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Integron-Associated Antibiotic Resistance and Phylogenetic Grouping of *Escherichia coli* Isolates from Healthy Subjects Free of Recent Antibiotic Exposure

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The study of integrons in 181 *Escherichia coli* isolates from three groups of healthy subjects who lived in communities and had not taken antibiotics for at least 1 month showed that the presence of integrons was associated with antibiotic resistance and phylogenetic grouping of the bacterial host and dependent on a subject's living environment.

Integrons play an important role in antibiotic resistance of clinical *Escherichia coli* strains because they are able to capture, integrate, and express gene cassettes encoding antibiotic resistance (11). The prevalence of integrons ranges from 22 to 59% in clinical *E. coli* (20, 26) and increases with the resistance of the isolates (17). Integrons are also present in resistant intestinal *E. coli* isolates from subjects living in a community (18). However, the subjects studied (18) were those admitted to a neurology ward and thus were, strictly speaking, not "perfectly healthy." Resistance in intestinal *E. coli* strains is promoted by recent exposure to antibiotics (23), diet (7), deficient hygiene, poor living conditions (28), and living in developing countries (16). *E. coli* populations are structured in four major phylogenetic groups, A, B1, B2, and D (13), and isolates from the B2 phylogenetic group appear to be the least resistant to antibiotics (14, 24).

We took advantage of collections of intestinal *E. coli* isolates from healthy adults free of recent direct exposure to antibiotics to investigate the epidemiology of integrons and the relationship between their prevalence and the isolates' phylogenetic grouping.

We studied a collection of 181 *E. coli* isolates of known phylogenetic groups (9) and known antibiotic susceptibilities (2, 10), originating from three age- and sex-matched groups of healthy subjects living in communities (25 subjects/group, four to five isolates/subject): (i) Wayampi Amerindians living in isolation in southern French Guyana (WA), (ii) pig farmers (PF), and (iii) bank or insurance workers (BIW); the last two groups were from western mainland France. Subjects had not taken antibiotics for at least 1 month before the fecal specimen was obtained. When several isolates from the same subject displayed indistinguishable antibiotic susceptibility patterns and belonged to the same phylogenetic group, they were considered replicates and only one was selected at random. It was noted that the prevalence of group B2 in these isolates (27/181 [15%]) was lower than that in isolates from healthy women in Michigan (42/88 [48%]) (30), a difference that has been discussed elsewhere (9).

DNA was extracted from each isolate, and plasmid DNA from *E. coli* K-12 DH1 containing plasmid PSU 2056 (21), PVC 2554 (6), or PSMB 731 (1) carrying, respectively, a class 1, 2, or 3 integron was used as a control. The *intI1*, *intI2*, and *intI3* genes were detected by triplex real-time PCR on an ABI Prism 7000 sodium dodecyl sulfate thermocycler (Applera, Courtaboeuf, France) using specific primer pairs (Table 1). Dissociation temperatures were 85, 80, and 90°C for the amplification products, corresponding to *intI1*, *intI2*, and *intI3*, respectively. All PCRs were performed in a volume of 25 μl containing 1× SYBR green PCR master mix (Applera) with 250 nM of each primer and 100 ng of DNA. A denaturation cycle of 10 min at 95°C was followed by 30 cycles of 15 s at 95°C and 60 s at 60°C. Each PCR series included positive controls in triplicate ranging from 0 to 10^7 copies of the targets.

To characterize the gene cassettes, a PCR was performed on isolates containing *intI1* using primer pair L1/R1 and two amplifications on isolates containing *intI2* using primer pair 986R/2021R and primer pair 1299F/TnsE-Tn*7* (Table 1). If the PCR using L1/R1 was negative, amplifications were performed using primer pairs Sul-833F/Sul-2602R and L1/TnsE-Tn*7* (Table 1) to detect two targets. PCR products were sequenced using the primers described in Table 1 and the ABI Prism sequencing kit (Applera).

Statistical analysis was done with SAS v.8.02 software (SAS Institute, Cary, NC) and with nQuery Advisor v.5.0 (Statistical Solutions, Boston, MA). Differences in the prevalences of integrons between phylogenetic groups and between groups according to antibiotic resistance were assessed using chi-square testing.

Twenty-seven isolates (15%) were integrase positive: 20

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TABLE 1. Primers used

Purpose	Primer	Target	Sequence (5′–3′)	Integron class	Reference
Real-time PCR	245 F	*intI1*	5′-TGAAAGGTCTGGTCATACATGTGA-3′	1	This work
	345 R	*intI1*	5′-CATTCCTGGCCGTGGTTCT-3′	1	This work
	312 F	*intI2*	5′-GCTAGGGCATTTAAAGCGATTTT-3′	2	This work
	412 R	*intI2*	5′-CAGACCATGGGCAGTGAAGA-3′	2	This work
	381 F	*intI3*	5′-TGCGCTCCAGTGCATGAG-3′	3	This work
	528 R	*intI3*	5′-GGCAAGGGCGACAAGGA-3′	3	This work
Standard PCR and sequencing	L1	5′Cs[a]	5′-GGCATCCAAGCAGCAAG-3′	1	20
	R1	3′Cs[b]	5′-AAGCAGACTTGACCTGA-3′	1	20
	Sul-833F	3′Cs[b]	5′-TGGTGACGGTGTTCGGCATTC-3′	1	This work
	Sul-2602R	3′Cs[b]	5′-GCGAAGGTTTCCGAGAAGGTG-3′	1	This work
	986R	5′Cs[c]	5′-GTAGCAAACGAGTGACGAAATG-3′	2	3
	2021R	*aadA*	5′-TCTTCCAACTGATCTGCGGGC-3′	2	5
	1299F	*aadA*	5′-ATGAGGGAAGCGGTGATCGCC-3′	2	5
	TnsE-Tn*7*	3′Cs[d]	5′-GAATTCGACATGTTTGGACGCCTTGGC-3′	2	3
	aadA1-283R	*aadA1*	5′-ATAACGCCACGGAATGATGTC-3′	1	This work
	dfr1-453F	*dfr1*	5′-CCAAATCTGGCAAAAGGGTTAA-3′	1	This work
	aadA2-283R	*aadA2*	5′-ATAACGCCACGGAATGATGTC-3′	1	This work
	dfr12-463F	*dfr12*	5′-TACACCCACTCCGTTTATGCG-3′	1	This work

[a] 5′ conserved class 1 integron segment.
[b] 3′ conserved class 1 integron segment.
[c] 5′ conserved class 2 integron segment.
[d] 3′ conserved class 2 integron segment.

TABLE 2. Prevalence of *intI1* and *intI2* genes in commensal *E. coli* isolates

Characteristic	No. of isolates	No. (%) of isolates with indicated gene				*P* value
		None	Any	*intI1*	*intI2*	
Source						
Pig farmers	54	41 (75.9)	13 (24.1)[a]	10 (18.5)	4 (7.4)	<0.05
Bank/insurance workers	49	42 (85.7)	8 (16.3)	5 (12.2)	2 (4.1)	
Amerindians	78	72 (92.3)	6 (7.7)	5 (6.4)	1 (1.3)	
Resistance						
Streptomycin						
Susceptible	119	117 (98.3)	2 (1.7)	2 (1.6)	0 (0)	<0.001
Resistant	62	37 (59.7)	25 (40.3)[a]	19 (30.6)	7 (11.2)	
Ampicillin						
Susceptible	140	129 (92.1)	11 (7.9)[a]	7 (5)	5 (3.6)	<0.0001
Resistant	41	25 (61)	16 (39)	14 (34.2)	2 (4.9)	
Co-trimoxazole						
Susceptible	149	140 (94)	9 (6)	6 (4)	3 (2)	<0.0001
Resistant	32	14 (43.8)	18 (56.2)[a]	15 (46.8)	4 (12.5)	
Tetracycline						
Susceptible	103	98 (95.2)	5 (4.8)	1 (0.9)	4 (3.9)	<0.0001
Resistant	78	56 (71.8)	22 (28.2)[a]	20 (25.6)	3 (3.8)	
Chloramphenicol						
Susceptible	140	125 (89.3)	15 (10.7)	11 (7.9)	4 (2.9)	<0.01
Resistant	41	29 (70.7)	12 (29.3)[a]	10 (24.3)	3 (8.5)	
Nalidixic acid						
Susceptible	177	153 (86.4)	24 (13.0)[a]	19 (10.7)	6 (3.3)	<0.05
Resistant	4	1 (25)	3 (75)	2 (50)	1 (25)	
Pefloxacin						
Susceptible	177	153 (86.4)	24 (13.0)[a]	19 (10.7)	6 (3.3)	<0.05
Resistant	4	1 (25)	3 (75)	2 (50)	1 (25)	
Phylogenetic group[b]						
A	74	62 (83.8)	12 (16.2)[a]	11 (14.8)	2 (2.7)	
B1	41	34 (82.9)	7 (17.1)	5 (12.1)	2 (4.8)	
B2	27	26 (96.3)	1 (3.7)	1 (3.7)	0	NS[c] (0.08)
D	39	32 (82.1)	7 (17.9)	4 (10.2)	3 (7.7)	

[a] Isolates with class 1 and class 2 integrons.
[b] Determined as described in reference 4.
[c] NS, not significant (B2 versus non-B2 values).

(11%) were positive for class 1 integrase, 6 (3%) for class 2, 1 (0.6%) for both classes 1 and 2, and none for class 3. All isolates harboring an integron were resistant to at least one antibiotic.

Integrons were more prevalent in isolates from PF than in those from BIW or WA ($P < 0.05$), and when each antibiotic was considered, integrons were more prevalent in the isolates resistant to each antibiotic than in the susceptible ones (Table 2). Isolates susceptible to all antibiotics were significantly more prevalent in the B2 phylogenetic group (15/27) than in the non-B2 groups (54/154) ($P < 0.05$). A single B2 isolate carried an integron. There was a trend towards lower integron prevalence among group B2 (Table 2) ($P = 0.08$).

Eighteen of 21 (85.7%) class 1 integrons carried either *dfr* (2/21 [9.5%]), *aadA* (8/21 [38.1%]), or both cassettes (8/21 [38.1%]). The remaining 3/21 (14.3%) integrons were resistant to both streptomycin and co-trimoxazole but probably carried truncated integrons lacking 3′-end-conserved segments since no class 1 or class 2 integron 3′-end-conserved regions were detected. All cassette sequences were identical to known GenBank sequences, except one that was closely related (96% identity) to *aadA2*. Four of the seven isolates containing class 2 integrons had *dfr-1*, *sat*, *aadA*, and *orfX* genes, in that order and were identical to the integron in the Tn*7* transposon; two had *sat*, *aadA*, and *orfX* genes and were identical to the integron in Tn*1826* (3). The remaining isolate contained two class 2 integrons, with three and four gene cassettes, respectively.

These results show that integrons can persist in commensal *E. coli* isolates, even in subjects who have not taken antibiotics for at least 1 month, a factor not analyzed previously (18). Antibiotic exposure is probably the major determinant of increased integron prevalence, as suggested by the prevalence of 22 to 59% in *E. coli* isolates from hospitalized patients (20, 26) who are more often exposed to antibiotics than subjects living in communities. Here, antibiotic exposure in the general population from which the included subjects were extracted was about twice as high in BIW as in WA (2, 10). This might explain why the prevalence of integrons was also twice as high in BIW as in WA (16.3% versus 7.7%) (Table 2). The observed differences in the integron prevalences between PF and BIW (Table 2) could be explained by differences in animal exposure. Indeed, PF and BIW were matched for age, gender, and country of residence, and their direct levels of antibiotic exposure were similar (2), but PF worked with animals receiving therapeutic and preventive antibiotics as well as antibiotics for growth promotion (8). As a result, it may be that the *E. coli* strains from swine harbored integrons and that these *E. coli* organisms were then transferred to the farmers, as has been demonstrated for enterobacteria and enterococci (19, 27).

As expected, class 1 integrons were more frequently observed than class 2 integrons (22). The rarely described class 3 integron was absent. All cassette sequences were identical to those of the most frequently described cassettes (20, 25), and integron structures were also as described previously (3, 12). We found only three unexpected structures corresponding to truncated class 1 integrons. Their role remains unclear. This low diversity of integrons and of their cassettes in commensal *E. coli* isolates from healthy subjects contrasts with the diversity previously found in clinical isolates (15, 22, 29) and may be due to the absence of direct recent antibiotic exposure of the source subjects. The *dfr* and *aadA* gene cassettes appeared stable, once inserted in integrons and even in the absence of selective pressure, for at least 1 month. This has not been tested for longer periods.

Finally, the phylogenetic group analysis may have revealed a new constraint to the dissemination of resistance. It has been suggested that clinical B2 *E. coli* strains were less resistant to antibiotics than non-B2 strains (14, 24). This was also true for commensal isolates in which a trend ($P = 0.08$) suggested that integrons were less frequent in B2 than non-B2 isolates, raising the hypothesis that the two phenomena are interrelated.

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REFERENCES

1. **Arakawa, Y., M. Murakami, K. Suzuki, H. Ito, R. Wacharotayankun, S. Ohsuka, N. Kato, and M. Ohta.** 1995. A novel integron-like element carrying the metallo-β-lactamase gene bla_{IMP}. Antimicrob. Agents Chemother. **39:** 1612–1615.
2. **Aubry-Damon, H., K. Grenet, P. Ndiaye-Sall, D. Che, E. Corderio, M. Bougnoux, E. Rigaud, Y. Le Strat, V. Lemanissier, L. Armand-Lefevre, D. Delzescaux, J. C. Desenclos, M. Liénard, and A. Andremont.** 2004. Increased prevalence of antibiotic resistant bacteria in commensal flora of pig farmers. Emerg. Infect. Dis. **10:**873–879.
3. **Biskri, L., and D. Mazel.** 2003. Erythromycin esterase gene *ere*(A) is located in a functional gene cassette in an unusual class 2 integron. Antimicrob. Agents Chemother. **47:**3326–3331.
4. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
5. **Collis, C. M., G. Grammaticopoulos, J. Briton, H. W. Stokes, and R. M. Hall.** 1993. Site-specific insertion of gene cassettes into integrons. Mol. Microbiol. **9:**41–52.
6. **Cordano, A. M., and R. Virgilio.** 1996. Evolution of drug resistance in *Salmonella panama* isolates in Chile. Antimicrob. Agents Chemother. **40:** 336–341.
7. **Corpet, D. E.** 1988. Antibiotic resistance from food. N. Engl. J. Med. **318:** 1206–1207.
8. **Cromwell, G. L.** 2002. Why and how antibiotics are used in swine production. Anim. Biotechnol. **13:**7–27.
9. **Escobar-Páramo, P., K. Grenet, A. Le Menac'h, L. Rode, E. Salgado, C. Amorin, S. Gouriou, B. Picard, M. C. Rahimy, A. Andremont, E. Denamur, and R. Ruimy.** 2004. Large-scale population structure of human commensal *Escherichia coli* isolates. Appl. Environ. Microbiol. **70:**5698–5700.
10. **Grenet, K., D. Guillemot, V. Jarlier, B. Moreau, S. Dubourdieu, R. Ruimy, L. Armand-Lefevre, P. Bau, and A. Andremont.** 2004. Antibacterial resistance, Wayampis Amerindians, French Guyana. Emerg. Infect. Dis. **10:**1150–1153.
11. **Hall, R. M., and C. M. Collis.** 1995. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol. Microbiol. **15:**593–600.
12. **Hansson, K., L. Sundström, A. Pelletier, and P. H. Roy.** 2002. IntI2 integron integrase in Tn*7*. J. Bacteriol. **184:**1712–1721.
13. **Herzer, P. J., S. Inouye, M. Inouye, and T. S. Whittam.** 1990. Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. J. Bacteriol. **172:**6175–6181.
14. **Johnson, J. R., M. A. Kuskowski, K. Owens, A. Gajewski, and P. L. Winokur.** 2003. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. J. Infect. Dis. **188:**759–768.
15. **Jones, M. E., E. Peters, A. M. Weersink, A. Fluit, and J. Verhoef.** 1997. Widespread occurrence of integrons causing multiple antibiotic resistance in bacteria. Lancet **349:**1742–1743.
16. **Lester, S. C., M. del Pilar Pla, F. Wang, I. Perez Schael, H. Jiang, and T. O'Brien.** 1990. The carriage of *Escherichia coli* resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu, China. N. Engl. J. Med. **323:**285–289.
17. **Leverstein-van Hall, M. A., H. E. M. Blok, A. R. T. Donders, A. Paauw, A. C. Fluit, and J. Verhoef.** 2003. Multidrug resistance among *Enterobacteriaceae* is strongly associated with the presence of integrons and is independent of species or isolate origin. J. Infect. Dis. **187:**251–259.

18. **Leverstein-van Hall, M. A., A. Paauw, A. T. A. Box, H. E. M. Blok, J. Verhoef, and A. C. Fluit.** 2002. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. J. Clin. Microbiol. **40:**3038–3040.
19. **Levy, S. B., G. B. FitzGerald, and A. B. Macone.** 1976. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. Nature **260:**40–42.
20. **Maguire, A. J., D. F. J. Brown, J. J. Gray, and U. Desselberger.** 2001. Rapid screening technique for class 1 integrons in *Enterobacteriaceae* and nonfermenting gram-negative bacteria and its use in molecular epidemiology. Antimicrob. Agents Chemother. **45:**1022–1029.
21. **Martinez, E., and F. de la Cruz.** 1990. Genetic elements involved in Tn21 site-specific integration, a novel mechanism for the dissemination of antibiotic resistance genes. EMBO J. **9:**1275–1281.
22. **Martinez-Freijo, P., A. C. Fluit, F. J. Schmitz, V. S. Grek, J. Verhoef, and M. E. Jones.** 1998. Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. J. Antimicrob. Chemother. **42:**689–696.
23. **Murray, B. E., E. R. Rensimer, and H. L. DuPont.** 1982. Emergence of high-level trimethoprim resistance in fecal *Escherichia coli* during oral administration of trimethoprim or trimethoprim-sulfamethoxazole. N. Engl. J. Med. **306:**130–135.
24. **Picard, B., and P. Goullet.** 1989. Correlation between electrophoretic types B1 and B2 of carboxylesterase B and sex of patients in *Escherichia coli* urinary tract infections. Epidemiol. Infect. **103:**97–103.
25. **Roe, M. T., E. Vega, and S. D. Pillai.** 2003. Antimicrobial resistance markers of class 1 and class 2 integron-bearing *Escherichia coli* from irrigation water and sediments. Emerg. Infect. Dis. **9:**822–826.
26. **Sallen, B., A. Rajoharison, S. Desvarenne, and C. Mabilat.** 1995. Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of *enterobacteriaceae*. Microb. Drug Resist. **1:**195–202.
27. **van Den Bogaard, A. E., N. London, and E. E. Stobberingh.** 2000. Antimicrobial resistance in pig faecal samples from the Netherlands (five abattoirs) and Sweden. J. Antimicrob. Chemother. **45:**663–671.
28. **Walson, J. L., B. Marshall, B. M. Pokhrel, K. K. Kafle, and S. B. Levy.** 2001. Carriage of antibiotic-resistant fecal bacteria in Nepal reflects proximity to Kathmandu. J. Infect. Dis. **184:**1163–1169.
29. **White, P. A., C. J. McIver, and W. D. Rawlinson.** 2001. Integrons and gene cassettes in the *Enterobacteriaceae*. Antimicrob. Agents Chemother. **45:** 2658–2661.
30. **Zhang, L., B. Foxman, and C. Marrs.** 2002. Both urinary and rectal *Escherichia coli* isolates are dominated by strains of phylogenetic group B2. J. Clin. Microbiol. **40:**3951–3955.