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Ciprofloxacin-Resistant Enterobacteria Harboring the *aac(6′)-Ib-cr* Variant Isolated from Feces of Inpatients in an Intensive Care Unit in Uruguay▽

The presence of *aac(6′)-Ib-cr* is associated with decreased susceptibility to aminoglycosides (kanamycin, amikacin, and tobramycin) and to norfloxacin and ciprofloxacin (9). This allelic variant of *aac(6′)-Ib* was found to be linked to the extended-spectrum β-lactamase (ESBL) gene $bla_{\text{CTX-M-15}}$ in isolates from many countries (4, 6, 7), while association of *aac(6′)-Ib* with the $bla_{\text{CTX-M-2}}$ ESBL gene has been widely reported in Uruguay and Argentina (3, 11).

In this work we looked for the presence of *aac(6′)-Ib* and the *aac(6′)-Ib-cr* variant and their putative ESBL coresistance markers in fecal isolates of enterobacteria resistant to ciprofloxacin and/or ceftazidime from inpatients in an intensive care unit (ICU) in Montevideo, Uruguay.

From 1 March to 31 October 2006, 106 patients were admitted to this ICU and followed daily until discharge. Rectal swabs obtained at 1, 4, 7, 10, 13, and 16 days after admission were plated on MacConkey agar plus ceftazidime (4 mg/liter) or ciprofloxacin (2 mg/liter). Enterobacterial isolates were identified by classical methods, including only the first isolate of each bacterial species per patient in this study.

Antibiotic resistance profiling, screening, and confirmatory testing for ESBL detection were performed by disk diffusion assay, and results were interpreted following the CLSI guidelines (2).

A total of 58/106 patients (55.2%) were colonized with ciprofloxacin- and/or ceftazidime-resistant enterobacteria, and 68 isolates were included in this study. Of these, 48 were resistant to gentamicin and 24 to amikacin (Table 1).

All aminoglycoside-resistant isolates were screened for *aac(6′)-Ib* by PCR; amplicons were analyzed by restriction with BstF5I, as described by Park et al. (8). PCR products that were not digested by the enzyme [tentatively assigned to *aac(6′)-Ib-cr*] were confirmed to contain *aac(6′)-Ib-cr* by double-strand sequencing. Only two *Escherichia coli* isolates were positive for *aac(6′)-Ib-cr* detection.

Recalling the observed links between $bla_{\text{CTX-M-15}}$ and *aac(6′)Ib-cr* (4, 6, 7) and between *aac(6′)Ib* and $bla_{\text{CTX-M-2}}$, the two *aac(6′)-Ib-cr*-positive isolates were further analyzed by PCR to detect CTX-M-1 and CTX-M-2 group ESBL genes using previously described primers (3, 5). Both isolates were positive only for CTX-M-1 group genes, identified as $bla_{\text{CTX-M-15}}$ after sequencing.

Both isolates were obtained at the time of patient admission into the ICU and showed identical pulsed-field gel electrophoresis patterns (10). Both patients were previously hospitalized before ICU admission, suggesting that this strain could be endemic in the hospital, where it could be horizontally transferred. All the other *E. coli* isolates yielded different pulsotypes (data not shown) compared with these.

PCR assays for the detection of class 1 integrons and IS*CR1* elements were performed according to the method of Di Conza et al. (3). Both isolates carried a class 1 integron containing the *dfr17* and *aadA5* gene cassettes, while IS*CR1* elements were not detected.

So far we have not been able to transfer these resistance genes, either by transformation or by conjugation.

This is the first report of *aac(6′)-Ib-cr* in Uruguay. In accordance with a previous report (6), $bla_{\text{CTX-M-15}}$ and *aac(6′)-Ib-cr* do not seem to be associated with class 1 integrons. Demonstration of a link to IS*26* as previously reported (1) is pending.

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TABLE 1. Main characteristics of the 68 studied isolates[a]

Species	No. of isolates											
	Total	Positive for ESBL[b]	Resistant to drug:						Positive for *aac(6′)-Ib*	With major resistance phenotype[d,e]:		
			CAZ	FOX	GEN	AMK	CIP	SXT		CAZ-GEN	CIP-GEN	CAZ-CIP-GEN
E. coli	22	6	6	0	12	2	21	17	4/2[c]	0	7 (1)	5/2[c] (3)
Klebsiella pneumoniae	13	7	11	10	8	5	7	7	8	2 (2)	0	6 (6)
Enterobacter spp.	27	6	27	27	23	17	19	22	17	5 (2)	0	18 (15)
Other	6	3	5	5	5	0	2	5	1	3 (1)	1	1
Total	68	22	49	42	48	24	49	51	30/2[c]	10 (5)	8 (1)	30 (24)

[a] Abbreviations: CAZ, ceftazidime; FOX, cefoxitin; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole.
[b] The screening test for ESBL detection was performed according to CLSI recommendations.
[c] The number after the shill is the number of positive *cr* variants.
[d] Numbers in parentheses are numbers of isolates positive by PCR for the presence of *aac(6′)-Ib*.
[e] Phenotypes: CAZ-GEN, resistance to ceftazidime and gentamicin and susceptibility to ciprofloxacin; CIP-GEN, resistance to ciprofloxacin and gentamicin and susceptibility to ceftazidime; CAZ-CIP-GEN, resistance to ceftazidime, gentamicin, and ciprofloxacin.

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