



**INCIDENCE AND DETECTION OF METALLO-BETA-LACTAMASE PRODUCING
KLEBSIELLA PNEUMONIAE IN A TERTIARY CARE HOSPITAL**

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Article Received on 25/12/2018

Article Revised on 15/01/2019

Article Accepted on 04/02/2019

ABSTRACT

Objective: To examine the distribution, emergence and spread of genes encoding beta-lactamase resistance in *Klebsiella pneumoniae* 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 659 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:** A total of 659 *K.pneumoniae* isolates were recovered, largest proportion of specimens were from UTIs 30%, followed by 25% in SSTIs, 21.3% in BSIs, 16% in RTIs, and 8% in IAIs and Misc. respectively. Among 659 tested isolates, **32.8%** isolates showed MIC >4µg/ml against imipenem and meropenem. Of the total number of samples, males contributed 68.1% while females contributed 31.9%. Of the total samples, highest 28.11% were from OBG, followed by 20.6% in surgery ward, 17.17% in ICU Surgery, 12.46% in Medicine ward and 10% in ICU Medical. Majority of Carbapenem resistant *K.pneumoniae* 28.83% were from UTIs, followed by 20% in SSTIs, 25.58% in BSIs, 19.4% in RTIs, and 6.51% in IAIs respectively. MHT was positive in **20.7%**, DDST in **24.3%**, CDST in **26.6%**, and MBL (IP/IPI) E-test in **29.3%** isolates. 100% *K.pneumoniae* isolates retained susceptible 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*_{VIM}, *bla*_{OXA-48} and *bla*_{NDM-1}, producing *K.pneumoniae* along with other β-lactamases genes carried on a single or multiple plasmids that serve as a driving force for the horizontal spread of carbapenem resistance. **Running title-** Metallo-beta-lactamase resistance in *Klebsiella pneumoniae*.

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

Klebsiella pneumoniae species are associated with human disease, capable of causing UTIs, liver abscess, and ventilator associated pneumonia. However, most infections caused by *K.pneumoniae* are acquired in the hospital and occur in those patients who are debilitated by various underlying conditions. In addition to this, nosocomial infection caused by *K.pneumoniae* includes wound infections, infections of intravascular and other invasive devices, biliary tract infections, peritonitis, and meningitis. *K.pneumoniae* can cause UTIs in individuals with normal as well as abnormal urinary tracts and is second only to *E. coli* as a cause of bacteremia resulting from UTI and of gram-negative bacteremia. Emergence of nosocomial *K.pneumoniae* with β-Lactamases resistance is a major health challenge. *K.pneumoniae* producing ESBL 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 *K.pneumoniae*.^[1-4] A number of risk factors are involved in association with MDR *K.pneumoniae* infections in an immunocompromised host such as invasive procedures including abdominal surgery, arterial and central venous catheterisation, urinary catheterisation, and mechanical ventilation; low birth weight in infants; in prolonged hospital stays; prior antibiotic use, particularly cephalosporins and aminoglycosides; and colonisation of the GI tract.^[1-4] Resistance to carbapenems is most frequently mediated by the enzymatic hydrolysis of the drugs by *K.pneumoniae*. 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 *K.pneumoniae* producing these Carbapenemases are disturbing as these multidrug-

resistant infections leave patients with very few or no antimicrobial options.^[5-7] Based on these considerations, this study was undertaken to detect prevalence of metallo-beta-lactamases resistance in *K.pneumoniae* 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 659 isolates were recovered from clinical specimens from different patients (one isolate per patient). Samples were collected from patients, using strict aseptic precautions and in accordance with standard protocols^[8,9] and immediately processed without any delay. *K.pneumoniae* 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.^[10] 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.^{[10] [11]} 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.^{[12] [13]}

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).^{[12] [13]} 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 *K.pneumoniae* 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 (30 µg/ml) and sodium azide (100 µg/ml). Plasmids were separated and compared by co-electrophoresis with plasmid of known sizes from *E.coli* (V517 and 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.^{[12] [13]}

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 *K.pneumoniae* strains recovered from patients.^{[12] [13]} 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.^{[12] [13]} 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 *K.pneumoniae* among clinical specimens

A total of 659 *K.pneumoniae* isolates were recovered from various clinical specimens in a prospective study that was conducted in a 1800 bedded tertiary care centre in Pune, India. The largest proportion of specimens were from UTIs 198 (30%), followed by 165(25%) in SSTIs, 140 (21.3%) in BSIs, 103(16%) in RTIs, and 53 (8%) in IAIs and Misc. respectively. Among 659 tested isolates, **216 (32.8%)** isolates showed MIC >4µg/ml against imipenem and meropenem. Of the total number of samples, males contributed significant number of samples 448 (68.1%) while females contributed 210 (31.9%) **Table-2**. Maximum number of isolates (113) was from the age group of 70-79yrs, followed by 40-49 yrs age group (97) and least number of isolates (21) was from 0-9years as shown in **Table-3**. Of the total samples, highest number of samples 185 (28.11%) were from OBG, followed by surgery ward 132 (20.06%), ICU Surgery 113 (17.17%), Medicine ward 82 (12.46%), ICU Medical 66(10%) and remaining were from Paedric, NICU, Urology, orthopedics and ENT. Number wise ward percentage distribution of *K.pneumoniae* shown in **Table-4**. Majority of Carbapenem resistant *K.pneumoniae* were from UTIs 62(28.83%), followed by SSTIs 43(20%), 55(25.58%) in BSIs, 41 (19.4%) in RTIs, and 14(6.51%) in IAIs respectively **Table-1**.

Antimicrobial susceptibility of *K.pneumoniae* isolates

Evaluation of antibiotic susceptibility pattern indicated that 21% *K.pneumoniae* were resistant against IPM, MEM, and ETP. Antibiogram and resistance percentage of *K.pneumoniae* 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(72%;R), AMC (57%;R), SAM (54;R), CRO (49%;R), ATM(47%;R), CTX(48%;R), CZ(48%;R), CPZ (48%;R), CPD (51%;R), CAZ (48%;R), PIP (52%;R), FEP (45%;R), FOX (46%;R), GEN (47%;R), TOB (48%;R), AMK (47%;R), TET (55%;R), SXT (57%;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 *K.pneumoniae* shown in Table-6.

Phenotypic detection of carbapenem-hydrolyzing-beta-lactamases

Out of 659 isolates, **216 (32.8%)** were found carbapenem resistant as MICs was ≥4µg/ml against IPM, MEM, and ETP as determined by the E-test and VITEK-2. Modified Hodge test for carbapenemase production was positive for **137(20.7%)**, DDST in **161 (24.3%)**, CDST in **176(26.6%)** isolates, MBL (IP/IPI) E-test was positive for **194(29.3%)** and **22(3.3%)** isolates were Non MBL. Results of different phenotypic tests of *K.pneumoniae* recovered from various clinical specimens are shown in **Table-7**. Out of total *K.pneumoniae* isolated from BSI (N=140), 39.28% (n=55) was found to be carbapenem resistant. Among 140, *K.pneumoniae* isolates from BSI, MBL E-test was found positive in 54, CDST in 45, DDST in 42 and MHT in 31 *i.e.* 38.57%, 32.14%, 30% and 22.14% respectively. Among 103, *K.pneumoniae* isolates from Endotracheal aspirate and BAL fluid, MBL E-test was positive in 37, CDST in 35, DDST in 33 and MHT in 22 isolates. *i.e.* 36%, 34%, 32% and 21.4%. Among 165, *K.pneumoniae* isolates from SSTIs, MBL E-test was positive in 41 isolates, CDST in 38, MHT in 34 and DDST in 31 isolates. *i.e.* 24.8%, 23%, 20.6% and 18.8% respectively. Among 196, *K.pneumoniae* isolates from urine MBL E-test was found positive in 54, CDST in 51, DDST in 48 and MHT in 43 isolates. *i.e.* 27.5%, 26%, 24.5% and 21.9 % respectively. Among 53, *K.pneumoniae* isolates from IAIs, MBL E-test was positive in 8 isolates, both CDST and DDST in 7 and MHT in 6 isolates. *i.e.* 15.1%, 13.2%, 13.2% and 11.3% respectively.

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

The prevalence of MBL-encoding genes among *K.pneumoniae* isolates was determined in the present study, MBL was present in 193. Among the tested genes, *bla*_{NDM-1} was the most prevalent gene as it was detected in 142, *bla*_{VIM-2} in **42**, *bla*_{VIM-6} in **9** and OXA-48 in 22 isolates, blaCTX-M was present in 165 isolates, followed by blaTEM-1 in 146 and blaSHV in 134. blaSHV-5, blaSHV-11, blaSHV-12, and blaSHV-28 are the commonest SHV genes detected in 19,19,52, and 44 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 112, 32 and 21 isolates. Molecular characterization of beta-lactamase genes in carbapenem resistant *K.pneumoniae* isolates recovered from clinical specimens shown in Figure 1-5.

Distribution of beta-lactamase genes Table-8

BSIs

NDM-1 gene was present in 36 isolates, co presence of NDM-1, TEM-1 and CTXM were found in 31 isolates while SHV, TEM-1, CTXM and NDM-1 all together

were present in 29 isolates Fig-1. *Bla*_{VIM-2} gene was present in 18 isolates, three isolates had only *bla*_{VIM-6} gene. Co presence of VIM, TEM-1 and CTXM were found in 8 isolates while SHV, CTXM and VIM gene all together were present in 14 isolates Fig-1.

RTIs

NDM-1, TEM-1 and CTXM were found together in 25 isolates while SHV, CTXM and NDM-1 were co-present in 30 isolates. While VIM gene was present in 6 isolates, VIM-6, CTXM-14 and TEM-1 co- present in 2 isolates and VIM-2 and CTXM-15 together were present in 4 isolates Fig-2.

SSTIs- A single NDM-1 gene was present in 7 isolates, NDM-1, TEM-1 and CTXM were found in 22 isolates while SHV, TEM-1, CTXM and NDM-1 were present in 16 isolates while A single VIM-2 gene was present in 4 isolates, SHV, TEM-1, CTXM and VIM-2 gene together were present in 2 isolates Fig-3.

UTIs -NDM-1, TEM-1, SHV, CTXM and OXA 48 together were found in 22 isolates while TEM-1, SHV, CTXM and VIM-2 gene were present in 8 isolates. Fig-4.

IAIs- NDM-1, TEM-1 and CTXM-15 were found in 4 isolates while SHV-28, TEM-1, CTXM-14 and NDM-1 were present in 2 isolates VIM-2, CTXM-15, SHV-28 and TEM-1 gene were present in 2 isolates Fig- 5.

Conjugation

For conjugational studies half the numbers of *K.pneumoniae* 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), Piperacillin-Tazobactam PTZ, CPZ, CTX, FOX, were high among transconjugants; (MIC, ≥ 64 $\mu\text{g/ml}$). The transconjugants were resistant to imipenem IMP, MEM, and ertapenem ETP; (MIC, ≥ 8 -

32 $\mu\text{g/ml}$), whereas MICs of AMK, GEN, TOB, CIP, MXF, LVX, TGC; (MIC, $\leq 2\mu\text{g/ml}$), CST; (MIC, $<1\mu\text{g/ml}$) and ATM fall within susceptible range as determined by E-test. Results of conjugational studies on *K.pneumoniae* isolates that were recovered from various clinical specimens are shown in Table-9.

Plasmid typing and characterization of Plasmid

Plasmid from both the *K.pneumoniae* parental strains and their transconjugants was characterized and found that *bla*_{NDM-1} gene was located on IncA/C in association with *bla*_{OXA-48} present on IncL/M type plasmids. *bla*_{TEM-1}, *bla*_{CTX-M-15}, *bla*_{SHV5}, *bla*_{SHV11}, *bla*_{SHV12}, *bla*_{SHV28}, *bla*_{CTX-M-14}, and *bla*_{CTX-M-28} was carried on plasmids belonging to IncFIA, IncT, IncP, IncW, IncFIB, IncFIC, and IncY replicons respectively where as *bla*_{VIM-2} on IncFII and *bla*_{VIM-6} was located and IncB/O and IncN type type associated with *bla*_{TEM-1}, *bla*_{SHV12}, *bla*_{SHV28}, *bla*_{CTX-M-14} and *bla*_{CTX-M-15} was carried on plasmids belonging to IncFIA, IncFIB, IncFIC, IncT, and IncY replicons respectively Table-10.

Plasmid size estimation-Plasmid size for NDM-1 gene was ranged from 30 kb to 180 kb while *bla*_{VIM-2} and *bla*_{VIM-6} were located on a 120 kb, 130kb, 150 kb and 160 kb size plasmid whereas *bla*_{SHV- 5}, *bla*_{SHV-11}, *bla*_{SHV-12}, *bla*_{SHV-28} were located on a 30-kb, 110kb, 130kb and 150kb plasmid respectively. Plasmid size for *bla*_{CTX-M-14}, *bla*_{CTX-M-28} & *bla*_{CTX-M-15} were ranged from 50kb to 150 kb in size while *bla*_{TEM-1} gene was located on a plasmid 70 kb, 95 kb and 125 kb in size.

Strain molecular typing

Molecular typing of 193 strains of *K.pneumoniae* by RAPD generated 18 cluster assigned as KP-A TO KP-R with an average of 7 to 15 fragments per *K.pneumoniae* strains Figure-6. ERIC PCR and REP PCR produced 15 clonal clusters with an average of 5 to 13 fragments per *K.pneumoniae* strains Figure-7.

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

Specimen	SSTIs	BSIs	RTIs	UTIs	IAIs	TOTAL
Carbapenem resistant	43	55	42	62	14	216
Total Isolated	165	140	103	198	53	659

Table 2: Showing Gender wise distribution of *K.pneumoniae*.

ORGANISM	Total Cases	Number of Males	Percentage	Number of Females	Percentage
<i>K.pneumoniae</i>	659	449	68.1	210	31.9

Table 3: Showing Chronological age wise distribution of *K.pneumoniae* patients.

Age in Years	Number of Patients
0-9	21
10-19	34
20-29	52
30-39	91
40-49	97
50-59	89
60-69	78
70-79	113
80 above	84
Total	659

Table 4: Showing number wise ward percentage distribution of *K.pneumoniae*.

Wards	Number of Cases(N=659)	Percentage of cases
OBG ward	185	28
Surgery ward	132	20
ICU Surgery	113	17
Medicine ward	82	13
ICU Medical	66	10
Urology	21	3
NICU	19	3
Orthopedics	18	3
Paedtrics ward	15	2
ENT	8	1

Table 5: Showing Antibiogram and resistance percentage of *K.pneumoniae* various infection sites.

Antibiotic	UTIs	IAs	SSTIs	RTIs	BSIs	Resistance	%	Sensitivity	%
AMK	98	14	89	42	65	308	47	351	53
AMC	111	45	88	62	70	376	57	283	43
AMP	130	51	134	76	85	476	72	183	28
SAM	111	43	74	62	68	358	54	301	46
ATM	98	24	67	56	62	307	47	352	53
FEP	98	22	62	56	58	296	45	363	55
CTX	98	24	78	56	62	318	48	341	52
FOX	98	24	68	56	58	304	46	355	54
CZ	98	24	78	56	62	318	48	341	52
SFP	98	15	62	56	55	286	43	373	57
CPZ	98	24	79	56	62	319	48	340	52
CPD	98	24	95	56	62	335	51	324	49
CAZ	98	24	78	56	62	318	48	341	52
CRO	98	24	85	56	62	325	49	334	51
CIP	98	24	72	42	64	300	46	359	54
ETP	62	21	43	42	55	223	34	436	66
GEN	98	24	67	59	62	310	47	349	53
IPM	62	21	43	42	55	223	34	436	66
LVX	55	21	56	56	55	243	37	416	63
MEM	62	21	43	42	55	223	34	436	66
PIP	111	24	85	61	60	341	52	318	48
TZP	98	21	62	56	55	292	44	367	56
TET	132	24	64	66	78	364	55	295	45
TGC		5	23	16	18	62	9	597	91
TOB	111	24	62	61	60	318	48	341	52
SXT	134	35	69	68	72	378	57	281	43
Total N=	198	53	165	103	140	659			

ampicillin(AMP), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), Piperacillin-Tazobactam (PIT), cefoperazone (CPZ), Cefotaxime (CTX), Cefoxitin (FOX), imipenem (IMP), meropenem (MEM), ertapenem (ETP), amikacin (AMK), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), moxifloxacin (MXF), levofloxacin

(LVX), tigecycline (TGC); colistin (CST); and azetronam (ATM).

Table 6: Showing MICs of imipenem, ertapenem and meropenem against *K.pneumoniae*.

Antibiotic concentrations (µg/ml)	Number of sample (N=659)	Percentage
0.25	83	12.61
0.5	98	14.89
1	149	22.64
2	113	17.17
4	5	0.75
8	58	8.81
16	39	5.92
32	68	10.33
64	46	6.9

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

Infection sites	Carbapenem resistant by MIC ^a	MBL E TEST ^b	DDST ^c	CDST ^d	MHT ^e	NON MBL ^f
SSTIs	43	41	31	38	34	2
BSIs	55	54	42	45	31	1
RTIs	42	37	33	35	23	5
UTIs	62	54	48	51	43	8
IAs	14	8	7	7	6	6
TOTAL	216(32.7%)	194(29.3%)	161 (24.3%)	176(26.6%)	137(20.7%)	22(3.3%)

a MIC values for imipenem, meropenem and ertapenem $\geq 4\mu\text{g/ml}$, **b** MBL (IP/IPI) E-test, **c** DDST- Double-disc synergy tests (DDST), **d** CDST- Combined-disc synergy test, **e** MHT - Modified Hodge test and **f** NON MBL^f

Table 8: Showing Distribution of beta-lactamase genes.

	NDM-1	TEM-1	CTXM-15	CTXM-14	CTXM-28	SHV-12	SHV-28	SHV-5	SHV-11	VIM-2	VIM-6	OXA-48
<i>K. pneumoniae</i>	142	146	112	32	21	52	44	19	19	42	9	22

Table 9: Showing Antibiogram of *K. pneumoniae* donor and Transconjugants.

From 36 NDM-1 producers 20 and from 18VIM producing <i>K. pneumoniae</i> strains seven were selected as a donor <i>K.pneumoniae</i> strains for conjugation studies																			
ISOLATE	IPM	MEM	ETP	ATM	CS	TGC	CAZ	CTX	CN	FEP	CPZ	PT	AMP	CIP	LVX	MOX	GEN	AMK	TOB
BACT72	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC72	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
BACT74	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC74	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
BACT109	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC109	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
BACT134	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC134	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
BACT113	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC113	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
BACT202	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC202	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
BACT203	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC203	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
BACT205	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC205	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
BACT241	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC241	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
BACT230	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC230	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
BACT239	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC239	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
BACT247	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC247	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.25	1	2	1
BACT349	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC349	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	2	1
BACT354	64	64	32	64	0.5	0.25	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC354	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	1
BACT177	64	64	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC177	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
BACT198	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC198	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2

BACT382	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC382	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
BACT403	64	64	32	64	0.125	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC403	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	1	1	1
BACT451	32	32	32	64	0.25	0.25	128	128	64	64	128	>128	256	8	8	4	>16	64	>16
TC451	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	2	2	2
BACT581	32	32	32	64	0.5	2	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC581	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	1	2	1
BACT562	32	32	32	64	0.125	0.5	256	256	64	64	256	>128	256	8	8	4	>16	64	>16
TC562	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
BACT541	32	32	32	64	0.125	1	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC541	8	16	16	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	1
BACT538	32	32	32	64	0.25	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC538	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	2	1	1
BACT524	32	32	32	64	0.5	2	256	256	64	64	256	>128	256	4	4	4	>16	64	>16
TC524	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	1	1
BACT510	64	64	32	64	0.125	1	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC510	16	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	1	1	1	2
BACT498	32	32	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC498	16	8	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
BACT476	64	64	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC476	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	0.5	0.25	1	2	1

From 30 NDM-1 producers 12 and from 6 VIM producers six were selected as a donor *K.pneumoniae* strains for conjugation studies

From 30 NDM-1 producers 12 and from 6 VIM producing *K. pneumoniae* strains six were selected as a donor *K.pneumoniae* strains for conjugation studies

ISOLATE	IPM	MEM	ETP	ATM	CS	TGC	CAZ	CTX	CN	FEP	CPZ	PT	AMP	CIP	LVX	MOX	GEN	AMK	TOB
ETB832	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC832	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
ETB877	32	32	32	64	0.25	2	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	0.5	0.5	2	2	2
ETB893	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC893	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
ETB905	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC905	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
ETB939	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC939	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
ETB1514	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC1514	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
ETB2015	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC2015	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2

ETB2242	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2242	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
ETB2578	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC2578	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
ETB2607	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2607	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
ETB2669	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC2669	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
ETB2728	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC2728	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.25	1	2	1
ETB764	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC764	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	2	1
ETB702	64	64	32	64	0.5	0.25	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC702	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	1
ETB791	64	64	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC791	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
ETB633	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC633	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
ETB641	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC641	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
ETB689	64	64	32	64	0.125	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC689	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	1	1	1
16 NDM-1 and 5 VIM producing K. pneumoniae strains were selected as a donor K.pneumoniae strains for conjugation studies																			
ISOLATE	IPM	MEM	ETP	ATM	CS	TGC	CAZ	CTX	CN	FEP	CPZ	PT	AMP	CIP	LVX	MOX	GEN	AMK	TOB
PC182	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC182	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
PC256	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC256	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
PC273	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC273	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
PC279	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC279	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
PC284	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC284	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
PC288	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC288	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
PC295	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC295	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
PC298	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16

TC298	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
PC308	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC308	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
PC359	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC359	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
PC370	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC370	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
PC374	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC374	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
PC404	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC404	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
PC407	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC407	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
PC408	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC408	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
PC415	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC415	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.25	1	2	1
PC418	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC418	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	2	1
PC475	64	64	32	64	0.5	0.25	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC475	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	1
PC514	64	64	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC514	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
PC538	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC538	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
PC566	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC566	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
20 NDM-1 and 8 VIM producing K. pneumoniae strains (N=28) 51% were selected as a donor K.pneumoniae strains for conjugation studies																			
ISOLATE	IPM	MEM	ETP	ATM	CS	TGC	CAZ	CTX	CN	FEP	CPZ	PT	AMP	CIP	LVX	MOX	GEN	AMK	TOB
UC1711	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC1711	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
UC1785	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC1785	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
UC1859	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC1859	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
UC1893	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC1893	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
UC2007	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC2007	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2

UC2086	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC2086	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
UC2067	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC2067	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
UC2387	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2387	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
UC2444	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC2444	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
UC2457	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2457	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
UC2473	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC2473	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
UC2573	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC2573	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.25	1	2	1
UC2581	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TC2581	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	2	1
UC2596	64	64	32	64	0.5	0.25	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2596	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	1
UC2661	64	64	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC2661	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
UC2719	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC2719	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
UC2824	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2824	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
UC2921	64	64	32	64	0.125	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC2921	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	1	1	1
UC2923	32	32	32	64	0.25	0.25	128	128	64	64	128	>128	256	8	8	4	>16	64	>16
TC2923	16	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	2	2	2
UC3027	32	32	32	64	0.5	2	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TC3027	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	1	2	1
UC3119	32	32	32	64	0.125	0.5	256	256	64	64	256	>128	256	8	8	4	>16	64	>16
TC3119	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	2	2	2
UC3123	32	32	32	64	0.125	1	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC3123	8	16	16	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	1
UC3274	32	32	32	64	0.25	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC3274	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	2	1	1
UC3311	32	32	32	64	0.5	2	256	256	64	64	256	>128	256	4	4	4	>16	64	>16
TC3311	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	1	1
UC3302	64	64	32	64	0.125	1	128	128	64	64	128	>128	256	4	4	8	>16	64	>16

TC3302	16	16	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	1	1	1	2
UC3397	32	32	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC3397	16	8	16	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
UC3433	64	64	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC3433	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	0.5	0.25	1	2	1
UC3450	32	32	32	64	0.125	0.75	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TC3450	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.5	0.5	1	1	1
6 NDM-1 and 2 VIM producing <i>K.pneumoniae</i> strains (N=8) were selected as a donor <i>K.pneumoniae</i> strains for conjugation studies.																			
IAIS1091	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC1091	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
IAIS1510	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC1510	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
IAIS1571	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC1571	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
IAIS1914	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC1914	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
IAIS1953	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC1953	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
IAIS2040	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC2040	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
IAIS2209	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TC2209	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
IAIS2522	32	32	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC2522	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2

Table 10: Showing characterization of MBL encoding Plasmid and its Typing.

ISOLATE	MBL	Plasmid	OXA-48	Plasmid	Other ESBL gene present			Plasmid type			Transferability
UC1711	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
UC1785	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
UC1859	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-28	SHV-5	FIA	Y	P	transferable
UC1893	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-28	SHV-11	FIA	Y	W	transferable
UC2007	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	transferable
UC2086	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	transferable
UC2067	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-14	SHV-11	FIA	Y	W	transferable
UC2387	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-28	SHV-5	FIA	Y	P	transferable
UC2444	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
UC2457	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-28	SHV-11	FIA	Y	W	transferable
UC2473	NDM-1	A/C	OXA-4 8	L/M	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	transferable
UC2573	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
UC2581	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-28	SHV-11	FIA	Y	W	transferable
UC2596	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	transferable
UC2661	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
UC2719	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	transferable
UC2824	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	transferable
UC2921	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	transferable
UC2923	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-28	SHV-5	FIA	Y	P	transferable
UC3027	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	transferable
UC3119	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-28	SHV-11	FIA	Y	W	transferable
UC3123	NDM-1	A/C	OXA-48	L/M	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
UC3274	VIM-6	B/O	ND	ND	TEM-1	CTXM-15	ND	FIA	T	ND	transferable
UC3311	VIM-2	FII	ND	ND	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
UC3302	VIM-2	FII	ND	ND	TEM-1	CTXM-14	SHV-28	FIA	Y	FIC	transferable
UC3397	VIM-2	FII	ND	ND	TEM-1	CTXM-14	SHV-28	FIA	Y	FIC	transferable
UC3433	VIM-2	FII	ND	ND	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
UC3450	VIM-2	FII	ND	ND	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	transferable
IAI1091	NDM-1	A/C	*	*	*	CTXM-14	SHV-28	*	B/O	FIC	Transferable
IAI1510	NDM-1	A/C	*	*	*	CTXM-14	SHV-28	*	B/O	FIC	Transferable
IAI1571	NDM-1	A/C	*	*	TEM-1	CTXM-15	*	FIA	T	*	Transferable
IAI1914	NDM-1	A/C	*	*	TEM-1	CTXM-15	*	FIA	T	*	Transferable
IAI1953	NDM-1	A/C	*	*	TEM-1	CTXM-15	*	FIA	T	*	Transferable
IAI2040	NDM-1	A/C	*	*	TEM-1	CTXM-15	*	FIA	T	*	Transferable
IAI2209	VIM-2	FII	*	*	TEM-1	CTXM-15	SHV-28	FIA	Y	FIC	Transferable
IAI2522	VIM-2	FII	*	*	TEM-1	CTXM-15	SHV-28	FIA	Y	FIC	Transferable
PC182	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIA	N	P	Transferable
PC256	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIB	N	W	Transferable
PC273	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIA	B/O	P	Transferable
PC279	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIB	N	FIC	Transferable
PC284	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIB	B/O	FIA	Transferable
PC288	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	N	FIC	Transferable
PC295	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIB	N	FIC	Transferable
PC298	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	B/O	FIC	Transferable
PC308	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIA	B/O	W	Transferable
PC359	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIA	B/O	FIA	Transferable
PC370	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIB	N	FIC	Transferable
PC374	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIB	B/O	FIA	Transferable
PC404	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIB	N	FIA	Transferable
PC407	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIB	B/O	P	Transferable
PC408	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIB	N	FIC	Transferable
PC415	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIB	B/O	W	Transferable
PC418	VIM-2	Y	*	*	TEM-1	CTXM-15	*	FIB	T	*	Transferable
PC475	VIM-2	Y	*	*	TEM-1	CTXM-15	*	FIB	T	*	Transferable

PC514	VIM-2	Y	*	*	TEM-1	CTXM-15	*	FIB	T	*	Transferable
PC538	VIM-2	Y	*	*	TEM-1	CTXM-15	SHV-28	FIB	T	FIC	Transferable
PC566	VIM-2	Y	*	*	TEM-1	CTXM-15	SHV-12	FIB	T	FIA	Transferable
ETB832	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIA	T	P	Transferable
ETB877	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
ETB893	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIA	T	P	Transferable
ETB905	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
ETB939	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
ETB1514	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIA	T	P	Transferable
ETB2015	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
ETB2242	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
ETB2578	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
ETB2607	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
ETB2669	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
ETB2728	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
ETB764	VIM-6	N	*	*	TEM-1	CTXM-14	*	FIA	Y	*	Transferable
ETB702	VIM-6	N	*	*	TEM-1	CTXM-14	*	FIA	Y	*	Transferable
ETB791	VIM-2	FII	*	*	*	CTXM-15	*	*	Y	*	Transferable
ETB633	VIM-2	FII	*	*	*	CTXM-15	*	*	Y	*	Transferable
ETB641	VIM-2	FII	*	*	*	CTXM-15	*	*	Y	*	Transferable
ETB689	VIM-2	FII	*	*	*	CTXM-15	*	*	Y	*	Transferable
BACT72	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIA	T	P	Transferable
BACT74	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIA	Y	W	Transferable
BACT109	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIA	T	P	Transferable
BACT134	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT113	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
BACT202	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT203	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
BACT205	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
BACT241	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-5	FIA	T	P	Transferable
BACT230	NDM-1	A/C	*	*	TEM-1	CTXM-14	SHV-11	FIA	Y	W	Transferable
BACT239	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
BACT247	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT349	NDM-1	A/C	*	*	TEM-1	CTXM-14	SHV-12	FIA	Y	FIB	Transferable
BACT354	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
BACT177	NDM-1	A/C	*	*	TEM-1	CTXM-14	SHV-11	FIA	Y	FIB	Transferable
BACT198	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT382	NDM-1	A/C	*	*	TEM-1	CTXM-14	SHV-12	FIA	Y	FIB	Transferable
BACT403	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV 11	FIA	T	W	Transferable
BACT451	NDM-1	A/C	*	*	TEM-1	CTXM-14	SHV-12	FIA	Y	FIB	Transferable
BACT581	NDM-1	A/C	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT562	VIM-2	FII	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
BACT541	VIM-2	FII	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
BACT538	VIM-2	FII	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
BACT524	VIM-2	FII	*	*	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
BACT510	VIM-6	HI2	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT498	VIM-6	HI2	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT476	VIM-6	HI2	*	*	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable

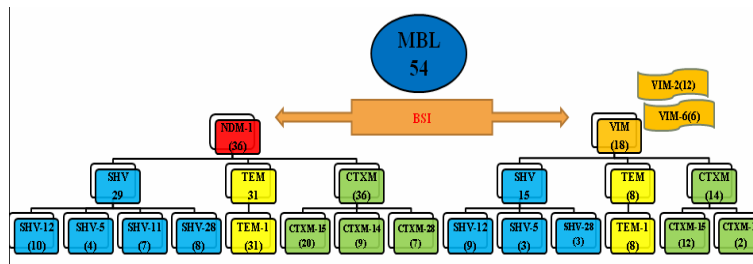


Fig. 1: Showing distribution of beta-lactamase genes in association with MBL genes in *K.pneumoniae* isolated from BSIs.

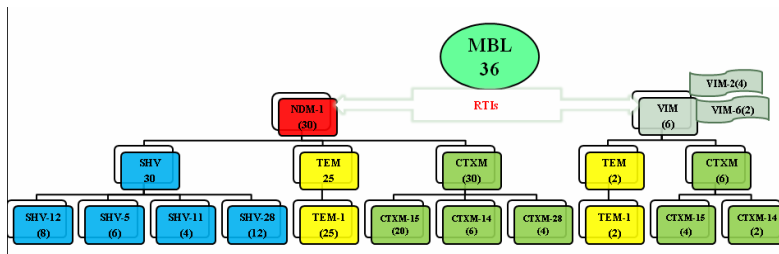


Fig. 2: Showing distribution of beta-lactamase genes in association with MBL genes in *K.pneumoniae* isolated from RTIs.

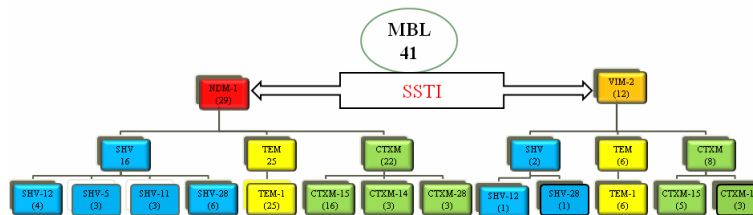


Fig. 3: Showing distribution of beta-lactamase genes in association with MBL genes in *K.pneumoniae* isolated from SSTI.

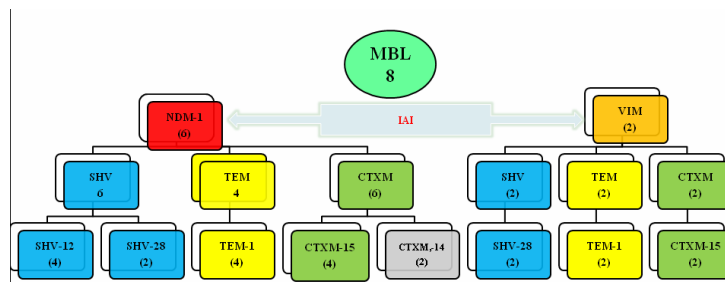


Fig. 4: Showing distribution of beta-lactamase genes in association with MBL genes in *K.pneumoniae* isolated from UTIs.

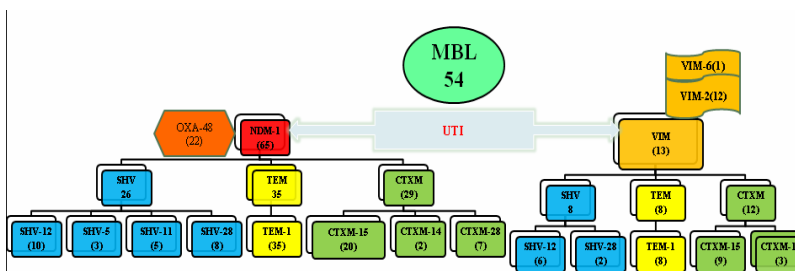


Fig. 5: Showing distribution of beta-lactamase genes in association with MBL genes in *K.pneumoniae* isolated from IAI.

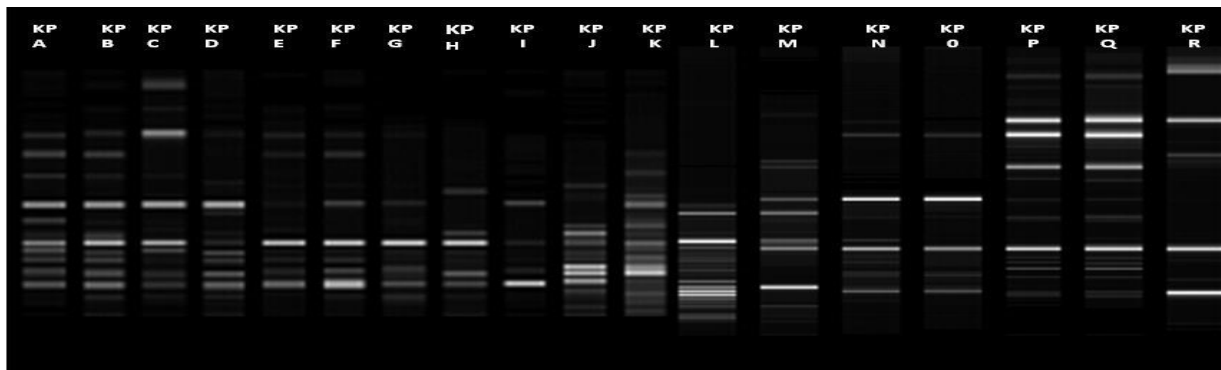


Figure 6: Showing RAPD among 18 clonal cluster of *K.pneumoniae*.

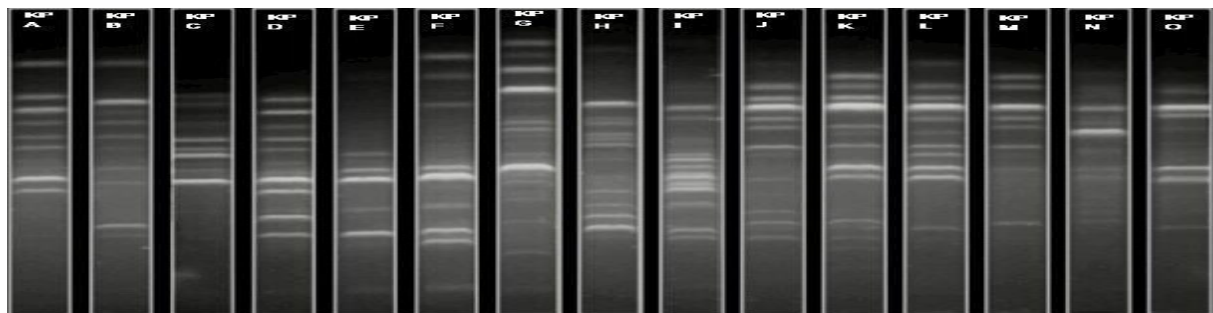


Figure 7: Showing ERIC & rep PCR among 15 clonal clusters of *K.pneumoniae*.

DISCUSSION

In this study majority of specimens 30% were from UTIs, followed by 25% in SSTIs, 21.3% in BSIs, 16% in RTIs and 8% in IAIs and Misc. respectively. Urinary tract infections generally occur as a complication, due to stone, stent, or catheter in the urinary tract or due to obstruction in the genitourinary system or after post instrumentation or surgery on the urinary tract. Carbapenem resistant *K.pneumoniae* is a serious cause of nosocomial infections at almost all sites of the body that result in number of different clinical syndromes, in the present study 28.8% of Carbapenem resistant *K.pneumoniae* were from UTIs, followed by 25.6% in BSIs, 20% in SSTIs, 19.4% in RTIs, and 6.51% in IAIs respectively. In this study 28.11% of samples were from OBG, followed by 20% in surgical ward, 17.2% in ICU Surgery, and 12.5% in ICU Medical. Our study revealed *K.pneumoniae* infection was significantly associated among hospitalized patients with an age group between 30-60 years of age, elderly (≥ 58 years) and in those who had already received any type of invasive procedure such as catheterization, intubation or ventilation. There is 50%-80% chances of colonization rates during hospitalization, especially among immunosuppressed, immunocompromised, debilitated, impaired immunity ICU patients who had experienced mechanical ventilation, tracheostomy, catheters, surgery or severe burns because it is present in rectum and anal area and chances of acquisition of genes are more from other GNB present in patient. Our study revealed, 32.8% *K.pneumoniae* isolates were (ETP, IPM and MEM) carbapenem resistant as per CLSI. Based upon antibiotic resistant pattern present study showed forty percent of *K.pneumoniae* isolates recovered from RTIs, BSIs and

IAIs, while 31% isolates recovered from UTIs whereas 26% isolates from SSTIs were resistant to carbapenem. These isolates were also 100% resistant to CAZ, CPZ, CRO, CZ, FOX, TOB, SXT, TZP, LVX, CIP, SFP, GEN and AMK. In the present study, *K.pneumoniae* had shown a prevalence of 29.3% in MBL production. *bla_{SHV-5}*, *bla_{SHV-11}*, *bla_{SHV-12}*, and *bla_{SHV-28}* are the commonest SHV genes detected in 14%, 14%, 38.2% and 32.8% of blaSHV 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 67.8%, 19.4% and 12% *bla_{CTX-M}* isolates respectively co associated with MBL genes and proved the existence of carbapenemases in *K.pneumoniae* isolates with other ESBL genes. Carbapenem are recently introduce for their use in hospitals, due to the consistent use of aminoglycosides and other higher antibiotics like tigecycline and Colistin in ICUs and emergency wards there is an increase in development of resistance against *K.pneumoniae* and lastly irrational and inappropriate use of higher antibiotics in medical settings and their selective pressure increased antimicrobial resistance towards commonly used drugs. In the present study, *bla_{NDM-1}*, *bla_{OXA-48}*, *bla_{CTX-M}*, *bla_{SHV}* and *bla_{TEM-1}* genes altogether were reported in 22 urinary isolates (11.3%) of MBL producing *K.pneumoniae* and 32.8% of *K.pneumoniae* isolates were XDR. In this study 32.8% of *K.pneumoniae* isolates were XDR. In the present study, among carbapenem producers *bla_{NDM-1}* was detected in 76.8% isolates, while *bla_{VIM}* was present in 23.2% isolates. *bla_{NDM-1}* gene was located on IncA/C while IncL/M associated with *bla_{OXA-48}*.

CONCLUSION

These findings highlights prevalence of *bla_{OXA-48}* and *bla_{NDM-1}*, producing *K.pneumoniae* 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 *K.pneumoniae* makes them refractory to the common antibiotics used in clinical practice.

Declarations

Funding: No grant/funding.

Competing Interests: None to declare.

Ethical Approval: Institutional Ethical Approval AFMC/7027.

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