A COMPARATIVE STUDY OF THE TYPE-3 FIMBRIAE OF *KLEBSIELLA* SPECIES

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SUMMARY. Type-3 fimbriae isolated from members of five different species of *Klebsiella* were 4–5 nm in diameter and agglutinated the tannic acid-treated erythrocytes of ox and, in some cases, the untanned erythrocytes of fowl. In sodium dodecyl sulphate-polyacrylamide gel electrophoresis, the type-3 fimbrial proteins had mol. wts in the range 19 500–21 500. Hydrophobic amino acids comprised 39.6% of all the amino acids of the type-3 fimbrial protein of *K. oxytoca* strain 70/1. The type-1 fimbrial protein of *Klebsiella* had a mol. wt of c. 18 000 and the type-1 fimbriae were serologically distinct from the type-3 fimbriae. Our results for the type-3 fimbriae of *Klebsiella* were compared with those of others for morphologically similar and serologically related thin fimbriae of *Salmonella* and *Yersinia*.

INTRODUCTION

The mannose-resistant *Klebsiella*-like haemagglutinin first described in strains of “*Klebsiella aerogenes*” and *Serratia marcescens* (Duguid, 1959), and designated MR/K-HA (Duguid and Old, 1980), is unusual in that it agglutinates erythrocytes of various animal species only after the erythrocytes have been treated with tannic acid. Examination of 131 strains of *Klebsiella* from seven species showed that MR/K-HA was the most commonly described haemagglutinin; it was produced by c. 67% of strains of *Klebsiella* but not by strains of *K. ozaenae* or *K. rhinoscleromatis* (Old and Adegbola, 1983 and 1984). When MR/K-HA⁺ strains of *K. oxytoca*, *K. planticola*, *K. pneumoniae sensu lato*, *K. terrigena* and *K. trevisani* were examined, they were found to produce MR/K-HA better at 30°C (and 20°C) than at 37°C. Furthermore, when incubated at 30°C, c. 50% of MR/K-HA⁺ strains of *Klebsiella* also gave weak mannose-resistant haemagglutination of untanned erythrocytes of fowl, a reaction classified as MR/P-HA (Duguid and Old, 1980; Old and Adegbola, 1983). That MR/P-HA activity demonstrable with fowl erythrocytes was never found independently of MR/K-HA; thus, it was never produced by strains that formed type-1 fimbriae only (and its associated mannose-sensitive HA, MS-HA) or by non-fimbriate strains (Old and Adegbola, 1983 and 1984).

Production of MR/K-HA by *Klebsiella* strains is associated with the presence of type-3 fimbriae (Duguid, 1959) shown in negatively stained preparations to be

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non-channelled, i.e., without an axial hole, and of estimated diameter 4–5 nm (Duguid, 1959; Old and Adegbola, 1983).

In recent studies (Adegbola *et al.*, 1983; Old and Adegbola, 1984), strong serological cross-reactivities were demonstrated by immunoelectronmicroscopy between the type-3 fimbriae of different species of *Klebsiella* and similar thin fimbriae found on a few strains of *Salmonella* and *Yersinia*, genera not generally considered to produce type-3 fimbriae (Duguid and Old, 1980). In the present paper our understanding of the serological relationships demonstrable among the type-3 fimbriae from different species and genera is extended by information obtained from enzyme-linked immunosorbent assay (ELISA). Again, apart from a report on one strain of *K. pneumoniae sensu lato* (Korhonen *et al.*, 1983), little is known about the chemistry of the type-3 fimbriae of *Klebsiella*. In this paper the properties of the type-3 fimbriae of several different *Klebsiella* species not previously reported are described and compared with those reported by other workers for the type-3 fimbriae of *Salmonella* and *Yersinia*.

MATERIALS AND METHODS

Bacteria. The bacterial strains used and their haemagglutinating and fimbrial properties are detailed in table I. Strains were stored on Dorset's egg slants at ambient temperature.

Haemagglutination tests. Preparation of suspensions (3% v/v in saline) of erythrocytes of

TABLE I
Haemagglutinins and fimbriae produced by strains of Klebsiella

Species and strain no.	Source	Haemagglutinins (and associated fimbriae)		
		MS-HA (type 1)	MR/K-HA (type 3)	MR/P-HA (type 3)
<i>K. oxytoca</i>				
70/1	JPD	—	+	—
U30	JGB	—	+	+
44	JPD	+	+	+
72	JPD	+	+	+
<i>K. planticola</i>				
11885	NCIB	+	+	+
<i>K. pneumoniae</i>				
ss. <i>aerogenes</i>				
4/20	JPD	—	+	—
69/1	JPD	—	+	+
J/B166	JPD	—	+	+
9/1	JPD	—	+	+
<i>K. terrigena</i>				
78-127	CUETM	—	+	—
77-177	CUETM	+	+	+
<i>K. trevisani</i>				
78-91	CUETM	—	+	—
78-111	CUETM	+	+	+

CUETM = Dr F. Gavini, Collection Unité d'Ecotoxicologie Microbienne, Villeneuve d'Ascq, France; JGB = Dr J. G. Barr, Royal Victoria Hospital, Belfast; JPD = Professor J. P. Duguid, University Medical School, Dundee; NCIB = National Collection of Industrial Bacteria, Aberdeen.

For definition of MS-HA, MR/K-HA and MR/P-HA, see text.

fowl and guinea pig and the tannic-acid treatment of erythrocytes of oxen (tanned ox red cells) have been described elsewhere (Duguid, 1959). The performance of haemagglutination tests with fimbriate bacterial suspensions in rocked porcelain tiles followed previously reported methods (Old and Adegbola, 1982). For the detection of the haemagglutinating activity of isolated fimbriae, equal volumes (50 μ l) of isolated fimbriae suspended in 50 mM Tris buffer (pH 7.2) and erythrocyte suspension were mixed for 15 min in depressions of a tile at ambient temperature.

Growth conditions. For primary culture, methods were chosen that favoured the expression of type-3 fimbriae and production of MR/K-HA. Thus, when the eight type-1 non-fimbriate strains (table I) were cultured serially at 48-h intervals in 10-ml volumes of peptone water (CM9, Oxoid) in tubes incubated statically in air at 30°C, haemagglutination tests confirmed that their cultures were strongly MR/K-HA⁺ after two or three such serial cultures. The two type-1 fimbriate strains of *K. oxytoca* cultured similarly produced little or no MS-HA. The other three type-1 fimbriate strains of *Klebsiella*, however, produced much MS-HA in static cultures at 30°C. Accordingly, *Klebsiella* strains 11885, 77-177 and 78-111 (table I) were grown in 10-ml volumes of peptone water in 50-ml flasks at 30°C on an orbital incubator, shaken at 80 rpm and cultured serially at 48-h intervals until their cultures rich in MR/K-HA produced little or no MS-HA. Such an MR/K-HA rich culture was used as inoculum for 1.5 L of peptone water (dispensed in 150-ml volumes in 500-ml flasks) which was incubated at 30°C for 48-72 h in the same conditions as described above for the primary culture of the strain.

Isolation of fimbriae. Bacteria were harvested by centrifugation (10 000 *g* for 30 min) at 4°C and the bacterial-cell pellet resuspended in 50 ml of 50 mM Tris buffer (pH 7.2) and homogenised at 10 000 rpm for 5 min on a blender (MSE Ltd, Crawley). The homogenate was centrifuged (10 000 *g* for 30 min) at 4°C and the fimbriae were precipitated from the supernate by the addition of solid ammonium sulphate to 50% w/v saturation. After standing overnight at 4°C, precipitated fimbriae were collected by centrifugation (10 000 *g* for 1 h) at 4°C, resuspended in 1 ml of 50 mM Tris buffer (pH 7.2) and extensively dialysed against this same buffer at 4°C.

Electronmicroscopy. Portions of the preparations of type-3 fimbriae, isolated as described above, were placed on copper grids with carbon-coated formvar, washed three times with distilled water *in situ* and negatively stained for 1 min with uranyl acetate 0.5% w/v in deionised water (pH 4.6) as previously detailed (Adegbola and Old, 1982). Stained preparations were examined with a Jeol 100CX electronmicroscope.

Immunoelectronmicroscopy. A drop of a preparation of isolated type-3 fimbriae, diluted 1 in 10 in 0.1 M phosphate-buffered saline (PBS), pH 7.4, was applied to carbon-coated, formvar-covered grids for 1 min and washed in water as described above. The grids were then floated on a drop of type-3 fimbrial antiserum (used routinely at a dilution of 1 in 10 in PBS at a titre of 640) and incubated for 1 h at 37°C in a moist chamber. Thereafter, the grid was rinsed through a series of several drops of distilled water on dental wax to remove unbound antibody (Adegbola and Old, 1982). The resultant, washed preparations were stained for 1 min with uranyl acetate as described above. The fimbrial antisera used in these tests were raised in rabbits against type-3 fimbriate strains of bacteria as described by Adegbola and Old (1982).

Polyacrylamide-gel electrophoresis (PAGE). Isolated fimbriae were boiled for 2 min in disruption buffer (0.05 M Tris-HCl, pH 6.7) containing 6 M urea, sodium dodecyl sulphate (SDS) 5% w/v, glycerol 20% w/v, 2-mercaptoethanol 1% w/v and a trace of bromophenol blue as tracking dye. Preparations were examined by SDS discontinuous PAGE, after the method of Laemmli (1970), on slabs of acrylamide 12% w/v, together with the mol. wt markers: ovalbumin (45 000), carbonic anhydrase (31 000), soya-bean trypsin inhibitor (20 100) and α -lactalbumin (14 200). Electrophoresis was performed at 20 mA at 4°C until the dye front had reached the bottom of the gel. Gels were stained overnight in Coomassie Brilliant Blue R250 0.1% w/v in a solvent mixture of isopropanol, acetic acid and water (25:10:65) and destained by gentle washing in several changes of isopropanol, acetic acid and water (10:10:80).

Amino-acid analysis. Purified fimbriae from *K. oxytoca* strain 70/1 were hydrolysed for 24 h and 72 h at 110°C in 6N HCl containing phenol 0.1% w/v and were analysed on an LKB Instruments 4400 amino-acid analyser. Amounts of valine and leucine were estimated from the 72-h hydrolysis reaction. Serine and threonine were estimated by extrapolation to zero time of the 24-h and 72-h results.

Isoelectric focussing. Purified fimbriae from *K. oxytoca* strain 70/1 were focussed initially on

ultrathin (100- μ m) polyacrylamide gels 5% w/v containing Bio-Lyte ampholytes (pH 3–10) (Bio-Rad Laboratories, Watford) and later on Servalyt precotes (pH 4–7) Uniscience Ltd, Cambridge) for 2 h at a constant power of 2.25 W. Gels were subsequently washed and fixed in Trichloroacetic acid (TCA) 20% w/v for 25 min, stained in Serva Violet 49, 0.04% w/v in a solvent mixture of methanol, acetic acid and water (40:10:50), and destained with the same solvent. When protein bands were difficult to visualise, gels were silver-stained according to the method of Merril *et al.* (1981).

Serological studies. In the ELISA technique, modified from that described by Clegg *et al.* (1980), plastic 96-well microtitration trays (Dynatech, M129B) were used. Each well received 200 μ l of fimbrial preparation suspended (0.5 μ g/ml) in 0.1 M sodium carbonate buffer (pH 9.6) containing sodium azide 0.02 w/v. After incubation at 37°C for *c.* 18 h, plates were washed four times with 0.1 M phosphate-buffered saline (pH 7.4), containing Tween 20 (Sigma) 0.05% v/v and bovine serum albumin (Sigma) 0.5% w/v (PBS-Tween-BSA) and incubated for 30 min at 37°C after the last wash to reduce non-specific binding of conjugate. All washings of plates were performed with a 96-channel washer-aspirator (Dynawasher II, Dynatech Instruments, Torrance, CA, USA). Type-3 fimbrial antiserum (200 μ l), diluted (1 in 10 to 1 in 51 200) in PBS-Tween-BSA, was added to the wells and, after incubation for 2 h at room temperature, plates were washed four times with PBS-Tween (without BSA). Then 200 μ l of donkey anti-rabbit IgG (Scottish Antibody Production Unit, Law Hospital, Carlisle, Scotland), conjugated to alkaline phosphatase (Sigma) by the method of Avrameas (1969) and diluted (1 in 5000) in PBS-Tween-BSA, was added to each well and the plates were incubated for 2 h at room temperature. Thereafter, plates were washed four times with PBS-Tween and to each well was added 20 μ l of *p*-nitrophenyl phosphate ("Sigma 104" phosphatase substrate) 1 mg/ml in 0.05 M sodium-carbonate buffer (pH 9.8) containing 1 mM MgCl₂. Plates were incubated at 37°C for 90 min and reactions stopped by the addition of 50 μ l of 3 M NaOH. Absorbances were read at 410 nm on a Microplate Reader MR6000 (Dynatech). Each dilution of antiserum was compared in quadruplicate in antigen-coated and -uncoated wells. The titre was recorded as the highest dilution of antiserum giving significant absorbance when evaluated by a Student's *t* test against the controls without antigen.

RESULTS

Properties of type-3 fimbriae

Electronmicroscopic examination of the isolated fimbriae from *K. oxytoca* strain 70/1 showed the presence of numerous, thin, non-channelled fimbriae of external diameter 4–5 nm (fig. 1a) typical of the type-3 fimbriae present on unhomogenised bacteria. The thin fimbriae harvested from the other type-3 fimbriate *Klebsiella* strains were of similar morphology, regardless of the species examined. Though *K. planticola* strain 11885, *K. terrigena* strain 77–177 and *K. trevisani* strain 78–111 had been grown in conditions sub-optimal for the production of MS-HA and type-1 fimbriae, the fimbrial preparations obtained from these three strains nevertheless contained numerous thick, channelled fimbriae the external diameter of which (8–9 nm) was typical of that of type-1 fimbriae. In immunoelectronmicroscopy tests, only the thin fimbriae were coated with type-3 fimbrial antibody against either homologous or heterologous *Klebsiella* strains. Thus, type-1 and type-3 fimbriae of *Klebsiella* were serologically distinct (fig. 1b).

The type-3 fimbriae isolated from the 13 different *Klebsiella* strains studied were analysed by SDS discontinuous PAGE; the results obtained for nine of them are compared in fig. 2. On repeated testing, the type-3 fimbrial proteins of the *K. pneumoniae* ss. *aerogenes* strains J/B166 and 4/20 consistently ran a little faster than those of the other strains (lanes *b* and *c*). The mol. wt of the protein of strain J/B166 (haemagglutination of tanned ox and untanned fowl red cells) and strain 4/20

FIG. 1.—*a*, Type-3 fimbriae, of external diameter 4-5 nm and non-channelled, isolated from *K. oxytoca* strain 70/1; *b*, type-3 fimbriae from *K. planticola* strain 11885, uncoated, and type-1 fimbriae, coated (arrow), after treatment with type-1 fimbrial antiserum against *K. pneumoniae* ss. *aerogenes* strain 55/1; *c*, type-3 fimbriae of *K. pneumoniae* ss. *aerogenes* strain 69/1 coated with type-3 fimbrial antibody against *K. oxytoca* strain 70/1. ($\times 60\,000$.)

FIG. 2.—SDS discontinuous polyacrylamide gel electrophoresis of type-3 fimbriae isolated from strains of *Klebsiella* of different species (and of different haemagglutination patterns): a, *K. pneumoniae* ss. *aerogenes* 69/1 (TO + F); b, *K. pneumoniae* ss. *aerogenes* J/B166 (TO + F); c, *K. pneumoniae* ss. *aerogenes* 4/20 (TO); d, *K. oxytoca* U30 (TO + F); e, *K. oxytoca* 70/1 (TO); f, *K. terrigena* 78–127 (TO); g, *K. terrigena* 77–177 (TO + F); h, *K. trevisani* 78–91 (TO); i, *K. planticola* 11885 (TO + F). Additional bands corresponding to type-1 fimbriae of mol. wt c. 18 000 shown in lanes g and i (→). Lanes 1–4 contain mol. wt markers: ovalbumin (45 000), carbonic anhydrase (31 000), soya-bean trypsin inhibitor (20 100) and α -lactalbumin (14 200), respectively. For haemagglutination patterns, see Materials and Methods.

(haemagglutination of tanned ox red cells only) was c. 19 500. The proteins of the type-3 fimbriae of the other strains (including *K. oxytoca* strains 44 and 72, *K. pneumoniae* ss. *aerogenes* strain 9/1, and *K. trevisani* strain 78–111, data not shown) ran as single major bands of mol. wt c. 21 000–21 500. The type-3 fimbrial preparations from the three strains known from electronmicroscopy and haemagglutination tests to contain some type-1 fimbriae gave an additional major band of mol. wt c. 18 000 when examined by SDS-PAGE (lanes g and i, fig. 2; data for *K. trevisani* strain 78–111 not shown).

The type-3 fimbriae of *K. oxytoca* strain 70/1 were examined in more detail after a further series of precipitations and resuspensions and were found to give, on isoelectric focussing, a single band of protein at pH 4.7. The amino-acid composition of the type-3 fimbriae of *K. oxytoca* strain 70/1 is presented in table II, along with similar data obtained by others for the type-3 fimbriae of *Salmonella* and *Yersinia* (Aleksić and Aleksić, 1979) and *K. pneumoniae* ss. *aerogenes* (Korhonen *et al.*, 1983). The mol. wt of the protein of the type-3 fimbriae of *K. oxytoca* strain 70/1 calculated from its amino-acid composition, was 20 495, a value in close agreement with that determined from its mobility on SDS-PAGE (21 000). The hydrophobic amino acids—alanine, isoleucine, leucine, methionine, phenylalanine, proline and valine—accounted for 39.6% of the amino acids detected (78 of 197 amino-acid residue, see table II). Though

TABLE II

Amino-acid composition of type-3 fimbriae from strains of Klebsiella, Salmonella and Yersinia

Amino acid	Residues per molecule in fimbriae from			
	<i>K. oxytoca</i> 70/1	<i>S. enteritidis</i> * 1380-36/71	<i>Y. enterocolitica</i> * 501.K1 ⁺	<i>K. pneumoniae</i> ss. <i>aerogenes</i> † 69/1
Alanine	23	22	23	34
Aspartic acid	23	30	28	39
Glycine	21	18	26	35
Glutamic acid	20	23	21	21
Threonine	18	21	22	37
Valine	17	11	10	25
Serine	14	24	16	20
Leucine	14	13	12	10
Lysine	9	6	15	14
Proline	9	NDt	NDt	9
Tyrosine	8	5	7	Tr
Isoleucine	7	6	6	5
Phenylalanine	6	5	5	NDt
Arginine	4	2	2	Tr
Methionine	2	1	2	1
Histidine	2	1	1	NDt
Tryptophane	ND	1	1	ND
Cystine/2	ND	1	1	NDt
Mol. wt of fimbrial proteins				
§	21 000‡ 20 495§	23 450 21 294	23 350 21 198	23 500 24 000

ND = not determined; NDt = no, detected; Tr = trace amounts.

* Data of Aleksic and Aleksic (1979).

† Data of Korhonen *et al.* (1983).

‡ Estimated from gel electrophoresis.

§ Estimated from amino-acid composition.

the amino acids present and their relative distributions in the type-3 fimbriae from these four organisms were remarkably similar, those of *K. oxytoca*, *Salmonella* and *Yersinia* seemed more alike than those of *K. pneumoniae*.

Serological relationships

The type-3 fimbriae obtained from some of the strains examined were used as antigens in an indirect ELISA system. In preliminary experiments, similar high titres were obtained with type-3 fimbriae of strains from four different *Klebsiella* species and type-3 fimbrial antiserum from *K. oxytoca* and *K. pneumoniae* ss. *aerogenes*. The titres obtained were generally in the range 25 600–51 200 with both homologous and heterologous sera (table III). Indeed, even in tests with the type-3 fimbriae of the *Klebsiella* strains and type-3 fimbrial antiserum of *Y. enterocolitica* strain 20·K1⁺ (Adegbola *et al.*, 1983), the titres obtained were generally within one doubling dilution of those given with the *klebsiella* antisera. Furthermore, differences in titre were not observed in relation to the different haemagglutination patterns (with tanned, ox and untanned, fowl red cells) associated with different type-3 fimbriate strains.

TABLE III
Serological relationships among type-3 fimbriae of *Klebsiella* species of different HA types

Type-3 fimbriae isolated from species and strain no.	Associated type-3 HA pattern:*	Titre of stated type-3 fimbriae in tests against type-3 fimbrial antiserum raised against		
		<i>K. oxytoca</i> 70/1 TO	<i>K. pneumoniae</i> ss. <i>aerogenes</i> 69/1 TO and F	<i>Y. enterocolitica</i> † 20.K1 ⁺ TO and F
<i>K. oxytoca</i> 70/1	TO	51 200	51 200	51 200
U30	TO + F	51 200	51 200	51 200
<i>K. pneumoniae</i> ss. <i>aerogenes</i> 4/20	TO	25 600	51 200	51 200
69/1	TO + F	...	51 200	12 800
<i>K. terrigena</i> 78-127	TO	51 200	12 800	51 200
<i>K. trevisanii</i> 78-91	TO	51 200	51 200	51 200

... = Not tested.

* Haemagglutination of: TO = tanned red cells of oxen; F = untanned red cells of fowl.

† See Adegbola *et al.* (1983).

DISCUSSION

Because we wished to make comparisons between type-3 fimbriae isolated from several strains of different species and different haemagglutination patterns, the isolation and purification methods used were chosen for simplicity and speed, though more elaborate methods suitable for work with small numbers of strains have been described (Korhonen *et al.*, 1980*a* and *b*). Nevertheless, our methods yielded fimbrial preparations which were found by electronmicroscopy to be free from contaminating cell structures and by gel electrophoresis to be free from gross contamination with other proteins. The mol. wt of the type-3 fimbriae examined was found to be *c.* 21 000 by both electrophoresis and amino acid analyses. This value, though a little less than that (*c.* 23 500) found by electrophoresis of similar fimbriae from other enterobacteria (Aleksić and Aleksić, 1979; Korhonen *et al.*, 1983), is very similar to the mol. wt of the type-3 fimbriae of *S. enteritidis* and *Y. enterocolitica* (*c.* 21 000) calculated from their amino-acid compositions (Aleksić and Aleksić, 1979). The finding that, on the same gels, type-1 fimbriae from strains of *K. planticola*, *K. terrigena* and *K. trevisanii* had mol. wts of 18 000, identical to that found by Korhonen *et al.* (1983) for the type-1 fimbriae of *K. pneumoniae* ss. *aerogenes*, gives credence to the size determinations of the type-3 fimbriae reported above.

It is probable that minor variations in size may exist between the same types of fimbriae in different strains and between different bacterial species. We found, for example, that the type-3 fimbriae of two of our strains of *K. pneumoniae* ss. *aerogenes* consistently ran faster than those of other strains examined by electrophoresis, and Korhonen *et al.* (1983) have reported similar variations with regard to type-1 fimbriae from some rodent-pathogenic strains of *K. pneumoniae*. This being so, the slight differences in size among type-3 fimbriae reported by different workers may not be significant; these differences did not reflect variations in haemagglutination patterns.

The amino-acid composition of the type-3 fimbriae of *K. oxytoca* 70/1 was very similar to that found by Aleksić and Aleksić (1979) for those type 3-like fimbriae of *S. enteritidis* and *Y. enterocolitica* that are serologically like the type-3 fimbriae of *Klebsiella* (Adegbola *et al.*, 1983); all contained phenylalanine and histidine residues. They appear to be somewhat different in composition from the type-3 fimbriae of *K. pneumoniae* ss. *aerogenes* 69/1 (Korhonen *et al.*, 1983) which, furthermore, were reported to lack phenylalanine and histidine residues. The slight differences in amino-acid composition are further reflected in our correspondingly greater estimates of hydrophobicity of the type-3 fimbriae of *K. oxytoca* (c. 40%, as compared to 34% for *K. pneumoniae* ss. *aerogenes* 69/1; Korhonen *et al.*, 1983), a level more like that reported for the type-1 fimbriae of several enterobacteria (Korhonen *et al.*, 1980a, and b, and 1983). Regardless of these differences, the type-3 fimbriae of *K. pneumoniae* ss. *aerogenes* 69/1 are coated well with type-3 fimbrial antibody prepared against *K. oxytoca* 70/1 and it is clear that they belong to the same broad serological group (fig. 1c).

Type-3 fimbriae have been described in many species of all genera of Klebsiellaceae and Proteaceae (Duguid and Old, 1980; Old and Adegbola, 1982). The adhesive properties of type-3 fimbriate bacteria are diverse. They include the agglutination of tanned red cells of different animal species and adhesion to candida, various moulds, cellulose fibres, plant-root hairs and glass (Duguid, 1959). Though it has been customary to describe type-3 fimbriate bacteria by their haemagglutination of tanned ox red cells, subsequent studies have shown that a majority (50 of 89) of type-3 fimbriate strains of different species of *Klebsiella* also agglutinated weakly the untanned red cells of fowl (Old and Adegbola, 1983). In typical richly type-3 fimbriate cultures, the relative strengths (as haemagglutinating powers) of these activities with tanned ox (TO) and untanned fowl (F) red cells were: TO=1600; F=200. Type-3 fimbriate strains of *Enterobacter* and *Serratia*, on the other hand, behaved like the minority of *Klebsiella* strains in agglutinating tanned, ox red cells only (Adegbola and Old, 1982 and 1983). The type-3 fimbriae isolated from all species of *Klebsiella* retained the ability to agglutinate tanned, ox cells. The ability to agglutinate fowl red cells was not shown by the isolated fimbriae of any strain of *K. pneumoniae* ss. *aerogenes*, but was demonstrable for those of all other strains originally possessing that ability.

Immunoelectronmicroscopy studies of type-3 fimbriate *Klebsiella* strains have demonstrated that the type-3 fimbriae of all species behaved serologically as a homogeneous group, suggesting that they are closely related though not necessarily identical (Adegbola and Old, 1983; Old and Adegbola, 1984). Preliminary results from the present study in which isolated fimbriae were used as antigens in ELISA tests are in good agreement with the observations from immunoelectronmicroscopy. Thus, findings indicating that the type-3 fimbriae of *Klebsiella* species are similar in morphology, size and antigenicity afford no ready explanation for the fact that some *Klebsiella* strains agglutinate untanned fowl red cells.

Narrow-spectrum, non-fimbrial mannose-resistant haemagglutinins have been described among that group of adhesins described as MRE (mannose-resistant and eluting) in *Escherichia coli* (Duguid *et al.*, 1955 and 1979; Duguid, 1964; Duguid and Old, 1980; Ip *et al.*, 1981). Moreover, recent studies have confirmed that mannose-resistant haemagglutination of the 'human only' type is associated with the production of protein aggregates at the cell surface of strains of *E. coli* of certain serogroups

(Knutton *et al.*, 1984; Williams *et al.*, 1984). Therefore, the mannose-resistant haemagglutination of the 'fowl only' type by some *Klebsiella* strains may represent the activity of a similar kind of non-fimbrial protein structure independent of type-3 fimbriae, though the available evidence suggests that this is not so. First, agglutination of untanned fowl red cells is never found independently of type-3 fimbriae and agglutination of tanned ox red cells. Second, when type-3 fimbriate strains of *Klebsiella* of haemagglutination type TO plus F were serially subcultured in broths containing type-3 fimbrial antiserum prepared against a strain of *Klebsiella* of haemagglutination type (TO), the only non-haemagglutinating cultures obtained had lost both haemagglutinating activities (Adegbola, 1983). Third, a few strains of *Salmonella* and *Yersinia* that produce type 3-like fimbriae (Rohde *et al.*, 1975; Aleksić *et al.*, 1976 and 1978; Aleksić and Aleksić, 1979) antigenically similar to the type-3 fimbriae of *Klebsiella* (Adegbola *et al.*, 1983) also show the TO plus F pattern of haemagglutination. When the gene or genes controlling type-3 fimbriation of *S. enteritidis* were transferred to other salmonellae by transduction or to *E. coli* by conjugation, the type-3 fimbriate recipient strains always expressed the TO plus F type of haemagglutination (D. C. Old, unpublished results). Thus, it seems more probable that these two haemagglutinating activities, TO and F, are related to each other and probably associated with some kind of type-3 fimbriae, slightly altered in biological activity but not in serological specificity. Similar changes have been reported for the type-1 and type-2 fimbriae of *S. paratyphi* B (Old and Payne, 1971) and the type-1 and type-6 fimbriae of *Klebsiella* (Old and Adegbola, 1984). Detailed amino-acid sequencing of the type-3 fimbrial proteins of the respective patterns will, no doubt, resolve the question in time.

A classification of fimbriae based on binding specificities and serological properties may be ideal, but when information about the fimbriae is limited, classifications based on their shared fimbrial properties are convenient and justified. Thus, all the fimbriae of Enterobacteriaceae classified as type 3 (Duguid *et al.*, 1966) are of similar morphology—thin, of external diameter 4–5 nm and without an axial hole (Duguid, 1959; Adegbola and Old, 1982 and 1983)—and their adhesive properties (*vide supra*) are similar. Nevertheless, immunoelectronmicroscopy studies have indicated their diversity. Thus, among the Klebsielleae, three major type-3 fimbrial antigenic groups are known, comprising: (i) type-3 fimbriate *Klebsiella* species, *Enterobacter gergoviae* and some strains of *Ent. aerogenes* (syn. *K. mobilis*); (ii) *Ent. intermedium* and type-3 fimbriate strains of most *Serratia* species; (iii) *S. marinorubra* (Adegbola and Old, 1982, 1983 and 1985; Old and Adegbola, 1983 and 1984; Old *et al.*, 1983). There is however, no cross-reactivity between the type-3 fimbriae of Klebsielleae and Proteae (D. C. Old and R. A. Adegbola, unpublished results). When the serological information is supplemented in the future with information about the molecular nature of their receptors, then will be the time for a redefinition of this broad group of fimbriae classed as type-3. In the meantime, the grouping, though clearly heterogeneous, is useful for ease of reference.

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