GENOTYPIC AND PHENOTYPIC CHARACTERISTICS OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* STRAINS ASSOCIATED WITH HUMAN DISEASES IN THE CZECH REPUBLIC (1965 – 2008)

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INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) are an important cause of food-borne infections worldwide. In this study we determined molecular and phenotypic characteristics of 42 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 April 2009.

CONCLUSIONS

1) The majority of EHEC strains associated with HUS in the Czech Republic belong to non-O157:H7 serotypes.
2) SF EHEC O157:NM account for a significant proportion (12%) of HUS associated EHEC.
3) EHEC O26 producing Stx2 only have been emerging as causes of HUS in this country since 1992.
4) Most of non-O157 EHEC produce EHEC-hemolysin on enterohemolysin agar and grow well on CT-SMAC. This can assist their isolation from patients´ stools.
5) stx_2 only is the predominant *stx* genotype in HUS-associated EHEC in the Czech Republic, whereas stx_1 (alone or together with stx_{2c}) is mostly detected in isolates from patients with diarrhea or bloody diarrhea (Figure 7).

MATERIALS AND METHODS

Origin of strains

The 42 EHEC strains originated mainly from stool samples and were sent to the National Reference Laboratory for *E. coli* and Shigella at the National Institute of Public Health (NRL NIPH) Prague from hospitals at different regions of the Czech Republic. Twenty-five strains were isolated from patients with D+ HUS and 17 from patients with bloody or nonbloody diarrhea (Figure 1).

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). Moreover, all strains were tested for cytotoxicity using Vero cell assay. Adherence of the EHEC strains to Caco-2 cells was assayed in the presence of D-mannose as assayed by the method described by Aldick et al. [1]. The adherence patterns were assigned according to Scaletsky et al. after 3 and 6h of incubation [2].

Molecular typing

PCRs for the detection of putative virulence genes were performed in MyCycler™ ThermalCycler (BIO-RAD) and PCR Express (Hybaid, Labsystems). The genes targeted are shown in Table 1, 2, 3 and Figure 5, 6.

stx_2 genes were subtyped according to Friedrich et al. [3]. stx_{2c} was distinguished from $stx_{2d\text{-}act}$ allele using PCR described by Zheng et al. [4] and stx_{1c} was detected using PCR (Zhang et al., 2001).

The flagellin subunit-encoding (*fliC*) genes were subtyped using *Hha*I RFLP [Zhang et al., 5].

RESULTS

PHENOTYPES

Serotypes. The 42 EHEC strains belonged to serotypes O157:H7/NM (nonmotile) (n = 16), O26:H11/NM (n = 16), O55:NM (n = 2), O111:NM (n = 6), ONT:NM (n = 1), and Orough:NM (n = 1) (Figure 2). Distribution of serotypes according to the diagnosis is shown in Figure 3.

Sorbitol fermentation. All strains of serogroups O26, O111 and O55 fermented sorbitol. Among the 16 EHEC O157, twelve 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 2).

Stx production. Production of Stx1 and/or Stx2 was detected in 41 strains and correlated with *stx* genotype. Only one strain (SF O157:NM) lost *stx* before Stx phenotyping. Fourteen (33 %) strains produced Stx1, 20 strains (48 %) Stx2 and 7 strains (17 %) both Stx1 and Stx2.

Moreover, supernatants of all strains were cytotoxic to Vero cells (titre range 1:16 - 1:2048).

Adherence to Caco-2 cells. Most of the strains displayed a localized adherence-like (LAL) pattern (Figure 4).

EHEC-hemolysin production was detected in majority of strains carrying the EHEC-*hlyA* gene. Exceptions were one strain of each serotype O26:NM, O157:H7, ONT:NM and Orough:NM, and two SF O157:NM strains, which did not express enterohemolytic phenotype (Table 2).

MOLECULAR CHARACTERISTICS

All EHEC O157:H7/NM shared $rfbE_{O157}$ gene and $O26_{wzy}$ gene was detected in all O26:H11/NM strains.

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

stx genotypes

Distribution of *stx* genes in EHEC of different serotypes is shown in Figure 5. Notably, majority (10 of 16 strains) of EHEC O26:H11/NM possessed stx_2 as the only *stx* allele. Most of the strains harboured stx_1, stx_2, stx_1+stx_2 or stx_1+stx_{2c} genotype. $stx_{2d\text{-}act}$ was found in none of the strains.

Putative non-*stx* virulence genes

All strains posssessed *eae*, but lacked *bfpA* (Table 1). EHEC-*hlyA*, alone or together with one or more of the other plasmid-encoded genes (*katP*, *espP*, *etpD*) was detected in 34 strains (81 %). The rest of the strains did not contain any of the plasmid genes, except of two strains O55 carying *etpD* (Figure 6). Three stx_2-harboring O26 strains 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 [Zhang et al., 6].

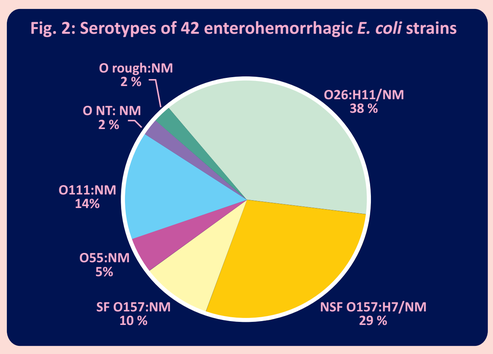
Each of the four SF EHEC O157:NM was positive for *sfpA* (Table 1).

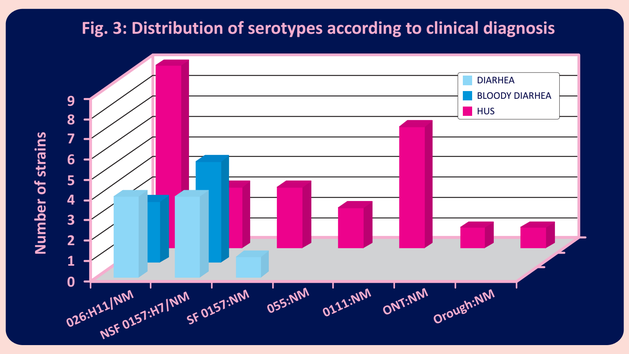
Presence of *terE* correlated with tellurite resistance. None of the SF O157 strains possessed *terE* and none grew on CT-SMAC (Table 2).

All 42 strains had a complete or incomplete OI-122 (Table 3).

The fimbrial gene $lpfA_{O157/OI\text{-}154}$ was present only in strains of serogroups O157 (n=16) and O55 (n=2). The other fimbrial gene $lpfA_{O26}$ was presented only in strains of serogroups O26 (n=16) and O111 (n=6). All of the strains of major EHEC serotypes possessed *iha*, while SF EHEC O157:NM, O55:NM, ONT and Orough lacked this gene (Table 1).

irp2 and *fyuA* genes were detected in all EHEC O26:H11/NM, but were absent in all strains of serogroups O157, O55, O111, ONT and Orough (Table 1).



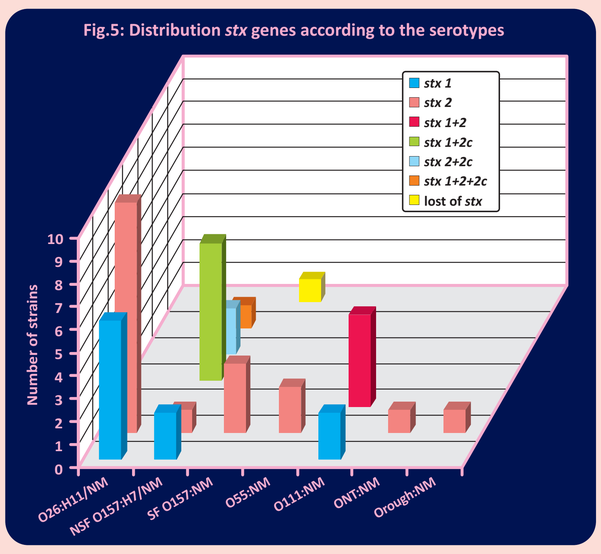


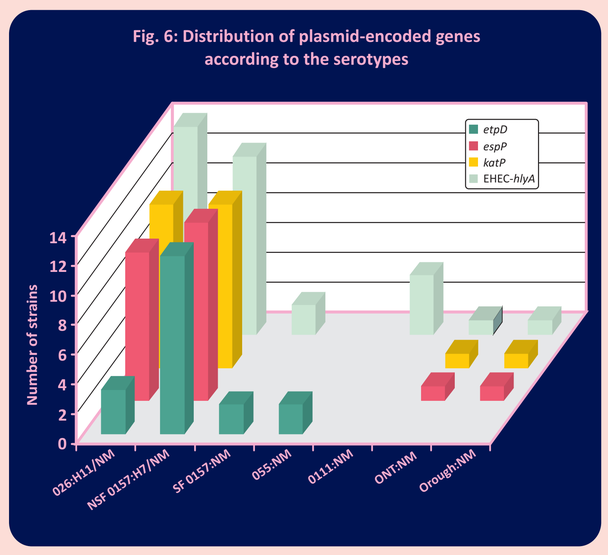
Tab. 1: Selected characteristics of 42 EHEC strains

Numberof strains	Phenotypes			Genotypes								
	Serotype	Sorbitol	α-Hly	*fliC*	*eae*	*bfpA*	*sfpA*	$lpfA_{O157/OI\ 154}$	$lpfA_{O26}$	*iha*	*irp2*	*fyuA*
16	O26:H11/NM	+	-	H11	+	-	-	-	+	+	+	+
12	NSF O157:H7/NM	-	-	H7	+	-	-	+	-	+	-	-
4	SF O157:NM	+	-	H7	+	-	+	+	-	-	-	-
2	O55:NM	+	-	H7	+	-	-	+	-	-	-	-
6	O111:NM	+	-	H8	+	-	-	-	+	+	-	-
1	ONT:NM	+	-	H25	+	-	-	-	-	-	-	-
1	Orough:NM	+	-	H25	+	-	-	-	-	-	-	-

Tab. 2: Comparison of selected phenotypic and genotypic characteristics according to the serotypes

Serotype	Number of tested strains	Number of positive strains			
		EHEC-hemolysin	EHEC-*hlyA*	CT-SMAC growth	*terE*
O26:H11/NM	16	13	14	15	15
NSF O157:H7/NM	12	11	12	12	12
SF O157:NM	4	0	2	0	0
O55:NM	2	0	0	0	0
O111:NM	6	4	4	5	5
ONT:NM	1	0	1	0	0
Orough:NM	1	0	1	0	0





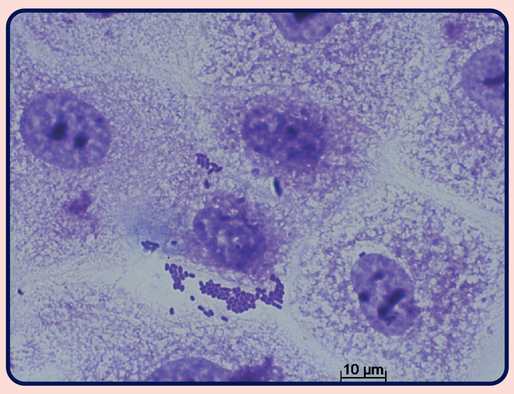


Fig. 4: LAL pattern, EHEC O26:H11

Tab.3: Distribution of OI-122-encoded genes (*pagC, sen, nleB, nleE, efa1*) in 42 EHEC strains of different serotypes

pagC+ sen+ nleB+ nleE+ efa1	OI-122 content	*sen+ nleB+ nleE+ efa1*
NSF O157:H7/NM (n=12)		O26:H11/NM (n=16)
SF O157:NM (n= 4)	serotype	ONT:NM (n= 1)
O55:NM (n= 2)		Orough:NM (n= 1)
O111:NM (n= 6)		

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