Isolation of *Escherichia albertii* from Raw Chicken Liver in Fukuoka City, Japan

Nanami Asoshima1*, Masanori Matsuda2, Kumiko Shigemura1, Mikiko Honda1, Hidehiro Yoshida1, Takahiro Oda1, and Hiroshi Hiwaki1

1Fukuoka City Institute for Hygiene and the Environment, Fukuoka 810-0065; 2Fukuoka City Minami Health and Welfare Center, Fukuoka 815-0032; 3Fukuoka City Meat Inspection Station, Fukuoka 812-0055; 4Food & Nutrition Division, College Faculty, Nakamura Gakuen University, Fukuoka 814-0198; and 5Fukuoka City Hall, Housing & Urban Planning Bureau, Greenery City Department, Fukuoka 810-8620, Japan

Communicated by Makoto Ohnishi

*Escherichia albertii* is an emerging diarrheagenic pathogen in humans (1,2), and there are a few reports on *E. albertii* isolation from outbreaks of food-borne or water-borne gastroenteritis in Japan (3–6). This pathogen was isolated from feces of wild or domestic birds which include chickens (7). These findings suggest that raw chicken is a potential source of *E. albertii* food poisoning. In Japan, there is a custom of eating sliced raw chicken meat and guts (chicken sashimi) as well as sliced raw fish (sashimi); however, the prevalence of *E. albertii* in chicken sashimi as well as raw chicken meat is currently unknown.

To investigate the incidence of *E. albertii* in chicken sashimi and raw chicken meat sold at retail stores in Fukuoka City, Japan, a total of 220 samples (comprising 114 samples of chicken sashimi and 106 samples of raw chicken meat) were collected from February through July 2013. These 114 chicken sashimi samples included 98 samples of chicken meat, 6 mixed samples of chicken meat and gizzard, 5 samples of gizzard, and 5 samples of chicken liver. An aliquot of 25 g of sample was suspended in 225 mL of EC broth. After 24 h incubation at 42°C, the enrichment cultures were screened by a PCR test to detect the *eae* gene (8). Of these 220 cultures, 3 cultures: 1 culture of the chicken liver sashimi sample and 2 cultures of the raw chicken meat samples were PCR positive for *eae*. These 3 PCR positive cultures were spread on 3 kinds of selective agar plates: Desoxycholate Hydrogen Sulfide Lactose agar, Salmonella Shigella agar, and MacConkey agar. After 24 h incubation at 35°C, 60–70 isolated colonies on the agar plates for each culture were again examined by PCR to confirm the presence of *eae*. An *eae*-positive colony was only found in the chicken liver sashimi sample; however, all colonies isolated from the 2 raw chicken meat samples were *eae*-negative.

In our present study, this *eae*-positive strain from chicken liver sashimi (laboratory designation FCI-ALB001) was analyzed by PCR and biochemical tests, and strains of *E. albertii* isolated from food-borne and water-borne outbreaks that occurred in Fukuoka City were used as reference strains (5,6). A multiplex PCR was performed to amplify the *lysP* and *mdh* genes, to detect unique polymorphisms of *E. albertii* (9), and the *clpX* gene, which is conserved in *E. coli*, *Shigella*, and the *E. albertii/S. boydii* lineage (7,9). The PCR test revealed that FCI-ALB001 strain harbored *lysP*, *mdh*, and *clpX* (Fig. 1). The biochemical characteristics of FCI-ALB001 strain are listed in Table 1. FCI-ALB001 strain was non-motile, indole-positive, lysine- and ornithine decarboxylase-positive, lactose-negative (but ONPG test [β-galactosidase]-positive), β-D-glucuronidase (MUG test)-negative and sucrose- and D-xylose-negative. These genetic and biochemical properties corresponded to features of *E. albertii* (3,5,10), and those biochemical properties were consistent to the strain isolated in the Fukuoka Prefecture (10). We therefore identified FCI-ALB001 strain as *E. albertii*.

FCI-ALB001 strain was examined for the presence and subtype of cytotoxinal distending toxin gene (*cdtB*) (11,12), because most *E. albertii* strains isolated con-
Fig. 2. PFGE patterns with Xba I for the 19 strains of Escherichia albertii. M: Salmonella Braenderup H9812, lane1-10: E. albertii strains from the water-borne outbreak in 2005, lane11: E. albertii strain (FCI-EC478) from the water-borne outbreak in 2005, lane12: E. albertii strain (FCI-EC447) from the food-borne outbreak in 2003, lane13–19: E. albertii from the food-borne outbreak in 2003.

Table 1. Characteristics of FCI-ALB001 isolated from a chicken liver sashimi sample and the representative Escherichia albertii strains isolated from the food-borne outbreak in 2003 and the water-borne outbreak in 2005

| Strain       | FCI-ALB001<sup>1)</sup> | FCI-EC447<sup>2)</sup> | FCI-EC478<sup>2)</sup> | FCI-EC479<sup>2)</sup> |
|--------------|------------------------|------------------------|------------------------|------------------------|
| Source       | Chicken                | Human                  | Human                  | Human                  |
| Year of isolation | 2013                  | 2003                    | 2005                    | 2005                    |
| Motility     | -                      | -                      | -                      | -                      |
| Indole       | +                      | +                      | +                      | +                      |
| Urea         | -                      | -                      | -                      | -                      |
| ONPG         | +                      | +                      | +                      | +                      |
| MUG          | -                      | -                      | -                      | -                      |
| Lysine decarboxylase | +                | +                      | +                      | +                      |
| Ornithine decarboxylase | +          | +                      | +                      | +                      |
| Arginine dihydrose | -                  | -                      | -                      | -                      |
| Citrate      | -                      | -                      | -                      | -                      |
| Acetate      | +                      | +                      | +                      | +                      |
| Malonate     | -                      | -                      | -                      | -                      |
| Voges-Proskauer | -                | -                      | -                      | -                      |
| Glucose, acid/gas | + /+            | +/+                     | +/+                     | +/+                     |
| Acid from    |                        |                        |                        |                        |
| Lactose      | -                      | -                      | -                      | -                      |
| Sucrose      | -                      | -                      | -                      | -                      |
| D-xylose     | -                      | -                      | -                      | -                      |
| Adonitol     | -                      | -                      | -                      | -                      |
| Cellobiose   | -                      | -                      | -                      | -                      |
| Salcin       | -                      | -                      | -                      | -                      |
| Dulcitol     | -                      | -                      | -                      | -                      |
| L-rhamnose   | -                      | -                      | -                      | -                      |
| D-sorbitol   | +                      | -                      | +                      | +                      |
| Raffinose    | -                      | -                      | -                      | -                      |
| L-arabinose  | +                      | +                      | +                      | +                      |
| Maltose      | +                      | +                      | +                      | +                      |
| D-mannose    | +                      | +                      | +                      | +                      |
| Mannitol     | +                      | +                      | +                      | +                      |
| Trehalose    | +                      | +                      | +                      | +                      |

<sup>1)</sup>: Tested strain.  
<sup>2)</sup>: Reference strain of E. albertii.
tained cdtB. FCI-ALB001 strain also possessed cdtB, and its subtype was II/III/IV. FCI-ALB001 strain was further examined for pulsed-field gel electrophoresis (PFGE) patterns with XbaI digestion. Fig. 2 shows that the PFGE pattern of FCI-ALB001 is quite different from the _E. albertii_ strains isolated from the 2 food poisoning outbreaks in 2003 and 2005 (5,6).

In this study, an _E. albertii_ strain was isolated from only 1 chicken liver sample among 114 chicken sashimi samples (positive rate: 0.88%). This finding suggests that the risk of _E. albertii_ food poisoning from eating chicken sashimi may be low.

On the other hand, we could not isolate any _E. albertii_ strains from 106 raw chicken meat samples; however, 2 EC broth cultures of raw chicken meat samples were PCR-positive for eae. Therefore, those 2 cultures were analyzed by PCR to detect lysP, _mdh_, and _clpX_. As a result, all 3 genes were found in the 2 cultures, suggesting that _E. albertii_ strains existed in the 2 raw chicken meat samples. We also attempted to isolate more than 60 suspected colonies grown on different selective agar plates from the 2 _eae_-positive EC broth cultures as much as possible; however, we could not find any _eae_-positive colonies. Therefore, the development of selective agar medium with good performance is strongly expected for effective isolation of the _E. albertii_ strain.

**Acknowledgments** We acknowledge Koichi Murakami of Fukuoka Institute of Health and Environmental Sciences for his invaluable advice.

**Conflict of interest** None to declare.

---

**REFERENCES**

1. Albert MJ, Alam K, Islam M, et al. _Hafnia alvei_, a probable cause of diarrhea in humans. Infect Immun. 1991;59:1507-13.

2. Huys G, Cnockaert M, Janda JM, et al. _Escherichia albertii_ sp. nov., a diarrhoeagenic species isolated from stool specimens of Bangladeshi children. Int J Syst Evol Microbiol. 2003;53:807-10.

3. Konno T, Yatsuyanagi J, Takahashi S, et al. Isolation and identification of _Escherichia albertii_ from a patient in an outbreak of gastroenteritis. Jpn J Infect Dis. 2012;65:203-7.

4. Ooka T, Tokuoka E, Furukawa M, et al. Human gastroenteritis outbreak associated with _Escherichia albertii_. Japan. Emerg Infect Dis. 2013;19:1446.

5. Asoshima N, Matsuda M, Shigemura K, et al. Identification of _Escherichia albertii_ as a causative agent of a food-borne outbreak occurred in 2003. Jpn J Infect Dis. 2014;67:139-40.

6. Baba A, Ebuchi S, Uryu K, et al. An outbreak of water-borne gastroenteritis caused by diarrhoeagenic _Escherichia coli_ possessing _eae_ gene. Jpn J Infect Dis. 2006;59:59-60.

7. Oaks JL, Besser TE, Walk ST, et al. _Escherichia albertii_ in wild and domestic birds. Emerg Infect Dis. 2010;16:638-46.

8. Kobayashi K, Seto K, Yatsuyanagi J, et al. Presence of the genes regarding adherence factors of _Escherichia coli_ isolates and a consideration of the procedure for detection of diarrhoeagenic strain. J Jpn Assoc Infect Dis. 2002;76:911-20. Japanese.

9. Hyma KE, Lacher DW, Nelson AM, et al. Evolutionary genetics of a new pathogenic _Escherichia_ species: _Escherichia albertii_ and related _Shigella boydii_ strains. J Bacteriol. 2005;187:619-28.

10. Murakami K, Etoh Y, Tanaka E, et al. _Escherichia albertii_ from a symptomatic human. Jpn J Infect Dis. 2014;67:204-8.

11. Ooka T, Seto K, Kawano K, et al. Clinical significance of _Escherichia albertii_. Emerg Infect Dis. 2012;18:488-92.

12. Toth I, Herrals F, Beutin L, et al. Production of cytolethal distending toxins by pathogenic _Escherichia coli_ strains isolated from human and animal sources: establishment of the existence of a new _cdt_ variant (type IV). J Clin Microbiol. 2003;41:285-91.