Outbreak of OXY-2 producing *Klebsiella oxytoca* in a renal transplant unit.

Running Title: *Klebsiella oxytoca* outbreak in a transplant unit

Mariela Soledad Zárate¹*
Ana C. Gales³
Renata C. Picão³
Gervasio Soler Pujol²
Alejandra Lanza¹
Jorgelina Smayevsky¹


**Keywords:** *Klebsiella oxytoca*, outbreak, OXY-2, kidney transplant

Corresponing author Mariela Soledad Zárate,

Guemes 3066 1º Dpto 4.

CP1425

Buenos Aires - Argentina

Phone: +54-11-15-54999139

+54-11-4822-6588

E-mail:*sole_z@hotmail.com, szarate@cemic.edu.ar
ABSTRACT

We describe a *Klebsiella oxytoca* outbreak in a renal transplant unit that involved 7 patients. All strains belonged to a single PFGE 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 gene bla_{OXY-2} promotor region.
Klebsiella oxytoca is an opportunistic pathogen responsible for causing healthcare associated infections (14,15). This species possess chromosomal genes encoding β-lactamases that are constitutively expressed at low levels and confer resistance to amino- and carboxypenicillins, but not to other β-lactams (4). K oxytoca β-lactamases were initially divided in two main groups: OXY-1 and OXY-2 that possessed distinct β-lactam hydrolytic profiles (11, 12). Recently, other OXY-type β-lactamases (OXY-3-6) 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 reason for OXY-hyperproduction in 10-20% K. oxytoca isolates, and led to a broader spectrum of β-lactam resistance (10, 13).

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

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

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

The isolates were associated with infection and were identified by conventional methods (5). Antimicrobial susceptibility testing was performed by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method and interpreted according to CLSI breakpoints [CLSI, 2006 and 2007]. Quality control (QC) was performed by testing Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853. All QC results were within the published MIC ranges [CLSI, 2007] (2, 3). PFGE was performed using the restriction endonucleases 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 Tenover’s criteria (20).

The detection of blaoxy group genes and promoter regions were carried out by PCR followed by DNA sequencing. PCR was performed under standard conditions using the primers OXY-F (5'-GATTTCACAAGCGCTCGGC-3') and OXY-R (5'-CCTGCTGCGGTGAAATATAA-3'), designed based on the nucleotide sequence of the blaoxy-1 and blaoxy-2 available at the GenBank, under the accession numbers Z30177 and Z49084, respectively. PCR products were analyzed by electrophoresis in 1.0% agarose gel and were sequenced on both strands by using AbiPrism 377 sequencer. The nucleotide sequences and deduced amino acid sequences were analyzed by using the Lasergene software package (DNASTAR, Madison, WI). Obtained sequences were compared to sequences available on the internet (http://www.cbi.ac.uk/fasta33/).

The outer membrane proteins of the isolates were studied according to the method described by Filip and colleagues (7). Wildtype K. oxytoca strains susceptible to penicillins and broad-spectrum cephalosporins were included as control strains.
The antimicrobial susceptibility profile of the studied *K. oxytoca* strains is presented in Table 1. The seven *K. oxytoca* strains were resistant to piperacillin, piperacillin/tazobactam, amoxicillin/clavulanic acid, cephalothin, cefuroxime, and aztreonam but 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. All seven *K. oxytoca* strains showed a unique PFGE pattern (pattern A) as displayed in Figure 1. The presence of *bla*<sub>OXY-2</sub> gene was detected in all strains. The *bla*<sub>OXY-2</sub> promoter sequence region of the clone A was P (-35[TGTCA]; -10[GATAAT]), which differed one base from the weak promoter, P (-35[TGTCA]; -10[GATAGT]), present in the *K. oxytoca* wild type carrying *bla*<sub>OXY-2</sub>. The outer membrane profiles were identical among the *K. oxytoca* isolates studied, except for the cefoxitin-resistant strain that exhibited reduced expression of the 36 KDa outer membrane protein on the SDS-page gel.

We described an outbreak of *K. oxytoca* among transplant patients (of a single institution) that overproduces OXY-2 due to a mutation in *bla*<sub>OXY-2</sub> promoter region. OXY β-lactamases are chromosomally encoded and 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 in our study. This feature helps to distinguish OXY-type overproducers from *K. oxytoca* isolates harboring plasmid-encoded ESBLs. (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 class 2be of the classification of Bush, Jacob and Medeiros due to their substrate profiles and inhibition patterns by clavulanic acid (1).
OXY-2-producing *K. oxytoca* strains are more commonly (53–74%) isolated from clinical specimens and are usually more resistant than OXY-1 producers. The substitution of guanine by adenine in the -10 *bla*<sub>OXY-2</sub> promoter region as observed in this study had already been described to enhance OXY-2 expression about 13-times in *K. oxytoca* strain SL911 (9, 11). This is the probable reason for the β-lactam susceptibility profile displayed by clone A.

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

In summary; a unique clone of *K. oxytoca* was responsible for causing infections in a renal transplant 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, cefoxitin resistance in one of these strains was possibly caused by the diminished expression of the 36 KDa outer membrane protein. Epidemiologic surveillance of transplant units is of major importance in order to prevent and early detect outbreaks caused by multidrug-resistant bacteria.

**ACKNOWLEDGMENTS:**

We would like to thank the personnel of ALERTA, LEMC and CEMIC for their contribution during the performance of this study, and, especially, Jussimara Monteiro for performing the PFGE technique. ACG is partially funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Process: 307714/2006-3).
REFERENCES


Table 1. Antimicrobial susceptibility profile of the wild type *K. oxytoca* and OXY-2 hyperproducers *K. oxytoca* strains evaluated in this study.

| Isolator number | MIC<sup>a</sup> (ug/ml) | AKN<sup>b</sup> | GEN<sup>b</sup> | AMP<sup>b</sup> | SAM<sup>b</sup> | AMC<sup>b</sup> | FEP<sup>b</sup> | CRO<sup>b</sup> | CAZ<sup>b</sup> | CXM<sup>b</sup> | CEF<sup>b</sup> | CIP<sup>b</sup> | MEZ<sup>b</sup> | TZP<sup>b</sup> | PIP<sup>b</sup> | TIM<sup>b</sup> | CTT<sup>b</sup> | SXT<sup>b</sup> | AZT<sup>b</sup> | IPM<sup>b</sup> | MER<sup>b</sup> |
|-----------------|--------------------------|---------------|----------------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 7               |                          | 16            | >8            | >16/8         | >16/8       | 1           | 1           | 2           | >16         | >16         | 2           | >64         | >64/2       | >16/2       | 4           | >2/38       | >256        | 0.25        | 0.25        |             |             |
| 6               |                          | 8             | 4             | >16/8         | >16/8       | 1           | 2           | 2           | >16         | >16         | 2           | >64         | >64/4       | >16/4       | 8           | >2/38       | >256        | 0.5         | 0.25        |             |             |
| 5               |                          | 16            | >8            | >16/8         | >16/8       | 2           | 8           | 2           | >16         | >16         | 8           | >64         | >64/4       | >16/4       | 32          | >2/38       | >256        | 0.25        | 0.25        |             |             |
| 4               |                          | 16            | 1             | >16/8         | >16/8       | 1           | 2           | 1           | >16         | >16         | 2           | >64         | >64/4       | >16/4       | 4           | >2/38       | >256        | 0.5         | 0.25        |             |             |
| 3               |                          | 2             | 4             | >16/8         | >16/8       | 1           | 2           | 1           | >16         | >16         | 2           | >64         | >64/4       | >16/4       | 4           | >2/38       | >256        | 0.5         | 0.25        |             |             |
| 2               |                          | 16            | 4             | >16/8         | >16/8       | 1           | 1           | 2           | >16         | >16         | 2           | >64         | >64/4       | >16/4       | 4           | >2/38       | >256        | 0.5         | 0.25        |             |             |
| 1               |                          | 8             | 2             | >16/8         | >16/8       | 1           | 1           | 2           | >16         | >16         | 2           | >64         | >64/4       | >16/4       | 4           | >2/38       | >256        | 0.5         | 0.25        |             |             |

<sup>a</sup>Minimal Inhibitory Concentration (ug/ml) determined by broth microdilution technique (CLSI, 2006)

<sup>b</sup>amikacin (AMK), amoxicillin/clavulanic acid (AMC), ampicillin/sulbactam (SAM), ampicillin (AMP), cefepime (FEP), ceftriaxone (CRO), ceftazidime (CAZ), cefuroxime (CXM), cephalothin (CEF), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IPM), meropenem (MER), aztreonam (AZT), mezlocillin (MEZ), piperacillin-tazobactam (TZP), piperacillin (PIP), ticarcillin/clavulanic acid (TIM), cefoxitin (CTT) and trimethoprim/sulfamethoxazole (SXT).
Figure 1. Pulsed-field gel electrophoresis patterns of Spe I-digested chromosomal DNA restriction fragments from *K. oxytoca* isolates resolved in 1% SeaKem Gold agarose. Lane 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).