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Virulence factors of bacteraemic *Escherichia coli* with particular reference to production of cytotoxic necrotising factor (CNF) by P-fimbriate strains

J. BLANCO, M. P. ALONSO†, E. A. GONZALEZ, M. BLANCO and J. I. GARABAL

Departamento de Microbiologia y Parasitologia, Facultad de Veterinaria, Universidad de Santiago, 27002 Lugo, and †Laboratorio de Microbiologica, Hospital de Corta, 27880 Burela, Lugo, Spain

Summary. Thirty-seven strains of *Escherichia coli* isolated from bacteraemia and 40 faecal strains isolated from healthy individuals were O serogrouped and investigated for the production of colicins, haemolysin (Hly), cytotoxic necrotising factor (CNF), lethal activity for mice, the expression of P fimbriae, mannose-resistant (MRHA) and mannose-sensitive (MSHA) haemagglutination, and relative cell surface hydrophobicity. Virulence factors significantly associated with bacteraemic strains were: serogroups O2, O4, O6, O7, O8 and O75 (54% *versus* 10%, $p < 0.001$), production of Hly (32% *versus* 8%, $p < 0.02$) and CNF (38% *versus* 10%, $p < 0.01$), expression of P fimbriae (27% *versus* 5%, $p < 0.02$), MRHA types III, IVa and IVb (51% *versus* 8%, $p < 0.001$), and possession of a moderate cell surface hydrophobic charge (35% *versus* 13%, $p < 0.05$). Virulence factors were strongly associated with strains expressing defined MRHA types. Thus, all strains belonging to MRHA types III and IVa were toxigenic, whereas only 11% of strains belonging to MRHA types IVb, V or VI were toxigenic ($p < 0.001$). Virulence factors were concentrated in strains belonging to O serogroups usually found in *E. coli* that cause extra-intestinal infections, especially in strains of O4 and O6 groups. The most interesting result of this study was that all 12 P-fimbriate strains expressed the MRHA type IVa and 11 of them synthesised CNF.

Introduction

Escherichia coli is one of the most common micro-organisms found in blood cultures. Bacteria reach the bloodstream from many different sites of infection, especially from the urinary tract (Kreger *et al.*, 1980; Ørskov and Ørskov, 1985). Adherence of bacteria to the host epithelium is an important virulence factor; in *E. coli* this is mediated by fimbriae (or pili), which usually express haemagglutinating activity (Gaastra and de Graaf, 1982; Ørskov and Ørskov, 1985). Two major groups of fimbriae have been found in extra-intestinal *E. coli*: common type 1 fimbriae which are responsible for mannose-sensitive haemagglutination (MSHA), and P, S, M, and X fimbriae that cause mannose-resistant haemagglutination (MRHA) (Väisänen *et al.*, 1981; Domingue *et al.*, 1985; Korhonen *et al.*, 1985; Ørskov and Ørskov, 1985; Hacker *et al.*, 1986; Brauner *et al.*, 1987; Johnson *et al.*, 1988). Many *E. coli* strains possess type 1 fimbriae, and generally no specific association has been demonstrated between the expression of these fimbriae and virulence. In contrast, mannose-resistant fimbriae are clearly associated with virulence (Ørskov and Ørskov, 1985). The most important mannose-resistant haemagglutinating fimbriae expressed by extra-intestinal *E. coli* are the P fimbriae, frequently detected in pyelonephritogenic *E. coli* (Väisänen *et al.*, 1981; Domingue *et al.*, 1985). Besides bacterial adherence, several virulence factors may contribute to the pathogenicity of bacteraemic *E. coli*. These include certain somatic (Vosti *et al.*, 1964; Ørskov and Ørskov, 1975) and capsular antigens (Evans *et al.*, 1981; Cross *et al.*, 1984; Korhonen *et al.*, 1985); resistance to the bactericidal activity of serum (Cross *et al.*, 1984) and to phagocytosis (Weinstein and Young, 1978; Cross *et al.*, 1984); expression of the siderophore aerobactin (Montgomerie *et al.*, 1984; Johnson *et al.*, 1988); and production of colicin V (Minshew *et al.*, 1978) and haemolysin (Hly) (Minshew *et al.*, 1978; Czirok, 1985). Furthermore, four toxins (LT, STa, VT and CNF) have been described in human *E. coli* strains. Enterotoxigenic *E. coli* synthesise heat-labile (LT) or heat-stable (STa) enterotoxin, or both. Verotoxin (VT) is produced by enterohaemorrhagic *E. coli* of serotype O157:H7 and some classical enteropatho-

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genic *E. coli* strains (Levine, 1987). Production of a cytotoxic necrotising factor (CNF) was found to be closely associated with *E. coli* strains causing urinary tract infections (Alonso *et al.*, 1987; Caprioli *et al.*, 1987).

We have developed (Blanco *et al.*, 1985; Blanco *et al.*, 1988*a*) a simple, rapid and economical MRHA typing system for *E. coli*, a modification of that originally described by Duguid *et al.* (1979) and Evans *et al.* (1980, 1981); it appears to be valid for the presumptive identification of pathogenic strains. MRHA strains were classified into six MRHA types (I–VI): (i) types I and II are expressed only by enterotoxigenic *E. coli* producing colonisation factors CFA/I and CFA/II respectively; (ii) types III and IVa are usually expressed by CNF- and Hly-producing strains belonging to serotypes commonly identified in *E. coli* that cause extra-intestinal infections; and (iii) types IVb, V and VI are not associated with pathogenic strains.

In this study the incidence of various virulence-associated factors in 37 *E. coli* strains isolated from blood and in 40 control strains isolated from faeces of healthy people was investigated.

Materials and methods

Bacterial strains

Thirty-seven *E. coli* strains were isolated from the blood of patients with bacteraemia in the Hospital Juan Canalejo in La Coruña (North-western Spain) from 1986 to 1988. The mean age of the patients was 64 years (range 3 days to 84 years). Underlying medical illnesses were present, singly or in combination, in 62% of the patients. The most common underlying diseases were neoplasia (24%) and diabetes (16%). Bacteraemias were associated with urinary tract infections (43%), abdominal infections (19%), and respiratory tract infections (11%), but 27% of the patients had bacteraemia without an identifiable source. Sixty-eight percent were community acquired and 32% hospital acquired bacteraemias. Forty strains isolated from stools of healthy individuals of widely varying age groups were used as controls. A single *E. coli* colony was chosen from each individual. Isolation and identification of *E. coli* were by standard bacteriological methods. Strains were stored at room temperature in Nutrient Broth with agar (Difco) 0·75% (w/v).

Toxin production

Erlenmeyer flasks of 250-ml volume containing 20 ml Tryptone Soya Broth (Oxoid) (pH 7·5), were inoculated with a loopful of the test strain taken from solid medium and incubated in an orbital shaker (200 rpm) at 37°C for 20 h. To obtain sonic extracts for testing, whole cultures (7×10^9 cfu/ml) were disintegrated in an ice bath with an MSE ultrasonic disintegrator at an amplitude of 10 μm peak for 5 min. After sonication, cultures were centrifuged (6000 *g* for 15 min at 4°C) and the supernatant fractions were filter sterilised. Sonic extracts were assayed the same day or stored at −20°C for a maximum period of 7 days (Alonso *et al.*, 1987; Blanco *et al.*, 1988*a*).

Detection of CNF, LT and VT on Vero cells

Sonic extracts from *E. coli* strains were assayed on Vero cell monolayers in plastic plates (Alonso *et al.*, 1987; Blanco *et al.*, 1988*a*); 0·05 ml of sonic extract was added to each well with Vero monolayers and 0·5 ml of fresh Eagle's Minimum Essential Medium (Flow Laboratories) without fetal calf serum and supplemented with polymyxin B sulphate (Gibco) 50 units/ml and 0·05 mM methyl-isobutyl-xanthine (Sigma) as phosphodiesterase inhibitor. Cultures were incubated at 37°C in an atmosphere of air+CO_2 5% and the morphological changes in cells were observed after incubation for 24 and 48 h. Specific morphological changes caused by CNF, LT and VT on Vero cells may be observed in fig. 1 (González and Blanco, 1985; Blanco *et al.*, 1988*b*).

Animal assays

Necrotising activity was detected by the skin test performed in New Zealand albino rabbits weighing 2·0–2·5 kg. Hair was shaved from the back of the animals and 0·1 ml of each sonic extract was injected intradermally into three rabbits following a random pattern. Forty-eight hours after inoculation, the presence of necrotic reactions was assessed by inspecting for ulceration and induration at the injection sites. Sonic extracts were considered to give positive reactions when they produced necrosis in at least two of the three animals. Mouse lethality was assayed by intraperitoneal injection of sonic extracts (0·5 ml per mouse) into five BALB/c mice (25–30 g). The number of mice that died during a week was scored. We considered the extract to have obvious lethal activity when at least three of the five mice died.

Haemolysin production

Haemolysis was assayed on Blood Agar Base medium (Merck, FRG) containing washed sheep erythrocytes 5% v/v. Strains that produced a clear zone of lysis (β-haemolysis) after incubation for 24 h at 37°C were considered to be haemolytic.

Colicin production

Tryptone Soya Medium (Oxoid) with agar 1·2% was inoculated with *E. coli* strains. After incubation for 24 h at 37°C, the organisms were killed by treatment with chloroform vapour (Merck) for 30 min and then opened to air for 1 h. Next, the medium was overlaid with soft agar (agar 0·4% and NaCl 0·5% in Nutrient Broth, Difco)

containing *E. coli* K12-711 or *E. coli* K12-711-Colicin V^+, 10^6 cfu/ml. After incubation for 24 h at 37°C, inhibition of the indicator strains by the test strain was scored. Strains that inhibited indicator strain K12-711 but not strain K12-711-Colicin V^+ were considered to be colicin V (Col V) positive. Strains that inhibited both indicator strains were considered to produce non-V colicins.

Haemagglutination test, P fimbriae and cell surface hydrophobicity

Bacteria were inoculated into Mueller Hinton Broth (Difco) and incubated statically at 37°C for 5 days until a pellicle was formed on the surface. From this pellicle, bacteria were recovered, inoculated on to CFA (Evans *et al.*, 1980) and Minca-Is (Guinée *et al.*, 1977) solid media and incubated at 37°C for 18 h. Haemagglutination was determined by the rocked-tile method (Blanco *et al.*, 1985, 1988*a*) with human group A, calf, guinea-pig, adult chicken, sheep and pig erythrocytes and dense suspensions (10^{12} cfu/ml in PBS, pH 7·4) of bacteria grown on CFA and Minca-Is media. One drop of bacterial suspension was mixed with one drop of erythrocytes (3% in PBS) and one drop of PBS (with or without D-mannose 3%) in a depression on a tile. The tile was rocked for 5 min at ambient temperature followed by 5 min at 4°C, and results were recorded after further incubation at ambient temperature and at 4°C. Haemagglutination was considered resistant to mannose (MRHA) when it occurred in the presence and absence of mannose, and sensitive to mannose (MSHA) when it was inhibited by the presence of mannose. Strains were grouped according to their MRHA patterns in six MRHA types (I–VI): (i) type I characterised by $MRHA^+$ with human, calf and adult chicken erythrocytes and $MRHA^-$ with the remaining three erythrocyte species; (ii) type II, $MRHA^+$ with calf and adult chicken erythrocytes and $MRHA^-$ with the remaining four erythrocyte species; (iii) type III, $MRHA^+$ with human erythrocytes only after incubation at 4°C and with sheep and pig erythrocytes, and MRHA variable (+ or −) with the remaining three erythrocyte species; (iv) type IVa, $MRHA^+$ with human erythrocytes at ambient temperatures and with sheep and pig erythrocytes, and MRHA variable with the remaining three erythrocyte species; (v) type IVb, $MRHA^+$ with human and pig erythrocytes. $MRHA^-$ with sheep erythrocytes and MRHA variable with the remaining three erythrocyte species; (vi) type V, $MRHA^+$ with human erythrocytes and $MRHA^-$ with the remaining five erythrocyte species; and (vii) type VI, $MRHA^-$ with human erythrocytes and MRHA variable with the remaining five erythrocyte species.

Detection of P fimbriae was performed by a particle agglutination test (PF test, Orion Diagnostica, Espoo, Finland) which was specific for the P receptor. The bacterial suspensions prepared for haemagglutination tests were employed for the detection of P fimbriae. Briefly, one drop of bacterial suspension was mixed with one drop of test solution containing latex particles coated with the P receptor α-D-Gal-(1–4)-β-D-Gal on a plastic slide. Latex particles devoid of P receptor served as a control. If P fimbriae were expressed by the organism, macroscopic agglutination was apparent, usually within 1 min.

Relative cell surface hydrophobicity was measured by the improved salt aggregation test (ISAT) with suspensions (5×10^9 cfu/ml in 0·02 M phosphate buffer, pH 6·8) of bacteria grown on CFA and Minca-Is media (González *et al.*, 1988). The final molar ammonium sulphate (Merck) concentrations used were 2·0, 1·4, 1·0, 0·4, 0·1, 0·06 and 0·02. Strains were considered hydrophobic when they aggregated in $\leqslant 1{\cdot}4$ M ammonium sulphate concentrations.

Serotyping

O serotyping of *E. coli* strains was performed in microtitration plates by the method of Guinée *et al.* (1972). The eight O antisera used (O1, O2, O4, O6, O7, O8, O18 and O75) were selected on the basis of their frequent occurrence in *E. coli* isolates from blood (Vosti *et al.*, 1964; Ørskov and Ørskov, 1975).

Statistical methods

Results were compared by the χ^2 test with Yates' correction for continuity.

Results

Incidence of the virulence-associated factors in E. coli isolated from blood and faeces: relation to mouse lethality

Serogroups O2, O4, O6, O7, O8 and O75, the production of Hly and CNF, the expression of P fimbriae, MRHA types III, IVa and IVb, and cell surface hydrophobicity were all more frequently detected in bacteraemic *E. coli* than in strains isolated from the faeces of healthy people (table I). In contrast, colicin production, mouse lethality, and the expression of MSHA and MRHA types V and VI were found in similar proportions in bacteraemic and control strains. When we examined the distribution of these characteristics in lethal and non-lethal strains, independent of their origin (blood or faeces), all characteristics except colicin production and the expression of MSHA and MRHA types IVb, V and VI, were closely associated with lethal strains (table II).

Haemagglutinating properties

All *E. coli* strains, grown on CFA and Minca-Is media, were tested for haemagglutination in the presence and absence of D-mannose. The results

Table I. Distribution of virulence characters in bacteraemic *E. coli* strains and in isolates from the faeces of healthy people

Character	Number (%) of strains isolated from		χ^2 p value
	blood (n = 37)	faeces (n = 40)	
O2, O4, O6, O7, O8 and O75 groups	20 (54)	4 (10)	<0·001
Production of colicin V	5 (14)	4 (10)	<0·90
Production of other colicins	11 (30)	16 (40)	<0·50
Production of Hly	12 (32)	3 (8)	<0·02
Production of CNF	14 (38)	4 (10)	<0·01
Mouse lethality	9 (24)	7 (18)	<0·70
Expression of P fimbriae	10 (27)	2 (5)	<0·02
MRHA types III, IVa and IVb	19 (51)	3 (8)	<0·001
MRHA types V and VI	3 (8)	9 (23)	<0·20
Expression of MSHA	20 (54)	21 (53)	<0·95
Moderate cell surface hydrophobicity	13 (35)	5 (13)	<0·05

Table II. Presence of virulence characters in lethal and non-lethal *E. coli* strains

Character	Number (%) of strains		χ^2 p value
	lethal (n = 16)	non-lethal (n = 61)	
O4, O6, O18 and O75 groups	9 (56)	2 (3)	<0·001
Production of colicin V	0 (0)	9 (15)	<0·30
Production of other colicins	5 (31)	22 (36)	<0·95
Production of Hly	10 (63)	5 (8)	<0·001
Production of CNF	11 (69)	7 (11)	<0·001
Expression of P fimbriae	7 (44)	5 (8)	<0·01
MRHA types III and IVa	11 (69)	5 (8)	<0·001
MRHA types IVb, V and VI	1 (6)	17 (28)	<0·20
Expression of MSHA	9 (56)	32 (52)	<0·99
Moderate cell surface hydrophobicity	9 (56)	9 (15)	<0·01

were as follows: (i) all 41 MSHA$^+$ strains expressed mannose-sensitive haemagglutination when grown on both media; (ii) 34 strains were MRHA$^+$, including 29 that expressed the property when grown on both media, three only when grown on Minca-Is medium and two only when grown on CFA agar; (iii) 25 of the 29 MRHA$^+$ strains expressed identical MRHA types when grown on CFA and Minca-Is media. *E. coli* strains were grouped according to their haemagglutinating phenotypes as shown in table III. Haemagglutinating strains were also grouped according to their MRHA patterns into six types (I–VI) (table IV) (Blanco *et al.*, 1988*a*).

Cell surface hydrophobicity

Of the 77 strains, 18 (23%) were moderately hydrophobic, aggregating in $\leqslant$1·4 M ammonium sulphate concentrations. Four strains aggregated in 0·4 M ammonium sulphate, and the other 14 strains in 1·0 M or 1·4 M solutions. Seven strains were hydrophobic when grown on CFA and Minca-Is media, five only when grown on CFA agar and six only when grown on Minca-Is medium. Hydrophobicity was found to be associated with haemagglutination; all 18 hydrophobic strains were haemagglutinating, whereas only 36 of the 59 non-hydrophobic strains were ($p < 0·01$). Furthermore, hydrophobic strains were more frequently detected among MRHA$^+$ MSHA$^+$ strains (43%) than in *E. coli* expressing either MRHA (31%) or MSHA (25%) alone (table III). Hydrophobic strains were more common in MRHA$^+$ strains belonging to types III and IVa (63%) than in strains of types IVb, V and VI (17%) ($p < 0·02$) (table IV).

Table III. Relationship of haemagglutinating phenotypes of *E. coli* strains with virulence factors

Haemagglutinating phenotypes	Number (%)* of strains from		Number (%)† of strains that were					
	blood (n=37)	faeces (n=40)	Hly$^+$	CNF$^+$	lethal$^+$	toxigenic$^+$‡	PF$^+$§	hydrophobic
MRHA$^+$ MSHA$^-$	6 (16)	7 (18)	4 (31)	6 (46)	4 (31)	6 (46)	4 (31)	4 (31)
MRHA$^+$ MSHA$^+$	16 (43)	5 (13)	9 (43)	11 (52)	8 (38)	12 (57)	8 (38)	9 (43)
MRHA$^-$ MSHA$^+$	4 (11)	16 (40)	2 (10)	1 (5)	1 (5)	3 (15)	0 (0)	5 (25)
MRHA$^-$ MSHA$^-$	11 (30)	12 (30)	0 (0)	0 (0)	3 (13)	3 (13)	0 (0)	0 (0)

* Percentages were calculated in relation to the total number of bacteraemic and faecal strains respectively.
† Percentages were calculated in relation to the total number of strains showing each haemagglutinating phenotype.
‡ Strains that were Hly$^+$, CNF$^+$ or lethal$^+$ were considered toxigenic.
§ P fimbriate.

Table IV. Virulence factors in *E. coli* strains belonging to different MRHA types

MRHA type*	MRHA with erythrocytes of†						Number (%)‡ of strains from		Number (%)‡ of strains that were					
	hA	Cl	Gp	Ck	Sh	Pg	blood (n=37)	faeces (n=40)	Hly$^+$	CNF$^+$	lethal$^+$	toxigenic$^+$‡	PF$^+$	hydrophobic
III	R§	V	V	V	R	R	3 (8)	1 (2)	4 (100)	4 (100)	4 (100)	4 (100)	0 (0)	4 (100)
IVa	R	V	V	V	R	R	10 (27)	2 (5)	8 (67)	11 (92)	7 (58)	12 (100)	12 (100)	6 (50)
IVb	R	V	V	V	–	R	6 (16)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)
V	R	–	–	–	–	–	1 (3)	5 (13)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)
VI	–	V	V	V	V	V	2 (5)	4 (10)	1 (17)	2 (33)	1 (17)	2 (33)	0 (0)	1 (17)

* Type I (RR–R––) and type II (–R–R––) are expressed by enterotoxigenic *E. coli* (ETEC) strains with CFA/I and CFA/II respectively.
† hA=Human group A; Cl=calf; Gp=guinea pig; Ck=chicken; Sh=sheep; Pg=pig. R=MRHA; – =no MRHA; V=some strains MRHA and others no MRHA.
‡ See footnote to table III.
§ Strains belonging to MRHA type III showed MRHA with human erythrocytes only after incubation at 4°C, whereas strains belonging to MRHA type IVa were MRHA with human erythrocytes at room temperature.

P fimbriae

The expression of P fimbriae was investigated in all 77 *E. coli* strains grown on CFA agar. Seven of the 77 strains were tested after being grown on both CFA and Minca-Is media because three of them were MRHA only when grown on Minca-Is and the other four developed different MRHA types on Minca-Is than on CFA agar. Twelve P-fimbriate strains were detected: 10 from blood (27%) and 2 (5%) from faeces ($p<0{\cdot}02$). All 12 P-fimbriate strains developed MRHA on both growth media used; 10 of them expressed the MRHA type IVa when grown on CFA and Minca-Is media, one strain expressed the MRHA type IVa when grown on CFA and the MRHA type V when grown on Minca-Is, and the remaining strain expressed the MRHA type V when grown on CFA and the MRHA type IVa when grown on Minca-Is. The correlation between expression of the P fimbriae and the MRHA type IVa was complete, because all P-fimbriate strains expressed the MRHA type IVa and all the strains belonging to the MRHA type IVa were P fimbriate (table IV).

Production of toxins

In assays for CNF, eleven strains gave positive results in both Vero cell and rabbit skin assays whereas five strains only produced necrosis in rabbit skin and two only caused the typical morphological transformation of Vero cells. Eighteen CNF strains were detected—14 (38%) from blood and 4 (10%) from faeces ($p<0{\cdot}01$) (table I).

Fifteen strains produced haemolysin (Hly), and

14 of them also synthesised CNF. Furthermore, 16 strains were lethal for mice. According to these three toxic markers (CNF, Hly and lethality), *E. coli* strains were grouped into six different toxic phenotypes (table V). The most frequent toxic phenotype, Hly^+ CNF^+ $lethal^+$, was exhibited by 9 (24%) of the bacteraemic strains and by 1 (3%) faecal strain ($p<0{\cdot}02$). This toxic phenotype was shown by all four strains belonging to MRHA type III and by six of the 12 P-fimbriate strains expressing MRHA type IVa. CNF production is clearly closely associated with haemolysin production and lethality for mice, because 14 (78%) of the 18 CNF strains were Hly^+ and 11 (61%) were lethal for mice. Another common toxic phenotype was Hly^- CNF^- $lethal^+$ which was shown by five strains, all of them isolated from the faeces of healthy people. Only one of these five strains was $MRHA^+$, belonged to the MRHA type IVa and expressed P fimbriae. Striking differences in the production of Hly, CNF and lethality were observed when *E. coli* strains were grouped according to their haemagglutinating properties (table III). Thus, the production of Hly, CNF or lethality for mice was detected in 53% of the $MRHA^+$ strains, whereas only 14% of the non-MRHA strains were Hly^+ CNF^+ or $lethal^+$ ($p<0{\cdot}001$). The production of toxins was a frequent property in strains belonging to specific MRHA types (table IV). Thus, all strains belonging to MRHA types III and IVa were Hly^+, CNF^+ or $lethal^+$, whereas no strain included in MRHA types IVb and V were toxigenic, and only 33% of strains of the MRHA type VI were toxigenic. Another interesting result was that all 12 P-fimbriate strains were toxigenic, 11 of them synthesising CNF. No LT-producing strain was detected and only one bacteraemic strain was found to synthesise VT. The VT-producing strain was also Hly^+ CNF^+ $lethal^+$, expressed the MRHA type III and belonged to serogroup O4.

O serogroups of E. coli

Thirty-one strains were typable with the eight O antisera used, 21 (57%) isolated from blood and 10 (25%) from faeces ($p<0{\cdot}01$). Furthermore, virulence factors were more frequently detected in typable strains than in non-typable strains. Thus, 92% of P-fimbriate strains, 87% of Hly^+ and 78% of CNF^+ strains were typable (table VI). All *E. coli* strains belonging to serogroups O4 (five strains) and O6 (five strains) were isolated from blood, and the majority were toxigenic (nine strains) and expressed P fimbriae (seven strains) or MRHA type III (two strains). In contrast, all five strains belonging to the O1 group were isolated from the faeces, only one of them was toxigenic and expressed the P fimbriae.

Discussion

We have investigated the association of virulence factors with bacteraemic *E. coli* strains, using strains isolated from the faeces of healthy people as controls. In most bacteraemic strains, several virulence factors were found. Thus, our results support the idea that pathogenicity of bacteraemic *E. coli* may be ascribed to a combination of virulence factors.

CNF was shown to be a thermolabile cell-associated protein that causes necrosis in rabbit skin and induces specific morphological transformation of tissue cultures (Caprioli *et al.*, 1983). Caprioli *et al.* (1987) and Bisicchia *et al.* (1985) found CNF production in 37% of *E. coli* strains from urinary tract infections, in 5% from diarrhoeic patients and in only 0·9% of strains isolated from the faeces of healthy people. In previous studies, we also detected a high prevalence of CNF-producing *E. coli* in urinary tract infections (50%) (Alonso *et al.*, 1987) and in $MRHA^+$ strains isolated from diarrhoeic patients (49%) (Blanco *et al.*,

Table V. Relationship of toxic phenotypes of *E. coli* strains with mannose-resistant haemagglutination and O groups

Toxic phenotype			Number of strains from		MRHA type (O groups)			Not-MRHA
			blood	faeces	III	IVa (P fimbriate)	VI	(O groups)
Hly^+	CNF^+	$Lethal^+$	9	1	4 (O4, O6, O75, NT)*	6 (2 O4, 3 O6, O18)	0	0
Hly^+	CNF^+		3	1	0	2 (O4, O6)	1 (O8)	1 (NT)
	CNF^+	$Lethal^+$	0	1	0	0	1 (NT)	0
Hly^+			0	1	0	0	0	1 (O8)
	CNF^+		2	1	0	3 (O2, O7, NT)	0	0
		$Lethal^+$	0	5	0	1 (O1)	0	4 (NT)

* NT = not typable.

Table VI. Relationship of *E. coli* serogroups with virulence factors

	Number (%)* of strains that were									
	isolated from						MRHA type			
Serogroup	blood	faeces	Hly$^+$	CNF$^+$	lethal$^+$	toxigenic$^+$	III	IVa (FP$^+$†)	others	hydrophobic
O1	0	5	0	0	1	1	0	1	1	0
O2	4	2	0	1	0	1	0	1	1	1
O4	5	0	4	4	3	4	1	3	0	3
O6	5	0	5	5	4	5	1	4	0	4
O7	1	0	0	1	0	1	0	1	0	0
O8	4	2	2	1	0	2	0	0	2	1
O18	1	1	1	1	1	1	0	1	1	0
O75	1	0	1	1	1	1	1	0	0	1
Total typable strains	21	10	13 (42)	14 (67)	10 (32)	16 (52)	3 (10)	11 (35)	5 (16)	10 (32)
Not typable strains	16	30	2 (4)	4 (9)	6 (13)	8 (22)	1 (2)	1 (2)	13 (28)	8 (17)
χ^2 p value	$p<0.01$		$p<0.001$	$p<0.001$	$p<0.1$	$p<0.01$	$p<0.5$	$p<0.001$	$p<0.5$	$p<0.3$

* Percentages were calculated in relation to the total number of typable and non typable strains.
† All strains with MRHA type IVa expressed P fimbriae.

1988*a*). In this study, we found CNF production in 38% of bacteraemic strains and 10% of control strains isolated from normal stools ($p<0.01$). The production of CNF has been reported to be closely associated with the production of Hly, the expression of mannose-resistant haemagglutination, and lethality (Bisicchia *et al.*, 1985; Alonso *et al.*, 1987; Caprioli *et al.*, 1987; Blanco *et al.*, 1988*a*). Our results corroborate these associations, since among the 18 CNF-producing strains, 14 (78%) were Hly$^+$, 17 (94%) expressed MRHA and 11 (61%) were lethal for mice. Furthermore, another virulence factor, the production of P fimbriae, was found to be associated with CNF-producing strains. Thus, 11 (61%) of the 18 CNF$^+$ strains expressed P fimbriae, whereas only one (2%) of the 59 CNF$^-$ strains were P fimbriate ($p<0.001$). In our study, 90% of the P-fimbriate bacteraemic strains were isolated from patients with confirmed urinary tract infections or with clinical evidence that a urinary infection was the probable source of the bacteraemia. P fimbriae were previously detected in the majority (90–100%) of *E. coli* strains isolated from pyelonephritis (Väisänen *et al.*, 1981; Domingue *et al.*, 1985).

A less than absolute correlation between the detection of CNF on Vero cells and in rabbit skin tests was observed. This may be due to a lack of sensitivity of the assays employed. However, it is also possible that more than one type of necrotising toxin may exist in bacteraemic *E. coli*. Two types of necrotising toxins, differentiated by means of seroneutralisation assays, have been previously detected in *E. coli* strains isolated from calves with diarrhoea. However, both necrotising toxins showed a similar transformation of Vero cells to that produced by CNF (De Rycke *et al.*, 1987; Blanco *et al.*, 1988*b*).

Previous studies have concluded that the majority of bacteraemic *E. coli* strains belong to a limited number of serogroups—O1, O2, O4, O6, O7, O8, O18 and O75 (Vosti *et al.*, 1964; Ørskov and Ørskov, 1975; Cross *et al.*, 1984; Brauner *et al.*, 1985). These O groups were also frequently detected in *E. coli* strains isolated from diverse extraintestinal infections, but they were rarely found in strains isolated from the stools of healthy people (Ørskov and Ørskov, 1985). The suggestion has been repeatedly made that *E. coli* strains belonging to those serogroups possess specific virulence factors which confer on them their special invasive ability. Our results support this suggestion. Thus, 92% of P-fimbriate strains, 87% of Hly$^+$, 78% of CNF$^+$ and 63% of lethal strains belonged to these serogroups. Virulence factors occurred especially in strains of serogroups O4 and O6, a result which is in agreement with those obtained by other authors (Czirók, 1985; O'Hanley *et al.*, 1985; Blanco *et al.*, 1988*a*).

As in previous studies (Ljungh and Wadström, 1982; González *et al.*, 1988), we have found that haemagglutinating strains are usually hydrophobic. However, the bacteraemic strains aggregating mostly at ammonium sulphate concentrations of

1·0–1·4 M, are less hydrophobic than enterotoxigenic strains with colonisation factor CFA/I (ISAT 0·04 M) or CFA/II (ISAT 0·1–0·4 M).

Minshew *et al.* (1978) showed an association of Col V production with *E. coli* isolates from blood. However, Quackenbush and Falkow (1979) found that the common occurrence of the Col V plasmid in invasive *E. coli* did not imply that Col V had any influence on the virulence of such strains. Our results indicate that production of Col V is neither more frequent in bacteraemic than in control strains, nor is it associated with lethality.

We have previously developed a simple, rapid and economical typing system of *E. coli* strains based on their mannose-resistant haemagglutination pattern that was found to be useful for the presumptive identification of pathogenic strains (Blanco *et al.*, 1985, 1988*a*). Results of this study confirm the utility of our MRHA typing system. Taking into account the results obtained in this study and those previously reported (Blanco *et al.*, 1985, 1988*a*), we now know that MRHA types I and II are expressed only by enterotoxigenic *E. coli* (producing LT or STa, or both, enterotoxins) with colonisation factors CFA/I and CFA/II respectively; MRHA type III appears in CNF-producing strains without P fimbriae; MRHA type IVa is specific for CNF producing P-fimbriate strains; and MRHA types IVb, V and VI are usually expressed by *E. coli* without virulence factors. Interestingly, in this study MRHA type IVb was only found in bacteraemic *E. coli* strains ($p < 0·05$). This merits further study.

In conclusion, we have found that bacteraemic *E. coli* strains show virulence factors similar to those shown by strains responsible for urinary tract infections, such as the production of CNF and Hly and the expression of P fimbriae. We have also confirmed that our modified MRHA typing system is a simple and reliable method for presumptive identification of pathogenic *E. coli*.

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