Bacteraemia due to OXA-48-carbapenemase-producing Enterobacteriaceae: a major clinical challenge

C. Navarro-San Francisco[1], M. Mora-Rillo[1], M. P. Romero-Gómez[2], F. Moreno-Ramos[3], A. Rico-Nieto[1], G. Ruiz-Carrascoso[2], R. Gómez-Gil[2], J. R. Arribas-López[1], J. Mingorance[2] and J. R. Paño-Pardo[1]

1) *Infectious Diseases and Clinical Microbiology Unit of the Internal Medicine and Microbiology Services, Hospital Universitario La Paz-IDIPAZ,* 2) *Microbiology Service, Hospital Universitario La Paz-IDIPAZ, and* 3) *Pharmacy Service, Hospital Universitario La Paz-IDIPAZ, Madrid, Spain*

Abstract

Bacteraemia due to carbapenemase-producing Enterobacteriaceae is an emerging medical problem. Management of this entity is complicated by the difficulty in identifying resistance patterns and the limited therapeutic options. A cohort study was performed including all episodes of bloodstream infection due to OXA-48-producing Enterobacteriaceae (O48PE), occurring between July 2010 and April 2012. Data on predisposing factors, clinical presentation, therapy and outcome were collected from medical records. There were 40 cases of bacteraemia caused by O48PE, 35 *Klebsiella pneumoniae* and five *Escherichia coli*. Patients were elderly with significant comorbidities (57.5% underlying malignancy). Thirty-five cases (87.5%) were nosocomial, and five (12.5%) were healthcare-associated. Patients had frequently been exposed to antibiotics and to invasive procedures during hospitalization. The most common source of bacteraemia was the urinary tract followed by deep intra-abdominal surgical site infection. Clinical presentation was severe sepsis or shock in 18 cases (45%). Extended-spectrum β-lactamase production was detected in 92.5% of isolates. MIC_{90} for ertapenem, imipenem and meropenem were 32, 16 and 16 mg/L, respectively. Most frequently preserved antibiotics were amikacin, colistin, tigecycline and fosfomycin. These antibiotics combined are the basis of targeted therapies, including carbapenem in selected cases. Median delay in starting clinically adequate and microbiologically appropriate treatment was 3 days. Crude mortality during admission and within 30 days from bacteraemia was 65% and 50%, respectively. Bloodstream infections caused by O48PE have a poor prognosis. Delay in diagnosis and in initiation of optimal antimicrobial therapy is frequent. Suspicion and rapid identification could contribute to improving outcomes.

Keywords: Bloodstream infection, carbapenemase, OXA-48
Original Submission: 16 August 2012; **Revised Submission:** 26 October 2012; **Accepted:** 26 October 2012
Editor: F. Allerberger
Article published online: 12 December 2012
Clin Microbiol Infect 2012; **19:** E72–E79
10.1111/1469-0691.12091

Corresponding author: C. Navarro-San Francisco, Unidad de Enfermedades Infecciosas y Microbiología Clínica, Servicio de Medicina Interna, Hospital Universitario La Paz-IDIPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain
E-mail: cnavarros.hulp@salud.madrid.org

Introduction

The emergence of carbapenem resistance among Enterobacteriaceae has been increasingly reported and is now a matter of major clinical concern [1–3]. OXA-48, a carbapenem-hydrolysing class D β-lactamase is one of the several carbapenemases that have been described so far [1,4]. This carbapenemase is encoded by the bla_{OXA-48} gene, which is part of the Tn*1999* composite transposon made of two copies of the insertion sequence IS*1999* [5].

OXA-48-producing isolates are frequently multidrug-resistant, because they combine multiple resistance mechanisms. This enzyme shows different hydrolysing activities against β-lactam antibiotics, with high activity against penicillins but only low activity against carbapenems. OXA-48 carbapenemase has very weak activity against third-generation and fourth-generation cephalosporins; however, these are seldom a therapeutic option because other β-lactamases, such as extended-spectrum β-lactamases (ESBL), are frequently associated [1].

The high prevalence of infections caused by OXA-48 producers in Turkey is well established [5,6], as it was first identified in a *Klebsiella pneumoniae* clinical isolate in 2001 [7]. From the Middle East (Turkey and Lebanon) and Northern

Africa, OXA-48 is spreading into Europe (France, Belgium, Germany, Spain) and elsewhere [1]. All previously described series of O48PE infections were found in hospital settings, and most of them were involved in nosocomial outbreaks [8,9].

The aim of this report is to describe the epidemiological, microbiological and clinical features of adult patients with OXA-48-producing Enterobacteriaceae (O48PE) bloodstream infections (BSI) occurring in the setting of a large outbreak in a Spanish Hospital.

Patients and Methods

Setting and design

Hospital La Paz is a 1328-bed university-affiliated hospital providing acute care for a population of 600 000 in Madrid, Spain. In December 2010 an outbreak of OXA-48-producing *K. pneumoniae* was first identified in our hospital; this prompted us to set up a prospective observational study with the aim of including all consecutive episodes of bacteraemia due to O48PE. The study period was from July 2010 to April 2012. Cases were detected through the daily review of blood culture results in adult patients (aged >18 years). Phenotypically similar isolates (those showing an ESBL phenotype and high resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam combined) stored at the microbiology laboratory were retrospectively evaluated for the presence of OXA-48, and one additional case of BSI due to O48PE was detected and included in our series. The study was approved by the local ethics committee.

Variables and data collection

We systematically collected from medical records demographic characteristics, underlying diseases, reason for admission, final diagnosis, source of bacteraemia, antimicrobial therapy and outcome. Antibiotic exposure preceding BSI (previous 30 days) was measured as defined daily dose (DDD); the DDD was defined by the WHO/ATC index (available at http://www.whocc.no).

Definitions

Bloodstream infections were primarily classified as nosocomial or community-acquired, in accordance with the classic CDC criteria [10]. Episodes of community-acquired bacteraemia, were further classified as healthcare-associated (HCA) if any of the following criteria were present [11]: 48-h hospital admission during the previous 90 days, receipt of haemodialysis, intravenous medication or home wound care in the previous 30 days, and residence in a nursing home or long-term care facility.

Charlson comorbidity index (range 0–37) [12] and McCabe–Jackson classification (non-fatal, ultimately fatal or rapidly fatal) [13] were used to evaluate comorbidity and prognosis. Acute severity of illness was evaluated with the use of the Pitt Bacteraemia Score [14]. In accordance with the criteria of systemic inflammatory response syndrome sepsis level was graded as sepsis, severe sepsis or septic shock [15].

The source of bacteraemia was determined according to the clinical presentation or by the evidence of an identical strain cultured near to, or on the same date as the onset of BSI from other body sites. If the source of bacteraemia could not be identified, it was classified as primary bacteraemia.

Antimicrobial therapy was considered microbiologically appropriate if the patient received one active agent against the isolate (MIC within the susceptible range), and clinically adequate if the patient received a combination of two active antibiotics, at the right dose (including loading dose and adjusted to renal function when necessary) and route according to the source of infection. Use of imipenem or meropenem if MIC was <4 mg/L was considered adequate if high-dosed and associated with a second microbiologically active agent [3,16].

Outcome parameters

Crude mortality during hospitalization and within 30 days after clinical onset of BSI episode was analysed. Relationship between death and BSI episode (directly related, indirectly related and non-related) was established by two different investigators by reviewing hospital medical records. In those patients who had been discharged, mortality was evaluated through HORUS, the regional electronic health records system, which includes data from primary care. Readmission within 1 month after discharge and its relatedness to the previous episode of bacteraemia were also collected as indicators of patient outcome.

Microbiological studies

Blood cultures were incubated in the Bactec automated blood culture device (BACTEC; Becton Dickinson, Franklin Lakes, NJ, USA) and BacT/ALERT® (BioMérieux, Marcy l'Etoile, France) blood culture bottle systems. All positive blood cultures were routinely subcultivated on three agar plates (Becton-Dickinson): sheep blood agar, chocolate blood agar and *Brucella* blood agar.

Identification was done using MALDI Biotyper (Bruker Daltonik GmbH, Bremen, Germany) and antimicrobial susceptibility testing was performed using the automated system (Vitek2®; BioMérieux). All Enterobacteriaceae isolates retrieved from blood cultures having MIC >1 mg/L to imipenem or $\geq$0.5 mg/L to ertapenem, according to the CLSI guidelines [17], were studied to rule out the production of

carbapenemase with a modified Hodge test. Carbapenem MIC was confirmed by *E*-test (BioMérieux); colistin and fosfomycin MICs were also tested by *E*-test. Tigecycline MICs were evaluated according to the interpretative criteria of the US Food and Drug Administration. Although Vitek2® is able to detect ESBL production, this was later confirmed by *E*-test ESBL stripes (BioMérieux).

As part of the microbiology laboratory work for identification of isolates, polymerase chain reaction amplification was used for the detection of the bla_{OXA-48} gene (using specific primers for OXA-48 and the insertion sequence IS*1999* of the Tn*1999* transposon [18]) and for the detection of other carbapenemase genes bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM-1} and β-lactamase-encoding ESBL genes bla_{TEM}, bla_{SHV}, bla_{OXA-1} and bla_{CTX-M} [19]. Polymerase chain reaction amplification products were sequenced using the dideoxynucleotide chain termination method.

As these infections were part of a hospital-wide outbreak, clonal relationships between strains were studied using the DiversiLab® (DL) System (BioMérieux) [20]. Multilocus sequence typing based on the *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB* genes was performed on all *K. pneumoniae* isolates [21].

Statistical analysis

Continuous variables were compared using the Mann–Whitney *U* test. Categorical variables were compared using the chi-square test or Fisher's Exact test, as appropriate. Statistical software program SPSS, version 17.0 for Windows (SPSS, Chicago, IL, USA) was used to perform all analyses.

Results

Clinical and epidemiological characteristics

During the study period, 40 patients with bacteraemia caused by O48PE (35 *K. pneumoniae* and five *Escherichia coli*), were detected and included (Table 1). Patients were elderly, with a mean age of 70 years (median 73, range 38–92) and predominantly male (57.5%). Comorbid conditions were frequent, with a median Charlson Index of 5 (range 0–11), and a high proportion of underlying malignancies (57.5%), including 12 patients (30%) with haematological malignancies.

No community-acquired cases were detected. Thirty-five (87.5%) cases were nosocomial; five cases (12.5%) were HCA (all of them had previous hospitalization at our institution). Each patient had a single episode of bacteraemia. At the time when BSI occurred, 17 (48.6%) of the 35 patients with nosocomially acquired disease were hospitalized in a medical service, ten (28.6%) were in a surgical service, and eight (22.8%) were in intensive care units. The median duration of hospital stay before onset of BSI in nosocomially acquired cases was 46.6 days (range 2–188 days).

During the hospital stay before the BSI episode, invasive procedures were frequent. Twenty-four patients (60%) had at least one surgery. Eight patients (20%) had a central venous catheter and 11 (27.5%) had a urinary catheter when BSI occurred.

Antibiotic exposure preceding BSI was high, with a median antibiotic DDD of 39.9 (range 0–251) for any antibiotic, 10.5 (range 0–105.2) for β-lactams (excluding carbapenems), 3.5 (range 0–135) for carbapenems and 0 (range 0–67.2) for quinolones.

The most common source of bacteraemia was the urinary tract, found in 12 patients (30%), followed by deep intra-abdominal surgical site infections in ten (25%) patients, primary bacteraemia in seven (17.5%) patients and catheter-related bacteraemia in four (10%) patients.

Regarding severity of disease, 11 patients with O48PE BSI (27.5%) presented as septic shock, seven as severe sepsis (17.5%) and nine as sepsis (22.5%); median Pitt score was 2 (range 0–11).

Microbiological results

All of the isolates included in the study were positive for bla_{OXA-48} and negative for other carbapenemases. Susceptibilities of OXA-48-producing *K. pneumoniae* isolates to antimicrobials are shown in Table 2. All of the isolates showed a high level of resistance to amoxicillin-clavulanate and piperacillin-tazobactam. Production of ESBL was detected in 36 (90%) isolates, and all non-ESBL-producing isolates were *E. coli*. Using DiversiLab® eight different clones were detected. The majority of ESBLs belonged to clone 1, which has been defined as sequence type (ST) 405, carrying CTX-M-15, as previously described [22]. Values of MIC_{50} for ertapenem, imipenem and meropenem were 16 (range 2 to >32), 4 (range 0.5 to >32) and 2 mg/L (range 1 to >32), respectively. Except for non-ESBL producers, which remained susceptible, the majority of the isolates were resistant to third-generation and fourth-generation cephalosporins and aztreonam. The most frequently preserved antibiotics were amikacin, colistin, tigecycline and fosfomycin, which remained active in 97.5% (39/40), 87.5% (35/40), 67.5% (27/40), 52.5% (19/40) of the isolates, respectively.

Treatment

Thirty-four of the 40 (85%) patients received microbiologically appropriate therapy and 70% were clinically adequately treated with at least two active antibiotics, at a proper dosage and route according to the source of infection. Different combinations of amikacin, fosfomycin, colistin, tigecycline and meropenem were used. Meropenem was chosen in five cases

TABLE 1. Clinical features of patients with bacteraemia due to OXA-48-producing Enterobacteriaceae

	Age	Sex	Charlson on admission day	Primary reason for admission	Associated malignancy	Previous surgery (*n*)	LOS prior to BSI	Microorganism	Source of BSI	SIRS	Pitt Index	Treatment	Days to appropiate treatment	Days to adequate treatment	Death during admission	Days from BSI to death or discharge	Atributable mortality
1	83	Female	5	Parotid abscess	No	Yes	2	*Klebsiella pneumoniae*	ENT	Septic Shock	11	Meropenem	0		Yes	0	Directly related
2	65	Male	3	Oesophageal cancer surgery	Oesophageal cancer	No	33	*Klebsiella pneumoniae*	Urinary tract	Septic Shock	3	Tigecycline + Amikacin	7	7	Yes	29	Indirectly related
3	92	Female	1	Spinal surgery	No	Yes	18	*Klebsiella pneumoniae*	Deep SSI	Severe Sepsis	1	Tigecycline + fosfomycin	3	3	Yes	15	Directly related
4	80	Male	9	Septic arthritis	Lymphoma	Yes	7	*Klebsiella pneumoniae*	Primary BSI	No SIRS	1	Tigecycline + Amikacin	4	4	Yes	13	Non-related
5	74	Male	1	Major burn	No	Yes (3)	30	*Klebsiella pneumoniae*	Skin and soft tissue	Septic Shock	11	Tigecycline + colistin	0	0	Yes	9	Indirectly related
6	77	Male	6	Aortic valve replacement	No	Yes	10	*Klebsiella pneumoniae*	Deep SSI	Septic Shock	4	Tigecycline + colistin	5	5	Yes	19	Directly related
7	81	Male	4	Leukaemia treatment	Leukaemia	No	81	*Klebsiella pneumoniae*	Catheter related BSI	No SIRS	0	Amikacin	2		No	11	
8	59	Female	10	Pancreatic adenocarcinoma Surgery	Pancreatic cancer	Yes (3)	29	*Klebsiella pneumoniae*	Catheter-related BSI	No SIRS	1	Tigecycline + Amikacin	7	7	Yes	64	Indirectly related
9	74	Male	2	Esophageal cancer surgery	Esophageal cancer	Yes (3)	104	*Klebsiella pneumoniae*	Deep Intra-abdominal SSI	Severe Sepsis	3	Meropenem + Amikacin	3	3	Yes	9	Directly related
10	70	Female	0	Bowel obstruction secondary to probable inflammatory intestinal disease	No	Yes (5)	130	*Klebsiella pneumoniae*	Deep Intra-abdominal SSI	Septic Shock	2	Colistin + Tigecycline + Amikacin	0	4	Yes	112	Non-related
11	73	Female	3	Lymphoma treatment	Lymphoma	No	46	*Klebsiella pneumoniae*	Primary BSI	No SIRS	2	Non-active antibiotic			Yes	0	Non-related
12	75	Male	4	Deep vein thrombosis in patient with moderate liver disease	No	Yes	21	*Klebsiella pneumoniae*	Primary BSI	Severe Sepsis	1	Meropenem + Colistin	8	8	Yes	18	Directly related
13	64	Male	5	Coronary acute syndrome	No	Yes (3)	98	*Klebsiella pneumoniae*	Urinary tract	Septic Shock	8	Non-active antibiotic			Yes	6	Directly related
14	64	Male	6	Nosocomial late pneumonia	Leukaemia	Yes	29	*Escherichia coli**	Deep Intra-abdominal SSI	Sepsis	2	Non active antibiotic			Yes	1	Directly related
15	86	Male	8	Decompensation of chronic heart failure and renal failure	Multiple myeloma	No	23	*Escherichia coli**	Urinary tract	No SIRS	0	Ceftriaxone + ciprofloxacine	3	3	Yes	5	Non-related
16	41	Male	6	Pancreatic adenocarcinoma surgery	Pancreatic cancer	Yes (3)	57	*Escherichia coli**	Deep Intra-abdominal SSI	No SIRS	1	Tigecycline	5		No	40	
17	87	Male	4	Urinary tract infection	No	Yes	0 (HCA)	*Klebsiella pneumoniae*	Urinary tract	Severe Sepsis	2	Colistin + fosfomycin	3	3	No	36	
18	84	Female	2	Spontaneous subdural haematoma	No	Yes (2)	60	*Klebsiella pneumoniae*	Primary BSI	No SIRS	2	Amikacin + Ciprofloxacin	3	3	Yes	112	Indirectly related
19	66	Male	1	Probable cholangiocarcinoma	Cholangio-carcinoma	Yes	112	*Klebsiella pneumoniae*	Deep Intra-abdominal SSI	No SIRS	0	Colistin + Amikacin	1	1	No	22	
20	63	Male	5	MRSA pneumonia and secondary bacteraemia	Leukaemia	No	167	*Klebsiella pneumoniae*	Catheter-related BSI	No SIRS	1	Amikacin	0		No	154	
21	49	Male	9	Metastatic pancreatic cancer	Pancreatic cancer	No	21	*Klebsiella pneumoniae*	Deep Intra-abdominal SSI	Severe Sepsis	2	Non-active antibiotic			Yes	6	Directly related
22	71	Female	6	Lymphoma treatment	Lymphoma	No	20	*Escherichia coli**	Urinary tract	Septic Shock	4	Tigecycline + Amikacin	0	0	Yes	48	Indirectly related

Table I (*Continued*)

	Age	Sex	Charlson on admission day	Primary reason for admission	Associated malignancy	Previous surgery (*n*)	LOS prior to BSI	Microorganism	Source of BSI	SIRS	Pitt Index	Treatment	Days to appropiate treatment	Days to adequate treatment	Death during admission	Days from BSI to death or discharge	Atributable mortality
23	81	Female	8	Late-stage cholangiocarcinoma	Cholangio-carcinoma	Yes	6	*Klebsiella pneumoniae*	Deep intra-abdominal SSI	Sepsis	1	Non active antibiotic			Yes	2	Directly related
24	70	Female	8	Urinary tract infection	Pancreatic cancer	Yes	0 (HCA)	*Klebsiella pneumoniae*	Urinary tract	Severe Sepsis	1	Colistin + Tigecycline + Amikacin	1	1	Yes	25	Directly related
25	77	Female	5	Hemiplegic patient with urinary tract infection	Multiple myeloma	No	0 (HCA)	*Klebsiella pneumoniae*	Urinary tract	Sepsis	1	Colistin + fosfomycin	0	3	No	14	
26	73	Male	7	Cholangiocarcinoma surgery	Cholangio-carcinoma	Yes	42	*Klebsiella pneumoniae*	Deep intra-abdominal SSI	No SIRS	0	Non-active antibiotic			No	3	
27	54	Female	2	Leukemia treatment	Leukemia	No	87	*Klebsiella pneumoniae*	Urinary tract	Septic Shock	4	Colistin + fosfomycin	0	0	Yes	6	Directly related
28	63	Male	8	Late-onset nosocomial pneumonia	Lymphoma	No	4	*Klebsiella pneumoniae*	Pneumonia	Sepsis	2	Tigecycline + colistin	2	4	Yes	22	Directly related
29	81	Female	7	Metastatic gastric cancer and pathological hip fracture	Gastric cancer	Yes (2)	19	*Klebsiella pneumoniae*	Urinary tract	No SIRS	1	Amikacin	5		Yes	12	Indirectly related
30	79	Female	2	Paralytic ileus in patient with dementia and previous hip surgery	No	Yes	5	*Klebsiella pneumoniae*	Primary BSI	Sepsis	3	Tigecycline + colistin	2	2	No	10	
31	38	Female	0	Postpartum brain intraparenchymal haemorrhage	No	No	21	*Klebsiella pneumoniae*	Urinary tract	Severe Sepsis	7	Imipenem + Amikacin	2	3	No	70	
32	68	Female	5	Gastric cancer with active bleeding	Gastric cancer	Yes (3)	67	*Klebsiella pneumoniae*	Deep Intra-abdominal SSI	Septic Shock	9	Tigecycline + colistin	2	3	Yes	29	Directly related
33	74	Female	2	Leukaemia treatment	Leukaemia	No	61	*Klebsiella pneumoniae*	Primary BSI	Sepsis	1	Colistin + fosfomycin	5	6	No	20	
34	81	Male	4	Nosocomial urinary tract infection	No	Yes	11	*Klebsiella pneumoniae*	Urinary tract	Sepsis	1	Meropenem + Amikacin	2	3	No	52	
35	53	Male	7	Arterial ischaemia and non-complicated MRSA bacteraemia	No	Yes	5	*Klebsiella pneumoniae*	Deep SSI	No SIRS	0	Colistin + Amikacin	3	3	No	52	
36	64	Male	11	HIV+ patient with pulmonary embolism and non-complicated SAMR bacteraemia	Lymphoma	No	1	*Klebsiella pneumoniae*	Primary BSI	Septic Shock	4	Meropenem + Colistin	6	6	No	10	
37	50	Male	5	Catheter-related bacteraemia	No	No	0 (HCA)	*Klebsiella pneumoniae*	Catheter-related BSI	Sepsis	2	Meropenem + Colistin	0	2	Yes	32	Indirectly related
38	71	Male	5	Nosocomial urinary tract infection	No	No	17	*Klebsiella pneumoniae*	Urinary tract	Septic Shock	3	Colistin	2		No	31	
39	77	Female	0	Hepatic hydatidosis surgery	No	Yes (3)	188	*Klebsiella pneumoniae*	Deep intra-abdominal SSI	Sepsis	2	Tigecycline + colistin	0	0	Yes	9	Directly related
40	77	Male	6	Schönlein–Henoch purpura Vasculitis and nephrotic syndrome	No	No	1 (HCA)	*Klebsiella pneumoniae*	Skin and soft tissue	No SIRS	1	Tigecycline	7		Yes	44	Directly related

LOS, length of stay; HCA, healthcare-associated episodes; ENT, ear, nose and throat; MRSA, methicillin-resistant *Staphylococcus aureus*; SIRS, systemic inflammatory response syndrome; SSI, surgical site infection.
*Non-extended-spectrum β-lactamase-producing isolates.

TABLE 2. Antimicrobial susceptibility of O48-producing *Klebsiella pneumoniae* isolates

	MIC (Range)	MIC_{50}	MIC_{90}
Amoxicillin/Clavulanate	>16/8	>16/8	>16/8
Piperacillin/Tazobactam	>64/4	>64/4	>64/4
Cefoxitin	≤8 to >16	≤8	>16
Cefotaxime	≤1 to >8	>8	>8
Cefotaxime/clavulanate	≤1/4 to >8/4	≤1/4	>8/4
Ceftazidime	≤1 to >16	>16	>16
Ceftazidime/clavulanate	≤1/4 to >8/4	≤1/4	>8/4
Cefepime	≤ 1 to >8	>8	>8
Aztreonam	≤1 to >8	>8	>8
Ertapenem*	2 to >32	16	> 32
Imipenem*	0.5 to >32	4	16
Meropenem*	1.0 to >32	2	16
Ciprofloxacin	1 to >4	>4	>4
Gentamicin	8 to >8	>8	>8
Tobramicin	≤4 to >8	>8	>8
Amikacin	≤2 to >16	≤2	16
Colistin*	0.25 to 24	0.5	4
Tigecycline	≤0.5 to >8	1	>8
Fosfomycin	8 to >128	128	>128
Trimethoprim/Sulfamethoxazol	≤2/38 to >4/76	>4/76	>4/76

*E-test results.

and imipenem in other one, as part of targeted therapy, when MIC was <4 mg/L, and was prescribed at high doses (2 g/8 h), adjusted to renal function if needed. In those patients receiving targeted treatment, the median delay in starting appropriate and adequate treatment was 3 days (range 0–8).

Outcome analysis

Crude mortality during hospital stay and within 30 days from onset of BSI was 65% (26/40) and 50% (20/40), respectively. Kaplan–Meier estimation was used to obtain the 30-day survival curve (Fig. 1). In 15 of the 26 (57.7%) patients who died during hospitalization, death was considered directly related to the BSI episode, based on clinical judgement.

The mean and median hospital stay of these patients after onset of BSI was 29.2 and 19 days (range 0–152 days), respectively.

Only two patients were readmitted within 30 days after hospital discharge for causes related to their previous episode of bacteraemia. The clinical and demographic features of patients and therapeutic options used, according to their outcome, are shown in Table 3. Specific data about the 34 treated patients are shown in Table 4. In univariate analysis we did not find any clinical or therapeutic factor associated with improved survival (Tables 3 and 4).

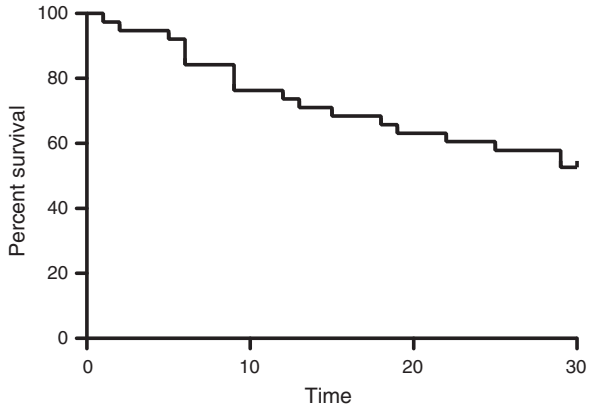

FIG. 1. Kaplan–Meier 30-day survival curve.

Discussion

There are few data regarding clinical features of infections caused by O48PE. Previous reports included a limited number of cases and focused mainly on microbiological and epidemiological features [1,5,8,9,23,24]. Our report, which to the best of our knowledge is the largest series of BSI caused by O48PE, provides information on clinical, epidemiological, microbiological and therapeutic features in the setting of a large outbreak occurring in a single centre [22].

O48PE are difficult to detect because a significant proportion of the isolates have carbapenem MICs that fall within or slightly above the normal range. Indeed, 50% of O48PE isolates included in this study had meropenem MIC ≤2 mg/L, and would have been considered to be within the susceptibility range before the last update of CLSI cut-off points [17] and current European Committee for Antibiotic Susceptibility Testing clinical break points (www.eucast.org). Epidemiological cut-off values of the European Committee for Antibiotic Susceptibility Testing are of value in the phenotypic detection of resistance to antimicrobial

TABLE 3. Distribution of clinical and demographical features and therapeutic options within patients with O48PE bloodstream infection grouped by clinical outcome

Characteristic	All patients (*n* = 40)	Patients alive at day 30 after onset of BSI (*n* = 20) (%)	Patients dead at day 30 after onset of BSI (*n* = 20) (%)
Source of infection			
Urinary tract	12	6 (30)	6 (30)
Deep intra-abdominal surgical site infection	10	4 (20)	6 (30)
Primary bloodstream infection	7	4 (20)	3 (15)
Catheter-related bloodstream infection	4	4 (20)	0 (0)
Others	7	2 (10)	5 (25)
Previous malignancy	23	10 (50)	13 (65)
Solid neoplasm	11	4 (20)	7 (35)
Haematopoietic malignancy	12	6 (30)	6 (30)
Previous surgery	24	10 (50)	14 (70)
Severity of presentation			
Mean Pitt Score	2.62	1.8	3.45
Septic shock	11	4 (20)	7 (35)
Severe sepsis	7	2 (10)	5 (25)
Septic shock or severe sepsis	18	6 (30)	12 (60)
Antimicrobial therapy			
Microbiologically appropriate therapy	34	19 (95)	15 (75)
Clinically adequate therapy	27	14 (70)	13 (65)

p value non-significant for all comparisons.

TABLE 4. Outcome of 34 treated patients according to antimicrobial regimens used

Treatment	All patients (n = 34)	Patients alive at day 30 after onset of BSI (n = 19) (%)	Patients dead at day 30 after onset of BSI (n = 15) (%)
Monotherapy			
Colistin	1	1 (5.2)	0 (0)
Tigecycline	2	2 (10.5)	0 (0)
Amikacin	3	2 (10.5)	1 (6.6)
Carbapenem	1	0 (0)	1 (6.6)
Total monotherapy	7	5 (26.3)	2 (13.3)
Combined therapy			
Two or more active drugs (carbapenem not included)	21	10 (52.6)	11 (73.3)
Two or more active drugs (carbapenem included)	6	4 (21.1)	2 (13.3)
Total combined therapy	27	14 (73.7)	13 (86.6)

p value non-significant for all comparisons.

agents as a biological phenomenon and may indicate the development of resistance at a level below the clinical breakpoint. To improve identification of carbapenem-resistant enterobacteria, CLSI recommends that production of carbapenemases should be suspected in enterobacterial isolates with ertapenem MIC values $\geq$0.5 mg/L, or in those with imipenem or meropenem MIC >1 mg/L [17]. This consideration is also supported by our data, showing that ertapenem resistance is the most sensitive indicator of OXA-48 activity, and meropenem the most frequently preserved carbapenem.

Bloodstream infections caused by O48PE were associated with a poor prognosis despite the urinary tract being the most common source of infection in half of the patients. Overall, 30-day mortality reached 50%. Of note, advanced age, significant comorbidity, active malignancies, long hospital stays and surgical interventions before the episode of bacteraemia were very frequent among these patients.

The best therapeutic approach to O48PE BSI is controversial and, in our opinion, selection of the appropriate therapy should be made on a case-by-case basis, balancing efficacy and toxicity, as therapeutic options are limited. Although amikacin, colistin, tigecycline and fosfomycin were the antibiotics that remained most frequently effective, no single antimicrobial was uniformly active against O48PE isolates, as it has been previously reported [25]. Third-generation cephalosporins, which remained active against OXA-48-producing but ESBL-negative isolates, have shown good clinical response in one animal model [26]. Unfortunately, as in our case, ESBLs are frequently associated with OXA-48, limiting the use of these agents. The use of carbapenems for the treatment of infections caused by carbapenemase-producing Enterobacteriaceae, if either meropenem or imipenem MICs are $\leq$4 mg/L, has been advocated [3]. Nevertheless, several failures of imipenem-containing regimens to treat infections caused by O48PE have been reported [6,9].

Although there is no clear evidence in the literature for the superiority of combination therapy, we, like others, tend to combine two active agents to treat serious infection such as BSI [1,27]. In our data, success rate seems to be higher in patients treated with a single agent treatment, but this finding should be interpreted with caution because patients who received monotherapy were carefully selected for such treatment because of less severe presentation or an easily treatable source of infection, mainly urinary tract infections. In addition to a non-β-lactam antibiotic, we believe that β-lactams, usually ceftazidime if ESBL-negative, or high-dose meropenem if MIC is $\leq$4 mg/L, should be included as part of the combined therapy.

Median interval between onset of BSI and initiation of microbiologically appropriate and clinically adequate antimicrobial therapy was 3 days, and could explain, in conjunction with a baseline-deteriorated patient condition, the high mortality observed in our series. In another study of BSI caused by carbapenem-resistant Enterobacteriaceae [28], a similar delay in the administration of appropriate therapy and a high mortality rate (40%) have also been reported.

Given the poor prognosis of BSI caused by O48PE, improvement in early detection and treatment is needed. Prompt initiation of optimal antimicrobial therapy in infections caused by carbapenem-resistant enterobacteria could be achieved if appropriate empirical antimicrobial protocols are applied in selected epidemiological settings. Patients with nosocomial infections, especially if they are known to be colonized by O48PE or other carbapenem-resistant enterobacteria, should be empirically treated with a combination of drugs with activity against these multi-drug-resistant bacteria. In addition, new methods for rapid detection of carbapenemases [29] could be useful to reduce the period of empirical antimicrobial therapy and to refine the antibiotic regimen used.

In conclusion, OXA-48 carbapenemase is an emergent resistance mechanism in Enterobacteriaceae, which can be involved in nosocomial and HCA bacteraemias. It is important to suspect and identify this mechanism of resistance, to offer targeted therapies or to change empirical treatment protocols in particular cases. Considering the often-unfavourable outcome, identification of carriers and reinforcement of infection control practices is of critical relevance to avoid the spread of these bacteria.

Acknowledgements

We thank Mr. Martin J. Smyth, B.A., for his help in correcting the English.

Partial results of this study were presented as an Oral Presentation at the 22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in London 2012 (O-497).

Conflict of Interest

No authors have conflicts of interest to declare with respect to the contents of this manuscript.

Transparency Declaration

No funding has been received by any of the authors for this work.

References

1. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother* 2012; 67: 1597–1606.
2. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis* 2011; 53: 60–67.
3. Daikos GL, Markogiannakis A. Carbapenemase-producing *Klebsiella pneumoniae*: (when) might we still consider treating with carbapenems? *Clin Microbiol Infect* 2011; 17: 1135–1141.
4. Poirel L, Castanheira M, Carrër A *et al.* OXA-163, an OXA-48-related class D β-lactamase with extended activity toward expanded-spectrum cephalosporins. *Antimicrob Agents Chemother* 2011; 55: 2546–2551.
5. Carrër A, Poirel L, Yilmaz M *et al.* Spread of OXA-48-encoding plasmid in Turkey and beyond. *Antimicrob Agents Chemother* 2010; 54: 1369–1373.
6. Carrër A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. Spread of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in Istanbul, Turkey. *Antimicrob Agents Chemother* 2008; 52: 2950–2954.
7. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004; 48: 15–22.
8. Pitart C, Solé M, Roca I, Fàbrega A, Vila J, Marco F. First outbreak of a plasmid-mediated carbapenem-hydrolyzing OXA-48 beta-lactamase in *Klebsiella pneumoniae* in Spain. *Antimicrob Agents Chemother* 2011; 55: 4398–4401.
9. Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P. Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in France. *Antimicrob Agents Chemother* 2011; 55: 2420–2423.
10. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. *Am J Infect Control* 1988; 16: 128–140.
11. Friedman ND, Kaye KS, Stout JE *et al.* Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. *Ann Intern Med* 2002; 137: 791–797.
12. Charlson ME, Charlson RE, Peterson JC, Marinopoulos SS, Briggs WM, Hollenberg JP. The Charlson comorbidity index is adapted to predict costs of chronic disease in primary care patients. *J Clin Epidemiol* 2008; 61: 1234–1240.
13. McCabe WR, Jackson GG. Gram-negative bacteremia: I. Etiology and ecology. *Arch Intern Med* 1962; 110: 847.
14. Chow JW, Yu VL. Combination antibiotic therapy versus monotherapy for gram-negative bacteraemia: a commentary. *Int J Antimicrob Agents* 1999; 11: 7–12.
15. Bone RC, Balk RA, Cerra FB *et al.* Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; 101: 1644–1655.
16. Tumbarello M, Viale P, Viscoli C *et al.* Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. *Clin Infect Dis* 2012; 55: 943–950.
17. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI M100-S22. CLSI, Wayne, PA, USA, 2012.
18. Aubert D, Naas T, Héritier C, Poirel L, Nordmann P. Functional characterization of IS*1999*, an IS4 family element involved in mobilization and expression of beta-lactam resistance genes. *J Bacteriol* 2006; 188: 6506–6514.
19. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 2007; 20: 440–458.
20. Healy M, Huong J, Bittner T *et al.* Microbial DNA typing by automated repetitive-sequence-based PCR. *J Clin Microbiol* 2005; 43: 199–207.
21. Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005; 43: 4178–4182.
22. Pano-Pardo JR, Ruiz-Carrascoso G, Navarro San Francisco C *et al.* Infections caused by OXA-48-producing *Klebsiella pneumoniae* in a tertiary hospital in Spain in the setting of a prolonged, hospital-wide outbreak. *J Antimicrob Chemother* 2012; [Epub ahead of print].
23. Potron A, Kalpoe J, Poirel L, Nordmann P. European dissemination of a single OXA-48-producing *Klebsiella pneumoniae* clone. *Clin Microbiol Infect* 2011; 17: E24–E26.
24. Pfeifer Y, Schlatterer K, Engelmann E *et al.* Emergence of OXA-48-type carbapenemase-producing Enterobacteriaceae in German hospitals. *Antimicrob Agents Chemother* 2012; 56: 2125–2128.
25. Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, Woodford N. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *Int J Antimicrob Agents* 2011; 37: 415–419.
26. Mimoz O, Grégoire N, Poirel L, Marliat M, Couet W, Nordmann P. Broad-spectrum β-lactam antibiotics for treating experimental peritonitis in mice due to *Klebsiella pneumoniae* producing the carbapenemase OXA-48. *Antimicrob Agents Chemother* 2012; 56: 2759–2760.
27. Akova M, Daikos GL, Tzouvelekis L, Carmeli Y. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. *Clin Microbiol Infect* 2012; 18: 439–448.
28. Daikos GL, Petrikkos P, Psichogiou M *et al.* Prospective observational study of the impact of VIM-1 metallo-beta-lactamase on the outcome of patients with *Klebsiella pneumoniae* bloodstream infections. *Antimicrob Agents Chemother* 2009; 53: 1868–1873.
29. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2012; 18: 1503–1507.