C2-647
ICAAC 2009
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Emergence of *bla*$_{OXA-48}$ among *Enterobacter cloacae* in Argentina and its Prevalence Among Carbapenem Non-susceptible Enterobacteriaceae

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: OXA-48 carbapenemase was described in *K. pneumoniae* from Turkey. We evaluated the occurrence of *bla*$_{OXA-48}$ among carbapenem non-susceptible Enterobacteriaceae from the SENTRY Antimicrobial Surveillance Program (2007-2008) and characterized OXA-48-producing strains from Argentina.

Methods: 14,893 Enterobacteriaceae consecutively collected in hospitals located in USA, Europe and Latin America showing elevated carbapenem MIC values (≥ 2 µg/ml) were evaluated. Susceptibility tests and Modified Hodge test (MHT) were performed by CLSI methods. *bla*$_{OXA-48}$ detection and association with IS*1999* was performed by PCR followed by sequencing. Plasmid and ICeuI preparations were hybridized with *bla*$_{OXA-48}$ probes. Isolates were typed by PFGE.

Results: Among 278 (1.9% overall) carbapenem non-susceptible Enterobacteriaceae, 12 strains carried *bla*$_{OXA-48}$ (4.3% of carbapenem non-susceptible). These isolates were recovered from one hospital in Ankara, Turkey (4 *K. pneumoniae* and 4 *E. coli*) and 2 in Buenos Aires, Argentina (3 *K. pneumoniae* and 1 *E. cloacae*). All but one isolate were positive by MHT. *bla*$_{OXA-48}$ was sequenced and strains from Turkey had IS*1999* upstream of the carbapenemase gene; however, among 6 strains (Turkey only) IS*1 tnpA* was observed between these structures. All but two strains displayed multiple plasmids. *E. coli* from Turkey were genetically distinct (3 types) and carried *bla*$_{OXA-48}$ in the same size plasmid. Two *K. pneumoniae* from the same country harbored the same size plasmid hybridizing with *bla*$_{OXA-48}$. *K. pneumoniae* were genetically unrelated. One *K. pneumoniae* from Turkey carried *bla*$_{OXA-48}$ in the chromosome. One *E. cloacae* strain carried multiple plasmids.

Conclusions: This is the first report of *bla*$_{OXA-48}$-carrying isolates outside of Turkey and in *E. cloacae*. Furthermore, Turkey and Argentina have a high prevalence of oxacillinases with carbapenemase activity among *Acinetobacter* spp., suggesting that the origin of these enzymes could be related.

INTRODUCTION

Carbapenems are important therapeutic options for the treatment of Enterobacteriaceae strains producing extended spectrum ß-lactamases (ESBLs) or multidrug resistant strains. However, the emergence of carbapenem resistance has complicated the treatment of serious nosocomial infections. Among carbapenem resistance determinants, carbapenemases, such as acquired metallo-ß-lactamases, serine-carbapenemases and oxacillinases have been demonstrated to be peculiarly threatening due to their potential for dissemination.

Several carbapenemases have been reported among Enterobacteriaceae species and whereas KPC- and VIM-variants seems to be the most common, other carbapenemases have recently emerged among these clinically important pathogens. OXA-48 was initially described among *K. pneumoniae* strains from Turkey and more recently this enzyme was detected among *E. coli* strains from this same country and in *K. pneumoniae* from Belgium. *bla*$_{OXA-48}$ was described as part of Tn*1999* composite transposons, carrying copies of IS*1999* flanking this carbapenemase gene on both sides. OXA-48-producing strains showing elevated carbapenem MIC values often possess a secondary non-enzymatic resistance mechanism, usually reduced expression of outer membrane proteins.

In this study, we describe the prevalence and molecular characterization of OXA-48-producing isolates among Enterobacteriaceae strains collected during 2008.

MATERIALS AND METHODS

Bacterial isolates. During 2007 and 2008, 14,893 Enterobacteriaceae isolates were collected from medical centers located in Europe, North and Latin America. These isolates were collected from bloodstream, respiratory tract and skin and soft tissue infections according to defined protocols. Only clinically significant isolates were included in the study, one per patient episode. Species identification was confirmed by standard biochemical tests and use of Vitek Systems (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing. All isolates were susceptibility tested against more than 25 antimicrobials by broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; 2008) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were by Clinical Laboratory Standard Institute (CLSI, 2009) criteria. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all results were in the published range.

Screening for carbapenemase encoding genes. All isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 µg/ml) were tested with the Modified Hodge test (MHT) using imipenem and meropenem disks. Isolates were screened for the presence of several carbapenemase-encoding genes by multiplex PCR reactions. Generic primers designed for *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{KPC}$, *bla*$_{SME}$, *bla*$_{GES}$ variants and for *bla*$_{IMI}$, *bla*$_{NMC-A}$, *bla*$_{OXA-48}$ were combined in two amplification reactions.

Genetic context of *bla*$_{OXA-48}$. Primers annealing on IS*1999* were used in combination with *bla*$_{OXA-48}$ primers to determine the genetic context of this gene. Plasmid extractions were performed using the Plasmid MIDI kit (QIAGEN, Hilden, Germany). Plasmid sizes were determined by comparison with plasmids harbored by *Escherichia coli* NCTC 50192. Agarose DNA preparations were treated with ICeuI and resolved as described elsewhere. DNA gels were transferred to nylon membranes by southern blotting. A digoxigenin (Roche Diagnostics GmbH, Mannheim, Germany) labeled *bla*$_{OXA-48}$-specific probe was used to identify the location of this gene.

DNA sequencing. Amplicons were sequenced on both strands. Results were analyzed using Lasergene® software (DNAStar, Madison, Wisconsin, USA) and compared to available sequences through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

Molecular typing. Isolates were evaluated for clonality by pulsed-field gel electrophoresis (PFGE). Genomic DNA was prepared in agarose blocks and digested with Spe I (New England, 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 23 h with the switch time ramped from 5 to 60 seconds.

RESULTS

- A total of 278 carbapenem non-susceptible Enterobacteriaceae clinical strains (1.9% of all strains; MIC at ≥2 µg/ml) were observed during the study period.
- *bla*$_{OXA-48}$ was detected among 12 strains (4.3% of carbapenem non-susceptible): seven *K. pneumoniae* (Turkey and Argentina – two hospitals), four *E. coli* (all from Turkey) and one *E. cloacae* (Argentina; Table 1).
- Ten isolates displayed positive MHT using imipenem and meropenem disks. Two *K. pneumoniae* had negative MHT for both compounds (Table 1).
- PFGE results showed that all *K. pneumoniae* strains were genetically unrelated (Figure 1a).
- Among four OXA-48-producing *E. coli* from the Turkish hospital, three genetic patterns were observed, two strains being genetically identical (Figure 1b).
- IS*1999* was detected upstream of *bla*$_{OXA-48}$ in all Turkish strains, but not among the Argentinean isolates. In six strains (two *K. pneumoniae* and four *E. coli*) *tnpA* from IS*1* was detected embedded between IS*1999* and *bla*$_{OXA-48}$ (Figure 2).
- Amplicons were observed for all but three strains (*K. pneumoniae* from Argentina) using primers to detect IS*1999* downstream of the carbapenemase gene. Two *E. coli* yielded two amplicons of 4- and 5.2-Kb (Table 2).
- *K. pneumoniae* strains from both Argentinean hospitals did not carry IS*1999* downstream or upstream of *bla*$_{OXA-48}$ (Table 2).
- Eleven strains carried *bla*$_{OXA-48}$ in plasmids of distinct sizes. One *K. pneumoniae* from Turkey harbored this gene in the chromosome (Table 2).

Table 1. Characteristics of strains carrying *bla*$_{OXA-48}$.

Isolate	Country	Organism	Specimen	Culture date	Service	MHT[a]	MIC (µg/ml) IMI[b]	MER[c]	ERT[d]
5928A	Turkey	*K. pneumoniae*	Blood culture	10-May-07	Internal Medicine	Positive	2	0.5	2
6011A	Turkey	*K. pneumoniae*	Blood culture	17-Jun-08	Hematology/Oncology	Positive	>8	>8	>8
10628A	Turkey	*K. pneumoniae*	Blood culture	27-Aug-08	Hematology/Oncology	Positive	4	1	2
14559A	Turkey	*K. pneumoniae*	Blood culture	17-Dec-07	Cardiothoracic/Pulmonary	Negative	2	1	1
2082D	Turkey	*E. coli*	Wound	23-Jun-08	Surgery	Positive	2	0.25	0.5
2084D	Turkey	*E. coli*	Wound	01-Jun-08	Orthopedics	Positive	2	0.5	2
10608A	Turkey	*E. coli*	Blood culture	14-Jul-08	Hematology/Oncology	Positive	2	0.5	0.5
10651A	Turkey	*E. coli*	Blood culture	29-Aug-08	General/GI	Positive	4	0.5	1
1559C	Argentina	*K. pneumoniae*	Sputum	29-Apr-08	General/GI	Positive	2	≤0.12	≤0.06
6299A	Argentina	*K. pneumoniae*	Blood culture	11-May-08	Internal Medicine	Positive	1	2	1
12007A	Argentina	*K. pneumoniae*	Blood culture	23-Sep-07	Internal Medicine	Negative	8	>8	>8
2185D	Argentina	*E. cloacae*	Peritoneal fluid	20-Apr-08	Internal Medicine	Positive	>8	>8	>8

a. MHT = Modified Hodge test.
b. IMI = Imipenem.
c. MER = Meropenem.
d. ERT = Ertapenem.

Table 2. Characteristics of the genetic elements carrying *bla*$_{OXA-48}$ detected in strains from Turkey and Argentina.

Isolate	Country	Organism	IS*1999* (band size) upstream	downstream	IS*1 tnpA*	*bla*$_{OXA-48}$ genetic location
5928A	Turkey	*K. pneumoniae*	4-Kb	5.2-Kb	Negative	plasmidic
6011A	Turkey	*K. pneumoniae*	4-Kb	5.2-Kb	Negative	chromosomal
10628A	Turkey	*K. pneumoniae*	5-Kb	5.2-Kb	Positive	plasmidic
14559A	Turkey	*K. pneumoniae*	5-Kb	5.2-Kb	Positive	plasmidic
2082D	Turkey	*E. coli*	5-Kb	5.2- and 4-Kb	Positive	plasmidic
2084D	Turkey	*E. coli*	5-Kb	5.2-Kb	Positive	plasmidic
10608A	Turkey	*E. coli*	5-Kb	5.2- and 4-Kb	Positive	plasmidic
10651A	Turkey	*E. coli*	5-Kb	5.2-Kb	Positive	plasmidic
1559C	Argentina	*K. pneumoniae*	Negative	Negative	Negative	plasmidic
6299A	Argentina	*K. pneumoniae*	Negative	Negative	Negative	plasmidic
12007A	Argentina	*K. pneumoniae*	Negative	Negative	Negative	plasmidic
2185D	Argentina	E. cloacae	Negative	1.2-Kb	Negative	plasmidic

Figure 1. PFGE pattern analysis of OXA-48-producing isolates. Panel (a) *K. pneumoniae* and (b) *E. coli*.

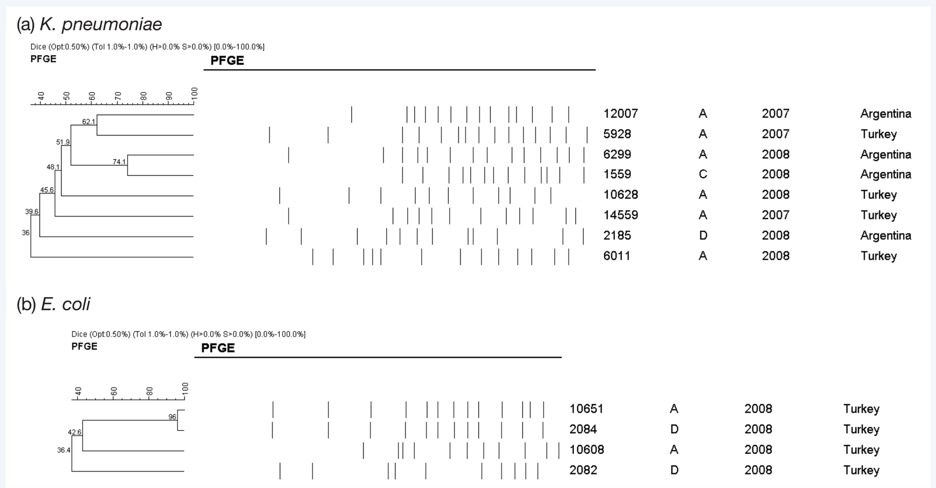
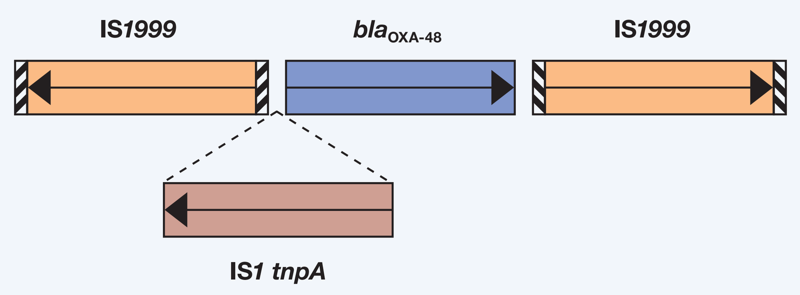


Figure 2. Schematic representation of *bla*$_{OXA-48}$-carrying element detected among Enterobacteriaceae strains detected during this study. Among six strains IS*1 was* detected upstream of the carbapenemase encoding gene. Genes are represented boxes with arrows and the direction of the arrows indicates the transcription orientation. Hashed boxes represent duplication sites.



CONCLUSIONS

- *bla*$_{OXA-48}$ was previously described only among Enterobacteriaceae strains from Turkey and Belgium. This is the first report of isolates carrying this carbapenemase gene in Argentina.
- Among the Argentinean strains, *bla*$_{OXA-48}$ was not located in the same genetic structure previously reported in the literature (Tn*1999*). Further investigations are needed to reveal the genetic element carrying *bla*$_{OXA-48}$ in these isolates.
- Genetic diversity was observed among *K. pneumoniae* strains from both countries, suggesting that *bla*$_{OXA-48}$ was mobilized on multiple occasions.
- OXA-48 was initially considered to have a low hydrolytic activity against carbapenems; however, the majority of isolates producing this enzyme can be detected by the MHT.

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