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Title: *Klebsiella pneumoniae* ESBL producers responsible for severe UTIs in a renal transplant unit

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Abstract: Infections caused by ESBL-producing *Klebsiella pneumoniae* are problematic because when coresistance to other antimicrobial classes is present, limited antibiotic options are available. *K. pneumoniae* is often associated to urinary tract infections (UTIs) in immunosuppressed patients after renal transplantation where cystitis and pyelonephritis are the most common forms of bacterial infections. The objective of this study was to characterize an outbreak of 10 severe UTIs caused by *K. pneumoniae* using molecular methods and to describe an epidemic diffusion of ESBL producing *K. pneumoniae*. All isolates were MDR (Multidrug-resistance) but sensitive to imipenem. PCR detection, using gene-specific primers, showed that all strains harbored the blaCTX-M gene, six cases associated with blaTEM, and only one strain carried the blaSHV together with blaTEM and blaCTX-M. Pulsed-field gel electrophoresis (PFGE) typing of all strains revealed 2 clonal types, A and B, where clone B was ciprofloxacin and amikacin susceptible.

Catania, July 13 , 2010

Prof. S. Christian Ruef
Editor in Chief
Infection
Switzerland

Dear Prof. **Christian Ruef**,

please find enclosed our manuscript entitled “***Klebsiella pneumoniae* ESBL producers responsible for severe UTIs in a renal transplant unit**” by Floriana Gona, Maria Lina Mezzatesta, Daniela Corona, Domenico Zerbo, Vanessa Scriffignano, Stefania Stefani, Pierfrancesco Veroux e Massimiliano Veroux for consideration in the journal.

All Authors have read and agreed to its submission and are responsible for its content and guarantee that the manuscript was not published previously, is not being considered or published elsewhere.

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Thank you very much

Yours sincerely
Maria Lina Mezzatesta

***Klebsiella pneumoniae* ESBL producers responsible for severe UTIs
in a renal transplant unit.**

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1 The emergence of extended-spectrum β -lactamase (ESBL)-producers, along with multidrug-resistant (MDR)
2 isolates, poses a serious problem in hospital settings. ESBLs are capable of hydrolysing penicillins, broad-
3 spectrum cephalosporins and aztreonam. Since the first report of ESBL-producing *Klebsiella* in 1983 from a
4 patient in Germany [1,2,3], a worldwide distribution of these enzymes has been reported [4], and in some
5 countries (e.g., Argentina, Greece, Japan, Spain and Taiwan), the CTX-M-type enzymes are more prevalent
6 than TEM- and SHV-type ESBLs [5, 6]. In Europe, where the TEM- and SHV-type ESBLs were first
7 reported and are widespread [7, 8], a rapid and massive dissemination of isolates producing CTX-M-type
8 ESBLs has recently been reported [9] and is a matter of major concern. In Italy the presence of CTX-M-type
9 ESBLs was previously reported in clinical isolates of *Enterobacteriaceae* from some hospitals [10], as well
10 as from pets [11]. In 2003, the second Italian nationwide survey on ESBL production among
11 *Enterobacteriaceae* was carried out, and the results showed that CTX-M-type enzymes were common
12 (around 20%) among ESBL producers [12]. The CTX-M family is recognized as a rapidly growing family of
13 ESBLs that selectively prefer to hydrolyze cefotaxime rather than ceftazidime [1]. However, variants of
14 CTX-M with increased hydrolyzing activity against ceftazidime have emerged. The widespread use of
15 antibiotics coupled with the transmissibility of resistant determinants mediated by plasmids, transposons and
16 gene cassettes in integrons are factors that contribute to the increase in antibiotic resistance in bacterial
17 pathogens[13]. In renal transplant recipients, urinary tract infections (UTIs), including asymptomatic
18 bacteriuria, cystitis and pyelonephritis, are the most common forms of bacterial infections [14]. UTIs are
19 frequently associated with early onset chronic rejection and may lead to reduced transplantation survival.
20 Susceptibility to bacterial infection in renal transplantation recipients is directly related to the level and
21 duration of the pharmacological immunosuppression [15].
22 From March to September 2009, 27 consecutive UTIs and 3 bacteremia were diagnosed in 60 patients who
23 had had renal transplantation at the University hospital of Catania. Twenty patients developed an
24 asymptomatic bacteriuria and were diagnosed only using a positive urine culture ($>10^5$ microorganisms/mm³)
25 (*E. coli* in 7 cases, *Enterococcus faecalis* in 6, *K. pneumoniae* in 4, *Enterobacter spp*, *Pseudomonas*
26 *aeruginosa* and *Staphylococcus coagulase negative* in one case each), and did not undergo any antibiotic
27 therapy.

1 Three patients developed bacteremia, seven patients developed symptomatic UTIs, due to the presence of
2 fever, urgency, frequency, dysuria, and suprapubic tenderness, caused by *K. pneumoniae* : 5 patients
3 presented with acute pyelonephritis (PN), 2 patients presented with acute graft dysfunction with anuria and
4 raised serum creatinine levels.

5 All *K. pneumoniae* isolates were identified by standard methods [16]. Antimicrobial susceptibility testing
6 was performed by using broth microdilution method and interpreted according to CLSI breakpoints (CLSI
7 2010). All strains were screened for ESBL production using ceftriaxone, ceftazidime and aztreonam discs as
8 recommended by the CLSI (2010). Double-disc synergy tests (DDSTs) were performed according to
9 previously published methods [2] using ceftazidime, ceftriaxone, aztreonam, cefepime and amoxicillin-
10 clavulanics discs. Quality control was performed by testing *Escherichia coli* ATCC 25922 and all quality
11 control results were within published MIC ranges [17]. ESBL-encoding genes were detected as previously
12 published [10] and clonal and epidemiological correlations were studied by macrorestriction analyses
13 (PFGE). Pulsed-field gel electrophoresis was performed using the restriction endonuclease *XbaI* enzyme as
14 previously described [10]. The isolates were classified according to previously described criteria [18].

15 Table 1 shows the characterization of the *Klebsiella pneumoniae* strains. All patients empirically underwent
16 an antibiotic therapy with imipenem 2 g/die for 15 days and the therapy was successful as confirmed by *in*
17 *vitro* susceptibility testing results. Using DDTs, 10 strains were found to be ESBL producers and were
18 confirmed by PCR. All strains showed positive amplification for *bla*-*CTX-M*, in six cases associated with *bla*-
19 *TEM*; only one strain carried the *bla*-*SHV* together with the *bla*-*TEM* and *bla*-*CTX-M*. The ten strains belonged to
20 two different clones, A and B, and clone A was an MDR clone, resistant to all β -lactams and amikacin,
21 piperacillin/tazobactam and ciprofloxacin. Clone B was susceptible to amikacin and ciprofloxacin. Subtle
22 differences in the *bla* gene content were observed among both clones, demonstrating that lateral gene
23 transfer drives the diffusion of many antibiotic resistance genes. In seven patients infection completely
24 resolved with restoration of a normal graft function. One patient lost his graft due to recurrent *K. pneumoniae*
25 infections six months after the first episode. Unfortunately two patients died: one patient developed a diffuse
26 Kaposi Sarcoma in both legs and finally developed a pulmonary infection caused by clone A, while the
27 other patient developed a hemophagocitic syndrome.

The rapid and massive spread of CTX-M-type ESBLs is rapidly changing the ESBL epidemiology in Italy and in some geographical areas these enzymes are now the most prevalent ESBLs in *Enterobacteriaceae* [19]. In our hospital *Enterobacteriaceae* carrying CTX-M as a prevalent ESBL is increasing (data not shown). In conclusion, from March to September 2009 , there was a diffusion of two MDR *K.pneumoniae* clones in the renal transplant unit, harbouring various ESBLs. These clones had never been isolated before and were responsible, for the first time, for severe upper UTIs with difficult resolution: one failure and one relapse. In conclusion, UTIs remain the most frequent infections among renal transplant recipients. Until recently infections were sustained by susceptible microorganisms in which complete resolution of infections was easily obtained; the increase of potentially life-threatening multi-resistant strains now emerging in hospital settings for renal transplant recipients, has changed the severity of infections and the corresponding outcome. It is mandatory to continue epidemiological surveillance of transplantation units in order to tailor a correct therapy to maintain potent antibiotics, such as carbapenem, that are losing their potency.

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Table 1

Table.1 Clinical findings and antibiotic susceptibility of patients with multi-resistant infections

Patients	Date	Type of infection	MIC (mg/liter) ^a								Mechanism of resistance	PFGE	Antibiotic	Outcome
			IMP	CEF	CTX	CAZ	ATM	TZP	CIP	AK				
1	7/18/2009	Complicated urinary tract infection	0.5	>64	32	>64	>128	>128	16	8	CTX-M	A	Imipenem	Cure
2	7/23/2009	Complicated urinary tract infection	0.25	8	64	>64	128	>128	>4	>8	TEM+CTX-M	A	Imipenem	Cure
3	5/1/2009	Bacteremia	0.25	>64	32	16	128	64	>4	4	TEM+CTX-M	A	Imipenem	Death, hemophagocitic syndrome
4	7/20/2009	Complicated urinary tract infection	0.5	>64	32	>64	>128	>128	16	8	CTX-M	A	Imipenem	Cure
5	7/25/2009	Complicated urinary tract infection	1	2	>64	64	128	64	>4	8	CTX-M	A	Imipenem	Cure
6	7/28/2009	Bacteremia	0.25	4	>64	>64	128	64	16	8	TEM+CTX-M	A	Imipenem	Failure, death
7	3/19/2009	Complicated urinary tract infection	0.25	8	64	>64	128	>128	>4	>8	TEM+CTX-M	A	Imipenem	Cure
8	4/30/2009	Complicated urinary tract infection	1	>64	>64	>64	64	128	32	4	TEM+CTX-M	A	Imipenem	Relapse, lost graft
9	5/24/2009	Bacteremia	1	2	16	8	64	32	0.25	<2	CTX-M	B	Imipenem	Cure
10	9/29/2009	Complicated urinary tract infection	0.5	4	64	16	>64	128	0.5	<2	SHV+TEM+CTX-M	B	Imipenem	Cure

^a Abbreviations. PFGE= pulsed field gel electrophoresis; IMP= imipenem; CEF= cefepime; CAZ= ceftazidime; CTX=cefotaxime; ATM= aztreonam; TZP= piperacillin/tazobactam; CIP= ciprofloxacin; AK= amikacin;