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Short Communication

Incidence of *bla*$_{IMP}$ and *bla*$_{VIM}$ Genes among Carbapenemase Producing *Escherichia coli* in Lahore, Pakistan

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SHK and SJ performed the lab work. IA provided chemicals and reagents. SR wrote the manuscript. TR supervised and design the experiments.

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ABSTRACT

Antibiotic drug resistance in *E. coli* is a worldwide health problem. Particularly, increasingly reported carbapenem resistance due to carbapenemases is highly worrisome. Literature regarding incidence of class B metallo-β-lactamases (MBLs) - producing *E. coli* has not yet judiciously been addressed. In this study, we investigated the occurrence of carbapenemase- and MBLs-producers among a collection of 100 carbapenem resistant *E. coli* obtained from a tertiary hospital at Lahore Pakistan. All carbapenemase producers were further investigated to identify frequency of *bla*$_{IMP}$ and *bla*$_{VIM}$ encoding genes. Results of modified hodge test identified 81% carbapenemase producers, while combined disc diffusion test identified 28 isolates as MBL producers among them. Of all carbapenemase producers, 25 isolates (30.8%) of carbapenemase-producing *E. coli* were found to harbor *bla*-$_{VIM}$ gene, while 13 isolates (16.04%) were found to carry *bla*$_{IMP}$ gene. We report on high incidence rate of carbapenemase producers among carbapenem resistant *E. coli* isolates.

All over the world, Antibiotic drug resistance (AMR) is emerging as a major health problem. Particularly resistant to carbapenem drug is highly worrisome as these drugs are considered to be the last resort for patients infected with multidrug resistant pathogens (Nordmann *et al*., 2012; Rahman *et al*., 2018a). The issue is even more serious in countries with less investment in health sectors and unrestricted regulations in drugs. In Pakistan, the irrational and indiscriminate use of antibiotics in hospitals and in general practices has led to the emergence of strains of bacteria that are resistant to one or more antibiotics (Ain *et al*., 2018; Amin *et al*., 2011; Khattak *et al*., 2018; Shah *et al*., 2017; Rahman *et al*., 2018b, c). There is a danger of the situation worsening because very few new antibiotic drugs are entering into clinical practice. Without effective action, it has been estimated that the worldwide death toll due to drug-resistant infections could reach 10 million by 2050 (https://amr-review.org/home.html).

Escherichia coli is one of the most important type of Enterobacteriaceae that causes urinary tract infections, respiratory tract infections, intestinal infections, septicemia and other hospital-acquired infections (Griffin and Tauxe, 1991). Empirical therapy using antibiotics has led *E. coli* to become resistant to different antibiotics, including carbapenems in *E. coli* recovered from human clinical settings, community and food animals (Adnan *et al*., 2017; Ali *et al*., 2016, 2017; Amin *et al*., 2011). Carbapenems are considered to be highly efficient against multidrug resistant *E. coli* and prescribed when other drugs do not respond. However, *E. coli* has developed various mechanisms of resistance including production of carbapenemase enzymes to inactivate carbapenem drugs (Nordmann *et al*., 2011; Aqil *et al*., 2018). The Ambler classification of β-lactamases (carbapenemases) describes four types (A, B, C, D) according to amino acid sequence homology with over 900 β-lactamases describes so far. Class B β-lactamases includes metallo β-lactamases

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enzymes that require zinc ions for enzymatic activity. All MBLs share a common feature of being inhibited by ethylenediaminetetraacetic acid (EDTA) and other metal chelating agents, due to their metal-dependent catalytic mechanism. The hydrolysis mechanism is unique for MBLs compared with other β-lactamases because no stable or pseudo-stable covalent intermediate is formed during hydrolysis. They are therefore not inhibited by classical serine β-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam (Meletis, 2016).

The first MBL was detected and studied in the environmental and opportunistic pathogenic bacterium *Bacillus cereus* (Lim *et al*., 1988). In Pakistan, the first MBL-producing strain was a *Pseudomonas aeruginosa* isolated from a patient with urinary tract infection in Rawalpindi (Butt *et al*., 2005). The $bla_{\text{-IMP}}$ and $bla_{\text{-VIM}}$ types of genes are considered to be important and widely reported MBLs (Nordmann *et al*., 2011; Toleman *et al*., 2005). Literature study suggested that the incidence $bla_{\text{-IMP}}$ and $bla_{\text{-VIM}}$ types of genes have been predominantly reported in Asia (Amudhan *et al*., 2012; Bahar *et al*., 2010). However, the prevalence of these two genes has not yet judiciously been highlighted in *E. coli*. Therefore, the current study was designed to investigate the occurrence of MBLs in a tertiary care hospital Lahore Pakistan. We report on high occurrence of carbapenemase-producing *E. coli* carrying $bla_{\text{-IMP}}$ and $bla_{\text{-VIM}}$ types of genes.

Materials and methods

This study was conducted between Sept 2017 and April 2018. A total of one hundred non duplicate *E. coli* isolates, which were found resistant to at least one carbapenem drug were obtained from collection of a tertiary care hospital at Lahore, Punjab Pakistan.. *E. coli* was reconfirmed on the basis of colonial characteristics and morphology and biochemically using an API 20E Test System for Enterobacteriaceae (bioMérieux, France). Antimicrobial susceptibility of isolates was tested by the Kirby-Bauer disc diffusion method using mueller-hinton agar (Oxoid, UK), according to Clinical Laboratory Standards Institute (CLSI). The antibiotic discs contained imipenem (10 µg) and meropenem (10 µg). Carbapenemase-producing *E. coli* were identified by the modified hodge test (Genc *et al*., 2016) and phenotypic detection of MBL was done by a combined disc diffusion method as per guidelines of Clinical laboratory Standard Institute (CLSI, 2016). In the combined disc test, if the increase in inhibition zone and imipenem-EDTA disc was ≥7 mm than the imipenem alone, the test was considered as MBL-positive.

Isolates found positive for carbapenemase production were subjected to PCR based identification for bla_{IMP} and bla_{VIM} using primers described in Table I. Plasmid DNA was extracted through plasmid isoaltion kit (TIANGEN Biotech Beijing, Co., Ltd.). The quality and quantity of extracted DNA was determined through nanodrop as well gel electrophoresis. Primers for PCR were synthesized by GeneLink, while master mix was purchased from Thermo Scientific. PCR reaction was performed in a total of 25µl reaction mixture. PCR was performed at a range of temperatures of 50-56°C and the optimum temperature was identified as 55°C.

Results and discussion

All *E. coli* isolates were picked up from carbapenem-resistant culture collection and were tested again against meropenem and imipenem antibiotics to ensure its resistance pattern against carbapenem drugs. Results showed that all of these 100 isolates of *E. coli* were carbapenem non-susceptible, all being resistant to imipenem (100%) and meropenem (100%). Out of these 100 isolates, 81 were found positive for carbapenemase production based on Modified Hodge Test, whilst only 28 isolates were phenotypically positive for MBL production. Our current observed incidence rate of carbapenemase production among imipenem-resistant *E. coli* is higher as compared to previous report of 37.1% from Lahore in 2015 (Ain *et al*., 2018). Furthermore, in the same study, Ain *et al*. (2018) observed an overall 63.38% frequency of occurrence of MBL among all clinical isolates as compared to 28% observed in our study (Ain *et al*., 2018). This suggests that in the current study, MBL incidence rate was found much lower as compared to previous study. This could be the strain difference, as we have only focused on *E. coli* while, Ain *et al*. (2018) studied overall prevalence among different strains including *E. coli*. Our results showed that 25 isolates (30.8%) of

Table I.- Primers used for PCR amplification of bla_{IMP} and bla_{VIM} genes.

Target gene	Primer	Primer sequence (5′- 3′)	Amplicon size (bp)	Reference
bla_{IMP}	IMP-F	CTACCGCAGCAGAGTCTTTG	587 bp	Senda *et al*. (1996)
	IMP-R	AACCAGTTTTGCCTTACCAT		
bla_{VIM}	VIM-F	AGTGGTGAGTATCCGACAG	261 bp	Mavroidi *et al*. (2000)
	VIM-R	ATGAAAGTGCGTGGAGAC		

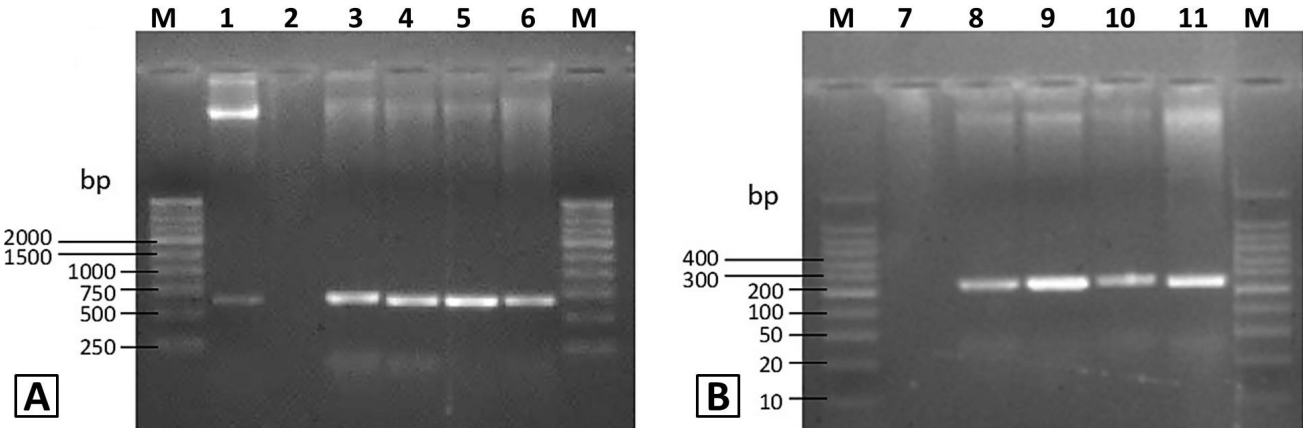

Fig. 1. PCR amplification of *bla*$_{IMP}$ and *bla*$_{VIM}$ genes from *E. coli* isolates. Ethidium bromide stained agarose gel demonstrating PCR amplification of the *bla*$_{IMP}$ gene (A) and the *bla*$_{VIM}$ gene (B) from *E. coli* isolates. Lane M, base pair size markers; Lane 1, positive control; Lane 2 and 7, negative control; Lanes 3 to 6, *bla*$_{IMP}$ gene positive samples; Lanes 8 to 11, *bla*$_{VIM}$ gene positive samples.

carbapenemase-producing *E. coli* were found to harbor *bla*-$_{VIM}$ gene, while 13 isolates (16.04%) were found to carry *bla*$_{IMP}$ gene (Fig. 1). All PCR amplicon of the targeted genes were sequenced and confirmed through BLAST. A recent study from district Lahore concluded that of 100 carbapenem resistant isolates, 93 and 89 isolates were positive for carbapenemase and β-lactamase production, respectively, while, 3.3% of these isolates were found positive for *bla*-$_{IMP}$ and 32.5% were found positive for *bla*-$_{VIM}$ (Akhtar *et al*., 2018). Our results are corroborates with the result of studies reported in India, Madagascar and Brazil where 94.4%, 89.8% and 88.2% carbapenemase positivity was described among gram-negative bacteria, respectively (Amudhan *et al*., 2012; Andriamanantena *et al*., 2010; Franco *et al*., 2010). Other studies suggest widespread presence of carbapenemase encoding genes in other parts of Paksitan suggesting to initiate steps to discourage dissemination of these features. This suggest that incidence of carbapenemase producers are quite high with considerably proportion of strains carrying *bla*-$_{IMP}$ gene. The higher incidence rate of MBL and subsequent higher occurrence of *bla*-$_{IMP}$ and *bla*-$_{VIM}$ genes in our clinical isolates is possibly due to the ability of *E. coli* to capture novel resistance genes through horizontal recombination located on the conjugative plasmids. The absence of *bla*-$_{IMP}$ and *bla*-$_{VIM}$ genes in the rest of carbapenem resistant strains does reflect that other types of carbapenemase encoding genes may also be present in the vicinity. It is speculated that other types of carbapenemase encoding genes such *bla*$_{NDM-1}$, *bla*$_{OXA-48}$ *etc*. may also be widespread in the area and should be investigated. In conclusion, the results describe here indicate high prevalence of carbapenemase-producing *E. coli* which, if not properly managed, will present a devastating challenge for the health care system of Pakistan.

Conclusion

It is concluded that *E. coli* isolated from different patients at a tertiary care hospital at Lahore indicate high prevalence of carbapenemase production stains comprising MBL. Majority of these isolates were encoding *bla*-$_{IMP}$ and *bla*-$_{VIM}$ genes, however, possibility of existence of other genes cannot be rule out. Notably, *bla*-$_{VIM}$ gene was found more prevalent (30.8%) than the *bla*-$_{IMP}$ gene (16.04%). This suggests to restrict the use of carbapenem drugs in order to restrict further dissemination of resistance.

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Statement of conflict of interest

The authors have no conflicts of interest to report.

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