Antimicrobial resistance pattern and phenotypic detection of Extended spectrum beta lactamase- and Metallo beta lactamase- producing *Pseudomonas aeruginosa* isolated from indoor-patients suffering ear discharge

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Abstract

A multidrug-resistant bacterium due to its intrinsic resistance nature and beta-lactamases production, *Pseudomonas aeruginosa* can colonize ubiquitously and is thus associated with life-threating bacterial infections. The study was aimed to inspect phenotypic detection and antimicrobial resistance pattern of extended spectrum beta lactamase- and metallo beta lactamase-producing *P. aeruginosa* strains. Clinical specimens (n=220) were collected from indoor patients with ear discharge were inoculated on suitable culture media. Antimicrobial resistance pattern of all isolates was investigated employing Kirby-Bauer disc diffusion method. Double-disc synergy test and imipenem-EDTA test were used respectively to detect ESBL- and MBL-producing strains. Of the n=220, n=37 (16.82%) clinical specimens confirmed the growth of *P. aeruginosa*. In comparison to female (43.24%), male population (56.76%) was more prevalent. Out of n=37 positive cases, n=16 (43.24%) strains were detected as ESBL-producers, while n=07 (18.92%) as MBL producers. Cefotaxime (100%) was found the most resisted antibiotic by isolates, followed by aztreonam (91.89%), gentamycin (86.49%), ceftriaxone (83.78%) and tazobactam/piperacillin (64.87%), while the lowest resistance was observed against imipenem (21.63%) and meropenem (51.36%). Furthermore, ESBL-producing strains revealed high resistance against cefotaxime (100%), ceftriaxone (93.75%), and carbenicillin (87.5%), while MBL-producing strains were completely resistance to imipenem, meropenem, cefotaxime and carbenicillin, followed by gentamicin (85.71%), amikacin aztreonam, ciprofloxacin and tazobactam/piperacillin (71.43% each). Our study concluded that strains of *P. aeruginosa* producing ESBL and MBL enzymes were mostly resistant to the drugs of choice, which puzzle the physicians to treat infections caused by *P. aeruginosa*. So, it is needed to study the resistant pattern of *P. aeruginosa* in order to recommend proper medication.

Key words: Antimicrobial resistance; ear discharge; ESBL; MBL; *Pseudomonas aeruginosa*

1. Introduction

Pseudomonas aeruginosa is one of opportunistic pathogens mostly associated with life-threating and hospital-acquired bacterial infections linked with immunocompromised patients

(Tewari *et al.*, 2020; Ali *et al.*, 2021). It threats public health around the world and can cause a wide class of infections including otitis media, burn, respiratory, blood and wound infections (Farhan *et al.*, 2019), due to its intrinsic resistance nature to a wide range of antibiotics and capability to acquire resistance either by horizontal gene transfer or mutation (Potron *et al.*, 2015). The rapid emerging resistance in the strains of *P. aeruginosa* against cephalosporin and penicillin threats a serious clinical challenge globally. So, imipenem and meropenem are being used as antipseudomonal agents for the treatment of infections associated with multidrug resistant (MDR) strains of *P. aeruginosa* (Ali *et al.*, 2021).

Strains of *P. aeruginosa* are well known for the production of extended spectrum beta lactamase (ESBL) and metallo beta lactamase (MBL) enzymes (Ali *et al.*, 2020). A wide range recommendation and consumption of third generation cephalosporin (ceftazidime, cefotaxime and ceftriaxone) have led to evaluate a newer class of β-lactamase enzymes i.e., ESBLs. These plasmid-mediated enzymes have potency to hydrolyze monobactam and oxyimino beta lactam antibiotics. Being a plasmid mediated, the genes encoding ESBL can easily be shared with offspring and other microorganisms (Okesola and Oni, 2012). MBL enzymes are plasmid as well as chromosomally mediated in some cases, which can easily hydrolyze almost all beta lactam antimicrobial agents except monobactam antibiotic (Chairat *et al.*, 2019). The current study was aimed to investigate the prevalence, phenotypic detection and antimicrobial resistance pattern of ESBL- and MBL-producing *P. aeruginosa* isolated from ear discharge specimens.

2. Methodology

2.1 Specimens collection and isolation

A total of n=220 ear discharge clinical specimens were collected from the indoor-patients of eye nose throat (ENT) ward of Ayub Medical Complex, Abbottabad, Pakistan within six-months duration from July-December 2019. The collected clinical specimens were inoculated onto blood agar (CM0055-Oxoid UK) and were incubated at 37°C for 20-24 hours. Growth was examined for the colonies of *P. aeruginosa*. All the suspected colonies were purified and confirmed via sub-culturing onto cetrimide agar (CM569-Oxoid UK), a selective medium for *P. aeruginosa*.

2.2 Detection of isolates

After overnight incubation, the grown colonies were confirmed via colonial morphology, growth pigmentation, biochemical activities (urease, oxidase, catalase, tri-sugar iron) and gram staining as recommended by (Okesola and Oni, 2012). The colonies were purified, labelled and were stored at -4°C for future use.

2.3 Antibiotic susceptibility testing

Prior to susceptibility test, all the bacterial isolates were inoculated into capped tubes containing 5ml tryptic soy broth (CM129-Oxoid UK) and were allowed for overnight incubation in hot incubator at 35±2°C. Growth of each sample was adjusted up to 0.5 McFarland index as recommended by CLSI (Clsi, 2016). Specimens were inoculated on Muller Hinton agar (CM0337-Oxoid UK) and different selected antibiotics (Oxoid) including ciprofloxacin, levofloxacin, carbenicillin, ceftriaxone, cefotaxime, ceftazidime, amikacin, gentamicin, meropenem, imipenem, aztreonam, tazobactam/piperacillin, were tested following Kirby-disc diffusion method to investigate susceptibility pattern of isolated strains (Cavallo *et al.*, 2007). After incubation at 35±2°C for 18 hours, the zone of inhibition (ZI) of each antibiotic against bacterial strain was examined, measured and was compared with Kirby-Bauer chart for *P. aeruginosa* (Patel *et al.*, 2015). The antibacterial susceptibility pattern of each isolated strain against each antibiotic was conducted in triplicate and the mean was calculated.

2.4 Phenotypic detection of ESBL-producing strains

ESBL-producing *P. aeruginosa* strains were phenotypically detected using double disc synergy test (DDST) as followed by Ali *et al.* (2020). Discs of third generation cephalosporin (cefixime, ceftriaxone, cefotaxime 30µg each) were placed on Mueller Hinton agar (MHA) at a distance of 25mm center-center from amoxi-clav (amoxicillin-clavulanic acid 20µg-10µg) antibiotic disc as described by Hawser *et al.* (2012). ESBL producing strains were detected by enhancing ZI of any of the third-generation cephalosporin towards amoxi-clav antibiotic disc after overnight incubation at 35±2°C.

2.5 Phenotypic detection of MBL-producing strains

MBL-producing *P. aeruginosa* strains were phenotypically detected via imipenem-EDTA disc method as described by Yong *et al.* (2002). Prior to test, 0.5M EDTA solution was prepared by dissolving 1.861g of EDTA in 10ml distilled water (pH 8.0) and the solution was autoclaved. All the imipenem- and meropenem-resistant strains of *P. aeruginosa* were inoculated on MHA medium. Two imipenem antibiotic discs were subjected at 25mm distance center-center and about 750µg EDTA was loaded to one of them and was labelled. Petri dishes were incubated at 35±2°C for 18-20 hours. After incubation, ZI of both imipenem and imipenem-EDTA were compared. ZI of imipenem-EDTA equal to or greater than 7mm ZI of imipenem without EDTA was considered as MBL-producing *P. aeruginosa*.

2.6 Statistical analysis

The resistance results were analyzed using Microsoft excel 2016 and SPSS version 20.0. The positive frequency of ESBL and MBL producing-*P. aeruginosa* was calculated by age and gender of the patients using Chi-square test (X^2 test) for checking the relation. The mean percentage of resistance of each isolated to all tested antibiotics was calculated as the number of resistance strains out of the total number of strains exposed to a particular antibiotic. The p-value statistically less than 0.05 was considered as significant value.

3. Results

Among n=220, only n=37 (16.82%) clinical specimens confirmed the presence of *P. aeruginosa* whereas, n=183 (83.18%) specimens either did not show or exhibited colonies other than *P. aeruginosa* on culture media. In contrast to female n=16 (43.24%), male population n=21 (56.76%) was more infected by *P. aeruginosa*. The age group 10-19 contributed the highest frequency rate (n=13), followed by the age group 20-29 (n=08). While the lowest frequency rate (n=03) was found in the aged group (≥40). Furthermore, of the n=37 (16.82%) specimens positive for *P. aeruginosa*, n=16 (43.24%) isolated strains were phenotypically detected as ESBL-producing *P. aeruginosa* employing double disc synergy test. Whereas, n=07 (18.92%) isolated strains were phenotypically detected as MBL-producing *P. aeruginosa* by imipenem-EDTA disc method. The highest frequency rate (n=05) of ESBL-producing *P. aeruginosa* was found in the age group 10-19, followed by 20-29 (n=04) and 30-39 (n=03) age groups. The maximum frequency rate (n=03) of MBL-producers was found in the age group 10-19, followed by 0-09 (n=02), though no MBL-producing *P. aeruginosa* was isolated from the aged group (≥40). The two variables, age groups and gender when cross tabulated employing X^2 test (Chi-square test), the p-value (p-value=0.02) found was statistically significant Table 1.

Table 1. Frequency distribution of positive strains, extended spectrum beta lactamase- and metallo beta lactamase-producing *Pseudomonas aeruginosa* and statistical relationship between gender and age groups.

Age group (Years)	**Total frequency (n)**			**Male frequency (n)**			**Female frequency (n)**			***p-value***
	Total Positive	ESBL	MBL	Total Positive	ESBL	MBL	Total Positive	ESBL	MBL	
0-09	06	02	02	04	01	01	02	01	01	
10-19	13	05	03	03	01	01	10	04	02	
20-29	08	04	01	07	03	01	01	01	00	0.020
30-39	07	03	01	04	02	00	03	01	01	
≥40	03	02	00	03	02	00	00	00	00	
Total	37	16	07	21	09	03	16	07	04	

Most of the *P. aeruginosa* isolated strains showed MDR pattern against the routinely used antibiotics. Figure 1 concluded the resistant pattern of the strains of *P. aeruginosa* was varied antibiotic to antibiotic. Moreover, strains of *P. aeruginosa* were 100% resistant to cefotaxime among the third-generation cephalosporin, followed by ceftriaxone (83.78%) and ceftazidime (64.87%). Similarly, aztreonam (91.89%) was found the second most antibiotic resisted by *P. aeruginosa*, followed by gentamycin (86.49%), tazobactam/piperacillin (64.87%), levofloxacin (62.17%) and ciprofloxacin (62.17%). Whereas imipenem (21.63%) and meropenem (51.36%) were found the lowest resisted among all selected antibiotics (Figure 1). Zones of inhibition and its measurement can be seen in Figure 2.

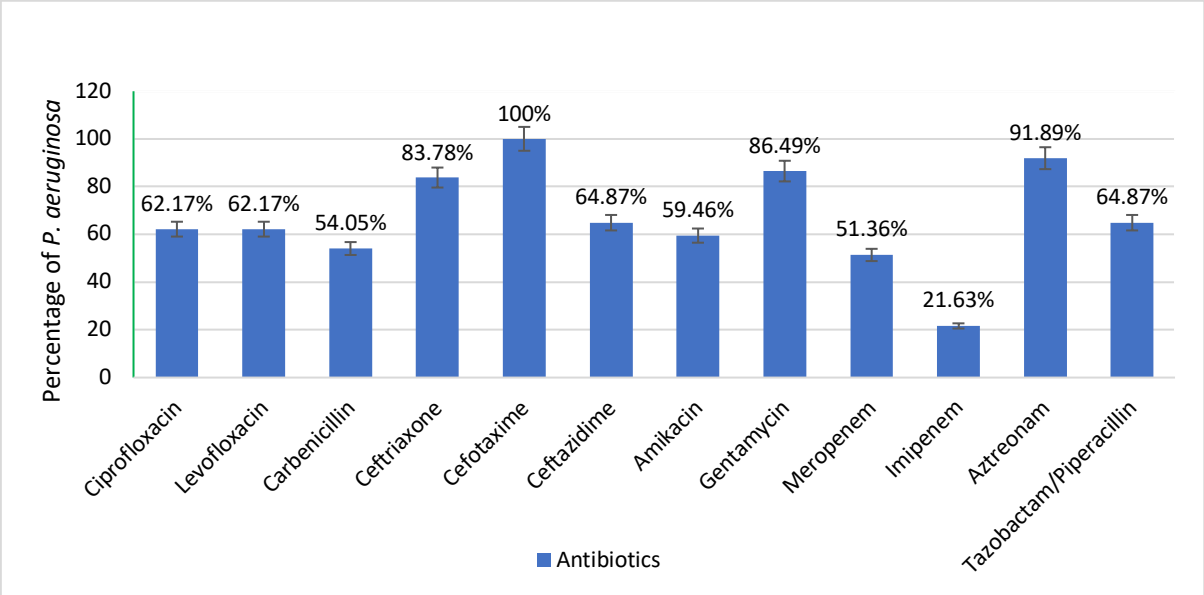

Fig. 1. Antimicrobial resistance pattern of *Pseudomonas aeruginosa* strains isolated from Ear secretion.

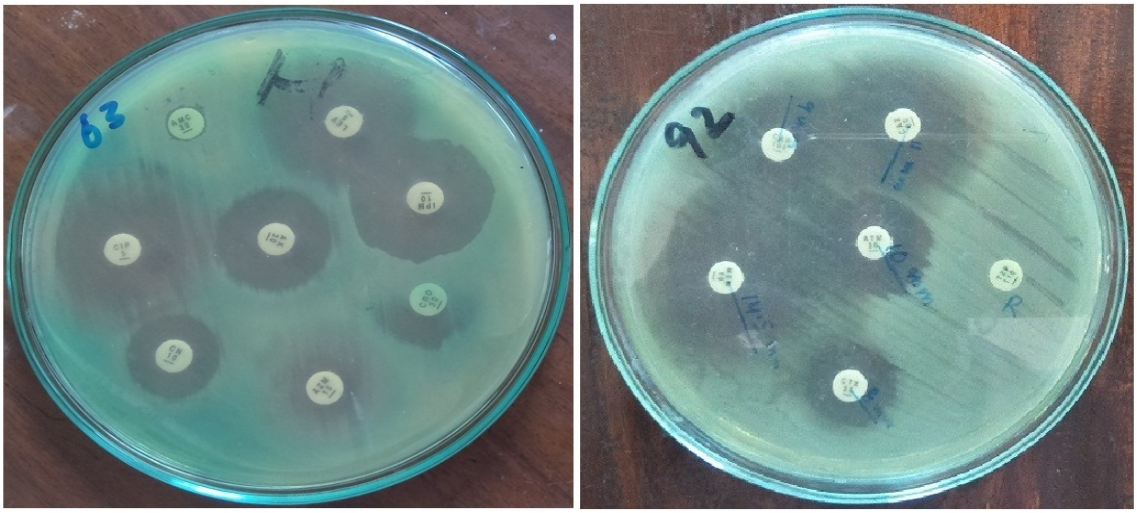

Fig. 2. Zones of inhibition of isolated *Pseudomonas aeruginosa* to antibiotics discs using Mueller Hinton agar.

Strains of ESBL-producing *P. aeruginosa* showed variance in their resistance pattern against the selected antibiotics. Table 2 revealed that these strains exhibited high resistance to cefotaxime (100%, p=0.001), ceftriaxone (93.75%, p=0.002), and carbenicillin (87.5%, p=0.327), followed by gentamicin (81.25%, p=0.795) and aztreonam (81.25%, p=0.599) and amikacin (75.0%, p=0.857). Ceftazidime (p=0.002) and tazobactam/piperacillin (p=0.898) were equally resisted (68.75%), while lowest resistance pattern of isolates was examined against imipenem (12.5%, p=0.001), meropenem (18.75%, p=0.001), ciprofloxacin (18.75%, p=0.015) and levofloxacin (50.0%, p=0.106). Moreover, unlike ESBL-producing strains, MBL-producing strains showed high resistance against the tested antibiotics. The MBL-producing strains found were completely (100%) resistant to imipenem (p=0.001), meropenem (p=0.001), cefotaxime (p=0.001) and carbenicillin (p=0.327), followed by gentamicin

(85.71%, p=0.795). Amikacin (p=0.857), ciprofloxacin (p=0.015), aztreonam (p=0.599) and tazobactam/piperacillin (p=0.898) were equally resisted (71.43%), whereas, ceftriaxone (p=0.002) and ceftazidime (p=0.002) were examined as 100% potent against MBL-producing strains of *P. aeruginosa* (Table 2).

Table 2. Antimicrobial resistance-pattern of extended spectrum beta lactamase- and metallo beta lactamase-producing *Pseudomonas aeruginosa*.

Antibiotics	ESBL-producing *P. aeruginosa* (N=16)		MBL-producing *P. aeruginosa* (N=07)		*p value*
	Sensitive n (%)	Resistance n (%)	Sensitive n (%)	Resistance n (%)	
Ciprofloxacin	13 (81.25)	03 (18.75)	02 (28.57)	05 (71.43)	0.015
Levofloxacin	08 (50.00)	08 (50.00)	06 (85.72)	01 (14.28)	0.106
Carbenicillin	02 (12.50)	14 (87.50)	0 (00.00)	07 (100.0)	0.327
Ceftriaxone	01 (06.25)	15 (93.75)	07 (100.0)	0 (00.00)	0.002
Ceftazidime	05 (31.25)	11 (68.75)	07 (100.0)	0 (00.00)	0.002
Cefotaxime	0 (00.00)	16 (100.0)	0 (00.00)	07 (100.0)	0.001
Amikacin	04 (25.00)	12 (75.00)	02 (28.57)	05 (71.43)	0.857
Gentamycin	03 (18.75)	13 (81.25)	01 (14.28)	06 (85.72)	0.795
Meropenem	13 (81.25)	03 (18.75)	0 (00.00)	07 (100.0)	0.001
Imipenem	14 (87.50)	02 (12.50)	0 (00.00)	07 (100.0)	0.001
Aztreonam	03 (18.75)	13 (81.25)	02 (28.57)	05 (71.43)	0.599
Tazobactam/Piperacillin	05 (31.25)	11 (68.75)	02 (28.57)	05 (71.43)	0.898

Abbreviations: Extended spectrum beta-lactamase (ESBL), Metallo beta-lactamase (MBL), *Pseudomonas aeruginosa* (*P. aeruginosa*)

4. Discussion

Due to the intrinsic resistance nature, ability to acquire resistance from environment and diverse nutrition choice, *P. aeruginosa* can survive everywhere and is mostly linked with the hospital-acquired bacterial infections globally (Gupta *et al.*, 2017; Farhan *et al.*, 2019). Like causing infections in other sites (pus, wound, sputum etc.), *P. aeruginosa* also infects otitis media and causes severe ear secretion (Ali *et al.*, 2020) and is noted as the most common causative agent for otitis media infection (Umar *et al.*, 2016), as examined in the current study. In our study, n=37 (16.82%) of the n=220 total clinical specimens showed colonies for *P. aeruginosa* when inoculated aseptically on suitable culture media, which was similar to that of results reported by the previous study (Umar *et al.*, 2016), which revealed that only 23.2% clinical specimens from ear secretion were positive for the growth of *P. aeruginosa*. Out of 37 (16.82%) positive specimens, male population contributed the most (56.76%) as compare to

female population (43.24%). The previous study also agreed with our finding, where they reported male population was more prevalent in comparison to female (Tewari *et al.*, 2020). It may be possible due to the frequent exposure of male population to environment in the study area. Table 1 also revealed that high frequency rate of *P. aeruginosa* was noted in the age group ranging from 0-39 years. A study conducted in 2016, is also in line with the results reported by the current study (Umar *et al.*, 2016).

It is believed since last decade that ESBL and MBL production spread widely amongst the strains of *P. aeruginosa*. Therefore, these strains resist a wide range of broad-spectrum antibiotics (Farhan *et al.*, 2019; Ali *et al.*, 2020), as indicated in our study. Of the total n=37 strains isolated in the current study, n=16 (43.24%) were phenotypically detected as ESBL-producing strains and n=07 (18.92%) were phenotypically detected as MBL- producing strains, this high frequency cannot be ignored. Moreover, ESBL-producing strains were high in the study area as compare to MBL producing (Table 1). Ahmad *et al.,* reported similar findings for the high production of ESBL and MBL. The study also reported that, in contrast to MBL production, ESBL-producing strains were quite high (Farhan *et al.*, 2019).

Antibacterial resistant pattern of all positive strains showed multidrug resistance pattern to all selected antibiotics as previously reported in 2017 (Gupta *et al.*, 2017). In the current study, the isolated strains highly resisted third-generation cephalosporin (100% cefotaxime, 83.78% ceftriaxone and 64.87% ceftazidime). Ali *et al*., studied the susceptibility pattern of *P. aeruginosa* isolated from hospitals. Their findings showed high resistance pattern of the strains of *P. aeruginosa* to third-generation cephalosporin (Ali *et al.*, 2020). Figure 1 also stated that 91.89% resistance was reported to aztreonam, 86.49% to gentamicin, 64.87% to tazobactam/piperacillin and 62.17% to each levofloxacin and ciprofloxacin. The previous study disagreed with our results and argued that these antibiotics are resisted but not too much (Farhan *et al.*, 2019). The current study showed that carbapenem (imipenem 21.63% and meropenem 51.36%) was the lowest resisted antibiotic amongst all. The potency of carbapenem antibiotics (imipenem and meropenem) against the strains of *P. aeruginosa* was supported by the two studies (Farhan *et al.*, 2019; Ali *et al.*, 2020).

Finding of the current study indicated that strains of ESBL-producing *P. aeruginosa* were vary in their resistant pattern to all tested antibiotics. These strains showed complete resistant against cefotaxime, while high resistance to ceftriaxone (93.75%), carbenicillin (87.5%), gentamicin and aztreonam (81.25 each) and amikacin (75.0%). (Table 2). Similarly, MBL-producing strains of *P. aeruginosa* exhibited complete resistance against carbenicillin, cefotaxime, imipenem and meropenem. They showed high resistance against gentamicin (85.71%), followed by amikacin aztreonam, ciprofloxacin and tazobactam/piperacillin (71.43% each). Some of the previous studies reported similar antimicrobial resistance pattern of ESBL and MBL-producing strains of *P. aeruginosa* against commonly available antibiotics (Gupta *et al.*, 2017; Farhan *et al.*, 2019; Ali *et al.*, 2020). Both Ali *et al*., and Farhan *et al*., reported that MBL- and ESBL-producing strains were highly resistant to cefotaxime, ceftazidime, ceftriaxone, aztreonam, levofloxacin, amikacin, ciprofloxacin, gentamicin and carbenicillin, while imipenem and meropenem were quite potent against these strains.

The emergence of ESBL and MBL mediated resistance and their spread in species of *P. aeruginosa* is a public health concern. Moreover, the distinction in resistance pattern among the species of *P. aeruginosa* isolated from different areas may permit the use of antibiotics, which may lead to horizontal gene transfer and the development of resistance against all potent antibiotics use for the treatment of *P. aeruginosa*. Thereby, it is very important to examine the isolated strains for the antimicrobial susceptibility pattern and the presence ESBL and MBL enzymes prior to antibiotic therapy. While comparing ESBL- and MBL-producing *P. aeruginosa* with each antibiotic statistically, some of the antibiotics showed high significance while others showed insignificance as mentioned in Table 2.

5. Conclusion

The selected antibiotics were initially recommended to treat infections caused by *P. aeruginosa* but finding of our study concluded that due to the wide recommendations, the strains of *P. aeruginosa* emerged high resistance against these commonly used antibiotics. So, the emergence of resistance obliges to investigate the resistance pattern and the presence of ESBL and MBL enzymes in isolated strains in order to formulate antibiotic therapy to treat those strains emerging resistance.

Running title: Phenotypic detection of ESBL- and MBL-producing *P. aeruginosa*.

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References

Ali, F., Kamal, S., Shakeela, Q. & Ahmed, S. (2021) Extended-spectrum and Metallo-beta lactamase enzymes mediated resistance in *Pseudomonas aeruginosa* in clinically isolated specimens. Kuwait Journal of Science, 48(**2**): 1-9.

Ali, F., Niaz, Z., Shah, P.T., Shakeela, Q., Uzma, B. & Ahmed, S. (2020) Antibiogram of ESBL and MBL producing *Pseudomonas aeruginosa* among the population of Hazara Division, KPK, Pakistan. Journal of the Pakistan Medical Association, 70(**11**): 1979-1984.

Cavallo, J., Hocquet, D., Plesiat, P., Fabre, R. & Roussel-Delvallez, M. (2007) Susceptibility of Pseudomonas aeruginosa to antimicrobials: a 2004 French multicentre hospital study. Journal of Antimicrobial Chemotherapy, 59(**5**): 1021-1024.

Chairat, S., Yahia, H.B., Rojo-Bezares, B., Sáenz, Y., Torres, C. & Slama, B.K. (2019) High prevalence of imipenem-resistant and metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in the Burns Hospital in Tunisia: detection of a novel class 1 integron. Journal of Chemotherapy, 31(**3**): 120-126.

Clsi (2016) Performance Standards for Antimicrobial Susceptibility Testing CLSI Document M100S-26 ed, Clinical and Laboratory Standards Institute, Wayne, Pa, USA, 36: 29-50

Farhan, S.M., Ibrahim, R.A., Mahran, K.M., Hetta, H.F. & Abd El-Baky, R.M. (2019) Antimicrobial resistance pattern and molecular genetic distribution of metallo-β-lactamases producing *Pseudomonas aeruginosa* isolated from hospitals in Minia, Egypt. Infection and Drug Resistance, 12: 21-25.

Gupta, R., Malik, A., Rizvi, M., Ahmed, M. & Singh, A. (2017) Epidemiology of multidrug-resistant gram-negative pathogens isolated from ventilator-associated pneumonia in ICU patients. Journal of Global Antimicrobial Resistance, 9: 47-50.

Hawser, S., Bouchillon, S., Lascols, C., Hackel, M., Hoban, D., *et al.* (2012) 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). Clinical Microbiology and Infection, 18(**3**): 253-259.

Okesola, A. & Oni, A. (2012) Occurrence of Extended-Spectrum Beta-Lactamase-Producing *Pseudomonas aeruginosa* Strains in South-West Nigeria. Research Journal of Medical Science, 6(**3**): 93-96.

Patel, J.B., Cockerill, F. & Bradford, P.A. (2015) Performance standards for antimicrobial susceptibility testing: twenty-fifth informational supplement, 1: 29-50.

Potron, A., Poirel, L. & Nordmann, P. (2015) Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. International Journal of Antimicrobial Agents, 45(**6**): 568-585.

Tewari, S., Kumar, A. & Rani, E. (2020) Prevalence of extended-spectrum β-lactamase in *Pseudomonas aeruginosa* in tertiary care centre. Indian Journal of Microbiology Research, 7(**3**): 226-229.

Umar, J.B., Ibrahim, M.M., Tom, I.M., Umoru, A.M. & Isa, T. (2016) *Pseudomonas aeruginosa* in otitis media. International Journal of Medicine, 4(**2**): 55-57.

Yong, D., Lee, K., Yum, J.H., Shin, H.B., Rossolini, G.M. & Chong, Y. (2002) Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas* spp. and Acinetobacter spp. Journal of Clinical Microbiology, 40(**10**): 3798-3801.

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