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Characterization of Plasmid-Mediated β-Lactamases in Fecal Colonizing Patients in the Hospital and Community Setting in Spain

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Aim: Active surveillance of plasmid-mediated β-lactamase-producing *Enterobacteriaceae* (PMBL-E) in fecal carriers in the hospital and in the community setting in a non-outbreak period of time. ***Methods:*** Patients were screened for carriage of *Enterobacteriaceae* resistant to expanded-spectrum cephalosporins and PMBL-E were characterized (extended-spectrum-β-lactamase [ESBL], plasmid-mediated AmpC β-lactamase [pAmpC], and carbapenemases) by PCR and sequencing. ***Results:*** The prevalence of ESBL and pAmpC carriers was 5.06% and 0.59%, respectively. Overall, CTX-M-like enzymes were the ESBL dominate enzymes (96.15%). The group CTX-M-9 was the most prevalent (81, 54%) [CTX-M-14 (74, 91.35%), CTX-M-9 (5, 6.17%), CTX-M-24 (1, 1.23%), and CTX-M-27 (1, 1.23%)] followed by the group CTX-M-1 (64, 42.67%) [CTX-M-15 (42, 65.63%), CTX-M-1 (13, 20.31%), CTX-M-32 (8, 12.5%), and CTX-M-3 (1, 1.56%)]. One CTX-M-10, one CTX-M-59, and three CTX-M-8 were also found. A very small representation of SHV or TEM ESBL enzymes was found (3.2% and 0.64%, respectively). pAmpC characterization revealed a predominance of CMY-2 (81.25%), followed by DHA-1 (18.75%). We did not detect the presence of carbapenemase producers. ***Conclusions:*** The prevalence of ESBL-producers from fecal carriers is stable in our area, but colonization by pAmpC producers has emerged recently as we have confirmed. Periodic active surveillance is useful to identify these human reservoirs and control the evolution of PMBL carriage in a community over time.

Introduction

The emergence of plasmid-mediated β-lactamase-producing *Enterobacteriaceae* (PMBL-E) is a great health concern.[17] Active surveillance of PMBL-E fecal carriage is suggested for hospital infection control, but only a few reports evaluated the fecal carriage of PMBL-E in the community setting.[20,21] Earlier colonization is a known risk factor for extended-spectrum-β-lactamase (ESBL) *Enterobacteriaceae* infections.[6] Among ESBL, CTX-M enzymes are the major concern because they have been spread worldwide, in the community setting as well as in hospitals.[4] Intestinal colonization seems the cornerstone of their dissemination, where plasmids carrying CTX-M enzymes can disseminate between *Escherichia coli* and other commensal enterobacteria.[1] There are little data about the rates of other PMBL-E fecal carriers as plasmid-mediated AmpC β-lactamases (pAmpCs) or carbapenemases in the hospital and community setting.[7,10] The objective of this work was to characterize the PMBL-E (ESBL, pAmpC, and carbapenemases) present in fecal carriers in a hospital and in a community setting in a non-outbreak period of time.

Materials and Methods

The study was performed at the University Teaching Hospital "Lozano Blesa" (Zaragoza, Spain), which has 803 beds and serves a population of 286,774 inhabitants, with 29,506 annual admissions. It includes an outpatient care facility that has 2,315,197 annual visits and an emergency department that has 127,694 annual visits.

During a 6-month prospective study (January 2010–June 2010), a total of 3,695 fecal samples from 2,508 patients (of all the patients who were requested for a stool culture, most of them were suffering from gastroenteritis) were analyzed (71.61% of whom were outpatients). The same patients could be re-enrolled only when a positive culture (*Enterobacteriaceae* isolates with a PMBL enzyme) was identified ≥30 days from

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the initial enrolment. The stool samples were cultured using standard methods for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Aeromonas*, and *Plesiomonas*.[2] One hundred and seventy-five isolates belonging to the *Enterobacteriaceae* family grew in the modified charcoal-cefazolin-deoxycholate agar (CCDA) containing cefoperazone 32 mg/L and amphotericin B supplement (Oxoid, Basingstoke, United Kingdom) incubated 48 hours under microaerobic conditions and used routinely in our laboratory for the isolation of *Campylobacter* spp. All the *Enterobacteriaceae* isolated from the CCDA medium were identified according to classical biochemical methods[13] and screened phenotypically for extended spectrum-β-lactamase (ESBL), plasmid-mediated AmpC (pAmpC), and carbapenemase production. More than one isolate from the same patient were also studied when different susceptibilities or different identifications were observed. ESBL production was studied using the CLSI confirmatory test[3] and/or E-test ESBL (AB Biodisk, Solna, Sweden). pAmpC production was tested by the E-test with cefotetan/cefotetan-cloxacillin[8] and carbapenemase production by testing susceptibility to ertapenem (10 μg disc), using a modified Hodge test,[15] determining the minimal inhibitory concentration (MIC) by E-test MBL IP/IPI (AB Biodisk) and showing synergism with dipicolinic acid, boronic acid, and cloxacillin 500 μg (Rosco Diagnostica, Taastrup, Denmark). The strains suspected of carrying a resistance pattern compatible with hyperproduction of the chromosomal enzymes (eight *Morganella morganii* strains) were disregarded. Finally, 167 isolates with a PMBL phenotype were selected from 142 patients.

Resistance genes, including $bla_{\text{CTX-M}}$ consensus, $bla_{\text{CTX-M-1}}$ group, $bla_{\text{CTX-M-9}}$ group, $bla_{\text{CTX-M-10}}$, $bla_{\text{CTX-M-8}}$, bla_{SHV}, and bla_{TEM} were amplified with specific primers according to phenotypic results as described elsewhere.[5,11,14] Cephalosporinase bla_{CMY} and bla_{DHA} were investigated using multiplex PCR, including the six families of bla_{AmpC}.[16] Amplification products were sequenced and submitted to the library of the National Center for Biotechnology Information for identification (http://blast.ncbi.nlm.nih.gov). Positive and negative controls were included in all PCR assays.

Statistical significance was calculated for comparison of proportions using the chi-square test, and a p-value of < 0.05 was considered statistically significant (SPSS V15.0; SPSS, Chicago, IL).

Results

A total of 167 *Enterobacteriaceae* isolates with a PMBL phenotype from 142 patients were included in the study, 151 of them showed an ESBL phenotype, and in the remaining 16 isolates, a pAmpC phenotype was detected. One hundred and fifty-six bla_{ESBL} genes were detected in the 151 ESBL-producing isolates of the 3,695 analyzed fecal samples (4.08%), resulting in a prevalence of ESBL carriers of 5.06%. Five ESBL-producing isolates presented two different ESBLs. The 151 ESBL-producing isolates were isolated from hospital patients (42, 5.89% of hospitalized patients) or outpatients (85, 4.73% of outpatients) from individuals in the following age groups: ≤14 years (53, 41.73%), 15–25 years (7, 5.51%), 26–50 years (26, 20.47%), 51–65 years (11, 8.66%), and >65 years (30, 23.63%).

The most frequent species isolated among the ESBL producers were *E. coli* (141, 93.37%), followed by *Klebsiella pneumoniae* (8, 5.29%) and *Klebsiella oxytoca* (2, 1.32%). Of the 156 ESBL detected, the most frequent were the CTX-M enzymes (150, 96.15%) followed by ESBL SHV (5, 3.2%) and ESBL TEM (1, 0.64%). The group CTX-M-9 was the most prevalent (81, 54%) [CTX-M-14 (74, 91.35%), CTX-M-9 (5, 6.17%), CTX-M-24 (1, 1.23%), and CTX-M-27 (1, 1.23%)] followed by the group CTX-M-1 (64, 42.67%) [CTX-M-15 (42, 65.63%), CTX-M-1 (13, 20.31%), CTX-M-32 (8, 12.5%), and CTX-M-3 (1, 1.56%)]. One CTX-M-10, one CTX-M-59, and three CTX-M-8 were also found. The association with other β-lactamases was studied in resistant strains to cefotaxime and ceftazidime. CTX-M-15 was detected alone (19, 45.2%) or in association with other ESBL SHV [SHV-2a (2), SHV-76 (1), SHV-108 (1)] and/or with other β-lactamases genes: TEM-1 (23), SHV-1 (1). One CTX-M-8 was associated with an ESBL TEM (TEM-52) and one ESBL SHV (SHV-12) was found alone. The characterized ESBL enzymes among hospital patients and outpatients are shown in Table 1. The results revealed that CTX-M-15 and CTX-M-14 were the two most prevalent enzymes in both patient groups. In hospital

Table 1. Characterization of PMBL Enzymes Among 167 Strains Isolated from Hospital Patients and Outpatients in Fecal Flora

Outpatient isolates (n = *108*)	*Type of PMBL (172, %) Type of PMBL (109, 63.37%)*	*PMBL enzymes (no. of isolates)*
Escherichia coli (104)	ESBL (97, 88.99%)	CTX-M-14 (53)
		CTX-M-9 (3)
		CTX-M-27 (1)
		CTX-M-15 (21)
		CTX-M-1 (10)
		CTX-M-32 (5)
		CTX-M-3 (1)
		CTX-M-8 (2)
		CTX-M-59 (1)
	pAmpC (7, 6.42%)	CMY-2 (7)
Klebsiella pneumoniae (2)	ESBL (3, 2.75%)	CTX-M-14 (1)
		CTX-M-15 (1)
		SHV-108 (1)
Klebsiella oxytoca (2)	ESBL (2, 1.83%)	CTX-M-14 (1)
		CTX-M-10 (1)
Hospital patient isolates (n = *59*)	*Type of PMBL (63, 36.63%)*	
E. coli (53)	ESBL (45, 71.43%)	CTX-M-14 (19)
		CTX-M-9 (2)
		CTX-M-24 (1)
		CTX-M-15 (14)
		CTX-M-1 (3)
		CTX-M-32 (3)
		CTX-M-8 (1)
		TEM-52 (1)
		SHV-12 (1)
	pAmpC (9, 14.29%)	CMY-2 (6)
		DHA-1 (3)
K. pneumoniae (6)	ESBL (9, 14.29%)	CTX-M-15 (6)
		SHV-2a (2)
		SHV-76 (1)

PMBL, plasmid-mediated-β-lactamase; ESBL, extended-spectrum-β-lactamase; pAmpC, plasmid-mediated AmpC β-lactamase.

patients, the prevalence of CTX-M-15 was higher than in outpatients (2.81% and 1.22%, respectively, p 0.005), whereas there was no significant difference in the prevalence of CTX-M-14 (2.67% and 3.06%, respectively, p 0.599).

pAmpC-producing isolates were detected in 16 of the 3,695 fecal samples analyzed (0.43%), all *E. coli*. These 16 isolates were harbored by 15 patients resulting in a 0.60% prevalence of pAmpC carriers.

The 16 pAmpC-producing strains were isolated from hospital patients (8, 1.12% of hospitalized patients) or outpatients (7, 0.39% of outpatients), from individuals in the following age groups: ≤14 years (3, 20%), 15–25 years (1, 6.67%), 26–50 years (3, 20%), 51–65 years (1, 6.67%), and >65 years (7, 46.67%). Of the 16 pAmpC-producing isolates, the most frequent was the CMY-2 enzyme (81.25%) followed by the DHA-1 enzyme (18.75%). The characterized pAmpC enzymes among hospital patients and outpatients are shown in Table 1. The results revealed that CMY-2 was the predominate enzyme in both patient groups. In hospital patients, the prevalence of CMY-2 was higher than in outpatients (0.84% and 0.39%, respectively, p 0.032). Carbapenemase-producing isolates were not detected.

Discussion

PMBL-E (ESBL, pAmpC, carbapenemases) have emerged worldwide and have been reported in hospital patients and in outpatients.[7,10] A threatening epidemiological problem is the dissemination of PMBL-E to healthy people in the community, which might depend on the frequency of PMBL fecal carriage as well as on the presence of PMBL-producing organisms in the food chain.[11,20,21] The rates of fecal carriage of ESBL-producing strains of the *Enterobacteriaceae* family were reported and that increased dramatically in our area from 2.3% in 2002 to 7.4% in 2004.[2] The prevalence of ESBL producers is currently between the two values obtained in the previous study. Colonization by pAmpC producers has emerged recently as we have confirmed. Overall, CTX-M-like enzymes are the dominate ESBL enzymes (96.15%), previously demonstrated in other countries.[10] ESBL characterization revealed a predominance of CTX-M-14 (47.43%) and CTX-M-15 (26.92%) alone or associated with SHV and TEM, and a small representation of SHV or TEM ESBL enzymes were found alone. The scarce presence of TEM and SHV ESBL in intestinal carriers is surprising since they are not uncommon in clinical strains in Spain: SHV-12-producing *E. coli* remained an important cause of community-acquired infection in 2006,[5] which seems to indicate differences in the reservoirs and the spread of different groups of ESBLs. pAmpC characterization revealed a predominance of CMY-2 (81.25%), followed by DHA-1 (18.75%). This distribution is similar to that found in patients with clinical infections identified in our environment.[9] No carbapenemase-producing enterobacteria were detected. These strains are identified very sporadically in the enterobacteria of clinical origin in our area.[12] The emergence of pAmpC is worrisome, because they appear to follow the same paths as ESBL although there may be some subtle differences.[19] As outpatients could be a reservoir not only of ESBL strains, but also of pAmpC producers, it suggests the possibility of their widespread emergence too within the community in the next few years. A high rate has been demonstrated of intestinal colonization of patients with community-acquired infections (*i.e.*, abdominal and urinary tract infection) due to ESBL-producing organisms, considering intestinal colonization a risk factor for infections with these bacteria.[20] The dynamics of gut pAmpC-producing *E. coli* as potentially pathogenic could be similar to ESBL-producing *E. coli*, where urinary tract infections accounted for 60% of all infections caused by pAmpC producers in our country.[18] Periodic active surveillance is useful to identify these community human reservoirs and control the evolution of PMBL carriage in a community over time.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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