

# CARBAPENEMASES IN URINARY ISOLATES OF *ENTEROBACTERIACEA* FROM CROATIA

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## INTRODUCTION AND PURPOSE

Recently, emergence of carbapenem-resistance was observed among *Enterobacteriaceae* causing urinary tract infections in Croatia. The aim of the study was to characterize the carbapenem-resistance mechanisms and the molecular epidemiology of carbapenem resistance in urine isolates of *Enterobacteriaceae* from Croatia.

## MATERIAL AND METHODS

The antimicrobial susceptibility to a wide range of antibiotics was determined by broth microdilution method. Double-disk-synergy test (DDST) was performed to detect ESBLs and modified Hodge test (MHT) was used to screen for production of carbapenemases. MBL E-test was used to screen for production of metallo-β-lactamases. Additionally the isolates were tested by combined disks tests using four disks of meropenem or imipenem one without and the other three with 3-aminophenylboronic acid test with 0.1 M EDTA or both 3-aminophenylboronic and EDTA to screen for KPC, MBLs or simultaneous production of MBL and KPC, respectively. The transferability of meropenem resistance was determined by conjugation (broth mating method) employing *E. coli* A15R<sup>r</sup> strain resistant to rifampicin. The presence of genes encoding broad and extended-spectrum β-lactamases (*bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub> and *bla*<sub>PER-1</sub>), plasmid-mediated AmpC β-lactamases, group A carbapenemases (*bla*<sub>KPC</sub>, *bla*<sub>SMEZ</sub>, *bla*<sub>IMI</sub>, *bla*<sub>NDM</sub>), metallo β-lactamases (*bla*<sub>VIM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>NDM1</sub>), and carbapenem hydrolyzing oxacillinases (*bla*<sub>OXA-48</sub>) was determined by PCR.

## RESULTS

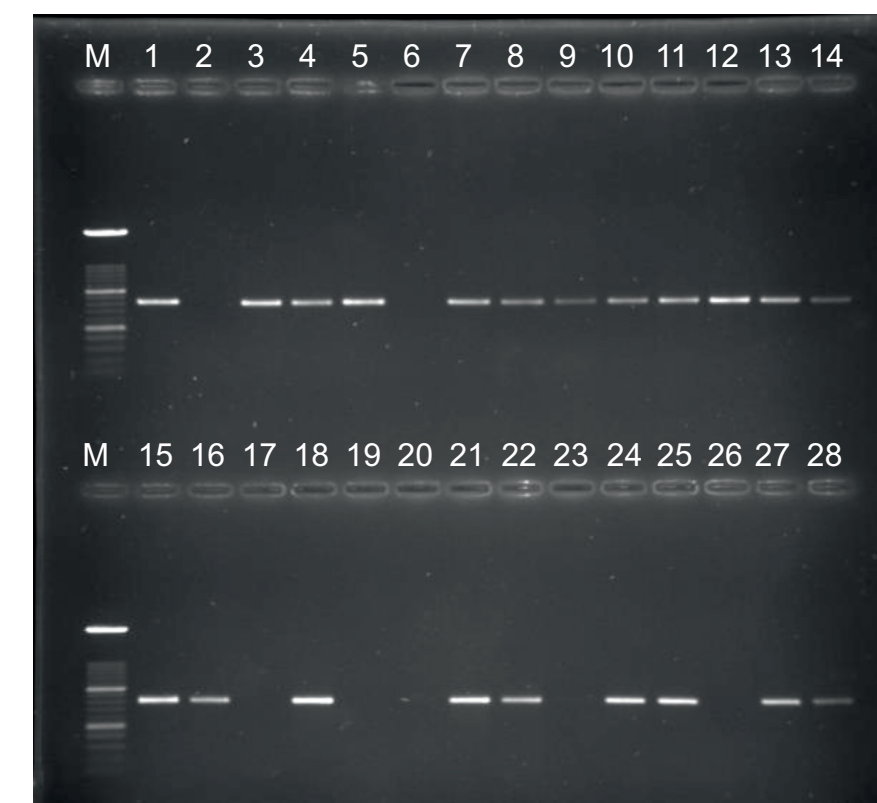
In total 47 strains (31 *Enterobacter* spp., 12 *Klebsiella pneumoniae*, 2 *Citrobacter* spp., and one *Escherichia coli* and *Serratia marcescens*, respectively) with reduced susceptibility to carbapenems, isolated from urine during 2012-2013 from two large hospital centres in Croatia were analyzed. All strains were tested to amoxicillin alone and combined with clavulanate, cefuroxime, ceftazidime, cefotaxime and ceftriaxone as shown in Table 1. The strains showed variable degree of susceptibility to cefepime, imipenem, meropenem, ertapenem, aminoglycosides and ciprofloxacin. Except colistin (all isolates were susceptible), potent activity against carbapenemase producing isolates exhibited also amikacin (50% susceptible isolates). Genes encoding carbapenemases were found in 38 isolates, with VIM-1 metallo-enzyme being the most prevalent and found in 34 strains (twenty five *E. cloacae* isolates; (one strain coharboured VIM-1 and NDM-1), six *K. pneumoniae* strains, two *C. freundii* and one *S. marcescens* isolate, respectively), NDM-1 was found in one *Enterobacter cloacae*, and KPC-2 in three *Klebsiella pneumoniae* strains. Nine strains were negative for true carbapenemases and harboured only ESBL belonging to CTX-M family (Table 1). Plasmids encoding VIM MBLs belonged to widespread IncA/C or

**Table 1.** Minimum inhibitory concentrations of various antibiotics and β-lactamase content of carbapenemase producing isolates of *Enterobacteriaceae* from urine.

PROTOCOL NUMBER	SAMPLE	DEPT	SPECIES	DATE	BETA LACTAMASE(S)	AMX	AMC	TZP	CZ	CAZ	CXM	CTX	CRO	FEP	IMI	MEM	ERT	GM	AMI	CIP	COL
1	ST-2322	URINE	ICU	16.02.2013.	<i>Enterobacter cloacae</i>	VIM-1, CTX-M-15-15	>128	>128	>128	>128	>128	>128	>128	8	0.5	2	8	1	2	1	0.5
2	ST-2417 (83)	URINE	PSYCHIATRIC	19.02.2013.	<i>Enterobacter cloacae</i>	VIM-1, TEM-1, CTX-M-15-15	>128	>128	>128	>128	>128	>128	>128	32	16	16	32	64	32	2	
3	ST-14730 (85)	URINE	MEDICAL	26.10.2012.	<i>Enterobacter cloacae</i>	VIM-1, TEM-1, CTX-M-15-15	>128	>128	>128	>128	>128	>128	>128	4	2	8	128	32	32	0.5	
4	KBC-45735 (89)	URINE	HAEMATATOLOGY	11.03.2013.	<i>Enterobacter cloacae</i>	VIM-1, TEM-1, CTX-M-15-15	>128	>128	>128	>128	>128	>128	>128	8	16	16	16	2	32	0.5	
5	KBC-96822	URINE	ICU	31.05.2013.	<i>Enterobacter cloacae</i>	VIM-1, TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	8	4	8	64	32	16	0.25	
6	KBC-113650	URINE	HAEMATATOLOGY	29.06.2013.	<i>K. pneumoniae</i>	KPC-2	>128	>128	>128	>128	>128	>128	>128	64	64	32	64	16	8	0.5	
7	KBC-114449	URINE	HAEMATATOLOGY	01.07.2013.	<i>K. pneumoniae</i>	VIM-1, TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	16	16	8	32	64	64	0.5	
8	KBC-117238	URINE	UROLOGY	05.07.2013.	<i>K. pneumoniae</i>	VIM-1, TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	8	16	8	32	64	32	0.5	
9	KBC-117238	URINE	UROLOGY	05.07.2013.	<i>K. pneumoniae</i>	VIM-1, TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	16	16	4	32	64	8	0.5	
10	KBC-123327	URINE	UROLOGY	15.07.2013.	<i>K. pneumoniae</i>	VIM-1, TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	8	8	16	128	32	16	0.5	
11	KBC-123349	URINE	MEDICAL	16.07.2013.	<i>K. pneumoniae</i>	KPC-2	>128	>128	>128	>128	>128	>128	>128	32	32	16	2	16	8	1	
12	KBC-133913	URINE	UROLOGY	06.08.2013.	<i>Enterobacter cloacae</i>	VIM-1, TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	4	8	16	128	32	16	0.5	
13	KBC-135007	URINE	HAEMATATOLOGY	07.08.2013.	<i>Enterobacter cloacae</i>	VIM-1, TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	4	8	16	32	64	16	0.12	
14	ST-UR 10638	URINE	NEUROLOGY	01.08.2013.	<i>Enterobacter cloacae</i>	VIM-1	>128	>128	>128	>128	>128	>128	>128	8	2	4	128	8	16	0.12	
15	ST-UR 11322	URINE	ICU	22.08.2013.	<i>Enterobacter cloacae</i>	VIM-1	>128	>128	>128	>128	>128	>128	>128	8	8	4	64	8	4	0.12	
16	KBC-50490+	URINE	OUTPATIENT	15.02.2012.	<i>Enterobacter sp.</i>	TEM-1, CTX-M-15-15, VIM-1-1	>128	>128	>128	>128	>128	>128	>128	16	2	8	32	32	64	2	0.5
17	KBC-69998	URINE	NEPHROLOGY	16.04.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1-1	>128	>128	>128	>128	>128	>128	>128	8	8	32	32	128	16	2	
18	KBC-79915	URINE	NEPHROLOGY	30.04.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1-1	>128	>128	>128	>128	>128	>128	>128	8	8	32	32	128	16	0.5	
19	KBC-79299	URINE	NEPHROLOGY	27.04.2012.	<i>Enterobacter sp.</i>	TEM-1, CTX-M-15, CMY, VIM-1-1	>128	>128	>128	>128	>128	>128	>128	32	2	16	16	32	8	16	0.5
20	KBC-96179	URINE	OUTPATIENT	24.05.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1-1	>128	>128	>128	>128	>128	>128	>128	256	2	8	16	64	32	8	0.25
21	KBC-95950	URINE	NEPHROLOGY	24.05.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, CMY, VIM-1	>128	>128	>128	>128	>128	>128	>128	256	4	16	16	32	16	16	0.5
22	KBC-112208	URINE	SURGERY	23.05.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	64	4	16	32	32	2	16	0.5
23	KBC-7115/2012	URINE	MEDICAL UNIT	29.05.2012.	<i>K. pneumoniae</i>	SHV-1, TEM-1-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	2	4	32	32	4	16	0.5	
24	KBC-7152/2012	URINE	NEUROLOGY	29.05.2012.	<i>K. pneumoniae</i>	SHV-1, TEM-1-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	2	4	32	64	8	16	0.5	
25	KBC-134580	URINE	UROLOGY	24.07.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1-1	>128	>128	>128	>128	>128	>128	>128	16	16	32	64	32	8	0.5	
26	KBC-126705	URINE	NEPHROLOGY	12.07.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1-1	>128	>128	>128	>128	>128	>128	>128	8	8	32	32	128	16	0.5	
27	KBC-16188	URINE	OUTPATIENT	20.07.2012.	<i>Serratia marcescens</i>	VIM-1	>128	>128	>128	>128	>128	>128	>128	8	32	4	16	4	32	8	0.5
28	KBC-105943	URINE	NEPHROLOGY	08.06.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, CMY, VIM-1	>128	>128	>128	>128	>128	>128	>128	16	4	8	16	32	32	1	0.06
29	KBC-105 648	URINE	PULMOLOGY	08.06.2012.	<i>Enterobacter amnigenus</i>	TEM-1, CTX-M-15, VIM-1	>128	>128	>128	>128	>128	>128	>128	2	4	16	32	16	1	0.12	
30	KBC-170 506	URINE	NEPHROLOGY	25.09.2012.	<i>K. pneumoniae</i>	SHV-1, TEM-1, DHA-1, VIM-1	>128	>128	>128	>128	>128	>128	>128	8	16	16	64	0.25	4	16	0.12
31	KBC-173 783	URINE	UROLOGY	30.09.2012.	<i>K. pneumoniae</i>	SHV-1, TEM-1, DHA-1, VIM-1	>128	>128	>128	>128	>128	>128	>128	8	1	8	16	4	16	4	0.12
32	KBC-175 841	URINE	UROLOGY	01.10.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1	>128	>128	>128	>128	>128	>128	>128	8	1	16	16	16	0.12	0.06	
33	KBC-180 073	URINE	HAEMATATOLOGY	07.08.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1	>128	>128	>128	>128	>128	>128	>128	32	32	64	32	16	32	0.06	
34	KBC-194142	URINE	MEDICAL ICU	30.10.2012.	<i>Enterobacter cloacae</i>	CTX-M-15, VIM-1	>128	>128	>128	>128	>128	>128	>128	32	4	1	16	2	8	16	0.12
35	KBC-196087	URINE	NEUROSURGERY	03.11.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1	>128	>128	64	>128	>128	>128	>128	8	2	2	16	2	8	1	0.12
36	KBC-199597	URINE	NEPHROLOGY	08.11.2012.	<i>Escherichia coli</i>	TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	16	0.12	0.5	8	2	8	0.5	0.12
37	KBC-200099	URINE	NEPHROLOGY	09.11.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	16	0.12	0.5	16	1	4	0.25	0.12
38	KBC-205461	URINE	UROLOGY	18.11.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1	>128	>128	>128	>128	>128	>128	>128	32	4	8	32	32	32	16	0.12
39	KBC-213750	URINE	ENDOCRINOLOGY	30.11.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, NDM-1	>128	>128	>128	>128	>128	>128	>128	32	4	8	32	32	8	32	0.12
40	KBC-219451	URINE	NEPHROLOGY	16.12.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1	>128	>128	>128	>128	>128	>128	>128	32	4	16	32	32	32	1	0.06
41	KBC-220652	URINE	ENDOCRINOLOGY	11.12.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15, VIM-1	>128	>128	>128	>128	>128	>128	>128	64	4	8	32	16	32	64	0.12
42	KBC-227816	URINE	NEPHROLOGY	21.12.2012.	<i>Enterobacter cloacae</i>	TEM-1, CTX-M-15	>128	>128	>128	>128	>128	>128	>128	8	2	2	16	1	8	0.12	0.12
43	KBC-69 098	URINE	MEDICAL	7.10.2012.	<i>K. pneumoniae</i>	KPC-2	>128	>128	>128	>128	>128	>128	>128	8	32	32	16	16	32	0.12	
44	KBC-169731	URINE	HAEMATATOLOGY	9.10.2012.	<i>Enterobacter cloacae</i>	VIM-1	>128	>128	>128	>128	>128	>128	>128	8	4	8	64	32	>128	0.12	
45	KBC-207315	URINE	HAEMATATOLOGY	10.12.2012.	<i>Citrobacter freundii</i>	VIM-1	>128	>128	>128	>128	>128	>128	>128	4	8	8	32	64	64	0.12	
46	KBC 208 601	URINE	UROLOGY	12.12.2012.	<i>Enterobacter cloacae</i>	VIM-1	>128	>128	>128	>128	>128	>128	>128	8	8	8	32	4	32	12	0.12
47	KBC 209 377	URINE	OUTPATIENT	13.12.2012.	<i>Enterobacter cloacae</i>	VIM-1, NDM-1, OXA-48	>128	>128	>128	>128	>128	>128	>128	2	4	8	32	8	>128	0.06	

Abbreviations: AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; FEP, cefepime; PIP, piperacillin; TZP, piperacillin/tazobactam; IPM, imipenem; MEM, meropenem; Ž, ertapenem, GM, gentamicin; AMI, amikacin, CIP, ciprofloxacin.

**Figure 1.** PCR for detection of VIM beta-lactamases



1. *E. cloacae* ST-2322 (+)
2. *K. pneumoniae* KBC-11365(-)
3. *E. cloacae* ST-2417 (+)
4. *E. cloacae* ST-4730 (+)
5. *E. cloacae* KBC-45735 (+)