WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 6, Issue 1, 22-30. Research Article ISSN 2277– 7105

ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF THE CARBAPENEMASE PRODUCING GRAM-NEGATIVE BACILLI

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Article Received on
26 Oct. 2016,
Revised on 16 Nov. 2016,
Accepted on 06 Dec. 2016
DOI: 10.20959/wjpr20171-7584

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ABSTRACT

The emergence and spread of Carbapenemase-Producing Enterobacteriaceae (CPE) has been identified as a public health threat, especially since recent studies on CPE and Carbapenem-Non-Susceptible Enterobacteriaceae (CNSE) have shown that infection or colonization has been associated with higher in-hospital mortality. The identification of Carbapenemase-producing Gram-negative bacilli (GNB) is mandatory, as they may cause severe infections, and carbapenems (imipenem - IMP and meropenem - MER) are the therapy of choice in many nosocomial infections. The objectives of the present study are the Isolation, identification and molecular characterization of the carbapenemase producing Gram-positive Bacilli. Six Gram-negative bacilli isolated in biological material cultures and Four *E. coli* strains were tested by disk-diffusion (Himedia) for DOR, MER, and IMP in Mueller-Hinton agar (Himedia). The interpretation criteria applied were those suggested by CLSI. All positive strains were subsequently analysed by PCR method using four different primers. Minimals Inibitory Concentrations (MICs) were determined by a broth macrodilution method with Mueller-Hinton broth (Himedia) and containing bacterial inoculum of 10^7 CFU per tube as per CLSI guidelines. All the six isolates of our study displayed resistance against carbapenem while *E. coli* control strains were observed to be sensitive against carbapenem. All the six isolates contain at least the NDM gene as the carbapemen resistance gene.

KEYWORDS: Carbapenemase, Carbapenem, Gram-negative Bacilli, PCR.

I-INTRODUCTION

Over the past decade carbapenemases, a group of clinically important β-lactamases have emerged and spread among Enterobacteriaceae (1-4). One of the milestones in the emergence of carbapenemases in Enterobacteriaceae was the detection of a novel carbapenemase, *Klebsiella pneumoniae* carbapenemase (KPC), in a *Klebsiella pneumoniae* isolate in North Carolina, USA, which later spread throughout the world.[1] Enterobacteriaceae strains acquiring carbapenemases are found and reported as Carbapenemase-Producing Enterobacteriaceae (CPE) globally.[2, 3]

Carbapenemases are enzymes that can efficiently hydrolyze most β-lactams, including carbapenems.[4,5] In addition, many CPE strains frequently carry additional resistance determinants to other non-β-lactam antibiotics, making these organisms resistant to most antibiotics. CPE commonly remain susceptible to only a few classes of antimicrobials, commonly the polymyxins, tigecycline, fosfomycin, and nitrofurantoin. There is no proven clinical efficacy against these strains and in fact there are reports of clinical failures[6] and emerging resistance to these antimicrobials.[7-10]

The emergence and spread of CPE has also been identified as a public health threat, especially since recent studies on CPE[11,12] and Carbapenem-Non-Susceptible Enterobacteriaceae (CNSE)[13,14] have shown that infection or colonization has been associated with higher in-hospital mortality. Similarly, prior studies of outcomes, involving patients infected with multidrug-resistant organisms (MDROs), show that an inadequate choice or the delayed administration of antimicrobial therapy is associated with poorer patient outcomes, increased morbidity, mortality, increased length of hospital stay and increased costs.[15-20] The risk to patients infected with these MDROs becomes even greater, given the very limited number of new antimicrobial agents that are in the developmental pipeline.[21, 22]

The identification of Carbapenemase-producing Gram-negative bacilli (GNB) is mandatory, as they may cause severe infections, and carbapenems (imipenem - IMP and meropenem - MER) are the therapy of choice in many nosocomial infections. In addition, the identification of carrier individuals allows controlling the dissemination of those agents. No precise phenotypic tool for their identification has yet been described, and the available tools are not able to differentiate resistance mechanisms.[23-24]

The objectives of the present study are the Isolation, identification and molecular characterization of the carbapenemase producing Gram-positive Bacilli.

II-MATERIALS AND METHODS

Six Gram-negative bacilli (W236, W248, W274, W276, U1410, U1672) isolated in biological material cultures and Four E. coli strains (2443, NGM1, NGM2, NGM10) were tested by disk-diffusion (Himedia) for DOR, MER and IMP in Mueller-Hinton agar (Himedia). The interpretation criteria applied were those suggested by CLSI.[25]

The strains that were considered intermediate or resistant to DOR, MER or IMP have a zone of inhibition diameter < 22mm. All positive strains were subsequently analysed by PCR method using four different primers listed in Table 1. Each PCR reaction contained: 5µl PCR buffer, 5µl dNTP, 1.5µl $MgSO_4$, 1µl forward primer, 1µl reverse primer, 0.5µlTaq polymerase, 3µl DNA template and 33µl distilled water. For PCR method, the initial denaturation phase for each PCR assay with different primers was established on 94°C for 5 min also denaturation was 94°C for 1 min. The annealing time was 1 min for all primers and temperature was 56°C. The extension time was 1 min at 72°C. The final extension was done at 72°C for 7 min. PCR products were visualized by electrophoresis on 0.8% agarose gels in Tris boric acid/EDTA buffer (pH 7.0) and stained with 1% ethidium bromide.

Minimals Inibitory Concentrations (MICs) were determined by a broth macrodilution method with Mueller-Hinton broth (Himedia) and containing bacterial inoculum of 10^7 CFU per tube as per CLSI guidelines. Results were recorded after incubation for 18 h at 37°C. *E. coli* CS109 is use as sensitive control. MIC determinations were performed in quadruplicate.

Table 1: Primers used in this study.

Primer	Sequence, 5' 3'	Gene	Product size, bp
NDM-F NDM-R	GGG CAG TCG CTT CCA ACG GT GTA GTG CTC AGT GTC GGC AT	bla_{NDM}	476
VIM-F VIM-R	GAT GGT GTT TGG TCG CAT A CGA ATG CGC AGC ACC AG	bla_{VIM}	390
IMP-F IMP-R	TTG ACA CTC CAT TTA CDG GAT YGA GAA TTA AGC CAC YCT	bla_{IMP}	139
SIM-F SIM-R	TAC AAG GGA TTC GGC ATC G TAA TGG CCT GTT CCC ATG TG	bla_{SIM}	571

III- RESULTS AND DISCUSSION

Bacterial screening

All the six isolates of our study displayed resistance against carbapenem while *E. coli* control strains were observed to be sensitive against carbapenem (Table 2).

Table: 2 Zone of inhibition diameters.

	IMIPENEM				MEROPENEM				DORIPENEM			
	Diameters (mm)	S	I	R	Diameters (mm)	S	I	R	Diameters (mm)	S	I	R
E. coli 2443	28	X			36	X			40	X		
E. coli NGM1	30	X			40	X			32	X		
E. coli NGM2	26	X			27	X			26	X		
E. coli NGM11	34	X			28	X			30	X		
U1410	12			X	10			X	11			X
U1672	20		X		18			X	19			X
W248	14			X	13			X	15			X
W236	13			X	13			X	13			X
W274	12			X	14			X	15			X
W276	10			X	12			X	20		X	

S: Susceptible I: Intermediate R: Resistant

Plasmids extractions

Plasmids extraction was performed and confirmed by agarose gel electrophoresis (Fig 1).

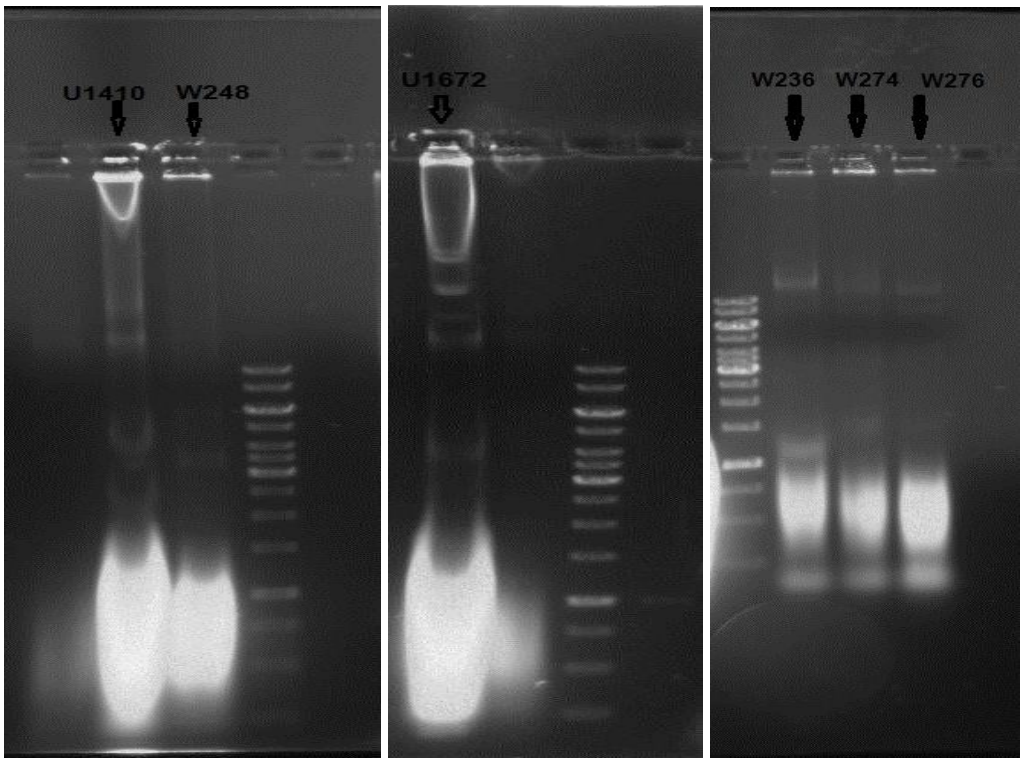
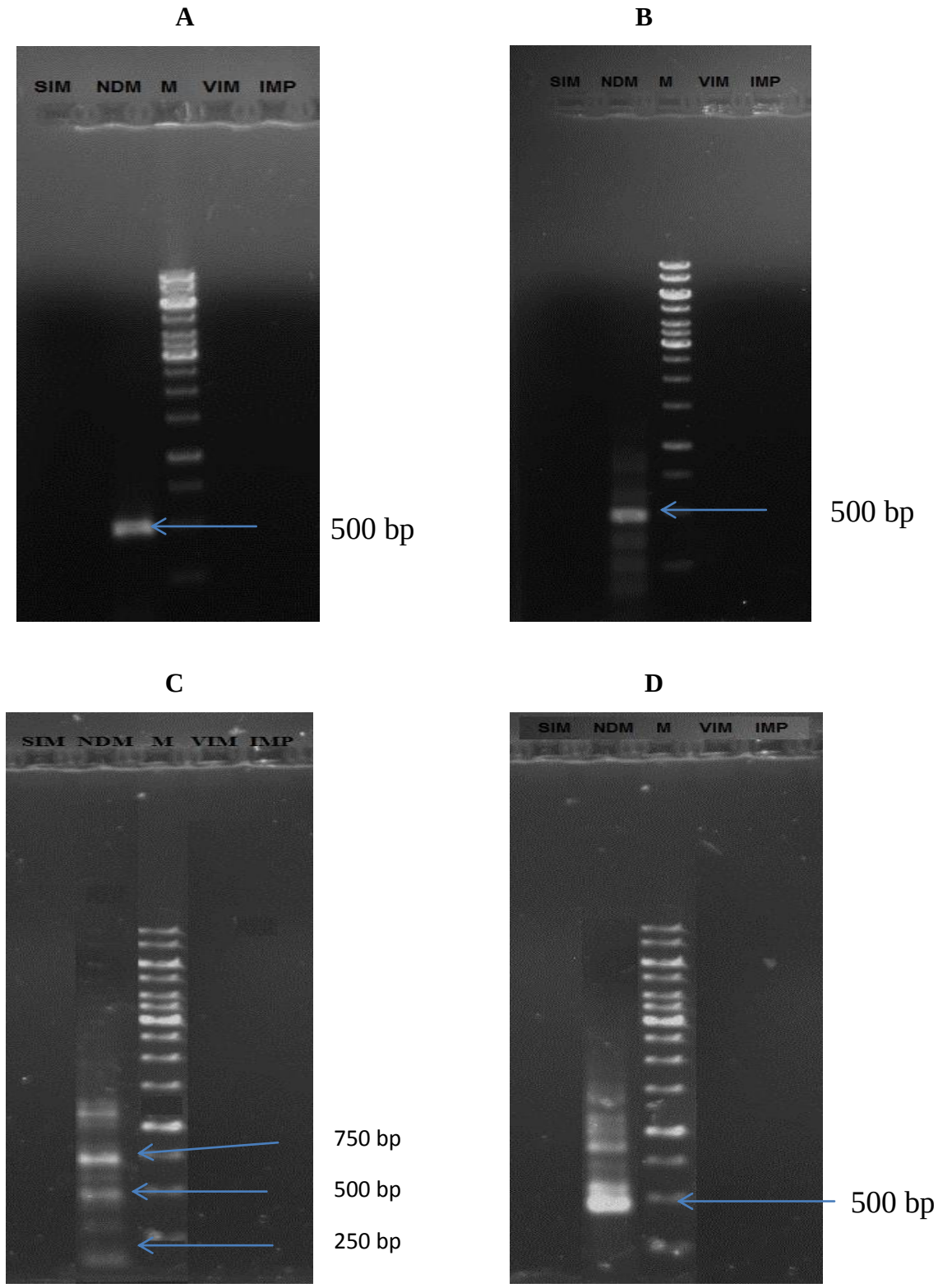


Fig.1. Agarose gel electrophoresis (0.8%) used to confirm plasmids extraction.

The appearance of bands under ultraviolet rays shows presence of plasmid DNA.

PCR products

The detection primers were used in PCR to identify the responsible genes for carbapenemase activity (Fig 2).



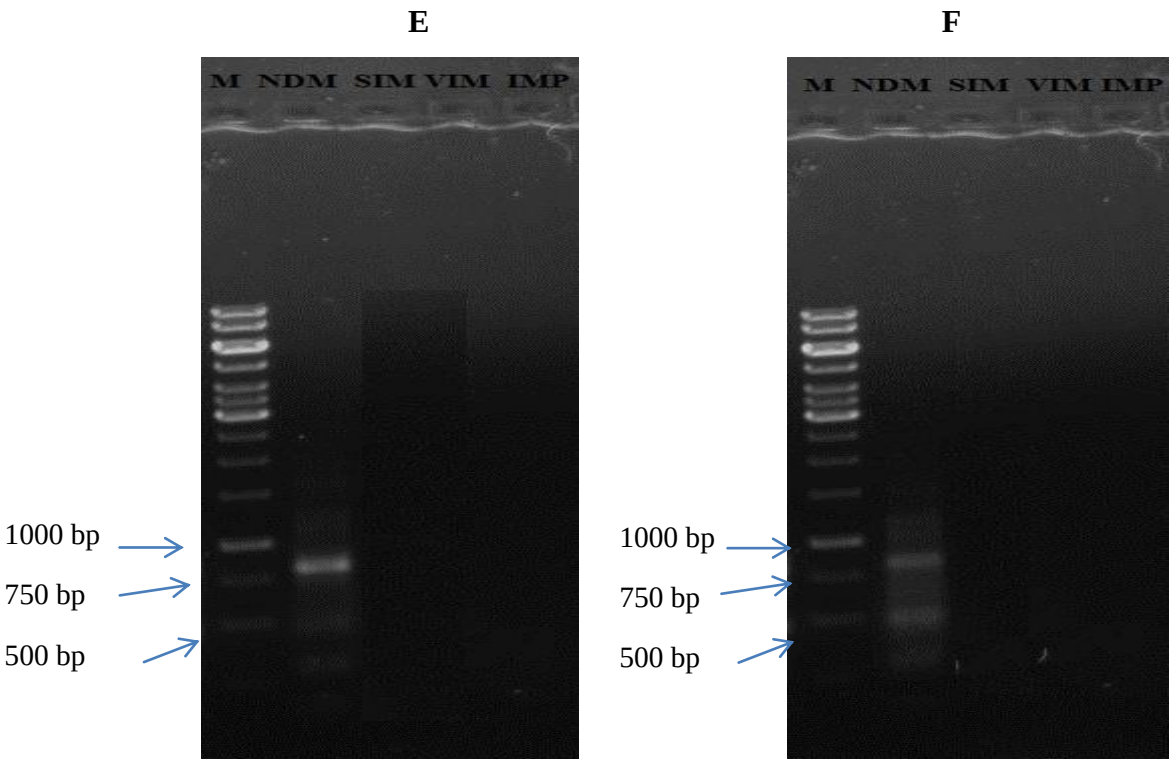


Fig.2. Agarose gel electrophoresis (0.8%) used for identification of the resistance gene.

(A) U1410 was containing blaNDM genes. (B) W248 was containing blaNDM genes (C) W236 was containing blaNDM genes. (D) U1672 was containing blaNDM genes; (E) W274 was containing blaNDM genes. (F)W276 was containing blaNDM genes.

All the six isolates contain at least the NDM gene as the carbapemen resistance gene. Only isolates U1410, U1672 and W248 are given exactly the expected size (476 bp) with the NDM primers. Others have given one or more bands having differents or equals size to the expected size.

Table 3: Minimal Inibitory Concentrations (MICs)

Antibiotic		MIC (µg/mL)		
	CS109	U1410	W248	W274
Imipenem	0.1	>50	>50	50
Faropenem	0.2	>50	>50	25

According to the CLSI[25], a strain is said resistant to carbapenems when its MIC is ≥ 4µg/mL. So the isolates (U1410, W248 and W274) are very resistant to Imipenem and Faropenem. That confirmed the results obtained above.

IV-CONCLUSION

The objective of this study was to perform the Isolation, identification and molecular characterization of the carbapenemase producing Gram-negative Bacilli. From a Gram negative bacilli groups, we were able to identify those who are resistant to carbapenems, which are the last resort in hospitals. The resistance of those bacteria is carried by bla_{NDM} genes. Further work will, by other techniques, find ways to work around this resistance.

ACKNOWLEDGMENTS

I am grateful to Indian government (Federation of Indian Chambers of Commerce and Industry) for the award of fellowship, to IIT Kharagpur, more precisely to Molecular Microbiology Lab directed by Dr. Anindya S. Ghosh, who accepted me within that laboratory.

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