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Correlation between uropathogenic properties of *Escherichia coli* from urinary tract infections and the antibody-coated bacteria test and comparison with faecal strains

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SUMMARY

Strains of *Escherichia coli* isolated from adult females with symptomatic urinary tract infection were found to possess the following properties significantly more frequently than faecal strains: (i) high K-antigen titre; (ii) haemolysin; (iii) type 1 pili; (iv) mannose-resistant haemagglutination; (v) fermentation of dulcitol and salicin; (vi) O serotype 2, 6 and 75; (vii) H serotype 1. *E. coli* isolated from urine specimens containing significant numbers of antibody-coated bacteria were richer in these seven properties than strains from urines without detectable antibody-coated bacteria.

The O and H serotypes of *E. coli* obtained from patients with urinary tract infection in two New Zealand cities were compared with those reported in the world literature and found to be similar.

INTRODUCTION

The presence of antibody-coated bacteria in the urine has been considered diagnostic for upper urinary tract infections since the work of Thomas, Shelokov & Forland (1974) and Jones, Smith & Sanford (1974). Some recent studies have failed to verify the accuracy of this test (Barnet & Abbot, 1978; Hellerstein *et al.* 1978; Forsum *et al.* 1978; Rumans & Vosti, 1978) but Fang, Tolkoff-Rubin & Rubin (1978) have used it as a basis for treatment. A recent investigation in England (Brooks *et al.* 1980) has shown that certain 'uropathogenic' properties are associated with *Escherichia coli* isolated from urinary tract infections. These include (i) high K antigen titre; (ii) production of haemolysin; (iii) production of type 1 pili (fimbriae); (iv) fermentation of salicin; (v) presence of certain O-antigens. The present study was undertaken to determine (*a*) whether these and other properties are prevalent in *E. coli* causing urinary tract infection amongst adult,

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female, domiciliary patients in New Zealand; and (*b*) whether strains isolated from urines containing significant numbers of antibody-coated bacteria are different from strains from urines without detectable antibody-coated bacteria. Faecal strains collected from healthy women of matched age-groups were also tested.

Although the O-antigen of *E. coli* appears to be related to virulence (Brumfitt & Heptinstall, 1960; van den Bosch, de Graaff & Maclaren, 1979), identifying 'uropathogenic' serotypes has proved difficult. This is partly because there is significant geographical variation amongst *E. coli* from urinary tract infections (Grüneberg & Bettelheim, 1969; Sietzen, 1979). In addition, little information is available for New Zealand strains, as studies have been carried out with only limited sets of antisera (Peddie & Little, 1978). A collection of strains randomly obtained from hospital patients from another city was also serotyped, in order to provide more information on the distribution of serotypes in New Zealand and to help distinguish between serotype prevalence due to geographical factors and that related to the patient-groups studied.

MATERIALS AND METHODS

Subjects

The adult, female, domiciliary patients studied were all aged 15 years or more, were resident in Palmerston North and had consulted their general practitioner because of symptoms of acute urinary-tract infection. The healthy controls were adult females of similar age distribution who were also resident in Palmerston North. They had no known history of urinary tract infection and were not taking antibiotics at the time of study.

Antibody-coated bacteria test

Mid-stream urine specimens from 200 domiciliary patients were examined for the presence of antibody-coated bacteria (ACB). The method of Thomas *et al.* (1974) was used with the following modifications:

(i) Five microlitre volumes of washed urine sediments were fixed onto glass slides by immersion in acetone for 10 min prior to treatment with fluorescein-conjugated anti-human gamma globulin (Wellcome Laboratories).

(ii) Preparations were counterstained with naphthalene black solution (0·1 mg/ml in phosphate buffer pH 7·6) at 37 °C for 30 min, rinsed in phosphate-buffered saline and mounted in buffered glycerol (pH 9·0).

Urines were regarded as truly positive for the ACB test only if 20% or more bacteria per microscope field fluoresced under ultra-violet light.

Collection and identification of E. coli *strains*

Using conventional techniques, 200 strains were isolated from mid-stream urine specimens of domiciliary patients with significant bacteriuria. Eighty-eight of the urines gave equivocal results for the ACB test in that they contained small numbers of ACB, and *E. coli* from these specimens were not included in the study. Strains isolated from the remaining 112 urines were divided into two groups.

(i) Fifty-three strains from patients with a clear-cut, positive ACB test, i.e. 20% or more bacteria per microscope field, were antibody-coated.

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(ii) Fifty-nine strains from patients with a clear-cut negative ACB test, i.e. no detectable ACB in their urine.

Forty faecal *E. coli* were collected from 30 healthy controls by plating faeces samples on McConkey's agar and incubating overnight at 37 °C. As more than one colony type was usually present a representative of each was chosen for further identification.

Two hundred and five strains were isolated from hospital patients with urinary tract infection. These were collected sequentially from mid-stream urines received by the microbiological laboratory at Wellington Hospital.

Identification of isolates was carried out according to Edwards & Ewing (1972). Strains were stored in glycerol broth at −10 °C during the period of study.

Uropathogenic properties

The methods used for quantifying K-antigen, detecting type 1 pili, H serotyping and sugar fermentation have been described previously (Brooks *et al.* 1980).

Cell bound and extracellular haemolysins were detected by the following method. Brain heart infusion broth (Difco) in 10 ml volumes was inoculated with 0·1 ml of overnight broth cultures and incubated on a shaker for 6 h at 37 °C. The cultures were divided into two portions, one of which was freed from bacterial cells by centrifugation and filtration through a millipore filter of 0·2 μm pore size. The whole cultures and cell-free supernatants were diluted 1/2 with physiological saline containing 0·02 M $CaCl_2$. Samples (0·5 ml) were placed in small test-tubes and 0·5 ml of thrice-washed, horse erythrocytes suspended in physiological saline added to each tube. Haemolytic activity was observed after incubation for 1 h at 37 °C. Duplicate tests were performed and sterile saline and brain heart infusion broths were used as controls.

Mannose-resistant haemagglutination was determined using human group O Rh-positive blood as described by Minshew *et al.* (1978) with one modification: duplicate tests were performed in the presence of 0·5% D-mannose.

Colicin V production was determined by the method of Minshew *et al.* (1978).

O serotype was determined with a complete set of antisera (01–0163) as described by Meekin, Bettelheim & Bacon (1979).

K^1 antigen was detected by slide agglutination using a suspension of live bacteria and K^1 antiserum prepared in rabbits.

Statistical tests. Data were analysed using the chi-squared test for two independent samples and the Mann–Whitney U test (Siegel, 1956).

RESULTS

Uropathogenic properties

Results for the strains isolated from domiciliary patients and healthy controls are shown in Fig. 1 (K antigen) and Table 1 (other properties). About one-third of the strains in all the groups belonged to K serotype 1 and only 4 strains produced colicin V.

Strains were awarded a score of one for each uropathogenic property they possessed, excluding K^1 serotype, which showed no obvious difference in distribution, and colicin V production which occurred in too few strains to be of any

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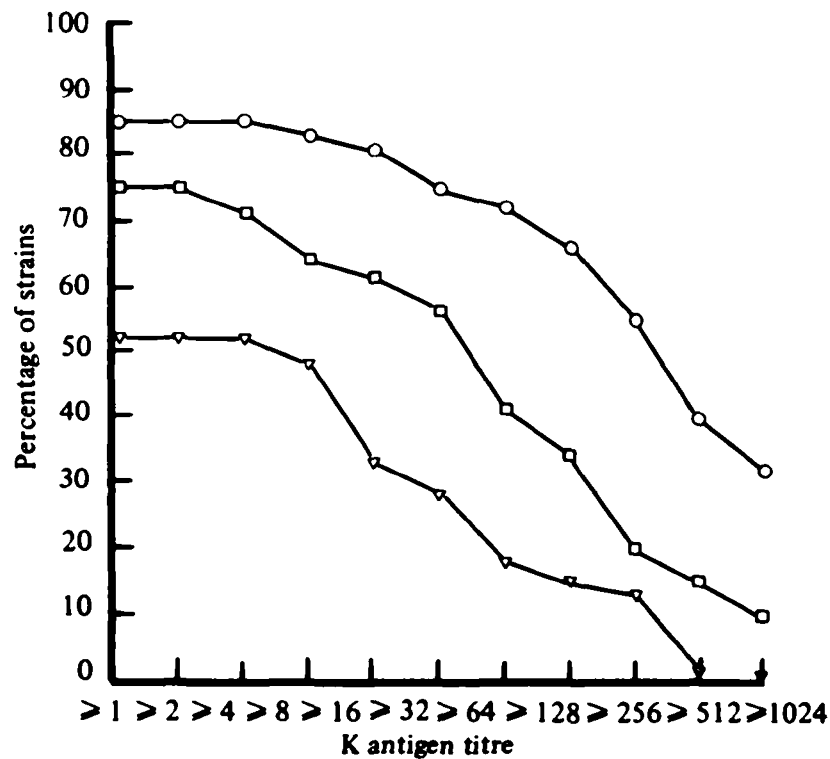

Fig. 1. K-antigen content of *Escherichia coli* isolated from urine containing antibody-coated bacteria (○—○), urine without antibody-coated bacteria (□—□) and faeces (▽—▽).

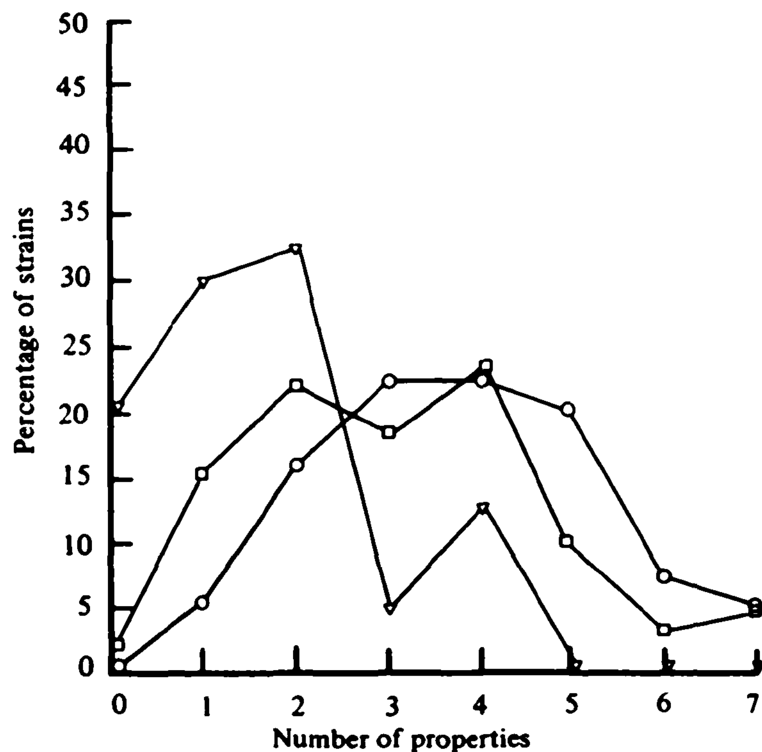

Fig. 2. Number of properties exhibited by *Escherichia coli* isolated from urine containing antibody-coated bacteria (○—○), urine without antibody-coated bacteria (□—□) and faeces (▽—▽).

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Table 1. *Incidence of uropathogenic properties in* Escherichia coli *isolated from urine and faeces*

	Sources of strains (number)		
	Urines (112)		Faeces (40)
	Antibody-coated bacteria test		
Uropathogenic properties	+ (53)	− (59)	
Haemolysin			
cell-bound only	15 (28 %)	13 (22 %)	3 (8 %)
extracellular + cell-bound	7 (13 %)	5 (9 %)	2 (5 %)
total haemolytic	22 (41 %)	18 (31 %)	5 (13 %)
		↑	↑
		$0{\cdot}05 > P > 0{\cdot}02$	
	↑		↑
		$0{\cdot}01 > P > 0{\cdot}01$	
Type 1 pili	46 (85 %)	49 (83 %)	23 (58 %)
		↑	↑
		$0{\cdot}01 > P > 0{\cdot}001$	
	↑		↑
		$0{\cdot}01 > P > 0{\cdot}001$	
Mannose-resistant haemagglutination factor	25 (47 %)	25 (42 %)	10 (25 %)
	↑		↑
		$0{\cdot}05 > P > 0{\cdot}02$	
Fermentation of dulcitol and salicin	39 (74 %)	32 (54 %)	12 (30 %)
	↑	↑ ↑	↑
	$0{\cdot}05 > P > 0{\cdot}02$	$0{\cdot}05 > P > 0{\cdot}02$	
Colicin V	1 (2 %)	2 (3 %)	1 (3 %)
*O serotypes 2, 6, 75	24 (57 %)	19 (41 %)	4 (13 %)
		↑	↑
		$0{\cdot}01 > P > 0{\cdot}001$	
	↑		↑
		$P < 0{\cdot}001$	
*H serotype 1	13 (36 %)	10 (32 %)	2 (9 %)
		↑	↑
		$0{\cdot}05 > P > 0{\cdot}02$	
	↑		↑
		$0{\cdot}05 > P > 0{\cdot}02$	
K serotype 1	17 (32 %)	20 (34 %)	14 (35 %)

* Percentages calculated out of total smooth, typable strains: ACB+ = 42, ACB− = 46, faeces = 30.

† Arrows adjoined by lines indicate that significant probability values were obtained for the pairs of groups arrowed when tested by the chi squared test.

significance. A 'high' K-antigen titre was arbitrarily taken to be > 1/32. Results are shown in Fig. 2. The faecal and ACB test negative strains exhibited bimodal distribution and were significantly different from each other ($P < 0{\cdot}001$) and from the ACB test positive strains ($P < 0{\cdot}001$ in both cases). The bulk of the faecal strains possessed fewer than 3 of the 7 uropathogenic properties; only 17 % possessed 3 or more. On the other hand, 79 % of ACB test positive and 61 % of ACB test negative strains possessed 3 or more properties.

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Table 2. *Number of different O and H serotypes of* Escherichia coli *isolated from urines of domiciliary and hospital patients and from faeces of healthy subjects*

Source	Total number of strains serotyped	Number of different O types	H types	OH types
Urinary tract infections in domiciliary patients (Palmerston North)	110	34	18	60
Urinary tract infections in hospital patients (Wellington)	205	39	25	80
Faeces of healthy subjects (Palmerston North)	40	25	16	32

Table 3. *Order of frequency of common O serotypes of* Escherichia coli*: comparison of urinary tract strains isolated in New Zealand with those reported in the world literature*

O serotypes in order of frequency		
Palmerston North	Wellington	World literature
06	06	06
02	075	075
075	018	04
07	04	02
01	02	07
04	025	01
068	01	018

Table 4. *Order of frequency of common H serotypes of* Escherichia coli*: comparison of urinary tract strains isolated in New Zealand with those reported in the United Kingdom and Hong Kong*

H serotypes in order of frequency			
Palmerston North	Wellington	United Kingdom	Hong Kong
H1	H1	H1	H4
H6	H5	H4	H6
H5	H31	H5	H10
H7	H6	H6	H1
H4	H7	H7	H31
H14	H4	H18	H40
H31	H10	H20	H5
H21	H14	H9	H21
H19	H21	H10	

Prevalence of serotypes in New Zealand

Table 2 shows the number of different serotypes isolated from urinary tract infections in hospital and domiciliary patients and from faeces. Table 3 lists the O serotypes prevalent in this study and compares them with those reported in the world literature. Table 4 similarly lists prevalent H serotypes and compares them with those most commonly found in cases of urinary tract infection in the U.K. and Hong Kong, data being unavailable for the other areas.

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DISCUSSION

In both the present and a previous study carried out in England (Brooks *et al.* 1980) *E. coli* causing urinary tract infections were found to possess the following properties significantly more frequently than commensal strains: (i) K-antigen titre of > 1/32; (ii) haemolysin; (iii) type 1 pili; (iv) O serotype 2, 6 and 75. In the present study, a further two properties were observed to occur more commonly amongst urinary strains: (v) fermentation of both dulcitol and salicin; (vi) H serotype 1. In addition, mannose-resistant haemagglutination was more common in the urinary tract group, but only the strains from patients with ACB in their urine were significantly different from the faecal *E. coli*. Some of these properties appear to endow *E. coli* with ability to resist host defence mechanisms and to produce disease in the urinary tract, but for others the relationship with virulence is unclear. Strains rich in K-antigen are more resistant to phagocytosis, antibody-binding and killing by complement (Glynn & Howard, 1970; Howard & Glynn, 1971) whilst strains producing type 1 pili are probably able to resist hydrokinetic clearance by adhering to the uroepithelium (Schaeffer, Amundsen & Schmidt, 1979; Edén & Hansson, 1978). Type 1 pili cause agglutination of erythrocytes which can be inhibited by the addition of D-mannose (Duguid *et al.* 1955 and Duguid, 1968). Mannose-resistant haemagglutination has been shown to be mediated by pili other than type 1 in some strains and by undefined components in others (Duguid, Clegg & Wilson, 1979). Possibly this latter property plays a role in adhesion to the uroepithelium, and studies to confirm this hypothesis are currently under way in our laboratory. Alternatively, the mannose-resistant haemagglutinating factor may be connected with invasiveness; Minshew *et al.* (1978) found a close correlation between haemagglutinating activity and virulence for chicken embryos. Although they did not ascertain whether the haemagglutination was inhibited by mannose, the culture conditions used were those which encourage production of the mannose-resistant factor and suppress type 1 pili production. The haemolysins elaborated by *E. coli* may damage the uroepithelium. Preliminary work in our laboratory indicates that both cell-bound and extra-cellular forms are cytotoxic for human uroepithelial cells and mouse kidney cells.

The relationship between O serotype and pathogenicity for the urinary tract remains unclear, although virulence of *E. coli* for experimental animals appears to depend partly on the O-antigen (Brumfitt & Heptinstall, 1960; van den Bosch *et al.* 1979). There is no obvious connexion between ability to ferment salicin, dulcitol and possession of H 1 antigen and uropathogenicity.

We initially hoped to compare *E. coli* causing kidney infections with strains confined to the bladder, and when this study was begun the ACB test was considered a reliable method for localizing infection in domiciliary patients. Fairley *et al.* (1971) showed that there was a poor correlation between symptoms and renal involvement, and the more invasive tests, such as ureteral catheterization, were not considered appropriate for this group of patients. However, the ACB test has since proved to be less accurate than was first anticipated (Mundt & Polk, 1979). It is now generally accepted that the presence of ACB in the urine indicates that subepithelial invasion has occurred (Smith, Jones & Kaijser, 1977; Riedasch *et al.* 1978; Thomas *et al.* 1978), which may in turn reflect the virulence of the infecting

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strain. In the present study, when the properties were examined singly, there were two which occurred significantly more often in *E. coli* isolated from urines containing antibody-coated bacteria; high K-antigen titre and ability to ferment both dulcitol and salicin. Therefore, it appears that these properties influence invasiveness. In view of the work of Howard & Glynn (1971), it is tempting to postulate that the K-antigen exerts the strongest influence, but there is evidence that dulcitol fermentation also correlates with the ability of strains to produce pyelonephritis in animal models (Guze *et al.* 1973; Kalmanson *et al.* 1975).

We have confirmed that uropathogenicity is a multifactorial phenomenon. The majority of the urinary tract strains possessed 3 or more of the properties examined, whilst such strains were in the minority in the faecal group. Presuming all faecal strains have the same opportunity to enter the bladder, those with > 3 properties appear to be more likely to cause infection. Conversely, it may be argued that colonization of the bowel by one of these strains predisposes the susceptible individual to infection. Furthermore, urinary strains which had invaded the subepithelial layer more frequently possessed 3 or more properties compared with those which had not.

Comparison of the orders of frequency of the O types found in Palmerston North and Wellington, and in the world literature (Table 3) suggests that the two New Zealand series fall within the world-wide results. However, most of the world-wide data have been obtained in temperate regions of the Northern Hemisphere (Europe and North America) and the few studies outside this area do show quite a different distribution as exemplified by the studies in Hong Kong (Wong & Bettelheim, 1976). The similarity of the New Zealand serotypes with Northern temperate groups is also seen when the H-antigens are compared (Table 4). Here again the Hong Kong figures show significant differences.

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