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Cluster of *Escherichia coli* Isolates Producing a Plasmid-Mediated OXA-48 β-Lactamase in a Spanish Hospital in 2012

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Three unrelated sequence type 131 (ST131), ST58, and ST83 *Escherichia coli* isolates with low-level resistance to imipenem and resistance to ertapenem were recovered in a Spanish hospital from July to October 2012. They were positive for bla_{OXA-48} carried by an IncL/M conjugative plasmid, which may have been acquired from *Klebsiella pneumoniae*.

Resistance to carbapenem antibiotics in Gram-negative bacteria has become an increasingly important problem worldwide over the last 2 decades (1). The most relevant mechanism for this resistance is the production of carbapenemases, with the responsible genes often carried by plasmids which contribute to their spread by horizontal gene transfer (2). OXA-48 is a class D β-lactamase which confers resistance to penicillins and weak, but nonetheless significant, resistance to carbapenems but not to extended-spectrum cephalosporins (3). This enzyme was first identified in 2004 in Turkey and since then has been reported in many other countries, mainly those of the Middle East, North Africa, and Europe, but also in the United States (4–6). OXA-48 is most often detected in *Klebsiella pneumoniae*, although other members of the *Enterobacteriaceae* family, including *Escherichia coli*, also produce it (3). In *K. pneumoniae*, the bla_{OXA-48} gene has been located in a conjugative plasmid of about 60 kb assigned to the IncL/M group (7). Here, we report the characterization of three *E. coli* OXA-48-producing isolates, each obtained from a different patient at a Spanish hospital (Hospital Universitario Central de Asturias [HUCA]) over a 3-month period (Table 1). These are the first OXA-48-producing *E. coli* isolates detected in our region, and only one was previously reported in Spain (8). The three patients underwent surgery, were hospitalized for long periods, were coinfected with other bacteria, and received prolonged treatment with numerous antimicrobials.

The first *E. coli* isolate (*Ec*-HUCA 1) was recovered in July 2012 from a colostomy specimen from a 46-year-old female (patient 1) with postsurgical septic shock. The colostomy specimen was also positive for *K. pneumoniae* (isolate *Kp*-HUCA 4, which was additionally detected in blood cultures for the same patient and partially characterized in this study) and *Morganella morganii*. The second isolate (*Ec*-HUCA 2) was recovered in October 2012 from a surgical wound in a 57-year-old male (patient 2) with intestinal obstruction. After empirical treatment with meropenem, the isolate was detected in the wound drainage, as was *Enterococcus faecalis*. The third isolate (*Ec*-HUCA 3) was found in a urine sample from a 68-year-old male (patient 3) with several complications after septic shock caused by multidrug-resistant *Pseudomonas aeruginosa* secondary to a surgical wound infection (Table 1).

Antimicrobial susceptibility tests were performed by disk diffusion assays (Becton Dickinson, Sparks, MD, USA). The Microscan system (Neg Combo Panel Type 53; Siemens Healthcare Diagnostics, Deerfield, IL, USA) was applied for the identification of bacterial isolates and the determination of MICs. The Etest (bioMérieux, Marcy-l'Étoile, France) was used to determine the MIC for tigecycline and to confirm the MIC for ertapenem. The results of the disk diffusion assays (not shown) and the MICs (Table 2) were interpreted according to CLSI breakpoints that

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TABLE 1 Characteristics of patients and clinical samples positive for *E. coli* or for *E. coli* and *K. pneumoniae* isolates carrying the bla_{OXA-48} gene and recovered in a Spanish hospital

Patient no. (sex[a]/age [yr])	Hospital unit	Disease/outcome	Sample origin	Isolate(s)[b]	Date of isolation (day/mo/yr)	Final therapy
1 (F/46)	General surgery	Surgical wound infection/discharged	Colostomy	*Ec*-HUCA 1, *Kp*-HUCA 4 (*Morganella morganii*)	16/7/2012	Ciprofloxacin
2 (M/57)	ICU[c]	Surgical wound infection/discharged	Wound exudate	*Ec*-HUCA 2 (*Enterococcus faecalis*)	15/10/2012	Ciprofloxacin, cefoxitin, vancomycin
3 (M/68)	Reanimation	Septic shock[d]/discharged	Urine	*Ec*-HUCA 3	26/10/2012	Colistin, meropenem, amikacin

[a] F, female; M, male.
[b] *Ec*, *Escherichia coli*; *Kp*, *Klebsiella pneumoniae*; HUCA, Hospital Universitario Central de Asturias.
[c] ICU, intensive care unit.
[d] Septic shock was caused by *Pseudomonas aeruginosa*, which was not recovered from urine.

TABLE 2 MICs for bla_{OXA-48}-positive isolates and their transconjugants[a]

Antimicrobial agent	MIC (μg/ml) for:											
	Ec-HUCA 1 (ST131)	Tc-1/1	Tc-1/2	*Ec*-HUCA 2 (ST58)	Tc-2/1	Tc-2/2	*Ec*-HUCA 3 (ST83)	Tc-3/1	Tc-3/2	*Kp*-HUCA 4	Tc-4/1	Tc-4/2
Amoxicillin	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Amoxicillin-clavulanic acid	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8
Piperacillin-tazobactam	>64	64	64	>64	>64	>64	>64	64	>64	>64	16	32
Cefazolin	>16	≤8	≤8	>16	>16	≤8	>16	≤8	>16	>16	≤8	≤8
Cefuroxime	>16	≤8	≤8	>16	>16	≤8	≤8	8	≤8	>16	≤8	≤8
Cefoxitin	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	< = 8	≤8	≤8
Cefotaxime	≤1	≤1	≤1	>32	>32	≤1	≤1	≤1	≤1	>32	≤1	≤1
Ceftazidime	≤1	≤1	≤1	>16	>16	≤1	≤1	≤1	≤1	>16	≤1	≤1
Cefepime	≤1	≤1	≤1	>16	>16	≤1	≤1	≤1	≤1	>16	≤1	≤1
Aztreonam	≤1	≤1	≤1	>16	>16	≤1	≤1	≤1	≤1	>16	≤1	≤1
Imipenem	2	≤1	≤1	2	2	2	2	≤1	2	≤1	≤1	1
Ertapenem	2	2	2	2	2	4	2	2	2	2	1	1
Gentamicin	≤2	≤2	≤2	8	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2
Tobramycin	≤2	≤2	≤2	>8	>8	≤2	≤2	≤2	≤2	>8	≤2	≤2
Amikacin	≤8	≤8	≤8	32	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8
Acid nalidixic	>16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	>16	≤16	≤16
Ciprofloxacin	1	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	>2	≤0.5	≤0.5
Trimethoprim-sulfamethoxazole	≤2/38	≤2/38	≤2/38	>2/38	>2/38	≤2/38	≤2/38	≤2/38	≤2/38	>2/38	≤2/38	≤2/38
Tigecycline	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1
β-Lactamase gene(s)	bla_{OXA-48}, bla_{TEM-1}	bla_{OXA-48}	bla_{OXA-48}	bla_{OXA-48}, bla_{TEM-1}, $bla_{CTX-M-15}$	bla_{OXA-48}, $bla_{CTX-M-15}$	bla_{OXA-48}	bla_{OXA-48}	bla_{OXA-48}	bla_{OXA-48}	bla_{OXA-48}, $bla_{CTX-M-15}$	bla_{OXA-48}	bla_{OXA-48}
Relevant plasmid(s) (kb)	ca. 60	ca. 60	ca. 60	ca. 60, <150	ca. 60, <150	ca. 60	ca. 60	ca. 60	ca. 60	ca. 60, >150	ca. 60	ca. 60

[a] Four and 20 transconjugants (Tc) were analyzed in matings using as donors clinical isolates of *Escherichia coli* (*Ec*) and *Klebsiella pneumoniae* (*Kp*) recovered in the Hospital Universitario Central de Asturias (HUCA). A rifampin-resistant derivative of *E. coli* J53 was used as the recipient, and selection was performed with rifampin and ertapenem. For each mating, results from two independent transconjugants are shown (see the text for details).

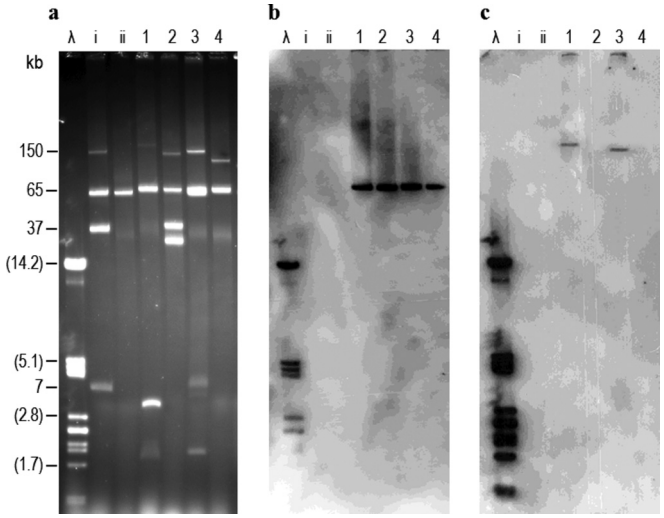

FIG 1 Plasmid profiles of *Klebsiella pneumoniae* (*Kp*) and *Escherichia coli* (*Ec*) isolates producing OXA-48 carbapenemase (a) and hybridization with bla_{OXA-48} (b) and $bla_{CTX-M-15}$ (c) probes. Lanes λ, phage λ DNA digested with PstI, used as reference for the hybridization experiments (the sizes of some of the linear fragments are shown in parentheses); lanes i and ii, plasmids obtained from *E. coli* V517 (NCTC 50192) and plasmid RP4 used as molecular size standards for undigested DNA; lanes 1, *Kp*-HUCA 4; lanes 2, *Ec*-HUCA 1; lanes 3, *Ec*-HUCA 2; lanes 4, *Ec*-HUCA 3.

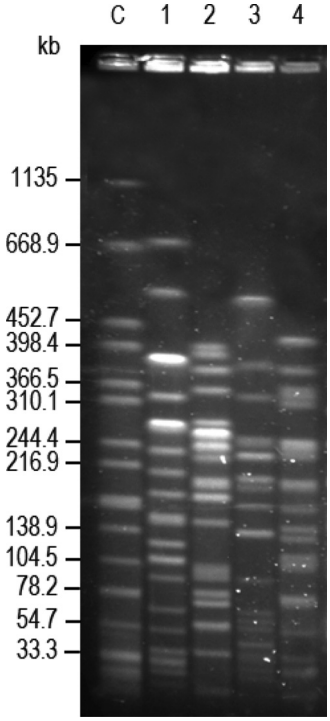

FIG 2 XbaI pulsed-field electrophoresis profiles of *Klebsiella pneumoniae* (*Kp*) and *Escherichia coli* (*Ec*) isolates producing OXA-48 carbapenemase. Lane C, XbaI-digested DNA of *Salmonella enterica* serovar Braenderup H9812 used as size standard; lane 1, *Kp*-HUCA 4; lane 2, *Ec*-HUCA 1; lane 3, *Ec*-HUCA 2; lane 4, *Ec*-HUCA 3.

were updated in January 2013 (9). As shown in Table 2, the resistance pattern was different for each of the three *E. coli* isolates. Note that each isolate showed intermediate susceptibility to imipenem (MICs of 2 mg/liter) and resistance to ertapenem (MICs of 2 mg/liter), and they were positive for the modified Hodge test recommended for the detection of carbapenemases (9). *Ec*-HUCA 2 was also resistant to third-generation cephalosporins. The *K. pneumoniae* isolate recovered from patient 1 was resistant to third-generation cephalosporins and ertapenem but susceptible to imipenem. Apart from resistance to β-lactam antibiotics, resistance to nalidixic acid, ciprofloxacin, tobramycin, and/or trimethoprim-sulfamethoxazole was detected in some of the isolates (Table 2).

The genes responsible for resistance to carbapenems and extended-spectrum cephalosporins were identified by PCR amplification (10, 11) followed by sequencing. The presence of bla_{OXA-48} was demonstrated in the three *E. coli* isolates and *Kp*-HUCA 4, and $bla_{CTX-M-15}$ was detected in *Ec*-HUCA 2 and *Kp*-HUCA 4. To establish the location of these genes, plasmid DNA extracted from each isolate (12) was hybridized with probes specific for the two genes amplified from *Kp*-HUCA 4 and labeled as previously reported (13). The same gel was also hybridized with phage λ DNA (Fermentas GmbH, Madrid, Spain), included as a control. The λ probe was generated by random-primed DNA labeling with digoxigenin-dUTP using the DIG DNA labeling kit (Roche Applied Sciences). As shown in Fig. 1a, each isolate displayed a distinct profile, including a common plasmid of ca. 60 kb accompanied by one or more plasmids of various sizes. The ca. 60-kb plasmid hybridized with the bla_{OXA-48} probe in the four isolates (Fig. 1b), while the $bla_{CTX-M-15}$ mapped on plasmids slightly larger or slightly smaller than 150 kb in *Kp*-HUCA 4 and *Ec*-HUCA 2, respectively (Fig. 1c).

To investigate the self-transfer ability of the bla_{OXA-48} plasmids, conjugation experiments were performed using each of the four isolates as donors and a rifampin-resistant derivative of *E. coli* J53 as the recipient. Transconjugants were selected on eosin methylene blue (EMB) agar (Oxoid, Madrid, Spain) containing rifampin (100 mg/liter) plus ertapenem (0.5 mg/liter). At least four independent transconjugants were tested per conjugation with regard to plasmid content and antimicrobial susceptibility (see Table 2 for representative examples). As expected, each transconjugant carried the ca. 60-kb plasmid, either alone or together with other plasmid(s), and each was PCR positive for the bla_{OXA-48} gene. These self-transferable bla_{OXA-48} plasmids were assigned to incompatibility group IncL/M by PCR amplification using transconjugants carrying only this plasmid as the source of the template DNA and primers targeting the *repA*, *traU*, and *parA* genes specific for this group (7). Three out of four transconjugants tested from crosses involving *Ec*-HUCA 2 harbored the bla_{OXA-48} plasmid together with the larger $bla_{CTX-M-15}$ plasmid, and they were resistant to cefotaxime and other extended-spectrum cephalosporins (Table 2). In contrast, all transconjugants analyzed from matings involving *Kp*-HUCA 4 (up to 20) were positive for the bla_{OXA-48} gene and plasmid only and showed a resistance pattern consistent with this (Table 2).

Multilocus sequence typing (MLST) (see mlst.warwick.ac.uk/mlst/dbs/Ecoli/) assigned *Ec*-HUCA 1, *Ec*-HUCA 2, and *Ec*-HUCA 3 to ST131, ST58, and ST83, respectively (Table 2). Pulsed-field gel electrophoresis (PFGE) performed with XbaI (14) revealed a different profile for each of the three *E. coli* isolates, and the XbaI profile of *Kp*-HUCA 4 was also different (Fig. 2). Note that the cluster of *E. coli* isolates carrying the bla_{OXA-48} gene was detected in our hospital during the national outbreak of *K. pneumoniae* producing the OXA-48 carbapenemase (4, 8). As the re-

sponsible gene appeared to be carried by the same conjugative IncL/M plasmid in the two species, it may have been transferred from *K. pneumoniae* into *E. coli* isolates which had been circulating at the time in the same hospital. Such a transfer may have independently occurred more than once, as the *E. coli* isolates were not clonally related according to their STs. The presence of bla_{OXA-48} in *Ec*-HUCA 1 (ST131) is particularly worrisome, considering the ability of the pandemic ST131 clone to acquire genes encoding extended-spectrum β-lactamases, especially bla_{CTX-M} (15). In Spain, the only reported OXA-48-producing *E. coli* isolate was also ST131 (8). Note that the detection of carbapenem resistance was difficult, because the three *E. coli* isolates showed intermediate resistance to imipenem, and that treatment of the patients was complicated by additional resistances accompanying OXA-48 production in two out of the three isolates and by coinfection of the patients with other bacteria. Altogether, these facts highlight the risk of high antimicrobial pressure, in conjunction with long-term hospitalization, for the emergence of new resistant bacteria. Nevertheless, after the therapy indicated in Table 1, further cultures tested negative for OXA-48-producing *Enterobacteriaceae*, and the three patients were eventually discharged from the hospital.

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