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Correlation between electrophoretic types B_1 and B_2 of carboxylesterase B and host-dependent factors in *Escherichia coli* septicaemia

By B. PICARD and PH. GOULLET*

Service de Microbiologie, Hôpital Beaujon (Centre Hospitalier Universitaire Bichat-Beaujon, Université Paris VII), 100 boulevard du Général Leclerc, 92110 Clichy, France

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SUMMARY

Electrophoretic types B_1 and B_2 of carboxylesterase B produced by strains of *Escherichia coli* isolated from 100 septicaemia cases were correlated with α-haemolysin and mannose resistant haemagglutinin (MRHA) production and with clinical data including eventual underlying diseases, origin of septicaemia and evolution. Electrophoretic type B_2 was phenotypically linked with α-haemolysin and MRHA production. The proportion of type B_2 isolates varied significantly with occurrence of an underlying illness (45% for patients without an underlying disease and 22% for compromised patients) and with the site of origin of the septicaemia (40% for those of urinary origin and 18% for infection of digestive origin). In the former infections, type B_2 isolates were obtained in the majority from male patients while type B_1 isolates predominated in women. The septicaemias associated with type B_1 were characterized by a lower proportion of isolates producing α-haemolysin and MRHA and by a greater frequency of septic shock and death than those associated with type B_2. These facts emphasize the importance of host-dependent factors in *E. coli* septicaemia.

INTRODUCTION

Escherichia coli strains from human extra-intestinal infections possess various phenotypic traits not usually found in faecal isolates from normal subjects, e.g. some O and K antigens (Orskov *et al.* 1977; Orskov & Orskov, 1985), resistance to serum bactericidal activity (Taylor, 1983; Cross *et al.* 1986), virulence to mice and to chicken embryo (Minshew *et al.* 1978; Van den Bosch, De Graaff & MacLaren, 1979; Van den Bosch, Emody & Ketyi, 1982), aerobactin production (Carbonetti *et al.* 1986), α-haemolysin production (Van den Bosch *et al.* 1981; Welch *et al.* 1981; Cavalieri, Bohach & Snyder, 1984) and mannose resistant haemagglutinin (Evans *et al.* 1980; Hagberg *et al.* 1981; Vaisanen *et al.* 1981). However, these infections not only depend on the intrinsic properties of the strains but also on various host-dependent factors, such as susceptibility linked to an underlying disease (Singer,

* Author for reprints.

Kaplan & Armstrong, 1977; Kreger *et al.* 1980) and site whereby the organisms gain entry (Minshew *et al.* 1978).

Electrophoretic analysis of esterases, which has been extensively used in studies of the biochemistry, population genetics and evolution of a wide range of living organisms, may also be used in studies on the taxonomy and epidemiology of bacteria. In *E. coli*, seven types of esterase designated A, B, C, D, I, F, and S and differing in their ability to hydrolyse synthetic substrates and their sensitivity to di-isopropyl fluorophosphate have been separated by polyacrylamide agarose gel electrophoresis (Goullet, 1973). The major component of this set of enzymes was carboxylesterase B (EC 3·1·1·1) (Goullet, Picard & Laget, 1984) which showed two types of electrophoretic mobilities: B_1 from $M_f \approx 74$ to $M_F \approx 66$ and B_2 from $M_F \approx 63$ to $M_F \approx 57$ (Goullet, 1973; Goullet, 1980; Goullet & Picard, 1986*a*, *b*). *E. coli* strains may be divided in two groups according to these two electrophoretic types. The proportion of isolates having type B_2 was found to be significantly higher in extra-intestinal invasive strains (40%) than in commensal intestinal strains (7%) (Goullet & Picard, 1986*b*). Moreover, the B_2 strains were characterized by frequent production of α-haemolysin and a mannose-resistant haemagglutinin (Goullet & Picard, 1986*b*) which are believed to be the main virulence factors of invasive bacterial strains (Hagberg *et al.* 1981; Van den Bosch *et al.* 1981; Welch *et al.* 1981; Van den Bosch *et al.* 1982; Gadeberg, Orskov & Rhodes, 1983; Hacker *et al.* 1983; Cavalieri, Bohach & Snyder, 1984). Consequently the suitability of using carboxylesterase B type B_2 as a molecular marker for highly pathogenic strains of *E. coli* was proposed.

This present work was performed to evaluate the relative importance of types B_1 and B_2 *E. coli* strains and host-related factors in *E. coli* septicaemia. To this end, the principal clinical features of 100 cases of septicaemia, including original infection site, existence of underlying disease and evolution, were correlated with the following parameters of 100 *E. coli* isolates from these cases: electrophoretic types B_1 and B_2 of carboxylesterase B, production of α-haemolysin and mannose-resistant haemagglutinin (MRHA).

PATIENTS AND METHODS

Patients. A series of 100 septicaemias were randomly chosen from the 368 cases of *E. coli* septicaemia which occurred at Beaujon hospital between July 1981 and September 1984. (A total of 848 cases of Gram-negative septicaemia occurred at the hospital during the same period.) *E. coli* infections were diagnosed by isolation of the bacteria in 2–10 blood cultures. Five major clinical data were recorded for each patient (Kreger *et al.* 1980). (i) Nosocomial infections: all septicaemias occurring after 5 days of hospitalization were considered nosocomial in origin unless they originated from a source of infection noted previously. Septicaemias occurring before 5 days were considered non-nosocomial unless the infection was related to a procedure performed during hospital admission. (ii) Site of origin of septicaemia: defined on the basis of evidence of inflammation and isolation from the local site of *E. coli*, as found in blood culture, or by clinical analysis (for digestive origin in cirrhosis). When no cultural confirmation of local sites of origin could be obtained, cases were recorded as being of an 'unknown' source. (iii)

Underlying disease: following the classification proposed by McCabe & Jackson (Kreger *et al.* 1980) recording rapidly fatal (leukaemia) and ultimately fatal diseases (i.e. cancer, cirrhosis, severe diabetes). (iv) Septic shock; defined by hypotension (systolic blood pressures less than 90 mmHg and diastolic pressures less than 60 mmHg) accompanied by tachycardia, peripheral vasoconstriction and oliguria or anuria. (v) Fatality: all deaths occurring within 7 days of the onset of septicaemia were considered due to *E. coli* septicaemia.

Esterase electrophoresis. Growth conditions of bacterial strains, preparation of extracts, horizontal slab polyacrylamide agarose gel eletrophoresis, estimation of electrophoretic mobility (M_f value), and esterase staining were described previously (Goullet, 1973; Goullet & Picard, 1985). B_1 and B_2 esterases exhibited electrophoretic mobilities extending from $M_F \approx 66$ to $M_F \approx 74$ and $M_F \approx 57$ to $M_F \approx 63$, respectively.

Haemolysin assay. α-haemolysin activity was routinely detected using horse erythrocyte agar (2% w/v erythrocytes) (Le Minor & Le Coueffic, 1975).

Mannose-resistant haemagglutinin assay. These were done on glass microscope slides using type A human erythrocytes (Vosti, 1979) that had been washed three times and resuspended at a final concentration of 3% in phosphate-buffered saline (M) (0·005 KH_2PO_4, 0·032 Na_2HPO4, 0·170 NaCl, 0·010 KCl, pH 7·2) containing 1% (w/v) methyl α-D-mannopyranoside (Sigma). Bacteria grown on agar were mixed with one 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.

Statistical analysis. Three major bacteriological variables characterizing the 100 *E. coli* strains (e.g. type B_1 or B_2 esterases, haemolysin production, MRHA production) were correlated with five major clinical variables characterizing the 100 septicaemia cases (sex of patient, origin of septicaemia, underlying disease, septic shock, fatality). Pearson χ^2 values were calculated between each bacteriological variable and each clinical variable using for some cases Yates correction.

Multidimensional analysis. Because of the exclusively qualitative character of the variables studied, analysis of correspondence (Benzecri *et al.* 1973) was chosen for statistical study of data. This analysis was conducted from a frequency table indicating, for each bacteriological variable, the number of cases exhibiting the different types of clinical variables.

RESULTS

Clinical data. The 100 patients examined (48 males and 52 females) ranged in age from 15 to 85 years. They were drawn from the following wards: hepatology (41 cases), internal medicine (20 cases), haematology (13 cases), intensive care (3 cases), gastrointestinal) surgery (7 cases), neurosurgery (7 cases), cardiovascular surgery (6 cases), gynaecology (2 cases), orthopaedics (1 case). Twenty-five septicaemias (25%) were considered nosocomial in origin. Fifty-eight patients were suffering from ultimately or rapidly fatal underlying disease during their bacterial infection: hepatic cirrhosis (31 cases), leukaemia (12 cases), solid tumour cancers (10 cases), chronic alcoholism (4 cases), diabetes (1 case). All patients

suffering from leukaemia had received cytotoxic therapy. The origin of the septicaemia could be traced in 85 cases, and was digestive in 44 patients (including 10 biliary origin), urinary in 25 patients and of varied origins in 16 cases (including 5 catheterizations, 3 pulmonary cases, 1 meningeal case, 2 gynaecological cases and 5 cases following surgical intervention or trauma). The origin could not be determined for 15 cases. Complications of septic shock occurred in 16 cases. Of the 100 patients, 77 recovered and there were 23 fatalities, often linked with septic shock. Nineteen deaths were the final lethal event of underlying disease: 15 cases of cirrhosis, 1 case of chronic alcoholism, 2 cancer cases and 1 case of leukaemia. The remaining 4 patients did not suffer from a specific underlying disease, but showed serious general debilitation: neurological coma, multiple trauma.

Relationship between carboxylesterase B types B_1 and B_2, α-haemolysin and MRHA productions and the septicaemia clinical profile. A total of 68 strains had type B_1 esterase and 32 had type B_2 (Table 1). Among the type B_1 strains, 8 (12%) produced α-haemolysin, 19 (28%) produced MRHA and 3 (4·5%) produced both virulence factors. An underlying disease was recorded for 45 patients (66%), septic shock was noted in 15 cases (22%) and there were 19 fatalities (28%). Eighteen septicaemia cases (33%) were considered as nosocomial in origin. For the B_2 strains, 28 (87·5%) were haemolytic, 22 (69%) were haemagglutinating and 20 (63%) were both haemolytic and haemagglutinating. However, an underlying disease was recorded for 13 patients (40·5%), septic shock occurred in 1 case (3%) and 4 patients dies (12·5%). Seven septicaemia cases (27%) were considered as nosocomial in origin. The B_2 strains were significantly more haemolytic ($\chi^2 = 53{\cdot}23$; $P < 0{\cdot}001$), haemagglutinating ($\chi^2 = 14{\cdot}74$; $P < 0{\cdot}001$) and concomitantly haemolytic and haemagglutinating ($\chi^2 = 40{\cdot}73$; $P < 0{\cdot}001$) than were the B_1 strains on the basis of χ^2 test calculated for each bacteriological and clinical characteristic. These latter strains were more often isolated from patients with underlying disease ($\chi^2 = 5{\cdot}65$; $P < 0{\cdot}02$) and in septicaemia with septic shock ($\chi^2 = 5{\cdot}88$; $P < 0{\cdot}02$) than were the B_2 strains.

Fig. 1 shows that, for the 100 cases studied, the proportion of type B_2 haemolytic and haemagglutinating strains was smaller in patients suffering from underlying disease. The greater proportion of strains having esterase of type B_1 migrating at $M_F \approx 70$ in this group of subjects corresponded to an increase in the number of non-haemolytic and non-haemagglutinating strains (Fig. 1*b*).

In 16 cases (23·5%) of the type B_1 infections, other bacteria (including other enterobacteria, streptococcus and staphylococcus) were isolated either from the same blood sample or from separate cultures of samples taken during the same infection episode. This situation occurred in only three cases (9%) of the type B_2 infections.

Considering the origin of the infection, Table 1 shows that the majority of the 44 strains of gastrointestinal origin came from patients suffering from underlying disease (79·5% of patients), especially those with cirrhosis. These isolates included a low percentage of haemolytic and/or haemagglutinating strains (20·5%) corresponding to the greatest percentage of non-haemolytic, non-haemagglutinating type B_1 strains (77%). The outcomes of these septicaemias were serious with 23% septic shock and 32% fatalities despite appropriate antibiotic therapy.

Septicaemias of urinary origin were distinguished from those of digestive origin

Table 1. *Relationships between electrophoretic types (B_1 and B_2) of carboxylesterase B, productions of α-haemolysin and mannose-resistant haemagglutinin of* E. coli *isolates and main clinical features of the 100 septicaemias*

Site of origin of septicaemia...	Total septicaemia (100 cases)		Digestive tract (44 cases)		Urinary tract (25 cases)		Divers (16 cases)		Unknown (15 cases)	
Type of carboxylesterase B...	B_1	B_2	B_1	B_2	B_1	B_2	B_1	B_2	B_1	B_2
Number of cases	68	32	36	8	15	10	9	7	8	7
α-haemolysin production	8	28	3	6	4	9	0	7	1	6
MRHA production	19	22	4	5	10	7	2	4	3	6
Male	30	18	21	3	1	7	6	4	2	4
Patient with rapidly or ultimately fatal underlying diseases	45	13	30	5	7	1	4	2	4	5
Septic shock	15	1	10	0	3	0	2	0	0	1
Death	19	4	13	1	1	0	2	2	3	1

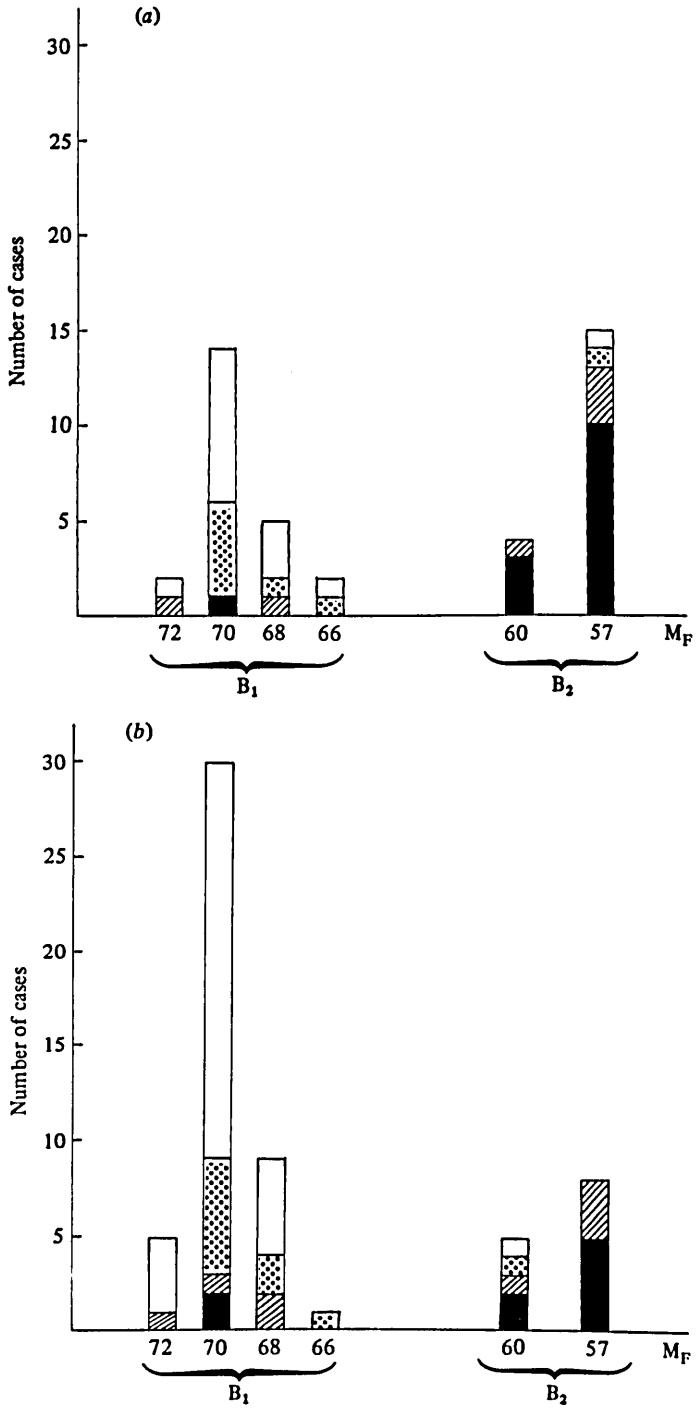

Fig. 1. Distribution of electrophoretic mobilities of carboxylesterase B produced by strains isolated from (*a*) Patient without an underlying disease and (*b*) patient with an underlying disease. In addition, number of strains producing α-haemolysin without MRHA (▨), MRHA without α-haemolysin (⊡), concomitantly α-haemolysin and MRHA (■) are indicated. M_F: relative mobility.

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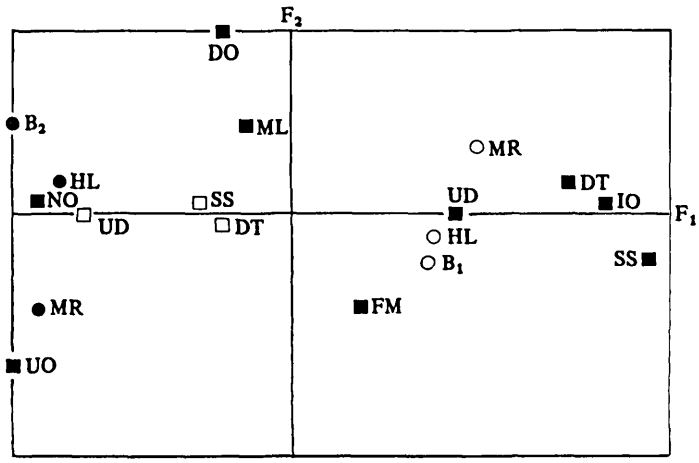

Fig. 2. Graphical representation of the results of analysis of correspondence carried out with the bacteriological variables from *E. coli* strains and the septicaemia clinical variables.

Projection of a bacteriological variable: ○ B_1, type B_1 isolate; ● B_2, type B_2 isolate; ● HL, production of α-haemolysin; ○ HL, absence of production of α-haemolysin; ● MR, production of MRHA: ○ MR, absence of production of MRHA.

Projection of a clinical variable: ■ ML, male patients; ■ FM, female patients; ■ UD, presence of underlying disease; □ UD, absence of underlying disease; ■ IO, intestinal tract origin; ■ UO, urinary tract origin; ■ DO, diverse origin; ■ NO, unknown origin; ■ SS, presence of septic shock; □ SS, absence of septic shock; ■ DT, death; □ DT, absence of death.

by a lower proportion of patients suffering from underlying disease (32%) ($\chi^2 = 14{\cdot}36$; $P < 0{\cdot}001$) and by a higher proportion of type B_2 strains (40%) ($\chi^2 = 3{\cdot}92$; $P < 0{\cdot}05$) and type B_1 haemagglutinating strains (66%) ($\chi^2 = 13{\cdot}73$; $P < 0{\cdot}001$). The type B_1 strains predominated in women while type B_2 strains were in the majority in the male patients ($\chi^2 = 8{\cdot}6$; $P < 0{\cdot}01$).

Compared to the infections of gastrointestinal or urinary origin, septicaemias of diverse origin involved an intermediate percentage of subjects suffering from underlying diseases (37·5%) and showed the highest percentage of type B_2 esterase (44%) and an intermediate percentage of haemolytic (43·75%) and haemagglutinating (37·5%) strains. Their outcomes were as serious as those of septicaemias of gastrointestinal origin.

Correspondence analysis. The first two principal axes defined by this analysis accounted for 97% of the total variance (Fig. 2). Projections of bacteriological variables on these first two axes revealed that axis F_1 (which accounted for 85·6% of the total variance) opposed B_2 type, production of α-haemolysin and of MRHA to B_1 type and absence of α-haemolysin and MRHA production. Projection of clinical variables revealed that axis F_1 opposed the urinary tract origin, unknown origin and absence of underlying disease to digestive origin, underlying diseases, septic shock and death. The first group of clinical variables was projected close to B_2 type and production of α-haemolysin and of MRHA whereas the second group was projected close to B_1 type and absence of α-

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haemolysin and MRHA production. The axis F_2 (which accounted for 11·5% of the total variance) opposed men to women and linked production of MRHA and urinary tract origin.

DISCUSSION

This work which extends our previous finding that the proportion of *E. coli* isolates showing electrophoretic type B_2 of carboxylesterase B was significantly higher in extra-intestinal infection than in the faeces of healthy subjects (Goullet & Picard, 1986*a*, *b*), established a significant correlation between types B_1 and B_2, α-haemolysin and MRHA productions and various host-dependent factors in *E. coli* septicaemia.

In agreement with the observations of other workers (Dupont & Spink, 1969; Bryant *et al.* 1971; Myerowitz, Medeiros & O'Brien, 1971; Singer, Kaplan & Armstrong, 1977; Kreger *et al.* 1980) *E. coli* was the most frequent aetiologic agent causing Gram-negative septicaemia in our hospital during the sampling period. Thirty-two per cent of isolates from septicaemia were of type B_2. This proportion is similar to those (40%) obtained from extra-intestinal infections of diverse site and of diverse geographical origin, including patients hospitalized in three separate hospitals and non-hospitalization patients (Goullet & Picard, 1986*b*); the similarities between these data of widely differing origins argue in favour of the clinical significance of the relative prevalence of B_2 isolates. The strains isolated from septicaemia were collected over a 3-year period in nine different wards of the hospital and the proportion of nosocomial infection was similar in both B_1 and B_2 septicaemias excluding any outbreak during the collection. The type B_2 could be considered as a molecular marker of virulence, but it is not known whether esterase type B_2 contributes directly or indirectly to the disease process or whether it is only associated with virulence factors. To evaluate this association, we have correlated types B_1 and B_2 with α-haemolysin and MRHA, which have been well documented as virulence factors (Hagberg *et al.* 1981; Van den Bosch *et al.* 1981; Welch *et al.* 1981; Van den Bosch *et al.* 1982; Gadeberg, Orskov & Rhodes, 1983; Hacker *et al.* 1983; Cavalieri, Bohach & Snyder, 1984) and considered to be genotypically linked (Berger *et al.* 1982; Low *et al.* 1984). The proportion of type B_1 strains and type B_2 strains producing a MRHA were comparable in septicaemia (28 and 68%, respectively) to those found in an earlier study (30 and 70%, respectively) (Goullet & Picard, 1986*b*). However the proportion of type B_1 and type B_2 strains producing α-haemolysin was higher in septicaemia (11·75 and 87·5%, respectively) than that found in earlier studies (5 and 60%, respectively). Thus type B_2 was phenotypically linked with α-haemolysin and MRHA productions. But genetic data are required to examine the possibility of genetic linkage between the locus coding for carboxylesterase type B_2 and loci coding for α-haemolysin and/or MRHA. The relationship between α-haemolysin production, the presence of fimbriae and enzyme electrophoretic polymorphism was evaluated by Selander *et al.* (1986) in strains of *E. coli* causing neonatal septicaemia and meningitis.

Since various host-related factors were implicated with the intrinsic properties of isolates to cause disseminating disease, we have compared the bacteriological

characteristic of the strains with the clinical features of septicaemia. The case fatality ratio of our study (23%) is comparable with those previously published (Dupont & Spink, 1969; Bryant *et al.* 1971; Myerowitz, Medeiros & O'Brien, 1971; Singer, Kaplan & Armstrong, 1977; Kreger *et al.* 1980). The difference in fatality rates between patients with either rapidly or ultimately fatal underlying diseases (33%) and those with non-fatal underlying diseases (9·5%) was significant ($\chi^2 = 7{\cdot}23$; $P < 0{\cdot}01$) as noted by Kreger *et al.* (1980). In the series reported by these authors the urinary tract was the most frequent site of origin of septicaemia, whereas in our work the digestive tract was the most common site. The digestive prevalence could be explained by 41 patients (including 31 with cirrhosis) being derived from a major hepatological-intensive unit at the hospital. The septicaemias associated with type B_1 strains which showed a lower proportion of α-haemolysin and MRHA were characterized by a greater frequency of septic shock and death than those associated with B_2 type. This apparent paradox emphasizes the importance of the host-dependent factors: underlying disease and site of origin of infections. The proportion of type B_2 isolates varied significantly both with the site of origin of the septicaemia (18% for infections of digestive origin, 40% for those of urinary origin and 44% for those of diverse origins) and with the underlying illness (22% for patients with an underlying disease and 45% for patients without an underlying disease).

To clarify the respective contribution and interaction of types B_1 and B_2 *E. coli* strains and of the host factors, a multidimensional analysis considering simultaneously the different variables appeared very useful for the statistical analysis of these data. The first factor of the correspondence analysis which accounted for the majority of the total variance separated the two types of isolates correlated with two clinical profiles: (i) type B_1 strains, which are generally non-haemolytic and non-haemagglutinating, were more frequently isolated from patients suffering from a rapidly or ultimately fatal underlying disease. These strains are responsible for septicaemia of digestive origin (especially in cirrhosis patients); their outcomes were often unfavourable because of the debilitated state of the patient. (ii) type B_2 strains which are often haemolytic and/or haemagglutinating were more frequently isolated from subjects without underlying disease during septicaemia of urinary origin; their outcomes were often favourable.

These results differentiate bacterial infections of digestive origin from those of urinary origin. In the first, which included the lowest percentage of type B_2 haemolytic and haemagglutinating isolates, the fact that outcomes were more often severe may be explained by the greater vulnerablity of patients (Singer, Kaplan & Armstrong, 1977; Kreger *et al.* 1980). Occurrence of septicaemia may be due to a weakening of the intestinal barrier brought on by mucosal lesions resulting from chemotherapy in cancer and leukaemia patients (Wolff, Wiseman & Kitchens, 1980), a reduction in the local immune defences, and/or disturbances of hepatic filtration in cirrhotic patients (Conn, 1984). Under these conditions, the non-haemolytic, non-haemagglutinating type B_1 strains of *E. coli* which are most frequently isolated from the gastrointestinal tract (Goullet & Picard, 1986*b*) may more readily be implicated in the septicaemia. The more opportunistic nature of type B_1 *E. coli* septicaemia is indicated by the frequent association (23%) of other

bacteria isolated from blood cultures. In septicaemias of urinary origin, the percentage of haemagglutinating type B_1 isolates was greater than that found in septicaemias of gastrointestinal origin and was similar to that of haemagglutinating type B_2 isolates. It has been suggested that these adhesive properties, as shown by haemagglutination, help to select bacteria capable of reaching and colonizing the normal urinary tract and influence the level of infection (Kallenius *et al.* 1981; Leffler & Svanborg-Eden, 1981). However, the greater percentage of type B_2 isolates from males suggests that virulence factors other than MRHA may be required for the invasive capacity of *E. coli* strains in these patients. Our recent work (in preparation) concerning 150 urinary tract infections confirms the prevalence of type B_2 strains in males.

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