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Original Research Article

Isolation, Identification and Molecular characterization of Carbapenemase producing Enterobacterales and Pseudomonas in a tertiary care hospital

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Conflict of interest: Nil

Abstract:

Background: Carbapenems are drug of choice for treatment of multidrug resistant hospital acquired infections due to the recent rise in carbapenem-resistant bacteria, treating these infections has become incredibly challenging because there are so few effective alternatives. In addition to increasing mortality and morbidity, this imparts great economic burden on the health care system. These infections can be contained with the aid of prompt detection of these carbapenemase producers, proper treatment, and measures to prevent infection.

Materials and Methods: A total of 100 Carbapenem resistant isolates consisting of Enterobacterales and Pseudomonas identified by automated ID/AST method (Vitek-2) were included in the study conducted from April 2023 to June 2023 at Infosys Central Laboratory, Department of Microbiology, Bangalore Medical College and Research Institute, Bengaluru and tested for carbapenemase production by Carba NP test and PCR for NDM and OXA-48 genes

Result: Among 100 isolates Klebsiella pneumoniae was predominant (56) followed by Escherichia coli (29), Pseudomonas aeruginosa (10), Enterobacter cloacae (3) Enterobacter aerogenes (1) and Citrobacter freundii (1).86 isolates were found to be positive by Carba -NP test. PCR revealed that 36 isolates harboured NDM gene and 18 isolates harboured OXA-48 gene.12 isolates harboured both genes, while neither NDM nor OXA-48 gene was detected in 34 isolates.

Conclusion: Wide adaptability of these tests by various laboratories will help in early identification of these potentially spreading carbapenemase producers, which in association with appropriate treatment and infection control practices will prevent emergence of Carbapenem resistant organisms and reduce mortality and morbidity.

Keywords: Carbapenemase, Enterobacterales, Pseudomonas aeruginosa, Carba NP test, PCR.

Introduction

Carbapenems due to their broad spectrum of activity, affect both Gram-positive and Gram-negative bacteria. Emergence and spread of Extended-spectrum β lactamase (ESBL) producers have led to a rise in the usage of carbapenems as an empirical therapy for the management of life-threatening infections. The frightening development of carbapenem resistance has brought on by drug abuse [1].

Carbapenemases are capable of hydrolyzing all β-lactam antibiotics including carbapenems. They fall under groups A, B, and D. The majority of carbapenemases have been found to be plasmid-mediated in the Enterobacterales and Pseudomonas [2].

Antibiotic resistance is still a significant global health issue that increases healthcare costs, lengthens hospital stays, and leads to unsuccessful treatments. An emerging issue is the prevalence and proliferation of Gram-negative bacteria that generate carbapenemase [3].

Detection of carbapenem-resistant Enterobacterales and Pseudomonas by phenotypic and genotypic methods is important for administering appropriate therapy and to control their spread among the patients in the hospital. Epidemiological data are

important to know about circulance of carbapenem resistant genes in our environment [4].

Materials and Methods

A prospective cross-sectional study was conducted from April 2023 to June 2023 after obtaining Institutional Ethical Committee clearance certificate. A total of 100 samples were included in the study derived from various samples namely pus, blood, CSF, urine, sputum, vaginal swab, ET aspirate, ascitic fluid.

Bacterial isolation and identification: Bacterial isolation was done according to standard Microbiological guidelines [5]. Identification and Antibiotic sensitivity testing were performed by automated ID/AST method (Vitek-2). Inclusion criterion was Carbapenem resistant Enterobacterales, Pseudomonas identified by automated ID/AST method (Vitek-2). Exclusion criterion was Carbapenem sensitive Enterobacterales and Pseudomonas identified by automated ID/AST method (Vitek-2).

Detection of carbapenemase by phenotypic method: A total of 100 Carbapenem resistant isolates consisting of Enterobacterales and Pseudomonas aeruginosa identified by automated ID/AST method (Vitek-2) were tested for carbapenemase production by Carba NP test

Solution A was prepared by adding phenol red (0.05%) and zinc sulphate (0.1mMol/L) to laboratory reagent water, pH was adjusted to 7.8. Solution B was prepared by adding 12mg/mL Imipenem- cilastatin injectable form to solution A. Two microcentrifuge tubes were labelled as 'a' and 'b' for each clinical isolate and 2 loopful of 18-24 hour bacterial isolate on blood agar was added to in-house prepared bacterial lysate buffer (tris- HCl 20mmol/L and 0.1% triton X -100) and vortexed for 5 secs and 100µL was added to each tube labelled 'a' and 'b'. 100 µL of solution A to tube 'a' and 100 µL of solution B to tube 'b' and incubated at 37°C and reading was taken at 30 mins and after 2 hours of incubation. When tube 'a' was red and tube 'b' was orange/ yellow test was considered positive and negative when both tubes were red in colour.[6] Uninoculated tube 'a' and 'b' containing Solution A and B respectively was used as control [7].

Detection of Carbapenemase by genotypic method: All the 100 Carbapenem resistant isolates were subjected for performance of polymerase chain reaction

DNA extraction: DNA extraction was done from boiling method.

Conventional Polymerase chain reaction: Two uniplex polymerase chain reactions (PCRs) (each 25 µL reaction) carried out for detecting NDM and OXA-48 genes separately [1].

Table 1: Showing primers used for PCR

Gene	Forward primer	Reverse primer	bp
NDM	CATTAGCCGCTGCATTGATG	GTCGCCAGTTTCCATTTGCT	83
Oxa-48	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG	438

PCR mixtures were prepared under laminar flow under strict precautions to prevent cross contamination.

Amplification in both PCRs was carried out with the following thermal cycling profile: Initial denaturation for 5 min at 95°C, 35 cycles of amplification consisting of 30 s at 95°C, 30 s at 45°C, and 30 s at 72°C, with 5 min at 72°C for the final extension.

Gel electrophoresis: Gel electrophoresis was carried out in 0.5X TAE using 1.5% agarose gel.

The gel was viewed under ultraviolet transilluminator and photographic image will be obtained in a Gel Documentation System.

Statistical Analysis: The data collected was entered into excel sheet. Distribution of different bacterial isolates, carbapenemase enzyme and carbapenem resistant genes was analysed.

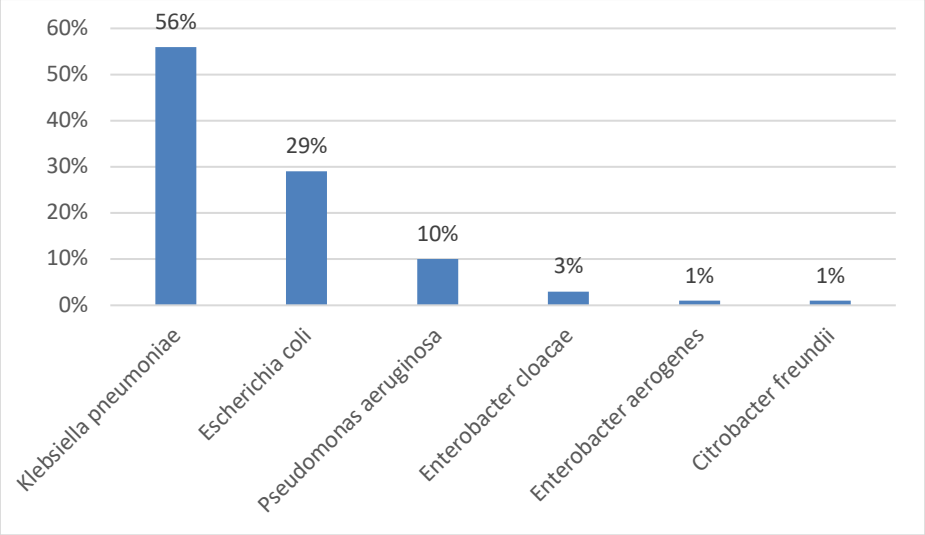
Results

Male patients were predominant in the study being 59 in number and females 41 in number

Table 2: Showing age distribution of patients included in the study

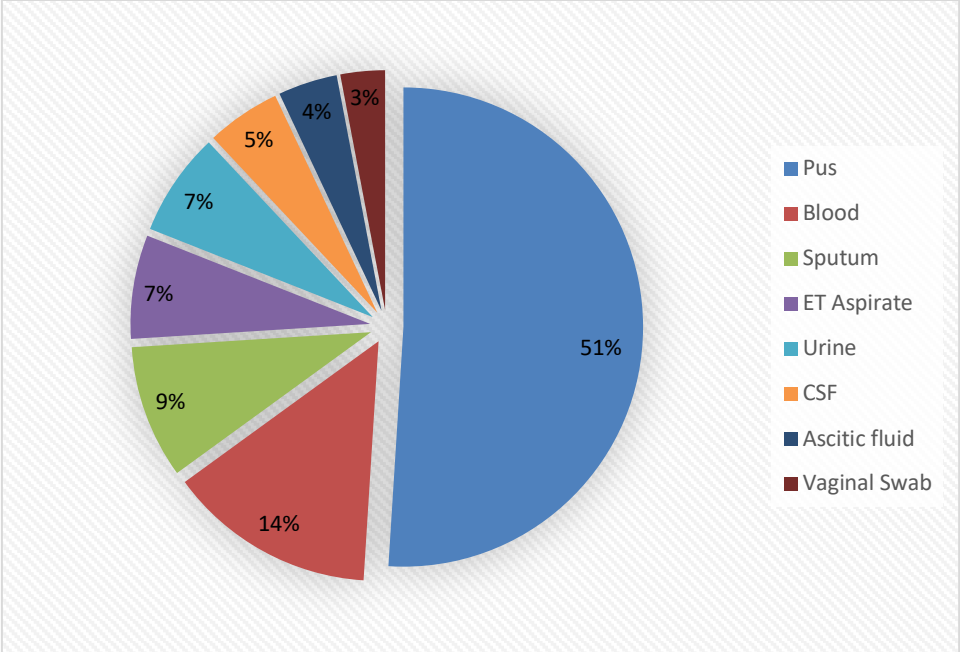
No of patients in the age group	
< 20 years	23
21 – 40 years	29
41 – 60	27
61 – 80	21

Among 100 isolates Klebsiella pneumoniae was predominant (56%) followed by Escherichia coli (29%), Pseudomonas aeruginosa (10%), Enterobacter cloacae (3%) Enterobacter aerogenes (1%) and Citrobacter freundii (1%).



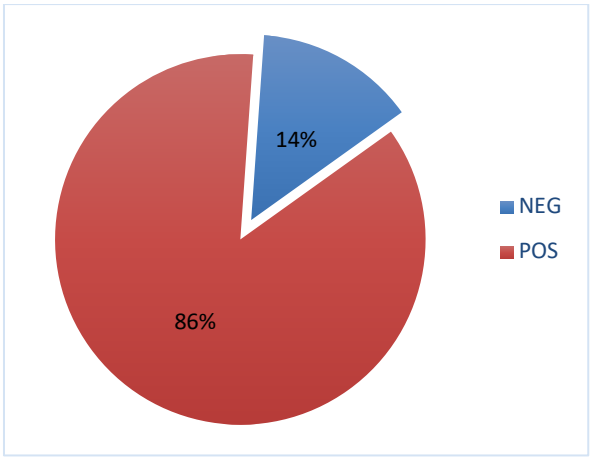
Graph 1: Showing distribution of organisms among 100 isolates

Among 100 isolates pus was maximum in number followed by blood and others



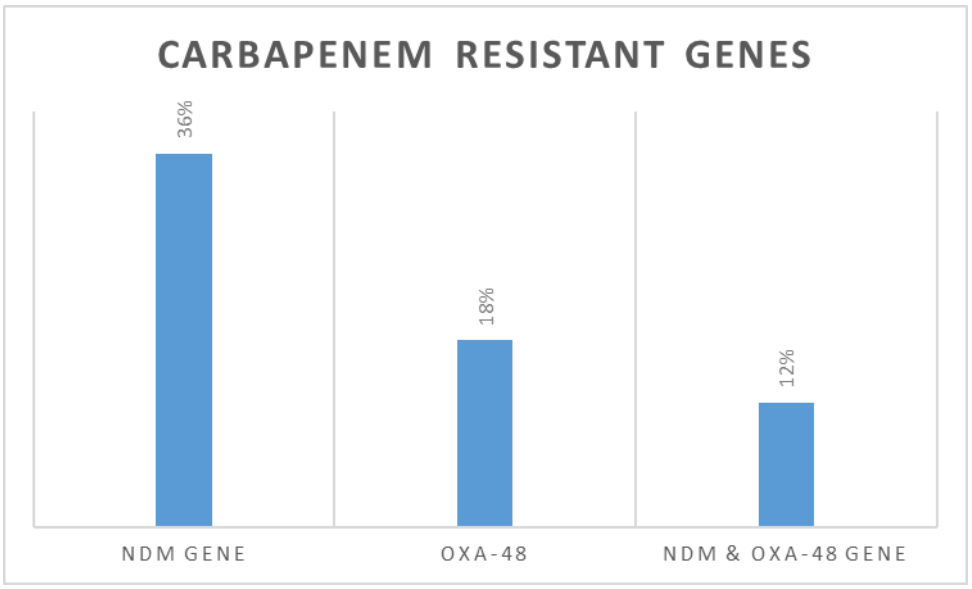
Graph 2: Showing distribution of samples among 100 isolates

86 isolates were found to be positive among 100 isolates by Carba NP test and rest were negative.



Graph 3: Showing percentage of positive and negative isolates by Carba NP test

PCR revealed that 36 isolates harboured NDM gene and 18 isolates harboured OXA-48 gene, 12 isolates harboured both genes, while neither NDM nor OXA-48 gene was detected in 34 isolates



Graph 4: Showing distribution of NDM and OXA – 48 genes

Table 3: Showing distribution of Carbapenem resistant genes among clinical samples

Clinical samples	NDM gene	OXA-48 gene
Pus	19	16
Blood	7	6
Urine	6	_
Sputum	4	2
CSF	4	3
ET aspirate	4	1
Ascitic fluid	2	2
Vaginal swab	2	_

Table 4: Showing distribution of NDM and OXA-48 genes among different isolates

Organisms	NDM gene	OXA-48 gene	Both NDM and OXA-48 gene
K. pneumoniae	18	18	12
E. coli	10	-	-
Pseudomonas aeruginosa	8	-	-

Positive result by Carba NP test

Image 1: Showing tube 'a' red and tube 'b' yellow colour

Negative result by Carba NP test

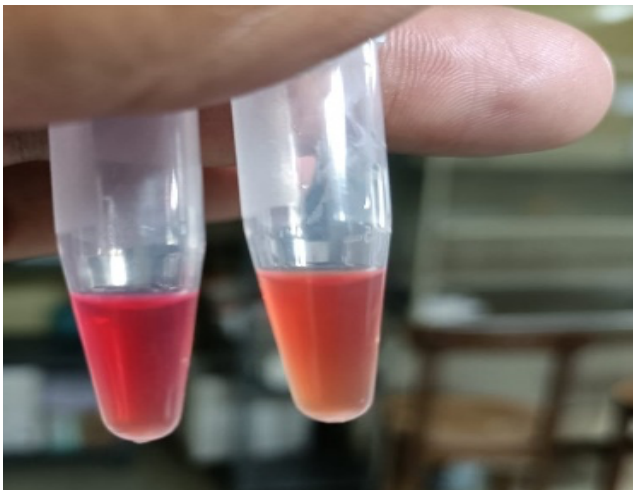


Image 2: Showing both tube 'a' and 'b' red colour

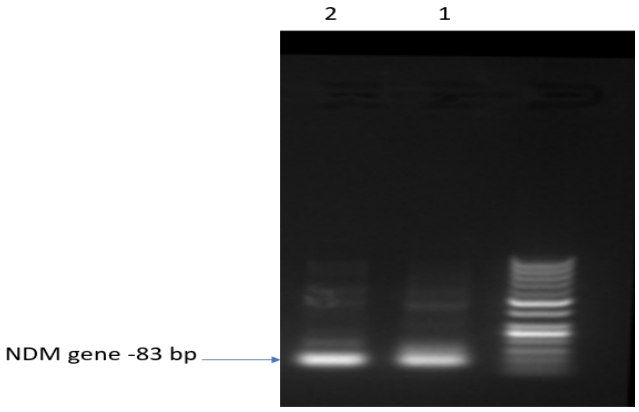


Image 3: Detection of NDM gene by Uniplex PCR. Isolates containing NDM gene lane 1,2

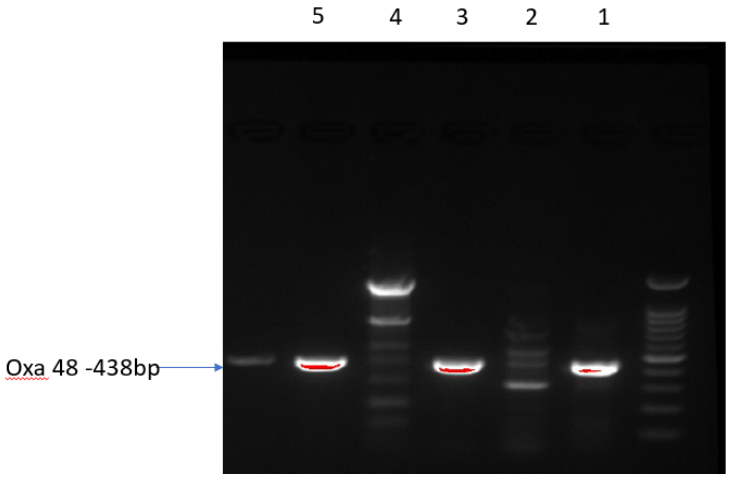


Image 4: Detection of OXA 48 gene by Uniplex PCR. Isolates containing Oxa 48 genes lane 1,3,5

Discussion

Carbapenems are the drug of choice to treat multidrug-resistant pathogens, which are emerging at an alarming rate and posing a threat to global health. Gram negative bacilli are becoming increasingly resistant to these potential drugs, primarily as a result of acquiring carbapenem resistant genes

Bhatt P published a study during August 2022, conducted at MAHE, Manipal, Karnataka involving 230 carbapenemase producing isolates, 150(65.2%) found to be positive by Modified Hodge test (MHT) and among 150 phenotypically confirmed isolates NDM gene was found in 85 isolates, OXA-48 in none of the isolates [1].

Study conducted by Rudresh SM during 2020 at Bengaluru, Karnataka involving 144 Carbapenem resistant Gram-negative bacilli, 76.7% were positive by Carba NP test, NDM gene was detected in 52, OXA-48 in 28 isolates respectively by multiplex PCR [6].

In a study conducted by Baran I and Aksu N during 2016 at Turkey involving 181 Carbapenem resistant Enterobacterales, MHT was performed with Meropenem and ertapenem disc ,110 were positive with MHT with meropemen and 109 were positive with MHT with ertapenem, OXA-48 gene was detected in 86 isolates and NDM-1 in 6 isolates by multiplex PCR [8].

Rajni et al conducted a study at Rajasthan, India during 2022 including 14 isolates consisting of E coli and K pneumoniae and subjected to Rapidec Carba NP test and multiplex PCR, all the 14(100%) isolates were positive by Rapidec Carba NP test, 86% isolates had OXA-48 gene and 79 % isolates had NDM-1 gene [9].

In a study conducted by Haji SH et al including 110 Gram negative bacilli during 2021 at Iraq 65(59%) isolates were positive for carbapenemase production by phenotypic method (Carba plus disc system) and among 65 isolates 44 isolates had NDM gene and 40 isolates had OXA- 48 gene detected by PCR. [10]

In our study when compared to above study 86% of isolates were positive by phenotypic method (Carba NP test), indicating the rise in resistance level and 36% of isolates were positive for NDM and 18% for OXA-48 gene respectively. NDM gene found to be more prevalent in our hospital environment. Detection of carbapenem resistant organisms is of utmost importance, for better patient care and also to prevent spread of resistance.

Conclusion

The present study reveals that carbapenemase producing genes are a significant factor responsible for emergence and spread of resistance among clinical isolates, with NDM gene being predominant in the demographic region. Wide adaptability of tests for detection of carbapenemase producers by various laboratories will help in early identification of these potentially spreading pathogens. Early detection in association with appropriate treatment and infection control practices will prevent emergence and spread of carbapenem resistant organisms and reduce mortality and morbidity.

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