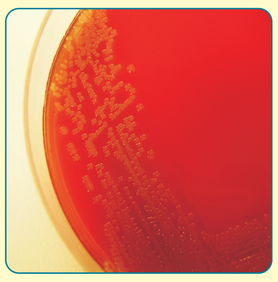
MOLECULAR AND PHENOTYPIC CHARACTERISTICS OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* STRAINS ASSOCIATED WITH HUMAN DISEASES IN THE CZECH REPUBLIC (1965 – 2007)

Monika Marejková, Hana Lhotová , Petr Petráš

National Institute of Public Health, Srobarova 48, Prague, Czech Republic;

monmarej@szu.cz



AIM OF THE STUDY

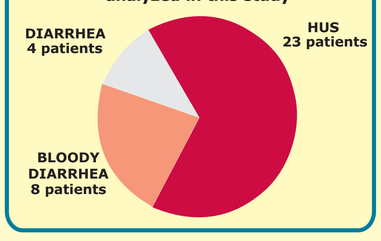
Enterohemorrhagic *Escherichia coli* (EHEC) cause human diseases worldwide. Here we determined molecular and phenotypic characteristics of 35 epidemiologically unrelated EHEC strains isolated from patients with diarrhea–associated hemolytic uremic syndrome (D+ HUS) or diarrhea without HUS in the Czech Republic between 1965 and 2007.

MATERIALS AND METHODS

Origin of strains

The 35 EHEC strains originated mainly from stool samples and were sent to the National Reference Laboratory for *E. coli* and Shigellae at the National Institute of Public Health (NRL NIPH) Prague from hospitals at different regions of the Czech Republic. Twenty-three strains were isolated from patients with D+ HUS and 12 from patients with bloody or nonbloody diarrhea (Figure 1).

Figure 1: Clinical outcomes in patients infected with 35 EHEC analyzed in this study



Phenotypic methods

The isolates were confirmed as *E. coli* using the API E set (BioMérieux), ENTEROtest 24 (Pliva Lachema) and complementary conventional tests, and serotyped (antisera from Denka Seiken, and SSI, Copenhagen, Denmark). Sorbitol fermentation (SF) was tested on SOR-MacConkey agar (Oxoid), tellurite resistance on CT-SMAC agar (Oxoid), and EHEC hemolysin production on enterohemolysin agar (Sifin). Beta-D-glucuronidase (GLR) activity was analyzed by COLItest (Pliva Lachema).The reverse passive latex agglutination (RPLA) method was used to detect production of Shiga toxin (Stx)1 and Stx2 (Denka Seiken kit VTEC – RPLA).

Molecular typing

PCRs for the detection of putative virulence genes were performed in MyCycler™ ThermalCycler (BIO-RAD) and PCR Express (Hybaid, Labsystems).
The stx_1 and stx_2 genes, *eae* encoding adhesin intimin [1], *bfpA* encoding bundle-forming pili structural subunit [2], plasmid-encoded genes EHEC-*hlyA* encoding EHEC-hemolysin [4], *espP* [5], *etpD* [6], *katP* [7], and *terE* used as a marker for the ter cluster encoding tellurite resistance [3] were detected by PCR.
stx_2 genes we subtyped according to Friedrich et al. [8]. stx_{2c} was distinguished from $stx_{2d\text{-}act}$ allele using PCR described by Zheng et al. [9].
The flagellin subunit-encoding (*fliC*) genes were subtyped using *Hha*I RFLP [10].

RESULTS

PHENOTYPES

Serotypes

The 35 EHEC strains belonged to serotypes O157:H7/NM (n = 12), O26:H11/NM (n = 14), O55:NM (n = 2), O111:NM (nonmotile) (n = 5), ONT:NM (n = 1), and Orough:NM (n = 1) (Figure 2).

Sorbitol fermentation

All strains of serogroups O26, O111 and O55 fermented sorbitol. Among the 12 EHEC O157, eight were classical non-sorbitol-fermenting, H7 or NM strains, whereas four were sorbitol-fermenting and nonmotile strains (Table 1).

Growth on CT-SMAC

Most of strains grew on CT-SMAC agar. Four SF O157:NM did not grow (Table 1).

RPLA

The production of Stx1 and/or Stx2 was detected in 34 strains. One strain (SF O157:NM) lost *stx* genes. Nine (25.5 %) strains produced Stx1, 19 strains (57.6 %) Stx2 and 6 strains (18.2 %) both Stx1 and Stx2.

EHEC-hemolysin production was detected in majority of strains carrying the EHEC-*hlyA* gene. Exception were strains No. 12 (O26:NM), No. 18 (NSF O157:H7), two strains SF O157:NM (No. 23 and 24), No. 34 (ONT:NM) and No. 35 (Orough:NM), which did not express enterohemolytic phenotype (Table 1).

Figure 2: Serotypes of 35 EHEC strains

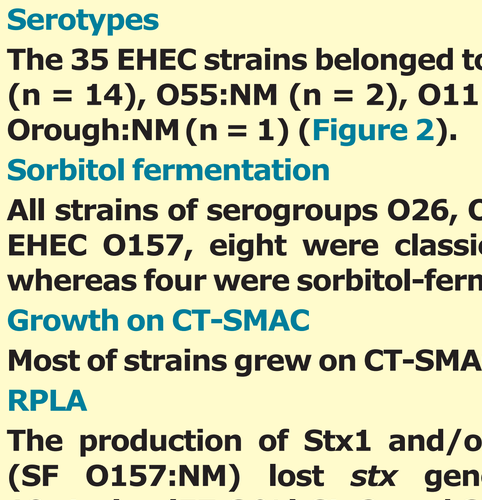
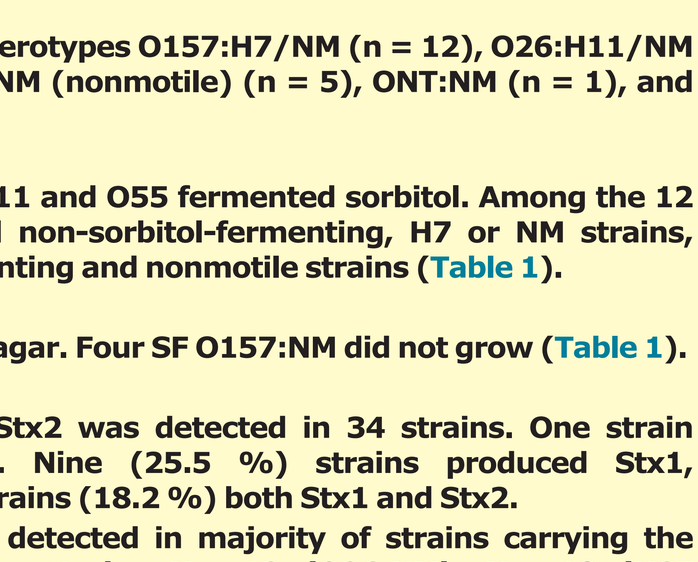


Figure 3: Distribution *stx* genes according serogroups



CONCLUSIONS

1) The majority of EHEC strains associated with HUS in the Czech Republic belong to non-O157:H7 serotypes.
2) Czech Republic is an additional country where SF EHEC O157:NM account for a significant proportion (13 %) of HUS-associated EHEC.
3) EHEC O26 producing Stx2 only have emerged as causes of HUS in this country since 1992.
4) All non-O157 EHEC produced EHEC-hemolysin on enterohemolysin agar and most of them grew well on CT-SMAC. This can assist their isolation from patients´ stools.

Table 1: Characteristics of 35 EHEC strains

No	Diagnosis	PHENOTYPES						GENOTYPES								
		Serotype	RPLA (Stx)	EHEC-hemolysin	CT-SMAC growth	Sorbitol	-Hly	*fliC*	*stx* genotype	*eae*	EHEC-*hlyA*	*katP*	*espP*	*etpD*	*bfpA*	*terE*
1	D	O26:H11	2	+	+	+	−	H11	2	+	+	−	−	+	−	+
2	BD	O26:H11	2	-	+	+	−	H11	2	+	−	−	−	−	−	+
3	BD	O26:H11	1	+	+	+	−	H11	1	+	+	+	+	−	−	+
4	HUS	O26:H11	2	+	+	+	−	H11	2	+	+	+	−	−	−	+
5	HUS	O26:H11	2	+	+	+	−	H11	2	+	+	+	+	−	−	+
6	HUS	O26:H11	2	+	+	+	−	H11	2	+	+	+	+	−	−	+
7	HUS	O26:H11	2	+	+	+	−	H11	2	+	+	-	-	+	−	+
8	HUS	O26:H11	1	+	+	+	−	H11	1	+	+	+	+	−	−	+
9	HUS	O26:H11	2	+	+	+	−	H11	2	+	+	−	−	+	−	+
10	BD	O26:NM	1	+	+	+	−	H11	1	+	+	+	+	−	−	+
11	HUS	O26:NM	2	+	+	+	−	H11	2	+	+	+	+	−	−	+
12	HUS	O26:NM	2	−	+	+	−	H11	2	+	+	+	+	−	−	+
13	D	O26:H11	1	+	+	+	−	H11	1	+	+	+	+	−	−	+
14	D	O26:H11	1	−	−	+	−	H11	1	+	−	−	−	−	−	−
15	BD	NSF O157:NM	1+2	+	+	−	−	H7	1+2+2c	+	+	+	+	+	−	+
16	BD	NSF O157:H7	1	+	+	−	−	H7	1	+	+	+	+	+	−	+
17	BD	NSF O157:H7	1	+	+	−	−	H7	1	+	+	+	+	+	−	+
18	BD	NSF O157:H7	1+2	-	+	−	−	H7	1+2c	+	+	+	+	+	−	+
19	HUS	NSF O157:H7	2	+	+	−	−	H7	2+2c	+	+	+	+	+	−	+
20	BD	NSF O157:H7	2	+	+	−	−	H7	2+2c	+	+	+	+	+	−	+
21	HUS	NSF O157:H7	2	+	+	−	−	H7	2	+	+	+	+	+	−	+
22	HUS	NSF O157:NM	1+2	+	+	−	−	H7	1+2c	+	+	-	+	+	−	+
23	HUS	SF O157:NM	2	−	−	+	−	H7	2	+	+	+	−	+	−	−
24	HUS	SF O157:NM	lost Stx	−	−	+	−	H7	lost stx	+	+	−	−	+	−	−
25	D	SF O157:NM	2	−	−	+	−	H7	2	+	−	−	−	−	−	−
26	HUS	SF O157:NM	2	−	−	+	−	H7	2	+	−	−	−	−	−	−
27	HUS	O55:NM	2	−	−	+	−	H7	2	+	−	−	−	+	−	−
28	HUS	O55:NM	2	−	−	+	−	H7	2	+	−	−	−	+	−	−
29	HUS	O111:NM	1	−	+	+	−	H8	1	+	−	−	−	−	−	+
30	HUS	O111:NM	1	−	−	+	−	H8	1	+	−	−	−	−	−	−
31	HUS	O111:NM	1+2	+	+	+	−	H8	1+2	+	+	−	−	−	−	+
32	HUS	O111:NM	1+2	+	+	+	−	H8	1+2	+	+	−	−	−	−	+
33	HUS	O111:NM	1+2	+	+	+	−	H8	1+2	+	+	−	−	−	−	+
34	HUS	ONT:NM	2	−	−	+	−	H25	2	+	+	+	+	−	−	−
35	HUS	Orough:NM	2	−	−	+	−	H25	2	+	+	+	+	−	−	−

MOLECULAR CHARACTERISTICS

All nonmotile strains within each serotype shared the ***fliC* gene** with the motile strains (Table 1). The two O55:NM strains possed the H7 - encoding *fliC* gene, and the ONT and Orough strains possed the H25 - encoding *fliC*.

stx genotypes

Distribution of *stx* genes in EHEC of different serotypes is shown in Figure 3. Notably, majority (9 of 14 strains) of EHEC O26:H11/NM possessed stx_2 as the only *stx* allele.

Putative non-*stx* virulence genes

All strains posssessed *eae*, but lacked *bfpA*. EHEC-*hlyA*, alone or together with one or more of the other plasmid-encoded genes (*katP, espP, etpD*) was detected in 27 strains (77 %). The rest of the strains did not posses any of the plasmid genes, except of two strains O55 carying *etpD* (Table 1). Three stx_2 - harboring strains of serogroup O26 possessed EHEC-*hlyA* and *etpD*, but lacked *katP* and *espP*, a combination of plasmid genes typical for the new O26 clone previously identified in Germany [11].

Other genes

Presence of *terE* correlated with tellurite resistance (Table 1). None of the SF O157 strains possed *terE* and none grew on CT-SMAC.

REFERENCES

[1] Schmidt H, Plaschke B, Franke S, *et al.* Med Microbiol Immunol Berl.1994; 183:23–31.
[2] Wieler, LH, Vieler E, Erpenstein C, *et al.* J. Clin. Microbiol 1996; 34: 2980–2984.
[3] Tailor DE, Rocker M, Keelan M, *et al.* J Bacteriol 2002; 184(17): 4690–4698.
[4] Schmidt H, Beutin L, and Karch H. Infect Immun 1995; 63(3): 1055–1061.
[5] Brunder W, Schmidt H, Karch H. Mol Microbiol 1997; 24:767–778.
[6] Schmidt H, Henkel B, Karch H. FEMS Microbiol Lett 1997; 148:265–272.
[7] Brunder W, Schmidt H, Karch H. Microbiology 1996; 142: 3305–3315.
[8] Friedrich AW, Bielaszewska M, Zhang WL, et al. J Infect Dis 2002; 185:74–84.
[9] Zheng, J, Shenghui C, Teel LD, et al. Appl Environ Microbiol 2008; in press.
[10] Zhang WL, Mellmann A, Sonntag AK, et al. Int J Med Microbiol 2007; 297: 17–26.
[11] Zhang WL, Bielaszewska M, Liesegang A, *et al.* J Clin Microbiol 2000; 38: 2134–2140.

ACKNOWLEDGEMENT

The authors thank Professor Dr. Helge Karch, Director of the Institute for Hygiene, University of Muenster, for the training in new molecular typing methods provided to Monika Marejková. Thanks are also due to Assoc. Prof. Dr. Martina Bielaszewska from the same Institute for generous assistance provided to the Czech NRL for *E. coli* and Shigellae, including the professional training and consultation on STEC strains. The authors thank the laboratory colleagues, in particular Miluška Vašáková, the collaborating microbiologists and clinicians for providing the patient data and ing. Jana Burjanková for graphical design of poster. Ing. Marejková´s participation in PEN – conference was partly supported by BIO-RAD, Ltd., Czech Republic.