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Case Report

Co-existence of *bla*$_{NDM-1}$ and *bla*$_{VIM-1}$ producing *Moellerella wisconsensis* in NICU of North Indian Hospital

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Abstract

Infections caused by carbapenemase-producing Enterobacteriaceae have become a major threat to public health, worldwide. Here we report clinically significant NDM-1 and VIM-1 producing *Moellerella wisconsensis* which has not yet been described in the literature; this is the first report of *M. wisconsensis* strain harbouring *bla*$_{NDM-1}$ and *bla*$_{VIM-1,}$ recovered from the rectal swab of a low birth weight female child admitted in NICU of the north Indian tertiary care hospital. A plasmid of IncW incompatibility with size of 154 kb was observed in AK-92 strain.

Key words: Carbapenemase; *Moellerella wisconsensis*; NICU.

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Introduction

Moellerella wisconsensis, a rare fermentative Gram-negative bacillus family of Enterobacteriaceae, was recognized as a new species in 1980 and belongs to enteric group 46 by the Centres for Disease [1]. We are passing through a phase which will end up to a sort of pre-antibiotic era due to resistance developed against all classes of antibiotics in bacterial strains, in both community and hospital settings. NDM-1 was first discovered in *Klebsiella pneumoniae,* from a Swedish patient previously hospitalized in India [2]. The resulting outbreaks in NDM-1-producing Enterobacteriaceae may put newborn population of Indian subcontinent at risk [3, 4]. Moreover, the discovery of VIM-1 (Verona integron-encoded MBL-1) in *Pseudomonas aeruginosa* from Italy in 1996 [5] led to the emergence of cases involving acquired VIM carbapenemases in both adults and children throughout South America, Europe and Asia [6]. The epidemiology of CRE within the neonatal population warrants separate consideration as they are among the mainly vulnerable pediatric patients. A previous report from India marked the levels of carbapenem-resistance up to 13% for *K. Pneumonia* and 33% for *E. coli* while 14% were reported as NDM-1 producing Enterobacteriaceae in NICU settings [4]. Further, a large number of NDM-1 producing Enterobacteriaceae were detected in the NICU setting of Nepal with 64% mortality rate [3]. The only tested antibiotic in the susceptibility range was colistin, whose action is now hampered by the emergence of MCR-1 marker [7]. It has become a great challenge for physicians to control such infections caused by these strains. The emergence of NDM-1 and its variants has become common in nosocomial as well as community-acquired infections, leading to difficulty in infection control [8].

The focus of this study was to characterize carbapenem-resistant genes in the north Indian pediatrics patients. Therefore, we have screened carbapenem-resistance bacterial strain in NICU from one of the north Indian hospitals to know if *bla*$_{NDM-1}$ and *bla*$_{VIM-1}$ are disseminating through any rare species.

Case Presentation

Herein, we reported co-existence of *bla*$_{NDM-1}$ and *bla*$_{VIM-1}$ among *M. wisconsensis* in a 14-days-old, low birth weight (1.395 kg) female child who was admitted to the neonatal intensive care unit (NICU) of tertiary care hospital in Aligarh town of India. The patient was diagnosed to have diarrhoea and was treated with cefotaxime (50 mg/kg body weight) with no recovery after a week. Amikacin (15 mg/kg body weight) was also added with cefotaxime for one more week. The baby was not recovered even after 10 days. A rectal

swab was collected after 10 days stay in NICU and the culture was found positive for NDM-1 and VIM-1 producing *M. wisconsensis* as a first report.

Species identification of the isolated strain (AK-92) was performed through BD Phoenix^{TM}-100 Automated Microbiology System using panel NMIC/ID-55 (Gram-negative susceptibility card) and further confirmed by 16S rRNA sequencing using primers as described perversely [9]. A carbapenemase activity was detected by Carba NP test as described earlier [10]. Antimicrobial susceptibility testing was performed on Mueller-Hinton agar plates by the disk diffusion method according to the (CLSI) guidelines [11]. The strain was found to be resistant to carbapenems (imipenem, meropenem and doripenem), extended-spectrum cephalosporins (ceftazidime, cefoxitin and cefotaxime), aminoglycoside (amikacin), monobactam (aztreonam), polymyxin-B and colistin. Moreover, the minimum inhibitory concentrations of these antibiotics were also determined against this strain and its transconjugants as shown in Table 1. Detection of Metallo-beta-lactamase activity was performed by using two imipenem discs (10 µg), one containing 10 µl of 0.1M anhydrous Ethylene Diamine Tetra-Acetic Acid (EDTA), following the protocol described in the earlier study [12]. PCR amplification of DNA from strain AK-92 and its transconjugant, using primers as described previously [13], revealed the presence of ($bla_{\text{NDM-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-9}}$, bla_{TEM}, bla_{SHV}, bla_{VIM} and $bla_{\text{CTX-M}}$). In this strain, only bla_{NDM} and bla_{VIM} were amplified. The amplified product was sent for sequencing (AgriGenom Labs Pvt. Ltd., Kerala, India) and nucleotide sequences were analyzed with software available at the National Centre for Biotechnology Information website (www.ncbi.nlm.nih.gov). The $bla_{\text{VIM-1}}$ was found to be associated with $bla_{\text{NDM-1}}$.

Conjugation assay was performed using *M. wisconsensis* AK-92 as a donor and azide-resistant *E. coli* J53 strain as a recipient of the selection medium with ceftazidime (10µg/mL) and sodium azide (100µg/mL). Conjugation confirmed $bla_{\text{NDM-1}}$ and $bla_{\text{VIM-1}}$ located on the plasmid. Isolation and characterization of plasmid revealed that the NDM-1 and VIM-1 producing *M. wisconsensis* strain harboured two plasmids of different molecular size (66 and 154 kb). Of two, 154 kb plasmids were found in transconjugant after conjugation. The plasmid incompatibility group was determined by PCR based replicon typing (PBRT) method [14], which revealed the presence of IncW type plasmid, carrying $bla_{\text{NDM-1}}$, and $bla_{\text{VIM-1}}$. The genetic environment of this strain (AK-92) was evaluated for the presence of an insertion sequence (IS), linked with the $bla_{\text{NDM-1}}$ in Enterobacteriaceae. The analysis was performed with four sets of PCR amplification reactions: Reaction 1: NDM forward and NDM reverse; Reaction 2: NDM forward and bleomycin reverse; Reaction 3: IS*Aba125*ext and NDM reverse; Reaction 4: IS*Aba125*A and NDM reverse; Primers: NDM forward: 5′-GGTTTGGCGATCTGGTTTTC-3′; NDM reverse: 5′-CGGAATGGCTCATCAGATC-3′; bleomycin reverse:5′-GGCGATGACAGCATCATCCG-3′; IS*Aba125*A: 5′-TGTATATTTCTGTGACCCAC-3′; IS*Aba125*ext: 5′-ACACCATTAGAGAAA TTTGC-3′.

Discussion

The emergence of NDM-1-producing Enterobacteriaceae has disseminated worldwide from the Indian subcontinent. bringing about problems regarding treatment and control. Our previous study showed that bla_{NDM} gene had disseminated in the NICU via different Gram-Negative Bacilli (*E. coli*, *Citrobacter freundii, Citrobacter braakii, Klebsiella oxytoca, Enterobacter cloacae, Enterobacter aerogenes*) harboring bla_{NDM} [15]. An earlier study reported that the bla_{NDM} is carried by various types of plasmids incompatibility such as IncA/C, IncF, IncN, IncL/M or untypable/IncR, and is rarely found to be chromosomally integrated [13]. Non-clonal Indian isolates from Chennai harbor $bla_{\text{NDM-1}}$, exclusively on plasmids ranging from 50 to 350 kb, whereas strain of *K. pneumonia* isolated in Haryana was found to have a plasmid size of either 118 kb or 50 kb, suggesting the wide spread of $bla_{\text{NDM-1}}$. Plasmid profiling showed that a plasmid of size 50 kb carries $bla_{\text{NDM-1}}$ in Enterobacteriaceae, which were found resistant to

Table 1. Phenotype and genotype characterization of NDM-1 and VIM-1 producing *Moellerella wisconsensis* isolated from NICU and its transconjugant.

Isolate Id	Organism name	Metallo-β-lactamase	Carba NP Test	Carbapenem Resistant gene	MIC (µg ml-1)									Plasmid size	Plasmid type	Genetic environment of blaNDM	
					IMP	MEM	CX	CFS	CIP	GEN	ATM	CL	PB			ISAba125	bleMBL
AK-92	*Moellerella wiscosensis*	Present	Positive	blaNDM-1, blaVIM-1	> 256	256	1024	512	1024	512	256	128	128	154 kb, 66kb	IncW	Present	Present
AK-92. T	Escherichia coli J53	present	positive	blaNDM-1, blaVIM-1	256	128	1024	256	256	512	128	128	128	154kb	IncW	Present	Present

IPM: imipenem, MEM: meropenem, CX: cefoxitin CFS: cefoparazone/sulbactam, CIP: ciprofloxacin GEN: gentamicin, ATM: aztreonam, CL: colistin, PB: polymyxin-B.

almost all antimicrobials except tigecycline and colistin [16]. Here, we report that the NDM-1 and VIM-1 producing *M. wisconsensis* strain harboured two plasmids of different molecular size (66 and 154 kb). Of two, 154 kb plasmids were found in transconjugant after conjugation and the plasmid harbouring the $bla_{\text{NDM-1}}$ and $bla_{\text{VIM-1}}$ belonged to the IncW type, which was different from previously replicon type reported in India.

Recent studies from Greece demonstrated the co-production of NDM-1 and VIM-1 in a *K. pneumoniae* [17]. While in our study, we found co-expression of $bla_{\text{NDM-1}}$ with $bla_{\text{VIM-1}}$ producing *M. wisconsensis* from neonate admitted in NICU of the north Indian hospital.

In genetic environment analysis, we found complete IS*Aba125* upstream and ble_{MBL} downstream of $bla_{\text{NDM-1}}$ in strain AK-92. The bleomycin resistance gene ble_{MBL} downstream of $bla_{\text{NDM,}}$ encodes a putative bleomycin (an antitumor drug resistance protein) as reported previously [13]. In earlier studies of our group on genetic analysis, a truncated IS*Aba125* with ble_{MBL} in *Cedecea lapagei* was observed [12] whereas, another study showed complete IS*Aba125* with ble_{MBL} in three different strains of *Enterobacter aerogenes* [18].

Conclusion

The study revealed detection of NDM-1 and VIM-1 in *Moellerella wisconsensis* in NICU, as a first report. It is alarming to the health care workers and hospital personals to control infections. Hence, it has become important to look into the matter more carefully in order to control its spread in the community as well as hospital settings, especially in NICU. Moreover, hospitals should work on infection control measurements.

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Nucleotide sequence accession number

The sequence of $bla_{\text{NDM-1}}$ determined in this study has been assigned Gene Bank accession no. KX999119.

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Ethical statement

All the isolates were collected after the approval of Ethical committee named as Institutional ethical Committee. The number of approval is 151/201517/PDFWM-2015-2017-UTT 31140 (SAII).

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