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Outbreak of OXY-2-Producing *Klebsiella oxytoca* in a Renal Transplant Unit[▽]

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We describe a *Klebsiella oxytoca* infection outbreak in a renal transplant unit that involved seven patients. All strains belonged to a single pulsed-field gel electrophoresis pattern and were resistant to amoxicillin-clavulanate, cefuroxime, piperacillin-tazobactam, and aztreonam but susceptible to ceftriaxone, ceftazidime, cefepime, and imipenem. Chromosomal β-lactamase hyperproduction was caused by a point mutation in the *bla*$_{OXY-2}$ gene promoter region.

Klebsiella oxytoca is an opportunistic pathogen responsible for causing health care-associated infections (14, 15, 17). These species possess chromosomal genes encoding β-lactamases that are constitutively expressed at low levels and that confer resistance to amino- and carboxypenicillins but not to other β-lactams (4). *K. oxytoca* β-lactamases were initially divided into the two main groups OXY-1 and OXY-2, which possessed distinct β-lactam hydrolytic profiles (11, 12). Recently, other OXY-type β-lactamases (OXY-3 to OXY-6) have been reported among *K. oxytoca* isolates (6). Distinct point mutations in the −35 and −10 promoter regions of these β-lactamase genes have been pointed out as a reason for OXY hyperproduction in 10 to 20% of *K. oxytoca* isolates and led to a broader spectrum of β-lactam resistance (10, 13).

Susceptibility to bacterial infection in renal transplantation recipients is related directly to the level and duration of the pharmacological immunosuppression. Bacterial urinary tract infections are frequently associated with early onset chronic rejection and may lead to reduced transplantation survival (8).

K. oxytoca infection outbreaks have been documented in multiple settings (4, 15, 18, 19, 21). However, *K. oxytoca* infection outbreaks in transplantation units have not yet been reported. The aim of this study was to evaluate the antimicrobial susceptibility profiles, the genetic relatedness, and the mechanisms of β-lactam resistance among clinical isolates of *K. oxytoca* that caused health care-associated infections in a renal transplantation unit of a teaching hospital located in Buenos Aires, Argentina.

Seven *K. oxytoca* strains were isolated from the urine, peritoneal fluid, and central venous catheters of renal and renal-pancreas transplantation patients hospitalized at the transplantation unit of the University Hospital, CEMIC, between March and August of 2005. According to an epidemiological investigation, the index case was a renal transplantation patient who developed a urinary tract infection caused by this strain during the hospitalization period. The outbreak of infection involved a total of seven patients (one isolate per patient). Horizontal transmission was suspected because at that time, the transplantation unit was located in a shared facility without individual rooms, and all patients were attended by a common group of health care workers.

The isolates were associated with infection and were identified by conventional methods (5). Antimicrobial susceptibility testing was performed by using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method and interpreted according to CLSI breakpoints (CLSI, 2006). Quality control was performed by testing *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All quality control results were within published MIC ranges (CLSI, 2006) (2, 3). Pulsed-field gel electrophoresis (PFGE) was performed using the restriction endonuclease SpeI as previously described (16). Analysis of PFGE patterns was performed by visual inspection of photographs of ethidium bromide-stained gels. The isolates were classified according to the criteria described by Tenover et al. (20).

Detection of the *bla*$_{OXY}$ group genes and promoter regions was carried out by PCR, followed by DNA sequencing. PCR was performed under standard conditions, using the primers OXY-F (5′-GATTTCACAAAGCGCTCGGC-3′) and OXY-R (5′-CCTGCTGCGGCTGGGTAAAA-3′), designed based on the nucleotide sequences of the *bla*$_{OXY-1}$ and *bla*$_{OXY-2}$ genes available at GenBank, under accession numbers Z30177 and Z49084, respectively. PCR products were analyzed by electrophoresis in 1.0% agarose gels and were sequenced on both strands by using an ABI Prism 377 sequencer unit. The nucleotide sequences and deduced amino acid sequences were analyzed by using Lasergene software (DNAStar, Madison, WI). The sequences obtained were compared to sequences available at http://www.ebi.ac.uk/fasta33/.

The outer membrane proteins of the isolates were studied according to the method described by Filip et al. (7). Wild-type *K. oxytoca* strains susceptible to penicillins and broad-spectrum cephalosporins were included as control strains.

The antimicrobial susceptibility profiles of the *K. oxytoca* strains studied are presented in Table 1. The seven *K. oxytoca* strains

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TABLE 1. Antimicrobial susceptibility profiles of wild-type *K. oxytoca* and *K. oxytoca* OXY-2 hyperproducer strains evaluated in this study

Isolate	MIC (μg/ml) of the indicated drugs to *K. oxytoca* isolates[a]																			
	AMK	GEN	AMP	SAM	AMC	FEP	CRO	CAZ	CXM	CEF	CIP	MEZ	TZP	PIP	TIM	CTT	SXT	ATM	IPM	MER
7	16	>8	>16	>16/8	>16/8	1	1	2	>16	>16	2	>64	>64/2	>64	>64/2	4	>2/38	>256	0.25	0.25
6	8	4	>16	>16/8	>16/8	1	2	2	>16	>16	2	>64	>64/4	>64	>64/2	8	>2/38	>256	0.5	0.25
5	16	>8	>16	>16/8	>16/8	2	8	2	>16	>16	8	>64	>64/4	>64	>64/4	32	>2/38	>256	0.25	0.25
4	16	1	>16	>16/8	>16/8	1	2	1	>16	>16	2	>64	>64/4	>64	>64/4	4	>2/38	>256	0.5	0.25
3	2	4	>16	>16/8	>16/8	1	2	1	>16	>16	2	>64	>64/4	>64	>64/4	4	>2/38	>256	0.5	0.25
2	16	4	>16	>16/8	>16/8	1	1	2	>16	>16	2	>64	>64/4	>64	>64/4	4	>2/38	>256	0.5	0.25
1	8	2	>16	>16/8	>16/8	1	1	2	>16	>16	2	>64	>64/4	>64	>64/4	4	>2/38	>256	0.5	0.25

[a] MIC values were determined by broth microdilution technique (3). AMK, amikacin; AMC, amoxicillin-clavulanic acid; SAM, ampicillin-sulbactam; AMP, ampicillin; FEP, cefepime; CRO, ceftriaxone; CAZ, ceftazidime; CXM, cefuroxime; CEF, cephalothin; CIP, ciprofloxacin; GEN, gentamicin; IPM, imipenem; MER, meropenem; ATM, aztreonam; MEZ, mezlocillin; TZP, piperacillin-tazobactam; PIP, piperacillin; TIM, ticarcillin-clavulanic acid; CTT, cefoxitin; SXT, trimethoprim-sulfamethoxazole.

were resistant to piperacillin, piperacillin-tazobactam, amoxicillin-clavulanic acid, cephalothin, cefuroxime, and aztreonam but were susceptible to ceftriaxone, ceftazidime, cefepime, and imipenem. A single isolate was resistant to cefoxitin (MIC, 32 μg/ml). No plasmids were found by phenotypic and molecular analyses. Seven *K. oxytoca* strains showed a unique PFGE pattern (pattern A), as shown in Fig. 1. The presence of the bla_{OXY-2} gene was detected in all strains. The bla_{OXY-2} promoter sequence region of clone A was P (−35[TTGTCA]; −10[GATAAT]), which differed by one base (underlined) from the weak promoter, P (−35[TTGTCA]; −10[GATAGT]), present in the *K. oxytoca* wild type carrying bla_{OXY-2}. The outer membrane profiles were identical among the *K. oxytoca* isolates studied, except for the cefoxitin-resistant strain, which exhibited reduced expression of the 36-kDa outer membrane protein on a sodium dodecyl sulfate-polyacrylamide gel.

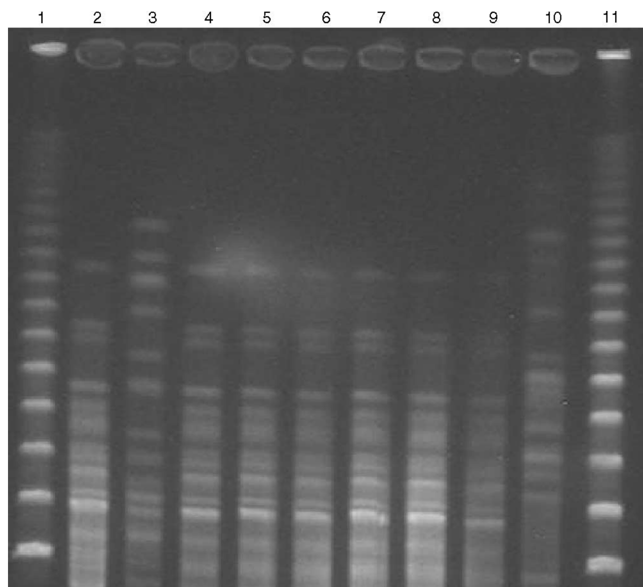

FIG. 1. PFGE patterns of SpeI-digested chromosomal DNA restriction fragments from *K. oxytoca* isolates resolved in 1% SeaKem Gold agarose. Lanes 1 and 11, molecular weight standards of lambda ladder. Lane 2, strain 1 (PFGE pattern A); lane 3, strain X (quality control strain); lane 4, strain 2 (PFGE pattern A); lane 5, strain 3 (PFGE pattern A); lane 6, strain 4 (PFGE pattern A); lane 7, strain 5 (PFGE pattern A); lane 8, strain 6 (PFGE pattern A); lane 9, strain 7 (PFGE pattern A); and lane 10, strain U (quality control strain).

We describe an outbreak of *K. oxytoca* infection among transplantation patients (hospitalized in a single institution), caused by an isolate that overproduces OXY-2 due to a mutation in the bla_{OXY-2} promoter region. OXY β-lactamases are chromosomally encoded and are usually synthesized at low levels, conferring resistance to amino- and carboxypenicillins. Overproduction of such enzymes results from a mutation in the β-lactamase gene promoter, enhancing the hydrolytic substrate profile and conferring resistance to penicillins and some extended-spectrum β-lactams, especially aztreonam (9). In contrast, these strains are susceptible to ceftriaxone, ceftazidime, and cefepime, as observed with our study. This feature helps to distinguish OXY-type overproducers from *K. oxytoca* isolates that harbor plasmid-encoded extended-spectrum β-lactamases (4, 6). In spite of being encoded by chromosomal DNA, this group of enzymes has been grouped with the extended-spectrum β-lactamases in the 2be class of the classification by Bush et al., due to the substrate profiles and inhibition patterns found by clavulanic acid (1).

OXY-2-producing *K. oxytoca* strains are more commonly (53 to 74%) isolated from clinical specimens and are usually more resistant than OXY-1 producers. The substitution of guanine with adenine in the −10 bla_{OXY-2} promoter region, as observed with this study, has already been described to enhance OXY-2 expression about 13-fold in *K. oxytoca* strain SL911 (9, 13). This is the probable reason for the β-lactam susceptibility profile displayed by clone A.

The outbreak of infection was controlled by an enhancement of standard biosafety precautions (i.e., hand-washing practice) through an educational program and contact isolation procedures. All the outbreak control procedures were supervised directly by the infection control department of the institution.

In summary, a unique clone of *K. oxytoca* was responsible for causing infections in a renal transplantation unit, suggesting that the patients might have acquired this clone from a common source. These isolates displayed enhanced resistance to β-lactams, including cefuroxime and aztreonam, which was attributed to a single base change in the −10 consensus sequence of the promoter. In addition, it is possible that cefoxitin resistance in one of these strains was caused by the diminished expression of the 36-kDa outer membrane protein. Epidemiologic surveillance of transplantation units is of major importance to the prevention and early detection of outbreaks caused by multidrug-resistant bacteria.

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