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Nosocomial Outbreak Due to Extended-Spectrum-Beta-Lactamase-Producing *Enterobacter cloacae* in a Cardiothoracic Intensive Care Unit▽

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***Enterobacter cloacae* has been associated with several outbreaks, usually involving strains that overproduce chromosomal β-lactamase or, uncommonly, strains expressing extended-spectrum β-lactamases (ESBL). Only sporadic cases of ESBL-producing *E. cloacae* have been identified in our hospital in recent years. We describe the epidemiology and clinical and microbiological characteristics of an outbreak caused by ESBL-producing *E. cloacae* in a cardiothoracic intensive care unit (CT-ICU). Prospective surveillance of patients with infection or colonization by ESBL-producing *E. cloacae* among patients admitted to the CT-ICU was performed during the outbreak. Production of ESBL was determined by decreased susceptibility to expanded-spectrum cephalosporins and a positive double-disk test result. Clone relatedness was determined by pulsed-field gel electrophoresis (PFGE). From July to September 2005, seven patients in the CT-ICU with ESBL-producing *E. cloacae* were identified (four males; median age, 73 years; range, 45 to 76 years); six patients had cardiac surgery. Four patients developed infections; three had primary bacteremia, one had ventilator-associated pneumonia, and one had tracheobronchitis. ESBL-producing *E. cloacae* showed resistance to quinolones and aminoglycosides. PFGE revealed two patterns. Five isolates belonged to clone A; two carried a single ESBL (pI 8.2 and a positive PCR result for the SHV type), and three carried two ESBLs (pIs 8.1 and 8.2 and positive PCR results for the SHV and CTX-M-9 types). Isolates belonging to clone B carried a single ESBL (pI 5.4 and a positive PCR result for the TEM type). Review of antibiotic consumption showed increased use of cefepime and quinolones during June and July 2005. The outbreak was stopped by the implementation of barrier measures and cephalosporin restriction. ESBL production could be increasingly common in nosocomial pathogens other than *Escherichia coli* or *Klebsiella pneumoniae*.**

Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated bacterial enzymes that confer resistance to a broad range of extended-spectrum β-lactam antibiotics (14). The most commonly encountered plasmid-mediated ESBLs are derived from the TEM and SHV enzyme families (14, 26). Large nosocomial outbreaks caused by ESBL-producing gram-negative bacilli have been reported, with *Escherichia coli* and *Klebsiella pneumoniae* being the most frequently involved pathogens (10, 15, 18, 27, 28). Selective antibiotic pressure, particularly that caused by the intensive use of expanded-spectrum cephalosporins and cross-transmission, has been associated with the emergence and dissemination of ESBL-producing members of the family *Enterobacteriaceae* (9, 13).

In *Enterobacter cloacae*, ESBLs have not often been reported (17, 21). In this species, the most important mechanism of expanded-spectrum cephalosporin resistance is overproduction of the chromosomal AmpC β-lactamase. However, ESBL-producing *E. cloacae* is currently increasing (8, 14, 26). Because of the "hide" expression of ESBL production in isolates with overproduction of AmpC, high suspicion by the microbiologist is needed to distinguish the constitutive AmpC phenotype from ESBL production, particularly when both mechanisms are present at the same time in the strain.

In our hospital, only sporadic and nonrelated cases of ESBL-producing *E. cloacae* were identified in 2004. In summer 2005, an outbreak involving seven patients caused by an epidemic strain of ESBL-producing *E. cloacae* was detected in the cardiothoracic intensive care unit (CT-ICU). This report describes the clinical and molecular epidemiology of this outbreak and the measures taken to manage it.

MATERIALS AND METHODS

Setting. The Hospital Universitari de Bellvitge is an 800-bed tertiary-care university hospital for adult patients that is located in the area of Barcelona, Spain. The CT-ICU is a 12-bed intensive-care unit for the care of cardiac surgery patients.

Definition and extent of the outbreak. All of the patients admitted to the CT-ICU who had ESBL-producing *E. cloacae* isolates from clinical or surveillance cultures were studied. The outbreak ran from July 2005, on a review of the medical record of the first patient admitted to the CT-ICU who developed an ESBL-producing *E. cloacae* infection, to September 2005, when the last isolate was identified.

Microbiological methods. (i) Bacterial strains and antimicrobial susceptibility testing. *E. cloacae* was isolated from clinical samples by conventional laboratory procedures. Fecal carriage screening was performed among CT-ICU patients from July to September 2005. Fecal swabs were inoculated onto two selective plates of MacConkey agar, one with 4 mg/liter cefotaxime and the other with 4 mg/liter ceftazidime. Microorganisms that grew in one or both selective media were identified. *E. cloacae* strains were identified and tested for antibiotic susceptibility by the MicroScan automated microdilution system (Dade International, West Sacramento, CA). The criteria of the Clinical and Laboratory

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Standards Institute (4) were used to define susceptibility and resistance to the antibiotics studied. All *E. cloacae* strains for which the MICs of cefotaxime, ceftazidime, aztreonam, or cefepime were >0.5 mg/liter were routinely screened for ESBL production by the double-disk synergy test (DDST). The DDST was performed with disks of cefotaxime, ceftazidime, aztreonam, and cefepime (30 μg each) at variable distances of 30 and 15 mm (edge to edge) from a disk containing amoxicillin plus clavulanate (20:10 μg) (36). The test result was interpreted as positive when an enlarged zone of inhibition was observed between the disk of amoxicillin plus clavulanate and disks of cefotaxime, ceftazidime, aztreonam, and/or cefepime.

Strains for which the MIC of imipenem was >0.5 μg/ml were screened for carbapenemase activity by the modified Hodge test and the EDTA disk synergy test. The modified Hodge test (22) was carried out on Mueller-Hinton agar. The plate was inoculated with *E. coli* ATCC 25922, and a disk of imipenem (10 μg) was placed at the center of the plate. Tested *E. cloacae* strains were streaked from the edge of the disk to the periphery of the plate. No distortion of the *E. coli* inhibition zone was interpreted as a negative result. To rule out the presence of metallo-beta-lactamases, the EDTA disk synergy test was performed with disks of imipenem (10 μg) and EDTA (1,900 μg) (23). The test result was considered negative when no enlargement of the inhibition zone was observed between disks of imipenem and EDTA.

(ii) ESBL characterization by isoelectric focusing. Isoelectric focusing analysis of crude extracts was done as previously described (28). Briefly, *E. cloacae* strains were grown for 4 h in Luria broth. Bacterial growth was centrifuged, resuspended in distilled water, and sonicated. Extract purification was performed by ultracentrifugation. Isoelectric focusing was performed with the PhastSystem apparatus (Pharmacia, Uppsala, Sweden) in polyacrylamide gels with a pH range of 3 to 9 (PhastGel 3-9; Pharmacia). Polyacrylamide gels were stained with nitrocefin (500 mg/ml; Oxoid), and the pIs were obtained by comparison with a set of different β-lactamases with known pIs (TEM-1, 5.4; SHV-2, 7.6; SHV-5, 8.2; CTX-M-9, 8.1; CTX-M-10, 8.1; kindly provided by R. Cantón) (3, 6).

(iii) PCR detection of genes coding for ESBLs. Detection of ESBL genes representative of the SHV, TEM, and CTX-M families was performed by PCR with DNA extracts from *E. cloacae* isolates with a positive DDST result. A single colony of each test isolate was resuspended in 500 μl of water and boiled for 15 min, and 3 μl of the supernatant was used as template DNA in a 50-μl PCR mixture. The primers used were 5′-GGG TTA TTC TTA TTT GTC GC-3′ and 5′-TTA GCG TTG CCA GTG CTC-3′ for detection of the bla_{SHV} gene and 5′-ATA AAA TTC TTG AAG AC-3′ and 5′-TTA CCA ATG CTT AAT CA-3′ for detection of the bla_{TEM} gene (31). Conditions for amplification were 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 10 min. The primers for CTX-M9 amplification were 5′-GTG ACA AAG AGA GTG CAA CGG-3′ and 5′-ATG ATT CTC GCC GCT GAA GCC-3′. For CTX-M10 PCR amplification, the primers used were 5′-CCG CGC TAC ACT TTG TGG C-3′ and 5′-TTA CAA ACC GTT GGT GAC G-3′ (33). Annealing temperatures were 62°C for CTX-M9 and 56°C for CTX-M10. The rest of the cycles were the same as those for bla_{SHV} and bla_{TEM} amplifications. The PCR products were examined in 1% agarose gels under UV light after staining with ethidium bromide.

(iv) Molecular typing. Molecular characterization of ESBL-producing *E. cloacae* was done by macrorestriction analysis of genomic DNA with XbaI (New England BioLabs). DNA fragments were separated by pulsed-field gel electrophoresis (PFGE) in a CHEF-DR III system (Bio-Rad). Electrophoresis conditions were pulse times ranging from 1 to 30 s for 23 h at 6 V/cm and 14°C. Restriction patterns were analyzed by following previously established criteria (34).

Infection control procedures. Patients from whom ESBL-producing *E. cloacae* was recovered at any site were visited by a member of the infection control team. Colonization or infection was determined according to the definition of the Centers for Diseases Control and Prevention for nosocomial infections.

In July 2005, the following infection control measures were implemented to limit the spread of the outbreak: (i) contact precautions for all patients infected or colonized by ESBL-producing *E. cloacae*, (ii) weekly screening of all patients admitted to the CT-ICU for detection of ESBL-producing *E. cloacae* fecal carriage, (iii) reinforcement of proper glove and gown use and reinforcement of hand disinfection with alcoholic gel, and (iv) review of antibiotic consumption during the period from January to June 2005 and restriction of the use of expanded-spectrum cephalosporins and cefepime from August 2005 on.

RESULTS

From January to September 2005, ESBL-producing *E. cloacae* was isolated from 14 hospitalized patients. In this period, 23.3% of the *E. cloacae* isolates identified in our hospital showed resistance to expanded-spectrum cephalosporins; in 16.7% of them, the mechanism involved was overproduction of AmpC and 6.7% carried ESBL. While no relationship was observed among the seven patients hospitalized in different wards from January to June 2005, 16 isolates of EBSL-producing *E. cloacae* were recovered during July and August 2005 from seven patients admitted to the CT-ICU (Fig. 1). Table 1 shows epidemiological and clinical data on these seven patients. Four patients were male; their median age was 73 (range, 45 to 76) years. The average previous hospital stay was 25 (range, 18 to 39) days, and the average prior CT-ICU stay was 13 (range, 7 to 21) days. Six of the seven patients had undergone cardiac surgery. The overall mortality was 42% (three patients). No death was related to infection.

Two patterns of *E. cloacae* were demonstrated by PFGE (clones A and B) (Fig. 2). Results of PFGE, pIs, and determination of PCR for the CTX-M-9, TEM, and SHV families in the isolates analyzed are shown in Table 2. Clone A was identified in five patients, and clone B was identified in two (patients 4 and 7) (Table 1). Two isolates that belonged to clone A carried a single ESBL (pI 8.2 and a positive PCR result for the SHV type), and the other three carried two different ESBLs (pIs 8.1 and 8.2 and positive PCR results for the SHV and CTX-M-9 types). In these strains, ESBL screening by DDST showed an enlarged zone of inhibition between a disk of amoxicillin plus clavulanate and disks of cefotaxime, ceftazidime, aztreonam, and cefepime. All of the isolates that belonged to clone B carried a single ESBL (pI 5.4 and a positive PCR result for the TEM type). In these strains, ESBL screening by DDST showed an enlarged zone of inhibition only between a disk of amoxicillin plus clavulanate and a disk of cefepime. In three patients, other ESBL-producing enterobacteria (*E. coli* and *K. pneumoniae*) were isolated at the same time. The antibiotic resistance profiles of all isolates of ESBL-producing *E. cloacae* showed, in addition to resistance to the expanded-spectrum penicillin and cephalosporins, including cefepime, resistance to quinolones, gentamicin, tobramycin, and trimethoprim-sulfamethoxazole. In addition, two patients, those with clone B, showed diminished sensitivity to carbapenems (imipenem MIC = 1 to 2 μg/ml; meropenem MIC = 1 μg/ml; ertapenem MIC = 2 to 12 μg/ml). Determination of carbapenemase production was negative.

Review of antibiotic consumption at the CT-ICU from January to July 2005 showed increased use of quinolones and cefepime just 2 months prior to the outbreak. No consumption of broad-spectrum cephalosporins was recorded from March to May, and in June this was 11 defined daily doses (DDD)/100 bed days. Consumption of quinolones increased from an average of 2 DDD/100 bed days per month in the first semester to 15.5 DDD/100 bed days in July. This increase was due to an antibiotic cycling strategy that was implemented in that unit during this period (Fig. 2).

In July 2005, all patients with ESBL-producing *E. cloacae* infection or colonization were placed in contact isolation. Fecal carriage screening was done from July 2005 to October 2005, 40 days after the last isolate of ESBL-producing *E. cloacae* was identified. Finally, 41 rectal swabs were obtained; however, only 3 were positive for ESBL-producing *E. cloacae* (only one patient was detected by screening). The outbreak

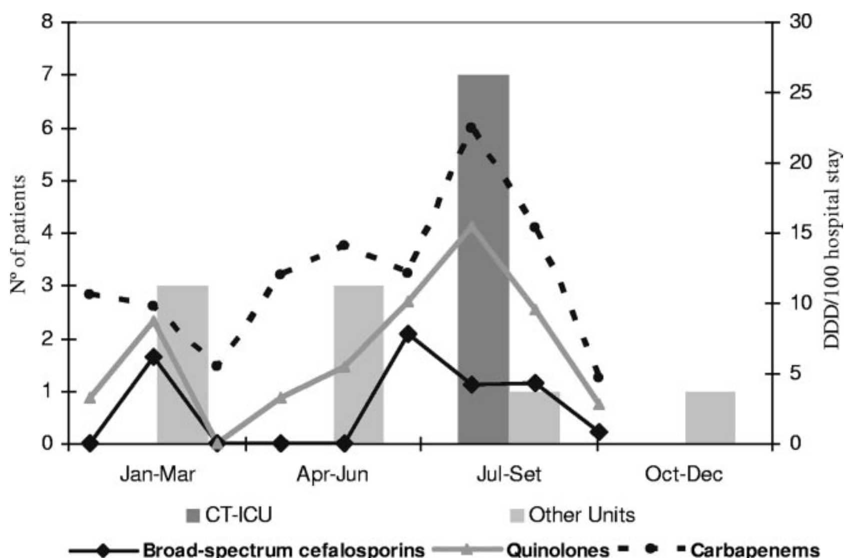

FIG. 1. Number of patients with ESBL-producing *E. cloacae* involved in an CT-ICU outbreak during 2005. The relationship with previous antibiotic consumption in the CT-ICU is shown.

was limited by the implementation of barrier measures and cephalosporin restriction to 3.4 and 1.2 DDD/100 bed days in August and September, respectively.

DISCUSSION

There is considerably less information on the clinical and microbiological epidemiology of ESBL-producing *E. cloacae* than on that of other ESBL producers such as *E. coli* or *K. pneumoniae*. In Europe, it is only mandatory to report invasive *E. coli* isolates as part of the European Antimicrobial Resistance Surveillance System. In 2005, detailed data from the European Antimicrobial Resistance Surveillance System identified a large increase in ESBL-producing *E. coli* isolates, although no data were available for *Enterobacter* spp. In Spain, a nationwide epidemiological study revealed that the prevalences of ESBL-producing *K. pneumoniae* and *E. coli* strains were 2.7% and 0.5%, respectively (10). The prevalence of ESBL-producing *E. cloacae* studied in a Spanish hospital after a 12-year period was 0.4% of all *Enterobacter* sp. isolates, while the prevalence of *Enterobacter* spp. with a constitutive phenotype of AmpC hyperproduction was approximately 20% (3). Studies performed in French (20), Greek (35), German (12), and Spanish (10) hospitals suggest a significant increase in fecal carriers, as well as in clinical isolates, of ESBL-producing *E. cloacae* during the last decade.

This article describes our experience with an outbreak caused by ESBL-producing *E. cloacae* at a CT-ICU in July and August 2005 that was temporally associated with increased use of cefepime and quinolones during the implementation of an antibiotic cycling strategy in that unit. This increase coincided with the identification of a postsurgical mediastinitis outbreak caused by *Staphylococcus aureus* in the same unit at the same time.

Identification of ESBL production in several strains of *E. cloacae*, still an unusual mechanism of resistance in this species, was the initial evidence of the outbreak. Spread through cross-transmission was suspected because of the kinds of infection—mostly device associated—and the low frequency of fecal carriers and was confirmed by the clonality of isolates detected by PFGE.

Three different β-lactamase types were characterized in the *E. cloacae* strains involved in the outbreak, SHV and CTX-M-9 (both clone A) and TEM (clone B). These β-lactamases are

TABLE 1. Clinical characteristics of patients involved in the outbreak

Patient no.	Date (day/mo)	Sex[a]/age (yr)	Type of infection	Previous antibiotic use	Treatment	Fecal carriage	Outcome
1	01/07	F/73	BSI[b]	Cefepime	Imipenem	No	Death
2	25/07	F/65	Colonization	Cloxacillin		Yes	Discharge
3	29/07	M/70	Tracheobronchitis	None	Ciprofloxacin	Yes	Discharge
	16/08		BSI		CVC[d] withdrawal		
4	03/08	M/75	VAP[c]	Cloxacillin	Imipenem		Death
5	12/08	F/76	Colonization	Cefepime		No	Death
6	24/08	M/45	Colonization	None		Yes	Discharge
7	31/08	F/76	Tracheobronchitis	Cefepime	Imipenem	No	Discharge

[a] M, male; F, female.
[b] BSI, bloodstream infection.
[c] VAP, ventilator-associated pneumonia.
[d] CVC, central venous catheter.

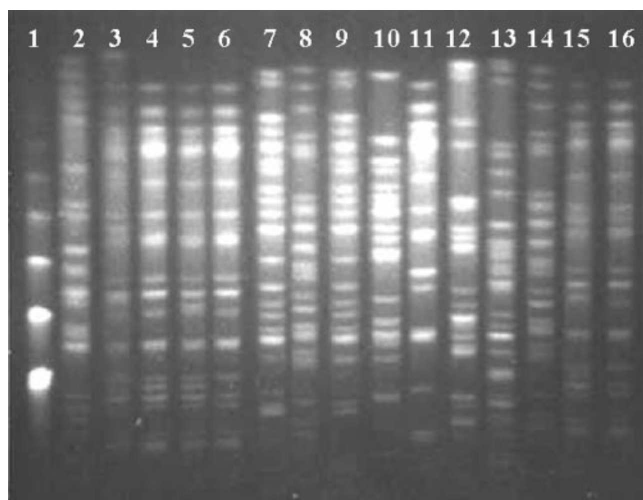

FIG. 2. PFGE patterns of genomic DNAs of *E. cloacae* isolates. Lane 1, molecular weight marker (lambda ladder PFG marker; New England BioLabs). ESBL-producing *E. cloacae* strains involved in the outbreak, lanes 3 to 6, 15, and 16 (belonging to clone A) and lanes 8 and 14 (designated clone B). Lanes 2, 7, and 9 to 13, unrelated strains of *E. cloacae*.

widely disseminated among strains of *K. pneumoniae* and *E. coli*. Simultaneous isolation of other ESBL-producing microorganisms from three of our patients may suggest a transferable plasmid encoding the production of ESBL as the initial trigger of the outbreak; these ESBLs were not characterized. The selective pressure exerted by extended-spectrum cephalosporins has been reported as a significant risk factor for the emergence of ESBL-producing members of the family *Enterobacteriaceae*, particularly among strains of *K. pneumoniae* (29). Antibiotic consumption in general and particularly the concentrated consumption of cefepime and ciprofloxacin seemed to play an important role in the emergence of ESBL-producing *E. cloacae* in the CT-ICU. This brings into question the efficacy of strategies, such as antibiotic cycling, that concentrate on the consumption of a particular antibiotic family (19).

The low incidence of fecal ESBL-producing *E. cloacae* carriage during active surveillance, even in patients who developed infections, was striking. This conflicts with our previous understanding of outbreaks of other gram-negative bacilli, such as ESBL-producing *K. pneumoniae* (28) or *Acinetobacter baumannii* (11), with high rates of intestinal carriage. Although there is no clear justification for this low rate of fecal carriage, it may be due to the limited sensitivity of swab samples in identifying colonized patients (7). The presence of undetected environmental reservoirs cannot be ruled out; environmental cultures were not performed. This was suggested during the investigation of an outbreak in a CT-ICU (16).

In *E. cloacae*, ESBL production needs to be distinguished from a constitutive AmpC hyperproduction phenotype, the most frequent mechanism of broad-spectrum cephalosporin resistance in this species. As screening methods recommended for *E. coli* and *K. pneumoniae* that are based on resistance to ceftazidime or cefotaxime may not be adequate for *Enterobacter* spp., an increased cefepime MIC may be a reliable marker for the presence of an ESBL in these species (1). This can be confirmed by the DDST. This distinction has only limited clinical impact because therapeutic options are similarly limited, but there is evident epidemiological significance.

It has been suggested that ESBLs are related to the acquisition of plasmids encoding resistance to non-β-lactam antibiotics, including aminoglycosides and trimethoprim-sulfamethoxazole, and recently described *qnrA* determinants that confer resistance to quinolones (24, 25, 30, 32). We also found increased MICs of carbapenems in two patients with ESBL-producing *E. cloacae* (clone B). In these strains, as screening for carbapenemases was negative, altered sensitivity to carbapenems may be due to distorted outer membrane permeability, although this was not further investigated. Decreased susceptibility to carbapenems is uncommon in ESBL-producing *E. cloacae*. Expression of AmpC or a class A ESBL plus loss of outer membrane proteins has been associated with carbapenem resistance in enterobacteria (21, 26). As carbapenems are considered the preferred agents for treating infections caused by ESBL-producing enterobacteria, diminished sensitivity would have more clinical impact if these strains became more prevalent in the future. Therefore, only a few antibiotic alternatives for severe infections, such as tigecycline, will be available.

Interventions such as contact isolation, antibiotic restriction, and active surveillance lead to rapid control of the outbreak. Because all interventions were applied simultaneously, the contribution of each one is difficult to assess. Patient-to-patient transmission of ESBL-producing *E. cloacae* by the hands of health care workers in the CT-ICU seemed to be common. In

TABLE 2. PFGE results, pIs, and PCR results for the CTX-M-9, TEM, and SHV families of ESBL-producing *E. cloacae*

Patient no.	Antibiotic resistance profile[a]	pI(s)	*bla* gene type[b]			PFGE result
			CTX-M-9	SHV	TEM	
1	GEN-TOB-CIP-SXT	8.1, 8.2	+	+	−	A
2	GEN-TOB-CIP-SXT	8.2	−	+	−	A
3	GEN-TOB-CIP-SXT	8.1, 8.2	+	+	−	A
	GEN-TOB-CIP-SXT	8.1, 8.2	+	+	−	A
4	GEN-TOB-CIP-SXT	5.4	−	−	+	B
5	GEN-TOB-CIP-SXT	8.2	−	+	−	A
6	GEN-TOB-CIP-SXT	8.1, 8.2	+	+	−	A
7	GEN-TOB-CIP-SXT	5.4	−	−	+	B

[a] GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole.
[b] TEM corresponds to a pI of 5.4. CTX-M9 corresponds to a pI of 8.1. SHV corresponds to a pI of 8.2.

this setting, implementation of barrier measures for all infected or colonized patients and reinforcement of hand hygiene with alcoholic gel were the keystone control measures. Although it has been considered controversial (5), restriction of wide-spectrum cephalosporins has been successfully applied in several outbreaks caused by ESBL-producing *K. pneumoniae* (2, 28). In our case, the circumstance of concomitant increased cefepime use and the emergence of ESBL-producing *E. cloacae* allowed us to introduce this measure with an effective result.

In conclusion, the increasing prevalence of ESBL-producing *E. cloacae* is becoming a concern, not only for infection therapy and empirical use of antibiotics but also for infection control programs. It is important to maintain active surveillance at microbiologic laboratories to detect ESBLs, even in species which still have a low prevalence of this mechanism of resistance.

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