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In vitro susceptibility and mechanisms of resistance in contemporary Citrobacter isolates in a university hospital in Crete, Greece

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Background: *Citrobacter* spp. are facultative anaerobic Gram negative bacilli that cause infections in both the community and the hospital. An increasing incidence in the antibiotic resistant *Citrobacter* isolates has been reported. In this study we sought to evaluate the activity of available antibiotics against clinical isolates of *Citrobacter* spp., resistance rates in time and phenotypic mechanisms of resistance

Material/methods: *Citrobacter* spp. isolated from outpatients and inpatients at the University Hospital of Heraklion, Crete, Greece during a six year period (2010-2015) were included in the study. Non-duplicate isolates from intensive care unit (ICU) and non-ICU patients were studied using automated systems (Advanced Expert System in conjunction with the VITEK 2). Phenotypic confirmatory tests were applied for detection of extended-spectrum beta-lactamases (ESBLs), AmpCs and carbapenemases.

Results: A total of 385 isolates were available. *C. freundii* (172, 44.7%) and *C. koseri* (166, 43.1%) were the most commonly isolated species. *C. braaki* (34), *C. amalonaticus* (6), *C. youngae* (6) and *C. sedlakii* (1) were the remaining isolates. Colistin and fosfomycin were the most active antibiotics (both

99.2%) followed by carbapenems (99%) aminoglycosides (96.6%-98.4%), tigecycline (96.1%), cefepime (94.8%), ciprofloxacin (94.3%), tetracycline (92.7%), trimethoprim/sulfamethoxazole (91.4%), chloramphenicol (88.1%), piperacillin/tazobactam (86.5%), and 3rd generation cephalosporins (85.7%). *C. freundii* were more resistant than *C. koseri*. Antibiotic resistance did not increase during the study period for most antibiotics. Lower susceptibility was observed among multi-drug resistant strains (table 1). AmpC was the most common resistant mechanism (10.9%); ESBLs (2.1%), carbapenemases (1.3%) and aminoglycoside modifying enzymes (2.9%) were also detected. All AmpC producers were resistant to cephalosporins but not to carbapenems. In all but one isolates aminoglycoside resistance was accompanied by acquired β-lactamases.

Conclusions: A significant proportion *Citrobacter* spp. isolates was resistant to several antibiotics, most notably β-lactams, but remained susceptible to fluoroquinolones, carbapenems, aminoglycosides, tetracyclines, fosfomycin and colistin.

Table 1. Suscepibility to selected antibiotics and MICs of all and MDR *Citrobacter* spp. isolated from clinical specimens during the period 2010-2015 in the University Hospital of Heraklion, Crete, Greece.

	All N=385					MDR N=72				
Antibiotic	**MIC range**	**MIC50**	**MIC90**	**% S**	**% R**	**MIC range**	**MIC50**	**MIC90**	**% S**	**% R**
piperacillin/ tazobactam	≤4 - >128	≤4	>128	86.5	12.2	≤4 - >128	>128	>128	33.3	66.1
ceftriaxone	≤1 - >64	≤1	32	85.7	14.3	≤1 - >64	32	>64	29.2	70.8
aztreonam	≤1 - >64	≤1	16	85.7	14.3	≤1 - >64	16	>64	29.2	70.8
imipenem	≤0.25 - >16	0.5	≤1	98.7	1.3	≤0.25 - >16	≤0.25	1	93.1	6.9
ciprofloxacin	≤0.5 - >8	≤0.5	2	96.1	0.3	≤0.25 - >4	≤0.25	>4	73.6	20.8
tigecycline	≤0.25 - >4	≤0.25	≤0.25	94.3	4.2	≤0.5 - 4	1	4	84.7	0.0
amikacin	≤2 - >64	≤2	≤2	98.4	1.6	≤2 - >64	≤2	8	91.7	7.3
colistin	≤0.25 - >16	0.5	0.5	99.2	0.8	0.5 - >16	0.5	0.5	95.8	4.2
fosfomycin	4 - ≥1024	12	32	99.2	0.5	4 - >1024	12	16	98.6	1.4

Abbreviations: MIC minimum inhibitory concentration, S susceptible, R resistant