Correspondence

Carriage of \(\text{bla}_{\text{NDM-1}}\) in \textit{Pseudomonas aeruginosa} through multiple \text{Inc} type plasmids in a tertiary referral hospital of northeast India

Sir,

\textit{Pseudomonas aeruginosa} is known to be a predominant opportunistic pathogen and also a frequent cause of nosocomial infection in patients with compromised immune system. Treatment option becomes complicated when this type of organism harbour resistance determinants such as New Delhi metallo-\(\beta\)-lactamase-1 (NDM-1). The genetic vehicles carrying this gene are often responsible for their horizontal spread, dissemination and maintenance within a broad host range\(^1\). Knowledge about transmission dynamics of \(\text{bla}_{\text{NDM-1}}\) is a key to succeed in the effort of infection control and slowing down the spread of multidrug resistance. This study was undertaken to characterize \(\text{bla}_{\text{NDM-1}}\) in clinical isolates of \textit{P. aeruginosa}, their transmission dynamics and plasmid \text{Inc} types responsible for their horizontal transfer in a tertiary referral hospital of northeast India.

The samples for the present study were collected from the patients who were admitted or attended outpatient department of Silchar Medical College and Hospital, Silchar, Assam, India from October 2012 to September 2013. The protocol was approved by Institutional Research and Ethical Committee. During this period, a total of 290 consecutive non-duplicate clinical isolates of \textit{P. aeruginosa} were collected, of which 88 isolates were found to be non-susceptible to carbapenem by minimum inhibitory concentration (MIC). Isolates with MIC above 4, 1, 8 \(\mu\)g/ml for imipenem, meropenem, and ertapenem, respectively were selected as per CLSI (Clinical Laboratory Standards Institute) guidelines\(^2\) and were subjected to modified Hodge test for detection of carbapenemase production\(^3\) and further confirmed for the presence of metallo-\(\beta\)-lactamase by imipenem-EDTA disc diffusion test\(^4\). PCR assay was performed to characterize the \(\text{bla}_{\text{NDM}}\) gene as well as other metallo-\(\beta\)-lactamase genes \(\text{bla}_{\text{VIM}}, \text{bla}_{\text{IMP}}, \text{bla}_{\text{SIM}}\) and \(\text{bla}_{\text{SMB}}\)^4-7, and amplified products were sequenced to confirm the presence of resistant genes. The linkage of \(\text{bla}_{\text{NDM-1}}\) with insertion sequence IS\text{Aba125} was determined by using forward primer of IS\text{Aba125} (5'-GAAACTGTCGCACCTCAT GTTTG-3') and reverse primer of \(\text{bla}_{\text{NDM-1}}\) (5'-GTAGTGCTCAGTGTCGGCAT-3'). The class of integron carried out by \(\text{bla}_{\text{NDM-1}}\) was determined by integrase gene PCR\(^8\). \(\text{bla}_{\text{NDM-1}}\) positive bacterial isolates were cultured in Luria-Bertani (LB) broth (Hi-Media, Mumbai, India) containing 0.25 \(\mu\)g/ml of imipenem. After overnight incubation, plasmids were extracted by QIAprep Spin Miniprep Kit (Qiagen, Germany). Plasmids of \(\text{bla}_{\text{NDM-1}}\) were subjected to transformation by heat shock method\(^10\) using \textit{Escherichia coli} JM107 as recipient. Transformants were selected on LB agar with 0.25 \(\mu\)g/ml of imipenem, which were then confirmed both by phenotypic as well as by PCR analysis. The plasmids were classified by PCR based replicon typing, carried out for determining the incompatibility group type of the plasmid in all \(\text{bla}_{\text{NDM-1}}\) harbouring strains. A total of 18 different replicon types such as FIA, FIB, FIC, HI1, HI2, I1/I\(\gamma\), L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA were targeted as described previously\(^11\). The antibiotic susceptibility was done by Kirby-Bauer disc-diffusion method\(^10\) against antibiotics \textit{viz.} piperacillin-tazobactam (100/10 \(\mu\)g), co-trimoxazole (25 \(\mu\)g), amikacin (30 \(\mu\)g), gentamicin (10 \(\mu\)g), ciprofloxacin (5 \(\mu\)g), polymixin B (300 units), netilmicin (30 \(\mu\)g), carbenicillin (100 \(\mu\)g) and faropenem (5 \(\mu\)g) (Hi-Media, Mumbai, India). MIC was performed by agar dilution method against imipenem (MSD, India), ertapenem (MSD, India), meropenem (Lupin, India) and the results were interpreted as per CLSI guidelines\(^2\). The clonal relatedness among the \(\text{bla}_{\text{NDM-1}}\) producing \textit{P. aeruginosa} isolates was determined by repetitive extragenic palindromic (REP) PCR\(^12\).
Of the 88 consecutive non-repetitive carbapenem non-susceptible (showing MIC of imipenem >4 μg/ml, meropenem >1 μg/ml and ertapenem >8μg/ml) Pseudomonas isolates, 16 were found carrying blaNDM-1 as confirmed by sequencing. The different clinical features of these 16 blaNDM-1 harbouring P. aeruginosa are given in Table I. In contrast, no other metallo-β-lactamase genes (blaVIM, blaIMP, blaSIM and blaGIM) were detected in any of the isolates. These blaNDM-1 positive isolates showed resistance towards most of the antibiotics including piperacillin/tazobactam, cotrimoxazole, faropenem, aminoglycoside and quinolone group of drugs. Seven isolates were resistant to polymixin B as well. Minimum inhibitory concentration results showed high MIC breakpoint (Table II) against all the tested antibiotics cephalosporins, monobactam and carbapenems. Integrase PCR showed that all the blaNDM-1 positive isolates were harbouring class 1 integron while in four isolates both class 1 and class 2 integrons were observed (Table I). In 11 isolates, ISAb125 was found in the upstream region of blaNDM-1. Plasmid analysis showed that the blaNDM-1 gene was located within the plasmid of approximately 25-40 Kb in size and in transformation assay, NDM-1 gene was found to be horizontally transferable and the resistance determinant was carried within diverse Inc group viz. FIA, FIC and K types. However, in six transformants, the plasmid was untypable (Table I).

After the first detection of NDM in Klebsiella pneumoniae it was also reported in Escherichia coli, Citrobacter freundii, Enterobacter and in Acinetobacter spp. Due to the rapid horizontal transmission this gene was also reported in P. aeruginosa from different parts of the world. In this study, we described the occurrence of blaNDM-1 gene among a number of MDR P. aeruginosa isolates indicating the spread of this resistant gene in the northeastern part of India. Toleman et al16 have described the association of insertion sequence ISAba125 in the upstream region of blaNDM-1 harbouring A. baumanni and it is also established that whole ISAb125 or a truncated portion of it is excised along with the resistant gene when it is horizontally transmitted among the members of Enterobacteriaceae family. In our study also, similar kind of association of ISAb125 with blaNDM-1 in the upstream region was observed, which may be due to the horizontal transmission of this gene along with ISAb125 at interspecies level. Thus, this mobile genetic element may act as a unit of interspecies transmission in our setting. This horizontal transmission may also

| Table I. Features of New Delhi metallo-β-lactamase (NDM-1) producing Pseudomonas aeruginosa and patients' characteristics |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Patient sex & age (yr) | Clinical specimen | Hospitalization unit | ISAba125 | Integron type | Plasmid Inc group |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| F-26             | Ear swab        | ENT             | -          | Class 1         | Untypable       |
| F-55             | Pus             | Surgery         | -          | Class 1 & 2     | FIA             |
| M-40             | Pus             | Orthopedics     | +          | Class 1         | Untypable       |
| F-40             | Urine           | Gynaecology     | +          | Class 1 & 2     | FIA             |
| F-20             | Pus             | FBU             | +          | Class 1         | FIC             |
| F-44             | Pus             | Surgery         | -          | Class 1         | Untypable       |
| F-40             | Pus             | Gynaecology     | +          | Class 1         | FIB             |
| F-12 days        | Nasal secretion | Paediatrics     | -          | Class 1         | K/B             |
| M-40             | Sputum          | Medicine        | +          | Class 1         | Untypable       |
| F-19             | Urine           | Paediatrics     | -          | Class 1         | K/B             |
| F-20             | Pus             | Surgery         | +          | Class 1         | Untypable       |
| M-9              | Urine           | Paediatrics     | +          | Class 1         | FreB            |
| M-13             | Urine           | Medicine        | +          | Class 1 & 2     | FIA & FIB       |
| M-12             | Urine           | Surgery         | +          | Class 1         | Untypable       |
| M-53             | Pus             | Surgery         | +          | Class 1         | K/B             |
| F-18             | Pus             | Surgery         | +          | Class 1 & 2     | FIA             |

ENT, Ear, nose and throat ward; FBU, female burn unit
be facilitated due to the association with gene capture mechanism as it is known to be an important mean of spreading resistance in clinical isolates of Gram-negative bacilli\textsuperscript{17}. An earlier study revealed that class 1 type of integron was mostly associated with clinical pathogens\textsuperscript{9}, as also supported by our study where all the isolates were found carrying class 1 integron. It is observed that different incompatibility types of plasmid act as a genetic vehicle for transmission of this resistance gene, which reflects acquisition of $\text{bla}_{\text{NDM-1}}$ harbouring plasmid from different sources. But in our study, in case of some transformants, we

| Serial no. | MIC (µg/ml) | Co-resistances |
|-----------|-------------|----------------|
|           | IMP | ERT | MER | CEF | AZT |                   |
| 1         | 16  | 16  | 16  | 16  | 32  | CXT, TGC, FAR     |
| 2         | >256 | >256 | 64  | >256 | >256 | PIT, CXT, AMK, GEM, CIP, PB, NET, CB, FAR |
| 3         | 64  | >256 | 64  | 128 | 128 | PIT, CXT, AMK, GEM, CIP, TGC, NET, CB, FAR |
| 4         | 64  | 128 | 32  | >256 | >256 | PIT, CXT, AMK, GEM, CIP, PB, NET, CB, FAR |
| 5         | >256 | >256 | 64  | >256 | >256 | PIT, CXT, AMK, GEM, CIP, PB, TGC, NET, CB, FAR |
| 6         | >256 | >256 | >256 | 128 | >256 | PIT, CXT, AMK, GEM, CIP, NET, CB, FAR |
| 7         | >256 | >256 | >256 | >256 | >256 | CXT, AMK, GEM, CIP, TGC, NET, CB, FAR |
| 8         | >256 | >256 | >256 | >256 | >256 | CXT, AMK, GEM, CIP, PB, NET, CB, FAR |
| 9         | 32  | 32  | 16  | 32  | 64  | CXT, TGC, FAR     |
| 10        | 16  | 32  | 16  | 32  | 32  | CXT, PB, CB, FAR  |
| 11        | 128 | 256 | 64  | 128 | 128 | PIT, CXT, AMK, GEM, CIP, NET, CB, FAR |
| 12        | 32  | 64  | 32  | >256 | >256 | CXT, CIP, PB, CB, FAR |
| 13        | >256 | >256 | >256 | >256 | >256 | PIT, CXT, AMK, GEM, CIP, PB, TGC, NET, CB, FAR |
| 14        | 32  | 64  | 32  | >256 | >256 | CXT, CB, FAR     |
| 15        | 64  | 64  | 32  | >256 | >256 | CXT, CB, FAR     |
| 16        | >256 | >256 | 128 | >256 | >256 | PIT, CXT, AMK, GEM, CIP, TGC, CB, FAR |

CXT, co-trimoxazole; TGC, tigecycline; FAR, faropenem; PIT, piperacillin-tazobactam; GEM, gentamicin; CIP, ciprofloxacin; AMK, amikacin; CB, carbenicillin; NET, neticillin; PB, polymyxin B; IMP, imipenem; ERT, ertapenem; MER, meropenem; CEF, cefepime; AZT, aztreonam
were unable to determine the incompatibility group of that plasmids. This may be due to the presence of any new incompatible type of plasmid that could not be detected by our target primers. Presence of new Inc type plasmids encoding \( \text{bla}_{\text{NDM-1}} \) corresponds their transplasmid expansion and diverse source of carriage. In an earlier study, it was reported that NDM-1 producing isolates were resistant to nearly all classes of antimicrobial agents except polymyxins and tigecycline\(^{13}\), but in our study the \( \text{bla}_{\text{NDM-1}} \) harbouring isolates showed resistance to all the antibiotics tested including polymyxin B and tigecycline.

In conclusion, carriage of \( \text{bla}_{\text{NDM-1}} \) in different Inc type plasmids within a single hospital setting and their expansion may be a serious matter of concern in combating the carbapenem resistance.

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