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BlaOXA-48 Genotypic Detection of Carbapenem Resistance in Isolates of *Pseudomonas Aeruginosa*

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ABSTRACT

Objective: To determine the prevalence of carbapenem resistance in strains of *Pseudomonas aeruginosa* at a molecular level by detecting OXA-48 gene which transcribe for resistance to the antibiotic carbapenem among indoor patients of a tertiary care hospital Karachi.

Study Design and Setting: This observational cross-sectional study was conducted from September 2018 to May 2019 at PNS Shifa hospital of Karachi.

Methodology: Total 140 strains of *Pseudomonas aeruginosa* were received and cultured from pus samples. These samples were collected from different wards like medicine, surgery, burn unit, ICU, ENT, plastic surgery, paedriatic and family ward. Carbapenem resistance was screened phenotypically by AST (Antibiotic susceptibility test), MHT (Modified Hodge test) and mCIM (Modified Carbapenem Inactivation Method) in all samples. Only in resistant cases OXA-48 gene was detected by real time PCR (polymerase chain reaction). Data was analyzed by following the proper loading sequence on product specification sheet. Data was statistically analyzed by SPSS version 23.0. Results were expressed as frequencies (percentages).

Results: Out of 140, 17 (12%) were found to be resistant to carbapenem by AST, 20 (14%) by MHT, 25 (17.8%) by mCIM. Out of 25 resistant cases, 4 (16%) presence of OXA-48 gene by real time PCR were detected.

Conclusion: OXA-48 gene showed 16% carbapenem resistance in this study. *Pseudomonas aeruginosa* is an opportunistic organism which causes multidrug resistance especially in hospitalized patients. Carbapenem is the last resort for serious infections.

Keywords: carbapenem resistance, OXA-48 gene, *Pseudomonas aeruginosa*, real time PCR,

INTRODUCTION:

Antibiotic resistance in microorganisms is the major challenge to global health all over the world. Several factors are involved in the emergence of resistance in microorganisms like *Pseudomonas aeruginosa*, *Acinetobacter bauminnii* and *Enterobacteriaceae* group. The major one is the non-judicious use of antibiotics in human beings and animals. These microorganisms exhibit resistance towards multiple drugs. Initially, few antibiotics were ineffective but resistance towards multiple antibiotics is observed all over the world. For the last twenty years, resistance as a result of β-lactamase enzymes occurred towards cephamycins, cephalosporins, carabapenems and monobactum. New resistance mechanisms are evolving and disseminating all over the world and reduce physician's abilities to treat common infections. Bacteria elaborated new type of β-lactamase; extended spectrum β-lactamase (ESBL) that is a carbapenemase and pan-drug resistance is expected in near future.[1]

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Pseudomonas aeruginosa is an opportunistic and sturdy microorganism that can cause disease in those individuals suffering from debilitating conditions in hospital settings like infections in burn patients and ventilator associated pneumonia. According to CDC (Center for disease control), the elderly, young and sick people are more vulnerable to diseases which are resistant to antibiotics. *Pseudomonas aeruginosa* exhibits both intrinsic and acquired resistance. Intrinsic resistance is caused by over-expressed efflux pumps and reduction of the uptake of antimicrobial drugs. Genotypic resistance is acquired by mutation in genes encoding influx pumps, efflux pumps, penicillin-binding protein and β-lactamases. Bacteria can acquire extensive genetic diversity through acquisition and deletion of genetic elements and this converts non pathogenic form of microorganisms into pathogenic form and plays important role in further dissemination of resistant genes among bacterial population. Antibiotic resistance mediated by β-lactamase is caused by many plasmid borne genes (KPC, IMI, GES, NDM, VIM, GIM and OXA-48) which encode enzymes that inactivate carbapenem.[2]

GENEVA WHO has provided the list of resistant pathogens in 2017. These bacteria are genetically equipped with resistant genes. According to this list, *Pseudomonas aeruginosa*, *Acinetobacterbauminnii and Enterobacteriaceae* exhibited resistance towards carbapenem. Carbapenems like

(meropenem, imipenem, doripenem and ertapenem) are the last option in some debilitating infections caused by this group of bacteria.[3] This drug is capable of treating life threatening infections of extended spectrum β-lactamase producing isolates of *Pseudomonas aeruginosa*. Excessive use of carbapenem has led towards a pattern of resistance. These resistant genes are grouped into different classes.[4] According to Ambler's classification, these are categorized into class A serine beta-lactam (KPC), class B metallo beta lactemases that contain zinc at active site (VIM,IMP,NDM), and class D carbapenemase serine beta-lactam (OXA-48). OXA-48 has ability to hydrolyze carbapenems by breaking its beta-lactam ring.[5] Detection of OXA-48 was first time reported in isolates of Enterobacteriaceae in Turkey *in 2001*.[6] Isolates carrying these genes have been reported all over the world. It was further reported in Europe and Middle East. OXA-48 gene has now also reported in gram negative bacteria in neighboring countries like India, China, Iran and Bangladesh.[7-11] Different phenotypic and molecular methods have been employed to detect carbapenem resistance. Molecular methods are gold standard for detection genotypic resistance. At molecular level genes NDM, KPC, VIP and IMP have been reported in Pakistan, but OXA-48 has not been detected in strains of *Pseudomonas aeruginosa* in our country. This resistant gene was detected with help of real time PCR. It is quantitative test with high level of accuracy, high sensitivity and high specificity.[12]

In this study, we detected bla_{OXA-48} transcribing resistance towards carbapenem 16% in strains of *Pseudomonas aeruginosa* in a tertiary care hospital of Karachi within time period of one year.

METHODOLOGY:

This observational cross sectional, study was conducted at PNS Shifa (tertiary care hospital) Karachi. Sample size was calculated by WHO calculator with prevalence rate 10.2%.[7]140 specimens of *Pseudomonas aeruginosa* were received from different wards of hospital from Jan 2018-Jan2019. This study was approved by both Ethical Review Committee of Bahria University Medical and Dental College and from PNS Shifa. Written consent was obtained from hospitalized patients after briefing the purpose of research work.

The strains of *Pseudomonas aeruginosa* were collected from pus swab of infected site from different body parts. These pus swabs were received at microbiology laboratory from different wards. Repeat samples, out-door patients and patients already on antibiotics were excluded. Samples were collected from several wards like Intensive care unit, Burn unit, Plastic surgery, General surgery, ENT (ear, nose and throat) ward, Paedriatic ward, medicine and family ward. Microorganisms were considered as gram negative *Pseudomonas aeruginosa* on basis of gram staining, blood culture[13], MacConkey agar[14] and oxidase test (Scien cell). Carbapenem like meropenem 10µg (oxoid) and imipenem 10µg (oxoid) susceptibility were checked by antibiotic susceptibility test (disc diffusion method) according to CLSI (clinical laboratory standard institute).[15] Screensing of carbamanimase producing *Pseudomonas aeruginosa* was done by MHT (Modified Hodge Test)[16] and mCIM (Modified carbapenemase inactivation method)[17].

Genotypic resistance of *Pseudomonas aeruginosa* was confirmed by detecting bla_{OXA-48} gene with help of real time PCR (polymerase chain reaction) as per Figure 1. Microbial DNA was extracted from culture of up to $2X10^9$ bacterial cells with help of Qiagen Medical DNA qPCR assay kit CATALOG NO 330033 BBXX-#####X (R/F) 330043: BBID-####Z (R/F)-3/4/5/6/7/America. Homogenous mixture was prepared by adding 400µl ATL buffer after vertexing at 14,000Xg speed. Mixture was lysed with 40µl proteinase K and vertexed for 10 seconds andIncubated at 56?C for 10 minutes. Buffer APL2 200ml was put into mixture, this mixture was vertexed for 30 seconds and incubated at 70?C for 10 minutes. Then it was spinned in order to remove the drops. Precipitation was done by adding 300µl ethanol and mixed by vertexing for 15-30 minutes. After passing through vacuum pump, the lysate was added into the tube extender of the QIA amp UCP Mini column. Whenever all lysate had been removed through the columns, its pressure was released to 0mbar by switching off the vacuum pump. 750µl Buffer APW2 was added to mini column of QIA amp UCP and pressure was released by switching off the vacuum pump. 2ml collection tube held the mini column and centrifuged at 20,000xg; 14,000 rpm for 3 minutes. Mini column was kept in into a new 2ml collection tube with open lid and incubated at 56?C for 3 minutes. Mini column was shifted from collection tube to 1.5ml elution tube. 20-100µl Buffer AVE was used at the center of mini membrane. After incubated at room temperature for a minute, it was centrifuged at 20,000xg; 14,000rpm for elution of DNA. Primers were available in Qiagen kit as per Table 1. OD (optical density) of extracted DNA was confirmed from Dow University of Health Sciences as per Table 2.

Real time PCR was performed for detection of resistant gene. Reaction mixture was arranged with DNA positive control (OXA-48), sample and negative template control .25µl reaction mixes were poured into PCR wells.20µl reaction mixes was added as per well only for Rotor-Gene. Data was analyzed by following the proper loading sequence on product specification sheet. The real time thermal cycler was used. Calculation of thermal cycle (C_T) was done for each cycle with help of cycler's software. Threshold 0.02 was considered as standard. The values of threshold cycles were shifted for each well to Excel spread sheet.

Data was statistically analyzed by SPSS version 23.0. Results were expressed as frequencies (percentages).

REULTS:

Among the total 140 samples of *Pseudomonas aeruginosa*, 17 cases (12%) exhibited resistance towards carbapenem by (AST method), 20 (14%) resistant cases by MHT and 25 (17.9%) cases by mCIM. These 25 resistant cases were further evaluated for detection of resistant gene OXA-48 as per Figure 2. We detected OXA-48(n=4) (16%) resistant genes, in strains of *Pseudomonas aeruginosa*.

Table 1: Primers for detection of OXA-48 in *Pseudomonas aeruginosa*

Targeted Gene	Kit Name	Primer Name	Sequence (5'-3' Direction)	Length Bases	Amplicon Size, bp	Tem in C?	Primer conc, pmol/µl
OXA-48	Qiagen Microbial DNA qPCR Assay Kit	Reverse	ACGACGGCATAGTCATTTGC	20	585 or 597	56	15pmo
		Forward	AACGGGCGAACCAAGCATTTT	21			

Table 2: Confirmation of extracted DNA (Optical density)

Samples	Yield
1	54.90g/µl
2	45.81g/µl
3	93.66g/µl
4	67.30g/µl
5	89.43g/µl
6	185.70g/µl
7	288.67g/µl
8	82.10g/µl

Figure 1: Cumulative result OXA-48 positive cases by real time PCR

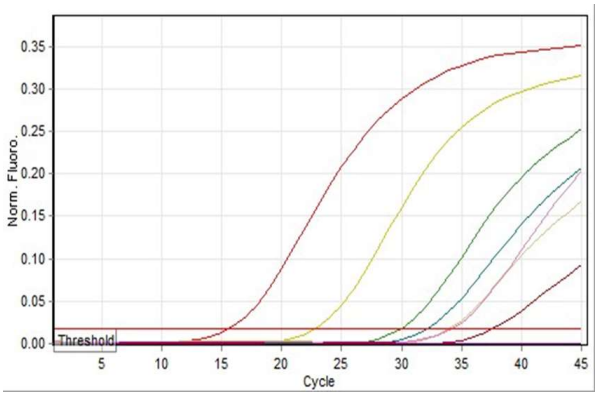
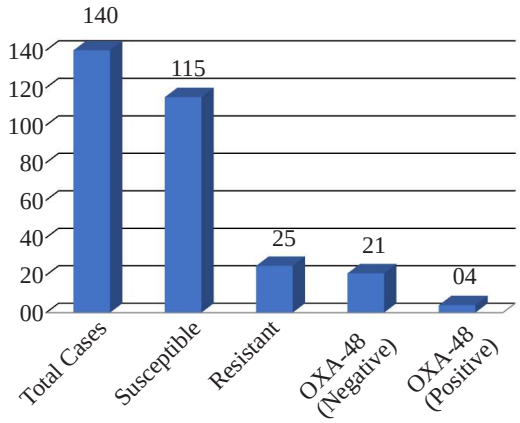


Fig 2 OXA-48 TRANSCRIBING RESISTANCE



DISCUSSION:

Pseudomonas aeruginosa is normal flora of human beings but whenever it gets opportunity it will become pathogenic and cause nosocomial infections. These infections are caused by imprudent utilization of antibiotics, surgical intervention and use of equipments. The resistance that is displayed by microorganisms towards multiple antibiotics can be both intrinsic and acquired or extrinsic. Resistant nosocomial infections do not respond to conventional antibiotics, and have for the last ten years inculcated a fear among health care facilitators.[18]

There are many phenotypic and genotypic methods for detection of carbapenamase producing *Pseudomonas aeruginosa*. Phenotypic methods are AST (Antibiotic sensitivity test), CDDT (combination disk diffusion test), MHT (Modified Hodge Test), mCIM (Modified carbapenem inactivation method), and genotypic methods are PCR amplification, MALDI TOFF (Matrix assisted laserdesorption / ionization-time of flight), real timePCR.[19] Carbapenem resistance towards *Pseudomonas aeruginosa*by phenotypic methods AST n=17 (12%) were detected according to CLSI (Clinical and laboratory standard institutes), MHT n=20 (14%) and mCIM n=25 (17.8%). Abbas et al exhibited carbapenem resistance 9.3% in isolates of *Pseudomonasaeruginosa* detected by AST which was higher as compared to our study.[20] Different studies described mCIM as a simple, accurate and reliable method for detection of carbapenemare in accordance with our study.[21-23] Phenotypic methods are screening methods. Genotypic methods are used for detection of multiple resistant genes. Multiple genes are involved in resistance like KPC, VIM, IMP, SIM, GES and NDM. In our study we only detected the presence of OXA-48 n=4 (16%) by using real time PCR. OXA-48 has been reported in different parts of the world. First OXA-48 gene was detected in Istanbul, Turkey. It has been found in African countries, Middle East, China, Afghanistan and India.[24] Our findings are in accordance with other studies like Bonnin et al recommended real time PCR as highly accurate and specific method.[25] This study focused on bla_{OXA-48} as it has not been found in strains of *Pseudomonas aeruginosa* in previous studies in Pakistan, while it is present in other neighbouring countries. Begum and Shamsuz zaman (2016) detected OXA-48 20% in Dhaka.[26] There is threat of

dissemination of this gene through horizontal gene transfer as Bangladesh is our neighboring country. According to study in Sudan Mohamed et al, OXA-48 exhibited resistance towards *Pseudomonas aeruginosa* was 22.4% which is quite close to our study.[27] Van der zee et al (2014) detected carbapenem resistant gene OXA-48 by real time PCR as accurate and reliable method in association with our study.[28]

Advances in diagnostic technologies have transformed the scenario of alleviating life threatening infections and have played pivotal role in contribution towards human health. It is the lack of motivation and interest of stake holders that now under minesserious problems such as nosocomial infections and antibiotic resistance by microorganisms like *Pseudomonasaeruginosa*. Although it is possible to overcome all these fatal infections through strict surveillance of resistant isolates economic problem in a resource poor country like ours hinders progress and allows dissemination of resistant genes. Hence Â-lactamases are transcribed by various genes like VIM, IMP, KPC, and so on. These genes are transferred from resistant strains to sensitive strains and have been reported in Pakistan. OXA-48 which transcribes class D was not previously reported in strains of *Pseudomonas aeruginosa* in Pakistan.

The foremost limitation of our study is that results cannot be generalized as we took data only from one Military set up where the subject population does not match the demograph of our country. OXA-48 gene showed 16% carbapenem resistance because of small sample size. Our study included only hospitalized patients. There are multiple carbapenemase genes and their allelic variants we looked for only OXA-48 gene using uniplex primers.

CONCLUSION:

Carbapenem resistance in strains of *Pseudomonas aeruginosa* due to the gene bla_{OXA-48} is accounted for 16% of tested cases, microorganisms can acquire extensive genetic diversity through acquisition of resistant genes and this converts a non pathogenic bacteria into pathogenic, which can disseminate at high rate, creating an antibiotic resistance crisis. Detection of these cases and establishing surveillance programs for control of antibiotic resistance will go a long way in resolving this problem.

REFERENCES:

1. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics. 2015; 40(4):277.
2. Chang HH, Cohen T, Grad YH, Hanage WP, O'Brien TF, Lipsitch M. Origin and proliferation of multiple-drug resistance in bacterial pathogens. Microbiol. Mol. Biol. Rev.. 2015;79(1):101-16.
3. World Health Organization. Implementation manual to prevent and control the spread of carbapenem-resistant organisms at the national and health care facility level: interim practical manual supporting implementation of the Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, Acinetobacter baumannii and Pseudomonas aeruginosa in health care facilities. World Health Organization; 2019.
4. Zanganeh Z, Eftekhar F. Correlation of oxacillinase gene carriage with the genetic fingerprints of imipenem-resistant clinical isolates of Acinetobacter baumannii. JJM. 2015 8(9).
5. Codjoe FS, Donkor ES. Carbapenem resistance: a rev. Med Sci. 2018; 6(1):1.
6. Aktaº Z, Kayacan ÇB, Schneider I, Can B, Midilli K, Bauernfeind A. Carbapenem-hydrolyzing oxacillinase, OXA-48, persists in Klebsiellapneumoniae in Istanbul, Turkey. Chemotherapy. 2008; 54(2):101-6.
7. Kazi M, Khot R, Shetty A, Rodrigues C. Rapid detection of the commonly encountered carbapenemases (New Delhi metallo-β-lactamase, OXA-48/181) directly from various clinical samples using multiplex real-time polymerase chain reaction assay. IJMS. 2018 1;36(3):369.
8. Mohanty S, Maurya V, Gaind R, Deb M. Phenotypic characterization and colistin susceptibilities of carbapenem-resistant of Pseudomonas aeruginosa and Acinetobacter spp. The Journal of Infection in Developing Countries. 2013; 7(11):880-7.
9. Guo L, An J, Ma Y, Ye L, Luo Y, Tao C, Yang J. Nosocomial outbreak of OXA-48-producing Klebsiellapneumoniae in a Chinese hospital: clonal transmission of ST147 and ST383. PLoS One. 2016; 11(8):e0160754.
10. Moghadampour M, Rezaei A, Faghri J. The emergence of bla OXA-48 and bla NDM among ESBL-producing Klebsiellapneumoniae in clinical isolates of a tertiary hospital in Iran. ActamicrobiologicaetimmunologicaHungarica. 2018; 65(3):335-44.
11. Khatun R, Shamsuzzaman SM. Detection of OXA-181/OXA-48 carbapenemase producing Enterobacteriaceae in Bangladesh. Ibrahim Medical College Journal. 2015;9(2):45-51.
12. Bakthavatchalam YD, Anandan S, Veeraraghavan B. Laboratory detection and clinical implication of oxacillinase-48 like carbapenemase: the hidden threat. Journal of global infectious diseases. 2016; 8(1):41.
13. Golle A, Janezic S, Rupnik M. Low overlap between carbapenem resistant Pseudomonas aeruginosa genotypes isolated from hospitalized patients and wastewater treatment plants. PloS one. 2017; 12(10):e0186736.
14. Al-Ahmadi GJ, Roodsari RZ. Fast and specific detection of Pseudomonas Aeruginosa from other pseudomonas species by PCR. Annals of burns and fire disasters. 2016 31; 29(4):264.
15. Wenzler E. Feature Article Applying Fluoroquinolone Pharmacokinetics, Pharmacodynamics, and Updated Clinical Breakpoints for Gram-Negative Pathogens to Determine Optimal Dosing. CLSI AST News Update.4 2019.
16. Song W, Hong SG, Yong D, Jeong SH, Kim HS, et al. Combined use of the modified hodge test and carbapenemase inhibition test for detection of carbapenemase-producing Enterobacteriaceae and metallo-β-lactamase-producing Pseudomonas spp. Annals of laboratory medicine. 2015; 35(2):212-9.
17. Walthall K, Anderson K, Reese N, Lonsway D, KamileRasheed J, Karlsson M. Evaluation of the RAPIDEC CARBA NP, Conventional CarbaNP, and the Modified Carbapenem Inactivation Method (mCIM) Tests for Phenotypic Detection of Carbapenemase-Producing Organisms. American Journal of Clinical Pathology. 2018; 150(suppl_1):S123-4.

18. Judd WR, Ratliff PD, Hickson RP, Stephens DM, Kennedy CA. Clinical and economic impact of meropenem resistance in Pseudomonas aeruginosa–infected patients. American journal of infection control. 2016; 44(11):1275-9.

19. Buehrle DJ, Shields RK, Clarke LG, et al. Carbapenem-resistant Pseudomonas aeruginosa bacteremia: risk factors for mortality and microbiologic treatment failure. Antimicrob agents and chemother. 2017; 61(1):e01243-16.

20. Abbas SH, Naeem M, Adil M, Naz SM, Khan A, Khan MU. Sensitivity patterns of Pseudomonas aeruginosa isolates obtained from clinical specimens in Peshawar. Journal of Ayub Medical College Abbottabad. 2015 ;27(2):329-32.

21. Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, et al. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. Journal of clinical microbiology. 2017; 55(8):2321-33.

22. Sfeir MM, Satlin MJ, Fauntleroy KA, et al. Blood-Modified Carbapenem Inactivation Method (Blood-mCIM): a Phenotypic Method for Detecting Carbapenemase-Producing Enterobacteriaceae Directly from Positive Blood Culture Broths. Journal of clinic microb. 2019.

23. Pawar S, Mohite ST, Datkhile K, et al. Closing the Gap Between Phenotypic and Genotypic Detection of Carbapenem Resistant Enterobacteriaceae by New Modified Carbapenem Inactivation Method. J of Clin and Diag. Res. 2018; 12(11).

24. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals of clinical microbiology and antimicrobials. 2016;15(1):20.

25. Bonnin RA, Bogaerts P, Girlich D, Huang TD, Dortet L, et al. Molecular characterization of OXA-198 carbapenemase-producing Pseudomonas aeruginosa clinical isolates. Antimicrobial agents and chemotherapy. 2018; 62(6):e02496-17.

26. Begum N, Shamsuzzaman SM. Emergence of carbapenemase-producing urinary isolates at a tertiary care hospital in Dhaka, Bangladesh. Tzu Chi Medical Journal. 2016; 28(3):94-8.

27. Mohamed SE, Alobied A, Hussien WM, Saeed MI. blaOXA-48 carbapenem resistant Pseudomonas aeruginosa clinical isolates in Sudan. Journal of Advances in Microbiology. 2018:1-5.

28. Van der Zee A, Roorda L, Bosman G, Fluit AC, et al. Multi-centre evaluation of real-time multiplex PCR for detection of carbapenemase genes OXA-48, VIM, IMP, NDM and KPC. BMC infectious diseases. 2014; 14(1):27.