

Full Length Research Paper

Prevalence and characterization of NDM-1 and OXA-48 carbapenemase gene harboring *Enterobacteriaceae* in a tertiary care hospital, South India

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The occurrence of carbapenem resistance among *Enterobacteriaceae* has reached critical levels worldwide. The present study was carried out to screen for carbapenem resistance among *Enterobacteriaceae* isolates from blood, surgical wound and tracheal samples in different wards and intensive care units between the period of 2010 through 2012 in a teaching hospital attached to JIPMER, south India. A total number of 425 meropenem-resistant isolates belonging to *Enterobacteriaceae* were included. Genes encoding β -lactamases (*bla*NDM-1 and *bla*OXA-48) and influx of β -lactams through the major porin OmpK36 were characterized. Multiplex PCR amplification assays indicated that out of 425 meropenem-resistant isolates, 75 and 187 isolates harbored *bla*NDM-1 with and without OmpK36, respectively. One and five *Klebsiella pneumoniae* isolates harbored *bla*OXA-48 with presence and absence of OmpK36, respectively. Transferability of the plasmids was tested by conjugation assays and it was found that 110 transconjugants carried *bla*NDM-1. *bla*OXA-48 gene was not transferable by conjugation analysis. The study highlights the emergence of *bla*NDM-1 and *bla*OXA-48 genes in this center.

Key words: *Enterobacteriaceae*; *bla*NDM-1; *bla*OXA-48; OmpK36; conjugation.

INTRODUCTION

Antibiotic resistance is one of the most important growing global challenges to humans in the environment (Korzeniewska and Harnisz, 2013; Prabaker and Weinstein, 2011; Rhomberg and Jones, 2009; Duijn et al., 2011). Carbapenems are very useful beta-lactams for treatment of infections due to MDR *Enterobacteriaceae*. Several investigations have been reported on the importance of long term care facilities especially hospitals as reservoirs of multidrug-resistant bacteria (MDRB) and colonization with these bacteria may last for an extended

period, and patients in long term care facilities represents a major route for the introduction of MDRB to acute-care facilities (Korzeniewska and Harnisz, 2013; Lewis et al., 2007; Mansouri and Abbasi, 2010; Pitout and Laupland, 2008).

Acquisition of carbapenemase enzymes and loss of porins are the main mechanism of resistance to this group of antibiotic (Cuenca et al., 2003; James et al., 2009). The most prevalent carbapenemases are the molecular class B metallo- β -lactamase (MBL), mainly

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Table 1. Primers used to identify *bla*NDM-1, *bla*OXA-48 and *OmpK36* genes by multiplex PCR analysis.

Name	Forward	Reverse	Size (bp)	Reference
NDM-1	GGTGCATGCCCGGTGAAATC	ATGCTGGCCTTGGGGAACGS	660	Michael et al., 2011
OXA-48	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG	438	Diana et al., 2012
<i>OmpK36</i>	CAGCACAATGAATATAGCCGAC	GCTGTTGTCGTCCAGCAGTTG	1,115	Frank et al., 2006

*bla*NDM-1 and *bla*OXA-48 (Castanheira et al., 2011; Kilic et al., 2011). Particularly, in last few years *bla*NDM-1 (Castanheira et al., 2013) and *bla*OXA-48 (Pitart et al., 2011) genes are carried on plasmids that enable their transfer between different species of Enterobacteriaceae (Mohamed et al., 2013). Outer membrane protein (OMP) serve as a major gateway for the passage of β -lactams and deficiency of this protein has emerged as a major mechanism in multidrug resistant (MDR) Enterobacteriaceae (Jacoby et al., 2004; Pfeifer et al., 2012). Furthermore, previous investigations have been reported that deficiency in OMP expression resulting in the elevation of MIC in *K. pneumoniae* than that of non-deficient strains (James et al., 2009; Jacoby et al., 2004; Pfeifer et al., 2012).

In this work, an attempt has been made to study the characteristics of the recent dissemination of *bla*OXA-48 and *bla*NDM-1-producing Enterobacteriaceae in *OmpK36* deficient strains and to determine its prevalence in strains isolated from patients.

MATERIALS AND METHODS

Bacterial isolates and susceptibility tests

A total of 425 meropenem-resistant Enterobacteriaceae isolates were collected between 2010 and 2012 from clinical specimens and tested for decreased susceptibility to carbapenems by agar dilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2011). According to CLSI recommendations, the production of carbapenemase among the isolates was phenotypically confirmed by the modified Hodge test and meropenem/ethylene diamine tetra-acetic acid (EDTA) double-disk synergy test as per CLSI guidelines 2011. *Escherichia coli* ATCC 25922 was used as a negative control.

PCR amplification

Total DNA was extracted from all strains by boiling lysis method. A multiplex polymerase chain reaction (PCR) was used to amplify genes encoding *bla*NDM-1 and *bla*OXA-48 and *OmpK36* (Table 1). A total of 30 cycles were performed with a thermal cycler (Master cycler gradient, eppendorf) consisting initial denaturation step for 15 min at 95°C, denaturation step for 1 min at 95°C, an annealing step for 1 min at 59°C and extension step for 1 min 30 s at 72°C. After the final cycle there was a step of final extension of 10 min at 72°C. The PCR products generated were visualized by ethidium bromide staining after electrophoresis in a gel containing 1.5% agarose.

Conjugation experiments

Conjugation was carried out at 30°C using sodium azide resistant

E. coli J53 as recipient. Transconjugants were selected on MacConkey agar plates supplemented with meropenem (4 mg/L) and sodium azide (100 mg/L) (Jamal et al., 2012). Plasmid was extracted from transconjugants, respectively and analyzed for *bla*NDM-1 and *bla*OXA-48 genes. Multiplex PCR was carried with respective *bla*NDM-1 and *bla*OXA-48 primers (Table 1).

RESULTS

The 425 meropenem-resistant isolates included *Klebsiella* spp. 229, *Enterobacter* spp. - 89, *E. coli* - 85, *Citrobacter* spp - 14, *Providencia* spp - 5, *Proteus* spp - 3. Figure 1 shows the presence of MBL-encoding genes such as *bla*NDM-1 and *bla*OXA-48 in both *OmpK36*-deficient and non-deficient strains, respectively.

Conjugation experiments were carried out on all 425 clinical isolates using sodium azide-resistant J53 *E. coli* strain as a recipient. Transconjugants were selected on MacConkey agar plate containing meropenem and sodium azide. 117 (27%) isolates had shown positive on agar plate with sodium azide and meropenem. Plasmid was extracted from all the selected transconjugants by alkaline lysis method, and *bla*NDM-1 and *bla*OXA-48 genes were analyzed by multiplex PCR assays (Figure 2). From this study, it was observed that 110 out of 117 transconjugants carried *bla*NDM-1 and none carried *bla*OXA-48.

DISCUSSION

The emergence of carbapenemase-producing Enterobacteriaceae has been reported in several studies worldwide (Castanheira et al., 2011; Mohamed et al., 2013; Duin et al., 2013; Castanheira et al., 2011; Poirel et al., 2011). The present study showed the emergence of *bla*NDM-1 and *bla*OXA-48 co-producers among Enterobacteriaceae isolates. It was also observed that there is loss of porin *OmpK36* in Enterobacteriaceae isolates contributing to the carbapenem resistance. Multiplex PCR analysis illustrated the presence of MBL-encoding genes such as *bla*NDM-1 and *bla*OXA-48 in both the *OmpK36* deficient and non-deficient Enterobacteriaceae isolates. Out of 425 isolates, 136 isolates had shown the presence *OmpK36*. A total number of 187 isolates harboured *bla*NDM-1 without *OmpK36* gene, 5 isolates harboured *bla*OXA-48 without *OmpK36*, 75 isolates harboured *bla*NDM-1 with *OmpK36* and one *Klebsiella pneumoniae* harboured *bla*OXA-48 with *OmpK36*. All the 5 isolates carrying *bla*OXA-48 without

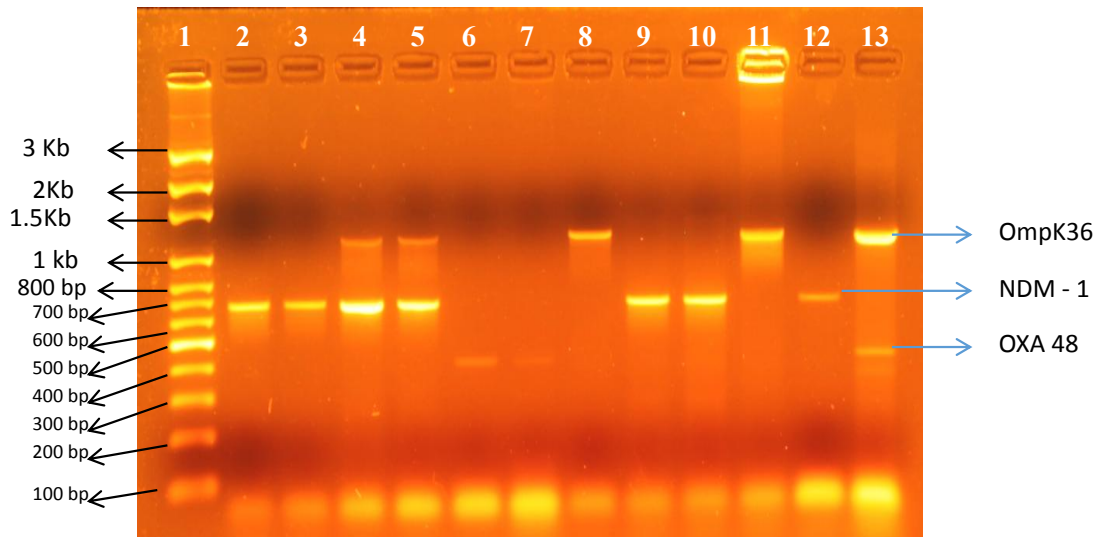


Figure 1. MBL-encoding genes (*bla*_{NDM-1} and *bla*_{OXA-48}) and OmpK36 gene were detected by PCR and visualized by agarose gel electrophoresis and ethidium bromide staining. Lane 1 shows the molecular weight marker (100 bp to 3 Kb). Lanes 2, 3, 9, 10 and 12 shows the presence of NDM-1 gene alone and Lanes 4 and 5 shows the presence of NDM-1 with OmpK36. Lanes 6 and 7 shows the presence of *bla*_{OXA-48} gene alone and Lane 13 shows the presence of genes of *bla*_{OXA-48} with OmpK36. Lanes 8 and 11 shows the presence of OmpK36 gene alone.

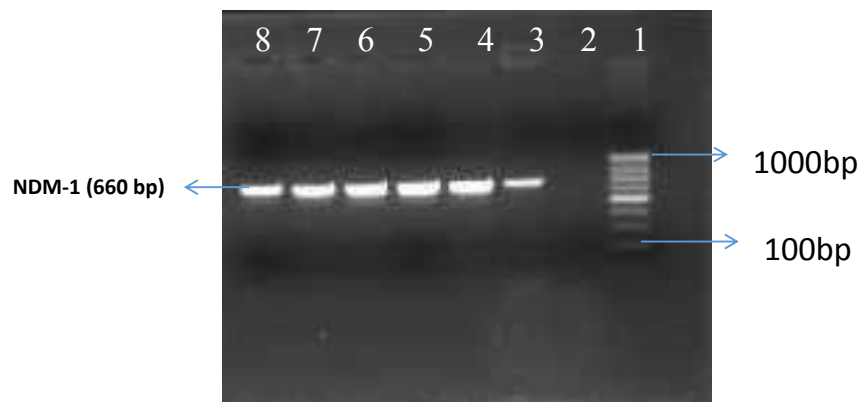


Figure 2. Detection of *bla*_{NDM-1} in plasmid preparations by PCR. The amplicons were separated by agarose gel electrophoresis and visualized with ethidium bromide staining. Lane 1 showing the molecular weight marker (100 to 1000 bp) and lanes 3 to 8 shows the presence of *bla*_{NDM-1}.

OmpK36 was found to be *K. pneumoniae*. Conjugation results using sodium azide-resistant recipient *E. coli* J53 with selection of 110 transconjugants on MacConkey agar carried only *bla*_{NDM-1} and no transconjugants were found to carry *bla*_{OXA-48}. Several studies worldwide have reported the prevalence of transconjugants bearing *bla*_{NDM-1} plasmids (Balm et al., 2013).

The present work highlights the prevalence of *bla*_{NDM-1} and *bla*_{OXA-48} in the clinical isolates of Enterobacteriaceae isolated in this centre. It is also remarkable to note that *bla*_{NDM-1} is found to be the most

prevalent in this centre accounting to nearly (264) 62% of the meropenem-resistant isolates, but the isolates with *bla*_{OXA-48} were only 1.4%. Furthermore, conjugation studies corroborated that 57 strains of *Klebsiella* spp, 18 strains of *Enterobacter* spp. and 21 strains of *E. coli* harboured *bla*_{NDM-1} plasmids and they were successfully transferred to the recipient strain *E. coli* J53 by conjugation. The results in this study indicated that there is increasing frequency of transmissible resistance genes such as *bla*_{NDM-1} and *bla*_{OXA-48} among the meropenem-resistant Enterobacteriaceae strains

isolated from the clinical samples.

Conclusion

*bla*NDM-1 and *bla*OXA-48 appear to be an emerging cause of carbapenem resistance in Enterobacteriaceae in India. The present study shows that *bla*NDM-1 is the most frequent resistance gene identified among the carbapenem-resistant isolates, and for some isolates, resistance to carbapenems is also related to the presence of *bla*OXA-48. The relationship between the occurrence of the resistance genes (*bla*NDM-1 and *bla*OXA-48) and the presence or absence of OmpK36 gene has been worked out in the study.

Conflict of interests

The author(s) did not declare any conflict of interest.

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