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# *traT* Gene Sequences, Serum Resistance and Pathogenicity-related Factors in Clinical Isolates of *Escherichia coli* and Other Gram-negative Bacteria

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The R6-5 plasmid-specified outer membrane protein, TraT protein, has previously been shown to mediate resistance to bacterial killing by serum. Colony hybridization with a 700 bp DNA fragment carrying most of the *traT* gene was used to examine the prevalence of *traT* in Gram-negative bacteria, particularly strains of *Escherichia coli*, isolated from clinical specimens. *traT* was found in isolates of *E. coli*, *Salmonella*, *Shigella* and *Klebsiella*, but not in *Pseudomonas*, *Aeromonas* or *Plesiomonas*, nor in the few isolates of *Enterobacter*, *Proteus*, *Acinetobacter*, *Citrobacter*, *Serratia* or *Yersinia* that were examined. It was detected in a significantly higher proportion of the *E. coli* strains isolated from the blood of patients with bacteraemia/septicaemia or from faeces of patients with enteric infections (50–70%) than in that of strains isolated from normal faeces (20–40%). The incidence of *traT* in strains isolated from cases of urinary tract infections was variable. *traT* was found to be frequently associated with production of the K1 capsule and with the carriage of ColV plasmids, but not with the carriage of R plasmids, nor with serum resistance or the production of haemolysin.

## INTRODUCTION

It is well established that normal animal serum is bactericidal for most types of bacteria (for recent review see Taylor, 1983). This bactericidal activity, the principal component of which is complement, is generally considered to be an important host defence against bacterial infections. As a consequence, resistance to serum killing is regarded as an important virulence property of invasive bacteria. Consistent with this notion is the fact that *Escherichia coli* strains isolated from the blood of patients with bacteraemia/septicaemia are more likely to be resistant to serum than are those isolated from the faeces of healthy individuals (Roantree & Rantz, 1960; Vosti & Randall, 1970). Moreover, serum resistant *E. coli* strains are more often associated with shock and death in bacteraemic patients than are serum sensitive strains (McCabe *et al.*, 1978).

Several distinct components of the bacterial cell surface have been implicated in serum resistance (for recent reviews, see Taylor, 1983; Timmis *et al.*, 1985), including the O-side-chains of certain types of lipopolysaccharide (LPS) (Taylor, 1976; Moll *et al.*, 1980; Joiner *et al.*, 1982; Goldman *et al.*, 1984), capsular antigens (Gemski *et al.*, 1980; Timmis *et al.*, 1981; Agüero & Cabello, 1983), and surface proteins (Hildebrandt *et al.*, 1978; Moll *et al.*, 1980; Munn *et al.*, 1982). In some instances, resistance does not result from a single attribute but rather from the combined effects of two or more cell components.

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*Abbreviation*: UTI, urinary tract infection.

The genetic determinants of some serum resistance factors of *E. coli* have been shown to be located on conjugative plasmids: the *iss* gene is carried by some ColV plasmids (Williams Smith, 1974; Binns *et al.*, 1979) whereas the *traT* gene, which encodes a major outer membrane protein, is carried by a variety of IncF plasmids (Timmis *et al.*, 1979; Moll *et al.*, 1980). These determinants have been cloned and subjected to genetic and functional analyses (Timmis *et al.*, 1979; Moll *et al.*, 1980; Timmis *et al.*, 1981; Ogata *et al.*, 1982; C. Parada, M. E. Fernandez, M. M. Binns & F. C. Cabello, unpublished).

The *traT* gene product is a major outer membrane protein having an apparent molecular weight of 25000 (Achtman *et al.*, 1978; Moll *et al.*, 1980), and is present in natural isolates of *E. coli* at 3000–10000 copies per cell (Agüero *et al.*, 1984; Bitter-Suermann *et al.*, 1984). It probably exists in the outer membrane as a lipoprotein (Perumal & Minkley, 1984) associated non-covalently with peptidoglycan (Manning *et al.*, 1980), and appears to be highly exposed on the cell surface, at least in cells of *E. coli* K12 (Timmis *et al.*, 1981). In addition to its ability to confer upon host bacteria resistance to killing by complement, TraT protein in combination with the product of an adjacent gene, *traS*, mediates the property of surface exclusion (Achtman *et al.*, 1977), the reduced ability of a strain carrying a conjugative plasmid to act as a recipient when mated with a donor strain carrying a related plasmid.

The mechanism by which TraT protein mediates resistance to complement has not yet been elucidated, although the possibility that it acts by inactivation of one or more complement components seems to have been ruled out (Timmis *et al.*, 1981). Studies on the binding of complement components to isogenic *traT*$^+$ and *traT* cells have failed to detect any significant differences in the amounts of individual components bound when bacteria are suspended in serum. Thus, the serum resistance property of *traT*$^+$ cells would seem not to result from a TraT protein-mediated reduction in the binding of complement, but rather by an interference with the correct assembly or functioning of the complement membrane attack complex. The finding that the presence of TraT protein in bacteria suspended in serum results in an altered distribution of C3b bound to the cell surface (Agüero *et al.*, 1984) would suggest that TraT protein may modify sites on the cell surface that are sensitive to membrane attack complex.

It should be noted that TraT protein also reduces the susceptibility of bacteria to phagocytosis by peritoneal macrophages (Agüero *et al.*, 1984) and increases to a limited but significant extent the pathogenicity of an *E. coli* strain for a mouse infection model (L. DeLuca & F. C. Cabello, unpublished observations).

A crucial aspect of any potential pathogenesis factor is its prevalence among pathogenic and non-pathogenic microbes. In this regard, the availability of cloned genes of such factors facilitates an analysis of their epidemiology in clinical isolates. In this communication, we report colony hybridization studies on the prevalence of *traT*-related sequences in strains of *E. coli* and other Gram-negative bacteria isolated from cases of septicaemia (sepsis), urinary tract infection (UTI) and enteric disease, and from faeces of healthy individuals. Furthermore, we have investigated the association of *traT* with several other putative pathogenesis factors of *E. coli*.

## METHODS

*Bacterial strains.* Bacterial isolates from patients or healthy individuals were obtained from the diagnostic laboratories of the Universities of: Mainz, FRG; Berlin, FRG; Geneva, Switzerland; Banaras, India; New York, USA; and Boston, USA.

*Plasmids.* The multiple antibiotic resistance plasmid R6-5 has been described previously (Timmis *et al.*, 1978). The pACYC184 hybrid plasmid pKT107 (Timmis *et al.*, 1979; Moll *et al.*, 1980), which carries the R6-5 *traT* gene, was used as the source of the *traT* gene probe. Plasmids belonging to the different incompatibility groups listed in Table 1 were obtained from N. Datta (Hammersmith Hospital, London, UK). Plasmid DNA was purified and subjected to fragmentation with restriction enzymes, and the fragments thereby generated were analysed by electrophoresis on 0·7% or 0·8% agarose gels in Tris/borate buffer, as described previously (Timmis *et al.*, 1978; Andres *et al.*, 1979). Plasmid DNA from natural isolates was isolated by the method of Kado & Lui (1981).

*Enzymes.* Restriction enzymes *Bst*EII, *Eco*RI and *Hin*dIII were purchased from New England Biolabs and used according to the conditions recommended by the supplier. DNA polymerase I and DNAase I used in the nick translation reaction were obtained from Boehringer-Mannheim.

Table 1. *Occurrence of sequences homologous to the traT gene in plasmids belonging to different incompatibility groups*

| Plasmid | Incompatibility group | *traT* | Plasmid | Incompatibility group | *traT* |
|---|---|---|---|---|---|
| RA1 | A | − | R144, R144-3, R64*drd*-11 | Iα | − |
| R16 | B − O | − | R621a | Iγ | − |
| R40a | C | − | R471a | L | − |
| R386 | FI | + | N3 | N | − |
| R1, R6-5, R100, R538-1 | FII | + | R478 | S | − |
| R124 | FIV | + | S-a | W | − |
| R27 | H1 | − | R6K | X | − |

*DNA probe.* The hybrid plasmid pKT107 consists of a 6 kb *Eco*RI fragment of the multi-resistant plasmid R6-5 linked to vector plasmid pACYC184 (Timmis *et al.*, 1979; Moll *et al.*, 1980). Digestion of this plasmid with *Bst*EII endonuclease generates two fragments, a large one and a 700 bp fragment which contains about 90% of the *traT* gene (Moll *et al.*, 1980; Ogata *et al.*, 1982). This latter fragment is the *traT*-specific probe used in all of the experiments described below. It was isolated by electroelution (Dretzten *et al.*, 1981) from 0·8% agarose gels, following electrophoresis of *Bst*EII-cleaved pKT107 DNA, and was labelled with [α-$^{32}$P]dCTP (Amersham) by nick translation (Rigby *et al.*, 1977).

*Hybridization experiments.* Colony hybridization was done essentially as described by Grunstein & Hogness (1975) and Moseley *et al.* (1980). The hybridization conditions were 50% (v/v) formamide/5 × SSC at 37 °C for 18 h (1 × SSC is 0·15 M-NaCl, 0·015 M-trisodium citrate, pH 7·0). Filters were washed in 5 × SSC at 65 °C, then in 2 × SSC at room temperature, and were then exposed to X-ray films.

Southern blotting was done essentially as described by Southern (1975). The hybridization conditions were 2 × SSC at 65 °C for 18 h. Prior to autoradiography, the filters were washed successively in 2 × SSC, 1 × SSC, and 0·2 × SSC, each solution at 65 °C and containing 0·1% SDS.

*Serum resistance, and production of colicin V and haemolysin.* The procedure followed for determination of bacterial resistance to killing by pooled rabbit serum was the viable count method described in Moll *et al.* (1979), except that equal volumes of serum and bacterial suspension were mixed to give a final serum concentration of 50% (v/v), and that the period of incubation of bacteria in serum was 120 min. Detection of the production of colicin V and haemolysin was as previously described (Agüero *et al.*, 1983).

## RESULTS

### *Specificity of the traT DNA probe and prevalence of the traT gene on plasmids of different incompatibility types*

The specificity of the DNA probe was investigated by Southern blot analysis of restriction endonuclease-cleaved and fractionated DNAs of plasmids belonging to different incompatibility groups. Figure 1 shows an autoradiograph of a blot of several different plasmids probed with the 700 bp fragment of pKT107. The probe hybridized to large *Hin*dIII fragments of plasmids R386 (IncFI) and R124 (IncFIV), in addition to the expected 6 kb *Eco*RI fragment of plasmid R6-5 (IncFII) and the small *Bst*EII fragment of pKT107. In contrast, no hybridization was observed to any *Hin*dIII fragment of plasmid R6K (IncX). Similar analysis of plasmids belonging to other incompatibility groups showed that none of them contain sequences homologous to the *traT* gene (Table 1). These experiments indicate that the probe DNA is highly specific and that the *traT* gene is carried only by the IncF plasmids. The *traT* gene was initially reported as being part of the transfer operon of the F plasmid (IncFI) (Achtman *et al.*, 1977) but has since also been found in other IncF plasmids (Achtman, 1978; Ogata *et al.*, 1982).

### *Prevalence of traT gene in non-E. coli Gram-negative clinical isolates*

About 300 non-*E. coli* Gram-negative clinical isolates were analysed by colony hybridization for carriage of the *traT* gene. Homology to the DNA probe was found in isolates of *Klebsiella* (22%), *Shigella* (35%) and *Salmonella*, particularly *Salmonella typhimurium* (95%), but not in isolates of *Citrobacter*, *Serratia*, *Enterobacter*, *Proteus*, *Acinetobacter*, *Pseudomonas*, *Aeromonas*, *Plesiomonas* or *Yersinia* (Table 2). The limited spectrum of bacteria carrying *traT* is consistent

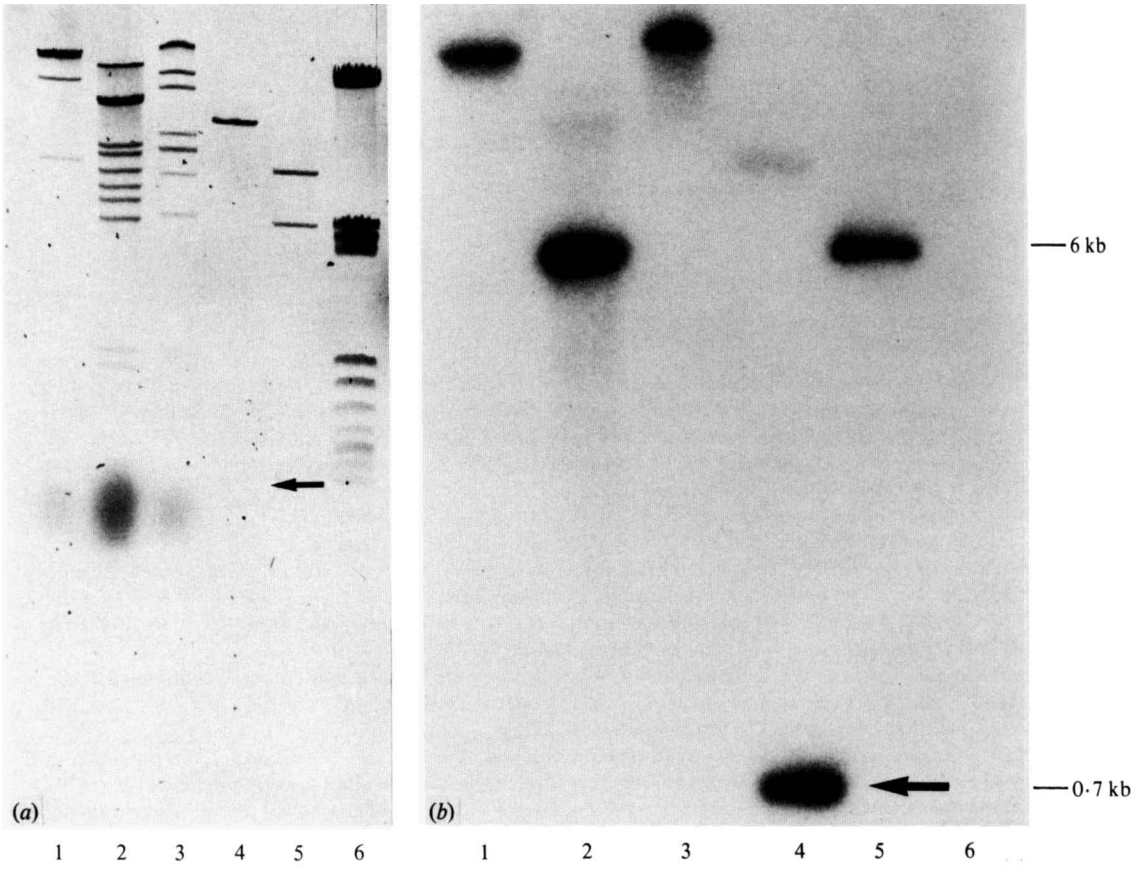


Fig. 1. The *traT* gene probe is specific for IncF plasmids. The plasmids listed below were digested with the indicated endonuclease and the fragments thereby generated were separated by electrophoresis through an agarose gel (*a*) and probed with the 700 bp *Bst*EII fragment of pKT107 (autoradiograph of nitrocellulose filter shown in *b*) as described in Methods. 1, R386/*Hin*dIII; 2, R6-5/*Eco*RI; 3, R124/*Hin*dIII; 4, pKT107/*Bst*EII; 5, pKT107/*Eco*RI; 6, R6K/*Hin*dIII. The arrows indicate the position of the 700 bp *Bst*EII fragment of pKT107.

Table 2. *Distribution of traT-related sequences among Gram-negative clinical isolates*

| Genus | *traT*⁺ strains/ total strains (percentage) | Genus | *traT*⁺ strains/ total strains (percentage) |
|---|---|---|---|
| *Shigella* | 8/23* (35) | *Pseudomonas* | 0/31 (<3) |
| *Salmonella* | 20/63† (32) | *Aeromonas* | 0/66 (<2) |
| *Klebsiella* | 15/69‡ (22) | *Plesiomonas* | 0/33 (<3) |
| *Enterobacter* | 0/12 (<8) | *Citrobacter* | 0/7 (<14) |
| *Proteus* | 0/5 (<20) | *Serratia* | 0/4 (<25) |
| *Acinetobacter* | 0/3 (<33) | *Yersinia* | 0/10 (<10) |

* 2/2 *dysenteriae* 1, 0/2 *dysenteriae* (other serotypes), 2/2 *schmitz*, 1/2 *sonnei*, 1/6 *boydii*, 0/2 *flexneri*, 2/7 undefined species.

† 19/20 *typhimurium*, 1/2 *dublin*, 0/1 *hato*, 0/1 *derby*, 0/1 *essen*, 0/1 *agona*, 0/3 *infantis*, 0/2 subgenus I, 0/1 *othmarshen*, 0/2 *enteridids*, 0/29 undefined species.

‡ 13/58 *pneumoniae*, 1/6 *oxytoca*, 1/5 undefined.

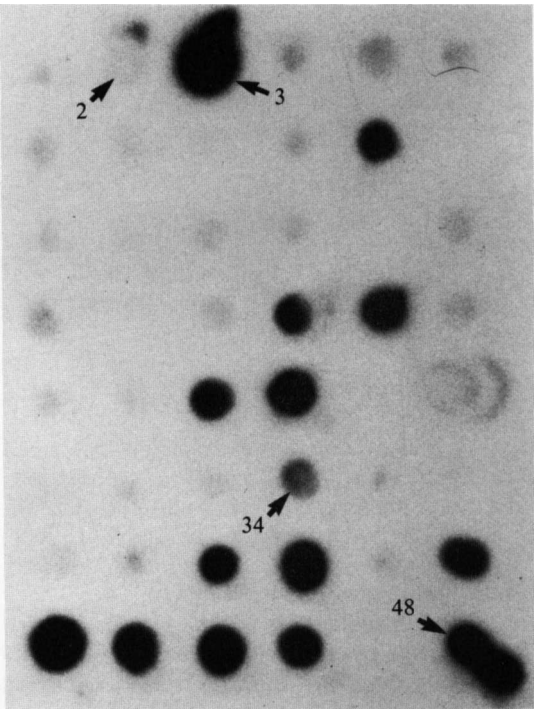


Fig. 2. Colony hybridization of isolates of *E. coli* with the *traT* gene probe. Control colonies are *E. coli* strains: C600 (2), C600(R6-5) (3) and C600(pKT107) (48) (Timmis *et al.*, 1978; Moll *et al.*, 1980). All dark spots on the autoradiogram were considered to be positive for the probe, whereas the lighter spot (34) was considered to be a weak but specific positive reaction.

with the fact that IncF plasmids exhibit narrow host range specificities, and that their propagation is therefore limited to some members of the family *Enterobacteriaceae*.

*Distribution of traT related sequences in isolates of E. coli*

Colonies of 680 *E. coli* strains from different clinical specimens and different geographical origins were analysed by hybridization with the $^{32}$P-labelled *traT* gene probe; for comparison 113 strains isolated from stools of healthy individuals were also analysed. Each group of strains was analysed on at least two separate occasions. An autoradiograph of one filter after hybridization is shown in Fig. 2. Among the colonies giving a positive reaction, signals of different strength could be distinguished. These differences could be due to different colony sizes, different cellular copy numbers of the *traT* gene in different strains, or different degrees of homology to the probe.

Table 3 summarizes the results of the analysis. The frequency of *E. coli* isolates that hybridized to the *traT* probe was markedly higher if they originated from blood cultures (sepsis; 50–67%) than if they were obtained from faeces of healthy individuals (23–38%). Of 119 *E. coli* strains isolated from blood cultures in Mainz, 54% hybridized with the *traT* probe, whereas only 38% of a comparably sized group of strains from faeces of healthy adults were positive. This observation is in good agreement with data obtained with an anti-TraT protein monoclonal antibody (Bitter-Suermann *et al.*, 1984) that was used to screen, by immunoblotting of electrophoretically fractionated total cell proteins, the same population of strains; 56% of sepsis strains and 38% of faecal strains reacted with the antibody. A direct comparison of the results of the two methods with this population of strains revealed an almost complete correlation, i.e. strains that reacted with the anti-TraT protein antibody also hybridized to the *traT* probe, and vice versa. Thus, *traT* genes identified by colony hybridization in natural isolates are expressed.

Table 3. *Frequency of traT-related sequences in clinical isolates of E. coli from diverse geographical origins*

Values are percentages with the number of strains examined given in parentheses. Isolates were taken from the blood of bacteraemia/septicaemia patients, the urine of UTI patients, the faeces of donors with enteric infections (Stool-e) and the faeces of healthy donors (Stool-n).

| Origin | Blood | Urine | Stool-e | Stool-n |
|---|---|---|---|---|
| Mainz | 54 (119) | — | 62 (24) | 38 (100) |
| Berlin | 54 (33) | 49 (65) | — | 23 (13) |
| Geneva | 50 (52) | 33 (60) | — | — |
| Freiburg | — | 58 (48) | — | — |
| Banaras | — | — | 53 (253) | — |
| New York | 60 (10) | 28 (7) | — | — |
| Boston | 67 (9) | — | — | — |
| Mean values | 54 (223) | 46 (180) | 54 (277) | 36 (113) |

A similar frequency of occurrence of the *traT* gene was found in smaller samples of isolates from cases of sepsis (54%) and from normal faecal strains (23%) collected in Berlin. Although in the case of sepsis strains isolated in Geneva, New York and Boston no direct comparisons with control groups of normal faecal strains of the same geographical origins were possible, the frequencies of *traT* positives in each group (50%, 60% and 67%, respectively) were comparable to those of sepsis strains from Mainz and Berlin (Table 3).

A large geographical variation was observed in the frequencies of carriage of *traT* (from 28% to 58%) by *E. coli* strains isolated from cases of UTI. Although this variation could be due to the existence of different populations of pathogens in the different geographical regions sampled, it is more likely to be due to variation in the frequencies of the type of strain analysed. All strains were obtained from positive urine cultures, but no information as to the type of infection caused was available. We might anticipate that invasive strains causing pyelonephritis would exhibit additional properties over and above those of strains causing lower urinary tract infections. Thus, clarification of the variation observed will require differentiation of the two types of strain which, in turn, will necessitate evaluation of clinical data of patients.

Of the *E. coli* strains isolated from cases of infantile diarrhoea in Mainz, 62% hybridized to the *traT* probe, a frequency comparable to that found for sepsis strains. A slightly lower, but still high, proportion of strains (53%) isolated from patients with diarrhoea in Banaras were also *traT* positive (Table 3).

### *The traT gene in clinical isolates is generally plasmid-borne*

Plasmid DNA was prepared by the procedure of Kado & Lui (1981) from 43 isolates, mostly strains of *E. coli*, from different sources, and was analysed by Southern blotting. All of the 22 *E. coli* isolates and four *S. typhimurium*, two *Klebsiella* and two *Shigella* isolates shown by colony hybridization to be *traT* positive contained large plasmids, and all of these plasmids hybridized to the probe (Fig. 3). Thus, at least in *E. coli* and probably also in *S. typhimurium*, *Klebsiella* and *Shigella traT* would seem to be exclusively a plasmid-borne gene. Of the remaining 20 *traT*-negative isolates, only eight contained large plasmids and of course none of these hybridized to the probe.

### *Lack of correlation of serum resistance and carriage of traT*

The ability of a selection of sepsis, UTI, and healthy stool isolates of *E. coli* to survive exposure to 50% normal rabbit serum was examined, and the relationship of the serum resistance property and carriage of the *traT* gene was analysed (Table 4). Serum resistance was found to occur at approximately the same frequencies as the carriage of the *traT* gene in these three groups of isolates, i.e. in 59% of sepsis strains, 40% of UTI strains, and 42% of isolates from normal stools. However, except for the relatively small population of UTI strains (80% of the serum resistant isolates were *traT*$^+$), there was not a high degree of correlation between the serum resistance property and production of TraT protein; only 58% of serum resistant sepsis strains were concomitantly *traT*$^+$.

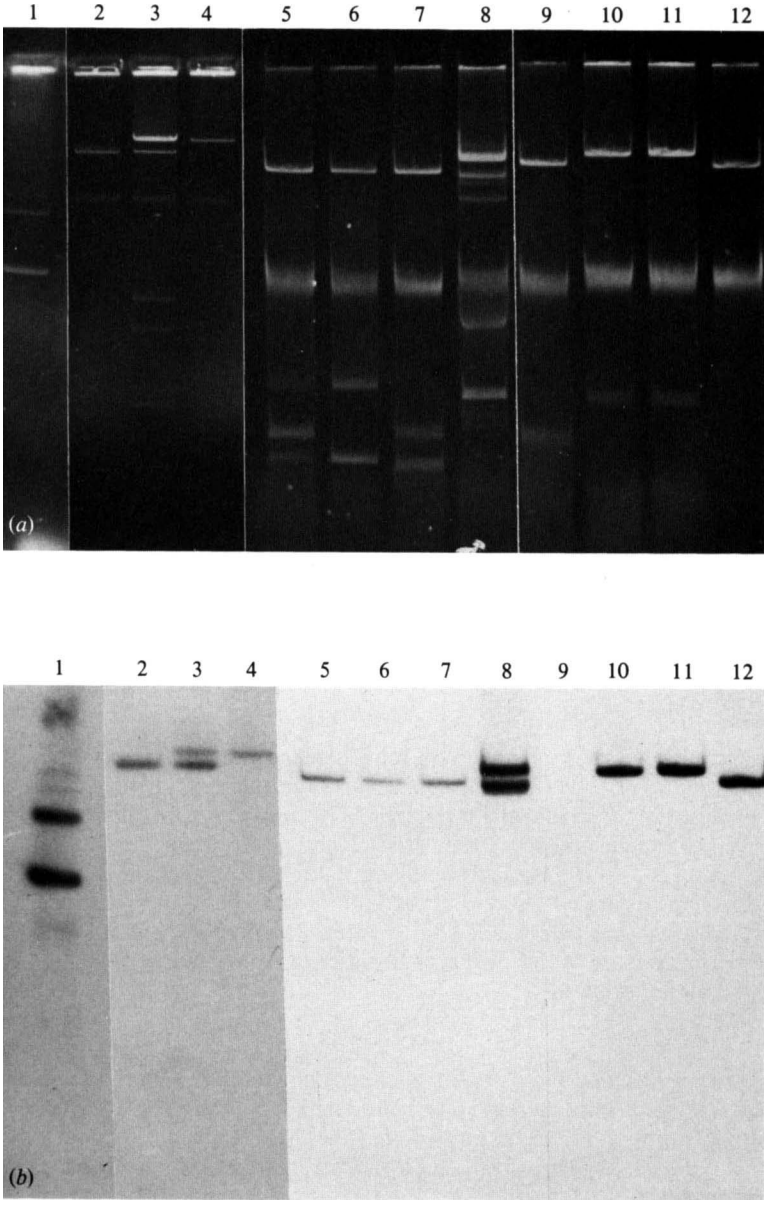


Fig. 3. Southern blot analysis of plasmid preparations from clinical isolates of *E. coli*, *Salmonella typhimurium*, *Klebsiella* and *Shigella*. Plasmid DNA was prepared from the strains listed below, subjected to electrophoresis on a 0·7% agarose gel (*a*) and blotted and hybridized to the *traT* gene probe (*b*), as described in Methods. All isolates were *traT* positive, as determined in the colony hybridization experiments described in the text. 1, *E. coli* C600 (pKT107) (control); 2, *E. coli* B2946-I (sepsis); 3, *E. coli* B2946-II (sepsis); 4, *E. coli* B3011 (sepsis); 5, *S. typhimurium* 4389/82 (sepsis); 6, *S. typhimurium* 5019/82 (sepsis); 7, *S. typhimurium* 3977/83 (diarrhoea); 8, *Klebsiella* 15 (diarrhoea); 9, *Klebsiella* 39 (diarrhoea); 10, *Shigella* 82 (diarrhoea); 11, *Shigella* 83 (diarrhoea); 12, *E. coli* C600 (R6-5) (control; Moll *et al.*, 1980). Hybridization to the *Klebsiella* 39 plasmid (lane 9) was weak, though reproducible, and detectable only on the original autoradiograms but not on prints made from them.

Table 4. *Frequency of resistance to serum and of traT-related sequences in clinical isolates of E. coli*

Isolates were taken from the blood of bacteraemia/septicaemia patients at Mainz and New York, the urine of UTI patients at New York and Freiburg and from faeces of healthy donors in Mainz.

| Source of isolate | No. of strains | Percentage serum resistant | Percentage of total serum resistant and *traT*$^+$ |
|---|---|---|---|
| Blood | 58 | 59 | 34 |
| Urine | 25 | 40 | 32 |
| Stools | 50 | 42 | 16 |

Table 5. *Frequency of pathogenicity-related characters in 139 E. coli isolates from cases of sepsis*

| Character* | No. of isolates (percentage) | No. of *traT*$^+$ isolates (percentage†) |
|---|---|---|
| ColV plasmid | 27 (19·4) | 21 (77·8) |
| R plasmid | 133 (95·7) | 69 (51·9) |
| K1 antigen | 20 (14·4) | 15 (75) |
| Haemolysin | 28 (20·1) | 7 (25) |

* Presence of ColV plasmids was detected as production of colicin V, and of R plasmids as the presence of a transferable antibiotic resistance plasmid.

† Percentage of the isolates positive for the indicated character that also hybridized to the *traT* gene probe.

*Association of traT, ColV and K1 antigen in E. coli strains isolated from cases of sepsis*

Possible associations of the *traT* gene with K1 capsular antigen, haemolysin, and ColV and R plasmids in 139 isolates of *E. coli* from cases of sepsis were analysed (Table 5); 96% of the strains carried R plasmids, 19% carried ColV plasmids, 20% produced haemolysin and 14% produced the K1 capsule. Although no association of *traT* and haemolysin production or R plasmid carriage was found (25% of the haemolysin positive strains and 52% of the R plasmid positive strains were *traT*$^+$), a high correlation between, on the one hand, *traT* and ColV (78% of the ColV positive isolates were *traT*$^+$) and, on the other, *traT* and K1 production (75% of K1$^+$ isolates were *traT*$^+$) was observed (see also Bitter-Suermann *et al.*, 1984).

## DISCUSSION

The IncF plasmid-specified TraT protein has been shown to confer upon selected strains of *E. coli* three properties that could increase their pathogenic potential, namely increased resistance to serum killing (Timmis *et al.*, 1979; Moll *et al.*, 1980), increased resistance to phagocytosis by peritoneal macrophages (Agüero *et al.*, 1984), and increased virulence for a mouse infection model (L. DeLuca & F. C. Cabello, unpublished observations). This protein is therefore a putative virulence factor of *traT*$^+$ strains of bacteria. In this study, by means of colony hybridization with a 700 bp fragment containing most of the *traT* gene of plasmid R6-5, we have analysed the prevalence of the *traT* gene in a variety of Gram-negative bacteria, particularly *E. coli*, isolated from clinical specimens.

The *traT* gene, which was shown to be carried exclusively on IncF plasmids, was found in 23–67% of *E. coli* isolates, in 95% of *Salmonella typhimurium* isolates, and in 35% of *Shigella* and 22% of *Klebsiella* isolates, but not in several other types of Gram-negative bacteria, including most other salmonellae. This is consistent with the known narrow host range property of IncF plasmids. Interestingly, studies with an anti-TraT protein monoclonal antibody (Bitter-Suermann *et al.*, 1984; Jürs *et al.*, 1985) revealed not only the presence of TraT protein in nearly all isolates of *E. coli* and *S. typhimurium* that were shown by colony hybridization to carry *traT*,

but also the presence of anti-TraT protein antibody-reacting proteins in 91% of isolates of *Enterobacter cloacae* and in 31% of isolates of non-*typhimurium* serotypes of *Salmonella*, neither group of which showed homology to the *traT* gene probe. The TraT-related proteins of these two latter groups of bacteria have, however, higher molecular weights than that of the TraT protein itself and may, except for the antibody-reacting epitope, be structurally distinct.

Within *E. coli* isolates, 54% of those from cases of bacteraemia/septicaemia, 54% of those from cases of enteric disease, and 36% of those from faeces of healthy individuals were found to carry *traT*. Thus, although *traT* is found in a relatively high proportion of isolates not associated with human disease, it is present in a markedly higher proportion of pathogenic strains. Carriage of the *traT* gene is thus correlated with pathogenicity of *E. coli*.

The K1 capsular antigen and ColV plasmids have been demonstrated in several studies to contribute significantly to the pathogenic potential of *E. coli* strains (e.g. see discussion in Timmis *et al.*, 1985). It is interesting to note that possession of the *traT* gene is highly associated with both of these characters. Although its association with ColV plasmids, which belong to incompatibility group IncF, is readily understandable, the basis of its association with the K1 antigen is not so apparent. Perhaps this association indicates an involvement of the TraT protein or some other product of IncF plasmids in the virulence of K1 strains. In any case, it would seem that the *traT* gene may serve as a useful marker for some aspect of virulence that is characteristic of the sub-population of pathogenic strains that have the K1 and ColV properties.

Surprisingly, there was not a high degree of correlation of *traT* and serum resistance; although 59% of *E. coli* isolates from cases of sepsis were serum resistant, only 34% were concomitantly *traT*$^+$ and serum resistant (i.e. 58% of serum resistant isolates were *traT*$^+$). At present we do not know if an association exists between *traT* gene carriage and resistance to phagocytosis, the second virulence-associated property of TraT protein, and this aspect is currently under investigation.

In conclusion, if TraT protein does play a role in the pathogenesis of some invasive pathogens, another cell surface component must fulfil an analogous function in others. Moreover, if the role of TraT protein in pathogenesis is to mediate resistance to complement, it obviously does not manifest this property in certain isolates, namely those serum sensitive strains that are *traT*$^+$. In order to determine conclusively whether or not the TraT protein plays a significant role in the pathogenesis of *traT*$^+$ invasive strains of *E. coli*, it will be necessary to generate isogenic pairs of such strains differing only in the TraT character, and to compare their virulence in appropriate animal infection models. Suitable deletion mutant derivatives of *traT*-carrying plasmids were recently generated (D. O'Connor, unpublished) and are currently being used to this end.

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