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Molecular Characterisation of Carbapenemase Resistance in Enterobacteriaceae by Detection of BLA NDM-1 Gene From A Tertiary Care Center

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ABSTRACT
Multidrug resistance refers to the ability of microorganisms, to resist multiple antimicrobial drugs. This phenomenon poses a significant challenge in treating infections and can lead to the failure of antibiotic therapies. It often arises due to genetic mutations or the acquisition of resistance genes through horizontal gene transfer. Present study is undertaken to identify the genotypic characterisation of carbapenemase resistant Enterobacteriaceae by the detection of BLA NDM-1 gene. Polymerase chain reaction is performed for the detection of the resistant gene in 121 carbapenemase producing strains of Enterobacteriaceae. 86% of the strains showed presence of BLA NDM-1genes. routine screening the hospital acquired strains need to be subjected for the detection of antibiotic resistant genes to prevent the spreading of Multiple drug resistant strains.

INTRODUCTION

Detection of antibiotic resistance mechanisms is always a serious challenge to clinical laboratories as many of them are not always detectable in routine susceptibility tests. Often, enzymes responsible for resistance are co-expressed in the same isolate which makes their detection and reporting even more complicating[1]. Detection of these mechanisms is important in targeting antimicrobial therapy and appropriate infection control, especially in the regions where carbapenem resistance is high or is increasing[2]. Carbapenemase is an enzyme under metallo-ß-lactamase (MBL) that break down ß- lactam drugs. The mechanism of carbapenem resistance is mainly due to the production of various Carbapenemase. A new metallo-ß-lactamase, named New Delhi metallo-ß-lactamase-1 (NDM-1) encoded by the BLA NDM-1 gene, was first identified in a Swedish patient who had traveled to India[3]. They confer resistance to all beta-lactamase including Carbapenemase, with the exception of Astronium. Highly mobile elements carry the genes for the same, helping easy dissemination of resistance[4]. Isolates of carbapenem-resistant Enterobacteriaceae were studied to determine the molecular mechanism for resistance and the blaNDM-1 gene was detected in the majority of them. Increasing reports of NDM-1-producing strains are seen in India and across the world. But molecular data regarding their rate of infection in hospital settings is scarce[5]. The current study will be conducted to determine various ß-lactamases along with the widely spreading blaNDM-1 gene among the isolates from various clinical samples from Index hospitals.

MATERIALS AND METHODS

Polymerase Chain Reaction[6]: 121 carbapenemase producing strains of Enterobacteriaceae are subjected for the detection of NDM-1 gene by polymerase chain reaction. Carbapenemase encoding gene, NDM-1 was amplified by Polymerase Chain reaction (PCR) using previously designed primers for NDM-1.

The Master Mix For PCR: The amplification was performed in a 50 µL PCR mixture consisting of

- Master Mix-25 µl-(Taq DNA Polymerase 2x Master mix RED 1.5mM MgCl2)-Synergy Scientific Services PVT LTD
- PCR grade water-18 µL
- Primer NDM-F-1 µL
- Primer NDM-R-1 µL
- DNA-5 µL

DNA was amplified in a Master cycler Eppendorf under the following conditions. PCR products were kept at 40C. A known blaNDM-1-producing laboratory strain of *E. coli* was used as a positive control. *E. coli ATCC* 25922 reference strain was used as the blaNDM-1 gene's negative strain.

Gel Electrophoresis:

- Steps of agar rose gel preparation
- Requirements
- 50x TAE
- Ethidium bromide
- Loading dye
- Agarose powder
- Aluminium foil
- Flask-250ml
- Parafilm
- Scotch tape

Preparation of TAE Buffer:

- For 50 ml of buffer-1ml 50 X TAE buffer to 49ml distilled water was added
- For 100 ml of buffer-2ml 50 X TAE buffer to 98ml distilled water was added
- Final concentration-1X TAE buffer was made

Agarose Gel Preparation:

- Agarose powder was weighed on the aluminum foil using the weighing scale
- For 50ml TAE-1gm agarose powder was used
- For 100ml TAE-2gm agarose powder was used
- Mixed well and boiled in microwave till no turbidity is seen approximately for 3 mins

Adding Ethidium Bromide:

- For 100ml of agarose mixture-2µl was used
- For 50ml of agarose mixture-1.5µl Ethidium bromide was used

Steps:

- Mixed well and poured into electrophoresis box after placing comb into it. Extra TAE buffer was poured into the box and the comb was removed
- 2µl of loading dye was placed on the paradigm and 8µl of the sample (extracted DNA) was added to it and mixed well. 10µl of this mixture was placed in the respective well of the electrophoresis gel. This was repeated for the other samples
- For the ladder 1µl of the ladder, 2µl of loading dye and 4µl of TAE buffer were mixed well and placed in the first well of the gel
- Anode and cathode plates were placed and connected to a high voltage current
- Results were read after there was a flow of around 60-70% by placing the gel under UV light

- The PCR products were analyzed by gel electrophoresis with 2% agarose gel in TAE (tris-acetate buffer) buffer with1.5µl ethidium bromide
- The PCR products were visualized and photographed under ultraviolet illumination

RESULTS AND DISCUSSIONS

The beta-lactamase emerged as the most common cause of antibiotic resistance among gram negative bacteria worldwide. ESBLs represent a major group of beta - lactamase, currently being identified world wide in large numbers along with inducible Amp C beta-lactamase. MBL genes have spread from *P. aeruginosa* to members of Enterobacteriaceae. Reports indicate that the carbapenemase producing Enterobacteriaceae seem to be increasing in number in recent years. These enzymes are phasmid mediated. Multidrug resistance and even pan drug resistance are characteristic feature of strains producing these enzymes. This leaves us with a very narrow spectrum of drugs for treatment. In our study, out of total 121 carbapenemase positive isolates, NDM-1 gene was isolated from 86% of isolates. This is in contrary to other studies in which NDM-1 gene was isolated from all carbapenemase positive isolates. Our study had higher prevalence rate of NDM-1 gene compared to a study by Nagaraj *et al.* where 72.5% of Carbapenem resistance Enterobacteriaceae were positive for NDM-1 gene[8]. Increasing prevalence of beta-lactam producing Enterobacteriaceae in clinical strains is alarming and reflects excessive use of beta-lactam. Therefore, judicious use of antibiotics, strict hand hygiene protocols and implementation of appropriate infection-control measures in the hospital are necessary in preventing the spread of these multidrug resistant Gram negative microorganisms. Screening procedures must be implemented worldwide for 'at risk' patients. Additionally, it is also important to follow antibiotic restriction policies to make use of carbapenem and other broad-spectrum antibiotics where it is necessary. Screening the at-risk patients for NDM-1 gene in case of international transfer of hospitalized patients on world wise scale may prevent the outbreaks in less NDM-1 prevalent areas.

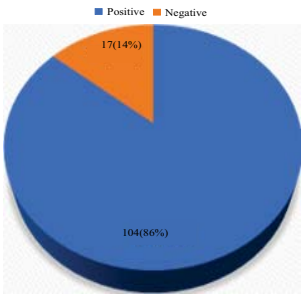

Fig. 1: Pie chart showing prevalence of NDM-1 gene among MBL producers.

Table 1: The primer sequence used in PCR and their product size[7].

Primer	Primer sequence (5-3')	Product size (bp)
bla NDM-1-F	GGTTTGGCGATCTGGTTTTC	621
bla NDM-1-R	CGGAATGGCTCATCACGATC	

Table 2: Cycling conditions for different gene identification.

Steps in PCR	NDM-1
Initial DNA denaturation	940C for 10 minutes
Final DNA denaturation	940C for 30 seconds
Primer annealing	520C for 40 minutes
Primer extension	720C for 50 seconds
Final extension or Holding temperature	720C for 5 minutes
Total Cycles	36

Table 3: Frequency of NDM-1 gene among carbapenemase producing isolates.

NDM-1 gene	Frequency (n = 121)	Percentage
Positive	104	86
Negative	17	14

CONCLUSION

Screening of carbapenemase producing Enterobacteriaceae for the presence of NDM-1 gene is essential for the identification and prevention of the spread of MDR strains.

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