Retrospective Search for NDM-1 Reveals Possible Indian Origin of DIM-1 Metallo-beta-lactamase

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Amended Abstract

Objectives: To assess the early occurrence of NDM-1 and other carbapenemases in a collection of Gram-negative bacilli (GNB) isolates collected in India during 2000. We previously demonstrated that NDM-1-producing isolates were present in India as early as 2006, but no data is available for prior sample years.

Methods: Among 220 GNB isolates collected in India during 2000, 22 strains showing elevated imipenem MIC values (≥0.5 mg/L) were further evaluated for the presence of carbapenemases. Modified Hodge test (MHT) was performed. Isolates were tested by PCR for genes encoding KPC, IMP, VIM, NDM, SPM, SIM, KHM, DIM, BIC, GIM, SME, IMI, NMC-A, GES and OXA-48. DIM-1-producer was compared to index strain (kindly supplied by L. Poirel, Bicetre Hospital, France) by PFGE and integron structures were amplified using primers located in the conserved sequences (CS).

Results: 22 GNB tested belonged to eight bacterial species, including 5 *E. cloacae*, 4 *P. aeruginosa*, 4 *P. fluorescens*, 2 each of *K. pneumoniae*, *A. baumannii*, *C. freundi*, *P. stutzeri* and one *P. vulgaris*. These strains were collected in 5 cities: Mumbai, Vellore, New Delhi, Lucknow and Indore. Only one strain yielded positive PCR results for bla_{DIM} primers. No isolates were positive for NDM or other carbapenemase-encoding genes. The *P. stutzeri* strain carrying bla_{DIM-1} was genetically distinct from the index *P. stutzeri* strain carrying this gene previously described in The Netherlands. Integron structure showed that bla_{DIM-1} was located in the second position of a class I integron downstream of *aadB*. In contrast the index strain that carried bla_{DIM-1} in the first position followed by *aadB* and *qacH*, and no 3'-CS.

Conclusions: NDM-producing strains were not detected in this bacterial collection from five Indian cities in 2000, narrowing the interval for the emergence of NDM-producing strains in India. On the other hand, the detection of a DIM-1-producing *P. stutzeri* from India collected many years prior to the finding of this gene in the Dutch strain, suggests that the Indian subcontinent could be the source of another metallo-beta-lactamase gene. Further studies should be performed to investigate the origin of DIM-1 and its prevalence in India.

Introduction

Several types of metallo-β-lactamases (MβL) have been reported to date. IMP- and VIM-types and a few NDM variants seem to be spread worldwide, whereas numerous other genes are limited to a single report or to certain geographic locations, including genes encoding SPM-1, GIM-1, SIM-1, AIM-1, KHM-1, DIM-1, BIC-1 and TMB-1. The NDM-1 (New Delhi Metallo-β-lactamase)-encoding gene was detected in a Swedish diabetic patient of Indian origin that traveled to New Delhi and acquired a urinary tract infection. Since this initial report, NDM-producing isolates have been reported in several countries among all continents and in various instances these reports are related to travels to the Indian sub-continent. Although much has been studied about NDM-producers, the timeline for the emergence of this gene has not been established prior to 2006.

DIM-1 was identified in a *Pseudomonas stutzeri* strain collected in 2007 from a Dutch patient. This gene, sharing only 45 to 52% gene amino acid identity with other MβL-encoding genes, hydrolyzes broad-spectrum cephalosporins and carbapenems and spared monobactams, as other MβLs. The DIM-1 encoding gene was embedded in a class 1 integron containing two other gene cassettes, confering resistance to aminoglycosides and disinfectants; carried by a 70-Kb plasmid.

This study was initiated to determine the presence of bla_{NDM} and other carbapenemases among a collection of Gram-negative bacilli collected during 2000 from hospitals located in India. During this process, we detected a DIM-1-producing *P. stutzeri* that was evaluated for the genetic context and compared to the index DIM-1-producing isolate.

Materials and Methods

Bacterial isolates and carbapenemase screening. A total of 220 Gram-negative isolates were collected from five hospitals in India during 2000. Only one isolate per patient from documented infections were included in the study. All strains displaying an imipenem MIC value ≥0.5 mg/L by broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, M07-A9; 2012) were screened for production of carbapenemases. PCR reactions targeting the following genes/groups were performed: bla_{IMP}, bla_{VIM}, bla_{SPM-1}, bla_{KPC}, bla_{SME}, bla_{IMI}, bla_{NMC-A}, bla_{GES}, bla_{KHM-1}, bla_{DIM-1}, bla_{BIC-1}, bla_{NDM}, and bla_{OXA-48}. *Acinetobacter* spp. were also screened for the genes encoding OXA-23, OXA-24/-40, OXA-51 and OXA-58. PCR 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/). Enterobacteriaceae strains were also evaluated using the Modified Hodge test (MHT) using imipenem and meropenem as substrates.

Methods-continued

Class 1 integron characterization. Primers annealing to the 5' and 3' conserved sequence (CS) regions of class 1 integrons were used in combination with the bla_{DIM-1} primers to determine the size and structure of the integron. Additional primers targeting the genes detected in the integron were used to complete sequencing. Sequencing was analyzed as described above.

Molecular typing. Pulsed-field gel electrophoresis (PFGE) was used to evaluate clonality among DIM-1-producing *P. stutzeri* from India and the index strain from the same bacterial species detected in The Netherlands (kindly supplied by L. Poirel, Bicetre Hospital, France). Genomic DNA was prepared in agarose blocks and digested with Spe I (New England Biolabs; Beverly, Massachusetts, USA). Electrophoresis was performed on the CHEF-DR II (BioRad, Richmond, California, USA), with the following conditions: 0.5 x TBE, 1% agarose, 13°C , 200V, for 24 h with the switch time ramped from 5 to 90 seconds.

Gene location analysis. Total cellular DNA embedded in 1% agarose plugs was subjected to partial digestion with S1 nuclease. Plasmids were resolved by electrophoresis performed on the CHEF-DR II (BioRad), with the following conditions: 0.5 x TBE, 1% agarose, 13°C, 200V, for 6 hours with switch time ramping from 5 to 25 seconds and 14 hours with the switch time from 30 to 45 seconds. ICeuI digested genomic DNA was also resolved on PFGE as described previously. DNA gels were transferred to nylon membranes by Southern blotting and hybridized with a digoxigenin labeled (Roche Diagnostics GmbH, Mannheim, Germany) bla_{DIM-1}-specific probe.

Results

- Among 220 Gram-negative clinical isolates collected from five hospitals/cities (Mumbai, Vellore, New Delhi, Lucknow and Indore) in India, 22 strains had imipenem MIC values ≥0.5 mg/L. These strains were: five *Enterobacter cloacae*, four *Pseudomonas aeruginosa*, four *Pseudomonas fluorescens*, two each of *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Citrobacter freundi*, *P. stutzeri* and one *P. vulgaris* (Table 1).
- Most Enterobacteriaceae strains had low imipenem MIC values (0.5-1 mg/L) and higher carbapenem MIC results were noted for *Pseudomonas* spp. (1-8 mg/L). The two *A. baumannii* strains had imipenem MIC values of 1 mg/L.
- No isolates were positive for bla_{NDM}, but one *P. stutzeri* strain yielded positive PCR results for bla_{DIM} primers. Other carbapenemase genes were not detected and all Enterobacteriaceae strains yielded negative MHT results with imipenem and meropenem. The two *A. baumannii* strains were positive for bla_{OXA-51} only.
- Sequencing demonstrated that the *P. stutzeri* strain carried bla_{DIM-1} and integron sequencing identified the MβL gene located in the second position of a class 1 integron with *aadB* located upstream and no 3'CS was detected using multiple primers. The comparison of the genetic environment of this strain and the index DIM-1-producing strain demonstrated differences in the genetic context of this MβL gene (Figure 1).
- DIM-1-producing *P. stutzeri* was resistant to cephalosporins, gentamicin, ciprofloxacin, but the imipenem MIC result was only marginally elevated to 2 mg/L (Table 1).
- PFGE analysis showed that the Indian DIM-1 was genetically distinct from the index *P. stutzeri* strain carrying this gene previously described in The Netherlands (Figure 2).
- The DIM-1-encoding gene was located on a 240-Kb plasmid, in contrast to the index strains that carried bla_{DIM-1} in a much smaller plasmid (70-Kb).

Figure 1. Schematic representation of the partial class 1 integron carrying bla_{DIM-1} from *P. stutzeri* 10627 compared to the integron carried by the index DIM-1-producing *P. stutzeri* isolated in The Netherlands (2007). Horizontal arrows indicate the gene cassette and their respective translation orientation.

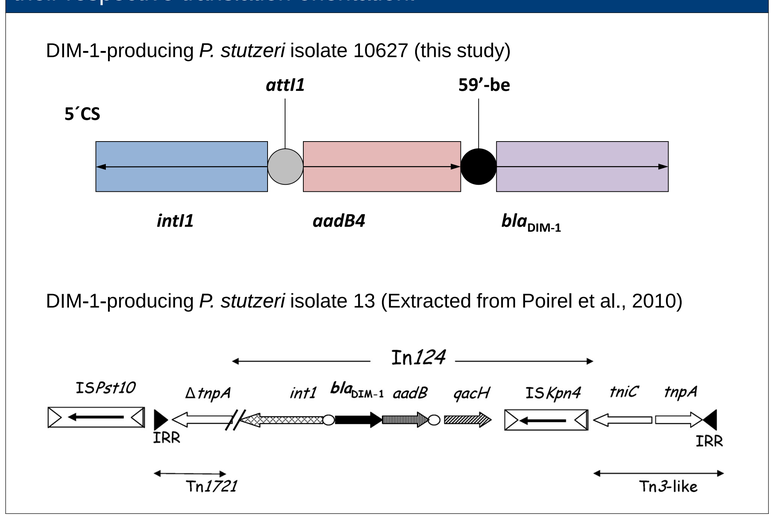
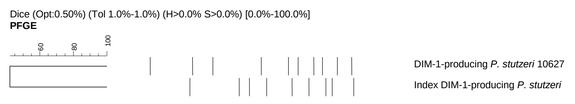


Figure 2. PFGE comparison of DIM-1-producing *P. stutzeri* 10627 from India and the index DIM-1-producing *P. stutzeri* isolated in The Netherlands (kindly provided by L. Poirel).



Conclusions

- This study was initiated in an attempt to determine the earliest emergence of bla_{NDM} in Indian hospitals. No NDM-producing strains were detected in this collection dating from 2000; however, we detected a DIM-1-producing *P. stutzeri* strain from New Delhi. The index DIM-1-producing *P. stutzeri* was only collected in 2007 and these results suggest that this gene most likely emerged in the Indian subcontinent.
- No other carbapenemase-encoding genes, including genes encoding VIM-types among *P. aeuruginosa* and OXA-181 among Enterobacteriaceae that have been reported from India were detected in this study.
- We previously demonstrated the presence of NDM-producing strains in Indian hospitals as early as 2006. Epidemiology studies from isolates from India collected between 2000 and 2006 may answer the question posed in this study regarding to the emergence of isolates carrying NDM-encoding genes as well as other metallo-enzymes.

Acknowledgment

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Table 1. Susceptibility profiles of the Gram-negative isolates displaying imipenem MIC values ≥0.5 mg/L collected in Indian hospitals during 2000.

Isolate	Organism[a]	City	MIC (mg/L)[b]								
			IMI	CTX	CFT	P/T	CIP	GEN	TET	TIG	T/S
10430	*A. baumannii*	Vellore	1	>32	>8	≤0.5	>4	>8	>8	0.25	>4
10456	*A. baumannii*	Indore	1	32	>8	>64	>4	>8	>8	0.5	≤0.5
10606	*C. freundii*	New Delhi	0.5	>32	>8	>64	>4	>8	1	0.25	≤0.5
10431	*C. freundii*	Indore	1	>32	>8	2	2	4	>8	0.5	>4
10525	*E. cloacae*	Lucknow	0.5	>32	>8	>64	2	>8	>8	0.5	>4
10418	*E. cloacae*	Vellore	0.5	>32	>8	32	≤0.03	>8	2	1	>4
10433	*E. cloacae*	Indore	0.5	>32	>8	>64	≤0.03	≤1	2	0.25	≤0.5
10476	*E. cloacae*	Mumbai	0.5	>32	>8	>64	0.06	>8	>8	0.5	>4
10488	*E. cloacae*	New Delhi	0.5	>32	>8	32	0.5	>8	>8	0.25	≤0.5
10447	*K. pneumoniae*	Indore	1	>32	>8	>64	>4	>8	4	0.25	>4
10480	*K. pneumoniae*	Mumbai	0.5	0.06	≤0.06	2	≤0.03	≤1	1	0.25	≤0.5
10432	*P. stuartii*	Vellore	1	>32	>8	2	2	4	>8	0.5	>4
10526	*P. aeruginosa*	Lucknow	8	2	>8	4	0.12	≤1	>8	4	4
10417	*P. aeruginosa*	Vellore	2	2	>8	16	>4	>8	>8	>4	>4
10550	*P. aeruginosa*	Lucknow	8	2	>8	4	0.12	≤1	>8	4	4
10552	*P. aeruginosa*	Lucknow	8	2	>8	4	0.12	≤1	>8	4	4
10528	*P. fluorescens*	Lucknow	8	4	>8	4	0.06	≤1	1	0.25	≤0.5
10540	*P. fluorescens*	Lucknow	8	4	>8	4	0.12	>8	2	0.12	2
10543	*P. fluorescens*	Lucknow	8	4	>8	8	0.12	>8	2	0.12	4
10545	*P. fluorescens*	Lucknow	4	4	>8	4	≤0.03	≤1	1	0.12	1
10627	*P. stutzeri*	New Delhi	2	16	>8	64	>4	>8	8	0.12	>4
10532	*P. stutzeri*	Lucknow	1	0.25	2	16	0.12	≤1	1	0.25	>4

a. The DIM-1-producing strain is underlined.
b. IMI=Imipenem; CTZ=Ceftazidime; CFT=Ceftriaxone; P/T=Piperacillin/Tazobactam; CIP=Ciprofloxacin; GEN=Gentamicin; TET=Tetracycline; TIG=Tigecycline; T/S=Trimethoprim/Sulfamethoxazole.