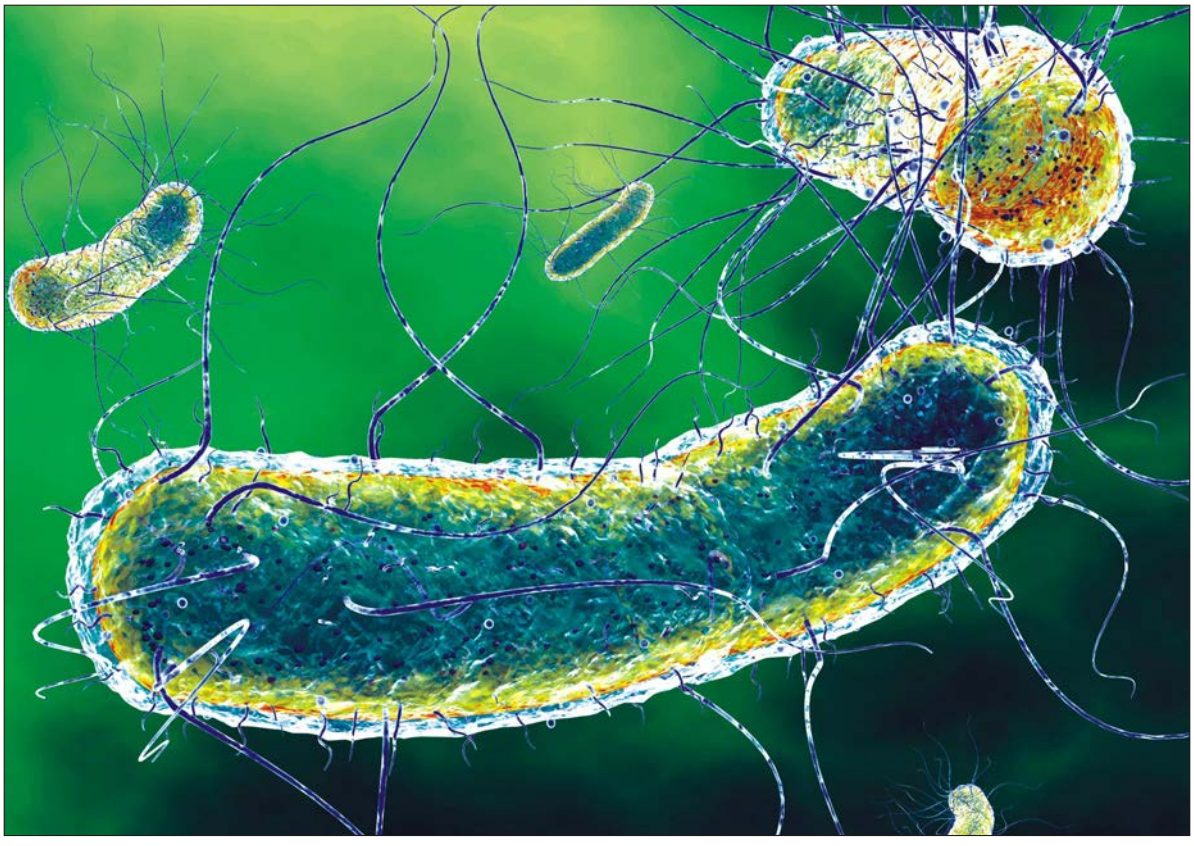

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Research Article
Prevalence of Carbapenem Resistant Gram Negative Bacilli Harboring *bla*$_{NDM-1}$ Gene Isolated in a Tertiary Care Hospital

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Abstract

Background: Increase in antimicrobial resistance is of major concern worldwide. This is largely attributed to broad spectrum β-lactamase production. New Delhi Metallo β-lactamase-1 (NDM-1) is a recently identified type of metallo-β-lactamase which has been increasingly viewed as a potential threat to global health. **Objective:** The aim of this study was to perform molecular detection of *bla*$_{NDM-1}$ gene to determine its occurrence among clinical isolates of Gram negative bacteria. **Methodology:** A total of 178 Gram negative bacilli isolated from different clinical samples including urine, tissue, sputum, blood, pus, endotracheal secretion (ET secretion), stool, pleural fluid, cup tip, Peripherally Inserted Central Catheter (PICC) tip, drain tube and bile were included in the study. The isolates were identified by Vitek 2 GN cards and antibiotic susceptibility testing was performed by using Vitek 2 AST-N280 and AST-N281 cards (bioMe'rieux, SA, France), as per manufacturer's instructions. The isolates were stocked and used for further study. PCR to detect the presence of *bla*$_{NDM-1}$ gene was performed with all the isolates. The ERIC-PCR was performed with 17 *bla*$_{NDM-1}$ positive representative isolates. Of the 178 isolates, a remarkably high incidence of 29.8% *bla*$_{NDM-1}$ gene was found. Of the 53 *bla*$_{NDM-1}$ positive cases, 17 representative isolates were studied for clonal relatedness by ERIC-PCR. **Results:** It was found that *Klebsiella pneumoniae* and *Acinetobacter baumannii* had 2 and 1 clonally related clusters, respectively. *Pseudomonas aeruginosa* and *Escherichia coli* were clonally divergent. We suggest that the genotypic detection of NDM-1 along with routine antimicrobial susceptibility test should be performed in all health centers worldwide. The *bla*$_{NDM-1}$ gene has "An alarming potential" to spread and diversify among bacterial populations. **Conclusion:** Hence early identification of cases of NDM-related infections and prevention of their spread by implementing screening, hygiene measures and the isolation of carriers is required.

Key words: New Delhi metallo β-lactamase-1, *bla*$_{NDM-1}$ gene, carbapenem resistant, Gram-negative isolates

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Increase in antimicrobial resistance during the past decade in Enterobacteriaceae has become a major concern worldwide. This is largely attributed to broad spectrum β-lactamase production (Menezes and Menezes, 2013). Further, the emergence and global spread of carbapenem-resistant Enterobacteriaceae (CRE) is of great distress (Zou *et al*., 2015). Among the metallo β-lactamase's (MBLs), Verona integron-encoded metallo-β-lactamase (VIM), imipenemase (IMP) and New Delhi metallo-β-lactamase (NDM) are the most prevalent types. The *bla*$_{NDM-1}$ was originally identified in *Klebsiella pneumoniae* and *Escherichia coli* in 2008 in Sweden from a traveller returning from India (Yong *et al*., 2009). The *bla*$_{NDM-1}$ encodes a broad-spectrum β-lactamase that inactivates all β-lactams except aztreonam (Shenoy *et al*., 2014). The gene *bla*$_{NDM-1}$ is mainly located on a plasmid and hence can transcend the genus/family barrier with ease and spread across the world (Menezes and Menezes, 2013). Currently, there are 16 variants of *bla*$_{NDM}$.

Appropriate and rapid detection of NDM-1 producers is critical in implementing infection control measures. To better control NDM-1 producers, it is essential to understand the mediating mechanisms and their molecular epidemiology. In this study, we performed the molecular detection of *bla*$_{NDM-1}$ gene among carbapenem resistant Gram negative clinical isolates cultured from clinical samples in a hospital in Mumbai, India. The ERIC-PCR was performed to study the clonal relatedness of *bla*$_{NDM-1}$ gene positive carbapenem resistant Gram Negative Bacilli (GNB).

MATERIALS AND METHODS

Place of study and study period: The study isolates were from S.L. Raheja Hospital, Mumbai, India cultured during March, 2013 to May, 2014.

Patient population: Both males and females attending OP and IP across all age groups were included in the study.

Samples: Bacterial isolates were obtained from urine, tissue, sputum, blood, pus, endotracheal secretion (ET secretion), stool, pleural fluid, cup tip, Peripherally Inserted Central Catheter (PICC) tip, drain tube and bile.

Clinical bacterial strains: A total of 178 GNB isolates resistant to carbapenem were included in the study.

Control strains: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* BA 2146 were used as controls where required.

Bacterial isolation, identification and antibiotic sensitivity testing: The isolates were identified by Vitek 2 GN cards and antibiotic susceptibility testing was performed by using Vitek 2 AST-N280 and AST-N281 cards (bioMe'rieux, SA, France), as per manufacturer's instructions. The isolates were stocked and used for further study. Tigecycline MIC breakpoints were as per European Committee on Antimicrobial Susceptibility Testing (EUCAST., 2011) clinical breakpoints. The MIC breakpoints for other antimicrobial agents were interpreted as per Clinical and Laboratory Standards Institute (CLSI., 2013) guideline. The cefoperazone MIC breakpoint used for cefoperazone/sulbactam was as described by Tunyapanit *et al*. (2014).

DNA extraction: For DNA extraction, a single bacterial colony from an overnight grown culture was suspended in 100 µL of sterile MilliQ water and boiled for 5 min. The suspension was centrifuged at 8,000 rpm for 10 min. The supernatant containing bacterial DNA was used as template for PCR (Harish and Menezes, 2015).

PCR amplification for the detection of *bla*$_{NDM-1}$ gene: PCR amplification of *bla*$_{NDM-1}$ gene was carried out by using primers as described in an earlier study (Nordmann *et al*., 2011). The primers used were NDM-Fm5'-GGTTTGGCGATCTGGTTTTC-3' and NDM-Rm5'-CGGAATGGCTCATCACGATC-3', which amplified a 621 bp internal fragment of the *bla*$_{NDM-1}$ gene. The DNA from known *bla*$_{NDM-1}$ positive and negative isolates were used as controls. The PCR was performed in a final reaction volume of 25 µL, containing 10 pmol each of forward and reverse primers, 2 µL of template DNA, 0.5 µL of 25 mM dNTPs, 2.5 µL of 10X amplification buffer, 0.5 U of *Taq* DNA polymerase. The PCR program consisted of following thermal cycling conditions: Initial denaturation step at 94°C for 10 min, followed by 36 cycles of 94°C for 30, 52°C for 40 and 72°C for 50 sec, followed by a final elongation at 72°C for 5 min. The application was performed using eppendorf thermocycler. The PCR products were run on 2% agarose (HI Media, Mumbai, India) gel, containing 1X tris-borate-EDTA (TBE) buffer and detected by ethidium bromide (Sigma) at 100 V for 30 min. The amplified PCR products were documented using Alpha Gel Imager (Alpha Innotech, USA) and the PCR band of 621 bp was visualized (Fig. 1).

Table 1: Distribution of occurrence of bla_{NDM-1} gene among clinical samples/isolates

	Isolates Positive for bla_{NDM-1} gene n = 35					
Clinical samples	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumoniae*	*Citrobacter freundii*	*Alcaligenes faecalis*
Blood (1)	1	-	-	-	-	-
Tissue (11)	5	4	1	1	-	-
Urine (19)	2	2	7	7	1	-
Sputum(9)	2	0	2	5	-	-
Pus (2)	-	-	1	1	-	-
ET secretion (6)	3	-	-	3	-	-
Pleural fluid (2)	-	1	-	-	-	1
Drain fluid (1)	-	-	-	1	-	-
Cup tip (1)	-	-	-	1	-	-
PICC tip (1)	-	-	1	-	-	-
Total (53)	13	7	12	19	1	1

ET: Endotracheal secretion, PICC: Peripherally inserted central catheter

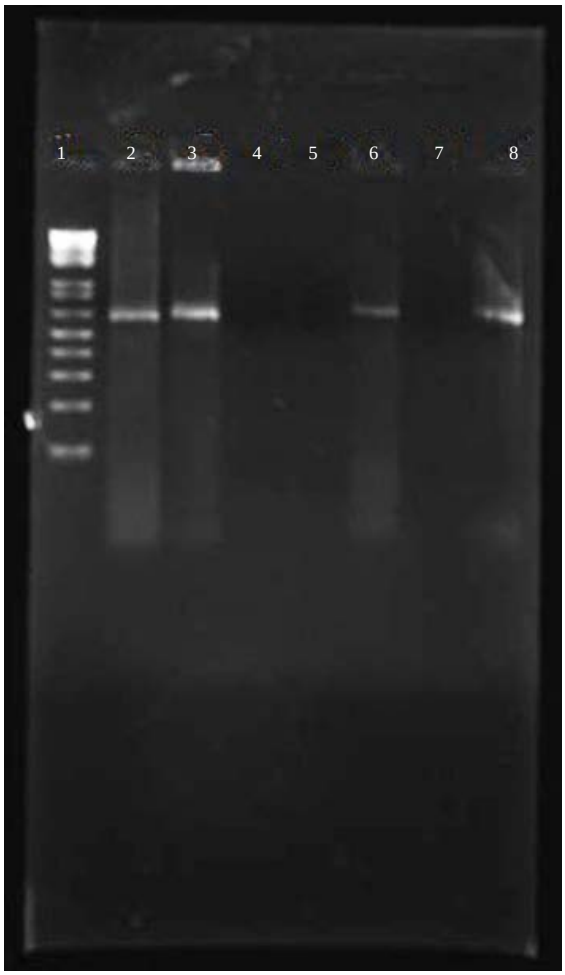

Fig. 1: Electrophoresis gel image demonstrating bla_{NDM-1} gene, Lane 1: Molecular mass marker (100 bp DNA ladder), Lane 2: Positive control, Lane 4: Negative control, Lane 3, 6 and 8: Test sample-positive (621 bp PCR product), Lane 5 and 7: Test sample-negative

Enterobacterial Repetitive Intergenic Concensus-Polymerase Chain Reaction (ERIC-PCR): The ERIC-PCR was performed as described by Dalla-Costa *et al*. (1998). Primers that were used are ERICIR, 5'ATGTAAGCTCCTGGGG-ATTCAC3' and ERIC2, S'AAGTAAGTGACTGGGGT-GAGCG3'. The PCR was performed in a final reaction volume of 25 µL, containing 10 pmol each of forward and reverse primers, 2 µL of template DNA, 0.5 µL of 25 mM dNTPs, 2.5 µL of 10X amplification buffer, 2.5 U of *Taq* DNA polymerase. The PCR program consisted of following thermal cycling conditions: Initial denaturation step at 95°C for 5 min, followed by 35 cycles of 92°C for 45 sec, 52°C for 1 min and 70°C for 10 min, followed by a final elongation at 70°C for 20 min. The application was performed using eppendorf thermocycler. The PCR products were run on 2% agarose (HI Media, Mumbai, India) gel, containing 1X tris-borate-EDTA (TBE) buffer and detected by ethidium bromide (Sigma) at 50 V for 1 h. The amplified PCR products were documented using Alpha Gel Imager (Alpha Innotech, USA).

RESULTS

Bacterial isolation and identification: During the 15 months study period, a total of 178 Gram negative isolates resistant to carbapenem were cultured. The isolates comprised of *Klebsiella pneumoniae* (86), *Pseudomonas aeruginosa* (33), *Acinetobacter baumannii* (24), *Escherichia coli* (22), *Enterobacter aerogenes* (3), *Enterobacter cloacae* (3), *Citrobacter freundii* (2), *Citrobacter Koseri* (1), *Alcaligenes faecalis* (1) and *Morganella morgani* (1).

Antibiotic sensitivity testing and PCR for bla_{NDM-1} gene: Of the 178 carbapenem resistant isolates, 53 (29.8%) were found to be positive for the bla_{NDM-1} gene (Table 1) by PCR. The 53 bla_{NDM-1} positive isolates included, *Acinetobacter*

Table 2: Demographic details of the subjects and antibiogram of the isolates positive for NDM-1 (n = 53)

						Antibiotic sensitivity						
Sample	Age/sex	History	Pathogen	Empiric therapy	PTO	CL	Tg	Cefo/sul	I	Mem	PT	NDM-1
Blood	44/M	Febrile neturopenia	*Acinetobacter baumannii*	Cef/taz	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Tissue	69/M	DFI	*Pseudomonas aeruginosa*	Pip/taz	Alive	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Sputum	67/M	DM/HTN/CKD	*Escherichia coli*	Pip/taz	Dead	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Pus	66/F	Breathlessness	*Klebsiella pneumoniae*	Mero, linezolid	Dead	S≤0.5	ND	R≥64	ND	R≥16	R≥128	Positive
Tissue	73/M	DM/HTN/Fever	*Pseudomonas aeruginosa*	Cefo/sul	Alive	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Cup tip	67/M	Lung cancer	*Klebsiella pneumoniae*	Mero, teico, colistin	Dead	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Urine	67/M	Fever with chills	*Klebsiella pneumoniae*	Azithro, ceftriaxone	Alive	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Urine	59/M	UTI	*Acinetobacter baumannii*	Mero, teico, amik	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Urine	86/F	Increase WBC count	*Escherichia coli*	Cefp	Alive	S≤0.5	S≤0.5	R≥64	ND	R (8)	R≥128	Positive
Tissue	65/M	Necrotising fascitis	*Acinetobacter baumannii*	Clindamycin, Pip/taz	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
ET secretion	59/F	DM/HTN/ Hypothyroidism	*Acinetobacter baumannii*	Pip/taz	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Tissue	69/M	DFI	*Acinetobacter baumannii*	Teico, mero	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Tissue	57/M	AKA	*Acinetobacter baumannii*	Imip	Alive	S≤0.5	ND	S≤8	R≥16	R≥16	R≥128	Positive
Tissue	76/M	DFI/amputation	*Acinetobacter baumannii*	Mero	Alive	S≤0.5	S≤0.5	S≤8	R≥16	R≥16	R≥128	Positive
Urine	61/M	DFI	*Escherichia coli*	Cefo/sul	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Urine	85/M	UTI	*Citrobacter freundii*	Cefo/sul	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Tissue	67/M	DFI	*Pseudomonas aeruginosa*	Cefo/sul	Alive	S (1)	R	R≥64	R≥16	R≥16	R≥128	Positive
Urine	64/M	DM/HTN/IHD	*Pseudomonas aeruginosa*	Cefo/sul	Alive	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Urine	85/F	Fever/UTI	*Acinetobacter baumannii*	Nil	Alive	S≤0.5	ND	S≤8	R≥16	R≥16	R≥128	Positive
Urine	47/M	BMC	*Klebsiella pneumoniae*	Cefo/sul	Alive	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Urine	75/F	DM/Fever	*Escherichia coli*	Cefo/sul	Alive	ND	S≤0.5	R≥64	R (8)	R≥16	R≥128	Positive
Urine	53/F	DM/HTN/CKD	*Klebsiella pneumoniae*	Cefo/sul	Dead	R	ND	R≥64	R≥16	R≥16	R≥128	Positive
Sputum	62/M	Appendix with PRES	*Acinetobacter baumannii*	Cefo/sul	Alive	S≤0.5	S≤1	R≥64	R≥16	R≥16	R≥128	Positive
Urine	78/M	Fever	*Escherichia coli*	Pip/taz, ofloxacin	Alive	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Picc tip	27 d/M	NRDS	*Escherichia coli*	Pip/taz	Dead	S≤0.5	S≤0.5	R≥64	R (8)	R≥16	R≥128	Positive
Sputum	72/M	Dry cough, MDS	*Klebsiella pneumoniae*	Cefo/sul	Dead	S≤0.5	1 [4]	R≥64	R≥16	R≥16	R≥128	Positive
Tissue	52/M	Fever/Foot amputation	*Acinetobacter baumannii*	Cefp	Alive	S≤0.5	S≤0.5	R≥64	I (8)	R≥16	R≥128	Positive
Urine	83/M	UTI	*Klebsiella pneumoniae*	Cefo/sul	Alive	S≤0.5	R	R≥64	R≥16	R≥16	R≥128	Positive
Sputum	53/M	Fever	*Acinetobacter baumannii*	Cefo/sul, ciplox, mero	Dead	S≤0.5	S [1]	R≥64	R≥16	R≥16	R≥128	Positive
Sputum	64/m	Cough	*Klebsiella pneumoniae*	Cefp	Alive	S≤0.5	R	R≥64	R≥16	R≥16	R≥128	Positive
Urine	70/F	Chronic UTI	*Escherichia coli*	Cefo/sul	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Pus	75/F	Abdominal resection	*Escherichia coli*	Cefotaxime	Alive	S≤0.5	S≤0.5	R≥64	S≤0.25	S≤0.25	R≥128	Positive
Urine	62/M	Fever with chills	*Escherichia coli*	Cefo/sul	Alive	S≤0.5	R	R≥64	R≥8	R≥16	R≥128	Positive
ET secretion	52/F	Acute febrile illness	*Klebsiella pneumoniae*	Cefotaxime	Alive	S≤0.5	R	R≥64	R≥16	R≥16	R≥128	Positive
ET secretion	46/M	Encephalitis	*Klebsiella pneumoniae*	Colistin, mero	Alive	S≤0.5	R	R≥64	R≥16	R≥16	R≥128	Positive
Sputum	40/M	Pancreatitis, septicemia	*Klebsiella pneumoniae*	Meropenem	Alive	S≤0.5	S [1]	R≥64	R≥16	R≥16	R≥128	Positive
Drain fluid	61/M	CA gall bladder	*Klebsiella pneumoniae*	Nil	Alive	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Urine	73/M	Urinary frequency	*Pseudomonas aeruginosa*	Cefo/sul	Alive	S≤0.5	S [1]	R≥64	R≥16	R≥16	R≥128	Positive
Tissue	76/M	DM, PVD SORES	*Klebsiella pneumoniae*	Cefo/sul	Alive	S≤0.5	ND	R≥64	R≥16	R≥16	R≥128	Positive
Tissue	68/M	DM	*Pseudomonas aeruginosa*	Cefo/sul, lz	Alive	S≤0.5	R	R≥64	R≥16	R≥16	R	Positive
Sputum	72/F	IHD	*Klebsiella pneumoniae*	Cefo/sul, Clari	Alive	S≤0.5	R	R≥64	R≥16	R≥16	R≥128	Positive
Sputum	93/F	COPD	*Klebsiella pneumoniae*	Cl, lz	Alive	S [2]	R	S≤8	R≥16	R≥16	R≥128	Positive
Pleural fluid	57/M	DM, breathlessness	*Klebsiella pneumoniae*	Mero	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	S≤4	Positive
Urine	34/F	UTI	*Klebsiella pneumoniae*	Cefo/sul	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive
Urine	34/F	Fever, sigmoid malignancy	*Escherichia coli*	Ciplox	Alive	S≤0.5	S≤0.25	R [32]	R≥16	R (8)	R≥128	Positive
ET	73/M	Acute febrile illness	*Acinetobacter baumannii*	Amik, metro	Alive	S≤0.5	R	R≥64	R≥16	R≥16	R≥128	Positive
Urine	78/F	Fever with uropathy	*Klebsiella pneumoniae*	Cefo/sul	Alive	S≤0.5	S≤0.5	R≥64	R≥16	R≥16	R≥128	Positive

Table 2: Continue

Sample	Age/sex	History	Pathogen	Empiric therapy	PTO	Antibiotic sensitivity						
						CL	Tg	Cefo/sul	I	Mem	PT	NDM-1
Sputum	86/M	Respiratory infection	*Escherichia coli*	Mero, clari	Alive	S [2]	R	R≥64	R≥16	R≥16	R≥128	Positive
ET secretion	65/F	Upper urinary tract bleed	*Klebsiella pneumoniae*	Mero, metro	Alive	S≤0.5	S [1]	R≥64	R≥16	R≥16	S≤4	Positive
Urine	73/F	Ca lung, dysurea	*Klebsiella pneumoniae*	Cefo/sul	Alive	S≤0.5	S [1]	R≥64	R≥16	R≥16	R≥128	Positive
Tissue	63/M	DM	*Escherichia coli*	Cefo/sul	Alive	S≤0.5	S [2]	R≥64	R≥16	R≥16	R≥128	Positive
ET secretion	65/M	GI bleed	*Acinetobacter baumannii*	Mero	Alive	S≤0.5	S [2]	R≥64	R≥16	R≥16	R≥128	Positive
Pleural fluid	52/M	Breathlessness	*Pseudomonas aeruginosa*	Nil	Alive	S≤0.5	R	R≥64	R≥16	R≥16	R≥128	Positive

Abbreviations: PTO: Post therapy outcome, ND: Not detected, ET: Endotracheal, DFI: Diabetic foot infection, DM: Diabetes mellitus, HTN: Hypertension, CKD: Chronic kidney disease, UTI: Urinary tract infection, AKA: Above-knee amputation, IHD: Ischemic heart disease, CA: Carcinoma, BMCA: Buccal mucosa carcinoma, PRES: Posterior reversible encephalopathy syndrome, NRDS: Neonatal respiratory distress syndrome, MDS: Myelodysplastic syndrome, PVD: Peripheral vascular disease, COPD: Chronic obstructive pulmonary disease, GI: Gastrointestinal, S: Susceptible, I: Intermediate, R: Resistant, Cd: Clindamycin, Clari: Clarithromycin, Cef/taz: Cefepime/tazobactam, Pt: Piperacillin/tazobactam, Cefo/sul: Cefoperazone+sulbactam, Mero: Meropenem, Teico: Teicoplanin , Azithro: Azithromycin, Amik: Amikacin, Cefp: Cefpodoxime, Imi: Imipenem, Cfn: Ceftriaxone, Oflox: Ofloxacin, Ciplox: Ciprofloxacin, Lz: Linezolid, Metro: Metronidazole, Cefot: Cefotaxime, Cl: Colistin and Tgc: Tigecycline

baumannii (13); *Pseudomonas aeruginosa* (7); *Escherichia coli* (12); *Klebsiella pneumoniae* (19), *Citrobacter freundii* (1) and *Alcaligenes faecalis* (1). These 53 isolates were obtained from different clinical samples-urine (19), tissue (11), sputum (9), blood (1), pus (2), ET secretion (6), pleural fluid (2), drain fluid (1), cup tip (1) and PICC tip (1). The bla_{NDM-1} was positive among 19 of 53 (35.8%) urinary isolates. Of the 53 bla_{NDM-1} cases, 16 (30.2%) were females. None of the stool and bile isolates were found to be positive for bla_{NDM-1}.

Among the 53 bla_{NDM-1} positive isolates, a single *K. pneumoniae* isolate from urine was found to be resistant to colistin and was responsible for the death of the patient. 13/53 isolates, (24.5%) were resistant to tigecycline of which 9 isolates were *K. pneumoniae*, 2 isolates were *P. aeruginosa* and one isolate each of *E. coli* and *A. faecalis* (Table 2). Of the 53 bla_{NDM-1} positive cases, 8 had succumbed to death. An isolate of bla_{NDM-1} positive *E. coli* was responsible for Neonatal Respiratory Distress Syndrome (NRDS) leading to death. Further, none of the *Enterobacter aerogenes* and *Enterobacter cloacae* was found positive for bla_{NDM-1} (Table 2).

ERIC-PCR: The ERIC-PCR was performed to study the clonal relatedness of bla_{NDM-1} positive Gram negative isolates. This was performed for 17 representative isolates, details of which are in Table 3 and Fig. 2. Manual typing of the isolates revealed that 6 out of 9 bla_{NDM-1} positive *K. pneumoniae* isolates belonged to two clonal cluster types i.e., 2 isolates were of clonal cluster type 1, 4 isolates were of clonal cluster type 2. Two isolates of *Acinetobacter baumannii* were also clonally related. None of the other isolates i.e., 3 isolates of *P. aeruginosa* and 3 isolates of *E. coli* shared any clonal relatedness.

DISCUSSION

The NDM-1 producing bacteria are the most frequent cause of urinary tract infections (UTIs). They can also cause wound infections, bloodstream infections and pneumonia (Menezes and Menezes, 2013). In this study, among the bla_{NDM-1} positive cases, majority [19 (35.8%)] were UTI cases followed by wound infections [08 (22.9%)]. Of the 53 bla_{NDM-1} cases, 16 (30.2%) were females, whereas 37 (69.8%) were males. The higher rate of NDM-1 positivity was similar to the study by Shenoy *et al.* (2014). In this study, age ranged from 44-86 years except for one case of neonate. Our study indicates a high incidence (29.8%) of bla_{NDM-1}. The reports of the incidence of NDM-1 has been highly variable. A recent study from China reported an incidence of 33.3% of NDM-1 among carbapenem resistant isolates (Qin *et al.*, 2014) similar to present study. The 53 bla_{NDM-1} positive isolates cultured in current study included, *A. baumannii* (13), *K. pneumoniae* (19), *E. coli* (12), *P. aeruginosa* (7), *A. faecalis* (1) and *C. freundii* (1). The finding was corresponding with the findings of Shenoy *et al.* (2014). The NDM-1 producing *A. baumannii* can lead to outbreak of infections (Decousser *et al.*, 2013).

Variable carbapenem resistance has been reported in NDM-1 positive isolates (Shenoy *et al.*, 2014). Of the bla_{NDM-1} positive isolates in present study, a single isolate was found resistant to colistin. Among the isolates, 13 (24.5%) were found resistant to tigecycline. Except for few, most of the NDM-1 producers have been reported to remain susceptible only to colistin and tigecycline. Nevertheless, a high rate of tigecycline resistance (43.2%) has been reported (Shenoy *et al.*, 2014). Due to limited therapeutic options, treatment of infections caused by NDM-1 producing pathogens is a major challenge for clinicians. These organisms

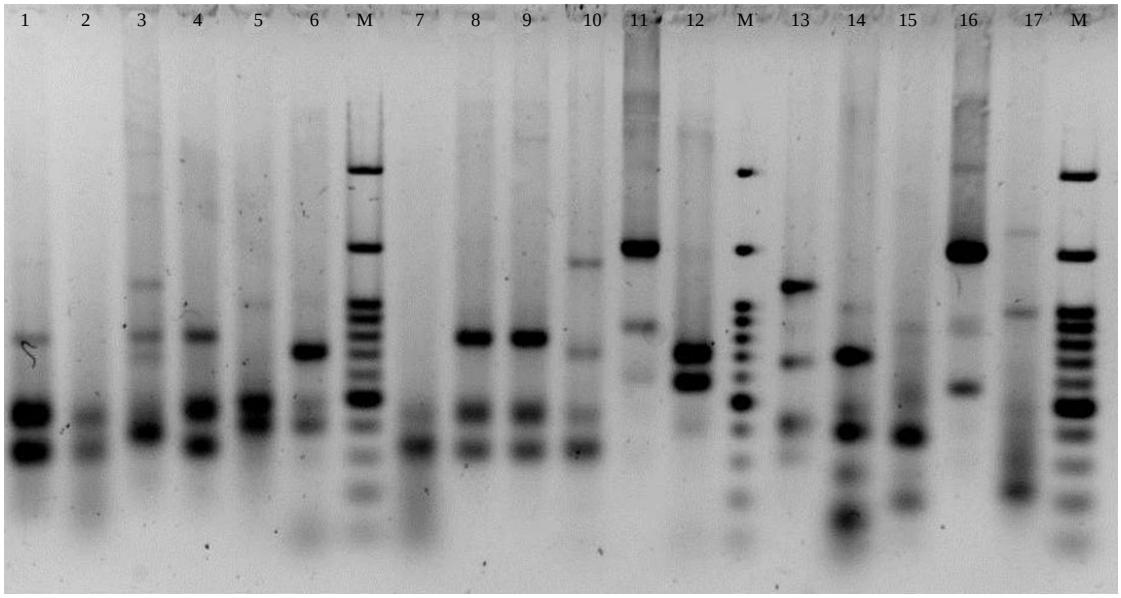

Fig. 2: DNA fingerprints of isolates generated by ERIC-PCR and separated in a 2% (w/v) agarose gel, 1: *K. pneumoniae*, 2: *K. pneumoniae*, 3: *P. aeruginosa*, 4: *K. pneumoniae*, 5: *P. aeruginosa*, 6: *K. pneumoniae*, 7: *K. pneumoniae*, 8: *K. pneumoniae*, 9: *K. pneumoniae*, 10: *E. coli*, 11: *A. baumannii*, 12: *K. pneumoniae*, 13: *E. coli*, 14: *K. pneumoniae*, 15: *E. coli*, 16: *A. baumannii*, 17: *P. aeruginosa* and M: *PCR Marker*-100 bp ladder

Table 3: Details of the 17 representative isolates chosen for ERIC-PCR

Lane	Sample	Organism	History	Age/Sex	Outcome
1	Sputum	*Klebsiella pneumoniae*	Pancreatitis, septicemia	40/M	Alive
2	Drain fluid	*Klebsiella pneumoniae*	CA gall bladder	61/M	Alive
3	Urine	*Pseudomonas aeruginosa*	Urinary frequency	73/M	Alive
4	Tissue	*Klebsiella pneumoniae*	DM, PVD sores	76/M	Alive
5	Tissue	*Pseudomonas aeruginosa*	DM	68/M	Alive
6	Sputum	*Klebsiella pneumoniae*	IHD	72/F	Alive
7	Sputum	*Klebsiella pneumoniae*	COPD	93/F	Alive
8	Pleural fluid	*Klebsiella pneumoniae*	DM, breathlessness	57/M	Alive
9	Urine	*Klebsiella pneumoniae*	UTI	34/F	Alive
10	Urine	*Escherichia coli*	Sigmoid malignancy	34/F	Alive
11	ET secretion	*Acinetobacter baumannii*	Acute febrile illness	73/M	Alive
12	Urine	*Klebsiella pneumoniae*	Fever with uropathy	78/F	Alive
13	Sputum	*Escherichia coli*	RTI	86/M	Alive
14	ET secretion	*Klebsiella pneumoniae*	Upper UT bleed	65/F	Dead
15	Tissue	*Escherichia coli*	DM	63/M	Alive
16	ET secretion	*Acinetobacter baumannii*	GI bleed	65/M	Alive
17	Pleural fluid	*Pseudomonas aeruginosa*	ND	52/M	Alive

CA: Carcinoma, DM: Diabetes mellitus, PVD: Peripheral vascular disease, IHD: Ischemic heart disease, COPD: Chronic obstructive pulmonary disease, UTI: Urinary tract infection, RTI: Respiratory tract infection, ET: Endotracheal and ND: Not documented, Cluster 1: Lane number 2 and 7, Cluster 2: Lane number 1, 8 and 9, Cluster 3: Lane number 11 and 16

frequently are found resistant to most antibiotics except colistin and, less consistently to tigecycline. Consequently, colistin and tigecycline (final resort antimicrobial agents) have been tried with limited success. The lack of effective antimicrobial agents demand newer agents for the treatment of infections caused by NDM-1-producing bacteria and other carbapenem resistant organisms (Menezes and Menezes, 2013). Rapid spread of CRE species in a hospital in Mumbai has been previously reported (Muir and Weinbren, 2010).

In our study, of the 53 $bla_{\text{NDM-1}}$ positive cases, 08 (15%) had succumbed to death which was in accordance of report by Shenoy *et al.* (2014), in which 03 of the 61 (4.9%) patients

had succumbed to death. Infections caused by NDM-1 producing pathogens are tough to treat leading to complications, but do not make pathogens more virulent or transmissible. Further, such infections range from mild to severe, while some have been fatal. The immuno-compromised status of the patient could be a predisposing factor for these infections (Menezes and Menezes, 2013).

Present study imparts insights into the intricate molecular epidemiology of bla_{NDM-1} gene in this tertiary care center. Among the different representative strains for which ERIC-PCR was performed, clonal relatedness was observed only with *K. pneumoniae* and *A. baumannii* isolates. Both the clonally related *A. baumannii* were isolated from endotracheal fluid. Clonal cluster type 1, consisting of 4 isolates of *K. pneumoniae*, were isolated from sputum, tissue, pleural fluid and urine. Clonal cluster type 2, consisting of 2 isolates of *K. pneumoniae*, were isolated from drain fluid from gall bladder and sputum. Though they were all isolated from different clinical samples, with the exception of *A. baumannii*, there is still a strong possibility of horizontal dissemination of bla_{NDM-1} gene within the healthcare setup. This calls for not only strict implementation of infection prevention and isolation protocols that could curtail possible outbreaks, but also routine surveillance of hospital environmental sampling and water supplies (Shanthi *et al*., 2013).

Clonal relatedness was however not observed in other isolates i.e., 3 isolates of *P. aeruginosa* and 3 isolates of *E. coli*. This is indicative of appearance of multiple clones with limited dissemination between patients, suggesting strong selection pressure on bacterial population, emphasizing the necessity for appropriate governance and administration of antibiotic therapy, within health-care units. Results demonstrate extensive diversity of bla_{NDM-1} producers which is consistent with the findings of previous studies (Shanthi *et al*., 2013; Kumarasamy *et al*., 2010; Nagaraj *et al*., 2012; Castanheira *et al*., 2011).

CONCLUSION

The bla_{NDM-1} gene has "An alarming potential" to spread and diversify among bacterial populations. This study demonstrated a high incidence of NDM-1-producing multi-drug resistant Gram negative bacilli from patients with different clinical diseases. There was clonal relatedness among *K. pneumoniae* and *A. baumannii* isolates and clonal diversity among *P. aeruginosa* and *E. coli*. This calls for increased alertness, continuous surveillance and strict enforcement of antibiotic policy with restricted use of inducer drugs. Carbapenem antibiotics should be treated as the last resort and reserved for severe infections. Spread of NDM-1 producing isolates seriously limit the options for clinical treatment. Thus, enhanced efforts are urgently needed to control the further spread of NDM-1-producing bacterial pathogens. It is very important to identify cases of NDM related infections early and prevent the spread by implementing screening, sanitation measures and isolation of the carriers. Hence, routine antimicrobial susceptibility testing along with genotypic characterization should be performed.

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