Original Article

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Microbiology Section

Detection of NDM-1 in Clinical Isolates of *Klebsiella Pneumoniae* from Northeast India

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ABSTRACT

Introduction: The growing incidence of the resistance to carbapenems among the members of *Enterobacteriaceae* is of major concern throughout the world, as these drugs are often used now-a-days as the last line of effective treatment against the serious infections which are caused by multidrug resistant *Enterobacteriaceae*. We detected bla_{NDM-1} in the *Klebsiella pneumoniae* isolates which were collected from a tertiary care referral hospital in northeast India.

Materials and Methods: A total of 219 non-duplicated *K. pneumoniae* isolates were recovered from various clinical samples between August 2009 and July 2010. These isolates were screened for the detection of carbapenemase production on the basis of their reduced susceptibility to meropenem or ertapenem by the disc diffusion method. The screened isolates were further phenotypically studied for carbapenemase production by the modified Hodge test and the detection of metallo-β-lactamase production was carried out by using the combined disc test. All the screened isolates were also subjected to PCR detection of the bla_{NDM-1} gene and the additional *bla* genes which coded for TEM, SHV, CTX-M, and AmpC.

Results: Out of the 219 isolates, 19 were screened for the detection of carbapenemase production on the basis of their reduced susceptibility to meropenem or ertapenem. Both the phenotypic confirmatory tests showed positive results for all the screening positive isolates, although few weakly positive reactions for the modified Hodge test were observed. All the screened isolates were found to be positive for bla_{NDM-1}. Each of the bla_{NDM-1} possessing isolates was also found to be positive for one or more additional *bla* genes.

Conclusion: This study indicated that the incidence of bla_{NDM-1}, together with that of one or more additional *bla* genes among the *K. pneumoniae* isolates from hospitalized and non hospitalized patients in northeast India.

Key Words: *Klebsiella pneumoniae*, Carbapenems, NDM-1, India

INTRODUCTION

Among the newly emerged β-lactamases in the planet, New Delhi metallo-β-lactamase-1 (NDM-1) represents the latest threat in the war between the bugs and the drugs. NDM-1 is a novel type of metallo-β-lactamase that hydrolyzes all the β-lactam antibiotics except aztreonam, which is usually inactivated by the co-production of the extended-spectrum or the AmpC β-lactamases. In addition, the gene which encodes NDM-1, which is known as bla_{NDM-1}, is located on a transmissible plasmid and its association with other resistant determinants leads to the extensive drug resistance which is exhibited by a majority of the NDM-1 producing *Enterobacteriaceae,* leaving only a few therapeutic options [1, 2]. Therefore, the NDM-1 producing organisms are now being recognized as the world's newest "superbugs". In general, the term "superbugs" is a colloquial reference to a bacterium that carries resistance genes for many antibiotics [3].

NDM-1 was first reported in 2009 in *Klebsiella pneumoniae* and *Escherichia coli*, both of which were recovered from a Swedish patient of Indian origin, who was previously admitted to a hospital in New Delhi, India [1]. Thereafter, NDM-1 has increasingly been reported from India as well as from several other parts of the world [2, 4-6]. Although, bla_{NDM-1} (gene encoding NDM-1) has been identified in different members of *Enterobacteriaceae*, *K. pneumoniae* and *E. coli* still remain the most frequent NDM-1 producing isolates throughout the world. In India, NDM-1 producing *K. pneumoniae* have emerged as important pathogens, causing a wide range of infections, both in the community as well as in hospital settings [2,7,8,9]. However, the data on the prevalence of NDM-1 producing *K. pneumoniae* isolates in Indian hospitals is limited. We therefore designed the present study to evaluate the occurrence of bla_{NDM-1} in the *K. pneumoniae* isolates at a tertiary care referral hospital in northeast India.

MATERIALS AND METHODS

The Bacterial Isolates

A total of 219 non-duplicated *K. pneumoniae* isolates were recovered from various clinical samples such as urine (*n* =111), sputum (*n* =59), blood (*n* =26) and pus (*n* =23) at a tertiary care referral hospital in Guwahati, northeast India. The samples were obtained from both hospitalized and non-hospitalized patients between August 2009 and July 2010. The standard microbiological techniques were used for the isolation and the identification of the isolates [10]. Prior to their testing, all the isolates were stored in 15% glycerol-supplemented Luria-Bertani medium at – 80^{0}C. This study was carried out with the consent of the institutional ethics committee.

Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method was performed to determine the susceptibilities of the different β-lactam and non-β-lactam antibiotics and the results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. All the

antibiotic discs and the media were procured from Hi-media, Mumbai, India, except doripenem, which was procured from BD Diagnostics, Franklin Lakes, NJ, USA. The *E.coli* ATCC 25922 and the *Pseudomonas aeruginosa* ATCC 27853 strains were used for quality control. The minimum inhibitory concentration (MIC) values for imipenem, meropenem, ertapenem, doripenem, tigecycline and colistin were determined by using Etest strips (bioMerieux, France) as per the manufacturer's protocol.

Screening for the Carbapenemase Production

By disc diffusion, all the isolates with a reduced susceptibility to meropenem or ertapenem (diameter of zones of inhibition, ≤ 21mm) were screened for the production of carbapenemase according to the CLSI guidelines [11].

Phenotypic Detection of the Carbapenemase Production

The phenotypic detection of the carbapenemase production was performed by the modified Hodge test by using a ertapenem disc (10 µg) as was described by CLSI [11]. The detection of metallo-β-lactamase production was also performed by the combined-disc test by using two imipenem discs (10 µg), one containing 10 µl of 0.1 M (292 µg) anhydrous EDTA (Sigma Chemicals, St. Louis, MO), which were placed 25 mm apart on a Mueller-Hinton agar plate [12]. An increase in the zone diameter of >4 mm around the imipenem-EDTA disc as compared to that of the imipenem disc alone was considered as positive for metallo-β-lactamase production.

Molecular detection of the β-lactamase genes:

Plasmid DNA was extracted from all the screening positive isolates by using the Sure Spin Plasmid Mini Kit (Genetix Biotech Asia Ptv. Lid., India) as per the manufacturer's protocol. The extracted plasmid DNA of each isolate was subjected to PCR detection of the bla_{NDM-1} gene by using the target specific primer set, NDM-Fm (5′-GGTTTGGCGATCTGGTTTTC-3′,) and NDM-Rm (5′-CGGAATGGCTCATCACGATC-3′) [13]. These isolates were also examined for the presence of additional plasmid-encoded β-lactamases genes such as bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{AmpC} by PCR amplification as has been described elsewhere [14-17]. The purified PCR product of the bla_{NDM-1} gene for one of the randomly selected representative isolates (Kb-43) was further sequenced with the same set of primers as was used for its amplification by using a 3730XL DNA analyzer (Applied Biosystems, CA, USA), based on Sanger's sequencing method.

The *K. pneumoniae* isolates which were positive for bla_{NDM-1} by PCR and were confirmed by gene sequencing were use as positive controls in this study.

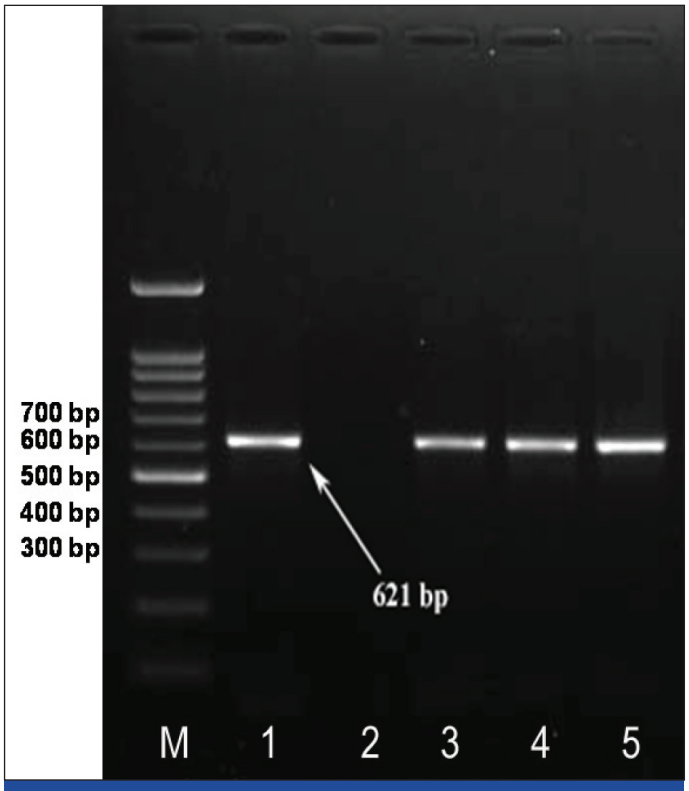
RESULTS

During the study period, out of the 219 *K. pneumoniae* isolates, a total of 19 isolates were screened for carbapenemase production on the basis of their reduced susceptibility to meropenem or ertapenem. Out of these 19 *K. pneumoniae* screen positive isolates, 11(57.89%) were from urine, 03 (15.79%) were from sputum, 02 (10.53%) were from blood and 03 (15.79%) were from pus. The screening positive isolates were also found to be positive for the modified Hodge test, although 3 (15.79%) isolates gave weakly positive reactions. The combined disc test showed positive results for all the screening positive isolates. The age of the patients with the NDM-1 positive isolates ranged from 5 days to 75 years and the male to female ratio was 1.37:1

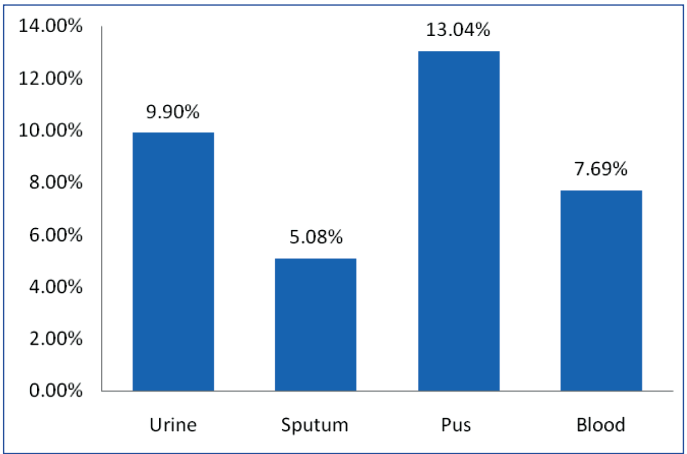
All the screening positive isolates (n =19) were found to be positive for the plasmid mediated bla_{NDM-1} gene by PCR [Table/Fig-1]. The nucleotide sequence of the bla_{NDM-1} gene from the Kb-43 isolate showed 99% identity with the previously reported bla_{NDM-1} genes which were present in the GenBank database. The deduced nucleotide sequence of the bla_{NDM-1} gene from the Kb-43 isolate has been submitted to the GenBank under the accession number, JN697592. The distribution of the NDM-1 producing isolates in the different clinical samples were 9.9% (11/111), 5.08% (3/59), 7.69 (2/26) and 13.04% (3/23) for urine, sputum, blood and pus respectively [Table/Fig-2].

Furthermore, these bla_{NDM-1} possessing isolates were found to also be positive for one or more additional plasmid mediated *bla* genes [Table/Fig-3-6]. The co-existence of bla_{NDM-1} with bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{AmpC} was established in 2 (10.53%) isolates, that of bla_{TEM}, bla_{SHV} and bla_{CTX} was established in 3 (15.79%) isolates, that of bla_{TEM}, bla_{CTX-M} and bla_{AmpC} was established in 3 (15.79%) isolates and that of bla_{TEM} and bla_{CTX} was established in 6 (31.58%) isolates . However, 5 (26.31%) isolates which possessed bla_{NDM-1} were found to be positive for only bla_{TEM}. The frequency of the additional *bla* genes in the NDM-1 producing *K. pneumoniae* isolates are shown in [Table/Fig-7].

By disc diffusion susceptibility testing, the susceptibility of the bla_{NDM-1} possessing isolates (n=19) for amikacin, gentamicin, ciprofloxacin, co-trimoxazole and nalidixic acid were found to be 21.05%, 15.79%, 21.05%, 21.05% and 15.79% respectively. All the bla_{NDM-1} possessing isolates were found to be resistant to ampicillin, cefpodoxime, cefotaxime, ceftazidime, ceftriazone, cefepime, cefoxitin and piperacillin/ tazobactam. None the isolates were found to be susceptible to imipenem, meropenem, ertapenem or doripenem by the disc diffusion method [Table/Fig-8]. The MIC values for the different carbapenems varied widely among the

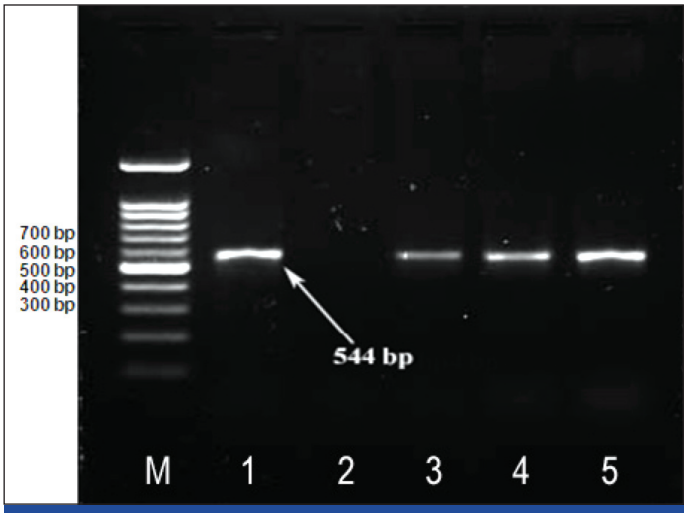


[Table/Fig-1]: Agarose gel showing PCR amplified product of bla_{NDM-1} genes, lane M= 100 bp DNA ladder (Biochem Life Sciences, India), lane 1 and 3-5= bla_{NDM-1} positive amplicons (621 bp), lane 2: negative control (no template DNA added).

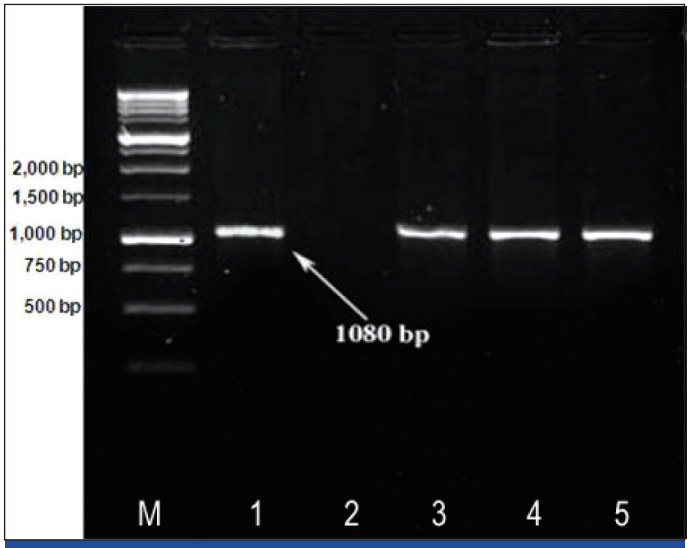


[Table/Fig-2]: Distribution of NDM-1 positive *K. pneumoniae* isolates in different clinical samples.

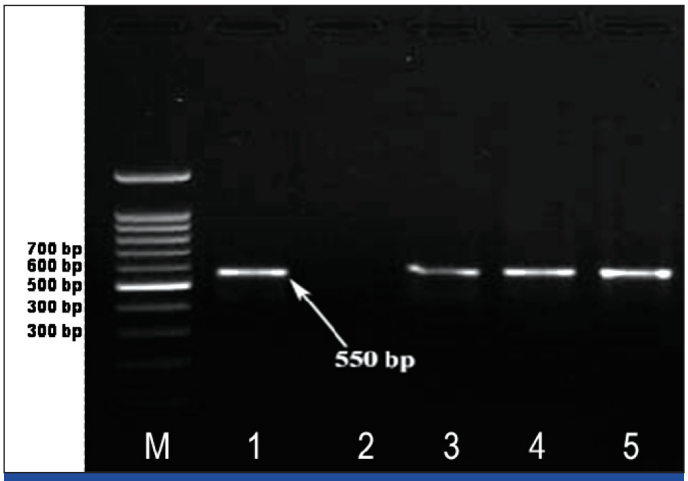
Numerical data on which [Table/Fig-2]is based : The distribution of NDM-1 producing isolates in different clinical samples were 9.9% (11/111), 5.08% (3/59), 13.04% (3/23) and 7.69 (2/26) for urine, sputum, pus and blood respectively.



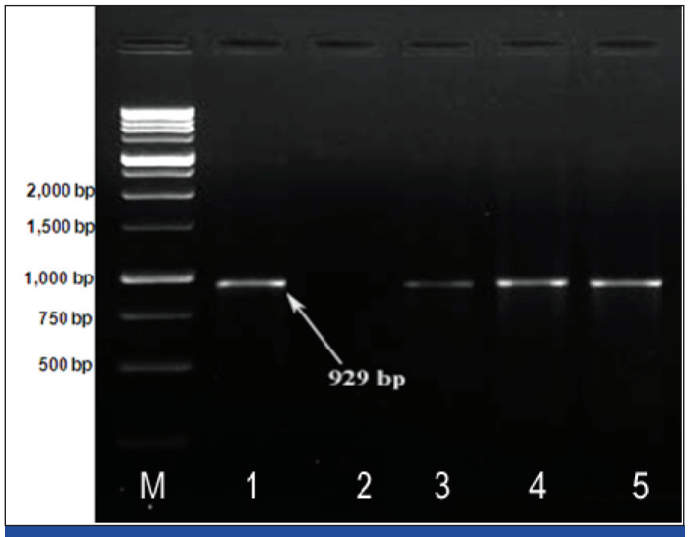
[Table/Fig-5]: Agarose gel showing PCR amplified product of bla_{CTX-M} genes, lane M= 100 bp DNA ladder (Biochem Life Sciences, India), lane 1 and 3-5= bla_{CTX-M} positive amplicons (544 bp), lane 2: negative control (no template DNA added).



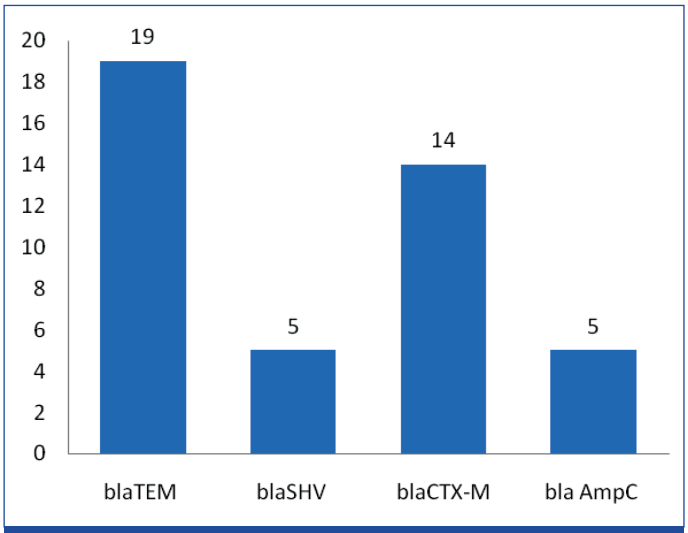
[Table/Fig-3]: Agarose gel showing PCR amplified product of bla_{TEM} genes, lane M= 1kb DNA ladder (Biochem Life Sciences, India), lane 1 and 3-5= bla_{TEM} positive amplicons (1080 bp), lane 2: negative control (no template DNA added).



[Table/Fig-6]: Agarose gel showing PCR amplified product of bla_{AmpC} genes, lane M= 100 bp DNA ladder (Biochem Life Sciences, India), lane 1 and 3-5= bla_{AmpC} positive amplicons (550 bp), lane 2: negative control (no template DNA added).



[Table/Fig-4]: Agarose gel showing PCR amplified product of bla_{SHV} genes, lane M= 1kb DNA ladder (Biochem Life Sciences, India), lane 1 and 3-5= bla_{SHV} positive amplicons (929 bp), lane 2: negative control (no template DNA added).



[Table/Fig-7]: Frequency of additional *bla* genes in NDM-1 producing *K. pneumoniae* isolates (n = 19).

Numerical data on which Table/Fig-7 is based : Among the NDM-1 producing *K. pneumoniae* isolates (n = 19), all the 19 isolates were positive for bla_{TEM} gene, whereas 5 isolates were positive for bla_{SHV}, 14 isolates for bla_{TEM}, and 5 isolates for bla_{AmpC} genes.

Isolate	Antimicrobial agent*																	
	A	AK	G	CF	CO	NA	CEP	CE	CA	CI	CN	AO	CPM	PT	IPM	MEM	ETP	DOR
Kb-04	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-07	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R
Kb-10	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R
Kb-20	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-24	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-37	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-43	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-64	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-94	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-108	R	S	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R
Kb-139	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-144	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-158	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-162	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-173	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-184	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-197	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-201	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
Kb-206	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

[Table/Fig-8]. Antimicrobial susceptibility profile of NDM-1 producing *K. pneumoniae* isolates by disc diffusion method*:*
*A=Ampicillin, AK= Amikacin, G= Gentamicin, CF= Ciprofloxacin, CO = Co-Trimoxazole, NA= Nalidixic Acid, CEP= Cefpodoxime, CE= Cefotaxime, CA= Ceftazidime, CI= Ceftriazone, CN= Cefoxitin, AO= Aztreonam, CPM= cefepime, PT= Piperacillin/ Tazobactam, IPM= Imipenem, MEM= Meropenem, ETP= Ertapenem, DOR= Doripenem ; R= Resistant, S= Sensitive.

Isolate	Year of isolation	Source of isolation	Sex of patient*	Age of patient	Hospital unit†	MIC (µg/mL)‡						Presence of other bla gene(s)
						IMP	MEM	ETP	DOR	TGC	CST	
Kb-04	2009	Urine	M	40 years	Medicine ward	2	4	12	4	0.50	0.25	$bla_{TEM,}$ $bla_{SHV,}$ bla_{CTX-M}
Kb-07	2009	Blood	M	05 days	NICU	2	2	8	2	0.38	0.25	$bla_{TEM,}$ bla_{CTX-M}
Kb-10	2009	Urine	M	50 years	Urology ward	2	4	8	2	1.0	0.125	$bla_{TEM,}$ bla_{CTX-M}
Kb-20	2009	Sputum	M	75 years	Medicine ward	2	4	6	2	0.75	0.50	bla_{TEM}
Kb-24	2009	Urine	F	40 years	ICU	8	3	16	4	1.0	0.25	bla_{TEM}, bla_{CTX-M}, bla_{AmpC}
Kb-37	2009	Urine	M	52 years	Urology OPD	2	4	12	4	0.50	0.38	bla_{TEM}, bla_{CTX-M}
Kb-43	2009	Pus	F	68 years	Surgical ward	3	4	12	3	2.0	0.25	bla_{TEM}, bla_{CTX-M}, bla_{AmpC}
Kb-64	2009	Urine	M	34 years	Urology ward	6	4	24	4	2.0	0.38	bla_{TEM}, bla_{CTX-M}
Kb-94	2009	Sputum	M	69 years	Medicine OPD	2	3	8	3	1.0	0.125	bla_{TEM}, bla_{SHV}, bla_{CTX-M}
Kb-108	2009	Urine	F	03 years	Pediatrics ward	2	4	6	2	0.50	0.25	bla_{TEM}
Kb-139	2010	Sputum	M	65 years	ICU	2	4	8	4	0.75	0.25	$bla_{TEM,}$ $bla_{SHV,}$ $bla_{CTX-M,}$ bla_{AmpC}
Kb-144	2010	Pus	F	62 years	Surgical ward	>32	>32	>32	>32	2.0	0.38	$bla_{TEM,}$ bla_{CTX-M}
Kb-158	2010	Urine	F	31 years	Urology OPD	4	4	12	3	1.0	0.50	bla_{TEM}
Kb-162	2010	Pus	F	48 years	Orthopedics ward	8	16	32	8	2.0	0.50	$bla_{TEM,}$ bla_{CTX-M}
Kb-173	2010	Blood	M	56 years	ICU	4	3	6	2	1.0	0.25	bla_{TEM}
Kb-184	2010	Urine	M	02 years	Pediatrics ward	2	4	6	3	1.0	0.25	$bla_{TEM,}$ $bla_{SHV,}$ $bla_{CTX-M,}$ bla_{AmpC}
Kb-197	2010	Urine	M	26 years	Surgical ward	2	2	6	3	0.38	0.25	$bla_{TEM,}$ $bla_{CTX-M,}$ bla_{AmpC}
Kb-201	2010	Urine	F	38 years	Medicine ward	2	4	12	4	0.50	0.38	bla_{TEM}
Kb-206	2010	Urine	F	50 years	ICU	4	4	8	3	0.75	0.50	$bla_{TEM,}$ $bla_{SHV,}$ bla_{CTX-M}

[Table/Fig-9]: Different characteristics of bla_{NDM-1} positive *K. pneumoniae* isolates
*M = male; F = female; †ICU = intensive care unit; NICU = neonatal intensive care unit.
‡MIC = minimum inhibitory concentration; IMP = imipenem; MEM = meropenem; ETP = ertapenem; DOR = doripenem; TGC = tigecycline; CST = colistin.

bla$_{NDM-1}$ possessing isolates [Table/Fig-9]. The range of the MIC values for imipenem, meropenem, ertapenem and doripenem were 2– >32 µg/mL, 2– >32 µg/mL, 6– >32 µg/mL and 2–>32 µg/mL respectively. The MIC value for tigecycline was ≥ 2 µg/mL (range, 0.38–2 µg/mL) and for colistin, it was > 1 µg/mL (range, 0.125–0.5 µg/mL).

DISCUSSION

The increasing reports on the NDM-1 producing *Enterobacteriaceae* is a major concern worldwide. Although, NDM-1 seems to be endemic in the Indian subcontinent, other reports have suggested that the Balkan countries may act as another reservoir of the NDM-1 producers [2,6,18]. Here, we are reporting the presence of NDM-1 producing *K. pneumoniae* isolates in various clinical samples from a tertiary care referral hospital in northeast India. The overall prevalence of the *bla*$_{NDM-1}$ possessing *K. pneumoniae* isolates in our hospital was found to be 8.67% (19/219). A pervious single-day point-prevalence study which was carried out in this geographical region, reported the incidence of *bla*$_{NDM-1}$ in a single *K. pneumoniae* isolate which was recovered from a surgical site infection [19]. Among the 22 NDM-1 producing *Enterobacteriaceae*, 10 *Klebsiella* spp. were found to be positive for *bla*$_{NDM-1}$ at a tertiary care centre in Mumbai [20]. Recently, a very high incidence of *bla*$_{NDM-1}$ (80%) has been reported in the *K. pneumoniae* strains which were collected from blood and an endotracheal aspirate from a hospital in Vellore [21]. However, it is difficult to make out the exact prevalence of NDM-1 in *K. pneumoniae,* as this study was confined to a single hospital.

In this study, we included the samples from both hospitalized and non-hospitalized patients. Among the 19 *bla*$_{NDM-1}$ positive *K. pneumonia* isolates, a majority of the isolates were recovered from the patients who were admitted to different wards and the intensive care unit (ICU). The incidence of *bla*$_{NDM-1}$ was 84.21% (16/19) in the hospitalized patients and it was 15.79% (3/19) in the non hospitalized patients. In addition, 26.31% (5/19) *bla*$_{NDM-1}$ positive isolates were recovered from ICU patients, of which one of the isolate was recovered from the neonatal intensive care unit (NICU). A previous study from another tertiary care centre in India also indicated the presence of NDM-1 producing *K. pneumoniae* in NICU patients [7].

The CLSI recommends the modified Hodge test as a phenotypic confirmatory test for the detection of carbapenemase production in *Enterobacteriaceae* isolates with elevated MICs for carbapenems or reduced inhibition zones in disc diffusion susceptibility testing, due to its acceptable sensitivity and specificity for carbapenemase detection. However, the sensitivity and the specificity of this test for detecting low-level metallo-β-lactamase production are not known [11]. In this study, 16 (84.21%) *bla*$_{NDM-1}$ positive *K. pneumonia* isolates showed clearly positive results and 3 (15.79%) isolates showed weakly positive results for the modified Hodge test. Different studies also reported the occurrence of weakly positive results for the modified Hodge test in the detection of NDM-1 producing *Enterobacteriaceae* [4, 22]. In contrast, other studies reported false positive or false negative results which were produced by the modified Hodge test in the detection of carbapenemase producing *Enterobacteriaceae* [23, 24]. On the other hand, the combined disc test which was performed to detect metallo-β-lactamase production was found to be highly sensitive (100%) for the NDM-1 detection, as this test showed positive results with all the *bla*$_{NDM-1}$ possessing *K. pneumoniae* isolates. The difference in the zone diameter was fairly more than 4mm for all the isolates with *bla*$_{NDM-1}$. Therefore, our results suggest that the combined disc test can be used for the detection of NDM-1 producing *K. pneumoniae* isolates in any clinical laboratory on a daily basis.

The *K. pneumonia* isolates which possessed the *bla*$_{NDM-1}$ gene were found to be resistant to several classes of β-lactam and non β-lactam antibiotics by disc diffusion susceptibility testing. All the isolates which possessed *bla*$_{NDM-1}$ were resistant to imipenem, meropenem, ertapenem and doripenem by disc diffusion susceptibility testing. These isolates also exhibited a high level of resistance to the penicillins, the third and fourth generation cephalosporins, cephamycin, and aztreonam, as well as to the β-lactam–β-lactamase inhibitor combination which was tested in the study. However, few *bla*$_{NDM-1}$ possessing isolates were found to be susceptible to amikacin, gentamicin, ciprofloxacin, co-trimoxazole and nalidixic acid. A similar type of susceptibility to the non β-lactam antibiotics in the NDM-1 producing organisms has been described in other studies from India [25, 26]. As a high-level of resistance was observed to the different classes of the β-lactam antibiotics, we also performed PCR detection for some of the important *bla* genes in the NDM-1 producing *K. pneumonia* isolates. It was observed that a majority of the isolates (73.68%) possessed two or more additional *bla* genes and that 26.32 % (5/19) isolates possessed only one additional *bla* gene in addition to *bla*$_{NDM-1}$. The frequency of *bla*$_{TEM}$, *bla*$_{SHV,}$ *bla*$_{CTX-M}$ and *bla*$_{AmpC}$ in the NDM-1 producing *K. pneumonia* isolates were 100%, 26.32%, 73.68% and 26.32% respectively. However, the sequencing of these additional *bla* genes could have given additional information. Previous studies from India had reported the presence of TEM-1, SHV-1, SHV-12, CTX-M-15, CMY-2 and DHA in the NDM-1 producing *K. pneumonia* isolates [5, 8]. Other studies from abroad also showed the presence of *bla*$_{TEM-1}$, *bla*$_{SHV-11}$, *bla*$_{SHV-28}$, *bla*$_{CTX-M-15}$, and *bla*$_{CMY-6}$ in the *bla*$_{NDM-1}$ possessing *K. pneumoniae* isolates [27,28].The incidence of *bla*$_{TEM}$ in all the *bla*$_{NDM-1}$ possessing isolates also indicated the high prevalence of *bla*$_{TEM}$ among the β-lactam resistant *K. pneumoniae* in this region.

In this study, we observed that the carbapenemase production was not always associated with elevated MIC values for the different carbapenems and that the MIC values of the carbapenems varied greatly among the *bla*$_{NDM-1}$ possessing isolates. The variations in the MIC values for the different carbapenems may be influenced by several factors, such as the type and the expression of the carbapenemase enzyme, the bacterial species and the presence of other resistance mechanisms (e.g., Extended spectrum and AmpC β-lactamases, reduced permeability and/or efflux pumps) [29]. The lowest MIC values for imipenem, meropenem, ertapenem and doripenem were 2µg/mL, 2µg/mL, 6 µg/mL and 2µg/mL respectively. There was only one *bla*$_{NDM-1}$ positive *K. pneumoniae* isolate in this study, for which the MIC values of all the four carbapenems were more than 32 µg/mL. In comparison to the other carbapenems, a higher MIC value for ertapenem was observed in all the *bla*$_{NDM-1}$ positive isolates. A previous study reported that a higher resistance for ertapenem than for imipenem or meropenem may result from the combination of a β-lactamase (CTX-M or the AmpC enzyme) in addition to a decreased permeability or an increased efflux [30]. In contrast, we observed a higher MIC value for ertapenem than for imipenem, meropenem and doripenem in few *bla*$_{NDM-1}$ positive isolates with *bla*$_{TEM}$ only and these isolates were found to be negative for *bla*$_{CTX-M}$ and *bla*$_{AmpC.}$

The MIC values for tigecycline and colistin were observed to be within the effective range for all the *bla*$_{NDM-1}$ possessing isolates, although 21.05% (4/19) isolates showed an MIC value of 2 µg/

mL for tigecycline. The isolates may show an *in vitro* susceptibility to tigecycline, but the clinical experience with the carbapenem-resistant strains is limited. Although some NDM-1-producing bacteria have found to be resistant to tigecycline, this drug is usually used as one of the first line agents against the carbapenemase-producing isolates [31]. During the initial studies, colistin was found to be effective in the treatment of carbapenemase producing *Enterobacteriaceae*, but recent reports have described the emergence of colistin resistant strains [31]. These reports have threatened the world community, as there is a possibility for turning the earth back to the pre-antibiotic era.

In conclusion, carbapenem resistant *K. pneumoniae* have been considered as one of the greatest threats to the global health care in this century. The emergence and the spread of NDM-1 producing *K. pneumoniae* have heightened this threat. It has been observed that all the screening positive isolates were found to harbour the plasmid mediated bla_{NDM-1} gene along with one or more additional *bla* genes, both in hospitalized and non-hospitalized patients. The dissemination of such plasmids between different clinically important bacterial species may lead to serious public health issues, as *K. pneumoniae* accounts for one of the the important bacterial species in the dissemination of antibiotic resistance genes, particularly in hospital environments. Therefore, the early detection of the bla_{NDM-1} possessing *K. pneumoniae* isolates with any reduced susceptibility to the carbapenems is indispensable for the choice of the most appropriate antibiotic therapy and the implementation of efficient infection control measures.

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