Electrophoretic type B₂ of carboxylesterase B for characterisation of highly pathogenic *Escherichia coli* strains from extra-intestinal infections

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Summary. The frequency of electrophoretic types B₁ (fast mobilities) and B₂ (slow mobilities) of carboxylesterase B, and α-haemolysin and mannose-resistant haemagglutinin (MRHA) production were compared in 705 strains of *Escherichia coli* isolated from cases of septicaemia, urinary tract infection (UTI) and other extra-intestinal infections from different geographical origins, in particular France, America (USA and Canada) and Oceania (Australia and New Zealand). In all groups of strains, whether classified according to their clinical or their geographical origin, electrophoretic type B₂ was phenotypically linked with α-haemolysin and MRHA production. Haemolytic type B₂ strains were isolated more frequently from France and Oceania than America whereas the proportions demonstrating production of MRHA were similar among the three groups. Type B₂ strains were more frequently isolated from UTI and other infections than from septicaemia. This is attributed to the high frequency of immunocompromised subjects in the septicaemia group. Our results establish the suitability of using the type B₂ of carboxylesterase B as a molecular marker for highly pathogenic *E. coli* strains implicated in extra-intestinal infections in man.

Introduction

Several phenotypic traits of *Escherichia coli* have been associated with strains isolated from extra-intestinal infections but rarely found in the normal intestinal flora. One of these traits, the production of mannose-resistant haemagglutinin (MRHA) correlates well with the capacity to adhere to epithelial cells and is important in the initiation of infections. α-Haemolysin is another factor that enhances the virulence of *E. coli* by aiding its proliferation. Many pathogenic strains simultaneously express these two main virulence factors which have been shown to be chromosomally linked.

Several types of electrophoretically variable esterases in *E. coli* have been characterised by their distinct spectra of activity on synthetic substrates. The major component of this set of enzymes was carboxylesterase B, which showed two types of electrophoretic mobility—B₁ (fast mobility) and B₂ (slow mobility). *E. coli* strains may be divided into two groups according to these two electrophoretic types. In a survey of *E. coli* strains isolated in France we have shown that strains of type B₂ were isolated considerably more frequently from extra-intestinal infections when compared to isolates from the commensal intestinal flora, and that a higher proportion of type B₂ strains produced MRHA and α-haemolysin than did type B₁ strains. For these reasons we considered the type B₂ strains to be highly pathogenic.

The present work compared MRHA and α-haemolysin production with electrophoretic mobility of carboxylesterase B in 705 *E. coli* strains isolated from episodes of septicaemia, urinary tract infection (UTI) and other extra-intestinal infections from diverse geographical areas, including Australia, Canada, France, Japan, New Zealand, UK and USA.

Materials and methods

*E. coli* strains

The carboxylesterase B of 735 strains of *E. coli* was characterised electrophoretically. For 30 of the strains, esterase B was not detected and these strains were excluded from the study. The remaining 705 strains comprised: 427 strains from four different sources in...
France which have been studied previously, 18-21 88 strains from America, from three sources each in the USA and Canada; 155 strains from Oceania, from 10 sources in Australia and one source in New Zealand; 20 strains from the UK and 15 strains from Japan. The 705 strains were obtained from 705 patients; 690 were from the following extra-intestinal infections: septicaemia (168), UTI (464) and other miscellaneous infections (58) including abscesses, lung infections, cholecystitis, and some other surgical infections. Episodes of septicaemia were diagnosed by isolation of the bacteria in several serial blood cultures22 and of UTI by pure bacterial counts of >10^5 cfu/ml of urine.23 The distribution of infections according to their geographical origin is shown in table I. The precise nature of the infections from which the 15 strains from Japan were isolated was not known.

**Esterase electrophoresis**

The conditions for bacterial growth, the preparation of extracts, horizontal slab polyacrylamide gel electrophoresis, estimation of electrophoretic mobility (M_F value) and esterase staining have been described previously.15,24

**Haemolysin assay**

a-Haemolysin activity was detected with horse erythrocyte 2% w/v agar.25

**Mannose-resistant haemagglutinin assay**

Assays were done on glass microscope slides with type A human erythrocytes36 that had been washed three times and resuspended at a final concentration of 3% in phosphate-buffered saline (0.005 M KH2PO4, 0.032 M Na2HPO4, 0.17 M NaCl, 0.01 M KCl, pH 7.2) containing methyl α-D-mannopyranoside (Sigma) 1% w/v. Bacteria grown on agar were mixed with one drop (50 μl) of the erythrocyte suspension at room temperature. The slides were agitated for 1 min and agglutination was read by comparison with positive and negative controls.

| Table I. Distribution of E. coli strains according to geographical origin and site of infection |
|---------------------------------|------------------|-----------------|-----------------|
| Origin (number of strains)      | Number (% of strains) | Number of strains | other site of infection |
| France (427)                    | 117 (27.4)        | 296 (69.3)       | 14 (3.3)         |
| America (88)                    | 8 (9)             | 62 (70.5)        | 18 (20.5)        |
| Oceania (155)                   | 31 (20)           | 99 (63.9)        | 25 (16.1)        |
| England (20)                    | 12                | 7                | 1                |

**Statistical analysis**

The major bacterial variables of the 705 E. coli strains (type B1 or B2 esterases, haemolysin and MRHA production) were compared within and between the three major geographical sources of strains (French, American and Oceania) and within and between the three major clinical origins of strains (septicaemia, urinary tract infection and other sites) by Pearson’s χ² test.

**Results**

**Correlation between carboxylesterase types B₁ and B₂ and the production of α-haemolysin and MRHA**

The figure shows the distribution of the electrophoretic mobilities of carboxylesterase B produced by the 705 strains of E. coli. Electrophoretic type B₁ corresponds to mobilities of M_F = 88-66 and carboxylesterase type B₂ to mobilities of M_F = 57-63. Types B₁ and B₂ were found in a similar percentage of strains (50-63% and 49-36%, respectively). However, type B₂ strains were significantly more often haemolytic (57-5%), haemagglutinating (66-4%) or both haemolytic and haemagglutinating (45-1%) than were B₁ strains (7-8%, 25-8% and 3-6%, respectively) (χ² = 191, p < 0.001 for haemolysin; χ² = 112, p < 0.001 for MRHA and χ² = 150, p < 0.001 for both haemolysin and MRHA). The percentage of type B₂ strains increased in accordance with the number of virulence factors (22% for any factor, 31% for one and 47% for two) whereas these percentages decreased with type B₁ strains (72%, 26% and 2%, respectively).

**Characteristics of type B₁ and B₂ strains according to the nature of infection**

Type B₂ strains were more frequent than type B₁ strains among septicaemia isolates compared to both UTI isolates (χ² = 11, p < 0.001) and isolates from the other sites of infection (χ² = 7-1, p < 0.01). For the three categories of infection, type B₂ strains were more frequently haemolytic, or haemagglutinating or both haemolytic and haemagglutinating than type B₁ strains. The relative proportions of these virulence factors were similar for type B₂ strains in the three types of infection (table II).

**Characteristics of type B₁ and B₂ strains according to their geographical origin**

Type B₁ strains were more frequent than type B₂ strains in the isolates from France, whereas type B₂ predominated in both American strains (χ² = 7, p < 0.01 compared to France) and Oceanic strains
PHENOTYPING OF *E. coli*

Figure. Histogram of electrophoretic mobilities of carboxylesterase B produced by 705 *E. coli* strains. The percentage of strains producing α-haemolysin without MRHA (□), MRHA without α-haemolysin (□), both α-haemolysin and MRHA (■) and neither of these properties (□) are indicated. $M_F$ = relative mobility.

($\chi^2 = 13$, $p < 0.001$ compared to France) (table III). These differences could be explained in part by the higher proportion of septicaemia isolates from France than from America and Oceania (table I). In all three geographical groups, type B$_2$ strains were more frequently haemolytic, or haemagglutinating or both haemolytic and haemagglutinating than type B$_1$ strains. However, type B$_2$ strains from France were significantly more frequently haemolytic than those isolated in America ($\chi^2 = 9$, $p < 0.01$) whereas the proportion of haemagglutinating type B$_2$ strains and of both haemagglutinating and haemolytic type B$_2$ strains was similar for each of the three groups.

Discussion

Most work analysing the phenotypic traits of *E. coli* isolated from human extra-intestinal infections has been performed with strains of relatively limited

Table II. Relationships between electrophoretic types B$_1$ and B$_2$ of carboxylesterase B and production of α-haemolysin and MRHA in *E. coli* isolates and type of infection

<table>
<thead>
<tr>
<th>Source (number of strains)</th>
<th>Type of carboxylesterase</th>
<th>Number (%)</th>
<th>α-haemolysin</th>
<th>MRHA</th>
<th>MRHA + α-haemolysin</th>
<th>neither MRHA nor α-haemolysin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septicaemia (168)</td>
<td>B$_1$</td>
<td>106 (63.1)</td>
<td>5 (4.7)</td>
<td>29 (27.4)</td>
<td>3 (2.8)</td>
<td>69 (65.1)</td>
</tr>
<tr>
<td></td>
<td>B$_2$</td>
<td>62 (36.9)</td>
<td>10 (16.2)</td>
<td>13 (21)</td>
<td>33 (53.2)</td>
<td>6 (9.6)</td>
</tr>
<tr>
<td>UTI (464)</td>
<td>B$_1$</td>
<td>222 (47.8)</td>
<td>10 (4.5)</td>
<td>44 (19.8)</td>
<td>9 (4.1)</td>
<td>159 (71.6)</td>
</tr>
<tr>
<td></td>
<td>B$_2$</td>
<td>242 (52.2)</td>
<td>29 (11.9)</td>
<td>49 (20.3)</td>
<td>105 (43.4)</td>
<td>59 (24.4)</td>
</tr>
<tr>
<td>Other sites (58)</td>
<td>B$_1$</td>
<td>25 (43.1)</td>
<td>1 (4)</td>
<td>4 (16)</td>
<td>0</td>
<td>20 (80)</td>
</tr>
<tr>
<td></td>
<td>B$_2$</td>
<td>33 (56.9)</td>
<td>3 (9.1)</td>
<td>8 (24.2)</td>
<td>15 (45.5)</td>
<td>7 (21.2)</td>
</tr>
</tbody>
</table>
geographic variety. In this study, large samples of strains from three continents, which had been isolated from various extra-intestinal infections, were compared for electrophoretic mobility of carboxylesterase B, and haemolysin and MRHA production, which were the two main virulence factors most routinely identified in clinical isolates. The latter encompasses P, AFA and S and Dr adhesins which all confer on E. coli strains the capacity to attach to human epithelial cells. In previous work, we showed that carboxylesterase mobility can be used to distinguish between two groups of E. coli strains, types B₁ and B₂, isolated from extra-intestinal infections in France, the strains of type B₂ being considerably more often haemolytic, haemagglutinating and lethal for mice than type B₁ strains. Moreover, in the faecal flora of healthy subjects, the latter group predominated whereas the former were very rare. A recent investigation has established that type B₂ strains correspond to the group B2 of ECOR strains characterised by Selander et al. on the basis of electrophoretic polymorphism of 35 enzymes, indicating that B₂ strains are a taxonomically distinct cluster within E. coli populations. The present work demonstrates the wide distribution of this highly pathogenic group of strains since it was represented by more than 50% of isolates obtained in America and Oceania from various types of extra-intestinal infections.

Among the strains isolated from UTI or other extra-intestinal infections, several virulent groups designated by type or clone were delineated on the basis of association of α-haemolysin and adhesins with other phenotypic traits—O and K serotypes, outer-membrane-protein patterns or enzyme electrophoretic types. The strong association of haemolytic and adhesive properties with the slow mobility of carboxylesterase B suggests that this electrophoretic type could be present in many strains of the above mentioned virulent groups.

<table>
<thead>
<tr>
<th>Source (number of strains)</th>
<th>Type of carboxylesterase</th>
<th>Number (%)</th>
<th>α-haemolysin</th>
<th>MRHA</th>
<th>α-haemolysin + MRHA</th>
<th>neither MRHA nor α-haemolysin</th>
</tr>
</thead>
<tbody>
<tr>
<td>France (427)</td>
<td>B₁</td>
<td>244 (57-2)</td>
<td>11 (4-5)</td>
<td>60</td>
<td>8</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>B₂</td>
<td>183 (43-8)</td>
<td>32 (17-5)</td>
<td>33</td>
<td>85 (46-5)</td>
<td>33</td>
</tr>
<tr>
<td>America (88)</td>
<td>B₁</td>
<td>36 (41)</td>
<td>4 (11)</td>
<td>9</td>
<td>2 (5-5)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>B₂</td>
<td>52 (59)</td>
<td>1 (2)</td>
<td>14</td>
<td>20 (38-5)</td>
<td>17</td>
</tr>
<tr>
<td>Oceania (155)</td>
<td>B₁</td>
<td>61 (39-9)</td>
<td>0</td>
<td>8</td>
<td>2 (3)</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>B₂</td>
<td>94 (60-7)</td>
<td>9 (9-6)</td>
<td>19</td>
<td>44 (47)</td>
<td>22</td>
</tr>
</tbody>
</table>

The clinical significance of the differentiation between strains of types B₁ and B₂ was emphasised by the distinct patient profiles corresponding to the two electrophoretic types observed in both septicemia and UTI. In E. coli septicemia, we have demonstrated that strains isolated from subjects without underlying disease were more frequently of type B₂ whereas strains isolated from immunosuppressed subjects were more frequently of type B₁. The higher proportion of type B₁ strains in septicemia compared to UTI and other sites of infection is explained by the high frequency of immunocompromised subjects among the former group of infections (58% for France). These facts emphasise the importance of host-dependent factors in E. coli septicemia. Our data agree with the studies of Johnson et al. which showed that E. coli strains causing septicemia of urinary origin in non-compromised patients produced chromosomally encoded aerobactin, P fimbriae and haemolysin, whereas these chromosomal virulence factors were largely absent from E. coli strains causing septicemia of urinary origin in compromised hosts. In UTI, the strains isolated from males were more frequently of type B₂, haemolytic and haemagglutinating than those isolated from females. These findings demonstrate the influence of the patient's sex on the host-parasite interaction during these infections and are consistent with the observation of Westerlund et al. that in lower UTI these virulence factors were found in a higher percentage of strains isolated from boys than in those isolated from girls. Johnson et al. distinguished antibiotic-susceptible strains with virulence traits and antibiotic-resistant strains lacking chromosomal virulence factors.
was also apparent in our studies, in which type B₂ strains were more frequently sensitive to antimicrobial agents than type B₁ strains, whether isolated from UTI₁¹ or septicaemia (Picard and Goullet, unpublished data).

Detection of MRHA and α-haemolysin is based on diverse methods of detecting interactions between bacterial cells and erythrocytes and involving variable expression of different adhesive proteins³, ⁷, ³³ or complex mechanisms of activation, secretion and inactivation of α-haemolysin.⁴²–⁴⁴ On the other hand, electrophoretic typing of carboxylesterase B is based on a physicochemical method involving stable expression of one protein,¹⁷ easily visualised by a specific reaction in a gel.¹⁵–¹⁶ Thus, electrophoresis of carboxylesterase B is a reliable procedure for distinguishing between two widely distributed groups of Escherichia coli strains which differ considerably in their pathogenicity. The type B₂ strains were clearly characterised as the more virulent group. In contrast, the pathogenicity of the type B₁ strains, characterised by a lack of virulence factors, may be due to their predominance in the faecal flora, their frequent resistance to antibiotics and to various host susceptibility factors that facilitate infection.

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