Detection of *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$ genes among Gram-negative bacteria isolated from human immunodeficiency virus patients in South India

Sir,

New Delhi metallo-β-lactamase (MBL)-1 (NDM-1) gene was first detected in extensively drug-resistant (XDR) *Klebsiella pneumoniae* from a Swedish patient of Indian origin.[1] Thereafter, NDM-1 emerged as a leading threat to the treatment of infections caused by Enterobacteriaceae. In this study, we aimed to study the positivity of *bla*$_{NDM-1}$ among Gram-negative bacteria (GNB) isolated from human immunodeficiency virus (HIV) patients attending YR Gaitonde Centre, Chennai, India. Antibiotic susceptibility of bacterial isolates was tested using Kirby–Bauer disc diffusion method.[2] Bacterial DNA extracted by boiling lysis method was used as template in polymerase chain reaction to detect the drug-resistant genes such as *bla*$_{NDM-1,}$ *bla*$_{NDM-5,}$[3] extended-spectrum β-lactamases, Class 1 integron, Class 2 integron, sulfamethoxazole (*sul*), and trimethoprim (*dfr*). In the present study, 45.1% (78/173) of GNB isolated from HIV patients showed resistance to imipenem (IPM) which was highly noted among *Escherichia coli* (73.1%; n = 57). In the E-test, 66.7% of IPM-resistant isolates were positive to MBL production. Among MBL producers, 17.3% (n = 9; $P < 0.001$) showed positive for *bla*$_{NDM-1}$ gene, and among *bla*$_{NDM-1}$ isolates, 77.7% showed XDR profile and 22.2% multidrug-resistant (MDR) profile. Phylogenetic analysis using Molecular Evolutionary Genetics Analysis Version 7.0 (The Pennsylvania State University, University Park, Pennsylvania, United States) revealed that 8 *bla*$_{NDM-1}$ (KU695556) gene sequences had fallen into *bla*$_{NDM-1}$ clad. One *bla*$_{NDM-1}$ fell into *bla*$_{NDM-5}$ (KU695557) clad due to two amino acid substitutions such as valine instead of leucine (Leu) in the 88th position and methionine instead of Leu in the 154th position. *bla*$_{NDM}$ positive isolates also exhibited co-positivity to other drug-resistant genes [Table 1].

Vignesh *et al.* in 2008 reported that IPM is the drug of choice against MDR bacteria and also that only 28% of GNB from HIV patients were resistant to IPM.[4] We found that 45% of GNB were resistant to IPM which indicates that IPM resistance rate has been increasing among HIV population. Another study demonstrated clonal similarity between *bla*$_{NDM-1}$ strains and difference in antibiotic resistance profiles based on 1–5 amino acid substitutions.[1] In our study, *bla*$_{NDM}$ isolates were found clonally different by random amplified polymorphic DNA analysis. In a study from Ecuador, *bla*$_{NDM}$-positive *K. pneumoniae* from HIV patients exhibited co-positivity to *bla*$_{CTX-M}$ and *bla*$_{SHV}$ genes.[5] Our study reports first time the positivity of *bla*$_{NDM-1}$ and its variant *bla*$_{NDM-5}$ among GNB from HIV patients in South India. Continuous monitoring of *bla*$_{NDM}$ genes among GNB is needed due to XDR and MDR profiles which could help in the timely treatment of bacterial infections in HIV patients.

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Conflicts of interest

There are no conflicts of interest.

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Table 1: Positivity of *bla*$_{NDM-1}$ and *bla*$_{NDM-5}$ along with other drug-resistant genes among Gram-negative bacteria isolated from HIV patients

Parameters	*bla*$_{NDM}$ gene positive isolates								
	8EC	10EC	62EC	124EC	137EC	142EC	144KO	156EC	161AB
bla$_{NDM}$ positivity	*bla*$_{NDM-1}$	*bla*$_{NDM-5}$	*bla*$_{NDM-1}$	*bla*$_{NDM-1}$	*bla*$_{NDM-1}$	*bla*$_{NDM-1}$	*bla*$_{NDM-1}$	*bla*$_{NDM-1}$	*bla*$_{NDM-1}$
Organism	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*K. oxytoca*	*E. coli*	*A. baumannii*
Sample	Urine	Pus	Urine	Vaginal swab	Blood	Urine	Urine	Urine	Pus
Age and sex of persons with HIV	52/male	34/female	56/female	33/female	50/male	42/male	47/male	40/male	46/male
CD4 cell count	106 cells/mm^3	351 cells/mm^3	47 cells/mm^3	47 cells/mm^3	503 cells/mm^3	15 cells/mm^3	06 cells/mm^3	145 cells/mm^3	72 cells/mm^3
Phenotypic detection of MBL production									
CDM									
IPM	17 mm	12 mm	18 mm	4 mm	0 mm	12 mm	0 mm	15 mm	13 mm
IPM + EDTA	31 mm	28 mm	28 mm	22 mm	12 mm	27 mm	12 mm	25 mm	24 mm
E-test (MIC)									
IPM	8 µg	8 µg	4 µg	>64 µg	12 µg	>64 µg	3 µg	12 µg	>64 µg
IPM + EDTA	>265 µg	>265 µg	>265 µg	>265 µg	>265 µg	>265 µg	32 µg	>265 µg	>265 µg
Molecular screening of drug resistance genes									
Positivity of ESBL genes	*bla*$_{TEM}$, *bla*$_{CTX-M}$	*bla*$_{TEM}$, *bla*$_{CTX-M}$	*bla*$_{TEM}$, *bla*$_{CTX-M}$	*bla*$_{TEM}$, *bla*$_{OXA}$	*bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{OXA}$	*bla*$_{TEM}$, *bla*$_{CTX-M}$	*bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{OXA}$	*bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{OXA}$	*bla*$_{TEM}$
Integrons	-	-	-	Class 2 integron	Class 2 integron	-	Class 1 Integron	-	-
Sulfamethoxazole resistance	*sul1, sul2*	*sul2*	-	*sul1, sul2*	*sul1, sul2*	*sul1, sul2*	*sul1, sul2*	*sul1, sul2*	*sul1, sul2*
Trimethoprim Resistance	-	-	-	-		*dfrA7*	*dfrA7*	*dfrA7*	*dfrA7*
Antibiotic resistance profile									
Resistance to antibiotics	pip, amp, ctx, caz, cpd, cpz, cro, cxm, at, dox, cip, sxt, ipm, tet, lvx	amk, gen, pip, tzp, amp, amx, ctx, caz, cpz, cpd, cro, cxm, fox, at, cip, sxt, tg, chl, etp, ipm, tet, lvx, nit	pip, tzp, amp, ctx, caz, cro, cpz, at, cip, sxt, ipm, ctx, caz, fox, ofx	amk, pip, tzp, amp, amx, ctx, caz, cro, cxm, fox, at, cip, sxt, tg, chl, ipm, lvx, ofx, nit	gen, pip, tzp, amp, amx, ctx, caz, cpd, cpz, cro, cxm, fox, at, dox, chl, cip, sxt, ipm, tet, nor, lvx	gen, pip, tzp, amp, amx, ctx, caz, cpd, cpz, cxm, fox, at, dox, chl, cip, sxt, ipm, tet, lvx, ofx, nit	amk, gen, pip, tzp, amp, amx, ctx, cpd, cpz, cro, cxm, at, cip, sxt, ipm, etp, tet, lvx	amk, gen, pip, tzp, amp, amx, ctx, caz, cpd, cpz, cxm, fox, at, dox, cip, sxt, ipm, etp, tet, nor, ofx	amk, pip, tzp, amp, amx, ctx, caz, cpd, cpz, cro, cxm, fox, at, chl, cip, sxt, ipm, etp, lvx, nit, ofx
Type of resistance	MDR	XDR	MDR	XDR	XDR	XDR	XDR	XDR	XDR

E. coli=Escherichia coli; K. oxytoca=Klebsiella oxytoca; A. baumannii=Acinetobacter baumannii; IPM=Imipenem, EDTA=Ethylenediaminetetraacetic Acid; MIC=Minimum Inhibitory Concentration; MDR=Multi-drug-resistant; XDR=Extensively drug-resistant; Amk=Amikacin; Amp=Ampicillin; At=Aztreonam; Cpd=Cefpodoxime; Cpz=Cefoperazone; Ctx=Cefotaxime; Fox=Cefoxitin; Caz=Ceftazidime; Cro=Ceftriaxone; Cxm=Cefuroxime; Chl=Chloramphenicol; Cip=Ciprofloxacin; Dox=Doxycycline; Gen=Gentamicin; Ipm=Imipenem; Pip=Piperacillin; Tzp=Piperacillin-tazobactam; Tet=Tetracycline; Tmp=Trimethoprim; Sxt=Trimethoprim-sulfamethoxazole; Lvx=Levofloxacin; Nit=Nitrofurantoin; Ofx=Ofloxacin; Nor=Norfloxacin; MBL=Metallo-Beta-Lactamase; CDM=Combination disk method; ESBL=Extended-Spectrum Beta -Lactamase

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