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# Occurrence and Genetic Analysis of OXA-48-Producing Strains in European Countries over a Four-Year Period (2007-2010)

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SENTRY ANTIMICROBIAL SURVEILLANCE

## Amended Abstract

**Objectives**: To evaluate the dissemination and genetically characterize OXA-48-producing strains in Europe. Increasing traveling and immigration among countries in the Mediterranean area seem to promote the spread of *bla*$_{OXA-48}$. We used the SENTRY Antimicrobial Surveillance Program network to assess the occurrence of OXA-48-producers among 16 European nations.

**Methods**: Among 201 (1.3%; 15,520 strains) carbapenem-resistant (R) Enterobacteriaceae strains collected during 2007-2010 in 16 European countries, 42 OXA-48-producing strains were detected using Modified Hodge Test (MHT) and PCR for carbapenemase-encoding genes. Clonality was assessed by PFGE. Gene location was determined by S1 endonuclease restriction, followed by hybridization. Genetic environment was amplified using primers targeting IS*1999* anchoring on the OXA-48 gene. Amplicons were digested with AluI, RsaI and Sau3AI and different types were sequenced.

**Results**: OXA-48-producers were collected in 2007 (3 strains), 2008 (6), 2009 (28) and 2010 (6). 41 strains were detected in Turkey (all years) and one *K. pneumoniae* (KPN) in Italy. The latter was collected in May/2010 from a 46 y/o female patient hospitalized in Genoa. Isolates belonged to five bacterial species: KPN (20 strains; all years), *E. coli* (EC; 14 strains; 2008 and 2009), *E. cloacae* (ECL: 4 strains; 2010 only), *K. oxytoca* (KOX; 3 strains; 2009 only) and one *E. aerogenes* (2009). Imipenem (IMI) MIC values ranged from 1 to >8 mg/L and meropenem (MER) from 0.25 to >8 mg/L (mode, 4 and 1 mg/L, respectively). One KPN strain was MHT negative (IMI and MER MIC, 2 and 1 mg/L, respectively). KPN displayed great genetic diversity by PFGE (14 patterns). Clonality was observed mainly in 2009 (1 cluster of 3 strains and 4 clusters of 2). Among EC, 8 patterns were noted and 7 of 10 strains from 2009 belonged to the one cluster. ECL strains displayed two patterns and KOX were identical. All strains carried *bla*$_{OXA-48}$ in plasmids and two different *bla*$_{OXA-48}$ genetic elements were observed: IS*1999*(± IS*1 tnpA* disruption)/ *bla*$_{OXA-48}$/IS*1999*.

**Conclusions**: OXA-48-producing strains were found to be disseminated in Turkey and one strain was detected in Italy. These strains were not observed on other European countries (5 in the Mediterranean region) surveyed by the SENTRY Program. High rates of OXA-48-producers in 2009 seemed to be related to clonal spread. This gene seems to disseminate via plasmid or genetic element with no boundaries among Enterobacteriaceae species.

## Introduction

OXA-48 was initially described among *Klebsiella pneumoniae* strains from Turkey; however, this class D carbapenemase has been identified in various Enterobacteriaceae species and recovered in several European countries and others in the Mediterranean area. The gene encoding OXA-48 is usually carried by Tn*1999* composite transposons that harbor copies of IS*1999* flanking both sides of this carbapenemase gene and other genes such as IS*1* and *lysR*. It has been recently demonstrated that *bla*$_{OXA-48}$ is usually carried by a unique IncL/M scaffold plasmid; different from other carbapenemases that are carried by a variety of plasmid structures/types.

OXA-48-producing strains showing elevated carbapenem MIC values often possess a secondary non-enzymatic resistance mechanism and most isolates have modestly elevated carbapenem MIC values. The low resistance levels encoded by this enzyme makes detection of strains harbouring this resistance mechanism difficult and facilitates the dissemination of this carbapenemase-encoding gene. Furthermore, recent animal studies demonstrated that infections caused by OXA-48-producing strains might be difficult to treat with clinically available β-lactams with or without available or investigational β-lactamase inhibitors.

In this study, we describe the prevalence and molecular characterization of OXA-48-producing isolates among Enterobacteriaceae strains collected during 2007 and 2010 from 16 European countries.

## Materials and Methods

Bacterial isolates. From 2007 to 2010, 201 carbapenem-non-susceptible Enterobacteriaceae isolates were collected from 16 medical centers located in European countries and Israel. These clinically significant isolates were collected from bloodstream, respiratory tract and skin and soft tissue infections according to defined protocols. Isolates were susceptibility tested by broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; M07-A9; 2012).

Screening for carbapenemase encoding genes. All isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 mg/L) were screened for production of carbapenemases by PCR and the Modified Hodge test (MHT) using imipenem and meropenem as substrates. 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*$_{OXA-48}$. 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/).

## Methods-continued

Genetic context of *bla*$_{OXA-48}$. Primers annealing on each copy of the IS*1999* located upstream and downstream of the carbapenemase gene were used in combination with *bla*$_{OXA-48}$ primers forward and reverse to determine the genetic context of this gene in two separate reactions. Amplicons were digested with AluI, RsaI and Sau3AI and different types were sequenced.

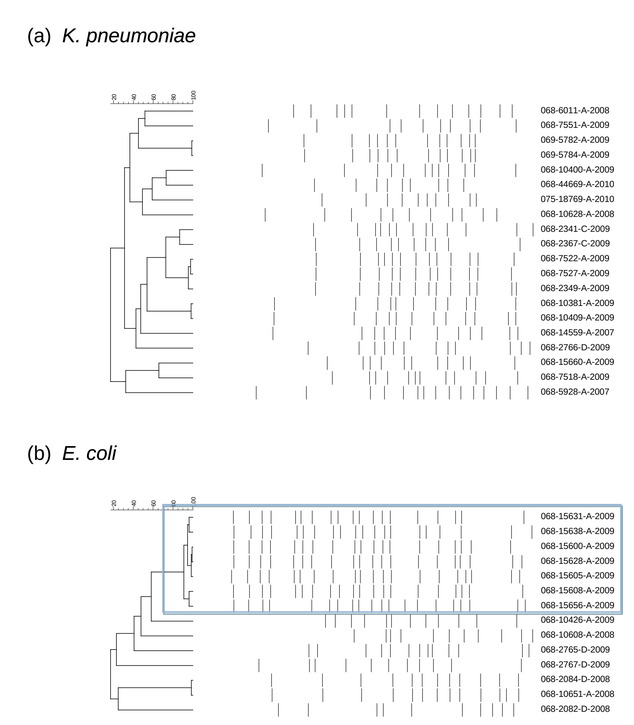
Total cellular DNA embedded in 1% agarose plugs was subjected to partial digestion with S1 nuclease. Plasmids were resolved by electrophoresis performed on the CHEF-DR II (BioRad, Richmond, California, USA), with the following conditions: 0.5 x TBE, 1% agarose, 13°C, 200V, for 6 hours with switch time ramping from 5 to 25 seconds and 14 hours with the switch time from 30 – 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*$_{OXA-48}$-specific probe.

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) as described elsewhere.

## Results

- Among 201 carbapenem-non-susceptible Enterobacteriaceae strains collected in European hospitals from 2007 to 2010, *bla*$_{OXA-48}$ was detected in 42 strains (20.9%). These isolates belonged to five bacterial species: *K. pneumoniae* (20 strains), *Escherichia coli* (14), *Enterobacter cloacae* (4), *K. oxytoca* (3) and *E. aerogenes* (1).
- OXA-48-producers were collected in 2007 (3 strains), 2008 (6), 2009 (28) and 2010 (6). Forty-one strains were detected in Turkey (all years; 39 in Ankara and 2 in Istanbul) and one *K. pneumoniae* from Italy. This latter strain was recovered in a medical center in Genoa (2010) from a blood culture of a 46 y/o female patient after 48h hospital admission.
- Isolates displayed diverse MIC patterns with imipenem MIC values varying from 1 to >8 mg/L and meropenem from 0.25 to >8 mg/L (mode, 4 and 1 mg/L, respectively). Thirteen strains displayed MIC values for broad spectrum-cephalosporins and/or aztreonam of ≤0.5 mg/L (≤0.12-0.5 mg/L), but elevated carbapenem MIC results with values ranging from 0.5 to 8 mg/L (Table 1).
- *K. pneumoniae* displayed great genetic diversity and 14 PFGE patterns were detected among 20 strains (Figure 1). Clones were detected in Ankara (4 clusters; 3 and 2 strains each) and one in Istanbul (2 strains; Table 1).
- One cluster (KPN-J) had two strains from different years (2007 and 2009) and interestingly, the MIC results for cephalosporins and aztreonam for the strain collected in 2007 were lower than the strain from 2009. One strain belonging to PFGE pattern KPN-H displayed negative MHT results, whereas an identical strain had a positive result for the carbapenemase screening test. The MIC values for the MHT-negative strain were low for cephalosporins and aztreonam (≤0.5 mg/L), but imipenem and meropenem MIC results were 2 and 1 mg/L, respectively.
- Two clusters were observed among *E. coli* strains and 7 of 10 strains from 2009 belonged to the one cluster (Figure 1). Four *E. coli* strains had low cephalosporins and aztreonam MIC results (≤0.12-0.5 mg/L; Table 1).
- *E. cloacae* strains displayed two PFGE patterns and were all collected in 2010 and highly resistant to all β-lactams tested (Table 1). Two identical *E. cloacae* strains also carried *bla*$_{IMP-1}$. All three *K. oxytoca* strains were genetically identical.
- All strains carried *bla*$_{OXA-48}$ embedded on Tn*1999* variants (Tn*1999* and Tn*1999.2*) and were plasmid located. Plasmid sizes varied from 60 to 120-Kb. A total of 31 strains carried plasmids ranging from 60 to 67.5-Kb.

**Figure 1**. PFGE profiles of the OXA-48-producing (a) *K. pneumoniae* showing that these strains were highly diverse and (b) *E. coli* displaying one large epidemiologic cluster detected in 2009 (7 strains; boxed).




## Conclusions

- Several OXA-48-producing strains had relatively low MIC values (≤0.5 mg/L) for broad-spectrum cephalosporins and aztreonam and although it has been suggested that strains carrying this carbapenemase might require other non-enzymatic resistance mechanisms to become fully resistant to β-lactam agents, our findings deserve further investigation, especially among those genetically identical strains having different MIC profiles.
- OXA-48 strains were dominantly detected in one hospital in Ankara, Turkey, where these organisms seem to be endemic. Furthermore, this is the first report of co-production of OXA-48 and IMP-1 with both genes prevalent in Turkey.
- The prevalence of OXA-48-producers in other European hospitals is still low (approximately 2%). However, the number of OXA-48 producing Enterobacteriaceae in 2010 was significantly reduced compared to 2009 (6 versus 28 occurrences; only 5 from Turkey).

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**Table 1**. Characteristics and susceptibility profiles of OXA-48-producing Enterobacteriaceae from Turkey (Ankara and Istanbul) and Genoa, Italy collected from 2007 to 2010. Isolates were grouped by species according to the PFGE profiles.

| Bacterial species (no. of strains) | PFGE (no. of strains) | City | Year | Specimen source[a] | MIC/MIC range (mg/L)[b]: IMI | MER | ERT | CAZ | CEF | CRO | AZT | P/T | TIG | AMI | LEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. aerogenes* (1) | NT[c] | Ankara | 2009 | RTI | 8 | 1 | 2 | 0.25 | 0.25 | 0.5 | 0.5 | >64 | 0.25 | 2 | ≤0.5 |
| *E. cloacae* (4) | ECL-A (2) | Ankara | 2010 | BSI | ≥8 | >8 | >8 | >32 | >16 | >8 | >16 | >64 | 0.25-0.5 | 4 | >4 |
| | **ECL-B (2)[d]** | **Ankara** | **2010** | **BSI** | **4-8** | **>8** | **8** | **>32** | **≥16** | **>8** | **>16** | **>64** | **0.25** | **8** | **≤0.5** |
| *E. coli* (14) | EC-A (7) | Ankara | 2009 | BSI | 2 | 0.5-1 | 2-4 | 8-16 | 8->16 | >32 | >16 | >64 | 0.12-0.25 | 4-8 | >4 |
| | EC-B (1) | Ankara | 2009 | BSI | 2 | 0.5 | 2 | 8 | >16 | >32 | >16 | >64 | 0.25 | 8 | >4 |
| | EC-C (1) | Ankara | 2008 | BSI | 2 | 0.5 | 0.5 | 0.25 | ≤0.12 | 0.5 | ≤0.12 | >64 | 0.12 | 2 | ≤0.5 |
| | EC-D (1) | Ankara | 2009 | SSSI | 2 | 0.5 | 1 | 16 | 16 | >32 | >16 | >64 | 0.25 | 2 | >4 |
| | EC-E (1) | Ankara | 2009 | SSSI | 4 | 2 | 4 | 32 | >16 | >32 | >16 | >64 | 0.25 | 4 | >4 |
| | EC-F (2) | Ankara | 2008 | BSI/SSSI | 2-4 | 0.5 | 2 | 0.25-0.5 | 0.5 | 1-2 | ≤0.12-0.25 | >64 | 0.12-0.25 | 4 | ≤0.5 |
| | EC-G (1) | Ankara | 2008 | SSSI | 2 | 0.25 | 0.5 | 0.12 | ≤0.12 | ≤0.25 | ≤0.12 | >64 | 0.06 | 4 | ≤0.5 |
| *K. oxytoca* (3) | KOX-A (3) | Ankara | 2009 | BSI/RTI | 2-8 | 1-2 | 4->8 | ≥32 | >16 | >32 | >16 | >64 | 0.5-1 | 4-8 | 2->4 |
| *K. pneumoniae* (21) | KPN-A (1) | Ankara | 2008 | BSI | >8 | >8 | >8 | 16 | >16 | >32 | >16 | >64 | 1 | 1 | 2 |
| | KPN-B (1) | Ankara | 2009 | BSI | 4 | 1 | 2 | 0.25 | 0.25 | 0.5 | ≤0.12 | >64 | 0.25 | 1 | ≤0.5 |
| | KPN-C (2) | Istanbul | 2009 | BSI | 4 | 2 | 8 | 32 | ≥16 | >32 | >16 | >64 | 2 | 2-4 | >4 |
| | KPN-D (1) | Ankara | 2009 | BSI | 2 | 1 | 8 | 32 | >16 | >32 | >16 | >64 | 4 | 4 | >4 |
| | KPN-E (1) | Ankara | 2010 | BSI | 4 | 1 | 2 | 0.5 | 0.5 | 1 | ≤0.12 | >64 | 0.25 | 2 | ≤0.5 |
| | KPN-F (1) | Genoa | 2010 | BSI | 4 | 2 | 4 | 0.5 | 0.5 | 1 | 0.25 | >64 | 0.25 | 1 | >4 |
| | KPN-G (1) | Ankara | 2008 | BSI | 4 | 1 | 2 | 0.25 | 0.25 | 0.5 | ≤0.12 | >64 | 0.25 | 2 | ≤0.5 |
| | KPN-H (2)[e] | Ankara | 2009 | BSI | 2 | 0.5-1 | 2-8 | ≤0.12-32 | ≤0.12->16 | 0.5->32 | ≤0.12->16 | >64 | 0.5-4 | 1-4 | ≤0.5->4 |
| | KPN-I (3) | Ankara | 2009 | BSI | 2->8 | 1->8 | 4->8 | >32 | >16 | >32 | >16 | >64 | 0.5 | 8 | >4 |
| | KPN-J (2) | Ankara | 2007 | BSI/SSSI | 2-4 | 1 | 1-4 | 0.25-32 | ≤0.12->16 | 0.5->32 | ≤0.12->16 | >64 | 0.12-0.25 | 1-2 | ≤0.5->4 |
| | KPN-K (2) | Ankara | 2009 | BSI | 2-4 | 0.5-2 | 2-8 | 0.25-0.5 | 0.25-0.5 | 0.5-1 | ≤0.12 | >64 | 0.25-2 | 0.5-2 | ≤0.5 |
| | KPN-L (1) | Ankara | 2007 | BSI | 2 | 0.5 | 2 | >32 | >16 | >32 | >16 | >64 | 1 | 4 | 1 |
| | KPN-M (1) | Ankara | 2009 | RTI | 4 | 1 | 8 | >32 | >16 | >32 | >16 | >64 | 0.5 | 8 | >4 |
| | KPN-N (1) | Ankara | 2009 | RTI | 4 | 1 | 8 | >32 | >16 | >32 | >16 | >64 | 0.5 | 8 | >4 |

a. BSI= bloodstream infection; RTI= respiratory tract infection; SSSI= skin and skin-structure infection.
b. IMI= imipenem; MER= meropenem; ERT= ertapenem; CAZ= ceftazidime; CEF= cefepime; CRO= ceftriaxone; AZT= aztreonam; P/T = piperacillin/tazobactam; TIG= tigecycline; AMI- amikacin; LEV= levofloxacin.
c. NT= not tested.
d. Strains in bold also produce IMP-1 metallo-β-lactamase.
e. One strain displaying PFGE profile KPN-H produced a negative MHT.