Correlation between esterase electrophoretic polymorphism and virulence-associated traits in extra-intestinal invasive strains of *Escherichia coli*

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(Accepted 13 July 1993)

SUMMARY

The electrophoretic variations of carboxylesterase B and of esterases A, C and I, the presence of mannose resistant haemagglutinin, α -haemolysin, cytotoxic necrotizing factor type 1 (CNF1) and certain O antigens were compared in 150 strains of *Escherichia coli* responsible for extra-intestinal infections. Electrophoretic mobilities of outer membrane proteins (OMP) were also studied for strains belonging to O4, O6, O7, O8 and O75 serogroups. Fast migrating allozymes of carboxylesterase B (pattern B₁) were correlated with slow migrating allozymes of esterase C, serogroups O7 and O8, lack of virulence factor, and particular OMP patterns, whereas slow migrating allozymes of carboxylesterase B (pattern B₂) were correlated with fast migrating allozymes of esterase C, serogroups O2, O4, O6, O18 and O75, virulence factor production, and distinct OMP patterns. Allozymes of esterases A and I were not clearly correlated with the distribution of virulence factors. The pattern B₂ was more strongly associated with CNF1 than with α -haemolysin and mannose resistant haemagglutinin. These results substantiate the view that the electrophoretic pattern B₂ of carboxylesterase B identified most of the highly pathogenic strains implicated in extra-intestinal infection of humans.

INTRODUCTION

Many studies have established that strains of *Escherichia coli* which cause extra-intestinal infections (EII) in humans possess several phenotypic traits which were rarely found in strains isolated from normal intestinal flora. Four of these traits,

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Table 1. *Characteristics of the strains*

Strain designation	Source of isolates*	Origin†	Sero-group‡	Esterase allozyme§				Virulence factor		
				B	A	C	I	MRHA	Hly	CNF1
				(M _F)	(M _F)	(M _F)	(M _F)			
B ₁ strains										
VANC9	S	C	O1	72	75	60	00	—	+	—
SA667	S	F	O1	70	81	62	70	+	—	—
AV748	U	F	O1	70	75	62	68	—	—	—
VANC12	D	C	O1	70	75	48	68	+	—	—
AV755	U	F	O1	70	75	00	00	+	—	—
SA641	U	F	O1	68	78	59	70	—	—	—
AV741	D	F	O1	68	00	60	70	—	—	—
SA637	S	F	O2	72	00	63	68	+	+	—
SA592	S	F	O2	70	78	57	72	+	+	+
SA604	U	F	O2	70	78	53	72	+	—	—
AUST35	U	A	O4	70	75	57	60	—	—	—
SA640	U	F	O4	70	00	57	73	—	—	—
SA595	U	F	O6	70	75	62	72	—	—	—
TIM31	U	F	O7	72	78	00	70	+	—	—
TIM30	U	F	O7	72	00	60	68	—	—	—
TIM36	U	F	O7	70	81	00	62	—	—	—
TIM27	U	F	O7	70	75	57	70	+	—	—
MELBR1	S	A	O7	70	75	57	68	+	—	—
AV2842	S	F	O7	70	75	57	00	—	—	—
AV757	U	F	O7	70	75	00	70	+	—	—
TIM29	D	F	O7	70	75	00	66	+	—	—
TIM28	U	F	O7	68	75	50	00	—	—	—
SA697	U	F	O8	73	78	55	70	—	—	—
SA658	U	F	O8	73	78	53	70	—	—	—
SA668	S	F	O8	72	78	55	70	—	—	—
AV2845	S	F	O8	70	78	55	74	—	—	—
AV2838	U	F	O8	70	78	55	70	—	—	—
AV2815	U	F	O8	68	81	53	70	—	—	—
TIM24	U	F	O8	68	78	50	00	—	—	—
AV2814	D	F	O8	66	75	48	70	—	—	—
JAP15	U	J	O18	72	75	60	68	+	+	—
TIM971	U	F	O18	70	75	53	00	+	—	—
TIM983	U	F	NT	74	00	59	70	—	—	—
SA648	U	F	NT	73	81	00	68	—	—	—
TIM977	U	F	NT	73	78	00	70	—	+	—
SA652	U	F	NT	72	81	57	00	—	—	—
TIM982	U	F	NT	72	81	55	74	—	—	—
SA598	U	F	NT	72	81	52	73	—	—	—
AV743	S	F	NT	72	78	60	70	—	—	—
AV738	U	F	NT	72	78	60	00	—	—	—
SA686	D	F	NT	72	78	55	73	—	—	—
AV768	U	F	NT	72	78	55	68	—	—	—
AV2809	U	F	NT	72	78	53	72	—	—	—
SA680	U	F	NT	72	78	00	68	—	—	—
TIM981	U	F	NT	72	78	00	00	—	—	—
SYDNEY76	U	A	NT	72	75	50	00	—	—	—
JAPON9	D	J	NT	72	75	00	62	—	—	—
TIM975	U	F	NT	72	00	00	68	—	+	—
TIM976	U	F	NT	72	00	00	60	—	—	—
TIM22	U	F	NT	70	81	53	70	+	—	—
VANC14	U	C	NT	70	81	57	68	—	—	—

Table 1 (cont.)

Strain designation	Source of isolates*	Origin†	Sero-group‡	Esterase allozyme§				Virulence factor		
				B (M _F)	A (M _F)	C (M _F)	I (M _F)	MRHA	Hly	CNF1
AUST38	U	A	NT	70	81	57	60	—	—	—
SA678	U	F	NT	70	81	55	70	—	—	—
SA635	U	F	NT	70	81	53	70	—	—	—
AV2830	U	F	NT	70	81	55	68	—	—	—
SYDNEY62	S	A	NT	70	78	65	65	—	—	—
SYDNEY72	D	A	NT	70	78	60	66	—	—	—
VANC20	U	C	NT	70	78	57	70	—	+	—
VANC1	D	C	NT	70	78	57	68	—	—	—
VANC13	U	C	NT	70	78	57	67	+	+	—
AV751	U	F	NT	70	78	55	72	—	—	+
TIM25	U	F	NT	70	78	00	62	—	—	—
SA703	S	F	NT	70	75	62	00	—	—	—
AUST22	U	A	NT	70	75	60	74	—	—	—
AV745	U	F	NT	70	75	60	70	—	—	—
AUST5	D	A	NT	70	75	60	65	—	+	—
AV765	U	F	NT	70	75	60	00	+	—	—
VANC15	U	C	NT	70	75	59	65	—	—	—
AV2841	U	F	NT	70	75	57	62	—	—	—
AV2817	U	F	NT	70	75	55	70	—	—	—
VANC11	D	C	NT	70	75	50	60	—	—	—
AV2833	U	F	NT	70	75	48	73	—	—	—
AV2840	U	F	NT	70	75	48	70	—	—	—
SA633	U	F	NT	70	75	48	00	—	—	—
FARMG3	U	US	NT	70	75	44	59	—	—	—
TIM980	U	F	NT	70	75	00	68	+	—	—
SA662	U	F	NT	70	75	00	62	+	—	—
SA685	U	F	NT	70	75	00	60	+	—	—
SA605	U	F	NT	68	75	57	70	—	—	—
TIM968	U	F	NT	68	75	55	75	—	—	—
B ₂ strains										
SYDNEY64	U	A	O1	63	78	62	68	+	—	—
AV19	U	F	O1	63	78	00	61	+	—	—
FARMG9	U	US	O1	62	78	60	00	+	—	+
AV1	S	F	O1	62	00	60	70	+	—	—
MELBR140	U	A	O2	63	75	60	60	+	—	—
SA85	U	F	O2	62	78	62	70	+	—	—
SA86	U	F	O2	62	78	60	70	+	+	—
MELBR7	U	A	O2	62	78	60	60	+	—	—
SA76	S	F	O2	60	78	60	70	+	+	—
AUST8	D	A	O2	57	78	61	68	—	+	+
AV4	D	F	O2	57	78	00	78	+	+	—
SA79	U	F	O2	57	75	62	75	+	—	—
AV59	U	F	O2	57	75	60	73	—	+	+
AV10	U	F	O2	57	75	60	00	+	+	+
AV90	S	F	O4	62	75	55	70	+	+	+
AV70	S	F	O4	62	75	55	61	—	+	+
SA29	U	F	O4	60	78	57	70	+	+	+
SA88	U	F	O4	60	78	55	70	—	—	—
AUST34	S	A	O4	60	78	00	60	+	+	+
MELBR141	D	A	O4	60	75	60	60	+	+	+
MELBR145	D	A	O4	60	75	59	59	+	+	+
VANC19	D	C	O4	60	75	57	68	+	+	+

Table 1 (*cont.*)

Strain designation	Source of isolates*	Origin†	Sero-group‡	Esterase allozymes§				Virulence factor		
				B	A	C	I	MRHA	Hly	CNF1
				(M _F)	(M _F)	(M _F)	(M _F)			
TIM43	U	F	O4	60	75	55	70	+	+	+
TIM40	U	F	O4	60	75	54	70	+	+	+
TIM7	U	F	O4	60	75	53	00	+	+	+
SA33	U	F	O4	60	75	00	00	+	—	+
SAND5	U	US	O6	60	78	60	57	+	—	+
SA23	U	F	O6	57	78	60	73	—	+	+
AUST2	S	A	O6	57	78	55	00	—	—	+
AV18	D	F	O6	57	78	00	62	+	—	+
AUST6	U	A	O6	57	75	62	62	+	+	+
AUST14	U	A	O6	57	75	61	00	+	+	+
MELBR134	U	A	O6	57	75	60	65	+	+	+
AUST44	U	A	O6	57	75	57	00	+	+	+
AV13	S	F	O6	57	75	00	62	+	+	+
AV51	D	F	O6	57	72	00	60	+	+	+
MELBR17	U	A	O15	62	75	59	65	+	—	—
AUST3	U	A	O15	57	78	44	68	—	+	—
TOR126	U	C	O18	62	00	60	00	+	—	+
JAPON7	U	J	O18	60	78	00	62	+	+	+
AUST13	S	A	O18	57	78	61	70	+	+	—
SA26	D	F	O18	57	78	60	75	+	+	—
FARMG2	U	US	O75	57	78	62	78	+	—	—
AUST51	U	A	O75	57	78	61	74	+	+	—
SA1	S	F	O75	57	78	60	78	+	—	—
MELBR8	S	A	O75	57	75	60	78	—	+	+
MELBR4	U	A	O75	57	75	60	75	+	+	+
BERK6	S	US	NT	62	81	57	00	—	—	—
SA75	S	F	NT	62	78	59	70	+	—	—
AV16	D	F	NT	62	78	00	70	—	—	—
AV60	U	F	NT	62	78	00	60	—	—	—
NEWZ95	S	NZ	NT	62	75	60	60	—	—	—
SA71	S	F	NT	62	75	59	70	+	+	—
MELBR144	U	A	NT	60	81	60	68	—	—	—
AV65	U	F	NT	60	75	62	67	—	—	—
AUST36	U	A	NT	60	75	60	68	—	—	—
JAPON16	D	J	NT	60	75	00	60	+	+	—
SA22	U	F	NT	60	75	00	58	+	—	—
NEWZ120	U	NZ	NT	57	78	62	75	—	—	—
TIM4	U	F	NT	57	78	60	72	—	+	+
SA74	U	F	NT	57	78	60	70	+	+	+
SA3	U	F	NT	57	78	60	68	+	+	—
SA34	U	F	NT	57	78	60	00	—	+	—
AV35	D	F	NT	57	78	00	72	—	—	+
SA38	U	F	NT	57	78	00	60	—	—	—
SA37	U	F	NT	57	75	62	00	+	+	+
SA44	U	F	NT	57	75	60	70	—	—	—
SA13	U	F	NT	57	75	60	62	+	+	+
SA20	U	F	NT	57	75	00	60	—	+	+
SA12	U	F	NT	57	75	00	59	—	—	—

* U, urinary tract infection; S, septicaemia; D, diverse.

† A, Australia; C, Canada; F, France; J, Japan; NZ, New Zealand; US, United States.

‡ NT, not typed with panel of sera used in this experiment.

§ B, esterase B; A, esterase A; C, esterase C; I, esterase I.

|| MRHA, mannose resistant haemagglutinin; Hly, α -haemolysin; CNF1, cytotoxic necrotising factor; + and — indicate the presence or absence of factor.

the production of mannose resistant haemagglutinin (MRHA), α -haemolysin (Hly), cytotoxic necrotizing factor type 1 (CNF1) [1, 2] and certain O antigens are frequently found together in pathogenic isolates. MRHA and Hly are simultaneously expressed in many strains and have been shown to be chromosomally linked [3, 4]. These two virulence factors or Hly plus CNF1 were more prevalent among strains belonging to a restricted number of serogroups [5-11].

In *E. coli*, four varieties of esterases designated A, B, C and I, differing in their ability to hydrolyse synthetic substrates and in their sensitivity to heat and to diisopropyl fluorophosphate, were found to be electrophoretically polymorphic [12, 13]. Esterase B (carboxylesterase B) [14], the principal component of this set of enzymes, exhibits two patterns of electrophoretic mobility: B₁ (fast mobilities) and B₂ (slow mobilities). We have demonstrated that strains having carboxylesterase pattern B₂ are considerably more frequent in *E. coli* from EII than in *E. coli* obtained from the stools of healthy individuals, and that B₂ strains produce more MRHA and Hly than do B₁ strains [15, 16]. The strong association between electrophoretic pattern B₂ and these virulence factors was found regardless of the geographical origins of EII and patient characteristics [16] and was confirmed by the prevalence of P-fimbriae and Hly genetic determinants in B₂ strains [17].

To substantiate the relationship between esterase polymorphism and virulence-associated traits, we have extended the analysis of strains causing EII by studying the electrophoretic variations of esterases A, C and I, determining CNF1 production, serotyping O antigens 1, 2, 4, 6, 7, 8, 15, 18 and 75, which are commonly detected in pathogenic strains [7, 18] and studying the outer membrane protein (OMP) patterns of some serotypes. This work compares the electrophoretic polymorphism of the four *E. coli* esterases with presence of MRHA, Hly, CNF1 and the O antigens listed above, in two representative panels of 80 B₁ and 70 B₂ strains.

MATERIALS AND METHODS

Bacterial strains

The 80 B₁ and the 70 B₂ strains (Table 1) were isolated from urinary tract infections, septicaemia and other EII in France ($n = 101$) and other parts of the world ($n = 49$). These strains were chosen from the 705 strains previously analysed [16] for their distinct combination of allozymes for the four esterases.

Electrophoretic mobility

The mobilities (M_F values) of esterases were determined as previously described [19]. Each M_F variant was designated as an allozyme.

Determination of the O serogroup

This determination was limited to the identification of O serogroups 1, 2, 4, 6, 7, 8, 15, 18 and 75, which are frequently associated with extra-intestinal human diseases [7, 18]. The bacteria grown in L broth were heated to 120 °C for 1 h to destroy capsular material. An aliquot (2 μ l) of heat-treated bacterial suspension was pipetted on to a nitrocellulose filter. The filters were dried, saturated with skimmed milk, and incubated for 1 h at 25 °C with rabbit *E. coli* O antisera (Difco) diluted 1/200 in gelatin buffer, pH 7.5. The filters were washed and the rabbit O

antibodies were detected using goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase, which reacted with hydrogen peroxide (substrate) and 4-chloronaphthol (chromogen). This serological O grouping was also evaluated by SDS-PAGE of extracts obtained by heating bacterial suspensions at 60 °C for 20 min [20]. After silver staining, the lipopolysaccharide (LPS) patterns of the strains were compared on a same gel with reference strains for O antigens. Strains showing a LPS pattern different to that of the reference strain for the O serogroup were discarded and the immunological result considered false positive.

Determination of cytotoxic necrotizing factor

CNF1 toxin was detected by its multinucleating effect on cell cultures as described by de Rycke and co-workers [21].

SDS-PAGE of outer membrane proteins [22]

Bacteria grown on Minca agar medium were collected in 2 ml of Tris buffer at 4 °C (Tris base, 0.05 M; EDTA, 1 mM, pH 7.8). After centrifugation for 15 min at 4500 g, the pellet was suspended in 10 ml Tris buffer at 4 °C. Fifteen-second ultrasonication was done four times in ice using 15% continuous cycle (New Brunswick). After centrifugation for 20 min at 1200 g, the supernatant was centrifuged at 50000 g for 1 h at 4 °C. The pellet was suspended in 10 mM Tris-buffer with 5 mM-MgCl₂, pH 8 containing 2% Triton X-100 to render the inner membrane soluble [23, 24]. After centrifugation at 50000 g for 1 h at 4 °C, the pellet was dissolved in buffer v/v (Tris base 0.0025 M, glycine 0.192 M, SDS 1%, pH 8.6).

Polyacrylamide gel electrophoresis was performed after addition of Laemmli buffer v/v and heating at 100 °C for 5 min. Ten percent polyacrylamide gel was used and electrophoresis was run at 20 mA for 5 h. Silver staining of the gel was done according to Oakley and colleagues [25].

Statistical analysis

The factorial analysis of correspondence method [26] was used with STAT-ITCF software [27] from a two-way table of 150 rows (the strains) and 62 columns corresponding to (i) the number of allozymes detected in all strains (including the null allozyme) for each esterase, (ii) 9 studied O serogroups and a column for the absence of O serogroup detected, (iii) the presence or absence of Hly, MRHA and CNF1. Pearson X² values were calculated between several data.

RESULTS

Distribution of esterase allozymes in B₁ and B₂ strains

Table 1 shows allozyme distribution of esterases B, A, C and I in the 80 strains of pattern B₁ and in the 70 strains of pattern B₂. The most frequently occurring allozyme of esterase B was M_F ≈ 70 for B₁ strains and M_F ≈ 57 for B₂ strains. For esterase A, the proportion of allozyme at M_F ≈ 81 was higher for B₁ strains and the proportion of allozyme at M_F ≈ 78 was higher for B₂ strains. The most frequently

Table 2. *Correlation between carboxylesterase B electrophoretic patterns B₁ and B₂ and presence of MRHA, Hly and CNF1*

Detection of virulence factor*			Carboxylesterase B electrophoretic pattern	
MRHA	Hly	CNF1	No. of strains	
			B ₁ (80)	B ₂ (70)
—	—	—	55 (68.75%)	12 (17.14%)
+	—	—	15 (18.75%)	12 (17.14%)
—	+	—	5 (6.25%)	2 (2.85%)
—	—	+	1 (1.25%)	2 (2.85%)
+	+	—	3 (3.75%)	9 (12.86%)
+	—	+	—	5 (7.14%)
—	+	+	—	7 (10%)
+	+	+	1 (1.25%)	21 (30%)

* + and — indicate the presence or absence of factor.

occurring allozyme of esterase C was $M_F \approx 57$ for B₁ strains and $M_F \approx 60$ for B₂ strains. The majority of B₁ strains had allozymes C of $M_F \approx 44$ to $M_F \approx 57$, while most B₂ strains had allozyme C with $M_F \approx 58$ to $M_F \approx 62$. Most B₁ strains had esterase I allozymes with mobilities of $M_F \approx 65$ to $M_F \approx 75$, whereas most B₂ strains had slower (M_F from 57 to 62) or faster mobilities (M_F from 67 to 78).

Correlation between esterase B allozymes and O serogroups

Forty-seven strains of pattern B₂ (67%) and only 32 strains of pattern B₁ (40%) fell in serogroups O1, O2, O4, O6, O7, O8, O15, O18 and O75 ($\chi^2 = 10.81$; $P < 0.01$) (Table 1). Thirty-seven strains (86%) belonging to serogroups O2, O4, O6 and O75 had the B₂ pattern. In contrast, all strains of serogroups O7 and O8 had the pattern B₁. Among the B₂ strains, serogroup O1 and O4 strains had allozyme B with an M_F from 60 to 63, whereas all serogroup O6 and O75 strains (with the exception of one strain of serogroup O6) had allozyme at $M_F \approx 57$. Among the B₁ strains, serogroup O7 and O8 strains appeared heterogeneous in esterase B allozyme distribution.

Correlation between electrophoretic patterns B₁ and B₂ and MRHA, Hly and CNF1

Table 2 shows the relationships between the electrophoretic patterns B₁ and B₂ and MRHA, Hly and CNF1. As was previously demonstrated for MRHA and Hly [15, 16], more B₂ pattern strains produced CNF1 than did B₁ pattern strains. The difference was 20-fold in terms of CNF1, 5-fold in terms of Hly and 2.8-fold in terms of MRHA. Thus, the B₂ pattern was more strongly associated with CNF1 than with Hly or MRHA. Since we have previously demonstrated that the percentage of pattern B₂ strains increases with the number of virulence factors while the percentage of type B₁ strains decreases [13, 16], we have compared in the present study the number of factors (MRHA, Hly and CNF1) in the B₁ and B₂ strains. The percentage of strains producing no factor was higher for pattern B₁ (68.75%) than for pattern B₂ (17.14%) ($\chi^2 = 39.73$; $P < 0.001$), whereas the

Fig. 1. Analysis of correspondance of the 150 *E. coli* strains. (a) The scores of allozymes of the four esterases plotted against virulence-associated traits for the first two factors F₁ and F₂. B57, esterase B allozyme at $M_F \approx 57$; +HL, production of Hly; OHL, absence of production of Hly; +MR, production of MRHA; OMR, absence of production of MRHA; +CN, production of CNF1; OCN, absence of production of CNF1; O1 to O75, serogroups O1, O2, O4, O6, O7, O8, O15, O18 or O75. Projections of esterase A and I allozymes were not indicated (see results). (b) Scores of strains for factors F₁ and F₂. ○, B₁ strains; ●, B₂ strains. In addition, serogroups O2, O4, O6, O18 and O75 were indicated for B₂ strains.

percentage of strains producing one factor were similar for the two patterns. More B₂ pattern strains produced two or three factors than did pattern B₁ strains. For strains producing two factors, the difference was 8-fold, while for strains producing the three factors the difference was about 30-fold.

Statistical analysis

Projections of different allozymes on the plan defined by the first two principal axes of the correspondance analysis (Fig. 1a) revealed that the first axis F₁ separated the B₁ allozymes of esterase B migrating at $M_F \approx 74$, $M_F \approx 72$, $M_F \approx 70$, $M_F \approx 68$, $M_F \approx 66$ (designated B-74, B-72, B-70, B-68, B-66) and the slow migrating allozymes of esterase C (C-44, C-48, C-52, C-55, C-57) from the B₂ allozymes of esterase B (B-57, B-60) and the fast migrating allozymes of esterase C (C-60, C-61, C-62, C-63). Projections of esterase A and I allozymes were less clearly separated (data not shown). Projections of the virulence factors and the O serogroups revealed that the first axis separated lack of MRHA, Hly and CNF1 production and serogroups O7 and O8 or non-O typable strains from MRHA, Hly and CNF1 production and serogroups O2, O4, O6, O18 and O75. The second axis separated serogroups O75, O6 and O2 from serogroup O4. The B₂ strains were clearly distinguished from the B₁ strains by the first axis (Fig. 1b) whereas axis F₂

Fig. 2. (a) OMP patterns of the B₁ and B₂ strains belonging to serogroups O4, O6, O7, O8 and O75. (b) photograph of OMP patterns [numbered as in (a)] for O4 isolates.

allowed the separation of several subclusters within the B₂ strains, each of them having a distinct serogroup.

Characterization by the OMP patterns of the B₁ and B₂ strains belonging to serogroups O4, O6, O7, O8, and O75

In order to refine the subclustering delineated within B₁ and B₂ groups by O serogrouping, the OMP patterns of the 30 strains belonging to serogroups O4, O6, O75 (27 B₂ strains), and the 17 strains of serogroups O7 and O8 (all B₁ strains) were studied. Nineteen OMP patterns were evidenced (Fig. 2). A relation between OMPs and B₁ or B₂ patterns was observed for OMP A which is the fastest migrating outer membrane protein. In the 27 B₂ strains, 26 had a fast OMP A compared to only 8 in the 20 B₁ strains ($\chi^2 = 22.6$; $P < 0.001$). Also, the OMPs were more diversified in the B₁ strains which had 13 different patterns compared to 7 only for 27 B₂ strains. The diversity was also O-serogroup associated. Eight O8 *E. coli* strains revealed 6 different OMP patterns. At the opposite, O4 *E. coli* strains were less heterogenous, 12 of 14 strains having a same OMP pattern.

DISCUSSION

The *E. coli* strains causing extra-intestinal infections were initially characterized using a limited number of virulence-associated traits, without considering the overall genetic diversity of bacterial populations. Since then, several studies have used enzyme or nucleic acid electrophoretic polymorphism to survey the genetic structure of *E. coli* clinical isolates [13, 15, 16, 28–31]. Electrophoretic polymorphism of esterases A, B, C and I was found to give reliable information on the overall genetic diversity of *E. coli* populations [13, 32]. Previous studies on strains causing human extra-intestinal infections focused on carboxylesterase B [15–17, 33, 34]. The present study show two correlations, one between the fast

migrating allozymes of carboxylesterase B (B_1 pattern) and the slow migrating allozymes of esterase C, and the other between the slow migrating allozymes of carboxylesterase B (B_2 pattern) and the fast migrating allozymes of esterase C. Slow migrating esterase B allozymes and fast migrating esterase C allozymes are correlated with the distribution of MRHA, Hly and CNF1, whereas esterase A and I allozymes which show distinct distributions in B_1 and B_2 strains, are not clearly correlated with the distribution of these factors. Thus, allelic variations of esterases B and C converge to delineate two groups of pathogenic strains differing considerably in the proportions of their virulence-associated traits. B_2 strains may well be particularly aggressive because they frequently carry two or three of these factors (Table 2). According to Caprioli and co-workers [9, 35], the haemolytic isolates of *E. coli* may be divided in two distinct classes on the basis of their ability to produce CNF1. The results of our study indicate that few strains produced Hly alone and they were not significantly associated with either the B_1 or the B_2 patterns, while 96.5% of haemolytic strains producing CNF1 or both CNF1 and MRHA belonged to B_2 electrophoretic patterns. Previous studies have established an association of serogroups O4 and O6 with Hly and MRHA or P-adhesin [4, 7, 8, 36], or with Hly and CNF1 [9, 10, 35]. The classification of strains according to their carboxylesterase B electrophoretic pattern used in this study provides a more detailed discrimination of pathogenic isolates, since 15 of the 22 strains of serogroups O4 and O6 having pattern B_2 were positive for the three factors, five were positive for two factors and one was positive for one factor. In contrast, the three strains of these serogroups having pattern B_1 lacked these factors (Table 1). On the other hand, the strains of serogroups O7 and O8 with low virulence factor levels were all in type B_1 . The present results are consistent with our earlier studies showing association of pattern B_2 with Hly, CNF1 and serogroups O4 or O6 [37, 38]. Several subclusters can also be identified within B_1 and B_2 strains by their esterase B allozymes, O serogroups and OMP patterns; the factorial plan of the CA (Fig. 1b) indicates that most of the B_2 strains are clustered according to serogroups and certain allozymes of esterases B and C. The divergent molecular structure of B_2 allozymes [39], which correlates with allelic variation of esterase C, is in agreement with the fact that the strains showing pattern B_2 correspond to a distinct phylogenetic group within the ECOR strains [40] as detected by the allelic variations of 38 enzymes [32, 41]. We have recently demonstrated that the restriction fragment length polymorphism (RFLP) of ribosomal DNA patterns of B_2 strains having MRHA, Hly and virulence in mice are clearly distinct from those of B_1 strains lacking these virulent characters [31]. The findings of esterase and ribosomal DNA polymorphism studies, coupled with virulence-associated traits, converge to demonstrate that the electrophoretic pattern B_2 of carboxylesterase B identifies most of the highly pathogenic strains implicated in extra-intestinal infections in humans.

REFERENCES

1. De Rycke J, Gonzalez EA, Blanco J, Oswald E, Blanco M, Boivin R. Evidence for two types of cytotoxic necrotizing factor in human and animal isolates of *Escherichia coli*. J Clin Microbiol 1990; **28**: 694-9.

2. Caprioli A, Falbo L, Roda G, Ruggeri FM, Zona C. Partial purification and characterization of an *Escherichia coli* toxic factor that induces morphological cell alterations. *Infect Immun* 1983; **39**: 1300-6.
3. Berger H, Hacker J, Juarez A, Hughes C, Goebel W. Cloning of the chromosomal determinants encoding haemolysin production and mannose-resistant haemagglutination in *Escherichia coli*. *J Bacteriol* 1982; **152**: 1241-7.
4. Low D, David V, Lark D, Schoolnik G, Falkow S. Gene clusters governing the production of hemolysin and mannose-resistant hemagglutination are closely linked in *Escherichia coli* serotypes O4 and O6 isolated from urinary tract infections. *Infect Immun* 1984; **43**: 353-8.
5. Evans DJ, Evans DG, Hohne C, Noble MA, Haldane EV, Lior H, Young LS. Hemolysin and K. antigens in relation to serotype and hemagglutination type of *Escherichia coli* isolated from extraintestinal infections. *J Clin Microbiol* 1981; **13**: 171-8.
6. Green CP, Thomas VL. Hemagglutination of human type O erythrocytes, hemolysin production and serogrouping of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis and asymptomatic bacteriuria. *Infect Immun* 1981; **31**: 309-15.
7. Hacker J, Schroter G, Schrettenbrunner A, Hughes C, Goebel W. Hemolytic *Escherichia coli* strains in the human fecal flora as potential urinary pathogens. *Zentralbl Bakteriol Mikrobiol Hyg* 1983; **254**: 370-8.
8. Czirok E, Miloh H, Csiszar K, Csik M. Virulence factors of *Escherichia coli*. *Acta Microbiol Hung* 1986; **33**: 69-83.
9. Caprioli A, Falbo V, Ruggeri FM et al. Cytotoxic necrotizing factor production by hemolytic strains of *Escherichia coli* causing extraintestinal infections. *J Clin Microbiol* 1987; **25**: 146-9.
10. Blanco J, Alonso MP, Gonzalez EA, Blanco M, Garabal JI. Virulence factors of bacteraemic *Escherichia coli* with particular reference to production of cytotoxic necrotising factor (CNF) by P-fimbriate strains. *J Med Microbiol* 1990; **31**: 175-83.
11. Johnson JR. Virulence factors in *Escherichia coli* urinary tract infection. *Clin Microbiol Rev* 1991; **4**: 80-128.
12. Goullet Ph. An esterase zymogram of *Escherichia coli*. *J Gen Microbiol* 1973; **77**: 27-35.
13. Goullet Ph, Picard B. Comparative esterase electrophoretic polymorphism of *Escherichia coli* isolates obtained from animal and human sources. *J Gen Microbiol* 1986; **132**: 1843-51.
14. Goullet Ph, Picard B, Laget PF. Purification and properties of carboxylesterase B of *Escherichia coli*. *Ann Microbiol (Institut Pasteur)* 1984; **135A**: 375-87.
15. Goullet Ph, Picard B. Highly pathogenic strains of *Escherichia coli* revealed by the distinct electrophoretic pattern of carboxylesterase B. *J Gen Microbiol* 1986; **132**: 1853-8.
16. Goullet Ph, Picard B. Electrophoretic type B₂ of carboxylesterase B for characterisation of highly pathogenic *Escherichia coli* strains from extra-intestinal infections. *J Med Microbiol* 1990; **33**: 11-6.
17. Johnson JR, Goullet Ph, Picard B, Moseley SL, Roberts PL, Stamm WE. Association of carboxylesterase B electrophoretic pattern with presence and expression of urovirulence factor determinants and antimicrobial resistance among strains of *Escherichia coli* that cause urosepsis. *Infect Immun* 1991; **59**: 2311-5.
18. Orskov I, Orskov F. *Escherichia coli* in extra-intestinal infections. *J Hyg* 1985; **95**: 551-75.
19. Goullet Ph, Picard B. A two-dimensional electrophoretic profile for bacterial esterases. *Electrophoresis* 1985; **6**: 132-5.
20. Stirn S, Orskov F, Mansa B. Episome carried surface antigen K₈₈ of *E. coli*. II. Isolation and chemical analysis. *J. Bacteriol* 1967; **143**: 731-9.
21. De Rycke J, Guillot JF, Boivin R. Cytotoxins in non-enterotoxigenic strains of *Escherichia coli* isolated from feces of diarrheic calves. *Vet Microbiol* 1987; **15**: 137-50.
22. Dassouli-Mrani-Belkebir A, Contrepois M, Girardeau JP, Der Vartanian M. Characters of *Escherichia coli* O78 isolated from septicaemic animals. *Vet Microbiol* 1988; **17**: 345-56.
23. Lutkenhaus JF. Role of a major outer membrane protein in *Escherichia coli*. *J Bacteriol* 1977; **138**: 969-75.
24. Schnaitman CA. Outer membrane proteins of *Escherichia coli*. III. Evidence that the major protein of *Escherichia coli* OIII outer membrane consists of four distinct polypeptides species. *J Bacteriol* 1974; **118**: 442-59.
25. Oakley BR, Kirsch DR, Morris NR. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal Biochem* 1980; **105**: 361-3.

26. Benzecri JP. In: L'analyse de correspondance. l'Analyse des données. Dunod, Paris. 1984.
27. Hill MO. Correspondence analysis: a neglected multivariate method. *Appl Stat* 1974; **3**: 237-51.
28. Selander RK, Korhonen TK, Vaisanen-Rhen V, Williams PH, Pattison PE, Caugant DA. Genetic relationship and clonal structure of strains of *Escherichia coli* causing neonatal septicemia and meningitis. *Infect Immun* 1986; **52**: 213-22.
29. Arthur M, Arbeit RD, Kim C, et al. Restriction fragment length polymorphisms among uropathogenic *Escherichia coli* isolates: *pap*-related sequences compared with *rrn* operons. *Infect Immun* 1990; **58**: 471-9.
30. Arbeit RD, Arthur M, Dunn R, Kim C, Selander RK, Goldstein R. Resolution of recent evolutionary divergence among *Escherichia coli* from related lineages the application of pulsed field electrophoresis to molecular epidemiology. *J Infect Dis* 1990; **161**: 230-5.
31. Picard B, Picard-Pasquier N, Krishnamoorthy R, Goullet Ph. Characterization of highly virulent *Escherichia coli* strains by ribosomal DNA restriction fragment length polymorphism. *FEMS Microbiol Lett* 1991; **82**: 183-8.
32. Goullet Ph, Picard B. Comparative electrophoretic polymorphism of esterases and other enzymes in *Escherichia coli*. *J Gen Microbiol* 1989; **135**: 135-43.
33. Picard B, Goullet Ph. Correlation between electrophoretic types B₁ and B₂ of carboxylesterase B and host-dependent factors in *Escherichia coli* septicaemia. *Epidemiol Infect* 1988; **100**: 51-61.
34. Picard B, Goullet Ph. Correlation between electrophoretic types B₁ and B₂ of carboxylesterase B and sex of patients in *Escherichia coli* urinary tract infections. *Epidemiol Infect* 1989; **103**: 97-103.
35. Caprioli A, Falbo V, Ruggeri FM, Minelli F, Orskov I, Donelli G. Relationship between cytotoxic necrotizing factor production and serotype in hemolytic *Escherichia coli*. *J Clin Microbiol* 1989; **27**: 758-61.
36. O'Hanley P, Low D, Romero I, Lark K, Vosti K, Falkow S, Schoolnik G. Gal-Gal binding and hemolysin phenotypes and genotypes associated with uropathogenic *Escherichia coli*. *N Engl J Med* 1985; **7**: 414-20.
37. Cherifi A, Contrepolis M, Picard B, Goullet Ph, De Rycke J, Fairbrother J, Barnouin J. Factors and markers of virulence in *Escherichia coli* from human septicemia. *FEMS Microbiol Lett* 1990; **70**: 279-84.
38. Cherifi A, Contrepolis M, Picard B, Goullet Ph, Orskov I, Orskov F, De Rycke J. Clonal relationships among *Escherichia coli* serogroup O6 isolates from human and animal infections. *FEMS Microbiol Lett* 1991; **80**: 225-30.
39. Picard B, Goullet Ph, Krishnanmoorthy R. A novel approach to study of the structural basis of enzyme polymorphism. Analysis of carboxylesterase B of *Escherichia coli* as model. *Biochem J* 1987; **241**: 877-81.
40. Ochman H, Selander RK. Standard reference strains of *Escherichia coli* from natural populations. *J Bacteriol* 1984; **157**: 690-3.
41. Herzer PJ, Inouye S, Inouye M, Whittam T. Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. *J Bacteriol* 1990; **172**: 6175-81.