Differentiation of faecal *Escherichia coli* from human and animal sources by random amplified polymorphic DNA–PCR (RAPD–PCR)

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Abstract In this study the assessment of randomly amplified polymorphic DNA (RAPD) analysis was established as a molecular epidemiological tool. RAPD analysis was performed to differentiate faecal *Escherichia coli* isolates from human and animal sources. *E. coli* strains (128) were isolated from human and animal faeces (from cattle and sheep). Genomic DNA was extracted and randomly amplified polymorphic DNA–PCR (RAPD–PCR) fingerprinting was performed. Seven arbitrary primers were tested with a view to discriminating between *E. coli* isolates from humans and *E. coli* isolates from animals. RAPD profiles were analysed with hierarchical cluster analysis using an unweighted pair group method. RAPD profiles obtained with three of the tested primers (1247, 1290 and 1254) established a distinct differentiation between *E. coli* isolates from humans and *E. coli* from animals. Low levels of misclassification and high levels of specificity make RAPD a sensitive, efficient and reliable means of distinguishing closely related strains.
Keywords Discrimination; *Escherichia coli*; faecal; RAPD typing

Introduction

Faecal pollution is a major concern with regard to many water resources, where it can originate from a variety of sources, including humans and animals. Its impact can degrade water quality and restrict its use. However, without knowing the precise source of faecal input, the human health risk cannot be accurately predicted. *Escherichia coli* is a ubiquitous bacterium in the intestines of warm-blooded animals and is used as an indicator of faecal pollution (Parveen *et al.*, 1999). In order to assess risk it is important to determine whether the source of faecal contamination is human or animal, as microorganisms of human origin are regarded as having greater potential to cause disease in humans (Guan *et al.*, 2002). DNA genotyping methods have been increasingly used for bacterial characterisation and for taxonomic studies (Bando *et al.*, 1998; Maurer *et al.*, 1998; Carson *et al.*, 2001; McLellan *et al.*, 2002). RAPD–PCR is one of the most promising of these methods as it has proven to be a reliable, practical, rapid, cost-effective strategy and it is suited for studying a large number of strains (Wang *et al.*, 1993; Seppala *et al.*, 1994; Pacheco *et al.*, 1997; Arias *et al.*, 1998; Bando *et al.*, 1998; Grif *et al.*, 1998; Maurer *et al.*, 1998; Chansiripornchai *et al.*, 2001; Dautle *et al.*, 2002; Khan *et al.*, 2002; Aslam *et al.*, 2003). The purpose of this study was to identify a procedure that could be used to differentiate *E. coli* isolates according to their origin (human–animal); thus we applied the RAPD method, and dendrograms were constructed based on the statistical analysis of fingerprint differences.

Materials and methods

Bacterial strains

The study included 128 *E. coli* isolates from human and animal faeces. All strains were isolated according to standard culture procedures on selective media: mEndo agar (Les-BBL) and TBX medium (Oxoid) and identified using API 20E (bioMerieux). Of the *E. coli* strains 60 were isolated from animal faeces (cows, sheep, bovine) and 68 from human faeces.

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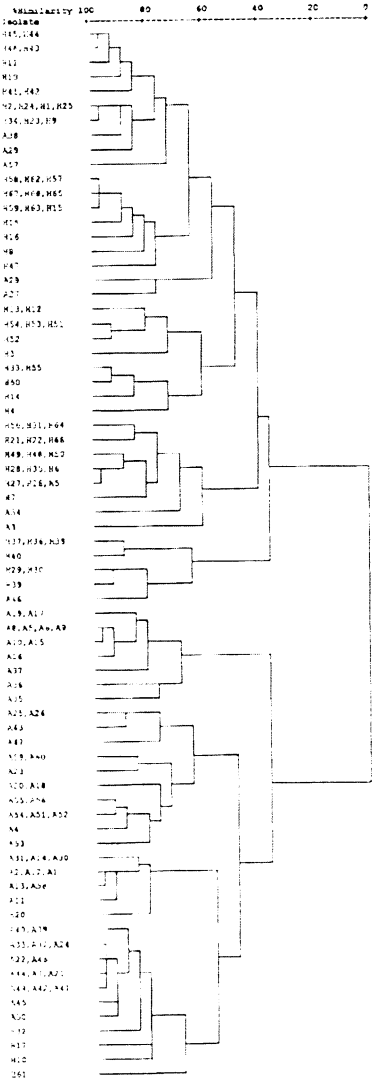

Figure 1 RAPD–PCR method analysis with primer 1247 of 128 *E. coli* isolates. Prefixes A and H indicate veterinary and human isolate respectively

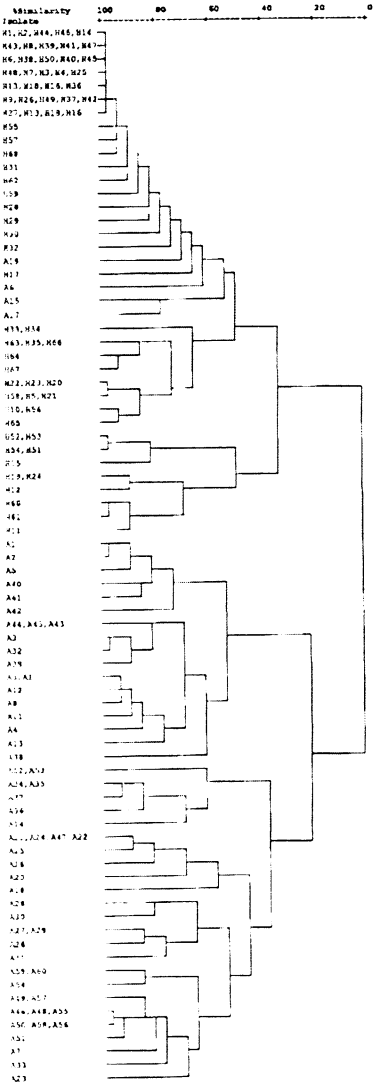

Figure 2 RAPD–PCR method analysis with primer 1254 of 128 *E. coli* isolates. Prefixes A and H indicate veterinary and human isolate respectively

DNA extraction

Genomic DNA was extracted from 1.5 mL stationary-phase *E. coli* cells using 20 mg/mL proteinase K and phenol–chloroform–isoamylalcohol (25:24:1) (Wang *et al.*, 1993). DNA samples were analysed using a spectrophotometer at 260/280nm for determination of purity.

RAPD fingerprinting

Several 10-nt primers were tested (Table 1). PCR was performed with the use of a Peltier Thermal Cycler (MJ Research) in 50 µL reaction volumes containing approximately 50 ng of bacterial DNA, $MgCl_2$ 2.5 mM, 10× PCR buffer, 20 pmol of primer, 1 U of AmpliTaq DNA polymerase and 250 µM of each dNTP (dATP, dCTP, dGTP and dTTP). All data used in the present analysis were generated in a cycling program of 45 cycles of the following: 94°C, 1 min; 38°C ramp to 72°C, 3 min; 72°C, 2 min. After PCR 20 µL aliquots of the products were electrophoresed in 1.5% agarose gels containing 0.5 µg/mL

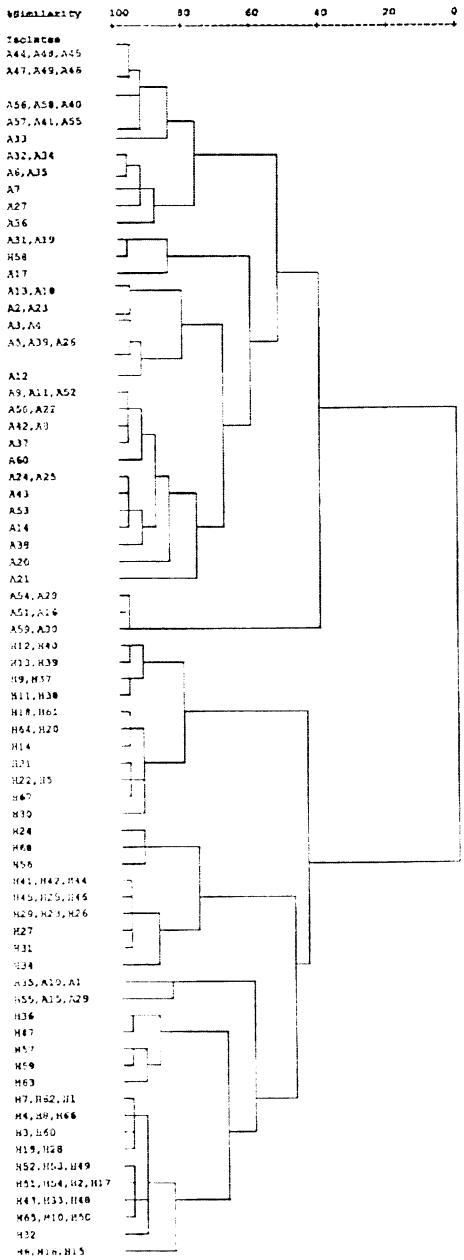

Figure 3 RAPD–PCR method analysis with primer 1290 of 128 *E. coli* isolates. Prefixes A and H indicate veterinary and human isolate respectively

ethidium bromide. Gels were photographed under UV light. A molecular size marker (100 bp, Biolabs) was used for reference in all gels.

Analysis of data

RAPD profiles were analysed with the Labworks Analysis Software UVP-Version 3.0.02.00. The statistical analysis of data was performed with the SPSS program, version 10.0, using Hierarchical Cluster Analysis (Unweighted pair-group method-UPGMA – Pearson correlation coefficient).

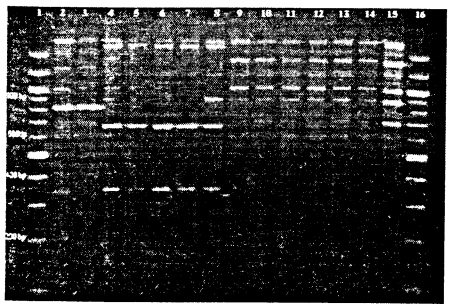

Figure 4 Representative RAPD profiles obtained with primer 1247. Lanes 1 and 16: ladder 100 bp (Biolabs: New England). Lanes 2–9: animal isolates. Lanes 10–15: human isolates

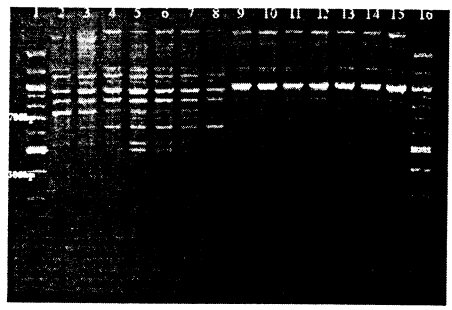

Figure 5 Representative RAPD profiles obtained with primer 1254. Lanes 1 and 16: ladder 100 bp (Biolabs: New England). Lanes 2–9: animal isolates. Lanes 10–15: human isolates

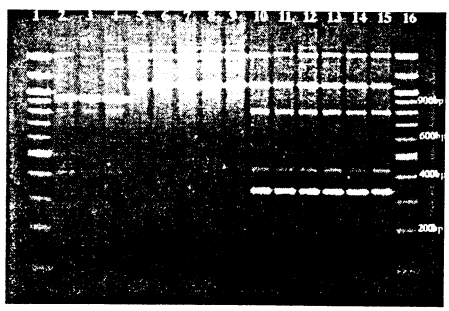

Figure 6 Representative RAPD profiles obtained with primer 1290. Lanes 1 and 16: ladder 100 bp (Biolabs: New England). Lanes 2–9: animal isolates. Lanes 10–15: human isolates

Table 1 Primers used for RAPD analysis

Primer	Nucleotide sequence	Primer	Nucleotide sequence
1247	5′-AAGAGCCCGT-3′	OPA6	5′-GGTCCCTGAC-3′
1254	5′-CCGCAGCCAA-3′	OPA10	5′-GTGATCGCAG-3′
1290	5′-GTGGATGCGA-3′	OPA14	5′-TCTGTGCTGG-3′
OPA5	5′-AGGGGTCTTG-3′	OPA15	5′-TTCCGAACCC-3′

Results and discussion

A total of eight primers were tested. Three primers that gave distinct differentiation between *E. coli* isolates from animal and human sources were chosen. Considering isolates with less than 75% of bands matching as different RAPD types, dendrograms were obtained according to different RAPD profiles of *E. coli* isolates. RAPD profiles obtained with primers OPA14, OPA15, OPA5, OPA6 and OPA10 did not establish a distinct differentiation between *E. coli* isolates according to their source. Data obtained with primers 1247, 1290 and 1254 separated bacteria into two major clusters according to their source (human–animal).

With primer 1247, *E. coli* isolates were divided into two major groups with a similarity level of 32%. Isolates generated 2–15 amplification bands. *E. coli* from animals showed 23 different RAPD types, whilst those from humans showed 15 RAPD types (Figures 1 and 4). The misclassifications observed were eight veterinary isolates within the major cluster of human isolates, and five human isolates within the major cluster of veterinary isolates. The specificity of the classification was 85%.

RAPD data with primer 1254 divided isolates in two major clusters with a similarity of

18%. Isolates generated 3–16 amplification bands. Human isolates generated 13 different RAPD types, while veterinary isolates generated 27 RAPD types (Figures 2 and 5). Only four veterinary *E. coli* isolates were found concentrated within the major cluster of human isolates. The specificity level of the method was 97%.

With primer 1290 we obtained two major clusters with bacteria from humans and animals respectively. The similarity level of the two clusters was 46%. Isolates generated 3–12 amplification bands. Animal isolates generated 13 different RAPD types and human isolates 10 RAPD types (Figures 3 and 6). Only four veterinary *E. coli* isolates were found concentrated within the major cluster of human isolates. The specificity level of the classification was 97%. Most of the misclassifications with the three primers used (1247, 1254 and 1290) concerned isolates from animals.

RAPD is a molecular tool that covers most of the genomic diversity and has been increasingly used in microbiology. In this study RAPD analysis using different primers revealed that the 128 *E. coli* isolates originating from different sources generated distinct amplification profiles. Moreover, many of the isolates from the same source showed similar or identical RAPD profiles. The greater diversity of RAPD profiles was observed among veterinary isolates. They revealed more RAPD subtypes than human isolates, which showed homology in their amplification patterns (Table 2).

Our dendrograms showed a good discrimination between veterinary and human *E. coli* isolates generating two major clusters that concentrated the two main groups of isolates (veterinary and human) (Figures 1–3). RAPD analysis has been used for similar classifications and differentiations of *E. coli* isolates (Wang *et al.*, 1993; Pacheco *et al.*, 1997; Grif *et al.*, 1998; Chansiripornchai *et al.*, 2001; Aslam *et al.*, 2003) or other microorganisms (Seppala *et al.*, 1994; Arias *et al.*, 1998). Concerning the classification of *E. coli* isolates and other bacteria, although many methods have been applied such as ribotyping (Grif *et al.*, 1998; Parveen *et al.*, 1999; Carson *et al.*, 2001; Guan *et al.*, 2002), PFGE (Grif *et al.*, 1998; McLellan *et al.*, 2002) and AFLP (Guan *et al.*, 2002; Aslam *et al.*, 2003), RAPD–PCR has been proposed as a method less laborious and time-consuming than other DNA-based techniques (Wang *et al.*, 1993; Pacheco *et al.*, 1997; Arias *et al.*, 1998; Bando *et al.*, 1998; Grif *et al.*, 1998; Maurer *et al.*, 1998; Chansiripornchai *et al.*, 2001). It seemed to be the fastest genetic typing method that could be employed for a rapid identification, and it has been widely used as a typing technique for both Gram-positive and Gram-negative bacteria (Arias *et al.*, 1998). Other techniques require relatively large amounts of DNA, expensive equipment and take days to obtain results. By contrast, RAPD results are generated within 4 h and hence are time and cost saving (Grif *et al.*, 1998; Chansiripornchai *et al.*, 2001). Furthermore, RAPD typing is more efficient than the other methods when numerous isolates are to be examined (Meunier and Grimont, 1993; Pacheco *et al.*, 1997). The main disadvantage of RAPD–PCR is its reproducibility, which can be overcome when the conditions are strictly defined (Meunier and Grimont, 1993). The RAPD method is reliable when used to answer limited problems, such as typing of a collection of unknown isolates, which do not necessitate between-laboratory comparison. In the present study the conditions and materials of all experiments were strictly identical and defined; thus we

Table 2 Human and animal isolates showing the same amplification bands of human and animal RAPD patterns, respectively, with the use of three primers

	Primer		
	1290	1254	1247
% human isolates sharing 70% of amplification bands of human RAPD pattern	81	69	44
% animal isolates sharing 70% of amplification bands of animal RAPD pattern	52	57	33

demonstrated that the RAPD fingerprints of isolates were reliable and enabled the compilation of a data bank of patterns for isolate classification.

Conclusions

The objective of this study was to evaluate the RAPD–PCR method as a molecular typing tool for the differentiation of *E. coli* isolates originating from animals and humans. Our results demonstrated that RAPD analysis provided great discriminatory ability and high rates of specificity, depending on the primers used, as *E. coli* isolates could be grouped and separated into two major clusters according to their source. Although RAPD analysis is not too laborious, and is fast, reliable and low cost, it needs high standardisation. Moreover, environmental studies with genetic comparisons to detect sources of *E. coli* contamination will require extensive isolation of strains to encompass the *E. coli* strain diversity found in host sources of contamination. This assay assists in the development of approaches to determine sources of faecal pollution, an effort important for protecting water resources and public health.

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