Lower Respiratory Tract Infection in Kolkata and Multidrug Resistant Pathogen- with a Focus on Carbapenem Resistant Organism

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ABSTRACT

Introduction: Lower respiratory tract infection (LRTI) is the commonest infectious cause of death in the world. Bacteria play an important role in LRTI. Present study aimed to show the resistance pattern in Enterobacteriaceae and non -fermenting gram negative bacilli (NFGNB) and molecular characterization of carbapenem resistant isolates in hospitalized LRTI patients.

Material and Methods: Present study (August 2012 - December 2014) identified 152 multidrug resistant gram negative bacilli (MDR-GNB) from validated sputum, endotracheal aspirate, bronchoalveolar lavage, pleural fluid collected from LRTI patients within 48 hours of admission. Their antimicrobial susceptibility test, MIC value determination and phenotypic detection of beta lactamases done. Enterobacteriaceae and NFGNB resistant to at least 3 antimicrobial groups were considered MDR-GNB. MIC value for ertapenem ≥0.5mg/L was set as screening breakpoint to detect carbapenemase for Enterobacteriaceae. Imipenem MIC breakpoints adopted to indicate MBL production were: Pseudomonas aeruginosa, ≥4µg/ml; Acinetobacter spp and other NFGNB other than P. aeruginosa, >2µg/ml. Molecular characterization of carbapenem resistant (CR) Enterobacteriaceae and NFGNB documented.

Results: Out of total MDR-GNB isolates, 32%,30% and 38% were co-resistant to 3,4, and 5 antimicrobial groups respectively. CR isolates (n=58, 28% among all GNB) harbour 31%(n=18) bla_{NDM}. Bla_{KPC} was conspicuous by its absence. There was a significantly higher incidence of LRTI caused by bla_{NDM} harbouring isolates in patients with neurological abnormality (CVA P=0.025). LRTI with bla_{NDM} harbouring isolates did not document higher mortality.

Conclusion: Common co-resistance pattern will help in empiric antimicrobial treatment. Presence of substantial percentage of CR isolates carrying bla_{NDM} gene in our clinical setting require effective infection control measures.

Keywords: Enterobacteriaceae, Klebsiella pneumoniae carbapenemase, Lower respiratory tract infection, Multidrug resistant gram negative bacilli, Non fermenting gram negative bacilli, New Delhi metallo beta lactamase

INTRODUCTION

Lower respiratory tract infection (LRTI) is a broad term indicating infection of anatomical lower respiratory tract, with or without lung involvement, as evidenced by imaging. Cough with or without expectoration, dyspnoea, wheeze and /or chest pain/discomfort are common presenting symptoms. LRTI in adults include community acquired pneumonia (CAP) acute bronchitis, influenza, and exacerbation of chronic and structural lung diseases like acute exacerbation of chronic obstructive pulmonary disease (AECOPD), bronchiectasis (AEBX).[1] Though LRTI patients are sometimes treated as outpatients frequent hospitalizations are needed in severe subgroup. According to WHO, LRTI is the most common infectious cause of death in the world (the 4th most common cause overall), and responsible for almost 3.5 million death yearly.[2] Surveillance studies documented the increasing number of infection by resistant organisms.[3]

An Indian study for evaluation of etiology and antimicrobial susceptibility patterns of LRTI revealed substantial proportion of multidrug resistant bacterial isolates. These included extended spectrum beta lactamases (ESBL) (75%) and metallo-betalactamases /carbapenemases (MBL) (25%) among gram negative bacterial isolates.[4] These multi drug resistant gram negative bacilli (MDR-GNB) need costlier antibiotic treatment, longer hospital stay and are associated with high mortality.[5]

Different beta lactamase enzymes like ESBL and Amp C producing Enterobacteriaceae, and other multidrug resistant gram negative bacilli (MDR-GNB) which were resistant to higher generations of cephalosporin (ceftriaxone, cefepime) were effectively treated by carbapenem group of antibiotics as preferred drug of choice until recently.[6] Emergence of carbapenemase producing organisms which could hydrolyze practically all beta-lactums, in association with faltering antimicrobial pipeline imparted serious management challenge. Organisms in which carbapenem resistance result from production of carbapenemase enzyme, also express resistance to other classes of antimicrobials like aminoglycosides, fluoroquinolones,beta- lactam beta lactamase inhibitor combinations. As a result, these carbapenemase producing isolates become extensively drug-resistant (XDR) or pandrug resistant (PDR).[7,8] Among the different carbapenemase enzymes, NDM-1 was identified locally as only carbapenemase type,

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in a recent five year consecutive study of sepsis in a tertiary care hospital.[9]

Detection of NDM-1 carbapenemase in *Klebsiella pneumoniae* is a serious concern. The gene encoding this novel beta-lactamase (NDM-1) was identified in a large plasmid that was easily transferrable to other Enterobacteriaceae with multiple transmissible resistance determinant gene in it. Most NDM-1producing isolates possess multiple beta lactamases, aminoglycoside resistant genes *armA* or *rmtB* and plasmid mediated quinolone resistant gene aac (69)-*Ib-cr*;*C-MY-4*,genes encoding inactivation of several other antibiotics including rifampicin, chloramphenicol, fluroquinolone and erythromycin. The organism identified initially harbouring this particular beta-lactamase was only susceptible to polymixins.[9,10]

The study of lower respiratory tract infection in two tertiary care hospitals in Kolkata revealed Enterobacteriaceae as important pathogen along with other gram negative non fermenting bacilli (NFGNB) in lower respiratory samples.[11] In the present work we studied the hospitalized LRTI patients harbouring MDR-GNB and their carbapenem resistance.

The present study aimed to get a cross sectional picture of epidemiological profile of MDR-GNB along with relative incidence of presence of bla_{NDM} and bla_{KPC} among the carbapenem resistant Enterobacteriaceae (EB) and other NF-GNB isolated from validated LRT samples and secondarily to compare the clinical profile of LRTI patients harbouring bla_{NDM} carrying isolates with those not having bla_{NDM} in carbapenem resistant isolates in the said patients group.

MATERIAL AND METHODS

Study design

We prospectively assessed patients aged >12 years hospitalized with a diagnosis of LRTI at two urban teaching hospitals from August 2012 to December 2014.The former one is an undergraduate teaching hospital with 750 beds while the latter one is a 150 bedded post graduate teaching hospital specialized for treatment of HIV infection and tropical diseases. The institutional ethics committee of both the hospitals approved the study. Informed written consent were taken from all the study participants or their accompanying relatives.

Sample Collection and bacterial Isolates

Total hospitalized LRTI patients assessed and enrolled in both the hospitals were 1829 (hospital 1,n=1432: hospital 2,n=397) during the study period according to study protocol.[11] Out of these, 1087 patients were excluded due to non production of validated lower respiratory tract sample and sputum containing acid fast bacilli (hospital 1, n=802 and n= 24: hospital 2, n=252 and n=9 respectively).

A sum of non duplicate 205 gram negative bacilli were identified from total 742 samples including validated sputum, endotracheal aspirate, bronchoalveolar lavage and pleural fluid.

Culture positive was accepted if the validated sample of sputum, endotracheal aspirate (>25/ lpf inflammatory cells and <10/ lpf epithelial cells) and BAL fluid showed semi quantitative growth (moderate to heavy growth) of pathogenic bacteria by standard culture methods.[12-14]

The demographic, clinical and laboratory data of patients were noted according to study protocol.[11]

MDR definitions

MDR-GNB was defined as resistance to at least 3 antimicrobial group. MDR in *K.pneumoniae, E.coli, Enterobacter cloacae, Enterobacter aerogenes, Citrobacter fruendii* was defined as resistant to at least 3 of the following groups of antimicrobials; third / fourth generation cephalosporins, aminoglycosides, fluoroquinolones and piperacillin-Tazobactum.[15] *Pseudomonas aeruginosa*, and other NFGNB were considered as MDR if resistant to at least 3 of the groups viz; third generation cephalosporin ceftazidime or the next generation cefepime, aminoglycosides, fluoroquinolones, carbapenem or piperacillin –tazobactum.[16]

Isolation and Identification of the gram negative bacteria

All isolates were identified by the standard culture method using standard media. Test kit product code KB001, and KB014 (Hi IMVIC™ and Hi Acinetobacter™ identification test kit) were used. Antibiotic susceptibility and minimum inhibitory concentration (MIC) were performed. Isolates exceeding screening breakpoints of respective carbapenem were studied for phenotypic tests and subjected to molecular characterization of MBL/KPC determinants.

Antimicrobial susceptibility test

Kirby Bauer standard disk diffusion method was adopted, and interpretation done following CLSI guidelines 2012.[17,18] Antimicrobial agents used were ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefoperazone (75µg) cefoxitin (30 µg), cefepime (30 µg), aztreonam (30 µg),gentamicin (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), levofloxacin (5µg), tetracycline (30 µg),tigecycline(15 µg) colistin (10 µg), ertapenem (10 µg) meropenem (10 µg), Imipenem (10 µg),piperacillin-tazobactam(75µg/10µg), ticarcillin- clavulanic acid(75µg/10µg) (Bio RAD,3,bd Raymond Poincare, France).

The MIC values of Ceftriaxone,Ceftazidime, Cefepime, Imipenem, ertapenem, meropenem, amikacin, gentamicin, levofloxacin and piperacillin- tazobactum were determined using E test method (AB Bio disk, Solna, Sweden) in one centre and by microbroth dilution by Microscan (Siemens, Germany)in another centre and interpreted following CLSI guidelines 2012.[18]

The clinical breakpoints for ertapenem were as follows: S ≤0.5 mg/ L, I: 1.0 mg/L, R ≥2 mg/L. For Enterobacteriaceae the MIC value for ertapenem ≥ 0.5 mg/L was set as the screening breakpoint to detect carbapenemases.[19] We adopted the following imipenem MIC breakpoints to indicate MBL production of *Pseudomonas aeruginosa*, ≥4µg/ml; For *Acinetobacter* spp and other NFGNB other than *P. aeruginosa*, >2µg/ml.[20] Phenotypic evaluation of ESBL production was done after screening test performed following CLSI guideline.[18]

Phenotypic identification

Phenotypic evaluation of ESBLs, carbapenemase

ESBL production were checked by double disc synergy method using ceftazidime and ceftazidime+ clavulanic acid and cefotaxime and cefotaxime+ clavulanic acid. (Bio RAD, 3,bd Raymond Poincare, France).

KPC and Metallo-β-Lactamase enzyme productions were screened by combination disc test. Meropenem (10 µg)/ boronic acid (300 µg) for KPC and imipenem (10 µg)/EDTA (750 µg) (Sigma-Aldrich, St Louis, MO, USA) for MBL were used respectively.[21] Zone of inhibition >5 mm of the combination discs indicated positive finding.

The Modified Hodge test (MHT)

Clinical and Laboratory Standards Institute (CLSI, 2012) recommended MHT as a confirmatory test for carbapenemase production. MHT performed in all EB Isolates fulfilling the CLSI criterion for performing carbapenemase detection by the MHT. Ertapenem 10-µg discs (Bio RAD, France) was used with *E. coli* ATCC 25922 and *K. pneumoniae* ATCC BAA-1705 as positive control and *K. pneumoniae* ATCC BAA-1706 as negative control.

Detection of *bla* $_{NDM}$ and *bla* $_{KPC}$ by PCR

A total of fifty eight isolates were subjected to PCR for *bla*$_{NDM}$ and *bla*$_{KPC}$ detection (Table 2).

Preparation of template DNA

All PCRs (polymerase chain reactions) in this study were carried out using DNA template, prepared by boiled lysate from the organisms. All PCRs were performed with an Applied Biosystems GeneAmp 9700 thermal cycler. PCR samples were resolved in 1-2 % agarose gel (as per requirement for various sizes of product) and stained in ethidium bromide (0.5 µg/ml). PCRs reactions were carried out in singlex. The gel was then visualized by a GelDoc 2000 (BioRad, Hercules, CA). DNA fragments with known molecular masses were included.

Primer sequence (NDM)

Forward-----5'- GTCTGGCAGCACACTTCCTA-3'
Reverse-----5'- TAGTGCTCAGTGTCGGCATC-3'

Primer sequence (KPC)

KPC forward 5' ATGTCACTGTATCGCCGTCT 3'
KPC reverse 5' TTTTCAGAGCCTTACTGCCC 3'

Storage

All isolates were stored in 20% glycerol supplemented tryptophane soy broth (-80^{0}C).

STATISTICAL ANALYSIS

A descriptive analysis was performed for demographic and clinical characteristics and results presented as mean ± standard deviation for continuous variables and numbers (percentages) for categorical variables. Association between outcomes of LRTI and its risk factors was assessed by using the Chi-square test. All tests were two tailed and a P<0.05 was

Gram negative pathogens n(%) n=205	MDR-GNB No(%) n=152(74)	Ertapenem (Enterobacteriaceae/Imipenem (NFGNB) non susceptible No(%) n=58 (28)	Ertapenem (Enterobacteriaceae/Imipenem (NF-GNB) susceptible No(%)= n=147(72)
Enterobacteriaceae n=123(60)	N=111(73)	N=38(65)	N=85(58)
Klebsiella pneumoniae N=86(42)	N=80(52)	N=28(48)	N=58(39)
Escherichi coli n=22(10.7)	N=18(12)	N=2(3)	N=20(14)
Enterobacter aerogenes n=7(3.4)	N=7(5)	N=3(5)	N=4(2)
Enterobacter cloacae n=5(2.4)	N=5(3)	N=5(9)	N=0
Citrobacter fruendii n=3(1.4)	N=1(0.6)	N=0	N=3(2)
Acinetobacter spps n=16(7.8)	N=14(9.2)	N=14(24)	N=2(1)
Pseudomonas aeruginosa n=66(32)	N=27(18)	N= 6(10)	N=60(41)
MDR-GNB-- Multidrug resistant gram negative pathogen,NFGNB—Non fermenting gram negative pathogen			

Table-1: Stratification of Enterobacteriaceae and non fermenting gram negative bacilli isolated from LRT samples of hospitalized LRTI patients

Total GNB isolated n=205	Total MDR-GNB n=152 (74%) 3 drug resistant, (n= 49, 32%), 4 drug resistant (n= 45, 30%) 5 drug resistant (n= 58, 38%))						
	Number (%) of MDR-GNB in different isolates						
Antimicrobial group co- resistance pattern	K.pneumoniae n (%) 80(93)	E.coli n(%) 18(81)	E.aerogenes n (%) 7(100)	E.cloacae n (%) 5(100)	C.fruendii n (%) 1(33)	P.aeruginosa n (%) 27(41)	Acinetobacter spps n (%) 14(88)
Resistance to 5 antimicrobial group including carbapenem							
	28(35%)	2(11)	3(43%)	5(100)	0	6(22)	14(100)
Resistance to 4 antimicrobial group							
Cephalo/Amino/Fluoro/Pip-Tazo	32(40)	4(22)	0	0	0	9(33)	0
Resistance to 3 antimicrobial group							
Pip-Tazo/Cephalo/Fluoro	17 (21)	8(44)	3(42)	0	1(100)	12(44)	0
Fluoro/Cephalo/Amino	0	4(22)	1(14)	0			0
Pip - Tazo/Cephalo/Aminoglycosides	3 (4)						
Note: Tazo = Piperacillin Tazobactum, Cephalo =Cephalosporins Fluoro= Fluoroquinolones, Amino=Aminoglycosides							

Table-2: Co-resistance pattern of 152 isolates of MDR- GNB identified from LRTI patients

considered statistically significant.

RESULTS

Bacterial Isolates

Enterobacteriaceae (EB) and NFGNB represented 60% and 40% of total 205 gram negative isolates respectively. Again EB and NFGNB accounted for 60% of the culture positive isolates (n=341) from the hospitalized LRTI patients.

Total EB included *K. pneumoniae* (70%), *E. coli* (18%), *Enterobacter aerogenes*(6%), *Enterobacter cloacae* (4%)and *Citrobacter fruendii* (2%). Again among all gram negative isolates identified 32% were *P. aeruginosa* and 8% were *Acinetobacter spps* (Table 1).

MDR-GNB

Overall prevalence of MDR-GNB was 74% (n=152). Out of total MDR-GNB, 38%, 30%and 32% revealed co resistance to 5, 4, and 3 antimicrobial groups respectively. The pattern and frequency of co resistance pattern against 3, 4, 5 antimicrobial groups of three species of MDR-GNB were shown in Table 2.

Overall susceptibility status

Meropenem demonstrated 70% and 91% coverage rate (% susceptible) among *K. pneumoniae* and *E. coli* respectively. Imipenem and ertapenem revealed 69% and 67% susceptibility for *K. pneumoniae* respectively. But a better coverage rate was observed for *E. coli* (93% and 91%) for the above antimicrobials respectively (Table 3).

All other broad spectrum antimicrobials showed variable susceptibility rates in *K. pneumoniae* and *E. coli*. Both of them showed a continued decline in coverage rate for higher generation cephalosporins. The former showed identical (7%) susceptibility rate for ceftriaxone and cefepime. All isolates were resistant to ceftazidime. *K. pneumoniae* revealed susceptibility rate 22% for gentamicin, 31%for amikacin,10% for levofloxacin and 7% for piperacillin- tazobactam. Whereas *E. coli* showed nearly identical, (9% and 14%) susceptibility rate towards Ceftriaxone and cefepime respectively without a single isolate being susceptible to ceftazidime in our study. *E. coli* showed comparatively varying coverage rate in contrast to *K. pneumoniae* in relation to gentamicin(44%), amikacin(54%), levofloxacin (18%) and piperacillin- tazobactum 36%, (Table 3).

The coverage rate observed in *P. aeruginosa* with carbapenem was 91%. But high level of resistance (88%) was observed among *Acinetobacter spps* against all 9 broad spectrum antimicrobials tested including carbapenem (Table 3).

Total carbapenem resistant isolates were 28% among all gram negative isolates (Table 1). MIC values of ertapenem and imipenem non- susceptible isolates were given in (Table 4) Enterobacteriaceae and NFGNB non susceptible to ertapenem/Imipenem were 100% susceptible to tigecycline and colistin. (By disc diffusion methods). No major difference was observed in antibiotic susceptibility between isolates with bla_{NDM} and isolates not harbouring bla_{NDM}. But both of them showed diminished susceptibilities to other classes of antibiotics. Bla_{NDM} were present in 18(31%) out of 58 isolate tested (Table 4).

Out of 28 ertapenem non susceptible isolates, *K. pneumoniae,* 10(36%) were harbouring bla_{NDM}. In *E. coli* and *E. cloacae* it was 50% and 20% respectively. *P. aeruginosa* and *Acinetobacter spps* were harbouring 33% and 29% bla_{NDM} respectively. No bla_{KPC} was identified in the present study (Table 4).

Evaluation of clinical profile of the patients depending on molecular characterization showed bla_{NDM} harbouring patients were older, and having significantly longer stay in the hospital than their non bla_{NDM} harbouring counterparts (14.75±3.67 days VS10.53±3.54 days; P=000 respectively). No significant difference observed in raised C-reactive protein value; 16(89%) vs 32(80%) P=0.709 or their lung imaging result e.g pleural effusion;7(39%) vs 20(50%) P= 0.734 and multilobar infiltrate; 12(66%) vs 26(65%) P= 0.992 in bla_{NDM} harbouring LRTI patients with their non bla_{NDM} counterparts respectively. Also no significant difference was observed in having relevant co morbidities between NDM +ve and NDM –ve isolates identified in LRTI patients e.g diabetes mellitus;8(44%) vs 22(55%) P= 0.995, heart failure 2(11%) vs 4(10%) P = 0.991, chronic obstructive pulmonary disease;10(55%) vs 20(50%) P=0.926 respectively. But bla_{NDM} containing carbapenemase producing isolates were present significantly more in numbers in LRTI patients with neurological abnormality (cerebrovascular accident patients);10(55%) vs 4(10%) P=0.000. No significant difference was observed in the severity of illness, as assessed from ICU admission; 15(83%) vs 33(82%) P= 0.997 or in hospital mortality 4(22%) vs 11(27%) P= 0.913 in bla_{NDM} +ve and bla_{NDM} –ve carbapenem resistant isolates identified in LRTI patients respectively.

DISCUSSION

Susceptibility pattern observed in the present study depicts a lower coverage rate towards carbapenem group of antimicrobials among *K. pneumoniae* when compared to *E. coli*. Finding of better coverage rate for the above antimicrobials among *E. coli* was not consistent with another Kolkata hospital study. Susceptibility rates for *K. pneumoniae* isolates were higher than *E. coli* isolates in respect to carbapenem during their study period.[9] However finding of the susceptibility rates of other different antimicrobials like third generation cephalosporins and aminoglycosides was somewhat similar with them where Dutta et al showed a lower susceptibility rates for cefotaxime, gentamicin and amikacin among *K. pneumoniae* isolates than *E. coli* isolates.[9,20-22] The present study showed high incidence of resistance in fluoroquinolone in all gram negative bacilli depicting similarity with another surveillance study abroad.[23]

Treatment of NFGNB is more challenging for the clinicians in the hospitalized patients than EB. In a ten year information collection programme in the United States where yearly meropenem susceptibility tests surveillance data was recorded till the year 2008, showed diminishing meropenem susceptibility among *Acinetobacter spps* (45.7%) rather than *P.aeruginosa* (85.4%), a finding having similarity with the present study.[23] Carbapenem resistant NFGNB were predominantly isolated from the ICU setting in our study.

No major difference was observed in antibiotic susceptibility pattern between isolates with bla_{NDM} and isolates not

harbouring bla_{NDM}. But both of them showed diminished susceptibilities to other classes of antibiotics. This result was consistent with other studies from Kolkata and abroad where carbapenem-non susceptible isolates showed reduced susceptibility to other classes of antibiotics.[9,22]

In the present study bla_{KPC} was conspicuous by its absence. The bla_{KPC} gene was also not found in another study of genes encoding carbapenem resistance in uropathogens from a tertiary care centre from north India and other studies in India and abroad.[24,9,26] But co-carriage of integron mediated bla_{K-PC-2} and bla_{NDM-1} in P. aeruginosa isolated from clinical specimens have been reported in tertiary care hospitals in India recently.[25]

The present study correlated the clinical profile of the patients with selective molecular characterization of the isolate identified. This work seems to be the first one in local clinical setting among adult population. No significant association of increased mortality or relevant comorbidity was observed with this novel beta lactamase gene NDM except neurological abnormality and increased mean hospital stay. In a neonatal sepsis study with carbapenem resistant isolates, no increased mortality was observed in patients associated

Organism/Antimicrobial agents	Susceptibility Range	Resistant Range	% susceptible
Klebsiella sps			
Imipenem	≤1	≥4 to ≥8	69
Meropenem	≤1	8 to ≥8	70
Ertapenem	≤0.25 to ≤2	≥2 to ≥8	67
Ceftriaxone	≤1	≥32 to≥64	7
Ceftazidime	nil	≥16 to≥32	0
Cefepime	≤8	≥32 to ≥64	7
Piperacillin Tazobactam	≤16/4	≥64 to≥128/4	7
Gentamicin	≤4	≥8 to≥32	22
Amikacin	≤8 to ≤16	≥16 to≥64	31
Levofloxacin	≤2	≥4 to≥8	10
E.coli			
Imipenem	≤0.5 to ≤1	≥4 to≥8	93
Meropenem	≤1	≥8	91
Ertapenem	≤1 to ≤2	≥2 to ≥4	91
Ceftriaxone	≤1	≥16 to≥32	9
Ceftazidime	nil	≥16 to ≥32	0
Cefepime	≤8	≥16 to ≥32	14
Piperacillin Tazobactum	≤16/4	≥64/4	36
Gentamicin	≤4	≥8 to≥16	44
Amikacin	≤16	≥32	54
Levofloxacin	≤1 to ≤2	≥4 to≥8	18
Pseudomonas aeruginosa			
Imipenem	<1to≤2	≥4 to≥8	91
Meropenem	≤2	≥4 to≥8	91
Ertapenem			
Ceftriaxone			
Ceftazidime	≤1 to≤4	≥16 to≥32	27
Cefepime	≤8	≥16 to≥32	30
Piperacillin Tazobactum	≤16/4	≥64 to ≤128/4	54
Gentamicin	≤4	≥8 to≥16	77
Amikacin	≤16	≥32 to≥64	80
Levofloxacin	≤2	≥4 to≥8	59
Acinetobacter sps			
Imipenem	≤1	>8 to>16	12%
Meropenem	≤1	>8 to>16	12%
Ertapenem			
Ceftriaxone	nil	≥32 to ≥64	0%
Ceftazidime	4	≥16 to ≥32	12
Cefepime	≤8	≥16 to ≥32	12
Piperacillin Tazobactum	≤16/4	≥64/4 to≥128/4	12
Gentamicin	≤4	≥8 to≥ 16	12
Amikacin	≤16	≥32	12
Levofloxacin	nil	≥4 to≥8	0%

Table-3: Activity of carbapenem and another seven broad spectrum antimicrobial agents tested against Enterobacteriaceae and non fermenting Gram-negative bacilli isolates collected from both the hospitals

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Isolate/ID	Specimen	Ceftriaxone	Ceftazidime	Cefepime	Gentamicin	Amikacin	Levofloxacin	Imipenem	Meropenem	Ertapenem	Piperacillin Tazobactum	bla_{NDM}	bla_{KPC}
K.pneumoniae1	Sputum	>32	>32	>32	>32	>64	>8	>8	>8	>4	≥128/4	+	_
K.pneumoniae2	ET	>32	>16	>32	>16	>32	≤2	>8	>8	>2	>128/ 4	_	_
K.pneumoniae3	ET	>32	>16	>16	>8	>32	>4	≤4	>8	>4	>64/4	_	_
K.pneumoniae4	ET	>32	>32	>32	>32	>64	>8	>8	>8	>4	≥128/4	+	_
K.pneumoniae5	ET	>32	>32	>32	>32	>64	>8	>8	>8	>4	≥128/4	+	_
K.pneumoniae6	BAL	>32	>16	>16	>8	>32	>4	≤4	>8	>4	>64/4	_	_
K.pneumoniae7	ET	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	+	_
K.pneumoniae8	ET	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	_	_
K.pneumoniae9	Sputum	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	_	_
K.pneumoniae10	ET	>32	>16	>16	>8	<16	>4	>4	>8	>4	>64/4	_	_
K.pneumoniae11	ET	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	_	_
K.pneumoniae12	Sputum	>32	>16	>16	>8	<16	>4	>4	>8	>4	>64/4	_	_
K.pneumoniae13	Sputum	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	_	_
K.pneumoniae14	Sputum	>64	>32	>64	>32	>32	>8	>8	>8	>8	>128/4	+	_
K.pneumoniae1	Sputum	>32	>32	>32	>32	>64	>8	>8	>8	>4	≥128/4	+	_
K.pneumoniae2	ET	>32	>16	>32	>16	>32	≤2	>8	>8	>2	>128/ 4	_	_
K.pneumoniae3	ET	>32	>16	>16	>8	>32	>4	≤4	>8	>4	>64/4	_	_
K.pneumoniae4	ET	>32	>32	>32	>32	>64	>8	>8	>8	>4	≥128/4	+	_
K.pneumoniae5	ET	>32	>32	>32	>32	>64	>8	>8	>8	>4	≥128/4	+	_
K.pneumoniae6	BAL	>32	>16	>16	>8	>32	>4	≤4	>8	>4	>64/4	_	_
K.pneumoniae7	ET	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	+	_
K.pneumoniae8	ET	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	_	_
K.pneumoniae9	Sputum	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	_	_
K.pneumoniae10	ET	>32	>16	>16	>8	<16	>4	>4	>8	>4	>64/4	_	_
K.pneumoniae11	ET	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	_	_
K.pneumoniae12	Sputum	>32	>16	>16	>8	<16	>4	>4	>8	>4	>64/4	_	_
K.pneumoniae13	Sputum	>32	>16	>16	>8	<16	>4	>4	8	>4	>64/4	_	_
K.pneumoniae14	Sputum	>64	>32	>64	>32	>32	>8	>8	>8	>8	**>128/4**	+	_
E.coli 1	Sputum	>32	>16	>16	>8	>32	>4	≥4	>8	>4	>64/4	_	_
E.coli 2	ET	>32	>16	>16	>8	>32	>4	>8	>8	>4	**>64/4**	+	_
E.aerogenes 1	ET	>32	>16	>16	>8	>32	>4	≤4	>8	>4	>64/4	_	_
E.aerogenes 1	ET	>32	>16	>16	>8	>32	>4	≤4	>8	>4	>64/4	_	_
E.aerogenes 1	ET	>32	>16	>16	>8	>32	>4	≤4	>8	>4	>64/4	_	_
E.cloacae1	ET	>32	>16	>16	>8	>32	>4	>8	>8	>4	>64/4	+	_
E.cloacae1	ET	>32	>16	>16	>8	>32	>4	>8	>8	>4	>64/4	_	_
E.cloacae1	ET	>32	>16	>16	>8	>32	>4	>8	>8	>4	>64/4	_	_
E.cloacae1	ET	>32	>16	>16	>8	>32	>4	>8	>8	>4	>64/4	_	_
E.cloacae1	ET	>32	>16	>16	>8	>32	>4	>8	>8	>4	>64/4	_	_

P.aeruginosa 1	ET		>32	>32	>16	>64	>8	>8		>128/4	–	–
P.aeruginosa 2	Sputum		>16	>16	>32	>32	>4	>8		>64/4	–	–
P.aeruginosa 3	ET		>32	>32	>16	>16	>8	>16		>128/4	+	–
P.aeruginosa 1	ET		>32	>32	>16	>64	>8	>8		>128/4	–	–
P.aeruginosa 2	Sputum		>16	>16	>32	>32	>4	>8		>64/4	–	–
P.aeruginosa 3	ET		>32	>32	>16	>16	>8	>16		>128/4	+	–
Acinetobacter spp 1	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	+	–
Acinetobacter spp 2	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 3	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 4	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 5	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 6	ET	>64	>32	>32	>16	>32	>8	>16		>128/4	+	–
Acinetobacter spp 7	Sputum	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 1	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	+	–
Acinetobacter spp 2	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 3	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 4	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 5	ET	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–
Acinetobacter spp 6	ET	>64	>32	>32	>16	>32	>8	>16		>128/4	+	–
Acinetobacter spp 7	Sputum	>32	>16	>16	>8	>32	>4	>8		>64/4	–	–

Table-4: MIC value of Ertapenem/Imipenem non susceptible bacterial isolates

with NDM-1 gene in Kolkata.[9] The finding was quite similar to the present work.

Our study had limitations. We did not identify the presence of other carbapenemase enzyme like OXA, VIM etc among identified carbapenem resistant isolates in our hospitals. Moreover PCR products were not confirmed by the sequencing in any case. So we cannot comment on the clonal relatedness among the identified isolates in our hospitals. But the identification of substantial percentage of presence of NDM gene in those isolates pose a significant management challenge to the clinician as well as infection control program. Routine and more elaborate surveillance and rapid laboratory protocol are needed to establish an effective control measure in infection in these clinical setting.

CONCLUSION

A total of 31% Enterobacteriaceae and other NFGNB were harbouring bla_{NDM} in our study. There was no isolate carrying bla_{KPC} in the present study. Susceptibility to other antimicrobials was decreased in carbapenemase producing isolates. Percentage susceptibility was highest in carbapenem group of antimicrobials other than Tigecycline and colistin in contrast to least coverage rate in cephalosporin group both in Enterobacteriaceae and *P. aeruginosa*. Carbapenem resistant isolates harbouring bla_{NDM} were equally distributed in LRTI patients irrespective of their co morbidities and laboratory findings (especially biomarkers for infection and Chest X -Ray) except in neurological (CVA) patients. Mean hospital stay of patients carrying isolates harbouring NDM gene were significantly longer than those who were not carrying these genes.

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