DETECTION AND SPREAD OF OXA-48-PRODUCING KLEBSIELLA OXYTOCA ISOLATES IN ISTANBUL, TURKEY

Hasan Nazik¹, Selda Aydin², Rüveyda Albayrak³, Esma A Bilgi³, Ismail Yıldız⁴, Nuray Kuvat¹, Fatih M Kelesoglu⁴, Nagehan Pakaşıçali¹, Fadime Yılmaz³ and Betigül Öngen¹

¹Department of Medical Microbiology, Istanbul Medical Faculty; ²Department of Infectious Diseases and Clinical Microbiology, Cerrahpasa Medical Faculty; ³Department of Medical Microbiology, Cerrahpasa Medical Faculty; ⁴Department of Pediatrics, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Abstract. Five OXA-48 producing Klebsiella oxytoca strains isolated in April-July 2010 were analyzed. Antibiotic susceptibility tests were performed using disc diffusion method and VITEK 2 system. Carbapenemase activity was investigated using the Modified Hodge test. Beta-lactamase genes were detected by PCR and blaOXA-48 was sequenced. Genetic relatedness between K. oxytoca isolates was investigated by pulse-field gel electrophoresis (PFGE). Carbapenemase activity was detected in 5 isolates by Modified Hodge test. Although all strains were resistant to ertapenem and imipenem, only one strain was also resistant to meropenem. BlaOXA-48 in 4 isolates harbored 2 or 3 other ESBL types, namely, blaTEM, blaSHV, blaCTX-M, or blaVEB. PFGE revealed 3 different pulso-types among the K. oxytoca isolates. The presence of OXA-48 carbapenemase in other species of clinical isolates should also be considered.

Keywords: Klebsiella oxytoca, beta-lactamase, carbapenem resistance, plasmid, OXA-48 carbapenemase

INTRODUCTION

Emergence and spread of carbapenemase-producing Enterobacteriaceae isolates represent a significant threat to the management of especially nosocomial infections. Carbapenem-hydrolyzing enzymes comprise of molecular classes A, B (metallo-β-lactamases), and D (oxa-

cilinases) (Queenan and Bush, 2007). Ambler class D OXA-48 beta-lactamase was initially identified in Klebsiella pneumoniae from Turkey in 2004 (Poirel et al, 2004), then outbreaks of OXA-48-producing Enterobacteriaceae have been reported in Turkey (Carrer et al, 2008), Tunisia (Mkaouar et al, 2008), Senegal (Moquet et al, 2011), Spain (Sahagun Pareja et al, 2005) and France (Cuzon et al, 2011). Additionally, OXA-48 producers belonging to Enterobacteriaceae have also been reported in several countries, such as Belgium, Lebanon, Egypt and Morocco (Carrer et al, 2008; Cuzon et al, 2008; Benouda et al, 2010; Carrer et al, 2010; Kalpoe et al, 2011).
In this study we describe the detection of _Klebsiella oxytoca_ isolates producing OXA-48 in Turkey.

**MATERIALS AND METHODS**

**Isolates**

Resistant or reduced susceptible isolates to carbapenems were obtained from clinical specimens in Istanbul Medical Faculty, Turkey a 1750 bed tertiary care teaching hospital, from April-July 2010. Isolates were identified by conventional methods and VITEK 2 System (bioMérieux, Marcy l’Etoile, France). Antimicrobial susceptibility tests were performed according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Carbapenemase activity was screened by the Modified Hodge test (CLSI, 2011). OXA-48 producing _Citrobacter freundii_ Lut strain from previous study and _E. coli_ ATCC 25922 were used as the control strains (Nazik _et al_., 2005). Minimal inhibitory concentrations (MICs) of isolates were determined using VITEK 2 System. Ethical approval was not required where only routine laboratory isolates were used. Consent to use isolates that had been provided from patients was acquired retrospectively from the patients, their legal guardians or their next of kin.

**Detection of _bla_**<sub>OXA-48</sub>

DNA extraction was performed as described previously (Mammeri _et al_., 2005; Nazik _et al_., 2009). PCR amplification of _bla_<sub>OXA-48</sub> was carried out using the following set of primers: OXA-48A (5’-TTGCTGGCATCGATTACG-3’) and OXA-48B (5’-GACCATCTTGGTATGCT-3’), producing 743 bp, in a 50 µl final volume containing 10x PCR buffer (5 µl), 2 mM deoxynucleoside triphosphates, 3.5 pmol of each primer, 2.5 mM MgCl₂ (5 µl), 1 U Taq DNA polymerase and 1 µl of genomic DNA of the test strain. PCR was performed in a thermal cycler (TaKaRa Thermal Cycler TP600; Otso, Shiga, Japan) using the following conditions: 94°C for 5 minutes; 35 cycles of 94°C for 60 seconds; 55°C for 45 seconds, and 72°C for 60 seconds; and a final heating at 72°C for 7 minutes (Nazik _et al_., 2005; Aktas _et al_., 2008). The _bla_<sub>TEM</sub>, _bla_<sub>SHV</sub>, _bla_<sub>CTX-M</sub>, _bla_<sub>VIM-1</sub>, _bla_<sub>VIM-2</sub>, _bla_<sub>IMP-1</sub>, _bla_<sub>IMP-2</sub>, _bla_<sub>KPC</sub> were screened by PCR as described previously (Poirel _et al_., 2004; Mammeri _et al_., 2005; Poirel _et al_., 2005; Pallecchi _et al_., 2007; Queenan and Bush, 2007). PCR products were separated by 1.5% agarose gel-electrophoresis, stained with ethidium bromide and visualized under UV light. _φ_174 HaeIII fragments (MBI Fermentas; St Leon-Rot, Germany) were used to assess PCR product size. Amplicons were purified (High-Pure Purification kit, Roche Diagnostics, Castle Hill, Australia) and both strands were sequenced in an Applied Biosystems sequencer (ABI 377; Applied Biosystems, Foster City, CA). Nucleotide and deduced protein sequences were analyzed with software from the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

**PFGE**

Genetic relatedness of the _K. oxytoca_ isolates was determined by PFGE. Following extraction of genomic DNA and digestion with _XbaI_, a CHEF DR2 system (Bio-Rad Laboratories, Hercules, CA) were used for performing PFGE, and the macrorestriction patterns were analyzed using GelCompar II software (Version 6.0; Applied Maths, Sint-Martens-Latem, Belgium). Relatedness was calculated using the unweighted pair group method with mathematical averaging (UPGMA). Cluster designation was determined according to criteria described previously and the strains categorized as indistin-
RESULTS

Three of the five patients were of pediatric age and two were hospitalized in pediatric intensive care unit. Although all patients were treated with beta-lactam antibiotics, only two patients were treated with carbapenems; all patients were cured (Table 1).

Modified Hodge test revealed carbapenemase activity in 5 isolates and $bla_{OXA-48}$ genes were demonstrated by PCR and sequence analysis. In addition to $bla_{OXA-48}$, 4 isolates harbored 2 or 3 other ESBL types, $bla_{TEM}$, $bla_{SHV}$, $bla_{CTX-M}$, or $bla_{VEB}$ (Table 2). PFGE revealed 3 different pulso-types among the *K. oxytoca* isolates (Fig 1). Three strains, two of which were isolated from the same patient hospitalized in pediatric intensive care unit, were clonally related.

Although all strains were resistant to ertapenem and imipenem, only one strain was also resistant to meropenem. Additionally, all 4 isolates were resistant to ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefazolin, and cefuroxime. Resistance to third generation cefalosporins, such as ceftriaxone or ceftazidime, was observed in all isolates. However, 4 isolates remained susceptible to cefepime. In addition to beta-lactam resistance, 4, 3 and 1 isolates were also resistant to gentamicin, Co-trimoxazole and levofloxacin, respectively (Table 2).

DISCUSSION

The global spread of ESBLs, particularly CTX-M enzymes, in clinical isolates of *E. coli* and *K. pneumoniae* is a public health problem due to limitation of the effectiveness of all β-lactams except carbapenems, which are mostly a last resort therapy (Livermore *et al*, 2007; Pitout, 2008; Nazik *et al*, 2011c). In addition to class A and class B carbapenemase, class D carbapenemase, OXA-48 type, might lead significantly to carbapenem resistance in *Enterobacteriaceae* and are involved in outbreaks in various locations and are increasingly reported in sporadic cases worldwide. Following detection of the first isolate of OXA-48-producing isolate from Turkey (Poirel *et al*, 2004), a number of OXA-48 producing isolates were reported occasionally (Nazik *et al*, 2005; Aktaş *et al*, 2008; Gulmez *et al*, 2008). However, two recent reports indicated
Table 1
Clinical features of patients with OXA-48-producing *K. oxytoca* isolates.

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Date of isolation</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Hospitalization unit</th>
<th>Source</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. oxytoca</em> 1</td>
<td>05/26/2010</td>
<td>F</td>
<td>1 m</td>
<td>RDS, PDA</td>
<td>Pediatric ICU</td>
<td>Tracheal aspirate</td>
<td>MEM, CTX</td>
<td>Cured</td>
</tr>
<tr>
<td><em>K. oxytoca</em> 2</td>
<td>07/28/2010</td>
<td>F</td>
<td>1 m</td>
<td>RDS, PDA</td>
<td>Pediatric ICU</td>
<td>Tracheal aspirate</td>
<td>MEM, CTX</td>
<td>Cured</td>
</tr>
<tr>
<td><em>K. oxytoca</em> 3</td>
<td>06/25/2010</td>
<td>M</td>
<td>1 m</td>
<td>Prematurity, RDS</td>
<td>Pediatric ICU</td>
<td>Tracheal aspirate</td>
<td>AMP, CTX</td>
<td>Cured</td>
</tr>
<tr>
<td><em>K. oxytoca</em> 4</td>
<td>07/13/2010</td>
<td>M</td>
<td>7 m</td>
<td>Epispadias</td>
<td>Pediatric surgery</td>
<td>Urine</td>
<td>SAM</td>
<td>Cured</td>
</tr>
<tr>
<td><em>K. oxytoca</em> 5</td>
<td>04/24/2010</td>
<td>M</td>
<td>50 y</td>
<td>DM</td>
<td>Emergency ICU</td>
<td>Urine</td>
<td>IMP</td>
<td>Cured</td>
</tr>
</tbody>
</table>

ICU, intensive care unit; PDA, patent ductus arteriosus; IPM, imipenem; MEM, meropenem; AMP, ampicillin; SAM, ampicillin-sulbactam; CTX, cefotaxime. *Strain 1 and 2 were isolated from the same patient and included in the study due their different colony morphology.

Table 2

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>PFGE</th>
<th>Related bla</th>
<th>βMIC for carbapenem (µg/ml)</th>
<th>Antibiotic resistance pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. oxytoca</em> 1</td>
<td>1</td>
<td>TEM, SHV, VEB</td>
<td>4/4/1</td>
<td>AMP, AMC, TZP, CZ, CXM, CAZ, CRO, GN, SXT</td>
</tr>
<tr>
<td><em>K. oxytoca</em> 2</td>
<td>1</td>
<td>TEM, SHV, VEB</td>
<td>4/8/1</td>
<td>AMP, AMC, TZP, CZ, CXM, CAZ, CRO, GN, SXT</td>
</tr>
<tr>
<td><em>K. oxytoca</em> 3</td>
<td>1</td>
<td>SHV, VEB</td>
<td>2/4/1</td>
<td>AMP, AMC, TZP, CZ, CXM, CAZ, CRO, GN</td>
</tr>
<tr>
<td><em>K. oxytoca</em> 4</td>
<td>2</td>
<td>-</td>
<td>2/4/≤0.25</td>
<td>AMP, AMC, TZP, CZ, CXM, CRO</td>
</tr>
<tr>
<td><em>K. oxytoca</em> 5</td>
<td>3</td>
<td>SHV, CTX-M, VEB</td>
<td>≥8/8/≥16</td>
<td>AMP, AMC, TZP, CZ, CXM, CRO, CRO, FEP, GN, LEV, SXT</td>
</tr>
</tbody>
</table>

ETP, ertapenem; IPM, imipenem; MEM, meropenem; AMC, amoxicillin-clavulanic acid; TZP, Piperacillin-tazobactam; CZ, cefazolin; CXM, cefuroxime; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; LEV, levofloxacin; SXT, Co-trimoxazole; GN, gentamicin; βMIC, range of antibiotics: for ETP: ≤0.25 susceptible, 0.5 intermediate, ≥1 resistant; for IMP and MEM: ≤1 susceptible, 2 intermediate, ≥4 resistant.

that the problem might actually be more serious than has been reported in Turkey. The first report was in 2008, an outbreak including 39 carbapenem-resistant, OXA-48 positive, *K. pneumoniae* strains isolated from mostly adults in intensive care units and emergency surgery in our hospital (Carrer *et al*, 2008). The second report was
in 2010, when 18 carbapenem-resistant, OXA-48-positive enterobacterial isolates were detected in Turkey, Lebanon, Egypt, France and Belgium (Carrer et al, 2010). Recently, we reported 22 multiresistant OXA-48 producing \(K\). \(pneumoniae\) isolates (Nazik et al, 2011a) and 10 multiresistant isolates including 9 \(K\). \(pneumoniae\) and 1 \(E\). \(coli\) isolates (Nazik et al, 2012) from the same hospital. Here, we presented another member of \(Enterobacteriaceae\), \(K\). \(oxytoca\), with carbapenemase activity.

The co-existence of \(bla_{OXA-48}\) together with other ESBL types, such as \(bla_{TEM}\), \(bla_{SHV}\), and \(bla_{CTX-M}\), is a threat to the use of all beta-lactams including carbapenems (Pitout, 2008; Queenan and Bush, 2007). In addition to TEM, SHV and CTX-M types, which are widespread worldwide, another type, beta-lactamases-VEB, has emerged in recent years (Poirel et al, 2005). In this study, in addition to previous beta-lactamases, the VEB type beta-lactamase was detected in 4 \(K\). \(oxytoca\) isolates. In our previous studies, \(Citrobacter freundii\) and \(K\). \(pneumoniae\) isolates producing \(bla_{OXA-48}\) and \(bla_{VEB}\) have been reported from same hospital in Istanbul (Nazik et al, 2005). This finding showed that VEB type beta-lactamase persists not only in \(C\). \(freundii\) and \(K\). \(pneumoniae\) but also in \(K\). \(oxytoca\) isolates in Turkey.

Moreover, the present study demonstrated that 3 strains isolated from pediatric intensive care unit were clonally related, but 2 distinct clones were also detected in different units. Thus the dissemination of \(bla_{OXA-48}\) was not spread by a single \(K\). \(oxytoca\) clone as shown previously for \(K\). \(pneumoniae\) isolates (Carrer et al, 2008, 2010; Nazik et al, 2011b), but that several OXA-48-producing clones were distributed in our hospital in Istanbul. The spread of \(K\). \(oxytoca\) isolates harboring \(bla_{OXA-48}\) but also ESBLs, such as TEM, SHV, CTX-M, and now VEB-1 type, may be a serious problem for treatment. Another concern is the difficulty to detecting these isolates by clinical laboratories as they may appear susceptible to carbapenems especially imipenem and meropenem according to the current CLSI breakpoints. Considerable but necessary efforts will have to be instituted in order to detect \(bla_{OXA-48}\) in enterobacteriaceal clinical isolates.

ACKNOWLEDGEMENTS

This work was presented as a poster at the 4th Eurasia Congress of Infectious Diseases, Sarajevo-Bosnia and Herzegovina, 1-5 June 2011. We thank Baris Otlu for technical assistance in preparing PFGE.

REFERENCES


Nazik H, Öngen B, Yildirim EM, Ermiş F. High prevalence of CTX-M type beta-lactamase in *E. coli* isolates producing extended spectrum beta-lactamase (ESBL) and displaying antibiotic co-resistance. *Afr J Microbiol Res* 2011c; (5); 1.


