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Frequency and Benefits of Oxa- 48-Like Gene Detection in Imipenem Resistant Enterobacterales Using Phenotypic Methods

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ABSTRACT

Objectives: To determine frequency of OXA-48 like gene in CRE and to find effectiveness of phenotypic methods for OXA-48 like gene detection.
Study Design: cross-sectional study.
Place and Duration of Study: Pakistan Railway hospital (PRH) Rawalpindi, Pakistan from Oct 2022 to Sep 2023.
Methodology: One hundred and four samples of pus, HVS, urine, sputum was included in the study, inoculated on suitable culture media, and then incubated for 48 hours. Microscopical examination was done after gram staining followed by biochemical testing. Susceptibility of isolates was tested by Disk Diffusion method and MIC of Imipenem was tested against all isolates by using E strips. Carba NP, mCIM, eCIm test were applied on CRE isolates. Isolates found negative for eCIM were tested with temocillin antibiotic disc. Isolates found resistant to temocillin with zone of Inhibition less than 11mm were labelled positive for OXA- 48 like gene.
Results: Out of total 104 CRE isolates 31 (30%) were resistant to temocillin with the sensitivity zone less than 11 mm and were labelled OXA-48 like positive. The frequency of OXA-48 like was determined 30%.
Conclusion: Frequency of OXA-48 like gene was found to be 30%. Phenotypic methods are useful and reliable in the early and speedy detection of OXA-48 like gene in CRE isolates.

Keywords: CRE, OXA-48 like, carbaNP, mCIM, eCIM

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INTRODUCTION

The two most common species detected in hospital intensive care units are Klebsiella pneumoniae and Escherichia coli. They belong to the Enterobacterales family of Gram-negative bacteria, which are responsible for both community and hospital acquired infections.[1] These organisms are often resistant to many commonly used antibiotics like Imipenem and Meropenem.[2] Enterobacterales showing resistance to at least one carbapenem antibiotic are labelled as Carbapenem - resistance Enterobacterales (CRE). Throughout the medical world, they are now considered a challenge. This resistance has important clinical and financial consequences, including increased hospital stay, higher antimicrobial cost, higher hospital cost and increased mortality.[3] The primary cause of Carbapenem resistance is the production of Carbapenemase enzymes by the bacteria which leaves fewer antibiotic options. Therefore, expensive, and hazardous medications like Tigecycline and Colistin are used.[4] All beta lactam and beta lactam inhibitor drugs, such as Clavulanic acid, Sulbactam, and Tazobactam, can be hydrolyzed by carbapenemases. These are the enzymes that can hydrolyze all beta lactams drugs, including Carbapenems, Cephalosporins, and Penicillin. Most frequently reported carbapenemases are Imipenem (IMP), New Delhi Metallo Lactamase (NDM), Guiana Extended Spectrum (GES), Oxacillin-48like (OXA-48like), Metallobeta lactamase, Serine carbapenemase, and Verona Integrin encode metallo beta lactamase (VM).[5] These are the enzymes that can hydrolyze any drug that contains beta lactams, including Carbapenems, Cephalosporins, and Penicillin. With a unique quality, OXA-48 spares some extended spectrum cephalosporins, such as Ceftazidime, and does not render them ineffective.[6] Patients still have the choice to use Ceftazidime instead of costly and hazardous drugs like Colistin and Tigecycline.

PCR is one of the most reliable techniques for identifying the various carbapenemase genes. Most of the laboratories, in particular those in developing nations like Pakistan, lack this facility. Phenotypic

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methods have been employed in place of molecular methods for gene detection as a way to address this issue. These phenotypic methods include Carbapenemase Nordmann-Poirel (CarbaNP) test for the detection of all types of carbapenemases. Modified carbapenem inactivation method (mCIM) to detect serine carbapenemase and metallo beta lactamase. EDTA modified carbapenem inactivation method (eCIm) for metallo beta lactamase detection and Temocillin susceptibility test.

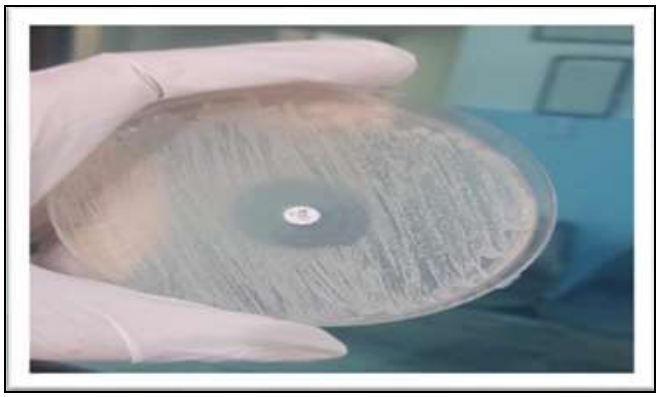

Figure:Temocillin Susceptibility By Disc Diffusion Method

The isolates found carbapenem resistant and showed negative result with eCIm test were then tested with Temocillin antibiotic disc for their susceptibility and those which were found resistant to Temocillin (Zone of inhibition is less than 11) were labelled positive for the gene OXA-48 like.[7]

This study aimed to detect OXA-48-like gene in local CRE isolates that will provide an opportunity to use less toxic and relatively cheaper antibiotics instead of more toxic and costly drugs like Colistin and Tigecycline and to explore the use of affordable phenotypic methods for carbapenemase detection that are suitable for the laboratories with limited funding and less technical expertise. It will ultimately help in the improvement of health care services.

METHODOLOGY

The study was descriptive cross- conducted in Pathology (Microbiology) Laboratory at Pakistan Railways Teaching Hospital, Islamic International Medical College Rawalpindi, Pakistan. Duration of the study was one year from October 2022 to Sep 2023 after the approval of synopsis from institutional ethical review committee of Riphah International University, Islamabad (Reference Number Riphah / IIMC /IRC /22/063).

Inclusion Criteria: The Enterobacrales (Klebsiella pneumoniae and E coli) isolated from clinical samples including pus, HVS, urine, sputum, body fluids like CSF, pleural fluid received at microbiology laboratory during the study period with no discrimination of age, gender, and type of specimen were included.

Exclusion Criteria: Duplicate samples from the same patient were excluded.

One hundred and four CRE isolates were included in the study by using nonprobability consecutive sampling technique. Sample size was calculated according to the Cochrane formula $n = Z^2 p (1-p)/ e^2$, where Z is the statistic for level of confidence and is equal to 1.96, p is the disease prevalence and it was 7.3% i.e, 0.07 and e is the margin of error and was chosen as 5.008 According to this formula, sample size calculated was 104.

The samples were collected and the organisms belonging to Enterobacterales K. pneumoniae, and E. coli were included in the study irrespective of age, gender, and type of specimen. A demographic Performa specially designed about patient's details was filled to avoid duplication of samples.

After collection two hundred and fifty samples were inoculated on suitable culture media depending upon the type of specimen i.e., Blood Agar, MacConkey Agar, Chocolate Agar, Cysteine lactose electrolyte deficient medium (CLED) and were incubated aerobically at 35°C +_ 2°C for 24 - 48 hours. Gram staining and then microscopy was carried out. Organisms belonging to Enterobacterales were identified by biochemical tests e.g oxidase test, catalase test and API 20 E. Antibiotic susceptibility of organisms was performed using modified Kirby Bauer Disk diffusion technique as recommended by CLSI guidelines. MIC of Imipenem was determined against all isolated Enterobacterales using E strips. The isolates resistant to Imipenem were sorted and carbapenemase production in those isolates was determined by using carbaNP and mCIM phenotypic method. Then the isolates showing positive result for mCIM were tested with phenotypic eCIm test. The isolates that tested positive for eCIm were assumed to be positive for metallo beta lactamase, while those that tested negative were further tested with antibiotic disc temocillin. The sensitivity was interpreted as having an OXA-48-like gene when the inhibition zone was less than 11mm (>11mm Resistant).

Data was analyzed by Statistical Package for the social sciences (SPSS) version 21.00 For qualitative

variables (type of samples, organisms isolated, antimicrobial susceptibility and their MICs) simple descriptive statistics (frequencies, percentages) were calculated.

RESULTS

Two hundred and fifty different types of samples including urine, pus, sputum, HVS, ETT received at Microbiology lab of Pakistan Railway Hospital for culture and sensitivity were collected. All were processed and 104 CRE isolates were collected. Highest percentage of CRE isolates was recoverd from urine samples. Different types of samples and the CRE isolates collected from those are given in Table-I.

Table-I: Percentage of Carbapenem Resistance Enterobacterales (Cre) Isolated From Different Types Of Samples (n=104)

Source of Samples	Total Samples	CRE (%)
Urine	75	35(46%)
Pus	76	30(39.4%)
HVS	60	20(33.3%)
Sputum	30	14(46.6)
ETT	9	5(55.5%)
Total	250	104(41.6%)

All the phenotypic tests applied on CRE isolates along with their outcome and the number and the percentage of OXA-48 like gene found given in the Table-II. Frequency of OXA-48 like gene was determined 30%. *p*-value was found to > 0.05 that is not significant.

Table-II: CarbaNP, mCIM,eCIM, temocillin suseptibility testing by Disk Diffusion method and percentage found of OXA-48 like gene (n=104)

Total Resistant Isolates	CarbaNP Test Positive %	mCIM Test Positive %	eCIM Test Negative %	Temocillin Suseptibility% (Resistant)	OXA 48 like gene Positive %	*p*-value
104	104(100%)	104(100%)	48(46.1%)	31(30.0%)	31(30.0%)	0.182 (p > 0.05)

DISCUSSION

In the present study one hundred and four CRE isolates were collected from different types of samples including urine, pus, sputum, HVS, ETT. Frequency of OXA-48 like gene was determined 30%. and *p*-value was found > 0.05 that is not significant.

In a study at Thailand both CarbaNP and mCIM test showed that 77.7% of CRE isolates were positive for carbapenemase. It was also concluded that CarbaNP can be considered a confirmatory test for carbapenemase production by CRE9. Our study also showed 100% effectiveness of both carbaNP and mCIM test for carbapenemase detetion.

In an Indian study carba NP and mCIM showed metallo-β-lactamases the most common carbapenemases followed by OXA-48-like enzymes Both CarbaNP and mCIM tests showed 80.6% and 62.1% positive results respectively.[10] Our study showed about 20% for carbaNP and almost 40% more positive result for mCIM.

In Egypt phenotypic approaches including, modified mCIM were used in a study to detect carbapenemases in CRE. Result revealed 68.65 % positive result with mCIM for carbapenemase detection in CRE. Our study showed mCIM 30% more positive for carbapenemase detection.[11] .In USA a study showed that only 2% of the results of Carba NP were found invalid.[12] Our study also supported the study by showing no invalid result.In Turkey both Carba NP and mCIM were used for carbapenemase detection in CRE.Result showed that out of 110 CRE isolates 90% were positive for carbapenemases while 9% had invalid results.[13] Our study had no invalid result by showing 100% result with both CarbaNP and mCIM. In a study at China CRE isolates were obtained from blood, rectal swabs, feces, urine, and respiratory secretions. For carbapenemase detection both mCIM and eCIm tests were used. Result revealed that 84.6% isolates were positive for carbapenemases. It was concluded that excellent results were shown by the tests .Because each assay has its own limitations, suitable methods should be combined to have better results.[14] In our study CRE isolates were collected from urine, HVS, pus, sputum and ETT. Study showed mCIM test highly effective with 100% and eCIm test with 51.9% positive results, respectively. We used a combination of Carba NP, mCIM ,eCIM and temocillin susceptibility testing by DD method. Results of the study showed 100% positive result for carbapenemase detection and 30% positive result for the presence of OXA-48 like gene.

In Belgium researchers used temocillin inhibition zone's diameter by DD susceptibility method to detect carbapenemases in CRE. Carbapenemase producing CRE isolates showed significant resistance for temocillin. It was concluded that temocillin resistance

was a sensitive surrogate measure for OXA-48 like production by CRE.[15] Our study supported this result by showing significant temocillin resistance for carbapenemase producing CRE isolates. (Susceptibility zone <11mm) that was considered positive result for OXA-48 like gene in CRE isolates.

In Germany results of a study indicated that 90% of CRE isolates with OXA-48 like gene were highly temocillin resistant (susceptibility zone < 12 mm).[16] Our study also showed high temocillin resistance for OXA-48 like producing CRE isolates. Results showed that 30% of CRE isolates had temocillin susceptibility zone size < 11 mm indicating high resistance for temocillin were considered positive for OXA-48 like gene.

Findings of a study conducted at Austria indicated that OXA-48 like positive isolates were highly temocillin resistant.[17] It is similar to our study showing high temocillin resistance for OXA-48 like producing CRE isolates. In another study in consensus with our study mCIM test showed 97.7% positive results for detecting CRE with different carbapenemases. Study concluded that mCIM test might be proven an effective and reliable test for carbapenemase detection in CRE.[18] Our study showed 100% results with mCIM test for the detection of carbapenemases in CRE. It revealed mCIM a reliable tool for carbapenemase detection in CRE. At Egypt mCIM, eCIM tests and temocillin susceptibility DD method were used in combination for the detection of carbapenemases in CRE isolates. Study concluded that 84.3% CRE isolates were detected carbapenemases positive with these methods.[19] Results of our study showed mCIM .eCIM tests and temocillin susceptibility DD method a reliable combination to detect carbapenemases in CRE isolates. Study showed 100% positive results with mCIM and 51.9% positive result with eCIm test by the carbapenemase producing CRE. Temocillin susceptibility DD method showed 30% of CRE isolates resistant (susceptibility zone < 11 mm) indicating the presence of OXA-48 like gene in them.

In a study at Turkey, temocillin disc diffusion test was used for the identification of K. pneumoniae producing OXA-48 like gene. Study concluded that carbapenemase producing CRE isolates showed significant temocillin resistance and temocillin disc diffusion test can be used in laboratories as a part of identification and screening of carbapenemase positive K. pneumoniae.[20] The current study supported temocillin susceptibility DD method a reliable tool for the detection of OXA-48 like gene in CRE isolates. Results showed 30% of CRE isolates positive for carbapenemase (OXA-48 like) production.

Research carried out in UK showed 60 % of the CRE isolates were OXA-48 like positive by showing elevated level of temocillin resistance (>128 mg/dl). Study concluded that OXA-48 like carbapenemases can be diagnosed by their elevated level of temocillin resistance.[21] In our study temocillin susceptibility DD method also showed high level of resistance for CRE isolates producing OXA-like gene. Results showed that 30% of CRE isolates had high temocillin resistance.

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LIMITATION OF STUDY

This study was single centered .There is need to do a larger multicenter study including different hospitals of the city. Results of this study were not compared with molecular method because of technical limitations.

RECOMMENDATION

Phenotypic methods for OXA-48-like gene detection should be used in all the microbiology laboratories especially those with limited resources .It will help in the early and speedy detection of OXA-48-like gene in local CRE isolates ultimately helping to avoid the use of colistin and tigecycline. An exclusive study should be done with CRE isolates by directly testing them with temocillin for the detection of OXA-48 like gene. It will reduce the time of testing procedure.

CONCLUSION

The frequency of OXA-48 like gene was found to be 30%. It was also concluded that the phenotypic methods are useful and reliable in the early and speedy detection of OXA-48 like gene in local isolates.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SB & SY: Conception, study design, drafting the manuscript, approval of the final version to be published.

TB & ST: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

NT & AB: Conception, data acquisition, drafting the manuscript, approval of the final version to be published. Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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