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COMPARISON OF TRADITIONAL AND MOLECULAR TYPING METHODS OF *ESCHERICHIA COLI* O157*

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An account is given using typing methods and detection of virulence genes of different serotypes of *Escherichia coli* isolated in Hungary. By hybridization using SLT-I and SLT-II probes and PCR method using stx1–2, eae and ehx primers we could differentiate O157 strains of different serotypes into eight (stx, eae, ehxA positive; stx, eae positive; stx, ehxA positive; stx positive; eae, ehxA positive; eae positive; ehxA positive; stx, eae, ehxA negative) types. The discriminatory power of phage typing proves to be much higher than that of the plasmid profile. RAPD typing with different primers could confirm or exclude the subtypes identity of the isolated *E. coli* O157 serotypes. *Escherichia coli* O157:HNM isolates could be sorted in six different phage types and six different RAPD types with ERIC-1, in five RAPD types with ERIC-2 and in seven types with M13 primers. *Escherichia coli* O157:H7 showed six different phage types and three RAPD types with ERIC-1 and ERIC-2 and five types with M13 primers. According to our results the standard PFGE protocol [32] gives the opportunity to differentiate epidemiologically independent but evolutionary related or unrelated isolates, but the practical value of PFGE method for epidemiological purposes must be confirmed by other or more restriction enzymes or using an other protocol. Summarizing our results we suggest the use of phage and RAPD typing and in doubtful cases the PFGE method.

Keywords: *E. coli* O157, pheno-genotyping, virulence genes

Introduction

Verotoxigen *E. coli* serogroup O157 is recognized as an important cause of both sporadic and epidemic disease in many regions of the world. Large outbreaks have occurred in the USA [1], Japan [2] and in some European countries [3–6]. No similar outbreaks occurred in Hungary, but on the basis of systematic, reasonable

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examinations of the incidence of this serogroup, several publications appeared on diagnostic and typing experiences of sporadic or family outbreaks [7–12].

In this paper an account is given of elaborated or applied pheno- and genotyping methods, comprising certain virulence genes detected in isolates originating from sporadic cases or family outbreaks in Hungary between 1990 and 2002.

Materials and methods

Bacterial strains: Out of 75 *E. coli* O157 strains isolated between 1990 and 2002 30 were of O157:HNM (non motile, H-)17 O157:H7, 5 of H:16, 1 of H:2, 22 of O157:HNT (non typable). Human strains originated from enteritis (E), haemorrhagic colitis (HC), or haemolytic uraemic syndrome (HUS) cases; the animal strains were isolated from calf, cattle and meat products, milk and lettuce. Culture conditions were described previously [13].

Phenotypic typing methods

Serotyping: method of Czirók and Herpay was used and carried out in their laboratory [8, 9, 13].

Phage typing: the methods of Kakhria et al. [14] and Milch [15] were used.

Colicin typing was carried out according to Lafont et al. [16].

Antibiotic resistance pattern was determined by disc diffusion test using Mueller–Hinton medium (Oxoid) with the following antibiotics: ampicillin (AM), streptomycin (SM), tetracycline (TE), gentamicin (GM), kanamycin (KM), chloramphenicol (CM), sulfamethoxazole (SXT), nalidixic acid (NA).

Genotypic typing methods

Plasmid profile analysis: Plasmid DNA was prepared according to Kado and Liu [17], using Wizard Miniprep-kit (Promega). Agarose gel electrophoresis was performed according to Meyers et al. [18].

Colony hybridization for Shiga-like toxins SLT-I and SLT-II as well as verocytotoxins VT-1 and VT-2 was performed according to Baldini et al. [19], using hybond-N-nylon hybridization membranes.

DNA probes: SLT-I and SLT-II probes provided by Newland et al. [20, 21], VT-1 and VT-2 probes provided by Willshaw et al. [22] were used. SLT probes were labelled by ^{32}P using a random priming method and Megaprime kit (Amersham). VT probes were labelled by digoxigenin dig-UTP [23].

Polymerase chain reaction (PCR) to detect virulence genes: multiplex PCR method was performed according to Fratamico et al. [24].

Primers to detect toxin genes: stx-1 and stx-2 were used, according to Karch and Meyer [25]. *Primers to detect intimin gene:* eae (attaching and effacing) was applied, according to Gannon et al. [26]. *Primers to detect haemolysin A gene:* ehx A was used, according to Fratamico et al. [24]. *Preparations of templates and PCR amplifications* for detection of PCR products were described by Gadó et al. [27].

Random amplified polymorphic DNA analysis was carried out according to Birch et al. [28] using ERIC-1, ERIC-2 and M13 as primers (Table I).

Amplification was made in thermocycler "progene" (Techne, Cambridge, UK).

Table I

Primer sequences used in RAPD typing

Primer	Sequence (5'–3')	References
ERIC-1	ATGTAAGCTCCTGGGGATTCAC	[29]
ERIC-2	AAGTAAGTGACTGGGGTGAGCG	[29]
M13	GAGGGTGGCGGTTCT	[30]

Detection of the products in gel electrophoresis was evaluated in Gel Doc 2000 apparatus by Quantity One program.

Pulsed field gel electrophoresis (PFGE)

To prepare the plugs for PFGE the rapid protocol of Gautom [31] was used. For restriction endonuclease digestion 2-mm thick slices of each plug were incubated overnight at 37ºC, with 50 U of XbaI, in 180 µl of the appropriate (1×) restriction enzyme buffer. The plug slices of the samples were loaded and electrophoresed in 1% Pulsed Field Certified Agarose (Bio-Rad Laboratories) with 4 liters of standard 0.5× TBE running buffer. The electrophoresis was performed with the CHEF-DR II (Bio-Rad) under conditions identical with those used in the standard procedure of Centers for Disease Control and Prevention [32]. The gels were stained for 30 minutes in 500 ml of sterile running buffer containing

50 µl of ethidium bromide (10 mg/ml), destained in 500 ml of sterile distilled water for 30 minutes and photographed on a UV light transilluminator of Gel Doc 2000 (Bio-Rad). A molecular weight standard (CHEF DNA Size Standards, Lambda Ladder, Bio-Rad) was applied in each gel.

Statistical analysis

The PFGE profiles were analysed with the softwer Quantity One Version 4 (Bio-Rad), that allows automatic band size determination with 1% precision. Heterogeneity with respect to the intensities and the shapes of bands was not considered as a difference.

The data sets for cluster analysis were generated as follows: each band was measured and designated with a band number. The presence or absence of a band in a given strain was indicated by 1 or 0, respectively (conversion of banding profiles to binary data) [33].

For clustering PFGE profiles Statistica for Windows, Version 4.5 (StatSoft, Inc., StatSoft Hungary Ltd.) was used to perform UPGMA analysis on these data sets [34].

Results

Pheno- and genotypes. Phage types, colicin types, antibiotic resistance patterns, plasmid profiles and virulence genes of the different serotypes of *E. coli* O157 isolates are demonstrated in Table II.

Out of the 30 *E. coli* O157:HNM strains 19 belonged to five different phage types, 11 were non typable by phages. Colicin production was detected in six strains. Only three strains were resistant to 1–3 antibiotics, two strains were multiresistant. Plasmid profiles of the strains were not very various, 16 carried only a single plasmid of about 60 Md, two that of 80 Md, besides small plasmids occurred in six strains. By multiplex PCR examinations stx-1 and stx-2 genes were detectable in 21 isolates. In one isolate genes stx 1-2, eae and ehxA occurred together, in nine isolates virulence genes were not detectable.

Out of 17 O157:H7 isolates 11 were typable by phages, they belonged to eight different phage types. Colicinogenic was one isolate and only one was resistant to 1–3 antibiotics. Five strains carried only a plasmid of 60Md, two strains that of 80Md, small plasmids were in seven isolates beside of the 60 or 80 Md plas-

Table II

Pheno- and genotypes of *Escherichia coli* O157 strains

Serotype	No. of strains examined	Phage type						Colici-nogenic	Antibiotic resistance			Plasmid profile (Md)				Virulence genes								
		8	14	31	55	others[1]	NT[2]		0	1–3	>3	60 60+	80 80+	others	Plas-mid free	stx 1–2 eae ehxA	stx 1–2 eae	stx 1–2 ehxA	eae ehxA	stx 1–2	eae	ehxA	–[3]
O157:HNM	30	6	6	–	4	3	11	6	25	3	2	25	3	2	–	19	–	1	–	1	–	–	9
O157:H7	17	–	3	2	1	5	6	1	16	1	–	16	1	–	1	11	1	–	2	–	1	1	1
O157:H2	1	–	–	–	–	–	1	–	–	–	1	–	–	1	–	–	–	1	–	–	–	–	–
O157:H16	5	–	–	–	–	–	5	1	3	2	–	3	2	–	–	–	–	–	–	–	2	–	3
O157:HNT	22	–	1	5	–	1	15	8	14	6	2	13	2	6	–	3	–	1	–	1	2	–	15
Total	75	6	10	7	5	9	38	16	58	12	5	57	8	9	1	33	1	3	2	2	5	1	28

[1] Other phage types: 2, 4, 23, 27, 48, 61, 63
[2] NT: Non typable, out of 38 NT strains 15 were typable by Hungarian phages [15]
[3] –: Virulence genes were not detectable

mids. Genes stx 1–2, eae and ehxA genes together were detectable in 11 isolates. Only one strain occurred without virulence gene.

Out of 22 O157:HNT isolates only seven were typable by phages, they belonged to three different phage types. Colicinogenic were eight strains, among them six originated from the same person. Six strains were resistant to 1–3 antibiotics, two were multiresistant. Thirteen strains carried a plasmid of 60 Md, two strains that of 80 Md, seven strains one of a 112 Md or one of 35.8 Md, 16 strains carried additionally small plasmids, too. In 15 strains virulence genes were not detectable, in five isolates were found stx 1–2 genes; while stx 1–2, eae and ehxA genes together only in three isolates. Gene stx 1–2 alone was in a single strain, which was isolated from lettuce.

Neither of the *E. coli* O157:H2 nor O157:H16 isolates were typeable by phages. One strain was colicinogenic, three were resistant to antibiotics. Four strains carried plasmids of 60 Md or 80 Md. Out of the six isolates virulence genes were detectable only in three one, eae gene alone in two, stx 1–2 and ehxA genes in one isolate.

Detection of virulence factors by hybridization and PCR methods

Escherichia coli O157:HNM, O157:H7 and O157:H16 strains originating from human cases of different diagnosis, examined by hybridization using SLT I and SLT II probes and by multiplex PCR using stx 1–2, eae and ehxA primers gave definite results concerning toxin genes. Positive results with stx 1–2, eae and ehxA primers together were obtained only with strains originating from cases of HC diagnosis (Table III).

Hybridization of eight *E. coli* O157 strains of different serotypes and one *E. coli* O26 strain with VT1 probe [22] labelled by ^{32}P-ATP and dig-UTP gave unequivocal results (Table IV).

RAPD typing patterns

Sixteen *E. coli* O157:HNM, 9 *E.coli* O157:H7 isolates were tested with three primers (ERIC-1, ERIC-2 and M13), while with two primers (ERIC-1 and ERIC-2) two *E. coli* O157:H16, one *E. coli* O157:H2 and five *E. coli* O157:HNT.

Sixteen *E. coli* O157:HNM isolates belonged to six different RAPD types with ERIC-1, to five types with ERIC-2 and in seven types with M13 primers. Out

Table III

Comparison of the results obtained by hybridization and PCR methods of *Escherichia coli* O157 strains of different origin

Strain	Serotype	Phage type	Plasmid profile (Md)	In situ hybridization		Multiplex PCR			Diagnosis
				SLT-I	SLT-II	stx 1–2	eae	ehx A	
276/95	H:NM	8	60	–	+	+	+	+	HC
19/96	H:NM	8	76, 31, 2, 7	–	+	+	+	+	HC
14/95	H:7	NT	60	–	+	+	+	+	HC
8/96	H:7	14	–*	–	+	–	–	+	SL
9/96	H:7	14	60	–	±	+	+	–	E
11/96	H:7	27	60	–	+	+	+	+	HC
256/95	H:16	NT	112, 60, 4, 8	–	–	–	–	–	E
12/95	H:2	NT	–*	–	–	–	–	–	E
12/96	H:NT	NT	–*	–	–	–	–	–	E

HC = haemorrhagic colitis
E = enteritis
SL = symptomless
—* = plasmid free
— = negativ

Table IV

Hybridization of *Escherichia coli* isolates with VT-1 probe [22] labelled by ^{32}P and dig-UTP

Strain/year	Serotype	VT-1 hybridization labelled	
		32P-ATP	Dig-UTP
10/90	O157:HNM	+	+
14/90	O157:H7	+	+
20/90	O157:H7	+	+
258/90	O157:H7	+	+
268/90	O157:H7	+	+
60R476[1]	O157:H7	+	+
576/90[2]	O157:HNT	+	+
12/88	O157:H16	–	–
3/77[3]	O26:H11	+	+
HB101[4]	•	–	–

[1] positive control strain, contained pACYC177 vector of VT-1 probe
[2] first isolate causing family outbreak in Hungary [9]
[3] first VT strain isolated in Hungary [50]
[4] negative control

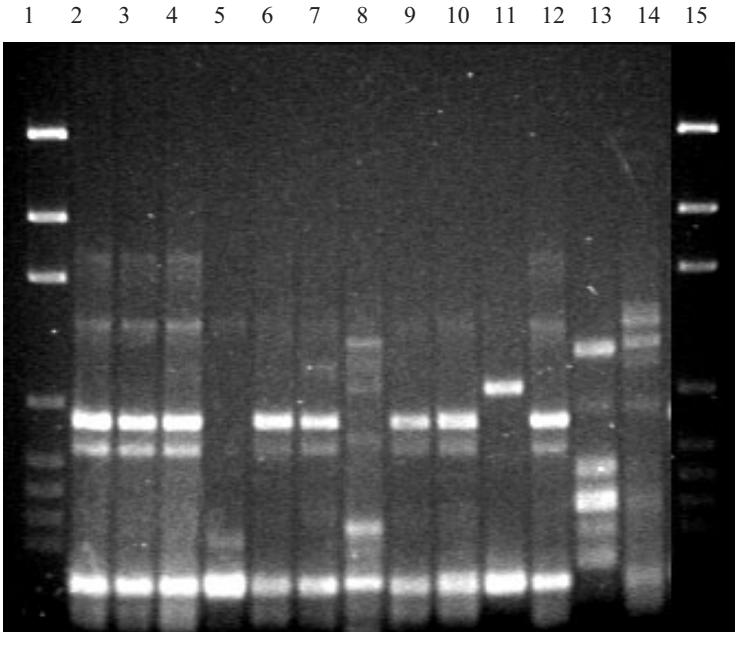

Figure 1. RAPD pattern of *Escherichia coli* O157:HNM strains with ERIC-1 primer. Marker (lanes 1 and 15). Lanes 2–14: 276/95, 97/96, 81/97, 71/98, 79/98, 234/98, 235/98, 114/01, 28/02, 62/02, 19/96, 68/99, 119/99

Table V

RAPD types of *Escerichia coli* O157:HNM using three primers comparing with phage types and virulence genes

Strain No./year	Phage-type	RAPD typing patterns ERIC-1	ERIC-2	M-13	Origin/ diagnosis	Virulence genes stx1–2	eae	ehx A
276/95	8	A	I	1	Pest/HC	+	+	+
97/96	14	A	I	1	Vas/HC	+	+	+
81/97	61	A	I	2	Veszprém/HC	+	+	+
71/98	NT	B	II	3	Baranya/HC	+	–	–
79/98	NT	A	I	1	Budapest/HUS	+	+	+
234/99	14	A	I	1	Hajdú/HC	+	+	+
235/99	14	C	III	4	Hajdú/HC	–	–	–
114/01	43	A	I	1	Zala/HC	+	+	+
28/02	NT	A	I	5	Budapest/HC	+	+	+
62/02	NT	D	IV	6	Pest/E	–	–	–
19/96	8	A	I	1	Pest/HC	+	+	+
68/99	8	E	V	7	Austria/HC	+	+	+
119/99	63	F	IV	3	Hajdú/E	–	–	–
244/99	14	A	I	8	Budapest/HC	+	+	+
83/00	55	A	I	9	Somogy/HC	+	+	+
150/00	NT	A1	I	X	Veszprém/E	+	–	–

E = human enteritis
HC = human haemorrhagic colitis
HUS = haemolytic uraemic syndrom

Table VI

RAPD types of Escerichia coli O157:H7 using three primers comparing with phage types and virulence genes

Strain No./year	Phage-type	RAPD typing patterns ERIC-1	ERIC-2	M-13	Origin/ diagnosis	Virulence genes stx1–2	eae	ehx A
41/97	61	A	I	1	Veszprém/HC	+	+	+
90/97	2	A	I	2	Somogy/HC	+	+	+
130/97	31	A	I	2	Budapest/row milk	+	+	+
57/97	31	A	I	3	Budapest/beef meat	–	+	+
75/99	14	A	I	2	Bács/HC	+	+	+
12/99	55	B	II	4	Budapest/beef meat	+	+	+
245/00	NT	A	I	2	Budapest/HC	+	+	+
11/96	27	A	I	5	Kuwait/HC	+	+	+
8/96	14	C	III	4	Kuwait/SL	–	–	+

SL = symptomless
HC = hemorrhagic colitis
NT = non typable

of 16 isolates 11 have shown identical RAPD types; out of three isolates of different RAPD type no virulence genes were detectable, one isolate of different RAPD type originated from an other country. Isolates 234/99 and 235/99 of different RAPD type originated from the same person, their serotype and phage type were identical, but virulence genes of 234/99 were non detectable in the 235/99 isolate (Table V, Figure1).

Out of nine *E. coli* O157:H7 isolates seven have shown identical RAPD pattern with ERIC-1 (A) and with ERIC-2 (I), two isolates were of different RAPD types with both ERIC primers (B, C; II, III) (Table VI, Figures 2 and 3). With M13 primer the strains could be grouped in five different RAPD types.

E. coli O157:HNT, *E. coli* O157:H16 and *E. coli* O157:H2 isolates could be sorted in four groups with ERIC-1 and in three groups with ERIC-2. Only one of the isolates carried the three virulence genes and the isolate have shown the same ERIC-1 and ERIC-2 RAPD patterns as the frequent *E. coli* O157:HNM and *E. coli* O157:H7 strains (strain 13/99, isolated from beef meat) (Table VII).

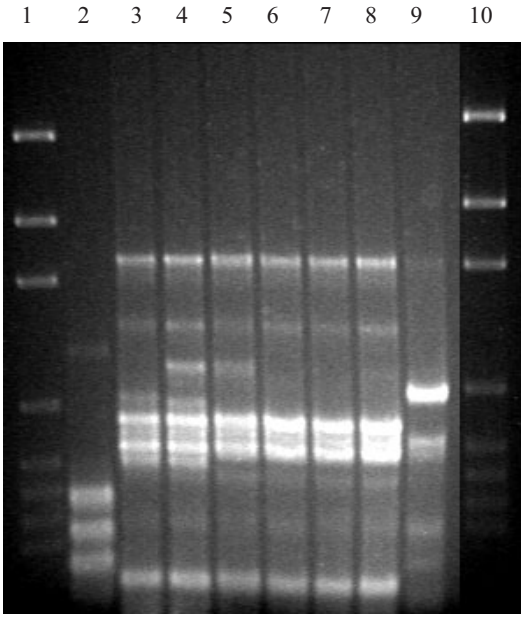

Figure 2. RAPD pattern of *Escherichia coli* O157:H7 strains with ERIC-1 primer. Marker (lanes 1 and 10). Lanes 2–9: 8/96, 11/96, 41/97, 90/97, 130/97, 57/97, 75/99, 12/99

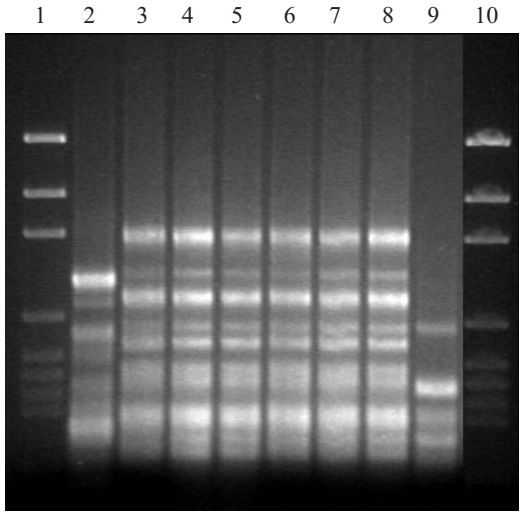

Figure 3. RAPD pattern of *Escherichia coli* O157:H7 strains with ERIC-2 primer. Marker (lanes 1 and 10). Lanes 2–9: 8/96, 11/96, 41/97, 90/97, 130/97, 57/97, 75/99, 12/99

Table VII

RAPD types of *Escherichia coli* O157:HNT and other H antigens using two primers comparing with phage types and virulence genes

Strain No./year	Sero type	Phage type	RAPD typing patterns ERIC-1	ERIC-2	Origin/ diagnosis	Virulence genes stx1–2	eae	ehx A
98/97	H:NT	NT	C1	Ia	beef faeces	–	–	–
231/99	H:NT	31	C	Ia	Csongrád/H	–	–	–
233/99	H:NT	NT	C	Ia	Csongrád/H	–	–	–
168/97	H:NT	NT	C	II	lettuce	+	–	–
13/99	H:NT	23	A	Ia	beef meat	+	+	+
256/95	H:16	NT	C1	Ia	Somogy/H	–	+	–
86/96	H:16	NT	C1	Ia	Zala/H	–	–	–
51/98	H:2	NT	D	Ia	Baranya/H	+	–	+

PFGE typing

Twenty eight strains belonging to identical and different serogroups and/or different ERIC-1, ERIC-2 and M13 patterns were examined by PFGE. Four isolates could not be subjected to PFGE analysis because despite repeated experiments their DNAs degraded easily. In agreement with Izumiya et al. [35] we found that by using the standard switching times [32] for PFGE DNA bands of less than 100 kb in size could not be separeted well, and because of this the minor differ-

encies of restriction fragment patterns previously described prominent in this zone [35] were not detecteble and pulsotypes – regarding Tenover's criteria [36] – could not have been established in a correct manner. Analyzing the bands of more than 100 kbs in size the restriction patterns of strains with identical ERIC-1 and ERIC-2 patterns showed more than 78% and 75% homology for the examined four and thirteen strains of C II and A I ERIC types, respectively. Controversely the two strains of ERIC type II B showed less than 66% homology just like the strains belonging to different ERIC I and/ or ERIC II types. There was only one pair of isolates signed as 51/ 98 and 71/ 98 that belonged to different ERIC-1 and ERIC-2 types, but showed 71% homology (Figures 4/A, 4/B).

Considering the ERIC PCR and PFGE results for 24 epidemiologically unrelated strains we can conclude that > = 75% homology of the *E. coli* O157 Xba I restriction fragment patterns confirms the clonal relationship between organisms of these serogroups (see Table VIII).

Table VIII

Sero-, RAPD- and pulsotypes of *Escherichia coli* O157

Strain No./Year	Origin/diagnosis	Serotype	RAPD types ERIC-1	RAPD types ERIC-2	Clonal origin PFGE, Xba I
256/95	Somogy/E	H: 16	C	II	EPID clone I
86/96	Zala/E	H: 16	C	II	EPID clone I
231/99	Csongrád/E	H: NT	C	II	EPID clone I
233/99	Csongrád/E	H: NT	C	II	EPID clone I
11/96	Kuwait/HC	H: 7	A	I	EPID clone II
41/97	Veszprém/HC	H: 7	A	I	EPID clone II
90/97	Somogy/HC	H: 7	A	I	EPID clone II
13/99	beef meat	H: NT	A	I	EPID clone II
97/96	Vas/HC	H: NM	A	I	EPID clone II
81/97	Veszprém/HC	H: NM	A	I	EPID clone II
79/98	Budapest/HUS	H: NM	A	I	EPID clone II
234/99	Hajdú/HC	H: NM	A	I	EPID clone II
244/99	Budapest/HC	H: NM	A	I	EPID clone II
83/00	Somogy/HC	H: NM	A	I	EPID clone II
150/00	Veszprém/E	H: NM	A	I	EPID clone II
114/01	Zala/HC	H: NM	A	I	EPID clone II
28/02	Budapest/HC	H: NM	A	I	EPID clone II
168/97	lettuce	H:NT	D	VIII	clone III
51/98	Baranya/E	H: 2	D	VII	clone IV
12/99	Budapest/beef meat	H: 7	B	II	clone V
71/98	Baranya/HC	H: NM	B	II	clone VI
235/98	Hajdú/HC	H: NM	C	III	clone VII
119/99	Hajdú/E	H: NM	F	VI	clone VIII
62/02	Budapest/E	H: NM	D	IV	clone IX

E = enteritis
HC = hemorrhagic colitis

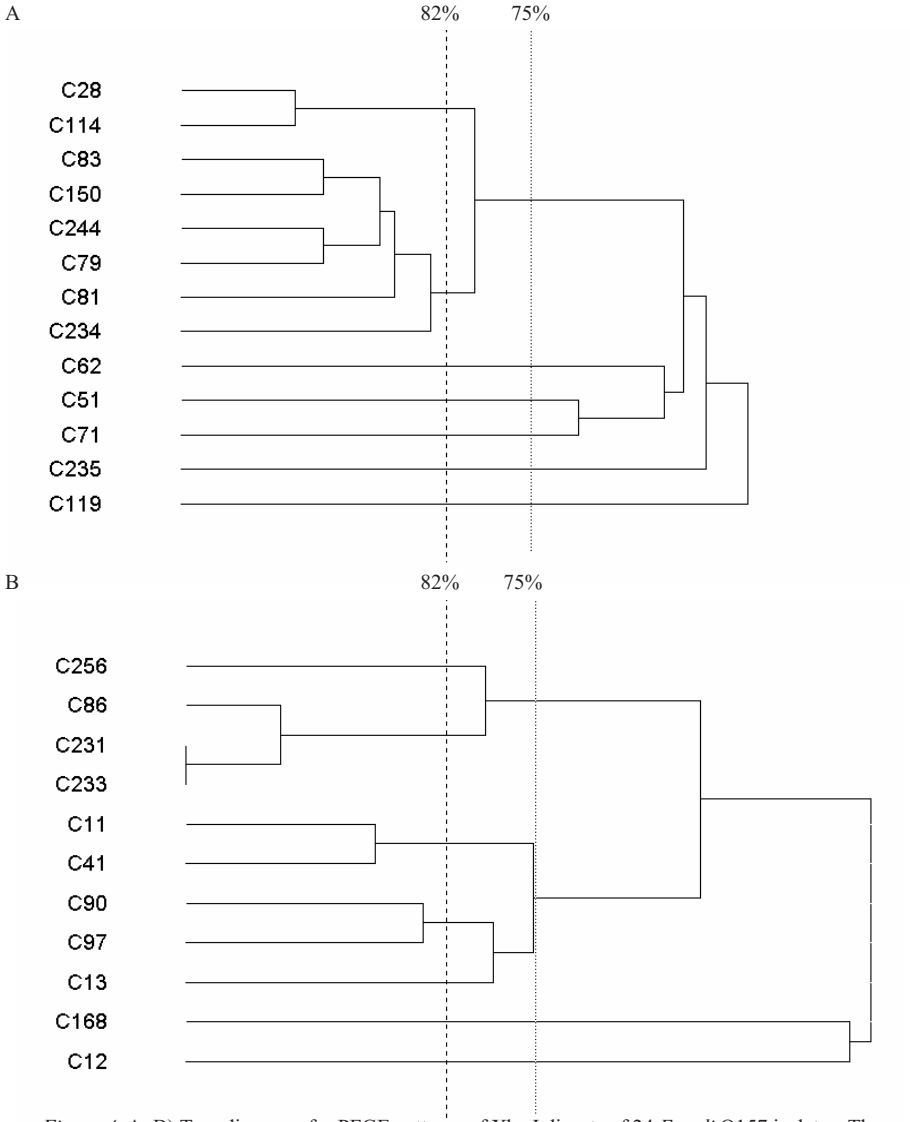

Figure 4. A–B) Tree diagrams for PFGE patterns of Xba I digests of 24 *E. coli* O157 isolates. The scattered and interrupted lines show the cut level of 75% and 82% homology, respectively. 75% homology was found to be the border for clonal origin, 82% homology was found to be equal with 6 band differencies by visual analysation as upper limit for evaluation strains as "closely related" [34]

A) C28: 28/ 02, C114: 114/ 01, C83: 83/ 00, C150: 150/ 00, C244: 244/ 99, C79: 79/ 98, C81: 81/ 97, C234: 234/ 99, C62: 62/ 02, C51: 51/ 98, C71: 71/ 98, C235: 235/ 99, C119: 119/ 99

B) C256: 256/ 95, C86: 86/ 96, C231: 231/ 99, C233: 233/ 99, C11:11/ 96, C41: 41/ 97, C90: 90/ 97, C97: 97/ 96, C13: 13/ 99, C168: 168/ 97, C12: 12/ 99

Discussion

In our earlier examinations concerning typing of *E. coli* O157 strains originating from different area of our country we used phenotypic methods completed accidentally by detection of virulence factors. These examinations led to the conclusions that the strains originated mainly from sporadic cases, rarely from family patients [37].

Smith and coworkers [38, 39] subdivided *E. coli* O157 strains of all the phage types except phage type 1 by hybridization with VT1 and VT2 probes – labelled radioactively and with digoxigenin or biotin. Willshow et al. [40] used two eae probes additional to VT probes and they found the VT probes and eae O157 probe valuable for differentiation of O157 strains. After comparison our results obtained by hybridization using two VT probes (SLT-I and SLT-II) and PCR method using VT primers (stx 1–2), the attaching and effacing primer (eae) and the haemolysin A primer (ehx) we could differentiate O157 strains of different serotypes into stx, eae, ehxA positive; stx, eae positive; stx, ehxA positive; stx positive; eae, ehxA positive; eae positive; stx, eae, ehxA negative (Tables III, V–VII).

Analysing the results of *E. coli* O157 typing of different authors proved that phage is useful but it has certain limits e.g. in the UK four phage types account for over 80% of isolates [41], however according to Birch et al. [28] RAPD typing is a potentially useful typing tool for the different *E. coli* O157 serogroups. Using the rapid and relatively simple method of Birch et al. [28] we completed our phenotypic typing methods (including serotyping) with the RAPD typing.

Our results with three primers (ERIC-1, ERIC-2 and M13) gave different banding patterns with the 33 isolates examined.

Experiences with RAPD typing by different primers revealed different banding patterns, as reported by several authors investigating several bacterial pathogens [42–44]. With respect to banding patterns out of 16 *E. coli* O157:HNM isolates 11 were identical according to ERIC-1 and ERIC-2 primers. With M13 primer only seven isolates displayed identical banding patterns. *E. coli* O157:HNM strains have showed six different banding patterns using ERIC-1 and five different patterns using ERIC-2 primers; according to M13 primer seven banding patterns could be observed. Out of 16 *E. coli* O157:HNM isolates five showed divergent patterns from the frequent 11 banding patterns with ERIC-1 and four different patterns with ERIC-2 primer. Virulence genes of this five isolates were different from the virulence genes of the frequent RAPD types in four cases.

In our complex typing examinations *E. coli* O157:HNM isolates (Table V) could be classified in six different phage types and in six different RAPD types

with ERIC-1, in five RAPD types with ERIC-2 primers and in seven types with M13 primer.

Strains of identical RAPD type with ERIC-1 and ERIC-2 displayed five different phage types, those with M13 primer could be grouped in three different phage types. Of the 5 isolates not typable by phages three different RAPD types were found.

Isolates of nine *E. coli* O157:H7 displayed six different phage types, three RAPD types with ERIC-1, ERIC-2 and five with M13 primers. One isolate was non typable by phages but was classified in the most frequent RAPD type with ERIC-1, ERIC-2 and M13 primers.

E. coli O157:H7 strains isolated from row milk (130/97 and beef meat (57/97) in the same year from the same town, were of identical phage type 31 and of the most frequent RAPD type A with the three primers. Another O157:H7 strain (12/99) isolated from beef meat two years later in the same town, was of different phage type and of different RAPD type (Table VI).

Our complex, pheno- and genotyping showed that type discriminating capacity of phage typing is very high, much higher than that of the plasmid profile (Table II). RAPD typing with different primers could confirm or exclude the identity of the isolated *E. coli* O157 strains (Tables V–VII).

Summarising our PFGE results it can be concluded – in agreement with Arbeit et al. [45] – that the standard running protocol [32] for the digests gives the opportunity to differentiate epidemiologically independent but evolutionary related and unrelated isolates, the discriminatory power, however, and consequently the value of it as an epidemiological typing method is limited, analysing genetically such closely related bacterial population as *E. coli* O157 strains [46]. It remained undecided whether the use of running protocol by Izumiya [35], separating the bands less than 100 kb in size as well, or the use of another restriction endonuclease, e.g. Sfi I [47] would increase the value of PFGE examinations for epidemiological purposes. On the other hand it was revealed by our pilot-study that a more detailed examination – similarly to those by Preston et al. [47] – of epidemiologically clearly related (more clusters of cases) and epidemiologically unrelated strains would be needed to determine the value of PFGE examinations for epidemiological purposes in Hungary.

According to our experience with the occurrence of antibiotic resistance among *E. coli* O157 strains isolated predominantly from human sources in Hungary, out of 75 strains 18 were resistant to one or more antibiotics, 14 out of them were VT negative. Our experiences are consistent with the studies of Bettelheim et al. [48] who found a much higher rate of resistance among the non VTEC strains

compared to VTEC ones isolated from animals and humans. Schroeder et al. [49] found antibiotic resistance in 40% among 195, other than *E. coli* O157 VT positive strains, mostly from animal sources. They emphasize the surveillance of antibiotic resistance of *E. coli* from food products.

Our strains examined were isolated from sporadic cases, rarely from family patients. Our study demonstrates the utility of phage typing and RAPD typing with ERIC-1, ERIC-2 and M13 primers to discriminate or identify *E. coli* O157 strains of identical serotypes. The use of detection of virulence genes is necessary in the estimation of the clinical significance of an *E. coli* O157 isolated from patients or food products.

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