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Molecular Characterization of *Escherichia coli* Strains Isolated from Different Food Sources

Marija Trkov[1], *Tatjana Rupel*[2], *Darja Žgur-Bertok*[3], *Sara Trontelj*[3], *Gorazd Avguštin*[4] *and Jerneja Ambrožič Avguštin*[3]*

[1]National Institute of Public Health of the Republic of Slovenia, Center for Laboratory Activities, Department of Medical Microbiology, Trubarjeva 2, SI-1000 Ljubljana, Slovenia

[2]Institute of Public Health Maribor, Department of Sanitary Microbiology, Unit Ljubljana, Prvomajska 1, SI-2000 Maribor, Slovenija

[3]Department of Biology, Biotehnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia

[4]Department of Animal Science, Biotehnical Faculty, University of Ljubljana, Groblje 3, SI-1230 Domžale, Slovenia

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Summary

Since food represents a possible source of pathogenic and antibiotic-resistant *Escherichia coli* strains, we analyzed 84 isolates from food samples identified in 2007 and 2008 at the National Institute of Public Health in Slovenia. Using polymerase chain reaction (PCR), the isolates were classified into phylogenetic groups and subgroups following the Clermont method. Forty-two (50 %) and thirty (35.7 %) isolates were classified into commensal gut phylogenetic groups A and B1, respectively. Only ten (11.9 %) and two (2.4 %) isolates were assigned to the phylogenetic groups D and B2, which include mainly extraintestinal pathogenic *E. coli* strains. The strains were further analyzed for the presence of various virulence genes and plasmid-mediated quinolone resistance *qnr* genes. Virulence genes *stx1*, *stx2*, both *stx1* and *stx2*, *ehxA* and *eae* associated with Shiga-toxin-producing *E. coli* were detected in one (1.2 %), five (6 %), five (6 %), eight (9.5 %) and three (3.7 %) isolates, respectively. Seventy-four (88.1 %) isolates carried the gene *fimH*, whereas virulence genes characteristic of extraintestinal pathogenic *E. coli*, *hra*, $ompT_{APEC}$ and *iha*, were detected in nine (11 %), eight (9.5 %) and six (7 %) isolates, respectively. Genes *kpsMT*II, *sfa*, *usp* and *vat* were discovered in single isolates, whereas *hlyA*, *bmaE*, *cnf*, *hbp* and *sat*, as well as plasmid-mediated quinolone resistance genes *qnr*, were not detected in the analyzed strains. Our results show that various food items are indeed a source of intestinal and, albeit to a lesser extent, of extraintestinal pathogenic *E. coli* strains.

Key words: *Escherichia coli*, food, virulence genes, phylogenetic groups, plasmid-mediated quinolone resistance genes

Introduction

Escherichia coli represents part of the intestinal microbiota in animals and humans. The majority of the gut-inhabiting *E. coli* strains are commensal microorganisms. However, strains equipped with virulence genes can cause various intestinal and extraintestinal diseases. Intestinal pathotypes are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent

*Corresponding author: Phone: +386 1 320 3391; Fax: +386 1 257 3390; E-mail: Jerneja.Ambrozic@bf.uni-lj.si

E. coli (DAEC) and Shiga-toxin-producing *E. coli* (STEC). The last may also be referred to as verocytotoxin-producing *E. coli* (VTEC) or enterohaemorrhagic *E. coli* (EHEC) (*1–3*). Strains of these pathotypes, except for STEC, are associated with mild to severe diarrhoea and colitis. STEC strains, on the other hand, cause a spectrum of human diseases, including bloody diarrhoea, haemorrhagic colitis (HC) and, the most severe, haemolytic uremic syndrome (HUS) (*4*). The main virulence genes contributing to this infection are *stx1* (Shiga toxin type 1), *stx2* (Shiga toxin type 2), *eae* (intimin), and *ehxA* (enterohaemolysin). Cattle are likely the main reservoir of STEC, including the serotype O157:H7, resulting in zoonotic transmission *via* the food chain (*5*). The major sources from which isolation of these strains is most successful are raw or undercooked meat, meat products and unpasteurized milk (*6*). Major STEC outbreaks in the last 10 years are summarized in Table 1 (*7–19*).

Recently it has been suggested that some foodborne *E. coli* strains, carrying genes commonly associated with extraintestinal pathogenic *E. coli* (ExPEC), also have the potential to cause extraintestinal infections (including urinary tract infections) (*20,21*). The major ExPEC virulence-associated genes encode for adhesins, toxins, iron acquisition systems, and protectins (*20*). ExPEC strains belong mainly to the phylogenetic groups B2 and D according to Clermont's classification, whereas commensal strains belong to groups A and B1 (*22–24*). The aim of our study is to determine the presence of VTEC and ExPEC virulence genes among *E. coli* strains isolated from various foods in Slovenia between January 2007 and December 2008. In addition, we also intend to clarify the virulence potential of foodborne strains, and to determine which *E. coli* phylogenetic groups and subgroups prevailed in the screened food samples.

Table 1. Major STEC-associated outbreaks in the last 10 years

Year	*E. coli* group	Location(s) (number of infected persons)	Suspected vehicle/mode of transmission	Ref.
2013	STEC O157	England and Wales (19)	watercress	(*7*)
2013	STEC O157:H7	USA (33)	ready-to-eat salads (RTE salads)	(*8*)
2013	STEC O121	USA (35)	frozen food products	(*8*)
2012	STEC O157:H7	USA (33)	organic spinach and spring mix blend	(*8*)
2012	STEC O145	USA (18)	not identified	(*8*)
2012	STEC O26	USA (29)	raw clover sprouts	(*8*)
2011	STEC O157:H7	USA (58)	Romaine lettuce	(*8*)
2011	STEC O157	Japan (146 confirmed, additional 136 suspected)	rice cakes	(*9*)
2011	Enteroaggregative STEC O104:H4	Germany (3816; 54 deaths)	fenugreek seeds	(*10,11*)
2011	Enteroaggregative STEC O104:H4	France (15)	fenugreek seeds	(*11*)
2011	STEC O157	France (8)	frozen beef burgers	(*11*)
2011	STEC O157:H7	USA (14)	Lebanon bologna*	(*8*)
2011	STEC O157:H7	USA (8)	in-shell hazelnuts	(*8*)
2010	STEC O157:H7	USA (38)	cheese	(*8*)
2010	STEC O145	USA (26)	shredded Romaine lettuce	(*8,12*)
2010	STEC O157:H7	USA (21)	beef	(*8*)
2009	STEC O157:H7	USA (26)	beef	(*8*)
2009	STEC O157:H7	USA (23)	beef	(*8*)
2009	STEC O157:H7	USA (72)	cookie dough	(8)
2009	STEC O157	UK (93)	animals (ruminants) in open farm	(*13*)
2009–2008	STEC O157	The Netherlands (20)	raw meat (steak tartare)	(*11,14*)
2008	STEC O157:H7	USA (48)	ground beef	(*8*)
2007	STEC O157:H7	USA (at least 21)	pizza	(*8*)
2007	STEC O157	USA (40)	Topps frozen ground beef patties	(*8*)
2007	STEC O145 and O26	Belgium (12)	ice cream	(*11,15*)
2006	STEC O157:H7	USA (199)	fresh spinach	(*8*)
2006	STEC O103:H25	Norway (17)	cured meat sausages (minced meat)	(*11,16*)
2005	STEC O157	Ireland (18)	water	(*17*)
2004	STEC O157	Sweden (11)	lettuce	(*18*)
2004–2003	STEC O157:H-	Denmark (25)	organic milk from a small dairy	(*19*)

*fermented semi-dry sausage

Materials and Methods

Bacteria were isolated and identified from various food items including raw meat, fish dishes, hash, salad, ham, steak tartare, grilled sausage, codfish spread, pastries, dough, pasta with meat, mayonnaise dressing and dry tea leaves using the IMViC tests according to ISO standards 16649-2:2001 and 16654:2001. Whenever necessary, API 20 E tests (bioMérieux, Marcy l'Etoiley, France) were employed for confirmation. A total of 84 *Escherichia coli* isolates were identified and further investigated. Crude genomic DNA was released from bacterial culture by boiling (*25*), and PCR amplifications were performed in a total volume of 50 μL, containing 2 μL of the bacterial lysate, 25 μL of PCR Master mix (Fermentas UAB, Thermo Fisher Scientific Baltics, Vilnius, Lithuania) and 20 pg of each of the used oligonucleotide primers. The phylogenetic groups A, B1, B2 and D were determined by multiplex PCR as previously described (*23*). Furthermore, the subgroups were determined as described by Escobar-Páramo *et al.* (*26*). Isolates were screened for the presence of STEC-associated genes *stx1*, *stx2* and *eae* by PCR using the DEC Primer Mix according to the manufacturer's instructions (Statens Serum Institut, SSI, Copenhagen, Denmark). The following cycling conditions were employed: initial denaturation for 2 min at 95 °C, 35 cycles of 50 s at 94 °C and 40 s at 62 °C, followed by an extension for 50 s at 72 °C. The *ehxA* genes were amplified using the primer pair hlyen1/hlyen2 (5'-GGTGCAGCAGAAAAAGTTGTA G-3'/5'-TCTCGCCTGATAGTGTTTGGTA-3') (*27*) and the following cycling conditions: initial denaturation for 5 min at 95 °C, 30 cycles of 30 s at 94 °C and 30 s at 60 °C, followed by an extension for 90 s at 72 °C. Extraintestinal virulence-associated genes including adhesins (*fimH*, *sfa*, *iha*, *hra*, *bmaE*), toxins (*hlyA*, *cnf, sat*, *vat*, *hbp*), protectin *kpsMT*II, and genotoxin *usp* were detected by PCR using amplification procedures as described elsewhere (*28–30*). The avian pathogenic *E. coli* (APEC)-associated outer membrane protease gene *ompT* was amplified using primer pair $ompT_{APEC}F/ompT_{APEC}R$ (5'-CAGAGTATCTGTCGGTGCCTCA-3'/5'-TACGGTTCCATGTTCCTTCGAC-3') and the following cycling conditions: initial denaturation for 5 min at 95 °C, 30 cycles of 30 s at 94 °C and 30 s at 64 °C, followed by an extension for 45 s at 72 °C. Plasmid-mediated quinolone resistance (PMQR) genes *qnrA*, *qnrB* and *qnrS* were screened by the multiplex PCR method as described by Cattoir *et al.* (*31*).

Results and Discussion

Food has recently been suspected to present a source of virulent and antimicrobial-resistant *Escherichia coli* strains which can, in addition to intestinal infections, also cause extraintestinal infections, such as cystitis, pyelonephritis, bacteraemia, and meningitis. Many efforts have been made in order to analyze antibiotic-resistant *E. coli* isolates, especially those producing extended-spectrum β-lactamases (ESBL), which were retrieved from food or food-producing animals. This has been reflected in an increasing number of scientific reports concerned with ESBL-producing isolates (*32–34*). Less information is available about the virulence potential of the non-ESBL *E. coli* strains isolated from different foods.

Phylogenetic group distribution

In our study a total of 84 *E. coli* strains were isolated from various foods between January 2007 and December 2008 in Slovenia. The distribution of these foodborne *E. coli* isolates into phylogenetic groups and subgroups is summarized in Table 2. The majority of the isolates belonged to the phylogenetic groups A (50 %) and B1 (35.7 %), whereas only 11.9 and 2.4 % belonged to the phylogenetic groups D and B2, respectively. Strikingly similar results were reported by Koo *et al.* (*35*), who assayed 162 *E. coli* isolates retrieved from different foods in South Korea, showing that 46.3, 43.2, 1.3 and 9.2 % isolates belonged to phylogenetic groups A, B1, B2 and D, respectively. Rúgeles *et al.* (*36*) analyzed *E. coli* isolates from 16 meat samples and 12 vegetable samples and placed them into phylogenetic groups A (75 %) and B1 (25 %). Among 32 meat isolates that were studied by Hannah *et al.* (*37*), 40 % belonged to group A, 17 % to group B1, 9 % to group B2 and 34 % to group D.

Table 2. Distribution of foodborne *E. coli* strains isolated from various foods in Slovenia, during the years 2007 and 2008, into different phylogenetic groups and subgroups

Phylogenetic group or subgroup	Year					
	2007		2008			
	N_1(isolate)	$\frac{N_1(\text{isolate})}{N(\text{sample})}$/%	N_2(isolate)	$\frac{N_2(\text{isolate})}{N(\text{sample})}$/%	N_{total}	$\frac{N_1+N_2}{N_{total}}$/%
A_0	14	31.1	8	20.5	22	26.2
A_1	15	33.3	5	12.8	20	23.8
B1	15	33.3	15	38.5	30	35.7
$B2_2$	0		1	2.5	1	1.2
$B2_3$	0		1	2.5	1	1.2
D_1	1	2.2	5	12.8	6	7.1
D_2	0		4	10.3	4	4.8
N(sample)	45		39		84	

Prevalence of STEC-associated genes

Altogether eleven (13.1 %) isolates retrieved in our study carried one or both of the STEC-associated *stx* alleles. One isolate from the phylogenetic subgroup $B2_2$ was positive for STEC virulence gene *stx1*, five isolates carried the *stx2* allele (two isolates from the phylogenetic subgroup A_1 and three from the group B1) and further five isolates were positive for both *stx* alleles (one from the subgroup A_0 and four from group B1). Four of these isolates were from minced meat, two from raw beef, two from steak tartare, two from salads, and one from dough. Strains that are positive for only the *stx2* allele are reported to be potentially more virulent and are more frequently associated with HUS than those harbouring only *stx1* or both alleles (*38*). The five *stx2*-positive isolates (almost 6 %) found in our study represent a rather large number, since Koo *et al.* (*35*) detected only one (0.6 %) such isolate originating from beef, whereas Rúgeles *et al.* (*36*) found two (7.1 %) STEC strains among *E. coli* isolates that were retrieved from screened food products, one originating from meat and one from vegetables. Magwedere *et al.* (*39*) screened only retail meat products. Among 138 *E. coli* isolates, belonging to the seven major STEC O-groups, they detected only one *stx*-positive isolate, retrieved from minced whitetail venison. Other available data deal with the prevalence of *stx* genes in *E. coli* from specific O-serogroups (*e.g.* O157), specific meat products (beef) or ecological niches (livestock faeces) (*5,40–43*).

Eight (9.5 %) isolates from our collection were found to carry the plasmid-encoded enterohemolysin gene *ehxA*. Two of them were also positive for *stx2* and three for *stx1* and *stx2*. The *ehxA* is an important STEC-associated virulence gene. It is part of three predominant virulence profiles (out of four), which comprised 78 % of all STEC isolates studied in Argentina, which has the highest incidence of haemolytic uremic syndrome (HUS) worldwide (*44*). Another virulence factor typical for STEC and enteropathogenic *E. coli* (EPEC) isolates is the *eae* gene, which encodes intimin, an outer membrane protein responsible for the intimate adherence between bacteria and enterocyte membranes (*45*). Three isolates from our collection were positive for *eae*. While one isolate carried also the *ehxA* gene, none of them were *stx* positive. A rather high percentage of strains carrying genes common for the STEC pathotype is not insignificant. According to the data collected by European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA), the number of reported cases of STEC infection in humans increased from 2007 to 2011 in the EU (*46*). Children under age 4 were at highest risk for HUS development, which is mostly associated with infections due to STEC O157 strains. It appears that STEC strains may be found in products from a range of different animal species (cattle, sheep) and food categories (meat, milk, fishery products, vegetables) (*46*).

Prevalence of ExPEC-associated genes

Of the 13 ExPEC virulence genes tested, we detected 8 in our strain collection. Seventy-four (88.1 %) isolates carried the type 1 fimbriae gene *fimH*, which is consistent with previous reports (*29,47*). The prevalence of other adhesins was as follows: nine isolates (10.7 %) contained *hra*, a gene encoding for heat-resistant agglutinin widely distributed among uropathogenic *E. coli*; six isolates (7.1 %) carried *iha*, a gene encoding the IrgA homologue adhesin, an adherence-conferring protein of *E. coli* O157:H7 which is also a catecholate siderophore receptor; and one isolate (1.2 %) carried *sfa*, a gene encoding the S fimbrial adhesin associated with urinary tract infections, meningitis, and septicemia in human patients. None of the isolates harboured the *bmaE* gene encoding the M-agglutinin subunit.

Among the five toxin genes examined, only two isolates from minced meat were positive, one for *vat* and one for *usp*. While eight (9.5 %) isolates carried the outer membrane protease gene $ompT_{APEC}$, the protectin gene *kpsMT*II was detected in only one (1.2 %). The number of ExPEC virulence genes detected in the 84 isolates ranged from zero to four. Ten (12 %), fifty-five (65.4 %), ten (12 %), eight (9.4 %) and one isolate (1.2 %) had 0, 1, 2, 3 and 4 virulence genes, respectively. All isolates from the phylogenetic subgroup A_0 were positive for *fimH*, but negative for all other tested virulence genes. On the other hand, four virulence genes were detected in an isolate belonging to the phylogenetic subgroup A_1 and three virulence genes were present in three isolates from B1, two isolates from A_1, and one from $B2_2$, $B2_3$ and D_2 group each.

All isolates carrying the ExPEC virulence genes other than *fimH*, except the $B2_2$ strain isolated from dough, originated from raw meat or meat products. ExPEC virulence genes *vat*, *kpsMT*II and *usp* were detected only in the isolates from the phylogenetic group D, which is in accordance with previous findings, and indicates that strains belonging to the phylogenetic groups B2 and D are more virulent in comparison with strains from the groups A and B1. Due to an overall low prevalence of ExPEC-associated virulence genes, the ability of the studied isolates to cause for example urinary tract infections is rather unlikely. However, it should be noted that none of the isolates was an ESBL-producing strain, and that isolates from poultry were not included in our study. The latter have been commonly associated with extraintestinal infections in recent studies (*48–50*). An overview of all isolates, their phylogenetic group assignment and virulence genes is given in Supplementary Table 1.

Plasmid-mediated quinolone-resistance (PMQR)

A serious threat to the public health is also the rising antimicrobial resistance of *E. coli* isolates originating from food-producing animals. Of particular concern are antimicrobial resistance determinants that are carried on mobile genetic elements. Non-virulent resistant *E. coli* strains ingested with food can become part of the commensal gut microbiota and thus a perfect reservoir of resistance genes for potential pathogens (*51*). Extraintestinal infections caused by virulent and antibiotic-resistant foodborne *E. coli* strains are much more difficult to treat. Particularly worrying is the resistance against antimicrobials which are crucial for the treatment of infectious diseases in humans, such as β-lactams (broad-spectrum cephalosporins) and fluoroquinolones. We therefore screened all 84 non-ESBL-producing isolates from our study for the presence of plasmid-mediated quinolone-resistance

gene *qnr*. None of the *qnr* alleles was detected, which is consistent with reports describing a low prevalence of this gene among non-ESBL producing isolates originating from food and a higher prevalence in foodborne ESBL-producing *E. coli* and isolates from food-producing animals (*52–55*).

Conclusions

In our study 84 non-ESBL foodborne *E. coli* strains isolated in years 2007 and 2008 in Slovenia were analyzed for the presence of various virulence-factor-coding genes. A larger proportion of strains carrying the Shiga-toxin-producing *E. coli*-associated virulence genes *stx* and other genes common for the STEC/VTEC pathotypes were detected as described in related studies from other countries. The distribution of strains into the phylogenetic groups was very much in agreement with these studies. It appears that the Slovenian foodborne isolates screened in this study do not carry the full potential for extraintestinal infections. Nevertheless, it should be noted that poultry meat samples were not included. Plasmid-mediated quinolone-resistance gene *qnr* was also not detected.

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Supplementary Table 1. Source, year of isolation, phylogenetic group and virulence genes of 84 strains isolated from various food

No.	Source	Year of isolation	Phylogenetic group or subgroup	Virulence genes																	
				Adhesins						Toxins								Others			
				fimH	*sfa*	*iha*	*hra*	*bmaE*	*eae*	*hlyA*	*cnf*	*sat*	*vat*	*hbp*	*stx1*	*stx2*	*ehxA*	*kpsMTII*	*ompT*	*usp*	
1	Meat	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
2	Mixed minced meat	2007	A_0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
3	Mixed minced meat	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
4	Minced meat	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
5	Fresh vegetable	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
6	Sausage	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
7	Meat	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
8	Vegetable salad	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
9	Mixed minced meat	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
10	Mixed minced meat	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
11	Sausage	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
12	Sausage	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
13	Sausage	2007	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
14	Vegetable salad	2007	A_0	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	
15	Minced meat	2008	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
16	Codfish spread	2008	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
17	Codfish spread	2008	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
18	Ready-made dish	2008	A_0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
19	Minced meat	2008	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
20	Minced meat	2008	A_0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
21	Delicatessen	2008	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
22	Mayonnaise dressing	2008	A_0	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
23	Minced meat	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	
24	Meat	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
25	Meat	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
26	Meat	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
27	Meat	2007	A_1	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	
28	Mixed minced meat	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
29	Mixed minced meat	2007	A_1	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	
30	Mixed minced meat	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
31	Mixed minced meat	2007	A_1	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–	
32	Meat	2007	A_1	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	
33	Beef	2007	A_1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
34	Vegetable salad	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
35	Sausage	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	
36	Meat	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
37	Vegetable salad	2007	A_1	+	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	
38	Minced meat	2008	A_1	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	
39	Prepared salad	2008	A_1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
40	Confectionery	2008	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
41	Salad	2008	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
42	Minced meat	2008	A_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
43	Minced meat sauce	2007	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
44	Minced meat sauce	2007	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
45	Steak tartare	2007	B1	+	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	
46	Mixed minced meat	2007	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
47	Meat	2007	B1	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–	
48	Meat	2007	B1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

Supplementary Table 1. – continued

No.	Source	Year of isolation	Phylogenetic group or subgroup	Virulence genes																
				Adhesins						Toxins								Others		
				fimH	*sfa*	*iha*	*hra*	*bmaE*	*eae*	*hlyA*	*cnf*	*sat*	*vat*	*hbp*	*stx1*	*stx2*	*ehxA*	*kpsMTII*	*ompT*	*usp*
49	Meat	2007	B1	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–
50	Meat	2007	B1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
51	Beef	2007	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
52	Minced meat	2007	B1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
53	Mixed minced meat	2007	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
54	Sausage	2007	B1	+	–		–	–	+	–	–	–	–	–	–	–	+	–	–	–
55	Beef	2007	B1	+	–	+	–	–	–	–	–	–	–	–	+	+	–	–	–	–
56	Steak tartare	2007	B1	+	–	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–
57	Beef	2007	B1	+	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
58	Minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
59	Minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–
60	Minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–
61	Minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–
62	Minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
63	Sausage	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
64	Sausage	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
65	Mixed minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
66	Minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
67	Sausage	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
68	Raw meat	2008	B1	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
69	Dough	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
70	Minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
71	Tea leaves	2008	B1	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
72	Minced meat	2008	B1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
73	Dough	2008	$B2_3$	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–	+	–
74	Minced meat	2008	$B2_3$	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–
75	Meat	2007	D_1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
76	Minced meat	2008	D_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
77	Salad	2008	D_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
78	Salad	2008	D_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
79	Spaghetti bolognese	2008	D_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
80	Minced meat	2008	D_1	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
81	Minced meat	2008	D_2	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
82	Minced meat	2008	D_2	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
83	Minced meat	2008	D_2	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
84	Minced meat	2008	D_2	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–