Antigenic relationships among type-1 fimbriae of Enterobacteriaceae revealed by immuno-electronmicroscopy

R. A. ADEGBOLA* and D. C. OLD

Department of Medical Microbiology, University of Dundee Medical School, Ninewells Hospital, Dundee DD1 9SY

Summary. Antigenic relationships among type-1 fimbriae of 40 strains of Enterobacteriaceae representing 19 species and seven genera were determined by immuno-electronmicroscopy. Ten distinct antigenic groups were distinguished. Intra- and inter-generic relationships were observed although some antigenic groups were species-specific only. Antigenic relationships among type-1 fimbriae are more complex than have been reported hitherto.

Introduction

Type-1 fimbriae are of common occurrence in the family Enterobacteriaceae (Duguid and Old, 1980), including the newly described species and genera of the tribe Klebsielleae (Adegbola, 1983). All type-1 fimbriae are similar morphologically and in adhesive properties, as suggested by their mannose-sensitive haemagglutination of certain erythrocyte species (Duguid and Gillies, 1957; Duguid, 1959; Duguid *et al.*, 1966; Salit and Gotschlich, 1977).

Type-1 fimbriae are antigenic (Gillies and Duguid, 1958) and share serological relationships with non-haemagglutinating type-2 fimbriae of salmonellae (Old and Payne, 1971) but not with the type-3 fimbriae of *Klebsiella* (Nowotarska and Mulczyk, 1977; Adegbola, 1983; Korhonen *et al.*, 1983; Old *et al.*, 1985). Agglutination tests have been used to demonstrate sharing of type-1 fimbrial antigens among: (i) *Escherichia*, *Shigella* and, to a lesser extent, *Klebsiella* (Gillies and Duguid, 1958); (ii) *Citrobacter* and *Salmonella* (Duguid and Campbell, 1969). There was, however, no sharing of type-1 fimbrial antigens between salmonellae on the one hand and *Escherichia coli* and *Shigella flexneri* on the other (Duguid and Campbell, 1969). Less is known, however, about the antigens of type-1 fimbriae on other strains of Enterobacteriaceae (Duguid, 1985).

Recent studies have revealed that multiple

fimbriation is a common phenomenon among enterobacteria (Duguid and Old, 1980; Adegbola and Old, 1982; Old and Adegbola, 1982; Adegbola *et al.*, 1983). Thus, previous serological reports based on agglutination tests should be interpreted with caution because agglutination tests are unlikely to be adequate for satisfactory antigenic characterisation of the different types of fimbriae that might be formed by a single bacterial strain.

In the present study, we used immuno-electron-microscopy (IEM), in which type-1 fimbriae were specifically labelled by attachment of type-1 fimbrial antibody, to assess the degree of relatedness among type-1 fimbriae present on strains from 19 species of Enterobacteriaceae.

Materials and methods

Bacteria

We examined 40 strains of 19 species of Enterobacteriaceae, six of which were also used as vaccine strains. All strains were identified by API-20E tests; in some cases, conventional tests such as carbon-source utilisation and growth in methahydroxybenzoate (Richard, 1977) were used to identify strains to species rank. Strains were stored at 4°C on slants of nutrient agar or Dorset's egg medium until subcultured for testing. Their sources, biotypes and haemagglutination status with corresponding fimbriae are detailed (table I).

Media and culture methods

These have been described before (Adegbola and Old, 1982 and 1983; Old and Adegbola, 1983).

Received 29 Jul. 1986; accepted 1 Sep. 1986.

* Present address: Department of Biological and Chemical Sciences, Lagos State University, Badagry Expressway, Ojo, PMB 1087, Apapa, Lagos, Nigeria.

Table I. Strains of Enterobacteriaceae

Bacterial species	Donor or source*	Strain no.	Type-1 fimbriae	Other fimbriae (and associated haemagglutinins†)
<i>C. freundii</i>	NCTC	6021	+	none
<i>C. freundii</i>	NCTC	6267	+	none
<i>C. freundii</i>	NCTC	6071	+	none
<i>C. freundii</i>	NCTC	9737	+	none
<i>Ent. aerogenes</i>	NCTC	10006	+	thin (MS, MR/P)
<i>Ent. aerogenes</i>	NCTC	10336	+	thin (MS, MR/P)
<i>Ent. amnigenus</i>	CUETM	78-77	+	none
<i>Ent. amnigenus</i>	CDC	2419-80	+	none
<i>Ent. cloacae</i>	JPD	035	+	none
<i>Ent. cloacae</i>	NCTC	10005	+	none
<i>Ent. intermedium</i>	CUETM	77-123	+	none
<i>Ent. intermedium</i>	CDC	1167/81	+	none
<i>Ent. sakazaki</i>	JPD	889	+	none
<i>Ent. sakazaki</i>	NCTC	8155	+	none
<i>K. pneumoniae</i>	JPD	K40/1	+	none
ss. <i>aerogenes</i>	JPD	K55/1	+	none
<i>K. pneumoniae</i> ss.	JPD	K3/8	+	none
<i>pneumoniae</i>	JPD	204	+	none
<i>P. vulgaris</i>	NCTC	4175	- ‡	thick (MS, MR/K, MR/P)
<i>P. vulgaris</i>	NCTC	10376	-	thick (MS, MR/K, MR/P)
<i>Prov. alcalifaciens</i>	JLP	248	-	thick (MS, MR/K)
<i>Prov. alcalifaciens</i>	CDC	9259	-	thin, thick (MS, MR/K)
<i>Prov. rettgeri</i>	NCTC	7477	-	thin, thick (MS, MR/K)
<i>Prov. rettgeri</i>	JLP	3881	-	thin, thick (MS, MR/K)
<i>Prov. stuarti</i>	JLP	CH5100	-	thick (MS, MR/K)
<i>Prov. stuarti</i>	NCTC	10318	-	thick (MS, MR/K)
<i>Prov. stuarti</i>	NCTC	10834	-	thick (MR/P)
<i>S. arizonae</i>	SA	3600-56	+	thin (MR/K)
<i>S. arizonae</i>	NCTC	7311	+	none
<i>S. enteritidis</i>	SA	224-36/73v	+	none
<i>S. enteritidis</i>	DU	41725/82	+	none
<i>S. typhimurium</i>	SA	162-36/72v	+	none
<i>S. typhimurium</i>	SA	221-36/73v	+	none
<i>S. virchow</i>	DU	79336	+	none
<i>S. virchow</i>	DU	79352	+	none
<i>Ser. marcescens</i>	PADG	5(A1)	+	thin (MR/K, MR/P)
<i>Ser. marcescens</i>	NCTC	2302	+	thin (MR/K)
<i>Ser. odorifera</i>	PHG	4013	+	thin (MR/K)
<i>Ser. odorifera</i>	PHG	4017	+	thin (MR/K)
<i>Ser. proteamaculans</i>	PADG	3894	+	thin (MR/P)

*CDC = Centers for Disease Control, Atlanta, GA, USA; CUETM = Collection Unité d'Ecotoxicologie Microbienne, Villeneuve-d'Ascq, France; DU = Department of Medical Microbiology, Dundee University; JLP = Dr J. L. Penner, University of Toronto, Ontario, Canada; JPD = Professor J. P. Duguid, University Medical School, Dundee; NCTC = National Collection of Type Cultures, Central Public Health Laboratory, London; PADG = Dr P. A. D. Grimont, Service des Entérobactéries, Unité INSERM, Institut Pasteur, Paris, France; PHG = Professor Ph. Goulet, Service de Microbiologie, Hospital Beaujon, Clichy, France; SA = Dr S. Aleksić, National Salmonella Laboratory, Hamburg, West Germany.

† The haemagglutinins were of the kinds described by Duguid and Old (1980) as: MS = mannose-sensitive; MR/K = mannose-resistant and klebsiella-like; MR/P = mannose-resistant and proteus-like.

‡ The production of MS-HA by Proteaceae has still to be definitively associated with the presence of thick, type 1-like fimbriae found on many strains; accordingly, these fimbriae have not been designated as type-1 (see Old and Adegbola, 1982).

Haemagglutination tests

Each bacterial culture was shown to be in the fimbriate state by haemagglutination tests with different species of erythrocytes in the absence and presence of D-mannose. Adequate preparation of erythrocyte suspensions and careful performance of haemagglutination tests are essential for full characterisation of the haemagglutinating properties of the strains. These and the characterisation of the mannose-sensitive haemagglutinin (MS-HA), the mannose-resistant and klebsiella-like haemagglutinin (MR/K-HA) and the mannose-resistant and proteus-like haemagglutinin (MR/P-HA) have been fully described elsewhere (Adegbola and Old, 1982 and 1983; Old and Adegbola, 1982; Old, 1985).

Preparation of type-1 fimbrial antiserum

Antisera were raised in adult rabbits as described by Adegbola and Old (1982). *O* and, where necessary, *H* antibodies were removed from crude antiserum by the methods of Old and Adegbola (1985). By these procedures antisera specific for type-1 fimbriae were prepared against fimbriate cultures of: *S. paratyphi* B strain S543; *K. pneumoniae* ss. *aerogenes* strain K55/1; and *Enterobacter cloacae* strain O35, each of which formed MS-HA and type-1 fimbriae only. The vaccine strains *Serratia marcescens* NCTC 2302 and *Ser. odorifera* PHG 4013 formed, in addition to type-1 fimbriae, type-3 fimbriae and MR/K-HA (table I). Their type-3 fimbriae are antigenically related to those of *Ser. plymuthica* strains which form type-3 fimbriae only (Adegbola and Old, 1982; Old *et al.*, 1983). To obtain specific type-1 fimbrial antisera against these latter two vaccine strains, type-3 fimbrial antibodies were removed by repeated absorptions at 37°C for 1 h with dense bacterial suspensions of type-3 fimbriae-rich cultures of *Ser. plymuthica* that had been grown in large volumes of nutrient broth at 30°C for 48 h and washed twice, and resuspended to $c. 5 \times 10^{11}$ bacteria/ml, in saline. Absorptions were continued until 1 in 10 and 1 in 20 dilutions of these latter antisera did not agglutinate type-3 fimbriate cultures of *Ser. plymuthica* or coat the type-3 fimbriae of these two vaccine strains as seen with the electronmicroscope.

Multiple fimbriation is a common phenomenon among strains of *Morganella*, *Proteus* and *Providencia* (Adegbola, 1981; Old and Scott, 1981; Old and Adegbola, 1982). However, "thick" and channelled fimbriae, morphologically similar to type-1 fimbriae but associated instead with a weak mannose-resistant agglutination of fowl and guinea-pig erythrocytes, were the only type of fimbriae produced by *Prov. stuarti* strain NCTC 10834 (Old and Adegbola, 1982). Fimbrial antiserum against these "thick" fimbriae of *Providencia* strains coated the "thick", channelled fimbriae of MS-HA⁺ strains of *Prov. stuarti* (Adegbola, 1981); antiserum against *Prov. stuarti* strain 10834 was included in this study.

Electronmicroscopy

The technique has been described before (Adegbola and Old, 1982; Old and Adegbola, 1982). Most bacterial cultures were negatively stained for 1 min with uranyl acetate (pH 4.6) 0.3% w/v (Adegbola and Old, 1982). Some kinds of fimbriae of strains of *Proteus* and *Providencia* are acid-labile (Adegbola, 1981; Old and Adegbola, 1982). Accordingly, methylamine tungstate (pH 6.5) (a commercial preparation from EMscope Laboratories Ltd, Ashford, Kent) was used for negative staining of cultures of these latter genera. The electronmicroscope was a Jeol 100CX model; micrographs of fimbriae, uncoated or coated with fimbrial antibody, were taken as described previously (Adegbola and Old, 1982; Old and Adegbola, 1982).

Immuno-electronmicroscopy

Fimbriate cultures were also negatively stained, as described by Adegbola and Old (1982), after treatment with fimbrial antiserum. Degrees of antibody coating of type-1 fimbriae were estimated with the electronmicroscope and scored on an arbitrary scale from 0 (no coating) to + + + (maximum coating) as described elsewhere (Adegbola and Old, 1982). Grids with bacteria alone or bacteria treated with pre-immunisation sera were included as controls.

Results

Haemagglutinins and fimbriae

Twenty-three of the 40 strains examined formed MS-HA only; 16 other strains formed MS-HA as well as MR/K-HA or MR/P-HA or both (table II). *Prov. stuarti* strain NCTC 10834 was the only one of the 40 strains that did not form MS-HA. All MS-HA⁺ strains had formed MS-HA, demonstrable in tests with red cells of fowl or guinea pig, by the third or fourth serial subculture in any series. However, the MS-HAs of strains of *Proteus* and *Providencia* were best demonstrated at the first or second subculture in any series because thereafter the simultaneous production of MR/P-HA masked the presence of MS-HA (see Old and Adegbola, 1982).

All MS-HA⁺ strains formed thick (c. 8 nm wide), channelled fimbriae observed with the electronmicroscope. *Prov. stuarti* strain NCTC 10834, though MS-HA⁻, also formed thick, channelled fimbriae morphologically indistinguishable from the type-1 fimbriae produced by MS-HA⁺ strains.

Immuno-electronmicroscopy

Haemagglutinating bacteria of the 39 strains which formed MS-HA and were rich in type-1, or

Table II. Serological relationships among type-1 fimbriae of Enterobacteriaceae revealed by immuno-electronmicroscopy

		Degree of antibody coating* of type-1 fimbriae of stated species in IEM tests with type-1 fimbrial antiserum against					
Bacterial species	Strain no.	<i>S. paratyphi</i> B S543	<i>K. pneumoniae</i> ss. <i>aerogenes</i> K55/1	<i>Ent. cloacae</i> 035	<i>Ser. marcescens</i> 2302	<i>Ser. odorifera</i> 4013	<i>Prov. stuarti</i> 10834
<i>S. arizonae</i>	3600-56	+++	0	0	0	0	0
<i>S. arizonae</i>	7311	+++	0	0	0	0	0
<i>S. enteritidis</i>	224-36/73v	+++	0	0	0	0	0
<i>S. enteritidis</i>	41725/82	+++	0	0	0	0	0
<i>S. typhimurium</i>	162-36/72v	+++	0	0	0	0	0
<i>S. typhimurium</i>	221-36/73v	+++	0	0	0	0	0
<i>S. virchow</i>	79336	+++	0	0	0	0	0
<i>S. virchow</i>	79352	+++	0	0	0	0	0
<i>C. freundii</i>	6021	+++	0	0	0	0	0
<i>C. freundii</i>	6267	+++	0	0	0	0	0
<i>C. freundii</i>	6071	0	+++	0	0	0	0
<i>C. freundii</i>	9737	0	+++	0	0	0	0
<i>K. pneumoniae</i> ss. <i>aerogenes</i>	K40/1	0	+++	0	0	0	0
<i>K. pneumoniae</i> ss. <i>aerogenes</i>	K55/1	0	+++	0	0	0	0
<i>K. pneumoniae</i> ss. <i>pneumoniae</i>	K3/8	0	+++	0	0	0	0
<i>K. pneumoniae</i> ss. <i>pneumoniae</i>	204	0	+++	0	0	0	0
<i>Ent. aerogenes</i>	10006	0	+++	0	0	0	0
<i>Ent. aerogenes</i>	10336	0	+++	0	0	0	0
<i>Ent. amnigenus</i>	78-77	0	0	+++	0	0	0
<i>Ent. amnigenus</i>	2419-80	0	0	+++	0	0	0
<i>Ent. cloacae</i>	035	0	0	+++	0	0	0
<i>Ent. cloacae</i>	10005	0	0	+++	0	0	0
<i>Ent. sakazaki</i>	889	0	0	+++	0	0	0
<i>Ent. sakazaki</i>	8155	0	0	+++	0	0	0
<i>Ent. intermedium</i>	77-123	0	0	0	0	0	0
<i>Ent. intermedium</i>	1167/81	0	0	0	0	0	0
<i>Ser. marcescens</i>	5(A1)	0	0	0	+++	0	0
<i>Ser. marcescens</i>	2302	0	0	0	+++	0	0
<i>Ser. proteamaculans</i>	3894	0	0	0	+++	0	0
<i>Ser. odorifera</i>	4013	0	0	0	0	+++	0
<i>Ser. odorifera</i>	4017	0	0	0	0	+++	0
<i>Prov. stuarti</i>	CH5100	0	0	0	0	0	+++
<i>Prov. stuarti</i>	10318	0	0	0	0	0	+++
<i>Prov. stuarti</i>	10834	0	0	0	0	0	+++
<i>P. vulgaris</i>	4175	0	0	0	0	0	0
<i>P. vulgaris</i>	10376	0	0	0	0	0	0
<i>Prov. alcalifaciens</i>	248	0	0	0	0	0	0
<i>Prov. alcalifaciens</i>	9259	0	0	0	0	0	0
<i>Prov. rettgeri</i>	7477	0	0	0	0	0	0
<i>Prov. rettgeri</i>	3881	0	0	0	0	0	0

*Coating of fimbriae was: +++ = maximum; 0 = absent.

other thick type 1-like, fimbriae were tested by IEM with each of the five type-1 fimbrial antisera (table II) and with the fimbrial antiserum specific for the thick (non-MS-HA) fimbriae of *Prov. stuarti* strain NCTC 10834. Several broad areas of serological relatedness were observed.

(i) Type-1 fimbrial antiserum against *S. paratyphi*

B strain S543 strongly coated the type-1 fimbriae of all serotypes of *Salmonella* tested and of two strains of *C. freundii* (table II). Moreover, this fimbrial antiserum had strongly coated the type-1 fimbriae of many other serotypes of *Salmonella* and those of another ten strains of *Citrobacter* in another study (results not shown). The type-1, and other kinds of

thick, fimbriae present on the other 29 MS-HA⁺ strains of Enterobacteriaceae examined in this study were not coated by this antiserum (table II).

(ii) Type-1 fimbrial antiserum against *K. pneumoniae* ss. *aerogenes* strain K55/1 strongly coated the type-1 fimbriae on bacteria from MS-HA⁺ cultures of homologous and heterologous strains of that subspecies (results for only two strains shown; table II). It also strongly coated the type-1 fimbriae of strains of *K. pneumoniae* ss. *pneumoniae* and two of the four strains of *C. freundii*, and the thick fimbriae on bacteria from multiply haemagglutinating cultures of *Ent. aerogenes* (table II). Furthermore, this type-1 fimbrial antiserum had also coated the type-1 fimbriae of strains of four other species of *Klebsiella* (Old and Adegbola, 1984). It did not coat the type-1, or other thick, fimbriae of the other 31 MS-HA⁺ strains tested (table II).

(iii) The type-1 fimbriae of homologous and heterologous strains of *Ent. cloacae* (results for only two strains shown; table II) and those of *Ent. amnigenus* and *Ent. sakazaki* were coated by type-1 fimbrial antiserum against *Ent. cloacae* strain 035; those of *Ent. intermedium* and the other 33 MS-HA⁺ enterobacteria tested were not coated (table II).

(iv) The two type-1 fimbrial antisera against strains of *Serratia* gave different reactions. Thus, the antiserum against *Ser. odorifera* strain PHG 4013 coated the type-1 fimbriae of that species only. The type-1 fimbrial antiserum of *Ser. marcescens* strain NCTC 2302, on the other hand, coated the type-1 fimbriae of strains of *Ser. marcescens* and *Ser. proteamaculans* but not those of *Ser. odorifera* (table II). This antiserum had also coated the type-1 fimbriae of four other species of *Serratia* strongly and those of *Ser. rubidaea* moderately (results not shown; Adegbola, 1983). Neither of the type-1 fimbrial antisera against *serratiae* coated the thick fimbriae of any other genus tested (table II).

(v) Antiserum against the thick (non-MS-HA) fimbriae of *Prov. stuarti* strain NCTC 10834 strongly coated the thick fimbriae of homologous and heterologous strains of that species only (table II).

(vi) The thick (type-1 or other) fimbriae of MS-HA⁺ strains of *Ent. intermedium*, *P. vulgaris*, *Prov. alcalifaciens* and *Prov. rettgeri* were unreactive in IEM tests with any of the six fimbriate antisera examined (table II).

Discussion

Our previous experience has shown that the technique of IEM was invaluable in distinguishing

different kinds of fimbriae formed by multi-fimbriated strains of several species of *Enterobacter*, *Klebsiella* and *Serratia* (Adegbola and Old, 1982 and 1983; Old and Adegbola, 1983 and 1984; Old *et al.*, 1983). Moreover, our success with the application of the technique of IEM that led to the establishment of nine antigenic groups among type-3 fimbriae of Enterobacteriaceae (Old and Adegbola, 1985) has encouraged us to employ the same technique for the determination of antigenic relationships among type-1 fimbriae. Our results showed some significant differences from those of earlier reports and it was of interest to find, for example, that the type-1 fimbriae of *Citrobacter* were not antigenically homogeneous as had been reported previously (Duguid and Campbell, 1969; Nowotarska and Mulczyk, 1977). Thus, whereas strains of *Salmonella* of different sub-genera and most strains of *C. freundii* examined in this and other studies (Adegbola, 1983) formed antigenically related type-1 fimbriae, two strains of *Citrobacter* were shown consistently to have type-1 fimbriae antigenically related to those of *Klebsiella*. Did this reflect the divergence in polynucleotide sequences of some members of *Citrobacter* from other members of the tribe Salmonelleae as revealed by Crosa *et al.* (1973) or was there a simpler explanation? Though the two strains in question, strains NCTC 6071 and NCTC 9737, were received originally, and are still listed, as *C. freundii*, that identification is now in doubt (Public Health Laboratory Service, 1983). Thus, strain NCTC 6071 behaves like *C. amalonaticus*, a species significantly different by DNA-DNA reassociation from the other two named species *C. freundii* and *C. koseri* (*syn. C. diversus*) (Crosa *et al.*, 1974). This apparently indicates, therefore, yet another example of antigenic diversity among the type-1 fimbriae of different species within a genus. Further studies, however, are needed for a more detailed analysis of the type-1 fimbriae of *Citrobacter* strains especially those of *C. amalonaticus* and *C. koseri*, the latter species not having been examined by us.

Strain NCTC 9737 (Public Health Laboratory Service, 1983) is probably a species of *Kluyvera*, a new (i.e., recently redefined) genus of Enterobacteriaceae (Farmer *et al.*, 1981). As judged by DNA-DNA reassociation, the closest relatives of *Kluyvera* were species of *Klebsiella*, *Enterobacter* and *Citrobacter* (Farmer *et al.*, 1981). Hence, it was of interest to find that the type-1 fimbriae of this strain of *Kluyvera* were antigenically like those of *Klebsiella* and the one strain of *C. amalonaticus* examined rather than like those of *Enterobacter* and *C. freundii*. Further information about the haemagglutinins

and fimbriae of the three species of *Kluyvera* (Farmer *et al.*, 1985) is clearly needed.

The type-1 fimbriae of different species of *Klebsiella* were antigenically similar and related to those of *Ent. aerogenes*, a further indication that *Ent. aerogenes* is probably more related to *Klebsiella* than to other species of *Enterobacter*. However, the thick fimbriae of *Ent. aerogenes* are probably not classical type-1 fimbriae because they are not principally associated with MS-HA activity in that species (Adegbola and Old, 1985). Moreover, *Ent. aerogenes* is itself heterogeneous as evidenced by reports from taxonomic studies (Bascomb *et al.*, 1971; Brenner *et al.*, 1972).

Of the other currently recognised species of *Enterobacter* only three—*Ent. amnigenus*, *Ent. cloacae* and *Ent. sakazaki*—produced antigenically related type-1 fimbriae. Those formed by *Ent. intermedium* were antigenically distinct and the strains of *Ent. gergoviae* and *Ent. agglomerans* previously examined (Adegbola and Old, 1983) were type-1 non-fimbriate. The newly described species—*Ent. taylorae*, *Ent. asburiae*, *Ent. nimipresuralis* and *Ent. dissolvens* (Farmer *et al.*, 1985; Brenner *et al.*, 1986)—have not yet been examined by us. These observations were not surprising in that similar antigenic heterogeneity has been demonstrated among the type-3, and other kinds of thin, fimbriae of species of *Enterobacter* (Adegbola and Old, 1985; Old and Adegbola, 1985).

Among the type-1 fimbriate species of *Serratia*, the type-1 fimbriae of *Ser. odorifera* formed a distinct group and those of *Ser. rubidaea*, whilst showing some antigenic sharing, were less related to the other type-1 fimbriate species of *Serratia*. The exact taxonomic relationship of these latter two species to other *serratiae* requires further clarification.

The MS-HA-associated fimbriae of *Proteus* and *Providencia* were not related to each other or to those of other Enterobacteriaceae. Indeed, they seemed to be species-specific rather than, as described by Nowotarska and Mulczyk (1977), genus-specific. The recent report of Old and Adegbola (1985) has also demonstrated that the type-3 fimbriae of the different species of Proteaceae are species-specific.

Previous studies (Duguid and Campbell, 1969; Nowotarska and Mulczyk, 1977) have reported antigenic relationships among the type-1 fimbriae of *Escherichia*, *Shigella* and *Klebsiella*. Although strains of *Escherichia* and *Shigella* were not tested by us, a recent study with an *E. coli*-derived *fim*-gene probe for detection of frequency of the DNA

sequences encoding type-1 fimbriation among Enterobacteriaceae (Buchanan *et al.*, 1985) reported that only *E. coli* and shigellae exhibited any degree of homology. Furthermore, that study revealed that type-1 fimbrial gene sequences were present in strains of *Sh. dysenteriae* and *Sh. boydi*, serotypes not generally reported as producing either MS-HA or type-1 fimbriae (Gillies and Duguid, 1958; Duguid and Old, 1980). Thus, these sequences are presumably either not expressed or are themselves insufficient for production of functional fimbriae. Equally intriguing is the demonstration that some isolates of *E. coli* produced three kinds of type-1 or type 1-like fimbriae (1A, 1B and 1C) with highly homologous N-terminal sequences (Klemm *et al.*, 1982). However, whereas type-1A fimbriae conferred MS-HA properties and adhered to substrates, such as urinary mucus, as described for type-1 fimbriae, types 1B and 1C were either non-haemagglutinating or adhered to distinct substrates or both (Ørskov and Ørskov, 1983; Klemm, 1985). Thus, even one strain of one species may produce a complexity of adhesins and fimbriae so that the picture is likely to be more complex than previously described.

Our strains were chosen for this IEM study either because they produced MS-HA and associated type-1 fimbriae alone or in simple combination with other fimbriae such as type-3 fimbriae or other haemagglutinins such as MR/P-HA. We do not know whether species of Enterobacteriaceae other than *E. coli* also produce several kinds of type 1-like fimbriae with different substrate affinities; if they do, the present IEM technique is unlikely to be sufficiently sensitive to detect them.

Type-1 fimbriae have been described as the "common" fimbriae because of their wide distribution among the species and genera of Enterobacteriaceae. Previous reports on antigenic relationships among type-1 fimbriae of Enterobacteriaceae were based essentially on findings from agglutination tests (Gillies and Duguid, 1958; Duguid and Campbell, 1969; Nowotarska and Mulczyk, 1977). Although agglutination studies were acceptable for cultures forming only one kind of fimbriae, a more sensitive technique was required for cultures that are multiply fimbriate, a common phenomenon among Enterobacteriaceae (Duguid and Old, 1980; Adegbola, 1981; Old and Scott, 1981; Adegbola and Old, 1982; Old and Adegbola, 1982; Ørskov and Ørskov, 1983; Old *et al.*, 1983). It is significant, therefore, that with the more sensitive technique of IEM we have observed antigenic relationships more complex than those previously described for the type-1 fimbriae of

Enterobacteriaceae. The importance of this observation on serological identification, sero-diagnosis and immunological preparations involving different enterobacteria should be noted.

We thank colleagues who donated strains and supplied information about them. Dr B. Holmes afforded helpful discussion regarding the strain identities of citrobacters. RAA, an Oyo State Scholar, thanks the Committee of Vice-Chancellors and Principals (UK) for an Overseas Research Student award.

REFERENCES

- Adegbola R A 1981 Multiple haemagglutinins in enterobacteria. MSc thesis, University of Dundee.
- Adegbola R A 1983 Fimbrial haemagglutinins of the tribe Klebsiellae. PhD thesis, University of Dundee.
- Adegbola R A, Old D C 1982 New fimbrial hemagglutinin in *Serratia* species. *Infection and Immunity* **38**: 306–315.
- Adegbola R A, Old D C 1983 Fimbrial haemagglutinins in *Enterobacter* species. *Journal of General Microbiology* **129**: 2175–2180.
- Adegbola R A, Old D C 1985 Fimbrial and non-fimbrial haemagglutinins in *Enterobacter aerogenes*. *Journal of Medical Microbiology* **19**: 35–43.
- Adegbola R A, Old D C, Senior B W 1983 The adhesins and fimbriae of *Proteus mirabilis* strains associated with high and low affinity for the urinary tract. *Journal of Medical Microbiology* **16**: 427–431.
- Bascomb S, Lapage S P, Willcox W R, Curtis M A 1971 Numerical classification of the tribe Klebsiellae. *Journal of General Microbiology* **66**: 279–295.
- Brenner D J, McWhorter A C, Kai A, Steigerwalt A G, Farmer J J 1986 *Enterobacter asburiae* sp. nov., a new species found in clinical specimens, and reassignment of *Erwinia dissolvens* and *Erwinia nimipressuralis* to the genus *Enterobacter* as *Enterobacter dissolvens* comb. nov. and *Enterobacter nimipressuralis* comb. nov. *Journal of Clinical Microbiology* **23**: 1114–1120.
- Brenner D J, Steigerwalt A G, Fanning G R 1972 Differentiation of *Enterobacter aerogenes* from klebsiellae by deoxyribonucleic acid reassociation. *International Journal of Systematic Bacteriology* **22**: 193–200.
- Buchanan K, Falkow S, Hull R A, Hull S I 1985 Frequency among *Enterobacteriaceae* of the DNA sequences encoding type 1 pili. *Journal of Bacteriology* **162**: 799–803.
- Crosa J H, Brenner D J, Ewing W H, Falkow S 1973 Molecular relationships among the *Salmonellae*. *Journal of Bacteriology* **115**: 307–315.
- Crosa J H, Steigerwalt A G, Fanning G R, Brenner D J 1974 Polynucleotide sequence divergence in the genus *Citrobacter*. *Journal of General Microbiology* **83**: 271–282.
- Duguid J P 1959 Fimbriae and adhesive properties in *Klebsiella* strains. *Journal of General Microbiology* **21**: 271–286.
- Duguid J P 1985 Antigens of type-1 fimbriae. In: Stewart-Tull D E S, Davies M (eds) *Immunology of the bacterial cell envelope*. Wiley, Chichester, pp 301–318.
- Duguid J P, Campbell I 1969 Antigens of the type-1 fimbriae of salmonellae and other enterobacteria. *Journal of Medical Microbiology* **2**: 535–553.
- Duguid J P, Gillies R R 1957 Fimbriae and adhesive properties in dysentery bacilli. *Journal of Pathology and Bacteriology* **74**: 397–411.
- Duguid J P, Old D C 1980 Adhesive properties of Enterobacteriaceae. In: Beachey E H (ed) *Bacterial adherence (Receptors and recognition, series B, vol. 6)*. Chapman and Hall, London, pp 185–217.
- Duguid J P, Anderson E S, Campbell I 1966 Fimbriae and adhesive properties in salmonellae. *Journal of Pathology and Bacteriology* **92**: 107–138.
- Farmer J J *et al.* 1981 *Kluyvera*, a new (redefined) genus in the family Enterobacteriaceae: identification of *Kluyvera ascorbata* sp. nov. and *Kluyvera cryocrescens* sp. nov. in clinical specimens. *Journal of Clinical Microbiology* **13**: 919–933.
- Farmer J J *et al.* 1985 Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. *Journal of Clinical Microbiology* **21**: 46–76.
- Gillies R R, Duguid J P 1958 The fimbrial antigens of *Shigella flexneri*. *Journal of Hygiene* **56**: 303–318.
- Klemm P 1985 Fimbrial adhesins of *Escherichia coli*. *Reviews of Infectious Diseases* **7**: 321–340.
- Klemm P, Ørskov I, Ørskov F 1982 F7 and type 1-like fimbriae from three *Escherichia coli* strains isolated from urinary tract infections: protein chemical and immunological aspects. *Infection and Immunity* **36**: 462–468.
- Korhonen T K, Tarkka E, Ranta H, Haahtela K 1983 Type 3 fimbriae of *Klebsiella* sp.: molecular characterization and role in bacterial adhesion to plant roots. *Journal of Bacteriology* **155**: 860–865.
- Nowotarska M, Mulczyk M 1977 Serologic relationship of fimbriae among Enterobacteriaceae. *Archivum Immunologiae et Therapiae Experimentalis* **25**: 7–16.
- Old D C 1985 Haemagglutination methods in the study of *Escherichia coli*. In: Sussman M (ed) *The virulence of Escherichia coli: reviews and methods*. Academic Press, Orlando, FL, pp 287–313.
- Old D C, Adegbola R A 1982 Haemagglutinins and fimbriae of *Morganella*, *Proteus* and *Providencia*. *Journal of Medical Microbiology* **15**: 551–564.
- Old D C, Adegbola R A 1983 A new mannose-resistant haemagglutinin in *Klebsiella*. *Journal of Applied Bacteriology* **55**: 165–172.
- Old D C, Adegbola R A 1984 A comparative immunoelectron-microscopical study of fimbriae of *Enterobacter* and *Klebsiella*. *Systematic and Applied Microbiology* **5**: 157–168.
- Old D C, Adegbola R A 1985 Antigenic relationships among type-3 fimbriae of Enterobacteriaceae revealed by immunoelectronmicroscopy. *Journal of Medical Microbiology* **20**: 113–121.
- Old D C, Payne S B 1971 Antigens of the type-2 fimbriae of salmonellae: "cross-reacting material" (CRM) of type-1 fimbriae. *Journal of Medical Microbiology* **4**: 215–225.
- Old D C, Scott S S 1981 Hemagglutinins and fimbriae of *Providencia* spp. *Journal of Bacteriology* **146**: 404–408.
- Old D C, Adegbola R A, Scott S S 1983 Multiple fimbrial haemagglutinins in *Serratia* species. *Medical Microbiology and Immunology* **172**: 107–115.
- Old D C, Tavendale A, Senior B W 1985 A comparative study of the type-3 fimbriae of *Klebsiella* species. *Journal of Medical Microbiology* **20**: 203–214.
- Ørskov I, Ørskov F 1983 Serology of *Escherichia coli* fimbriae. *Progress in Allergy* **33**: 80–105.
- Public Health Laboratory Service 1983 Catalogue of the National

- Collection of Type Cultures, 6th edn. Public Health Laboratory Service, London, p 22.
- Richard C 1977 Présence chez *Enterobacter aerogenes* d'antigènes capsulaires apparentés à ceux de *Klebsiella*. Intérêt de l'utilisation du méthahydroxybenzoate dans le diagnostic différentiel *E. aerogenes*-*K. pneumoniae*. *Annales de Microbiologie* **128A**: 289–295.
- Salit I E, Gotschlich E C 1977 Hemagglutination by purified type 1 *Escherichia coli* pili. *Journal of Experimental Medicine* **146**: 1169–1181.