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Cell surface hydrophobicity, adherence to HeLa cell cultures and haemagglutination pattern of pyelonephritogenic *Escherichia coli* strains

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SUMMARY

Cell surface hydrophobicity, haemagglutination pattern and adherence to HeLa cells were examined in 230 strains of *Escherichia coli* collected from women (n = 61 strains) and children (n = 65 strains) with non-obstructive acute pyelonephritis and in 104 faecal control strains of *E. coli* from healthy adults (n = 71 strains) and children (n = 33 strains). Pyelonephritogenic *E. coli* strains showed a significantly increased incidence of hydrophobic properties (90%) and mannose resistant haemagglutination (MRHA) of human erythrocytes (83%) than faecal control strains (64 and 23% respectively, $P < 0{\cdot}001$ in both cases). Mannose sensitive haemagglutination (MSHA) was observed in 48% of the pyelonephritogenic *E. coli* strains and in 50% of the faecal control strains (NS). The incidence of adherence to HeLa cells was low both in pyelonephritogenic and faecal control strains, 6 and 7% respectively (NS). The bacterial phenotypes MRHA+MSHA+ and MRHA+MSHA− appeared significantly more often in pyelonephritogenic *E. coli* strains (35 and 48% respectively) than in faecal control strains (5 and 17% respectively, $P < 0{\cdot}001$ in both cases). The phenotype MRHA−MSHA+ occurred significantly more often in control strains (45%) than in pyelonephritogenic strains (13%, $P < 0{\cdot}001$). Eighty-three per cent of the pyelonephritogenic *E. coli* strains expressing hydrophobic properties showed MRHA and 50% of the hydrophobic strains showed MSHA. There were no significant correlations between cell surface hydrophobic properties and haemagglutination pattern or adherence to HeLa cells in pyelonephritogenic *E. coli* strains nor in faecal control strains.

INTRODUCTION

The urinary bladder is normally sterile and most bacteria introduced into the normal human bladder rapidly disappear, probably because cells of the bladder

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are resistant to the attachment of microorganisms in the healthy host. The role of adherence in the ability of organisms to induce urinary tract infections (UTI) has been extensively studied. *Escherichia coli* strains isolated from the urinary tract possess two distinct mechanisms of adherence mediated by filamentous appendages. These structures mediate bacterial haemagglutination of different erythrocytes and this reaction is either mannose sensitive (MSHA) or mannose resistant (MRHA) [1]. MSHA is mediated by type 1 fimbriae and MRHA is caused by P-fimbriae [2, 3]. The role of P-fimbriae as a virulence factor in UTI is well documented. Several epidemiologic surveys have shown that the majority of pyelonephritogenic *E. coli* isolates express P-fimbriae, whereas this bacterial phenotype is observed less frequently among isolates from patients with cystitis and faecal isolates [4, 5]. The role of type 1 fimbriae in UTI has not been as thoroughly studied as that of P-fimbriae, and investigations have led to conflicting results [6–9]. Nevertheless, several studies have demonstrated that type 1 fimbriae contribute to *E. coli* bladder colonization in animal models [10, 11].

Bacterial binding to uroepithelial cell surfaces is however not only mediated by bacterial fimbriae but also by hydrophobic interactions between enterobacteria and various host cells [12–14]. The attachment to HeLa cell cultures by *E. coli* strains is either by a diffuse or localized pattern [15].

We have earlier reported that pyelonephritogenic *E. coli* strains more often express hydrophobic properties than faecal control strains [14]. In the present study, the incidence of different haemagglutination patterns and HeLa cell adherence were examined and related to the expression of cell surface hydrophobic properties of pyelonephritogenic and faecal control strains of *E. coli*.

PATIENTS AND METHODS

Patients with acute non-obstructive pyelonephritis

Urine specimens were collected from 65 children below the age of 2 years with their first episode of acute pyelonephritis. The diagnostic criteria were positive urine culture ($>10^8$ colony-forming units (c.f.u.)/l urine), fever above 38·5 °C, leucocyturia, increased C-reactive protein (CRP > 20 mg/l) without concurrent symptoms or signs of any other infection. Radiological examinations revealed vesico-ureteric reflux grade II–IV in 11 of 49 examined children (22%).

Midstream urine specimens were collected from 58 women (mean age 33 years, range 17–70 years) with acute non-obstructive *E. coli* pyelonephritis. Three patients had two episodes of acute pyelonephritis with an interval of >6 months with different strains of *E. coli*. Acute pyelonephritis was diagnosed by loin pain, temperature above 38 °C, substantial bacteriuria ($>10^8$ c.f.u./l urine), increased CRP without signs or symptoms of other infection. Parenchymal renal scarring, i.e. calyceal clubbing in combination with a corresponding renal parenchymal reduction on i.v. urography, was found in 14% of the patients.

Controls

Faecal isolates were collected from 33 healthy children below the age of 1 year without a history of UTI or diarrhoeal disease and from 71 adults in connection with a routine out-patient health examination. None of the healthy controls had

a history of symptomatic UTI or recent gastrointestinal disease. Their urine did not yield *E. coli* on culture.

BACTERIOLOGICAL METHODS

All urine samples were collected after washing the external genitalia with water and was kept at 4 °C until examined within 24 h. The number of bacteria in the urine was estimated by the standard loop technique. Faecal samples were spread on CLED agar (Oxoid) and six colonies were selected, using a method that gives 99% probability that at least one colony belongs to the dominant aerobic faecal flora [15]. Bacteria were characterized as *E. coli* by their biochemical characteristics established by means of the API 20E system (API La Balme Les Grottes, France).

Storage and subculturing

All strains were kept in −70 °C until assayed. Strains were subcultured on Tryptic Soy agar (Difco) on receipt and stored at 4 °C. Fresh cultures of these strains were prepared on suitable media in each experiment as described below. All experiments were done without replicates unless indicated.

Mannose sensitive haemagglutination (MSHA)

Presence of type 1 fimbriae were detected according to the method described by Duguid and co-workers [1]. Red blood cells from laboratory-bred guinea-pigs were washed three times in 85% (w/v) NaCl solution and adjusted to a concentration of 3% (v/v) in fresh saline. Test strains were grown on 1·5% agar slants containing 1% casamino acid (Difco) and 0·15% yeast extract plus 0·005% $MnCl_2$ and 0·05% $MgSO_4$ (CFA agar) [17] after an overnight incubation at 37 °C. Bacteria were then harvested and concentrations of 5×10^8 c.f.u./ml were prepared in saline. To test inhibition by D-mannose, samples were also prepared in saline containing 2% D-mannose. Equal volumes (25 μl) of the bacterial suspensions and erythrocytes were mixed on microscope slides at room temperature with gentle rocking and agglutination was detected visually within 2 min.

Mannose resistant haemagglutination (MRHA)

Bacterial adhesion was evaluated according to a modification of the method described by Duguid and co-workers [1], using human erythrocytes of blood group A (Rh+). Erythrocytes were washed three times in PBS (pH 7·2) and suspended to a concentration of 3% (v/v). Using the same suspension of the bacteria as described above, equal volumes (25 μl) of the bacterial suspension and erythrocytes were mixed on a microscope slide and tilted for 2 min at room temperature and observed for agglutination.

HeLa cell adhesion

Adhesion to HeLa cells was performed according to the method described by Scaletsky and colleagues [15]. HeLa cells were grown in medium 199 with Eagle salt, supplemented with 100 units of penicillin per ml and 100 μg of streptomycin

per ml, 2 mM L-glutamine and 10% fetal bovine serum. Monolayer cells were grown in 1 ml of the growth media in each well of plastic dishes (24 wells) covered with cover slips. Twenty-five microlitres of a suspension of the bacteria grown in Tryptic soy broth at 37 °C overnight, was inoculated in each well and plates incubated for 30 min in 5% CO_2 atmosphere. Cells were then washed six times in sterile PBS (pH 7·2) and inoculated with fresh medium. Plates were then reincubated for another 3 h and washed three times in PBS. Finally, cells were fixed with methanol and stained with May–Grünwald stain (for 5 min) followed by Giemsa stain (20 min). After rinsing, cover slips were mounted on microscope slides and searched for bacterial attachment to the HeLa cells under light microscope.

Cell surface hydrophobicity

Cell surface hydrophobic properties of the strains in the present study have previously been determined [14]. The salt aggregation test (SAT) was performed as previously described by Ljungh and Wadström [18]. Bacteria cultured on a blood agar plate were suspended in 0·001 M sodium phosphate buffer, pH 6·8, at a concentration of 10^8 bacteria/ml. From each bacterial suspension 10 μl were mixed on a glass slide with 10 μl ammonium sulphate (pH 6·8) of varying molarities (1·6, 0·9, 0·1, 0·01 and 0·001 M). The SAT value noted was represented as the lowest concentration of ammonium sulphate at which aggregation was observed after 1 min at 20 °C. Bacteria of higher surface hydrophobicity aggregated at lower salt concentrations.

Statistical analysis

The χ^2 test with continuity correction, Fisher's exact test and Stepwise regression analysis were used.

RESULTS

MRHA was observed in 83% of the pyelonephritogenic *E. coli* strains compared to 23% of the faecal control strains ($P < 0{\cdot}001$, χ^2 test, Table 1). There was no significant difference in the incidence of MSHA in pyelonephritogenic (48%) and faecal control strains (50%). *E. coli* strains exhibiting both MRHA and MSHA occurred significantly more often in pyelonephritogenic strains (35%) compared to control strains (5%, $P < 0{\cdot}001$, Table 1). Thirteen per cent of the pyelonephritogenic isolates showed only MSHA and not MRHA compared to 45% of the faecal control strains ($P < 0{\cdot}001$). Of the pyelonephritogenic *E. coli* strains 48% expressed only MRHA and not MSHA compared to 17% of the faecal isolates, a significant difference ($P < 0{\cdot}001$). Thirty-one per cent of all faecal *E. coli* strains (32/104) showed neither MRHA nor MSHA compared to 5/126 (4%) of the pyelonephritogenic isolates ($P < 0{\cdot}001$).

Adherence to HeLa cell cultures was observed in 7 of 126 (6%) pyelonephritogenic *E. coli* strains of which 4 showed only diffuse adherence, 1 only localized adherence and 2 strains showed both diffuse and localized adherence on the same preparation. Seven per cent of the faecal control strains adhered to HeLa cell cultures (Table 1).

Table *Cell surface hydrophobicity, haemagglutination and adherence pattern of pyelonephritogenic and faecal* E. coli *st*

	Hydrophobicity			MRHA			MSHA			MRHA⁺MSHA⁺			MRHA⁺MSHA⁻			MRHA⁻MSHA⁺			HeLa	ace
	n	(%)	*P*	*n*	(%)	*P*	*n*	(%)	*P*	*n*	(%)	*P*	*n*	(%)	*P*	*n*	(%)	*P*	*n*	*P*
All pyelo										44/125	(35)									
(*n* = 126	s strains	114	(90)		105	(83)		61	(48)				60	(48)		16	(13)		7	NS
All contr						<0·001			NS			<0·001			<0·001			<0·001		
(*n* = 104	us	66/103	(64)		24	(23)		52	(50)		5	(5)		18	(17)		47	(45)		7	
Pyelonep										25/64	(39)									
children	rains from	64	(98)*		55	(85)		34	(52)				29	(45)		8	(12)		3	NS
Control s				<0·001			<0·001			NS		<0·001			<0·05			<0·001		
(*n* = 33)	rom children	27	(82)†		18	(54)		22	(67)		2	(6)		6	(18)		20	(61)		1	
Pyelonep										19	(31)									
adults (*	rains from	50	(82)		50	(82)		27	(44)				31	(51)		8	(13)		4	NS
Control st				<0·001			<0·001			NS		<0·001			<0·001			<0·001		
(*n* = 71)	rom adults	39/70	(56)		16	(23)		30	(42)		3	(4)		12	(17)		27	(38)		6	

The χ^2 test with continuity correction and Fisher's exact test were used for statistical analyses.
* $P < 0{\cdot}01$ *vs.* corresponding strains from adults.
† $P < 0{\cdot}05$ *vs.* corresponding strains from adults.

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Cell surface hydrophobicity was observed in 90% of the pyelonephritogenic *E. coli* strains compared to 64% of the faecal control strains ($P < 0{\cdot}001$) (Table 1). The highest frequency of hydrophobic *E. coli* strains (98%) was found among pyelonephritogenic *E. coli* strains from children which was significantly higher than in pyelonephritogenic isolates from adults (82%, $P < 0{\cdot}01$).

Stepwise regression analysis of expression of MRHA, MSHA, cell surface hydrophobicity and HeLa cell adherence showed that firstly MRHA ($r^2 = 0{\cdot}37$) and secondly cell surface hydrophobicity (cumulative $r^2 = 0{\cdot}40$) significantly contributed to the differences between pyelonephritogenic and faecal *E. coli* strains.

Eighty-three per cent (94/113) of the hydrophobic pyelonephritogenic *E. coli* strains showed MRHA, 57/112 (50%) showed MSHA and 40/112 (36%) expressed the phenotype MRHA+MSHA+. Forty-seven per cent (53/113) of the hydrophobic pyelonephritogenic strains were MRHA+MSHA−, 16/113 (14%) were MRHA−MSHA+ and 3/113 (3%) were MRHA−MSHA−. There were no significant correlations between cell surface hydrophobicity and haemagglutination pattern or adherence to HeLa cell cultures between all *E. coli* strains or between isolates obtained from the different diagnostic groups (data not shown).

DISCUSSION

There is general agreement that bacterial adhesins act as essential virulence factors in urinary tract infections by facilitating colonization of the uroepithelium. In the present study we examined the occurrence of MRHA of human erythrocytes, MSHA of guinea-pig erythrocytes (representing type 1 fimbriae), adherence to HeLa cell cultures and cell surface hydrophobicity of 230 *E. coli* strains obtained from children and women with acute non-obstructive pyelonephritis and from healthy controls. Pyelonephritogenic *E. coli* strains from both children and women showed MRHA significantly more often than faecal control strains from each group respectively. Several prior observations have stated that surface-associated adhesins and particularly fimbriae which mediate MRHA of human erythrocytes are virulence factors important in non-intestinal infections [19, 20]. MRHA positive strains are more common among *E. coli* strains causing urinary tract infections and especially in strains causing acute pyelonephritis [1, 4, 5, 7, 9].

Type 1 fimbriae, mediating MSHA, were observed in 48% of all pyelonephritogenic strains and in 50% of the faecal control strains of the present study. These figures compare well to previous observations [7, 21, 22]. In the present study, strains expressing both MRHA and MSHA were more common among strains causing acute pyelonephritis compared to faecal control strains. Strains expressing MRHA but not MSHA were also more common in pyelonephritogenic strains compared to control strains. In contrast, strains expressing only MSHA and not MRHA appeared significantly more often among faecal control strains of the present study than in clinical isolates of *E. coli*. Duguid and colleagues observed a higher incidence of the MRHA+MSHA+ phenotype among *E. coli* strains isolated from patients with urinary tract infections compared to faecal control strains but a similar incidence of strains expressing only MSHA in urinary

and faecal isolates [1]. Hopkins and colleagues examined strains from patients with urinary tract infections and found type 1 fimbriae on 61% of the isolates and coexpression of type 1 and P-fimbriae on 14% of the strains [21]. Similarly, Latham and co-workers observed a coexpression of type 1 and P-fimbriae on 14% of *E. coli* strains isolated from adult women with cystitis [23]. Differences in patient selection as to the type of patients studied are the probable reasons for the differences in phenotypic expression of *E. coli* from different centres. Type 1 fimbrial expression by *E. coli* seems to play an important role in the colonization of the urinary bladder [24, 25] whereas P-adhesins confer a selective advantage to *E. coli* in colonizing the upper urinary tract [7]. The present study supports this concept.

The incidence of adherence to HeLa cells was low and similar in pyelonephritogenic and faecal control strains of the present study. Scaletsky and colleagues [15] observed diffuse mannose resistant adherence to HeLa cell cultures in 30% of urinary *E. coli* strains isolated from patients with symptomatic bacteriuria and in 37% of enteropathogenic or enterotoxigenic *E. coli* isolates.

Stepwise regression analysis showed that expression of MRHA was the most important characteristic in the differentiation between pyelonephritogenic and faecal strains. Cell surface hydrophobicity also independently contributed to the differences between pyelonephritogenic and faecal strains but to a lesser extent.

The bacterial adhesion process is complex and involves both specific interactions between adhesins and epithelial cell surface receptors and non-specific physicochemical interactions [26–28]. Both bacterial fimbriae and cell surface hydrophobic properties promote attachment of *E. coli* to epithelial cells [1, 12–14, 20, 29, 30]. We have previously reported that pyelonephritogenic *E. coli* strains significantly more often express hydrophobic cell surface properties than faecal control strains [14]. There was no correlation between cell surface hydrophobicity and haemagglutination pattern of the pyelonephritogenic and faecal control strains in the present study. There are contradictory reports on the contribution made by fimbrial expression to cell surface hydrophobicity [29–31]. Jann and coworkers [29] and Sobel and Obedeanu [30] found that type 1 fimbriae were more hydrophobic than mannose resistant fimbriae while Ljungh and Wadström [31] found that mannose resistant fimbriae expressed a higher surface hydrophobicity than type 1 fimbriae.

It is conceivable that both bacterial adhesins and non-specific physicochemical interactions play an important role in the establishment of UTI.

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