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DETECTION OF CARBAPENEM RESISTANCE GENES IN *E.COLI IN* A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: To examine the distribution, emergence and spread of genes encoding beta-lactamase resistance in *E.coli* recovered from hospitalized patients in a tertiary care hospital. **Methods:** A prospective study was conducted in an 1800 bedded tertiary care centre in Pune, India from October 2013 to October 2017. A total of 1070 *E.coli* isolates were recovered from clinical specimens of hospitalized patients admitted to the Medical and Surgical intensive care units (one isolate per patient). Polymerase chain reaction (PCR) assays and sequencing was used to determine the presence of beta-lactamase encoding genes and conjugation experiments were performed to determine the transferability. Isolate relatedness were determined by REP PCR, ERIC PCR and RAPD. **Results:** Majority of Carbapenem resistant *E.coli* were from UTIs 90 (40%), followed by SSTIs 45(20%), 18% (40) in RTIs, 30(13%) in BSIs and 21(9%) in IAIs respectively. Evaluation of antibiotic susceptibility pattern indicated that 21% *E.coli* were resistant against IPM, MEM, and ETP. Out of 1070 isolates, 226 were found carbapenem resistant as MICs was >4µg/ml against IPM, MEM, and ETP as determined by the E-test and VITEK-2, 185 were MBL producers and 41 were non MBL producers. In the present study, among carbapenem producers $bla_{NDM-1}$was detected in 67.7% isolates, while bla_{VIM} was present in 14.3% isolates, bla_{CTX-M} was present in 89% isolates, followed by bla_{TEM-1} in 81% and bla_{SHV} in 69.2%. In this study, bla_{SHV-5}, bla_{SHV-11}, bla_{SHV-12}, and bla_{SHV-28} are the commonest SHV genes detected in 8%, 10.1%, 37.5% and 48.5% of bla_{SHV} producing isolates respectively whereas $bla_{CTX-M-15}$, $bla_{CTX-M-14}$ and $bla_{CTX-M-28}$ are the commonest CTX-M ESBLs that were present in 64.2%, 23% and 12.8% of bla_{CTX-M} producing isolates

respectively suggesting co association of multiple variants of ESBL along with MBLs that aids in marked increase in MICS against cephalosporins and other beta lactams including carbapenems and thereby increasing antimicrobial resistance towards commonly used drugs. Susceptibility profiling of the isolates indicated that 100% retained susceptibility to colistin. Conjugation experiments indicated that bla_{NDM-1},bla_{VIM}, $bla_{OXA-48,}$ bla_{SHV-5}, bla_{SHV-11}, bla_{SHV-12}, $bla_{SHV-28,}$ $bla_{CTX-M-15}$, $bla_{CTX-M-14}$ were transferable via plasmid. **Conclusion:** This study highlights prevalence of bla_{OXA-48} and bla_{NDM-1}, producing *E.coli* in multiple combinations with other β-lactamases which are commonly found a single or multiple plasmids to serve as driving force for the horizontal spread of emerging carbapenem resistance that are critically important for treatment of human bacterial infections.

KEYWORDS: bla_{NDM-1}, bla_{VIM}, bla_{SHV-5}, bla_{SHV-11}, bla_{SHV-12}, $bla_{SHV-28,}$ $bla_{CTX-M-15}$, $bla_{CTX-M-14,}$ REP PCR, ERIC PCR and RAPD.

INTRODUCTION

Emergence of nosocomial bacterial pathogens with β- Lactamases resistance is a major health challenge. ESBL-producing organism had severely threatened therapeutic choices. Carbapenems belong to beta-lactam class of antibiotics that are frequently used for treating such type of infections caused by multidrug-resistant pathogens. *E.coli* is one of the most common causative agent of UTIs, leading causes of neonatal bacteremia, sepsis, and meningitis, second only to Streptococcus agalactiae and can cause a wide variety of other extraintestinal infections, such as nosocomial pneumonia, cholecystitis and cholangitis, peritonitis, cellulitis, osteomyelitis, and infectious arthritis. [1-5] Resistance to carbapenems is most frequently mediated by the enzymatic hydrolysis of the drugs by *E.coli*. Carbapenemases belong to three molecular classes: the Ambler class A (including KPC and GES), Ambler class B (including IMP, VIM, SIM, and NDM), and Ambler class D (CHDLs or OXA-48) beta-lactamases. Reports of *E.coli* producing these Carbapenemases are disturbing as these multidrug-resistant infections leave patients with very few or no antimicrobial options. [1-5] Based on these considerations, this study was under taken to detect prevalence of metallo-beta-lactamases resistance in a tertiary care centre in India. This study provides an insight into the acquisition and spread of the MBL genes and emphasizes its transmission capability through plasmids.

MATERIALS AND METHODS

Bacterial isolates

A prospective study was conducted in a 1800 bedded tertiary care centre in Pune, India from October 2013 to October 2017. A total of 1070 *E.coli* isolates were recovered from clinical specimens of hospitalized patients admitted to the Medical and Surgical intensive care units (one isolate per patient). Samples were collected from patients, using strict aseptic precautions and in accordance with standard protocols [6] and immediately processed without any delay. *E.coli* was identified up to the species level using VITEK-GNI cards (bioMérieux, Marcy l'Etoile, France) and molecular-based methods.

Antimicrobial susceptibility testing

The antimicrobial susceptibility test was performed by the Kirby Bauer's disc diffusion technique on Mueller–Hinton agar, as per Clinical Laboratory Standard Institute (CLSI) guidelines.[7] The antibiotics tested were as follows (potency in µg/disc): Ampicillin(10), Cefuroxime (30), Cefpodoxime(30), Ceftazidime (30), Cefepime (30), Cefotaxime (30), Piperacillin(100), Ticarcillin (75), Piperacillin-Tazobactam (100/10), Ticarcillin-Clavulanic acid (75/10), Aztreonam (30), Imipenem (10), Meropenem (10), Ertapenem (10), Colistin (10), Gentamicin (10), Tobramycin (10), Amikacin (30), Netilmicin (30), Ciprofloxacin (5), Levofloxacin (5), Lomefloxacin (10) and Ofloxacin (5) (Hi Media Laboratories Pvt. Ltd., Mumbai, India). *P. aeruginosa* ATCC 27853, *E.coli* ATCC 25922, *E. coli* ATCC 35218 *and K. pneumoniae* ATCC 700603 were used as quality control strains.

MIC Determination

Minimum inhibitory concentrations (MIC) of antibiotics were determined by VITEK-2 AST-GN25 and AST-GN280 susceptibility cards in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations and manufacturers' instructions, except tigecycline and colistin, for which the 2012 European Committee on Antimicrobial Susceptibility Testing break points were used. [7] [8] MICs were further determined by the E-test (bioMérieux, Marcy l'Etoile, France).

Phenotypic Screening for Carbapenemase Production

Isolates with reduced susceptibility to meropenem and imipenem (diameter of zones of inhibition ≤13mm) by disc diffusion method were screened for the production of carbapenemase. MHT, DDST, CDST and MBL (IP/IPI) E-test was performed to detect Carbapenemase as well as Metallo-beta-lactamase production as described previously.[9,10]

DNA extraction and Molecular detection

DNA was extracted from the bacterial isolates using the spin column method (QIAGEN; GmbH, Hilden, Germany) as per manufacturer's instructions. PCR-based detection of beta lactamase (ESBL) genes (*bla*$_{CTXM}$, *bla*$_{SHV}$, *bla*$_{TEM}$ and *bla*$_{OXA}$), Ambler class B MBLs (*bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{SPM}$, *bla*$_{GIM}$, *bla*$_{SIM}$ and *bla*$_{NDM-1}$), Ambler class D (*bla*$_{OXA-23}$, *bla*$_{OXA-24}$ and *bla*$_{OXA48}$) and serine carbapenemases (*bla*$_{KPC,}$ *bla*$_{GES}$ and *bla*$_{NMC}$) were carried out on the isolates by using Gene Amp 9700 PCR System (Applied Biosystems, Singapore).[9] [10] PCR products were run on 1.5% agarose gel, stained with ethidium bromide visualized under UV light and photographed. The amplicons were purified using QIAquick PCR purification kit (QIAGEN; GmbH, Hilden, Germany)

DNA sequencing and sequence analysis

Automated sequencing was performed on an ABI 3730XL DNA analyzer using the Big Dye system (Applied Biosystems Foster City, CA, USA). Sequences were compared with known sequences using the BLAST facility (http://blast.ncbi.nlm.nih.gov).

Conjugation experiments

Transfer of resistance genes by conjugation was assayed by mating experiments in Luria–Bertani broth using *E. coli* isolates (Parental strains) as donors and an azide-resistant *E. coli* J53 as the recipient strain using 1:10 ratio. The transconjugants were selected on Luria–Bertani agar with selection based on growth on agar in the presence of ceftazidime (30µg/ml) and sodium azide (100µg/ml). Plasmids were separated and compared by co-electrophoresis with plasmid of known sizes from *E.coli* (V517and 39R861) on a horizontal 0.5% agarose gel at 50 volts for 3 Hrs. Bands were visualized with UV transilluminator after staining with 0.05%ethidium bromide.[9,10]

Strain molecular typing

Repetitive element based PCR (REP-PCR), Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) and Randomly Amplified Polymorphic DNA (RAPD) assays were performed to characterize *E.coli* strains recovered from patients.[9,10]Similarity clustering analysis was performed using unweighted pairgroup method with arithmetic mean and Dice coefficient. Clinical isolates with a similarity coefficient >85% were considered clonal.

Plasmid analysis

Plasmid from the parental strains and their transconjugants was extracted by using Qiagen plasmid mini kit (GmbH, Hilden, Germany) as per manufacturer's Instructions. Extracted plasmid DNA were subjected to Plasmid based replicon incompatibility (Inc) typing by using eighteen pairs of primers to perform five multiplex and three single PCRs which recognized F, FIA, FIB, FIC, B/O, X, Y, N, P, W, T, A/C, HI1, HI2, I1-Ic, L/M, K and FII replicons as described previously.[9,10] Plasmid replicons were determined for the ESBL as well as carbapenemase producing clinical isolates.

Statistical analysis

The prevalence of resistance to each antimicrobial agent, phenotypic detection of carbapenem hydrolyzing beta-lactamases, and prevalence of resistance determinants were recorded as percentage. Each conjugation experiment was repeated twice. The mean of the readings was calculated and interpreted according to each experiment specification. All data were reported and analyzed using SPSS software (version 20.0).

RESULT

Prevalence of *E.coli* among clinical specimens

A total of 1070 *E.coli* were obtained from various clinical specimens in a prospective study that was conducted in a 1000 bedded tertiary care centre in Pune, India. The largest proportion of specimens were from UTIs 64.8% (693), followed by 11.68% (125) in SSTIs, 10% (112) in RTIs, 9% (97) in BSIs, and 4% (43) in IAIs and Misc. respectively Table-1. Of the total number of samples, females contributed significant number of samples 695(65%) while males contributed 375(35%) shown in Table-2. Age of the subjects varies from 0 to 80 years. Maximum number of isolates (272) was from the age group of 20-29 yrs, followed by 30-39 yrs age group (238) and least number of isolates (11) was from ≥80 yrs. Chronological age wise distribution shown in Table-3. Of the total samples, highest number of samples 519 (48.50%) were from OBG, followed by 156 (15%) in ICU surgery, 103 (10%) in Surgery ward, 91 (8.5%) in Medicine ward, 87(8.1%) in ICU medical and remaining were from NICU, Urology, Orthopedics and ENT shown in Table-4. Majority of Carbapenem resistant *E.coli* were from UTIs 90 (40%), followed by SSTIs 45(20%), 18% (40) in RTIs, 30(13%) in BSIs and 21(9%) in IAIs respectively Table-1.

Antimicrobial susceptibility of *E.coli* isolates

Evaluation of antibiotic susceptibility pattern indicated that 21% *E.coli* were resistant against IPM, MEM, and ETP. Antibiogram and resistance percentage of E.coli various infection sites shown in Table-5 as determined by VITEK-2 and E-test. The proportions of resistance to other beta lactam group and to other classes of antibiotics was distributed as follows: AMP(61%;R), AMC (45%;R), SAM (43;R), CRO (43%;R), ATM(42%;R), CTX(42%;R), CZ(42%;R), CPZ (42%;R), CPD (42%;R), CAZ (42%;R), PIP (42%;R), FEP(41%;R), FOX (40%;R), GEN (34%;R), TOB (32%;R), AMK (31%;R), TET (35%;R), SXT (33%;R), SFP (31%;R). All isolates were sensitive to polymyxin B and colistin. MICs of IPM, MEM, and ETP in µg/ml as determined by VITEK-2 and E-test against *E.coli* shown in Table-6.

Phenotypic detection of carbapenem-hydrolyzing- beta-lactamases

Out of 1070 isolates, 226 were found carbapenem resistant as MICs was >4µg/ml against IPM, MEM, and ETP as determined by the E-test and VITEK-2, 185 were MBL producers and 41 were non MBL producers. Modified Hodge test for carbapenemase production was positive for 144 (13.5%), DDST in 157(14.7%), CDST in 158 (14.8%) isolates, MBL (IP/IPI) E-test was positive for 185(17.3%) and 3.8% isolates were Non MBL. Results of different phenotypic tests of *E.coli* recovered from various clinical specimens are shown in **Table-7**. Out of total E.coli isolated from blood (N=97), 30.92% (n=30) was found to be carbapenem resistant. Among 97 E.coli isolates from BSI, MBL E-test was found positive in 23 isolates, DDST in 23, CDST in 22 and MHT in 21 isolates. *i.e.* 23.71%, 23.71%, 22.68% and 21.64. Among *E.coli* (N=112) isolates from Endotracheal Aspirate and BAL fluid 35% (n=40) found to be carbapenem resistant, MBL E-test was found positive in 22 isolates, DDST in 20, CDST in 18 and MHT in 15 isolates i.e. 19.6%, 17.9%, 16.1% and 13.4% respectively. Among 125 E.coli isolates from SSTIs, MBL E-test was found positive in 39 isolates, DDST in 36, CDST in 32 and MHT in 18 isolates i.e. 31.2%, 28.8%, 25.6% and 14% respectively. Among 693 E.coli isolates from urine, MBL E-test was found positive for 80 isolates, MHT was positive in 75, CDST in 68 and DDST in 62.i.e. 11.5%, 10.8%, 9.8% and 8.9% respectively. Among 43 E.coli isolates from IAIs, MBL E-test was found positive in 21 isolates, CDST in 18, DDST in 16 and MHT in 15 isolates i.e. 48.8%, 41.8%, 37.2% and 34.8 % respectively.

Molecular characterization of carbapenem-hydrolyzing-beta-lactamases-encoding genes

The prevalence of MBL-encoding genes among *E.coli* isolates was determined in the present study, MBL *was* present in 185.Among the tested genes, bla_{NDM-1} was the most prevalent gene as it was detected in 153, bla_{VIM-2} in 29, and bla_{VIM-6} in 3 isolates.blaCTX-M was present in 165 isolates, followed by blaTEM-1 in 141 and blaSHV in 126.blaSHV-5, blaSHV-11, blaSHV-12, and blaSHV-28 are the commonest SHV genes detected in 13,10,55, and 48 of blaSHV producing isolates respectively whereas blaCTX-M-15, blaCTX-M-14 and blaCTX-M-28 are the commonest CTX-M ESBLs that were present in 106, 38 and 21 isolates. Molecular characterization of beta-lactamase genes in carbapenem resistant *E.coli* isolates recovered from clinical specimens shown in Figure 1-5.

Distribution of beta-lactamase genes

BSIs -NDM-1 gene was present in 21 isolates, NDM-1, TEM-1 and CTXM were found in 17 isolates while TEM-1, SHV, CTXM and NDM-1were present in 8 isolates Fig-20. WhileVIM-2 in association with CTXM-15 gene was present in 2 isolates, one isolate had co presence of VIM-2 with TEM-1 Fig-1.

RTIs -NDM-1, TEM-1 and CTXM were found together in 18 isolates while TEM-1, SHV, CTXM and NDM-1were co-present in 13 isolates While VIM-2 gene was present in 2 isolates Fig- 2.

SSTIs- A single NDM-1 gene was present in 9 isolates while TEM-1, SHV, CTXM and NDM-1 together were present in 15 isolates while VIM-2 together with CTXM and TEM-1gene was present in 7 isolates, while 4 isolates had co presence of TEM-1, SHV, CTXM andVIM-2.

UTIs

In *E.coli* NDM-1, TEM-1, SHV, CTXM and OXA-48 together were found in 32 isolates while NDM-1, TEM-1, SHV, and CTXM were found in 55 isolates, co presence of VIM-2, SHV, TEM-1 and CTXM were found in 8 isolates Fig-4.

IAIs- NDM-1 gene was present in 15 isolates, NDM-1, TEM-1 and CTXM were found together in 12 isolates while SHV, TEM-1, CTXM and NDM-1were Co-present in 6 isolates.VIM-2 and SHV gene Co-present in 6 isolates,while TEM-1, VIM-2 and CTXM-15 together were found in 2 isolates (Fig-5).

Conjugation

For conjugational studies half the numbers of *E.coli* isolates were selected from different infection sites and for further PCR based molecular typing of the plasmids. Bacterial identification of the transconjugants from Luria-Bertani agar was performed by using VITEK-GNI cards and MICs of antibiotics were determined by VITEK-2 AST susceptibility cards. MICs values of AMP, ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), Pipera-Tazobactam PTZ, CPZ, CTX, FOX, were high among transconjugants ; (MIC, ≥64 µg/ml). The transconjugants were resistant to imipenem IMP, MEM, and ertapenem ETP; (MIC, ≥8-32µg/ml), whereas MICs of AMK, GEN, TOB,CIP, MXF, LVX, TGC; (MIC, ≤2µg/ml), CST; (MIC, <1µg/ml) and ATM fall within susceptible range as determined by E-test. Results of conjugational studies on E.coli isolates that were recovered from various clinical specimens are shown in Table-8.

Plasmid Typing, and characterization of Plasmid

Plasmid from both the *E.coli* parental strains and their transconjugants was characterized and found that $bla_{\text{NDM-1}}$ gene was located on IncA/C, and IncHI1 plasmids. blaVIM-2 and $bla_{\text{VIM-6}}$ gene was carried on plasmids belonging to IncFII, IncY and IncB/O replicons. $Bla_{\text{CTX-M-15}}$,$bla_{\text{CTX-M-14}}$and $bla_{\text{CTX-M-28}}$was associated with multiple replicon(IncTand IncY) & (IncFIA and IncFIB) in association with $bla_{\text{NDM-1}}$ gene while $bla_{\text{SHV-5}}$, $bla_{\text{SHV-11}}$ $bla_{\text{SHV-28}}$, $bla_{\text{SHV-12}}$ and $bla_{\text{TEM-1}}$ was located on IncP, IncW IncB, IncFIC and IncFIA type replicons respectively. Plasmid Typing and its characterization are shown in Table-9.

Strain molecular typing

Molecular typing of 182 strains of E.coli by RAPD generated15 cluster assigned as E.c-A to E.c-O with an average of 4 to 14 fragments per E.coli strains Figure-67. ERIC PCR produced 16 clonal clusters with an average of 7 to 13 fragments per E.coli strains Figure-7. REP PCR produced 16 clonal clusters with an average of 11 to 15 fragments per E.coli strains Figure-8.

Table 1: Showing distribution of Carbapenem resistant *E.coli* from total isolated from various sites of infections.

Specimen	SSTIs	BSIs	RTIs	UTIs	IAIs	Total
Carbapenem resistant	45	30	40	90	21	**226**
Total Isolated	125	97	112	693	43	**1070**

Table 2: Showing Gender wise distribution of *E.coli.*

Organism	Total Cases	Number of Males	Percentage	Number of Females	Percentage
E.coli	1070	375	35.04	695	64.95

Table 3: Showing Chronological age wise distribution of *E.coli* patients.

Age in Years	Number of Patients
0-9	35
10-19	62
20-29	272
30-39	238
40-49	165
50-59	118
60-69	106
70-79	63
80 above	11
Total	1070

Table 4: Showing number wise ward percentage distribution of *E.coli.*

Wards	Number of Cases (N=1070)	Case Percentage
OBG ward	519	48
ICU Surgery	156	15
Surgery ward	103	10
Medicine ward	91	8.5
ICU Medical	87	8.1
Urology	68	6.3
Paediatrics ward	17	1.5
Orthopedics	13	1.2
NICU	9	0.8
ENT	7	0.6

IPM, imipenem; MEM, meropenem; ETP, ertapenem; AMK, amikacin; GEN,gentamicin; TOB, tobramycin; CIP, ciprofloxacin; MXF, moxifloxacin; TGC,tigecycline; SXT, trimethoprim-sulfamethoxazole; SAM, ampicillin-sulbactam; TZP,piperacillin-tazobactam; FEP, cefepime; AMP, ampicillin; CRO, ceftriaxone; CAZ,ceftazidime; CS, colistin.

Table-5: Showing Antibiogram and resistance percentage of *E.coli* various infection sites.

Antibiotics	BSIs	RTIs	SSTIs	UTIs	IAIs	Resistance	%	Sensitivity	%
AMK	40	48	70	155	21	334	31	736	69
AMC	56	56	95	240	35	482	45	588	55
AMP	52	89	103	369	35	648	61	422	39
SAM	45	45	98	240	30	458	43	612	57
ATM	52	48	80	240	30	450	42	620	58
FEP	43	48	75	240	30	436	41	634	59

CTX	48	48	81	240	30	447	42	623	58
FOX	43	48	67	240	30	428	4	642	6
CZ	51	48	80	240	30	449	42	621	58
SFP	40	48	72	155	21	336	31	734	69
CPZ	51	48	80	240	30	449	42	621	58
CPD	51	48	81	240	30	450	42	620	58
CAZ	49	48	81	240	30	448	42	622	58
CRO	51	48	89	240	30	458	43	612	57
CIP	49	48	87	155	30	369	34	701	66
ETP	**30**	**40**	**45**	**90**	**21**	**226**	21	844	79
GEN	45	48	79	155	35	362	34	708	66
IPM	**30**	**40**	**45**	**90**	**21**	**226**	**21**	844	79
LVX	42	42	69	155	21	329	31	741	69
MEM	**30**	**40**	**45**	**90**	**21**	**226**	**21**	844	79
PIP	52	48	81	240	30	451	42	619	58
TZP	40	44	67	155	21	327	31	743	69
TET	49	59	56	176	30	370	35	700	65
TGC	12	6	34	N T	2	54			
TOB	45	48	69	155	30	347	32	723	68
SXT	30	67	75	155	30	357	33	713	67
TOTAL	97	112	125	693	43	**1070**			

Table 6: Showing MICs of IPM, MEM, and ETP against *E.coli.*

Antibiotic concentrations (µg/ml)	Number of sample (N=1070)	Percentage
0.25	142	13.27
0.5	179	16.72
1	213	19.90
2	310	28.97
4	20	1.86
8	40	3.73
16	70	6.54
32	63	5.88
64	33	3.08

Table 7: Showing percentage and result of different phenotypic tests of *E.coli* recovered from various infection sites.

Infection sites	Carbapene m resistant by MIC[a]	MBL E Test[b]	DDST[c]	CDST[d]	MHT[e]	NON MBL[f]
SSTIs	45	39	36	32	18	06
BSIs	30	23	23	22	21	07
RTIs	40	22	20	18	15	18
UTIs	90	80	62	68	75	10
IAIs	21	21	16	18	15	00
TOTAL	226(21.1%)	185(17.3%)	157(14.7%)	158(14.8%)	144(13.5%)	41(3.8)

a MIC values for imipenem, meropenem and ertapenem ≥ 4µg/ml, b **MBL (IP/IPI)** E-test, **c DDST**- Double-disc synergy tests (DDST)**, CDST**- Combined-disc synergy test, **e MHT** - Modified Hodge test **and f NON MBL[f]**

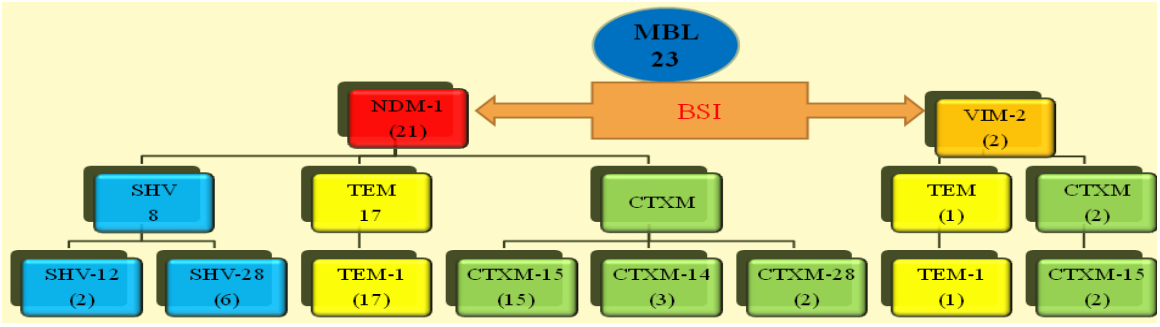

Fig. 1: Showing distribution of beta-lactamase genes in association with MBL genes in *E.coli* isolated from BSIs.

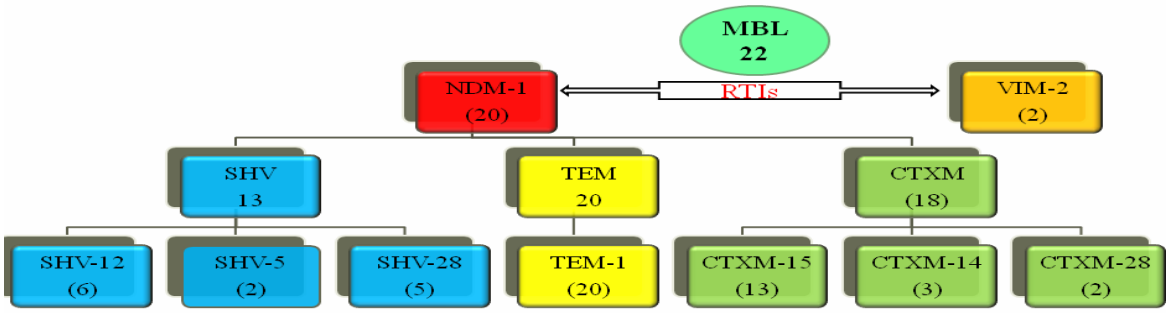

Fig. 2: Showing distribution of beta-lactamase genes in association with MBL genes in E.coli isolated from RTIs.

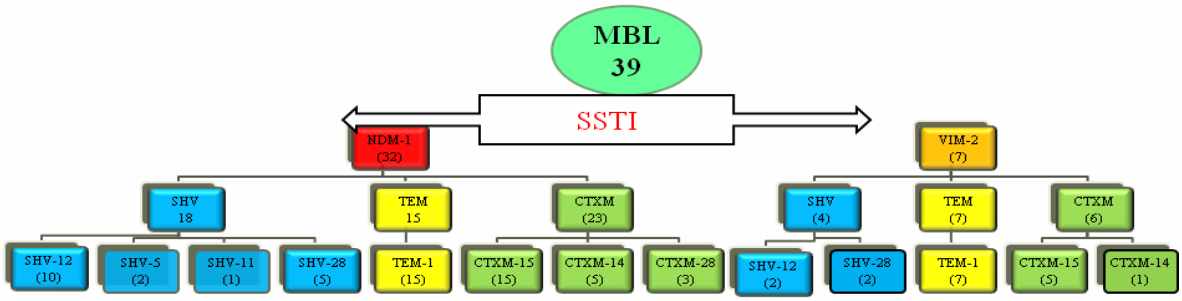

Fig. 3: Showing distribution of beta-lactamase genes in association with MBL genes in *E.coli* isolated from SSTIs.

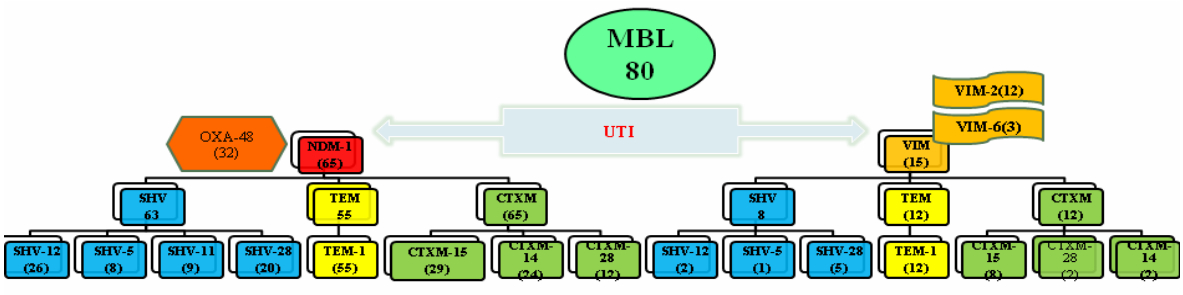

Fig. 4: Showing distribution of beta-lactamase genes in association with MBL genes in *E.coli* isolated from UTIs.

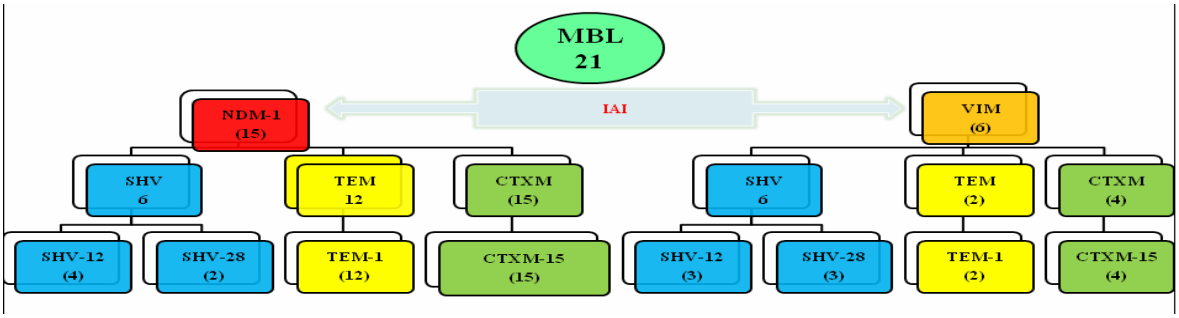

Fig. 5: Showing distribution of beta-lactamase genes in association with MBL genes in *E.coli* isolated from IAIs.

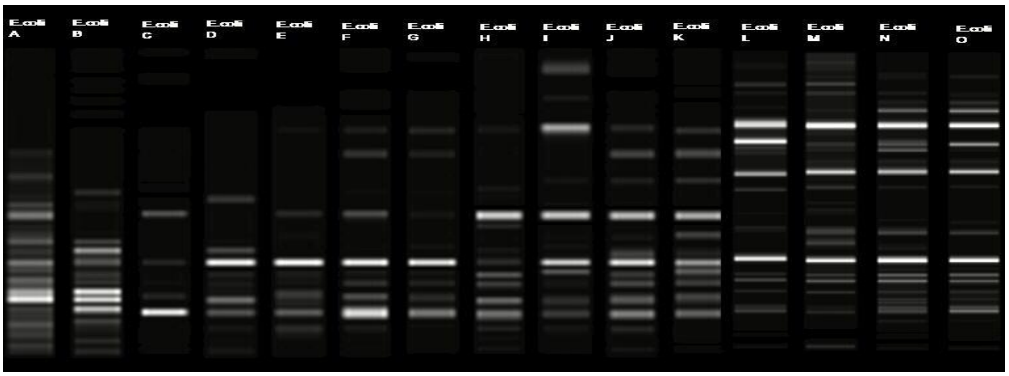

Figure 6: Showing RAPD PCR banding pattern among 15 clonal cluster of *E.coli.*

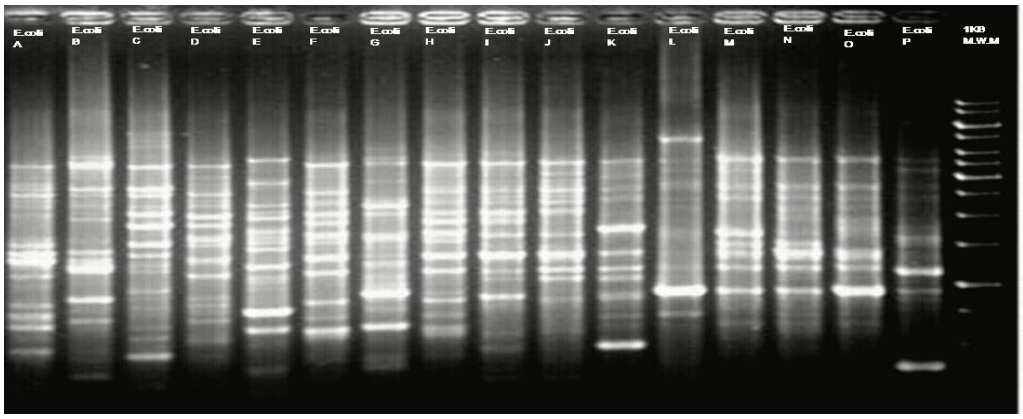

Figure 7: Showing ERIC PCR banding pattern among 16 clonal cluster of *E.coli.*

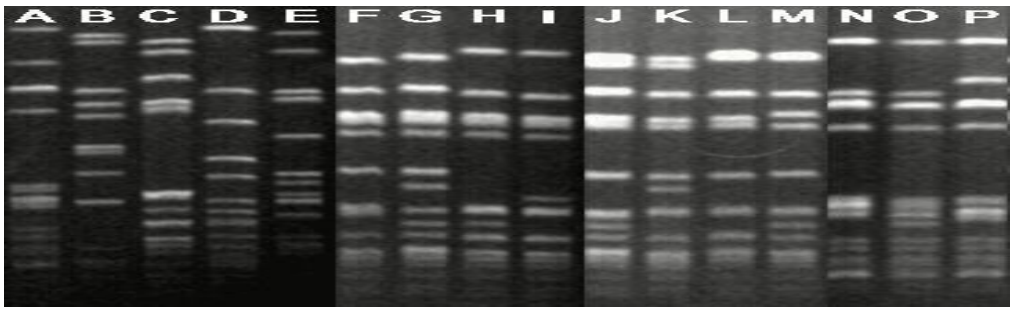

Figure 8: Showing REP PCR band among 16 clonal cluster of *E.coli.*

Table 8: Showing Antibiogram of donor and Transconjugants.

ISOLATE	IPM	MEM	ETP	ATM	CS	TGC	CAZ	CTX	CN	FEP	CPZ	PT	AMP	CIP	LVX	MOX	GEN	AMK	TOB
BACT 188	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC188	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
BACT403	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC403	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
BACT454	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC454	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
BACT692	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC692	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
BACT722	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC722	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
ISOLATE	**IPM**	**MEM**	**ETP**	**ATM**	**CS**	**TGC**	**CAZ**	**CTX**	**CN**	**FEP**	**CPZ**	**PT**	**AMP**	**CIP**	**LVX**	**MOX**	**GEN**	**AMK**	**TOB**
BACT1036	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC1036	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
BACT1223	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC1223	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
BACT1280	32	32	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC1280	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
BACT1403	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC1403	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
BACT1519	32	32	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC1519	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
ISOLATE	**IPM**	**MEM**	**ETP**	**ATM**	**CS**	**TGC**	**CAZ**	**CTX**	**CN**	**FEP**	**CPZ**	**PT**	**AMP**	**CIP**	**LVX**	**MOX**	**GEN**	**AMK**	**TOB**
ETB744	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC744	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
ETB811	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC811	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
ETB831	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC831	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1

ETB844	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC844	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
ETB877	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC877	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
ETB893	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC893	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
ETB945	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC945	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
ETB1015	32	32	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC1015	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
ETB1072	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC1072	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
ETB1145	32	32	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC1145	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
ETB1242	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC1242	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
ETB1770	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC1770	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
ETB2064	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC2064	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
ETB2114	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC2114	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
ETB2204	32	32	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2204	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
ISOLATE	**IPM**	**MEM**	**ETP**	**ATM**	**CS**	**TGC**	**CAZ**	**CTX**	**CN**	**FEP**	**CPZ**	**PT**	**AMP**	**CIP**	**LVX**	**MOX**	**GEN**	**AMK**	**TOB**
PC232	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC232	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
PC237	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC237	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
PC251	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16

TC251	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
PC260	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC260	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
PC275	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC275	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
PC282	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC282	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
PC291	32	32	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC291	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
PC311	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC311	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
PC331	32	32	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC331	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
PC346	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC346	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
PC305	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC305	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
PC368	32	32	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC368	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
PC383	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC383	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
PC485	32	32	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC485	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
PC459	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC459	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
PC504	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC504	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
PC517	32	32	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC517	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
PC549	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC549	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2

PC573	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC573	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
ISOLATE	**IPM**	**MEM**	**ETP**	**ATM**	**CST**	**TGC**	**CAZ**	**CTX**	**FOX**	**FEP**	**CPZ**	**PT**	**AMP**	**CIP**	**LVX**	**MOX**	**GEN**	**AMK**	**TOB**
UC10	64	64	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC10	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
UC53	64	64	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC53	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
UC66	64	64	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC66	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
UC251	64	64	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC251	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
UC320	64	64	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC320	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
UC405	64	64	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC405	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
UC480	64	64	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC480	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
UC1387	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC1387	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
UC1445	64	64	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC1445	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
UC1632	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC1632	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
UC1686	64	64	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC1686	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
UC1880	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC1880	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.25	1	2	1
UC2044	64	64	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC2044	16	16	16	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	2	1
UC2330	64	64	32	64	0.5	0.25	128	128	64	64	128	>128	256	4	8	4	>16	64	>16

TC2330	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	1
UC2454	64	64	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC2454	16	8	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	1
UC2632	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC2632	16	8	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
UC2778	64	64	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2778	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	0.5	0.25	1	2	1
UC2981	64	64	32	64	0.125	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC2981	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	1	1	1
UC3028	64	64	32	64	0.25	0.25	128	128	64	64	128	>128	256	8	8	4	>16	64	>16
TC3028	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	2	2	2
UC3239	64	64	32	64	0.5	2	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC3239	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	1	2	1
UC3521	64	64	32	64	0.125	0.5	256	256	64	64	256	>128	256	8	8	4	>16	64	>16
TC3521	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
UC3649	64	64	32	64	0.125	1	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC3649	8	16	16	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	1
UC3741	64	64	32	64	0.25	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC3741	8	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	2	1	1
UC4008	64	64	32	64	0.5	2	256	256	64	64	256	>128	256	8	8	4	>16	64	>16
TC4008	16	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	1	1
UC4330	64	64	32	64	0.125	1	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC4330	16	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	1	1	1	2
UC2044	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC2044	16	8	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
UC2050	64	64	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2050	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	0.5	0.25	1	2	1
UC2078	64	64	32	64	0.125	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC2078	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	1	1	1
UC3017	64	64	32	64	0.25	0.25	128	128	64	64	128	>128	256	8	8	4	>16	64	>16
TC3017	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	2	2	2

UC3003	64	64	32	64	0.5	2	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC3003	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	1	2	1
UC3156	64	64	32	64	0.125	0.5	256	256	64	64	256	>128	256	8	8	4	>16	64	>16
TC3156	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
UC3168	64	64	32	64	0.125	1	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC3168	8	16	16	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	1
UC3233	64	64	32	64	0.25	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC3233	8	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	2	1	1
UC3244	64	64	32	64	0.5	2	256	256	64	64	256	>128	256	8	8	4	>16	64	>16
TC3244	16	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	1	1
UC3290	64	64	32	64	0.125	1	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC3290	16	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	1	1	1	2
UC3332	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC3332	16	8	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
UC3422	64	64	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC3422	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	0.5	0.25	1	2	1
UC3458	64	64	32	64	0.125	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC3458	16	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	1	1	1	2
UC3449	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC3449	16	8	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
UC3522	64	64	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC3522	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	0.5	0.25	1	2	1
ISOLATE	**IPM**	**MEM**	**ETP**	**ATM**	**CS**	**TGC**	**CAZ**	**CTX**	**CN**	**FEP**	**CPZ**	**PT**	**AMP**	**CIP**	**LVX**	**MOX**	**GEN**	**AMK**	**TOB**
IAIS83	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC831	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
IAIS112	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC112	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
IAIS241	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC241	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
IAIS309	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16

TC309	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
IAIS383	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC383	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
IAIS401	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC401	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
IAIS428	32	32	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC428	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
IAIS493	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC493	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
IAIS502	32	32	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC502	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
IAIS513	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC513	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2

Table 9: Showing Plasmid Typing and characterization of Plasmid among donor and Transconjugants.

***E.coli* (N=18)**												
ISOLATE	**MBL**	**Plasmid**	**Transfer**	**Other ESBL gene present**			**Plasmid type**			**Transfer**		
ETB744	NDM-1	A/C	transferable	TEM-1	CTXM-14	SHV12	FIA	T	FIB	transferable		
ETB811	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable		
ETB831	NDM-1	A/C	transferable	TEM-1	CTXM-14	SHV12	FIA	T	FIB	transferable		
ETB844	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV05	FIA	T	P	transferable		
ETB877	NDM-1	A/C	transferable	TEM-1	CTXM-14	SHV12	FIA	T	FIB	transferable		
ETB893	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable		
ETB945	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable		
ETB1015	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV05	FIA	T	P	transferable		
ETB1072	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable		
ETB1145	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable		
ETB1242	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable		
ETB1770	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable		
ETB2064	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable		
ETB2114	VIM-2	FII	transferable	*	*	*	*	*	*	transferable		

ETB2204	VIM-2	FII	transferable	*	*	*	*	*	*	transferable		
E.coli (N=10) isolates.												
ISOLATE	MBL	Plasmid	Transfer	Other ESBL gene present			Plasmid type			Transfer		
BACT 188	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable		
BACT403	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	P	transferable		
BACT454	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable		
BACT692	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable		
BACT722	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	P	transferable		
BACT1036	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	Transferable		
BACT1223	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	Transferable		
BACT1280	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	Transferable		
BACT1403	VIM-2	FII	transferable	*	CTX-M-15	*	*	T	*	Transferable		
BACT1519	VIM-2	FII	transferable	TEM-1	CTX-M-15	*	FIA	T	*	Transferable		
E.coli (N=19) isolates.												
PC232	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIB	FIA,FIB	P	transferable		
PC237	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	FIA,FIB	FIC	transferable		
PC251	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIB	FIA,FIB	FIB	transferable		
PC260	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIB	FIA,FIB	FIA	transferable		
PC275	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	FIA,FIB	P	transferable		
PC282	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIB	FIA,FIB	FIC	transferable		
PC291	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIB	FIA,FIB	FIA	transferable		
PC311	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	FIA,FIB	FIC	transferable		
PC331	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIB	FIA,FIB	FIB	transferable		
PC346	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	FIA,FIB	FIC	transferable		
PC305	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	FIA,FIB	P	transferable		
PC368	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIB	FIA,FIB	FIC	transferable		
PC383	NDM-1	HI1	transferable	TEM-1	CTX-M-15	SHV12	FIB	FIA,FIB	FIB	transferable		
PC485	NDM-1	HI1	transferable	TEM-1	CTX-M-15	SHV28	FIA	FIA,FIB	FIC	transferable		
PC459	NDM-1	HI1	transferable	TEM-1	CTX-M-15	SHV12	FIA	FIA,FIB	FIB	transferable		
PC504	VIM-2	Y	transferable	TEM-1	CTX-M-15	SHV28	FIB	B/O	FIC	transferable		
PC517	VIM-2	Y	transferable	TEM-1	CTX-M-15	SHV12	FIB	B/O	FIA	transferable		

PC549	VIM-2	Y	transferable	TEM-1	CTX-M-15	SHV12	FIB	B/O	FIA	transferable		
PC573	VIM-2	Y	transferable	TEM-1	CTX-M-15	SHV28	FIB	B/O	FIC	transferable		
E.coli (N=40) isolates.												
UC10	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIA	transferable	OXA-48	L/M
UC53	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	Y	FIC	transferable	OXA-48	L/M
UC66	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIA	transferable	OXA-48	L/M
UC251	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable	OXA-48	L/M
UC320	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable	OXA-48	L/M
UC405	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	Y	FIC	transferable	OXA-48	L/M
UC480	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable	OXA-48	L/M
UC1387	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable	OXA-48	L/M
UC1445	NDM-1	A/C	transferable	ND	CTX-M-15	SHV12	ND	T	FIB	transferable	OXA-48	L/M
UC1632	NDM-1	A/C	transferable	ND	CTX-M-15	SHV12	ND	T	FII	transferable	OXA-48	L/M
UC1686	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIA	transferable	OXA-48	L/M
UC1880	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable	OXA-48	L/M
UC2044	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	Y	FIC	transferable	OXA-48	L/M
UC2330	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	Y	FIC	transferable	OXA-48	L/M
UC2454	NDM-1	A/C	transferable	ND	CTX-M-15	SHV05	ND	T	W	transferable	OXA-48	L/M
UC2632	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	I1-Ic	transferable	OXA-48	L/M
UC2778	NDM-1	A/C	transferable	TEM-1	CTX-M-28	SHV11	FIA	Y	W	transferable	OXA-48	L/M
UC2981	NDM-1	A/C	transferable	TEM-1	CTX-M-28	SHV05	FIA	Y	P	transferable	OXA-48	L/M
UC3028	NDM-1	A/C	transferable	TEM-1	CTX-M-28	SHV11	FIA	Y	W	transferable	OXA-48	L/M
UC3239	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	Y	FIC	transferable	OXA-48	L/M
UC3521	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	Y	FIC	transferable	OXA-48	L/M
UC3649	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	Y	FIC	transferable	OXA-48	L/M
UC3741	NDM-1	A/C	transferable	ND	CTX-M-15	SHV11	ND	T	P	transferable	OXA-48	L/M
UC4008	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable	OXA-48	L/M
UC4330	NDM-1	A/C	transferable	TEM-1	CTX-M-28	SHV05	FIA	Y	P	transferable	OXA-48	L/M
UC2044	NDM-1	A/C	transferable	TEM-1	CTX-M-28	SHV11	FIA	Y	W	transferable	OXA-48	L/M
UC2050	NDM-1	A/C	transferable	TEM-1	CTX-M-28	SHV11	FIA	Y	W	transferable	OXA-48	L/M
UC2078	NDM-1	A/C	transferable	TEM-1	CTX-M-28	SHV11	FIA	Y	W	transferable	OXA-48	L/M

UC3017	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	T	FIC	transferable	OXA-48	L/M
UC3003	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	T	FIC	transferable	OXA-48	L/M
UC3156	NDM-1	A/C	transferable	TEM-1	CTX-M-14	SHV28	FIA	T	FIC	transferable	OXA-48	L/M
UC3168	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV05	FIA	T	P	transferable	OXA-48	L/M
UC3233	VIM-2	FII	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable	ND	ND
UC3244	VIM-6	B/O	transferable	TEM-1	CTX-M-14	SHV28	FIA	Y	FIC	transferable	ND	ND
UC3290	VIM-2	FII	transferable	TEM-1	CTX-M-14	SHV12	FIA	Y	FIB	transferable	ND	ND
UC3332	VIM-2	FII	transferable	TEM-1	CTX-M-15	SHV05	FIA	Y	P	transferable	ND	ND
UC3422	VIM-6	B/O	transferable	TEM-1	CTX-M-28	SHV12	FIA	Y	FIB	transferable	ND	ND
UC3458	VIM-6	B/O	transferable	TEM-1	CTX-M-28	SHV12	FIA	Y	FIB	transferable	ND	ND
UC3449	VIM-2	FII	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	transferable	ND	ND
UC3522	VIM-2	FII	transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	transferable	ND	ND
E.coli (N=10) isolates.												
IAI83	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	P	transferable		
IAI112	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	Y	FIC	transferable		
IAI241	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	P	transferable		
IAI309	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	Y	FIC	transferable		
IAI383	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	P	transferable		
IAI401	NDM-1	A/C	transferable	TEM-1	CTX-M-15	SHV28	FIA	Y	FIC	transferable		
IAI428	VIM-2	FII	transferable	TEM-1	CTX-M-15	SHV12	FIA	T	P	transferable		
IAI493	VIM-2	FII	transferable	TEM-1	CTX-M-15	SHV28	FIA	Y	FIC	transferable		
IAI502	VIM-2	FII	transferable	*	CTX-M-15	SHV12	*	T	P	transferable		
IAI513	VIM-2	FII	transferable	*	CTX-M-15	SHV28	*	Y	FIC	transferable		

DISCUSSION

In the present study, *E.coli* had shown a prevalence of 17.3% in MBL production. Prevalence of MBL in E.coli ranged from 4% to 70%.[3,4,5] Our results was in concordance with various other authors throughout India and abroad while some previously reported studies had lower values as compared to this study[3,4,5,11,12] this variation was due to antibiotic usage. Previously cephalosporins were used tremendously and know carbapenem are in a use. In a study by Seema K et al from February 2010 to July 2010, out of 528 *E.coli* 6% were MBL producers and all were NDM-1 producers by PCR,[12] while Kazi M et al had showed prevalence of 27% in carbapenem resistant *E.coli* and among them 75.22 % were blaNDM-1 producers and 80% concordance was observed.[13] In the present study, among carbapenem producers *bla*NDM-1was detected in 67.7% isolates, while *bla*VIM was present in 14.3% isolates whereas Nagaraj S from south India had reported a prevalence of *bla*NDM-1 gene in 66% while 15% had incidence of *bla*VIM gene in E.coli these results are in concordance with our study the only variation is small sample size as compared to our study[14] while In a single centre study in Mumbai, 22% of E.coli have been blaNDM-1 producers all these data from INDIA as reported by various authors had shown concordance with the findings of our study.[15] Moreover, emergence of blaNDM-1 gene in *E.coli* from Mumbai had been reported by Deshpande P et al in 2009 and proved MBL producers in Indian sub continent with a prevalence of 40% MBL producing *E.coli* in total GNB.[16] In a multi centric study, out of 75 *E.coli* isolates from Chennai 25.3% were found to be MBL producers whereas out of 37 UK *E.coli* isolates, 18.91% were MBL producers and all the MBL producing isolates were NDM-1 producers by PCR.[17] In the present study, among carbapenem producers blaNDM-1was detected in 67.7% isolates, while blaVIM was present in 14.3% isolates, blaCTX-M was present in 89% isolates, followed by blaTEM-1 in 81% and blaSHV in 69.2%. In this study, blaSHV-5, blaSHV-11, blaSHV-12, and blaSHV-28 are the commonest SHV genes detected in 8%, 10.1%, 37.5% and 48.5% of blaSHV producing isolates respectively whereas blaCTX-M-15, blaCTX-M-14 and blaCTX-M-28 are the commonest CTX-M ESBLs that were present in 64.2%, 23% and 12.8% of blaCTX-M producing isolates respectively suggesting co association of multiple variants of ESBL along with MBLs that aids in marked increase in MICS against cephalosporins and other beta lactams including carbapenems and thereby increasing antimicrobial resistance towards commonly used drugs. In the present study, bla_{NDM-1}, bla_{OXA-48}, bla_{CTX-M}, bla_{SHV}, bla_{TEM-}, bla_{OXA-1}, bla_{OXA-2}, bla_{OXA-9} and bla_{OXA-10} genes altogether were reported in 32 urinary isolates (17.3%) of MBL producing *E.coli.* Though, the strain remains sensitive for tigecycline in vitro but it is not recommended for use

in UTI infections. Colistin is the main stay of therapy. Furthermore, bla_{IMP}, bla_{KPC}, bla_{GES}, bla_{SIM}, bla_{SPM} and bla_{GIM} were not detected in any of the study isolates.

CONCLUSION

These findings highlights prevalence of bla_{OXA-48} and bla_{NDM-1}, producing *E.coli* in multiple combinations with other β-lactamases which are commonly found a single or multiple plasmids to serve as driving force for the horizontal spread of emerging carbapenem resistance that are critically important for treatment of human bacterial infections. It further indicates dissemination of NDM-1in multidrug resistant *E.coli* makes them refractory to the common antibiotics used in clinical practice.

Declarations

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