

Detection of carbapenemases and other mechanisms of enzymatic resistance to β -lactams in Enterobacteria with diminished susceptibility to carbapenems in a tertiary care hospital in Zaragoza, Spain

OBJECTIVE

Carbapenemase-producing *Enterobacteriaceae* (CPE) have emerged in recent years, causing important outbreaks in hospitals worldwide. In the absence of carbapenemases, carbapenem resistance can also be achieved through other mechanisms (AmpC β -lactamases, extended spectrum β -lactamases (ESBLs) or porin loss).

Our objective was to characterize the enzymatic mechanisms of carbapenems and other β -lactams resistance in clinical *Enterobacteriaceae* with diminished susceptibility to carbapenems in the last two years (2013-2014) at Hospital Universitario Miguel Servet.

MATERIALS AND METHODS

Antimicrobial susceptibility testing was determined by MicroScan WalkAway® (Beckman Coulter).

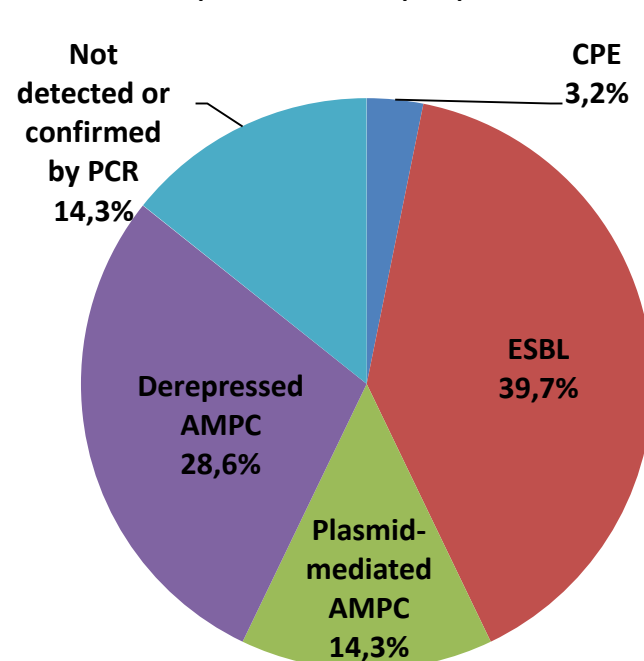
Isolates with reduced susceptibility to at least one carbapenem (imipenem, meropenem or ertapenem according to EUCAST breakpoints for CPE screening) were analysed for the presence of carbapenemases (KPC, OXA-48 and MBL), ESBLs and AmpC enzymes (according to Eucast criteria for ESBLs and AmpC screening respectively) by combined disk methods, followed by PCR confirmation according to Poirel *et al* 2011, Montsein *et al* 2007 and Perez Perez *et al* 2002.

CONCLUSIONS

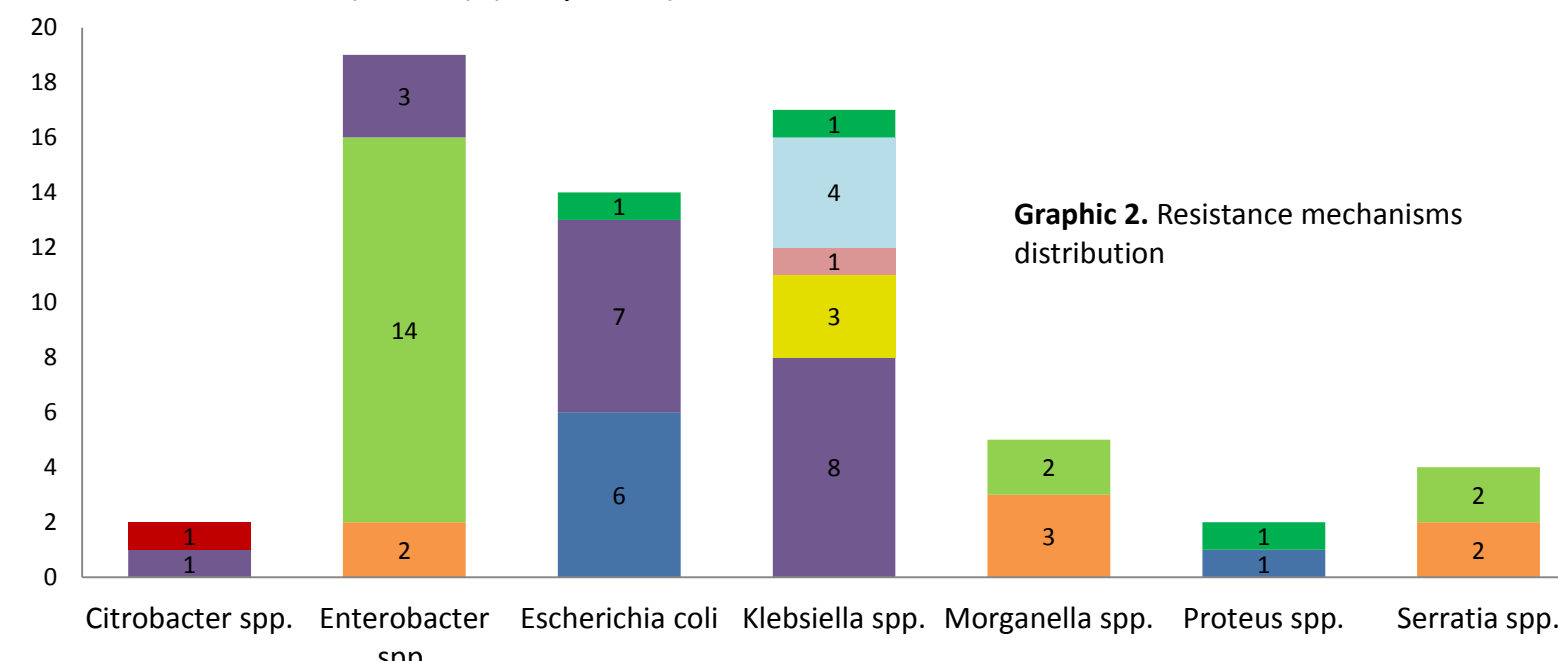
- ✓ The decreased susceptibility to carbapenems in *Enterobacteriaceae* in our area is not mainly due to true carbapenemases but rather to β -lactamase activity probably combined with decreased permeability of the outer membrane.
- ✓ Most of the isolates studied (82.5%) were ESBL or AmpC producers.
- ✓ For the present only two cases of OXA-48 carbapenemase-producing *Enterobacteriaceae* have been detected in our hospital, unlike other Spanish or European regions.
- ✓ Given the existing situation in surrounding areas and the easy and rapid spread of these strains it is necessary to further strengthen their surveillance.

RESULTS

- Retrospectively, 88 clinical isolates presented reduced susceptibility to carbapenems (one isolate per patient): 63 were stored and available for the study.
- The most prevalent *Enterobacteriaceae* were *Enterobacter* spp. (n=19, 30%), followed by *Klebsiella* spp (n=17, 27%), *Escherichia coli* (n=14, 22%) and others (n=13, 21%).
- Phenotypic detection of carbapenemases was positive in 15 strains (Table 1); two of these were PCR confirmed as OXA-48 producers (*Klebsiella pneumoniae* and *Citrobacter koseri*).
- ESBL detection was positive in 25 isolates (39.7%); TEM and CTX-M were the most prevalent families (Graphic 1).
- Plasmid-mediated AmpC was detected in 9 isolates (14.3%), most of them amplified with CIT primers (families LAT-1 to LAT-4, CMY-2 to CMY-7 and BIL-1) (Graphic 1).
- Derepressed AmpC β -lactamase was present in 18 isolates (28,6%) (Graphic 1).



Graphic 1. Distribution of isolates producing ESBL, AmpC and carbapenemases



Graphic 2. Resistance mechanisms distribution

		CARBAPENEMASE PHENOTYPIC DETECTION		
		POSITIVE	NEGATIVE	Total
PCR	POSITIVE	2	0	2
	NEGATIVE	13	48	61
		15	48	63

Table 1. Carbapenemase phenotypic detection compared to PCR results

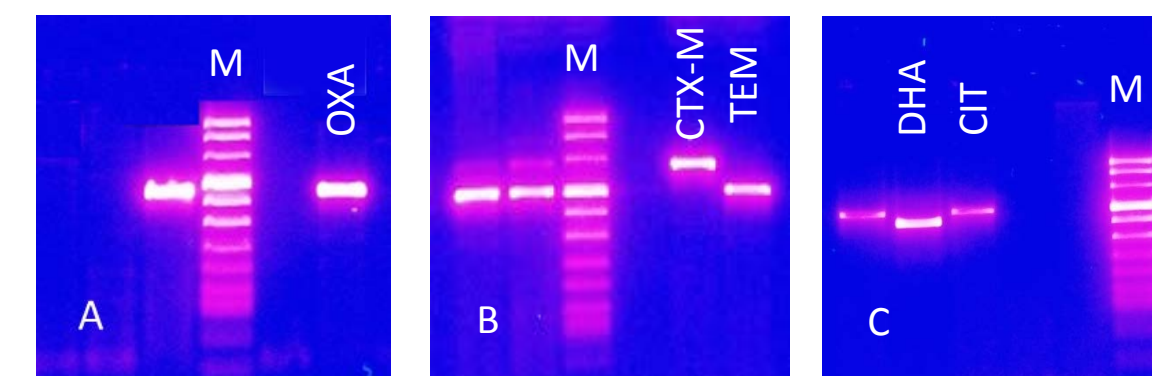


Figure 1. Examples of multiplex PCR for molecular confirmation. A) CPE Poirel *et al*, B) ESBL Monstein *et al*, C) AmpC Perez-Perez *et al*
M: DNA Molecular Weight Marker; OXA, CTX-M, TEM, DHA, CIT: positive controls