

EMERGENCE OF OXA-48 β -LACTAMASE IN CROATIA

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Background

Previous studies found VIM-1, NDM-1 and KPC-2 among CRE in Croatia with VIM-1 being the most prevalent. Recently OXA-48 was identified in three hospital centers.

Material and methods

Since 2012 surveillance system for CRE was implemented in University Hospital Center (UHZ) in Croatia which included phenotypic and molecular identification of carbapenemases in *Enterobacteriaceae*. In total four carbapenem non-susceptible strains of *K. pneumoniae* were found to be positive for OXA-48 in three hospital centers (University Hospital Center Zagreb-UHZ, University Hospital Center Sisters of Mercy-UHM, University Hospital Split-UHS). The antimicrobial susceptibility 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 metallo- β -lactamases (MBLs). Additionally the isolates were tested by combined disks test with EDTA or 3-aminophenylboronic to screen for KPC, MBLs or simultaneous production of MBL and KPC respectively. The transferability of meropenem resistance was determined by conjugation employing *E. coli* A15R^r strain resistant to sodium-azide. The presence of genes encoding broad and extended-spectrum β -lactamases (*bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{PER-1}), plasmid-mediated AmpC β -lactamases, group A carbapenemases (*bla*_{KPC}, *bla*_{SME}, *bla*_{IMI}, *bla*_{NDM}), metallo β -lactamases (*bla*_{VIM}, *bla*_{IMP} and *bla*_{NDM}), and carbapenem hydrolyzing oxacillinases (*bla*_{OXA-48}), was determined by PCR.

Results

The strains were uniformly resistant to amoxicillin alone and combined with clavulanate, cefazoline, cefuroxime, ceftazidime, cefotaxime, ceftriaxone, but uniformly susceptible to colistin and had variable resistance patterns to carbapenems. Modified Hodge test was positive indicating the production of carbapenemase. Phenotypic testing was positive for ESBL but negative for, MBL, KPC and AmpC. Meropenem resistance was not transferred to *E. coli* recipient strain. PCR revealed the strains to be positive also for CTX-M-15 and TEM-1. The strains from UHZ coproduced OXA-48 and VIM-1. Two *E. cloacae* strains from UHZ belonged to the same ST-418. The patients did not have a travel history to the countries where OXA-48 is endemic.



Conclusions

- The study demonstrated emergence of OXA-48 β -lactamase in three hospital centers located in different geographic regions in Croatia
- OXA-48 was first report in Turkey in 2001 but later spread in Mediterranean and western European countries such as Israel, France, Italy, Spain, Germany, Switzerland, Belgium and the Netherlands.
- Most of the strains in western Europe were imported from Turkey, Morocco, Egypt, Algeria or Lybia.

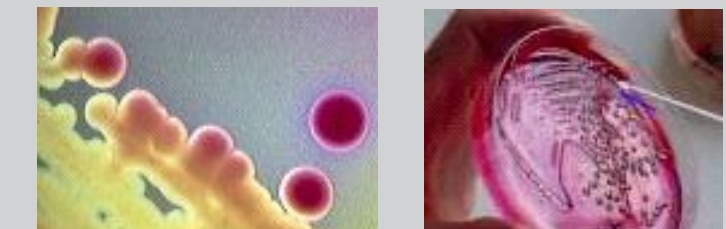


Table 1. Phenotypic tests, MICs, β -lactamase content, fluoroquinolone resistance genes, plasmid types and rep-PCR clusters of carbapenem-resistant *Enterobacteriaceae*

Protocol number	specimen	department	species	date	ESBL	AmpC	Hodge	EDTA	PBA	BL	qnr	ST	AMX	AMC	TZP	CZ	CAZ	CXM	CTX	CRO	FEP	FOX	IMI	MEM	ERT	GM	AMI	CIP	COL
KBC-209377 (281)	urine	Outpatient (previously hospitalized in UHZ)	<i>E. cloacae</i>	13. 12. 2013.	+	-	+	+	-	VIM-1 OXA-48 TEM-1 CTX-M-15	neg	ST418I	>128	>128	>128	>128	>128	>128	>128	>128	>128	16	16	32	32	8	>128	0.12	
KBC-8391(299)	urine	Outpatient (previously hospitalized in UHZ)	<i>E. cloacae</i>	17. 01. 2014.	+	-	+	+	-	VIM-1 OXA-48 TEM-1 CTX-M-15	neg	ST418I	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	16	16	32	32	16	16	0.25
KBCSPLIT UR17555	urine	Dpt. Of Medicine	<i>K. pneumoniae</i>	11.11. 2014.	+	-	-	-	-	OXA-48 CTX-M15 TEM-1	neg	ST37	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	32	32	32	16	16	0.12
Sisters of Mercy-866	urine	urology	<i>K. pneumoniae</i>	19.02. 2015.	+	-	+	-	-	OXA-48 CTX-M15 TEM-1	neg	NT	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	1	0.5	16	>128	16	32	0.25

Abbreviations: AMX-amoxicillin; AMC-amoxicillin/clavulanate; CZ-cefazoline, CXM-cefuroxime; CAZ-ceftazidime; CRO-ceftriaxone; CTX-cefotaxime, FEP-cefepime; TZP-piperacillin/tazobactam; FOX-cefoxitin; COL-colistin; IMP-imipenem; MEM-meropenem; ETP-ertapenem; GM-gentamicin; AMI-amikacin; CIP-ciprofloxacin; ESBL-combined disk test using cephalosporins alone and combined with clavulanate for detection of ESBLs; AmpC- combined disk test using cephalosporins alone and combined with phenylboronic acid for detection of AmpC β -lactamases; PBA: combined disk test using carbapenems alone and combined with phenylboronic acid for detection of AmpC β -lactamases; EDTA: combined disk test using carbapenems alone and combined with phenylboronic acid for detection of MBLs; PBA-combined disk test using carbapenems alone and combined with phenylboronic acid for detection of KPC β -lactamase; BL- β -lactamase content; qnr-qnr genes- -encoding resistance to fluoroquinolones; ST-sequence type ; NT-not tested