Genotypic Comparison of OXA-23 and OXA-48 in Carbapenem Resistant vs. Carbapenem Susceptible Gram Negative Bacteria

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Abstract

Objective: To compare the distribution of OXA-23 and OXA-48 genes in Carbapenem resistant vs. Carbapenem susceptible gram negative bacteria by PCR.

Material and Methods: It is a comparative cross sectional study conducted in Pathology Department, King Edward Medical University Lahore. A total of 76 samples were collected, of which 38 were Carbapenem susceptible and 38 were Carbapenem resistant Gram negative bacteria. Samples were cultured on Blood and MacConkey agar followed by biochemical testing. Phenotypic Carbapenem susceptibility was evaluated, and finally, all samples underwent Polymerase Chain Reaction (PCR) for detection of OXA-23 and OXA-48 genes.

Results: In this study OXA-23 gene was isolated in 22 (28.9%) samples and OXA-48 gene was isolated in 5 (6.6%) samples only. OXA-23 gene was found only in Carbapenem resistant samples. However, among Carbapenem susceptible samples no OXA-23 gene was isolated i.e. p-value<0.001. Similarly, OXA-48 gene was detected among Carbapenem resistant samples and no OXA-48 gene was found in Carbapenem sensitive samples i.e. p-value=0.021.

Conclusion: Results of this study show a higher frequency of OXA-23 gene as compared to OXA-48 genes in Carbapenem resistant gram negative bacteria. More importantly, none of the Carbapenem susceptible samples had OXA-23 or OXA-48 genes. Meaning that the phenotypic response of bacteria to Carbapenems was in correspondence with the genotypic results; indicating the reliability of disc diffusion method to determine Carbapenem resistance in patient samples. This can be very helpful for clinicians for prudent usage of antibiotics and controlling antimicrobial resistance.

Keywords: OXA-23, OXA-48, Carbapenemase, Antimicrobial resistance, PCR

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Introduction

Internationally, antimicrobial resistance (AMR) is emerging as a chronic public health problem. Number of deaths per year is expected to reach 10 million deaths by 2050 due to AMR. A study conducted in UK showed that the rate of AMR increased by 10% in just one year.[1,2]

In Pakistan, the situation is also alarming. One study done in Pakistan in 2023 proved that a majority of the respiratory tract infections were being caused by multi-drug resistant gram negative bacteria, such as Klebsiella, Pseudomonas etc.[3] Moreover, to add on to this; another study published from Services Hospital in 2020 showed that one in every 3 species of clinically isolated gram negative bacteria is in fact Carbapenem resistant.[4]

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For this reason, Carbapenems are the newest class of beta lactam antibiotics that are being utilized widely nowadays. Since they are somewhat resistant to most β-lactamases, they are one of the most effective antibiotics. In fact, with high mortality rates and scarcity of effective antibiotic treatments, the spread of carbapenem-resistant bacteria immediately becomes a global health emergency.[5,6] As is known, most gram-negative bacteria are resistant to beta lactam antibiotics because they produce β-lactamases that inactivate them. There are four distinct categories of β-lactamases, designated as A, B, C, and D. Class D is also known as OXA enzymes and play a crucial role in antimicrobial resistance.[7]

The OXA-23 gene was the first to be identified as encoding a carbapenem hydrolyzing Class D Lactamase (CHDL), and it is commonly associated with CRAB or carbapenem resistant Acinetobacter Baumanni throughout Asia.[8]

There has been a dramatic rise in the number of OXA-48 carbapenemase producing enterobacteriaceae in Asia during the past decade.[9] Some isolates with in-vitro susceptibility to carbapenems have been shown to produce OXA-48 enzymes. Unfortunately, the therapeutic efficacy of carbapenems against carbapenemases producing bacteria may not be accurately predicted by the current phenotypic methods of detecting carbapenem sensitivity. Therefore, it is crucial to understand the prevalence of OXA enzymes in a given region.[10] The goal of the study is to understand Carbapenem resistance in Pakistan, and compare its phenotypic detection with its genotype OXA genes, in order to interpret therapeutic failures using carbapenems and improve antimicrobial resistance in Pakistan.

Material and Methods

It is a comparative cross sectional study conducted in Pathology Department, King Edward Medical University Lahore. Ethical approval was received from Institutional Review Board under No.150/RC/KEMU dated 10-02-2020. The study was conducted from June 2020 to December 2020. Sample collection was done after ethical approval. Gram negative bacteria susceptible and resistant to Carbapenems (Imipenem, Meropenem or Ertapenem) were included in this study that were randomly collected. Any other microorganisms that were not Gram negative bacteria were excluded. A total of 76 samples were collected from KEMU's central diagnostic lab, out of which 38 were Carbapenem susceptible and 38 were Carbapenem resistant Gram negative bacteria. Samples were first cultured on Blood and MacConkey agar. Biochemical tests, including TSI, citrate, urease and indole were run for identification. Phenotypic detection of Carbapenem susceptibility was done using Kirby-Bauer disc diffusion method. Zone diameters were referenced according to Clinical & Laboratory Standards Institute guidelines 2019. Polymerase Chain Reaction (PCR) was performed on all isolates. DNA extraction was done using TRIzol reagent, followed by amplification of OXA-23 and OXA-48 genes using gene-specific primers, and lastly gel electrophoresis was carried out to identify the amplicons. Comparative analysis of data including Chi-square test was done using SPSS version 26.

Results

In this study, patients had a mean age of 42.10 (SD ±14.76) years old. The ages of our patients ranged from 15 to 80. There were 57 male patients (75%) and 19 female patients (25%). 35 patients were from emergency department (46.1%), 21 from the surgical department (26.6%), 8 from the outpatient department (10.5%), 3 from the medical department (3.9%), and 9 from the intensive care unit (11.8%). Pseudomonas aeruginosa was isolated in 25 samples (32.9%), followed by Escherichia coli from 22 samples (28.9%), Acinetobacter baumannii in 14 samples (18.4%). (Table 1) In a test for susceptibility to Carbapenem antibiotics, 50% of the samples were found sensitive and 50% were resistant. The Carbapenem drugs tested were Imipenem (6%) and Meropenem (92%). Out of total 76 samples OXA-23 gene was isolated in 22 (28.9%) samples; whereas OXA-48 gene was isolated in 5 (6.6%) samples only. Bands of both genes were recovered on 1% Agarose Gel Electrophoresis. (Figure 1) Moreover, both genes were only present in Carbapenem resistant samples. Prevalence of OXA-23 genes was approximately 5 times higher than that of OXA-48 among Carbapenem resistant samples, and no OXA-23 gene was isolated among Carbapenem susceptible samples, p-value<0.001. (Table 2) Similarly, frequency of OXA-48 gene was significantly higher among Carbapenem resistant samples and no OXA-48 gene was found among Carbapenem sensitive samples i.e. p-value=0.021. (Table 3)

Table 1: *Isolated Organisms*

	Frequency	Percent
Acinetobacter baumannii	14	18.40%
Citrobacter koseri	1	1.30%
Citrobacter Species	3	3.90%
E.coli	22	28.90%
Enterobacter cloacae	1	1.30%
Klebsiella pneumoniae	4	5.20%
Proteus mirabilis	4	5.30%
Providencia stuartii	1	1.30%
Pseudomonas aeruginosa	25	32.90%
Salmonella typhi	1	1.30%
Total	**76**	**100%**

Table 2: *Frequency of OXA-23 gene among Carbapenem resistant and susceptible gram negative bacteria by PCR*

OXA-23	Carbapenem		Total
	Sensitive	Resistant	
Positive	0(0%)	22(57.9%)	**22**
Negative	38(100%)	16(42.1%)	**54**
Total	**38**	**38**	**76**
p-value		**<0.001 significant**	

Table 3: *Frequency of OXA-48 gene among Carbapenem resistant and susceptible gram negative bacteria by PCR*

OXA-48	Carbapenem		Total
	Sensitive	Resistant	
Positive	0(0%)	5(13.2%)	**5**
Negative	38(100%)	33(86.8%)	**71**
Total	**38**	**38**	**76**
p-value		**0.021 Significant**	

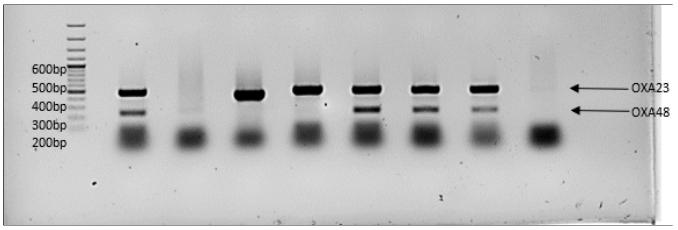

Figure-1: *OXA-23 on 1% Agarose Gel Electrophoresis*

Discussion

As is evident by the results of this study, OXA-23 and OXA-48 can be easily detected among Carbapenem resistant gram negative bacteria. OXA-23 was in fact more prevalent in the population of this study. The reason for this contrast can be attributed to the type of Gram negative bacteria isolated in this study. Indeed, according to a review published by the American Society of Microbiology, there are five subgroups of Oxacillinases, of which OXA-23 are more frequently isolated from Acinetobacter species; whereas OXA-48 is more commonly found among Enterobacterales.[11] Acinetobacter baumannii constituted the majority of our Carbapenem resistant isolates, hence explaining the higher frequency of OXA-23 gene as compared to OXA-48. While considering the limitations of this study, a greater sample size and more varied profile of microorganisms may help overcome this difference in future research. Furthermore, in our study approximately 58% of Carbapenem resistant samples had prevalence of OXA-23 gene. This number is relatively low when compared to that of China (91.5%) and India (97%).[12,13] In contrast, the good news is that Carbapenem susceptible samples did not possess these genes, meaning that the rate of carriage among susceptible population is relatively low. From international studies, OXA-48 has shown prevalence as high as 77% among Carbapenem resistant isolates reported in Middle Eastern countries such as Egypt.[14] With regards to domestic studies, an investigation done at Biomedical Research center at KEMU in 2020 found that 55 out of 117 Carbapenem resistant Klebisella Pneumoniae strains tested positive for carbapenemase resistance genes through resistome profiling. Carbapenemase genes encoding blaNDM-1 (23%) and blaOXA-48 (22.2%) were found most frequently.[15] Moreover, in a joint collaboration done between Institute of Antibiotics at Fudan University, Shanghai China and Department of Microbiology GCU Faisalabad, a similar study was done in 2020 that also confirm the results that blaOXA-48 and blaOXA-23 are the most common Carbapenem resistance genes found in Klebisella pneumoniae and Acinetobacter baumannii.[16] Our results support prior studies indicating that the countries of the Subcontinent are a hotspot for the spread of carbapenemase genes like blaNDM-1 and blaOXA-48.[17] Another important point to note from this study is that the phenotypic detection methods done in the

lab did in fact correspond with the genotypic results. This means that in low-and middle-income countries such as Pakistan and low resource settings, phenotypic detection may be used as a good indicator to detect Carbapenem resistance in patients. However, confirmatory tests still need to be done through molecular analysis. Not to mention, the importance of keeping in place stringent infection control practices including the implementation of antimicrobial stewardship, surveillance, and prudent usage of antibiotics.

Conclusion

Results of this study showed higher frequency of OXA-23 gene as compared to OXA-48 gene in Carbapenem resistant gram negative bacteria. Neither OXA-23 nor OXA-48 was found in Carbapenem susceptible bacteria. The phenotypic response of bacteria to carbapenems correlated with our genotyping results hence these findings can help choose appropriate carbapenems by relying on disc diffusion method.

Conflict of Interest *None*

Funding Source *None*

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Authors Contribution

SM, HB: Conceptualization of Project

SM, ZZ: Data Collection

SM, SM: Literature Search

SM: Statistical Analysis

SM, HB, SM: Drafting, Revision

SM, ZZ: Writing of Manuscript