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Occurrence of NDM-1 and other Carbapenemases among *Acinetobacter* spp. and *Pseudomonas* spp. Collected from India

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: Genes encoding NDM-1 and NDM-2 have been reported from *Acinetobacter* spp. (ASP). We evaluated the presence of bla_{NDM} and other carbapenemases among ASP and *Pseudomonas* spp. (PSP) strains collected in India during 2006, 2007 and 2010.

Methods: 324 imipenem [IMI] and meropenem [MER] non-susceptible (MIC, ≥8 µg/mL; CLSI susceptibility method) ASP and PSP clinical strains collected in Indian hospitals during 2006, 2007 and 2010 were screened for bla_{NDM} and other carbapenemases (2010 only) by PCR and sequencing. NDM-producers were further characterized. Clonality was assessed by PFGE. Gene location was determined by S1 endonuclease restriction and hybridization with bla_{NDM-1} probe. Isolates were screened for genes encoding ESBLs, plasmidic (p) AmpC and 16S rRNA methylases (rRNAm). bla_{NDM}-carrying plasmids (2006-2007) were transferred to *E. coli* host.

Results: Among 153 PSP and 171 ASP tested, bla_{NDM-1} was detected in 6 *A. baumannii* (ACB). NDM-1-producers were detected in 2006 (1 Indore), 2007 (1 Mumbai) and 2010 (2 Bangalore, 2 Mumbai [different hospital from 2007]). NDM-producers from 2010 were positive for bla_{TEM}. Other ß-lactamases and rRNAm genes were not detected in bla_{NDM-1}-carrying ACB. All but two (2010; different hospitals) NDM-producing strains were genetically diverse. bla_{NDM-1} was located in plasmids of different sizes. All 40 ASP from 2010 were positive for bla_{OXA-51} (all ACB) and 37 were positive for bla_{OXA-23}, including 3 NDM-1-producers (2 clonal). Among 30 PSP from 2010, 13 carried bla_{VIM-2}. These strains were collected in all three hospitals studied. *E. coli* host carrying bla_{NDM-1} plasmids were resistant to most ß-lactams but IMI and MER MICs were low (1 and 0.12 µg/mL, respectively).

Conclusions: VIM types and carbapenem hydrolyzing oxacillinases have been documented among PSP and ASP from India collected during 2006 and 2007 in the SENTRY Program. We extended this knowledge with the occurrence of bla_{NDM-1} and evaluated strains collected in 2010. NDM-1-producing ACB strains were detected in all years and genetic diversity was observed, suggesting that spread of bla_{NDM-1} is due to genetic mobilization.

INTRODUCTION

Production of acquired metallo-ß-lactamases (MßLs) is not considered the most prevalent resistance mechanism against carbapenems found in *Pseudomonas* and *Acinetobacter* species; however, these enzymes constitute one of the most important resistance mechanisms clinically and epidemiologically. MßLs have the ability to hydrolyze the vast majority of available ß-lactam agents and are not inhibited by the ß-lactamase inhibitors currently marketed or in development. Additionally, the acquired genes encoding these enzymes are carried in mobile structures, which facilitate its dissemination and usually harbor other resistance elements.

The MßL NDM-1 was characterized from *Klebsiella pneumoniae* and *Escherichia coli* strains in a Swedish diabetic patient of Indian origin that traveled to New Delhi and acquired a urinary tract infection. After this initial report, NDM-1 was also detected in other Enterobacteriaceae species. More recently, NDM-1 and the single aminoacid variant, NDM-2 have been described from *Acinetobacter baumannii*. NDM-1 has also been detected among two *Pseudomonas aeruginosa* from Serbia.

In this study, we screened *Acinetobacter* and *Pseudomonas* spp. clinical strains collected from Indian hospitals for the presence of NDM encoding genes (2006-2007) and other carbapenemase genes (2010).

MATERIALS AND METHODS

Bacterial isolates. A total of 891 *Acinetobacter* spp. and *Pseudomonas* spp. isolates were collected from hospitals in India as part of the SENTRY Antimicrobial Surveillance Program (2006, 2007 [14 hospitals] and 2010 [3 hospitals]). Only one isolate per patient from documented infections were included in the study. Isolates were collected from bloodstream, respiratory tract, and skin and skin-structure infections according to a common protocol. Species identification was confirmed by standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, Missouri, USA), when necessary.

Antimicrobial susceptibility testing. All isolates were susceptibility tested using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, M07-A8). Categorical interpretations for all antimicrobials were those found in M100-S21 (2011) and quality control (QC) was performed using *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents (M100-S21).

Genotypic detection of resistance encoding genes. Carbapenem non-susceptible isolates (imipenem and meropenem MIC, ≥8 µg/mL) were PCR screened using primers targeting bla_{NDM-1} (all years) and bla_{IMP}, bla_{VIM}, bla_{SPM-1}, bla_{KPC}, bla_{SME}, bla_{IMI}, bla_{NMC-A}, bla_{GES} and bla_{OXA-48} for 2010 strains that were not screened previously. *Acinetobacter* spp. (2010 only) were also screened for the genes encoding OXA-23, OXA-24/-40, OXA-51 and OXA-58. NDM-1-producing isolates were screened for ESBL (bla_{CTX-M}, bla_{OXA-2}, bla_{OXA-10}, bla_{PER} and bla_{VEB}), plasmidic AmpC and 16S rRNA methylase (*armA*, *rmtA-D* and *npmA*) encoding genes. Amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin, USA). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

Molecular typing. Pulsed-field gel electrophoresis (PFGE) was used to evaluate clonality among strains producing NDM-1. Genomic DNA was digested with Apa I (New England Biolabs; Beverly, Massachusetts, USA) and resolved on the CHEF-DR II apparatus.

Transference of the bla_{NDM-1}-encoding plasmids. Plasmid extractions were performed by alkaline lyses and extra-chromosomal DNA was transferred to *E. coli* DH5α by electroporation. Selection was performed on agar plates containing 4 µg/mL of ceftazidime.

RESULTS

- Among 590 *Pseudomonas* spp. and 301 *Acinetobacter* spp. clinical isolates collected in Indian hospitals during 2006, 2007 and 2010, 153 and 171 strains of each species, respectively, displayed elevated carbapenem MIC values and were selected for carbapenemase screening.
- PCR and sequencing demonstrated that six *A. baumannii* strains carried bla_{NDM-1} (Table 1). This MßL gene was detected across all years (2006 [one strain], 2007 [one strain] and 2010 [four strains]), and in medical sites located in Indore (2006), Mumbai (2007 and 2010 [different institutions]) and Bangalore (2010).
- NDM-1-producing *A. baumannii* were highly resistant to the majority of the antimicrobial agents tested (Table 1). Tigecycline and colistin were the only compounds showing acceptable in vitro activity against these strains (MIC values ranging from 0.25-1 and ≤0.5-1 µg/mL, respectively).
- NDM-1-producers were tested for the presence of several other ß-lactamases: four strains were positive for bla_{OXA-23} and three carried bla_{TEM-1} (Table 1). Genes encoding 16S rRNA methylases were not detected among NDM-1-producing *A. baumannii*.
- Molecular typing showed that two isolates from different hospitals were genetically related. All remaining strains were genetically distinct (Figure 1).
- Among remaining *Acinetobacter* spp. strains from 2010, bla_{OXA-51} was detected in all strains (confirming identification as *A. baumannii*) and bla_{OXA-23} was detected in 34 of the 36 strains.
- NDM-encoding genes were not detected among *P. aeruginosa* strains. However, 13 strains recovered during 2010 carried bla_{VIM-2}. VIM-2-producing strains were observed in Bangalore (seven strains), Chennai (four) and Mumbai (two).
- *E. coli* transformants harboring bla_{NDM-1} plasmids displayed modestly elevated MIC results for imipenem and doripenem (MIC results 1 and 0.25 µg/mL for the two strains, respectively; Table 1); however these strains were susceptible to meropenem and ertapenem (MIC results, ≤0.12 µg/mL).
- Other resistance markers were not transferred with bla_{NDM-1}.

Figure 1. PFGE patterns of NDM-1-producing *A. baumannii* from India.

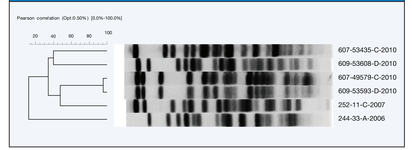

Table 1. Demographic information, antimicrobial profile and molecular results from NDM-1-producing *A. baumannii* from India. Antimicrobial susceptibility profile and molecular results for two transconjugant *E. coli* transformant strains harboring bla_{NDM-1} carrying plasmids isolated from *A. baumannii* clinical strains (2006 and 2007).

	Isolate							
Parameter	244-33A	252-11C	607-49579C	607-53435C	609-53593D	609-53608D	*E. coli* 8858J (p252-11C)	*E. coli* 8859J (p244-33A)
Collection year	2006	2007	2010	2010	2010	2010	NA[d]	NA
City	Indore	Mumbai	Bangalore	Bangalore	Mumbai	Mumbai	NA	NA
Source[a]	BSI	unknown	RTI	RTI	SSSI	SSSI	transformant	transformant
Age	10	unknown	27	74	90	49	NA	NA
Sex	M	unknown	F	M	M	M	NA	NA
Service	Pediatric	unknown	Neurosurgery	Geriatric	Surgery	Surgery	NA	NA
MIC (µg/mL)								
Imipenem	>8	>8	>8	>8	>8	>8	1	1
Meropenem	>8	>8	>8	>8	>8	>8	≤0.12	≤0.12
Ertapenem	>8	>8	>8	>8	>8	>8	≤0.06	≤0.06
Doripenem	>8	>8	>8	>8	>8	>8	0.25	0.25
AMP/SUL[b]	>16	>16	>16	>16	>16	>16	>16	>16
PIP/TAZ[c]	>64	>64	>64	>64	>64	>64	16	8
Ceftazidime	>16	>16	>16	>16	>16	>16	>16	>16
Aztreonam	16	>16	>16	>16	>16	>16	≤0.12	≤0.12
Amikacin	>32	>32	>32	>32	>32	>32	≤0.25	0.5
Gentamicin	>8	>8	>8	>8	>8	>8	≤2	≤2
Tobramycin	>16	>16	>16	16	>16	>16	≤0.12	≤0.12
Ciprofloxacin	>4	>4	>4	>4	>4	>4	≤0.5	≤0.5
Tetracycline	≤2	>8	>8	>8	>8	8	≤2	≤2
Tigecycline	0.25	0.5	1	1	1	0.5	0.12	0.12
Colistin	1	≤0.5	1	≤0.5	1	1	≤0.5	≤0.5
bla_{OXA-23}	neg	pos	pos	neg	pos	pos	neg	neg
bla_{OXA-51}	pos	pos	pos	pos	pos	pos	neg	neg
bla_{TEM-1}	neg	neg	pos	pos	pos	neg	NT[e]	NT

a. SSSI= skin and skin-structure infection; BSI= bloodstream infection; RTI= respiratory tract infection.
b. AMP/SUL= ampicillin/sulbactam.
c. PIP/TAZ= piperacillin/tazobactam.
d. NA= not applicable.
e. NT= not tested.

CONCLUSIONS

- NDM-1-producing *P. aeruginosa* were recently described in Eastern Europe; however, this MßL gene was not detected among the *P. aeruginosa* from India evaluated in the present study. The absence of NDM-1-producing *Pseudomonas* spp. in India during the three studied years supports the recent hypothesis of a secondary source of bla_{NDM-1} that was mobilized in the Serbian *P. aeruginosa*.
- We previously reported the presence *P. aeruginosa* strains producing several VIM-variants in Indian hospitals (VIM-2, -5, -6, -11 and -18; 2006-2007 strains). In this study, we show that VIM-2 is likely to be the dominant and most stable VIM-type across India.
- Six bla_{NDM-1}-carrying *A. baumannii* strains were detected in four Indian institutions in the three years sampled. These strains that seem to be geographically dispersed; were detected in small numbers. Furthermore, NDM-1-producers were multidrug resistant and treatment of infections caused by these organisms could be challenging.

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