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Epidemiologic Characterization of Carbapenemase-producing Enterobacteriaceae in India

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SENTRY ANTIMICROBIAL SURVEILLANCE

Amended Abstract

Background: ESBL- and carbapenemase (CARBase)-encoding genes are important β-lactam resistance mechanisms. This study reports on the occurrence and molecular characterization of CARBase-producing Enterobacteriaceae (ENT) pathogens from India.

Methods: 1,443 ENT were collected from 14 hospitals across India during 2006 – 2007. Isolates were susceptibility (S) tested by CLSI methods. Those with carbapenem MICs at ≥2 µg/mL were screened for CARBase by Modified Hodge Test (MHT) and PCR (NDM-1, IMP, VIM, GES, KPC, OXA-48). CARBase producers were also PCR-screened for CTX-M-, PER-, VEB-, OXA-2-, OXA-10-encoding genes. Clonality was assessed by PFGE. Gene location was determined by Southern blot/hybridization.

Results: 39 (2.7%) ENT met the screening criteria. 15 were PCR-positive for bla_{NDM-1}, 9 for $bla_{OXA-181}$ and one for bla_{VIM-6}. One *K. pneumoniae* (KPN) carried $bla_{OXA-181}$ and bla_{VIM-5}. Most (84.6%) CARBase producers harbored bla_{CTX-M}. bla_{NDM-1}-carrying *E. coli* (EC) clustered within two PFGE groups (EC-A and -B). EC-A strains (2) were recovered from New Delhi (ND), while those belonging to EC-B (4 strains) were from 4 sites in ND (2), Mumbai (1) and Pune (1). NDM-1-producing KPN (6 strains) clustered within 5 clones, while remaining strains (9) had $bla_{OXA-181}$ and belonged to 5 clones, mostly from Kolkata (55.6%). One NDM-1- (Pune) and one OXA-181- (ND) producing KPN shared similar genetic background (subtypes). Two *E. cloacae* (ECL) from Pune had bla_{NDM-1} or bla_{VIM-6}, and belonged to the same clone (2 subtypes). Other ECL harbored $bla_{OXA-181}$ (1 strain from Kolkata) or bla_{NDM-1} (2 related strains from ND). CARBase genes were predominantly plasmid-located.

Conclusion: CARBases genes were largely associated with bla_{CTX-M}. NDM-1-producers were commonly isolated in ND, Mumbai or Pune, while OXA-181-producing strains were mostly from Kolkata. bla_{NDM-1}-carrying EC and ECL shared similar genetic background within each species, however bla_{NDM-1}-carrying KPN were more clonally diverse, suggesting greater genetic exchange.

Introduction

Several carbapenemases have been reported among Enterobacteriaceae species worldwide. Although KPC- and VIM-variants seems to be the most common enzymes, other carbapenemases have emerged among these clinically important pathogens. OXA-48 was initially described among *Klebsiella pneumoniae* strains from Turkey. More recently, this enzyme was detected in Enterobacteriaceae species in Turkey, Belgium, Argentina and the United Kingdom (UK). Additionally, OXA-162 showing one amino acid difference compared to OXA-48 was described in a *K. pneumoniae* from Turkey.

More recently, a new MβL was reported in *K. pneumoniae* and *Escherichia coli* strains from India. This enzyme, called NDM-1 (New Delhi Metallo-β-lactamase) was detected in a Swedish diabetic patient of Indian origin that traveled to New Delhi and acquired a urinary tract infection. NDM-1 was also detected in various Enterobacteriaceae species (*K. pneumoniae*, *E. coli*, *Enterobacter cloacae* and *Citrobacter freundii*) in the UK and the United States and these cases were closely linked to receipt of medical care in India or Pakistan.

In this study, we report occurrences and characterization of carbapenemase producing Enterobacteriaceae collected from Indian hospitals during the SENTRY Antimicrobial Surveillance Program (2006 – 2007), including isolates producing NDM-1 and a new OXA-48-variant, OXA-181.

Methods

Bacterial isolates. A total of 1,443 Enterobacteriaceae isolates were collected from 14 hospitals in India as part of the SENTRY Program (2006-2007). 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 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-S20-U and quality control (QC) was performed using *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents (M100-S20-U, 2010).

Screening for carbapenemases. All Enterobacteriaceae isolates with reduced susceptibility to imipenem, meropenem or ertapenem (MIC, ≥2 µg/mL) were screened for production of carbapenemases. Modified Hodge test (MHT) was performed using imipenem and meropenem as substrates.

Genetic detection of β-lactamase-encoding genes. Carbapenem non-susceptible isolates were PCR screened using primers targeting bla_{NDM-1}, bla_{IMP}, bla_{VIM}, bla_{GES}, bla_{KPC}, bla_{OXA-48}. Isolates were also screened for ESBL production using primers amplifying bla_{CTX-M}, bla_{OXA-2}, bla_{OXA-10}, bla_{PER} and bla_{VEB}. PCR amplicons were sequenced on both strands and analyzed.

Molecular typing. Pulsed-field gel electrophoresis (PFGE) was used to evaluate clonality among carbapenemase-producing Enterobacteriaceae isolates, as described elsewhere. NDM-1 producing *E. coli* isolates were additionally evaluated by multilocus sequence typing (MLST) according to the protocols described previously. Sequencing results were analyzed using internet resources (http://mlst.ucc.ie).

Genetic location of carbapenemase encoding genes. Total DNA was subjected to partial digestion with S1 nuclease or ICeuI and DNA fragments were resolved in agarose gels. Nucleic acid bands from gels were transferred to nylon membranes by Southern blotting and hybridized with a digoxigenin labeled (Roche Diagnostics GmbH, Mannheim, Germany) bla_{NDM-1}-, bla_{VIM}- or $bla_{OXA-181}$ probes.

Results

- Among 1,443 Enterobacteriaceae isolates, 39 (2.7%) were carbapenem resistant and 26 (1.8% overall) of those carried carbapenemase encoding genes. PCR and sequencing revealed that 15 strains carried bla_{NDM-1}, 10 harbored a new bla_{OXA-48}-like gene (94.4% homology) and one *E. cloacae* carried bla_{VIM-6} (Table 1). One *K. pneumoniae* strain carried bla_{VIM-5} and bla_{OXA-48}-like.
- The gene encoding NDM-1 was detected in *E. coli* (six strains), *E. cloacae* (three strains) and *K. pneumoniae* (six strains) whereas bla_{OXA-48}-like was found in *K. pneumoniae* (nine strains; one also carrying bla_{VIM-5}) and one *E. cloacae*.
- The gene encoding OXA-48-like was sequenced and 45 nucleotide substitutions were noted when compared to bla_{OXA-48}. Deduced amino acid sequence revealed that this β-lactamase differed from OXA-48 by four amino acids (T104A, N110D, E168Q and S171A; Figure 1) and was named OXA-181 (GenBank accession number HM992946).
- Carbapenemase-producing strains exhibited elevated MICs to β-lactams, including imipenem, meropenem (MIC ranges, 1 - >8 µg/mL), ertapenem (2 - >8 µg/mL), cefepime (8 - >16 µg/mL), ceftazidime (>16 µg/mL), aztreonam (8 - >16 µg/mL) and piperacillin/tazobactam (>64 µg/mL). These isolates were also resistant to amikacin (MIC range 8 to >32 µg/mL) and all but one *E. cloacae* had elevated ciprofloxacin MIC values (MIC range ≤0.03 to >4 µg/mL).
- *E. coli* strains produced NDM-1 and clustered within two PFGE groups (EC-A and -B; Table 1). EC-A strains (two) were recovered from New Delhi, while those belonging to EC-B (four strains) were from four sites, two in New Delhi and one each in Mumbai and Pune. EC-A strains were ST-167, while EC-B subtypes were ST-101.
- *K. pneumoniae* carrying bla_{NDM-1} (six strains) belonged to five types (Table 1). Two identical strains were detected in Pune and Mumbai. The remaining nine *K. pneumoniae* strains harbored $bla_{OXA-181}$, were mostly from Kolkata (55.6%) and belonged to five clones. Clonality within medical sites was observed. The strain carrying $bla_{OXA-181}$ and bla_{VIM-5} had a unique genetic background.
- One OXA-181- producing *K. pneumoniae* from New Delhi was genetically related to NDM-1-carrying *K. pneumoniae* type detected in Pune and Mumbai (KPN-E; Table 1).
- *E. cloacae* produced NDM-1, OXA-181 or VIM-6. Two NDM-1-producers from New Delhi were identical and isolates from Pune harboring bla_{NDM-1} or bla_{VIM-6} were genetically related.
- A large percentage (22/26; 84.6%) of carbapenemase producers also harbored $bla_{CTX-M-15}$. Three CTX-M-15- producing isolates were also positive for bla_{OXA-2} and one for bla_{OXA-10} (Table 1).
- Carbapenemase genes were predominantly plasmid-located (data not shown).
- bla_{VIM-6} in *E. cloacae* was located in the first position of a 3.9-Kb class 1 integron carrying also *aacA4* and bla_{OXA-10}. This genetic arrangement was similar to one described previously in *P. aeruginosa* isolated from this medical center.

Table 1. Distribution of β-lactamase enzymes and molecular typing profiles of 26 carbapenemase-producing Enterobacteriaceae isolated from Indian medical centers in 2006-2007.

Bacterial Species[a]	City	Carbapenemase	PFGE pattern[b]	Other β-lactamases
Escherichia coli (6)	Mumbai (1)	NDM-1 (1)	EC-B1	CTX-M-15, OXA-2
	New Delhi (4)	NDM-1 (4)	**EC-A (2 strains)**	none
			EC-B, EC-B2	CTX-M-15, OXA-2
	Pune (1)	NDM-1 (1)	EC-B3	CTX-M-15, OXA-2
E. cloacae (5)	Kolkata (1)	OXA-181 (1)	ECL-C	CTX-M-15
	New Delhi (2)	NDM-1 (2)	ECL-A	CTX-M-15 (only 1 strain)
	Pune (2)	NDM-1 (1)	ECL-B	CTX-M-15
		VIM-6 (1)	ECL-B1	CTX-M-15
K. pneumoniae (16)	Kolkata (5)	OXA-181 (4)	**KPN-F (2), KPN-H (2)**	CTX-M-15
		OXA-181 and VIM-5 (1)	KPN-G	CTX-M-15, OXA-10
	Mumbai (4)	NDM-1 (2)	KPN-D, KPN-E	CTX-M-15
		OXA-181 (2)	KPN-I	CTX-M-15
	New Delhi (5)	NDM-1 (3)	KPN-A, KPN-B, KPN-C	CTX-M-15
		OXA-181 (2)	KPN-E1, KPN-I	CTX-M-15
	Pune (1)	NDM-1	KPN-E	CTX-M-15

a. Number of isolates in each category indicated in parenthesis.
b. Clonality within medical centers is demonstrated in bold. Underlined PFGE patterns demonstrate clonality among sites.

Figure 1. Alignment of amino acid sequences of novel OXA-181 detected in this study and related enzymes OXA-48 and OXA-162.

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OXA-48. pro     MRVLALSAVFLVASIIGMPAVAKEWQENKSMNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIA 80
OXA-162. pro    MRVLALSAVFLVASIIGMPAVAKEWQENKSMNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIA 80
OXA-181. pro    MRVLALSAVFLVASIIGMPAVAKEWQENKSMNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPASTFKIPNSLIA 80

OXA-48. pro     LDLGVVKDEHQVFKWDGQTRDIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDG 160
OXA-162. pro    LDLGVVKDEHQVFKWDGQTRDIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDG 160
OXA-181. pro    LDLGVVKDEHQVFKWDGQTRDIAÂWNRDHÐLITAMKYSVVPVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDG 160

OXA-48. pro     GIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEANGDYIIRAKTGYSTRIEPKIGWWVGWVELDNNVWFAMNMD 240
OXA-162. pro    GIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEANGDYIIRAKTGYSTRIEPKIGWWVGWVELDNNVWFAMNMD 240
OXA-181. pro    GIRISATQQIÂFLRKLYHNKLHVSERSQRIVKQAMLTEANGDYIIRAKTGYSTRIEPKIGWWVGWVELDNNVWFAMNMD 240

OXA-48. pro     MPTSDGLGLRQAITKEVLKQEKIIP. 266
OXA-162. pro    MPTSDGLGLRQAITKEVLKQEKIIP. 266
OXA-181. pro    MPTSDGLGLRQAITKEVLKQEKIIP. 266
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Conclusions

- Carbapenem-resistant Enterobacteriaceae strains from Indian hospitals showed a great variety of genes, including NDM-1, VIM-types and new OXA-181. These enzymes were detected in three bacterial species emphasizing the potential for interspecies dissemination. Additionally, carbapenemase genes were largely associated with the extended spectrum β-lactamase $bla_{CTX-M-15}$.
- bla_{NDM-1}-carrying *K. pneumoniae* displayed greater clonal diversity when compared to *E. coli* and *E. cloacae* harboring the same enzyme. This could suggest greater genetic exchange among strains of this bacterial species.
- NDM-1-producing isolates appear to be an emerging problem. Further studies are needed to elucidate the mechanism of dissemination of this β-lactamase gene and the newly described OXA-181.

References

1. Detection of *Enterobacteriaceae* isolates carrying metallo-beta-lactamase - United States, 2010. *MMWR Morb Mortal Wkly Rep* 59: 750.
2. Castanheira M, Bell JM, Turnidge JD, Mathai D, Jones RN (2009). Carbapenem resistance among *Pseudomonas aeruginosa* strains from India: Evidence for nationwide endemicity of multiple metallo-beta-lactamase clones (VIM-2, -5, -6, and -11 and the newly characterized VIM-18). *Antimicrob Agents Chemother* 53: 1225-1227.
3. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: eighth edition.* Wayne, PA: CLSI.
4. Clinical and Laboratory Standards Institute (2010). *M100-S20-U. Performance standards for antimicrobial susceptibility testing: 20th informational supplement (June 2010 Update).* Wayne, PA: CLSI.
5. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *Lancet Infect Dis* 10: 597-602.
6. Livermore DM, Woodford N (2010). Carbapenemase-producing *Enterobacteriaceae* in the UK: NDM (New Delhi Metallo-)beta-lactamase: Repeated importation from Indian subcontinent. HPA. Available at http://www.hpa.org.uk/web/HPAweb/HPAweb_C/1248854045473. Accessed April, 2010.
7. Poirel L, Castanheira M, Carrer A, Jones RN, Parada Rodriguez CA, Smayevsky J, Nordmann P (2010). Oxa-163, an oxa-48-related class D beta-lactamase with an extended activity to expanded-spectrum cephalosporins Abstr. C1-675 *50th ICAAC, September 12-15, 2010,* Boston, Massachusetts, USA.
8. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR (2009). Characterization of a new metallo-beta-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother* 53: 5046-5054.

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