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First sporadic case of pathogenic *Escherichia coli* infection in Black swan in China

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ABSTRACT

A strain of bacteria was isolated from the diseased black swan (*Cygnus atratus*) died from enteritis diarrhea, and designated tentatively as B-1 strain. Morphological and biochemical tests, as well as phylogenetic analysis derived from 16S rRNA and fimC gene sequencing both strongly indicated that B-1 strain is identical to *Escherichia coli*. Furthermore, the polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) profile of the isolate was different from that of two reference strains. Antibiotic sensitivity testing of B-1 strain was carried out by the standard Kirby-Bauer disc diffusion method. Animal experiments demonstrated that B-1 strain is pathogenic to mice and chickens. This is first sporadic case of pathogenic *E. coli* infection in Black swan in China.

1. Introduction

The genus *Escherichia*, a member of the family *Enterobacteriaceae* is composed of several species, but only *Escherichia coli* is an important pathogen of animals [1]. *E. coli* is gram-negative bacterium that is regarded as a member of the commensal intestinal flora of humans and animals. While most *E. coli* strains are commensal, the certain pathogenic strains have the ability to cause severe disease [1,2]. *E. coli* is also considered to be an important opportunistic cause of disease in mammals, birds and reptiles [3]. It is known that the *E. coli* O157:H7 is an emerging cause of foodborne and waterborne illness [4,5]. Recently, the new enterohaemorrhagic *E. coli* outbreak strain was firstly reported in Germany [6]. However, the knowledge on the colibacillosis in wild birds remains very limited.

Here in this study, we describe fatal infection in black swan associated with *E. coli*. To our knowledge, this is the first report of a sporadic case of *E. coli* infection from wild birds in China, which was identified by isolation and identification of bacteria, 16s rRNA gene sequencing, and animal experiment. The results from our work will contribute to the understanding of the pathogenesis of *E. coli*, which have important scientific and practical significance for the prevention and treatment of colibacillosis in wild birds.

2. Materials and methods

The bacterium B-1strain was isolated from the liver of died black swan in Xuzhou City, Jiangsu Province, P. R. China. Isolation of this strain was done in eosin methylene blue (EMB) agar and incubated aerobically at 37 °C for 24 h. The strain was confirmed as *E. coli* using morphological and biochemical tests. The bacterial strain was cultivated in LB medium and then the Gram staining test was performed using the Hucker method, and SEM observation was conducted under the HITACHI S-3400N scanning electron microscopy. Biochemical tests were performed to confirm the *E. coli* using commercial microtest systems (Tianhe, Hangzhou,China).

Total genomic DNA of all isolates was extracted by using the UNIQ-10 column genomic DNA extraction kit (Sangon, Shanghai,-China) according to the instructions of the manufacturer. The nearly full-length 16S rRNA, V3 region and *fimC* genes were amplified with the bacterial universal primers based on *E. coli* sequences, respectively. The PCR primers were synthesized by Sangon, and the three PCR products were amplified with the primers

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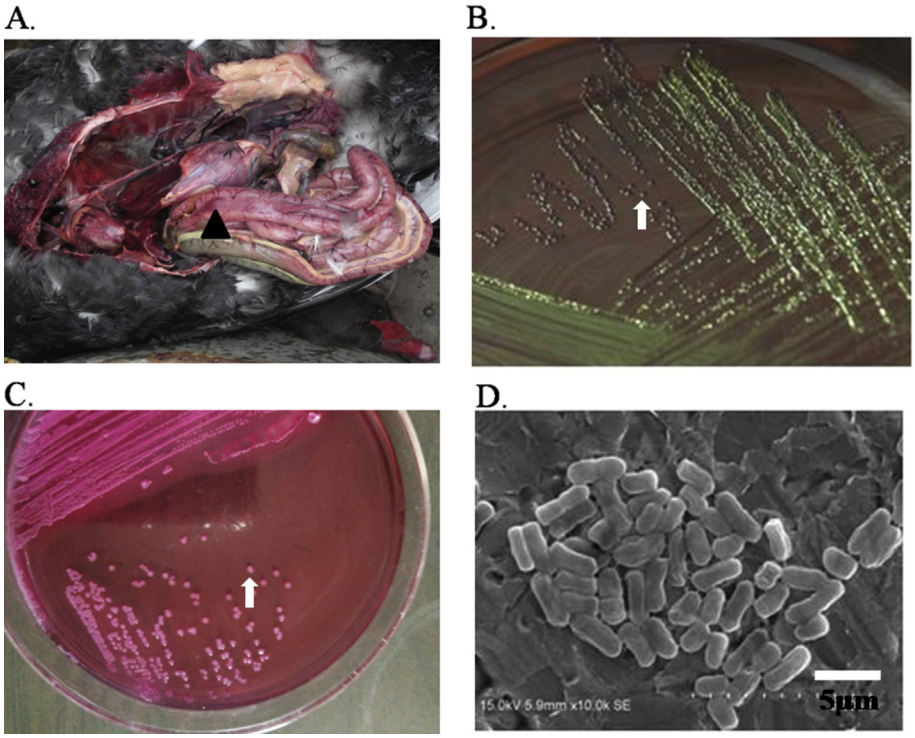

Fig. 1. *E. coli* strain B-1 isolation. (A) Clinical signs in the dying black swan (*Cygnus atratus*). The white arrow represent the air sacculitis, black triangle represent the hemorrhages; (B) Colonial characteristics on the LB plate and the MacConkey Agar Medium (C); Scanning electron micrograph (SEM) of the B-1 strain (D).

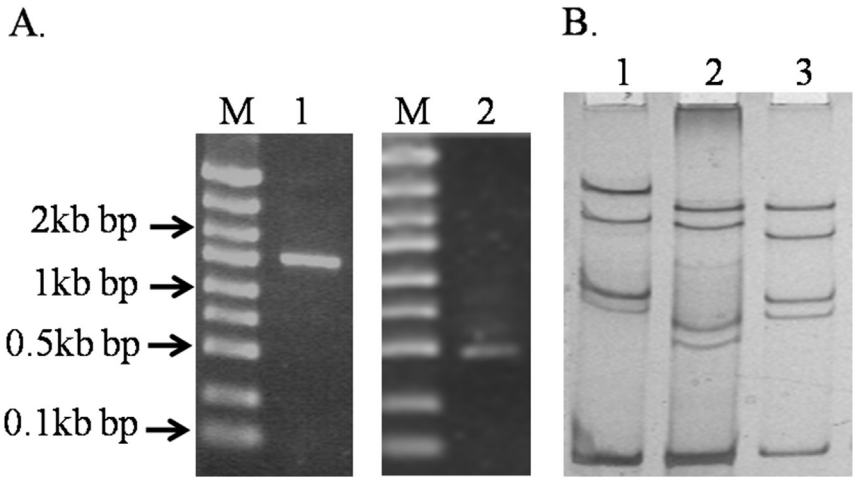

Fig. 2. PCR amplification for 16S rRNA and fimC gene of B-1. (A) 16S rRNA (left) and fimC (right) gene. M, DL250 + DNA marker; (B) PCR-SSCP analysis of the 16S rRNA V-3 region of three strains. (1) B-1 strain; (2) *Acinetobacter* sp.; (3) *Aeromonas veronii.*

sequences (5′ to 3′) as follows: 16S rRNA-F:AGAGTTTGATCATGGCTCAG, 16S rRNA-R:TACGGTTACCTTGTTACGACTT (Tm = 57.0 °C); V3-F:CCTACGGGAGGCAGCAG, V3-R:ATTACCGCGGCTGCTGG (Tm = 51.0 °C); fimC-F:GGGTAGAAAATGCCGATGGTG, fimC-R:CGTCATTTTGGGGGTAAGTGC (Tm = 59.0 °C). The PCR products were evaluated by electrophoresis in 1% agarose gel by staining with ethidium bromide. Amplification of the V3 region of the 16S rRNA for PCR-single-strand conformational polymorphism (PCR-SSCP) analysis was performed according to the method of Nair et al., 2002 [7].

The BLAST search was done at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nih.gov/BLAST/). Alignment was performed using CLUSTAL W method in MEGA 4.1 software. Phylogenetic trees were constructed using the neighbour-joining algorithm of MEGA4.1 software, with 1000 Bootstrap replicates.

Antibiotic susceptibilities were assessed by the standard Kirby-Bauer disc diffusion method using commercially available antimicrobial discs from Hangzhou Microbial Reagent Co,Ltd, China. The reference strain *Escherichia coli* ATCC.

25,922 served as a quality control. Results were interpreted as susceptible, medium susceptible or resistant based on zone diameters of inhibition, including the diameter of the disc (mm).

B-1strain was tested for pathogenicity according to Koch's postulates, and mice and chickens were used for the animal