Adhesion of clinical and environmental *Aeromonas* isolates to HEP-2 cells

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Summary. A total of 63 *Aeromonas* strains isolated from diarrhoeal faeces or water samples were tested for adhesion to HEP-2 cells. An association between diarrhoea and high level adhesion was observed in that 12 of the 34 faecal isolates and none of the 29 environmental isolates yielded > 20 bacteria per HEP-2 cell in the adhesion assay. The proportion of high adherers was significantly greater for *A. sobria* (57%) than for *A. hydrophila* isolates (19%). Three of the eight faecal *A. caviae* isolates were also found to be high adherers. All of the environmental isolates were heavily pilated with pili having a mean diameter of 5 nm and a mean length of 420 nm; these were termed type-S pili. Of the 34 faecal isolates, 32% possessed S pili, and 68% were lightly pilated with up to 15 thin, flexible type-L pili, of mean diameter 2.5 nm and mean length 960 nm. Type-L pilation was associated with a high level of HEP-2 cell adhesion, and was more common in *A. sobria* and *A. caviae* than in *A. hydrophila* isolates. These results suggest that adherence to HEP-2 cells is a useful model for the investigation of *Aeromonas* enteropathogenicity, and that adhesion may be pilus-mediated in this organism.

Introduction

Recent studies have implicated *Aeromonas* species as important enteric pathogens in man, both in children (Gracey *et al.*, 1982; Janda *et al.*, 1983) and adults (Pitarangsi *et al.*, 1982; Goodwin *et al.*, 1983). Strains of *A. hydrophila* and *A. sobria* possess characteristics associated with virulence of the better-defined enteropathogenic bacteria, including a cholera toxin cross-reactive factor (Campbell and Houston, 1985), cytotoxic enterotoxin, cytotoxin and haemolysin (Pitarangsi *et al.*, 1982; Chakraborty *et al.*, 1984). The role of these toxins in the pathogenicity of *Aeromonas* spp. is not yet clear. *Aeromonas* strains have also been shown to invade HEP-2 cells grown in culture, and this property of invasiveness is most common among isolates from patients with dysentery-like infections (Lawson *et al.*, 1985; Watson *et al.*, 1985). The latter *Aeromonas* strains may thus resemble *Shigella* spp. or enteroinvasive *Escherichia coli* in their pathogenesis, while the former may be more similar to enterotoxigenic *E. coli* or to *Vibrio cholerae* (Levine *et al.*, 1983).

For both invasive and non-invasive, enterotoxi-

genic bacteria, the ability to adhere to the intestinal mucosa is an essential early step in colonisation and development of diarrhoeal disease (Levine *et al.*, 1983). One of the most commonly found mechanisms of bacterial adhesion is mediated by the filamentous bacterial structures termed fimbriae or pili. Other mechanisms of adhesion depend on bacterial LPS, flagella, or non-fimbrial proteins (Levine *et al.*, 1983). *Aeromonas* strains are capable of adhesion to human erythrocytes and buccal epithelium (Atkinson and Trust, 1980; Daily *et al.*, 1981) and to rabbit intestinal brush borders (Levett and Daniel, 1981), the former type of adhesion being associated with the presence of pili. Haemagglutination of human erythrocytes by *Aeromonas*, which is not inhibited by fucose, galactose or mannose, shows a strong correlation with diarrhoeal isolates (Burke *et al.*, 1984a), but the nature of the cell-associated haemagglutinin is not known.

Adhesion to HEP-2 cells has recently provided a useful model for the study of pathogenicity in *E. coli* (Cravioto *et al.*, 1979; Knutton *et al.*, 1984; Tavendale and Old, 1985). Tissue culture adhesion assays are also reported for *Salmonella* (Mintz *et al.*, 1983; Tavendale *et al.*, 1983), *Campylobacter* (Cinco *et al.*, 1984) and *Yersinia* (Lassen and Kapperud, 1986). We have studied the ability of *Aeromonas* spp. to adhere to HEP-2 cells, and have

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examined the association between this property and the presence of pili.

Materials and methods

Bacterial strains

Sixty-three strains of motile aeromonads comprising 32 *A. hydrophila*, 23 *A. sobria*, and 8 *A. caviae* isolates, identified according to Popoff's criteria (Popoff, 1984), were used in this study. There were 34 faecal isolates obtained from patients with diarrhoea and 29 isolates from water samples, all from Western Australia.

Bacterial preparations

Stock cultures of *Aeromonas* spp. were stored at room temperature in maintenance medium containing: agar 5 g, sodium chloride 5 g, Bacto-Peptone 01118 (Difco) 2.5 g, and peptone L34 (Oxoid) 2.5 g, in 200 ml of phosphate buffer (Na_2HPO_4 2.8 g, K_2HPO_4 1.3 g/L, pH 6.7). All isolates were grown on blood agar (BA) plates incubated at 37°C for 18–24 h. To prepare overnight cultures, single colonies were seeded into 10 ml of heart infusion broth (HIB) (Difco) and grown statically for 16–18 h at 37°C. Log-phase cultures were prepared by adding 0.5 ml of the overnight culture to 10 ml of HIB followed by static incubation for 3–3.5 h at 37°C.

HEp-2 cell culture

The HEp-2 cell line (Commonwealth Serum Laboratories, Melbourne, Australia) was maintained in Dulbecco's Modified Eagle Medium (Gibco Laboratories, OH, USA) without antibiotics but with fetal calf serum 10% in 250 ml of Lux tissue culture flasks (Miles Laboratories Inc., USA).

Adhesion assay

Log-phase cultures of *Aeromonas* spp. were diluted in phosphate buffer solution supplemented with Dulbecco's Mineral Salt Solution (Oxoid) 0.5% (PBSS) to a concentration of $(2-3) \times 10^6$ cfu/ml as determined by serial dilution and plating on to BA. Semiconfluent monolayers of HEp-2 cells grown for 20 h on glass coverslips in 24-well trays (Costar Tissue Culture Cluster, Cambridge, MA, USA) were washed with 2 ml of PBSS. Bacterial cultures were added in 1-ml volumes to the wells, and three wells were used for each test. After incubation for 90 min at 37°C in an atmosphere of CO_2 5% in air, non-adherent bacteria were removed from the infected monolayers by washing four times *in situ* with 2 ml of PBSS. The cells were fixed in 1 ml of a methanol:acetic acid (3:1) mixture for 5 min; the coverslips were then mounted on glass microscope slides and stained by Gram's method.

Adhesion was assessed by bright-field microscopy at a

magnification of $1000\times$ under oil immersion. For each assay bacteria adhering to 25 randomly selected infected cells from each of three monolayers were counted. The number of bacteria per infected cell was expressed as the mean $\pm$ standard error of the mean (SEM), of a total of 75 cells for each assay. The proportion of HEp-2 cells with adherent bacteria was also determined by viewing 100 cells from each of the three monolayers. The average number of bacteria/cell for the three monolayers was calculated from these results by multiplying the number of bacteria/infected cell by the proportion of infected cells.

Haemagglutination

Patterns of haemagglutination of human group-O cells with L-fucose, D-galactose and D-mannose (Sigma) were determined as described by Burke *et al.* (1984a).

Removal of pili

Piliated *Aeromonas* strains were treated by a mechanical blending process as described by Daily *et al.* (1981). One-half of a log-phase suspension of each isolate was assayed for binding capability in the HEp-2 cell adhesion assay as previously described. The remainder was subjected to four 30 s blending cycles at top speed in a Tosco blender (Thomas Optical and Scientific Co. Pty. Ltd.). Between each cycle, the cultures were cooled for 15 s intervals in an ice bath. After centrifugation at 12000 *g* for 15 min the bacteria were washed once in PBSS, diluted and assayed for adhesion to HEp-2 cells and haemagglutination. Bacterial viability was confirmed by viable counts on BA at 37°C. Loss of pili, and cell-surface integrity were confirmed by transmission electronmicroscopy.

Effect of trypsin treatment

To investigate the nature of the external polymer mediating the adhesion of *Aeromonas* spp., trypsin treatment was employed. Bacteria prepared as for an adhesion assay were incubated in trypsin (Difco) 1 mg/ml for 30 min at 37°C. Residual trypsin was removed by washing the bacteria once in 10 ml of PBSS and the resuspended bacteria were assayed for adhesion to HEp-2 cells and haemagglutination. Adhesion of the treated bacteria was compared to that of control bacteria incubated in PBSS for 30 min.

Electronmicroscopy

All strains of *Aeromonas* were examined for the presence of pili by transmission electronmicroscopy. Formvar-coated grids were placed for 1 min on a drop of bacterial suspension from a log phase culture. The grids were blotted dry with filter paper and stained with sodium silicotungstate (3%, pH 7.3) for a further 1 min. They were again blotted and examined in a Philips 301 transmission electronmicroscope.

Bacterial hydrophobicity

Bacterial adherence to polystyrene was used as a simple method of screening for bacterial hydrophobicity, as described by Rosenberg (1981). Three drops of an overnight bacterial culture were placed separately on a BA plate. After incubation for 24 h at 37°C, a flat polystyrene disk cut from a petri dish (Bunzl Australia Ltd., Hendon, S.A., Australia) was pressed on to the BA plate. The replica of the colonies obtained on the polystyrene surface was vigorously washed for 1 min with tap water, fixed in methanol and stained with crystal violet. Non-adherent cells were removed during the washing procedure so that the disk was not stained by the crystal violet where these had been, whereas adherent cells remained bound to the polystyrene and the disk was clearly stained in these areas.

Results

HEp-2 adhesion assay

Preliminary experiments revealed that adhesion of *Aeromonas* was maximal at 37°C and pH 7.8, and no Ca⁺⁺ or Mg⁺⁺ requirement was detected for two test strains (*A. sobria* 3385 and *A. sobria* 8565). Adhesion was most conveniently quantitated with a bacterial inoculum of 10⁶ cfu/ml and an incubation time of 90 min. With higher bacterial numbers or with longer incubation times, as reported for recent HEp-2 adhesion assays (Cravioto *et al.*, 1979; Mintz *et al.*, 1983) some *Aeromonas* strains adhered in clumps to the glass coverslips, and counting of bacteria adhering to HEp-2 cells was difficult. Also, for some strains, cytopathic effects became evident on the HEp-2 monolayers, with cells rounding up or even detaching from the coverslip as time or bacterial inoculum increased. Thus for all subsequent adhesion assays the conditions detailed above were used. Log-phase (3–3.5 h) cultures were used in all experiments because for seven test strains adherence of late exponential phase bacteria was on average 76% higher than that of overnight cultures.

All the 63 *Aeromonas* isolates tested adhered to HEp-2 cells to some degree, as shown in table I. The majority of isolates were classified as low adherers, with < 10 bacteria/HEp-2 cell; the mean level was 5 SEM 2 bacteria/cell. High adherers had ≥ 10 bacteria/cell; the maximum adhesion level detected was 46 bacteria/cell. The mean level was 21.5 SEM 7.5 bacteria/cell. To ensure the reliability of this classification, adhesion assays were performed on four separate occasions with three low adherers and four high adherers. An analysis of variance showed that, for each strain, there was no

Table I. Adhesion of *Aeromonas* to HEp-2 cells in relation to species and source of isolate

Adhesion (bacteria/cell)	Number of strains				
	<i>A. hydrophila</i>		<i>A. sobria</i>		<i>A. caviae</i>
	Faecal	Water	Faecal	Water	Faecal
> 10*	10	16	2	8	5
10–20†	1	3	3	2	1
> 20†	2	0	8	0	2

* Low level adhesion to HEp-2 cells.

† High level adhesion to HEp-2 cells.

significant difference in adhesion levels on different days ($p > 0.05$).

Adhesion in relation to species and source of isolate

The distribution of adhesion in relation to species is shown in table I. Of the 23 *A. sobria* isolates, 13 (57%) were high adherers, with eight (35%) giving > 20 bacteria/HEp-2 cell. Only six (19%) of the 32 *A. hydrophila* isolates were high adherers, and two (6%) adhered in the > 20 bacteria/cell range. The proportion of high adherers was significantly greater for *A. sobria* than for *A. hydrophila* strains (χ^2 test, $p < 0.005$). For *A. caviae*, three of eight isolates were high adherers.

Table I also summarises adhesion in relation to source of isolate. The majority (83%) of the 29 isolates from water samples were low adherers, regardless of species. Five water isolates were high adherers, but none adhered above the 20 bacteria/cell level. A significantly greater proportion of the 34 faecal isolates were highly adhesive (χ^2 test, $p < 0.01$); 17 (50%) were high adherers with 12 (35%) showing > 20 bacteria/cell.

Piliation of *Aeromonas* isolates

All of the 63 isolates examined by transmission electronmicroscopy were pilated. Two patterns of piliation were observed. Twenty three (37%) of the isolates were lightly pilated with long, thin, apparently flexible pili, which were designated type-L pili. The mean pilus length of four isolates chosen for detailed measurements was 962 SEM 44 nm, and the mean pilus diameter was 2.5 SEM 0.1 nm. There were between 1 and 15 type-L pili visible per bacterial cell (fig. 1). In contrast, in the other 40

Fig. 1. Electronmicrographs showing type-L-piliated isolates of *Aeromonas* negatively stained with sodium silicotungstate 3% (pH 7.3). (A) *A. sobria* strain 3700, (B) *A. sobria* strain 3754, (C) *A. sobria* strain 3385. Arrows indicate type-L pili. Bar, 0.2 μ m.

isolates (63%) hundreds of pili per cell were seen. These were shorter, thicker and straighter and were designated type-S pili (fig. 2). The mean pilus length of four heavily pilated strains was 422 SEM 18 nm, and the mean pilus diameter was 4.9 SEM 0.2 nm.

The distribution of pilus type in relation to species and source of isolate is shown in table II. All 29 environmental isolates were heavily pilated with type-S pili, irrespective of the species of *Aeromonas*. Strains exhibiting type-L light piliation were all faecal isolates, comprising four (31%) of the 13 *A. hydrophila*, 11 (85%) of the 13 *A. sobria*, and all of the eight *A. caviae* isolates. Significantly more *A. sobria* and *A. caviae* isolates than *A. hydrophila* isolates possessed type-L pili (χ^2 test, $p < 0.005$).

Piliation and adhesion of *Aeromonas* to HEP-2 cells

Pilus morphology correlated well with adhesion levels (table III). Seventeen (74%) of the strains with light, type-L piliation were classified as high adherers, with ≥ 10 bacteria/HEP-2 cell. Conversely, type-S heavy piliation was associated with low HEP-2 cell adhesion. Thirty-five (88%) of the type-S strains were low adherers while of the five (12%) high adherers, none adhered at a level of

> 20 bacteria/cell. The difference in adhesion ability between the L- and S-piliated isolates was significant (χ^2 test, $p < 0.005$).

To assess the possible role of pili in HEP-2 cell adhesion, pili were removed from three of the type-L-piliated, highly adherent strains by mechanical shearing. Adherence was reduced by between 74% and 82% (table IV). The same three strains were treated with trypsin to determine the susceptibility of the adhesin(s) to a protease. Adhesion ability was reduced in each case, by between 59% and 83% (table IV).

Haemagglutination pattern

The haemagglutination patterns for all isolates are shown in table V. The most common pattern for both L and S pilated strains was $F^+ G^- M^+$. There was a significantly greater proportion of non-

Table II. Piliation of *Aeromonas* spp. in relation to species and source of isolate

Pilus type	Number of strains				
	<i>A. hydrophila</i>		<i>A. sobria</i>		<i>A. caviae</i>
	Faecal	Water	Faecal	Water	Faecal
L	4	0	11	0	8
S	9	19	2	10	0

L = long, thin, flexible pili, with < 15 pili/cell.
S = short, thick, straight pili, with many pili/cell.

Table III. Relation of piliation to adhesion ability of *Aeromonas*

Adhesion	Number (%) of strains with	
	type-L pili	type-S pili
Low	6 (26)	35 (88)
High	17 (74)	5 (12)

Low level adhesion = < 10 bacteria/HEP-2 cell.
High level adhesion = ≥ 10 bacteria/HEP-2 cell.

Table IV. Effects of shearing and trypsin treatment on adhesion of *Aeromonas* to HEP-2 cells

<i>A. sobria</i> strain no.	Adhesion expressed as number of bacteria/HEP-2 cell*			
	Before shearing	After shearing†	Before trypsin	After trypsin‡
3385	19.9	5.1 (74)	22.1	5.2 (76)
3767	36.6	9.5 (74)	34.3	13.9 (59)
8565	25.6	4.6 (82)	26.8	4.5 (83)

* Numbers in parentheses indicate the percentage reductions in adhesion.

† Bacteria were subjected to four 30-s cycles in a blender at 4°C.

‡ Trypsin 1 mg/ml for 30 min at room temperature.

Table V. Haemagglutination patterns in relation to piliation of *Aeromonas*

Agglutination pattern	Number (%) of strains with	
	type-L pili	type-S pili
$F^- G^- M^-$	0 (0)	1 (2)
$F^+ G^- M^-$	1 (4)	0 (0)
$F^- G^+ M^-$	1 (4)	0 (0)
$F^+ G^- M^+$	11 (48)	33 (83)
$F^- G^+ M^+$	0 (0)	1 (2)
$F^+ G^+ M^+$	2 (9)	2 (5)
No agglutination	8 (35)	3 (8)

F = L-fucose; G = D-galactose; M = D-mannose.

+ Inhibition of haemagglutination; - no inhibition.

Fig. 2. Electronmicrographs showing type-S-piliated isolates of *Aeromonas* negatively stained with sodium silicotungstate 3% (pH 7.3). (A) *A. hydrophila* strain 5365, (B) *A. hydrophila* strain L15, (C) *A. hydrophila* strain L7. Many pili project from the surface of each bacterium. Bar, 0.2 μ m.

agglutinating strains among the L-pilated bacteria (35%) than among the S-pilated bacteria (8%) (χ^2 test, $p < 0.05$). Shearing and trypsin treatment did not affect the level of haemagglutination for any of the 25 isolates tested, which included both L and S pilated bacteria.

Cell surface hydrophobicity

Sixty-one isolates were screened for cell-surface hydrophobicity, by determining their adhesion to a polystyrene surface (table VI). Isolates with type-L piliation tended to be non-hydrophobic (87%), while type-S piliation was equally distributed between hydrophobic and non-hydrophobic isolates. The majority (75%) of the type L-pilated, non-hydrophobic isolates were highly adhesive to HEP-2 cells. Of the *A. sobria* isolates, 83% were non-hydrophobic, regardless of pilus type, compared with 43% of *A. hydrophila* isolates.

Discussion

In this study we have shown that *Aeromonas* strains adhere to HEP-2 cells in tissue culture, in an adhesion assay that is relatively rapid, easy to perform, and highly reproducible. Such tissue culture assays have been used to identify and characterise the adhesive mechanisms of various bacteria, including *E. coli* (Knutton *et al.*, 1984; Tavendale and Old, 1985), *Salmonella* (Mintz *et al.*, 1983; Tavendale *et al.*, 1983), *Campylobacter* (Cinco *et al.*, 1984) and *Yersinia* (Lassen and Kapperud, 1986). The clinical relevance of these adhesins is sometimes unclear, firstly because bacterial interaction with the intestinal mucosa is complex, and secondly because it cannot be assumed that tissue culture cells derived by cell transformation possess the same surface receptors for bacterial adherence

as those found on human intestinal cells *in vivo* (Freter and Jones, 1983). However, in several cases a correlation between adhesion *in vitro* and bacterial pathogenicity has been demonstrated (Cravioto *et al.*, 1979; Bergman *et al.*, 1981; Mathewson *et al.*, 1985).

For *Aeromonas* strains, our data suggest an association between the ability to cause diarrhoea and high-level adhesion to HEP-2 cells. A significantly greater proportion of diarrhoeal isolates than environmental isolates were highly adhesive—none of the isolates from water samples adhered at above the 20 bacteria/HEP-2 cell level. Similarly, in some studies, haemolysin and enterotoxin activity in *Aeromonas* correlate with origin of the strains from faeces rather than from the environment (Daily *et al.*, 1981; Burke *et al.*, 1984b).

An association between species and adhesive ability was also evident from this study. A significantly greater proportion of *A. sobria* than *A. hydrophila* isolates were high adherers. It has been suggested previously that the pathogenicity of these species may not be identical. Daily *et al.* (1981) suggested that *A. sobria* is more virulent, because of mouse lethality, production of cytotoxin and other exoenzymes, and increased adherence to buccal cells. The ability to invade HEP-2 cells has been suggested to be an important virulence factor in *Aeromonas* spp. isolated from patients with dysenteric symptoms (Lawson *et al.*, 1985), and *A. sobria* strains are more commonly invasive than are *A. hydrophila* strains (Watson *et al.*, 1985). Selective distribution of the two species is also reported; in one case 85% of diarrhoeal isolates of *Aeromonas* were *A. sobria* (Burke *et al.*, 1984b), and in another study *A. sobria* was most commonly isolated from the gastro-intestinal tract, whereas *A. hydrophila* was equally common in wounds (Janda *et al.*, 1984). Thus the high adhesive ability of *A. sobria* faecal isolates we observed may reflect a greater enteropathogenic potential in this species.

A. caviae has been considered to be of little enteropathogenic significance, due to the lack of any known virulence factor such as enterotoxin, cytotoxin, or invasive ability (Gracey *et al.*, 1982; Janda *et al.*, 1983; Watson *et al.*, 1985). However, a recent report suggests that in some geographical areas *A. caviae* isolation may be strongly associated with diarrhoeal disease (Altwegg, 1985). It is interesting in this regard that we found three out of eight faecal isolates of *A. caviae* to be highly adherent to HEP-2 cells. These isolates may possess virulence mechanisms analogous to those of the enteropathogenic *E. coli* strains that neither produce enterotoxin nor are invasive (Levine *et al.*,

Table VI. Hydrophobicity in relation to piliation and HEP-2 cell adhesion ability of *Aeromonas*

Hydrophobicity	Number of strains with			
	Pilus type L†		Pilus type S	
	Low adherers†	High adherers†	Low adherers	High adherers
+	1	2	19	1
—	5	15	16	2

Hydrophobicity (+) = adhesion to a polystyrene surface.

† See legend to table III.

1983) but that bind to HEP-2 cells (Cravioto *et al.*, 1979).

All 63 *Aeromonas* strains in this study were pilated, either with type-S pili or type-L pili. This has not been reported previously; in the two other studies mentioning pili of *Aeromonas*, only two of 11 (Atkinson and Trust, 1980) and 19 of 23 (Daily *et al.*, 1981) isolates were pilated. This may be due to the differences in bacterial growth conditions used. These workers used plate-grown instead of the broth-grown log-phase cultures that we used.

Numerous studies have shown that bacterial pili are involved in bacterial adhesion. For enterotoxigenic *E. coli* various pilus adhesins have been identified (Levine *et al.*, 1983). In studies of *Aeromonas* adhesion to buccal epithelium, Daily *et al.* (1981) associated the presence of pili on an enterotoxigenic *A. sobria* wound isolate with adherence ability, while Atkinson and Trust (1980) reported a similar finding for a faecal *A. hydrophila* isolate.

The correlation we found between type-L pilation and high adhesion levels suggests that these pili play a role in adhesion to HEP-2 cells. Removal of pili by shearing resulted in a marked decrease in adhesive ability, as did trypsin treatment of the pilated bacteria. These pili may resemble the flexible 2-nm fibrils of *E. coli* which have recently been associated with attachment to HEP-2 cells (Tavendale and Old, 1985).

The type-S pili were not associated with high level adhesion to HEP-2 cells. All water isolates examined possessed type-S pili, which suggests that these pili may play a role in the survival of *Aeromonas* spp. in their environmental habitats.

It is believed that *Aeromonas* spp. in water may be a source of diarrhoeal disease (Le Chevalier *et al.*, 1982; Burke *et al.*, 1984b). About one third of faecal isolates in this study were type-S-pilated, and may thus have originated from a water-borne infection. The origin of the type-L-pilated *Aeromonas* spp. remains in doubt. An as yet unidentified environmental source may be involved. Alternatively, adherence to and colonisation of the small intestine in patients with diarrhoea may select subpopulations of type-L-pilated bacteria that already

exist within the environmental populations. Our electron microscopy techniques would not detect a pilus type present on <1% of bacterial cells. Alternatively the dense covering of S pili may obscure a few L pili present on some cells.

Neither pilus type appeared to mediate haemagglutination of human group O cells; removal of pili by shearing or trypsin treatment had no effect on the degree of haemagglutination observed. Furthermore, pilus type could not be associated with a specific haemagglutination pattern. Burke *et al.* (1984a) reported that fucose-resistant haemagglutination is most frequently found in *Aeromonas* spp. isolated from faeces of patients with diarrhoea. Among the isolates tested in this study, fucose-resistant haemagglutination was rare; the most common pattern was F⁺G⁻M⁺ for both L- and S-pilated strains.

Recent studies have demonstrated that the hydrophobic character of bacteria plays a role in their interaction with the intestinal epithelium and that different pilus types contribute to cell surface hydrophobicity to varying degrees (Rosenberg, 1981; Sherman *et al.*, 1985). No strict correlation between L or S pilation and surface hydrophobicity was found although the L-pilated strains were most commonly non-hydrophobic. This suggests that neither pilus type consists of a uniform population. However, the screening test for hydrophobicity we used lacks sensitivity. Further studies are needed, with methods, such as hydrophobic interaction chromatography, which are sensitive to small differences in hydrophobicity of relatively hydrophilic bacteria.

These studies suggest that adhesion to HEP-2 cells is a useful in-vitro model for investigating *Aeromonas* gut infection. Type-L pili are implicated as bacterial adhesins, but proof of their role requires further experiments, such as purification of pili and their use in adhesion blocking assays.

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