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Integrons and Antibiotic Resistance in Phylogenetic Group B2 *Escherichia coli*

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It has been reported that *Escherichia coli* B2 phylogenetic group strains are more susceptible to antibiotics, especially to quinolones, and tend to carry less integrons than other phylogenetic groups in commensal environments. To gain a better understanding of the relationships between antibiotic resistance, integrons, and phylogenetic groups in an environment with high antibiotic selective pressure, we compared these characteristics in three selected groups of urinary tract infection *E. coli* isolated in a university hospital (G1, G2, and G3). The isolates were fully susceptible to antibiotics, resistant to amoxicillin and cotrimoxazol, or resistant to amoxicillin, cotrimoxazol, and nalidixic acid in the G1, G2, and G3 group, respectively. The prevalence of B2 isolates was significantly lower in the most resistant G3 group (22.6%) than in susceptible G1 (57.8%, $p < 0.001$) and G2 groups (50%, $p < 0.01$). In contrast, the prevalence of B2 isolates was not significantly different between G1 and G2 groups. The prevalence of integrons was nil in G1 isolates but very high in G2 (94.3%) and G3 (87.5%) isolates, and integrons were equally distributed among the phylogenetic groups. We propose a step-by-step mechanism for the emergence of antibiotic resistance in *E. coli*. Under very low selective pressure, resistance emerges without integrons. When the antibiotic pressure increases, quinolone and integron-mediated resistance occurs outside phylogenetic group B2. With strong antibiotic selective pressure, integrons are highly prevalent and widespread regardless of the phylogenetic group.

Introduction

The emergence of antibiotic resistance in bacteria is the result of complex interactions with the environment of the strain, including the exposure to antibiotics, the genomic constraints, and the fitness of the strain. *Escherichia coli* is particularly well suited to study such interactions, as it is a widespread commensal of the gut of vertebrates, including humans, that is inevitably excreted into the external environment. It is also a major pathogen involved in intestinal and extraintestinal diseases.[14] During these various lifestyles, *E. coli* is subjected to various selective pressures. Population genetic studies have shown that the species has a mainly clonal population structure[4,22] with four main phylogenetic groups (A, B1, B2, and D),[9,16] with strains of the four different groups differing in their phenotypic and genotypic characteristics. Strains of the B2 and, at a lesser extent D, phylogenetic groups are predominant among extraintestinal isolates,[1,11,21] whereas differences in prevalence of the four phylogenetic groups in commensal strains are observed according to the strain host.[5–7]

By studying commensal *E. coli* strains from various hosts exposed to various humans and/or human activities, we were able to show an increase in resistance linked to human exposure, probably related to increasing antibiotic selective pressure.[24] Further, the previously reported link between integron (a highly efficient genetic tool for the expression and spread of antibiotic resistance[18]) prevalence and antibiotic resistance was present in the strains isolated from pets and farm animals, but not in resistant strains from wild animals, which had less contact with humans. This suggests that the presence of integrons may constitute a process of genomic adaptation to environments when antimicrobial pressure exceeds a certain threshold. In addition to the environment of the host, the genetic background of the strain also seems to be involved in the emergence of resistance. B2 group strains appear more sensitive to antibiotics than strains of the other phylogenetic groups,[10,20] especially to quinolones.[12,13,15,19,25,27] Likely, in the

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human community commensal environment strains from the B2 group would tend to carry fewer integrons than the others.[23]

To gain a better understanding of the relationships between resistance, integrons, phylogenetic groups, and host environment, we selected three groups of human urinary tract infection (UTI) *E. coli* isolates that are known to comprise a high rate of B2 strains,[11] for the present study. The first included isolates susceptible to amoxicillin (AMX), cotrimoxazol (TMP-SMZ), and quinolones; the second included isolates resistant to AMX and TMP-SMZ but sensitive to quinolones; and the third included isolates resistant to these three antibiotics. The patients of the three groups were matched for age and sex to avoid potential bias linked to the phylogenetic distribution of *E. coli* related to these characteristics.[8] We then determined and compared their integron content and phylogenetic grouping.

Materials and Methods

Bacterial isolates

The strains were selected among the UTI *E. coli* isolated from patients hospitalized in the Bichat-Claude Bernard Hospital in Paris between January 2005 and July 2006. Isolates were routinely identified by the clinical microbiology laboratory of the hospital as *E. coli* using API 20E strips (API, La Balme-les-Grottes, France) and tested for antibiotic susceptibility by the disk diffusion technique, as previously described (www.sfm.asso.fr). During the period of the study, 2391 strains of *E. coli* were isolated from urine samples of patients, including 940 hospitalized for more than 48 hours (nosocomial infections). Among the 2391 strains, we selected three groups: the first (G1) comprised all the strains ($n=1143$) susceptible to nalidixic acid (NA), AMX, and TMP-SMZ; the second (G2) comprised all the strains ($n=621$) susceptible to NA, but resistant to AMX and TMP-SMZ, and the third (G3) included all the strains ($n=191$) resistant to NA, AMX, and TMP-SMZ. For this study, UTI was defined as urine cultures yielding *E. coli* in pure culture in counts $\geq 10^5$ colony-forming units (CFU)/ml, with $>10^4$ leukocytes/ml. Strains from patients with urinary catheters or out of the age range (20–85 years) were excluded. These criteria were met for 74 strains from 74 patients in the G3 group. To avoid potential bias linked to the phylogenetic distribution of *E. coli* related to sex and age of patients,[11] 74 patients from the G3 group were matched for their characteristics with the patients from the G2 and G1 groups. When a patient from the G3 group was matched with more than one from another group, only one of these patients (randomly chosen) was selected. When no match was found, the patient was removed from the study. These last steps resulted in 66 strains from 66 patients (16 males and 50 females) in each group. The 198 strains were then further studied.

DNA extraction

DNA extraction was performed using MagnaPure LC (Roche, Mannheim, Germany), following the manufacturer's recommendations. DNA was quantified at an optical density of 260 nm with a Gene Quant II spectrophotometer (Amersham-Pharmacia Biotech, Orsay, France).

Typing of isolates by enterobacterial repetitive intergenic consensus

To eliminate replicates within each group of strains, all isolates were typed using enterobacterial repetitive intergenic consensus (ERIC)–PCR with the ERIC-2 primer (5′-AAGT AAGTGACTGGGGTGAGCG-3′) as described.[26] PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized using ultraviolet (UV) radiation. The patterns obtained were analyzed using Bionumerics software V1.5 (AppliedMaths, Ghent, Belgium). Single-band differences between isolate patterns led to the definition of separate genotypes. Only the strains in each group with different genotypes were included in the analysis.

Phylogenetic grouping

Strains were assigned to the *E. coli* phylogenetic groups using a triplex PCR based on the presence or absence of three DNA fragments: *chuA*, *yjaA*, and TspE4C2, as previously described.[3]

Integron detection

For the detection of class 1, 2 and 3 integrons, isolates were tested for the presence of genes *intI1*, *intI2*, and *intI3* by a triplex real-time PCR on an ABI Prism 7000 SDS thermocycler (Applied BioSystems, Courtaboeuf, France) using specific primer pairs, as previously described.[23]

Statistical analysis

Each isolate was regarded as independent sample, since each was derived from a single host and had a single ERIC-PCR pattern. Rates were compared using the chi-square test or, when samples were small, Fisher's exact test. A p-value of <0.05 was considered significant.

Results

ERIC-PCR patterns

After processing by ERIC-PCR, the 198 isolates studied displayed 173 different patterns. We found only a small number of strains that had a similar pattern in each group. Among the 66 strains in the G1 group, only two identical patterns were found for two strains each (data not shown). In the G2 group, identical patterns were found for six pairs and two triplets of strains (Fig. 1A). Lastly, in the G3 group, identical patterns were found for five pairs and two triplets of strains, and one for five strains (Fig. 1B). Since the goal of our study was to assess the relationships between resistance, integrons, phylogenetic groups, and host environment, we randomly selected only one strain from each clone to avoid

FIG. 1. Comparison of ERIC-PCR patterns from 66 strains belonging to G2 (**A**) and G3 (**B**) groups. Dendograms were constructed using the unweighted pair group method using arithmetic averages with similarity matrices generated from pairwise comparisons of ERIC-PCR patterns. The strains displaying the same pattern are framed. The scale bar on the top indicates the similarity values.

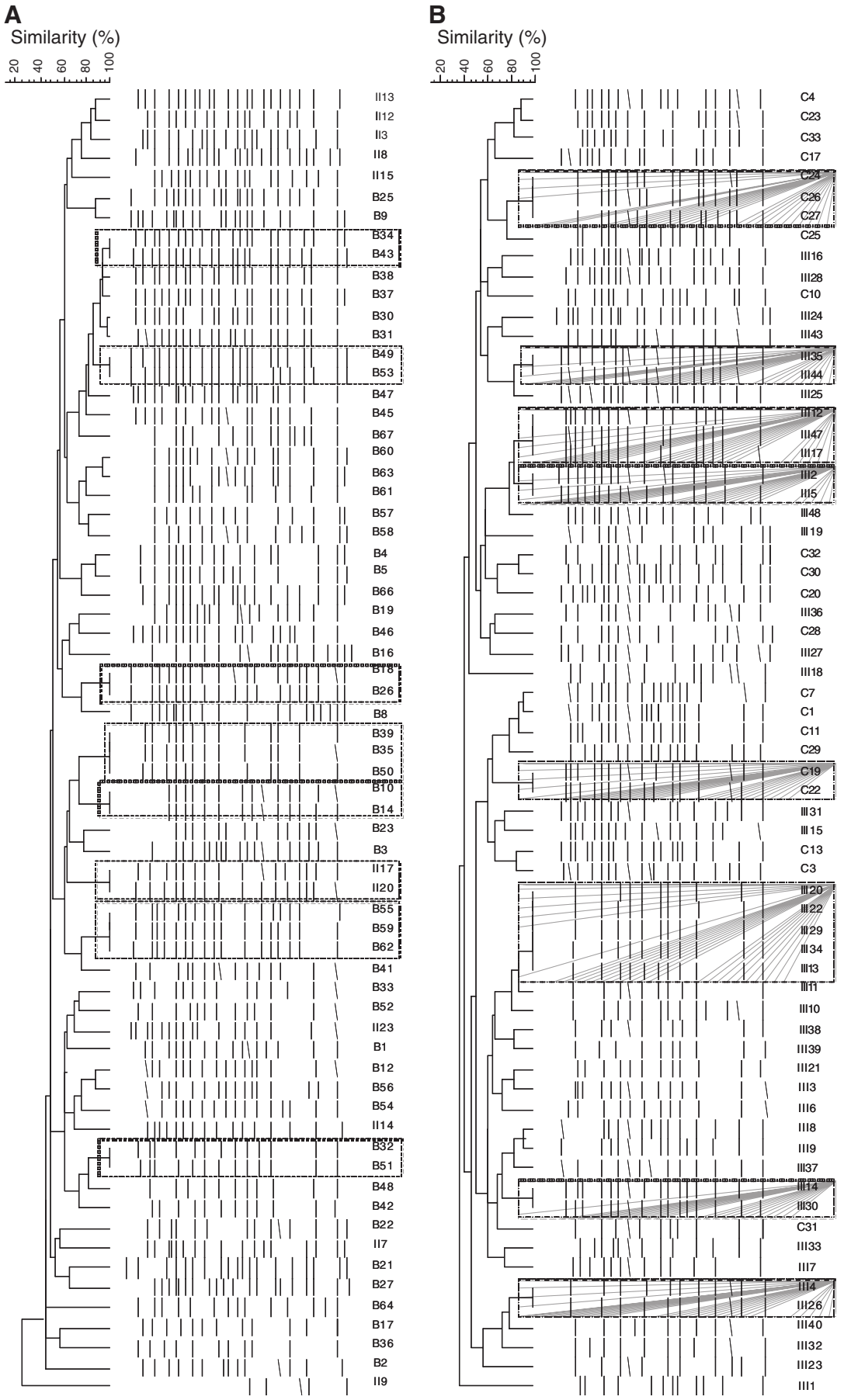
A
Similarity (%)
20
40
60
80
100
II13
II12
II3
II8
II15
B25
B9
B34
B43
B38
B37
B30
B31
B49
B53
B47
B45
B67
B60
B63
B61
B57
B58
B4
B5
B66
B19
B46
B16
B18
B26
B8
B39
B35
B50
B10
B14
B23
B3
II17
II20
B55
B59
B62
B41
B33
B52
II23
B1
B12
B56
B54
II14
B32
B51
B48
B42
B22
II7
B21
B27
B64
B17
B36
B2
II9
B
Similarity (%)
20
40
60
80
100
C4
C23
C33
C17
C24
C26
C27
C25
III16
III28
C10
III24
III43
III35
III44
III25
III12
III47
III17
III2
III5
III48
III19
C32
C30
C20
III36
C28
III27
III18
C7
C1
C11
C29
C19
C22
III31
III15
C13
C3
III20
III22
III29
III34
III13
III11
III10
III38
III39
III21
III3
III6
III8
III9
III37
III14
III30
C31
III33
III7
III4
III26
III40
III32
III23
III1

Table 1. Distribution of the Phylogenetic Groups and Prevalence of Integrons in the Populations of *Escherichia coli* Tested

	Repartition of the phylogenetic groups (%)				*Prevalence of integrons (%)*		
Resistance groups	*A*	*B1*	*D*	*B2*	intI1	intI2	intI
G1[a] ($n=64$)	20.3	6.3	15.6	57.8	0	0	0
G2[b] ($n=56$)	19.7	8.9	21.4	50	83.9	10.7	87.5
G3[c] ($n=53$)	39.6	15.1	22.6	22.6	83	24.5	94.3
All ($n=173$)	26	9.8	19.7	44.5	52.6	11	57.2

[a]G1 comprises strains susceptible to AMX, TMP-SMZ, and NA.
[b]G2 comprises strains resistant to AMX and TMP-SMZ but susceptible to NA.
[c]G3 comprises strains resistant to AMX, TMP-SMZ, and NA.
AMX, amoxicillin; TMP-SMZ, cotrimoxazol; NA, nalidixic acid.

the bias due to the presence of repetitive strains in the analysis. After discarding the duplicates, 64, 56, and 53 isolates from the G1, G2, and G3 groups, respectively, were further analyzed.

Phylogenetic group distribution

In all, 77/173 (44.5%) of the strains tested belonged to phylogenetic group B2, 45/173 (26%) to group A, 17/173 (9.8%) to group B1, and 34/173 (19.7%) to group D. Phylogenetic group distribution in *E. coli* strain groups G1, G2, and G3 is shown in Table 1. This distribution differed significantly between G1 and G3 ($p < 0.05$) and between G2 and G3 ($p < 0.05$) but not between G1 and G2. The prevalence of group B2 was significantly lower in the most resistant G3 strains (12/53, 22.6%) than in susceptible G1 strains (37/64, 57.8%; χ^2 $p < 0.001$) and in G2 strains (28/56, 50%; $p < 0.01$). Interestingly, G3 and G2 only differed by their susceptibility to NA. The prevalence of phylogenetic group B2 isolates was not significantly different between strains of the G1 and G2 groups, despite their difference in resistance to AMX and TMP-SMZ (Table 1). The prevalence of phylogenetic group A was significantly higher in the most resistant G3 strains (21/53, 39.6%) than in G1 or G2 strains (13/64, 20.3% and 11/56, 19.6%, respectively; $p < 0.01$).

Integron prevalence and distribution

In all, an integrase-encoding gene was detected in 99/173 (57.2%) of the strains tested: 80/99 (80.8%) had *intI1*, 8/99 (8.1%) had *intI2*, and 11/99 (11.1%) had both *intI1* and *intI2*. No strain included *intI3*. The prevalence of integrons was very high in the resistant G2 and G3 strains (49/56, 87.5% and 50/53, 94.3%, respectively). No integrons were found in the susceptible G1 strains (Table 1). When all strains were analyzed together, integron prevalence was significantly lower in phylogenetic group B2 strains (44.2%) than in group A, B1, and D strains (66.7, 76.5, and 64.7%, respectively; $p < 0.05$ in each instance), but these differences disappeared when samples were grouped according to resistance patterns (Table 2).

Nosocomial UTI and community-acquired UTI

As presented in Table 3, no difference between the prevalence of integrons and the phylogenetic distribution of the strains was observed when nosocomial and community-acquired UTIs were studied separately, with a prevalence of integrons of 86.3% and 88.2%, respectively, in the G2 strains and 93.5% and 95.4%, respectively, in the G3 strains; the prevalence of the phylogenetic group B2 was 58.5% and 61.9% in the G1 strains, 50% and 50% in the G2 strains, and 19.3% and 27.2% in the G3 strains, respectively.

Discussion

With a carefully age- and sex-matched cohort of hospitalized UTI patients, we show, as previously reported,[12,13,15,19,25,27] that the B2 *E. coli* strains are less resistant to antibiotics than other phylogenetic *E. coli* groups and that this concerns quinolones in particular. Although the molecular mechanism is still unknown, these data indicate a role of the genomic background in the emergence of the resistance gained by point mutations, as also reported for extended β-lactamases, which are mobile elements that are horizontally transferred.[2]

As expected, we found no integrons in the sensitive strains in the G1 group. On the other hand, in the resistant G2 and G3 strains, almost all the isolates (except 10) carried an integron. These 10 strains were isolated from 10 patients who were not of a particular age or sex, and who were not from a

Table 2. Distribution of Integrons Between the Four Phylogenetic Groups in the Three Groups with Different Antibiotic Phenotype Studied

Resistance groups	*G1*[a] (n = *64*)				*G2*[b] (n = *56*)				*G3*[c] (n = *53*)				*G1 + G2 + G3* (n = *173*)			
Phylogenetic groups	*A*	*B1*	*D*	*B2*	*A*	*B1*	*D*	*B2*	*A*	*B1*	*D*	*B2*	*A*	*B1*	*D*	*B2*
Prevalence of integron (%)																
Class 1 integron	0	0	0	0	90.9	100	91.6	75	76.2	83.3	83.3	83.3	57.7	71.3	64.7	40.2
Class 2 integron	0	0	0	0	0	0	0	21.4	33.3	33.3	8.3	25	15.6	12.3	2.9	11.6
Class 1 and/or class 2 integron	0	0	0	0	90.9	100	91.6	82.1	95.2	100	91.6	91.6	66.7	76.5	64.7	44.2

[a]G1 comprises strains susceptible to AMX, TMP-SMZ, and NA.
[b]G2 comprises strains resistant to TMP-SMZ and AMX but susceptible to NA.
[c]G3 comprises strains resistant to TMP-SMZ, AMX, and NA.

Table 3. Distribution of the Phylogenetic Groups and Prevalence of Integrons in the Populations of *Escherichia coli* Comparing Community-Acquired Urinary Tract Infection and Nosocomial Urinary Tract Infection

	G1		*G2*		*G3*	
	Community acquired UTI (n = *41*)	*Nosocomial UTI* (n = *23*)	*Community acquired UTI* (n = *34*)	*Nosocomial UTI* (n = *22*)	*Community acquired UTI* (n = *22*)	*Nosocomial UTI* (n = *31*)
Prevalence of integron (%)	0	0	88.2	86.3	95.4	93.5
Repartition of the phylogenetic group (%)						
Group phylogenetic A	21.9	17.3	20.5	18.1	31.8	45.1
Group phylogenetic B1	4.8	8.7	5.8	13.6	13.6	16.1
Group phylogenetic B2	58.5	56.5	50	50	27.2	19.3
Group phylogenetic D	14.6	17.3	23.5	18.1	27.2	19.3

UTI, urinary tract infection.

particular department with a particular antibiotic prescription protocol.

This is the higher proportion of integrons reported in the literature,[17] probably because all the strains tested were from hospitalized UTI patients submitted to high antibiotic selective pressure. In this environment, we did not observe the pattern resistance/absence of integrons reported for wild animal strains in which the antibiotic pressure is very low.[24] We statistically confirmed, with UTI strains exhibiting a 57% prevalence of integrons, the trend of the underrepresentation of B2 integron-positive strains that had been previously observed for commensal strains in which the prevalence of integrons was 15%.[23] However, when considering only resistant strains, a reflection of strong antibiotic selective pressure, integrons are a widespread and common means enabling bacteria-causing infections to adapt to the highly selective environment, regardless of their phylogenetic background.

In conclusion, our results shed further light on the complex interactions between the environment, represented partly by the antibiotic selective pressure, and the genomic constraints, represented by the genetic background, in the emergence of the resistance to antibiotics, revealing a step-by-step strategy. Under very low selective pressure, resistance emerges without integrons. When the antibiotic pressure increases, quinolone and integron-mediated resistance occurs outside phylogenetic group B2. With strong antibiotic selective pressure, integrons are highly prevalent and widespread regardless of the phylogenetic group.

Disclosure Statement

No competing financial interests exist.

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