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Characterization of enteroaggregative *Escherichia coli* isolated from outbreaks of diarrhoeal disease in England

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SUMMARY

Twenty-two strains of enteroaggregative *Escherichia coli* (EAggEC), isolated from four outbreaks of diarrhoeal disease in England, were examined for a range of phenotypic attributes including the ability to produce fimbriae, haemolysins and siderophores, and cell-surface properties such as surface charge and hydrophobicity. Strains of EAggEC isolated from two of these outbreaks belonged to a diverse range of serotypes and were heterogeneous in phenotype. Strains of EAggEC isolated from the other two outbreaks belonged predominantly to serotypes O86:H34 and O98:H-, respectively. Only two strains expressed fimbriae and two strains produced an 18 kDa membrane associated protein (MAP), suggesting that EAggEC express a range of adhesion mechanisms to produce the cell arrangement recognized as the 'stacked brick' formation. The possible explanation for the diversity of EAggEC serotypes is discussed.

INTRODUCTION

Strains of enteroaggregative *Escherichia coli* (EAggEC) are a major cause of chronic infantile diarrhoea in certain developing countries [1–3], although EAggEC have been isolated from cases of diarrhoeal disease in developed countries including England, Italy and Japan [4–7]. Strains of EAggEC belong to a wide range of different serotypes [8–11] and share the ability to adhere to HEp-2 cells in a pattern known as a 'stacked brick' formation, a property usually encoded on a 60 MDa plasmid [12], from which an aggregative adhesion (AA) DNA probe has been constructed [12]. For certain strains of EAggEC, the aggregative pattern of adhesion has been attributed to the expression of fimbriae. For example, aggregative adherence fimbriae (AAF)/ I have been described with a subunit size of 14 kDa [13], and also AAF/II which are antigenically distinct from AAF/I [14]; DNA probes have also been prepared to genes encoding production of both AAF/I and AAF/II. However, other studies have shown that for certain strains of EAggEC, adhesion to HEp-2 cells does not involve fimbriae [15–18] but outer membrane-associated proteins (MAPs) [15, 16, 18, 19]. Two forms of MAP have been recognized, which migrate on SDS-PAGE gels as proteins of 18 kDa [15, 16] or 20 kDa [19]. Strains which express the 18 kDa MAP hybridize with a DNA probe for the diffuse adhesion phenotype (DA) [18] and the AAF/II probe, in addition to the AA probe, whilst strains expressing a 20 kDa MAP hybridise with the AA gene probe only [19]. Both forms of MAP are highly charged and are involved directly in the adhesion of these bacteria to HEp-2 cells [16].

In a previous study [18], we showed that strains of EAggEC belong to a diverse range of serotypes and express considerable heterogeneity in surface properties such as cell surface hydrophobicity and charge, and the ability to haemagglutinate and form a pellicle. In the present study, strains of EAggEC, isolated from four outbreaks of diarrhoeal disease in England, were examined for a range of physical properties.

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MATERIAL AND METHODS

Bacterial strains

The 22 strains of EAggEC used in this study had been isolated from 4 outbreaks (A–D) of diarrhoeal disease in England, in 1994 [20]. Four strains were isolated from 4 patients involved with outbreak A (Table 1), 3 of these patients were male and 1 was known to have experienced a diarrhoeal illness. Nine strains were isolated from 9 patients from outbreak B (Table 1); 6 of these patients were female and 1 was male, and 5 patients were known to have experienced a diarrhoeal illness. Four strains were isolated from 4 patients from outbreak C (Table 1); 3 of these patients were female. Five strains were isolated from 5 patients from outbreak D (Table 1); 3 of these patients were female and 2 were male; all had experienced a diarrhoeal illness and 4 reported abdominal pain. All strains were serotyped by established methods [21], and stored on Dorset's egg agar slopes at room temperature.

HEp-2 adhesion tests

HEp-2 cell adhesion tests were performed as described previously [18]. Strains causing HEp-2 cell detachment were tested using formalin-fixed HEp-2 cells [17].

DNA probe tests

E. coli strains were tested by colony hybridization with the AA probe [12] and DA probe [22]. The AA probe consisted of a 1 kb *Pst*I–*Eco*RI fragment from pCVD432 and the DA probe was a 370 kb *Pst*I fragment within the *daa*C gene from pSLM852 [22]. The AAF/I probe was 0·9 kb *Pst*I–*Hin*cII fragment from pJPN61 [13] and AAF/II was a 1·7 kb *Eco*RI fragment from pJC2 [14]. Probes were labelled with fluorescein-dUTP and hybridizations were carried out at 68 °C for 18 h. The membranes were washed twice at room temperature with 2×SSC (sodium chloride/sodium citrate), 0·1% w/v/ SDS. Subsequent washes for the AA probe were 15 min at 54 °C with 5×SSC/0·1% (w/v) SDS and the conditions for the DA, AAF/I and AAF/II probes were 0·5% SSC/-0·1% (w/v) SDS at 68 °C for 15 min. Detection of hybrids was performed as described by the manufacturer (Amersham International, UK).

Bacterial charge

Bacterial cell surface charge was determined based on the method of Krishna and colleagues [23] as described previously [18]. Briefly, bacteria were grown overnight in L-broth and 1 μl volumes placed on 1 cm wide strips of Whatman No 1 filter paper. Strips were placed in a Multiphor 2117 electrophoresis apparatus (LKB, Uppsala, Sweden), with PBS electrode buffer, a current of 5 mA applied along the paper strips for 5 min. Following electrophoresis, strips were placed face down on to dried agar plates before incubation (37 °C, 18 h).

Table 1. *Strains of EAggEC used in this study*

			Hybridization		
Strain	Serotype	Outbreak	AA*	DA‡	AAF/II
E98529	O?:H18	A	+	–	–
E98527	O19:H-	A†	+	–	–
E97622	O113:H-	A	+	–	–
E96393	O125:H27	A	+	–	–
E97820	O62:H30	B§	+	–	–
E97590	O73:H13	B§	+	–	–
E96386	O73:H18	B	+	–	–
E96485	O134:H27	B	+	–	–
E96390	O?:H-	B§	+	–	–
E96487	O?:H-	B	+	–	–
E97900	O?:H-	B	+	–	–
E97819	O?:H27	B	+	–	–
E96483	O?:H33	B	+	–	–
E97472	O86:H34	C	+	–	–
E97462	O86:H34	C	+	–	–
E97470	O86:H34	C	+	–	–
E97474	O116:H27	C	+	+	+
E101408	O98:H-	D	+	–	–
E101621	O98:H-	D	+	–	–
E101396	O98:H-	D	+	–	–
E101406	O98:H-	D	+	–	–
E101402	O110:H-	D	+	+	+

* Strains hybridized with an aggregative adhesion probe.
† Strains causing detachment of HEp-2 cells during adhesion test.
‡ Strains hybridized with an aggregative adhesion fimbriae/II probe.
§ Strains hybridized with a diffuse adhesion probe.

Outer membrane proteins

Outer membranes (OMs) were prepared by sonicating bacteria and selectively solubilizing inner membrane proteins with Sarkosyl (Merck Ltd, Poole, UK)[15].

SDS–PAGE

Outer membrane proteins were separated by SDS–PAGE using the method of Laemmli [24]. Gels were cast and electrophoresis performed with an Atto mini-

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gel system (Genetic Research Instrumentation Ltd.). Outer membrane protein preparations (10 γ/lane) were loaded onto gels consisting of a 4·5% acrylamide stacking gel and a 12·5% acrylamide separation gel [15]. Protein profiles were either stained with Coomassie blue [18] or used for Western blotting.

Western blotting with rabbit antibodies

Outer membrane protein profiles were transferred onto nitrocellulose paper by the method of Towbin and colleagues. [25] and reacted with rabbit antibodies. Sera were prepared in New Zealand white rabbits to *E. coli* strains E57157A (O126: H27, AA-probe positive) and E57144A (O111: H21, AA probe-positive) which expressed 18 kDa and 20 kDa OMPs respectively [15]. The serum prepared to *E. coli* E57157A was adsorbed with *E. coli* strain E57157B (O126: H27, AA probe-negative), to enrich for antibodies to the 18 kDa protein [15]. Similarly, the serum prepared to *E. coli* E57144A was adsorbed with *E. coli* strain E57144B (O111: H21, AA probe-negative), to enrich for antibodies to the 20 kDa OMP [15].

Haemagglutination

Agar grown bacteria were suspended in PBS to give a concentration of 1 mg of cell mass per ml. Suspensions (50 μl) were placed in the wells of a white ceramic tile and mixed with an equal volume of sheep or rat erythrocytes (10% heparinized blood in PBS), with and without the addition of 1% (w/v) mannose. Agglutination was assessed following mixing on a gyrating shaker after 2 min.

Salt aggregation test

Bacterial hydrophobicity was determined by observing cell clumping in ammonium sulphate salt solutions with a range of concentration (0·02, 0·05, 0·5, 1·0, 2·0, 4·0 M) [26]. Agar grown bacteria were suspended in PBS to give a concentration of 1 mg of cell mass per 100 ml, and 50 μl volumes were mixed with an equal volume of ammonium sulphate solution in a 96 V-shaped well microtitre plate. Bacterial clumping was assessed after mixing by gyration for 30 min at room temperature. Strains of *E. coli* clumping in all concentrations of ammonium sulphate were considered hydrophobic.

Pellicle formation

Strains were examined for the ability to produce a pellicle following static growth in L-broth (37 °C, 16 h) [18, 27].

Haemolysin production

Strains were assessed for the ability to express a haemolysin, by preparing 'streak' plates on nutrient agar containing washed sheep erythrocytes (5% v/v). Plates were examined following incubation at 37 °C for 16 h.

Verocytotoxin production

Strains of *E. coli* were grown in trypticase soy broth (37 °C, 16 h) and used to prepare sterile culture supernatants. An aliquot of culture supernatant was incubated at 100 °C to produce a heat-treated control sample. These were added (25 μl) to wells in a 96 well tissue culture plate containing a Vero cell monolayer [28]. After a 4-day incubation (37 °C), Vero cells were stained with 10% (v/v) Giemsa stain (Merck Ltd.) [28] and examined for death and detachment of Vero cells.

E. coli heat-labile toxin production

Sterile culture supernatants were prepared as described for the Vero cell assay (above); however, 25 μl volumes were added to wells in a 96 well tissue culture plate containing a Y1 cell monolayer [29]. After overnight incubation (37 °C), Y1 cells were stained with 10% (v/v) Giemsa stain (Merck Ltd.) [29] and examined for morphological changes indicative of *E. coli* heat-labile toxin.

Transmission electron microscopy (TEM)

Bacteria were grown on nutrient agar and suspended in 1% (v/v) formalin in PBS prior to transfer to copper electron microscopy grids and 'negative' staining with 1% (w/v) ammonium molybdate and examination by transmission electron microscopy [16].

Heat-extraction of cellular proteins

Bacteria were grown on nutrient agar (37 °C, 16 h) and the cell mass from two agar plates (15 cm

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Table 2. *Properties of strains of EAggEC isolated from outbreaks A to D*

		Haemagglutination*						Siderophore production	
Strain	Serotype	Rat	Sheep	Pellicle†	Haemolysin‡	Charge§	Hydrophobicity¶	Enterochelin	Aerobactin
Outbreak A									
E98529	O?:H18	+	+	+	+	± ‖	4–0·05	+	+
E98527	O19:H-	−	−	−	−	±	4–0	+	−
E97622	O113:H-	−	−	−	−	±	4–0	No growth	
E96393	O125:H27	−	−	+	−	±	4–0·5	+	+
Outbreak B									
E97820	O62:H30	−	−	−	−	−	−	+	−
E9759	O73:H13	+	−	+	+	±	4–0	+	+
E96386	O73:H18	−	−	−	−	±	4–0	+	−
E96485	O134:H27	−	−	−	−	±	−	+	−
E96390	O?:H-	+	−	+	+	±	4–0	+	+
E96487	O?:H	−	+	−	−	±	4–2	+	−
E97900	O?:H	−	+	−	−	±	4 only	+	−
E97819	O?:H27	−	−	−	−	−	4–2	+	−
E96483	O?:H33	−	−	−	−	−	4 only	+	−
Outbreak C									
E97472	O86:H34	+	−	+	−	±	4–0·5	+	+
E97462	O86:H34	+	−	−	−	±	4–0·5	+	+
E97470	O86:H34	+	−	+	−	−	4–0	+	+
E97474**	O116:H27	+	−	+	−	±	4–0	+	−
E101408	O98:H-	+	−	−	−	−	4 only	+	−
E101621	O98:H-	+	−	+	−	±	4–0	+	−
E101396	O98:H-	+	−	−	−	−	4 only	+	−
E101406	O98:H-	+	−	−	−	±	4–0	No growth	
E101402**	O110:H-	+	−	+	+	±	4–0	+	+

* Haemagglutination of erythrocytes following growth on nutrient agar; †Pellicle formation in L-broth; ‡Haemolysis on sheep blood agar; §Bacterial surface charge; ‖Bacteria migrated to anode and cathode; ¶Molar concentrations of ammonium sulphate causing bacterial aggregation; **Expressed an 18 kDa MAP.

diameter) was suspended in 500 μl PBS prior to incubation at 60 °C for 30 min. Whole cells were sedimented (12 500 g, 5 min) and proteins remaining in the supernatant were examined by SDS–PAGE.

Siderophore production

Siderophore production was induced by growing strains of *E. coli* were grown in Tris-succinate medium (TSM) or TSM containing nicotinic acid (Sigma; 20 mg/litre; 48 h, 37 °C, shaking 120 rpm). The siderophore enterobactin was detected using the Arnow assay [30] and involved mixing equal volumes of sterile culture supernatant with 0·5M HCl, nitrite molybdate reagent and 1M NaOH . The pink colour, indicative of the presence of enterobactin, was quantified by measuring absorbance at 505 nm. The siderophore aerobactin was detected using ferric perchlorate [31]. A volume of sterile culture supernatant was mixed with an equal volume of ferric perchlorate reagent, a reddish-brown colour, indicative of the presence of aerobactin, was quantified by measuring the absorbance at 480 nm.

Plasmid profiling

Plasmid DNA was prepared by the alkaline extraction method of Birnboim & Doly [32], separated on 0·7% agarose gels with a borate buffer (100 V, 4 h) and stained with ethidium bromide. Plasmid sizes were estimated using *E. coli* strain 39R621, carrying plasmids of 98, 42, 23·9, 4·6 MDa, and *Salmonella typhimurium* strain P100836 carrying a plasmid of 60 MDa [33].

Antimicrobial resistance typing

Antibiotic resistance typing was performed on agar plates by a breakpoint method [33]. The antibiotics used in routine screening were ampicillin (A), chloramphenicol (C), colomycin (Co), ciprofloxacin (Cp), furazolidone (Fu), gentamicin (G), kanamycin (K), nalidixic acid (Nx), streptomycin (S), sulphathiazole (Su), tetracycline (T), trimethoprim (Tm). Strains were grown on agar plates containing a range of antibiotic concentrations up to 128 μg/l [34].

RESULTS

Of the 22 strains expressing an aggregative phenotype (Table 1), only strain E97474 (O116:H27) from outbreak C and strain E101402 (O110:H-) from outbreak D hybridized with probes for the diffuse adhesion phenotype (DA) and expression of the AAF/II genotype (Table 1). Four strains, one from group A and three from group B, caused detachment of HEp-2 cell monolayers. None of the strains expressed VT or LT.

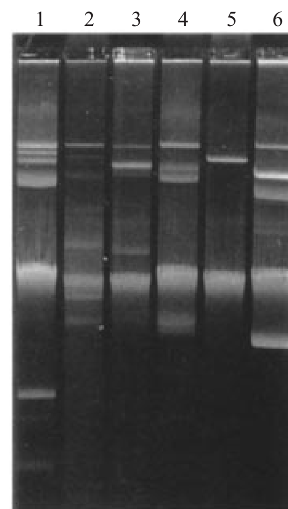

Fig. 1. Plasmid profiles prepared from strains isolated from outbreak A. Profiles shown in lanes 1, 2 and 4 contained plasmids of 126 MDa which hybridized with the DNA probe for the AA phenotype, this probe hybridized with a 63 MDa plasmid in lane 3. Lanes 5 and 6 contain standard plasmid profiles prepared from *S. typhimurium* strain P100836 (60 MDa plasmid) and *E. coli* 39R621 (98, 42, 26, 4·6 MDa).

Outbreak A

The four strains of EAggEC isolated from this outbreak belonged to four distinct serotypes (Table 2). One strain (E98529) agglutinated rat and sheep erythrocytes in the presence of mannose, formed a pellicle, and produced haemolysin and aerobactin; this strain was also hydrophobic and comprised a population of bacteria with positive and negative charge (Table 2). Strains E98527 and E97622 were hydrophobic, did not haemagglutinate and did not express a haemolysin or aerobactin. Strain E96393 was hydrophobic, formed a pellicle and produced aerobactin (Table 2). All four strains carried plasmids (Fig. 1, Table 3). Three strains carried a plasmid of 126 MDa, hybridising with the AA gene probe (Table 3). *E. coli* strain E96393 carried a plasmid of 63 MDa hybridizing with the AA probe (Fig. 1, Table 3).

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Table 3. *Plasmid carriage and antibiotic resistance properties of EAggEC isolated from outbreaks A to D*

Strain	Serotype	Plasmids (MDa)*	Antibiotic resistance
Outbreak A			
E98527	O19:H-	126†, 93, 66, 35, 3·8, 3·5, 2·9	A
E97622	O113:H-	126†, 66, 40, 14·4, 5	A, C, Su
E96393	O125:H27	126, 63†, 11·5	A, C, Su
E98529	O?:H18	126†, 46, 12, 5	A, C, Su, pS
Outbreak B			
E96386	O73:H18	44·7†	None
E97820	O62:H30	61·7†, 14·4, 4	A, pS, Su, T, Tm
E97819	O?:H27	61·7†, 55, 51·3, 3·7	A, pS, Su
E96483	O?:H33	61·7†, 55, 51·3, 35·5, 3·5	A, pS, Su
E96485	O134:H27	61·7†, 55, 51·3, 12·9, 5·4, 3·8	A, C, pS, Su
E96390	O?:H-	44·7, 39·8†, 19, 12·9, 4·5, 4	PS
E97590	O73:H13	35·4†, 19, 12·9, 4·5, 4	PS
E96487	O?:H-	61·7†, 58·9, 51·3, 39·8, 3·7, 3·4, 3·2	A, pS, Su
E97900	O?:H-	61·7†, 58·9, 51·3, 39·8, 3·7, 3·4, 3·2	PS
Outbreak C			
E97472	O86:H34	44·7†, 6·3	None
E97468	O86:H34	36·3†, 6·3	None
E97470	O86:H34	44·7†, 6·3	None
E97474	O116:H27	89·1, 74·1, 56·2†, 6·3	A
Outbreak D			
E101408	O98:H-	67·6†, 35·5	None
E101621	O98:H-	56·2†, 35·5	None
E101396	O98:H-	67·6†, 35·5	None
E101406	O98:H-	67·6†, 35·5	None
E101402	O110:H-	85·1†, 47·9	None

* Estimated sizes of plasmids carried by each strain (MDa), †Plasmid hybridizing with probe for aggregative adherence. A, ampicillin; C, chloramphenicol; pS, partial streptomycin ($< 128 \mu g/l$ but $> 64 \mu g/l$); Su, sulfathiazole; T, tetracycline; Tm, trimethoprim.

Outbreak B

Of the 9 strains of EAggEC isolated from outbreak B, 4 strains belonged to 1 of 3 serogroups (Table 2). The remaining five strains expressed unidentifiable somatic antigens. Four strains agglutinated rat erythrocytes in the presence of mannose. Two of these strains formed a pellicle, expressed haemolysin and aerobactin (Table 2). Six strains comprised a mixed population of bacteria carrying either positive or negative charge, and three strains were hydrophobic. Strains E97820 and E96390 expressed fimbriae, as observed by electron microscopy, with protein subunits of 19 kDa (Fig. 2). These fimbrial subunits did not bind rabbit antibodies prepared to the 18 kDa MAP [15, 18]. The nine strains possessed a variety of plasmids (Fig. 3, Table 3). Six strains possessed plasmids of 61·7 MDa hybridizing with the AA probe. Strains E96487 and E97900 had identical plasmid profiles. Three strains carried plasmids of 35·4, 44·7 and 39·8 MDa respectively, which hybridized with the AA probe. Strain E96386 was sensitive to all the antibiotics tested, the remaining strains were resistant to at least one antibiotic (Table 3).

Outbreak C

Three of the four strains of EAggEC isolated from this outbreak belonged to serotype O86: H34 and produced aerobactin. All four strains agglutinated rat erythrocytes in the presence of mannose and were hydrophobic, but only three formed a pellicle and comprised populations of bacteria with either positive or negative charge (Table 2). Strain E97470 carried a negative charge only. Strain E97474 expressed an 18 kDa MAP, which bound rabbit antibodies prepared to the 18 kDa MAP [15, 18]. The four strains possessed plasmids of 44·7, 36·3 or 89·1 MDa. which hybridized with the AA gene probe (Fig. 4, Table 3). Two strains belonging to serotype O86:H34 had 44·7 MDa plasmids and all three strains belonging to this

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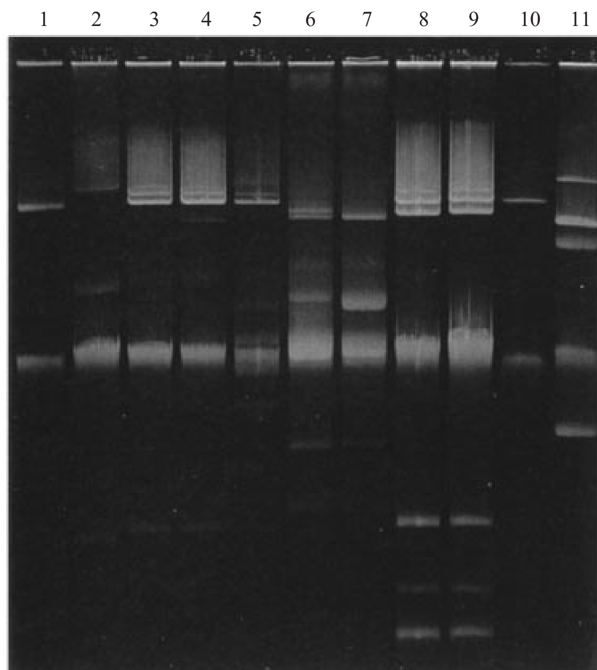

Fig. 2 (a) Electron micrograph showing fimbriae expressed by *E. coli* strain E97590 (bar = 10 nm). (b) SDS–PAGE profile showing the 19 kDa protein subunit (arrowed) of fimbriae expressed by *E. coli* strain E97590.

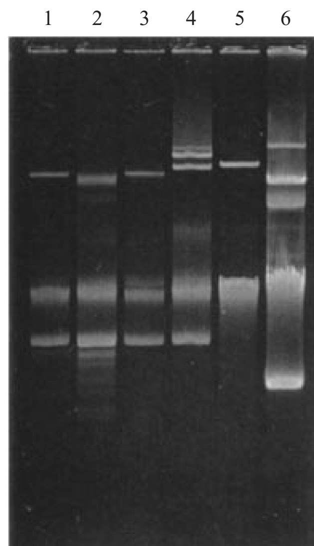

Fig 3. Plasmid profiles prepared from strains of EAggEC isolated from outbreak B. Lanes 2–5, 8 and 9 contained plasmid of 61·7 MDa, which hybridized with the AA gene probe. This probe hybridized with plasmid of 44·7 MDa in lanes 1 and 6, and a plasmid of 35·4 in lane 7. Lanes 8 and 9 contain standard plasmid profiles prepared from *S. typhimurium* strain P100836 (60 MDa plasmid) and *E. coli* 39R621 (98, 42, 26, 4·6 MDa).

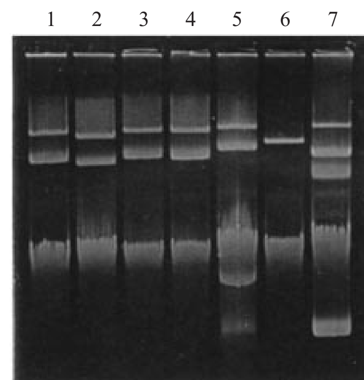

Fig. 4. Plasmid profiles prepared from strains of EAggEC isolated from outbreak C. Lanes 1 and 3 contained plasmids of 44·7 MDa, which hybridized with the AA gene probe. This probe hybridized with plasmids of 36·3 and 56·2 MDa in lanes 2 and 4. Lanes 5 and 6 contain standard plasmid profiles prepared from *S. typhimurium* strain P100836 (60 MDa plasmid) and *E. coli* 39R621 (98, 42, 26, 4·6 MDa).

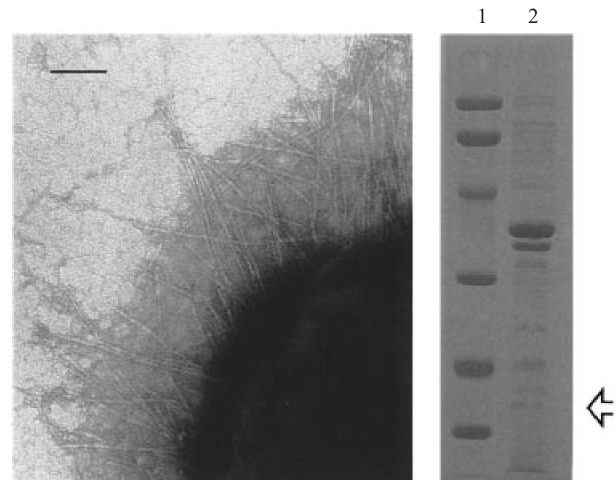

Fig. 5. Plasmid profiles prepared from strains of EAggEC isolated from outbreak D. Lanes 1, 3 and 4 contained plasmids of 67·6 MDa, which hybridized with the AA gene probe. This probe hybridized with plasmids of 56·2 and 85·1 MDa in lanes 2 and 5 Lanes 6 and 7 contain standard plasmid profiles prepared from *S. typhimurium* strain P100836 (60 MDa plasmid) and *E. coli* 39R621 (98, 42, 26, 4·6 MDa).

serotype carried a plasmid of 6·3 MDa and were drug sensitive.

Outbreak D

Four of the five strains of EAggEC isolated from outbreak D belonged to serotype O98:H-. All five strains agglutinated rat erythrocytes in the presence of

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mannose and two formed a pellicle (Table 2). Strain E101402 made aerobactin and expressed a haemolysin, and produced an 18 kDa MAP, which bound rabbit antibodies, prepared to the 18 kDa MAP as described previously [15, 18]. All strains carried two plasmids each (Fig. 5, Table 3). Strains E101408, E101396 and E101406 (all O98:H-) carried a plasmid of 67·6 MDa, which hybridized with the AA gene probe. The four strains belonging to serotype O98:H- carried a plasmid of 35·5 MDa and were drug sensitive. Strains E101621 and E101402 carried plasmids of 56·2 and 85·1 MDa respectively, which hybridized with the AA probe.

DISCUSSION

Although all four strains from outbreak A were hydrophobic, this did not correlate with the ability of strains to agglutinate rat and sheep erythrocytes, or express a pellicle. Two strains were able to produce the siderophore aerobactin, a property usually associated with strains of *E. coli* causing extra-intestinal infections [35]. The nine strains of EAggEC isolated from outbreak B, also belonged to diverse serotypes. Two of the three non-motile strains with untypable somatic antigens were thought to be similar since strains E96487 and E97900 had similar plasmid profiles; however, these strains expressed distinct antibiotic resistance patterns.

Strains of EAggEC isolated from outbreaks C and D each comprised EAggEC with a single predominant serotype. However, within both major serotypes strains expressed variation in properties such as pellicle formation, bacterial charge and plasmid carriage.

The fimbriae detected during this study were not genetically related to AAF/I or AAF/II since these strains did not hybridize with the specific DNA probes. However, the observation that only 2 of the 22 strains of EAggEC expressed fimbriae suggests that fimbriae do not play a major role in the HEp-2 adhesion process, although the importance of inducible fimbrial expression remains to be determined. Nevertheless, since only two strains of EAggEC expressed MAPs of 18 kDa, it would seem likely that EAggEC express a range of adhesion mechanisms, in addition to fimbriae and MAPs, as a prelude to the cell arrangement recognized as the 'stacked brick' formation.

Previous studies have demonstrated the diversity of serotypes express by EAggEC, and this heterogeneity was also observed with the present study. One possible explanation for this diversity may be the rapid transfer of the genes encoding the EAggEC phenotype to strains of commensal *E. coli*. Since we continue to use a single attribute to categorize the EAggEC, the relationship between pathogenic EAggEC and commensal *E. coli* acquiring the EAggEC genes will remain obscure. The identification of a single EAggEC attribute, other than the adhesion pattern, would greatly facilitate our understanding of the enteroaggregative *E. coli*.

From the studies presented here and elsewhere [18, 19], it would appear that the only common attribute is the ability to adhere to HEp-2 cells in an aggegative pattern.

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