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EMERGENCE OF CARBAPENEM RESISTANT RAOULTELLA ORNITHINOLYTICA ISOLATES CO-HARBORING *BLANDM-1* AND *BLA*OXA-48

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ABSTRACT

Objective: To examine the distribution, emergence and spread of genes encoding beta-lactamase resistance in *R.ornithinolytica* 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 13isolates 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 13 *R.ornithinolytica* isolates were recovered, largest proportion of specimens were from UTIs 38.5%, followed by 23% in IAIs, 15.4% in both BSIs, and SSTIs and 7.7% in RTIs, respectively. Among 13 tested isolates, **84.6%** isolates showed MIC >4µg/ml against imipenem and meropenem. Of the total number of samples, males contributed 38.5% while females contributed 61.5%. Of the total samples, highest **61.5%** were from ICU Surgery followed by 38.5% in ICU Medical. Majority of Carbapenem resistant *R.ornithinolytica* 45.6% were from UTIs, followed by 18.2% in both BSIs, and SSTIs, and 9% in both IAIs, and RTIs, respectively. MHT, DDST, CDST, and MBL(IP/IPI) E-test was positive in **84.6%** isolates. 100% *R.ornithinolytica* isolates retained susceptible to polimixin and colistin. Conjugation experiments indicated that bla_{NDM-1}, bla_{OXA-48}, bla_{SHV-12}, bla_{SHV-28}, $bla_{CTX-M-15}$, $bla_{CTX-M-14}$ were transferable via plasmid. **Conclusion:** This study highlights prevalence of bla_{OXA-48} & bla*NDM-1* producing *R.ornithinolytica* 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.

Raoultella ornithinolytica is an encapsulated Gram-negative aerobic bacillus belonging to new genus of the Enterobacteriaceae family formerly known as Klebsiella ornithinolytica. In 2001, Raoultella was excised from Klebsiella, the bacterium was reclassified as Raoultella based on genetic approaches.[1-3] *R.ornithinolytica* is known to inhabit aquatic environments and can be found in hospital environments. *R.ornithinolytica* isolates that could have been erroneously reported as Klebsiella are sometimes be underestimated due to difficulties in its identification that may have led to uncertainty in its incidence, epidemology and its pathogenic role in human infections.[1-5] The pathogenic potential of *R.ornithinolytica* isolates in human disease has become increasingly important. Recently, clinical reports of Raoultella in clinical microbiology laboratories have been increased, probably through the introduction of accurate molecular techniques to identify. Many cases of infection caused by *R.ornithinolytica* have been reported so far.[1-5] However, the clinical features, antimicrobial susceptibility, and clinical outcomes of *R.ornithinolytica* infection have not yet been well elucidated. There are very few studies in Medical literature, regarding MBL detection among *R.ornithinolytica* in India and abroad as compared to other members of family *Enterobacteriaceae*.[3-8] In this prospective study conducted in a tertiary care centre in Pune, seventeen *R.ornithinolytica isolates* were recovered from various clinical specimens, among them 13 were found to be Imipenem, Meropenem, Ertapenem (carbapenem) resistant based upon Minimum inhibitory concentrations (MIC) of antibiotics as determined by VITEK-2 AST-^{GN25} and AST-^{GN280} susceptibility cards. MICs were further determined by the E-test (bioMérieux, Marcy l'Etoile. MHT, DDST, CDST and MBL (IP/IPI) E-test was performed to detect Carbapenemase as well as Metallo-beta-lactamase production as described previously.[2] In the present study, some important cases associated *with* bla_{NDM-1} producing *R.ornithinolytica* are reported below herein- Case 1-A 67-year-old male patient was transferred to our tertiary care centre; he was presented with hypotension, sensory impairment, and poor general condition, poor peripheral perfusion, desaturation and crackling rales. He was undergoing

chemotherapy with methotrexate (20 mg/week) and prednisone (150mg/day) for acute myeloid leukemia. Two days after his admission, *R.ornithinolytica* isolate was obtained from his blood culture and a tracheal aspirate also yielded the same organism, the patient was treated with carbapenem and an amino glycoside Concurrently, a hypermucoviscous *R.ornithinolytica* strain was recovered from a second tracheal aspirate culture (135 CFU/ml). The isolate was resistant to all beta-lactams including carbapenems, aminoglycosides, fluoroquinolones, trimethoprim sulfamethoxazole, doxycycline, fosfomycin, but remained susceptible to colistin, and tigecycline. MHT, MBL E-test and bla_{NDM-1} was positive.

Case 2- A 68-year-old female patient was transferred from a nursing home to our ICU with symptoms of pneumonia, respiratory failure and shock, clinical examination revealed bronchopneumonia in the right lower lung field. Tracheal aspirate culture yielded carbapenem-resistant *R.ornithinolytica*, and the patient received fosfomycin, and cefepime. Due to progressive leukocytosis, unstable hemodynamics, and increased O2 demand, colistin was prescribed instead of cefepime. The patient recovered well and was discharged alive from the ICU after 14 days. The isolate was resistant to all beta-lactams, carbapenems, aminoglycosides, fluoroquinolones, trimethoprim sulfamethoxazole, doxycycline, fosfomycin, but remained susceptible to colistin, and tigecycline. MHT, MBL E-test and bla_{NDM-1} was found to be positive.

Case 3-A 62-year-old female patient had bilateral mediastinal lymph nodes and multiorgan metastasis, catheter-related bacteremia was suspected, and blood cultures grew carbapenem- resistant *R.ornithinolytica* positive for MHT, MBL E-test and bla_{NDM-1}.

Case-4 A 57-year-old female who had been on peritoneal dialysis for the past six years because of end-stage renal disease secondary to diabetes mellitus was admitted for kidney transplant. The patient at the time of admission complained of dysuria, fever and chills. On physical examination, her general condition was moderate and she had a blood pressure of 150/70 mmHg, a heart rate of 115 beats/min, a respiratory rate of 21 breaths/min, and his body temperature was 38 °C. She had suprapubic tenderness, white blood cell count was 19,400 cells/ µL (absolute neutrophil count, 16,811 cells/ µL), and urinalysis showed a high number of white blood cells and erythrocytes. Her biochemical test results were as follows: plasma creatinine 12.8 mg/dL, blood urea nitrogen 92 mg/dL, sodium 139 mEq/dL, potassium 5.2 mEq/dL, and C-reactive protein (CRP) 110 mg/L (reference range, 0–5 mg/L). The peritoneal fluid cell count was 15/mm3. Two peripheral blood cultures, urinary culture and a peritoneal fluid culture sent to microbiology lab. The blood cultures and peritoneal fluid culture turned out negative but her urine culture yielded $bla_{\text{OXA-48}}$ & bla_{NDM-1} producing *R.ornithinolytica* resistant to all beta-lactams, aminoglycosides, fluoroquinolones, trimethoprim sulfamethoxazole, doxycycline, fosfomycin, but remained susceptible to colistin. The patient was treated with empirical broad-spectrum intravenous colistin for 10 days.

Case-5 was a 63-year-old man who underwent coronary bypass surgery, without complications. 8TH days after the surgery, the patient presented with dysuria and urgency without fever. A urinary tract infection was diagnosed. The urine analysis showed 800,000 leukocytes/mL, and the culture yielded $bla_{\text{OXA-48}}$ & bla_{NDM-1} producing *R.ornithinolytica*.

Case-6 was a 58-year-old woman who underwent cardiac surgery, owing to surgical complications; she was admitted to the ICU. 7th day post-discharge patient presented with dysuria and pelvic discomfort. The urine analysis showed 739,000 leukocytes/ mL, and the culture yielded $bla_{\text{OXA-48}}$ & bla_{NDM-1} producing R.ornithinolytica resistant to all beta-lactams, aminoglycosides and fluoroquinolones, but remained susceptible to colistin.

Case-7 was a 58-year-old man with chronic renal failure on peritoneal dialysis who was admitted to the hospital with a lower UTI. The urine analysis showed 562,000 leukocytes/mL, and the culture yielded $bla_{\text{OXA-48}}$ & bla_{NDM-1} producing R.ornithinolytica resistant to all beta-lactams, aminoglycosides and fluoroquinolones, but remained susceptible to colistin.

A urinary catheter was not used (Case 4-7) in later four patients. Treatment for all patients included colistin for 10 days, without adverse effects. Urine analyses were conducted, and cultures were collected at the end of the treatment and again one week later. No leukocyturia was evident, and all cultures remained negative. Upon admission, the urine cultures of all four patients showed colony counts >100,000 CFU/mL (colony-forming units per milliliter), with growth of *R.ornithinolytica* identified by using the Vitek 2 Compact® automated system. The MHT, MBL E-test and PCR for both $bla_{\text{OXA-48}}$ & bla_{NDM-1} genes were positive for all urinary isolates. Antibiogram of 11 $bla_{\text{NDM-1}}$ producing *R.ornithinolytica* as a donor strains and its Transconjugants shown in Table-1. REP PCR, ERIC PCR and RAPD PCR showed that Isolates were clonally different. *bla*NDM-1 gene was located on In cA/C and IncHI2while IncL/M associated with *bla*OXA-48. blaCTX-M-15 was located on IncY, IncP and IncT whereas *bla*TEM-1 was associated withInc FIA. *bla*SHV-12 located on IncFIB while *bla*SHV-28 associated with In cFIC. **Plasmid Typing and characterization of MBL gene shown in Table-2.**

Table-1: Showing Antibiogram of 11 *R.ornithinolytica bla*$_{NDM-1}$ producers selected as a donor strains for conjugation studies.

ISOLATE	IPM	MEM	ETP	ATM	CST	TGC	CAZ	CTX	FOX	FEP	CPZ	PIT	AMP	CIP	LVX	MOX	GEN	AMK	TOB
BACT 721	32	32	32	64	0.5	1	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TC 721	8	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	2	2	4
BACT 732	32	32	32	64	0.25	2	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TC732	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
PC-RO-18	32	32	32	64	0.5	2	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TCRO18	8	8	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	1	2	2	1
PC-RO-23	32	32	32	64	0.25	0.75	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TCRO23	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.25	1	1	2	4	1
ETB –521	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	8	8	>16	64	>16
TCRO521	16	16	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	1	4	4	2
IAI -RO-8	32	32	32	64	0.25	0.5	128	128	64	64	128	>128	256	4	4	4	>16	64	>16
TC -RO-8	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	2	4	4
UC-RA05	32	32	32	64	0.125	2	256	256	64	64	256	>128	256	4	8	8	>16	64	>16
TCRO5	16	16	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	0.25	1	2	2	2
UC-RA-13	64	64	32	64	0.5	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCRA13	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-RA-25	32	32	32	64	0.25	1	128	128	64	64	128	>128	256	8	4	8	>16	64	>16
TCRA25	16	32	8	0.5	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	1	0.5	1	2	2
UC-RA-34	64	64	32	64	0.125	0.5	128	128	64	64	128	>128	256	4	8	4	>16	64	>16
TCRA34	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	0.5	1	0.25	1	2	2
UC-RA-56	32	32	32	64	0.5	0.75	256	256	64	64	256	>128	256	4	4	8	>16	64	>16
TCRA56	8	8	8	1	<0.25	<0.5	>64	>64	>64	>64	>64	>64	>64	1	0.5	1	1	2	2

Imipenem (IMP), meropenem (MEM), ertapenem (ETP), azetronam (ATM), colistin (CST); tigecycline (TGC); ampicillin(AMP), ceftazidime (CAZ), Cefotaxime (CTX), Cefoxitin (FOX), cefepime (FEP), ceftriaxone (CRO), cefoperazone (CPZ),Piperacillin-Tazobactam (PIT), ciprofloxacin (CIP), levofloxacin (LVX), moxifloxacin (MXF), gentamicin (GEN), amikacin (AMK), and tobramycin (TOB),

Table-2: Showing Plasmid Typing and characterization of beta-lactamase genes present in *R.ornithinolytica*.

ISOLATE	MBL	Plasmid	Transfer	Other ESBL gene present			Plasmid type			Transfer	Class D	Plasmid
BACT 721	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-28	FIA	T	FIC	Transferable	*	*
BACT 732	NDM-1	HI2	Transferable	TEM-1	CTXM-15	SHV-12	FIA	T	FIB	Transferable	*	*
PC-RO-18	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-28	FIA	P	FIC	Transferable	*	*
PC-RO-23	NDM-1	HI2	Transferable	TEM-1	CTXM-15	SHV-12	FIA	P	FIB	Transferable	*	*
ETB –521	NDM-1	HI2	Transferable	TEM-1	CTXM-15	SHV-28	FIA	Y	FIC	Transferable	*	*
IAI -RO-8	NDM-1	A/C	Transferable	TEM-1	CTXM-15	SHV-28	FIA	P	FIC	Transferable	*	*
UC-RA05	NDM-1	HI2	Transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	Transferable	OXA-48	L/M
UC-RA-13	NDM-1	HI2	Transferable	TEM-1	CTX-M-15	SHV28	FIA	T	FIC	Transferable	OXA-48	L/M
UC-RA-25	NDM-1	A/C	Transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	Transferable	OXA-48	L/M
UC-RA-34	NDM-1	A/C	Transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	Transferable	OXA-48	L/M
UC-RA-56	NDM-1	A/C	Transferable	TEM-1	CTX-M-15	SHV12	FIA	T	FIB	Transferable	OXA-48	L/M

The presence of a urinary catheter is considered a complicating factor probably due to the biofilm production that may be related with microbiological failure, catheter absence might have contributed to the successful treatment of these four patients.

Furthermore, colistin might be able to eradicate biofilms caused by *R.ornithinolytica*. Over the past decade, *R.ornithinolytica* has emerged as an infrequent, but important causal agent of human infections. To our knowledge, our study reports $bla_{\text{OXA-48}}$ & *blaNDM-1* producing R.ornithinolytica resistant to all beta-lactams, aminoglycosides, fluoroquinolones, trimethoprim sulfamethoxazole, doxycycline, fosfomycin, but remained susceptible to colistin. There are few reports regarding Carbapenem resistance bla_{NDM-1} producing *R.ornithinolytica* from India and especially from Indian sub continent.[1-5] Our findings are similar to the findings of other studies,[1-5] since this variation in genes is due to the fact that carbapenems are recently being introduced for their use in hospitals, previously being only used for nonfermenters, and due to their consistent use along with aminoglycosides and other antibiotics in ICUs, there is an increase in development of resistance against R.ornithinolytica due to irrational and inappropriate use of higher antibiotics in medical settings and their 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 mechanical ventilation, tracheostomy, catheters, surgery or severe burns also aid in development and dissemination of resistant genes. In this study, we found have that blaNDM-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*NDM-1gene, the bleomycin resistance gene bleMBL, and a truncated transposon Tn125. This study provides an insight into the acquisition and emergence of $bla_{\text{OXA-48}}$ & bla_{NDM-1} *producing R.ornithinolytica*, and emphasizes its transmission capability through plasmids. It is a cause for great concern as treatment options are virtually exhausted.

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