Numerical interpretation of TRS-PCR profiling results for *Escherichia coli* strains isolated from patients with bacteriuria in Lodz region, Poland

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Abstract

With the multiplicity of existing methods to track *E. coli* infections, it still seems necessary to seek new, better and/or complementary ways for epidemiological investigations. Particularly, fast, cheap, effective and reproducible methods providing easily comparable results are needed. Our previous studies showed that the use of TRS-PCR is an effective molecular tool in *E. coli* epidemiology. In this paper, we have developed a unique classification scheme in which an individual TRS-PCR pattern is assigned a numerical value. This approach allows for rapid interpretation of the results obtained from several similarity dendrograms. Using this approach, based on CAC-PCR, GTG-PCR and CGG-PCR, we obtained 52, 86 and 99 different numerical types for the 124 analyzed uropathogenic *E. coli* strains, respectively. This allowed for the identification of 121 unique isolates differing in at least one TRS-PCR class. In this approach, we got numerical results, easy to sort and interpret, allowing easier analysis of these strains.

Keywords Microsatellites · TRS-PCR profiling · *E. coli*

Abbreviations

TRS-PCR	Trinucleotide repeat sequences-based PCR
CAC	GTG-, CGG-PCR – CAC, GTG or CGG repeats-based PCR, respectively
IPEC	Intestinal pathogenic <i>E. coli</i>
ExPEC	Extraintestinal pathogenic <i>E. coli</i>
UPEC	Uropathogenic <i>E. coli</i>
NMEC	Neonatal meningitis <i>E. coli</i>
APEC	Avian pathogenic <i>E. coli</i>
SEPEC	Septicemia causing <i>E. coli</i>
UTI	Urinary tract infection
VF	Virulence factors

MLVA	Multi-locus variable-number tandem repeat analysis
MLST	Multi-locus sequence typing
PFGE	Pulsed-field gel electrophoresis

Introduction

Pathogenic *Escherichia coli* is an extremely versatile and heterogeneous group of strains which poses a threat to people's health and life from all over the world. Based on the site of induced infection and virulence attributes, this group can be subdivided into intestinal pathogenic *E. coli* (IPEC) and extraintestinal pathogenic *E. coli* (ExPEC) strains [1–3]. The most important ExPEC pathotypes, that are associated with human diseases are uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), avian pathogenic *E. coli* (APEC) and septicemia causing *E. coli* (SEPEC) [1–8].

Majority of urinary tract infections (UTI) are caused by uropathogenic *E. coli* strains, which are highly adapted not only to live in the urinary tract but also to evade the host's defense mechanisms and immune responses [2, 7, 9]. UPEC pathotype constitutes very diverse group of strains with a wide range of virulence factors [1, 2, 4, 6, 10]. Moreover, not only bacterial factors determine such infections but also

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host factors are involved in this process [2, 7]. As the number of UPEC strains resistant to antibiotics is increasingly growing and many people are immunocompromised or become sensitive to these pathogens, costs of medical care and other indirect costs escalate every year [7, 10, 11]. It was estimated that every year 130–170 million cases of UTI were registered, in addition to that, over 80% of them were UPEC induced [5, 11].

Molecular epidemiology of *E. coli* infections is based mainly on multi-locus variable-number tandem repeat analysis (MLVA), multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) [8, 12–14]. PFGE is considered as a “gold standard” for epidemiology and strain typing for many bacterial species, including *E. coli*, but is also time-consuming and results obtained from different laboratories are problematic to compare [13, 14].

In our laboratory, we have developed an effective method for the differentiation and genotyping of bacterial strains called TRS-PCR [15–19]. Trinucleotide repeat sequences are kind of microsatellites dispersed in genome of every organism. This test was successfully used to detect associations between CGG-PCR and GTG-PCR fingerprints and specific *E. coli* properties such as phylogroups and virulence profiles [15–19]. The method is simple, fast, reproducible and inexpensive. However, a large diversity of fingerprint profiles obtained from various TRS-PCR tests and the need to compare them, in our opinion, required development of a new strategy to facilitate the interpretation of results. Here, we propose a new approach for strain typing of *E. coli* based on three separate TRS-PCR tests using CAC-PCR, GTG-PCR and CGG-PCR profiling. In this technique, each unique TRS-PCR profile is assigned a number. Based on the high reproducibility and also the stability of fingerprints, each strain can be assigned a set of three numbers corresponding to three TRS classes (Table 1). The effectiveness of the proposed approach was tested on a collection of 124 *E. coli* strains isolated from patients with urinary tract infections.

Materials and methods

Bacterial strains

One hundred and twenty-four *E. coli* strains were isolated from patients with urinary tract infections between June 2005 and September 2006 and originated from various wards of Military Teaching Hospital No. 2, Medical University of Lodz, Poland. Information about the date of isolation, ward/department, sex of the patient, the scale of bacteriuria and specified serotype are listed in Table 2. This collection of strains has been redefined as the spectrum of virulence factors had been extended [15, 16], phylogenetic affiliation had been performed according to the new Clermont's

protocol [20]. Also, isolates were serotyped according to the manufacturer's protocols for *E. coli* (O pool and O single antisera, Statens Serum Institut SSI Diagnostica, Denmark) (Fig. 1S.B).

Bacterial growth and genomic DNA isolation

All *E. coli* strains were grown overnight in a liquid LB broth at 37 °C with agitation at 120 RPM. For genomic DNA isolation and purification, GenElute Bacterial Genomic DNA Kit was used according to the manufacturer's protocol (Sigma-Aldrich, St. Louis, MO). Each of the genomic DNA sample was measured spectrophotometrically at 260 nm (BioPhotometer, Eppendorf, Germany) in order to estimate its quantity and quality. The concentration of DNA samples used in the study was 20 ng/μl.

Phylogenetic structure

Phylogenetic analyses were carried out exactly according to the Clermont's *E. coli* phylo-typing scheme [16]. The whole methodology, such as, sequences of primers, their concentrations and phylo-group detection schemes was performed as described [20] (Fig. 1S.A).

Virulence factors repertoire

Detection of the virulence factors was performed exactly as it was published elsewhere [16]. The presence of uropathogenic virulence factors was determined using sequences of primers previously published [15]. Detection of intestinal virulence factors typical for different pathovars was performed according to the Müller et al. [21]. The presence of additional five virulence genes—*iroN*, *fyuA*, *iutA*, *sat* and *tsh* was determined. Two separate PCRs were performed. The first multiplex PCR reaction was designed to determine the presence of *iroN*, *fyuA* and *iutA* genes. Sequences of primers for *iroN* detection were published by Bonacorsi et al. [22] and sequences of primers for detection of *fyuA* and *iutA* were published by Johnson and Stell [4]. The second multiplex PCR was designed to determine the presence of *sat*, as described by Restieri et al. [23] and *tsh*, as described by Moulin-Schouleur et al. [24] (Fig. 1S.C).

TRS-PCR profiling and reproducibility analysis

For each strain, three separate TRS-PCR profiles were generated based on the presence of CAC, GTG, and CGG motifs in used primers. Conditions for GTG-PCR and CGG-PCR reactions, primers sequences, electrophoresis, reproducibility analysis, determination of diversity indices and bioinformatic analyses were performed as described elsewhere [15–19]. Conditions for CAC-PCR were the same as for GTG-PCR

Table 1 Assignment of three separate TRS-PCR classes for uropathogenic *E. coli* strains based on CAC-, GTG- and CGG-PCR profiling. Strains sharing identical TRS-PCR profiles and strains derived from the same patients were shown separately

Key	CAC class	GTG class	CGG class	Phylotype	Virulence profile
The differentiated group of strains					
U 032	1	2	71	A	None of the tested
U 104	2	16	40	B1	papC, fimG/fimH, fyuA, iutA, iroN
U 001	3	1	80	A	None of the tested
U 037	3	51	51	C	fimG/fimH, astA, fyuA
U 038	3	66	5	C	fimG/fimH, astA, fyuA
U 043	4	43	36	A	iutA, iroN, tsh
U 034	4	53	77	A	fimG/fimH, astA
U 029	5	36	84	D	fimG/fimH, astA, fyuA
U 091	6	59	24	A	fyuA
U 128	8	35	75	B1	fimG/fimH, iutA, iroN
U 003	9	54	23	A	papC
U 076	10	32	50	B1	fimG/fimH, iroN
U 092	11	58	6	A	None of the tested
U 025	12	40	96	F	fimG/fimH, fyuA, iutA, iroN, tsh
U 116	13	40	34	F	fimG/fimH, astA, fyuA, iutA, iroN, tsh
U 023	14	38	40	B1	papC, sfaD/sfaE, cnf1, hly1, fimG/fimH, astA, fyuA, iroN
U 052	15	34	78	B1	fimG/fimH, astA, iutA, iroN
U 004	16	62	91	A	fimG/fimH
U 107	17	21	27	B1	hly1, fimG/fimH
U 068	18	15	52	B1	fimG/fimH
U 009	19	3	30	A	fimG/fimH, astA, iroN
U 011	19	42	38	A	fimG/fimH, fyuA
U 044	19	51	45	C	fimG/fimH, astA, fyuA, iutA, iroN
U 073	19	57	32	A	fimG/fimH, iroN
U 079	19	57	39	A	papC, sfaD/sfaE, cnf1, usp, fimG/fimH, fyuA, iroN
U 041	19	58	75	A	fimG/fimH
U 060	19	62	74	A	fimG/fimH, fyuA, iutA
U 099	19	67	82	A	fimG/fimH, fyuA, iroN
U 005	20	64	32	A	None of the tested
U 106	21	65	72	A	fimG/fimH
U 062	22	49	31	A	fimG/fimH
U 113	22	61	37	A	fimG/fimH, astA
U 071	23	48	86	A	fimG/fimH, iutA, iroN
U 074	24	18	32	A	fimG/fimH
U 059	25	68	43	F	None of the tested
U 117	25	69	29	F	fimG/fimH, fyuA, iutA, iroN, tsh
U 015	26	27	74	F	fimG/fimH, fyuA, iutA
U 007	27	22	83	F	fimG/fimH, iutA
U 100	27	23	85	F	fimG/fimH, astA, iutA, iroN
U 012	27	24	89	F	papC, fimG/fimH, fyuA, iutA
U 082	28	80	64	B2	sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 114	28	81	58	B2	papC, sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN
U 018	29	72	59	B2	sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN
U 090	30	74	12	B2	papC, sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, astA, fyuA, iroN
U 086	30	83	88	B2	sfaD/sfaE, usp, fimG/fimH, pic, fyuA, iutA, iroN
U 127	31	9	95	B2	usp, fimG/fimH, fyuA, iutA, iroN
U 055	31	10	93	A	fimG/fimH, fyuA
U 130	31	20	26	B1	fimG/fimH

Table 1 (continued)

Key	CAC class	GTG class	CGG class	Phylotype	Virulence profile
U 045	31	39	25	B1	fimG/fimH
U 119	31	47	51	C	papC, hly1, fimG/fimH, fyuA, iutA
U 028	32	76	4	B2	papC, fimG/fimH, fyuA
U 108	32	77	18	B2	papC, fimG/fimH, fyuA, iutA, iroN
U 095	32	82	56	B2	papC, fimG/fimH, fyuA, iutA, iroN
U 064	33	19	44	B1	fimG/fimH, iroN
U 072	33	25	62	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 026	33	71	67	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 087	33	72	6	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 089	33	72	10	B2	sfad/sfaE, usp, fimG/fimH, fyuA, iroN
U 013	33	72	60	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 046	33	72	61	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 033	33	73	62	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 054	33	74	63	B2	sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 006	33	74	97	B2	sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 049	33	85	68	B2	sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN
U 047	33	85	69	B2	sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 063	33	86	1	B2	papC, cnf1, usp, hly1, fimG/fimH, fyuA
U 105	34	74	97	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 050	35	9	94	B2	usp, fimG/fimH, fyuA, iutA, iroN, tsh
U 080	35	33	35	B1	fimG/fimH, iroN
U 057	36	13	48	C	fimG/fimH, fyuA, iutA
U 019	36	17	73	A	None of the tested
U 027	36	29	8	B2	papC, sfad/sfaE, cnf1, usp, fimG/fimH, fyuA, iutA, iroN
U 124	36	37	16	B2	fimG/fimH, fyuA, iutA, sat
U 010	36	41	66	E	papC, fimG/fimH
U 121	36	52	48	C	fimG/fimH, iutA, iroN
U 083	36	63	15	B2	papC, cnf1, hly1, fimG/fimH, fyuA, iutA, iroN
U 088	36	72	7	B2	sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA
U 016	36	72	59	B2	papC, sfad/sfaE, usp, fimG/fimH, fyuA, iutA, iroN, sat
U 014	36	74	98	B2	sfad/sfaE, cnf1, hly1, fimG/fimH, fyuA, iroN
U 030	36	75	3	B2	sfad/sfaE, cnf1, hly1, fimG/fimH, fyuA, iroN
U 085	36	75	11	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, astA, fyuA, iroN
U 125	36	75	12	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, astA, fyuA, iroN
U 098	36	75	66	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, astA, fyuA, iroN
U 093	36	76	4	B2	papC, fimG/fimH, fyuA, iutA, iroN
U 021	36	77	17	B2	papC, fimG/fimH, fyuA, iutA, iroN
U 040	36	78	2	B2	papC, sfad/sfaE, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 035	36	80	59	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 036	36	82	55	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN
U 067	36	84	13	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 110	37	11	14	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, pic, fyuA, iroN
U 111	37	75	1	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 084	37	75	9	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 102	37	78	1	B2	papC, sfad/sfaE, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 101	37	84	57	B2	papC, sfad/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 048	38	12	54	C	fimG/fimH, astA, fyuA, iutA, iroN
U 024	38	28	49	C	fimG/fimH
U 077	39	17	42	A	astA, iutA, iroN
U 039	40	26	1	B2	papC, sfad/sfaE, fimG/fimH, fyuA, iutA, iroN

Table 1 (continued)

Key	CAC class	GTG class	CGG class	Phylotype	Virulence profile
U 097	40	26	57	B2	papC, sfaD/sfaE, fimG/fimH, fyuA, iutA, iroN
U 066	40	79	1	B2	papC, sfaD/sfaE, cnf1, hly1, fimG/fimH, fyuA, iroN
U 115	41	46	46	C	fimG/fimH, iroN
U 002	42	4	33	B1	fimG/fimH
U 109	42	6	87	B1	fimG/fimH
U 042	42	44	53	B1	fimG/fimH, fyuA, iutA, iroN
U 120	42	45	79	B1	fimG/fimH, iutA, iroN
U 031	42	50	28	B1	fimG/fimH, iutA, iroN
U 096	42	56	76	B1	fimG/fimH, iroN
U 017	43	70	92	A	fimG/fimH
U 094	44	7	41	B1	fimG/fimH, iutA, iroN, tsh
U 051	45	34	47	B1	fimG/fimH, iutA, iroN
U 126	46	8	99	A	astA, fyuA, iroN
U 103	47	30	20	D	papC, fimG/fimH, iutA, sat
U 061	47	31	21	D	fimG/fimH, iutA, iroN
U 053	48	60	81	D	fimG/fimH, astA, iutA, iroN
U 070	49	30	19	D	fimG/fimH
U 118	50	30	22	D	fimG/fimH, iutA, iroN
U 008	51	5	70	D	fimG/fimH, fyuA
U 081	52	14	90	D	fimG/fimH, pic, aggR, fyuA, iutA
Pairs of strains identical in TRS-PCR analysis					
U 020	7	55	32	A	astA, iutA, iroN
U 022	7	55	32	A	fyuA, iutA
U 058	33	71	62	B2	sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 112	33	71	62	B2	papC, sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 069	33	72	65	B2	papC, sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 075	33	72	65	B2	papC, sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
Strains derived from the same patients					
U 004	16	62	91	A	fimG/fimH
U 020	7	55	32	A	astA, iutA, iroN
U 006	33	74	97	B2	sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 021	36	77	17	B2	papC, fimG/fimH, fyuA, iutA, iroN
U 011	19	42	38	A	fimG/fimH, fyuA
U 031	42	50	28	B1	fimG/fimH, iutA, iroN
U 013	33	72	60	B2	papC, sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 046	33	72	61	B2	papC, sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iutA, iroN, sat
U 014	36	74	98	B2	sfaD/sfaE, cnf1, hly1, fimG/fimH, fyuA, iroN
U 054	33	74	63	B2	sfaD/sfaE, cnf1, usp, hly1, fimG/fimH, fyuA, iroN
U 032	1	2	71	A	None of the tested
U 039	40	26	1	B2	papC, sfaD/sfaE, fimG/fimH, fyuA, iutA, iroN
U 097	40	26	57	B2	papC, sfaD/sfaE, fimG/fimH, fyuA, iutA, iroN
U 037	3	51	51	C	fimG/fimH, astA, fyuA
U 050	35	9	94	B2	usp, fimG/fimH, fyuA, iutA, iroN, tsh
U 042	42	44	53	B1	fimG/fimH, fyuA, iutA, iroN
U 124	36	37	16	B2	fimG/fimH, fyuA, iutA, sat
U 055	31	10	93	A	fimG/fimH, fyuA
U 109	42	6	87	B1	fimG/fimH
U 121	36	52	48	C	fimG/fimH, iutA, iroN
U 062	22	49	31	A	fimG/fimH
U 096	42	56	76	B1	fimG/fimH, iroN

Table 1 (continued)

Key	CAC class	GTG class	CGG class	Phylotype	Virulence profile
U 113	22	61	37	A	fimG/fimH, astA
U 116	13	40	34	F	fimG/fimH, astA, fyuA, iutA, iroN, tsh

except for the primer sequence. Reproducibility of each clustering analyses was calculated by a comparison of the densitometric curve, characteristic of three separate band patterns obtained for the three chosen strains (Pearson coefficient, UPGMA). Amplifications were repeated with the same strain on a different day. The average similarity (SA) of the band pattern obtained for these strains and the standard deviation (SD) values were estimated. The reproducibility rate of the band pattern (R) for each primer was calculated as follows: $R = SA - SD$ [%] (Table 1S). The PCR amplifications were accomplished using a T-3000 thermocycler (Biometra, Goettingen, Germany). The PCR products were separated by horizontal electrophoresis on 1.6% agarose gel in a $1 \times$ TAE buffer at 6 V/cm operating within 2 h, stained with ethidium bromide and photographed. Subsequently, gels were optimized according to the recommendations provided by BioNumerics 5.0 software (Applied Maths, Belgium) and normalized with regard to a 100 bp Plus DNA size marker (Fermentas, Thermo Scientific Waltham, MA, USA). The three separate fingerprint similarity analyses based on CAC-PCR, GTG-PCR and CGG-PCR profiles using Pearson correlation (optimization 1%, position tolerance 1%) and grouping according to the UPGMA algorithm were performed. The reproducibility analysis of CAC-PCR was performed as previously described for CGG and GTG [15–19] and obtained by comparing the three separate fingerprints from three different PCR reactions for the three chosen UPEC strains.

Stability analyses of CAC-, GTG-, CGG-PCR profiles

Two strains were selected for this study: U 036 isolated from urine and representative of another collection, K 157 + isolated from stool. Stability analysis for each of the used primers with the TRS motif was obtained by comparing TRS-PCR fingerprints performed for several passages (initial, fifth, twenty-fifth and thirty-ninth) from a series of consecutive subcultures of these strains. The fingerprint similarity analyses based on CAC-PCR, GTG-PCR and CGG-PCR profiles (Fig. 2S) were performed exactly as for TRS-PCR profiling described above and published elsewhere [15–19].

Results

In previous analyses of *E. coli* strains, we analyzed CGG- and GTG-PCR profiles [16]. In this approach, for the first time we further performed CAC-PCR profiling on genomic DNAs obtained from a collection of 124 *E. coli* strains. The fingerprint similarity analysis for this assay was performed. The analysis of reproducibility was also performed and it exhibited 95.0%. Fingerprint patterns with similarity above this value, were treated as identical. Based on analyses using BioNumerics software, each unique profile was assigned a class number (Fig. 1). Next, the GTG-PCR and CGG-PCR tests were conducted on the examined strains. The fingerprint similarity analysis and assignment of class numbers were done in an analogous way. Thereby, 52 CAC-PCR classes, 86 GTG-PCR classes and 99 CGG-PCR classes were obtained. This allowed the separation of 121 unique isolates differing in at least one TRS class (Table 1). The TRS pattern stability studies conducted on multi-generational re-cultivations have shown that the profiles were almost identical, starting from the first re-cultivation to the last one (Fig. 2S). This allowed us to conclude that assigning numbers of classes in such a way was reliable.

In parallel, these results were also confronted with the belonging of strains to phylogenetic groups by applying the Clermont's *E. coli* phylo-typing improved method scheme [4] and with their repertoire of pathogenic factors [15, 16, 20–24]. The results are shown in the Table 1.

Discussion

Until now, we have focused more closely on grouping strains in dendrogram clusters due to the presence of the examined traits (VFs, phylogroups). Currently, our new approach focuses on the maximum distinction of the strains in the collection. The proposed assay for *E.*

Table 2 Epidemiological information about *E. coli* strains used in the study

Key	Department	Date [D-M-Y]	Bacteriuria	Sex	Serotype/O-group
U 001	A	05.07.2005	10 ⁵	F	NT
U 002	B	02.07.2005	10 ⁶	M	NT
U 003	B	30.06.2005	10 ⁶	F	O-rough
U 004	D	29.06.2005	10 ⁶	F	O7
U 005	B	20.06.2005	10 ⁶	F	HH-pool
U 006	B	25.07.2005	10 ⁶	F	NT
U 007	B	01.08.2005	10 ⁶	M	O1
U 008	D	19.08.2005	10 ⁶	F	NT
U 009	B	17.08.2005	10 ⁶	F	NT
U 010	D	29.08.2005	10 ⁶	M	NT
U 011	B	17.08.2005	10 ⁵	F	O-rough
U 012	B	30.08.2005	10 ⁶	M	SS-pool
U 013	C	12.09.2005	10 ⁶	M	O2
U 014	C	28.09.2005	10 ⁶	F	NT
U 015	E	27.09.2005	10 ⁵	F	O1
U 016	E	25.10.2005	10 ³	M/CH	O6
U 017	F	22.10.2005	10 ⁶	F	HH-pool
U 018	B	20.10.2005	10 ⁶	F	O22
U 019	G	20.10.2005	10 ⁶	F	O9
U 020	D	10.10.2005	10 ⁶	F	NT
U 021	B	10.10.2005	10 ⁶	F	O2
U 022	B	10.10.2005	10 ⁶	M	NT
U 023	I	19.10.2005	10 ⁶	F	NT
U 024	B	18.10.2005	10 ⁶	M	O8
U 025	B	17.10.2005	10 ⁶	F	O-rough
U 026	B	17.10.2005	10 ⁶	F	O-rough
U 027	E	13.10.2005	10 ⁵	F	O-rough
U 028	B	14.10.2005	10 ⁶	F	O2
U 029	C	12.10.2005	10 ⁵	F	O7
U 030	B	26.10.2005	10 ⁶	F	NT
U 031	B	27.10.2005	ND	F	O-rough
U 032	B	27.10.2005	10 ⁵	M/CH	NT
U 033	D	28.10.2005	10 ⁵	F	O6
U 034	B	30.10.2005	10 ⁶	F	O7
U 035	H	04.11.2005	10 ⁵	F	O6
U 036	H	04.11.2005	10 ⁵	F	O6
U 037	I	07.11.2005	10 ⁵	F	NT
U 038	E	10.11.2005	10 ⁶	M	NT
U 039	E	10.11.2005	10 ³	M/CH	O6
U 040	F	11.11.2005	10 ³	F	O-rough
U 041	G	14.11.2005	10 ⁶	F	NT
U 042	C	14.11.2005	10 ⁵	F	O6
U 043	H	05.12.2005	10 ⁶	F	O15
U 044	B	06.12.2005	10 ⁵	M	NT
U 045	E	07.12.2005	10 ⁶	F	O-rough
U 046	E	02.12.2005	10 ⁶	M	O2
U 047	E	30.11.2005	10 ⁶	F	O22
U 048	B	26.11.2005	10 ⁶	M	NT
U 049	C	21.11.2005	10 ⁶	M	O-rough
U 050	I	08.01.2006	10 ⁵	F	OO-pool
U 051	E	23.11.2005	10 ⁶	F	O-rough

Table 2 (continued)

Key	Department	Date [D-M-Y]	Bacteriuria	Sex	Serotype/O-group
U 052	J	18.11.2005	10 ⁶	F	PP-pool
U 053	B	07.01.2006	10 ⁶	F	NT
U 054	C	05.01.2006	10 ⁶	F	NT
U 055	C	04.01.2006	10 ⁵	F	FF-pool
U 057	C	04.01.2006	10 ⁶	F	NT
U 058	C	04.01.2006	10 ⁴	M	O22
U 059	B	27.12.2005	10 ⁶	M	NT
U 060	N	20.12.2005	10 ⁶	F	NT
U 061	E	21.12.2005	10 ⁶	F	O17
U 062	E	31.01.2006	10 ⁶	F	WW-pool
U 063	D	14.02.2006	10 ⁶	M	O4
U 064	E	08.03.2006	10 ⁶	F	NT
U 066	C	31.01.2006	10 ⁶	F	O6
U 067	E	03.02.2006	10 ⁵	F	O75
U 068	E	13.01.2006	10 ⁶	F	O8
U 069	G	06.09.2006	10 ⁶	F	O2
U 070	E	16.01.2006	10 ⁶	F	NT
U 071	D	06.09.2006	10 ⁵	F	O-rough
U 072	B	23.01.2006	10 ⁵	F	O6
U 073	H	31.01.2006	10 ⁶	F	NT
U 074	C	28.01.2006	10 ⁶	F	NT
U 075	C	08.03.2006	10 ⁶	F	O2
U 076	B	10.02.2006	10 ⁵	M	UU-pool
U 077	F	07.03.2006	10 ⁶	F	NT
U 079	D	15.02.2006	10 ⁶	M	O-rough
U 080	B	21.02.2006	10 ⁶	M	O8
U 081	D	22.02.2006	10 ⁶	M	NT
U 082	E	24.02.2006	10 ⁶	F	NT
U 083	C	27.02.2006	10 ⁶	F	O25
U 084	C	06.03.2006	10 ⁶	F	O75
U 085	D	13.03.2006	10 ⁵	F	O6
U 086	B	14.03.2006	10 ⁵	M	O2
U 087	B	14.03.2006	10 ⁶	M	O2
U 088	D	22.03.2006	10 ⁶	M	O6
U 089	B	23.03.2006	10 ⁵	F	O6
U 090	M	25.03.2006	10 ⁵	M	O2
U 091	B	27.03.2006	10 ⁶	M	O-rough
U 092	K	29.03.2006	10 ⁶	F	O15
U 093	D	31.03.2006	10 ⁶	F	O1
U 094	E	04.04.2006	10 ⁶	F	PP-pool
U 095	E	18.04.2006	10 ⁶	F	NT
U 096	E	19.04.2006	10 ⁶	F	KK-pool
U 097	E	19.04.2006	10 ⁵	M/CH	O6
U 098	D	05.04.2006	10 ⁵	F	O6
U 099	D	06.04.2006	10 ⁶	F	O-rough
U 100	C	26.04.2006	10 ⁶	F	MM-pool
U 101	C	26.04.2006	10 ⁴	F	O75
U 102	E	28.04.2006	10 ⁶	F/CH	NT
U 103	E	28.04.2006	10 ⁵	F/CH	O17
U 104	E	28.04.2006	10 ⁶	F	NT
U 105	E	28.04.2006	10 ⁵	F	O6

Table 2 (continued)

Key	Department	Date [D-M-Y]	Bacteriuria	Sex	Serotype/O-group
U 106	E	12.05.2006	10 ⁶	F	SS-pool
U 107	H	17.05.2006	10 ⁶	F	O-rough
U 108	C	17.05.2006	10 ⁶	F	O1
U 109	C	05.09.2006	10 ⁶	F	O-rough
U 110	C	18.05.2006	10 ⁶	F	O2
U 111	E	18.05.2006	10 ⁶	F	O-rough
U 112	C	21.04.2006	10 ⁵	F	O6
U 113	E	10.05.2006	10 ⁵	F/CH	NT
U 114	ND	ND	ND	ND	O6
U 115	ND	ND	ND	ND	O-rough
U 116	E	01.08.2006	10 ⁶	F/CH	NT
U 117	L	17.07.2006	10 ⁶	K	O1
U 118	K	02.08.2006	10 ⁶	K	HH-pool
U 119	F	26.07.2006	10 ⁶	K	O8
U 120	C	25.07.2006	10 ⁶	K	KK-pool
U 121	C	24.07.2006	10 ⁶	K	O78
U 124	C	03.07.2006	10 ⁶	K	NT
U 125	C	14.07.2006	10 ⁶	K	O-rough
U 126	ND	ND	ND	ND	O-rough
U 127	C	13.07.2006	10 ⁶	K	OO-pool
U 128	C	06.09.2006	10 ⁶	K	GG-pool
U 130	ND	ND	ND	ND	O-rough

Department: *A* Clinic of cardiology and hypertension, *B* Urology clinic, *C* Urology office, *D* Clinic of internal medicine, *E* Primary medical care, *F* Intensive care unit, *G* Clinic of interventional cardiology, cardiac diabetology and cardiac rehabilitation, *H* Clinic of neurology, *I* Department of laboratory diagnostics, *J* Clinic of neurosurgery, *K* Trauma clinic, *L* General surgery office, *M* Emergency unit, *N* Paid examination, private order

Sex: *F* Female, *M* Male, *CH* Child, *ND* No data obtained, *NT* Non typable

O-pool detects the following serogroups: *FF-pool* O10, O11, O41, O100, O110, O148, O154, O159, O168, O179, *GG-pool* O49, O51, O52, O58, O61, O160, O176, O178, *HH-pool* O13, O17, O44, O62, O66, O73, O77, O106, *KK-pool* O24, O27, O29, O36, O56, O81, O169, O186, *MM-pool* O28ab, O28ac, O42, O112ab, O112ac, O142, O136, O144, *OO-pool* O34, O46, O85, O130, O134, O140, O167, O171, *PP-pool* O37, O39, O48, O59, O88, O91, O155, O165, O184, *SS-pool* O82, O87, O96, O105, O120, O137, O170, O183, *UU-pool* O86, O111, O114, O119, O125ab, O125ac, *WW-pool* O97, O98, O99, O104, O132, O139, O147

Fig. 1 Scheme depicting the assignment of numeric classes based on TRS-PCR profiles (here for CAC-PCR). The reproducibility for CAC-PCR exhibited 95,0% (see Table 1S.), fingerprint patterns with

similarity above this value, were treated as identical. Based on analyses using BioNumerics software, each unique profile was assigned a class number—for these 14 strains 10 CAC classes was assigned

coli sub-typing based on the multiple TRS-PCR profiling, allowed the separation of 121 unique isolates from a collection of 124 strains. In this approach, we obtained numerical results which were easy to sort and interpret, allowing for easier analysis of these strains. Each test based on another TRS primer can bring something new to the analysis, but comparing the growing number of similarity dendrograms, it becomes difficult to interpret. Additionally, averaging them does not show detailed differences. In our new approach, we can test the effectiveness of several TRS markers at the same time, without the need to average the analyses from all TRS-PCR tests.

By analyzing the contents of the Table 1, it can be seen that strains U 058 and U 112 are considered to be identical in TRS-PCR profiles despite the lack of one pathogenicity factor—*papC* (strain U 058). It can be inferred that these strains have lost one of their adhesines, without disturbing the structure of genome in the area we were investigating [1, 2, 8]. However, it cannot be ruled out that differentiation with an additional TRS marker would separate those strains. A more interesting case we observed for strains U 020 and U 022, which have identical TRS-PCR profiles, despite differences in virulence composition. Strain U 020 possess *astA*, *iutA* and *iroN*, whereas strain U 022 possess *fyuA* and *iutA* (Table 1). Apparently, there are no sequences in the genomic region where these genes are encoded, allowing for the generation of three specific types of TRS fragments.

In the group of tested strains, there were also isolates obtained from the same patient. Strains U 013 and U 046 isolated at different times had the same pathogenicity but differed within the CGG class. Such a small difference does not exclude the possibility of a re-infection with the same strain. The difference in one class may suggest a slight genetic change in the same strain, perhaps under the influence of antibiotic therapy, due to infection with a very similar strain or insufficient sensitivity of the method [1, 2, 8]. Among strains U 032, U 039 and U 097, also isolated from the same patient at different time points, the isolate U 032 differs from the other two strains. In case of U 039 and U 097 strains, there is a similar situation as for U 013 and U 046 isolates. For such strains (U 013, U 046 and U 039, U 097), we recommend further subtyping, perhaps with an additional TRS marker.

Similarity dendrograms based on composite analysis of many tests usually show better resolution, but their interpretation may increase difficulties. We think that the proposed strategy in rendering TRS-PCR data, facilitates the interpretation of the obtained outcome. In addition, it could be done rapidly and results may be easier to compare, even between laboratories. It should be emphasized, with the method we used, we are able to demonstrate even small genetic differences among bacterial strains. Because the results are promising, the next stage of the research should include validation

of the method: repeatability among laboratories working on different equipment and reagents.

In our research, we have repeatedly demonstrated the usefulness of the TRS-PCR profiling in bacterial genotyping [15–19]. However, strain differentiation, based on comparing single TRS-PCR results, or tracing differences resulting from averaged affinity trees (based on several TRS-PCR reactions), could also lead to evolutionary conclusions in addition to epidemiological investigations. To be sure, the results should be confronted with the results obtained from MLST analyses. Interestingly, we have shown in our recent studies that based on the analysis of TRS-PCR profiles, it is possible to predict directions of pathogenicity development in the *E. coli* population [25].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human and animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

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