Highly Pathogenic Strains of *Escherichia coli* Revealed by the Distinct Electrophoretic Patterns of Carboxylesterase B

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One hundred and ninety one strains of *Escherichia coli* isolated from extra-intestinal infections and 85 strains isolated from the stools of healthy human beings were compared for electrophoretic mobility and isoelectric point of carboxylesterase B, and for production of α -haemolysin and the presence of mannose resistant haemagglutinin. Fast and slow electrophoretic mobilities were distinguished among the strains. The frequency of strains showing slow mobilities was considerably higher when they originated from extra-intestinal infections (40%) than when they were obtained from the stools of healthy individuals (7%). In a two-dimensional electrophoretic profile, the fast and slow mobility variants of carboxylesterase B were resolved into two patterns, B₁ and B₂, respectively. The frequency of pathogenic strains that concomitantly produced α -haemolysin and mannose resistant haemagglutinin was 48.7% for strains of pattern B₂ but only 2.8% for strains of pattern B₁. Thus, the electrophoretic pattern B₂ of carboxylesterase B appears to be a molecular marker for a group of highly pathogenic *E. coli* strains which are frequently implicated in extra-intestinal infections.

INTRODUCTION

In the preceding paper (Goullet & Picard, 1986) we showed by conventional electrophoresis (CE) that *Escherichia coli* strains producing slow mobility variants of carboxylesterase B were more frequently isolated from extra-intestinal infections than from human stools.

To substantiate this electrophoretic discrimination between commensal and pathogenic strains of *E. coli*, isoelectrofocusing (IEF) was used with CE to establish two-dimensional electrophoretic profiles (Goullet & Picard, 1985*a, b*; Picard & Goullet, 1985) of carboxylesterase B. These data correlate with production of α -haemolysin (Smith, 1963; van den Bosch *et al.*, 1981; Hughes *et al.*, 1983; Cavalieri *et al.*, 1984) and the presence of a mannose resistant haemagglutinin (MRHA) (Evans *et al.*, 1980; Vaisanen *et al.*, 1981). These are believed to be virulence factors of invasive bacterial strains (Hagberg *et al.*, 1981; Welch *et al.*, 1981; van den Bosch *et al.*, 1981, 1982; Cavalieri & Snyder, 1982; Gadeberg *et al.*, 1983; Hacker *et al.*, 1983). Using a random collection of isolates obtained from extra-intestinal infections and the faeces of healthy human beings, highly pathogenic strains were delineated by a distinct electrophoretic pattern of the esterase and by the frequent production of α -haemolysin and a MRHA.

METHODS

Sources of the bacterial strains. Commensal strains of *Escherichia coli* were obtained from faecal samples collected in Paris from 85 healthy human beings. Pathogenic strains of *E. coli* were recovered during the acute phase of 191 cases of extra-intestinal infections. Essentially the patients were grouped as follows: (i) two groups of hospitalized subjects (81 patients at St Antoine Hospital, Paris and 66 patients at Durance Hospital, Avignon) and (ii) one

Abbreviations: CE, conventional electrophoresis; HR, haemolysin release; IEF, isoelectrofocusing; MRHA, mannose resistant haemagglutinin; M_F , electrophoretic mobility.

group of 44 non-hospitalized patients who were treated by general practitioners in Paris. To avoid bias, strains from each category were taken randomly without considering the nature of the infection. Thus, 160 of the 191 pathogenic strains (83.5%) were derived from urinary tract infection as confirmed by the presence of significant bacteriuria ($>10^5$ bacteria ml^{-1}) determined using a calibrated loop (Isenberg *et al.*, 1985). Of the remaining organisms, 17 strains (9%) were obtained from cases of septicaemia and 14 strains (7.5%) were from other miscellaneous infections, including abscesses, lung infections, cholecystitis and some surgical infections. In all cases, the strains were obtained in pure culture by isolation on tryptic soy agar.

Electrophoretic analysis. Growth conditions, preparation of extracts, protein estimation, horizontal slab PAGE, estimation of electrophoretic mobility (M_F value), polyacrylamide gel isoelectrofocusing, determination of isoelectric points and esterase staining were described by Goullet & Picard (1985a).

α -Haemolysin assays. α -Haemolysin activity was routinely detected using horse erythrocyte agar (2%, v/v, erythrocytes) (Le Minor & Le Coueffic, 1975) and was subsequently confirmed by titration of α -haemolytic activity (van den Bosch *et al.*, 1980).

During the exponential phase of growth in fresh alkaline veal meat broth (Smith, 1963), supernatants were filtered and diluted from 1:2 to 1:256 in Tris buffered saline (0.01 M-Tris/HCl, 0.135 M-NaCl, pH 7.5) (van den Bosch *et al.*, 1980). Fresh sheep erythrocytes, washed three times and suspended to 2% in Tris buffered saline, were then added. The mixtures were incubated and centrifuged. Haemolysin release (HR) was measured with a Beckman spectrophotometer (model 24) at 540 nm. The titre was estimated as the highest dilution giving an HR of at least 25% of the maximal HR of the erythrocytes.

MRHA assays. These were done on glass microscope slides using type A human erythrocytes (Vosti, 1979) that had been washed three times and resuspended to 3% in phosphate buffered saline (0.005 M- KH_2PO_4 , 0.032 M- Na_2HPO_4 , 0.170 M-NaCl, 0.010 M-KCl, pH 7.2) containing 1% (w/v) methyl α -D-mannopyranoside (Sigma). Bacteria grown on agar were mixed with a drop (50 μl) of the erythrocyte suspension at room temperature. Agglutination was read after agitation for about 1 min, and was compared with positive and negative controls.

RESULTS

M_F distribution

The M_F distribution patterns of carboxylesterase B produced by commensal and pathogenic strains of *E. coli* are shown in Fig. 1. For both commensal and pathogenic strains, fast (ranging from $M_F \simeq 74$ to $M_F \simeq 66$) and slow (ranging from $M_F \simeq 63$ to $M_F \simeq 57$) electrophoretic mobilities may be distinguished. The two types of mobilities were not simultaneously observed in the same strain. Most (90.5%) of the commensal strains showed fast mobilities; 7% showed slow mobilities and 2.5% (two strains) produced no detectable carboxylesterase B activity. For pathogenic strains, 56.5% of the isolates showed fast mobilities which were distributed around $M_F \simeq 70$; 40% showed slow mobilities and 3.5% (seven strains) produced no detectable carboxylesterase B activity.

pI distribution

The pI distribution of carboxylesterase B produced by commensal strains and by pathogenic strains is shown in Fig. 2. For both types, the pI values of the esterase ranged from 4.5 to 5. In general the proportion of strains with pI values from 4.8 to 5 was higher for pathogenic isolates (45.5%) than for commensal isolates (18.8%).

Two-dimensional electrophoretic profile distribution

Two-dimensional electrophoretic profiles defined by mobility and the pI of each electrophoretic variant of carboxylesterase B as produced by commensal and pathogenic strains are shown in Fig. 3. Several mobility variants occurred for a single pI, and conversely, several pI variants were recovered in a single mobility. The number of electrophoretic variants resolved by two-dimensional electrophoretic profiles was higher than the number of electrophoretic variants resolved by CE or by IEF. Thus CE, IEF and two-dimensional electrophoretic profiles detected 7, 6 and 13 electrophoretic variants for commensal strains and 11, 8 and 26 electrophoretic variants for pathogenic strains, respectively.

The electrophoretic variants may be classified according to two distinct electrophoretic patterns, namely B_1 , which was defined by mobilities ranging from $M_F \simeq 74$ to $M_F \simeq 66$ and by

Fig. 1. Histograms of the electrophoretic mobility distribution of carboxylesterase B. □, Commensal strains; ■, pathogenic strains.

Fig. 2. Histograms of the isoelectric point distribution of carboxylesterase B. □, Commensal strains; ■, pathogenic strains.

pI ranging from 4.5 to 4.9, and B₂, which was defined by mobilities ranging from $M_F \approx 63$ to $M_F \approx 57$ and by pI ranging from 4.8 to 5. The frequency of strains showing the electrophoretic variant $M_F \approx 57$ and pI 5 was higher among pathogenic isolates (17.3%) than among commensal isolates (2.3%). Moreover, among the strains producing detectable carboxylesterase B activity, 77 commensal isolates showed electrophoretic pattern B₁ (these isolates were designated as CB₁) but only six commensals showed electrophoretic pattern B₂ (these isolates were designated as CB₂). In contrast, 108 pathogenic isolates (56.5%) showed electrophoretic pattern B₁ (these strains were designated as PB₁) and 76 of the pathogens (40%) showed electrophoretic pattern B₂ (these isolates were designated as PB₂).

Strains with the electrophoretic pattern B₂ (Fig. 3) were isolated from all three groups of patients, and from the diverse extra-intestinal infections. However, the frequency of PB₂ strains was slightly higher among the organisms isolated from the two groups of hospitalized patients (44.5% and 41%, respectively) than among those derived from non-hospitalized patients (29.5%). This also applied to strains derived from cases of septicaemia and other extra-urinary infections (48.5%) as compared with strains from urinary tract infections (38%).

Correlation between electrophoretic pattern and production of α -haemolysin and MRHA

The production of α -haemolysin and/or the presence of MRHA was much higher in pathogenic than in commensal strains (Table 1). The relationship between α -haemolysin and MRHA and electrophoretic patterns B₁ and B₂ is shown in Table 2. In all cases, haemolysis was

Fig. 3. Two-dimensional electrophoretic profile established by plotting electrophoretic mobility against isoelectric point of carboxylesterase B for 83 commensal strains (a) and for 184 pathogenic strains (b). The number of strains producing each electrophoretic variant is included.

Table 1. Production of α -haemolysin and MRHA in commensal and pathogenic strains of *E. coli*

Characteristic	No. of strains	
	Commensal	Pathogenic
Producing α -haemolysin without MRHA	2	14
Producing MRHA without α -haemolysin	4	46
Producing concomitantly α -haemolysin and MRHA	2	41
Producing neither α -haemolysin nor MRHA	77	90
Totals	85	191

Table 2. Correlation between the two electrophoretic patterns of carboxylesterase B and production of α -haemolysin and MRHA in commensal and pathogenic strains of *E. coli*

For CB_1 , CB_2 , PB_1 and PB_2 designations, see text.

Characteristic	No. of strains			
	CB_1	CB_2	PB_1	PB_2
Producing α -haemolysin without MRHA	1	1	3	9
Producing MRHA without α -haemolysin	3	1	29	17
Producing concomitantly α -haemolysin and MRHA	0	2	3	37
Producing neither α -haemolysin nor MRHA	73	2	73	13
Totals	77	6	108	76

confirmed by the production of α -haemolysin in alkaline meat broth (titres were in the range of 1:2 to 1:256). The percentage of strains producing α -haemolysin and/or MRHA was higher in group PB_1 than in group PB_2 . In the case of strains producing MRHA the increase was about 2.4-fold; for strains producing α -haemolysin the increase was 11-fold, and for strains producing α -haemolysin and MRHA concomitantly the increase was 17.6-fold. Although the number of CB_2 isolates (only six) was too low to allow statistical analysis of the data, it appeared that these strains were similar in the production of α -haemolysin and MRHA to PB_2 strains. Thus, the two commensal strains that concomitantly produced α -haemolysin and MRHA, were both CB_2 isolates.

DISCUSSION

As in previous investigations on the diversity of bacterial esterases (Goullet & Picard, 1985*a*, *b*; Picard & Goullet, 1985), the number of electrophoretic variants of carboxylesterase B resolved by two-dimensional electrophoretic profiles was higher than the number of electrophoretic variants defined by CE or by IEF. Two distinct electrophoretic patterns – B₁ (from $M_F \simeq 74$ to $M_F \simeq 66$) and B₂ (from $M_F \simeq 63$ to $M_F \simeq 57$) were clearly defined by CE whereas IEF showed a continuous spectrum of pI variants from 4.75 to 5 (Fig. 3). The poorer discrimination obtained by IEF is due to the fact that several mobility variants of patterns B₁ and B₂ fall into a single pI. Carboxylesterase B showing electrophoretic pattern B₂ was more prevalent among clinical isolates from extra-intestinal infections (40%) than in faecal isolates from healthy subjects (7%).

Since the isolates were taken randomly from a variety of infections and sources, esterase electrophoretic pattern B₂ may be considered as a molecular characteristic often associated with virulence. The production of α -haemolysin and MRHA was much higher in isolates from extra-intestinal infections than in commensal isolates as previously reported (van den Bosch *et al.*, 1982; Hagberg *et al.*, 1981; Cavalieri *et al.*, 1984). The electrophoretic pattern of carboxylesterase B correlates with the presence of α -haemolysin and MRHA (Table 2) in two ways. Firstly, the proportion of isolates producing MRHA but not α -haemolysin was six times higher in totally pathogenic strains (24%) than in CB₁ strains (4%) [the percentage of isolates producing MRHA without α -haemolysin was not significantly different in PB₁ (27%) and in PB₂ (22.5%) strains]. Secondly, the proportion of isolates producing concomitantly α -haemolysin and MRHA was 17.6 times greater in PB₂ (48.68%) than in PB₁ (2.77%) strains. The association of electrophoretic pattern B₂ with both clinical virulence and production of α -haemolysin and MRHA suggests the suitability of using carboxylesterase B as a molecular marker for highly pathogenic organisms.

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