Dramatic Increase in Prevalence of Fecal Carriage of Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* during Nonoutbreak Situations in Spain

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The occurrence of extended-spectrum β-lactamase (ESBL)-producing isolates has increased worldwide. Fecal carriage of ESBL-producing isolates has mainly been detected in nosocomial outbreaks, and few studies have evaluated fecal carriage during nonoutbreak situations and among patients in the community. We have studied the prevalence of ESBLs in 1,239 fecal samples from 849 patients (64.1% of whom were ambulatory) in 1991 and have compared the prevalence data with those obtained in 2003 for 400 fecal samples from 386 patients (75.9% of whom were ambulatory) and 108 samples from independent healthy volunteers. Samples were diluted in saline and cultured in two MacConkey agar plates supplemented with ceftazidime (1 µg/ml) and cefotaxime (1 µg/ml), respectively. Colonies were screened (by the double-disk synergy test) for ESBL production. The clonal relatedness of all ESBL-producing isolates was determined by pulsed-field gel electrophoresis with XbaI digestion; and the ESBLs of all ESBL-producing isolates were characterized by isoelectric focusing, PCR, and sequencing. The rates of fecal carriage of ESBL-producing isolates increased significantly ($P < 0.001$) in both hospitalized patients and outpatients, from 0.3 and 0.7%, respectively, in 1991, to 11.8 and 5.5%, respectively, in 2003. The rate of occurrence of ESBL-producing isolates among healthy volunteers was 3.7%. All ESBL-producing isolates recovered in 2003 were nonepidemic clones of *Escherichia coli*. ESBL characterization revealed an increasing diversity of ESBL types: TEM-4 and CTX-M-10 were the only enzymes detected in 1991, whereas TEM-4, TEM-52, SHV-12, CTX-M-9, CTX-M-10, CTX-M-14, and a CTX-M-2-like enzyme were recovered in 2003. The ESBL-producing isolates recovered from outpatients in 2003 corresponded to a CTX-M-9-type cluster (62.5%) and SHV-12 (31.2%), whereas TEM-4 was detected only in hospitalized patients. The frequencies of coresistance in isolates recovered in 2003 were as follows: sulfonamide, 75%; tetracycline, 64.3%; streptomycin, 57.1%; quinolones, 53.5%; and trimethoprim, 50%. The increased prevalence of fecal carriage of ESBL-producing isolates during nonoutbreak situations in hospitalized patients and the establishment of these isolates in the community with coresistance to non-β-lactam antibiotics, including quinolones, represent an opportunity for these isolates to become endemic.

β-Lactamase production is the most common mechanism of bacterial resistance to β-lactam antibiotics. Many new β-lactam antibiotics have been developed in the last few decades. However, with the introduction of each new β-lactam antibiotic a new β-lactamase class causing resistance to that drug has emerged (27). One of these new classes is the extended-spectrum β-lactamases (ESBLs), which hydrolyze oximino-β-lactams, such as the expanded-spectrum cephalosporins, but not the carbapenems, and which are highly susceptible to inhibition by clavulanic acid and tazobactam (24). ESBLs were first reported in the mid-1980s, and most of them have been found in *Klebsiella pneumoniae* and *Escherichia coli* (7).

Many ESBLs arise from simple point mutations in existing plasmid-mediated β-lactamase genes, like TEM- and SHV-derived ESBLs; however, members of a new group of ESBLs (the CTX-M type), derived from chromosomal class A β-lac-

tamases, have been identified in the past 10 years. The CTX-M enzymes are not related to TEM or SHV enzymes, as they share only 40% identity with these ESBLs. The first CTX-M enzyme was described in Germany in 1989 and nearly simultaneously in Argentina (4). They have been detected in a variety of species of the family *Enterobacteriaceae* in very distant regions, such as Europe, the Near and Far East, South America, and, more recently, North America (4, 7, 30). Although one specific enzyme could be detected in different regions, it seems that there are predominant enzymes in each region (4). It has been suggested that these ESBLs are responsible for the recent huge increment in ESBLs throughout the world.

As ESBLs are frequently encoded by genes located on different transferable genetic elements, a variety of epidemiological situations have been identified, ranging from sporadic cases to large outbreaks (10). Whereas ESBLs were initially associated with nosocomial outbreaks caused by single enzyme-producing strains, recent studies have revealed more complex situations, with a significant increase in community isolates (11, 13, 14, 20, 36, 39).

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Patients with fecal carriage of ESBL-producing bacterial isolates have been investigated previously during nosocomial outbreaks (12, 17, 38), but the number of prospective longitudinal studies conducted in the hospital setting during nonoutbreak situations or in the community remains scarce (5, 28, 29). Moreover, to the best of our knowledge, the rate of fecal carriage of ESBL-producing bacterial isolates during different and distant periods of time in the same location has not been investigated previously.

The aim of the present study was to investigate the prevalence of *Enterobacteriaceae* producing different types of ESBLs in the human fecal flora in both hospitalized and nonhospitalized patients in a nonoutbreak scenario and during two different periods of times more than a decade apart, 1991 and 2003. A supplementary group with healthy volunteers was included in the study in 2003.

MATERIALS AND METHODS

Detection of ESBL-producing isolates in fecal samples. A total of 1,239 fecal samples from 849 patients (64.1% of whom were ambulatory) prospectively collected during 1991 and 400 fecal samples from 386 patients (75.9% of whom were ambulatory) prospectively collected during 2003 were screened for the presence of ESBL-producing *Enterobacteriaceae*. Sampling was carried out during nonoutbreak periods that were at least 1 year from the time of a recorded epidemic. None of the outpatients studied were residents of a skilled care facility or lived in a nursing home or a health care center. In 2003, 108 fecal samples from an equivalent number of healthy volunteers without recent hospitalization and/or without recent exposure to antibiotics (within the previous 3 months) were studied.

The procedure used to screen isolates for ESBL production was identical during both experimental periods. Samples were collected and processed within 4 h of sampling. A total of 0.5 g of each fecal sample was suspended in 5 ml of saline, and aliquots of 200 μl were seeded into two MacConkey agar plates (Oxoid Ltd., Basingstoke, England), one supplemented with 1 μg of cefotaxime per ml and one supplemented with 1 μg of ceftazidime per ml, and incubated for 48 h. One colony representing each distinct colonial morphotype was regrown in the same selective plate and further analyzed. All isolates that grew were screened for ESBL production by using both the resistance phenotype and the double-disk synergy test (19, 24). One isolate of each morphotype from each patient was selected for ESBL characterization. Different isolates from the same patient were also studied in case of different susceptibilities or isoelectric focusing patterns.

Identification and antimicrobial susceptibility testing. Bacterial identification and initial antibiotic susceptibility testing were performed by using the semiautomated PASCO system (Difco, Detroit, Mich.) or the WIDER system (Soria-Melguizo, Madrid, Spain). The susceptibility patterns were confirmed by the standard NCCLS microdilution method (32). The double-disk synergy test was performed with conventional amoxicillin-clavulanate, cefotaxime, ceftazidime, and cefepime disks that were applied 20 and 30 mm apart (19). The standard disk diffusion method (31) was used to investigate the ESBL-associated resistance profiles. Disks were purchased from Oxoid.

Isoelectric focusing. Bacteria exponentially growing at 37°C in Luria-Bertani medium were harvested, and cell-free lysates were prepared by sonication. Isoelectric focusing was performed by applying the crude sonic extract to Phast gels (pH 3 to 9) in a Phastsystem apparatus (Pharmacia AB, Uppsala, Sweden) (18). β-Lactamases with known pIs (pIs 5.9, 5.4, 7.6, and 8.1) were used in parallel with the controls. Gels were stained with 500 μg of nitrocefin (Oxoid) per ml to identify β-lactamase bands.

PCR detection and sequencing of ESBLs. Genomic DNA from wild-type isolates was used as the template for PCR. ESBL amplification was performed with the appropriate primers and cycling conditions for the TEM, SHV, CTX-M-2, CTX-M-10, CTX-M-9, OXA-1, OXA-2, and OXA-10 ESBL types, as described previously (1, 11, 25, 33, 34). The PCR products were separated in 0.8% agarose gels and visualized under UV light after the gels were stained with ethidium bromide. The PCR products were purified with a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and were sequenced on an ABI Prism 377 automated sequencer (Perkin-Elmer, Norwalk, Conn.).

Pulsed-field gel electrophoresis (PFGE). Bacterial DNA was prepared as described previously (21), and XbaI (Roche GmbH, Mannheim, Germany) was used as the restriction enzyme. Digested DNA was separated in a CHEF-DRIII system (Bio-Rad, La Jolla, Calif.), and the conditions were as follows: 14°C, 6 V/cm, 10 to 40 s, 27 h. The patterns obtained were interpreted according to the criteria established by Tenover et al. (42).

Statistical analysis. Statistical significance for comparison of proportions was calculated by the chi-square test (a *P* value <0.05 was considered statistically significant).

RESULTS

The rates of patient fecal carriage of ESBL-producing strains of the family *Enterobacteriaceae* recovered during two nonoutbreak periods separated by 12 years increased in Madrid, Spain, from 0.6% (5 of 849 patients) in 1991 to 7.0% (27 of 386 patients) in 2003 ($P < 0.001$). The corresponding figures when hospitalized and ambulatory patients were considered separately were 0.3% (1 of 305 patients) and 0.7% (4 of 544 patients), respectively, in 1991 and 11.8% (16 of 293 patients) and 5.5% (11 of 93 patients), respectively, in 2003 ($P < 0.001$). The rate of fecal carriage of ESBL-producing isolates of the *Enterobacteriaceae* was slightly lower in healthy volunteers (3.7%; 4 of 108 volunteers), who were enrolled during 2003.

The characterization of the ESBLs, the identities of the ESBL-producing isolates of the *Enterobacteriaceae*, and the patients' hospital locations are shown in Table 1. All 32 isolates recovered during 2003 (12 from hospitalized patients, 16 from outpatients, and 4 from healthy volunteers) were *E. coli*, whereas isolates recovered in 1991 were *E. coli* ($n = 4$), *Klebsiella pneumoniae* ($n = 1$), and *Citrobacter freundii* ($n = 1$). In 2003 the distribution of ESBL-positive isolates by patient gender was similar among both males and females, whereas in 1991 a higher proportion of males harbored ESBL-producing isolates. In addition, the mean ages of the patients were higher in 1991 (73 ± 28.7 years) than in 2003 (32.5 ± 28.7 years) (Table 1).

A high degree of diversity in the PFGE patterns of the *E. coli* isolates was found during both periods (Fig. 1). None of the hospitalized patients shared an identical ESBL-producing *E. coli* PFGE type, denoting the absence of epidemic isolates within the population studied. In contrast, four outpatients in 2003 shared two clones (one clone each in two patients): clone ECF250S produced an SHV-12 ESBL and clone ECF245C produced a CTX-M-14 ESBL. The clone that produced SHV-12 was isolated from two outpatients from the same family (two brothers), whereas the clone that produced CTX-M-14 was isolated from two outpatients with no apparent epidemiological link.

Two different *E. coli* clones (clones ECF284C and ECF284S) producing two different ESBLs (CTX-M-9 and SHV-12) were recovered from a fecal sample from a patient hospitalized in the Infectious Diseases ward of the Hospital Universitario Ramón y Cajal during 2003. An outpatient from the 1991 period was simultaneously colonized with *K. pneumoniae* and *E. coli* clones that produced the same ESBL (CTX-M-10). Interestingly, no evidence of any invasive infection in patients harboring ESBL-producing isolates was found in any of our series.

The ESBL characterization revealed an increasing diversity of enzyme types: TEM-4 (50%) and CTX-M-10 (50%) were

TABLE 1. Characteristics of ESBL-producing isolates from fecal samples of hospitalized and nonhospitalized patients in 1991 and 2003 at the University Hospital Ramón y Cajal

Yr and strain	Organism	PFGE pattern	Patient gender (age [yr])[c]	Ward of origin	pI(s)	ESBL sequence
1991						
Ec13	*E. coli*	EC6T	F (78)	Pneumology	5.9	TEM-4
Ec14	*E. coli*	DEG[d]	M (77)	Outpatient	5.9 + 5.4	TEM-4
Ec15	*E. coli*	DEG	M (75)	Outpatient	5.9 + 5.4	TEM-4
Ec16	*E. coli*	EC7C	M (64)	Outpatient	8.1	CTX-M-10
Kp30[a]	*K. pneumoniae*	Kp30C	M (71)	Outpatient	8.1	CTX-M-10
Cf1[a]	*C. freundii*	CF1C		Outpatient	8.1	CTX-M-10
2003						
Fec 231	*E. coli*	ECF231T	M (67)	Gastroenterology	5.9	TEM-4
Fec 355	*E. coli*	ECF355T	F (80)	Gastroenterology	5.9	TEM-4
Fec 295	*E. coli*	ECF295T	F (71)	Gastroenterology	5.9 + 5.4	TEM-52
Fec 394	*E. coli*	ECF394S	M (2)	Pediatric	7.6 + 5.4	SHV-2
Fec 284Z[b]	*E. coli*	ECF284S	F (58)	Infectious Diseases	8.2	SHV-12
Fec 335	*E. coli*	ECF335	F (63)	Gastroenterology	8.2	SHV-12
Fec 222	*E. coli*	ECF222S	F (38)	Outpatient	8.2	SHV-12
Fec 250	*E. coli*	ECF250S	M (13)	Outpatient	8.2	SHV-12
Fec 251	*E. coli*	ECF250S	M (12)	Outpatient	8.2	SHV-12
Fec 310	*E. coli*	ECF310S	F (2)	Outpatient	8.2 + 5.4	SHV-12
Fec 340	*E. coli*	DEG	F (8)	Outpatient	8.2 + 5.4	SHV-12
Fec 147	*E. coli*	ECF147C	M (UK)	Outpatient	8.1 + 5.4	CTX-M-10
Fec 284X[b]	*E. coli*	ECF284C		Infectious Diseases	8.1	CTX-M-9
Fec 395	*E. coli*	DEG	F (75)	Cardiology	8.1 + 5.4	CTX-M-9
Fec 5	*E. coli*	ECF5C	M (2)	Outpatient	8.1	CTX-M-9
Fec 87	*E. coli*	ECF87C	F (4)	Outpatient	8.1	CTX-M-9
Fec 311	*E. coli*	ECF311C	F (UK)	Outpatient	8.1 + 5.4	CTX-M-9
Fec 386	*E. coli*	ECF386C	F (43)	Outpatient	8.1 + 5.4	CTX-M-9
Fec 325	*E. coli*	ECF325C	M (31)	Infectious Diseases	8.1 + 5.4	CTX-M-14
Fec 327	*E. coli*	ECF327C	M (6)	Infectious Diseases	8.1 + 5.4	CTX-M-14
Fec 397	*E. coli*	ECF397C	F (18)	Nephrology	8.1	CTX-M-14
Fec 166	*E. coli*	DEG	M (UK)	Outpatient	8.1	CTX-M-14
Fec 216	*E. coli*	ECF216C	M (2)	Outpatient	8.1	CTX-M-14
Fec 245	*E. coli*	ECF245C	F (5)	Outpatient	8.1	CTX-M-14
Fec 268	*E. coli*	ECF245C	F (UK)	Outpatient	8.1	CTX-M-14
Fec 336	*E. coli*	ECF336C	F (41)	Outpatient	8.1 + 5.4	CTX-M-14
Fec 383	*E. coli*	ECF383C	M (8)	Outpatient	8.1 + 5.4	CTX-M-14
Fec 38	*E. coli*	ECF38	M (73)	Hematology	7.6	Unknown

[a] Isolated from one patient.
[b] Isolated from one patient.
[c] F, female; M, male; UK, unknown.
[d] DEG, degrade digested DNA.

the only ESBLs detected in 1991, whereas TEM-4 (7.1%), TEM-52 (3.6%), CTX-M-10 (3.6%), SHV-2 (3.6%), SHV-12 (25.0%), CTX-M-9 (21.4%), CTX-M-14 (32.1%), and an unknown ESBL type of pI 7.6 (3.6%) were recovered in 2003 (Table 1). Outpatient fecal carriage during 2003 was mainly due to organisms producing the CTX-M-9-type enzyme cluster (62.5%) and the SHV-12 enzyme (31.2%). TEM ESBL types were mainly detected in hospitalized patients (Table 1). The ESBL-producing isolates obtained from healthy volunteers are described in Table 2. SHV-12 was detected in two volunteers, and CTX-M-14 and a CTX-2-like enzyme were each detected in one volunteer (Table 2).

The β-lactam susceptibility profiles and the associated antimicrobial resistance of the ESBL-producing isolates are shown in Table 3. When NCCLS breakpoints are considered, all isolates were susceptible to imipenem, whereas all of them were resistant to cefotaxime. Tazobactam restored the piperacillin susceptibilities (piperacillin-tazobactam MIC range, ≤16/4 to 32 μg/ml) of all except one of the isolates (piperacillin-tazobac-

tam MIC, >64/4 μg/ml). The frequencies of resistance among the isolates recovered in 2003 were as follows: streptomycin, 57.1%; nalidixic acid, 53.5%; tetracycline, 64.3%; sulfonamide, 75%, and trimethoprim, 50%. The frequencies were lower among the small number of ESBL-producing isolates recovered in 1991 (Table 3), which can be related to the shift in the types of enzymes. Isolates recovered from outpatients were mainly responsible for the increased frequency of the associated resistance, particularly isolates producing the SHV-12, CTX-M-9, and CTX-M-14 ESBLs.

DISCUSSION

Since 1987, the Microbiology Department of the Hospital Universitario Ramón y Cajal has continuously screened all isolates of the family *Enterobacteriaceae* recovered from all routinely studied clinical samples for ESBL production. Like other institutions (16, 40), patients' fecal carriage of ESBL-producing isolates was investigated only when the emergence
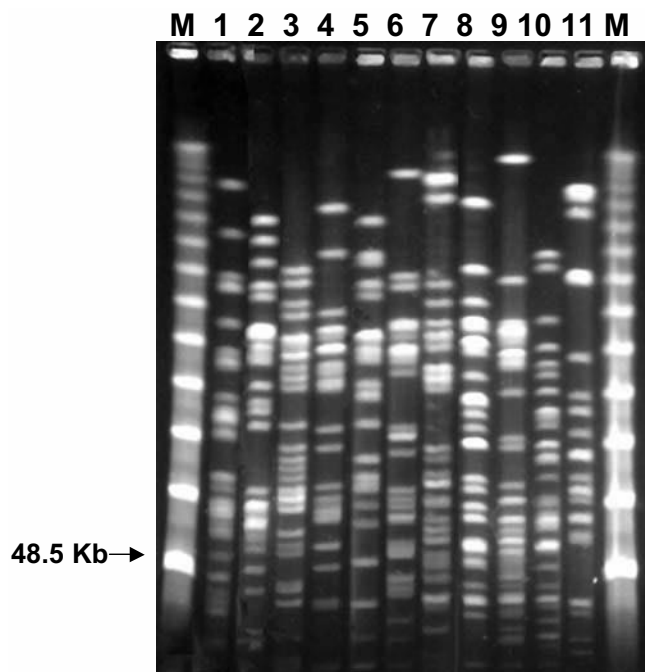
FIG. 1. Diversity of ESBL-producing *E. coli* isolates recovered from fecal samples (lanes 1 to 11, different *E. coli* isolates). Lanes M, molecular marker (λ phage 48.5 concatemers).

of an outbreak situation was suspected. Fecal colonization status was not routinely determined for all patients, even if they were admitted to an intensive care unit. In 1991, we investigated the prevalence of fecal carriage of ESBL-producing *Enterobacteriaceae* in our institution during a nonoutbreak situation. In that study, we also included fecal samples from community patients submitted to our laboratory for stool culture. The results revealed that the prevalence of fecal carriers among ambulatory patients was 0.7%, more than twofold that among hospitalized patients (0.3%). In 2003, we repeated the study, using identical methods and consecutive stool specimens submitted to our laboratory. A dramatic, significant increase in the frequency of fecal carriage of ESBL-producing isolates was demonstrated in 2003. In contrast to the results obtained in 1991, in 2003 the prevalence of fecal carriage of ESBL-producing isolates among outpatients (5.5%) was lower than that among hospitalized patients (11.8%). The rate of fecal carriage among outpatients was consistent with but slightly higher than that observed among healthy volunteers (3.7%) enrolled during the same period of time. It should be noted that this group

TABLE 2. Characteristics of ESBL-producing isolates from fecal samples from healthy volunteers in 2003

Strain[a]	PFGE pattern	Patient gender (age [yr])[b]	pI	ESBL sequence
VS27	ECVS27C	M (24)	8.1	CTX-M-2 like
VS41	ECVS41S	F (23)	8.2	SVH-12
VS85	ECVS85S	F (25)	8.2	SHV-12
VS62	ECVS62C	F (25)	8.1	CTX-M-14

[a] All isolates were *E. coli*.
[b] M, male; F, female.

of healthy volunteers had no history of antibiotic consumption or admission to a hospital during at least the 3 months before sampling.

Asymptomatic colonization of the intestinal compartment with ESBL-producing isolates has been described previously (12, 17, 38). Most of those studies were performed at the time of nosocomial outbreak situations and showed the dispersion and transmission of specific clones in specific wards or even in the same institution. In 2002, a study carried out in a hospital in Barcelona, Spain, revealed that the incidence of strains of the *Enterobacteriaceae* producing ESBLs in the stools of outpatients was up to 7.5% (29). This value is even higher than those reported in the present work for outpatients (5.4%) and healthy volunteers (3.7%). In 2001 and 2002, 2% of outpatients admitted to an intensive care unit in a hospital in Baltimore, Md., were colonized with ESBL-producing isolates (16). A higher proportion, 4.2%, was found in hospitalized neonates on admission to a neonatal intensive care unit in Washington, D.C. (40). Conversely, other investigators did not detect ESBL-producing isolates in stool specimens from 1993 to 1997 in Finland (35) or from 1997 to 1999 in Spain (9), but those studies did not include antibiotic-supplemented selective agar plates. The cefotaxime or ceftazidime concentration in our MacConkey agar plates was 1 μg/ml, which corresponds to that recommended by the NCCLS for initial screening of *K. pneumoniae*, *Klebsiella oxytoca*, and *E. coli* for ESBL production. In our experience, the use of MacConkey selective broth supplemented with 2 μg of ceftazidime per ml or 1 μg of cefotaxime per ml in order to detect carriers with low bacterial loads did not increase the rate of detection of ESBL-producing organisms (data not shown).

Colonization with multiresistant isolates, including ESBL-producing isolates, is considered a prerequisite for infection. The importance of the detection of carriers of antimicrobial-resistant bacteria has recently been highlighted not only in patient populations but also in healthy people (41). The increase in the proportion of carriers in the community increases the risk that other individuals will become carriers as a consequence of human-to-human transmission of resistant bacteria or through the environment (22), enriching the resistance gene pool and thus facilitating the acquisition of resistance mechanisms by susceptible bacteria (10). The reduction in the proportion of susceptible microbiota in the community reduces the possibility that the proportion of resistant bacteria in the nosocomial setting will decrease (23). Finally, the admission of carriers harboring resistant bacteria to hospitals increases the risk of infection for other hospitalized patients (6, 16).

Antibiotic selective pressure in hospitals may amplify the number of carriers harboring resistant bacteria (6) and enhance the opportunity for these bacteria to cause infections. This fact could also be responsible for the higher prevalence of fecal carriage of ESBL-producing *Enterobacteriaceae* in the nosocomial setting than in the community (this study) or the finding that the rate of ESBL colonization is higher among patients admitted to high-risk units with high levels of antibiotic consumption (15). A similar situation can be applicable to nursing homes and residents of health care or skilled care facilities, among whom the rates of colonization with multiresistant pathogens, including ESBL producers, is higher than

TABLE 3. Antimicrobial susceptibility profiles of ESBL-producing isolates of the family *Enterobacteriaceae* from fecal samples and associated resistance patterns

Yr and isolate	ESBL	MIC (µg/ml)[a]											Resistance phenotype
		AMC	PIP	P/T	FOX	CTX	CTX-C	CAZ	CAZ-C	CPM	ATM	IMP	
1991													
Ecl3	TEM-4	≤4/2	>64	≤16/4	≤2	>8	≤0.12/4	4	≤0.12/4	16	8	≤0.5	St Sp Gm Tb Net Nm Nal Cip Tet Sul Tp
Ecl4	TEM-4	8/4	>64	32/4	4	>8	≤0.12/4	16	≤0.12/4	8	8	≤0.5	St Gm Tb Nal Cip Tet Sul
Ecl5	CTX-M-10	8/4	>64	≤16/4	≤2	>8	≤0.12/4	16	≤0.12/4	16	16	≤0.5	
Ecl6	CTX-M-10	8/4	>64	≤16/4	≤2	>8	≤0.12/4	2	≤0.12/4	32	64	≤0.5	
Kp30	CTX-M-10	8/4	>64	≤16/4	≤2	>8	≤0.12/4	8	≤0.12/4	>64	>64	≤0.5	St Gm Tb Nal Cip Tet Sul
CF	CTX-M-10	8/4	>64	≤16/4	>16	>8	≤0.12/4	8	≤0.12/4	32	64	≤0.5	St Clo
2003													
Fec 231	TEM-4	≤4/2	>64	≤16/4	4	>128	≤0.12/4	16	≤0.12/4	32	8	≤0.5	Tet Sul Tp Clo
Fec 355	TEM-4	≤4/2	>64	≤16/4	4	>128	≤0.12/4	8	≤0.12/4	8	16	≤0.5	Tet Sul Clo
Fec 295	TEM-52	≤4/2	>64	≤16/4	4	>128	≤0.12/4	32	≤0.12/4	>32	8	≤0.5	St Nal Tet Sul Tp Clo
Fec 394	SHV-2	≤4/2	>64	≤16/4	4	>128	≤0.12/4	8	≤0.12/4	16	16	≤0.5	Tet Sul Tp Clo
Fec 284Z	SHV-12	≤4/2	>64	≤16/4	≤2	64	≤0.12/4	128	≤0.12/4	32	64	≤0.5	Sul Clo
Fec 335	SHV-12	≤4/2	>64	≤16/4	4	>128	≤0.12/4	64	≤0.12/4	64	64	≤0.5	St Clo
Fec 222	SHV-12	≤4/2	>64	≤16/4	4	>128	≤0.12/4	>128	≤0.12/4	>32	>64	≤0.5	Nal Sul Clo
Fec 250	SHV-12	≤4/2	>64	≤16/4	4	128	≤0.12/4	128	≤0.12/4	64	64	≤0.5	Nal Sul Clo
Fec 251	SHV-12	≤4/2	>64	≤16/4	4	128	≤0.12/4	128	≤0.12/4	32	32	≤0.5	Nal Sul Clo
Fec 310	SHV-12	8/4	>64	≤16/4	≤2	128	≤0.12/4	128	≤0.12/4	32	64	≤0.5	Nal Cip Tet Sul Clo
Fec 340	SHV-12	≤4/2	>64	≤16/4	≤2	>128	≤0.12/4	128	≤0.12/4	64	>64	≤0.5	St Sp Nal Sul Tp
Fec 147	SHV-12	8/4	>64	≤16/4	4	>128	≤0.12/4	2	≤0.12/4	>32	>64	≤0.5	Nal
Fec 284X	CTX-M-10	≤4/2	>64	≤16/4	4	128	≤0.12/4	1	≤0.12/4	>32	>64	≤0.5	St Sp Nal Sul Tp
Fec 395	CTX-M-9	≤4/2	>64	≤16/4	4	>128	≤0.12/4	4	≤0.12/4	>64	32	≤0.5	Nal Cip Tet Sul Tp
Fec 5	CTX-M-9	≤4/2	>64	≤16/4	8	>128	≤0.12/4	1	≤0.12/4	32	64	≤0.5	St Sp Nal Cip Tet Sul Tp
Fec 87	CTX-M-9	8/4	>64	≤16/4	4	>128	≤0.12/4	1	≤0.12/4	64	64	≤0.5	St Sp Tet Sul Tp
Fec 311	CTX-M-9	≤4/2	>64	≤16/4	4	>128	≤0.12/4	1	≤0.12/4	>64	>64	≤0.5	St Tet Sul
Fec 386	CTX-M-9	≤4/2	>64	≤16/4	≤2	>128	≤0.12/4	≤0.5	≤0.12/4	>32	4	≤0.5	Nal
Fec 325	CTX-M-9	≤4/2	>64	≤16/4	4	128	≤0.12/4	≤0.5	≤0.12/4	32	2	≤0.5	St Sp Nal Cip Tet Sul Tp
Fec 327	CTX-M-14	≤4/2	>64	≤16/4	4	>128	≤0.12/4	≤0.5	≤0.12/4	16	4	≤0.5	St Sp Nal Cip Tet Sul Tp
Fec 397	CTX-M-14	≤4/2	>64	≤16/4	≤2	>128	≤0.12/4	1	≤0.12/4	>32	32	≤0.5	St Sp Nal Cip Tet Sul Tp
Fec 166	CTX-M-14	≤4/2	>64	≤16/4	≤2	>128	≤0.12/4	≤0.5	≤0.12/4	8	4	≤0.5	St Nal Tet Sul Tp
Fec 216	CTX-M-14	≤4/2	>64	≤16/4	4	>128	≤0.12/4	≤0.5	≤0.12/4	>32	8	≤0.5	St Nal Cip Tet Sul Tp Clo
Fec 245	CTX-M-14	≤4/2	>64	≤16/4	4	>128	≤0.12/4	2	0.25/4	>32	>64	≤0.5	Nal
Fec 268	CTX-M-14	8/4	>64	≤16/4	4	>128	≤0.12/4	2	0.25/4	>32	>64	≤0.5	St Sp Tet Sul Tp Clo
Fec 336	CTX-M-14	≤4/2	>64	≤16/4	4	>128	≤0.12/4	1	≤0.12/4	>32	>64	≤0.5	Nal Tet Sul
Fec 383	CTX-M-14	≤4/2	>64	≤16/4	≤2	>128	≤0.12/4	1	≤0.12/4	>64	>64	≤0.5	St Sp Nm Km Nal Cip Tet Sul Tp
Fec 38	Unknown	>16/8	>64	>64/4	8	128	4/4	1	0.25/4	16	≤0.5	≤0.5	St Tet
2003, healthy volunteers													
VS 27	CTX-M-2 like	≤4/2	>64	≤16/4	≤2	>128	≤0.12/4	8	≤0.12/4	>32	>64	≤0.5	Nal Cip Tet Sul
VS 41	SHV-12	≤4/2	>64	≤16/4	≤2	128	≤0.12/4	>128	≤0.12/4	>32	>64	≤0.5	
VS 85	SHV-12	≤4/2	>64	≤16/4	≤2	128	≤0.12/4	128	≤0.12/4	32	32	≤0.5	Tet Sul
VS 62	CTX-M-14	>4/2	>64	≤16/4	4	>128	≤0.12/4	2	≤0.12/4	>32	>64	≤0.5	St Tet Sul

[a] AMC, amoxicillin-clavulanate; PIP, piperacillin; P/T, piperacillin-tazobactam; CTX, cefotaxime; CTX-C, cefotaxime-clavulanate; CAZ, ceftazidime; CAZ-C, ceftazidime-clavulanate; CPM, cefepime; ATM, aztreonam; IMP, imipenem.

that among true community patients or healthy volunteers (44, 45).

It is worth noting that in our study the majority of ESBL-producing isolates (and all isolates recovered in 2003) were *E. coli* and there was a high degree of genetic diversity among the different isolates, which is indicative of an allodemic situation rather than the dissemination of specific clones (3). This suggests that the huge increment in ESBL production is a consequence of horizontal gene transfer rather than the spread of specific clones, particularly for the CTX-M β-lactamases. In a recent Spanish study on the epidemiology and clinical features of infections caused by ESBL-producing *E. coli* isolates in nonhospitalized patients, Rodríguez-Baño et al. (39) also detected clonally unrelated isolates from each patient studied. Those investigators found the CTX-M-9-type enzymes to be the most prevalent ESBLs, with a prevalence of up to 64%. In our study, nearly 60% of the patients studied during 2003 were colonized with isolates harboring CTX-M-type enzymes, mainly CTX-M-9 and CTX-M-14 (Table 1). Although many CTX-M ESBL types are widely distributed geographically, both the CTX-M-9 and the CTX-M-14 enzymes are also widespread in other countries (4). In our study, nearly 70% of patients colonized with CTX-M ESBL types were not hospitalized, demonstrating that the community compartment is essential for the maintenance of these enzymes. Moreover, the community can be a reservoir of ESBLs not yet detected in clinical isolates. For example, a CTX-M-2 like enzyme was found only in healthy volunteers and not in patients. ESBLs from the CTX-M-2 group have mainly been found in South America and other European countries but have not yet been found in Spain (4).

The SHV-12 enzyme was also prevalent in our study both in the community and in healthy volunteers. This enzyme has risen to prominence in recent years and has been detected in different species of the *Enterobacteriaceae* in many parts of the world, including typical opportunistic environmental isolates and healthy animals (8, 26, 36). TEM-type ESBLs were found only in hospitalized patients in 2003. It should also be noted that a *K. pneumoniae* strain harboring TEM-4 was responsible for a clonal and plasmid outbreak in our hospital from 1997 to 1999 (2, 11).

Multidrug resistance profiles involving non-β-lactam antibiotics in ESBL-producing isolates may also contribute to the increase in colonization pressure. Fluoroquinolone resistance is becoming a common feature rather than an exception in ESBL-producing isolates (43, 37, 46, 39). In our study, the decrease in the rate of fluoroquinolone susceptibility was high both in the community (62.5%) and in the hospital (41.6%) isolates recovered from patients during 2003 (Table 3). Previous fluoroquinolone use has been demonstrated to be a risk factor for the acquisition of ESBL-producing isolates, particularly isolates producing the CTX-M-type enzymes in the community setting (39, 45). Other antimicrobial agents such as trimethoprim–co-trimoxazole or tetracyclines may also contribute to the acquisition of ESBL-producing isolates. In 2003, the prevalence of tetracycline, streptomycin, trimethoprim, and sulfonamide resistance among ESBL-producing *E. coli* fecal isolates was greater than 50%, similar to that observed among ESBL-producing *E. coli* clinical isolates (46, 47). In our case, this could be related to the locations of bla_{ESBL} genes in

the integron structures encoding these resistances (E. Machado et al., 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-53, 2003).

In summary, a dramatic increase in the prevalence of fecal carriage of ESBL-producing isolates has been observed over the last decade. This increase was associated with a higher diversity of ESBLs, although more than 50% of the ESBLs corresponded to CTX-M-type enzymes. Our results denote the importance of the intestinal tract as a reservoir for ESBL-producing isolates and also illustrate the possibility that colonized patients may act as a source of ESBLs for clinical strains. This work also points out the increasing trend toward the endemic nature of these isolates.

ACKNOWLEDGMENTS

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