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Propagation Of *bla*$_{IMP}$ Within IncHI1 In *Escherichia Coli* From A Tertiary Referral Hospital Of India

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INTRODUCTION

Among multidrug-resistant Enterobacteriaceae, imipenem resistant carbapenemase (IMP), which encodes the ***bla***$_{IMP}$ gene, which is a significant plasmid-mediated carbapenemase and one of the most widely distributed metallo-beta-lactamase (MBL) in the world. The World Health Organization has identified *Escherichia coli* as a critical concern due to its global dissemination in the human gut and its prevalence in the environment. The current study characterizes the presence of *bla*$_{IMP}$ producing *Escherichia coli* of multidrug resistant phenotype and its carriage within a single study centre in India.

METHODOLOGY

Table 1: List of oligonucleotide primer pairs used for amplification of *bla*$_{IMP}$ gene:

Target	Primer pairs	Sequence (5'-3')	Amplified product size
bla$_{IMP}$	Forward	GTGGGGCGTTGTTCCTAAAC	139 bp
	Reverse	CAGGCAACCAAACCACTACG	

Plasmid isolation kit (by Qiagen, Germany)

Horizontal gene transferability (Transformation) was checked by heat shock method

Plasmid incompatibility typing was done

Table 2: List of oligonucleotide primer pairs used for plasmid incompatibility typing:

Primer pairs	Sequence (5'-3')	Amplified product size(bp)
HI 1-FW	5'-GGAGCGATGGATTACTTCAGTAC-3'	471
HI 1-RV	5'-TGCCGTTTCACCTCGTGAGTA-3'	
HI 2-FW	5'-TTTCTCCTGAGTCACCTGTTAACAC-3'	644
HI 2-RV	5'-GGCTCACTACCGTTGTCATCCT-3'	
I 1-FW	5'-CGAAAGCCGGACGGCAGAA-3	139
I 1-RV	5'-TCGTCGTTCCGCCAAGTTCGT-3'	

RESULTS

Among 70 clinical isolates, 45 of them were confirmed to be *Escherichia coli* and out of them, 41 were non-susceptible to Imipenem antibiotics. Amongst them, 21 showed MIC above ≥64 µg/mL of Imipenem concentration, while rest showed MIC below ≤64 µg/mL

Out of the 41 multi drug resistant carbapenem non-susceptible isolates, 5 of them harboured the ***bla***$_{IMP}$ gene after PCR assay. For the positive isolates, plasmid was isolated and horizontal gene transferability was checked by transformation assay with 0.25µg/mL Imipenem concentration on LB agar plates by using *E. coli* DH5α as recipient strain

For the transformants, plasmid isolation was done further to know the plasmid incompatibility typing group responsible for carriage of ***bla***$_{IMP}$ gene

Fig 1: *Escherichia coli* on Mac Conkey agar

Fig 2: *Escherichia coli* on EMB agar

Fig 3: MIC plate

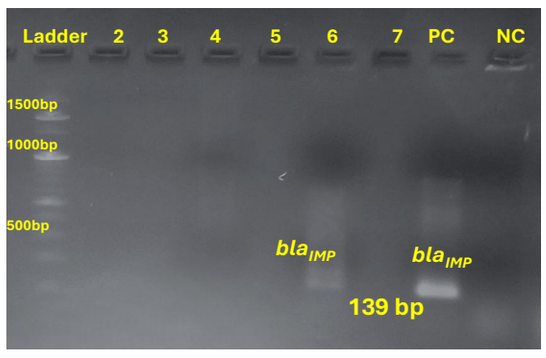

Fig 4: Gel image showing PCR amplification of *bla*$_{IMP}$ gene(139bp)

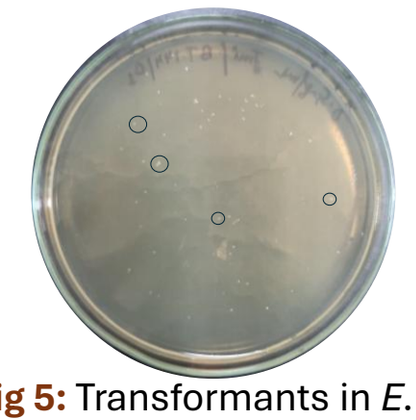

Fig 5: Transformants in *E. coli* DH5α with antibiotic stress

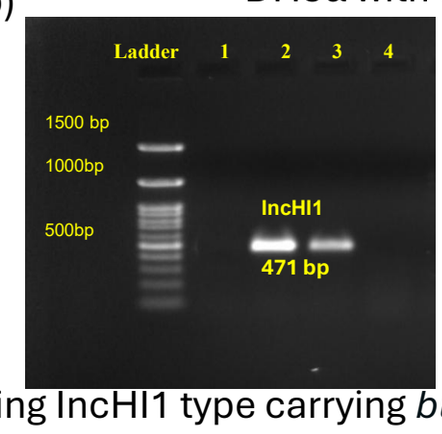

Fig 6: Gel image showing IncHI1 type carrying *bla*$_{IMP}$ gene

CONCLUSION

The current study highlighted the occurrence of *bla*$_{IMP}$ producing *Escherichia coli* and also IncHI1 type plasmid have the potential to be the primary vector facilitating the spread of the *bla*$_{IMP}$ genes within a single study centre in India.