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Dissemination of carbapenem resistance and plasmids encoding carbapenemases in Gram-negative bacteria isolated in India

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Background: Carbapenem resistance in Gram-negative bacteria is an ongoing public health problem of global dimensions leaving very few treatment options for infected patients.

Objectives: To study the dissemination of plasmid-borne carbapenemase genes in Gram-negative bacteria from a diagnostic centre in Tamil Nadu, India.

Methods: A total of 151 non-repetitive isolates belonging to 10 genera were collected between January 2015 and December 2016 from a diagnostic centre in Tamil Nadu. The isolates included *Escherichia coli* ($n = 57$), *Klebsiella pneumoniae* ($n = 45$), *Pseudomonas aeruginosa* ($n = 10$), *Salmonella* Typhi ($n = 8$), *Enterobacter cloacae* ($n = 8$), *Acinetobacter baumannii* ($n = 7$), *Serratia marcescens* ($n = 5$), *Achromobacter xylosoxidans* ($n = 5$), *Proteus mirabilis* ($n = 5$), *Klebsiella oxytoca* ($n = 5$) and *Elizabethkingia meningoseptica* ($n = 1$).

Results: Of the 151 isolates, 71% ($n = 107$) and 68% ($n = 103$) were found to be resistant to meropenem and imipenem, respectively. The most prevalent β-lactamase gene was $bla_{\text{NDM-1}}$ ($n = 22$), followed by $bla_{\text{OXA-181}}$ ($n = 21$), $bla_{\text{GES-1}}$ ($n = 11$), $bla_{\text{OXA-51}}$ ($n = 9$), $bla_{\text{GES-9}}$ ($n = 8$), $bla_{\text{OXA-23}}$ ($n = 7$) and $bla_{\text{IMP-1}}$ ($n = 3$). We also observed $bla_{\text{OXA-23}}$ in *E. coli* ($n = 4$), and three *K. pneumoniae* were positive for both, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$. Plasmid incompatibility (inc/rep) typing results showed that the resistance genes ($n = 11$) were present in the isolates carrying plasmid-types IncX, IncA/C, IncFIA-FIB and IncFIIA. The plasmid-borne resistance genes in *E. coli* and *K. pneumoniae* were transferred to susceptible *E. coli* AB1157.

Conclusions: This study highlights the prevalence of carbapenem resistance and the acquisition of plasmid-borne carbapenemase genes in Gram-negative bacteria isolated at this centre.

Introduction

Antibiotic resistance is an emerging global health problem due to the injudicious use of antibiotics.[1] It is considered as a major clinical and public health problem because of the limited treatment options available to treat infections caused by antibiotic-resistant bacteria. The increasing bacterial resistance rates to most available antibiotics, including penicillin, cephalosporins, carbapenems, and colistin pose a serious threat.[1] The WHO recently listed carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and ESBL-producing Enterobacteriaceae as pathogens of critical importance.[2] Gram-negative bacteria (GNB), especially Enterobacteriaceae, have developed resistance to a broad-spectrum of antibiotics responsible for significant mortality around the globe.[3] Carbapenems are considered as one of the last resort antibiotics against infections caused by multidrug-resistant GNB.[4] The emergence of carbapenem resistance in Enterobacteriaceae is a major clinical problem, particularly for patients with complex infections, especially when they are immunocompromised or suffering from multiple diseases.[5] Pathogens that are resistant to carbapenems often show high levels of resistance to other

commonly used antibiotics. This not only leads to high mortality rates, but often the patient's time in the hospital is prolonged and medical expenses accumulate, placing an emotional, economic and financial burden on families, especially in resource-limited countries.[6]

The assessment of the worldwide rise in antibiotic resistance has become very difficult due to the increasing rates of multidrug resistance shown by pathogens and the lack of harmonized surveillance systems.[7] Moreover, the coexistence of carbapenem resistance genes with other genes such as plasmid-mediated AmpC or plasmid-mediated quinolone resistance has resulted in an increased acquisition of resistance, causing community- and hospital-acquired infections.[8,9] The carbapenem-hydrolysing oxacillinases (CHDL) are the major source of carbapenem resistance in *A. baumannii*. The first report of OXA-23-type β-lactamase in *A. baumannii* was in 1985 in Edinburgh, UK.[10] Recently, OXA-23 was also reported in members of the Enterobacteriaceae family.[11–14] The OXA-51-like β-lactamase was first reported by the same laboratory in Edinburgh from isolates collected from three hospitals in Buenos Aires, Argentina. At present, more than 150 variants of OXA-51 have been reported worldwide.[15] These intrinsic enzymes (OXA-51-like) in *A. baumannii* are naturally chromosome-borne, but in rare cases are also reported to be encoded on plasmids.[16] Previously, we reported the distribution of colistin resistance in the study region, and investigated the importance of integrons in disseminating antibiotic resistance.[17,18] In the present study, dissemination of carbapenem resistance among Gram-negative bacteria was evaluated, and the role of plasmid transfer in developing carbapenem resistance was also explored in further detail.

Materials and methods

Ethics approval

Ethics approval was from the Institutional Ethical Committee for studies on Human subjects (IECH), ref. no. VIT/IECH/004/Jan2015.

Isolate collection and classification

During January 2015 and December 2016, a total of 151 Gram-negative bacterial isolates were collected from Hi-Tech diagnostic centre in Chennai, Tamil Nadu, India. Bacteria were isolated from urine, blood, pus, bronchial secretion, CSF, pulmonary secretion and bile fluid. The collected isolates were received at the Antibiotic Resistance and Phage Therapy Laboratory, VIT, Vellore, for further analyses. Bacterial identification was carried out using the VITEK identification system (bioMérieux) and 16S rRNA gene nucleotide sequence analysis using universal primers 27 F and 1492 R.[18] DNA was extracted from all the isolates using a boiling lysis method. Briefly, overnight-grown bacterial cultures were centrifuged at 8000 **g** for 10 min, and the bacterial pellet was resuspended in 100 μL of sterile distilled water. The cells were boiled at 100°C for 10 min and the mixture was centrifuged at 2000 **g** for 2 min. The supernatant was extracted and used as a source of template for PCR. The PCR products were sequenced and identified to the species level using the BLASTN tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

Antibiotic susceptibility testing and MICs

Antibiotic resistance profiling was performed using the disc diffusion method according to CLSI guidelines.[19] The antibiotic discs used for this study were gentamicin (10 μg), co-amoxiclav (30 μg), cefotaxime (30 μg), ertapenem (10 μg), amikacin (30 μg), meropenem (10 μg), colistin (10 μg) and cefepime (30 μg). Briefly, on the Muller-Hinton (MH) agar plate, a lawn culture of bacteria was prepared by adjusting the bacterial culture to 0.5 McFarland turbidity standards. The antibiotic discs were placed on the bacterial lawn and the MH plates were incubated at 37°C for 18 h. Based on the zone of inhibition, the results were interpreted as susceptible, intermediate or resistant. MICs were determined by the broth microdilution method for meropenem and imipenem, as described previously.[18] Briefly, in the 96-well microtitre plate, 100 μL of cation-adjusted MH broth was added to each well. Meropenem or imipenem was added at concentrations ranging from 0.06 to 128 mg/L in columns 1 to 11, whereas column 12 served as growth control. The bacterial culture at 5×10^5 dilutions from the overnight grown cells was added and the plates were incubated at 37°C for 20 h. *Escherichia coli* ATCC 25922 was used as a control strain and the results were interpreted according to CLSI guidelines.[19]

Molecular analysis of resistance-related genes

The isolates were screened for the presence of the carbapenem resistance genes bla_{NDM}, $bla_{OXA-48-like}$, bla_{KPC}, bla_{IMP} and bla_{VIM}.[18] A second multiplex PCR was also performed for bla_{DIM}, bla_{BIC}, bla_{GIM}, bla_{SIM} and bla_{AIM}.[20] The bla_{OXA-1}, bla_{OXA-4}, bla_{OXA-30}, $bla_{GES-1-9}$ and bla_{GES-11} were screened as described earlier.[21] The $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$ and $bla_{OXA-58-like}$ were screened for according to Woodford *et al*.[22] The primers and PCR conditions used for analyses are given in Tables S1 to S5 (available as Supplementary data at *JAC-AMR* Online). The PCR amplicons of the resistance genes were sequenced and genes were confirmed using NCBI BLASTN program (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

Plasmid isolation and plasmid incompatibility grouping

Plasmid isolation was performed for all the isolates harbouring resistance genes. The isolation of plasmid DNA was performed using HiPurA Plasmid DNA Miniprep Purification Kit (Himedia, India). Chromosomal DNA contamination was checked using the 16S rRNA primers as described earlier.[23] The purified plasmid DNA was used for screening β-lactamase genes. Plasmid incompatibility (*inc/rep*) typing (FIA, FIB, FIC, HI1, HI2, I1-Ig, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA replicons) was performed using multiplex PCR following the primers and PCR conditions as described by Carattoli *et al*.[24] The primers and PCR conditions used for analysis are given in Table S6.

Conjugation studies

Representative carbapenem-resistant isolates harbouring plasmid-borne resistance were tested for conjugation using the broth-mating method.[18] Briefly, the donor strain (strains carrying resistance genes) and the recipient strain (*E. coli* AB1157, Str^r) were grown overnight in MH broth at 37°C and mixed in 9:1 ratio each of donor and recipient. The cells were kept undisturbed for 6 h at 37°C and plated onto antibiotic-containing medium. The isolates which grew on both meropenem and streptomycin were considered as transconjugants. All the donor strains were tested for streptomycin resistance and MIC values (<2 mg/L) were found to be suitable for the assay. The transconjugants were confirmed for the presence of respective carbapenem resistance genes using PCR. The list of isolates used for conjugation studies is given in Table S7.

Results

Bacterial identification

In this cross-sectional study, a total of 151 non-duplicate, Gram-negative bacteria belonging to 10 genera were studied which included *E. coli* ($n = 57$, 37.7%), *Klebsiella pneumoniae* ($n = 40$, 26.4%), *Klebsiella oxytoca* ($n = 5$, 3.3%), *P. aeruginosa* ($n = 10$, 6.6%), *Salmonella* Typhi ($n = 8$, 5.2%), *Enterobacter cloacae* ($n = 8$,

Table 1. Antibiotic susceptibility testing employing the disc diffusion method and the prevalence of MDR isolates among 151 Gram-negative bacteria isolated from clinical samples

	No. of resistant isolates (%)								
Bacteria/antibiotic	GEN	AMC	IPM	ETP	AMK	MEM	CST	FEP	Total MDR isolates ($n = 151$)
E. coli ($n = 57$)	51 (89)	45 (79)	46 (81)	38 (67)	49 (86)	43 (75)	35 (61)	45 (79)	54 (95)
K. pneumoniae ($n = 40$)	33 (83)	31 (78)	32 (80)	28 (70)	36 (90)	32 (80)	29 (73)	30 (75)	32 (80)
P. aeruginosa ($n = 10$)	10 (100)	10 (100)	9 (90)	6 (60)	8 (80)	10 (100)	7 (70)	8 (80)	10 (100)
S. Typhi ($n = 8$)	6 (75)	7 (88)	5 (63)	5 (63)	6 (75)	7 (88)	5 (63)	7 (88)	7 (88)
E. cloacae ($n = 8$)	7 (88)	8 (100)	7 (88)	6 (75)	8 (100)	8 (100)	6 (75)	7 (88)	8 (100)
A. baumannii ($n = 7$)	7 (100)	6 (86)	7 (100)	6 (86)	7 (100)	7 (100)	5 (71)	7 (100)	7 (100)
S. marcescens ($n = 5$)	5 (100)	5 (100)	5 (100)	3 (60)	5 (100)	5 (100)	4 (80)	5 (100)	5 (100)
A. xylosoxidans ($n = 5$)	5 (100)	5 (100)	4 (80)	2 (40)	5 (100)	5 (100)	4 (80)	5 (100)	5 (100)
K. oxytoca ($n = 5$)	4 (80)	5 (100)	5 (100)	4 (80)	5 (100)	5 (100)	4 (80)	5 (100)	5 (100)
P. mirabilis ($n = 5$)	5 (100)	5 (100)	4 (80)	4 (80)	5 (100)	5 (100)	4 (80)	5 (100)	5 (100)
E. meningoseptica ($n = 1$)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)

Values represent the number of resistant isolates, % is listed in brackets. Isolates were defined as MDR only when the isolates are resistant to three or more antibiotics. Abbreviations: GEN, gentamicin; AMC, co-amoxiclav; IPM, imipenem; ETP, ertapenem; AMK, amikacin; MEM, meropenem; CST, colistin; FEP, cefepime.

5.2%), *A. baumannii* ($n = 7$, 4.6%), *Serratia marcescens* ($n = 5$, 3.3%), *Achromobacter xylosoxidans* ($n = 5$, 3.3%), *Proteus mirabilis* ($n = 5$, 3.3%) and *Elizabethkingia meningoseptica* ($n = 1$, 0.6%). Most of the isolates were isolated from urine (37%; 56/151) and blood (28%; 42/151) and from other sources such as pus (7%), bronchial secretion (2%), CSF (1%), pulmonary secretion (1%), bile fluid (5%) or from sources that were not documented (19%).

Antibiotic susceptibility studies

Table 1 summarizes the antibiotic susceptibility pattern of all the isolates tested against eight different antibiotics. Meropenem MICs showed that 107/151 (71%) isolates were resistant (Figure 1), whereas 128 (84.7%) isolates were meropenem-resistant when analysed by the disc diffusion method. For imipenem, 68% ($n = 103$) were resistant by microbroth dilution method whereas 83% ($n = 125$) were resistant according to the disc diffusion method. MIC_{50} and MIC_{90} values for meropenem were 4 mg/L and 16 mg/L, respectively, and for imipenem the MIC_{50} was 4 mg/L and the MIC_{90} was 16 mg/L.

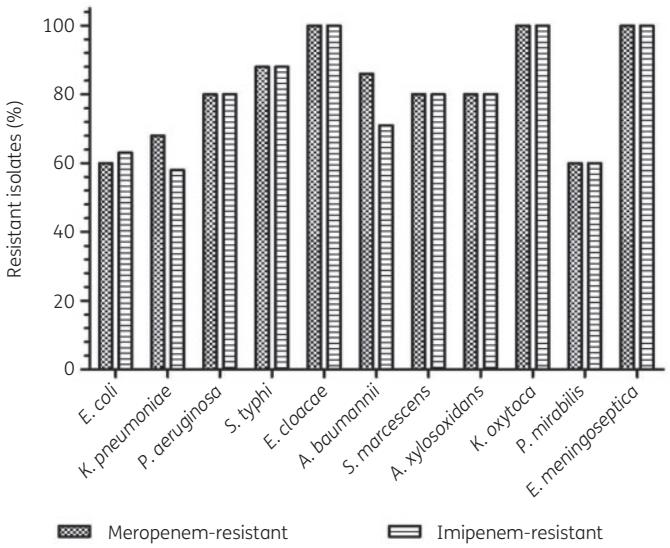

Figure 1. The distribution of Gram-negative bacteria and comparison of imipenem and meropenem resistance.

Distribution of carbapenemase resistance genes

Of the 57 *E. coli*, 32 isolates carried carbapenemases (bla_{NDM}, $bla_{OXA-48-like}$, bla_{GES-1}, bla_{GES-9}, $bla_{OXA-23-like}$ and bla_{IMP}) and five *E. coli* isolates carried more than one of the carbapenem resistance genes (Figure 2). Among the *K. pneumoniae* strains, 19/40 carried the studied genes (bla_{NDM}, $bla_{OXA-48-like}$, bla_{GES-1}, bla_{GES-9}, $bla_{OXA-23-like}$, $bla_{OXA-51-like}$), and one isolate was positive for both bla_{NDM} and $bla_{OXA-48-like}$. Carbapenem resistance genes were detected in 70/151 by PCR, and 10 isolates had more than one gene type. The most prevalent resistance genes were bla_{NDM} ($n = 22$), $bla_{OXA-48-like}$ ($n = 21$), bla_{GES-1} ($n = 11$), bla_{GES-9} ($n = 8$), $bla_{OXA-23-like}$ ($n = 7$), $bla_{OXA-51-like}$ ($n = 9$) and bla_{IMP} ($n = 3$). None of the β-lactamase genes bla_{KPC}, bla_{VIM}, bla_{BIC}, bla_{GIM}, bla_{DIM}, bla_{SIM} or bla_{AIM} were detected in the isolates. Sequencing of genes showed that all the amplified bla_{NDM} genes were bla_{NDM-1}, $bla_{OXA-48-like}$ genes were $bla_{OXA-181}$, and bla_{IMP} genes were bla_{IMP-1}.

Plasmid incompatibility typing and conjugation

Plasmid DNA was isolated from 70 isolates that carried resistance genes (Table 2). In total, of the 151 isolates studied, 70 isolates carried resistance genes, of which 11 were plasmid-borne and 59 were chromosomal. Of the 37 *E. coli* isolates, 32 isolates carried resistance genes, of which six were plasmid-encoded. Among the 40 *K. pneumoniae* strains, only 19 isolates carried resistance genes, of which three were encoded on plasmids. In *E. cloacae*, one isolate carried bla_{NDM-1} on a plasmid and one *P. mirabilis* carried plasmid-borne bla_{IMP-1}. Plasmid incompatibility/replicon (inc/rep) typing

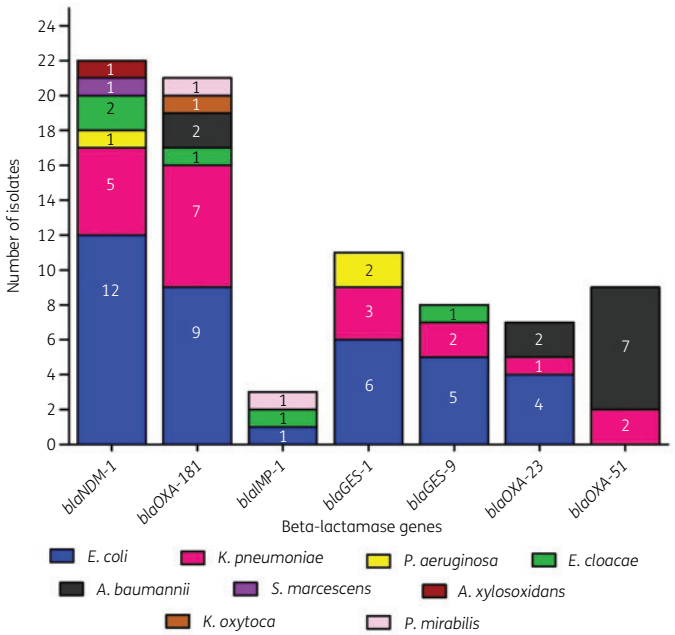

Figure 2. The distribution of carbapenemase genes among Gram-negative bacteria isolated from the clinical samples. A total of 20 resistance genes were studied that include bla_{NDM}, $bla_{OXA-48-like}$, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{DIM}, bla_{BIC}, bla_{GIM}, bla_{SIM}, bla_{AIM} bla_{OXA-1}, bla_{OXA-4}, bla_{OXA-30}, $bla_{GES-1-9}$, bla_{GES-11}, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$, $bla_{OXA-58-like}$. The genes bla_{KPC}, bla_{VIM}, bla_{DIM}, bla_{BIC}, bla_{GIM}, bla_{SIM}, bla_{AIM}, bla_{OXA-1}, bla_{OXA-4}, bla_{OXA-30}, bla_{GES-11}, $bla_{OXA-24-like}$ and $bla_{OXA-58-like}$ were not observed in any of the isolates.

results showed that the plasmids belonged to inc/rep types: IncX, IncA/C, IncFIA-FIB and IncFIIA (Table 2). *E. coli* isolates that carried IncX (EC10), IncA/C (EC21) and IncFIA-FIB (EC29) -type plasmids harboured bla_{NDM-1} genes. *E. coli* strains carrying IncFIIA (EC39) and IncFIA-FIB (EC29) harboured $bla_{OXA-181}$ genes and IncFIA-FIB (EC47) type plasmids carried $bla_{GES-1/9}$ genes. *K. pneumoniae* isolates carrying IncFIA-FIB (KP10) -type plasmids carried bla_{NDM-1} genes, and IncA/C (KP31 and KP39) carried bla_{GES-1}, $bla_{OXA-23/51-like}$ genes. One *E. cloacae* isolate with IncFIIA (EL3)-type plasmid harboured bla_{NDM-1} gene and one *P. mirabilis* isolate carrying IncFIA-FIB (PM5)-type plasmid had the bla_{IMP-1} gene.

In total, 11 carbapenem-resistant isolates harbouring plasmid-encoded resistance were subjected to conjugation studies (Table 2). The six *E. coli* isolates EC10, 21, 29, 39, 44, and 47 were found to facilitate the transfer of plasmid-mediated resistance to susceptible *E. coli* AB1157. Inter-generic transfer of NDM-1 was observed in one *K. pneumoniae* isolate (KP10) (Table 2).

Discussion

In India, carbapenem-resistant Gram-negative bacteria have been reported as becoming more frequent.[17,18] In this study, the distribution of carbapenem-resistant isolates in 10 genera of Gram-negative bacteria isolated at a diagnostic centre in Tamil Nadu, India, has been investigated. Previous studies describe the increasing prevalence of ESBL and MBL producers among Gram-negative bacteria in India.[25–28]

In this study, experiments determining MIC values show that 107/151 (71%) isolates were resistant to meropenem, correlating with the observation made by the disc diffusion method ($n = 128$). All the 70 isolates harbouring carbapenem resistance genes were resistant according to the results of both the methods (MIC and disc diffusion). As carbapenems are one of the last-resort antibiotics available to treat infections caused by Gram-negative bacteria, the prevalence of carbapenem resistance is of worldwide concern. Our previous studies had reported the dissemination of carbapenem-resistant bacteria and carbapenem resistance genes among Gram-negative bacteria in Tamil Nadu.[17,18] Here, we report the prevalence (71%) of carbapenem-resistant isolates among 10 genera of Gram-negative bacteria. β-Lactamase genes such as bla_{NDM-1} ($n = 22$), $bla_{OXA-181}$ ($n = 21$), bla_{GES-1} ($n = 11$), bla_{GES-9} ($n = 8$), bla_{OXA-23} ($n = 7$), bla_{OXA-51} ($n = 9$) and bla_{IMP-1} ($n = 3$) were found in 70 isolates (with 10 isolates carrying more than one gene type), in contrast to our earlier study which reported a lower prevalence (27%) of bla_{NDM-1} and $bla_{OXA-181}$ genes among carbapenem-resistant isolates.[18] The coexistence of bla_{NDM-1} and $bla_{OXA-181}$ in *E. coli* is a reason for major concern from the healthcare perspective. All the *A. baumannii* isolates ($n = 7$) were found to have either OXA-23 or OXA-181 along with OXA-51 intrinsic β-lactamase.[29] Earlier reports from India showed the presence of OXA-23 and OXA-51 in carbapenem-resistant *Acinetobacter* causing serious healthcare problems.[12] Enterobacteriaceae carried OXA-48-like genes, which are carbapenem-hydrolysing class D β-lactamases.[13,30] The unusual occurrence of bla_{OXA-23} in *E. coli*, and plasmid-encoded bla_{OXA-23} and bla_{OXA-51} in *K. pneumoniae* are very important findings of this study, as only very few earlier studies have reported the presence of the bla_{OXA-23} gene in *E. coli*.[31,32] OXA-23-like genes in Enterobacteriaceae may be embedded within a transposon but were not characterized in this study. The resistance reports on *E. meningoseptica* are very rare in India,[31,32] and in this study it was found that one isolate of *E. meningoseptica* was resistant to imipenem and meropenem. Although earlier studies showed the presence of carbapenemase genes in *E. meningoseptica*, in this study no carbapenem resistance genes were found.

Carbapenem resistance among Gram-negative bacteria is becoming very common in Tamil Nadu, India. The isolates producing carbapenemases are mostly MDR and the rapid spread of carbapenem resistance genes is highly concerning. These resistance genes are located adjacent to mobile genetic elements (integrons and transposons), which facilitates the easy transposition between replicons.[33] The extrachromosomal plasmids are the primary carriers of antibiotic resistance genes and can spread horizontally between strains or species. The recent molecular and genomic surveillance studies are also focused to track the clonally evolving lineages, besides plasmids being the primary focus.[34] The most common plasmid replicon types for carbapenem resistance genes are IncF, IncA/C_2, IncX3, IncL/M and IncH.[35] In this study, bla_{NDM-1} was found in the isolates that carried IncX, IncA/C, IncFIA-FIB and IncFIIA; $bla_{OXA-181}$ in IncA/C, IncFIA-FIB and IncFIIA; $bla_{GES-1/9}$ in IncFIA-FIB and IncA/C; bla_{IMP-1} in IncFIA-FIB; and $bla_{OXA-23/51}$ in IncA/C. The presence of plasmid-encoded $bla_{OXA-23/51}$ is an important finding, considering the rapid spread of carbapenem resistance among Gram-negative bacteria. Interestingly, the isolates we investigated (such as *P. aeruginosa*, *Salmonella* Typhi, *A. baumannii*, *S. marcescens*, *A. xylosoxidans*, *K. oxytoca*, and *E. meningoseptica*) do not carry any plasmids harbouring

Table 2. Distribution of resistance genes, plasmid incompatibility grouping and transconjugation studies on Gram-negative isolates that were harbouring resistance genes

Isolate	Source	MIC (mg/L)		Resistance gene	Plasmid *inc/rep* typing	Conjugative plasmid
		Meropenem	Imipenem			
E. coli EC1	Urine	16	8	bla_{NDM-1}	–	–
E. coli EC2	Urine	0.25	0.12	ND	–	–
E. coli EC3	Blood	1	1	ND	–	–
E. coli EC4	Pus	32	32	bla_{NDM-1}	–	–
E. coli EC5	Urine	8	16	bla_{NDM-1}	–	–
E. coli EC6	Pus	2	0.5	ND	–	–
E. coli EC7	Urine	8	8	bla_{NDM-1}	–	–
E. coli EC8	Urine	1	2	ND	–	–
E. coli EC9	Urine	0.25	0.5	ND	–	–
***E. coli* EC10**	**Blood**	**32**	**8**	**bla_{NDM-1}**	**IncX**	+
E. coli EC11	Unknown	0.5	0.25	ND	–	–
E. coli EC12	Urine	64	32	bla_{NDM-1}	–	–
E. coli EC13	Unknown	0.5	1	ND	–	–
E. coli EC14	Unknown	2	2	ND	–	–
E. coli EC15	Urine	32	32	ND	–	–
E. coli EC16	Urine	0.5	1	ND	–	–
E. coli EC17	Urine	8	4	bla_{NDM-1}	–	–
E. coli EC18	Urine	0.25	0.25	ND	–	–
E. coli EC19	Unknown	4	4	ND	–	–
E. coli EC20	Urine	0.12	0.25	ND	–	–
***E. coli* EC21**	**Blood**	**16**	**8**	**bla_{NDM-1}**	**IncA/C**	+
E. coli EC22	Unknown	>128	>128	bla_{NDM-1}	–	–
E. coli EC23	Urine	64	8	bla_{NDM-1}	–	–
E. coli EC24	Unknown	1	1	ND	–	–
E. coli EC25	Urine	>128	128	bla_{NDM-1}	–	–
E. coli EC26	Urine	2	8	ND	–	–
E. coli EC27	Urine	0.25	0.5	ND	–	–
E. coli EC28	Bile fluid	1	1	ND	–	–
***E. coli* EC29**	**Unknown**	**8**	**32**	**bla_{NDM-1}, $bla_{OXA-181}$**	**IncFIA-FIB**	+
E. coli EC30	Urine	4	4	$bla_{OXA-181}$	–	–
E. coli EC31	Unknown	16	32	$bla_{OXA-181}$	–	–
E. coli EC32	Blood	2	2	ND	–	–
E. coli EC33	Blood	8	8	$bla_{OXA-181}$	–	–
E. coli EC34	Bile fluid	16	8	$bla_{OXA-181}$	–	–
E. coli EC35	Urine	0.5	0.25	ND	–	–
E. coli EC36	Urine	32	16	$bla_{OXA-181}$	–	–
E. coli EC37	Bile fluid	2	8	ND	–	–
E. coli EC38	Urine	1	0.5	ND	–	–
***E. coli* EC39**	**Blood**	**64**	**>128**	**$bla_{OXA-181}$**	**IncFIIA**	+
E. coli EC40	Blood	8	8	$bla_{OXA-181}$	–	–
E. coli EC41	Blood	16	16	$bla_{OXA-181}$	–	–
E. coli EC42	Blood	32	16	bla_{IMP-1}	–	–
E. coli EC43	Urine	0.5	1	ND	–	–
***E. coli* EC44**	**Pus**	**16**	**64**	**bla_{GES-1}**	**IncFIA-FIB**	+
E. coli EC45	Unknown	32	32	bla_{GES-1}	–	–
E. coli EC46	Urine	16	8	bla_{GES-1}	–	–
***E. coli* EC47**	**Blood**	**>128**	**>128**	**bla_{GES-1}, bla_{GES-9}**	**IncFIA-FIB**	+
E. coli EC48	Pus	0.06	0.06	ND	–	–
E. coli EC49	Blood	64	64	bla_{GES-1}, bla_{GES-9}	–	–
E. coli EC50	Pus	16	32	bla_{GES-1}, bla_{GES-9}	–	–

Continued

Table 2. *Continued*

Isolate	Source	MIC (mg/L)		Resistance gene	Plasmid *inc/rep* typing	Conjugative plasmid
		Meropenem	Imipenem			
E. coli EC51	Bile fluid	4	4	bla_{GES-9}	–	–
E. coli EC52	Unknown	>128	64	bla_{GES-9}, bla_{OXA-23}	–	–
E. coli EC53	Blood	64	>128	bla_{OXA-23}	–	–
E. coli EC54	Urine	8	8	bla_{OXA-23}	–	–
E. coli EC55	Unknown	1	0.5	ND	–	–
E. coli EC56	Urine	32	64	bla_{OXA-23}	–	–
E. coli EC57	Blood	0.5	0.5	ND	–	–
K. pneumoniae KP1	Urine	1	1	ND	–	–
K. pneumoniae KP2	Urine	0.5	0.25	ND	–	–
K. pneumoniae KP3	Blood	>128	128	bla_{NDM-1}	–	–
K. pneumoniae KP4	Bile fluid	2	1	ND	–	–
K. pneumoniae KP5	Urine	8	32	ND	–	–
K. pneumoniae KP6	Blood	0.25	0.12	ND	–	–
K. pneumoniae KP7	Blood	8	16	bla_{NDM-1}	–	–
K. pneumoniae KP8	Blood	0.5	0.25	ND	–	–
K. pneumoniae KP9	Urine	32	32	bla_{NDM-1}	–	–
***K. pneumoniae* KP10**	**Blood**	**64**	**>128**	$\boldsymbol{bla_{NDM-1}}$	**IncFIA-FIB**	+
K. pneumoniae KP11	Unknown	8	8	bla_{NDM-1}	–	–
K. pneumoniae KP12	Bile fluid	1	1	ND	–	–
K. pneumoniae KP13	Urine	32	64	ND	–	–
K. pneumoniae KP14	Urine	0.06	0.12	ND	–	–
K. pneumoniae KP15	Pulmonary secretion	8	4	ND	–	–
K. pneumoniae KP16	Urine	2	2	ND	–	–
K. pneumoniae KP17	Blood	16	16	$bla_{OXA-181}$	–	–
K. pneumoniae KP18	Unknown	0.5	0.25	ND	–	–
K. pneumoniae KP19	Blood	32	8	$bla_{OXA-181}$	–	–
K. pneumoniae KP20	Unknown	128	64	$bla_{OXA-181}$	–	–
K. pneumoniae KP21	Unknown	8	8	$bla_{OXA-181}$	–	–
K. pneumoniae KP22	Unknown	16	2	ND	–	–
K. pneumoniae KP23	Blood	16	8	ND	–	–
K. pneumoniae KP24	Blood	0.25	0.25	ND	–	–
K. pneumoniae KP25	Unknown	32	64	$bla_{OXA-181}$	–	–
K. pneumoniae KP26	Blood	8	2	ND	–	–
K. pneumoniae KP27	Unknown	64	>128	$bla_{OXA-181}$	–	–
K. pneumoniae KP28	Blood	32	8	$bla_{OXA-181}$	–	–
K. pneumoniae KP29	Unknown	4	0.5	ND	–	–
K. pneumoniae KP30	Urine	1	2	ND	–	–
K. pneumoniae KP31	Blood	16	4	bla_{GES-1}	IncA/C	–
K. pneumoniae KP32	Unknown	32	32	bla_{GES-1}	–	–
K. pneumoniae KP33	Urine	128	>128	bla_{GES-1}	–	–
K. pneumoniae KP34	Blood	8	1	ND	–	–
K. pneumoniae KP35	Unknown	0.5	1	ND	–	–
K. pneumoniae KP36	Urine	64	16	bla_{GES-9}	–	–
K. pneumoniae KP37	Bile fluid	64	64	bla_{GES-9}	–	–
K. pneumoniae KP38	Blood	0.5	2	ND	–	–
K. pneumoniae KP39	Urine	>128	>128	bla_{OXA-23}, bla_{OXA-51}	IncA/C	–
K. pneumoniae KP40	Urine	32	8	bla_{OXA-51}	–	–
P. aeruginosa PA1	Pus	8	*16*	bla_{NDM-1}	–	–
P. aeruginosa PA2	Pus	1	0.5	ND	–	–
P. aeruginosa PA3	Pus	16	32	ND	–	–
P. aeruginosa PA4	Bronchial secretion	0.25	0.25	ND	–	–
P. aeruginosa PA5	Urine	>128	128	bla_{GES-1}	–	–

Continued

Table 2. *Continued*

Isolate	Source	MIC (mg/L)		Resistance gene	Plasmid *inc/rep* typing	Conjugative plasmid
		Meropenem	Imipenem			
P. aeruginosa PA6	Unknown	8	4	ND	–	–
P. aeruginosa PA7	Blood	32	32	bla_{GES-1}	–	–
P. aeruginosa PA8	Pus	64	128	ND	–	–
P. aeruginosa PA10	Pus	>128	64	ND	–	–
S. Typhi ST1	Blood	8	64	ND	–	–
S. Typhi ST2	Unknown	32	32	ND	–	–
S. Typhi ST3	Urine	0.5	1	ND	–	–
S. Typhi ST4	Blood	32	64	ND	–	–
S. Typhi ST5	Urine	128	64	ND	–	–
S. Typhi ST6	Blood	16	8	ND	–	–
S. Typhi ST7	Blood	4	4	ND	–	–
S. Typhi ST8	Unknown	8	32	ND	–	–
E. cloacae EL1	Urine	64	>128	ND	–	–
E. cloacae EL2	Blood	16	8	bla_{NDM-1}	–	–
E. cloacae EL3	Urine	4	64	bla_{NDM-1}	IncFIIA	–
E. cloacae EL4	Bronchial secretion	32	128	ND	–	–
E. cloacae EL5	Blood	32	32	$bla_{OXA-181}$	–	–
E. cloacae EL6	Urine	16	8	–	–	–
E. cloacae EL7	Urine	128	128	bla_{IMP-1}	–	–
E. cloacae EL8	Urine	32	8	bla_{GES-9}	–	–
A. baumannii AB1	CSF	8	8	$bla_{OXA-181}$, bla_{OXA-51}	–	–
A. baumannii AB2	Urine	16	64	$bla_{OXA-181}$, bla_{OXA-51}	–	–
A. baumannii AB3	Unknown	0.5	1	bla_{OXA-51}	–	–
A. baumannii AB4	Pus	8	64	bla_{OXA-23}, bla_{OXA-51}	–	–
A. baumannii AB5	Blood	32	32	bla_{OXA-23}, bla_{OXA-51}	–	–
A. baumannii AB6	Urine	32	>128	bla_{OXA-51}	–	–
A. baumannii AB7	Urine	16	2	bla_{OXA-51}	–	–
S. marcescens SM1	Bronchial secretion	8	4	ND	–	–
S. marcescens SM2	Blood	32	64	bla_{NDM-1}	–	–
S. marcescens SM3	Unknown	128	64	ND	–	–
S. marcescens SM4	Urine	2	2	ND	–	–
S. marcescens SM5	Unknown	32	8	ND	–	–
A. xylosoxidans AY1	Unknown	4	8	ND	–	–
A. xylosoxidans AY2	Blood	128	128	ND	–	–
A. xylosoxidans AY3	Urine	32	32	ND	–	–
A. xylosoxidans AY4	Urine	1	0.5	ND	–	–
A. xylosoxidans AY5	Urine	64	128	bla_{NDM-1}	–	–
K. oxytoca KO1	Blood	32	128	ND	–	–
K. oxytoca KO2	Urine	8	16	ND	–	–
K. oxytoca KO3	Blood	32	32	ND	–	–
K. oxytoca KO4	Blood	128	128	$bla_{OXA-181}$	–	–
K. oxytoca KO5	Urine	8	2	ND	–	–
P. mirabilis PM1	Unknown	1	2	ND	–	–
P. mirabilis PM2	Blood	128	128	$bla_{OXA-181}$	–	–
P. mirabilis PM3	Urine	8	8	ND	–	–
P. mirabilis PM4	Blood	0.06	0.25	ND	–	–
P. mirabilis PM5	Urine	64	32	bla_{IMP-1}	IncFIA-FIB	–
E. meningoseptica EM1	CSF	64	64	ND	–	–

ND, not detected; '–' denotes absence; '+' denotes conjugation positive; bold text indicates the isolates were carrying resistance genes on conjugative plasmids. The resistance breakpoint (CLSI) for both meropenem and imipenem is MIC $\geq$ 4 mg/L.

resistance genes. This clearly showed that the β-lactamase or carbapenemase genes were confined to certain strains and present in the different replicon types (plasmids) in the study region. Earlier, the bla_{NDM} IncFII plasmids were reported from India,[35] and IncFIA-FIB plasmids carrying carbapenem resistance genes such as bla_{NDM} were described in samples collected from river and sewage treatment plants in India.[7,35] This study also showed that some isolates with plasmids were carrying more than one resistance gene, an alarming public health threat. Conjugative plasmids are known to spread their resistance among the bacteria of the same or of different genera. This study showed that all the six *E. coli* isolates carrying plasmid-encoded resistance genes (bla_{NDM-1}, $bla_{OXA-181}$, bla_{GES-1}, and bla_{GES-9}) were conjugative and one *K. pneumoniae* plasmid (IncFIA-FIB with bla_{NDM-1}) was transferable, illustrating how resistance genes rapidly spread in clinically relevant bacteria.

We acknowledge several limitations of our study. First, the clinical samples or isolates were collected randomly from the diagnostic centre, which receives clinical samples from multiple hospitals (both in- and out-patient) in the study region. Second, the presence of insertion sequence (IS) elements was not studied. Finally, the transfer of resistance genes between the bacteria was studied using simple conjugation experiments but we did not confirm the results using Southern hybridization or sequencing techniques.

Conclusions

The increasing frequency of antibiotic resistance in bacteria is a major healthcare problem. This study highlights the distribution of carbapenem-resistant isolates in the region we studied, with the emphasis on the existence of bla_{NDM-1}, $bla_{OXA-181}$, bla_{IMP-1}, bla_{GES-1}, bla_{GES-9}, $bla_{OXA-23-like}$, and $bla_{OXA-51-like}$ among the clinical pathogens. The unusual presence of an *E. coli* strain carrying bla_{OXA-23}, and *K. pneumoniae* isolates carrying bla_{OXA-23} and bla_{OXA-51} require targeted antibiotic resistance surveillance programmes. The development of alternative therapeutic options should be undertaken immediately to be able to combat the problem of resistance, especially to treat carbapenem-resistant infections in the future. Our study shows that conjugative plasmids are a major contributor to the transfer of resistance in pathogens leading to further dissemination of resistance genes. A One-Health approach is necessary to combat the problem of resistance both at the local and international level.

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Transparency declarations

None to declare. All the datasets are presented in the main manuscript. The raw datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

Authors P.M. and R.N. collected the isolates from the clinical samples. Authors P.M. and R.N. undertook the laboratory work, R.N. and B.S.L. interpreted the data, and P.M. and R.N. wrote the initial manuscript. Authors S.L., R.N. and B.S.L. revised and finalized manuscript. All the authors read and approved the manuscript.

Supplementary data

Tables S1 to S7 are available as Supplementary data at *JAC* Online.

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