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Prevalence and diversity of integrons and associated resistance genes in faecal *Escherichia coli* isolates of healthy humans in Spain

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***Objectives*: To analyse the prevalence and diversity of integrons in faecal *Escherichia coli* isolates from healthy humans in Spain.**

***Methods*: One hundred *E. coli* isolates were obtained in Levine agar plates from faecal samples of 100 healthy humans during March to October 2007. Susceptibility to 16 antimicrobial agents was determined by the disc diffusion method. The presence and characterization of class 1, 2 and 3 integrons, as well as the presence of other antimicrobial resistance genes, were performed by PCR and DNA sequencing.**

***Results*: Integrases associated with class 1 and/or class 2 integrons were identified in 29 *E. coli* isolates (*intI1* gene in 26 isolates, *intI2* in 1 isolate and *intI1* + *intI2* in 2 isolates), the remaining 71 isolates being free of these integrons. Seven different gene cassette arrangements were demonstrated in 27 of the 28 *intI1*-positive isolates and were as follows (number of isolates): *dfrA1* + *aadA1* (12), *aadA* (8), *dfrA17* + *aadA5* (3), *dfrA7* (1), *dfrA5* (1), *dfrA1* (1) and *dfrA12* + *orfF* + *aadA2* (1). Four isolates presented defective class 1 integrons lacking the 3′-conserved region. The three isolates containing class 2 integrons harboured the *dfrA1* + *sat* + *aadA1* gene cassette array in their variable region. Integron-positive isolates showed higher percentages of resistance to streptomycin, ampicillin, tetracycline, trimethoprim, sulfamethoxazole, chloramphenicol and nalidixic acid than integron-negative isolates. Sixty-five percent of the integron-positive isolates belonged to phylogenetic groups A or D.**

***Conclusions*: A high prevalence of integrons was detected in faecal *E. coli* of healthy humans. Individuals in the community could be a reservoir of integron-containing *E. coli* isolates.**

Keywords: *E. coli*, antimicrobial resistance, gene cassettes

Introduction

Integrons play an important role in the antimicrobial resistance of clinical *Escherichia coli* strains because they are able to capture, integrate and express gene cassettes encoding proteins associated with antimicrobial resistance. Furthermore, the capture of genes is particularly important when these integrons are mobilized by broad-host-range conjugative plasmids or transposons. The presence of integrons in clinical multiresistant *E. coli* isolates recovered from the hospital environment is frequently reported. However, there are relatively few reports about the presence of integrons in healthy individuals.[1–3] The objective of our study was to determine the prevalence and diversity of class 1, 2 and 3 integrons in faecal *E. coli* isolates of healthy humans and to analyse the associated resistance genes and the phylogenetic groups of the integron-positive isolates.

Materials and methods

One hundred faecal samples were recovered between March and October 2007 from healthy humans living in two regions of Spain,

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Madrid (23 samples) and La Rioja (77 samples), located in the centre and in the north of the country, respectively. Eighty-nine percent of the individuals lived in urban areas. The age of healthy humans ranged from 3 to 85 years (<10 years, 5%; 20–40 years, 50%; 40–60 years, 29% and >60 years, 16%), and none of them were exposed to antimicrobial agents or to a hospital environment in the 3 months prior to sample recovery. All individuals (or their parents in the case of children) gave informed consent for participation in this study.

Faecal samples were seeded on Levine agar plates and were incubated at 37°C for 24 h. One colony per sample with a typical *E. coli* morphology (black and metallic green colonies) was recovered and identified by classical biochemical methods (Gram, TSI, indole, Methyl-Red-Voges-Proskauer and urease) and by PCR amplification of the *uidA* gene, which encodes a β-glucuronidase protein specific for *E. coli*.

Susceptibility to 16 antimicrobial agents (ampicillin, amoxicillin/clavulanic acid, cefoxitin, cefotaxime, ceftazidime, imipenem, aztreonam, gentamicin, streptomycin, kanamycin, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, sulphonamides, tetracycline and chloramphenicol) was determined for all isolates by the disc diffusion method.[4] *E. coli* ATCC 25922 was used as a control strain.

The presence of class 1, 2 and 3 integrons was analysed in all *E. coli* isolates obtained in this study. PCR amplification was used to detect the *intI1*, *intI2* and *intI3* genes, as well as the 3′-conserved region (3′ CS) of class 1 integrons (*qacΔE*+*sul1* genes).[5] The characterization of the variable region of class 1 and 2 integrons was performed by PCR and subsequent DNA sequencing.[5] The presence of genes associated with ampicillin (bla_{TEM}, bla_{SHV} and bla_{OXA-1}), tetracycline [*tet*(A)–*tet*(E), *tet*(G) and *tet*(M)], streptomycin (*aadA*), sulphonamide (*sul1*, *sul2* and *sul3*), kanamycin [*aph(3′)-Ia* and *aph(3′)-IIa*] and chloramphenicol resistance (*cmlA* and *floR*) was also analysed by PCR.[5] Chloramphenicol–acetyltransferase activity was studied, as described previously.[5] The identification of the major phylogenetic groups of the integron-positive isolates was carried out by PCR.[6] Positive and negative controls from our *E. coli* strain collection of the University of La Rioja were included in all PCRs.

Results and discussion

One hundred *E. coli* isolates were obtained from the studied faecal samples (one isolate per sample), and the presence of integrons was demonstrated in 29 of them (29%). The *intI1* gene was identified in 26 of these isolates, the *intI2* gene in 1 isolate and both the *intI1* and *intI2* genes in 2 additional isolates (Table 1). The *intI3* gene was not identified in our bacterial collection, and similar results were also previously reported by others.[1,3]

Seven different gene cassette arrangements were demonstrated in 27 of the 28 *intI1*-positive isolates and were as follows (number

Table 1. Type of integrons and associated resistance genes detected in 29 faecal *E. coli* isolates recovered from healthy humans

E. coli strain ($n = 29$)	Phylogenetic group	Class of integrase	*qacΔE* + *sul1*	Gene cassettes inside the variable region[a]	Phenotype of antimicrobial resistance[b]
Pn345	D	*intI1*	+	*aadA*[c]	NAL-SUL-TET-STR
Pn79	B_2	*intI1*	+	*aadA*[c]	AMP-AMC-SUL-TET-STR
Pn250	B_2	*intI1*	+	*aadA*[c]	AMP-NAL-SUL-TET-STR-SXT-CHL
Pn130	A	*intI1*	+	*aadA*[c]	AMP-SUL-TET-STR[d]
Pn213	A	*intI1*	+	*aadA*[c]	AMP-NAL-SUL-TET-STR
Pn132	B_1	*intI1*	+	*aadA*[c]	AMP-SUL-TET-STR
Pn343	A	*intI1*	−	*dfrA1*	AMP-SUL-TET-STR[d]-SXT
Pn131	B_2	*intI1*	−	*dfrA5*	AMP-SUL-STR-SXT
Pn84	A	*intI1*	+	*dfrA7*	AMP-SUL-TET-STR-SXT
Pn342	A	*intI1*	+	*dfrA1* + *aadA1*	AMP-SUL-TET-STR[d]-SXT
Pn218	A	*intI1*	+	*dfrA1* + *aadA1*	AMP-SUL-TET-STR-SXT
Pn242	A	*intI1*	+	*dfrA1* + *aadA1*	SUL-TET-STR-SXT
Pn217, Pn352	B_1	*intI1*	+	*dfrA1* + *aadA1*	AMP-SUL-TET-STR-SXT-CHL
Pn221, Pn257	B_2	*intI1*	+	*dfrA1* + *aadA1*	AMP-SUL-TET-STR-SXT
Pn225, Pn239, Pn354, Pn355, Pn249	D	*intI1*	+	*dfrA1* + *aadA1*	AMP-SUL-TET-STR-SXT
Pn353	B_1	*intI1*	−	*dfrA12* + *orfF* + *aadA2*	AMP-AMC-NAL-SUL-TET-STR-SXT-CHL
Pn351	A	*intI1*	+	*dfrA17* + *aadA5*	SUL-TET-STR-SXT
Pn356	A	*intI1*	+	*dfrA17* + *aadA5*	SUL-TET-STR[d]-SXT
Pn235	B_1	*intI1*	+	*dfrA17* + *aadA5*	SUL-STR[d]-SXT
Pn240	D	*intI1*	−	unknown	AMP-TET-STR[d]-SXT[d]
Pn253	D	*intI2*		*dfrA1* + *sat* + *aadA1*	AMP-SUL-KAN-TET-STR-SXT
Pn205, Pn209	D	*intI1*	+	*aadA*	AMP-SUL-STR-SXT-CHL
		intI2		*dfrA1* + *sat* + *aadA1*	

[a]Genes included inside the variable region of class 1 integrons (isolates with *intI1* gene) or class 2 integrons (isolates with *intI2* gene).
[b]AMP, ampicillin; AMC, amoxicillin/clavulanic acid; NAL, nalidixic acid; KAN, kanamycin; STR, streptomycin; SUL, sulphonamides; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; CHL, chloramphenicol.
[c]This gene cassette was determined by PCR using specific primers for *aadA1* or *aadA2* genes, but it was not confirmed by sequencing.
[d]Intermediate category according to the CLSI standards.

Table 2. Genes of resistance detected among our antimicrobial-resistant *E. coli* isolates with and without integrons

	Integron-positive isolates ($n = 29$)		Integron-negative isolates ($n = 71$)	
Antibiotics	number	genes detected (no. of isolates)	number	genes detected (no. of isolates)
Ampicillin	24	bla_{TEM} (11) bla_{SHV-1} (1)	11	bla_{TEM} (6)
Tetracycline	25	*tet*(A) (15) *tet*(B) (1) *tet*(D) (1) *tet*(A) + *tet*(C) (1)	6	*tet*(A) (2) *tet*(B) (3)
Sulphonamide	28	*sul1* (15) *sul2* (3) *sul3* (1) *sul1* + *sul2* (9)	12	*sul2* (9) *sul1* + *sul2* (1)
Kanamycin	1	*aph(3′)-Ia* (1)	2	*aph(3′)-Ia* (1)
Chloramphenicol	6[a]	*cmlA* (1)	1	ND[b]

[a]Four of these isolates presented chloramphenicol–acetyltransferase activity.
[b]ND, no gene detected.

of isolates): *dfrA1* + *aadA1* (12), *aadA* (8), *dfrA17* + *aadA5* (3), *dfrA7* (1), *dfrA5* (1), *dfrA1* (1) and *dfrA12* + *orfF* + *aadA2* (1) (Table 1). It is interesting that the *qacΔE* + *sul1* 3′ CS fragment was missing in 4 of the 28 *intI1*-positive isolates (14.3%). The genetic composition of the variable region of these four isolates was studied in detail by PCR amplification using the primer walking strategy; *dfrA1*, *dfrA5* and *dfrA12* + *orfF* + *aadA2* gene cassette arrangements were found in three of these isolates, but all the PCRs performed were negative for the remaining isolate (*E. coli* Pn240). The presence of these 3′ CS-lacking integrons has also been reported at low frequencies in *E. coli* recovered from healthy humans and animals.[2,5] The three isolates of our study containing class 2 integrons presented the same gene cassette array in their variable region, i.e. *dfrA1* + *sat* + *aadA1*, also being frequent in other studies.[1,3,5]

Most of the gene cassettes found within the variable region of class 1 integrons in our *E. coli* isolates corresponded to different variants of *dfrA* and *aadA* genes (43% and 54%, respectively), and similar results were previously obtained in commensal *E. coli* isolates from healthy subjects (33% and 55%, respectively).[1] These genes are associated with trimethoprim and streptomycin resistance, respectively, and *dfrA1* + *aadA1* was the combination most frequently detected not only in our study but also in *E. coli* isolates recovered from healthy and sick humans, animals and foods in other studies.[1–3,5,7–9] The reason for the wide distribution of some successful integrons with a specific arrangement is not known, although the possible inclusion of these integrons in transposons and/or plasmids could explain their wide dissemination in different environments.

The antimicrobial resistance phenotypes were studied in all *E. coli* isolates of our study, and the percentages of resistance detected were as follows (% among integron-positive/% among integron-negative isolates): sulphonamides (97/17), trimethoprim/sulfamethoxazole (79/7), streptomycin (79/17), tetracycline (86/8), ampicillin (83/16), amoxicillin/clavulanic acid (7/3), chloramphenicol (21/1), nalidixic acid (14/6), kanamycin (3/3) and gentamicin (0/3). All isolates were susceptible to cefoxitin, ceftazidime, cefotaxime, imipenem, aztreonam and ciprofloxacin. All 29 integron-positive isolates showed resistance to at least three antimicrobial agents, as found in other studies,[1,8,9] but only 10 of our 71 integron-negative isolates presented this characteristic (14%). Higher percentages of resistance to some antimicrobial agents (streptomycin, ampicillin, tetracycline, trimethoprim, sulfamethoxazole, chloramphenicol and nalidixic acid) were observed among integron-positive isolates with respect to integron-negative isolates. This fact could be explained by the presence of resistance genes in the conserved or variable region of integrons (as is the case for genes associated with sulfamethoxazole, trimethoprim and streptomycin resistance) or by the inclusion of resistance genes in the same mobile elements that carry integrons.

Table 2 shows the antimicrobial resistance genes detected in our isolates. A bla_{TEM} gene was detected in 17 of the 35 ampicillin-resistant isolates and the bla_{SHV-1} gene in 1 additional isolate. Regarding tetracycline resistance, a diversity of *tet* genes was found in the resistant integron-positive isolates [*tet*(A), *tet*(B), *tet*(C) and *tet*(D)], the *tet*(A) gene being the predominant one. It has been previously suggested that the *tet*(A) gene and class 1 integrons are often located on the same conjugative plasmid.[10]

The following genes were identified among the 40 sulphonamide-resistant isolates (integron-positive/integron-negative isolates): *sul1* (15/0), *sul2* (3/9), *sul3* (1/0) and *sul1*+*sul2* (9/1). The prevalence of *sul1* and *sul2* genes in our integron-positive isolates (86% and 43%, respectively) was similar to previously reported prevalences (67% and 56%, respectively) in class 1 integron-containing *E. coli* isolates from healthy children.[2] The *aph(3′)-Ia* gene was found in two of the three kanamycin-resistant *E. coli* isolates. Among the six chloramphenicol-resistant integron-positive isolates, the *cmlA* gene was found in one isolate and chloramphenicol–acetyltransferase activity was demonstrated in four other isolates.

The phylogenetic groups D and A were the most prevalent ones among our integron-containing isolates (10 and 9 isolates, respectively; 65.5%), and groups B_1 and B_2 were each identified

in five isolates. Similar to our data, class 1 integrons were detected more frequently among *E. coli* isolates of the phylogenetic group A, and some authors suggest that B_2 *E. coli* isolates could be less resistant to antimicrobials than non-B_2 isolates.[1,3,7]

In conclusion, a high prevalence of integrons was detected in faecal *E. coli* isolates of healthy humans (29%), the *dfrA1* + *aadA1* gene cassette combination being most frequently found in their variable region, and this fact suggests that individuals in the community could be a reservoir of integron-containing *E. coli* isolates.

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Transparency declarations

None to declare.

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