OPEN ACCESS
Potravinarstvo Slovak Journal of Food Sciences
Crossref Similarity Check Powered by iThenticate

potravinárstvo®

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 12, 2018, no. 1, p. 304-312
doi: https://doi.org/10.5219/897
Received: 10 February 2018. Accepted: 16 March 2018.
Available online: 24 April 2018 at www.potravinarstvo.com

ISSN 1337-0960 (online)

CHARACTERIZATION OF ESCHERICHIA COLI STRAINS ISOLATED FROM RAW VEGETABLES

Magda Janalíková, Pavel Pleva, Silvie Pavlíčková, Martin Lecomte, Tanguy Godillon, Ivan Holko

ABSTRACT

Vegetables are an important part of the human diet. Sometimes, contamination by pathogenic *Escherichia coli* can be underestimated; moreover there is a risk of antibiotic resistance spreading via the food chain. The purpose of this study was to examine the prevalence of *Escherichia coli* in fresh vegetables sold in retail market in the Czech Republic and to evaluate the risk to human health. Antibiotic resistance against 12 antibiotics, the presence of 12 virulence and 15 resistance genes were determined among 15 isolated strains. Most of tested strains belonged to B1 phylogenetic group, less frequently represented was B2 and D phylogroup. These results indicate that most strains are probably of human origin. All *E. coli* strains were resistant to at least one of twelve tested antibiotics. A multidrug resistance was observed in four strains. In this study, the presence of virulence factors *Einv* and *papC* and also genes encoding toxins (*CNF1, CNF2*) was detected. Nevertheless, none strain can be considered as STEC or EHEC. The widespread appearance of a growing trend associated with the prevalence of antibiotic resistance among enterobacterial isolates is undeniable and the possibility of transfer to humans cannot be ignored. Nevertheless, these results indicate that raw vegetables sold in the retail market can constitute a potential health risk for consumers.

Keywords: vegetables, *Escherichia coli*, virulence, resistance, safety

INTRODUCTION

Escherichia coli, a common inhabitant of intestinal tract of humans and animals, has been used as an indicator of fecal contamination in food. Fecal pollution can indicate the presence of enterobacterial pathogens. *E. coli* can be easily disseminated in different ecosystems through the food chain **(Ryu et al., 2012)**. Raw vegetables can harbour many different pathogens including *Escherichia coli* and may become contaminated through animals, people, animal manures, and contaminated irrigation water **(Harapas et al. 2010)**.

Escherichia coli strains can be assigned to one of the main phylogenetic groups: A, B1, B2 or D. These phylogroups apparently differ in their ecological niches, life-history and some characteristics, such as their ability to exploit different sugar sources, and their antibiotic-resistance profiles **(Carlos et al., 2010)**. Strains from phylogroups B2 and D often contained more virulence factors than strains from the phylogroups A and B1. The extraintestinal pathogenic strains usually belong to groups B2 and D, the commensal strains to groups A and B1, whilst the intestinal pathogenic strains may belong to groups A, B1 and D. Fecal strains isolated from birds were assigned to groups D and B1; A and B1 were isolated from non-human mammals; and A and B2 were found in humans **(Carlos et al., 2010)**. **Clermont et al. (2005)** have developed a PCR based method to characterize the phylogroups using the genetic markers chuA, yjaA and the DNA fragment TspE4.C2.

The consumption of vegetables has risen over the last decades in many countries **(Franz and van Bruggen, 2008)**. Lettuce, radish and salads are the most common causes of *E. coli* outbreaks, where vegetable was proved as a source of infection all over the world **(ICMSF, 2005)**. A large epidemic caused by shigatoxigenic *E. coli* in spring 2011 in Germany resulted in reduction of trust in the health safety of raw vegetables and sprouted seeds. It has been found that the number of outbreaks of EHEC infections associated with the consumption of contaminated vegetables has increased over the last years **(Tzschoppe et al., 2012)**. STEC and EHEC have been isolated from animal faeces, soil, water, sewage and manure and can persist in the environment over long periods of time **(Fremaux et al., 2008)**. Vegetables can become contaminated with STEC and EHEC at all stages of food production. Unlike food of an animal origin, vegetables have rarely been examined as a possible source of human infections with EHEC **(Tzschoppe et al., 2012)**.

The broad group of *E. coli* known as enterohemorrhagic *E. coli* (EHEC), including *E. coli* O157:H7, contain the LEE (locus of enterocyte effacement) encoded intestinal colonisation mechanism (*eae* gene). The *Einv* gene is responsible for encoding enteroinvasive mechanisms. The *Eagg* gene is known for its encoding enteroaggregative

mechanisms **(Kaper and O'Brien, 1998)**. Cytonecrotic factors (CNF1, CNF2) are often produced by *E. coli* strains, which were isolated from intestinal and also extraintestinal infections of humans and animals. The *iss* (increased serum survival) gene and its protein product (ISS) of avian pathogenic *E. coli* (APEC) are important characteristics of resistance to the complement system. The *iss* gene is probably located in a conserved portion of some plasmids **(Buchanan and Doyle, 1997)**. The *papC* gene encodes fimbrial adhesin. Genes encoding temperature-sensitive haemagglutinin (*tsh*), aerobactin (*iucD*), and serum resistance protein (*iss*) are known for their frequent or exclusive localization on large transmissible R plasmids in APEC **(Buchanan and Doyle, 1997)** and thus these genes are clearly associated with APEC strains **(Yu and Kaper, 1992)**.

The major virulence factors of *E. coli* are Shiga toxins encoded by Shiga toxin genes *stx1* and *stx2*. The ability to produce Stx1 and/or Stx2 is a critical determinant of whether a bacterium can cause the clinical syndrome associated with STEC. Unlike O157:H7, most non-O157 STEC strain cannot be easily distinguished from non-pathogenic *E. coli* strains. The infection of non-O157 STEC in human beings is also considered to be underestimated **(Li et al., 2016)**.

Apart from Shiga-toxin, epidemic strains can produce hydrolysing enzymes known as extended spectrum β-lactamases (ESBLs) causing resistance to several antimicrobial agents. Strains possessing ESBLs, have emerged in the last few decades as a global risk for human health and have been shown to contribute to increased morbidity and mortality **(Said et al., 2015)**. Especially, when they also possess other resistance determinants represent these strains a growing public health threat **(Tzschoppe et al., 2012)**. The high use of antibiotics not only in human medicine, but also in veterinary medicine or even in agriculture, could constitute a selective pressure for the spread of antibiotic resistant bacteria, including ESBL-Eb **(Durso and Cook, 2014)**. ESBL-Eb could persist on the surface of plants or even reach their interior, and they could be transmitted to humans or animals. It is very important to highlight the potential role of consumption of uncooked vegetables in gastrointestinal bacteria acquisition **(Hamilton-Miller and Shah, 2001)**. Future studies should be carried out to evaluate the real risk for human health of this potential transmission linked to the consumption of vegetables **(Said et al., 2015)**.

Scientific hypothesis

The study was focused on the isolation of *E. coli* strains from fresh vegetables sold in the retail market in the Czech Republic. The main objective was to evaluate the presence of antibiotic resistance and virulence factors among these *E. coli* strains and determine a potential risk of fresh vegetables to human health.

MATERIAL AND METHODOLOGY

Samples and *E. coli* isolation

A total of 108 various fresh vegetable samples were bought in the Czech retail markets and investigated for the presence of *E. coli* during year 2015 and other 108 various fresh vegetable samples were investigated during 2016.

Ten grams of sample were homogenized in 90 ml of sterile saline solution, inoculated on the plates with the selective media for *E. coli* - Endo agar or Hi-chrome agar (Oxoid Ltd., United Kingdom) and incubated at 37 °C / 24 h.

Identification of strains

Suspected colonies were identified to the species level by using commercial identification microsystem ENTEROtest24 (ErbaLachema Brno, Czech Republic). This test is designed for routine, definitive identification of important strains of family Enterobacteriaceae. The kit contains 24 biochemical tests (dehydrated substrates for biochemical reactions, e.g, IND, ONP), which are placed in microplate. Obtained data were evaluated by software TNW Lite 6.5 (ErbaLachema Brno, Czech Republic).

Bacterial isolates were cultivated on Nutrient Agar (beef extract 10 g, peptone 10 g, agar 15 g, NaCl 5 g, distilled water 1000 mL, pH 7,2) or on Mueller Hinton Agar. Subsequently, the strains were stored at −80°C in glycerol.

Phylogenetic group determination

A previously described multiplex PCR method was used for determination of phylogenetic groups according to presence of three genes (chuA, yjaA, TspE4.C2) **(Clermont et al. 2000)**. A few colonies of each strain were solved in 100 µL of 1x PCR buffer (ThermoPol Buffer, NEB, USA) and heated up to 95°C for 20 min. Supernatant after centrifugation (10.000 g/L min) was taken as DNA template for all PCR testing. The amplification products were visualized by 1% agarose gel electrophoresis and strains were assigned to the phylogenetic groups **(Carlos et al., 2010)**.

Antibiotic resistance determination

Antimicrobial susceptibility testing to twelve antibiotics was performed by disc diffusion method according to EUCAST methodology **(Matuschek et al., 2014)**. Antibiotic discs (Oxoid Ltd., United Kingdom): amoxicillin/clavulanic acid (AMC 30 µg), ampicillin (AMP 10µg), ceftazidime (CAZ 10µg), cephalotine (CEF 30µg), ciprofloxacin (CIP 5 µg), doxycycline (D 10 µg), chloramphenicol (C 30 µg), gentamicine (CN 10 µg), sulbactam/cefoperazone (SCF 10 µg), streptomycine (S 10µg), sulfamethoxazole/trimethoprim (SXT 25 µg), and tetracycline (T 10 µg) were used. The diameters of the inhibition zones were evaluated (susceptible, intermediate, resistant) according to EUCAST breakpoints.

Detection of virulence and resistance genes

Ten virulence genes (*stx1, stx2, tsh, CNF1, CNF2, papC, neuC, iss, iucD, EinV, Eagg* and *eaeA*) were assessed using PCR as was described previously **(Holko et al., 2006; Ewers et al., 2007)**. The primer sequences of virulence genes, the expected size of their products, and the cycling conditions for the determination of the presence of selected ten genes are listed in Table 1.

The genes encoding antibiotic resistance: *qnrS, tet(A), tet(B), sul1, sul3 qac, merA, qepA, int*, and the genes responsible for the ESBL phenotype (*blaTEM, blaSHV, blaOXA-1, blaOXA-7, blaPSE-4*, and *blaCTX-M-3*) were identified by polymerase chain reaction (PCR). The cycling conditions varied according to the specific gene determination **(Table 1)**.

Table 1 Oligonucleotides sequences of primers used and PCR conditions for detection of virulence and resistance genes in *E. coli* strains.

Description	Virulence gene	Oligonucleotide sequence (5´- 3´)	Annealing temperature (°C)	Size of amplified product (bp)	Reference
temperature-sensitive haemagglutinin	*tsh*	ACTATTCTCTGCAGGAAGTC CTTCCGATGTTCTGAACGT	58	824	Ewers et al. 2007
cytotoxic necrotising factor	*CNF1*	GGCGACAAATGCAGTATTGCT GACGTTGGTTGCGGTAATTTT	63	552	Holko et al. 2006
cytotoxic necrotising factor	*CNF2*	GTGAGGCTCAACGAGATTATG CCACGCTTCTTCTTCAGTTGT	63	839	
shiga toxin 1	*stx1*	TTTCCCCTCTTTTAGTCAGTCAACTG GGCAGGATTACAACAAAGTTCACAG	63	160	Holko et al. 2006
shiga toxin 2	*stx2*	CCCCCTCTCTTTTGCACTTCTTTCC TGCTCCAGCAGTACCATCTCTAACCC	63	423	
pilus associated with pyelonephritis	*papC*	TGATATCACGCAGTCAGTAGC CCGGCCATATTCACATAAC	58	501	
K1 capsular polysaccharide	*neuC*	GGTGGTACATTCCGGGATGTC AGGTGAAAAGCCTGGTAGTGT	58	670	Ewers et al. 2007
increased serum survival	*iss*	ATCACATAGGATTCTGCCG CAGCGGAGTATAGATCCCA	58	309	
aerobactin siderophore synthesis	*iucD*	ACAAAAAGTTCTATCGCTTCC CCTGATCCAGATGATCCTC	58	714	
enteroinvasive mechanisms	*EinV*	TTCTGATGCCTGATGGACCAG TGGAAAAACTGAGTGCCTCTG	63	140	
enteroaggregative mechanisms	*Eagg*	AGACTCTGGCGAAAGACTGTA ATGGCTGTCTGTAATAGATGA	63	194	Holko et al. 2006
intimin	*eaeA*	TGAGCGGCTGGCATGAGTCAT TCGATCCCCATCGTCAACAGA	63	241	
resistance genes					
Beta-lactams	bla_{TEM}	GAGTATTCAACATTTTCGT ACCAATGCTTAATCAGTGA	50	857	Maynard et al. 2004
	bla_{SHV}	TCGCCTGTGTATTATCTCCC CGCAGATAAATCACCACAATG	50	768	
	bla_{OXA-1}	GCAGCGCCAGTGCATCAAC CCGCATCAAATGCCATAAGTG	50	198	
	bla_{OXA-7}	AGTTCTCTGCCGAAGCC TCTCAACCCAACCAACCC	50	591	
	bla_{PSE-4}	CTGCTCGTATAGGTGTTTCC TCGCATCATTTCGCTCTTC	50	705	
	$bla_{CTX-M-3}$	AATCACTGCGTCAGTTCAC TTTATCCCCCACAACCCAG	50	701	

Table 1 (Continue).

Resistance genes					
plasmid-mediated quinolone resistance	*qnrS*	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	58	600	Cattoir et. al 2007
tetracycline	*tet*(A)	GGCCTCAATTTCCTGACG AAGCAGGATGTAGCCTGTGC	55	372	Guillaume et al. 2000
	tet(B)	GAGACGCAATCGAATTCGG TTTAGTGGCTATTCTTCCTGCC	55	228	
sulphonamides	*sul*1	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	63	433	Perreten, Boerlin, 2003
	*sul*3	GAGCAAGATTTTTGGAATCG CATCTGCAGCTAACCTAGGGCTTTG GA	55	880	Kerrn, 2002
quarternary ammonium compounds	*qac*	GCCCCTTCCGCCGTTGTCATAATC CGGCCTCCGCAGCGACTTCC	63	250	Johnson et al. 2012
mercury resistant genes	*merA*	GATCCGCGCCGCCCATATCGCCCAT CTG CACGCGCTCGCCGCCGTCGTTGAGT TG	60	250	
plasmid-mediated gene responsible for reduced fluoroquinolones	*qep*	GCAGGTCCAGCAGCGGGTAG CTTCCTGCCCGAGTATCGTG	60	199	Yamane et al. 2008
integron mediated antibiotic resistance	*int*	GGGTCAAGGATCTGGATTTCG	60	483	Mazel et al. 2000

Statistical analysis

The results of antibiotic resistance and virulence factors were evaluated by Pearson's correlation coefficient between the measured variables. The analysis was performed using statistical software STATISTICA CZ (StatSoft, Inc. 2007).

RESULTS AND DISCUSSION

The prevalence of *Escherichia coli* in various raw vegetables bought in Zlín region (Czech Republic) is shown in Table 2. *E. coli* was detected in 15 out of 108 samples bought in 2015 (13.9%). In contrast, among 108 fresh vegetable samples bought in 2016, there was found no *Escherichia coli* strain. Thus, the prevalence of *E. coli* strains in vegetable during two years is 15 out of 216 (6,9%). In 2013, the incidence of *E. coli* in raw vegetable in the Czech Republic was 26.4% **(Skočková et al., 2013)**. This study proves a decreasing trend in the occurrence of *E. coli* in retail market in the Czech Republic. In comparison, data from Canada reported 8.2% of *E. coli* recovered from fresh produce (lettuce, spinach, carrots, and green onions), which represents low levels of enteric pathogen contamination in vegetables sold in North America **(Bohaychuk et al., 2009)**. On the other hand, a total of 90 samples of raw salad vegetables (parsley, lettuce, radish) were collected in Lebanon and *E. coli* was present in almost half (45.5%) of samples **(Faour-Klingbeil et al., 2016)**.

All positive samples in this study came from European countries (Table 2). The most commonly contaminated vegetable samples were mung sprouts (4/12), radish (2/7), tomatoes (2/10), and spring onion (2/4). The occurrence of *E. coli* in the other types of vegetable was less frequent. **Skočková et al. (2013)** indicated leafy vegetable and sprouted seeds as the most common sources of *E. coli.*

Mukherjee et al. (2004) who analysed fresh fruits and vegetables produced by organic and conventional farmers in Minnesota have shown *E. coli* prevalence rates of 1.6 and 9.7%, respectively. **Tzschoppe et al. (2011)** have detected *E. coli* in five (12.5%) of 40 salad and sprouted seed samples. Lower prevalence of *E. coli* may also be a result of a different methodology of detection with no enrichment **(Skočková et al., 2013)**.

Phylogenetic groups

In this study, the most *E. coli* strains (73.3%) belonged to B2 phylogroup, much less of them was assigned into B1 (13.3%) and D (13.3%) phylogroup. None of the strains can be determined as A phylogroup. It is known **(Carlos et al., 2010)** that B2 phylogroup *E. coli* strains are highly probably human extraintestinal pathogenic strains. On the other hand, *E. coli* strains isolated from chicken meat mostly belong to A, less to B1 phylogroup **(Pavlíčková et al., 2015)**. This study is also in accordance with the statement that strains from phylogroups B2 and D contained more virulence factors than strains from the phylogroups A and B1 **(Carlos et al., 2010)**.

Antibiotic resistance

All 15 strains (100.0%) isolated from raw vegetables in this work were resistant to at least one antibiotic, even more 4 of these strains (26.7%) were multiresistant (3 or more). The most frequent antibiotic resistance is ampicillin resistance (93.3%) and resistance to cephalotine (53.3%) and ceftazidime (40.0%). Also, resistance against gentamicine (13.3%) and streptomycine (6.7%) were

Table 2 Prevalence of *E. coli* in raw vegetable samples sold in retail market.

Vegetable	Country of origin	Number of samples positive for *E. coli* /number of samples
mung sprouts	Czech Republic, Italy	4/12
lentils sprouts	Czech Republic	0/1
alfalfa sprouts	Czech Republic	0/1
broccoli	Spain	0/4
strawberry	Spain	0/4
carrot	Czech Republic	1/4
zucchini	Spain	1/3
spinach	Italy, Spain	0/3
ice lettuce	Italy	0/3
cucumber	Slovakia	0/6
mix of lettuces	Hungary, Italy	0/9
tomato	Morocco	2/10
eggplant	Italy	0/3
chickpeas sprouts	Italy	0/1
baby carrot	Czech Republic, Netherlands	1/3
leek	Czech Republic	0/1
radish	Czech Republic, Italy	2/7
spring onion	Germany	2/4
pepper	Poland	0/2
lettuce little gem	Netherlands	1/2
rucola	Italy	0/3
lettuce	Czech Republic	0/1
lamb's lettuce	Italy	0/1
wallflower	Netherlands	0/1
celery	Spain	0/1
champignon	Poland	0/1
parsley	Czech Republic	1/2
avocado	unknown	0/1
beet	Czech Republic	0/1
turnip	unknown	0/1
cauliflower	Czech Republic	0/1
green beans	unknown	0/1
potato	Czech Republic	0/1

present. High prevalence of resistance to aminopenicillins is also proved by presence of *bla* genes (**Table 3**).

ESBL-Eb that contaminates vegetables can be transmitted to human consumers via the food chain. In the work of **Said et al. (2015)**, ESBL-Eb were detected in 4 of 45 vegetable samples of market origin tested and in 3 of 13 markets. ESBL-Eb positive isolates were also detected among vegetables samples in the Netherlands (**Reuland et al., 2013**).

Fluoroquinolone antibiotics inhibit two bacterial enzymes, DNA gyrase and topoisomerase IV, both of which play essential roles in DNA replication. Resistance to quinolone is often linked to amino acid substitutions in the quinolone-resistance-determining regions of DNA gyrase and DNA topoisomerase IV subunits, leading to target modification (**Rezazadeh et al., 2016**). However, recent reports indicate that quinolone resistance can also be mediated by mobile genetic elements such as plasmids.

Table 3 Characterization of *E. coli* strains isolated from fresh vegetable.

Isolate	Source	Country of origin	Resistance phenotype	Resistance genes	β-lactamase genes	Virulence genes	PG
F10	mung sprouts	Italy	CAZ, AMP	-	*blaOXA-1*	*EinV*	B2
F52	mung sprouts	Czech Republic	S, CEF, AMP	*qac*	*blaTEM, blaSHV, blaOXA-1, blaPSE-4, blaCTX-M-3*	*CNF1, EinV*	B2
F53	mung sprouts	Czech Republic	CEF, AMP	*qac*	*blaTEM, blaOXA-1*	*papC, EinV*	D
F77	spring onion	Czech Republic	CAZ, AMP	*sul1*	*blaTEM*	*EinV*	B2
F78	tomato	Morocco	CAZ, AMP	-	-	*papC, EinV*	B2
F81	lettuce little gem	Italy	AMP	*tetA, int, sul1, sul3, mer*	*blaTEM*	*EinV*	B1
F84	baby carrot	Netherlands	CEF, AMP	*qnrS, qac*	*blaTEM, blaSHV, blaOXA-1, blaCTX-M-3*	*papC, CNF1, EinV*	B2
F87	mung sprouts	Czech Republic	CAZ, CEF, AMP	*qnrS, qac, mer*	*blaTEM, blaSHV, blaOXA-1, blaPSE-4, blaCTX-M-3*	*EinV*	B2
F94	tomato	Czech Republic	AMP	*sul1*	-	*CNF1, EinV*	B2
F103	parsley	Czech Republic	AMP	*qnrS, qac*	*blaOXA-1*	*EinV*	B2
F104	radish	Czech Republic	CAZ, CEF, AMP	*qnrS, qac*	*blaOXA-1*	*papC, EinV*	B2
F105	carrot	Czech Republic	AMP	*tetB*	*blaTEM, blaCTX-M-3*	*EinV*	B2
F106	radish	Czech Republic	CEF, CN	*qac*	*blaOXA-1*	*papC, CNF2, EinV*	D
F107	spring onion	Czech Republic	CAZ, CEF, AMP, CN	*tetB*	*blaTEM*	*EinV*	B1
F108	zucchini	Spain	CEF, AMP	*qac*	*blaTEM, blaSHV, blaOXA-1, blaPSE-4, blaCTX-M-3*	*papC, CNF2*	B2

Note: AMP – ampicillin; CAZ – ceftazidime; CEF – cephalotine; CN – gentamycine; S – streptomycine; PG – phylogroup.

Plasmid-mediated quinolone resistance is mediated by the genes (*qnr*) encoding proteins that protect DNA gyrase and topoisomerase IV against quinolone compounds. The three major groups of *qnr* determinants are *qnrA, qnrB*, and *qnrS*.

The first plasmid-mediated quinolone-resistance gene (*qnrA*) was identified in a clinical strain of *Klebsiella pneumoniae* isolated in Alabama in 1998. The other two determinants of qnr (qnrB and qnrS) have subsequently been observed in other enterobacteria including *E. coli*, *Enterobacter*, *Salmonella*, and *Klebsiella pneumoniae* **(Rezazadeh et al., 2016)**.

It is of interest to remark that vegetables containing ESBL-Eb of market origin are commonly eaten uncooked, and the possibility of transfer to humans cannot be ignored. Different authors also indicate the potential problems derived from these uncooked food samples containing antibiotic resistant bacteria (**Hamilton-Miller and Shah, 2001; Veldman et al., 2014)**. In fact, foodborne outbreaks due to ESBL-positive *E. coli* isolates related to vegetables have been previously reported **(Reuland et al., 2013)**. Obviously, the widespread appearance of a growing trend associated with the prevalence of antibiotic resistance among enterobacterial isolates is undeniable.

Detection of virulence factors

In this study, 15 out of 15 isolates (100.0%) possessed some virulence factor and 2 of these strains (13.3%) were even multivirulent (more than 3 factors). The most frequently found virulence factors were *Einv* gene (93.3%) and *papC* (40.0%). The *Einv* gene is responsible for encoding enteroinvasive mechanisms (EIEC) and *papC* gene encodes an adhesin. Presence of these two factors much more resemble origin in wildlife **(Pavlíčkova et al., 2017)** than in e.g. poultry meat **(Pavlíčková et al., 2015)**. Moreover, five strains were positive for cytotoxic necrotizing factors *CNF* toxins (20.0% *CNF1*; 13.3%

CNF2). These results may represent potential threat to human health. CNF1 is a major virulence factor of UPEC strains and it was found in bacteria isolated from meningitis affected children. Moreover, CNF1 is produced in some extraintestinal *E. coli* (ExPEC). There are hints that CNF1 may be involved in cancer development: CNF1 induces the expression of cyclooxigenase-2 (COX-2), activates nuclear factor-kappa B (NF-κB), increases cell motility and inhibits apoptosis. CNF2 has been demonstrated in *E. coli* isolated in calves and lambs with diarrhoea **(Knust and Schmidt, 2010)**.

Plasmid-mediated resistance is of growing clinical concern as they may transfer resistance genes to other species via horizontal gene transfer. Resistance of *eaeA*-positive STECs to fluoroquinolones constitute health threat to consumers, where resistance determinants can spread among non-pathogenic bacteria in the gastrointestinal tract due to plasmid mobility **(Khalil and Gomaa, 2016)**. Moreover, the simultaneous presence of extended-spectrum beta-lactamases (ESBLs), AmpC, and *qnr* genes on the same plasmid has been well documented and this highlights the complexity of determinants involved in plasmid-mediated resistance among the enterobacterial isolates **(Rezazadeh et al., 2016)**. It can be observed that at least four strains isolated in this study represent plasmid mediated resistance **(Table 3)**. Fortunately, neither *eaeA*-positive strains (EHEC) nor STEC strains were proved among *E. coli* strains found in the Czech raw vegetable, thus it can be summarised that vegetables sold in the Czech Republic are not enteropathogenic/shigatoxigenic.

In this work, statistically significant correlation (p <0.05) was not proved between presence of antibiotic resistance and virulence factors.

CONCLUSION

It can be concluded that presence of *Escherichia coli* on raw vegetables sold in retail market in the Czech Republic is decreasing. In 2015 it was found 13.9% positive samples and a year later there were no *E. coli* strain among 108 samples. It was observed that all 15 *E. coli* isolates were resistatnt at least to one antibiotic, especially against aminopenicillins and cephalosporines. Four strains carry resistance to three or more antibiotics. Furthermore, all strains also encode at least one of virulence factors – *Einv, papC* and even *CNF1/CNF2*, which are toxins and may represent pathogenic bacteria. In contrast, neither STEC nor EHEC were determined. There exists a possibility of antibiotic resistance transfer from enterobacteria to humans and it should not be ignored, as well as there is a small hazard of intestinal infection. These results indicate that raw vegetables sold in the retail market can represent a potential health risk for consumers.

REFERENCES

Bohaychuk, V. M., Bradbury, R. W., Dimock, R., Fehr, R. M., Gensler, G. E., King, R. K., Rieve, R., Romero Barrios, P. 2009. A Microbiological Survey of Selected Alberta-Grown Fresh Produce from Farmers' Markets in Alberta, Canada. *Journal of Food Protection*, vol. 72, no. 2, p. 415-420. https://doi.org/10.4315/0362-028X-72.2.415 PMid:19350990

Buchanan, R. L., Doyle, M. P. 1997. Foodborne Disease Significance of *Escherichia coli* O:157 and Other Enterohemorrhagic *E. coli*. *Food Technology*, vol. 51, no. 10, p. 69-76.

Carlos, C., Pires, M. M., Stoppe, N. C. C., Hachich, E. M., Sato, M. I., Gomes, T. A. T., Amaral, L. A., Ottoboni, L. M. M. 2010. Escherichia coli phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. *BMC Microbiology*, vol. 10, no. 1, p.161. https://doi.org/10.1186/1471-2180-10-161 PMid:20515490

Cattoir, V., Poirel, L., Rotimi, V., Soussy, C. J., Nordmann, P. 2007. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *Journal of Antimicrobial Chemotherapy*, vol. 60, no. 2, p. 394-397. https://doi.org/10.1093/jac/dkm204 PMid:17561500

Clermont, O., Bonacorsi, S., Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. 2000. *Applied Environmental Microbiology,* vol. 66, no. 10, p. 4555-4558. https://doi.org/10.1128/AEM.66.10.4555-4558.2000 PMid:11010916

Durso, L. M., Cook, K. L. 2014. Impacts of antibiotic use in agriculture: what are the benefits and risks? *Current Opinion in Microbiology*, vol. 19, p. 37-44. https://doi.org/10.1016/j.mib.2014.05.019 PMid:24997398

Ewers, C., Li, G., Wilking, H., et al. 2007. Avian pathogenic, uropathogenic, and newborn meningitis-causing Escherichia coli: How closely related are they? *International Journal of Medical Microbiology*, vol. 297, no. 3, p. 163-176. https://doi.org/10.1016/j.ijmm.2007.01.003 PMid:17374506

Faour-Klingbeil, D., Murtada, M., Kuri, V., Todd, E. C. D. 2016. Understanding the routes of contamination of ready-to-eat vegetables in the Middle East. *Food Control*, vol. 62, p. 125-133. https://doi.org/10.1016/j.foodcont.2015.10.024

Franz, E., Van Bruggen, A. H. C. 2008. Ecology of *E. coli* O157:H7 and Salmonella enterica in the Primary Vegetable Production Chain. *Critical Reviews in Microbiology*, vol. 34, no. 3-4. https://doi.org/10.1080/10408410802357432 PMid:18728991

Fremaux, B. C., Prigent-Combaret, C., Vernozy-Rozand, C. 2008. Long-term survival of Shiga toxin-producing Escherichia coli in cattle effluents and environment: An updated review. *Veterinary Microbiology*, vol. 132, no. 1-2, p. 1-18. https://doi.org/10.1016/j.vetmic.2008.05.015 PMid:18586416

Guillaume, G. 2000. PCR typing of tetracycline resistance determinants (Tet A–E) in Salmonella enterica serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. *FEMS Microbiology Ecology,* vol. 32, no. 1, p. 77-85. PMid:10779622

Hamilton-Miller, J. M., Shah, S. 2001. Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables. *Int. J. Antimicrob. Agents,* vol. 18, no. 1, p. 81-83. https://doi.org/10.1016/S0924-8579(01)00353-3

Harapas, D., Premier, R., Tomkins, B., Franz, P., Ajlouni, S. 2010. Persistence of Escherichia coli on injured vegetable plants. *International Journal of Food Microbiology*, vol. 138,

no. 3, p. 232-237. https://doi.org/10.1016/j.ijfoodmicro.2010.01.022

Holko, I., Bisova, T., Holkova, Z., Kmet, V. 2006. Virulence markers of Escherichia coli strains isolated from traditional cheeses made from unpasteurised sheep milk in Slovakia. *Food Control*, vol. 17, no. 5, p. 393-396. https://doi.org/10.1016/j.foodcont.2005.01.007

ICMSF. 2005. *Microbial ecology of food commodities*. 2nd ed. NEW YORK : Kluwer Academic/Plenum Publishers, c2005. ISBN 0-306-48675-x.

Johnson, T. J., Logue, C. M., Johnson, J. R., et al. 2012. Associations between multidrug resistance, plasmid content, and virulence potential among extraintestinal pathogenic and commensal Escherichia coli from humans and poultry. *Foodborne Pathogens and Disease*, vol. 9, no. 1, p. 37-46. https://doi.org/10.1089/fpd.2011.0961 PMid:21988401

Kaper, J. B., O'Brien, A. D. 1998. *Escherichia coli O157:H7 and other shiga toxin-producing E. coli strains*. WASHINGTON, DC : ASM Press, xxii, 465 p. ISBN 1555811299.

Kerrn, M. B. 2002. Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. *Journal of Antimicrobial Chemotherapy,* vol. 50, no. 4, p. 513-516. https://doi.org/10.1093/jac/dkf164 PMid:12356795

Khalil, R. K. S., Gomaa, M. A. E. 2016. Prevalence and characterization of Shiga toxin-producing Escherichia coli (STEC) in fruits and vegetables sold at local street markets in Alexandria, Egypt. *LWT - Food Science and Technology*, vol. 74, p. 199-210. https://doi.org/10.1016/j.lwt.2016.07.041

Knust, Z., Schmidt, G. 2010. Cytotoxic Necrotizing Factors (CNFs)−A Growing Toxin Family. *Toxins*. vol. 2, no. 12, p. 116-127. https://doi.org/10.3390/toxins2010116 PMid:22069550

Matuschek, E., Brown, D. F. J., Kahlmeter, D. 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection*, vol. 20, no. 4, p. O255-O266. https://doi.org/10.1111/1469-0691.12373 PMid:24131428

Maynard, C., Bekal, S., Sanschgrin, F., LEVESQUE, R. Brousseau, R. C., Masson, L., Lariviere, S., Harel, J. 2004. Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal Escherichia coli isolates of animal and human origin. *Journal of Clinical Microbiology,* vol. 42, no. 12, p. 5444-5452. https://doi.org/10.1128/JCM.42.12.5444-5452.2004 PMid:15583263

Mazel, D., Dychinco, B., Webb, V. A., Davies, J. 2000. Antibiotic resistance in the ECOR collection: Integrons and identification of a novel aad gene. *Antimicrobial Agents and Chemotherapy*, vol. 44, no. 6, p. 1568-1574. https://doi.org/10.1128/AAC.44.6.1568-1574.2000 PMid:10817710

Mukherjee, A., Speh, D., Dyck, E., Diez-Gonzalez, F. 2004. Preharvest evaluation of coliforms, Escherichia coli, Salmonella, and Escherichia coli O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.,* vol. 67, no. 5, p. 894-900. https://doi.org/10.4315/0362-028X-67.5.894 PMid:15151224

Pavlíčková, S., Doležalová, M., Holko, I. 2015. Resistance and virulence factors of Escherichia coli isolated from chicken. *Journal of Environmental Science and Health, Part B* vol. 50, no. 6, p. 417-421. https://doi.org/10.1080/03601234.2015.1011959 PMid:25844863

Pavlíčková, S., Klancik, A., Doležalová, M., Smole Možina, S., Holko, I. 2017. Antibiotic resistance, virulence factors and biofilm formation ability in Escherichia coli strains isolated from chicken meat and wildlife in the Czech Republic. *Journal of Environmental Science and Health, Part B*, vol. 52, no. 8, p. 570-576. https://doi.org/10.1080/03601234.2017.1318637 PMid:28494209

Perreten, V., Boerlin, P. 2003. A New Sulfonamide Resistance Gene (sul3) in Escherichia coli Is Widespread in the Pig Population of Switzerland. *Antimicrobial Agents and Chemotherapy*, vol. 47, no. 3, p. 1169-1172. https://doi.org/10.1128/AAC.47.3.1169-1172.2003 PMid:12604565

Reuland, E. A., Overdevest, I. T., Al Naiemi, N., et al. 2013. High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch community patients with gastrointestinal complaints. *Clinical Microbiology and Infection*, vol. 19, no. 6, p. 542-549. https://doi.org/10.1111/j.1469-0691.2012.03947.x PMid:22757622

Rezazadeh, M., Baghchesaraei, H., Peymani, A. 2016. Plasmid-mediated quinolone-resistance (qnr) genes in clinical isolates of Escherichia coli collected from several hospitals of Qazvin and Zanjan provinces, Iran. *Osong Public Health and Research Perspectives*, vol. 7, no. 5, p. 307-312. https://doi.org/10.1016/j.phrp.2016.08.003

Ryu, S. H., Park, S. G., Choi, S. M., et al. 2012. Antimicrobial resistance and resistance genes in Escherichia coli strains isolated from commercial fish and seafood. International *Journal of Food Microbiology*, vol. 152, no. 1-2, p.14-18. https://doi.org/10.1016/j.ijfoodmicro.2011.10.003 PMid:22071288

Skočková, A., Karpíšková, R., Koláčková, I., Cupáková, Š. 2013. Characteristics of Escherichia coli from raw vegetables at a retail market in the Czech Republic. *International Journal of Food Microbiology*, vol. 167, no. 2, p. 196-201. https://doi.org/10.1016/j.ijfoodmicro.2013.09.011 PMid:24135675

Tzschoppe, M., Martin, A., Beutin, L. 2012. A rapid procedure for the detection and isolation of enterohaemorrhagic Escherichia coli (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104: H4 strain from ready-to-eat vegetables. *International Journal of Food Microbiology*, vol. 152, no. 1-2, p. 19-30. https://doi.org/10.1016/j.ijfoodmicro.2011.10.009 PMid:22071287

Veldman, K., Kant, A., Dierikx, C., Van Essen-Zandbergen, A., Wit, B., Mevius, D. 2014. Enterobacteriaceae resistant to third-generation cephalosporins and quinolones in fresh culinary herbs imported from Southeast Asia. *International Journal of Food Microbiology*, vol. 177, p. 72-77. https://doi.org/10.1016/j.ijfoodmicro.2014.02.014 PMid:24607424

Yamane, K., Wachino, J., Suzuki, S., Arakawa, Y. 2008. Plasmid-mediated qepA gene among Escherichia coli clinical isolates from Japan. *Antimicrobial Agents and*

Chemotherapy, vol. 52, no. 4, p. 1564-1566. https://doi.org/10.1128/AAC.01137-07

Yu, J., Kaper, J. B. 1992. Cloning and characterization of the eae gene of enterohaemorrhagic *Escherichia coli* O157: H7. *Molecular Microbiology*, vol. 6, no. 3, p. 411-417. https://doi.org/10.1111/j.1365-2958.1992.tb01484.x

Contact addresses:

Magda Janalíková, Department of Environmental Protection Engineering, Faculty of Technology, Tomas Bata University in Zlín, Vavrečkova 275, 760 01 Zlín, Czech Republic, corresponding author, Tel.: 00420 576 031 020; E-mail: mdolezalova@ft.utb.cz

Pavel Pleva, Department of Environmental Protection Engineering, Faculty of Technology, Tomas Bata University in Zlín, Vavrečkova 275, 760 01 Zlín, Czech Republic, Tel.: 00420 576 031 209; E-mail: ppleva@utb.cz

Silvie Pavlíčková, Department of Environmental Protection Engineering, Faculty of Technology, Tomas Bata University in Zlín, Vavrečkova 275, 760 01 Zlín, Czech Republic; E-mail: silvie.pavlickova@seznam.cz

Martin Lecomte, Institute Universitaire de Technologie, Université d'Angers, 40, rue de Rennes - BP 73532, 49035 - Angers cedex 01, France; E-mail: martin.lcmt@gmail.com

Tanguy Godillon, Institute Universitaire de Technologie, Université d'Angers, 40, rue de Rennes - BP 73532, 49035 - Angers cedex 01, France; E-mail: tanguygodillon79@live.fr

Ivan Holko, Vetservis s.r.o., Kalvária 715/3, 949 01 Nitra, Slovak Republic; Tel.: 00421 376 519 742, E-mail: ivan.holko@gmail.com