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# Correlation between electrophoretic types $B_1$ and $B_2$ of carboxylesterase B and sex of patients in *Escherichia coli* urinary tract infections

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SUMMARY

One hundred and sixty-eight strains of *Escherichia coli* isolated from 84 men and 84 women who had urinary tract infections (134 cases) or bacteremia of urinary tract origin (34 cases) were assessed for their carboxylesterase B electrophoretic types $B_1$ and $B_2$, $\alpha$-haemolysin production, the presence of mannose resistant haemagglutinin (MRHA) and antibiotic susceptibility. Electrophoretic type $B_2$ was phenotypically linked with $\alpha$-haemolysin and MRHA productions. The strains isolated from males were more frequently of type $B_2$, haemolytic and both haemolytic and haemagglutinating than those isolated from females. The strains isolated during bacteremia were more frequently haemolytic and haemagglutinating than those obtained from urinary tract infections. Type $B_1$ strains were more frequently resistant to antimicrobial agents than type $B_2$ strains. The results reinforced the distinction, in terms of virulence and antibiotic sensitivity, between $B_1$ and $B_2$ strains and demonstrated the influence of the sex of patients on the host-parasite interaction during urinary tract infections.

## INTRODUCTION

Several recent reports have demonstrated the influence of host factors on the characteristics of bacteria causing urinary tract infections (UTI) (Lomberg *et al.* 1984; Brauner *et al.* 1985; Johnson *et al.* 1988). The anatomical and physiological differences between men and women (Sobel & Kaye, 1986) suggest that the host-parasite interactions during UTI could be different according to the sex of patients.

We have previously established that the *Escherichia coli* strains responsible for extra-intestinal infections may be divided into two groups according to the electrophoretic types of their carboxylesterase B; type $B_2$ (fast moving) and type $B_2$ (slow moving). Type $B_2$ strains are considerably more haemolytic and haemagglutinating and lethal in mice than are type $B_1$ strains (Goullet & Picard,

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1986; Goullet, Picard & Sevali Garcia, 1986). Also, in patients suffering from septicaemia following UTI, women were found to have principally type $B_1$ strains while male patients mostly had type $B_2$ strains (Picard & Goullet, 1988).

This work was carried out to examine the relationships between the sex of patients, the electrophoretic mobilities of carboxylesterase B and the production of α-haemolysin and mannose resistant haemagglutination in *E. coli* strains isolated during UTI and bacteremia arising from the urinary tract in male and female patients.

## PATIENTS AND METHODS

### *Patients*

A total of 84 male and 84 female patients were randomly selected from patients on 14 surgical and medical wards at Beaujon hospital (Clichy, France) between July 1981 and July 1988. One hundred and sixty-eight strains of *E. coli* including 34 strains (17 from males and 17 from females) concomitantly isolated from both blood and urine cultures were examined. All patients had bacterial counts of $> 10^5$ per ml in freshly voided midstream urine samples.

### *Esterase electrophoresis*

The conditions for bacterial growth, the preparation of extracts, horizontal slab polyacrylamide agarose gel electrophoresis, estimation of electrophoretic mobility ($M_F$ value) and esterase staining have been described previously (Goullet, 1973; Goullet & Picard, 1985).

### *Haemolysin assay*

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

### *Mannose-resistant haemagglutinin assay*

Assays 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 $Na_2HPO_4$, 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. The slides were agitated for 1 min and agglutination was read and compared to positive and negative controls.

### *Determination of antibiotic susceptibility*

A disk diffusion method (Bauer *et al.* 1966) in Muller Hinton agar was used to test susceptibility to the following antibiotics: ampicillin 10 μg), ticarcillin (75 μg), chloramphenicol (30 μg), minocycline (30 international units), nalidixic acid (30 μg) and sulfamethoxazole-trimethoprim (23·75 μg and 1·25 μg, respectively). Antibiotic disks were purchased from Institut Pasteur Production.

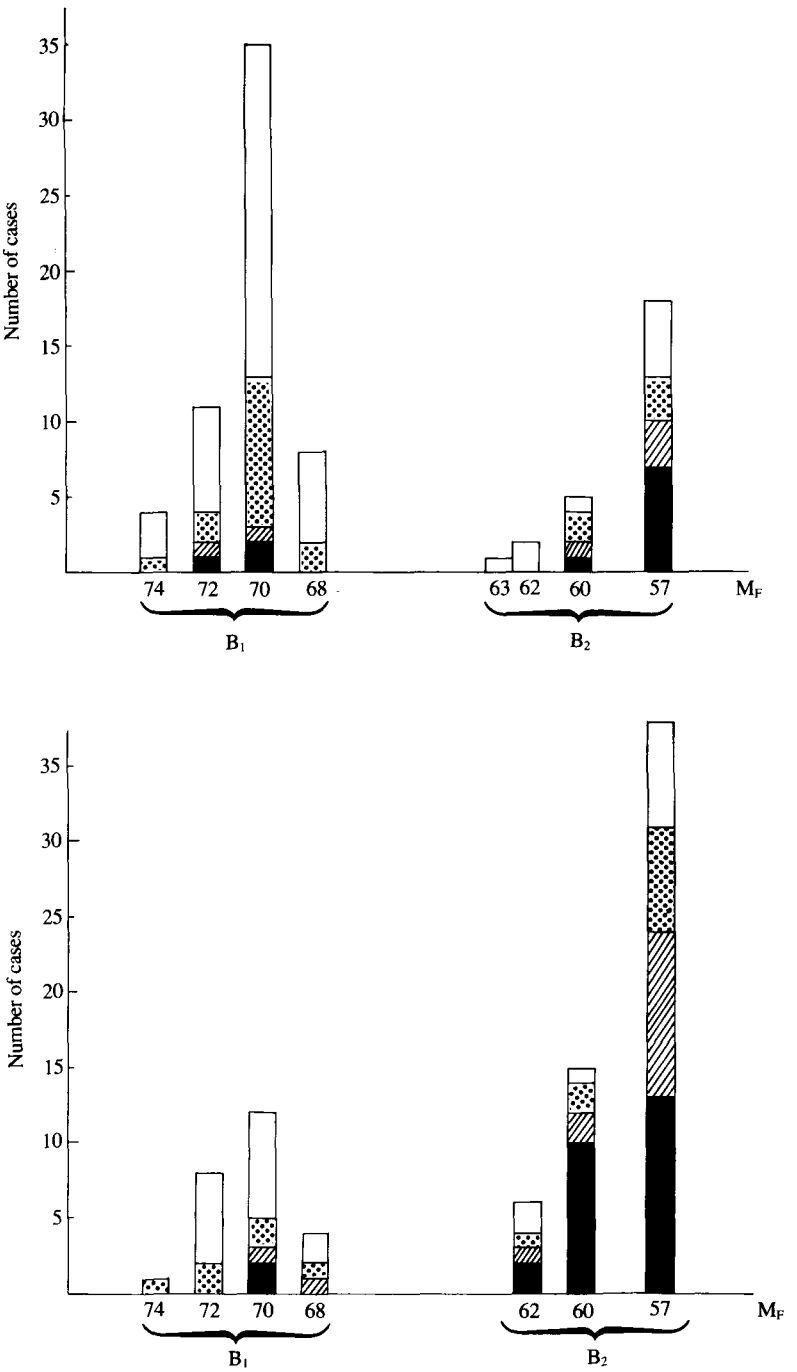


Fig. 1. The electrophoretic mobilities of carboxylesterase B produced by *E. coli* strains isolated from (*a*) females and (*b*) males. The numbers of strains producing α-haemolysin without MRHA (▨), MRHA without α-haemolysin (⊡), and both α-haemolysin and MRHA (■) are indicated. $M_F$: relative mobility.



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Table 1. *The electrophoretic types ($B_1$ and $B_2$) of carboxylesterase B and the productions of α-haemolysin and mannose resistant haemagglutinin in* E. coli *strains isolated from patients with bacteremia and urinary tract infections.*

| | Bacteremia (34 strains) | Urinary tract infections (134 strains) |
|---|---|---|
| $B_1$ type strains | 15 (44%) | 68 (51%) |
| $B_2$ type strains | 19 (56%) | 66 (49%) |
| Haemolytic strains | 19 (56%) | 42 (31%) |
| Haemagglutinating strains | 21 (62%) | 52 (39%) |
| Haemolytic-haemagglutinating strains | 13 (38%) | 26 (19%) |

*Statistical analysis*

The major bacterial variables of the 168 *E. coli* strains (e.g. types $B_1$ or $B_2$ esterases, haemolysin and MRHA productions and antibiotic susceptibility) were compared with patient characteristics (sex, bacteremia, UTI) using the Pearson $\chi^2$ test, plus in some cases, Yale correction.

## RESULTS

*Correlations between electrophoretic types $B_1$ and $B_2$ of carboxylesterase B, the production of α-haemolysin and MRHA and patient sex*

Fig. 1 shows the electrophoretic distribution of carboxylesterase B produced by the 168 strains. Electrophoretic type $B_1$ had mobilities between $M_F \approx 68$ and $M_F \approx 74$ and electrophoretic type $B_2$ from $M_F \approx 57$ to $M_F \approx 63$. Electrophoretic type $B_1$ was the major component of strains isolated from females (69%) (Fig. 1*a*), whereas type $B_2$ predominated in strains isolated from males (70%) (Fig. 1*b*). $\chi^2$ analyses indicated that the strains isolated from males were significantly more of type $B_2$ ($\chi^2 = 25{\cdot}9$) haemolytic ($\chi^2 = 17{\cdot}5$) and both haemolytic and haemagglutinating ($\chi^2 = 8{\cdot}68$) than the strains isolated from females.

Both male and female patients showed similar correlations between the frequency of electrophoretic type $B_2$ and α-haemolysin and MRHA productions. Thus type $B_2$ strains were significantly more haemolytic ($\chi^2 = 15{\cdot}6$), and more haemolytic and haemagglutinating ($\chi^2 = 10{\cdot}3$) than type $B_1$ strains.

*Comparison between strains isolated from UTI with and without bacteremia* (Table 1)

The strains isolated during bacteremia of urinary tract origin were more frequently haemolytic ($\chi^2 = 6{\cdot}93$), haemagglutinating ($\chi^2 = 5{\cdot}76$) and haemolytic and haemagglutinating ($\chi^2 = 5{\cdot}36$) than those isolated from UTI whereas the proportions of $B_1$ and $B_2$ strains were comparable in the two series.

*Resistance to antibiotics in $B_1$ and $B_2$ strains*

For each antibiotic, type $B_1$ strains were significantly more frequently resistant than were type $B_2$ strains (Table 2). The difference was most marked for sulfamethoxazole/trimethoprim and chloramphenicol. On the other hand, type $B_1$ strains more frequently exhibited a resistance to multiple antimicrobial agents

Table 2. *The antibiotic resistance of type $B_1$ and type $B_2$ strains of* E. coli.

| | AMP | TIC | CHL | MIN | NAL | SMX/TMP | S | R ⩾ 4 |
|---|---|---|---|---|---|---|---|---|
| $B_1$ strains (83) | 40 (48%) | 36 (43%) | 31 (37%) | 23 (28%) | 5 (6%) | 31 (37%) | 34 (41%) | 27 (32·5%) |
| $B_2$ strains (85) | 24 (28%) | 22 (26%) | 15 (17%) | 16 (19%) | 3 (3%) | 12 (14%) | 51 (60%) | 11 (13%) |

AMP, ampicillin; TIC, ticarcillin; CHL, chloramphenicol; MIN, minocyclin; NAL, nalidixic acid; SMX/TMP, sulfamethoxazole/trimethoprim.

S, sensitive to all antibiotics; R ⩾ 4, multiply-resistant (resistance ⩾ 4 antimicrobial agents).

Table 3. *Correlation between α-haemolysin and MRHA productions and antibiotic resistance in the type $B_1$ and type $B_2$ strains.*

| | $B_1$ strains | | $B_2$ strains | |
|---|---|---|---|---|
| | R ⩾ 1 (49 strains) | S (34 strains) | R ⩾ 1 (34 strains) | S (51 strains) |
| Haemolytic strains | 3 (6%) | 6 (17·5%) | 13 (38%) | 38 (74·5%) |
| Haemagglutinating strains | 12 (24·5%) | 14 (41%) | 17 (50%) | 32 (62·7%) |
| Haemolytic-haemagglutinating strains | 1 (2%) | 4 (12%) | 10 (29·5%) | 24 (47%) |

R ⩾ 1, strains resistant to at least one antibiotic; S, strains sensitive to all antibiotics.

(resistance ⩾ 4 antimicrobial agents) than type $B_2$ strains (32·5% and 13%, respectively).

In $B_2$ strains, antibiotic sensitivity appeared to be correlated with α-haemolysin production. Thus 38 strains (74%) sensitive to all the antibiotic tested were haemolytic and only 13 strains (38%) resistant to at least one antibiotic were haemolytic. This correlation was less marked for MRHA production, which was detected in 32 (62%) sensitive strains and 17 (50%) resistant strains (Table 3).

## DISCUSSION

Most of the studies published to date show that virulence factors are less frequently present in *E. coli* strains causing UTI in patients with abnormalities of the urinary tract or with serious medical illness, than in strains infecting normal hosts (Lomberg *et al.* 1984, Brauner *et al.* 1985; Johnson *et al.* 1987; Sandberg *et al.* 1988; Johnson *et al.* 1988). In urosepsis, Johnson *et al.* (1988). have differentiated between antibiotic-sensitive *E. coli* strains producing chromosomal virulence factors and infecting normal subjects, and antibiotic-resistant strains lacking these factors and more frequently isolated from immunodepressed subjects. We recently described a group of highly virulent *E. coli* strains characterized by slow mobilities (type $B_2$) of carboxylesterase B which were more frequent in human extra-intestinal infections than in the stools of healthy subjects, (Goullet & Picard, 1986). Moreover in septicaemia we have demonstrated that type $B_2$ strains were more frequently isolated from subjects without

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underlying disease, whereas type $B_1$ strains were more frequently isolated from immunodepressed subjects (Picard & Goullet, 1988). A recent investigation has established that type $B_2$ stains corresponded to the B2 group characterized by Selander *et al.* (1987) on the basis of the electrophoretic polymorphism of 35 enzymes and thus constituted a taxonomically distinct cluster within *E. coli* (Goullet & Picard, 1989). The present study reinforce the correlation between the type $B_2$ strains and the production of haemolysin and/or haemagglutinin previously demonstrated (Goullet & Picard, 1986), and shows that male patients were more frequently infected with haemolytic type $B_2$ strains than were females (Fig. 1). There was no significant correlation between the sex of patient and MRHA. This virulence factor was closely linked to the gravity of the urinary tract infection (Lomberg *et al.* 1984). In the present study the proportion of haemagglutinating strains was lower in non-septicemic urinary infections than in septicemia of urinary origin (Table 1). With these latter infections, the proportions of haemagglutinating strains were similar to those reported by Johnson *et al.* (1987).

The usual antibiotic sensitivity of type $B_2$ strains concords with the greater sensitivity of strains producing chromosomal virulence factors reported by Johnson *et al.* (1987). The higher level of antibiotic resistance exhibited by type $B_1$ strains could be explained by selection pressure on the gut flora of patients.

Our findings provide further evidence for the differing virulences of type $B_1$ and $B_2$ strains and demonstrate the influence of patient sex on the host-parasite interaction during urinary tract infections.

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