INTRODUCTION

Extended-spectrum β-lactamase (ESBL)-producing members of the Enterobacteriaceae have spread worldwide. During the past decade, the CTX-M type has been the most rapidly growing group of ESBLs, with CTX-M-15 being the dominant type, found mainly in Escherichia coli and Klebsiella pneumoniae (Oteo et al., 2006; Diestra et al., 2009).

Enterobacter cloacae is a significant cause of nosocomial infections; in this species, the most important mechanism of expanded-spectrum cephalosporin resistance is overproduction of the chromosomal AmpC β-lactamase, but ESBL production is increasingly reported in this pathogen (Manzur et al., 2007; Iabadene et al., 2008; Garza-González et al., 2011).

In neonatal intensive care units (NICUs), the spread of ESBL-producing Enterobacter species has been reported very rarely (Kartali et al., 2002; Moriguchi et al., 2007; Mshana et al., 2011), and to our knowledge, significant nosocomial outbreaks due to the clonal dissemination of CTX-M-15-producing Enterobacter cloacae have not been described in newborns.

In this study, we have described the clinical and molecular features of a NICU outbreak caused by CTX-M-15-producing Enterobacter cloacae.

Abbreviations: ESBL, extended-spectrum β-lactamase; HGUGM, Hospital General Universitario Gregorio Marañón; NICU, neonatal intensive care unit.

METHODS

Study design and bacterial isolates. In September 2010, a newborn female baby admitted to the NICU of the Hospital General Universitario Gregorio Marañón (HGUGM) had an episode of bacteraemia caused by an ESBL-producing Enterobacter cloacae isolate. Between December 2010 and January 2011, six additional cases of infection or colonization due to ESBL-producing Enterobacter cloacae isolates were detected; this observation prompted the present investigation. All newborns admitted to this NICU, either infected or colonized by ESBL-producing Enterobacter cloacae between September 2010 and December 2011, were included in the study.

Rectal swabs were taken from the newborns and cultured on MacConkey agar plates. Samples from the surfaces surrounding the newborns and from common areas and materials used for the newborns, as well as from their wash basins, were taken with sterile gauzes, which were introduced into brain–heart infusion broth tubes and incubated for 18–24 h at 37°C. All brain–heart infusion cultures showing turbidity were subcultured on MacConkey agar plates. After incubation of the plates for 18–24 h at 37°C, all isolates that grew were identified by standard procedures.

Antibiotic susceptibility testing and ESBL detection were performed using an automated MicroScan microdilution system (Siemens Healthcare Diagnostics) following the manufacturer’s recommendations. The Clinical and Laboratory Standards Institute breakpoints were applied (CLSI, 2012).

Molecular epidemiology. The genetic relationship between the ESBL-producing Enterobacter cloacae isolates was determined by PFGE after total chromosomal DNA digestion with XbaI (Oteo et al., 2006).

Characterization of antibiotic resistance genes. Genes encoding ESBLs belonging to groups CTX-M-1, CTX-M-9, SHV and TEM were
amplified by PCR. The blacTX-M genes were amplified and sequenced using specific primers (Oteo et al., 2006). In addition, the following resistance genes were also investigated by PCR and DNA sequencing: aac(3)-IIa (aminoglycoside resistance), aac(6’)-Ib-cr (aminoglycoside-quinolone resistance), dfr (trimethoprim resistance), qnr (quinolone resistance) and blaoXA-1 (amoxicillin/clavulanic acid resistance) (Oteo et al., 2006; labadene et al., 2008).

Conjugation assay and plasmid characterization. Conjugation experiments were performed with the kanamycin–azide-resistant Escherichia coli BM101 as a recipient. Putative transconjugants were selected on Mueller–Hinton agar plates containing kanamycin (100 mg l⁻¹) and cefotaxime (4 mg l⁻¹).

The number and size of plasmids were determined by PFGE after S1 nuclease digestion of whole genomic DNA. Plasmids were classified according to their incompatibility group using a PCR-based replicon-typing scheme (Carattoli et al., 2005).

RESULTS

Patients and bacterial isolates

Fourteen newborns were either infected (n=7, 50 %) or colonized (rectal culture positive; n=7, 50 %) by ESBL-producing Enterobacter cloacae. Of these, seven (50 %) were female. Four of the infected patients were also colonized. The clinical diagnoses were bacteraemia (three cases) and one case each of urinary, respiratory, abdominal and conjunctiva infection. A total of 30 ESBL-producing Enterobacter cloacae isolates were available for molecular studies, as some newborn patients had repeated positive cultures.

Table 1 shows the characteristics of the CTX-M-15-producing Enterobacter cloacae infection and colonization cases in the 14 newborns.

ESBL characterization and molecular epidemiology

CTX-M-15 was positively identified in all 30 ESBL-producing Enterobacter cloacae isolates. Analysis of PFGE profiles consistently revealed two well-differentiated PFGE clusters (Figs 1 and 2). The most important was cluster 1 (C1), which comprised 21 indistinguishable CTX-M-15-producing isolates from 11 newborns. Cluster 2 (C2) comprised nine CTX-M-15-producing Enterobacter cloacae affecting three newborns (Figs 1 and 2). In both the C1 and C2 clusters, the insertion sequence ISecp1 was identified 48 bp upstream of blacTX-M-15, as described previously (Diestra et al., 2009), but none had the IS26 element flanking blacTX-M-15 (Diestra et al., 2009).

Susceptibility testing and additional mechanisms of antibiotic resistance

The CTX-M-15-producing Enterobacter cloacae isolates of clusters C1 and C2 were resistant to ampicillin, amoxicillin/clavulanic acid, cefazolin, cefoxitin, cefuroxime, cefotaxime, cefazidime, cefepime, gentamicin, tobramycin and cotrimoxazole, and were susceptible to imipenem, meropenem, ertapenem and amikacin. Ciprofloxacin susceptibility was variable: in C1 isolates, the MIC ranged from 2 μg ml⁻¹ (intermediate susceptibility) to >2 μg ml⁻¹ (resistant), whilst in C2 isolates, the MIC ranged from ≤0.12 μg ml⁻¹ (susceptible) to >2 μg ml⁻¹ (resistant).

All 30 isolates producing CTX-M-15 also carried the aac(3)-IIa, aac(6’)-Ib-cr, dfrA14 and blaoXA-1 resistance genes. In addition, the qnrB gene was also identified in C1 isolates.

Conjugation assays and plasmid characterization

CTX-M-15-producing transconjugants were obtained from the four donor isolates assayed representing clusters C1 and C2 (two donor isolates each). Plasmids of the incompatibility group F carrying blacTX-M-15 were identified in the C1 transconjugants (~300 kb) and C2 transconjugants (~70 kb).

Clinical risk factors, treatment and outcome

All 14 newborns infected or colonized by CTX-M-15-producing Enterobacter cloacae had underlying medical conditions including prematurity (ten cases), respiratory distress syndrome (two cases), congenital malformations (two cases), duodenal atresia (one case) and cerebrovascular accident (one case) (Table 1). Five were treated with meropenem as monotherapy (20–40 mg kg⁻¹ twice a day) and one with ciprofloxacin eye drops; six patients were cured, and one newborn with multiple congenital malformations who did not receive specific antibiotic treatment died (Table 1).

Outbreak evolution and infection control measures

The NICU of the HGUGM has a total of 16 beds, and had a mean occupancy rate of 85 % from September 2010 to April 2011 (8 months). During this period, 413 newborns were admitted to the NICU as follows: 146 were admitted from September 2010 to December 2010 with a 2 % mean occupancy rate of 85 % from September 2010 to December 2010 with a 2 % incidence of ESBL-producing Enterobacter cloacae, and 267 were admitted from January 2011 to April 2011 with a 4.11 % incidence of ESBL-producing Enterobacter cloacae.

The first identified case was a pre-term female baby. After 7 days with enteral nutrition, the patient suffered a necrotizing enterocolitis with bacteraemia caused by CTX-M-15-producing Enterobacter cloacae. The origin of this isolate remained unknown, as none of the cultures from the body surfaces of the patient, faecal cultures from her mother or environmental cultures were positive for CTX-M-15-producing Enterobacter cloacae. Unfortunately, the enteral nutrition system was not available for culture.

Fig. 2 shows the monthly evolution of the 14 infected or colonized patients caused by C1 or C2 PFGE clusters.

Rectal swabs were taken at least once a week from all newborns hospitalized in the same unit in which a colonization or an infection case was present, until no more cases were observed because of discharge, death or
Table 1. Characteristics of the CTX-M-15-producing Enterobacter cloacae infection and colonization cases

F, Female; M, male; BL, blood; UR, urine; REC, rectal; BAS, bronchial aspirates; PER, peritoneal; CAT, catheter; UTI, urinary tract infection; LRTI, lower respiratory tract infection; PNI, pre-term newborn infant; DA, duodenal atresia; MCM, multiple congenital malformations; CAV, cerebrovascular accident; CHD, congenital heart disease; RDS, respiratory distress syndrome.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Samples (n)</th>
<th>Date of first isolation</th>
<th>PFGE cluster</th>
<th>Localization of infection</th>
<th>Underlying condition(s)</th>
<th>Antimicrobial therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>BL (2)</td>
<td>25/09/2010</td>
<td>C1</td>
<td>Bacteraemia</td>
<td>PNI</td>
<td>Meropenem</td>
<td>Cure</td>
</tr>
<tr>
<td>M</td>
<td>BL (1), UR (1), REC (1), BAS (1)</td>
<td>02/12/2010</td>
<td>C2</td>
<td>Bacteraemia, UTI</td>
<td>PNI, DA</td>
<td>Meropenem</td>
<td>Cure</td>
</tr>
<tr>
<td>F</td>
<td>REC (2), PER (1)</td>
<td>06/12/2010</td>
<td>C1</td>
<td>Intra-abdominal infection</td>
<td>PNI</td>
<td>Meropenem</td>
<td>Cure</td>
</tr>
<tr>
<td>M</td>
<td>BAS (2)</td>
<td>03/02/2011</td>
<td>C1</td>
<td>LRTI</td>
<td>RDS</td>
<td>Meropenem</td>
<td>Cure</td>
</tr>
<tr>
<td>M</td>
<td>EYE (1), REC (1)</td>
<td>08/02/2011</td>
<td>C1</td>
<td>Conjunctival infection</td>
<td>PNI</td>
<td>Ciprofloxacin eyedrops</td>
<td>Cure</td>
</tr>
<tr>
<td>F</td>
<td>BL (1), CAT (1)</td>
<td>14/02/2011</td>
<td>C1</td>
<td>Bacteraemia</td>
<td>PNI</td>
<td>Meropenem</td>
<td>Cure</td>
</tr>
<tr>
<td>F</td>
<td>UR (1), REC (1), BAS (1)</td>
<td>26/04/2011</td>
<td>C2</td>
<td>UTI, LRTI</td>
<td>PNI, MCM</td>
<td>No specific treatment</td>
<td>Death*</td>
</tr>
<tr>
<td>M</td>
<td>REC (2)</td>
<td>06/01/2011</td>
<td>C2</td>
<td>Colonization</td>
<td>PNI</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>REC (2), BAS (1)</td>
<td>14/01/2011</td>
<td>C1</td>
<td>Colonization</td>
<td>CHD</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M</td>
<td>CAT (1)</td>
<td>25/01/2011</td>
<td>C1</td>
<td>Colonization</td>
<td>PNI</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>REC (2)</td>
<td>27/01/2011</td>
<td>C1</td>
<td>Colonization</td>
<td>PNI</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M</td>
<td>REC (2)</td>
<td>17/01/2011</td>
<td>C1</td>
<td>Colonization</td>
<td>RDS</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>10/03/2011</td>
<td>C1</td>
<td>Colonization</td>
<td>CAV</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M</td>
<td>REC (1)</td>
<td>29/03/2011</td>
<td>C1</td>
<td>Colonization</td>
<td>PNI</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*The contribution of the Enterobacter cloacae infection to death is not clear, as this patient had multiple congenital malformations.
transfer to other wards. All newborns with ESBL-producing Enterobacter cloacae were identified and assigned contact precautions, including the use of disposable gowns and gloves. Reinforcement of standard precautions was performed for all NICU patients, including improvement of hand hygiene compliance by the use of alcohol rub before and after care of the patients. None of the environmental cultures was positive for CTX-M-15-producing Enterobacter cloacae. Between April 2011 and December 2011, no more cases of infection or colonization by CTX-M-15-producing Enterobacter cloacae isolates were detected.

**DISCUSSION**

To our knowledge, this is the first documented outbreak due to the clonal spread of Enterobacter cloacae producing CTX-M-15 in a NICU. The outbreak was in fact caused by two different bacterial clones in which two transferable plasmids of very different sizes, both carrying bla\textsubscript{CTX-M-15}, were identified.

In 2002, the prevalence of Enterobacter species over-producing AmpC in a Spanish hospital was 20\%, whilst ESBL production was only 0.4\% (Cantón et al., 2002). CTX-M-15 is the most prevalent ESBL type produced by Escherichia coli and K. pneumoniae worldwide, including in Spain (Oteo et al., 2006; Diestra et al., 2009); other members of the Enterobacteriaceae such as Enterobacter cloacae sharing similar ecological niches could acquire bla\textsubscript{CTX-M-15}, which is frequently linked to epidemic IncF plasmids (Diestra et al., 2009), as occurred in our Enterobacter cloacae isolates. Recently, CTX-M-15 was the most frequently detected ESBL in Enterobacter cloacae in Algeria and Mexico in adults (Iabadene et al., 2008; Garza-Gonzalez et al., 2011).
There is little information available about outbreaks caused by ESBL-producing Enterobacter cloacae in newborns. In a recent series from Tanzania, 17 newborns had blood infections caused by a clonal strain of a novel Enterobacter species carrying \(bla_{CTX-M-15}\); the same strain was isolated from a milk bucket (Mshana et al., 2011). In a Greek hospital NICU, 18 newborns were infected by IBC-1-producing Enterobacter cloacae (Kartali et al., 2002). Finally, in 2002, an outbreak of CTX-M-3-producing Enterobacter cloacae was described in a paediatric ward of a Japanese hospital (Moriguchi et al., 2007). A noteworthy aspect of this study is the existence of two different clusters of an infrequent micro-organism, CTX-M-15-producing Enterobacter cloacae. We could not establish the origin of the outbreak isolates, as a thorough cleaning and disinfection of environmental surfaces was carried out before bacterial samples could be obtained. Also, no cultures were taken from the babies’ mothers other than from the mother of the index case patient, so we could not discard any of these options as a possible origin of the outbreak.

The clonal dissemination of ESBL-producing micro-organisms in newborns is a matter of serious clinical and epidemiological concern because they are usually resistant to almost all \( \beta \)-lactams and are co-resistant to other antibiotic families, leaving carbapenems as almost the only reliable \( \beta \)-lactams for treatment of serious infections. In this study, 71.4% of the newborns (five out of seven) were treated with meropenem as monotherapy, and all five were cured. It has been demonstrated that the main risk factor for acquisition of carbapenem-resistant Enterobacteriaceae infection is the use of carbapenem antibiotics during the 30 days prior to the isolation of the carbapenem-resistant strain (Hyle et al., 2010). One outbreak of carbapenem-resistant VIM-1-producing Enterobacter cloacae in a paediatric ward was described recently (Oteo et al., 2010). Although the mortality rate in the current study was low and limited to a single case with severe underlying diseases, high mortality rates have been described in newborns infected by ESBL-producing Enterobacter species (Mshana et al., 2011).

In summary, in this study, we reported an outbreak caused by multidrug-resistant CTX-M-15-producing Enterobacter cloacae in a NICU. Infections caused by multidrug-resistant members of the Enterobacteriaceae are increasingly being reported in adult patients, but, as shown in this study, newborns with severe underlying conditions are also at high risk.

The implementation of effective hygiene measures for the prevention and control of multidrug-resistant Enterobacteriaceae isolates, mainly in high-risk patients such as newborns, is critical. In addition, prompt and adequate treatment is essential for recovery of the patient.

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REFERENCES


