
30. Sasirekha B, Manasa R, Ramya P, Sneha R. Frequency and antimicrobial sensitivity pattern of extended spectrum β-lactamases producing *E. coli* and *Klebsiella pneumoniae* isolated in a tertiary care hospital. Al Ameen J Med Sci 2010; 3: 265-71.
31. Turner PJ. Meropenem and imipenem activity against *Pseudomonas aeruginosa* isolates from the MYSTIC Program. Diagn Microbiol Infect Dis 2006; 56: 341-4.
32. Chen H, Yuan M, Livermore D. Mechanisms of resistance to β-lactam antibiotics amongst *Pseudomonas aeruginosa* isolates collected in the UK in 1993. J Med Microbiol 1995; 43: 300-9.
33. Cavallo J, Hocquet D, Plesiat P, Fabre R, Roussel-Delvallez M. Susceptibility of *Pseudomonas aeruginosa* to antimicrobials: a 2004 French multicentre hospital study. J Antimicrobial Chemotherapy 2007; 59: 1021-4.
34. Haque R, Salam M. Detection of ESBL producing nosocomial gram negative bacteria from a tertiary care hospital in Bangladesh. Pak J Med Sci 2010; 26: 887-91.
35. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility pattern and ESBL prevalence in *Klebsiella pneumoniae* from urinary tract infections in the North-West of Pakistan. Afr J Microbiol Res 2009; 3: 676-80.
36. Nisha KC, Singh S, Ghosh S. A Study of Metallo beta lactamase Producing *Pseudomonas aeruginosa* in Patients attending D.Y. Patil Hospital and Research Centre, Kolhapur. IOSR J Dental Med Sci 2016; 15: 51-5.