Research Article

Report on carbapenemase-producing rare sequence types of *Escherichia coli* and *Enterobacter hormaechei*

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ABSTRACT

Introduction and Aim: Carbapenem Resistant *Enterobacteriaceae* (CRE) have emerged at an alarming rate. Multi locus sequence typing (MLST) is an important parameter for identifying drug resistant organisms. The present study was carried out for elucidating the mechanisms of CRE and MLSTs associated with CRE.

Materials and Methods: CRE (n=14) were obtained from various clinical samples and subjected to Rapidec Carba NP (CNP) test and multiplex polymerase chain reaction (M-PCR) and five isolates proceeded for whole genome sequencing (WGS). β-lactamase (*bla*) genes were analysed using Resfinder and CARD tool. Bioinformatics tools: mlplasmids, plasmid finder, mobile element finder, and Center for Genomic Epidemiology (CGE) toolbox were used.

Results: All isolates (n=14) were positive for CNP and *bla* genes using M-PCR. Isolates (J21, J22, J23, J27) were identified as *Escherichia coli* while (J34) was *Enterobacter hormaechei.* MLST showed *E. coli* isolates (J21& J22) as ST648; *E. coli* (J23) was ST940; *E. coli* (J27) was ST 2851, and *E. hormaechei* (J34) was closest to ST1325. Genes bla_{TEM}, bla_{NDM} & bla_{ampC} were found to be present in all isolates; bla_{CTX-M} was present in all *E. coli* isolates but not in *E. hormaechei*. bla_{OXA} was present in *E. coli* (J23) and in *E. hormaechei* (J34); while ESBL bla_{SFO-1} in *E. hormaechei* (J34).

Conclusion: ESBLs (bla_{TEM} & bla_{CTXM}) and metallo beta-lactamase -MBL (bla_{NDM}) cause carbapenem resistance in rare sequence types of *E. coli* while; ESBL (bla_{SFO-1}) and MBL (bla_{NDM}) cause carbapenem resistance in *E. hormaechei*.

Keywords: β-lactamases; multi drug resistance; *Enterobacteriaceae;* MLST; whole genome sequencing.

INTRODUCTION

Enterobacteriaceae are a large family of Gram-negative bacteria commonly present as normal commensal flora in the gastrointestinal (GI) tract of humans. There is a growing trend of resistance seen to commonly used antibiotics amongst these isolates (1). Carbapenems are last resort agents used to treat infections caused by organisms that are resistant to other classes of antibiotics. However, Carbapenem Resistant *Enterobacteriaceae* (CRE) are emerging at an alarming rate and pose a significant global threat (2). Infections commonly associated with CRE are due to *Klebsiella* spp., *Escherichia* spp., and *Enterobacter* spp(3). *Enterobacter cloacae* complex is considered an emerging pathogen responsible for causing the second most common nosocomial infections; while *Escherichia* spp. is reported to spread in the environment (drinking water, soil, poultry farms, etc)(4). Resistance to carbapenems occurs because of various mechanisms. These include hyperproduction of AmpC type or extended-spectrum β-lactamases (ESBLs) and carbapenemases coupled with outer membrane porin loss, hyperproduction of efflux pumps, and decreased affinity of penicillin-binding proteins. Carbapenem resistance due to the acquisition of carbapenemases is widely reported. Three types of carbapenemases are commonly identified in the *Enterobacteriaceae* family. These are mainly; Ambler class A – *Klebsiella pneumoniae* carbapenemase (KPC), class β-Metallo-beta-lactamase (MBL), and class D-Oxacillinase (OXA) types (1). Originally, carbapenem resistance was found in *K. pneumoniae* isolates harboring the KPC, however, clinical isolates of *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, etc. have been identified to be carbapenem-resistant due to the presence of KPC, MBLs, and OXA(3). The emergence of carbapenem resistance among *Enterobacteriaceae* isolates due to carbapenemases is particularly worrisome because this is plasmid-mediated. These bacteria are a leading cause of

hospital-associated infections as they easily disseminate among indoor patients (2).

There are few reports on the prevalence of CRE, their mechanisms of resistance, and antimicrobial susceptibility pattern from the Western part of India. The present study was carried out to do a complete phenotypic and genotypic analysis of CRE strains from a tertiary level care institute in Western India. Further, five CRE isolates were randomly selected for whole genome sequencing to find the β-lactamase (*bla*) genes, plasmids, MLST type, and variation among the genes.

MATERIALS AND METHODS

Sample collection, identification, and antibiotic susceptibility testing

This was a prospective observational multicentric study conducted over three months (-October to December'2018. All clinical samples received from outpatients and hospitalized patients during the study period were processed in the department of microbiology. The samples included pus, urine, stool, bronchoalveolar fluid (BAL), wound swab, blood, sputum, endotracheal aspirate (ET), etc. Standard microbiological guidelines were followed for bacterial identification and antimicrobial susceptibility testing (5). All isolates were identified using biochemical tests and tentatively grouped in the *Enterobacteriaceae* family. The antibiotics tested included Amikacin (30µg), Cefoperazone-sulbactam (75/30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg), Cefepime (30µg), Meropenem (10µg) by disc diffusion method. E test (AB Biodisk, Solna, Sweden) was used for determining colistin and tigecycline susceptibility. A total of 14 nonduplicate carbapenem-resistant (meropenem zone size <23 mm) clinical isolates of the *Enterobacteriaceae* family were included in the study and subjected to Rapidec CARBA NP (CNP) (bioMerieux, France) and Multiplex PCR (M-PCR). CNP is based on direct detection of carbapenem hydrolysis by carbapenemase-producing bacteria and was done as per the manufacturer's instructions (6).

Detection of beta-lactamases using multiplex PCR (M-PCR)

Genomic DNA isolation was done using the standard protocol. M-PCR was performed for detection of β-lactamase genes in carbapenemase positive isolates using three PCR reactions and conditions mentioned by Poirel *et al*(7). Beta-lactamase genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{AIM}, bla_{DIM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{BIC}, and bla_{OXA-48}) were amplified using primers listed in **Table 1**. Amplified products were visualized using the DNA agarose gel electrophoresis. PCR amplified products from three random isolates were subjected to Sanger sequencing for bla_{NDM} and bla_{OXA-48}.

Whole genome sequencing (WGS) and bioinformatics analysis

Genomic DNA extraction and library preparation: Genomic DNA for randomly selected five isolates (J21, J22, J23, J27, and J34) was extracted using XpressDNA Bacterial kit (MagGenome), and library preparation was done by using Ion Xpress™ Plus gDNA Fragment Library Preparation Kit (ThermoFisher Scientific) as per given instructions in manual. Loading of genomic libraries was done onto the Ion 530Tm chip and using the Ion S5TM system (Iontorrent, Thermo Fisher Scientific) sequencing was done by following the standard protocols.

Bioinformatics analysis for whole genome sequence

Quality of each raw file generated after sequencing was checked by using FastQC (v. 0.11.5) tool. Ambiguous bases and low-quality reads were deflected using the FASTX toolkit (available at: http://hannonlab.cshl.edu/ fastx_toolkit/). SPAdes (v. 3.11.1) tool was used for de novo assembly of high-quality reads. Quality assessment for the assembled sequence was done using QUAST (v. 5.0.2) tool. Prokka (v. 1.13.30 available at: https://github.com/tseemann/prokka) tool was used for the gene annotation. Multilocus sequence typing (MLST) for each isolate based on seven housekeeping genes were determined using MLST (v. 2.0.4 available at: https://cge.cbs.dtu.dk/ services/MLST/)) of Centre for Genomic Epidemiology (CGE) toolbox.

Screening of β-lactamase genes and translated sequence from each draft genome was performed using Resfinder (v. 4.0 available at: https://cge.cbs.dtu.dk/services/ResFinder/) of CGE toolbox along with Geneious prime (v. 2020.2 created by Biomatters, available from https://www.geneious.com). The location of the β-lactamase gene in the genome was detected using mlplasmids tool (v. 1.0.0 available at: https://sarredondo.shinyapps.io/mlplasmids/).

Screening of plasmid was done using plasmid Finder (v.2.0.1available at: https://cge.cbs.dtu.dk/ services/ PlasmidFinder/) of CGE toolbox. Mobile Element finder (v. 1.0.3available at: https://cge.cbs.dtu.dk/ services/MobileElementFinder/) of CGE toolbox was used for finding mobile genetic elements and their association with antimicrobial resistance genes. iTOL was used to represent the beta-lactamase genes and plasmids of our isolates (n=5). Phylogenetic trees built previously were used as input files to generate figures using iTOL for comparative analysis. iTOL

v3 (https://itol.embl.de/), is an online tool to display genomic features of the genomes.

RESULTS

Antimicrobial susceptibility, carbapenem resistance, and multiplex PCR

A total of 1240 samples received in the Department of Microbiology during the study period were processed and 58 *Enterobacteriaceae* isolates were obtained. When subjected to antibiotic susceptibility testing, 14/58 isolates (24%) were found to be meropenem resistant by the disc diffusion method. The susceptibility profile to various antibiotics is shown in **Table 2**. All isolates (n=14) tested positive for carbapenemase using the CNP test. Gel electrophoresis of PCR amplified products is shown in **Figure 1**. Gel purified amplified products for all genes were sent for Sanger sequencing; however, the sequences only for bla_{NDM} could be confirmed. All isolates were found to harbor one or more than one resistance gene as shown in Table 3.

Table 2: Antimicrobial susceptibility profile of carbapenem resistant *Enterobacteriaceae* isolates

S.No.	Name of Antibiotics	Number of resistant isolates (n=14)	Percentage of resistant isolates (n=14)
1	Amikacin	9/14	64
2	Cefoperazone-sulbactam	13/14	93
3	Ceftazidime	14/14	100.00
4	Ceftriaxone	14/14	100.00
5	Ciprofloxacin	14/14	100.00
6	Colistin	0/14	0.00
7	Cotrimoxazole	12/14	86
8	Cefepime	10/14	71
9	Meropenem	14/14	100.00
10	Tigecycline	0/14	0.00

Evaluation of beta-lactamase genes using whole genome sequencing

Five isolates (J21, J22, J23, J27, and J34) were randomly selected for whole genome sequencing (WGS) and the WGS summary for these isolates (J21, J22, J23, J27, and J34) along with genomic data are presented in Table 3. From the respective16srDNA sequence and MLST of the isolates, they were identified as *E. coli* (n=4; J21, J22, J23, J27) while; J34 was identified to be *E. hormaechei*. To our knowledge, this is the first isolate of *E. hormaechei* from India to be the whole genome sequenced. Quality assessment of draft genome was done using Quast tool and number of contigs, N50 value (defined as the sequence length of the shortest contig at 50% of the total genome length), and percentage (%) of GC-content for each isolate were defined. Multilocus sequence typing (MLST) characterization was done based on seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA, recA*) for *E. coli* isolates and ST types - ST648, ST940 & ST2851 were identified for J21& J22, J23, and J27 respectively. The housekeeping genes of *Enterobacter cloacae* (*dnaA, fusA, gyrB, leuS, pyrG, rplB, rpoB*) were used for determining the MLST of *E. hormaechei* (J34) as MLST of *E. hormaechei* was not available on pubMLST and CGE toolbox. However, the *rplB* gene in MLST profile of *E. hormaechei* was missing and therefore exact match with any ST type was not found; hence the closest-ST1352; is mentioned.

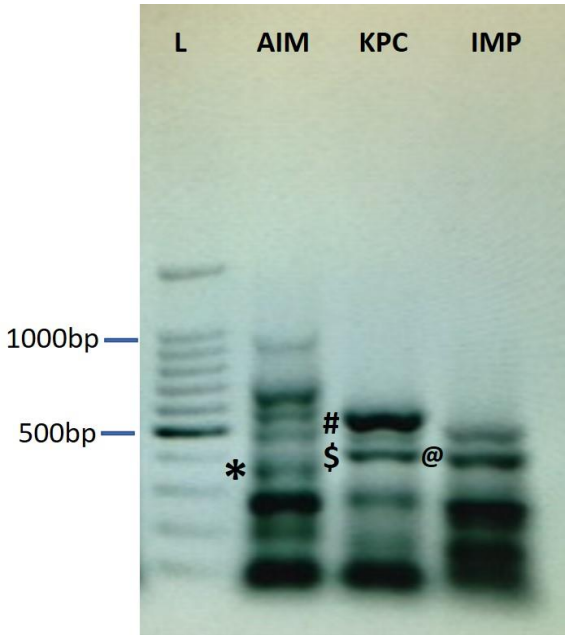

Figure 1: 1% agarose gel electrophoresis showing PCR amplified products of multiplex PCR for AIM (bla_{AIM}, bla_{DIM}, bla_{SIM}, bla_{GIM}), KPC (bla_{KPC}, bla_{NDM-1}, bla_{OXA-48}, bla_{BIC}) and IMP (bla_{IMP}, bla_{VIM}, bla_{SPM}) set of primers. L corresponds to the ladder and band sizes matching the respective bla genes are marked as- #NDM-1(621bp), @VIM(390bp), $Oxa-48(438bp), *AIM(322bp)

Antimicrobial resistance gene determination was done for β-lactamase genes. Figure 2 shows the summary of *bla* genes and plasmids in all isolates. Beta-lactamase genes; bla_{TEM}, bla_{NDM} & bla_{AmpC} were found as most prevalent in all five isolates; while $bla_{CTX-M15}$ was present in all four *E. coli* isolates but not in *E. hormaechei*. bla_{OXA-1} was present in one *E. coli* (J23), bla_{OXA-9} was present in *E. hormaechei* (J34); while ESBL $bla_{SFO-1}$was only present in *E. hormaechei* (J34). Further analysis determined the location of each *bla* gene; whether they are present on plasmid or chromosome. In all *E. coli* isolates; bla_{TEM}, bla_{NDM} were present on plasmids; while bla_{AmpC} was present on the chromosome. In all *E. coli*

having *bla*$_{CTX-M}$; it was plasmid-borne except J23 where *bla*$_{CTX-M}$ was on the chromosome. In contrast to *E. coli*; the isolate *E. hormaechei* (J34) had four *bla* genes (OXA, NDM, AmpC, and SFO-1) on chromosome while only one *bla*$_{TEM}$ was borne on the plasmid. Plasmid profile and their presence in isolates were also done and we found IncFII is present in all four *E. coli* isolates rest are distributed in isolates (Figure 2). Analysis of mobile genetic elements showed the presence of many IS elements (Table 4); among which two IS elements namely, IS6100 (isolate J27) and Tn6082 (isolate J34) were associated with *bla*$_{TEM-1B}$ and *bla*$_{SFO-1}$ respectively.

Table 3: Occurrence of beta-lactamase genes

Primer set	Gene	Presence	Presence of more than one bla gene	N (%)
AIM set	AIM	8/14	NDM+OXA	11/14(79%)
	DIM	0/14		
	GIM	1/14		
	SIM	3/14	NDM+OXA+BIC	8/14(57%)
KPC set	KPC	0/14	NDM+OXA+AIM	6/14(43%)
	NDM-1	11/14		
	OXA	12/14	NDM+OXA+SPM	5/14(36%)
	BIC	8/14		
IMP set	IMP	2/14	NDM+OXA+SIM	3/14(21%)
	SPM	6/14		
	VIM	1/14		

Table 3: Summary of whole-genome analysis:

Isolates	No.of Contigs	N50	%GC	CDS	rRNA	tRNA
J21	204	151099	50.39	4947	61	4
J22	211	159690	50.42	4888	62	3
J23	174	86769	50.55	4677	61	3
J27	132	110197	50.65	4594	63	5
J34	236	61956	50.39	5461	59	4

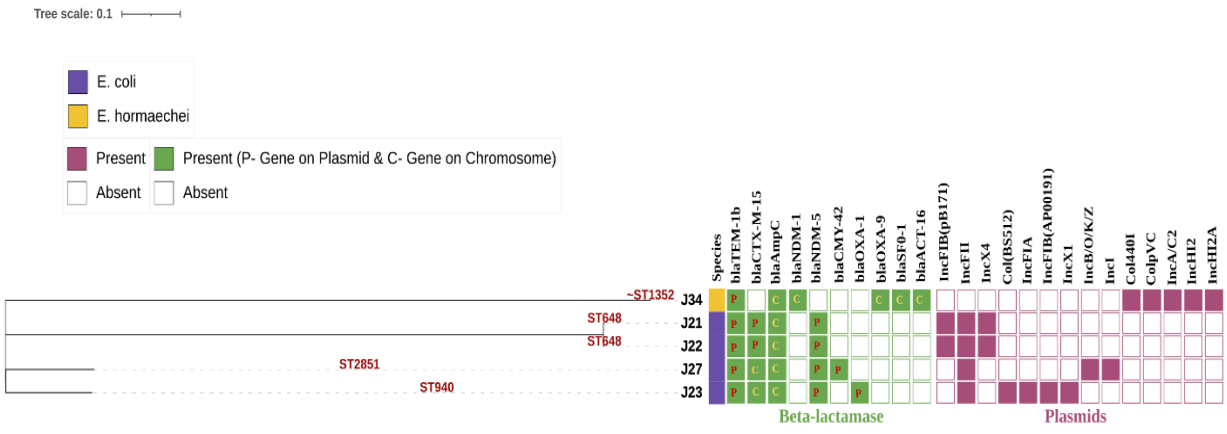

Figure 2: Comparative analysis of all isolates. Analysis of phylogenetic and genomic features of n=5 whole genome sequences. The branch length of the tree indicates the divergence (substitution/site) of the genomes. Resistance phenotype and genotype of the isolates along with *bla* gene and plasmids are shown in the figure. Empty boxes only with the outline indicate absence, and color-filled boxes indicate the presence of the respective genes (names are shown on the top). Legends for the strip data type are also included in the figure. The was used to generate the phylogenetic tree and iTOL v5 was used to exhibit the genotypes. The maximum-likelihood phylogenetic tree was made using IQ-TREE v1.6.11. The branch length indicates the divergence of the genomes.

Table 4: Summary of Mobile genetic elements

Isolates	IS Elements	Association of IS element with *bla* gene
J21 (*E. coli)*	MITEEc1, MITEEc1, ISEc81, ISSen4, ISEc30, IS30, IS6100, ISEc38, ISKpn8, IS26	No association with any beta-lactamase genes
J22 (*E. coli)*	MITEEc1, MITEEc1, ISEc81, ISSen4, ISEc30, IS30, IS6100, ISEc38, ISKpn8, IS26, ISKpn26, IS4	No association with any beta-lactamase genes
J23 (*E. coli)*	Tn6024, MITEEc1, IS629, ISSfl10, ISEc30, IS5075, ISSfl8, MITEEc1, ISEc38, IS6100, IS682, IS26	No association with any beta-lactamase genes
J27 (*E. coli)*	MITEEc1, MITEEc1, ISEc38, MITEEc1, IS6100, ISKpn8, IS3, IS30, IS26	IS6100 which belongs to family IS6is associated with $bla_{\text{TEM-1B}}$
J34 (*E. hormaechei)*	ISEc9, ISEc29, Tn6082	Tn6082 is associated with $bla_{\text{SFO-1}}$

DISCUSSION

There has been a trend of growing antimicrobial resistance in *Enterobacteriaceae* worldwide(1). The global spread of resistance mechanisms like extended-spectrum β-lactamases (ESBLs) has resulted in all penicillins and cephalosporins losing their clinical utility. This has in turn led to an increase in carbapenem consumption, thereby increasing the selection pressure and facilitating the emergence and spread of CRE. It is particularly important to consider the type of carbapenemase imparting resistance because novel drugs are developed with a unique spectrum of activity for a specific carbapenemase. Ceftazidime/avibactam is active against KPC and OXA-48 producers while meropenem/ tazobactam shows activity against mainly KPC producers. Some isolates particularly OXA-48 producers have been reported to be susceptible to carbapenem themselves(8). Keeping the above points in mind, this pilot study was carried out to find the prevalence of CRE, identify the various types of carbapenemases among these CRE isolates, and study their susceptibility profile to other classes of drugs. In addition, complete molecular profiling was also done.

The present study has revealed a 24.1%prevalence of CRE, which is in agreement with other reports recording CRE prevalence to be varying from 3-65%(9). Higher rates from some centers may be explained by the fact that these studies are done on isolates collected from high-risk areas like intensive care units(10).

In the present study, resistance to carbapenem was confirmed using the CNP test, and further M-PCR was done for *bla* genes. Also, WGS was done to identify the MLST, *bla* genes along with their location on chromosome/plasmid and presence of plasmids. M-PCR was able to detect the presence of bla_{NDM} and bla_{OXA}. However, the detection of other genes (e.g. AIM, VIM) appears to be false positive as WGS did not confirm their presence. This could be because we did not design the multiplex assay based on information on Indian isolates. This indicates that multiplex can be readily used for the most found genes but is not reliable for detecting rare genes. We recommend the CNP test for diagnosis of carbapenemase production as it provides rapid results, it is easy to use, easy to implement, and interpret. Further corroborative evidence is provided by the fact that all isolates that tested positive by this test also showed the presence of ≥1 carbapenemase gene by PCR, thereby implying 100% agreement between the two. Analogous supporting results have been reported by other studies too(11).

Genome analysis of multidrug-resistant organisms provides us with information about phylogroup and the number of genes and plasmids on bacteria. The MLST aids in identifying region specific STs and their association with AMR genes. The random selection of isolates for WGS in the present study resulted in the identification of four *E. coli* and one *E. hormaechei;* the genome analysis is discussed separately for both genera as below.

The MLST of our isolates grouped the *E. coli* into ST 648 (J21, J22), ST 940 (J23), and ST 2851 (J27). A recent study from India on the first genome wide comparison of 60 MDR *E. coli* from bloodstream infections identified ST167, ST 405 (global clone ST131 equivalent), and ST410 (fast-spreading high-risk clone) as the most prevalent STs in their study(12). ST 648 and ST 2851 were also found in their study, but as rare sequence types. It is surprising that the MLST of isolates in the present study neither matched the global clones (ST405/ST131) nor the

fast-spreading clones. In another study from Lebanon also, ST648 and ST940 were reported as rare STs(13). This warrants a need to undertake ST analysis of Indian *E. coli* isolates on a high throughput scale and from different geographic locations. Determination of MLST is highly important to correlate possible clonal dissemination and association of AMR genes with a particular ST. E.g. ST131 clones spread worldwide carried $bla_{\text{CTX-M}}$ while; ST 405 another global clonal group is reported to carry the bla_{NDM} gene. In both the above-mentioned studies (India and Lebanon) the ST648, ST940 and ST2851 did not carry $bla_{\text{NDM-5}}$; while we found $bla_{\text{NDM-5}}$ in these rare STs in the present study. The shortcoming of our study is that WGS was done only for a few isolates. The presence of $bla_{\text{NDM-5}}$on these rare STs is worrisome but, further analysis of a large number of such rare STs is needed to confirm the severity of the problem.

In the present study, $bla_{\text{CTX-M-15}}$ was found on the chromosomes in two isolates. In the past two decades, CTXM type ESBLs have replaced TEM and SHV and CTXM-15 is currently the most common ESBL found in *E. coli*(14). Further, it is suggested that the spread of CTX-M has followed an allodemic pattern rather than an epidemic one. Such global community dissemination of the CTX-M type ESBLs has primarily been attributed to the extremely mobilizable genetic elements, of both narrow host range (IncFI, IncFII, IncHI2, IncI) and broad host range (IncN, IncP-1, Inc L/M, Inc A/C) which harbor blaCTX-M genes(15). We found that some of our isolates carried both narrow (IncF) and broad (IncP) host range plasmids. Recently, alike observations have been made in a study on plasmid profiles of ESKAPE pathogens by Raghupathi *et al*., 2019(16). These plasmids have also been reported in *E. coli* strains isolated from sewage, animals, livestock, humans, and food products(15). Chromosomal integration of antibiotic resistance genes is fairly considered a rare event. However, raising reports of chromosomal localization of CTXM, indicate cephalosporin resistance may spread via clonal expansion(17). While most chromosomal integration in the above studies occurred in ST130 and ST410; occasional chromosomal integration was reported in ST176 also. In the present study, a similar chromosomal integration of CTXM-15 occurred in ST940 and ST2851. These results suggest that the chromosomal integration of the $bla_{\text{CTX-M-15}}$ gene happened in numerous independent incidents and highlight that a chromosomal location of this gene might be more common than anticipated(18). Findings have stated a very recurrent association of $bla_{\text{OXA-1}}$ with the global-spread CTX-M-15 ESBL element observed among human and *E. coli* isolates from different origins. Though all four *E. coli* isolates in the present study had the $bla_{\text{CTX-M}}$ gene, only one showed the presence of $bla_{\text{OXA-1}}$.

The third important gene found in all isolates in the present work was AmpC lactamase which comprises another important group of β-lactamases from *E. coli*. Also, one isolate showed the presence of two copies of AmpC; one each on plasmid and chromosome. AmpC being chromosomally incorporated is the most common gene found in several reports (19). The hyperproduction of AmpC is attributed to the lack of an AmpR repressor, but the existence of single or many AmpC genes mediated by plasmid has regularly been observed as a well-known mechanism of the making of high levels of AmpC.

Enterobacter hormaechei is a negative bacterium in Gram staining that generally causes a nosocomial infection that comes within the *Enterobacter cloacae* complex. So far, many ESBLs (CTX-M-15, 9, 2;SHV-12; TEM-1B) and carbapenemases (KPC-2,4,7; NDM-1,7; GIM-1; IMP26) have been reported in *E. hormaechei*(20). Further, important plasmids (IncHI2/2A) carrying these β-lactamases have also been characterized(21). The identification and whole genome sequence analysis of *E. hormaechei* occurred by chance and lead to two important findings; (i) except $bla_{\text{TEM-1}}$ all other genes ($bla_{\text{OXA-9}}$, $bla_{\text{NDM-1}}$, $bla_{\text{SFO-1}}$, bla_{ampC}) were located on the chromosome and (ii) presence of rare ESBL, $bla_{\text{SFO-1}}$ with Tn6082. The rare ESBL, $bla_{\text{SFO-1}}$in *E. hormaechei* was recently reported from China (22) and was shown to be associated with IS26; while the same ($bla_{\text{SFO-1}}$) was found to be with Tn6082 in our isolate. Additionally, in China, a recent study showed the cohabitation of $bla_{\text{NDM-1}}$, $bla_{\text{SFO-1}}$, and mcr-9 on a transmissible plasmid in an MDR *E. hormaechei* isolated from clinical samples (23). Frequently, in *E. hormaechei*, ESBL activity is attributed to $bla_{\text{CTX-M}}$ genes; however, the same was absent in our isolate.

This study has a few limitations. It has been conducted on a small number of isolates, thus it may not be possible to generalize the results. However, the authors want to highlight that this was conducted as a pilot study aimed at getting a preliminary phenotypic and genotypic profile of carbapenem-resistant enterobacteriaceae isolates in this geographical area. Additional studies using more isolates are needed to give a better understanding of the same. The strength of our study lies in the fact that it is the first from Western India to present a detailed account of various resistance mechanisms seen in CRE. It will serve to sensitize clinicians and microbiologists alike to sharpen the surveillance mechanisms to detect this superbug and in developing guidelines for empirical therapy.

CONCLUSION

Carbapenem resistance in rare STs of *E. coli* and *E. hormaechei* is due to the presence of ESBLs (bla_{TEM}, bla_{CTX-M}, bla_{SFO-1}) and MBL (bla_{NDM}). This pilot study highlights the importance of conducting a prospective analysis of CRE isolates and their complete characterization.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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