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CONTRIBUTION OF THE *traT* GENE TO SERUM RESISTANCE AMONG CLINICAL ISOLATES OF ENTEROBACTERIACEAE

USHA KANUKOLLU, SANDRA BIELER, SHEILA HULL AND R. HULL*

Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030, USA

SUMMARY. Antimicrobial resistance plasmids containing the *traT* gene confer resistance to serum bactericidal activity upon some laboratory strains of *Escherichia coli*. We have examined the DNA of Enterobacteriaceae from human extraintestinal infections to determine the frequency of *traT*-like genes. DNA sequences homologous with *traT* were found among 58% of Escherichieae but among none of the Klebsielleae or Proteeae tested and were found as frequently among serum-sensitive *E. coli* as among serum-resistant strains. Sequences related to *traT* were always associated with large plasmids. The potential contribution of *traT*-containing plasmids to serum resistance of clinical isolates is discussed.

INTRODUCTION

The sensitivity of bacteria to human serum was first observed more than 90 years ago by Nuttall (1888) and implicated as a factor in determining bacterial virulence by Roantree and Rantz (1960). Resistance to these bactericidal effects has since been shown to be correlated with virulence of gram-negative bacteria in various infections, including burn infections (Lowbury and Ricketts, 1957), urinary tract infections (Vosti and Randall, 1970; Gower *et al.*, 1972; Olling, 1977; Bjorksten and Kaijser, 1978; Hughes, Phillips and Roberts, 1982), endocarditis (Archer and Fekety, 1976; Durack and Beeson, 1977) and with the severity of bacteraemia (McCabe *et al.*, 1978; Clumeck *et al.*, 1982). Most studies have been conducted with *Escherichia coli* isolates from various sources but serum resistance has also been reported among other genera of Enterobacteriaceae including *Klebsiella*, *Serratia* and *Proteus* (Roantree and Rantz, 1960; Olling, 1977; Clumeck *et al.*, 1982).

Although the mechanism of resistance to serum bactericidal activity has been the subject of considerable study, no single bacterial factor has been found consistently to confer serum resistance. Several cell-surface antigens, including certain O and K serotypes, and an *E. coli* outer-membrane protein have been shown to contribute to serum resistance, and at least two plasmid gene products confer low-level resistance in particular genetic backgrounds (see Taylor, 1983, for recent review). Only plasmids capable of self-transfer, i.e., plasmids of mol. wt $>(20–25) \times 10^6$ (Jacob *et al.*, 1977),

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have been implicated as able to confer serum resistance. One of the plasmid products is associated with the conjugative antibiotic-resistance plasmids R100 and R6-5, and is encoded by the *traT* gene (Mol, Manning and Timmis, 1980; Ogata and Levine, 1980). This gene is part of the conjugal transfer system of these plasmids and encodes a surface protein responsible for entry exclusion. Both R6-5 and R100 are assigned to entry exclusion group IV; group specificity is determined by the *traS* and *traT* gene products (Willets and Skurray, 1980). Some Col V plasmids (group I) also confer serum resistance but the mechanism of resistance is different from R6-5 and R100 (see *Discussion*). The F plasmid (group I) is also reported to confer low-level serum resistance but the molecular basis for resistance has not been determined (Fietta, Romero and Siccardi, 1977). The purpose of the work reported here was to determine the frequency of plasmids containing DNA sequences homologous with the R100 *traT* gene among serum-sensitive and serum-resistant strains of Enterobacteriaceae isolated from sites of extraintestinal infections in man.

METHODS

Bacterial strains. Isolates of Enterobacteriaceae used in this study were collected from various extraintestinal sites including respiratory tract infections, urinary tract infections, blood infections and wound exudates. They were obtained from Ben Taub County Hospital, Houston, Texas and stored in peptone 1%—glycerol 30% at −20°C. *E. coli* strain J62 containing the recombinant plasmid pOW3 was obtained from R. Ogata.

Preparation of dot blots. Bacteria were collected from 30-ml brain-heart infusion broth cultures and resuspended in 3 ml of TES (50 mM Tris, 10 mM EDTA, 50 mM NaCl, *p*H8). Cells were lysed by addition of lysozyme 3 mg, proteinase K 10 μg, 75 μM EDTA and sodium lauroyl sarcosine 40 μg. Lysates were incubated at 50°C for 1 h before storage at 4°C. Samples (5 μl) of each DNA preparation were denatured in 20 μl of 0·5N NaOH, partially neutralised by addition of 25 μl of 1M Tris (*p*H7) and then spotted on to a nitrocellulose filter. The filter was then transferred for 10 min to a double thickness of Whatmann 3MM filter paper soaked with 1M Tris (*p*H7)-1·6 M NaCl, dried at room temperature and baked for 2 h *in vacuo* at 80°C.

Probe DNA. A probe DNA fragment containing part of the *traT* gene was prepared from the recombinant plasmid pOW3 described by Ogata, Winters and Levine (1982). This plasmid contains the *traT* gene from plasmid R100 and parts of IS2 ligated to the vector pBR322. We used for our probe a 258-base-pair fragment flanked by *PstI* sites and encoding 86 amino acids of the *traT* protein beginning at position 69. Plasmid pOW3 was cut to completion with *PstI* and the desired fragment was twice purified by electrophoresis through 10% acrylamide gels and radiolabelled with α^{32}P CTP by nick translation (Maniatis, Jeffrey and Kleid, 1975).

Hybridisation conditions. Blots and gels were hybridised with probe overnight at 37°C in a solution containing formamide 50%, 5 × SSC (SSC is 0·15 M NaCl, 0·015 M sodium citrate), SDS 0·1%, 1mM EDTA, Ficoll 400 0·02%, polyvinyl pyrrolidone 360 0·02% and bovine serum albumin 0·02%, washed for 1 h at 65°C in 5 × SSC, dried and placed against Kodak XAR-5 film overnight at −70°C (Moseley *et al.*, 1983).

Plasmid DNA purification. Plasmid DNA was isolated from bacteria by the method described by Portnoy, Moseley and Falkow (1981).

Agarose gel hybridization. Agarose gels containing plasmid DNA were probed directly for *traT*-related sequences by the method described by Kidd *et al.* (1983). Gels were prepared with Tris-borate buffer as described by Maniatis, Fritsch and Sambrook (1982) except that melted agarose was passed through a 0·45-μm filter before casting the gel. Plasmid DNA was electrophoresed through the gels for 2–3 h at 100 V and DNA bands were visualised with ethidium bromide and photographed. The gels were then soaked at 4°C in 500 ml of 0·5 M NaOH, 0·15M NaCl for 30 min, neutralised in 500 ml of 0·5 M Tris (*p*H8), 0·15 M NaCl for 30 min at 4°C, transferred to a double thickness of Whatmann 3MM paper and then dried on to the

paper at 60°C until flat (1–2 h). Dried gels were floated off the paper in distilled water, blotted dry and stored at −70°C until used.

Serum resistance assay. The colorimetric assay of Moll, Cabello and Timmis (1979) was used to distinguish serum-sensitive and serum-resistant isolates. Pooled normal human serum was used at a final concentration of 20%.

Statistical analyses. Comparisons of the frequency of *traT* among various isolates were made by the Chi-square test.

Results

We collected 211 isolates of Enterobacteriaceae from various extraintestinal sites and tested each for resistance to the bactericidal effects of human serum and for the presence of gene sequences homologous with *traT*. The dot-blot hybridisation procedure described in *Methods* was used to determine *traT* gene frequency because results with the colony-blot method (Grunstein and Hogness, 1975) with non-*E. coli* clinical isolates were inconsistent. The results shown in the table indicate that *traT*-homologous DNA sequences are found among both serum-resistant and serum-sensitive *E. coli* isolates but not among any other tribe of bacteria tested. The frequency of *traT*-related sequences among *E. coli* isolates from stools was significantly lower ($\chi^2_{df=1} = 4{\cdot}5046$, $0{\cdot}05 > p > 0{\cdot}025$) than among extraintestinal isolates. No significant difference was found in the frequency of *traT* among serum-resistant and serum-sensitive isolates ($\chi^2_{df=1} = 0{\cdot}5766$, $0{\cdot}50 > p > 0{\cdot}30$).

Plasmid DNA was partially purified from each dot-blot-positive (*traT*$^+$) isolate and electrophoresed through 0·7% agarose gels. The gels were then probed with radiolabelled *traT*-gene fragment to identify plasmid or chromosomal bands containing *traT*-hybridising sequences (figure). For all strains tested, *traT* was found to be encoded on large plasmids of various sizes but on only one plasmid per isolate.

Discussion

Bacterial resistance to the lethal effects of human serum seems to contribute to increased virulence in extraintestinal infections. Several properties, including the plasmid encoded gene *traT* have been shown to contribute to serum resistance of laboratory *E. coli* strains. In this study we have measured the potential contribution of *traT* to serum resistance among extraintestinal isolates by determining the frequency of the gene among these strains. No isolates of the tribes Klebsielleae or Proteeae were found to contain *traT*-related sequences which suggests that this gene has no role in serum resistance of these strains. Among Escherichieae from extraintestinal sites, *traT* was found in about half (58%) of serum-resistant isolates and was found as frequently among serum-sensitive strains. Previous studies have also shown that some serum-sensitive wild strains carry R factors that contribute to serum resistance when they are transferred to certain *E. coli* recipients (Taylor and Hughes, 1978). Our results suggest that *traT* makes only a minor contribution to serum resistance of clinical isolates.

A significantly greater number of extraintestinal isolates than stool isolates contained *traT*. This finding may be attributed to the association of *traT* with antimicrobial-resistance plasmids and the use of antimicrobials in treatment of extraintestinal infections. The incidence of transferable drug resistance among human faecal *E. coli* and *E. coli* from infections, and the increase in frequency of transferable

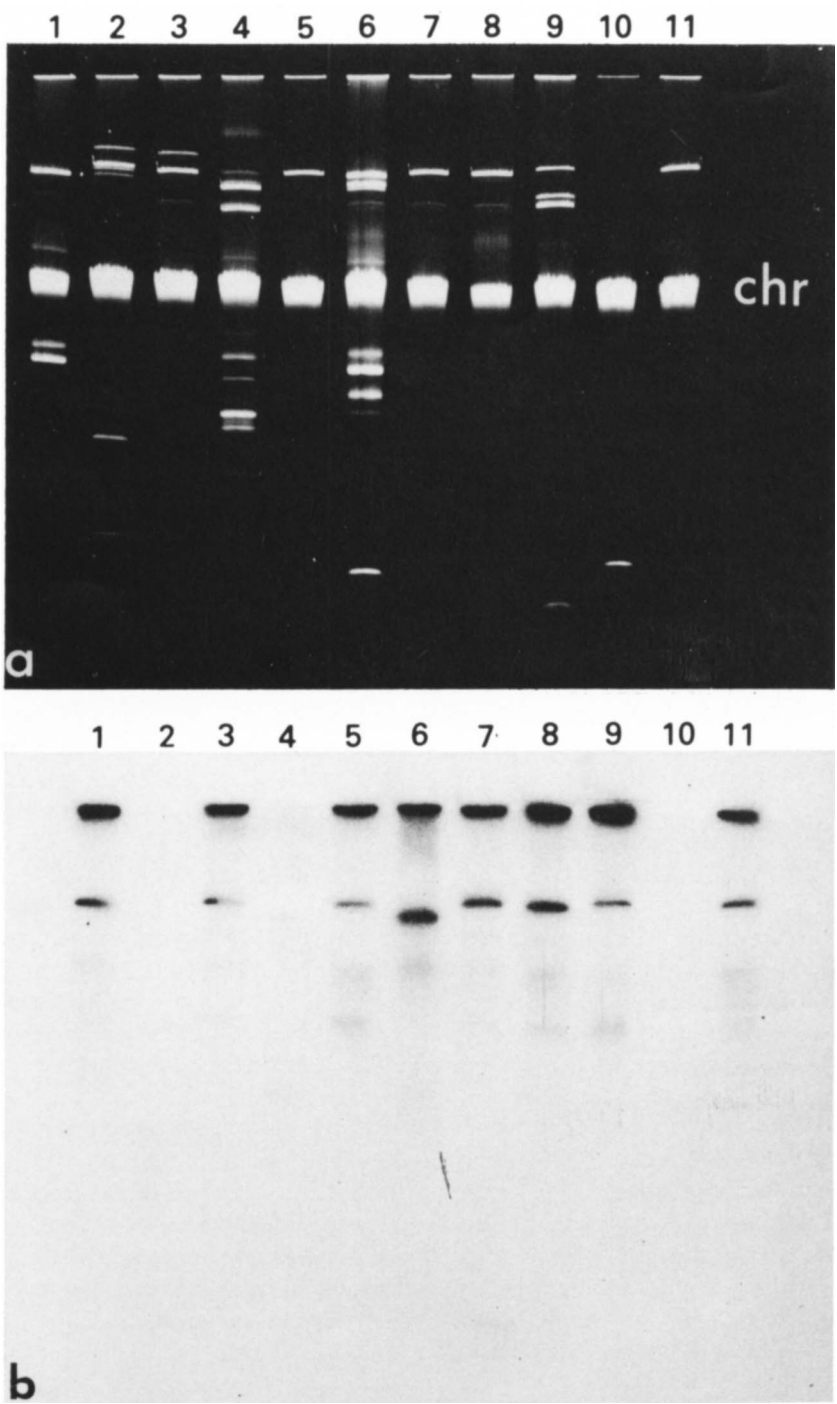

FIG.—(a) Agarose gel electrophoresis of plasmid DNA from 11 representative *E. coli* extraintestinal isolates. CHR = chromosomal DNA. (b) Autoradiograph of gel shown in (a) after hybridisation with radiolabelled *traT* probe DNA. DNA from isolates 2, 4 and 10 shows little or no homology with *traT*.

TABLE

Frequency of traT gene among extraintestinal isolates of Enterobacteriaceae

Tribe	Serum resistance	Total number of isolates	Number of isolates* with *traT*	Number of isolates with plasmids $>25 \times 10^6$ mol. wt
Escherichieae	R	43	25	31
Escherichieae	S	19	11	14
Klebsielleae†	R	96	0	64
Klebsielleae	S	14	0	9
Proteeae‡	R	21	0	4
Proteeae	S	6	0	2
Escherichieae§	R	40	14	36
Escherichieae	S	10	5	10

* Whole-cell DNA was purified from each isolate; samples were applied to nitrocellulose filters and hybridised with a radiolabelled *traT*-specific probe DNA fragment. DNA from isolates that possess *traT* bind the probe and are detected by autoradiography.
† Includes 48 *Klebsiella*, 47 *Enterobacter* and 15 *Serratia* isolates.
‡ Includes *Proteus*, *Providencia* and *Morganella* solates.
§ These bacteria were isolated from stools of healthy individuals not currently undergoing antimicrobial therapy.

resistance after treatment with antimicrobials, has been well documented (Datta, 1971; Shaw *et al.*, 1973; Harkness, Anderson and Datta, 1975; Datta and Richards, 1981). All *traT*$^+$ extraintestinal isolates tested were resistant to one or more antimicrobial although none has an antibiogram characteristic of the known *traT*$^+$ plasmids R100 or R6-5.

Another gene, *iss* (increased serum survival) associated with some Col V plasmids has also been shown to confer serum resistance upon laboratory *E. coli* strains (Binns, Mayden and Levine, 1982). Because no DNA probe specific for *iss* is currently available for hybridisation studies the frequency of this gene among clinical isolates cannot be determined. However Minshew *et al.* (1978) and Davis, Falkiner and Hardy (1981) have shown that Col V plasmids are present in only 10–30% of *E. coli* isolates from extraintestinal infections and we have found (table) that 40% of our serum-resistant Enterobacteriaceae contain either no plasmid or plasmids much smaller (mol. wt $< 25 \times 10^6$) than Col V (94×10^6) (Hardy, 1975). These results suggest that the Col V-associated *iss* gene may also have a secondary role in serum resistance of clinical isolates.

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