Detection of *Escherichia albertii* from chicken meat and giblets

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ABSTRACT. *Escherichia albertii* occasionally causes food-borne outbreaks of gastroenteritis in humans; however, little is known about the vehicle of transmission. To screen retail chicken products for the presence of *E. albertii*, 104 retail chicken products were investigated. Portions of enrichment cultures that were PCR-positive for *E. albertii* (n=3) were sub-cultured on agar medium. Only 2 strains obtained from 2 chicken giblet samples were identified as *E. albertii* by multi locus sequence typing. Antimicrobial susceptibility testing showed that 1 strain was resistant to streptomycin and sulfisoxazole. Both strains harbored the virulence genes *cdt* and *eae*. This study is the first description of *E. albertii* isolation from retail food, suggesting that chicken products are a potential vehicle of *E. albertii* transmission.
KEY WORDS: chicken giblet, chicken meat, chicken products, *Escherichia albertii*, infection source

Escherichia albertii can infect humans and birds, and was first described in 2003 [5]. *E. albertii* occasionally causes outbreaks of gastroenteritis in humans [1, 9, 15], yet the vehicle of this presumably foodborne pathogen remains unknown. Oaks *et al.* [12] reported that chickens harbor *E. albertii*; therefore, we suspected that chicken meat may be a vehicle for transmission of this enteropathogen to humans. It is important to identify the sources of contamination to help develop effective food hygiene protocols. The aim of the present study was to screen retail chicken meat and giblet samples for the presence of *E. albertii*.

A total of 104 retail chicken meat (n=54: 15 thigh, 8 skin, 6 inner fillet, 5 mix of thigh and breast, 5 neck meat, 4 mince, 3 breast and 8 uncertain meat) and giblet (n=50: 42 liver, 4 crop, 2 heart and 2 reproductive tract) samples were collected from 10 supermarkets in Fukuoka Prefecture, Japan, between June and October 2014. Samples were incubated in 9 volumes of buffered peptone water (BPW) (Oxoid, Basingstoke, U.K.) at 35°C for 18 hr. Aliquots of each BPW culture were then centrifuged at 13,800 × *g* for 5 min, and the supernatant was discarded. The pellet was resuspended in normal saline (1 m*l*) and centrifuged at 13,800 × *g* for 5 min. The supernatant was discarded, and the pellet was resuspended in 1 m*l* of 0.1× TE buffer with 5% Chelex 100 (Bio-Rad Laboratories, Hercules, CA, U.S.A.) before being boiled for 10 min, and then centrifuged at 13,800 × *g* for 10 min. The supernatant was used as a template for a

PCR assay to detect *E. albertii* using primer pair lysP107F/lysP358R, as described previously [6]. To isolate the pathogen, the remainder of the BPW cultures of the PCR-positive samples were streaked onto deoxycholate-hydrogen sulfide-lactose agar (Eiken Chemical Co., Tokyo, Japan) and incubated at 37°C overnight. Presumptive *E. albertii* colonies (50–100/sample) were isolated on nutrient agar plates (Eiken Chemical Co.) and incubated at 37°C overnight. The isolates were then re-tested using the PCR method described above. The confirmed PCR-positive isolates were then examined for fermentation of glucose, motility and hydrogen sulfide production on triple-sugar iron agar (Eiken Chemical Co.) and sulfide indole motility medium agar (Eiken Chemical Co.). Isolates with positive glucose fermentation, negative motility and negative hydrogen sulfide production test results were then confirmed as *E. albertii* using multi locus sequence typing (MLST) as previously described [9]. Sequences of isolates in the present study were submitted to MLST databases run by the University of Warwick (http://mlst.warwick.ac.uk/mlst/, accessed 5th October, 2014), and isolates were assigned sequence types (ST) defined by the database (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/GetTableInfo_html). Allele sequences for each isolate were then concatenated in the order *adk − fumC − gyrB − icd − mdh − purA − recA*, for a final composite length of 3,423 bp. The concatenated sequences were aligned using ClustalW [21], and a phylogenetic tree was constructed using the neighbor-joining method in MEGA5 software [20] to compare the results with other published STs.

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc-diffusion method, in accordance with the guidelines of the Clinical and Laboratory Standards Institute [2, 3]. Ampicillin, chloramphenicol, ciprofloxacin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, gentamicin, tetracycline and cefixime discs used for the test were purchased from Becton Dickinson (Franklin Lakes, NJ,

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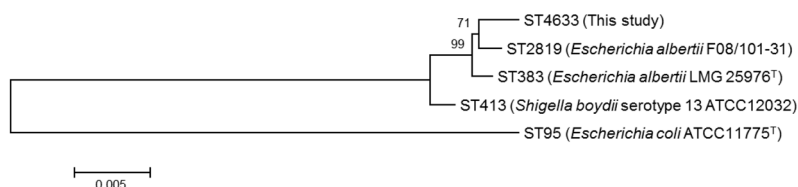
Fig. 1.   Phylogenetic tree showing nucleotide sequence clusters of the tested *Escherichia albertii* strains obtained using multi-locus sequence typing. ST383, ST413, ST95 and ST2819 were included as outgroups. Reference sequences were tested in a previous study [9]. *Shigella boydii* serotype 13 ATCC 12032 has been reclassified into the *Escherichia albertii* lineage. The scale bar indicates the number of nucleotide substitutions per site.

Table 1.   Features of *Escherichia albertii* isolates in this study and in previous studies

E. albertii isolates	Origins	Number of strains tested	Virulence-related genes[a]			Antimicrobial resistance (ampicillin, chloramphenicol, ciprofloxacin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, gentamicin, tetracycline and cefixime)	References
			eae	*cdt*	stx_{2f}		
This study							
ID 3428	Chicken giblet		+[b]	+	–[c]	Susceptible to all antimicrobials tested	This study
ID 3483	Chicken giblet		+	+	–	Streptomycin and sulfisoxazole	This study
Other studies							
	Birds	29	100%	100%	NT	NT[d]	[12]
	Birds	9	100%[e]	100%	NT	NT	[13]
	Birds	11	100%[e]	100%	9.1%	NT	[14]
	Feline	1	100%[e]	0%	0%	NT	[14]
	Swine	1	100%	100%[f]	NT	NT	[4]
	Humans	4	NT	NT	NT	Ampicillin (0%), chloramphenicol (0%), kanamycin (0%) and tetracycline (100%)[g]	[5]
	Humans	48	100%	85%	4.2%	NT	[11]
	Humans	14	100%[e]	100%	7.1%	NT	[14]
	Humans	21	NT	NT	NT	Ampicillin (14%), chloramphenicol (62%), ciprofloxacin (0%), kanamycin (0%), streptomycin (76%), gentamicin (0%), tetracycline (100%) and cefixime (0%)[h]	[19]
	Humans	1	100%[e]	100%	100%	NT	[9]
	Environmental water	18	0%	83%	0%	NT	[8]
	Uncertain	3	NT	NT	NT	Ampicillin (0%), chloramphenicol (0%), gentamicin (0%) and tetracycline (100%)[i]	[17]

a) *eae*: Intimin gene, *cdt*: Cytolethal distending toxin gene, stx_{2f}: Shiga toxin 2f gene. b) +: Detected. c) –: Not detected. d) NT: Not tested. e) Only *eae*-positive isolates were used in this study. f) Only *cdt*-positive isolates were used in this study. g) Did not test with ciprofloxacin, nalidixic acid, streptomycin, sulfisoxazole, gentamicin or cefixime. h) Did not test with nalidixic acid or sulfisoxazole. i) Did not test with ciprofloxacin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole or cefixime.

U.S.A.). The presence of the virulence-related genes stx_{2f} (Shiga toxin 2f gene) [10], *cdt* (cytolethal distending toxin gene) [18] and *eae* (intimin gene) [16] was also examined by PCR as described previously.

PCR results showed that 2 enrichment cultures of chicken giblets (liver) and 1 chicken meat sample were positive for *E. albertii*. However, only 2 *E. albertii* strains, from the 2 chicken giblet samples, were subsequently isolated from the 104 chicken product samples tested. These samples were from different manufacturers and were obtained from different supermarkets. Both strains were identified as ST4633, and the phylogenetic tree confirmed that this ST is an *E. albertii* lineage (Fig. 1). The 2 *E. albertii* strains showed different resistance patterns, although both strains harbored the virulence-related genes *eae* and *cdt*, but not stx_{2f} (Table 1).

These results show that retail chicken can be contaminated by *E. albertii*. The inability to isolate *E. albertii* from 1 of the 3 PCR-positive samples might be caused by a low density of the bacterium in the sample and/or non-specific annealing of the primer pair lysP107F/lysP358R, as described previously [7]. The virulence-related gene patterns of the 2 strains, showing positive for *eae* and *cdt*, and negative for stx_{2f}, were similar to those of previous reports that showed that most reported *E. albertii* strains carry *eae* (114/132) [4, 8, 9, 11–14] and *cdt* (121/132) [4, 8, 9, 11–14], and several contain stx_{2f} (5/93) [8, 9, 11, 14], including bird isolates (Table 1). Interestingly, both *E. albertii* strains from the current study were susceptible to tetracycline, although only one of the 2 strains was resistant to streptomycin and sulfisoxazole. This is in contrast to 3 previous studies [5, 17, 19], which reported that *E. albertii* was resistant to tetracycline (Table 1). The results of the current study show that *E. albertii* contamination of

chicken giblets might pose a potential health risk to humans because of the presence of virulence-related and antimicrobial resistance factors.

To the best of our knowledge, the present study is the first description of *E. albertii* isolation from retail food, although *E. albertii* has previously been isolated from birds, including chickens [12], feline [14] and swine [4]. Because only 104 samples collected from a small area were tested in the present study, further analyses are needed to determine the prevalence of *E. albertii* in chicken products. However, this study supports the hypothesis that chicken products might be a potential vehicle of *E. albertii* transmission.

Sequences of strains in the present study were submitted to MLST databases run by the University of Warwick (http://mlst.warwick.ac.uk/mlst/) as ST4633.

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