Published in "International Journal of Antimicrobial Agents
46(4): 469–474, 2015"
which should be cited to refer to this work.

Dissemination of multiresistant *Enterobacter cloacae* isolates producing OXA-48 and CTX-M-15 in a Spanish hospital

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Twenty-one multiresistant *Enterobacter cloacae* isolates producing OXA-48 ($n = 10$), CTX-M-15 ($n = 7$) or both ($n = 4$) β-lactamases were detected in a Spanish hospital during a 1-year period (June 2013 to June 2014). The isolates were also resistant to non-β-lactam antimicrobials, further complicating the therapeutic options. Genotyping of the isolates identified two major clones (ST74 and ST66) that caused prolonged outbreaks in different buildings of the hospital as well as some sporadic isolates (ST78, ST45 and ST295). Isolates belonging to clone 1 ($n = 7$) were carbapenem-resistant and carried the bla_{OXA-48} gene on a conjugative IncL/M plasmid of ca. 65 kb. Clone 2 isolates ($n = 11$) were resistant to cefepime and harboured the $bla_{CTX-M-15}$ gene on an ca. 150-kb, non-conjugative plasmid of the IncF group, co-harbouring the *qnrB* and *aac(6′)-Ib*-cr genes encoding quinolone resistance. Four clone 2 isolates were also resistant to carbapenems owing to the co-production of OXA-48. Most of the isolates were recovered from critically ill patients and were admitted to intensive care units; a single patient was transferred from another Spanish hospital. Intrahospital and interhospital dissemination of multiresistant *E. cloacae* isolates is of major clinical concern as it could lead to endemic nosocomial situations.

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1. Introduction

Enterobacter cloacae is an opportunistic pathogen frequently involved in nosocomial infections [1]. This enterobacterial species is intrinsically resistant to aminopenicillins, amoxicillin/clavulanic acid (AMC) and cephalosporins of early generations owing to a chromosomally encoded AmpC β-lactamase. Isolates overproducing AmpC are also resistant to broad-spectrum cephalosporins. Furthermore, *E. cloacae* has the ability to acquire additional resistance mechanisms, including plasmid-encoded extended-spectrum β-lactamases (ESBLs) and carbapenemases [1]. Phenotypic detection of ESBL production may be difficult since it can be masked by overexpression of AmpC. Some *E. cloacae* isolates may also exhibit permeability defects due to the absence of porins or porin mutations, further increasing the minimum inhibitory concentrations (MICs) of β-lactams and carbapenems, particularly ertapenem [1].

In members of the *Enterobacteriaceae* family, CTX-M-15 and OXA-48 are among the most common enzymes involved in resistance to broad-spectrum cephalosporins and carbapenems, respectively [2,3]. CTX-M-15 is a class A ESBL encoded by the $bla_{CTX-M-15}$ gene, which has been identified in plasmids of varying size (85–200 kb) and structure, and often belonging to the FII incompatibility (Inc) group [4]. OXA-48 is an Ambler class D carbapenemase that confers resistance to penicillins and reduced susceptibility to carbapenems but does not significantly hydrolyse broad-spectrum cephalosporins [3]. The bla_{OXA-48} gene has been almost always located on a conjugative plasmid of ca. 62 kb assigned to the IncL/M group and has been identified only in enterobacterial species [5,6]. Both CTX-M-15 and OXA-48 have been detected in several enterobacterial species, being particularly frequent in *Klebsiella pneumoniae*, whilst they are relatively uncommon in *E. cloacae* [2,3]. Here we report the clinical and genetic features of 21 *E. cloacae* isolates producing CTX-M-15, OXA-48 or both, which were mainly recovered from critically ill patients, in a Spanish hospital during a 1-year period.

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2. Patients and methods

2.1. Setting, patients and bacterial isolates

'Hospital Universitario Central de Asturias' (HUCA) (Oviedo, Spain) is a 1100-bed university hospital providing care for a population of ca. 342,000 inhabitants in the Asturias region of northern Spain. During the 1-year period from June 2013 to June 2014, all *E. cloacae* isolates recovered at the hospital ($n = 448$) were analysed phenotypically for production of carbapenemases and ESBLs. A total of 21 multiresistant isolates, all recovered from different patients, produced a carbapenemase, an ESBL or both. Most *E. cloacae* isolates were obtained from critically ill patients admitted to two intensive care units (ICU1 and ICU2) located in different buildings or to other hospital wards. Relevant features regarding the patients are shown in Table 1.

2.2. Susceptibility testing

All *E. cloacae* isolates underwent antimicrobial susceptibility testing by the disc diffusion assay (Becton Dickinson, Sparks, MD). The MicroScan® system (Neg Combo Panel Type 53; Siemens Healthcare Diagnostics, Deerfield, IL) was used for determination of MICs and for bacterial identification, and the latter was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (microflex™; Bruker Daltonik GmbH, Bremen, Germany). Results of disc diffusion assays and MICs were interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints updated in January 2014 [7]. Etest strips (bioMérieux, Marcy-l'Étoile, France) were used for determining the MICs of ertapenem and imipenem in those isolates suspected of carbapenemase production. The Carba NP test recommended for detection of carbapenemases [8] was also performed for isolates with elevated MICs of ertapenem and imipenem. All *E. cloacae* suspected of ESBL production (double-disc synergy test positive and/or elevated MIC to cefepime) were tested with the cefepime/cefepime + clavulanic acid (PM/PML) Etest (bioMérieux).

2.3. Identification of genes encoding extended-spectrum β-lactamases and carbapenemases, and plasmid analysis

Genes encoding resistance to broad-spectrum cephalosporins (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) and carbapenems (bla_{OXA-48}) were identified by PCR amplification [5] followed by sequencing. Transposon Tn*1999* (known to carry the bla_{OXA-48} gene) and plasmid-mediated quinolone resistance genes [*qnrA*, *qnrB*, *qnrS* and *aac(6′)-Ib*-cr] were searched by PCR amplification followed by sequencing of the obtained products [3,9]. Plasmid DNA was extracted by the Kado and Liu method [5] and was visualised by agarose gel electrophoresis.

Plasmid Inc groups were determined by PCR-based replicon typing [5]. Conjugation experiments were performed using as donor isolates representatives of three antibiotic resistance phenotypes, namely resistance to carbapenems but susceptibility to cefepime (Ecl10), resistance to cefepime but susceptibility to carbapenems (Ecl8 and Ecl9), or resistance to both (Ecl15). A rifampicin-resistant *Escherichia coli* J53 strain was used as recipient for conjugation assays. Transconjugants were selected on eosin–methylene blue agar (Oxoid, Madrid, Spain) containing rifampicin (100 mg/L) plus ertapenem (0.5 mg/L) or rifampicin plus ceftriaxone (10 mg/L). Plasmid DNA extracted from each of the donor *E. cloacae* and their transconjugants was transferred to a nylon membrane and was then hybridised with probes specific for the $bla_{CTX-M-15}$ and bla_{OXA-48} genes. Probes were labelled using the PCR DIG Labeling Mix (Roche Applied Science, Penzberg, Germany), followed by gel extraction with the GFX™ DNA and Gel Band Purification Kit (Amersham Biosciences, Little Chalfont, UK).

2.4. Pulsed-field gel electrophoresis (PFGE) analysis

Total DNA from the 21 *E. cloacae* isolates was digested with *Xba*I (Takara Bio Europe, Saint-Germain-en-Laye, France; 30 U, 4 h at 37 °C). The generated fragments were separated by PFGE using a CHEF-DR III System (Bio-Rad Laboratories, Hercules, CA) under the conditions recommended by PulseNet (http://www.pulsenetinternational.org). Similarity between *Xba*I profiles was evaluated by the Jaccard's coefficient (*S*), and cluster analysis was performed by the unweighted pair-group method with arithmetic averages (UPGMA) using the software program MVSP (Multivariate Statistical Package for PCs; RockWare Inc., Golden, CO).

2.5. Multilocus sequence typing (MLST)

MLST was performed for representative isolates with different PFGE profiles (Ecl10, Ecl3, Ecl9, Ecl8, Ecl21, Ecl5 and Ecl7) as described previously [10]. The database available at http://pubmlst.org/ecloacae was used for assigning sequence types (STs).

3. Results

3.1. Antimicrobial susceptibility and genetic basis for resistance to broad-spectrum cephalosporins and carbapenems

As shown in Table 2, 20 of the 21 *E. cloacae* isolates were resistant to cefotaxime and 11 were also resistant to cefepime. Moreover, 14 isolates displayed intermediate susceptibility or resistance to carbapenems. In addition to β-lactam antibiotics, all *E. cloacae* isolates were variably resistant to other antimicrobial groups, including quinolones (nalidixic acid and ciprofloxacin), aminoglycosides (gentamicin and tobramycin) and trimethoprim/sulfamethoxazole.

The $bla_{CTX-M-15}$ gene was detected in all isolates resistant to cefepime and positive for the PM/PML test ($n = 11$), whilst the bla_{OXA-48} gene was found in all isolates with elevated MICs of imipenem and positive for the Carba NP test ($n = 14$). Both $bla_{CTX-M-15}$ and bla_{OXA-48} were identified in four isolates. Overall, isolates harbouring $bla_{CTX-M-15}$, bla_{OXA-48} or both accounted for 1.6%, 2.2% and 0.9% of the total number of *E. cloacae* recovered in HUCA over the 1-year study period ($n = 448$).

The genetic location of $bla_{CTX-M-15}$ and bla_{OXA-48} was established by plasmid analysis, hybridisation and conjugation experiments. As shown in Fig. 1A and Table 2, all ESBL-producers carried a plasmid of ca. 150 kb hybridising with the $bla_{CTX-M-15}$ probe and belonging to the IncF group. Furthermore, all imipenem-resistant isolates harboured a common plasmid of ca. 65 kb that gave a positive hybridisation signal with the bla_{OXA-48} probe and belonged to the IncL/M group. Noteworthy, the bla_{OXA-48} gene was always identified in transposon Tn*1999.1*.

To investigate the potential of transfer of the IncF and IncL/M plasmids carrying the $bla_{CTX-M-15}$ and bla_{OXA-48} genes, conjugation experiments were performed using Ecl8 and Ecl9 ($bla_{CTX-M-15}$), Ecl10 (bla_{OXA-48}) and Ecl15 ($bla_{CTX-M-15}$ and bla_{OXA-48}) as donors. When selection was performed with ertapenem, *E. coli* transconjugants were readily obtained both from Ecl10 and Ecl15 donor strains. Three independent *E. coli* transconjugants analysed from each mating experiment showed resistance to ampicillin, AMC and ertapenem, were positive by PCR for the bla_{OXA-48} gene and carried an ca. 65 kb plasmid (Fig. 1A; Table 2). By contrast, no transconjugants were obtained after three independent conjugations using Ecl8 and Ecl9 as donors and ceftriaxone as selective agent, and only two transconjugants after the same number of

Table 1
Features of the 21 patients infected or colonised by multiresistant *Enterobacter cloacae* isolates.

Patient	Sex/age (years)	Hospital unit	Admission diagnosis/colonisation or infection type	Sample origin	Date of isolation	Treatment	Outcome
1	M/46	ICU1	Pancreatitis/surgical wound infection	Wound exudate	25/06/2013	Tobramycin	Discharged
2	F/56	ICU1	Lung cancer/pneumonia	Lung necropsy	26/06/2013	TZP	Died
3	F/77	ICU2	Cardiac surgery/respiratory colonisation	Tracheobronchial aspirate	5/07/2013	Untreated	Discharged
4	M/19	ICU1	Severe head injury/urinary tract infection	Urine	12/08/2013	Ciprofloxacin	Died
5	M/81	Reanimation	Vascular surgery/respiratory infection	Tracheobronchial aspirate	23/12/2013	Ciprofloxacin/ gentamicin/meropenem	Died
6	M/81	ICU2	Vascular surgery/pneumonia	Tracheobronchial aspirate	26/12/2013	Imipenem	Discharged
7	M/76	General surgery	Abdominal surgery/surgical wound infection	Wound exudate	27/12/2013	TZP	Discharged
8[a]	F/57	Pneumology unit	Lung transplantation follow-up/respiratory infection	Sputum	17/01/2014	Amikacin/meropenem	Discharged
9	M/41	ICU2	Septic shock/pneumonia	Tracheobronchial aspirate	21/01/2014	Meropenem	Discharged
10	F/33	ICU1	H1N1 flu pneumonia/respiratory colonisation	Tracheobronchial aspirate	23/01/2014	Untreated[b]	Discharged
11	F/53	ICU1	H1N1 flu pneumonia/respiratory colonisation	Tracheobronchial aspirate	27/01/2014	Untreated[b]	Discharged
12	M/79	Cardiac surgery	Cardiac surgery/urinary tract infection	Urine[c]	29/01/2014	SXT	Discharged
13	M/36	ICU2	H1N1 flu pneumonia/pneumonia	Pleural fluid	11/02/2014	TZP/colistin[b]	Died
14	M/62	Cardiac surgery	Cardiac surgery/asymptomatic bacteriuria	Urine	17/02/2014	Untreated	Discharged
15	F/30	Cardiology	Heart transplantation/surgical wound infection	Wound exudate	19/02/2014	Colistin/tigecycline	Discharged
16	F/61	ICU2	Cardiac surgery/surgical wound infection	Wound exudate	17/03/2014	Imipenem	Discharged
17	F/66	Internal medicine	Respiratory failure/asymptomatic bacteriuria	Urine	24/03/2014	Untreated	Discharged
18	M/69	Neurology	Epileptic attack/urinary catheter colonisation	Urine	24/04/2014	Untreated	Discharged
19	F/92	Maxillofacial surgery	Maxillofacial surgery/urinary tract infection	Urine	29/04/2014	Meropenem	Discharged
20	M/66	Cardiac surgery	Cardiac surgery/surgical wound infection	Wound exudate	13/05/2014	Unknown[d]	Discharged
21	M/79	ICU2	Vascular surgery/catheter sepsis	Catheter tip	14/05/2014	Imipenem	Discharged

ICU, intensive care unit; TZP, piperacillin/tazobactam; SXT, trimethoprim/sulfamethoxazole.
[a] Patient transferred from another hospital in a nearby region, where the *E. cloacae* infection had already been diagnosed.
[b] Patients 10, 11 and 13 were treated with oseltamivir.
[c] Another *E. cloacae* isolate with a different resistance pattern and not producing extended-spectrum β-lactamase (ESBL) or OXA-48 was detected in the same sample.
[d] The sample from this patient was collected in a primary care centre a few days after have being discharged of the Cardiac Surgery Unit of the hospital. There are no treatment data for this patient.

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matings and ceftriaxone selection using Ecl15 as donor. Both Ecl15 transconjugants were resistant to ampicillin, AMC, broad-spectrum cephalosporins and aminoglycosides, had an elevated MIC of ciprofloxacin and carried two plasmids, one of ca. 150 kb and the other slightly larger than 65 kb, which hybridised with the $bla_{CTX-M-15}$ and bla_{OXA-48} probes, respectively (Fig. 1A; Table 2). The latter could have derived from the ca. 65 kb plasmid through acquisition of additional DNA. Taken together, these results strongly suggest that the ca. 150 kb IncF plasmid carrying $bla_{CTX-M-15}$ is not self-transferable, but it could be mobilised by the ca. 65 kb plasmid though at a rather low frequency. The *qnrB* and *aac(6′)-Ib-*cr genes were detected in the Ecl15 transconjugants as well as in the *E. cloacae* isolates carrying the IncF plasmid, all with decreased susceptibility to ciprofloxacin.

3.2. Genotyping of the CTX-M-15- and/or OXA-48-producing Enterobacter cloacae isolates

Typing of the 21 *E. cloacae* isolates by PFGE yielded seven profiles (X1–X7) (Fig. 1B). A dendrogram of similarity based on these profiles revealed two main clusters of *E. cloacae* (clusters I and II) that were strongly associated with the two separate buildings of HUCA (Fig. 1C). Cluster I comprised two profiles (X1 and X5) with seven and one isolates, respectively. A representative X1

Table 2
Microbiological characteristics of clinical *Enterobacter cloacae* isolates and their transconjugants.

Isolate[a,b]	Antimicrobial resistance phenotype	ESBL/CARB gene	ERT/IPM MIC (mg/L)	Corresponding plasmids	PFGE profile/ cluster/building[c]	MLST
Ecl1, Ecl2, Ecl4, **Ecl10**, Ecl11, Ecl17, Ecl18	AMX-AMC-FOX-CTX-ERT-IPM-NAL-CIP-SXT	$bla_{\text{OXA-48}}$	4/2	65 kb[d]	X1/I/1	ST74
Tc-Ecl10-ERT (1, 2 and 3)	AMX-AMC-ERT	$bla_{\text{OXA-48}}$	2/0.5	65 kb[d]	N/A	N/A
Ecl3	AMX-AMC-FOX-CTX-GEN-ERT-IPM-NAL-CIP-SXT	$bla_{\text{OXA-48}}$	3/2	65 kb[d]	X5/I/2	ST78
Ecl6, Ecl12, Ecl14, Ecl16, Ecl20	AMX-AMC-FOX-CTX-FEP-GEN-TOB-NAL-CIP[e]	$bla_{\text{CTX-M-15}}$	≤0.5/≤1	150 kb[f]	X2/II/2	ST66
Ecl9					X3/II/2	
Ecl8	AMX-AMC-FOX-CTX-FEP-GEN-TOB-NAL-CIP[e]-SXT	$bla_{\text{CTX-M-15}}$	≤0.5/≤1	150 kb[f]	X4/II/1	ST66
Ecl19, Ecl21	AMX-AMC-FOX-CTX-FEP-ERT-IPM-GEN-TOB-NAL-CIP[e]	$bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-48}}$	4–6/2–4	150 kb[f], 65 kb[d]	X2/II/2	ST66
Ecl13, **Ecl15**					X3/II/2	
Tc-Ecl15-ERT (1, 2 and 3)	AMX-AMC-ERT	$bla_{\text{OXA-48}}$	2/0.5	65 kb[d]	N/A	N/A
Tc-Ecl15-CRO (1 and 2)	AMX-AMC-CTX-FEP-ERT-GEN-TOB-CIP[e]	$bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-48}}$	1/0.38	150 kb[f], >65 kb[d]	N/A	N/A
Ecl5	AMX-AMC-FOX-ERT-IPM-NAL-CIP-SXT	$bla_{\text{OXA-48}}$	4/4	65 kb[d]	X6/–/1	ST45
Ecl7	AMX-AMC-FOX-CTX-ERT-IPM-GEN-TOB-NAL-CIP-SXT	$bla_{\text{OXA-48}}$	8/4	65 kb[d]	X7/–/1	ST295

ESBL, extended-spectrum β-lactamase; CARB, carbapenemase; MIC, minimum inhibitory concentration; PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence type; AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; FOX, cefoxitin; CTX, cefotaxime; ERT, ertapenem; IPM, imipenem; NAL, nalidixic acid; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; FEP, cefepime; TOB, tobramycin; CRO, ceftriaxone; N/A, not applicable.

[a] *Enterobacter cloacae* (Ecl) isolates with numbers corresponding to patient numbers in Table 1. Ecl isolates used as donors in conjugation experiments are shown in bold. Ecl isolates for which the MLST was determined are underlined.

[b] *Escherichia coli* transconjugants designated with Tc followed by the *E. cloacae* donor isolate and the antimicrobial used for selection: ERT (ertapenem) or CRO (ceftriaxone); the number of transconjugants tested is shown in parenthesis. No transconjugants were obtained for Ecl9 and Ecl8 after selection with CRO.

[c] See Fig. 1C and text for details.

[d] IncL/M plasmids.

[e] Isolates positive for the *qnrB* and *aac(6′)-Ib*-cr genes.

[f] IncF plasmids.

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isolate and the X5 isolate belonged to ST74 and ST78, respectively, within the same clonal complex (Fig. 1C; Table 2). All X1 isolates were recovered in building 1, which houses the main ICU of the hospital (ICU1), where five of them were detected. The remaining two came from the internal medicine and neurology services. All isolates within cluster I were resistant to carbapenems owing to production of OXA-48 and all were also resistant to broad-spectrum cephalosporins, consistent with overexpression of the chromosomal bla_{ampC} gene. Cluster II was associated with a second building of the hospital encompassing the cardiac and maxillofacial surgery units and another ICU (ICU2). It included 11 isolates with identical (X2; 7 isolates) or closely related (X3 and X4, with 3 and 1 isolates, respectively) profiles assigned to ST66, and all except one (Ecl8; X4 profile) were recovered in building 2 (Fig. 1C; Table 2). Interestingly, Ecl8 was identified from a single patient who had been transferred from a hospital in the nearby region of Santander, where the *E. cloacae* infection had already been diagnosed. Isolates within cluster II were all resistant to broad-spectrum cephalosporins owing to the production of CTX-M-15, and 4 of the 11 isolates also harboured $bla_{\text{OXA-48}}$ and were therefore resistant to carbapenems. Two distantly related profiles (X6 and X7) were shown by OXA-48-producers belonging to ST45 and ST295, respectively, and found in building 1 (Fig. 1C; Table 2).

4. Discussion

Enterobacter cloacae isolates producing OXA-48, CTX-M-15 or both β-lactamases emerged as important pathogens in HUCA, a general university hospital in northern Spain. In fact, they accounted for 4.7% (21/448) of the total *E. cloacae* isolates recovered over a 1-year period, although they were not previously reported in this region. Together with some sporadic isolates, two major clones (ST74 and ST66, falling in two different clusters according to PFGE analysis) are causing prolonged outbreaks in separate buildings of the hospital. The ST74 clone consisted of carbapenem-resistant isolates carrying the $bla_{\text{OXA-48}}$ gene. This is the first outbreak caused by OXA-48-producing *E. cloacae* reported in Europe, but a previous outbreak has been noticed in a neonatal ICU in Jerusalem [11]. In contrast to that observed here, isolates from Israel carried a derivative of the OXA-48 plasmid that had acquired the $bla_{\text{CTX-M-14}}$ gene. The second clone identified (ST66) corresponded to $bla_{\text{CTX-M-15}}$-positive isolates with the ESBL gene located on an ca. 150-kb, non-conjugative plasmid of the IncF group. Some of them co-harboured the IncL/M plasmid and co-produced OXA-48. Interestingly, one of the clone 2 isolates was apparently transferred from another hospital located in a nearby region of Spain, so in addition to intrahospital dissemination, interhospital spread is contributing to the emergence of CTX-M-15-producing *E. cloacae* in HUCA. Additional outbreaks of *E. cloacae* producing CTX-M-15 have occurred in a newborn ICU in Spain as well as in a children's hospital in Finland [12,13].

Noticeably, some isolates belonging to clone 2 also carried the $bla_{\text{OXA-48}}$ plasmid, which could have been acquired from other OXA-48-producing *Enterobacteriaceae* circulating in the hospital [5; our unpublished results]. This might be in accordance with the high frequency of dissemination of the $bla_{\text{OXA-48}}$-carrying plasmid as previously demonstrated [14].

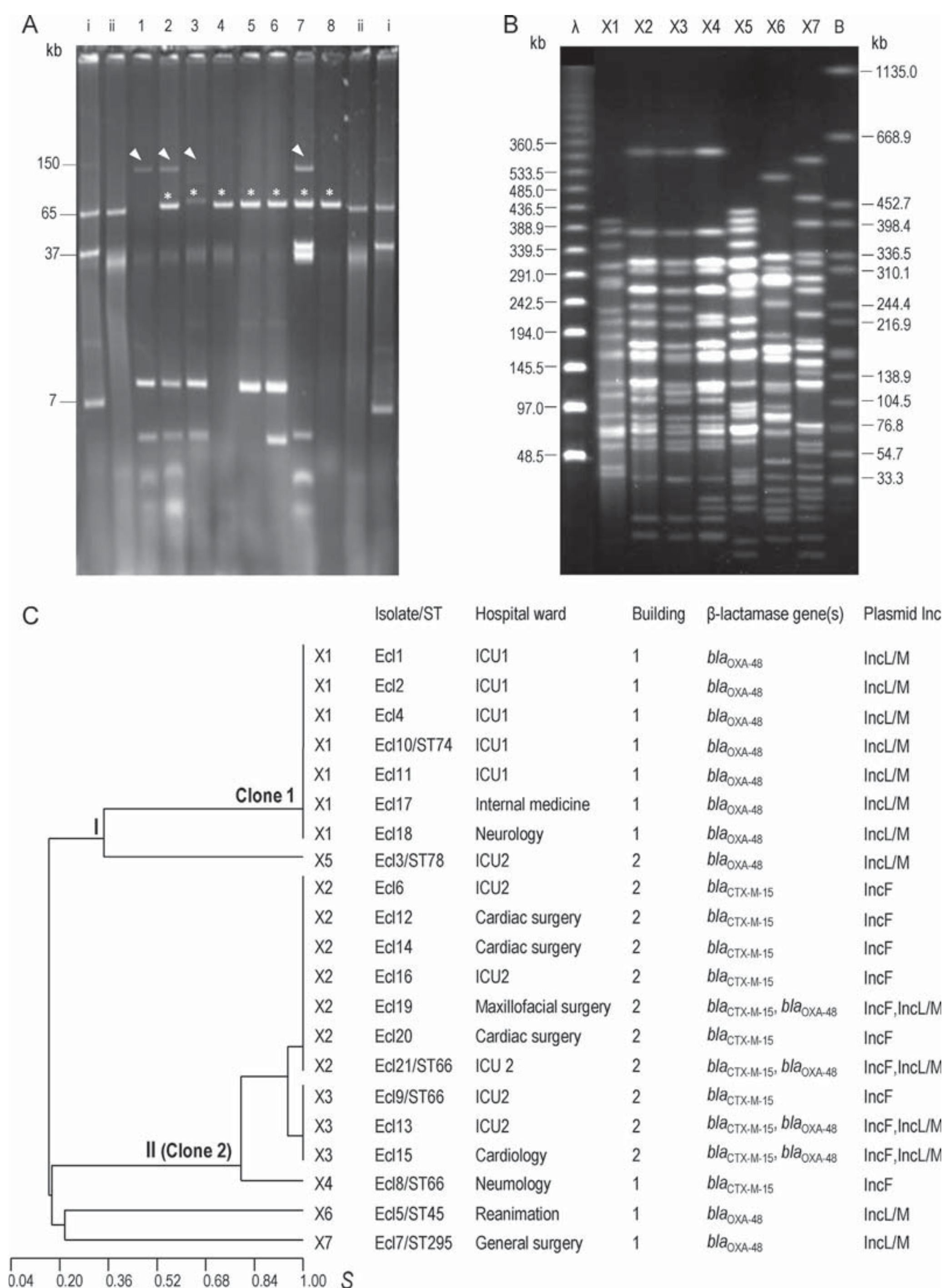

Fig. 1. Molecular typing and genetic relationships of *Enterobacter cloacae* isolates producing OXA-48 and CTX-M-15 β-lactamases in a Spanish hospital. (A) Plasmid profiles of representative *E. cloacae* isolates and their transconjugants. Lanes i and ii, plasmids obtained from *Escherichia coli* 39R861 (NCTC 50192) and plasmid RP4 used as molecular size standards for uncut DNA; lane 1, Ecl9; lane 2, Ecl15; lane 3, Tc-Ecl15-CRO (1); lane 4, Tc-Ecl15-ERT (1); lane 5, Tc-Ecl15-ERT (2); lane 6, Tc-Ecl15-ERT (3); lanes 7, Ecl10; and lane 8, Tc-Ecl10-ERT (1). Plasmids hybridising with $bla_{CTX-M-15}$ and bla_{OXA-48} probes are marked with arrowheads and asterisks, respectively. (B) Pulsed-field gel electrophoresis (PFGE) profiles generated from *E. cloacae* isolates by *Xba*I digestion. Lane λ, Lambda Ladder PFG Marker (New England Biolabs, Beverly, MA) and lane B, DNA from *Salmonella enterica* serovar Braenderup H9812 digested with *Xba*I, both used as size standards. Lane X1, Ecl10; lane X2, Ecl6; lane X3, Ecl9; lane X4, Ecl8; lane X5, Ecl3; lane X6, Ecl5; and lane X7, Ecl7. (C) Dendrogram of similarity showing the relatedness between the *Xba*I profiles. Isolates assigned to each profile as well as the hospital ward and building in which they were collected are shown. Relevant genes and plasmids carried by each isolate are also indicated. Clusters I and II comprise ST74 (clone 1)/ST78 (positive for bla_{OXA-48}) and ST66 (clone 2, positive for $bla_{CTX-M-15} \pm bla_{OXA-48}$) isolates, respectively. X, *Xba*I profile; ST, sequence type; ICU, intensive care unit; Inc, incompatibility group; $\underline{S}$, Jaccard's coefficient of similarity.

Occurrence of the *qnrB* and *aac(6′)-Ib*-cr genes in enterobacterial isolates producing OXA-48 or ESBLs, including some of the CTX-M-15-producing *E. cloacae* isolates causing the outbreak in a neonatal unit in Spain, was previously noticed [12].

Most *E. cloacae* reported in this study were assigned to STs previously associated with ESBL- and carbapenemase-producers and considered as high-risk international clones (ST45, ST66, ST74 and ST78), with the latter two belonging to the same clonal complex [10,15]. Noteworthy, none of these clones had been previously

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associated with OXA-48 production. Since phylogenetic studies on multiresistant *E. cloacae* have been initiated only recently, further studies are required to expand the knowledge of these multiresistant bacteria.

Although the number of *E. cloacae* producing carbapenemases or ESBLs has increased in recent years, their real prevalence could be underestimated because of difficulties in detecting both β-lactamases. In the present study, the Carba NP test proved to be very efficient for accurate identification of carbapenemase-producers.

5. Conclusions

Intrahospital and interhospital dissemination of multiresistant *E. cloacae* isolates producing CTX-M-15, OXA-48 or both β-lactamases is a cause of concern due to the limited options remaining for the treatment of affected patients, most with critical underlying conditions. Interestingly, as observed for *K. pneumoniae*, emergence of both broad-spectrum resistance traits in single *E. cloacae* isolates corresponds to the transfer of genes having disseminated from community pathogens into nosocomial pathogens. Considering that the community reservoir of some of these resistance traits is significantly growing nowadays, the likelihood of witnessing an increased number of nosocomial outbreaks due to multiresistant *E. cloacae* is very high.

Funding: This work has been supported by project FIS-PI11-00808 (Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Ministerio de Economía y Competitividad, Spain), co-funded by the European Regional Development Fund of the European Union: a way to Making Europe, and by the University of Fribourg (Fribourg, Switzerland). A short stay of JF at the University of Fribourg was supported by a grant from the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). IM was the recipient of a predoctoral grant from the Fundación para el Fomento en Asturias de la Investigación Científica Aplicada y la Tecnología [FICYT BP09-069].

Competing interests: None declared.

Ethical approval: Not required.

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