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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 MIC_{90}s 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 bla_{TEM-1}, bla_{SHV-76}, $bla_{CTX-M-15}$ and bla_{OXA-1} genes and the insertion sequence IS1999 of the Tn1999 transposon were associated with bla_{OXA-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 East[10–12] 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-β-lactamases were discarded if carbapenemase activity was not inhibited by EDTA. PCR analyses detected *bla*$_{OXA-48}$, which was confirmed by sequencing and PCR detection of the associated insertion sequence IS*1999*. 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 *bla*$_{OXA-48}$. 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 with culture-proven infection or colonization by O48KP. On 8 February 2011 a total of 15 cases had been identified.

Patients known to be infected/colonized by O48KP were transferred to single rooms, contact precautions were reinforced and surveillance cultures were done.[18] When single rooms were not available these patients were cohorted in shared rooms. Readmission of patients with previously known O48KP infection/colonization was automatically detected through an automated alert system in order to expedite the initiation of contact precautions.[19,20] Environmental cleaning practices were reviewed and twice-daily cleaning in rooms occupied by patients infected/colonized with O48KP was enforced. Universal precautions, mainly hand hygiene practices, were globally encouraged through a proactive campaign in which the healthcare staff was informed of the outbreak and the implemented measures. In order to optimize the clinical management of patients with O48KP infections, the attending physician in charge of the patient received assistance from the Infectious Diseases consult service, which was systematically informed of all CRE isolates detected in clinical cultures. In October 2011 the local antimicrobial stewardship programme began to assess all non-ICU carbapenem prescriptions on day 3 as well as all antimicrobial non-ICU prescriptions on day 7. If prescriptions were found to be optimizable according to hospital antimicrobial protocols, prescribing advice was given to the clinician in charge.

Inclusion criteria and clinical and demographic data

Only patients with O48KP isolated in clinically driven cultures were included. Demographic characteristics, comorbidity, duration and allocation during present and previous recent hospitalizations, clinical presentation, source of infection, exposure to antimicrobials, and clinical outcomes of patients with infections caused by O48KP were retrieved from the medical record by the investigators. Allocations within the hospital were retrospectively grouped in six different areas considering major patient flow circuits: (i) Cardiology–Heart Surgery–Nephrology; (ii) Department of Surgery; (iii) Department of Medicine (including the Medical Intensive Care Unit); (iv) Haematology; (v) Department of Traumatology, Orthopaedic and Plastic Surgery; and (vi) Cantoblanco Hospital, which is an affiliated hospital 15 km from HULP, with 160 beds, including a specific isolation unit with 16 beds. Investigators considered clinical specimens to represent infection or colonization based on definitions outlined by the CDC.[21] Infections were primarily classified as nosocomial or community acquired, in accordance with the classic CDC criteria.[22] Episodes of community-acquired infections were further classified as healthcare-associated (HCA) if any of the following criteria were present:[23] >48 h hospitalization in an acute care hospital during the previous 90 days, attendance 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'Étoile, France) systems. Isolates were categorized as susceptible or resistant to all the antibiotics tested according to the guidelines of the CLSI.[24] Tigecycline MICs were evaluated according to the interpretative criteria of the FDA.[25] 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.[26,27] 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 bla$_{OXA-48}$ 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 *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{NDM-1}$ and *bla*$_{OXA-48}$ and the β-lactamase-encoding genes *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{OXA-1}$.[28–30] Positive controls confirmed by sequencing were included in all the PCR assays. PCR amplification products were sequenced using the dideoxynucleotide chain termination method. The genetic environment of the *bla*$_{OXA-48}$ gene was determined by PCR using specific primers for IS*1999* of the Tn*1999* transposon.[31]
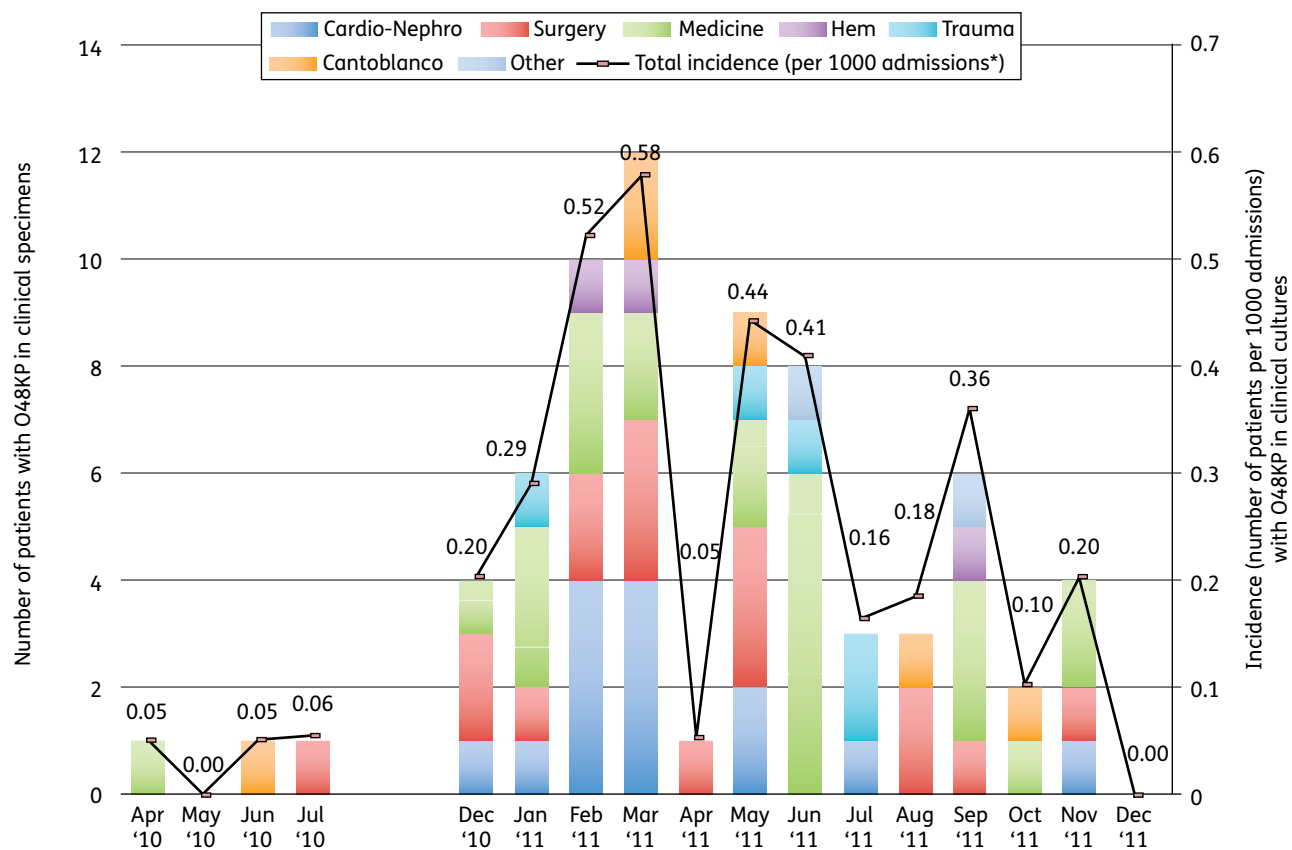
Figure 1. Epidemic curve of infections caused by O48KP between April 2010 and December 2011. Nephro, Nephrology; Hem, Haematology. *Children's and Maternal Hospital admissions have not been considered since no OXA-48 infections were identified in these areas during the study period. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

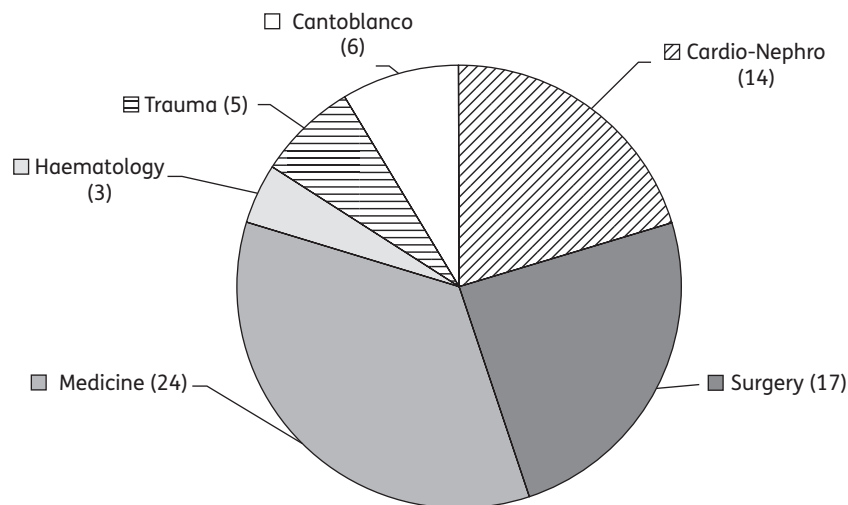**Figure 2.** Distribution of cases within the hospital. Nephro, Nephrology.

Molecular typing

The genetic relationships between the isolates were determined by automated repetitive-sequence-based PCR using the DiversiLab® (bioMérieux) system.[32] 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.[33]

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 patients (42.25%) received at least one dose of any carbapenem prior to the detection of O48KP. The median DDD of carbapenem prior to O48KP detection was 8.5 (range 1–46.5).

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).
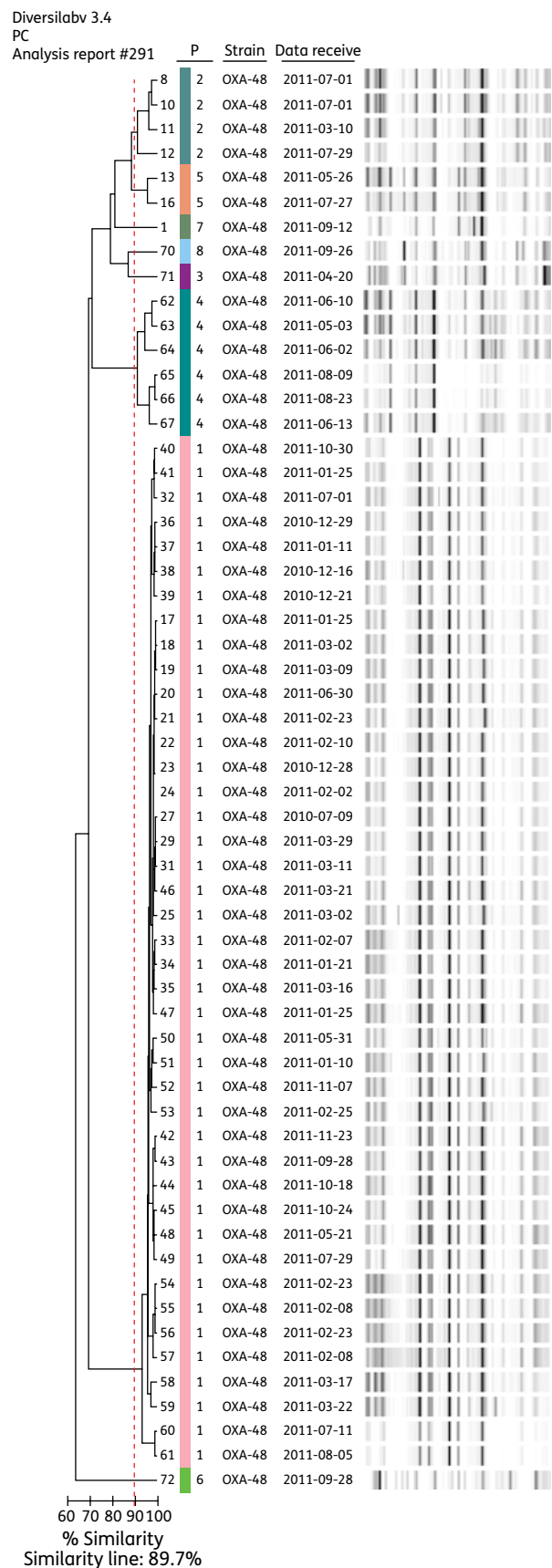
Clinical features

In nine patients (12.7%), O48KP was considered not to be causing a clinical infection. Three of them had asymptomatic bacteriuria. The rest of them had positive skin swabs (decubitus ulcers and wounds) in the absence of clear signs of infection. Two of the nine patients developed serious infections caused by O48KP afterwards.

Among those patients with nosocomial infections, the median time from admission to documented O48KP infection was 33 days (range 2–167 days). Five patients with nosocomial infections became infected within the first week of hospitalization. The most frequent source of infection was urine (22/62 patients), followed by surgical site infections (17/62), of which three were intra-abdominal organ/space infections. The respiratory tract was the source of infection in nine patients, while four patients had intra-abdominal infections, three had a catheter-related infection and four had primary bacteraemia. Twenty-three (37.1%) patients had an O48KP bloodstream

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

	Infected	Colonized
Patients (*n*)	62	9
Age (years), median (range)	74 (16–96)	66 (58–83)
Male, *n* (%)	40 (56.3)	3 (33.3)
Charlson's score, median (range)	4 (0–10)	4 (0–6)
Nosocomial, *n* (%)	44 (71)	7 (77.8)
LOS (days) prior to O48KP isolation (nosocomial), *n* (range)	33 (2–167)	26 (3–61)
Source of infection, *n* (%)		
urinary tract infection	22 (35.5)	
surgical site infection	17 (27.4)	
respiratory tract infection	9 (14.5)	
Intra-abdominal infection	4 (6.5)	
intravenous catheter-related infection	3 (4.8)	
primary BSI	4 (6.5)	
total BSI	23 (37.1)	
Clinical presentation		
septic shock/severe sepsis at presentation, *n* (%)	20 (32.8)	1 (11.1)
Pitt score at presentation, median (range)	1 (0–11)	0 (0–1)
Outcomes		
death during hospitalization, *n* (%)	27 (43.5)	2 (22.2)
LOS (days) after O48KP isolation, median (range)	15 (0–140)	12 (0–103)

LOS, length of hospital stay.

Table 2. Susceptibilities of O48KP isolates to several antimicrobials

	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Amoxicillin	>32	>32	>32
Amoxicillin/clavulanate	>16/8	>16/8	>16/8
Piperacillin	>64	>64	>64
Piperacillin/tazobactam	>64/4	>64/4	>64/4
Cefoxitin	≤8->16	≤8	>16
Cefotaxime	≤1->8	>8	>8
Cefotaxime/clavulanate	≤1/4->8/4	≤1/4	>8/4
Ceftazidime	≤1->16	>16	>16
Ceftazidime/clavulanate	≤1/4->8/4	≤1/4	>8/4
Cefepime	≤1->8	>8	>8
Aztreonam	≤1->8	>8	>8
Ertapenem[a]	0.5->32	8	> 32
Imipenem[a]	1->32	4	16
Meropenem[a]	0.25->32	2	16
Ciprofloxacin	1->4	>4	>4
Trimethoprim/sulfamethoxazole	≤2/38->4/76	>4/76	>4/76
Gentamicin	8->8	>8	>8
Tobramycin	≤4->8	>8	>8
Amikacin	≤4->16	≤4	16
Colistin[a]	0.25–16	0.5	2
Tigecycline	≤0.5->8	1	>8
Fosfomycin	≤16->128	≤16	>128

[a]Etest results.

Diversilabv 3.4
PC
Analysis report #291

	P	Strain	Data receive
8	2	OXA-48	2011-07-01
10	2	OXA-48	2011-07-01
11	2	OXA-48	2011-03-10
12	2	OXA-48	2011-07-29
13	5	OXA-48	2011-05-26
16	5	OXA-48	2011-07-27
1	7	OXA-48	2011-09-12
70	8	OXA-48	2011-09-26
71	3	OXA-48	2011-04-20
62	4	OXA-48	2011-06-10
63	4	OXA-48	2011-05-03
64	4	OXA-48	2011-06-02
65	4	OXA-48	2011-08-09
66	4	OXA-48	2011-08-23
67	4	OXA-48	2011-06-13
40	1	OXA-48	2011-10-30
41	1	OXA-48	2011-01-25
32	1	OXA-48	2011-07-01
36	1	OXA-48	2010-12-29
37	1	OXA-48	2011-01-11
38	1	OXA-48	2010-12-16
39	1	OXA-48	2010-12-21
17	1	OXA-48	2011-01-25
18	1	OXA-48	2011-03-02
19	1	OXA-48	2011-03-09
20	1	OXA-48	2011-06-30
21	1	OXA-48	2011-02-23
22	1	OXA-48	2011-02-10
23	1	OXA-48	2010-12-28
24	1	OXA-48	2011-02-02
27	1	OXA-48	2010-07-09
29	1	OXA-48	2011-03-29
31	1	OXA-48	2011-03-11
46	1	OXA-48	2011-03-21
25	1	OXA-48	2011-03-02
33	1	OXA-48	2011-02-07
34	1	OXA-48	2011-01-21
35	1	OXA-48	2011-03-16
47	1	OXA-48	2011-01-25
50	1	OXA-48	2011-05-31
51	1	OXA-48	2011-01-10
52	1	OXA-48	2011-11-07
53	1	OXA-48	2011-02-25
42	1	OXA-48	2011-11-23
43	1	OXA-48	2011-09-28
44	1	OXA-48	2011-10-18
45	1	OXA-48	2011-10-24
48	1	OXA-48	2011-05-21
49	1	OXA-48	2011-07-29
54	1	OXA-48	2011-02-23
55	1	OXA-48	2011-02-08
56	1	OXA-48	2011-02-23
57	1	OXA-48	2011-02-08
58	1	OXA-48	2011-03-17
59	1	OXA-48	2011-03-22
60	1	OXA-48	2011-07-11
61	1	OXA-48	2011-08-05
72	6	OXA-48	2011-09-28

60 70 80 90 100
% Similarity
Similarity line: 89.7%

infection (BSI). The median Pitt's score among patients with infections caused by O48KP was 1 (range 0–11). Twenty-seven patients (43.5%) with infections caused by O48KP died during hospitalization. Among patients with O48KP BSIs, 16 died (69.5%). The median time from hospital admission to death was 13 days (range 0–140 days). The total number of hospital stays of patients with infections caused by O48KP since the isolation of the pathogen was 1776.

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 MIC_{50}s and MIC_{90}s 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 bla_{TEM-1}, bla_{SHV-76}, $bla_{CTX-M-15}$ and bla_{OXA-1} genes, as well as IS*1999* of the Tn*1999* 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

Figure 3. Clonal relationship of 56 isolates performed using Diversilab®. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.
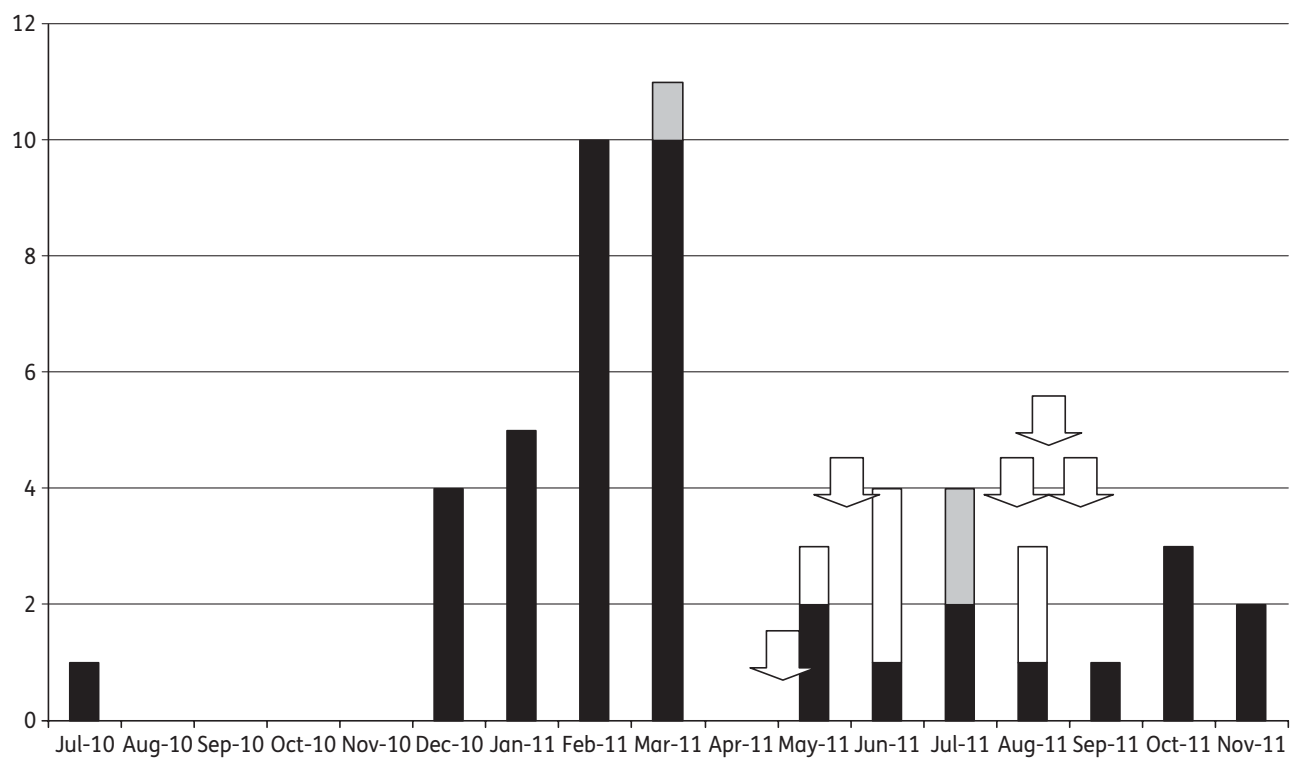
Figure 4. Distribution of clones during the study period. Bars represent the number of patients with identification of O48KP in clinical specimens. Black, grey and white represent clones 1, 2 and 4, respectively. Arrows indicate the distribution of sporadic unique clones.

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.[34] So far a single, small outbreak of KPC-producing *Citrobacter freundii* has been detected at our institution.[35]

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%,[36] 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.[37] 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.[16] 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.[16,38,39]

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

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[40] 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.

Transparency declarations

None to declare.

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