ORIGINAL ARTICLE

Epidemiologic typing of *Escherichia coli* using RAPD analysis, ribotyping and serotyping

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Objective To compare random amplified polymorphic DNA (RAPD) analysis and ribotyping with serotyping for epidemiologic typing of *Escherichia coli*.

Methods Thirty-two epidemiologically unrelated strains, nine cerebrospinal fluid isolates with the O7K1 serotype from nine patients, and nine sets of epidemiologically related *E. coli* isolates from nine patients were typed by RAPD analysis, ribotyping and serotyping.

Results Among the 32 epidemiologically unrelated *E. coli* isolates, 29 types were distinguished by RAPD analysis, 25 by ribotyping and 27 by serotyping. Indistinguishable patterns were obtained by RAPD analysis and ribotyping within the collection of nine cerebrospinal fluid isolates. For the epidemiologically related isolates, intrapatient variation was only found by RAPD analysis among the isolates of one set and by ribotyping among the isolates of two sets. No interpatient variation was observed between three sets of isolates. With serotyping, the epidemiologically related isolates yielded similar typing relationships to those obtained by RAPD analysis and ribotyping.

Conclusions RAPD analysis had the highest discriminatory capacity for typing *E. coli* isolates. RAPD analysis, ribotyping and serotyping can all be used for assessment of strain relationships.

Keywords *E. coli*, RAPD, ribotyping, serotyping

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INTRODUCTION

Most healthy individuals carry *Escherichia coli* in the intestine. Nevertheless, members of this species can give rise to severe infections, including diarrhoea, urinary tract infection, sepsis and neonatal meningitis [1]. Efficient methods for strain identification are required to study the epidemiology of infections caused by *E. coli* and to screen for possible spread of *E. coli* strains in hospital wards. Traditionally, *E. coli* isolates have been characterized by serotyping with the lipopolysaccharide (O-type), the capsule antigen (K-antigen) and flagellar (H-type) antigen [2]. However, not all *E. coli* isolates can be typed with serotyping and strains within a serotype cannot be distinguished [3–6]. In addition, most diagnostic laboratories are dependent for serotyping on reference laboratories. In general, genotypic methods are considered to be more discriminatory than phenotypic methods and are increasingly being used in diagnostic laboratories. It has been suggested that combining data obtained by different typing methods will give an optimal insight into strain relatedness [7].

To test the usefulness of genotypic typing methods, this study compared random amplified polymorphic DNA (RAPD) analysis [8–11] and ribotyping [12,13] with conventional serotyping for three collections of well-defined *E. coli* isolates.

Typing results obtained by using the three methods were compared and evaluated with respect to discriminatory capacity, stability and intra- and interpatient variation.

MATERIALS AND METHODS

Isolates

Three collections of *E. coli* isolates were included in the study. The collection of epidemiologically unrelated isolates included 20 *E. coli* urinary isolates from adult patients of general practitioners in Leiden, 10 epidemiologically unrelated *E. coli* isolates from neonatal cerebrospinal fluid samples from several

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bacteriological laboratories in The Netherlands [14], and *E. coli* strains ATCC 11775^T and ATCC 25922. These isolates were considered unrelated on the basis of their epidemiologic origin. In addition, a collection of nine epidemiologically unrelated *E. coli* isolates with the same serotype (O7K1), isolated from cerebrospinal fluid samples from nine neonates [14], was included in the study.

The collection of epidemiologically related isolates included 21 *E. coli* isolates from five neonates (patients I, II, III, IV and VII; Table 1) and 19 isolates from four adult hospitalized patients (patients V, VI, VIII and IX; Table 1) in the Leiden University Medical Center. Within these collections, sets of isolates were considered epidemiologically related because they were isolated from the same patient or because they were isolated from patients hospitalized in the same ward during the same period (Table 1).

All isolates included in the study were identified as *E. coli* by the API-20E system (bioMérieux, Marcy l'Etoile, France) and by a negative oxidase reaction (DrySlide; Difco, Detroit, MI, USA).

Serotyping

O-, OK- and H-antisera were prepared as described previously [15,16]. O-antigen suspensions were prepared by heating an overnight broth culture for 1 h at 100 °C to inactivate the K-antigen. The antigens, diluted ($OD_{600\,nm}$ 0.22) with formalinized (0.5%) phosphate-buffered saline (PBS), were stained with gentian violet (0.005% w/v) and tested against all 173 O-antisera in an agglutination test [17]. Positive reactions were titrated in microtitre plates. If all reactions with a strain were negative, an overnight broth culture of that strain was autoclaved at 121 °C for 2 h to inactivate a possible heat-stable K antigen (A antigen). The antigen was then stained and titrated against antisera of O8, O9, O20 and O101.

Growth taken from fresh agar slants (37 °C and 18 °C) was used for K-antigen determination with 71 OK-antisera [16]. The cultures were tested by slide agglutination against a 1 : 5 dilution of homologous O-antisera. Agglutination indicated the absence of a K-antigen. If no agglutination was observed, the cultures were tested against K-antisera known to be associated with that O-group. If a positive reaction was observed, the K-antigens were provisionally established. If negative, the results were recorded as 'K?', indicating a surface structure causing O inagglutinability. The influence of growth temperature on the development of the K-antigen [18] was tested against the homologous O and OK antisera after incubation at 18 °C on a fresh agar slant. Temperature-independent K-antigens do not agglutinate with the homologous O-antiserum, whereas temperature-dependent K-antigens should agglutinate with the homologous O-antiserum.

For H-antigen determination [16], the cultures were checked for motility in U-tubes with semisolid medium (0.1–0.4% agar) and, if motile, passaged through U-tubes on five occasions. After the last passage, the cultures were grown in broth overnight at 37 °C and then formalinized (0.5%). The suspensions were tested against 10 pools of a total of 56 H-antisera in tube agglutination tests. The tubes were incubated at 45 °C and read

Table 1 Typing data for the clinically related strains

Patient	Department	Period	Serotype (*n*)[a]	RAPD type (*n*)[a]	Ribotype (*n*)[a]
I	NEON	12/96	O8K(A)28H-(1)	A (1)	a (1)
II	NEON	01/97	O8K-H-(1)	A (1)	a (1)
III	NEON	12/96	O2K-H6 (6)	B (6)	b (6)
		–	O7K-H? (1)	C (2)	c (2)
		01/97	O7K7H?(1)		
IV	NEON	12/96	O8K(A)28H-(6)	A (8)	a (8)
			O8K-H? (1)		
			O8K28H-(1)		
V	NEPH	09/96	O nontyp.:Hauto (1)	D (8)	d (7)
		–	O nontyp.:H19 (2)		e (1)
		10/96	O nontyp.:H- (4)		
			O nontyp.:H? (1)		
VI	ENDO	09/96	O24K-H? (1)	E (3)	f (3)
			O auto.:H? (2)		
VII	NEON	07/96	O9K?H? (3)	F (3)	g (3)
VIII	ICHK	10/96	O6K1H- (4)	G (4)	h (4)
IX	NITR/NEPH	07/96 -	O7K1Hauto (3)	H (4)	j (4)
		08/96	O7K1H6 (1)		

NEON, neonatology; NEPH, nephrology; ENDO, endocrinology; ICHK, surgical intensive care; NITR, kidney transplantation.
[a] Number of isolates is shown in parenthesis.

after 1–2 h. One or a combination of two pools gave a key for the established H-antigen. Positive reactions were titrated.

RAPD analysis

Template DNA was prepared from bacteria grown overnight at 37 °C on blood agar plates. Crude DNA extracts were obtained by suspending four colonies in 1 mL distilled water and boiling at 95 °C for 15 min. Each polymerase chain reaction mixture consisted of a Ready-To-Go RAPD Analysis bead (Pharmacia Biotech, Freiburg, Germany), 50 pmol M13-core primer (5′-GAG GGT GGC GGT TCT-3′) (Eurogentec Nederland b.v., Maastricht, The Netherlands) or DAF4 primer (5′-CGG CAG CGC C-3′) (Eurogentec), and 2 μL of template DNA suspension, all in a final volume of 25 μL. Amplification was performed in a Progene thermocycler (Techne, Cambridge, UK), starting with a denaturation step of 5 min at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C. RAPD products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide (0.5 mg/L) with TAE running buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). A 100 bp ladder was included on all gels. After electrophoresis, the gels were photographed with a Polaroid camera and the RAPD banding patterns were evaluated visually.

Ribotyping

DNA was extracted by the method of Pitcher et al. [19]. Restriction endonuclease digestion and hybridization was performed as described by Van Soolingen et al. [20]. In short, DNA was digested with *Eco*RI (10 U/μL) in a final volume of 20 μL [21]. The restriction fragments were separated by electrophoresis at 25 V for 18 h on a 0.8% agarose gel [19]. Subsequently, the DNA fragments were transferred to a nylon membrane (Hybond-N^+; Amersham, Little Chalfont, UK) by vacuum blotting (Vacuum blotter Model 785; Bio-Rad, Veenendaal, The Netherlands) and the membrane was incubated overnight at 42 °C with a 16S and 23S rRNA probe (product 206938; Boehringer Mannheim, Lewes, UK) labelled with horseradish peroxidase. Detection of hybridized DNA was performed using the enhanced chemiluminescence-based ECL™ system [12,20]. The banding patterns were evaluated visually.

Interpretation of molecular typing results

For both RAPD analysis and ribotyping, banding patterns with a difference of $\geqslant 2$ bands were considered to represent different strains, while isolates with <2 bands difference were regarded as the same strain.

Determination of reproducibility, stability and discriminatory capacity

The stability of the RAPD typing and the ribotyping method was tested by typing 11 subcultures of three strains as described previously [22]. In short, one subculture was frozen at −80 °C and recultured after 6 days. One subculture was stored at 4 °C for 1 week and three at 21 °C for 1, 3 and 7 days, respectively. In addition, each isolate was tested after one, two and four subcultures and after subsequent storage at 4 °C for 2 days followed by one, two and five subcultures. The stability was defined as the number of indistinguishable patterns divided by the number of subcultures.

The discriminatory capacity of the molecular typing methods was determined by calculating Simpson's index of diversity, as described by Hunter [23], on the basis of type distribution among the 32 epidemiologically unrelated strains.

RESULTS

RAPD analysis

In total, 23 banding patterns were distinguished among the 32 epidemiologically unrelated isolates with the M13-core primer and the DAF4 primer, giving a Simpson's index of diversity of 0.98 and 0.97, respectively. When the results obtained with both primers were combined, 29 types were distinguished, which corresponds with a Simpson's index of diversity of 0.99. Examples of banding patterns are shown in Figure 1.

The banding patterns of three distinguishable isolates were unchanged following the subculture procedure (data not shown), demonstrating that the stability of the banding patterns of these isolates was 100% over the period tested. Indis-

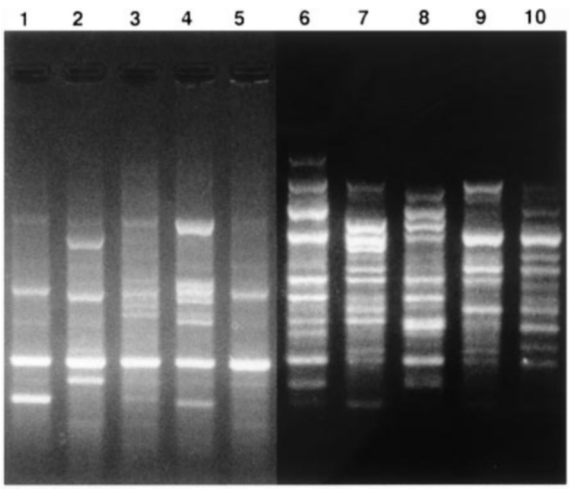

Figure 1 Examples of banding patterns obtained by RAPD analysis of *E. coli* isolates with M13-core primer (lanes 1–5) and DAF 4 primer (lanes 6–10).

tinguishable banding patterns were also observed within the collection of nine cerebrospinal fluid isolates with the O7K1 serotype.

The results for the epidemiologically related isolates are shown in Table 1. For the patients with multiple isolates, no intrapatient variation was observed among the isolates from two neonates (patients IV and VII) and from four adult patients (patients V, VI, VIII and IX). Two profiles were distinguished among the isolates from another neonate (patient III). No interpatient variation among the isolates from three neonates (patients I, II and IV) was observed. The sets of epidemiologically related isolates from the remaining six patients had different banding patterns.

Ribotyping

Twenty-five ribotypes were distinguished among the 32 epidemiologically unrelated isolates, corresponding to a Simpson's index of diversity of 0.98. Examples of banding patterns are shown in Figure 2. Indistinguishable types were obtained from subcultures of three isolates (data not shown), demonstrating that the stability of the ribotyping was 100% over the period tested. The nine cerebrospinal fluid isolates with the O7K1 serotype from nine neonates also had indistinguishable patterns. The results for the epidemiologically related isolates are shown in Table 1. For the patients with multiple isolates, no intrapatient variation was observed among the sets of isolates from two neonates (patients IV and VII) and from three adult patients (patients VI, VIII and IX). Among the isolates from the remaining adult patient (patient V) and one neonate (patient III), two profiles (d,e and b,c, respectively) were distinguished. No interpatient variation was observed between the isolates from patients I, II and IV (type a). The sets of isolates from the remaining six patients had different patterns.

Serotyping

Seven epidemiologically unrelated *E. coli* isolates were O-nontypable. Twenty-seven serotypes were distinguished among the 32 epidemiologically unrelated *E. coli* isolates, corresponding to a Simpson's index of diversity of 0.98. The results for the series of epidemiologically related isolates are shown in Table 1. The isolates from patient V were O-nontypable. Patients VI and IX had two serotypes within the sets of isolates; patients III and IV, three serotypes; and patient V, four serotypes. The isolates from three neonates (patients I, II and IV) all had the O8 serotype, but similar H and K serotypes were also found among the isolates from these patients. Distinct serotypes were found among the isolates from the remaining patients.

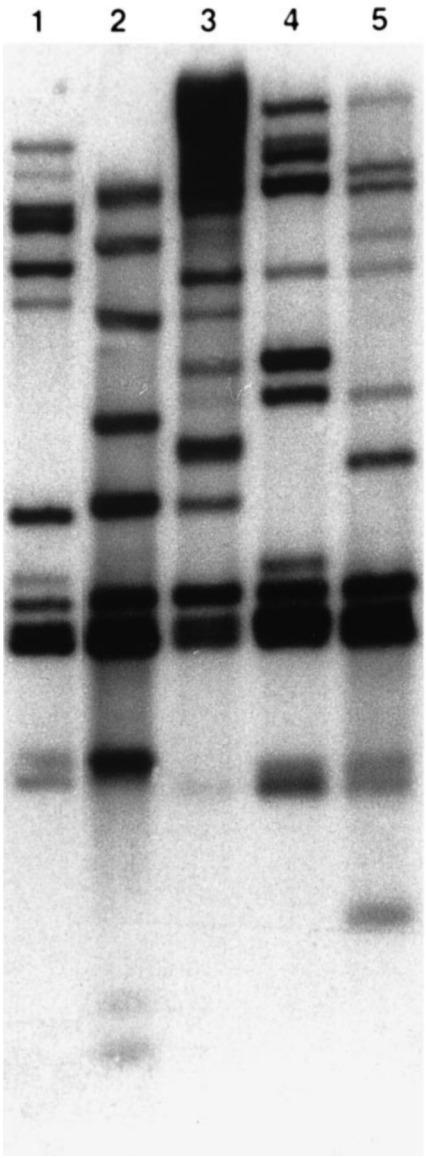

Figure 2 Examples of banding patterns obtained by ribotyping from *E. coli* isolates.

DISCUSSION

Although *E. coli* is a common inhabitant of the human intestine, this bacterial species is also a causative agent of infections [1,14]. To study the transmission of these bacteria between patients, typing methods that allow for discrimination at the strain level are required. Therefore, this study assessed the performance of RAPD analysis and ribotyping for 32 well-described epidemiologically unrelated *E. coli* isolates, a collection of nine *E. coli* isolates with the O7K1 serotype from cerebrospinal fluid samples from nine patients and nine sets of epidemiologically related isolates, in comparison with results obtained by sero-

typing and in association with known data regarding the origin of the isolates.

Struelens et al. [7] recommended that 100 isolates should be used to test the discriminatory capacity of a typing method. The present study used 32 strains since it was considered that this number was sufficient for comparing the typing methods, provided that the strains were carefully selected on the basis of their distinctness in time and space. RAPD analysis had the highest discriminatory capacity for typing *E. coli* isolates, whereas the discriminatory capacity of ribotyping was comparable with that of serotyping. The results obtained with the epidemiologically unrelated isolates indicated that RAPD analysis, ribotyping and serotyping may be used as single typing methods since the Simpson's index of diversity was >0.95 in each case [7].

The banding patterns obtained with RAPD analysis or ribotyping were not influenced by subculturing or storage conditions, since no differences were observed in the banding patterns from the subcultures of each of the three tested isolates.

Sets of isolates from three adult patients (patients VI, VIII and IX) and two neonates (patients IV and VII) showed similar banding patterns within each set by both molecular typing methods, supporting the assumed epidemiologic relatedness of the isolates within each set. One neonate (patient III), having distinct isolates with different banding patterns, was apparently infected with different strains. The similar banding patterns of the isolates from three neonates (patients I, II and IV) indicated transmission of one *E. coli* strain within the neonatology department.

Overall, the results obtained with the epidemiologically related strains indicated that RAPD analysis and ribotyping of *E. coli* isolates are applicable in clinical epidemiology since the typing data correlated with the epidemiologic origin of the strains. RAPD analysis and ribotyping may be preferable to serotyping for investigating cross-infection or epidemic spread, since more serotypes were distinguished among the isolates within the sets from patients III, IV, V, VI and IX than were RAPD or ribotypes. Moreover, in contrast to serotyping, RAPD analysis and ribotyping are applicable in any laboratory. However, since RAPD analysis is faster and less laborious than ribotyping, RAPD analysis seems an acceptable option to screen for spread of *E. coli* in a hospital ward in the case of suspected cross-infection or epidemic spread.

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