Prevalence and diversity of extended-spectrum β-lactamases in faecal *Escherichia coli* isolates from healthy humans in Spain

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Abstract

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates were detected in seven of 105 faecal samples from healthy humans, from two Spanish cities, during 2007. In these isolates, five ESBLs were detected, CTX-M-14 (*n* = 2), CTX-M-1 (*n* = 2), CTX-M-32 (*n* = 1), CTX-M-8 (*n* = 1) and TEM-52 (*n* = 1). Both $bla_{\text{CTX-M-14a}}$ (surrounded by IS*Ecp1*-IS*903*) and $bla_{\text{CTX-M-14b}}$ variants (included in an integron structure) were identified in this study. This is the first time that the $bla_{\text{CTX-M-8}}$ gene and ESBLs of the CTX-M-8 group have been found in Europe and Spain, respectively. Faecal *E. coli* of healthy humans therefore constitute a reservoir of $bla_{\text{CTX-M}}$ genes with different surrounding genetic elements.

Keywords: CTX-M, *E. coli*, ESBL, faecal samples, healthy humans, Spain

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A great increase in the prevalence of extended-spectrum β-lactamases (ESBL) among clinical *Escherichia coli* isolates has been observed worldwide in the last few years, with CTX-M-producing *E. coli* especially prevalent as a cause of community-acquired infections [1,2]. Faecal carriage of ESBL-positive *E. coli* isolates has been reported in community and hospital patients in only few studies [3–6]. In addition, ESBL-positive *E. coli* isolates have also been found among healthy and sick animals and also in food in various countries [7–10], suggesting its spread in different ecosystems. Relatively few data exist thus far on faecal colonization by ESBL-positive *E. coli* isolates in healthy humans [4–6,11–13]. The objective of our study was to characterize and determine the prevalence of ESBL genes in faecal *E. coli* isolates from healthy humans, and to analyse the surrounding regions and their possible inclusion into integrons.

Between March and October 2007, 105 faecal samples were collected from healthy humans (age range 3–85 years) living in two regions in the Centre and in the North of Spain, Madrid (23 samples) and La Rioja (82 samples), respectively. None of the individuals included in the study had been exposed to antimicrobial agents or to a hospital environment in the 3 months prior to sample recovery. Samples were seeded onto Levine agar plates supplemented with cefotaxime (CTX, 2 μg/mL). After incubation at 37°C for 48 h, two colonies per sample showing *E. coli* morphology were recovered, identified by classical biochemical methods and by species-specific PCR (amplification of *uidA* gene), and screened for ESBL production according to CLSI criteria [14].

All ESBL-positive *E. coli* isolates obtained from this screen were included in this study for further characterization. Susceptibility testing to 17 antimicrobials [ampicillin, amoxicillin-clavulanic acid, cefoxitin, CTX, ceftazidime (CAZ), imipenem, aztreonam, gentamicin, streptomycin, kanamycin, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazole, sulphonamides, tetracycline, rifampicin and chloramphenicol] was performed using the disc-diffusion and agar dilution methods [14]. Genes encoding CTX-M, SHV, TEM, OXA and CMY type β-lactamases, and the genetic environment of $bla_{\text{CTX-M}}$ genes, were analysed by gene-specific PCR and DNA sequencing [15]. The presence of class 1, 2 and 3 integrons, as well as the characterization of the gene cassette arrangements were studied by PCR and DNA sequencing [16]. The presence of genes associated with tetracycline [*tet*(A)-*tet*(E), *tet*(M)], streptomycin [*aadA*], sulphonamides [*sul1*, *sul2*, *sul3*], kanamycin [*aph(3′)-Ia*, *aph(3′)-IIa*], and gentamicin resistance [*aac(3)-II*, *aac(3)-IV*] were also analysed [16]. In addition, amino acid changes in GyrA and ParC proteins were studied by PCR and sequencing of the corresponding genes in quinolone-resistant isolates [16]. The identification of the major phylogenetic groups of ESBL-positive isolates was determined by PCR [17].

TABLE 1. Characteristics of the seven extended-spectrum β-lactamase (ESBL)-positive *Escherichia coli* isolates recovered from faecal samples of healthy humans

E. coli isolate[a]	Type of ESBL	Phylogenetic group	MIC (μg/mL)		Resistance to non-β-lactams	Other genes detected[b]	Class 1 integron		Amino acid changes in[c]	
			CTX	CAZ			*intI1*	Gene cassettes	GyrA	ParC
Pn244-L	CTX-M-1	B_1	>128	4	STR[d]	*aadA*	–	–		
Pn248-L	CTX-M-1	D	>128	>128	STR[d]	*aadA*	–	–		
Pn215-L	CTX-M-8	A	>128	1	TET–NAL	*tet* (B)	–	–	S83L	Wild-type
Pn219-L	CTX-M-14a	B_2	>128	2	TET–STR–SUL	*tet* (B), *sul2*	–	–		
Pn138-M	CTX-M-14b	D	>128	>128	STR[d]–SXT–SUL	*sul1*	–[e]	*dfrA16, aadA2*		
Pn137-M	CTX-M-32	B_2	>128	>128	STR–KAN–GEN–SUL–NAL–CIP	*aph(3′)-Ia, aac(3)-II, sul3*	+	*estX, psp,aadA2*	S83L + A84P	S80I
Pn357-L	TEM-52	A	>128	8	STR[d]	–	–	–		

CTX, cefotaxime; CAZ, ceftazidime; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; SUL, sulphonamides; SXT, trimethoprim-sulfamethoxazole, TET, tetracycline.
[a]The last letter of the isolate names shows the geographical origin of the samples: L, La Rioja and M, Madrid.
[b]Detected outside integron structure.
[c]Amino acid changes in GyrA and ParC have been studied only in quinolone-resistant isolates.
[d]Intermediate category according to the CLSI standards.
[e]The *intI1* gene is interrupted at the 3′ end by the insertion of the IS*26* element.

ESBL-producing *E. coli* isolates were detected in seven of the 105 analysed faecal samples (6.6%). Two *E. coli* isolates were recovered per positive sample, but only one of each pair was kept for further studies due to the identical antimicrobial resistance phenotypes and ESBL genes exhibited by both isolates. Table 1 shows the characteristics of the isolates recovered from these positive samples. All of them were found to be unrelated clonally when studied by repetitive sequence-based PCR (data not shown). They presented MIC values for CTX of >128 μg/mL and for CAZ from 1 to >128 μg/mL. The ESBL genes found were $bla_{\text{CTX-M-14}}$ (in two isolates), $bla_{\text{CTX-M-1}}$ (in two), $bla_{\text{CTX-M-32}}$ (in one), $bla_{\text{CTX-M-8}}$ (in one), and $bla_{\text{TEM-52}}$ (in one). The $bla_{\text{CTX-M}}$ genes detected in these isolates are included in three CTX-M groups: $bla_{\text{CTX-M-14}}$ in the CTX-M-9-group, $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-32}}$ in the CTX-M-1-group, and $bla_{\text{CTX-M-8}}$ in the CTX-M-8-group. All PCR tests performed to detect the presence of other *bla* genes were negative.

According to previous reports, ESBLs of the CTX-M-8 group are very unusual in Europe and have been previously detected in *E. coli* only in the UK, as the CTX-M-40 variant [18], and never before in Spain.

An increase in the rate of ESBL-positive *E. coli* in commensal microbiota of healthy children has been previously observed in Latin America (0.1% in 2002 vs. 1.7% in 2005), and diversification of the type of CTX-M β-lactamases has also been reported [13]. In 2003, levels of faecal colonization by ESBL-positive *E. coli* isolates of 2.4% and 3.7% were found in Lebanon [12] and Spain [4], respectively. The higher rate found in our study (6.6% in 2007) is, however, less than that recently detected in non-related healthy individuals in the South of Spain (7.4%) [6] or in Saudi Arabia (13.1%) [5], and less than the 16.7% and 27.4% detected in household contacts of community patients in two further studies in Spain [6,11]. The prevalence of faecal carriage of ESBL-positive *E. coli* strains has significantly increased in recent years, suggesting that this is an emerging problem that is worsening with time.

Different resistance phenotypes and genotypes were identified among the *E. coli* isolates studied. The *tet*(B), *aadA, sul1*/*sul2*/*sul3*, *aph(3′)-Ia* and *aac(3)-II* genes were found in most of the tetracycline-, streptomycin-, sulfamethoxazole-, kanamycin- and gentamicin-resistant ESBL-producing isolates. Only the CTX-M-32-producing isolate showed ciprofloxacin resistance and presented an unusual amino acid change in GyrA (Ser83Leu and Ala84Pro), in addition to a change in ParC (Ser80Ile). The B_2 or D phylogenetic groups comprised four of the isolates, and the remaining three isolates were assigned to the A or B_1 phylogenetic groups (Table 1).

The region surrounding the $bla_{\text{CTX-M}}$ genes detected in our isolates is shown in Fig. 1. The $bla_{\text{CTX-M-14a}}$ genetic variant, flanked by IS*Ecp1* and IS*903* sequences, was found in one of the isolates (Pn219), similar to isolates described in previous reports [9,15,21]. Moreover, another isolate (Pn138) possessed the unusual $bla_{\text{CTX-M-14b}}$ variant, included into an In60 integron with a *dfrA16* and *aadA2* gene cassette combination in its variable region and with the *intI1* gene truncated at the 3′ end by the insertion of the IS*26* element. The integron-associated $bla_{\text{CTX-M-14b}}$ variant has been described very recently in four human clinical *E. coli* isolates in Spain and in Korea [19,20], but has not previously been identified from faecal *E. coli* isolates from healthy humans.

The IS*26*/IS*Ecp1* and *orf477* sequences were detected on either side of the $bla_{\text{CTX-M-1}}$ gene in two of our *E. coli*

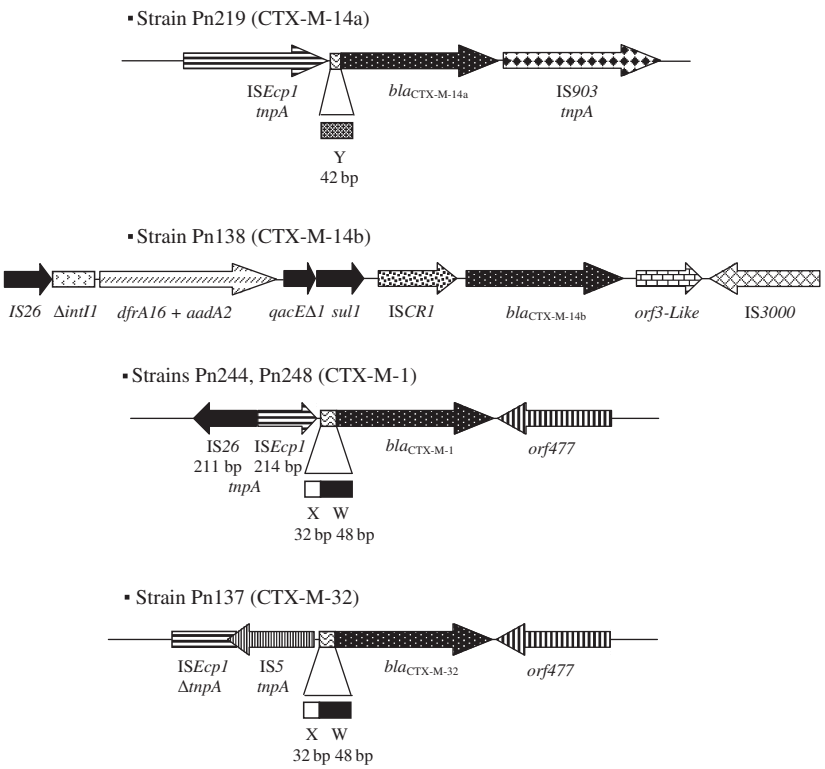

FIG. 1. Genetic environments of the $bla_{CTX\text{-}M}$ genes detected among the extended-spectrum β-lactamase-positive *Escherichia coli* strains studied (the intergenic X, Y and W regions have been previously reported [9,21]).

isolates (Pn244 and Pn248); a similar arrangement has been described by others [9,21]. In another isolate (Pn137), the IS*Ecp1* truncated transposase and the IS*5* sequence were detected upstream of the $bla_{CTX\text{-}M\text{-}32}$, and the *orf477* downstream of this *bla* gene, as previously reported [22]. The surrounding region of the $bla_{CTX\text{-}M\text{-}8}$ gene could not be identified. The $bla_{CTX\text{-}M\text{-}32}$-positive isolate (Pn137) harboured an unusual class 1 integron with the *estX*, *psp*, *aadA2* arrangement in its variable region (Table 1), although we could not demonstrate the inclusion of the ESBL gene inside the integron structure.

In conclusion, a moderate prevalence and high diversity of ESBLs were detected in faecal *E. coli* isolates of healthy humans, mainly of the CTX-M type. This is remarkable as the first detection of CTX-M-8 group determinants in Spain, and of the $bla_{CTX\text{-}M\text{-}8}$ gene in Europe. The community could be a reservoir of ESBL-producing *E. coli* isolates. This is the first time that the $bla_{CTX\text{-}M\text{-}14}$ gene has been found included into the In60 integron structure in commensal *E. coli* from healthy humans.

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Transparency Declaration

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Seasonal variation of *Pneumocystis jirovecii* infection: analysis of underlying climatic factors

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Abstract

Pneumocystis jirovecii causes severe pneumonia (PCP) in immunocompromised patients. Seasonal changes of PCP incidence may be associated with climate changes. In this first study using multiple linear regression statistics to assess monthly climatic data and *Pneumocystis*, PCP incidence was positively correlated with mean temperature, but not with rainfall or wind strength.

Keywords: Climate, environment and public health, epidemiology, incidence, infection, meteorological factors, *Pneumocystis jirovecii*, *Pneumocystis* pneumonia

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Pneumocystis jirovecii pneumonia (PCP) is one of the most frequent and serious opportunistic infections. However, the modes of transmission of PCP remain unknown. Air-borne