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Ribotyping as an epidemiologic tool for *Escherichia coli*

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SUMMARY

Restriction fragment length polymorphism of ribosomal RNA genes was analysed among 133 *Escherichia coli* strains predominantly from blood and urine, including 21 isolates from faeces of healthy persons. The strains had also been characterized for their O:K:H serotypes, for the presence of P, S and type 1C fimbriae, non-P, non-S mannose-resistant haemagglutinins and haemolysin production. *Hin*d III-digested genomic DNA was subjected to Southern blot analysis with either plasmid pKK3535 containing *E. coli* rRNA operon or purified rRNA as a probe. Among the 133 strains 20 ribotypes were obtained. The distribution of strains into different ribotypes generally correlated with their O:K:H serotype. Ribotype variation within serotypes was mainly seen among strains with the K5 capsule. The origin of the strains or the presence of virulence-associated factors did not correlate with the ribotype. In conclusion, ribotyping appears to be a valuable method in epidemiologic studies especially when the serotyping methods are not available.

INTRODUCTION

Escherichia coli is the most abundant facultative bacterium in the normal flora of the large intestine. From that reservoir with thousands of different *E. coli* types [1], only a small subset of strains cause extraintestinal infections, such as urinary tract infection (UTI) [2], septicaemia or neonatal meningitis [3–5]. These strains usually belong to a limited number of serotypes and produce adhesins, haemolysin and other molecules that contribute to their virulence. Different serotypes have been found associated with different infections [3, 6, 7].

Epidemiology of *E. coli* has been based on variation in the above-mentioned phenotypic characteristics, and has been studied, largely because of the large numbers of different methods and non-commercial reagents required, in only a few major centres. Recently, generally applicable molecular methods based on nucleotide sequence variation in the chromosomal DNA of the isolates have been developed and used in epidemiological investigations. These methods include identification of the pattern of restriction endonuclease digests of the genome by pulsed field gel electrophoresis [8, 9] or detection of the restriction fragment length polymorphism (RFLP) at specific loci, such as fimbrial adhesin or ribosomal RNA

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Table 1. *Phenotypic characteristics and* Hind *III ribotype patterns of* E. coli *strains*

Serotype			Presence of*					*Hind* III ribotype	Origin†	Dg‡	No of strains	Reference
O	K	H	P	S	X	1C	HL					
1	1	1	+	–	–	–	–	A	CSF	Men	1	[4]
1	1	–	+	–	–	–	–	A	CSF	Men	1	[4]
1	1	–	+	–	–	–	–	B	Blood	PN	1	[4]
1	1	–	–	–	–	–	–	A	Blood	Sept	1	[4]
1	NT§	7	–	–	–	–	–	A	Blood	PN	1	[4]
1	1	7	–	–	–	–	–	B	Blood	PN	1	[4]
1	1	7	+	–	–	–	–	B	Blood	PN	2	[4]
1	1	7	+	–	–	–	+	B	Blood	Sept	1	[4]
1	1	7	+	–	–	–	–	B	Urine	Cys	1	[16]
1	1	7	+	ND‖	–	–	+	B	Urine	PN	1	[7]
1	1	7	+	–	–	–	–	B	Urine	PN	2	[7]
1	1	7	+	–	–	–	–	B	A		1	¶
1	1	7	+	–	–	–	–	B	F	Healthy	1	[17]
2	1	3	+	–	–	–	–	B	Blood	Sept	1	[4]
2	1	5	+	–	–	–	–	B	Blood	PN	1	[4]
2	1	5	–	–	+	–	–	B	Blood	PN	1	[4]
2	1	5	+	–	–	–	–	B	A		1	¶
2	1	5	+	–	–	–	–	B	F	Healthy	1	[17]
2	1	7	+	ND	–	–	–	B	Urine	ABU	1	[15]
2	1	6	–	–	–	–	–	C	Blood	Sept	1	[4]
2	1	6	–	ND	–	–	–	C	Urine	Cys	1	[16]
2	NT	–	+	–	–	–	–	D	Urine	Cys	1	[16]
4	12	1	+	–	–	–	+	C	Blood	PN	1	[4]
4	12	1	+	–	–	–	+	C	Blood	Sept	1	[4]
4	12	1	+	–	–	–	+	C	Urine	PN	2	[7]
4	12	5	+	–	–	+	+	E	Blood	PN	1	[4]
4	12	5	+	–	–	–	+	E	Blood	PN	1	[4]
4	12	5	+	ND	–	+	+	E	Urine	PN	2	[7]
4	12	–	+	–	–	–	+	E	Blood	PN	1	[4]
4	NT	5	+	–	–	–	+	C	Blood	PN	1	[4]
4	–	1	+	–	–	–	+	C	Blood	PN	3	[4]

6	2	1	+	–	–	+	+	F	Blood	PN	1	[4]
6	2	1	+	ND	–	+	+	F	Urine	PN	1	[7]
6	5	1	–	–	–	+	–	F	Blood	Sept	4	[4]
6	5	1	–	ND	–	+	–	F	Urine	ABU	1	[15]
6	5	1	+	–	–	+	+	K	Urine	ABU	1	[15]
6	5	1	–	–	–	–	–	F	Urine	Febrile UTI	1	[15]
6	5	1	+	–	–	+	–	F	Urine	PN	1	[7]
6	5	1	+	–	–	+	+	K	Urine	PN	1	[7]
6	5	1	–	ND	–	+	–	F	A		1	¶
6	5	1	–	–	–	–	–	F	F	Healthy	2	[17]
6	5	1	–	ND	–	+	–	F	F	Healthy	1	[17]
6	NT	1	–	ND	+	–	+	C	Urine	Cys	1	[15]
6	NT	31	–	–	+	–	+	C	Blood	Sept	1	[4]
6	NT	31	–	–	+	–	–	C	Blood	PN	1	[4]
6	NT	31	–	–	+	–	+	C	Urine	Cys	1	[16]
6	NT	31	–	–	+	+	+	C	Urine	Cys	4	[16]
6	NT	31	–	–	–	+	+	C	Urine	ABU	1	[15]
6	NT	31	–	–	+	–	+	C	F	Healthy	1	[17]
6	NT	31	+	–	–	–	–	C	F	Healthy	1	[17]
6	NT	–	–	ND	+	–	+	C	Urine	Cys	1	[15]
18	1	7	–	–	–	–	–	G	Blood	Sept	1	[4]
18	1	7	–	+	–	–	–	G	Blood	Sept	4	[4]
18	1	7	–	ND	–	–	–	G	Urine	Cys	2	[15, 16]
18	1	7	–	–	–	–	–	G	A		1	¶
18	1	7	–	+	–	–	–	G	F	Healthy	1	[17]
18	5	7	+	–	–	–	+	H	Blood	PN	1	[4]
18	5	–	+	ND	–	+	+	C	Urine	Cys	1	[16]
18	5	–	+	–	–	–	+	C	Urine	Cys	1	[16]
18	5	–	+	–	–	–	+	C	Urine	ABU	1	[15]
18	5	–	+	–	–	+	+	L	Urine	Cys	1	[16]
18	5	–	+	–	–	+	+	C	Urine	Cys	1	[16]
18	5	–	+	–	–	–	–	N	Urine	Cys	1	[16]
18	5	–	+	–	–	+	+	C	A		1	¶
18	5	–	+	–	–	–	+	O	F	Healthy	1	[17]
18	NT	–	+	–	–	–	+	C	Blood	PN	1	[4]
18	NT	–	–	–	–	–	–	I	Blood	PN	1	[4]

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Table 1 (*cont.*)

Serotype			Presence of									
O	K	H	P	S	X	1C	HL	*Hin*d III ribotype	Origin†	Dg‡	No of strains	Reference
22	13	1	−	−	−	+	+	F	Blood	Sept	1	[4]
22	13	1	−	−	−	+	+	F	Urine	ABU	1	[15]
22	13	1	−	−	−	+	+	N	Urine	Febrile UTI	1	[15]
22	13	1	−	−	−	+	+	F	Urine	Cys	2	[16]
22	NT	1	−	−	−	+	+	N	Urine	Cys	1	[16]
25	5	1	−	−	−	+	−	F	Blood	Sept	1	[4]
25	5	1	−	ND	−	−	−	F	Urine	Cys	1	[16]
25	5	1	−	ND	−	+	+	F	Urine	Febrile UTI	1	[15]
25	5	1	+	−	−	+	+	N	Urine	Cys	1	[16]
25	5	1	−	ND	−	−	−	F	A		1	¶
25	5	1	+	−	−	+	+	F	F	Healthy	1	[17]
25	5	1	+	−	−	−	−	F	F	Healthy	1	[17]
25	5	1	−	−	−	−	−	F	F	Healthy	3	[17]
25	5	1	−	−	−	+	+	F	F	Healthy	1	[17]
25	5	1	+	−	−	+	−	F	F	Healthy	1	[17]
25	5	6	+	−	−	−	−	F	Blood	PN	1	[4]
75	1	7	−	−	−	−	+	C	Blood	Sept	1	[4]
75	1	7	−	ND	−	−	+	C	Urine	Cys	1	[16]
75	1	7	−	ND	−	−	+	C	Urine	Febrile UTI	1	[15]
75	1	7	−	ND	−	−	+	C	A		1	¶
75	1	7	−	ND	−	−	+	C	F	Healthy	1	[17]
75	5	5	−	−	+	+	+	M	Urine	Cys	1	[16]
75	5	5	−	−	−	+	+	M	Urine	Cys	1	[16]
75	5	5	−	−	−	+	+	P	Urine	Cys	1	[16]
75	5	5	−	−	+	+	+	Q	Urine	Febrile UTI	1	[15]
75	5	5	−	−	+	−	+	C	Urine	Cys	1	[16]
75	5	5	−	ND	+	−	+	J	Urine	ABU	1	[15]
75	5	7	−	−	−	−	+	C	Blood	Sept	1	[4]
75	5		+	−	−	−	−	C	Blood	PN	1	[4]
75	5	−	−	−	−	−	−	C	Blood	Sept	1	[4]
75	5	−	−	−	−	−	−	C	Blood	PN	1	[4]
75	5	−	−	−	−	−	−	R	F	Healthy	1	[17]

R	1	7	+	–	–	–	B	Blood	Sept	1	[4]
R	1	7	+	–	–	–	B	Blood	PN	1	[4]
R	1	7	+	–	–	+	B	Blood	Men	1	[4]
R	1	7	–	+	–	–	G	Blood	Sept	1	[4]
R	1	7	–	+	–	–	–**	Blood	PN	1	[4]
R	1	7	–	–	–	–	B	Blood	Sept	1	[4]
R	1	7	–	–	–	–	F	Blood	Men	1	[4]
R	1	7	–	+	–	–	–	F	Healthy	1	[17]
R	1	7	+	–	–	–	B	F	Healthy	1	[17]
R	1	–	+	–	–	–	N	Blood	Sept	1	[4]
R	1	–	–	–	–	–	T	F	Healthy	1	[17]
R	1	33	–	–	–	–	S	Blood	Men	1	[3]
R	1	33	–	–	–	–	B	Blood	Men	2	[3]

* P, S, or type 1C fimbriae, X adhesin (non-P, non-S mannose-resistant haemagglutinin), HL, haemolysin.
† CSF, cerebrospinal fluid; A, appendix; F, faeces.
‡ Dg, diagnosis; Men, meningitis; PN, pyelonephritis; Sept, septicaemia; Cys, cystitis; ABU, asymptomatic bacteriuria; UTI, urinary tract infection.
§ NT, not typable, capsule present, not of type 1, 2, 5, 7, 12, 13, 27 or 30.
‖ ND, not determined.
¶ unpublished data, manuscript in preparation.
** –, not digestible with *Hind* III.

(rRNA) operons (ribotyping) [10, 12]. Ribotyping has been successfully used to evaluate the relatedness of certain *E. coli* isolates of clinical importance [13, 14].

In this study, we used a non-radioactively labelled rRNA probe to ribotype *E. coli* strains isolated from different extraintestinal infections and from stools of healthy persons. We were interested in the ability of this method to identify and possibly to differentiate further between strains of the same serotype and of similar phenotype. We therefore selected for the study 133 isolates representing homogeneous groups of strains that had been previously characterized by their O:K:H serotypes, expression of P, S, and type 1C fimbriae, non-P, non-S mannose-resistant adhesins, and haemolysin (3, 4, 7, 15–17].

MATERIALS AND METHODS

E. coli *strains*

A total of 133 *E. coli* strains were from the culture collection of the National Public Health Institute, Helsinki (Table 1). Of the strains, 54 were isolated from blood and 2 from cerebrospinal fluid of the patients with invasive infection (7 patients had meningitis, 24 non-focal septicaemia, 25 invasive pyelonephritis), 49 from urine of patients with urinary tract infection (UTI) (10 patients had non-invasive pyelonephritis, 28 cystitis, 4 febrile UTI, 7 asymptomatic bacteriuria), 7 from inflamed appendix, and 21 from stools of healthy persons. The strains had been characterized for their O, K and H antigens, and for the presence of P, S and type 1C fimbriae, non-P, non-S mannose-resistant (MR) adhesins, and haemolysin production [3, 4, 7, 15–17].

Extraction of chromosomal DNA

Chromosomal DNA was extracted by a DNA extractor (Applied Biosystems, Foster City, California), when large amounts of DNA were required, or for routine analysis using guanidium thiocyanate (Fluka BioChemica, Fluka Chemie AG, Buchs, Switzerland) [18] starting from 2 ml of an overnight culture in Luria-broth.

Restriction enzyme digestion

2–5 μg samples of chromosomal DNA, as estimated from agarose gel, were digested with restriction endonucleases (*Ava* I, *Bam* H I, *Bgl* II, *Cla* I, *Eco*R I, *Hin*d III, *Pst* I, *Sal* I, *Sma* I, Boehringer Mannheim GmbH, Mannheim, Germany, or New England Biolabs Inc., USA). The enzymes were used according to the instructions of the manufacturer in excess amounts (20 U/digest). Digests were analysed in 1% agarose gels (Sea KemME agarose, FMC BioProducts, Rockland, USA), 1xTAE (40 mM Tris, 1 mM-EDTA, pH adjusted to 8·0 with acetic acid) as running buffer. After staining with ethidium bromide, the gels were photographed.

Southern blotting

Gels were depurinated in 0·25 M-HCl in an orbital shaker for 15 min, denatured in 0·5 M-NaOH, 1·5 M-NaCl for 20 min, and neutralized in 0·5 M Tris, 1·5 M-NaCl pH 8·0 for 25 min. The DNA was transferred to a positively charged nylon membrane Hybond™-N+ (Amersham International plc, Amersham, UK) by capillary blotting with 20 × SSC (0·3 M-Na_3-citrate, 3 M-NaCl pH 7·0) overnight.

After blotting, the DNA was fixed with short alkaline treatment, by placing the membrane onto filter paper pad soaked in 0·4 M-NaOH for 2–4 min. The membrane was gently rinsed (max 1 min) in 5 × SSC.

Probes and hybridization

As a probe, either plasmid pKK3535 harbouring the 5S, 16S and 23S rRNA genes [19] or purified 16S + 23S ribosomal RNA (Boehringer Mannheim) was used. We used two different commercial kits in the labelling of the probe, hybridization and detection. With both rRNA and plasmid probe we used an ECL labelling and detection kit (Amersham International) based on enhanced chemiluminescence reaction. Blots were prehybrized in the hybridization buffer supplied with the kit, with 5% blocking agent and 0·42 M-NaCl, at 40 °C. After 30 min prehybridization, labelled probe was added to the buffer, and allowed to hybridize at 40 °C overnight. Washing and detection were as recommended. The membrane was exposed to an autoradiography film (Hyperfilm-ECL; Amersham International) for 1–10 min. With the plasmid probe also DIG DNA labelling and detection kit (Boehringer Mannheim) was used, based on random primed incorporation of deoxyuridin triphosphate with digoxigenin hapten. The membranes were prehybridized for 1–2 h and hybridized overnight at 60 °C. The hybrids were detected with anti-digoxigenin alkaline phosphatase conjugate by subsequent colour reaction on the membrane.

Statistical analysis

The χ^2-test was used to assess the significance of differences in the proportions of different ribotypes within serotypes.

RESULTS

A total of 133 *E. coli* strains isolated from various extraintestinal infections or stools from healthy persons (Table 1) were analysed. Southern blots of restriction endonuclease digests of the genomic DNA were hybridized with the plasmid pKK3535 that encoded the 5S, 16S and 23S rRNA operon of *E. coli*, or with purified *E. coli* 16S + 23S rRNA. In general, hybridization with either the plasmid or the rRNA probe gave identical banding patterns. However, in some strains few additional bands were detected with the plasmid probe, most probably due to the pBR322-derived vector region of the plasmid probe hybridizing with plasmid sequences in the test strains.

Preliminary experiments of various restriction enzymes (*Ava* I, Bam H I, *Bgl* II, *Cla* I, *Eco*R I, *Hin*d III, *Pst* I, *Sal* I, *Sma* I) with a panel of strains showed that *Hin*d III gave potentially the most discriminating profile of 9–10 bands over a size range of 3 to > 23 kb (Fig. 1). Among the 133 strains representing 9 O groups and 34 O:K:H serotypes 20 different hybridization patterns were detected. The patterns were marked with letters A to T, and are shown schematically in Fig. 2. Of the patterns, 17 had a similar basic structure and had at least 6 bands in common. Three patterns were entirely different: pattern A that comprised 3 of the 4 O1:K1:H1/H- and 1 O1:KNT:H7 (the only isolate of this serotype) strains, pattern S that included only 1 of the 3 OR:K1:H33 strains, and pattern T, that

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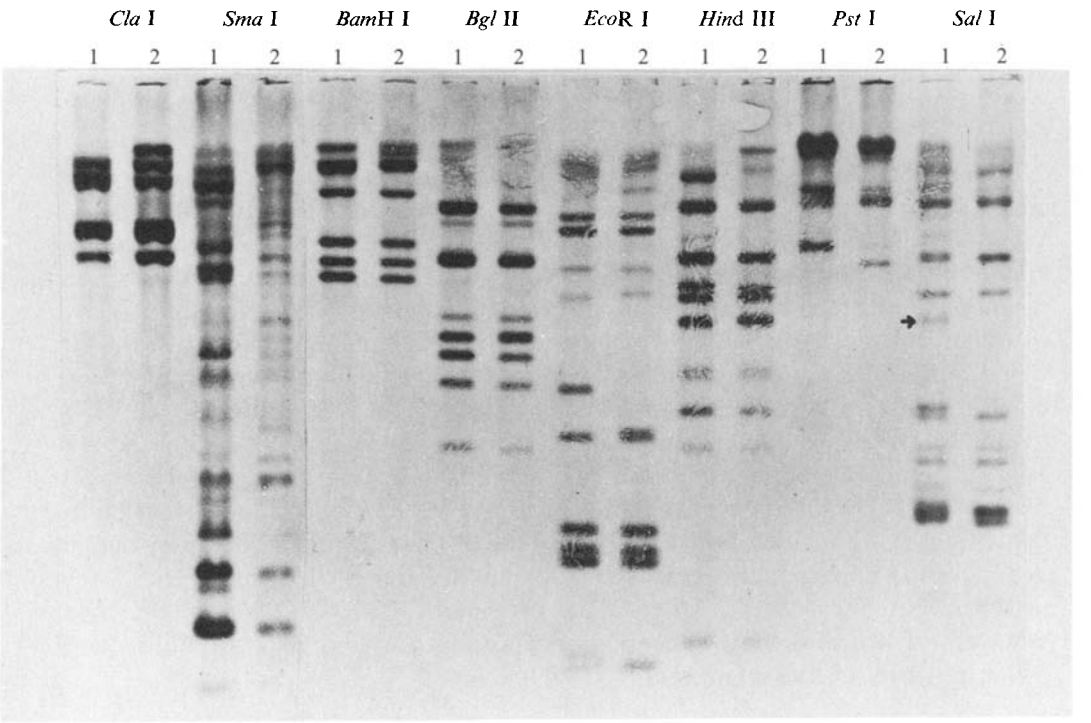

Fig. 1. Hybridization patterns of two representative *E. coli* strains obtained with various restriction enzymes and DIG labelled plasmid probe. Lane 1, O1:K1:H7 and lane 2, O4:K12:H1 strain. The arrow in *Sal* I/lane 1 (pattern a) indicates a 5·4 kb band that is replaced by a 4·8 kb band in *Sal* I pattern b (see Table 2).

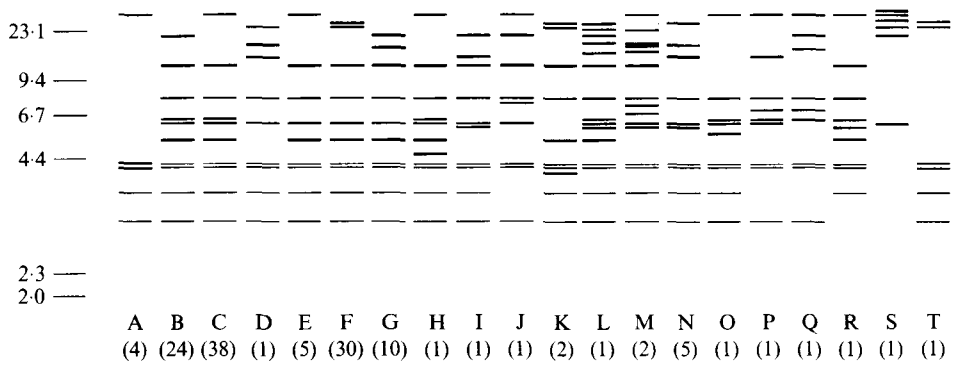

Fig. 2. Schematic representation of the ribotype patterns of *E. coli* obtained by *Hind* III digestion. The total number of strains belonging to each pattern is shown in parentheses. Molecular weight markers (kb) are shown on the left.

included only 1 OR:K1:H- strain. Four patterns (B, C, F and G) each encompassed 10–38 strains, whereas 11 patterns each included only 1 strain. The chromosomal DNAs from 2 of 9 OR:K1:H7 strains could not be digested with the *Hind* III enzyme, even though they could be digested with other restriction enzymes.

The distribution of the strains into different ribotypes correlated with their O:K:H serotypes (Table 1). Some ribotypes were restricted to strains of single or

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closely related serotypes; pattern A consisted of O1:K1:H1/H- (3/4 isolates) and O1:KNT:H7 (1 isolate) strains and pattern E of O4:K12:H5/H- (5/5 isolates) strains. Patterns B, C, F and N were common to several serotypes, e.g. all strains of serotypes O1:K1:H7 (10 isolates) and O2:H1:H5 (4 isolates) belonged to pattern B, and all strains of serotypes O4:K12:H1 (4 isolates), O6:KNT:H31 (10 isolates) and O75:K1:H7 (5 isolates) belonged to pattern C, whereas all O6:K2:H1 (2 isolates), and most O6:K5:H1 (10/13 isolates), O22:K13:H1 (4/5 isolates) as well as O25:K5:H1 (11/12 isolates) strains belonged to pattern F. Pattern N was found only occasionally among strains of various serotypes.

One hundred of 120 isolates with an identical O:K:H-antigenic structure belonged to the same ribotype (13 serotypes represented by a single isolate were not included) but a change in only one of these antigens could lead to an altered pattern. Thus K1:H7 strains with different O antigens belonged to 4 different ribotypes (B, C, F or G), and O1:K1 strains with different H antigens belonged to 2 different ribotypes (A or B). In some cases, two or more ribotypes were seen within strains of the same serotype, e.g. 2 phenotypically identical O6:K5:H1 strains (pattern K) differed from the bulk of that serotype (pattern F) in respect to a 6·2 kb band that was replaced by a 4·1 kb fragment. One O22:K13:H1 and one O25:K5:H1 strain showed a totally different banding pattern (pattern N) from the other strains of the same serotypes (pattern F). The majority of OR:K1:H7 (5/9) strains belonged to the same pattern as O1:K1:H7 strains (pattern B). However, 1 of the 3 OR:K1:H7 strains that expressed S fimbriae belonged to pattern G, thus resembling O18:K1:H7 strains.

Considerable heterogeneity was seen among O18:K5:H- (8 isolates) and O75:K5:H5 (6 isolates) strains. Within these 14 strains 8 different patterns were seen, whereas among the remaining 106 strains (serotypes represented by only 1 isolate were excluded from this analysis) only 10 patterns were seen ($P < 0{\cdot}001$). This variability was not explained by differences in other characteristics, e.g. the presence of various adhesins and haemolysin or the origin of the strains. Some strains with an identical set of those virulence-associated factors and from similar origin belonged to different patterns, e.g. 2 phenotypically identical O18:K5:H- strains belonged to patterns L and C, and 2 O75:K5:H5 strains to patterns M and P.

Strains within the same serotype and ribotype often possessed a heterogeneous set of virulence-associated factors, e.g. in O6:KNT:H31 strains, all belonging to ribotype C, there were 5 different combinations of adhesins and haemolysin. On the other hand, the 2 O6:K5:H1 strains, that possessed an identical set of virulence factors, belonged to ribotype K whereas the remaining 11 O6:K5:H1 strains all belonged to ribotype F. Furthermore, within a serotype, faecal isolates of healthy persons did not differ from clinical isolates: e.g. O1:K1:H7 strains, isolated from invasive infections, non-invasive UTI or from faeces, all belonged to pattern B. Only 2 faecal isolates, 1 O18:K5:H- and 1 O75:K5:H-, had a unique pattern different from the others of the same serotypes. However, among strains with the K5 antigen there was also variation between strains of similar origin.

Strains of ribotypes F (O6:K2:H1, O6:K5:H1, O22:K13:H1, O25:K5:H1/H6, total 29 of 30 type F strains) and G (O18:K1:H7 and OR:K1:H7, total 10 strains) were additionally analysed with *Sal* I (Table 2). *Sal* I gave a distinctive

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Table 2. *Analysis of* E. coli *strains belonging to* Hin*d III ribotype patterns F and G with* Sal *I enzyme*

Serotype	Number of strains	Ribotype pattern obtained with *Hind* III	*Sal* I
O6:K2:H1	2	F	a*
O6:K5:H1	11	F	a
O22:K13:H1	4	F	a
O25:K5:H1/H6	12	F	a
O18:K1:H7	9	G	b
R:K1:H7	1	G	b
R:K1:H7	2	−†	b

* a, pattern as in Fig. 1 in *Sal* I/lane 1, 5·4 kb fragment marked with an arrow replaced by a 4·8 kb fragment; b, pattern as in *Sal* I/lane 1.

† Not digestible with *Hind* III.

hybridization pattern for each group, but did not divide them further. The two OR:K1:H7 strains, that were not cleaved by *Hind* III, both expressed the S adhesin, and showed a hybridization pattern similar to the O18:K1:H7 strains.

DISCUSSION

The material in this study comprised a large set of extensively analysed *E. coli* strains isolated from patients with specific extraintestinal infections and from stools of healthy persons. Ribotyping, a genome-based typing method, was tested for its ability to replace or complement serotyping or other phenotypic typing methods. Two different probes, ribosomal RNA genes cloned in a plasmid vector or rRNA purified from *E. coli*, and two commercial non-radioactive DNA labelling and detection kits, based on enhanced chemiluminescence (ECL) or incorporation of digoxigenin labelled dUTP (DIG), were used in this study. With the DIG system we obtained sharper bands which made it easier to separate adjacent bands that could be misinterpreted as a single band with the ECL system. The advantage of the ECL method was that the same kit could be used with both the plasmid DNA and the rRNA probe, in contrast to DIG where separate kits would be needed for DNA and RNA labelling, and thus it enabled us to distinguish the interfering plasmid-derived bands.

In general the banding patterns obtained by the hybridization correlated with the serotype of the strains. Each of the O, K, and H antigens was of importance in identifying the serotype corresponding to a ribotype. Strains of one serotype usually belonged to the same ribotype, with only some exceptions. It has been suggested [6] that the H antigen should not be included in the definition of a clone, because of the ununiformity of H antigens within some serotypes (in particular within O2:K1). However, in this previous study all strains within one O:K serotype (except O2:K1) possessed identical H antigens. In contrast, in the present study, most of the O:K serotypes studied included strains differing in respect to their H serogroup. We found that differences in the H antigen were indeed correlated with differences in the ribotype.

An exceptional amount of variation of ribotypes was seen within groups of strains with the K5 antigen (O18:K5:H- and O75:K5:H5). This variability was not explained by differences in phenotypic characteristics (adhesins, haemolysin) or by different origins of the strains. The K5 capsule was identified by the sensitivity of strains to the K5 capsule-specific phage. However, cross-sensitivity to K5-specific phage has been reported in O18:K77:H7 and O75:K95:H5 strains [20]. Thus, the capsule detected by the phage may actually in some cases have not been K5 – this could explain at least part of this variation. Furthermore, the H- phenotype (non-motile strains) is probably a heterogeneous group of strains, in which the absence of flagella may be due to different reasons, e.g. mutations in a number of separate flagellation genes [21]. Achtman and Pluschke [6] have also reported variation in outer membrane protein and isoenzyme patterns among O18:K5 strains.

Our finding that strains of the same O:K:H serotype have identical ribotypes is in agreement with the clone concept [1] and gives further support to the hypothesis of a common ancestry of a clone. Recombination between chromosomal genes is thought to be a rare event that does not prevent the preservation of such clones [22]. However, strains of the same serotype and identical ribotype were found to possess quite heterogeneous sets of virulence factors. This is suggestive of horizontal transfer of genes encoding virulence determinants between genetically distant clones as suggested for the *pap*-region encoding P-fimbriae of *E. coli* [23]. On the other hand, there is evidence that at least fragments of genes encoding fimbriae (and possibly other virulence-associated characteristics) may be present in the genome even though they are incompletely expressed [9, 24, 25]; thus the strains actually might be less heterogeneous at the genetic level.

It should be pointed out that although a difference in the ribotype pattern shows that two strains are of different origin, similar ribotype patterns of isolates do not prove them identical. We observed that strains with totally different serotypes could belong to the same ribotype pattern. This indicates that ribotyping cannot replace serotyping, but it is a useful tool complementing serotyping, and can also be used for epidemiologic evaluations where serotyping methods are not available. Furthermore, ribotyping is especially valuable in the case of nontypable strains, where the full O:K:H serotype cannot be determined.

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