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Antimicrobials: Surveys of molecular epidemiology of resistance and resistance genes, strains or serotypes

Prevalence of plasmid-mediated quinolone resistance and aminoglycoside resistance determinants in *Enterobacter cloacae* complex producing extended-spectrum beta-lactamase (ESBL)

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Objectives: In our institution, a tertiary hospital in Northern Spain, ESBL-producing *E. cloacae* complex (Eclo) is highly prevalent, with a clonal group harboring CTX-M-15 coexisting with a polyclonal distribution of CTX-M-9 producing isolates, as defined by PFGE (XbaI restriction). The present study was performed to characterize the prevalence of plasmid mediated-quinolone resistance (PMQR) and aminoglycoside-modifying enzymes (AMEs) among ESBL- Eclo isolates.

Methods: PCR and sequencing were performed to detect TEM, SHV and CTX-M type ESBLs, PMQR genes [including *qnrA*, *qnrB*, *qnrS*, *aac(6´)-Ib-cr*, and *qepA*], and AME genes [including *aac(6´)-Ib*, *aac(3)-IIa*, *ant(2')-Ia*, *aph(3´)-Ia*] among 132 ESBL-Eclo isolates from 130 patients recovered in the 2005-2011 period (2 patients were infected with 2 isolates harboring different ESBL enzymes).

Results: The percentages of strains with the investigated genes are presented in the Table. 100% of the 68 isolates harboring CTX-M-15 (all but one belonging to a unique clonal group) carried *aac(6´)-Ib-cr*, 66 (97%) contained *qnrB1* (3 of them were additionally *qnrS1* positive), 51 (75%) *aac3´-IIa* and 1 *ant2´-Ia*. Among the 29 CTX-M-9 isolates (corresponding to 20 PFGE pulsotypes) 26 (89.7%) carried *qnrA1*, 1 *qnrS1*, 1 *aac3´-IIa*, 27 (93.1%) *ant2´-Ia*, and 2 (6.9%) *aph3´-Ia*. One isolate harboring both CTX-M-9 and M-15, carried *qnrA1*, *qnrB1*, *aac(6´)-Ib-cr*, *aac3´-IIa* and *ant2´-Ia*. Among the 11 SHV-12 isolates (4 pulsotypes) 7 harbored *qnrB2*, 1 *qnrB4*, 2 *qnrS1*, 3 *aac(6´)-Ib*, 1 *ant2´-Ia*, 1 *aph3´-Ia*. The single isolate harboring both CTX-M9 and SHV-12, contained *qnrA1*, *aac(6´)-Ib* and *ant2´-Ia*. 3 out of 4 CTX-M-3 isolates belonged to same pulsotype and harbored *aac3´-IIa*, the other one carried *qnrA2* and *aac(6´)-Ib-cr*. One isolate produced TEM-12, and was *aac(6´)-Ib* positive. No *qepA* harboring isolates were found.

Table. Number of isolates with specific ESBL type and percentages of PMQR and AME genes detected

ESBL type	n° of isolates (n° of PFGE pulsotypes)	Percentages of isolates containing						
		qnrB1	*qnrA1*	other *qnr*	*aac(6´)-Ib / aac(6´)-Ib-cr*	*aac(3)-IIa*	*ant(2")-Ia*	*aph(3´)-Ia*
CTX-M-15	68 (2)	97.1	0	S1: 4.4	100 / 100	75	1.5	0
CTX-M-15 + CTX-M-9	1	100	100	0	100 /100	100	100	0
CTX-M-9	29 (20)	0	89.7	S1: 3.4	0	3.4	93.1	6.9
CTX-M-9 + SHV-12	1	0	100	0	100 / 0	0	100	0
SHV-12	11 (4)	63.7	0	S1: 18.2 B4: 9.1	23.3 / 0	0	9.1	9.1
CTX-M-3/22	4 (2)	0	0	A2: 25	25 / 25	75	0	0
TEM-12	1	0	0	0	100 / 0	0	0	0
Undefined ESBL*	17 (7)	17.6	0	S1: 17.6	41.2 / 17.6	5.9	47.1	35.3

* PCR negative for CTX-M, TEM and SHV

Conclusions: PMQR and AME genes were highly prevalent among ESBL-Eclo isolates from our center. The predominant clone with CTX-M-15 harbored qnrB1 and diverse AMEs (particularly *aac(6´)-Ib-cr*) while CTX-M-9 harboring isolates were closely associated with *ant2´-Ia* and *qnrA*.