Shiga toxin-producing *Escherichia coli* (STEC) in the Czech Republic: Characterization of pathogenic strains isolated from pig and cattle carcasses

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Summary

This study was performed in order to monitor the occurrence of Shiga toxin-producing *Escherichia coli* (STEC) in swabs from the carcasses of pigs and cattle at slaughterhouses in the Czech Republic. Altogether, 38 out of 819 (4.6 %) swabs were positive for the presence of STEC. Overall, 41 isolates of shigatoxigenic *E. coli* and 3 strains with the characteristic of enterohemorrhagic *E. coli* (EHEC) were obtained. Fourteen (34.2 %) STEC isolates were resistant to at least one antimicrobial agent and 5 (12.2 %) of them were considered the multi-resistant isolates, while resistance to tetracycline and ampicillin was found most frequently. Highly pathogenic serogroups O91, O113, O146 and O157 were detected, but only the serogroup O157 strain harboured additional virulence factors (stx_{1a}, stx_{2c} and stx_{2e}). Although raw meat has not been considered an important source of STEC for humans, our results point out that this route of infection cannot be excluded.

Keywords

Shiga toxin; serogroup O157; multi-resistant isolates; raw meat; Czech Republic

Escherichia coli is not only a harmless inhabitant of the intestinal tract that is frequently used as an indicator organism for gaps in hygiene but it is also a potential pathogenic bacterial species [1, 2]. To date, six categories of pathogenic *E. coli* that affect the human intestine have been reported [3]. Shiga toxin-producing *E. coli* (STEC) belonging to diarrhoeagenic *E. coli* species have emerged as an important zoonotic food-borne pathogen over the last few decades [4, 5]. STEC are characterized by the production of Shiga toxins (Stxs), which are divided into two main types (Stx1 and Stx2) and several subtypes. In particular, three subtypes of Stx1 and seven subtypes of Stx2 were described, while only some of them have a potential to cause human illness [6].

Pathogenicity of *E. coli* is a multi-factorial feature depending on a number of virulence genes in addition to those encoding Shiga toxins [7]. Therefore, the production of Stx alone is deemed to be insufficient to cause severe disease. Most notable is the production of intimin protein encoded by *eaeA* gene, product of which enables *E. coli* cells to adhere to intestinal cells and thus facilitates the development of attaching and effacing lesions [6, 8]. Simultaneous presence of *eaeA* and *stx* genes is typical for enterohemorrhagic *E. coli* (EHEC), a subset of STEC. These strains can cause gastrointestinal diseases, such as hemorrhagic colitis (bloody diarrhea), and can be responsible for life-threatening hemolytic-uremic syndrome (HUS) [9]. However, classification of pathogenic *E. coli* into individual categories is not always unambiguous. As an example, during 2011, a large outbreak caused by an unusual *E. coli* strain was reported in Germany. The pathotype combined the virulence potential of STEC (stx_{2a} – encoding bacteriophage) and enteroaggregative *E. coli* (EAggEC). The aggregative adherence fimbriae colonization mechanism substituted for the locus of enterocyte effacement functions normally encoded by *eae* gene in EHEC strains. Clinical presentation of the infection included also bloody diarrhoea and HUS [3]. EAggEC strains pro-

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duce a number of virulence factors. Among them, aggregative adherence fimbriae (AAF) regulated by a transcription factor encoded by the *aggR* gene and the presence of *aaiC* gene encoding a secreted protein are most frequently used for diagnostic purposes [10].

Several hundred STEC serotypes have been described but not all of them are considered as pathogenic [1, 11]. Serotype O157:H7 is the one most frequently associated with both outbreaks and sporadic cases of severe disease. However, other non-O157 STEC serogroups are also emerging as leading causes of infection. According to the European Food Safety Authority report [12], the major serotypes of public concern are also O26, O103, O145, O111, and O104. Moreover, other serotypes have also been described in connection with human infection in the United States (O45, O121) and in Europe (O55, O113, O128, O146 O91) [13–17].

Animals, particularly ruminants, are natural reservoirs of STEC, being common asymptomatic carriers shedding these microorganisms in their faeces [18]. The food chain represents a major route of animal-to-human infection and thus the abattoir is one of the primary steps in the "farm to fork" process [4, 9]. Contamination of meat with STEC during slaughter is the principal route through which these pathogens enter the meat supply chain. Therefore, the main objectives of this study were the following: (i) to investigate the prevalence of Shiga toxin-producing *E. coli* in swabs from the carcasses at slaughterhouses from different parts of the Czech Republic; (ii) to characterize STEC isolates with an emphasis on the detection of virulence factors and serological analysis; (iii) to assess antimicrobial resistance of obtained samples and thus try to estimate the risk level of infection in primary meat production in the Czech Republic.

MATERIALS AND METHODS

Sampling procedure and *E. coli* isolation

Sampling was conducted from June to August of 2013 and 2014 by the State Veterinary Administration (Prague, Czech Republic). A total of 331 swabs from the carcasses of cattle (168 in 2013 and 163 in 2014) and 488 swabs from the carcasses of pigs (318 in 2013 and 170 in 2014) were sampled at 158 different slaughterhouses in the Czech Republic. Preferably, each farm was sampled only once a year.

Basic processing of the samples was carried out in the State Veterinary Institutes in Prague, Jihlava and Olomouc according to the methodical process coordinated by the National Reference Laboratory for *Escherichia coli* (NRL for *E. coli*, Veterinary Research Institute, Brno, Czech Republic). The procedure was based on the guideline ISO TS 13136 [19]. In the laboratory, swabs were placed into plastic bags with buffered peptone water (BPW; Oxoid, Basingstoke, United Kingdom), homogenized by a Stomacher device (IUL, Barcelona, Spain), and incubated at 37 °C. After a 24 h incubation, 1 ml of bacterial suspension was used for DNA isolation. The presence of $stx_{1,2}$ genes and the gene *eaeA* was investigated using polymerase chain reaction (PCR) [19]. Enriched BPW originating from *stx*-positive samples was inoculated on selective media (tryptone bile X-glucuronide medium, TBX, Oxoid and MacConkey, Oxoid) and incubated at 37 °C overnight. Up to 50 colonies from one sample with *E. coli* morphology were investigated for the presence of *stx* and *eaeA* genes. Positive isolates were sent to NRL for *E. coli* for confirmation and further typing.

Confirmation and typing of isolates

In NRL for *E. coli*, PCR was used for the detection of genes encoding selected virulence markers – Shiga toxins 1 and 2 (stx_1 and stx_2) with subtypes, intimin (*eaeA*) and genes typical of EAggEC – *aggR* and *aaiC*. Bacterial DNA was isolated from an overnight culture on blood agar (BioRad, Hercules, California, USA) by lysis of the bacterial cell suspension at 95.5 °C for 10 min, followed by centrifugation at $14\,000 \times g$ for 2 min. The supernatant was used as a DNA template. PCR assays were performed according to BOISEN et al. [20], FAGAN et al. [21] and SCHEUTZ et al. [22]. Primer sequences and amplicon sizes are listed in Tab. 1. PCR products were analysed by gel electrophoresis in 1.5% agarose (Serva, Heidelberg, Germany), followed by visualization on a transilluminator after being stained with ethidium bromide.

Serotyping of *E. coli* isolates

Typing of somatic O-antigen was performed using a U-type microplate agglutination assay [23]. Agglutination was performed with a set of 70 types of O-antisera (Veterinary Research Institute, Brno, Czech republic), including the most common O-serogroups. With strains where unclear results were obtained, PCR serotyping was performed [13–17].

Antimicrobial susceptibility testing

The susceptibility to antibiotics commonly used in clinical treatments was tested by disk diffusion method according to the Clinical Labora-

Tab. 1. List of primers for the detection of virulence genes used in the study.

Primer	Primer sequence (5'-3')	Amplified gene	Amplicon size [bp]	Reference
Screening of virulence genes				
stx_1 F	ACA CTG GAT GAT CTC AGT GG	stx_1	614	[21]
stx_1 R	CTG AAT CCC CCT CCA TTA TG			
stx_2 F	CCA TGA CAA CGG ACA GCA GTT	stx_2	779	
stx_2 R	CCT GTC AAC TGA GCA GCA CTT TG			
eaeA F	GTG GCG AAT ACT GGC GAG ACT	eaeA	890	
eaeA R	CCC CAT TCT TTT TCA CCG TCG			
aggR F	GCA ATC AGA TTA ARC AGC GAT ACA	aggR	426	[20]
aggR R	CAT TCT TGA TTG CAT AAG GAT CTG G			
aaiC F	TGG TGA CTA CTT TGA TGG ACA TTG T	aaiC	313	
aaiC R	GAC ACT CTC TTC TGG GGT AAA CGA			
Subtyping of _stx_ genes				
vtx_{1a} F1	CCT TTCCAG GTA CAA CAG CGG TT	stx_{1a}	478	[22]
vtx_{1a} R2	GGA AAC TCA TCA GAT GCC ATT CTG G			
vtx_{1c} F1	CCTTTCCTGGTACAACTGCGGTT	stx_{1c}	252	
vtx_{1c} R1	CAAGTGTTGTACGAAATCCCCTCTGA			
vtx_{1d} F1	CAGTTAATGCGATTGCTAAGGAGTTTACC	stx_{1d}	203	
vtx_{1d} R2	CTCTTCCTCTGGTTCTAACCCCATGATA			
vtx_{2a} F2	GCG ATA CTG RGB ACT GTG GCC	stx_{2a}	349, 347	
vtx_{2a} R3	CCG KCA ACC TTC ACT GTA AAT GTG			
vtx_{2a} R2	GGC CAC CTT CAC TGT GAA TGT G			
vtx_{2b} F1	AAA TAT GAA GAA GAT ATT TGT AGC GGC	stx_{2b}	251	
vtx_{2b} R1	CAG CAA ATC CTG AAC CTG AGC			
vtx_{2c} F1	GAA AGT CAC AGT TTT TAT ATA CAA CGG GTA	stx_{2c}	177	
vtx_{2c} R2	CCG GCC ACY TTT ACT GTG AAT GTA			
vtx_{2d} F1	AAA RTC ACA GTC TTT ATA TAC AAC GGG TG	stx_{2d}	179, 280	
vtx_{2d} R1	TTY CCG GCC ACT TTT ACT GTG			
vtx_{2d} R2	GCC TGAT GCA CAG GTA CTG GAC			
vtx_{2e} F1	CGG AGT ATC GGG GAG AGG C	stx_{2e}	411	
vtx_{2e} R2	CTT CCT GAC ACC TTC ACA GTA AAG GT			
vtx_{2f} F1	TGG GCG TCA TTC ACT GGT TG	stx_{2f}	424	
vtx_{2f} R1	TAA TGG CCG CCC TGT CTC C			

tory Standards Institute (CLSI) protocol [24] on Mueller-Hinton agar (Oxoid) using the following antimicrobials and concentrations: ampicillin (AMP, 10 µg), amoxicillin/clavulanic acid (AMC, 30 µg), cefotaxime (CTX, 30 µg), chloramphenicol (CMP, 30 µg), streptomycin (STR, 10 µg), kanamycin (KAN, 30 µg), gentamicin (GEN, 10 µg), sulfamethoxazole/trimethoprim (SXT, 25 µg), trimethoprim (TMP, 5 µg), tetracycline (TET, 30 µg), nalidixic acid (NAL, 30 µg), ciprofloxacin (CIP, 5 µg) and colistin (COL, 10 µg). Antibiotic disks were obtained from Oxoid. The _E. coli_ strains were evaluated based on the size of the zones of inhibition and classified as susceptible, intermediate and resistant according to the CLSI criteria for _Enterobacteriaceae_ [24].

In isolates found to be resistant to tetracycline, β-lactams and quinolones, genes responsible for these resistances were detected, using PCR. PCR assays were performed according to BRIÑAS et al. [25], CATTOIR et al. [26], GAY et al. [27], LEWIS et al. [28] and NG et al. [29]. All primers are listed in Tab. 2.

RESULTS AND DISCUSSION

Prevalence of STEC on the carcasses

Altogether 58 out of 331 samples of the cattle carcasses (17.5 %) revealed positive PCR results indicating the presence of stx_1 and/or stx_2 in BPW. However, only 13 (3.9 %) yielded positive isolates.

One of the samples yielded two types of strains. This meant that 14 STEC isolates were obtained from swabs of cattle carcasses. These results are similar to those of MONAGHAN et al. [7], who reported that 27 % of bovine carcass samples in their study were positive for Shiga toxin genes, with culture-based prevalence of only 1.1 %. ETCHEVERRÍA et al. [8] detected 12.3 % of beef carcasses as STEC-positive at slaughter but these authors did not perform the corresponding strain isolation. In our study, the occurrence of STEC on the cattle carcasses in 2013 and 2014 was similar (4.7 % and 3.6 %, respectively; Tab. 3).

PCR screening of 488 swab samples from pig carcasses revealed a positive reaction in 85 (17.4 %) cases, which is very similar to the results of our cattle carcass investigation. The culture-based method confirmed the presence of STEC in 25 (5.1 %) samples, two of them yielding two different *E. coli* strains. The 27 STEC isolates from pig carcasses were used for further typing. In accordance with our study, BOUVET et al. [30] reported that 15 % of pig carcass samples after chilling were positive for some *stx* gene but STEC isolates were obtained from only 3.3 % of samples. A low prevalence (2.2 %) of STEC was also detected by MARTINS et al. [4] in the intestines of pigs during slaughter in Brazil, and OPORTO et al. [31] reported even absence of STEC in swine faeces from Spanish herds. As with the cattle carcasses, STEC occurrence on the pig carcasses did not change between 2013 and 2014. The detailed information is presented in Tab. 3 and Tab. 4.

The detection of *stx* gene in samples where

Tab. 2. List of primers for the detection of resistance genes used in the study.

Primer	Primer sequence (5'-3')	Amplified gene	Amplicon size [bp]	Reference
tetA/F	GCT ACA TCC TGC TTG CCT TC	*tet*(A)	210	[29]
tetA/R	CAT AGA TCG CCG TGA AGA GG			
tetB/F	TTG GTT AGG GGC AAG TTT TG	*tet*(B)	659	
tetB/R	GTA ATG GGC CAA TAA CAC CG			
tetC/F	CTT GAG AGC CTT CAA CCC AG	*tet*(C)	418	
tetC/R	ATG GTC GTC ATC TAC CTG CC			
tetG/F	CAG CTT TCG GAT TCT TAC GG	*tet*(G)	844	
tetG/R	GAT TGG TGA GGC TCG TTA GC			
TEM-F	TTC TTG AAG ACG AAA GGG C	*bla*TEM	1150	[25]
TEM-R	ACG CTC AGT GGA ACG AAA AC			
SHV-F	CAC TCA AGG ATG TAT TGT G	*bla*SHV	885	
SHV-R	TTA GCG TTG CCA GTG CTC G			
PANCTX-M.F	TTT GCG ATG TGC AGT ACC AGT AA	*bla*CTX-M	554	
PANCTX-M.R	CGA TAT CGT TGG TGG TGC CAT A			
QnrAm-F	AGA GGA TTT CTC ACG CCA GG	*qnrA*	580	[26]
QnrAm-R	TGC CAG GCA CAG ATC TTG AC			
QnrBm-F	GGM ATH GAA ATT CGC CAC TG	*qnrB*	264	
QnrBm-R	TTT GCY GYY CGC CAG TCG AA			
QnrS-1	ACG ACA TTC GTC AAC TGC AA	*qnrS*	417	[27]
QnrS-2	TAA ATT GGC ACC CTG TAG GC			

Tab. 3. Numbers of collected and STEC-positive samples.

							Detection of *stx* gene			
	Number of samples		Number of slaughterhouses		Number of farms		In the broth		Number of isolated strains	
Year	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Cattle	168	163	72	74	109	128	29	29	8	6
Pig	318	170	85	84	132	133	47	38	15	12
Total	486	333	157	158	241	261	76*	67*	23	18

* – data were provided by the State Veterinary Institutes in Prague, Jihlava and Olomouc.

the corresponding strain isolation had not been performed is incomplete and is regarded as a presumptive diagnosis [8]. The pattern of virulence genes, exhibited by STEC which are considered to be pathogenic for humans, is complex and it is possible that different strains presenting only part of the virulence gene pattern may be present in a sample at the same time. Therefore, isolation of a STEC strain is required to confirm that the positive PCR signals are generated from genes that are simultaneously present in the same live bacterial cell [19].

Overall, 38 samples were confirmed as STEC-positive. This meant that the prevalence of Shiga-toxin producing *E. coli* in the swabs from the car-

casses in our study was 4.6 %. Strains belonging to the pathogenic sub-group of enterohemorrhagic *E. coli* were isolated only twice (once in 2013 and once in 2014). These strains were positive not only for *stx* gene but also for *eaeA* gene encoding the production of intimin protein.

Although ruminants, in particular cattle, are considered as the main reservoir of STEC [11, 30], swabs obtained from the pig carcasses were more often the sources of this pathotype compared to the results from the cattle carcass swabs. However, we found the prevalence in both cases as comparatively low and comparable with the results of other studies [4, 7, 8, 30].

Tab. 4. Characteristics of STEC isolates by serogroup, virulence and resistance to antimicrobials.

Sample		Serogroup	*stx1*	*stx2*	*eaeA*	Number of isolates	Resistance phenotype
Pig carcass swabs	2013	O91**	1a		–	1	–
		O112	–	2e	–	1	–
		O146**	1d		–	1	–
		O-	–	2e	–	7	–
		O-	–	2e	–	1	AMP, SXT, TMP, TET
		O-	–	2e	–	1	AMP, STR, SXT, TMP, TET
		O-	–	2e	–	2	AMP
		O-	–	2e	–	1	TET
	2014	O8	–	2e	–	2	–
		O8†	–	2e	–	1	AMP, SXT, TMP, TET, NAL
		O30	–	2e	–	1	–
		O156††	–	2e	–	1	TET
		O157**	1a	F2e	+	1	GEN
		O-	–	2e	–	2	–
		O-†	–	2e	–	1	TET
		O-	–	2e	–	1	CMP, STR, KAN, GEN, SXT, TMP, TET
		O-††	–	2e	–	1	STR, TET
		O-	–	2e	–	1	AMP, STR, TET
Cattle carcass swabs	2013	O8	–	2a	–	1	–
		O113**	–	2c	–	1	–
		O141	–	2e	–	1	–
		O152†	–	2e	–	1	TET
		O-	1a		+*	1	–
		O-	1a	2a	–	1	–
		O-†	–	2e	–	1	–
		O-	–	2e	–	1	TET
	2014	O30	–	2a	–	1	–
		O91**	–	2d	–	1	–
		O153	1a	2a	–	1	–
		O-	–	2e	–	2	–
		O-	–	2d	–	1	–

* – potentially enterohemorrhagic strain, ** – epidemiologically significant serogroup, †, †† – isolates from the same sample, AMP – ampicillin, CMP – chloramphenicol, GEN – gentamicin, KAN – kanamycin, NAL – nalidixic acid, STR – streptomycin, SXT – sulfamethoxazole/trimethoprim, TET – tetracycline, TMP – trimethoprim.

Characterization of STEC isolates

The genes stx_1, stx_2 and the combination of both were present in 7.3 %, 85.4 % and 7.3 % of isolates, respectively. The isolates of the same origin (farms or slaughterhouses) and the same virulence profile collected in different time periods were recorded only twice during the course of the study (altogether 4 similar samples were obtained from pig carcasses in 2013). Therefore, persistent contamination can be assumed in these two farms and slaughterhouses, although subsequent analyses (e.g. pulsed field gel electrophoresis, repeated sampling) were not performed because of the low number of samples.

Detection of stx_1 gene

Gene stx_1 was detected in three samples (1 isolated from cattle carcasses and 2 from pig carcasses). Simultaneous presence of stx_1 and genes encoding aggregative adherence fimbriae *aaiC*, *aggR* was not found. An isolate from the cattle carcasses carried gene stx_{1a} and also gene *eaeA*. The latter encodes intimin, an outer membrane protein required for intimate attachment to the host intestinal mucosa [2]. The combination of detected virulence factors is typical of EHEC strains associated with severe disease in humans [4]. The obtained *E. coli* isolate did not belong to any of the 70 serogroups tested (including highly significant serogroups). Nevertheless, it is an important finding of a strain potentially pathogenic for humans.

One of the stx_1-positive isolates (of pig origin) belonged to serogroup O91, subtype 1a. Serogroup O146 with subtype 1d was also identified (Tab. 4). Both serogroups belong to the epidemiologically significant serogroups. However, the isolates without the presence of any other virulence factor (*eaeA*, *aaiC*, *aggR*) are not considered dangerous to human health [6].

Detection of stx_2 gene

The presence of stx_2 was confirmed in 35 out of the 41 obtained isolates. Simultaneous production of stx_2 gene and genes *aaiC*, *aggR* or *eaeA* was not confirmed.

Altogether, 24 stx_2-positive isolates were detected in swab samples from pig carcasses. All of these isolates revealed the subtype stx_{2e}. Serogroup O8 was identified in three isolates and serogroups O30, O112 and O156 were identified in three isolates, one in each. The rest of isolates did not belong to any of 70 serogroups tested. The results are summarized in Tab. 4. In accordance with our study, BOUVET et al. [30] detected the subtype stx_{2e} in 87 % of stx_2-positive isolates from pig car-

casses. The strains carrying stx_2 are potentially more virulent than those carrying stx_1 or even strains carrying both stx_1 and stx_2 genes [8]. Nevertheless, the stx_{2e} subtype is commonly associated with edema disease in pigs and only rarely found in human STEC infections [32]. Because this subtype has not been associated with diarrhoea or any severe illness [14, 32], strains carrying the stx_{2e} gene most likely do not present a health risk for humans.

The stx_2 gene was detected also in 11 isolates originating in cattle carcasses. Most of these isolates belonged to the stx_{2e} subtype (54.6 %). A specific serogroup was identified in two stx_{2e} isolates (O141 and O152, respectively). The stx_{2c} subtype and epidemiologically significant serogroup O113 were identified in one isolate simultaneously. The stx_{2a} subtype and O8 serogroup were present in one sample, and *E. coli* strain carrying stx_{2d} gene with an unknown serogroup was detected in our study. In two isolates, the subtype of stx_2 gene was not identified. One of these strains belonged to the epidemiologically significant serogroup O91 and the other carried O-antigen specific to serogroup O30. Both epidemiologically important serotypes O113 and O91 belong to atypical EHEC strains, which have a potential to cause HUS even though they lack the *eaeA* gene [33]. Nevertheless, these *eaeA*-negative EHEC strains usually have other putative adherence and virulence-associated factors, e.g. STEC agglutinating adhesin and subtilase cytotoxin [6], which were not investigated in our study.

Detection of both stx_1 and stx_2 genes

Both stx_1 and stx_2 genes were detected in two isolates from cattle and in one isolate from pig carcasses. Cattle isolates revealed the stx_{1a} and stx_{2a} subtypes and none of them belonged to any of the epidemiologically significant serogroups. Strains with the simultaneous presence of stx_1 and stx_2 genes are rare. However, the presence of any other virulence factor was not detected and, therefore, these strains are not considered as a threat to human health.

Simultaneous presence of stx_1, stx_2 and *eaeA* genes was detected in one isolate obtained from a pig carcass. Further typing of this isolate revealed the presence of stx_{1a} and stx_{2e} genes in association with serogroup O157. As was previously described, the O157:H7 serotype is most frequently associated with food and waterborne outbreaks and also with sporadic cases of the severe course of the disease accompanied by HUS [9, 11]. Although cattle is considered as the major reservoir of *E. coli* O157, contamination of pig carcasses

by *E. coli* O157 was detected also in Italy (0.7 %) [34] and in Ireland (0.2 %) [35]. The prevalence of *E. coli* O157 at slaughterhouses varies from very low to high levels in different countries [36-39]. These variations in the prevalence are mainly attributed to a range of factors such as sampling strategies, detection techniques, geographical location and animal management practices [40, 41]. Various routes of *E. coli* O157 infection in humans have been reported, these being consumption of contaminated meat, unpasteurized dairy products, fresh fruits and vegetables and also environment-mediated and direct transmission of the pathogen [42].

Resistance to antimicrobial agents

Fourteen (34.2 %) out of 41 STEC isolates from the swab samples were resistant to at least one antimicrobial agent and 5 of them (12.2 %) were resistant to three or more groups of antimicrobial agents (multi-resistant isolates). Most of the resistant isolates were recovered from the pig carcasses (12/14), while only 2 isolates from the cattle carcasses. All multi-resistant isolates originated from the pig carcasses. These results are in accordance with our earlier study in which retail meat was investigated. In that study, significantly higher numbers of multi-resistant *E. coli* isolates were obtained from pork compared to beef [43].

STEC isolates were most frequently resistant to tetracycline (11/14) and ampicillin (6/14). Resistance to other antimicrobial agents such as trimethoprim, sulfamethoxazole/trimethoprim, streptomycin, kanamycin, chloramphenicol, gentamicin or nalidixic acid was also recorded, mostly in multi-resistant isolates (Tab. 4).

In most of the tetracycline-resistant isolates (72.7 %), the gene *tet*(A) was detected. One isolate was positive for the presence of *tet*(B) gene while, in another isolate, the gene responsible for tetracycline resistance was not identified. Both *tet*(A) and *tet*(B) genes were reported by many authors as the most common genetic markers of resistance to tetracycline [44–46]. These genes encode an energy-dependent efflux pump system, one of the most frequently used mechanisms of tetracycline resistance in bacteria of the family *Enterobacteriaceae* [47, 48].

In five out of six ampicillin-resistant STEC isolates, the *bla*TEM gene, which encodes the production of β-lactamase enzymes and deactivates β-lactams [49, 50], was identified. In one ampicillin-resistant isolate, no gene responsible for the resistance to β-lactam antibiotics was detected.

Neither resistance to the third generation of cephalosporins (cefotaxime) nor resistance to fluoroquinolones (ciprofloxacin) was found in any isolate. Resistance to quinolones (nalidixic acid) was found in one isolate. Bacterial resistance to fluoroquinolones is conventionally attributed to chromosomally encoded mechanisms that allow the alteration of quinolone targets (quinolone resistance determining region, QRDR) [51]. In our study, we focused on the detection of plasmid-mediated quinolone resistance genes (PMQR) that are horizontally transferable, but none of the investigated genes (*qnrA*, *qnrB*, *qnrS*) was found.

CONCLUSION

Bovine faeces and hides have been established as major sources of Shiga toxin-producing *E. coli* presenting numerous opportunities for contamination and cross-contamination of meat during the slaughtering process [52]. A considerable collection of analysed samples enabled us to review STEC contamination rate of cattle and pig carcasses in Czech slaughterhouses. Results of our study showed that the prevalence of STEC in cattle (3.9 %) and pig (5.1 %) carcasses in the Czech Republic is not high and thus the risk of alimentary infection for the final consumers is comparatively low. Nevertheless, the presence of a potentially enterohemorrhagic strain was detected in three cases, namely, in two positive swabs originating from cattle and one from a pig carcass. The infection dose of these strains is very low, in the case of O157:O7 even fewer than 700 cells [53]. Moreover, we can assume possibility of enrichment of STEC-positive isolates in butcheries. Therefore, it is highly important to keep to hygienic rules at handling of raw meat. The screening of antimicrobial resistance showed that the presence of resistant isolates is higher in pigs compared to cattle. A third (34.2 %) of STEC isolates was resistant to at least one antimicrobial agent and 5 (12.2 %) isolates were multi-resistant, while resistance to tetracycline and ampicillin was detected most frequently. Our results indicate that the role of raw meat in the epidemiology of Shiga toxin-producing *E. coli* in the Czech Republic should not be underestimated.

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