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INCIDENCE AND DETECTION OF METALLO-BETA-LACTAMASE PRODUCING *P.MIRABILIS IN* A TERTIARY CARE HOSPITAL *AT CENTRAL INDIA*

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ABSTRACT

Objective: To examine the distribution, emergence and spread of genes encoding beta-lactamase resistance in ***P.mirabilis*** 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 115 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 115 ***P.mirabilis*** isolates were recovered, largest proportion of specimens were from SSTIs 39.1%, followed by 29.6% in UTIs, 14.8% in IAIs 9.6% in BSIs, and 6.9% in RTIs, respectively. Among 115 tested isolates, **64.3%** isolates showed MIC >4µg/ml against imipenem and meropenem. Of the total number of samples, males contributed 71.3% while females contributed 28.7%. Of the total samples, highest **26.9**% were from Medicine ward followed by 24.3% in ICU Medical, 21.7% in ICU Surgery, 14% in surgery ward 7.9% in Urology and 5.2% in orthopedics. Majority of Carbapenem resistant ***P.mirabilis*** 40.5% were from SSTIs, followed by 27% in UTIs, 16.2% in IAIs, 12.2% in BSIs, and 4.1% in RTIs, respectively. MHT was positive in **31.3%,** DDST in **48.7%**, CDST in **49.6%**, and MBL (IP/IPI) E-test in **53%** isolates. 100% ***P.mirabilis*** 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 ***P.mirabilis*** 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* ***P.mirabilis.***

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

INTRODUCTION

Proteus mirabilis and *Proteus vulgaris* account for majority of clinical isolates in the genus Proteus that are lactose negative, motile and produce phenylalanine deaminase. Both produce urease, H2S, are capable of swarming motility and the latter is indole positive. P.mirabilis is the most frequently encountered species of the tribe in human infections and is responsible for 70–90 percent of all Proteus infections in man. The most common site of Proteus infection is the urinary tract and P.mirabilis, the species most frequently implicated, is possibly the organism after Escherichia coli most frequently associated with urinary tract infections, occurs commonly in those with indwelling catheters or anatomic or functional abnormalities of the urinary tract. UTIs caused by Proteus spp. tend to be more severe than those caused by E.coli, with a higher proportion representing pyelonephritis.

Proteus spp. are secondary to UTI, *P.mirabilis* commonly isolated from the bloodstream, often associated with urinary catheters. Second only to *E.coli* as a cause of bacteremia from a urinary source, and its fimbriae also play a major role in the adhesion of Proteus to catheters and the persistence of infection in catheter-associated Proteus bacteriuria. *P.mirabilis* tend to produce a potent Hemolysin and urease that also has been confirmed to contribute to both colonization and stone formation.[1-4]

Carbapenems demonstrate broader antimicrobial spectrum in vitro than the available penicillins, cephalosporins, and β-lactam/ β-lactamase inhibitor combinations. Because of their broad antibacterial spectrum covering gram-positive, gram-negative, and anaerobic bacteria, carbapenems are useful for treatment of a wide variety of infections, including respiratory tract infections. bacteremia, Skin and soft tissue infections, obstetric and gynaecologic infections, complicated urinary tract infections, and intra-abdominal infections.

Resistance to carbapenems is most frequently mediated by the enzymatic hydrolysis of the drugs by *P.mirabilis*.[1-5] 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.[5,6] Reports of *P.mirabilis* producing these carbapenemases are disturbing as these multidrug-resistant infections leave patients with very few or no antimicrobial options.[5-6] Based on these considerations, this study was aimed to detect infections caused by *P.mirabilis* its incidence and an insight into the acquisition and spread of the MBL genes in *P.mirabilis* and emphasizes its transmission capability through plasmids.

MATERIALS AND METHODS

Study design & 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 115 *P.mirabilis all non-duplicate clinical* isolates were recovered from clinical specimens of hospitalized patients admitted to the Medical and Surgical intensive care units (one isolate per patient). Samples were collected from patients, using strict aseptic precautions and in accordance with standard protocols[7] and immediately processed without any delay. *P.mirabilis* 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.[8] 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.[8,9] MICs of imipenem, ertapenem, and meropenem 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.[10,11]

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).[10,11] 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.mirabilis* 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* (V517and 39R861) on a horizontal

0.5% agarose gel at 50 volts for 3 Hrs. Bands were visualized with UV transilluminator after staining with 0.05%ethidium bromide.[10,11]

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.mirabilis* strains recovered from patients.[9,10] Similarity clustering analysis was performed using unweighted pairgroup method with arithmetic mean and Dice coefficient. Clinical isolates with a similarity coefficient >85% were considered clonal.[12,13]

Plasmid analysis

Plasmid from the parental strains and their transconjugants was extracted by using Qiagen plasmid maxi 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.[14] Plasmid replicons were determined for the ESBL as well as carbapenemase producing clinical isolates.

STATISTICAL ANALYSIS

The prevalence of *P.mirabilis* 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.mirabilis* among clinical specimens

A total of 115 *P.mirabilis* isolates were recovered from various clinical specimens in a prospective study that was conducted in a 1800 bedded tertiary care centre in Pune, India. A total of 115 ***P.mirabilis*** isolates were recovered, largest proportion of specimens were from SSTIs 39.1%, followed by 29.6% in UTIs, 14.8% in IAIs 9.6% in BSIs, and 6.9% in RTIs, respectively **Table-1**. Of the total number of samples, males contributed 71.3% while females contributed 28.7% **Table-2**. Of the total samples, highest **26.9**% were from Medicine ward followed by 24.3% in ICU Medical, 21.7% in ICU Surgery, 14% in surgery ward 7.9% in

Urology and 5.2% in orthopedics **Table-3**. Majority of Carbapenem resistant ***P.mirabilis*** 40.5% were from SSTIs, followed by 27% in UTIs, 16.2% in IAIs, 12.2% in BSIs, and 4.1% in RTIs, respectively **Table-1**.

Antimicrobial susceptibility of *P.mirabilis* isolates

Evaluation of antibiotic susceptibility pattern indicated that 21% ***P.mirabilis*** were resistant against IPM, MEM, and ETP. Antibiogram and resistance percentage of ***P.mirabilis*** various infection sites shown in **Table-4** 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: TET (75%;R), SXT (77%;R), AMP(90%;R), AMC (87%;R), SAM (81;R), CRO (82%;R), ATM(81%;R), CTX(81%;R), CZ(82%;R), CPZ (80%;R), CPD (81%;R), CAZ (81%;R), PIP (81%;R), FEP (75%;R), FOX (78%;R), GEN (72%;R), TOB (75%;R), AMK (77%;R), SFP (73%;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 ***P.mirabilis*** shown in **Table-5**.

Phenotypic detection of carbapenem-hydrolyzing- beta-lactamases

Out of 115 isolates, **74 (64.3%)** 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 **36 (31.3%),** DDST in **56 (48.7%)**, CDST in **57(49.6%)** isolates, MBL (IP/IPI) E-test was positive for **61(53%)** and **13(11.3%)** isolates were Non MBL. Results of different phenotypic tests of ***P.mirabilis*** recovered from various clinical specimens are shown in **Table-6.** 100% ***P.mirabilis*** isolates retained susceptible to colistin.

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

The prevalence of MBL-encoding genes among ***P.mirabilis*** isolates was determined in the present study, MBL *was* present in 61.Among the tested genes, $bla_{\text{NDM-1}}$ was the most prevalent gene as it was detected in 52, $bla_{\textbf{VIM-2}}$ was present in 9, $bla_{\text{CTX-M-15}}$, and $bla_{\text{CTX-M-14}}$ are the commonest CTX-M ESBLs that were present in 52, and 7 isolates. $bla_{\text{SHV-5}}$, $bla_{\text{SHV-12}}$, and $bla_{\text{SHV-28}}$ are the commonest SHV genes detected in 4, 22, and 26 of bla_{SHV} producing isolates respectively whereas $bla_{\text{TEM-1}}$ was present in 61 isolates Table-7A&7B.

Conjugation

For conjugational studies and PCR based molecular typing 34 ***P.mirabilis*** isolates were selected. 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, CAZ, CRO, FEP, PTZ, CPZ, CTX, FOX, were high among transconjugants; (MIC, ≥64 µg/ml). The transconjugants were resistant to imipenem IMP, MEM, and ETP; (MIC, ≥8-32µg/ml), whereas MICs of AMK, GEN, TOB, CIP, MXF, LVX, TGC; (MIC, ≤2µg/ml), CST; (MIC, <1µg/ml) and ATM fall within susceptible range as determined by E-test. Results of conjugational studies are shown in Table-8.

Plasmid Typing and characterization of Plasmid: Plasmid from both the ***P.mirabilis*** parental strains and their transconjugants was characterized and found that $bla_{\text{NDM-1}}$ gene was located on IncA/C, IncHI1 and IncHI2 plasmids. $Bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ gene was carried on plasmids belonging to IncP, IncT and IncY replicons, $bla_{\text{TEM-1}}$ was associated with IncFIA type replicons while $bla_{\text{SHV-5}}$,$bla_{\text{SHV-28}}$, and $bla_{\text{SHV-12}}$ gene in association with $bla_{\text{NDM-1}}$ located on IncP, IncW, IncFIC and IncFIB type replicons respectively. Plasmid Typing and its characterization are shown in Table-9.

Strain molecular typing: Molecular typing of *P.mirabilis* by RAPD, ERIC PCR and REP PCR generated 10 cluster pattern assigned as P.r.-A TO P.r.-J produced an average of 12-14 fragments per *P.mirabilis* strains.

Plasmid size estimation: Plasmid size for NDM-1 gene was ranged from 30 kb to 180 kb while b$la_{\text{VIM-2}}$ was located on a 80 kb to 130kb, size plasmid whereas $bla_{\text{SHV- 5}}$, $bla_{\text{SHV-12}}$, $bla_{\text{SHV-28}}$ were located on a 30-kb, 110kb, and 130kb size plasmid respectively. Plasmid size for $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-28}}$ & $bla_{\text{CTX-M-15}}$ were ranged from 50kb to 100 kb in size while $bla_{\text{TEM-1}}$ gene was located on a plasmid 70 kb to 120 kb in size. Plasmid size for $bla_{\text{OXA-48}}$ was50kb.

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

Specimen	BSIs	RTIs	SSTIs	UTIs	IAIs	TOTAL
Carbapenem resistant	9	3	30	20	12	74
Total Isolated	11	8	45	34	17	115

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

Organism	Total Cases	Number of Males	Percentage	Number of Females	Percentage
P.mirabilis	115	82	71.3	33	28.7

Table. 3: Showing number wise ward percentage distribution of *P.mirabilis* in Wards.

Wards	Number of Cases(N=115)	Percentage of org
Surgery ward	**16**	**14**
ICU Surgery	**25**	**21.7**
Medicine ward	**31**	**26.9**
ICU Medical	**28**	**24.3**
Urology	**9**	**7.9**
Orthopedics	**6**	**5.2**
Total	115	100

Table. 4: Showing Antibiogram and resistance percentage of P.mirabilis various infection sites.

Antibiotic	BSIs	RTIs	SSTIs	UTIs	IAIs	Resistance	%
AMK	9	3	35	27	15	89	77.3
AMC	9	5	39	32	15	100	87
AMP	9	5	40	32	17	103	90
SAM	9	5	36	29	15	94	81.7
ATM	9	5	39	29	12	94	81.7
FEP	9	5	36	25	12	87	75.6
CTX	9	5	39	29	12	94	81.7
FOX	9	5	39	25	12	90	78.2
CZ	9	5	40	29	12	95	82.6
SFP	9	5	34	25	12	85	73.9
CPZ	9	5	39	27	12	92	80
CPD	9	5	40	28	12	94	81.7
CAZ	9	5	39	29	12	94	81.7
CRO	9	5	40	29	12	95	82.6
CIP	9	5	32	27	12	85	73.9
ETP	9	3	30	20	12	74	64.3
GEN	9	5	32	25	12	83	72
IPM	9	3	30	20	12	74	64.3
LVX	9	5	32	20	12	78	68
MEM	9	3	30	20	12	74	64.3
PIP	9	5	39	29	12	94	81.7
TZP	9	5	36	20	12	82	71.3
TET	9	5	32	29	12	87	75.6
TIC	9	5	32	25	12	83	72
TCC	9	5	30	20	12	76	66
TOB	9	5	36	25	12	87	75.6
SXT	9	5	34	29	12	89	77.3
TGC	3	2	14	0	3	22	19
TOTAL	**11**	**8**	45	**34**	**17**	**115**	

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. 5: Showing MICs of imipenem, ertapenem and meropenem against *of P.mirabilis.*

Antibiotic concentrations (µg/ml)	Number of sample (N=115)	Percentage
0.25	14	12
0.5	8	7
1	9	7.9
2	9	7.9
4	1	0.8
8	18	15.6
16	27	23.5
32	19	16.6
64	10	8.7
Total	115	100

Table. 6: Showing showing percentage and result of different phenotypic tests of *P.mirabilis* recovered from various infection sites.

Infection sites	Isolated	CR MIC[a]	Mbl ETEST[B]	DDST[c]	CDST[d]	MHT[e]	Non Mbl[f]
BSIs	11	9	7	7	7	5	2
RTIs	8	3	3	3	3	3	0
SSTIs	45	30	28	28	28	15	2
UTIs	34	20	15	12	12	8	5
IAIs	17	12	8	6	7	5	4
Total	115	74**(64.3%)**	61**(53%)**	56**(48.7%)**	57**(49.6%)**	36**(31.3%)**	13**(11.3%)**

Table. 7A Showing Distribution of beta-lactamase genes.

P. Mirabilis	NDM	TEM	SHV	CTXM	VIM	TEM	CTXM
IAIs	8	8	8	8			
UTIs	15	15	15	15			
SSTIs	19	19	19	19	9	9	9
RTIs	3	3	3	3			
BSIs	7	7	7	7			
TOTAL	52	52	52	52	9	9	9

Table. 7: B showing Distribution of beta-lactamase genes.

Gene	NDM-1	TEM-1	CTXM-15	CTXM-14	CTXM-28	SHV-12	SHV-28	SHV-5	VIM-2	OXA-48
P.mirabilis	52	61	52	7	2	22	26	4	9	15

Table. 8: Showing Antibiogram of *P.mirabilis* donor and Transconjugants From 52 bla_{NDM-1} producers 34 and from 9 bla_{VIM} producers FIVE were selected as a donor *P.mirabilis* strains for conjugation studies.

ISOLATE	IPM	MEM	ETP	ATM	CS	TGC	CAZ	CTX	CN	FEP	CPZ	PT	AMP	CIP	LVX	MOX	GEN	AMK	TOB
BACT 89	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR89	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
BACT 78	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TCPR78	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
BACT 132	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TCPR132	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
BACT 209	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TCPR209	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
ETB-248	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TCPR248	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
ETB -316	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TCPR316	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
ETB -324	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TCPR324	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
PC-PR09	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR09	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
PC-PR13	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TCPR13	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
PC-PR18	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR18	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
PC-PR21	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TCPR21	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
PC-PR28	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	8	8	8	>16	64	>16

TCPR28	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.25	1	2	1
PC-PR35	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TCPR35	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	2	1
PC-PR43	64	64	32	64	0.5	0.25	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR43	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	1
PC-PR47	64	64	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TCPR47	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
PC-PR52	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TCPR52	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
PC-PR72	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TCPR72	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
PC-PR39	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR39	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
PC-PR44	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TCPR44	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
PC-PR57	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR57	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
PC-PR62	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TCPR62	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
UC-PR22	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TCPR22	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.25	1	2	1
UC-PR29	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TCPR29	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	2	1
UC-PR36	64	64	32	64	0.5	0.25	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR36	16	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	1
UC-PR47	64	64	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	8	>16	64	>16
TCPR47	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
UC-PR54	64	64	32	64	0.125	1	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TCPR54	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	2
UC-PR62	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TCPR62	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2

UC-PR73	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR73	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	0.5	1	2	2
UC-PR82	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TCPR82	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
IAI-PR05	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCPR05	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
IAI-PR12	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TCPR12	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2
IAI-PR19	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TCPR19	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.25	1	2	1
IAI-PR28	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TCPR28	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	1	2	1

Table. 9: Showing characterization of beta-lactamase encoding Plasmid and its Typing.

ISOLATE	MBL	Plasmid	Transfer	Other ESBL gene present			Plasmid type			Transfer	Class D	Plasmid
BACT 89	**NDM-1**	**A/C**	transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
BACT 78	**NDM-1**	**HI2**	transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**P**	Transferable	*	*
BACT 132	**NDM-1**	**HI2**	transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
BACT 209	**NDM-1**	**HI2**	transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
ETB-248	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**P**	Transferable	*	*
ETB -316	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
ETB -324	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**P**	Transferable	*	*
PC-PR09	**VIM-2**	**FII**	Transferable	**TEM-1**	**CTXM-15**	**ND**	**FIA**	**P**	**ND**	Transferable	*	*
PC-PR13	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
PC-PR18	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**FIB**	Transferable	*	*
PC-PR21	**VIM-2**	**FII**	Transferable	**TEM-1**	**CTXM-15**	**ND**	**FIA**	**P**	**ND**	Transferable	*	*
PC-PR28	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
PC-PR35	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**FIB**	Transferable	*	*
PC-PR43	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**FIB**	Transferable	*	*
PC-PR47	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
PC-PR52	**VIM-2**	**FII**	Transferable	**TEM-1**	**CTXM-15**	**ND**	**FIA**	**P**	**ND**	Transferable	*	*

PC-PR72	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**FIB**	Transferable	*	*
PC-PV39	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
PC-PV44	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**FIB**	Transferable	*	*
PC-PV57	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**FIB**	Transferable	*	*
PC-PV62	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
UC-PR22	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	**OXA-48**	**L/M**
UC-PR29	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	**OXA-48**	**L/M**
UC-PR36	**NDM-1**	**HI1**	Transferable	**TEM-1**	**CTX-M-14**	**SHV-5**	**FIA**	**Y**	**P**	Transferable	**OXA-48**	**L/M**
UC-PR47	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**FIB**	Transferable	**OXA-48**	**L/M**
UC-PR54	**NDM-1**	**HI1**	Transferable	**TEM-1**	**CTX-M-14**	**SHV-5**	**FIA**	**Y**	**P**	Transferable	**OXA-48**	**L/M**
UC-PR62	**NDM-1**	**HI1**	Transferable	**TEM-1**	**CTX-M-14**	**SHV-5**	**FIA**	**Y**	**P**	Transferable	**OXA-48**	**L/M**
UC-PR73	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-5**	**FIA**	**T**	**W**	Transferable	**OXA-48**	**L/M**
UC-PR82	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-12**	**FIA**	**T**	**FIB**	Transferable	**OXA-48**	**L/M**
IAI-PR05	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-14**	**SHV-28**	**FIA**	**Y**	**FIC**	Transferable	*	*
IAI-PR12	**NDM-1**	**HI2**	transferable	**TEM-1**	**CTXM-14**	**SHV-28**	**FIA**	**Y**	**FIC**	Transferable	*	*
IAI-PR19	**NDM-1**	**A/C**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*
IAI-PR28	**NDM-1**	**HI2**	Transferable	**TEM-1**	**CTXM-15**	**SHV-28**	**FIA**	**T**	**FIC**	Transferable	*	*

DISCUSSION

In this study, $bla_{\text{NDM-1}}$ was the most prevalent gene as it was detected in 45.2% isolates along with $bla_{\text{CTX-M,}}$ bla_{SHV} and $bla_{\text{TEM-1}}$ and $bla_{\text{VIM-2}}$ was present in 8% isolates along with $bla_{\text{CTX-M,}}$ and $bla_{\text{TEM-1.}}$ $bla_{\text{CTXM-28,}}$ $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ was the most prevalent CTX-M ESBLs that were present in 45.2%, 8% and 2% isolates respectively whereas $bla_{\text{SHV-5}}$, $bla_{\text{SHV-12}}$, and $bla_{\text{SHV-28}}$ are the commonest SHV genes detected in 4%, 19%, and 26% isolates respectively Table-7A&7B. **Our study is the first study from India to describe co-association of $bla_{\text{OXA-48}}$ with $bla_{\text{NDM-1}}$ $bla_{\text{CTX-M,}}$ bla_{SHV} and $bla_{\text{TEM-1}}$ in fifteen *P.mirabilis* urinary isolates that leads to high level of resistance (MICs ≥128 mg/L) against beta-lactam, beta-lactam-beta-lactamase inhibitor combinations, aminoglycosides and fluoroquinolones.** In this study, we found have that $bla_{\text{NDM-1}}$ gene located on IncA/C plasmids was immediately bracketed by a truncated insertion sequence ISAba125 upstream and the bleomycin resistance gene bleMBL downstream using a PCR mapping approach and sequencing and demonstrated that these plasmids are highly conserved, particularly in regions encoding proteins involved in stability and conjugal transfer. We also have found that IncHI2 type broad-host-range plasmid carrying insertion sequence ISAba125 fragment containing the 145 promoter region, the $bla_{\text{NDM-1}}$ gene, the bleomycin resistance gene bleMBL, and a truncated transposon Tn125.

Carbapenem selective pressure along with factors such as lengthy ward-stay, debilitating clinical condition especially among imunosuppressed, immunocompromised, impaired immunity in ICU patients, and frequent exposure to medical interventions such as tracheostomy, mechanical ventilation, surgery, catheters, or severe burns also aid in development and dissemination of resistant genes in hospital environment.

CONCLUSION

This study provides an insight into the acquisition and emergence of $bla_{\text{OXA-48}}$ & $bla_{\textit{NDM-1}}$ *producing P.mirabilis*, and emphasizes its transmission capability through plasmids. Presence of NDM‑1 and other resistance genes makes the organisms refractory to most of the common antibiotics used in clinical practice. It is a cause for great concern as treatment options are virtually exhausted.

DECLARATIONS

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