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Association of 16S rRNA Methylases and NDM-1 in Isolates from Indian Hospitals Collected in 2006-2007

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objective: To evaluate the association of 16S rRNA methylase genes and NDM-1 metallo-β-lactamase among 15 Enterobacteriaceae isolates collected in India during 2006 and 2007.

Methods: Fifteen NDM-1 producing Enterobacteriaceae (*Escherichia coli* [EC; 6], *Enterobacter cloacae* [ECL; 3] and *Klebsiella pneumoniae* [KPN; 6]) displaying resistance to amikacin (AMK), gentamicin (GEN) and tobramycin (TOB; >32, >8, >16 mg/L, respectively) were screened for the presence of *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD* and *npmA* by PCR. Amplicons were sequenced on both strands. Isolates were further tested by CLSI reference broth microdilution MIC methods against arbekacin, apramycin, kanamycin, neomycin, streptomycin and extended ranges for AMK, GEN, TOB. Clonality was accessed by PFGE.

Results: Thirteen of the 15 (87.0%) NDM-1-producing isolates were positive for 16S rRNA methylase-encoding genes (6 EC, 3 ECL and 4 KPN; 2 KPN were negative). Isolates were collected in four hospitals located in three cities (New Delhi, Mumbai, Pune). Nine strains carried *armA* and three harboured additional genes: *rmtC* (2 EC) and *rmtA* (1 ECL). Three strains carried *rmtC* alone (1 each species). Gene sequences showed 100% homology with *armA*, *rmtA* and *rmtC* published sequences. Genetically identical EC and KPN carried *armA* and ECL possessed *rmtC*. EC strains with similar PFGE profiles harboured *armA*, *rmtC* or both. One ECL carried *armA* and *rmtA*. Susceptibility profiles showed that all strains had AMK, GEN, TOB and kanamycin MIC results at: ≥1024, ≥512, ≥512, ≥1024 mg/L, respectively. Arbekacin MIC results varied from 256 to ≥1024 mg/L and most isolates had streptomycin MIC values between 16 and 512 mg/L; however, one isolates had an MIC of only 4 mg/L (*rmtC*-carrying ECL). Apramycin and neomycin MIC results were lower compared to other aminoglycosides tested (4-16 and 1-32 mg/L, respectively).

Conclusions: 16S rRNA methlylases confer high-level of aminoglycoside resistance, and the association of genes encoding 16S rRNA methylases and beta-lactamases have been reported. In this study, we expand these results to a large collection of strains producing NDM-1, showing that these isolates carried one or two 16S rRNA methylase genes, and displayed high levels of resistance to most aminoglycosides tested. NDM-1-producing isolates were multidrug-resistant and the presence of 16S rRNA methylases further limits the therapeutic options to treat infections caused by these emerging pathogens.

INTRODUCTION

Aminoglycosides play an important role in the treatment of serious infections caused by Gram-negative bacteria. Resistance to aminoglycoside compounds among these organisms can be attributed to modifying enzymes and 16S ribosomal RNA (rRNA) methylases, both usually carried by mobile genetic structures that are often associated with other resistance genes, such as those encoding β-lactamases.

Although less prevalent than modifying enzymes, 16S rRNA methylases confer high resistance levels to most aminoglycoside compounds, including commonly used amikacin, tobramycin and gentamicin, that cannot be overcome by dose adjustments. Two sites of methylation in the 16S rRNA 30S ribosome subunit have been identified: residue A1408 that can be caused by *armA* and *rmtA-E* and G1405 caused by *npmA*.

The metallo-β-lactamase (MβL) NDM-1 (New Delhi Metallo-β-lactamase) has been detected in various Enterobacteriaceae species and *Acinetobacter* spp. in several countries. Strains carrying this MβL gene appear to also harbor genes encoding 16S rRNA methylases, mostly *armA*, *rmtB* and *rmtC*.

In this study, we screened a collection of NDM-1-producing Enterobacteriaceae isolated during 2006-2007 in hospitals located in India for the presence of 16S rRNA methylases genes. These isolates were susceptibility tested by reference broth microdilution methods against several aminoglycosides using extended dilution ranges.

MATERIALS AND METHODS

Bacterial isolates. Fifteen Enterobacteriaceae strains carrying bla_{NDM-1} were identified as part of the SENTRY Antimicrobial Surveillance Program (2006-2007) in hospitals located in India. These isolates were detected among 1,443 isolates from 14 hospitals. Only one isolate per patient episode 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 2 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 *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents (M100-S21).

Screening for 16S rRNA methylases. Isolates were screened for *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD* and *npmA* using custom primers (**Table 1**). Amplicons were sequenced on both strands and nucleotide sequences obtained were analyzed using Lasergene® software package (DNAStar; Madison, Wisconsin, USA) and compared to available sequences via NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS

- Among 15 NDM-1-producing isolates tested (three bacterial species; six *E.* coli, three *Enterobacter cloacae* and six *Klebsiella pneumoniae*), 13 (87.0%) carried 16S rRNA methylase encoding genes.
- Strains carrying 16S rRNA methylase genes belonged to the three bacterial species (**Table 2**): six *E. coli*, three *E. cloacae* and four *K. pneumoniae* and were detected in four different hospitals located in New Delhi (two institutions), Mumbai and Pune.
- Among the *E. coli* strains, three carried *armA*, one harboured *rmtC* and two carried both *armA* and *rmtC* (**Table 2**).
- Genetically identical *E. coli* strains (PFGE profile EC-A, two strains) carried *armA*. Four strains displaying similar, not identical PFGE profile carried different 16S rRNA methylase genes (**Table 2**).
- Among 16S rRNA methylase-producing *E. cloacae*, two identical strains carried *rmtC* (profile ECL-A; same hospital) and a genetically distinct strain harboured *armA* and *rmtA* (**Table 2**).
- Four *K. pneumoniae* strains carrying 16S rRNA methylase-encoding genes showed three PFGE patterns: KPN-A carrying *rmtC* and, KPN-B and KPN-E harboring *armA*. Two *K. pneumoniae* (profiles KPN-C and -D) displayed negative results with the primers used in this study.
- All isolates, including strains that did not carry 16S rRNA methylase-encoding genes, were highly resistant (MIC results ≥512 mg/L) to amikacin, arbekacin, gentamicin, kanamycin and tobramycin. Streptomycin MIC values varied from 4 to 1024 mg/L. Apramycin and neomycin MIC results were lower compared to other aminoglycosides tested (4-16 and 1-32 mg/L, respectively; **Table 2**).
- All isolates tested were negative for oligonucleotides targeting *rmtB*, *rmtD* and *npmA*.

Table 1. Oligonucleotides used in this study.

Oligonucleotide name	Sequence (5' to 3')	Amplicons size
armA-F	TAT GGG GGT CTT ACT ATT CTG CCT AT	514-bp
armA-R	TCT TCC ATT CCC TTC TCC TTT	
rmtB-F	TAC GAG CGC GGC ATT GCA TC	301-bp
rmtB-R	CGT AGT TCG CCT CCA TGC CT	
rmtC-F	GCC AAA GTA CTC AGA AGT GG	752-bp
rmtC-R	CTC AGA TCT GAC CCA ACA AG	
rmtA-F	GGT TGG CCG AAT TGG ACT G	201-bp
rmtA-R	TTA GGC CGC AAG CGA TAT CC	
rmtD-F	GAG CGA ACT GAA GGA AAA AC	732-bp
rmtD-R	CAG CAC GTA AAA CAG CTC	
npmA-F	CTC AAA GGA ACA AAG ACG G	641-bp
npmA-R	GAA ACA TGG CCA GAA ACT C	

Table 2. Demographic, antimicrobial profile and 16S rRNA methylase screen results from clinical Enterobacteriaceae isolates producing NDM-1 from India (New Delhi, Mumbai and Pune).

	Isolate														
Parameter	246-49D	246-34D	246-17D	252-26D	257-25A	258-03D	246-14A	246-05A	258-18A	257-36D	257-42D	246-61A	252-38C	252-41C	258-14C
Species[a]	EC	EC	EC	EC	EC	EC	ECL	ECL	ECL	KPN	KPN	KPN	KPN	KPN	KPN
Collection year	2006	2006	2007	2007	2007	2007	2007	2007	2007	2007	2007	2007	2007	2007	2007
City	New Delhi	New Delhi	New Delhi	Mumbai	New Delhi	Pune	New Delhi	New Delhi	Pune	New Delhi	New Delhi	New Delhi	Mumbai	Mumbai	Pune
Source[b]	SSSI	SSSI	SSSI	SSSI	BSI	SSSI	BSI	BSI	BSI	SSSI	SSSI	BSI	RTI	RTI	RTI
MIC (mg/L)															
Amikacin	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	1024	1024	>1024	>1024
Apramycin	8	16	8	16	16	16	8	8	8	4	4	8	4	4	4
Arbekacin	1024	1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	1024	512	1024	256
Gentamicin	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	512	>512
Kanamycin	>1024	>1024	>1024	>1024	>1024	>1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
Neomycin	16	16	16	32	32	32	16	32	8	16	1	8	4	16	8
Streptomycin	256	256	128	256	64	256	4	512	16	128	256	1024	128	512	512
Tobramycin	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	512	>512	512
Imipenem	8	8	32	32	64	128	2	2	32	2	8	64	16	64	32
Meropenem	2	4	8	8	16	64	2	2	2	1	2	16	8	8	8
PFGE	EC-A	EC-A	EC-B	EC-B1	EC-B2	EC-B3	ECL-A	ECL-A	ECL-B	KPN-A	KPN-B	KPN-C	KPN-D	KPN-E	KPN-E
rmtA	neg	neg	neg	neg	neg	neg	neg	neg	pos	neg	neg	neg	neg	neg	neg
rmtC	neg	neg	neg	pos	pos	pos	pos	pos	neg	pos	neg	neg	neg	neg	neg
armA[c]	pos	pos	pos	neg	pos	pos	neg	neg	pos	neg	pos	neg	neg	pos	pos

a. EC= *Escherichia coli*; ECL= *Enterobacter cloacae*; KPN= *Klebsiella pneumoniae*.
b. SSSI= skin and skin-structure infection; BSI= bloodstream infection; RTI= respiratory tract infection.
c. All isolates were negative for *rmtB*, *rmtD* and *npmA*.

CONCLUSIONS

- The association of 16S rRNA methylases- and NDM-1-encoding genes was confirmed in numerous instances; however, further studies evaluating transformant strains carrying NDM-1 plasmids from isolates 258-03-C and 258-18-A reported in this study, demonstrated that 16S rRNA methylase encoding genes were not harboured in the same plasmid as the MβL gene.
- Isolates carrying multiple plasmids with different arrays of resistance genes are a threat to the treatment of patients with serious Enterobacteriaceae infections. New therapeutic options that overcome these resistance mechanisms are urgently needed.

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