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IDENTIFICATION AND DISTRIBUTION OF THE CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA CARRYING METALLOBETALACTAMASE GENES IN A TERTIARY CARE CENTRE

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ABSTRACT

Objective: The aim of this study is to evaluate the expression of metallobetalactamase genes in Pseudomonas aeruginosa recovered from hospitalized patients in a tertiary care hospital. ***Materials and Methods:*** A prospective study was conducted in an 1800 bedded tertiary care centre in Pune, India from October 2013 to October 2017. Carbapenem resistant strains were isolated and presence of the metallo carbapenemase enzyme was confirmed by Polymerase chain reaction (PCR) assays and sequencing. Transferability of genes was determined by conjugation experiments. REP PCR, ERIC PCR and RAPD PCR assays carried out to check Isolate relatedness. **Results:** Out of 525 isolates, MHT for carbapenemase production was positive for 68(13%)**,** DDST in 126(24%), CDST in 130(24.8%) isolates, MBL (IP/IPI) E-test was positive for 157(30%) and 88(16.8%) isolates were Non MBL. MHT, DDST and CDST assay for P.aeruginosa showed sensitivity of 43.31%, 80.25% and 82.80 %, Specificity of 100%, its PPV was 100% and its NPV was 80.53%, 92.23% and 93.16% respectively. In the present study, *bla*NDM-1was detected in 36 *P.aeruginosa* isolates, while blaVIM in 121 isolates. Furthermore, *bla*IMP, *bla*SIM, *bla*SPM and *bla*GIM were not detected in any of the study isolates. Among ESBLs genes, blaCTX-M was present in 162 isolates, followed by blaTEM-1 in 154, and blaSHV in 151 isolates. **Conclusion:** The results of this study, highlights prevalence of bla_{VIM}, and bla_{NDM-1}, producing *Pseudomonas aeruginosa* 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 especially metallobetalactamase resistance in patients that suffer nosocomial infections.

KEYWORDS: *P.aeruginosa*, bla_{NDM-1}, bla_{VIM-2}, bla_{VIM-6}, bla_{SHV-5}, bla_{SHV-11}, bla_{SHV-12}, bla_{SHV-28}, $bla_{CTX-M-15}$, $bla_{CTX-M-14}$, REP PCR, and RAPD PCR.

INTRODUCTION

P.aeruginosa has been reported to cause infections of respiratory and urinary tract, wounds, blood stream, and even the central nervous system. For immunocompromised patients, subjected to injury, such infections are often serious and frequently life-threatening. Nutritional status of patient, old age, pre existing infection, other co-morbid conditions are some of the patient related factors illness) and (inadequate sterilization of surgical instruments, prolonged duration of surgery, poor surgical technique, pre operative part preparation) are the some of the procedure related factors can influence the risk of *P.aeruginosa* infections.[1-4]

P.aeruginosa shows high level of intrinsic resistance to a number of structurally unrelated antimicrobial agents and these results in prolonged illness, deaths, and health care cost. *P. aeruginosa* acquires resistance to antibiotics through chromosomal mutations or it is plasmid mediated that can easily transferred between two different strains. Carbapenems, such as imipenem and meropenem used to treat multidrug resistant strains of *P.aeruginosa however*, *Carbapenem-resistant* strains have been emerged worldwide.[1-4] *The* present study is performed using PCR based molecular techniques and standard identification focused on determining the antibiotic resistance pattern and prevalence of metallo-beta-lactamase genes in carbapenem resistant *P.aeruginosa* in a tertiary care centre.

MATERIALS AND METHODS

Study design & Bacterial isolates

A prospective cross sectional study was conducted in a 1800 bedded tertiary care centre in Pune, India from October 2013 to October 2017. A total of 525 clinical isolates were recovered from various specimens from

different patients (one isolate per patient). Samples were collected from patients, using strict aseptic precautions and in accordance with standard protocols[5,6] and immediately processed without any delay. *P.aeruginosa* 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), 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), 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.[5] [6]

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).[5] [6] 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 *P.aeruginosa* 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 *P.aeruginosa* strains recovered from patients.[5] [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.[5] 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 *P.aeruginosa* among clinical specimens

A total of 525 clinical isolates **of *P.aeruginosa*** were recovered from various sites of infections in a 1800 bedded tertiary care centre in Pune, India. The largest proportion of specimens were from UTIs 160 (30%), followed by 145(28%) in SSTIs, 78(15%) in IAIs, 75(14%) in RTIs, and 67(13%) in BSIs respectively shown in **Table-1.** Among 525 tested isolates, 245(46.7%) isolates showed MIC ≥8µg/ml against imipenem and meropenem. Of the total number of samples, males contributed significant number of samples 357 (68%) while females contributed 168 (32%) shown in **Table-2.** Age of the subjects in the SMaximum number of isolates (184) was from the age group of 40-59yrs, followed by 20-39 yrs age group (138) and least number of isolates (05) was from 0-9years. Chronological age wise distribution is shown in **Table-3**. Of the total samples, highest number of samples 197(38%) were from ICU Surgery, followed by 169 (32%) in Surgery ward, 62(12%) in OBG ward, 44(8%) in ICU Medical, 35(7%) in Medicine ward and remaining 18(3%) were from Paedtrics ward (Fig-10). Number wise ward percentage distribution of *P.aeruginosa* shown in **Table-4.** Majority of Carbapenem resistant *P.aeruginosa* were from UTIs 85(35%), followed by 69 (28%) in SSTIs, 57(23%) in RTIs and 17(7%) in both BSIs and IAIs respectively shown in **Table-1.**

Antimicrobial susceptibility of *P.aeruginosa* isolates

Evaluation of antibiotic susceptibility pattern indicated that 47% *P.aeruginosa* were resistant against IPM, and MEM. Antibiogram and resistance percentage of *P.aeruginosa* various infection sites shown in **Table-5** as The proportions of resistance to other beta lactam group and to other classes of antibiotics was distributed as follows: AMK (65%; R), ATM (66%; R), FEP (65%; R), CTX(69%; R), FOX (67%; R), CAZ (66%; R), CRO (71%; R), GEN (70%; R), PIP (70%; R), TZP(60%; R), TET(70%; R), TIC(69%; R), TCC(53%; R), **CIP**(64%; R), TOB (66%; R), and SXT (70%; R). All isolates were sensitive to polymyxin B and colistin. MICs of IPM, and MEM, in **µg/ml** as determined by VITEK-2 and E-test against *P.aeruginosa* shown in **Table-6**.

Phenotypic detection of carbapenem-hydrolyzing-beta-lactamases

Out of 525 isolates, MHT for carbapenemase production was positive for 68(13%)**,** DDST in 126(24%), CDST in 130(24.8%) isolates, MBL (IP/IPI) E-test was positive for 157(30%) and 88(16.8%) isolates were Non MBL. Results of different phenotypic tests of ***P.aeruginosa*** recovered from various clinical specimens are shown in **Table-7**. Out of total ***P.aeruginosa*** isolated from BSI (N=67), 25.37% (n=17) was found to be carbapenem resistant. Among 67, *P.aeruginosa* isolates from Blood, MBL E-test positive was positive in 12 isolates, DDST in 10, CDST in 10 and MHT in 5 isolates. *i.e.* 17.91%, 14.92%, 14.92%, and 7.46% respectively. Among 75 *P.aeruginosa* isolates from Endotracheal aspirate and BAL fluid, MBL E-test was positive in 48, CDST in 40, DDST in 40 and MHT in 20 isolates. *i.e.* 64%, 53%, 53% and 26% respectively. Among 145 *P.aeruginosa* isolates from SSTIs, MBL E-test was positive in 41, CDST in 36, DDST in 28 and MHT in 12. *i.e.* 28.2%, 24.8%, 19.3% and 8.2% respectively. Among 160 *P.aeruginosa* isolates from urine, MBL E-test was positive in 42 isolates, DDST in 36, CDST in 32 and MHT in 28 isolates. *i.e.* 26.2%, 22.5%, 20%, and 17.5% respectively. Among 78 *P.aeruginosa* isolates from IAIs, MBL E-test was positive in 14, CDST in 12, DDST in 12 and MHT in 03 isolates. *i.e.* 17.9%, 15.4%, 15.4% and 3.8% respectively.

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

The prevalence of MBL-encoding genes among ***P.aeruginosa*** 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 36, $bla_{\mathbf{VIM-2}}$ in **106,** and $bla_{\mathbf{VIM-6}}$ **in 15.**$bla_{\mathbf{CTX-M}}$ was present in 157 isolates, followed by bla_{TEM-1} in 154 and $bla_{\mathbf{SHV}}$ in 151.$bla_{\mathbf{SHV-5}}$, $bla_{\mathbf{SHV-11}}$, $bla_{\mathbf{SHV-12}}$, and $bla_{\mathbf{SHV-28}}$ are the commonest SHV genes detected in 11,13,75, and 52 of $bla_{\mathbf{SHV}}$ producing isolates respectively whereas $bla_{CTX-M-15}$, $bla_{CTX-M-14}$ and blaCTX-M-$_{28}$ are the commonest CTX-M type ESBLs that were present in 127, 27 and 3 isolates. Molecular characterization of beta-lactamase genes in carbapenem resistant ***P.aeruginosa*** isolates recovered from clinical specimens shown in Figure 1-5.

Distribution of beta-lactamase genes Table-8A and 8B

BSIs

Co presence of VIM-2, SHV, TEM-1 and CTXM-15 were detected in 10 isolates while NDM-1 and CTXM-15gene was present in 2 isolates Fig-1.

RTIs

VIM-2, SHV, TEM-1 and CTXM were found together in 36 isolates while SHV, TEM-1, CTXM and NDM-1 gene were co-present in 12 isolates Fig-2.

SSTIs-VIM, SHV, TEM-1 and CTXM gene together were present in 31 isolates while NDM-1, SHV, TEM-1 and CTXM were together found in 10 isolates while TEM-1, CTXM and NDM-1 gene were present in10 isolates Fig-3.

UTIs-VIM, SHV, TEM-1 and CTXM together were found in 34 isolates while 22 isolates had VIM-2 and 12 isolates had VIM-6. Six isolates had co presence of SHV, TEM-1, CTXM and NDM-1 gene Fig-4.

IAIs-NDM-1, TEM-1 and CTXM-15 were found in 03 isolates while SHV, CTXM-15 and NDM-1 were present in 04 isolates. VIM-2 gene was present in 10 isolates, VIM-2, TEM-1 and CTXM were found in 10 isolates while SHV, TEM-1, CTXM and VIM-2 gene were present in 08 isolates **Fig-5**.

Conjugation

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 ampicillin(AMP), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), Piperacillin-Tazobactam (PIT), cefoperazone (CPZ), Cefotaxime (CTX), Cefoxitin (FOX), were high among transconjugants; (MIC, ≥64 µg/ml). The transconjugants were resistant to imipenem (IMP), and meropenem (MEM), MIC, ≥8-32µg/ml), whereas MICs of amikacin (AMK), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), moxifloxacin (MXF), levofloxacin (LVX), tigecycline (TGC); MIC, <2µg/ml), colistin (CST); MIC, <1µg/ml) and azetronam (ATM) fall within susceptible range as determined by E-test. Results of conjugational studies on ***P.aeruginosa*** isolates that were recovered from various clinical specimens are shown in Table-9A-9E.

Plasmid typing and characterization of Plasmid

Plasmid from both the ***P.aeruginosa*** parental strains and their transconjugants was characterized and found that *bla*VIM-2 and *bla*NDM-1 gene was carried on plasmids belonging to IncFII and IncN replicons. *bla*CTX-M-14 &*bla*CTX-M-15 in association with *bla*VIM-2 was carried on IncT replicons while replicons associated with *bla*NDM-1 showed IncY.bla_{SHV-5}, bla_{SHV-11}, *bla*SHV12, *bla*SHV28 and *bla*TEM-1 gene was located on IncP, IncW, IncFIB, IncFIC and IncFIA type replicons respectively Table-10.

Plasmid size estimation-Plasmid size for NDM-1 gene was ranged from 30 kb to 180 kb while b*la*VIM-2 and b*la*VIM-6 were located on a120 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 157 strains of P.aeruginosa by RAPD generated 10 RAPD pattern assigned as Ps-A TO Ps-J with an average of 8 to 15 fragments per strains Figure-6 and REP PCR produced 10 clonal clusters with an average of 4 to 8 fragments per P.aeruginosa strains Figure-7.

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

Specimen	SSTIs	BSIs	RTIs	UTIs	IAIs	TOTAL
Carbapenem resistant	69	17	57	85	17	**245**
Total Isolated	145	67	75	160	78	**525**

Table 2: Showing Gender wise distribution of *P.aeruginosa.*

ORGANISM	Total Cases	Males	Percentage	Females	Percentage
P.aeruginosa	**658**	**357**	**68**	**168**	**32**

Table 3: Showing Chronological age wise distribution of *P.aeruginosa* from patients.

Age in Years	Number of Patients
0-9	**5**
10-19	**28**
20-29	**52**
30-39	**86**
40-49	**91**
50-59	**93**
60-69	**64**
70-79	**69**
80 above	**37**
Total	**525**

Table 4: Showing Distribution of *P.aeruginosa* in various wards.

Wards	Number of Cases(N=525)	Percentage of cases
ICU Surgery	**197**	**38**
Surgery ward	**169**	**32**
OBG ward	**62**	**12**
ICU Medical	**44**	**8**
Medicine ward	**35**	**7**
Paedtrics ward	**18**	**3**

Table 5: Showing Antibiogram and resistance percentage of P.aeruginosa various infection sites.

Antibiotics	BSIs	RTIs	SSTIs	UTIs	IAIs	R	%	S	%
AMK	34	65	89	129	28	345	65	180	34
ATM	40	65	87	129	28	349	66	176	33
FEP	45	65	75	129	28	342	65	183	34
CTX	45	65	81	145	28	364	69	161	30
FOX	45	65	89	129	28	356	67	169	32
CAZ	45	65	83	129	28	350	66	175	33
CRO	45	65	91	145	28	374	71	151	28
CIP	28	65	72	145	28	338	64	187	35
GEN	48	65	89	145	24	371	70	154	29
IPM	17	57	69	85	17	245	46	280	53
LVX	34	57	71	98	28	288	54	237	45
MEM	17	57	69	85	17	245	46	280	53
PIP	45	65	89	145	28	372	70	153	29
TZP	32	60	72	129	17	310	59	215	40
TET	45	71	89	145	28	378	72	147	28
TIC	45	60	89	145	28	367	69	158	30
TCC	28	65	72	98	17	280	53	245	46
TOB	45	60	89	129	24	347	66	178	33
SXT	52	60	79	145	28	364	69	161	30
TOTAL	**67**	**75**	**145**	**160**	**78**	525		525	

Ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), Piperacillin-Tazobactam (PIT), Cefotaxime (CTX), Cefoxitin (FOX), imipenem (IMP), meropenem (MEM), 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 and meropenem against *P.aeruginosa* (µg/ml).

Antibiotic concentrations	Number of sample	Percentage
1 µg/ml	**72**	**13.7**
2 µg/ml	**68**	**13**
4 µg/ml	**140**	**26.7**
8 µg/ml	**12**	**2.2**
16 µg/ml	**28**	**5.3**
32 µg/ml	**47**	**9**
64 µg/ml	**76**	**14.5**
128 µg/ml	**82**	**15.6**

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

Infection	CR MIC[a]	MBL[b]	DDST[c]	CDST[d]	MHT[e]	NONMBL[f]
SSTIs	69	41	28	36	12	28
BSIs	17	12	10	10	5	5
RTIs	57	48	40	40	20	9
UTIs	85	42	36	32	28	43
IAIs	17	14	12	12	3	3
Total	**245(46.7%)**	**157(30%)**	**126(24%)**	**130(24.8%)**	**68(13%)**	**88(16.8%)**

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]**

Table 8A: showing Distribution of beta-lactamase genes.

ORGANISM	NDM-1	TEM-1	CTXM-15	CTXM-14	CTXM-28	SHV-12	SHV-28	SHV-5	SHV-11	VIM-2	VIM-6
P. aeruginosa	**36**	**154**	**127**	**27**	**3**	**75**	**52**	**11**	**13**	**106**	**15**

Table 8B: Showing Distribution of beta-lactamase genes.

P.aeruginosa	NDM	TEM	SHV	CTXM	VIM	TEM	SHV	CTXM
IAIs	4	3	4	4	10	10	8	10
UTIs	8	8	6	8	34	34	34	34
SSTIs	10	10	10	10	31	31	31	31
RTIs	12	12	12	12	36	36	36	36
BSIs	2			2	10	10	10	10
TOTAL	36	33	32	36	121	121	119	121

Table 9A: Showing strains selected for conjugational studies (N=06), MICs of multidrug resistant P.aeruginosa and their transconjugants E.coli J53 (µg/ml)recovered from BSI.

Isolate	IPM	MEM	AZM	CRO	CAZ	CST	TIC	TIC/CA	PIP	PIP/TZ	AMK	GEN	TOB	NET	CIP	LEV	MXF
BACT368	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC368	32	64	0.25	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	0.25	1	1
BACT342	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC342	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5
BACT381	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC381	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5
BACT392	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC392	64	64	0.5	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	1	0.25	0.25
BACT412	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC412	64	64	0.5	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	1	0.25	0.25
BACT460	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC460	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5

Table 9B: Showing strains selected for conjugational studies (N=06), MICs of multidrug resistant P.aeruginosa and their transconjugants E.coli J53 (µg/ml) recovered from IAI.

Isolate	IPM	MEM	AZM	CRO	CAZ	CST	TIC	TIC/CA	PIP	PIP/TZ	AMK	GEN	TOB	NET	CIP	LEV	MXF
IAIs252	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC252	32	64	0.25	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	0.25	1	1
IAIs254	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC254	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5
IAIs271	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC271	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5
IAIs813	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC813	64	64	0.5	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	1	0.25	0.25
IAIs971	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC971	64	64	0.5	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	1	0.25	0.25
IAIs1134	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC134	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5
IAIs1571	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC1571	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5
IAIs1703	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC1571	64	64	0.5	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	1	0.25	0.25
IAIs1801	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC1801	64	64	0.5	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	1	0.25	0.25
IAIs1854	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC1854	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5

Table 9C: Showing strains selected for conjugational studies (N=20), MICs of multidrug resistant P.aeruginosa and their transconjugants E.coli J53 (µg/ml) recovered from RTI.

Isolate	IPM	MEM	AZM	CRO	CAZ	CST	TIC	TIC/CA	PIP	PIP/TZ	AMK	GEN	TOB	NET	CIP	LEV	MXF
ETB1993	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC1993	64	64	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.5	0.3	0.5	0.25	0.5
ETB2054	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2054	32	32	0.5	128	128	0.5	32	32	32	32	0.5	0.5	0.5	0.5	1	1	1
ETB2063	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2063	32	32	0.5	64	64	0.25	32	16	32	16	0.5	0.5	0.5	0.5	0.5	0.5	0.5
ETB2064	32	32	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2064	8	8	0.25	32	32	0.25	16	16	16	16	0.5	0.5	0.5	0.5	1	1	1
ETB2085	32	32	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2085	16	16	0.25	64	64	0.5	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
ETB2090	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2090	32	32	0.5	32	32	0.25	32	32	32	32	1	1	1	1	1	1	1
ETB2111	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2111	16	16	0.5	32	32	0.75	32	32	32	32	0.25	0.25	0.25	0.3	0.25	0.25	0.25
ETB2142	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2142	16	16	0.5	32	32	0.5	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
ETB2141	32	32	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2141	8	8	0.5	64	64	0.75	64	64	64	64	1	0.5	0.25	0.3	1	2	1
ETB2142	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2142	32	32	0.5	64	64	0.5	64	64	64	64	0.25	0.25	0.25	0.3	1	1	1
ETB2160	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2160	8	8	0.25	64	64	0.5	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
ETB2161	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2161	16	16	0.25	64	64	0.5	64	64	64	64	0.5	0.5	0.5	0.3	0.25	0.25	0.25
ETB2180	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2180	64	64	0.5	64	64	0.75	64	64	64	64	0.25	0.25	0.25	0.3	0.75	0.75	0.75
ETB2223	32	32	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2223	32	32	0.5	64	64	0.5	64	64	64	64	0.25	0.25	0.25	0.3	0.25	0.25	0.25
ETB2346	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2346	64	64	0.25	64	64	0.25	64	64	64	64	0.25	0.5	0.5	0.5	1	1	1
ETB2514	32	32	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2514	32	32	0.25	64	64	0.75	64	64	64	64	0.25	0.25	0.25	0.3	0.25	0.25	0.25
ETB2577	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2577	64	64	0.5	64	64	0.125	64	64	64	64	0.5	0.5	0.5	0.5	1	1	1
ETB2669	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2669	64	64	0.25	64	64	0.25	64	64	64	64	0.25	0.25	0.25	0.3	0.25	0.25	0.25

ETB2701	32	32	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2701	32	32	0.5	64	64	0.5	64	64	64	64	0.5	1	1	1	0.5	0.5	0.5
ETB2723	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2723	32	32	0.25	64	64	0.75	64	64	64	64	0.25	0.25	0.25	0.3	0.25	0.25	0.25

Table 9D: Showing strains selected for conjugational studies, MICs of multidrug resistant P.aeruginosa and their transconjugants E.coli J53 (µg/ml) recovered from SSTIs.

Isolate	IPM	MEM	AZM	CRO	CAZ	CST	TIC	TIC/CA	PIP	PIP/TZ	AMK	GEN	TOB	NET	CIP	LEV	MXF
PC241	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC241	32	32	0.25	128	128	0.125	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.5	0.5
PC243	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC243	32	64	0.25	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	0.5	0.5	0.5
PC260	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC260	32	64	0.25	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	0.25	1	1
PC265	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC265	64	64	0.5	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	1	0.25	0.25
PC302	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC302	32	32	0.25	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	0.5	0.5	0.5
PC322	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC322	32	32	0.25	128	128	0.25	128	128	128	128	0.5	o.5	0.25	0.25	1	1	1
PC342	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC342	64	64	0.5	128	128	0.25	128	128	128	128	0.5	0.5	0.25	0.5	1	1	0.25
PC373	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC373	32	32	0.25	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PC389	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC389	64	64	0.25	64	64	0.75	64	64	64	64	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PC393	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC393	64	64	0.5	64	64	1	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PC417	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC417	32	32	0.25	64	64	0.5	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PC424	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC424	32	32	0.25	64	64	0.5	64	64	64	64	0.25	0.25	0.25	0.25	1	0.5	0.5
PC434	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC434	32	64	0.5	128	128	1	128	128	128	128	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PC450	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC450	64	64	0.5	128	128	0.5	128	128	128	128	0.25	0.25	0.25	0.25	1	1	1
PC463	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC463	8	8	0.25	128	128	0.5	128	128	128	128	0.25	0.25	0.25	0.25	1	2	1
PC484	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8

TC484	64	64	0.5	64	64	1	64	64	64	64	0.25	0.25	0.25	0.25	2	2	1
PC493	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC493	16	32	0.25	64	64	0.25	64	64	64	64	0.25	0.25	0.25	0.25	1	1	1
PC511	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC511	64	64	0.5	128	128	0.25	128	128	128	128	0.25	0.25	0.25	0.25	2	2	2

Table 9E: Showing strains selected for conjugational studies, MICs of multidrug resistant P.aeruginosa and their transconjugants E.coli J53 (µg/ml) recovered from UTIs.

ISOLATE	IPM	MEM	AZM	CRO	CAZ	CST	TIC	TIC/CA	PIP	PIP/TZ	AMK	GEN	TOB	NET	CIP	LEV	MXF
UC1773	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC1773	64	64	0.12	128	128	0.25	64	64	64	64	0.5	1	0.25	1	2	2	1
UC2524	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2524	64	64	0.25	128	128	0.125	64	64	64	64	0.25	0.5	0.5	0.25	1	1	1
UC2603	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2603	64	64	0.5	128	128	0.5	64	64	64	64	1	1	1	1	1	1	1
UC2789	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2789	64	64	0.25	128	128	0.25	64	64	64	64	0.25	0.25	0.25	0.25	0.25	0.25	0.25
UC2929	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC2929	64	64	0.5	128	128	0.25	64	64	64	64	0.25	0.25	0.25	0.25	0.25	0.25	0.25
UC3009	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3009	64	64	0.5	128	128	0.25	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
UC3082	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3082	64	64	0.25	128	128	0.25	64	64	64	64	0.25	0.25	0.25	0.25	0.25	0.25	0.25
UC3251	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3251	64	64	0.5	128	128	0.25	64	64	64	64	0.25	0.25	0.25	0.25	0.25	0.25	0.25
UC3309	32	32	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3309	32	32	0.25	128	128	0.25	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
UC3371	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3371	64	64	0.5	128	128	0.25	64	64	64	64	0.5	0.5	1	0.5	1	1	1
UC3689	64	64	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3689	32	32	0.5	128	128	0.25	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
UC3764	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3764	64	64	0.25	128	128	0.25	64	64	64	64	1	1	1	1	1	1	1
UC3830	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3830	64	64	0.5	128	128	0.25	64	64	64	64	0.25	0.25	0.25	0.25	0.25	0.25	0.25
UC3831	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3831	64	64	0.5	128	128	0.25	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5
UC3841	128	128	128	128	128	≤2	128	≥128/2	128	≥128/4	≥64	≥16	≥16	≥32	≥4	≥8	≥8
TC3841	64	64	0.25	128	128	0.25	64	64	64	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5

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

P.aeruginosa (N=06) isolates.										
ISOLATE	MBL	Plasmid	Transfer	Other ESBL gene present			Plasmid type			Transfer
BACT368	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable
BACT342	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT381	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable
BACT392	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
BACT412	NDM-1	N	transferable	*	CTXM-15	*	*	Y	*	Transferable
BACT460	NDM-1	N	transferable	*	CTXM-15	*	*	Y	*	Transferable
P.aeruginosa (N=20)										
ETB1993	VIM-2	FII	Transferable	TEM-1	CTXM-14	SHV-5	FIA	Y	P	Transferable
ETB2054	VIM-2	N	Transferable	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
ETB2063	VIM-2	FII	Transferable	TEM-1	CTXM-14	SHV-5	FIA	Y	P	Transferable
ETB2064	VIM-2	FII	Transferable	TEM-1	C TXM-14	SHV-5	FIA	Y	P	Transferable
ETB2085	VIM-2	FII	Transferable	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
ETB2090	VIM-2	N	Transferable	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
ETB2111	VIM-2	N	Transferable	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
ETB2142	VIM-2	N	Transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
ETB2141	VIM-2	N	Transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
ETB2142	VIM-2	FII	Transferable	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
ETB2160	VIM-2	FII	Transferable	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
ETB2161	VIM-2	FII	Transferable	TEM-1	CTXM-15	SHV-11	FIA	T	W	Transferable
ETB2180	NDM-1	A/C	Transferable	-	CTXM-14	SHV-28	-	Y	FIC	Transferable
ETB2223	NDM-1	A/C	Transferable	-	CTXM-14	SHV-28	-	Y	FIC	Transferable
ETB2346	NDM-1	A/C	Transferable	-	CTXM-15	SHV-12	-	T	FIB	Transferable
ETB2514	NDM-1	A/C	Transferable	-	CTXM-15	SHV-12	-	T	FIB	Transferable
ETB2577	NDM-1	A/C	Transferable	-	CTXM-15	SHV-12	-	T	FIB	Transferable
ETB2669	NDM-1	A/C	Transferable	-	CTXM-15	SHV-12	-	T	FIB	Transferable
ETB2701	NDM-1	A/C	Transferable	-	CTXM-15	SHV-12	-	T	FIB	Transferable
ETB2723	NDM-1	A/C	Transferable	-	CTXM-14	SHV-28	-	Y	FIC	Transferable
Plasmid typing of P.aeruginosa (N=18) isolates										
PC241	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIA	FIA,FIB	FIA	Transferable
PC243	VIM-6	N	transferable	TEM-1	CTXM-14	SHV-28	FIC	Y	FIC	Transferable
PC260	VIM-6	FII	transferable	TEM-1	CTXM-14	SHV-28	FIB	Y	FIC	Transferable
PC265	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	FIA,FIB	FIC	Transferable
PC302	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIB	FIA,FIB	FIA	Transferable
PC322	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIC	FIA,FIB	FIA	Transferable
PC342	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIB	FIA,FIB	FIC	Transferable
PC373	VIM-6	N	transferable	TEM-1	CTXM-14	SHV-28	FIA	Y	FIC	Transferable
PC389	VIM-2	FII	transferable	TEM-1	CTXM-14	SHV-12	FIB	Y	FIA	Transferable
PC393	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	FIA,FIB	FIC	Transferable
PC417	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIB	FIA,FIB	FIA	Transferable
PC424	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-28	FIC	FIA,FIB	FIC	Transferable
PC434	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-12	FIB	FIA,FIB	FIA	Transferable
PC450	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	FIA,FIB	FIC	Transferable
PC463	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	FIA,FIB	FIC	Transferable
PC484	NDM-1	A/C	transferable	TEM-1	CTXM-14	SHV-12	FIB	Y	FIA	Transferable
PC493	NDM-1	A/C	transferable	TEM-1	CTXM-14	SHV-12	FIB	Y	FIA	Transferable
PC511	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-28	FIA	FIA,FIB	FIC	Transferable
`*P.aeruginosa* (N=15) isolates										
UC1773	NDM-1	A/C	transferable	TEM-1	CTXM-14	SHV-12	FIA	Y	FIB	Transferable
UC2524	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIA	Transferable
UC2603	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
UC2789	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
UC2929	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
UC3009	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
UC3082	VIM-6	B/O	transferable	TEM-1	CTXM-14	SHV-11	FIA	Y	W	Transferable

UC3251	VIM-6	B/O	transferable	TEM-1	CTXM-14	SHV-11	FIA	Y	W	Transferable
UC3309	VIM-6	B/O	transferable	TEM-1	CTXM-14	SHV-05	FIA	Y	P	Transferable
UC3371	VIM-6	B/O	transferable	TEM-1	CTXM-14	SHV-05	FIA	Y	P	Transferable
UC3689	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
UC3764	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable
UC3830	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
UC3831	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
UC3841	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable
P.aeruginosa (N=10) isolates										
IAI252	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable
IAI254	NDM-1	A/C	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable
IAI271	NDM-1	N	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable
IAI813	NDM-1	N	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable
IAI971	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	Y	FIC	Transferable
IAI1134	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable
IAI1571	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable
IAI1703	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	Y	FIC	Transferable
IAI1801	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-28	FIA	Y	FIC	Transferable
IAI1854	VIM-2	FII	transferable	TEM-1	CTXM-15	SHV-12	FIA	T	P	Transferable

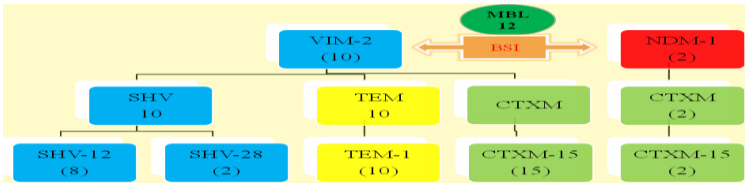

Fig. 1: Showing distribution of beta-lactamase genes in association with MBL genes in *P.aeruginosa* isolated from BSIs.

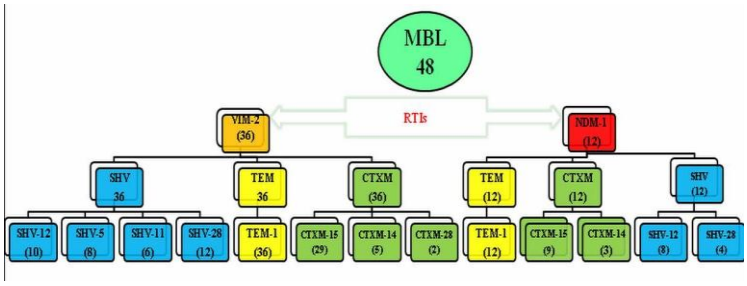

Fig. 2: Showing distribution of beta-lactamase genes in association with MBL genes in *P.aeruginosa* isolated from RTIs.

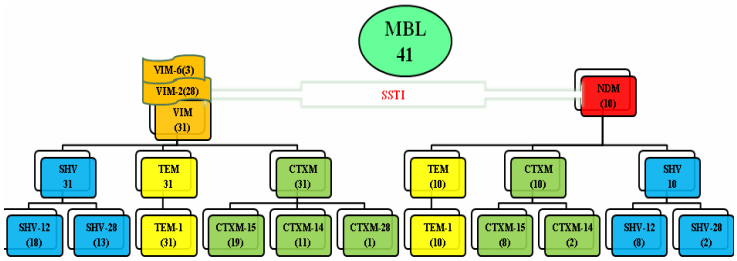

Fig. 3: Showing distribution of beta-lactamase genes in association with MBL genes in *P.aeruginosa* isolated from SSTIs.

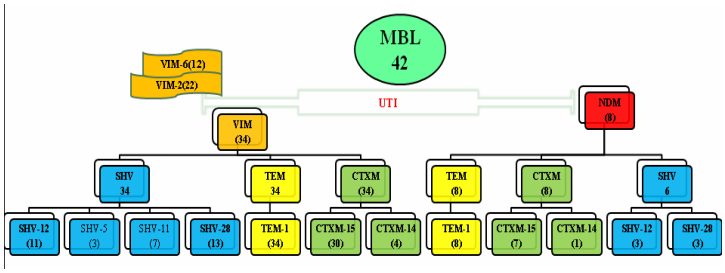

Fig. 4: Showing distribution of beta-lactamase genes in association with MBL genes in *P.aeruginosa* isolated from UTIs.

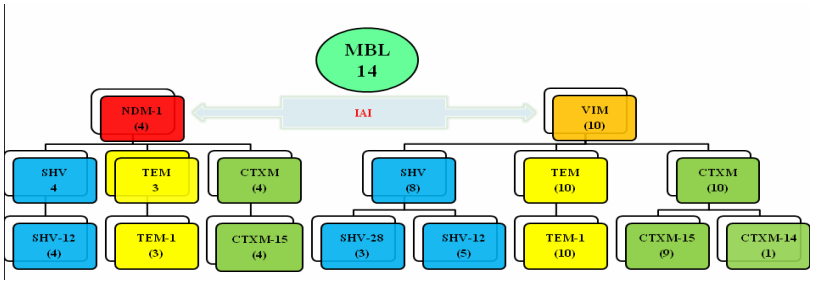

Fig. 5: Showing distribution of beta-lactamase genes in association with MBL genes in P.aeruginosa isolated from IAIs.

Molecular typing of 157 strains of *P.aeruginosa* by RAPD generated 10 RAPD pattern assigned as Ps-A TO Ps-J with an average of 08 to 15 fragments per strains **Figure-6**. REP PCR produced 10 clonal clusters with an average of 4 to 8 fragments per *P.aeruginosa* strains **Figure-7**.

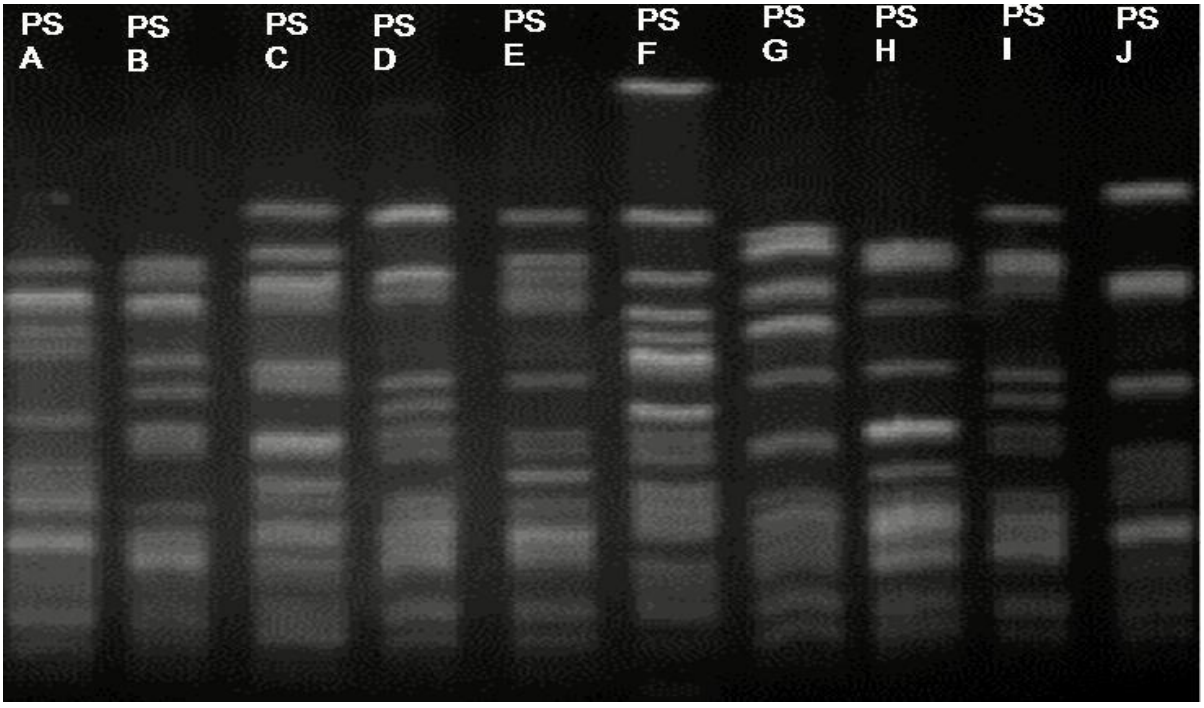

Figure 6: Showing RAPD PCR among 10 clonal cluster of *P.aeruginosa*.

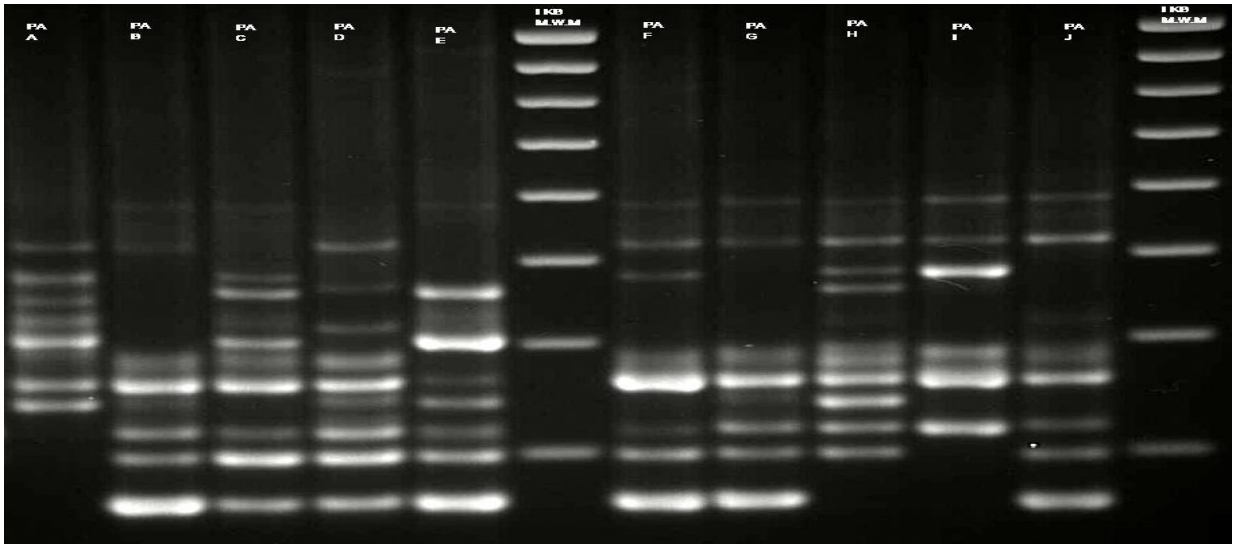

Figure 7: Showing REP PCR among 10 clonal cluster of *P.aeruginosa.*

DISCUSSION

Carbapenem resistant *P.aeruginosa* is a serious cause of nosocomial infections at almost all sites of the body that result in number of different clinical syndromes that are often manifestations of its opportunism. Urinary tract infections generally occur as a complication of the presence of a foreign body such as a stone, stent, or catheter in the urinary tract or the presence of an obstruction within the genitourinary system or after instrumentation or surgery on the urinary tract. In the present study, the major source of MDRPA was found to be Urine, followed by 28% in wound swabs and 23% in endotracheal aspirate implying that *P.aeruginosa* is more frequent in males due to particular characteristics inherent to the patient, including sex, previous use of antimicrobials, previous interventions in the urinary tract and patients with neurogenic bladder followed by wound infections and respiratory tract infections are the most significant infections caused by MDRPA in our settings. Our study revealed *P.aeruginosa* infection was significantly associated among hospitalized, elderly (≥58 years) and in those patients who had already received any type of invasive procedure such as catheterization, intubation or ventilation. *P.aeruginosa* is rarely seen as a member of human normal flora. However, colonization rates may exceed 50%-70% during hospitalization, especially among imunosuppressed, immunocompromised, debilitated, impaired immunity ICU patients who had experienced mechanical ventilation, tracheotomy, catheters, surgery or severe burns. These isolates were also 100% resistant to CAZ, CPZ, CRO, CZ, FOX, TOB, SXT, TZP, LVX, CIP, SFP, GEN and AMK. In this study 45% of *P.aeruginosa* isolates were XDR. In the present study, *P.aerugiosa* had shown a prevalence of 30% in MBL production. In this study, these resistant isolates further tested by MHT revealed 13% isolates as positive for carbapenemase production, MBL E-test identified 30% as MBL producers while IPM EDTA-DDST identified 24% whereas 24.8% were MBL positive by IPM EDTA-CDST method. MHT, DDST and CDST assay for P.aeruginosa showed sensitivity of 43.31%, 80.25% and 82.80%, Specificity of 100%, its PPV was 100% and its NPV was 80.53%, 92.23% and 93.16% respectively. P.aeruginosa in comparison of DDST assay with MBL E-Test showed sensitivity of 80.25%, Specificity of 100%, and NPV of 92.23% and When comparing CDST assay with MBL E-Test P.aeruginosa showed sensitivity of 82.80%, Specificity of 100%, and NPV of 93.16%. Carbapenem resistant isolates are generally resistant to most other classes of antibiotics, while usually retaining susceptible to colistin. In this study, MHT for P.aeruginosa showed sensitivity of 43.31%, and its NPV was 80.53% respectively. Among the other fallacies, the qualitative nature of the MHT causes subjective variations while interpreting the results. The results produced are not always binary, indeterminate results are a common occurrence, which can neither be considered positive, nor negative. In the present study, *bla*NDM-1was detected in 36 *P.aeruginosa* isolates, while blaVIM in 121 isolates. Furthermore, *bla*IMP, *bla*SIM, *bla*SPM and *bla*GIM were not detected in any of the study isolates. Among ESBLs genes, blaCTX-M was present in 162 isolates, followed by blaTEM-1 in 154, and blaSHV in 151 isolates. blaSHV-5, blaSHV-11, blaSHV-12, and blaSHV-28 are the commonest SHV genes detected in 11, 13, 75 and 52 isolates respectively whereas blaCTX-M-15, blaCTX- M-14 and blaCTX-M-28 are the commonest CTX-M ESBLs that were present in 132, 27 and 3 isolates respectively. This study is first from India to highlight the presence of *bla*NDM-1 in carbapenem resistant *P.aeruginosa*. Considering that of the 157 carbapenem resistant MBL producing *P. aeruginosa* isolates tested, only 36 were found to harbour NDM-1, it can be reasonably assumed that NDM-1 is not a major mechanism mediating MBL carbapenem resistance in *P.aeruginosa* in India. Co-association of blaNDM-1 with blaTEM-1 was detected in 33, blaNDM-1 with blaCTX-M-15 present in 30 and blaNDM-1 with blaCTX-M-14 in 6 whereas blaNDM- 1 with blaSHV-12and blaSHV-28

was detected in 23 and 9 isolates respectively. NDM-1 was first identified in *Enterobacteriaceae* and recently being reported in *A.baumannii* also. Detection of *bla*NDM- 1 in *P.aeruginosa* as demonstrated in our study indicates that this gene can spread at a high rate from fermentative GNB to non-fermenters. The genes encoding NDM are heterogeneous on the basis of molecular size and location. In *Enterobacteriaceae*, it is plasmid-borne, while in *A.baumannii* it is present both chromosomally or plasmid located. NDM-1 firstly being reported revealed in seven clinical isolates of *P.aeruginosa* from Military Medical Academy in Serbia, from hospitalized patients that had no history of travel to other country. After our findings in India,[6] NDM-1 producing *P.aeruginosa* had being reported in 2012 from France in a case of recurrent pyelonephritis.[9,10,11] This patient had history of prior hospitalization in Serbia and hence it was hypothesized by some authors that the Balkan States may be endemic for NDM-1.[9] But our report is against this hypothesis since it is also found in Indian sub-continent. Our study has proved that, NDM-1 is disseminated with plasmids from one bacterial species to another. This study being a single center, further studies are required at national or regional levels to understand the magnitude and prevalence of NDM-1 in P.aeruginosa.

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