Infections caused by OXA-48-producing Klebsiella pneumoniae in a tertiary hospital in Spain in the setting of a prolonged, hospital-wide outbreak

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Objectives: We describe clinical and microbiological features of infections caused by OXA-48-producing Klebsiella pneumoniae (O48KP) in the setting of a prolonged, hospital-wide outbreak detected in January 2011.

Methods: Clinical, demographic and microbiological data of patients with growth of O48KP in clinical specimens were collected until December 2011. PCR was used to detect carbapenemase and β-lactamase genes. The genetic relationships were determined by automated repetitive-sequence-based PCR.

Results: Seventy-one patients with clinically guided cultures showing growth of O48KP were identified. Nine were considered to be colonizing rather than causing infection. The most frequent source of infection was the urinary tract (22/62), followed by surgical site infections (17/62). Blood cultures were positive in 23/62 patients. Many patients had significant comorbidity and prolonged hospital stays. In-hospital mortality among patients with O48KP infections was 43.5%. The MIC90s of ertapenem, imipenem and meropenem were 32, 16 and 16 mg/L, respectively. No single antimicrobial was active against all the isolates. The antibiotics most active against O48KP were amikacin (97.2% susceptible), colistin (90.1%), tigecycline (73%) and fosfomycin (66.2%). Although eight clones were identified, a predominant clone caused 73.2% of the infections. Multilocus sequence typing (MLST) of the predominant clone gave sequence type (ST) 405 and blaTEM-1, blaSHV-76, blaCTX-M-15 and blaOXA-1 genes and the insertion sequence IS1999 of the Tn1999 transposon were associated with blaOXA-48 in this clone.

Conclusions: To our knowledge, this is the largest reported series of infections caused by O48KP in the setting of a single-centre outbreak and provides further input on the clinical relevance of infections caused by O48KP and the difficulties associated with its detection and control.

Keywords: carbapenemases, nosocomial, hospital spread

Introduction

The spread of carbapenem-resistant Enterobacteriaceae (CRE) constitutes a significant threat to healthcare systems.1 –3 In the setting of a dry antimicrobial pipeline, carbapenems remain the last frontier of optimal antimicrobial therapy for a significant and growing proportion of patients with severe infections caused by Enterobacteriaceae. For many patients infected with CRE, second-choice antimicrobials, or combinations thereof, offer lower probabilities of clinical cure, which in many instances directly translates into death.4 –6

First described in a clinical isolate of Klebsiella pneumoniae from a patient hospitalized in Turkey in 2001, OXA-48 is a carbapenem-hydrolysing oxacillinase with strong carbapenem-hydrolysing activity.7 Since then, OXA-48-producing Enterobacteriaceae have been isolated in several countries in northern Africa,8,9 the Middle East10–12 and Europe.13–16 OXA-48-producing Enterobacteriaceae have been involved in nosocomial outbreaks.14–16
The aim of this report is to describe infections caused by OXA-48-producing K. pneumoniae (O48KP) in the setting of a hospital-wide outbreak in a single tertiary Spanish hospital between April 2010 (isolation date of the first detected O48KP) and December 2011, following the recommendations of the ORION statement.17

Methods

Setting

Hospital Universitario La Paz (HULP) is a 1328 bed tertiary academic centre belonging to the Spanish National Public Health Service, which provides medical assistance to a mixed urban and rural population of around 600000 people in Madrid, Spain, with ~48000 hospital admissions per year. Most of the rooms are double occupancy. Infection surveillance and control is conducted by the Division of Preventive Medicine. Surveillance cultures are performed weekly for all patients hospitalized in intensive care units and in patients known to be infected and/or colonized with multidrug-resistant organisms in order to define duration of contact precautions. The Microbiology and Preventive Medicine departments share daily data about targeted multidrug-resistant isolates.

Definition and extent of the outbreak

In December 2010 four isolates of K. pneumoniae were obtained that showed an extended-spectrum β-lactamase (ESBL) phenotype accompanied with high resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam combinations, variable decreased susceptibility to carbapenems, and resistance to fluoroquinolones, co-trimoxazole and aminoglycosides (gentamicin and tobramycin). Carbapenemase production was suggested because of the positive modified Hodge test (MHT). Metallo-b-lactamases were discarded if carbapenemase activity was not inhibited by EDTA. PCR analyses detected blaOXA-48, which was confirmed by sequencing and PCR detection of the associated insertion sequence IS1999. Active surveillance for similar phenotypes was started then, since carbapenem resistance was found to be variable and sometimes difficult to detect. In addition, records of the Microbiology laboratory were reviewed and all available frozen isolates of phenotypically similar K. pneumoniae were evaluated, searching for carbapenemase production and the presence of blaOXA-48. Three isolates could be detected retrospectively dating back to May 2010. In January 2011 an interim epidemiological study was conducted. For epidemiological purposes, a case was defined as any inpatient attending at a haemodialysis clinic, receipt of intravenous medication or home wound care in the previous 30 days, and residence in a nursing home or long-term care facility.

Bacterial isolates and determination of ESBL and carbapenemase production

Antibiotic susceptibility was determined using the Wider® (Fco. Soria Melguizo, Madrid, Spain) or Vitek® (bioMérieux, Marcy l’Etoile, France) systems. Isolates were categorized as susceptible or resistant to all the antibiotics tested according to the guidelines of the CLSI. Antibiotic MICs were evaluated according to the interpretative criteria of the FDA. Both the Vitek and Wider automated systems are capable of ESBL detection tests. Nevertheless, ESBL production was confirmed later by Etest ESBL strips (bioMérieux). Carbapenem MICs were confirmed by Etest (bioMérieux).

To rule out carbapenemase production, an MHT was performed on all Enterobacteriaceae isolates retrieved from clinical cultures having an MIC ≥1 mg/L to any carbapenem. The inhibition tests with boronic acid and EDTA were used to screen for the production of class A and class B carbapenemases. Those isolates of K. pneumoniae with a carbapenem MIC within the normal range and an ESBL phenotype, but resistant to β-lactamase inhibitors, were retrospectively studied (Etest and MHT). A PCR-confirmed, VIM-producing K. pneumoniae was used as the control for the MHT. An O48KP isolate confirmed by PCR and gene sequencing was also included as a positive control.

Characterization of blatOXA-48 by PCR and DNA sequencing

All the isolates in which carbapenemase production had been confirmed by the MHT were further characterized by molecular methods. PCR was used for the identification of the carbapenemase genes blatOXA, blatVIM, blatIMP, blatNDM-1 and blatOXA-48 and the β-lactamase-encoding genes blatTEM, blatSHV, blatCTX-M and blatOXA-1. Positive controls confirmed by sequencing were included in all the PCR assays. PCR amplification products were sequenced using the dyeoxynucleotide chain termination method. The genetic environment of the blatOXA-48 gene was determined by PCR using specific primers for IS1999 of the Tn1999 transposon.
Molecular typing

The genetic relationships between the isolates were determined by automated repetitive-sequence-based PCR using the DiversiLab™ (bioMérieux) system. Multilocus sequence typing (MLST) based on the gapA, infB, mdh, pgI, phoE, rpoB and tonB genes was performed on a representative of the major clone.
**Ethics**

The Institutional Review Board at HULP evaluated and approved the study protocol (PI-1289).

**Results**

**Outbreak extent and epidemiological and demographical features**

From April 2010 to December 2011, 71 patients were identified to have O48KP in clinically guided cultures. The peak incidence occurred in March 2011 (12 cases), as shown in the epidemic curve (Figure 1). Then the number of incident cases decreased. To date, O48KP-infected/-colonized patients continue to be identified. The distribution of cases within the hospital is shown in Figures 1 and 2. Only patients with clinically guided cultures are included in this report. Forty patients (56.3%) were men and the median age was 74 years (range 16–96 years) and the median non-age-adjusted Charlson’s score was 4 (range 0–10) (Table 1). Fifty-nine patients (83.1%) had received systemic antimicrobials in the previous 2 months. The median total antimicrobial defined daily doses (DDDs) received prior to isolation of O48KP was 25 (range 0.6–187). Regarding carbapenem use, 30 microbiological defined daily doses (DDDs) received prior to isolation of O48KP was 25 (range 0.6–187). Regarding carbapenem use, 30 DDDs of carbapenem were documented prior to the detection of O48KP. The median DDD of carbapenem prior to O48KP detection was 8.5 (range 1–46.5).

**Clinical features**

Fifty-one (71.83%) of the O48KP infections were nosocomially acquired. Twenty patients had HCA O48KP, all of them having had prior recent hospitalization at our institution, with the exception of a patient with an HCA urinary tract infection diagnosed in July 2011 who had had a prolonged hospitalization in Melilla (northern Africa).

**Table 1. Epidemiological and clinical data of patients with O48KP in clinically driven cultures**

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Infected</th>
<th>Colonized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>74 (16–96)</td>
<td>66 (58–83)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>40 (56.3)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>Charlson’s score, median (range)</td>
<td>4 (0–10)</td>
<td>4 (0–6)</td>
</tr>
<tr>
<td>Nosocomial, n (%)</td>
<td>44 (71)</td>
<td>7 (77.8)</td>
</tr>
<tr>
<td>LOS (days) prior to O48KP isolation (nosocomial), n (range)</td>
<td>33 (2–167)</td>
<td>26 (3–61)</td>
</tr>
<tr>
<td>Source of infection, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>22 (35.5)</td>
<td></td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>17 (27.4)</td>
<td></td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>9 (14.5)</td>
<td></td>
</tr>
<tr>
<td>Intrabdominal infection</td>
<td>4 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Intravenous catheter-related infection</td>
<td>3 (4.8)</td>
<td></td>
</tr>
<tr>
<td>Primary BSI</td>
<td>4 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Total BSI</td>
<td>23 (37.1)</td>
<td></td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septic shock/severe sepsis at presentation, n (%)</td>
<td>20 (32.8)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Pitt score at presentation, median (range)</td>
<td></td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>Outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death during hospitalization, n (%)</td>
<td>27 (43.5)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>LOS (days) after O48KP isolation, median (range)</td>
<td>15 (0–140)</td>
<td>12 (0–103)</td>
</tr>
</tbody>
</table>

**Table 2. Susceptibilities of O48KP isolates to several antimicrobials**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC range (mg/L)</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/L)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≤8–16</td>
<td>≤8</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ceftazidime/ clavulanate</td>
<td>≤1/4–&gt;8/4</td>
<td>≤1/4</td>
<td>&gt;8/4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≤1–16</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ceftazidime/ clavulanate</td>
<td>≤1/4–&gt;8/4</td>
<td>≤1/4</td>
<td>&gt;8/4</td>
</tr>
<tr>
<td>Ceftazidime/ clavulanate</td>
<td>≤1–16</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8–&gt;8</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>≤4–&gt;8</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≤4–&gt;16</td>
<td>≤4</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Colistina&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25–16</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤0.5–&gt;8</td>
<td>1</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>≤16–&gt;128</td>
<td>≤16</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

<sup>a</sup>Etest results.
Hospital-wide OXA-48 Klebsiella pneumoniae outbreak

Microbiology results

One-hundred-and-seventy-three O48KP were isolated from the 71 patients. The majority of the isolates had a phenotype consistent with ESBL production activity with susceptibility to cefoxitin and resistance to cefotaxime, ceftazidime, cefepime and aztreonam, but with associated high-level resistance to amoxicillin/clavulanate and piperacillin/tazobactam. Only four isolates recovered from a single patient were non-ESBL producers. The MICs of several antimicrobials are shown in Table 2. The isolates were resistant to all antibiotics tested except amikacin (97.2% of the patients with susceptible strains), colistin (90.1%), tigecycline (73%) and fosfomycin (66.2%). Five patients were infected with strains also resistant to colistin and tigecycline (n = 2), colistin and fosfomycin (n = 1), tigecycline and fosfomycin (n = 1), and amikacin and fosfomycin (n = 1), respectively. Three patients were infected with O48KP susceptible only to amikacin. One patient was co-infected with both OXA-48-producing K. pneumoniae and Enterobacter cloacae. The E. cloacae isolated also displayed a derepressed AmpC phenotype and was susceptible to all aminoglycosides, cefepime, quinolones, co-trimoxazole, colistin and fosfomycin, the MICs for ertapenem, meropenem and imipenem were 4, 2 and 1 mg/L, respectively. In addition to O48KP, during the study period six patients had non-K. pneumoniae OXA-48-producing Enterobacteriaceae (five Escherichia coli and one E. cloacae) isolated in clinical samples, four of which (E. coli) were retrieved from blood cultures.

One isolate of the predominant clone was further tested for the presence of bla genes. The DNA amplification and subsequent sequencing of bla genes showed the presence of blaTEM-1, blaSHV-76, blaCTXM-15 and blaOXA-1 genes, as well as IS1999 of the Tn1999 transposon. The analysis of 58 O48KP isolates from 56 patients by automated repetitive-sequence-based PCR revealed eight different clones with 41 strains belonging to the major clone (Figure 3). The temporal distribution of clones is shown in Figure 4. MLST of the O48KP predominant clone gave the allelic profile 2-1-62-3-10-4-110, which has been defined as sequence type (ST) 405.

Discussion

In this article, we describe a large, widespread cluster of infections caused by O48KP in a Spanish tertiary hospital. To the best of our knowledge this is the largest outbreak that has
been described so far. This outbreak occurred in the epidemiological setting of high rates of ESBL-producing *K. pneumoniae* (~20%, data not shown) and low-level sustained detection of VIM metallo-β-lactamase-producing microorganisms in clinical and surveillance samples at HULP since 2006. So far a single, small outbreak of KPC-producing *Citrobacter freundii* has been detected at our institution.

The most frequent source of infections caused by O48KP is the urinary tract, followed by surgical site infections, with a significant proportion of these patients having BSIs. As with other infections caused by CRE, the observed mortality during hospitalization was high, nearly 50%, being expectedly higher, roughly 70%, among the 23 patients in which BSI was documented. Nevertheless, it is quite difficult to appropriately estimate the attributable mortality since an elevated proportion of patients had significant comorbidity and an otherwise protracted and eventful hospitalization course.

A significant number of the O48KP isolates in the outbreak described here showed a low-level resistance to carbapenems in the routine automated susceptibility testing systems. It should be noted that most of the isolates were associated with ESBL and OXA-1 production. For these reasons, it is important to consider the possibility of OXA-48 production in Enterobacteriaceae showing an ESBL phenotype and high-level resistance to β-lactam plus β-lactamase inhibitor combinations. Among carbapenems, ertapenem MIC was the most sensitive indicator of OXA-48 activity, supporting the value of ertapenem in the routine phenotypic detection of carbapenemase production.

Although this study was focused on O48KP isolated in clinical specimens, it can also provide some input from an epidemiological standpoint. First, while five of the eight identified clones were present in only one patient each, a single clone (clone 1) accounted for more than 70% of O48KP infections and clearly prevailed. This should bring into consideration the variable epidemic fitness of different clones and its relevance in outbreak extent and control. This major clone was ST405, originally identified in an isolate from Casablanca, Morocco. This had the same profile of β-lactamase genes (*bla*TEM-1, *bla*SHV-76, *bla*CTX-M-15 and *bla*OXA-1) except for *bla*OXA-48, which was not present. A small ST405 O48KP outbreak has been described in Belgium. We believe that this was the original clone of this outbreak and the source of *bla*OXA-48 that was subsequently transferred to other clones. The high transmissibility of the mobile genetic element encoding *bla*OXA-48 has been suggested by several authors and poses an increased potential of spread as compared with other carbapenemases.

Interestingly, neither paediatric nor cases among patients hospitalized in the Obstetrics and Gynaecology wards were identified during the study period. The geographically scattered pattern of distribution in combination with the preservation of certain areas of the hospital, which had specific patient-flow circuits, suggests that infected/colonized patients might be acting as reservoirs from which horizontal transmission occurred. In addition, it should be noted that in many cases the onset of infection happened once the patient left the hospital, as in the case of HCA infections. These patients, unknowingly colonized at discharge, constituted a pool of O48KP-colonized individuals, which could have potentially contributed to perpetuate the outbreak, mainly in the setting of recurrent hospitalizations. Nevertheless, this remains hypothetical since no extensive

![Figure 4. Distribution of clones during the study period. Bars represent the number of patients with identification of O48KP in clinical specimens. Black, grey and white represent clones 1, 2 and 4, respectively. Arrows indicate the distribution of sporadic unique clones.](https://academic.oup.com/jac/article-abstract/68/1/89/672919)
environmental data are available to exclude other potential reservoirs. In spite of the duration and the widespread distribution of the cases, we believe that this set of cases represents a cluster of outbreaks associated with an outbreak of a major high-risk clone rather than an endemic situation, encouraging current efforts to intensify surveillance and strict compliance with standard and contact precautions.

In summary, this large and widespread cluster of infections is an example of the potential harm that this emerging and highly transmissible mechanism of resistance can cause in a healthcare institution. The clinical presentations of these infections, which were associated with significantly high in-hospital mortality, pose an important burden at a hospital level. Active surveillance of OXA-48-producing Enterobacteriaceae in healthcare institutions is as relevant as the reinforcement of contact precautions.

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Transparency declarations
None to declare.

References

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