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Letter to the Editor

First identification of clinical isolate of a Novel "NDM-4" producing *Escherichia coli* ST405 from urine sample in Pakistan

Dear Sir,

New Delhi metallo-β-lactamase producing *Enterobacteriaceae* are a serious threat to the public health sectors worldwide. NDM producing pathogens generally display resistance against several different classes of antibiotics including carbapenems; considered last resort to treat infections caused by such pathogens.[1,2] Until now 19 NDM variants (NDM-1 to NDM-19) have been identified from different parts of the world http://www.lahey.org/Studies/other.asp. NDM-4 differs from NDM-1 by a single point mutation at position 154 (M–L) and is associated with high carbapenemase activity.[3] We are first time reporting the description of NDM-4 producing *Escherichia coli* isolated from urine sample in Pakistan.

A 4-year-old female patient was hospitalized in a tertiary care hospital Lahore, Pakistan in March 2014. After admission, her urine culture revealed the presence of *E. coli* which was confirmed by VITEK® 2 system (bioMerieux, France) and MALDI-TOF (Bruker, Germany). Isolate was also carbapenemase and metallo-β-lactamase producer identified by modified Hodge's test and double disk synergy method respectively.[4] Minimum inhibitory concentration (μg/mL) of antibiotics using GN XN05 card in VITEK® 2 compact system (bioMerieux, France) displayed pan-drug resistance to commonly used antibiotics including meropenem and only effective drug was colistin. Furthermore, colistin susceptibility was determined by broth microdilution assay in 96 microtiter Plate[5] (Table 1). A previous study reported that NDM-4 producing bacteria has higher MIC (μg/mL) and hydrolytic activity as compared to NDM-1 producing bacteria.[3] Genotyping of bla_{NDM-4} was accomplished by PCR (NDM-F-TGGCTTTTGAAACTGTCGCACC, NDM-R- CTGTCACATCGAAATCGCGCGA) and DNA sequence analysis. Plasmid characterization was performed as previously reported.[4] S1 nuclease pulse field gel electrophoresis and in gel DNA hybridization revealed the presence of bla_{NDM-4} on 120 kb of plasmid (data not shown). As per Carratoli[6] procedure of plasmid typing, the isolate contained the Incompatible FII group of plasmids. Multilocus sequence typing (MLST) was performed as described earlier and *E. coli* belonged to the sequence type (ST) 405.[7] ST405 NDM producing *E. coli* has also been isolated from two inpatients in Italy.[8] It is documented that the Indian sub-continent is the main reservoir and source of NDM producing bacteria globally. Present study also supported this fact in that the patient had no previous travel history. Moreover, NDM-4 producing *E. coli* has also been reported from different parts of the world including India and Italy.[3,8] This study indicates, the emergence of NDM-4 producing *E. coli* ST405 which can lead to therapeutic failure and deaths particularly in children. This isolate could be transferred through risk factors such as bed sharing, substandard infection control practices and most importantly urinary catheters.

The bla_{NDM} containing IncFII plasmids might have become the common vehicle for the spread of various NDM alleles

Table 1 – MIC (μg/mL) of NDM-4 producing *Escherichia coli*.

Isolates	SAM	PIP	CXM	CXA	CFM	CRO	FEP	ATM	MEM	LEV	MXF	MNO	TE	TMP	C	TGC	CS
NDM-4 producing *E. coli*	≥32	≥128	≥64	≥64	≥4	≥64	≥64	≥64	≥16	≥8	≥8	≥16	≥16	≥16	≥64	4	1

SAM: ampicillin/sulbactam, PIP: piperacillin, CXM: cefuroxime, CXA: cefuroxime axetil, CFM: cefixime, CRO: ceftriaxone, FEP: cefepime, ATM: aztreonam, MEM: meropenem, LEV: levofloxacin, MXF: moxifloxacin, MNO: minocycline, TE: tetracycline, TMP: trimethoprim, C: chloramphenicol, TGC: tigecycline, CS: colistin.

among *Enterobacteriaceae*. Furthermore, they could also play a relevant role in the spread of such strains and other resistance genes, especially if the isolate is present as part of the dominant microbiota and the hygienic conditions are suboptimal particularly in low-resource settings.

Nucleotide sequence accession number: The nucleotide sequence of bla_{NDM-4} producing *E. coli* strain has been deposited in the BankIt/GenBank/NCBI data base under accession number KY912035.

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Conflicts of interest

All authors declared that there is no conflict of interest.

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