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

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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 Proteae 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),

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, pH8). 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 (pH7) 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 (pH7)-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 *Pst*I sites and encoding 86 amino acids of the *traT* protein beginning at position 69. Plasmid pOW3 was cut to completion with *Pst*I and the desired fragment was twice purified by electrophoresis through 10% acrylamide gels and radiolabelled with $\alpha^{32}\text{P}$ 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%, $5\times\text{SSC}$ (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 $5\times\text{SSC}$, 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 (pH8), 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 ($\chi^2_{df=1} = 4.5046$, $0.05 > p > 0.025$) than among extraintestinal isolates. No significant difference was found in the frequency of *traT* among serum-resistant and serum-sensitive isolates ($\chi^2_{df=1} = 0.5766$, $0.50 > p > 0.30$).

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 Klebsiellae or Proteaeae were found to contain *traT*-related sequences which suggests that this gene has no role in serum resistance of these strains. Among Escherichiaeae 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 <i>traT</i>	Number of isolates with plasmids > 25 × 10 ⁶ mol. wt
Escherichiae	R	43	25	31
Escherichiae	S	19	11	14
Klebsiellae†	R	96	0	64
Klebsiellae	S	14	0	9
Proteeae‡	R	21	0	4
Proteeae	S	6	0	2
Escherichiae§	R	40	14	36
Escherichiae	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* isolates.

§ 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 R 100 or R 6-5.

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 × 10⁶) than Col V (94 × 10⁶) (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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REFERENCES

- Archer G, Fekety F R 1976 Experimental endocarditis due to *Pseudomonas aeruginosa*. I. Description of a model. *Journal of Infectious Diseases* 134:1-7.
- Binns M M, Mayden J, Levine R P 1982 Further characterization of complement resistance conferred on *Escherichia coli* by the plasmid genes *traT* of R100 and *iss* of ColV, I-K94. *Infection and Immunity* 35:654-659.
- Bjorksten B, Kaijser B 1978 Interaction of human serum and neutrophils with *Escherichia coli* strains: differences between strains isolated from urine of patients with pyelonephritis or asymptomatic bacteriuria. *Infection and Immunity* 22:308-311.

- Clumeck N, Steens M, Englert Y, Butzler J P 1982 Serum sensitivity of strains isolated and antibodies against O antigen in gram negative bacteraemia. *Scandinavian Journal of Infectious Diseases* 14:283-288.
- Datta N 1971 R factors in *Escherichia coli*. *Annals New York Academy of Science* 182:59-64.
- Datta N, Richards H 1981 Trimethoprim-resistant bacteria in hospital and in the community: spread of plasmids and transposons, In: Levy S B, Clowes R C, Koenig E L (eds) *Molecular biology, pathogenicity and ecology of bacterial plasmids*, Plenum Press, New York, pp 21-30.
- Davies D L, Falkiner F R, Hardy K G 1981 Colicin V production by clinical isolates of *Escherichia coli*. *Infection and Immunity* 31:574-579.
- Durack D T, Beeson P B 1977 Protective role of complement in experimental *Escherichia coli* endocarditis. *Infection and Immunity* 16:213-217.
- Fietta A, Romero E, Siccardi A G 1977 Effect of some R factors on the sensitivity of rough Enterobacteriaceae to human serum. *Infection and Immunity* 18:278-282.
- Gower P E, Taylor P W, Koutsaimanis K G, Roberts A P 1972 Serum bactericidal activity in patients with upper and lower urinary tract infections. *Clinical Science* 43:13-22.
- Grunstein M, Hogness D S 1975 Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene. *Proceedings of the National Academy of Sciences* 72:3961-3965.
- Hardy K G 1975 Colicinogeny and related phenomena. *Bacteriological Reviews* 39:464-515.
- Harkness J L, Anderson F M, Datta N 1975 R factors in urinary tract infection. *Kidney International Suppl* 4:130-133.
- Hughes C, Phillips R, Roberts A P 1982 Serum resistance among *Escherichia coli* strains causing urinary tract infection in relation to O Type and the carriage of hemolysin, colicin, and antibiotic resistance determinants. *Infection and Immunity* 35:270-275.
- Jacob A E, Shapiro J A, Yamamoto L, Smith D I, Cohen S N, Berg D 1977 Plasmids studied in *Escherichia coli* and other enteric bacteria, In: Bukhari A I, Shapiro J A, Adhya S L (eds) *DNA insertion elements, plasmids and episomes*, Cold Spring Harbor Laboratory. pp 607-638.
- Kidd V J, Wallace R B, Itakura K, Woo S L C 1983 Alpha₁-antitrypsin deficiency detection by direct analysis of the mutation in the gene. *Nature* 304:230-234.
- Lowbury E J L, Ricketts C R 1957 Properdin and the defence of burns against infection. *Journal of Hygiene* 55:266-275.
- Maniatis T, Fritsch E F, Sambrook J 1982 *Molecular cloning. A laboratory manual*. Cold Spring Harbor Laboratory. pp 150-158.
- Maniatis T, Jeffrey A, Kleid D G 1975 Nucleotide sequence of the rightward operator of phage lambda. *Proceedings of the National Academy of Sciences* 72:1184-1188.
- McCabe W R, Kaijser B, Olling S, Uwaydah M, Hanson L A 1978 *Escherichia coli* in bacteremia: K and O antigens and serum sensitivity of strains from adults and neonates. *Journal of Infectious Diseases* 138:33-41.
- Minshew B H, Jorgensen J, Swanstrum M, Grootes-Reuvecamp G A, Falkow S 1978 Some characteristics of *Escherichia coli* strains isolated from extraintestinal infections of humans. *Journal of Infectious Diseases* 137:648-654.
- Moll A, Cabello F, Timmis K N 1979 Rapid assay for the determination of bacterial resistance to the lethal activity of serum. *FEMS Microbiology Letters* 6:273-276.
- Moll A, Manning P A, Timmis K N 1980 Plasmid-determined resistance to serum bactericidal activity: a major outer membrane protein, the *traT* gene product, is responsible for plasmid-specified serum resistance in *Escherichia coli*. *Infection and Immunity* 28:359-367.
- Moseley S L, Hardy J W, Huq M I, Echeverria P, Falkow S 1983 Isolation and nucleotide sequence determination of a gene encoding a heat-stable enterotoxin of *Escherichia coli*. *Infection and Immunity* 39:1167-1174.
- Nuttall G 1888 Experimente über die bacterienfeindlichen Einflüsse des thierischen Körpers. *Zeitschrift für Hygiene und Infektions-Krankheiten* 4:353-394.
- Ogata R T, Levine R P 1980 Characterization of complement resistance in *Escherichia coli* conferred by the antibiotic resistance plasmid R100. *Journal of Immunology* 125:1494-1498.

- Ogata R T, Winters C, Levine R P 1982 Nucleotide sequence analysis of the complement resistance gene from plasmid R100. *Journal of Bacteriology* 151:819-827.
- Olling S 1977 Sensitivity of gram-negative bacilli to the serum bactericidal activity: a marker of the host-parasite relationship in acute and persisting infections. *Scandinavian Journal of Infectious Diseases Suppl* 10:7-40.
- Portnoy D A, Moseley S L, Falkow S 1981 Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. *Infection and Immunity* 31:775-782.
- Roantree R J, Rantz L A 1960 A study of the relationship of the normal bactericidal activity of human serum to bacterial infection. *Journal of Clinical Investigation* 39:72-81.
- Shaw E J, Pursell R E, Datta N, Brumfitt W 1973 R factors in Enterobacteriaceae causing urinary-tract infection in general practice in 1962-63 and 1968-69. *Journal of Medical Microbiology* 6:451-454.
- Taylor P W 1983 Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. *Microbiological Reviews* 47:46-83.
- Taylor P W, Hughes C 1978 Plasmid carriage and the serum sensitivity of enterobacteria. *Infection and Immunity* 22:10-17.
- Vosti K L, Randall E 1970 Sensitivity of serologically classified strains of *Escherichia coli* of human origin to the serum bactericidal system. *American Journal of Medical Science* 259:114-119.
- Willetts N, Skurray R 1980 The conjugation system of F-like plasmids, *Annual Review of Genetics* 14:41-76.