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Prevalence and Molecular Characterization of Carbapenemase Producing *P. aeruginosa* from Clinical Isolates in Tertiary Care Hospital

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Abstract

Carbapenemases are β-lactamases with hydrolytic capacities and can hydrolyze penicillin, monobactams, carbapenems, and cephalosporins. Bacteria producing these β-lactamases renders many β-lactams ineffective and thus may cause serious infections. This study was designed to investigate the prevalence and molecular characterization of Carbapenemase producing *Pseudomonas aeruginosa* from clinical isolates in tertiary hospital to identify its prevalence. This study was conducted at the department of Microbiology, Microbiology Research Laboratory of Quaid-i-Azam University. Antibiotic susceptibility testing and phenotypic screening for MBLs were performed on 150 *P. aeruginosa* isolates which were collected from department of Microbiology Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad Pakistan. MICs were determined and Carbapenemase genes were sought by PCR. The resistant rate to imipenem was 34.6 %. The resistance rates of *P. aeruginosa* to levofloxacin, ciprofloxacin, tobramycin, Ceftazidime, Meropenam, Aztreonam, Amikacin, cefaperazone/sulbactam and piperacillin/tazobactam were 43.3%, 40.1%, 40.1%, 34.6%, 34.6%, 26.6%, 25.3%, 20.1% and 15.3% respectively. Piperacillin+tazobactum (15.3%) were observed to be the most effective. The point prevalence of Multi-Drug-Resistant (MDR) *P. aeruginosa* showed 56%, The MIC of 35 isolates showed no zone of inhibition under the potential range of E-test strips and thus have MIC > 32 µg/ml showing the high level of resistance to imipenem. Modified Hodge Test (MHT) was used for screening of Carbapenemase which showed 71% (n=37 out of 52) and for Metallo-beta-lactamase (MBL) screening with EDTA it showed 100% positive. PCR was used for the confirmation of 52 positive samples which indicated that 23 (44%) of the isolates were positive for (IMP) gene. A total of 11 isolates (21%) were positive for (VIM) resistant gene while 7 isolates were identified positive for IMP and VIM genes showing a high prevalence of MDR strains.

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Introduction

In the last few decades, antibiotic resistance to pathogenic bacteria; especially among the non-fermenting bacteria, has been one of the alarming issues for the physicians to treat health problems. *Pseudomonas aeruginosa* is a Gram-negative aerobe and falls in the category of "opportunistic pathogens". It has been a common causative pathogen in the health care related infections. *P. aeruginosa* has been reported to cause all-inclusive morbidity and mortality not only in the developed countries but also under-developed countries. *P. aeruginosa* has been a major cause of nosocomial infection in health *facilities* (Vahdani, Azimi *et al.* 2012). *P. aeruginosa* usually causes bacteremia, urinary tract infections, pneumonias in the surgical patients who are on invasive devices (Khan JA 2008). Among the other gram negative bacteria it has been observed that *P. aeruginosa* causes a higher mortality (Yang, Lee *et al.* 2011). Increase in the nosocomial infections due to *P. aeruginosa* multi-drug-resistant (MDR-PA) has been reported in the last two decades (Kohlenberg, Weitzel-Kage *et al.* 2010).

Due to versatility in temperature 42°C, low oxygen conditions, high concentration of salts and antiseptics *P. aeruginosa* has the ability to adapt resistance to several antimicrobial agents persistently in hospital setting. In hospital environment including medical equipment, immunocompromised and seriously ill patients are high at risk (Engel and Balachandran 2009). According to the Centre for Disease Control and Prevention (CDC), (Nosocomial Infection Surveillance System of (USA), it has been drawn in that *P. aeruginosa* causes a variety of nosocomial infections like bacteraemia, urinary tract infections, pneumonia etc (Hirsch and Tam 2010). Infections that occur due to *P. aeruginosa* are usually intense and dangerous to life, because these microorganisms have adopted antimicrobial agent resistance, and secondly there is shortage of development of new drugs. Some of the mechanisms by which *P. aeruginosa* is developing resistance to antibiotics are production of enzymes including metallo- β -lactamases (MBL) and Broad-spectrum β-lactamases, alteration in protein binders of penicillin (PBP), plasmid enzymatic modification, porin mutations, mutation in DNA gyrase and efflux pumps (Mulcahy, Isabella *et al.* 2014). Along with the new generation of antibiotics, the pathogens are acquiring resistance against these antimicrobials. An indiscriminate use of antimicrobial agents is one of the major reasons for the increased resistance of MDR. It has been reported that extensive use of third-generation cephalosporins resulted in various outbreaks of Gram-negative in the world (Kohlenberg, Weitzel-Kage *et al.* 2010). This study aimed to find the prevalence of multidrug resistant *P. aeruginosa* in clinical specimen and the detection of drug resistant genes in its MDR strains through PCR to highlight its risks.

Materials and methods

Study design

Clinical samples were collected "between" November 2014 to March 2015. Total 150 *P. aeruginosa* strains *were* isolated from urine, sputum, pus, blood, wound swabs, endotracheal tube secretions, catheter tips and other body fluids. The sources of these clinical samples were indoor as well as outdoor patients from the Department of Clinical Microbiology of PIMS which is a tertiary care hospital. Out of 150 isolates, 84 were multi drug resistant *P. aeruginosa* (MDRPA) to three or more groups of antibiotics, and the remaining were sensitive. Out of 84 MDRPA, 52 were resistant to Carbapenem which were included for further characterization.

Identification of Isolates

The identification and confirmation of isolates were performed using colony morphology, gram's staining, motility tests and biochemical tests (Oxidase test) the presence of pigments (pyoverdin and pyocyanin), and growth at 42°C.

Pigments Production and Colony Morphology

The strains (pure culture) were streaked on Pseudomonas Cetrimide agar and incubated for 24h at 37°C to analyze the pigment production (pyocyanin, pyoverdin) and colony morphology, adapted from (Jácome, Alves *et al.* 2012).

Antibiotic Susceptibility Test

Antibiotic sensitivity test to a variety of antimicrobial agents was performed for 150 strains of *P. aeruginosa u*sing Mueller-Hinton agar plates by Kirby-Bauer disk diffusion method. According to Clinical Laboratory Standard Institute guidelines (CLSI, 2010). A using sterile swabs, lawn culture of the test strain whose turbidity was adjusted to 0.5 McFarland standards was made on Mueller Hinton agar plate. Ten antibiotic disks were placed aseptically on the inoculated plate by disk diffusion method. Sensitivity tests were carried out for aminoglycosides, fluoroquinolones, carbapenems, β-lactams; tobramycin (10µg), ceftazidime (30µg), amikacin (Ak-30µg), aztreonam (30 µg), ciprofloxacin (5µg) and levofloxacin (5µg), carbapenems such as meropenem (10µg), imipenem (10µg), piperacillin/tazobactam (100/10µg), cefaperazone/sulbactam (30 /10µg), etc.

Phenotypic Detection of Metallo-β-lactamases (MBL)

A preparation of EDTA (0.5 M) solution was carried out by dissolving 186.1 g of disodium EDTA (Sigma Chemicals, St. Louis, MO) in 1000 ml of distilled water was poured on imipenem disk to get a required concentration of 750 µg per disk. The EDTA impregnated antibiotic disks were dried without delay in a drier and stored at −20°C in an air-tight vial until used. McFarland (0.5) equivalent overnight broth inoculation of test strain culture was carried out on a plate of Mueller Hinton agar. One of the 10 µg imipenem disk was positioned on the agar plate. On same agar plate each of EDTA impregnated imipenem disk was also placed. The plates were incubated at 37°C for 16 to 18 h. An increase in the zone size of ≥7 mm around the imipenem-EDTA disk compared to imipenem disk without EDTA was recorded as MBL producing strain (Bashir, Thokar *et al.* 2011).

Phenotypic Detection of Carbapenemase

Modified Hodge Test

The lawn of control *Escherichia coli* strain ATCC 25922 was prepared on MHA plates, the indicator organism *E. coli* ATCC 25922. Sterile swabs were used for the inoculation by dipping into standardized suspension of bacteria at a turbidity of 0.5 McFarland standards. Inoculum was spread uniformly over the whole surface of MHA plates by swabbing back and forth across the agar surface in three directions. The plates were left open and allowed to dry out. Then a 10µg Carbapenem disc was applied in a straight line, from the edge of the disc to the edge of the plate. The Carbapenem resistant test organisms were streaked; on single plate three organisms were tested. At 35˚C for 18 hours the plates were then incubated in an inverted position. The plates were then examined for the zone of inhibition and interpreted for the Carbapenem hydrolysis screening (Lee, Chong *et al.* 2001).

MIC Determination for MBL-Producing P. aeruginosa

Minimum inhibitory concentration of imipenem was checked using E strip (Epsilometer test strips) against imipenem resistant *P. aeruginosa* strains. E test strip also called MIC evaluator, is a measuring system used for the quantitative determination of minimum inhibitory concentration (MIC) of a particular antibiotic against the specific test organism.

The MIC imipenem were determined to *P. aeruginosa* isolates that phenotypically produce MBL using E test the results were interpreted using CLSI criteria for the susceptibility testing (CLSI 2010) *P. aeruginosa* ATCC 27853 was used as the reference strain (Wayne 2006).

Molecular Characterization

Extraction of DNA

Genomic DNA extraction of wild type of *Pseudomonas aeruginosa* from different clinical samples was done by CTAB/NaCl method (Chen and Kuo 1993).

Amplification of Carbapenemase Genes

All the reagents of PCR were provided by Promega Corporation (Madison, USA) and primers (Kalaivani *et al*, 2014) for *bla IMP*, (Yan *et al,* 2001) (Zafer *et al*, 2014) for *bla VIM* and (Camila *et al*,2014) for *KPC* (Table 1) were used. PCR products were analyzed by gel electrophoresis.

Results

Overall 150 bacterial isolates were recovered from different clinical specimen from outdoor and indoor patients who visited or were admitted at PIMS. Colony morphology and various biochemical tests were used to identify these isolates primarily. Of the total 101(67 %) and 49 (33%) isolates were collected from male and female respectively. Out of these 49 (58.3%) were resistant to male and 35 (41.6%) were resistant in female.

Table 1. Primer sequence genes.

Target	Primer sequence Genes	Product length	Source
IMP	FP: CTACCGCAGCAGAGTCTTTGC RP: GACAACCAGTTTTGCCTTACC	640 BP	Kalaivani *et al*, 2014
VIM	FP: TTATGGAGCAGCAACGATGT RP: CAAAAGTCCCGCTCCAACGA	920 BP	Yan *et al*, 2001
KPC	FP: ATGTCACTGTATCGCCGTCT RP: TTTTCAGAGCCTTACTGCCC	893 BP	Camila *et al*,2014 Patricia *et al*, 2004

Prevalence of Multi-Drug Resistant P. aeruginosa Among Patients

Out of total 150 clinical isolates, 84 were identified as multi-drug-resistant *P. aeruginosa* while 66 samples were found to be sensitive, thereby giving hospital-based prevalence of 56 and 44 percent respectively. The distribution is manifested in (Figure 1).

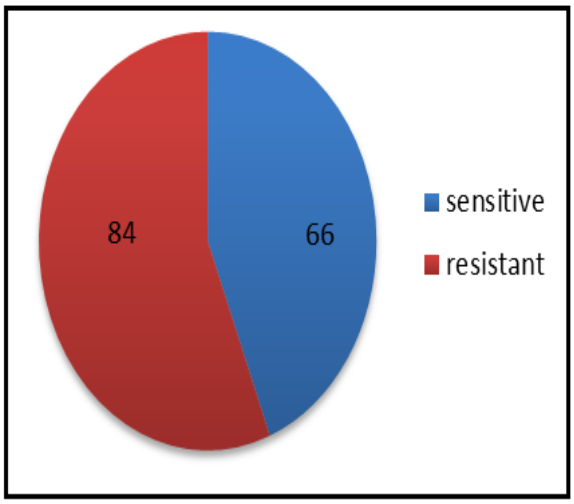

Fig. 1. Prevalence of *P. aeruginosa* multi-drug resistant among patients

Prevalence of Multi-Drug Resistant P. aeruginosa in Different Wards

Prevalence of *P. aeruginosa* at different hospital sites including medical ICU, emergency surgical ICU, Medical ward, ENT, General surgery, General medicine, surgical ward, Neurology, Orthopaedics, Private ward second floor, Dermatology, Cardiology, was observed and was not alike in all the examined hospital sites the highest prevalence was found in M.ICU (n=39, 46.4%) followed by Emergency (n=18, 21.4%) and General medicine (n=6, 7.14%) (Figure 2).

Prevalence of P. aeruginosa Multi-Drug Resistant in Different Specimens

Among the 84 samples of *P. aeruginosa*, the highest prevalence was observed in catheter Tips (n=32, 38.0%) followed by pus (n=22, 26.1%) endotracheal tube secretion (n=19, 22.6%) and blood (n=5, 5.95%) Urine (n=4, 4.76%) (Figure 3).

Antibiotic Resistant Patterns of P. aeruginosa

The following (Figure 4) explains the resistant patterns of *P. aeruginosa* against a variety of antibiotics were determined by disc diffusion method. In current study the highest resistant (n=84, 56%) against fluoroquinolones, Aminoglycosides, carbapenems, monobactams, β-lactam. In aminoglycosides, amikacin (n=38, 25.333%) showed better activity than tobramycin (n=60, 40%).

In fluoroquinolones, levofloxacin showed highest resistance (n=65, 43%) carbapenems (n=52, 34.666), Cephems (n=52, 34%) monobactams (n=40, 26.66%) while β-lactam/ β lactamase inhibitor combination showed a good antibacterial activity (n=23,15.333%). In the present study among all antibiotics used Piperacillin+tazobactam were found to be the most effectual.

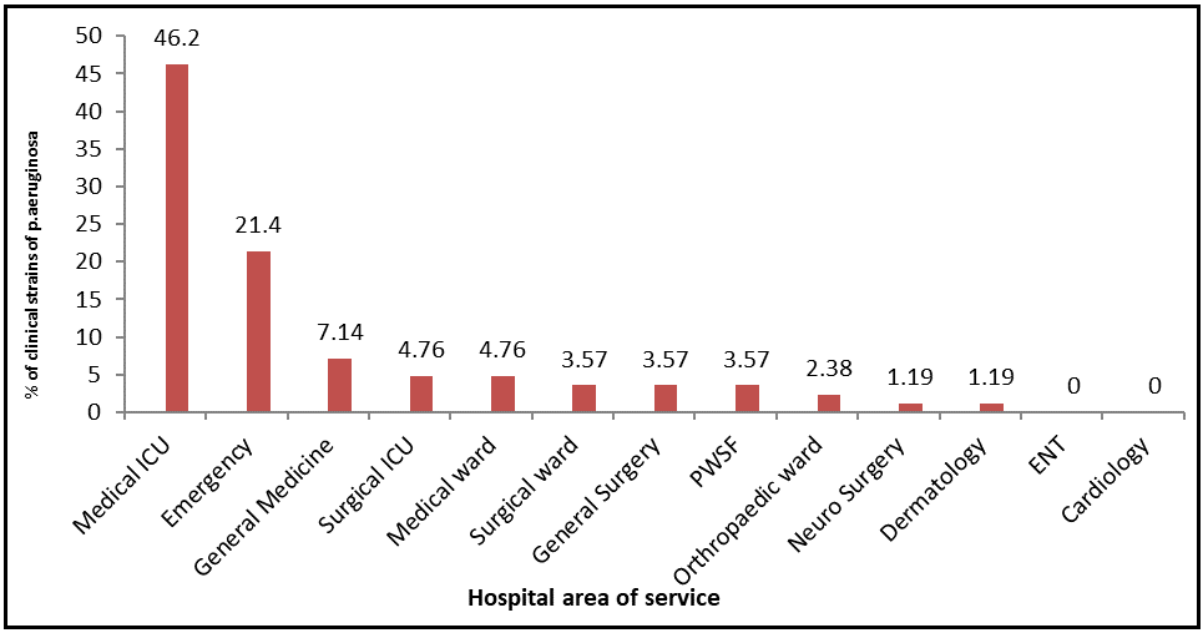

Fig. 2. Prevalence of Multi-Drug Resistant *P. aeruginosa* in different service area of tertiary care hospital.

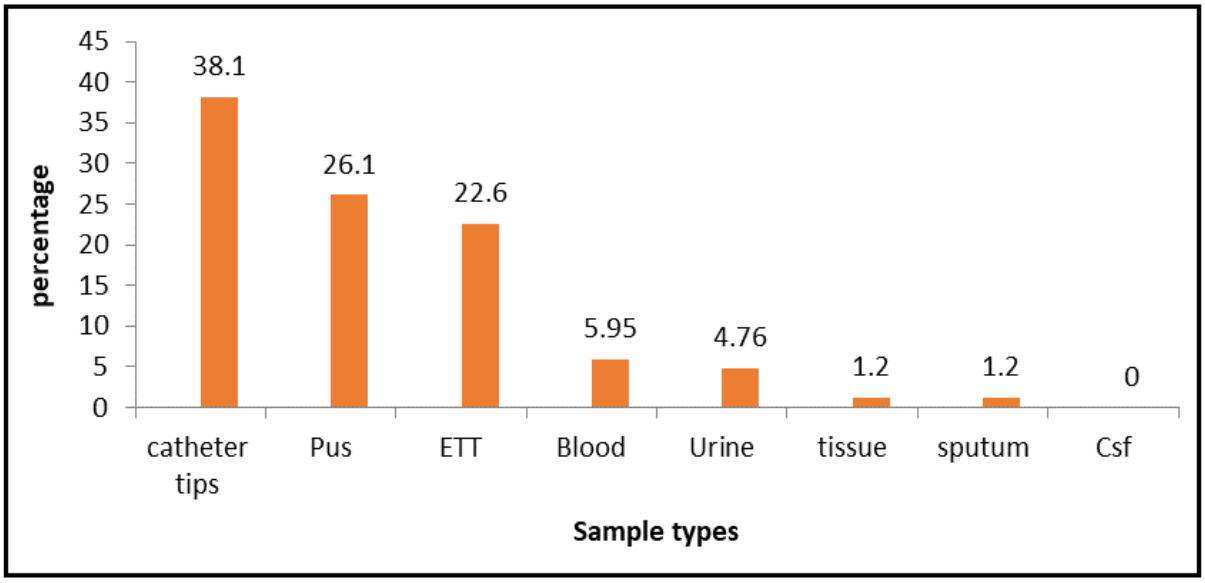

Fig. 3. Prevalence of Multi-Drug Resistant *P. aeruginosa* in different specimens.

Antibiotics Used and Sensitivity to Different Antibiotics

Antibiotics discs were used to perform the antibiotic susceptibility profile for MDR P. Aeruginosa strains. The antibiotics concentration were; Tobramycin (10µg), amikacin (Ak-30µg), aztreonam (30 µg), ciprofloxacin (5µg) and levofloxacin (5µg), carbapenems such as meropenem (10µg), imipenem (10µg), piperacillin/tazobactam (100/10µg), cefaperazone/sulbactam (30 /10µg) ceftazidime (30µg). (Figure 5) shows the resistance/sensitivity among all the examined clinical samples.

Phenotypic Detection of Carbapenemase: Modified Hodge Test

Among the 52 Carbapenem resistant *P. aeruginosa* isolates (n=37, (71.1%) were found to be Carbapenemase producers (Figure 6a).

Phenotypic Detection of Metallo-beta-lactamase

All the 52 isolates of Carbapenem resistant were tested for the production of MBL test.

All isolates belong to the Metallo-beta-lactamase producers 52 (100%) (Figure 6b).

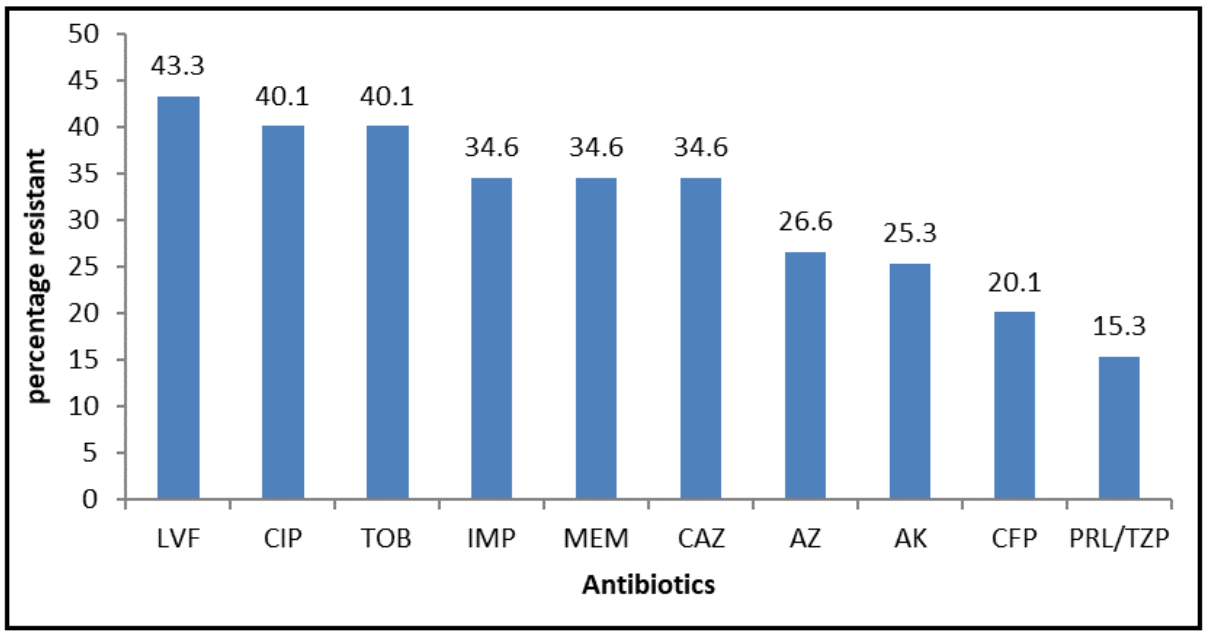

Fig. 4. Antibiotic resistant patterns of *P. aeruginosa*.

Minimum Inhibitory Concentration Determination

For the determination of inhibitory concentration of imipenem, E tests strip (epsilometer test strips) also called MIC. Evaluator, was used with potential range from .002 µg/ml to 32 µg/ml. MIC was determined against 52 *P. aeruginosa* Imipenem resistant clinical isolates. Out of total 52 Carbapenem resistant, 35 isolates gave no zone under the potential range of E test strips and thus have MIC > 32 µg/ml (high level imipenem resistance), 07 isolates have MIC= 1 µg/ml, 06 isolates have MIC= 8 µg/ml, 04 isolates have MIC= 4 µg/ml (Figure 6c).

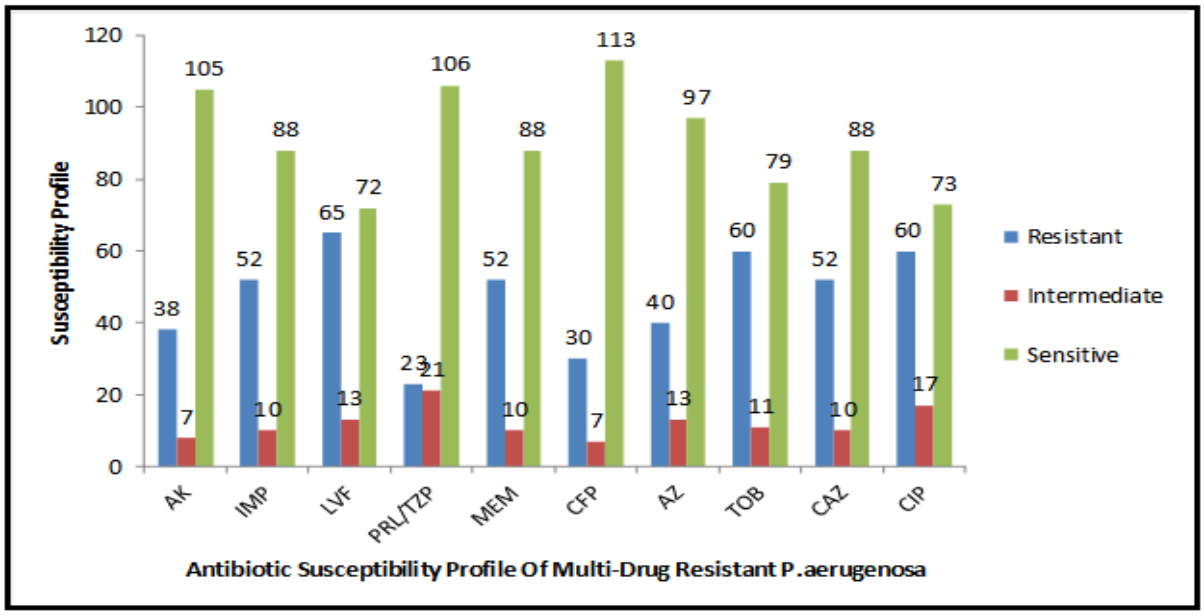

Fig. 5. Resistance/sensitivity among all the examined clinical samples.

PCR Amplification

The extraction of DNA from the *Pseudomonas aeruginosa* from different clinical samples was further conformed for it's used in PCR by amplifying gene responsible for resistance in these isolates of *Pseudomonas aeruginosa*. The Resistant genes VIM, IMP and KPC were analyzed by PCR using their respective set of primers. The PCR products gave an

expected band of about 920 bp from VIM primer, 640 BP IMP (Figure 7) and 893 bp with KPC primer from *Pseudomonas aeruginosa* from different clinical samples. PCR confirmation of 52 positive samples were done which indicate that 23 (44%) of the isolates were positive for IMP gene. A total of 11 isolates (21%) were positive for VIM resistant gene while no isolate was positive for KPC gene. 7 isolates of the 52 were positive both for IMP and VIM genes.

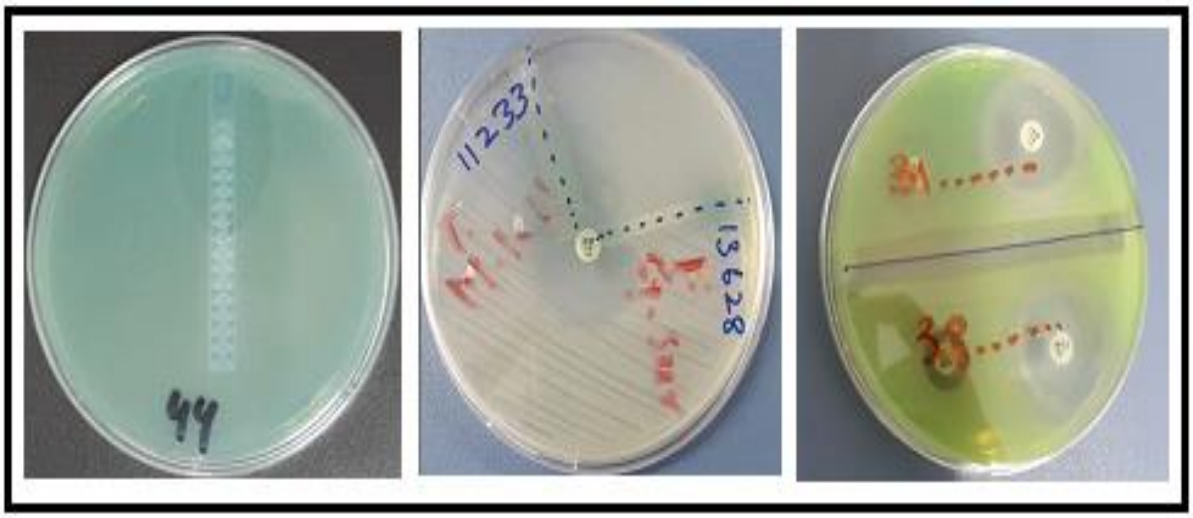

Fig. 6. (a) Positive Carbapenemase producer (b) ESBL MBL producer (c) MIC IMP resistant isolates with clear zone of inhibition.

Discussion

In our study the susceptibility profile of antimicrobials and the prevalence of MDR *P. aeruginosa* were determined to be 56 %. A similar prevalence (49.5%) was reported earlier (Ameen, Memon *et al.* 2015). However, Naqvi *et al* (2005) and Ullah *et al* (2009) demonstrated in their studies the prevalence of MDR *P. aeruginosa* (32%) and (29%) conducted at Karachi and Peshawar respectively. (Naqvi, Hashmi *et al.* 2005) (Ullah, Malik *et al.* 2009). In an another study in Islamabad an incidence of MDR *P. aeruginosa* was reported (22.7%) (Gill, Usman *et al.* 2011).

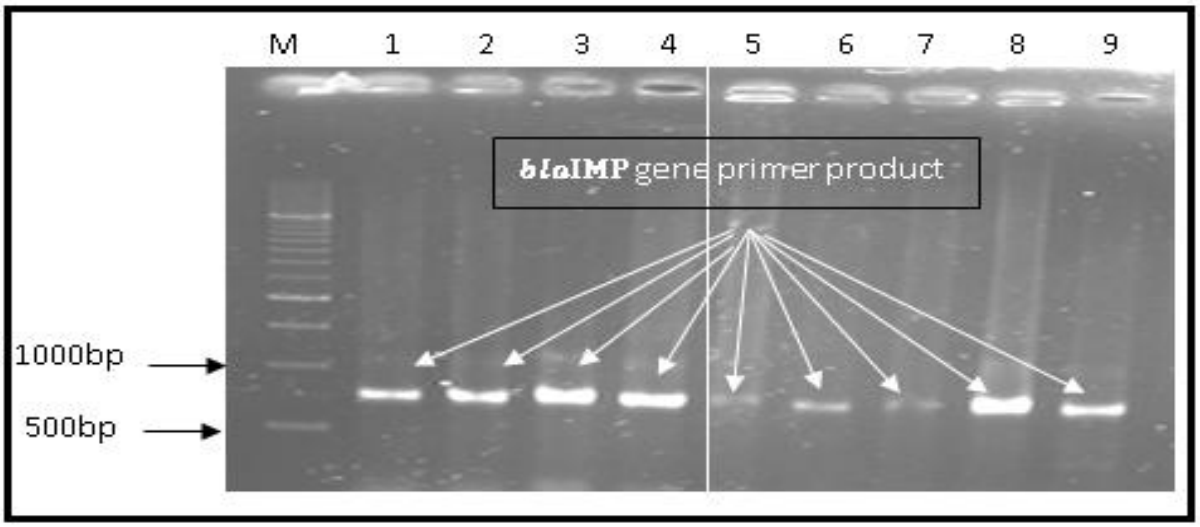

Fig. 7. PCR amplification of *Pseudomonas aeruginosa* genomic DNA with bla IMP primer. Lane 1-9 is *Pseudomonas aeruginosa* DNA amplified with a bla IMP gene primer. M is O'RangeRuler™ 500 bp DNA Ladder (Fermentas Lithuania UAB).

Prevalence of MDR P. aeruginosa

Out of total 150 clinical isolates, collected from different wards and units, 84 were identified as multi-drug-resistant P. aeruginosa, thereby giving hospital-based prevalence of 56 percent. It was observed the infection caused *Pseudomonas aeruginosa* was more prevalent in male (53 %) compared to female (41 %). Ali *et al* (2015) also reported higher prevalence rate in males.(Ali, Mumtaz *et al.* 2015). It was observed that the highest number (46%) of Multi-Drug Resistant *P. Aeruginosa* (MDRPA) was collected from medical ICU followed by emergency (21 %) and surgical (13%) wards. These findings may indicate that the patients in ICU are more prone to nosocomial infections; the reason being that they are more frequently subjected to medical devices such as catheters and blood lines. However, there was no MDRPA observed in ENT and Cardiology.

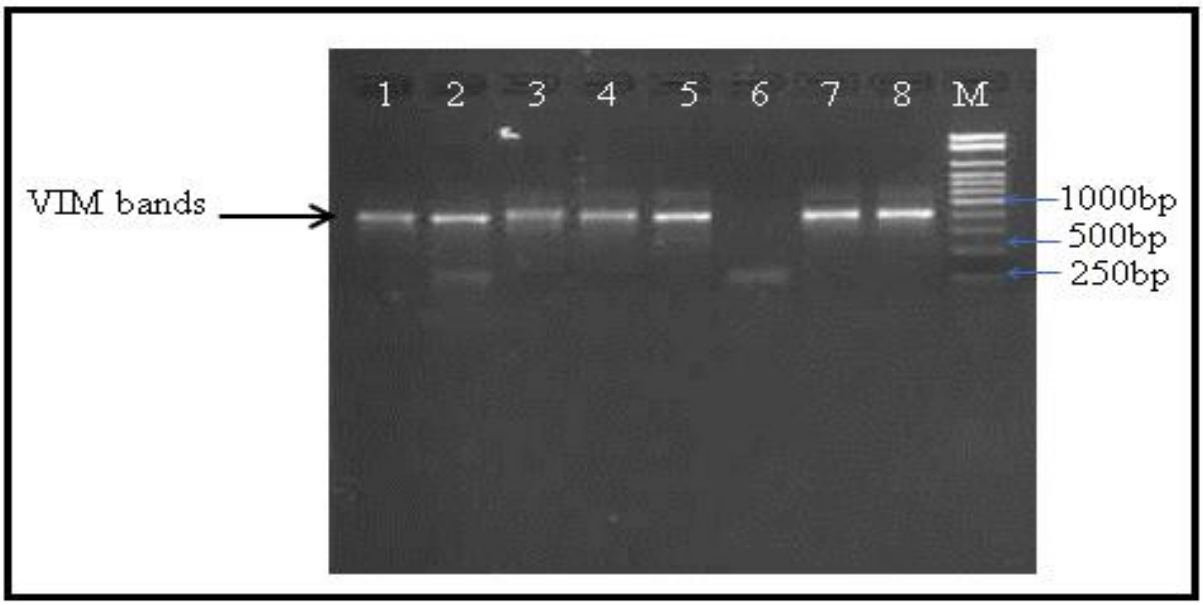

Fig. 8. PCR amplification of *Pseudomonas aeruginosa* genomic DNA with VIM specific primer. Lane 1 – 8 is *Pseudomonas aeruginosa* DNA amplified with a VIM gene primer. M is the DNA Ladder.

Prevalence in hospital equipment

Further, of the 84 *P. Aeruginosa* samples, the highest infection prevalence was observed in catheter tips (38 %) followed by pus (26 %) endo-tracheal tube secretion (22 %) and the lowest was observed in blood (6 %), urine (4.7%), sputum (1.2%) and CSF (0 %) , suggesting that the catheters are one of the main reasons for nosocomial infection. In contrast Gill *et al* (2011) reported in their study conducted at one of the hospitals in Rawalpindi, higher prevalence of *Pseudomonas aeruginosa in* urine (32%), sputum (19.5%) whereas lower in catheter tips (9.7%), blood (2.4%) and pus (19.5%). Among all the antibiotics (Fluoroquinolones, Aminoglycosides, Carbapenems, Monobactams, β-lactam) used in this study. The Fluoroquinolones showed highest resistant (43%) followed by (40%) Carbapenem (34%) and the minimum resistant were observed in (β-lactams) Piperacillin+tazobactam (15 %) showing the most effective among others. However, contrary to our findings (Khan, Khan *et al.* 2014) reported resistant to Fluoroquinolones (26%), Aminoglycosides (30%), Carbapenems (40%) whereas for (β-lactams) Piperacillin+tazobactam reported the highest (56%). It shows that the efficacy of Piperacillin+tazobactam and Carbapenem is decreasing.

Modified Hodge Test

Modified Hodge Test (MHT) was used for screening of Carbapenemase which showed 71% (n=37 out of 52) and for Metallo-beta-lactamase (MBL) screening with EDTA it showed 100% positive (Amudhan, Sekar *et al.* 2012) reported 94% and 80% respectively. By this shows that in the recent past MBL producing isolates have emerged and linked with outbreaks worldwide in health-care settings. They are also

causing serious infections such as bacteraemia and catheter and ventilator associated infections and pneumonia respectively in mostly ICU patients.

Antibiotic susceptibility

The antibiotic susceptibility was additionally confirmed by determining the Minimum Inhibitory concentration (MIC) as recommended by CLSI; where *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as control strains.

The MIC out of total 52 Carbapenem resistant, 35 isolates showed no zone of inhibition under the potential range of E-test strips and thus have MIC > 32 µg/ml showing the high level of resistance of imipenem, 7 isolates showed MIC= 1 µg/ml, 6 isolates MIC= 8 µg/ml, and 4 isolates MIC= 4 µg/ml.

Molecular characterization

In the present study, MDR *P. aeruginosa* isolates showed the resistance to carbapenems such as imipenem (34 %), which were found to be the most effective against MDRPA infections. PCR was used for the confirmation of 52 positive samples which indicated that 23 (44%) of the isolates were positive for (IMP) gene. A total of 11 isolates (21%) were positive for (VIM) resistant gene while no isolate was positive for KPC gene. Seven isolates of the 52 were positive both for IMP and VIM genes. In a similar study conducted in Iran (Doosti, Ramazani *et al.* 2013) showed that 23/41 (56%) carried bla_{VIM} and 10/41 (24.3%) possessed bla_{IMP} gene, showing bla_{VIM} higher than in our results while bla_{IMP} is lower than our results. Therefore, in our study it is indicated that the ratio of bla_{IMP} is higher than others, while the frequency of bla_{VIM} is lower.

Conclusion

From the study the maximum resistance in Levofloxacin (n=65, 43%), Tobramycin (n=60, 40%) Imipenem (n=52, 34%) and the minimum was observed in Piperacillin+tazobactum (n=23, 15%) showing to be the most effective antimicrobial. It was also observed that the genes *bla VIM* and *IMP* were predominantly present among the *IPM* resistant *P. aeruginosa* in our capital and prevalence of *bla IMP* was higher than bla VIM type. The findings of this study showed high prevalence of *P. aeruginosa* strains in some of the wards and units which warrants taking some strategic measurements for the control of bacterial infection in general and more specifically for *P. aeruginosa*. Irrational prescription of antibiotics coupled with non-compliance of patients for their use has been one of the main reasons that we are witnessing for high mortality and morbidity in this part of the region. I would suggest that the aspect of molecular epidemiology may also be explored in future research studies in Pakistan.

References

Ali Z. 2015. "Multi-Drug Resistant Pseudomonas Aeruginosa: A threat of nosocomial infections in tertiary care hospitals." Journal of Pakistan Medical Association **65(12).**

Ameen N. 2015. "Imipenem Resistant Pseudomonas aeruginosa: The fall of the final quarterback." Pakistan journal of medical sciences." **31(3),** 561-570. http://dx.doi.org/10.12669/pjms.313.7372.

Amudhan MS. 2012. "blaIMP and blaVIM mediated carbapenem resistance in Pseudomonas and Acinetobacter species in India." The Journal of Infection in Developing Countries **6(11),** 757-762.

Bashir D. 2011. "Detection of metallo-beta-lactamase (MBL) producing Pseudomonas aeruginosa at a tertiary care hospital in Kashmir." African Journal of Microbiology Research **5(2),** 164-172.

Chen WP, Kuo T. 1993. "A simple and rapid method for the preparation of gram-negative bacterial genomic DNA." Nucleic acids research **21(9),** 2260.

Doosti M. 2013. "Identification and characterization of metallo-β-lactamases producing Pseudomonas aeruginosa clinical isolates in University Hospital from Zanjan Province, Iran." Iranian biomedical journal **17(3),** 129.

Engel J, Balachandran P. 2009. "Role of Pseudomonas aeruginosa type III effectors in disease." Current opinion in microbiology **12(1),** 61-66.

Gill MM. 2011. "Frequency and antibiogram of multi-drug resistant Pseudomonas aeruginosa." J Coll Physicians Surg Pak **21(9),** 531-534.

Hirsch EB, Tam VH. 2010. "Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes."

Jácome PRLDA. 2012. "Phenotypic and molecular characterization of antimicrobial resistance and virulence factors in Pseudomonas aeruginosa clinical isolates from Recife, State of Pernambuco, Brazil." Revista da Sociedade Brasileira de Medicina Tropical **45(6),** 707-712.

Khan F. 2014. "Prevalence and susceptibility pattern of multi drug resistant clinical isolates of Pseudomonas aeruginosa in Karachi." Pakistan journal of medical sciences **30(5),** 951.

Khan JA IZ, Rahman SU, Farzana K, Khan A. Report. 2008. " prevalence and resistance pattern of Pseudomonas aeruginosa against various antibiotics. ." Pak J Pharm Sci.

Kohlenberg A. 2010. "Outbreak of carbapenem-resistant Pseudomonas aeruginosa infection in a surgical intensive care unit." Journal of Hospital Infection **74(4),** 350-357.

Lee K. 2001. "Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of Pseudomonas and Acinetobactet species." Clinical microbiology and infection **7(2),** 88-91.

Mulcahy LR. 2014. "Pseudomonas aeruginosa biofilms in disease." Microbial ecology **68(1),** 1-12.

Naqvi ZA. 2005. "Multidrug resistant Pseudomonas aeruginosa: a nosocomial infection threat in burn patients." Pakistan J Pharma **22(2),** 9-15.

Ullah F. 2009. "Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan." Burns **35(7),** 1020-1025.

Vahdani M. 2012. "Phenotypic screening of extended-spectrum ß-lactamase and metallo-ß-lactamase in multidrug-resistant *Pseudomonas aeruginosa* from infected burns." Annals of burns and fire disasters **25(2),** 78. http://dx.doi.org/10.5897/AJMR2013.6194.

Wayne P. 2006. Clinical and Laboratory Standarts Institute: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow Aerobically, Approved Standart M7-A7, CLSI, USA.

Yang M. 2011. "*Pseudomonas aeruginosa* bacteremia in children over ten consecutive years: analysis of clinical characteristics, risk factors of multi-drug resistance and clinical outcomes." Journal of Korean medical science **26(5),** 612-618.