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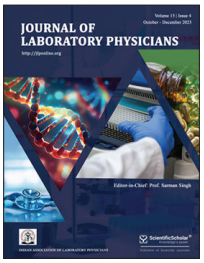

Original Article

Molecular characterization of extended-spectrum beta-lactamases and carbapenemases producing *Enterobacteriaceae* isolated from North Eastern region of India

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ABSTRACT

Objectives: This study is aimed to investigate the prevalence of genes encoding extended-spectrum β-lactamases (ESBLs) and carbapenemases production among *Enterobacteriaceae* isolated from North East India.

Materials and Methods: A total of 210 non-duplicate multi-drug resistant *Enterobacteriaceae* (MDRE) strains were included in this investigation. The isolates were resistant to third-generation cephalosporins, aminoglycosides, and fluoroquinolones. First, the strains were subjected to phenotypic assays to determine ESBLs and carbapenemases production; then, multiplex polymerase chain reaction (mPCR) assays were done to detect ESBLs and carbapenemases genes. In addition, efflux pump activity was determined by phenylalanine-arginine b-naphthylamide assay.

Statistical Analysis: The frequency of ESBLs and carbapenemase genes among MDRE strains was shown as percentages. The data analysis was done using Microsoft Excel computer software.

Results: Among 210 MDRE clinical isolates, ESBLs production was observed in 72.86% (153) isolates. During mPCR assay, gene encoding ESBLs were detected in 55.24% (116) MDRE strains beta-lactamase Temoniera (*bla*TEM) (26.67%, 56), beta-lactamase Cefotaxime-Munich (*bla*CTX-M) (19.52%, 41), and beta-lactamase sulfhydryl reagent variable (*bla*SHV) (9.05%, 19)]. In addition, 55 (26.2%) and 53 (25.26%) strains were found to be meropenem and imipenem resistant, respectively. Carbapenemase nordmann-poirel (Carba-NP) test for carbapenemases activity was found to be positive in 18.58% (39) MDRE strains. The genes encoding carbapenemases production was observed in 18.58% (39) MDRE [beta-lactamase New Delhi metallo-β-lactamases-1(*bla*NDM-1) (8.10%, 17), beta-lactamase oxacillinase-48 (*bla*OXA-48) (2.86%, 6), beta-lactamase Verona imipenemase (*bla*VIM) (1.43%, 3), and *bla*OXA-48 and *bla*VIM (6.19%, 13)]. Efflux pump activity was observed in 5 (2.3%) of Carbapenem-resistant *Enterobacteriaceae* isolates.

Conclusions: For the first time in this region, we have detected the presence of *bla*OXA-48 and *bla*VIM in a single MDRE isolate as high as 6.1%. Therefore, clinicians need to detect the ESBLs and carbapenemases producing *Enterobacteriaceae* on priority in hospital settings for therapeutic options as well as stringent infection control strategies to be adopted as precautions.

Keywords: *Enterobacteriaceae*, Extended-spectrum β-lactamases, Carbapenemases, Multiplex polymerase chain reaction

INTRODUCTION

The increasing rate of antimicrobial resistance (AMR) has become a severe threat to public health globally. The emergence of extended-spectrum β-lactamases (ESBLs) producing *Enterobacteriaceae* (ESBL-E) and carbapenemase-producing *Enterobacteriaceae* (CPE) is responsible for higher morbidity and mortality rates worldwide.[1] The World Health Organization included ESBL-E and CPE in the prioritized pathogen group.[2] ESBLs are plasmid mediated and capable of hydrolyzing the amide bond of four membered β-lactamase ring, inactivates the wide variety of beta-lactams including penicillins, cephalosporins, and monobactams except for cephamycins and carbapenems but are inhibited by clavulanic acid.[3] ESBLs are mostly effective against third-generation cephalosporins,including ceftazidime, ceftriaxone, and cefotaxime. Temoniera (TEM), sulfhydryl reagent variable (SHV) and Cefotaxime-Munich (CFX-M) are the most common families among ESBLs and are associated with the nosocomial infections outbreak.[4] Several studies have also reported that the genes encoding ESBLs were frequently located on large plasmids, which also carry genes mediating resistance to aminoglycosides and fluoroquinolones.[5] The increasing rate of ESBL-E strains were treated and controlled using carbapenem antibiotics,including meropenem, imipenem, ertapenem, and doripenem.[6] However, among *Enterobacteriaceae*, the emergence of an increasing rate of carbapenem resistance in clinical settings is worrisome, and it is alarming.

Carbapenems are β-lactam antibiotics that have the potential to inhibit transpeptidases enzymes (penicillin-binding proteins), prevent peptidoglycan synthesis, and cause lytic cell death.[6] Carbapenem resistance is mediated by the production of various carbapenemases enzymes. Carbapenemases are β-lactamases enzymes differentiated into three major classes according to Ambler classification (A, B, and D) based on the hydrolytic mechanisms at their active sites. Carbapenemases hydrolyses β-lactam antibiotics,including penicillins, cephalosporins, monobactams, and carbapenems. Class A carbapenemases include *Klebsiella pneumoniae* carbapenemase (KPC), which could be inhibited by clavulanic acid. The Metallo-β-lactamases are *bla*NDM (New Delhi metallo-β-lactamases), *bla*IMP (imipenemase), and *bla*VIM(Verona imipenemase) belonging to class B. The class D carbapenemases are referred as Oxacillinase (OXA)- type carbapenemases.[7] So far, the therapeutic options for Carbapenem-resistant *Enterobacteriacea*e (CRE) infections remain challenging and are very limited. However, recent studies have reported that novel β-lactamase inhibitor combinations may play an important role in providing new treatment options against CRE infections.[6] Hence, it is important for clinicians and researchers to screen and highlight the carbapenemase-producing bacteria in clinical settings. Although there are fewer studies on CRE in the North Eastern region of India, Tripura, Nagaland, Meghalaya, and Mizoram have reported <5% of carbapenem resistance prevalence due to poor development in healthcare.[8] Hence, it is important to clinicians in various regions of North East India for earlier detection of ESBLs and carbapenemases-production in *Enterobacteriaceae* clinical strains to decide or choose appropriate therapeutic options. Based on this knowledge and background, we aimed to highlight the emergence of ESBLs and carbapenemases production in multidrug-resistant *Enterobacteriaceae* (MDRE) strains isolated from patients hospitalized in a tertiary care hospital in the North Eastern region of India.

MATERIALS AND METHODS

A total of 210 different MDRE isolates were collected from patients in outpatient departments, different wards, and intensive care units of a tertiary care hospital in Gangtok, Sikkim, for a period from January 2019 to December 2021. This study was reviewed and approved by the Institutional Ethics Committee (IEC), SMIMS (SMIMS/IEC/2018–033). The MDRE strains were found to be resistant to the antibiotics including Ceftriaxone (30 μg), Ciprofloxacin (5 μg), Gentamicin (10 μg), and Amikacin (30 μg). *Escherichia coli (E. coli)* American type culture collection (ATCC) 25922 and *Klebsiella pneumoniae (K. pneumoniae)* ATCC 700603 were used as control strains. In addition, out of the total of 210 MDRE, 53 (25.26%), and 55 (26.2%) strains were found to be Imipenem (10 μg) and Meropenem (10 μg) resistant, respectively.[9] The antimicrobial susceptibility testing assays were carried out by adopting Clinical and Laboratory Standards Institute (CLSI) the described Kirby Bauer disk diffusion method.

Disc diffusion test for ESBLs

The double disk diffusion method was carried out in Mueller–Hinton agar with inoculum (0.5 McFarland), to determine the ESBLs production among the MDRE stains using ceftazidime (30 μg) and ceftazidime-clavulanic acid (30 μg/10 μg) disks, respectively. The ESBLs production was interpreted by zone diameter of ≥5 mm in ceftazidime-clavulanic acid than ceftazidime disc alone.[10]

Carba-NP (CNP) test

The CNP test was done to determine the production of carbapenemases among the MDRE strains. The inoculum of test strains was prepared by growing the test strains in 500 μL peptone water (pH 7) for 2 h. The inoculum was subjected to CNP test,according to the Nordmann *et al*.[11]

Deoxyribonucleic acid (DNA) extraction

All the study isolates (MDRE, $n = 210$) were subjected to DNA extraction using the boiling lysis method.[12] Briefly, a loop full of freshly subcultured MDRE was emulsified in nuclease-free

water (NFW), 200 µL,and centrifuged at 10,000 rpm (10 min). The washing step was carried out two times accordingly at 10,000 rpm (10 min). The pellet was then re-suspended in NFW (200 µL) and incubated at 100°C for five minutes in the water bath. After boiling, the total reaction mixture was removed from the water bath, cooled on ice immediately for five minutes, and centrifuged at 10000 rpm (10 min). The supernatant was collected and used as a DNA template for multiplex polymerase chain reaction (mPCR) assays.

Phenotypic testing of efflux pump activity

Phenotypic testing was performed to determine the presence of efflux pump-mediated carbapenem resistance in MDRE. The test procedure was carried out as per the study by Ellappan *et al.*[7] We excluded MDRE isolates for efflux pump activity assays, which were found to be harboring with carbapenemase genes. The microbroth dilution method was used for efflux pump activity with Meropenem alone (32 µg/mL) and in combination with the efflux inhibitor phenylalanine-arginine b-naphthylamide (PAbN) at 50 µg/mL. The enhanced expression of efflux pump activity was determined if the addition of PAbN caused a four-fold decrease in minimum inhibitory concentration (MIC). *E. coli* ATCC 25922 *and K. pneumoniae* ATCC 700603 were used as control strains.

Molecular detection for carbapenemase and ESBL genes

The mPCR assay was carried out to detect the presence of ESBLs encoding genes [beta-lactamase Temoniera (*bla*TEM), beta-lactamase sulfhydryl reagent variable (*bla*SHV) and beta-lactamase Cefotaxime-Munich (*bla*CTX-M)] in MDRE strains that were found to be phenotypically positive for ESBLs, and also for detection of carbapenemase genes [beta-lactamaseVerona imipenemase [beta-lactamase Verona imipenemase (*bla*VIM), beta-lactamase New Delhi metallo-β-lactamases-1 (*bla*-NDM-1), beta-lactamase imipenemase (*bla*-IMP), beta-lactamase Klebsiella pneumoniae carbapenemase (*bla*KPC), beta-lactamase oxacillinase-48 (*bla*OXA-48)].[12] in CRE strains. The primers used for the detection of ESBLs and carbapenemases genes as well as the mPCR parameters are shown in Tables 1 and 2, respectively.

Statistical analysis

The frequency of ESBLs and carbapenemase genes among MDRE strains was exhibited as percentages. The data analysis was done using Microsoft Excel computer software.

RESULTS

A total of 210 MDRE strains were included in this study. All the study isolates were found to be resistant to third-generation cephalosporins, aminoglycosides, and fluoroquinolones. Of the total of 210 MDRE, 39 (18.58%) strains were found to be carbapenemase producer and 153 (72.86%) strains were producing ESBLs phenotypically [Table 3]. Most of the strains were collected from urine (90, 42.86%), followed by sputum (29, 13.81%), pus (13, 6.19%), endotracheal aspirate (12, 5.71%), and blood (9, 4.29%).

ESBLs production was found to be predominant in *E. coli* (96, 45.71%), followed by *K. pneumoniae* (45, 21.43%), *Morganella morganii* (5, 2.38%), *Enterobacter cloacae* (3, 1.43%), *Serratia marcescens* (3, 1.43%), and *Providencia rettgeri* (1, 0.48%). mPCR assays revealed that genes encoding ESBLs were detected in 55.24% (116) of MDRE strains, highlighting that *bla*TEM (26.67%, 56) was predominant, followed by *bla*CTX-M (19.52%, 41) and *bla*SHV (9.05%, 19) [Table 4]. Most of the ESBLs encoding genes were observed in *E. coli* (*bla*TEM, 32 (15.24%), *bla*CTX-M (21, 10%), and *bla*SHV (12, 5.71%)) and *K. pneumoniae* (*bla*TEM, 22 (10.48%), *bla*CTX-M (20, 9.52%) and *bla*SHV (7, 3.3%)). Of the total of 55 CRE, 39 (18.58%) strains were found to be

Table 1: Multiplex primers used in this study: ESBL (*bla*TEM, *bla*CTX-M, and *bla*SHV) and carbapenemases genes (*bla*VIM, *bla*IMP, *bla*KPC, *bla*NDM-1, and *bla*OXA-48).

Genes	Primers (5'–3')	Reverse (5'–3')	Size (bp)
*bla*TEM	CATTTCCGTGTCGCCCTTATT	CGTTCATCCATAGTTGCCTGAC	800
*bla*CTX-M	TTAGGAAATGTGCCGCTGTA	CGATATCGTTGGTGGTACCAT	878
*bla*SHV	AGCCGCTTGAGCAAATTAAA	ATCCCGCAGATAAATCACCAC	713
*bla*VIM	GTTTGGTCGCATATCGCAAC	AATGCGCAGCACCAGGATAG	390
*bla*IMP	GGAATAGAGTGGCTTAAYTCTC	GGTTTAAYAAAACAACCACC	232
*bla*KPC	TGTCACTGTATCGCCCGTC	CTGAGTGCTCTACAGAAAACC	1011
*bla*NDM-1	CACCTCATGTTTGAATTCGCC	CTCTGTCACATCGAAATCGC	984
*bla*OXA-48	TATATTGCATTAAGCAAGGG	CACACAAATACGCGCTAACC	800

ESBL: Extended-spectrum β-lactamase, *bla*TEM: beta-lactamase Temoniera, *bla*CTX-M: beta-lactamase Cefotaxime-Munich, *bla*SHV: beta-lactamase sulfhydryl reagent variable, *bla*VIM: beta-lactamase Verona imipenemase, *bIa*IMP: beta-lactamase imipenemase, *bla*KPC: beta-lactamase Klebsiella pneumoniae carbapenemase, *bla*-NDM-1: beta-lactamase New Delhi metallo-β-lactamases-1, *bla*OXA-48: beta-lactamase oxacillinase-48.

Table 2: PCR parameters used in amplifying the ESBL and carbapenemases genes.

β-lactamases genes	Parameters
ESBL genes (*bla*TEM, *bla*CTX-M and *bla*SHV)	• Initial denaturation at 94°C for 10 min. • 30 cycles of: - denaturation at 94°C for 40 s, - annealing at 60°C for 40 s - elongation at 72°C for 1 min • Final elongation at 72°C for 7 min and hold at 4°C
Carbapenemases genes (*bla*VIM, *bla*IMP, *bla*KPC, *bla*NDM-1 and *bla*OXA-48)	• Initial denaturation at 95°C for 10 min. • 30 cycles of: - denaturation at 94°C for 1 min - annealing at 59°C for 30 s - elongation at 72°C for 2 min • Final elongation at 72°C for 10 min and hold at 4°C

PCR: Polymerase chain reaction, ESBL: Extended-spectrum β-lactamase, *bla*TEM: beta-lactamase Temoniera, *bla*CTX-M: beta-lactamase Cefotaxime-Munich, blaSHV: beta-lactamase sulfhydryl reagent variable, *bla*VIM: beta-lactamase Verona imipenemase, *bIa*IMP: beta-lactamase imipenemase, *bla*KPC: beta-lactamase Klebsiella pneumoniae carbapenemase, *bla*-NDM-1: beta-lactamase New Delhi metallo-β-lactamases-1, *bla*OXA-48: beta-lactamase oxacillinase-48.

Table 3: Phenotypic identification of carbapenemases and ESBLs production in *Enterobacteriaceae*.

Organisms	*n*	%
Carba-NP		
E. coli	17	8.1
K. pneumoniae	22	10.48
ESBLs		
E. coli	96	45.71
K. pneumoniae	45	21.43
M. morganii	5	2.38
E. cloacae	3	1.43
S. marcescens	3	1.43
Pr. rettgeri	1	0.48

E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, ESBLs: Extended-spectrum β-lactamases, *M. morganii: Morganella morganii, E. cloacae: Enterobacter cloacae, S. marcescens: Serratia marcescens, P. rettgeri: Providencia rettgeri*

producing carbapenemases phenotypically. Among 55 CRE, *K. pneumoniae* (27, 12.86%) was predominant followed by *E. coli* (21, 10%), *E. cloacae* (2, 0.95%), *Serratia marcescens (S. marcescens)* (2, 0.95%), *Providencia rettgeri (P. rettgeri)* (1, 0.48%), *Morganella morganii (M. morganii)* (1, 0.48%), *Proteus vulgaris* (1, 0.48%), and *Shigella sonnei*. (1, 0.48%) [Table 5]. Among 39 CRE, *bla*NDM-1 was observed in 17 (8.10%) strains, *bla*OXA-48 was observed in 6 (2.86%), and *bla*VIM was observed in 3 (1.43%). Furthermore, 13 (6.19%) CRE strains were found to be co-occurrence of both *bla*OXA-48 and *bla*VIM [Table 4]. We observed that most of the carbapenemases producing strains were

Table 4: Genotypic analysis of ESBLs and Carbapenemases in multi drug resistant *Enterobacteriaceae* strains.

Genes	*n*	%
ESBLs		
TEM	56	26.67
CTX-M	41	19.52
SHV	19	9.05
Carbapenemases		
NDM-1	17	8.10
OXA-48	6	2.86
VIM	3	1.43
OXA+VIM	13	6.19

*bla*TEM: beta-lactamase Temoniera, *bla*CTX-M: beta-lactamase Cefotaxime-Munich, *bla*SHV: beta-lactamase sulfhydryl reagent variable, *bla*-NDM-1: beta-lactamase New Delhi metallo-β-lactamases-1, *bla*OXA-48: beta-lactamase oxacillinase-48, *bla*VIM: beta-lactamase Verona imipenemase.

Table 5: Resistance pattern of imipenem and meropenem against *Enterobacteriaceae*.

Organisms	Meropenem		Imipenem	
	n	%	*n*	%
Klebsiella pneumoniae	26	12.38	27	12.86
Escherichia coli	21	10.00	20	9.52
Enterobacter cloacae	2	0.95	1	0.48
Serratia marcescens	2	0.95	1	0.48
Providencia rettgeri	1	0.48	1	0.48
Morganella morganii	1	0.48	1	0.48
Proteus vulgaris	1	0.48	1	0.48
Shigella sonnei	1	0.48	1	0.48

found to be *K. pneumoniae* (22, 10.4%) followed by *E. coli* (17, 8.0%). Carbapenemases production was not observed in other *Enterobacteriaceae* species. In addition, efflux pump activity was observed in 5 (2.3%) of CRE isolates *K. pneumoniae* (3, 1.4%) and *E. coli* (2, 0.9%).

DISCUSSION

Enterobacteriaceae infections, with the occurrence of multidrug resistance mechanisms, have become an emerging threat in hospital settings. ESBLs and CPE have become a very serious problem, and remain a challenge for diagnostics and therapeutic management. In this investigation, we described the prevalence of ESBLs and carbapenemases production in MDRE strains isolated from North Eastern region of India by phenotypic and genotypic methods. The present study highlighted that 72.86% and 18.58% of MDRE isolates were found to be producing ESBLs and carbapenemases, respectively. This displays an alarming and worrisome scenario. One possible reason could be the upsurge in the pharmaceutical industries in this region, especially in Sikkim, which could have contributed to a higher rate of antibiotic resistance due to the amount of waste reaching the various

waterways that may indirectly act as a continuous source of AMR.[9,13] Other associated reasons could be the increasing rate of diseases, inadequate hospitals or healthcare centers, lack of appropriate diagnostic methods, poor infection control practices, and the affinity of clinicians with the empirical treatment practices may have further supported the global crisis of AMR.[14] The emergence of ESBLs production in *Enterobacteriaceae* has become a serious concern worldwide,including India, China, Pakistan, Korea, and Japan.[15] In India, the prevalence rate of ESBLs ranges from 60 to 80%[16],and in our study, the magnitude of ESBLs producing MDRE was found to be 72.86%. We also highlighted that the majority of ESBLs producing strains were *E. coli* (96, 45.71%) followed by *K. pneumoniae* (45, 21.43%). Similar to our study, various reports from several parts of India highlighted the predominance of ESBLs production in *E. coli* and *K. pneumoniae*. In India, a multi-centric study reported that ESBLs production was seen in 33% in *E. coli* and 42% in *K. pneumoniae*.[17] The incidence of ESBLs fluctuates extensively between geographical locations. Low occurrence rates have been reported in USA, Europe, and North America,[18,19] whereas high rates are generally detected in Asian countries, South America,[20] and some African countries.[21] Compared with our investigation, the incidence of ESBLs producing *Enterobacteriaceae* in Europe is lower; 0.7% in Austria, 23.8% in Turkey,[22] and 6.3% in Italy.[23] The difference might be due to infection control strategies in those countries. Other possible reasons which can attribute to the higher incidence of ESBLs and their spread could be non-prescription antimicrobial use, self-medication, poor hygiene, high burden of infectious diseases, consumption of counterfeit drugs, and lack of proper implementation of AMR detection systems.[24-26] Therefore, it further confirms the spread of such bacteria in this region. In this regard, a conceivable account for such a high magnitude of ESBLs incidence could be due to the selective pressure produced by the significant use of β-lactam antibiotics in this region, where they are commonly anticipated as the first line of therapy for bacterial infections caused by *Enterobacteriaceae*.[27] Infectious Diseases Society of America has listed *E. coli* and *K. pneumoniae* among six pathogens in urgent need of new drugs to combat the development of drug resistance.[28] A study from Ethiopia reported the prominence of *E. coli* 228 (53.5%) and *K. pneumoniae* 103 (24.1%) among ESBLs producing MDRE.[29] Mahamat *et al*. also reported that most *E. coli* (63.8%) were found to be ESBL-producers followed by *K. pneumoniae* (21.2%).[27] In addition, our results highlighted that the ESBLs producing MDRE strains were mainly associated with the occurrence of *bla*TEM (26.67%, 56), followed by *bla*CTX-M (19.52%, 41) and *bla*SHV (9.05%, 19). Similarly, Verma *et al*. highlighted that *bla*TEM was most predominant in both *E. coli* and *K. pneumoniae,* followed by *bla*CTX-M and *bla*SHV.[30] However, a previous study from North East India reported a higher prevalence of *bla*SHV (63.4%), followed by *bla*CTX-M (60.86%) and *bla*TEM (54.3%).[31] The spread of mobile genetic elements, mostly conjugative plasmids belonging to classic incompatibility groups, and the diffusion of specific clones have been accountable for the upsurge in ESBL-producing isolates and for the spread of TEM, SHV, and CTX-M, in particular.[32] CTX-M is being considered endemic in various countries and is swiftly disseminating among different *Enterobacteriaceae* species.[27] India, being a densely populated country with meager sanitation and drinking water glitches, denotes the leading reservoir of CTX-M ESBL genes alongside China.[31] In our study, it was highlighted that most of the ESBLs strains were isolated from urine samples. Similar to this, a study by Shashwati *et al*. from North India observed that the majority of ESBL-producing strains were obtained from urine samples. In addition, they also revealed that *bla*TEM gene was predominantly observed in *E. coli* isolates.[33] Ravikant *et al*. observed that *bla*SHV (63.04%) is predominant in ESBLs producing *E. coli* followed by *bla*TEM (60.86%) and *bla*CTX-M (54.34%).[31] Urinary tract infection (UTI) is considered as the most recurrent bacterial infection throughout the world in patients with nosocomial and community-acquired infections,and *Enterobacteriaceae* (primarily *E. coli* and *K. pneumoniae*) are commonly the causative agent.[9,27] In this context, it is pertinent to state that *E. coli* represents the leading pathogen isolated from urine specimen worldwide that is responsible for UTI infection.[31] The dissemination of ESBLs compromises the potential of broad-spectrum antibiotics resulting in main therapeutic complications with a substantial impact on the outcomes for patients.[34] Carbapenem has become the last resort and first-line empirical treatment against ESBLs-producing MDRE. The increasing usage of carbapenem against any known or unknown case of hospital infections leads to the emergence of CRE. The emergence and rapid spread of CRE has become a serious threat in hospital settings. This is worrisome and alarming. In the present study, we highlighted that 18.58% of MDRE was found to be carbapenem-resistance. Among 18.58% of CRE, *K. pneumoniae* was predominant, followed by *E. coli, E. cloacae*, *S. marcescens*, *P. rettgeri*, *M. morganii, P. vulgaris,* and *S. sonnei*. The emergence of novel β-lactamases with direct carbapenem-hydrolyzing activity has contributed to an upsurge in the incidence of CRE. Thus, CRE becomes problematic given the rate with which *Enterobacteriaceae* cause infections, the high mortality linked with infections caused by CRE, and the potential for extensive spread of carbapenem resistance through mobile genetic elements.[35] In 2017 and 2018, Modi *et al*. reported that 34.74% and 29.34% of *Enterobacteriaceae* strains were found to be carbapenem-resistant, respectively,[36] which is a much higher percentage compared to our study. *Enterobacteriaceae* being the popular cause of health care as well as community infections, raises the likelihood of transmission of CRE into the community. These factors, combined with the finite therapeutic options available to treat patients infected with these bacteria, have made CRE troublesome.[35] A study from North Eastern India by Ralte *et al*. reported that about 11.3% of Gram-negative strains were found to be carbapenem-resistant in which *K. pneumoniae* and *E. coli* were found to be predominant.[8] Another study from North Eastern India by Chellapandi *et al*. also revealed the occurrence of 5% of carbapenem resistance, especially

in the *Enterobacteriaceae*.[37] In our study, it is worth noting that several drug resistance mechanisms have been incorporated in the CRE, including carbapenemases production and efflux pump activity. Our study revealed that *bla*NDM-1 and *bla*OXA-48 were mostly detected in CRE strains in our hospital environment and are spreading rapidly, with less occurrence of *blaVIM*. Several studies from India also reported that *bla*NDM and *bla*OXA-48 were the most common carbapenemase genes reported in several states of India. A study by Sharma *et al*. revealed that about 48% and 19% of *E. coli* strains were found to be harboring with *bla*NDM and *bla*OXA-48, respectively. In addition, 27% and 36% of *K. pneumoniae* strains were found to be harboring *bla*NDM and *bla*OXA-48, respectively.[38] A study from Mohanty *et al*. reported that *bla*NDM (65.6%) and *bla*OXA-48 (24.7%) were observed among 93 (24.03%) CRE isolates.[39] In contrast, a study from China by Han *et al*. observed that *bla*KPC-2 (51.6%) producing *Enterobacteriaceae* strains were mostly isolated, followed by *bla*NDM (35.7%) and *bla*OXA-48 (7.3%).[40] Hence, increasing spread of *bla*NDM and *bla*OXA-48 like carbapenemases in MDRE is worrisome as they are progressively highlighted with nosocomial infection outbreaks across the world. The epidemiological spreading of carbapenemase producers fluctuates enormously. In Asian subcontinents, *bla*OXA-48-like and *bla*NDM-types are more common, while *bla*KPC and other *bla*OXA-types are recorded quite often in the European countries.[8] In India, the incidence of carbapenem-resistant bacterial infections is widespread, especially in the Southern and Northern regions, where the population density is high.[12] The finding of the present study, wherein the occurrence of 18.58% CRE previously unknown in Sikkim, exemplifies the emergence of CRE in this region that forms an indicator of disseminating resistance to the northeastern parts of India, where the population is not dense. Overuse of antibiotics in healthcare settings leads to selection pressure for resistant strains, whereas lack of early detection of infection with CRE and poor infection control practices promote its spread. Infections with CRE are tough to treat and the limited treatment options as well as the costliness, impose extra financial burden on the patients. Under these circumstances, it becomes important to curb the rise of CRE by formulating and implementing antimicrobial policy that is based on the local antibiogram as well as ensure adherence to infection control practices. In addition to carbapenemases production, we also observed that about 5 (2.3%) of CRE isolates (*K. pneumonia* (3, 1.4%) and *E. coli* (2, 0.9%) were found to be exhibiting efflux pump activity. Several studies from India have reported that the efflux pump inhibition has played an important role in decreasing of carbapenems MIC to *K. pneumonia* and *E. coli*.[41]

Limitation of the study

Our study has some limitations. The study was conducted in the Microbiology Laboratory of Central Referral Hospital, Gangtok, Sikkim; hence, the results may not be generalized to the entire state or the North Eastern Region of India. We are unable to see the possible risk factors, clinical features,and the outcome of the patients infected with ESBLs and CPE due to lack of adequate resources.

CONCLUSIONS

Our results highlighted the higher prevalence and rapid emergence of ESBLs and carbapenemases production in clinical MDRE strains isolated from the North Eastern region. The high MDRE rate detected in such a small populated and remote region highlights a peak of danger in bigger populated cities of India. This situation is worrisome. Our study also highlighted the increasing spread of NDM and OXA-48 with co-occurrence of VIM and efflux pump activity among clinical isolates in hospital environment, which remains a challenge for clinicians to choose appropriate treatment against MDRE infections. There are very limited cases of prevalence studies about drug resistance investigated in the North Eastern part of India, which is still underdeveloped in healthcare sectors compared to other parts of India. This finding further reiterates the imperative need for rational use of antibiotics in hospital settings and to develop empirical treatment strategies. In this regard, the present study may contribute to the development of new strategies to control the spread of ESBLs and carbapenemases production in India, including the North Eastern region.

Author contributions

Salvia, T: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing the original draft, Writing review; Laishram Shantikumar S. Conceptualization, design, data acquisition, data analysis and interpretation, writing the original draft, review, revising the manuscript, gave the final approval for publishing; Khati, R: Data acquisition and analysis, drafting, editing, Methodology; Ellappan, K: Data curation, Methodology, drafting, editing, interpretation, review writing; Dolma, K.G: Design and conceptualization, overall supervision, data curation, gave the final approval for publishing; Dhakal O.P.: Contributed to data analysis and interpretation, editing.

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Ethical approval

Approved by the Institutional Ethics Committee at SMIMS, number SMIMS/IEC/ 2018–033, dated -26 May 2018.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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