BRIEF COMMUNICATION

Isolation and Identification of *Escherichia coli* O157:H7 in a Taiwanese Patient with Bloody Diarrhea and Acute Renal Failure

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Abstract: *Escherichia coli* O157:H7 is a frequent foodborne pathogen in many developed countries. Outbreaks of this infection have been reported in countries all over the world. The first clinical case of *E. coli* O157:H7 infection from Taiwan was reported in a 6- year-old boy who had returned from USA in August 2001. In this paper, we describe the results of the isolation and identification of this strain, and molecular typing for comparison with previously reported strains. Biochemical and molecular biological tests were used to confirm that this patient, who developed bloody diarrhea and kidney failure as a result of the infection, was indeed infected with *E. coli* O157:H7. None of the patients' close contacts were affected. Molecular typing by use of pulsed-field gel electrophoresis revealed this clinical strain to have a unique genotype, which is different from all available clinical strains reported from Japan and environmental strains reported from Taiwan. America Type Culture Collection reference strains and an out-break strain from USA had the nearest relationships with this clinical isolate. Molecular typing showed that this infection by *E. coli* O157:H7 was not derived from the local environmental strains and was acquired during overseas travel.

Key words: *Escherichia coli* O157; Polymerase chain reaction; Pulsed-field gel electrophoresis

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Enterohemorrhagic *Escherichia coli* O157:H7 is a commonly encountered pathogen worldwide. Outbreaks of this infection have been reported in Australia, Canada, Japan, the United States, and various countries in Europe and southern Africa. This particular strain, first recognized as a human pathogen in 1982, is now known to cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS).[1,2] Since 1992, reported outbreaks of enterohemorrhagic *E. coli* O157:H7 infection were mostly derived from consumption of contaminated hamburgers prepared by fast-food restaurants in the northwest part of the United States.[3] *E. coli* O157:H7 infection has been attributed to a variety of sources, including ground beef, milk, alfalfa spouts, apple cider,[4,5] lettuce, game meats, cheese curd,[6] and water.[7]

In the years after its discovery, this pathogen has become increasingly prominent, causing in 1995 an estimated 20,000 illnesses and 250 deaths in the United States.[8] In Japan, there were 3 major outbreaks in 1996, resulting in more than 17,000 illnesses and 13 deaths. In Taiwan, *E. coli* O157:H7 infection has been designated a statutory communicable disease but no human cases were reported until 2001. In the summer of 2001, a 6-year-old boy who had been born in Taiwan, but was now living in the United States developed a sudden illness about 6 weeks after returning to Taiwan. Symptoms included bloody diarrhea, acute renal failure. HUS was suspected. Fecal samples were collected from the patient and people in close contact with him, and samples were also taken of suspected contaminated foods, and environmental items (soil and water). Standard and molecular biologic methods were applied to isolate and identify the possible causal agent. This was the first human case of *E. coli* O157:H7 infection reported in Taiwan. In this paper, we

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describe the molecular fingerprint of this isolate and the comparison of its molecular typing with other strains isolated from other areas worldwide.

Methods

A total of 35 rectal swabs were collected from the patient and his close contacts. Another 5 specimens were taken from some suspected food items and 8 specimens from environmental soil and water at a restaurant as well as at the patient's residence in Taipei in August 2001.

Each of the human rectal swabs, the related foods and environmental specimens were examined according to the procedures published by Center for Disease Control Taiwan.[9] Polymerase chain reaction (PCR) was used to check the O and H serotype of the isolates and for shiga-like toxin genes. The primers and conditions of the PCR used were as previously reported.[10]

A somewhat modified pulsed-field gel electrophoresis (PFGE) based on the method originally reported by Barrett et al[11] was used. After PFGE, the gel was stained with ethidium bromide (0.2 μg/mL), photographed under ultraviolet transillumination and analyzed using the Bioprofil Biogene software (Vilber Lourmat, France).

The agar dilution method was used to determine the minimal inhibitory concentration of this isolate, performed in accordance with National Committee for Clinical Laboratory Standards guidelines. The antibiotics used in this analysis are listed in the Table.

Results and Discussion

Forty eight samples were collected from people who had close contact with the patient, as well as from environmental water and food. However, the stool sample collected from the patient was the only one that had gray to white colonies identified on sorbitol MacConkey agar and CHROM agar. The results of biochemical test and the rapid kit, API-20E, showed that this was an *E. coli* strain.The O and H serotypes of this strain were determined by latex agglutination, which revealed that this isolate had the 157th and seventh antisera. PCR was performed using a Centers for Disease Control and Prevention (CDC) in the USA standard procedure to identify the shiga-like toxin genes, and the results showed that this isolate contained both shiga-like toxin I (475 bp) and II (862 bp). A PCR using primers designed by Pan et al[10] was also performed and this test confirmed the results of the previous PCR (Fig. 1). The results also showed that the O and H serotype were O157 and H7, respectively. Thus we concluded that this isolate was indeed *E. coli* O157:H7 and the infection was caused by this pathogen.

Table. Minimum inhibitory concentrations (mg/mL) from antimicrobial susceptibility testing of *E. coli* O157:H7 strains isolated from Taiwan.

	TWE01	TWC01
Cefepime	< 0.5/S	< 0.5/S
Amoxicillin-clavulanic acid	32/R	32/R
Chloramphenicol	32/R	32/R
Ciprofloxacin	< 0.5/S	< 0.5/S
Aztreonam	< 0.5/S	< 0.5/S
Enrofloxacin	< 0.5/S	< 0.5/S
Cefazolin	< 0.5/S	< 0.5/S
Ampicillin-sulbactam	8/SR	8/SR
Nitrofurantoin	32/S	32/S
Sulfamethoxazole-trimethoprim	2/S	2/S
Streptomycin	4/S	4/S
Gentamicin	8/SR	8/SR
Oxytetracycline	< 4/S	< 4/S
Ampicillin	64/R	64/R

S = sensitive; R = resistant; SR = semi-resistant.

Our previous studies found that the prevalence of *E. coli* O157:H7 in cattle was 0.13% and 0.6% in northern and southern Taiwan, respectively.[12,13] Thus, *E. coli* O157:H7 exists in the environment and might be associated with outbreaks of *E. coli* O157:H7 infection. There are no laws or regulations to prevent and control *E. coli* O157:H7 infection. This study used a standard procedure for detection formulated by the Center for Disease Control of Taiwan. Furthermore, we were also able to detect *E. coli* O157:H7 using a PCR with primers designed by Pan et al.[10] This combination of standard pre-enrichment, acidity treatment and PCR greatly improves the efficiency of detection.

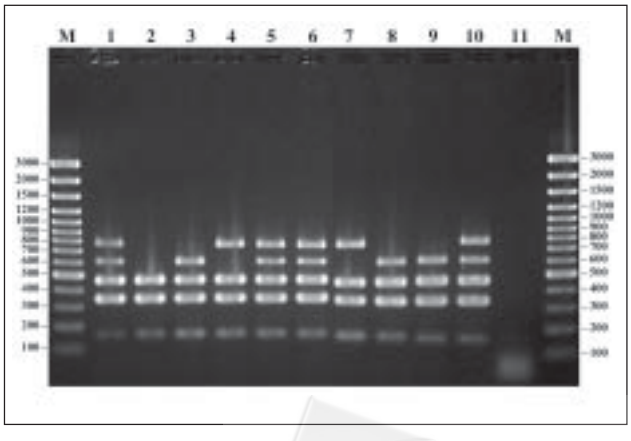

Fig. 1. Pulsed-field gel electrophoresis results for the detection of O157, H7, VTI and VTII gene of *Escherichia coli* O157:H7 in the clinical isolate. Lane M: 100 bp marker. Lane 1-10: ATCC35150, ATCC 43888, ATCC 43889, ATCC 43890, ATCC43894, ATCC43895, HER1258, HER1261, HER1269, and TWC01. Lane 11: negative control. ATCC = America Type Culture Collection.

Pan et al used 6 combinations of primer pairs to detect and identify *E. coli* O157:H7 and showed that these primer pairs could correctly identify this pathogen, with specificity and rapidity better than biochemical tests.[10] The molecular methods used to detect *E. coli* O157:H7 were more efficient than traditional biochemical test methods. In this study we tried to develop more rapid and accurate molecular methods to identify this pathogen.

Molecular typing was performed using PFGE. Included in the PFGE were 5 strains of *E. coli* O157:H7 from the America Type Culture Collection (ATCC35150, ATCC43889, ATCC43890, ATCC43894 and ATCC43895), 2 strains from Japan isolated from the outbreak in 1996 (JP01 and JP02), and 2 Taiwan strains isolated from dairy herds and a clinical case (TWE01 and TWC01). Two restriction enzymes, *Xba*I and *Avr*II, were used to compare the genotype of this first clinical strain isolated in Taiwan with these 8 previously identified strains. The results showed that this clinical strain had a unique genotype which was different from all the ATCC reference and standard strains, Japan outbreak strains, and environmental strains from Taiwan (Fig. 2A). There were 5-6 bands different from the USA strains using *Xba*I restriction enzyme and 6-7 bands using *Avr*II. This strain is also different from the 2 Taiwan strains, showing 8 differing bands using *Xba*I restriction enzyme and 11 bands using *Avr*II. Dendrogram analysis using *Xba*I restriction enzymes (Fig. 2B) revealed 67% similarity between the clinical strain and 2 reference strains (ATCC43894 and 43895). Use of *Avr*II restriction enzyme (Fig. 2C) revealed that the similarity with reference strains (ATCC43894 and 43895) was 73%, while the similarity was about 33-58% with other strains. Molecular typing of other environmental strains isolated from Taiwan showed these to be significantly different from the infecting strain (Fig. 2D). ATCC reference strains had the nearest relationships with this isolate.

Comparison of this clinical strain with strains in the databank of the CDC in the USA revealed that this strain had high similarity with an outbreak strain from the USA (data not shown). Our patient's recent return from the USA about 40 days before symptom onset suggests that the infection may have resulted from contaminated food consumed in the USA. PFGE is the gold standard molecular typing technique for analyzing the difference between organisms. In epidemiologic studies, this technique is often applied to investigate the connection between outbreaks. The CDC in the USA established PulseNet, the national molecular subtyping network for foodborne disease surveillance, in 1996 after a large outbreak of foodborne illness caused by the bacterium *E. coli*

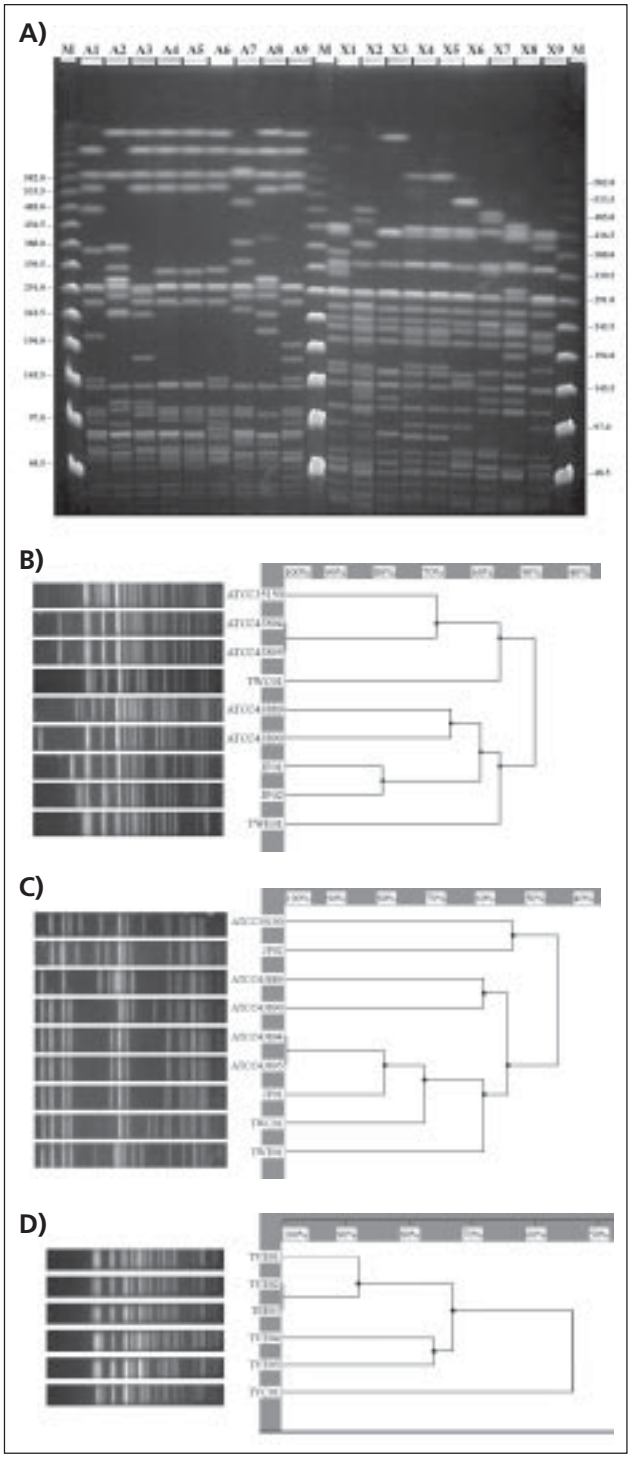

Fig. 2. A) Pulsed-field gel electrophoresis patterns and dendrogram of 9 strains of *Escherichia coli* O157:H7 isolated in the USA, Japan and Taiwan using *Xba*I (B), *Avr*II restriction enzyme (C), and 6 strains isolated in Taiwan using *Xba*I restriction enzyme (D). X1-X9: ATCC35150, ATCC43889, ATCC43890, ATCC43894, ATCC43895, JP01, JP02, TWE01, and TWC01 were restricted by *Xba*I restriction enzyme. A1-A9: ATCC35150, ATCC43889, ATCC43890, ATCC43894, ATCC43895, JP01, JP02, TWE01, and TWC01 were restricted by *Avr*II restriction enzyme. ATCC = America Type Culture Collection.

O157:H7 occurred in the western United States. Scientists at the CDC performed PFGE to characterize clinical and food isolates of *E. coli* O157:H7 and demonstrated its utility in outbreak investigation. About 22 countries/areas have joined the PulseNet in order to cooperate in the control and prevention of foodborne outbreaks.[11,14] In Taiwan, no organization has participated in this network so far, so it is difficult to trace the source of a foodborne pathogen, especially when the origin of infection is a foreign country. To establish and join a global network database for control and prevention the foodborne outbreaks is an urgent mission in Taiwan.

We used 13 antibiotics to compare the sensitivity of this clinical strain with those of isolates from the environment. The results showed that this clinical strain (TWC01) was resistant to amoxicillin-clavulanic acid, chloramphenicol and ampicillin, semi-resistant to ampicillin-sulbactam and gentamicin, and sensitive to other antibiotics. The environmental strain TWE01 showed the same results as this clinical strain and both of these *E. coli* O157:H7 strains were sensitive to most of the antibiotics tested.

In this study, we performed a comparative molecular fingerprinting of a strain of *E. coli* O157:H7 causing infection in a 6-year-old boy who presented with bloody diarrhea and renal failure in Taiwan. The results revealed that this clinical strain had a unique fingerprint map and also suggested that this case had no relationship with the local environmental strains previously isolated in Taiwan.

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