Defining the Genetic Features of O-Antigen Biosynthesis Gene Cluster and Performance of an O-Antigen Serotyping Scheme for Escherichia albertii

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Escherichia albertii is a newly described and emerging diarrheagenic pathogen responsible for outbreaks of gastroenteritis. Serotyping plays an important role in diagnosis and epidemiological studies for pathogens of public health importance. The diversity of O-antigen biosynthesis gene clusters (O-AGCs) provides the primary basis for serotyping. However, little is known about the distribution and diversity of O-AGCs of E. albertii strains. Here, we presented a complete sequence set for the O-AGCs from 52 E. albertii strains and identified seven distinct O-AGCs. Six of these were also found in 15 genomes of E. albertii strains deposited in the public database. Possession of wzy/wzx genes in each O-AGC strongly suggest that O-antigens of E. albertii were synthesized by the Wzx/Wzy-dependent pathway. Furthermore, we performed an O-antigen serotyping scheme for E. albertii based on specific antisera against seven O-antigens and a high throughput xTAG Luminex assay to simultaneously detect seven O-AGCs. Both methods accurately identified serotypes of 64 tested E. albertii strains. Our data revealed the high-level diversity of O-AGCs in E. albertii. We also provide valuable methods to reliably identify and serotype this bacterium.

Keywords: Escherichia albertii, O-AGC, serotype, antiserum, xTAG Luminex

INTRODUCTION

Lipopolysaccharide (LPS) molecules form the outer leaflet of the outer membrane of many Gram-negative bacteria and are essential components of the bacterial cell envelope. The O-antigen polysaccharide constitutes the exterior part of the LPS and consists of oligosaccharide repeats (O-units) containing three to six sugar residues. The O-antigen plays an important role in resistance to phagocytosis and complement-mediated lytic action (Murray et al., 2003, 2006; Duerr et al., 2009; Saldias et al., 2009). Meanwhile, the O-antigen is a major surface antigen and is responsible for serological diversity of Gram-negative bacteria which are clinically and
epidemiologically important to classify various strains. O-antigen has also provided a basis for development of vaccine against many pathogens. The genes required for O-antigen biosynthesis are clustered at a chromosomal locus, named the O-antigen biosynthesis gene cluster (O-AGC) in many bacteria. Generally, the genes in O-AGC are clustered into three major classes: sugar synthesis genes, glycosyltransferase genes, and O-unit processing genes. Polymerization of the O-units into an O-antigen is mostly mediated through two of three pathways in Gram-negative species: Wzx/Wzy-dependent pathway and ABC transporter-dependent pathway (Valvano, 2003). Synthase-dependent pathway, the third pathway, is rarely present in Gram-negative species. O-AGC is always located between the conserved galF (encoding UTF-glucose-1-phosphate uridylyltransferase) and gnd (encoding 6-phosphogluconate dehydrogenase) genes in many species of the Enterobacteriaceae, such as E. coli (Iguchi et al., 2015) and Cronobacter sakazakii (Mullane et al., 2008). Two genes, encoding the O antigen flippase (wzx) and O antigen polymerase (wzy), are unique in most of the O-AGCs, and have been used as targets for molecular O serogrouping (DebRoy et al., 2016).

Escherichia albertii is a newly described and emerging diarrheagenic pathogen, which is associated with both sporadic infections and outbreaks in humans (Ooka et al., 2013; Asoshima et al., 2014; Murakami et al., 2014; Brandal et al., 2015; Inglis et al., 2015). It was initially identified as Hafnia alvei and later proposed as E. albertii, a new species within the genus Escherichia (Huys et al., 2003). E. albertii strains were often misidentified as E. coli, Hafnia, Salmonella, or Yersinia ruckeri as the lack of specific biochemical characteristics (Abbott et al., 2003). Thus, the prevalence of E. albertii may be underestimated owing to the lack of effective methods to discriminate E. albertii from other members of the Enterobacteriaceae. To date, little information on the E. albertii O-antigen is available. Only several chemical structures of the O-specific polysaccharide (OPS) of E. albertii were reported in previous study (Eserstam et al., 2002; Naumenko et al., 2017; Zheng et al., 2017). There is no comprehensive scheme for O-antigen classification of E. albertii. This study was aimed to investigate the prevalence and characteristics of O-AGCs in E. albertii strains and develop an O-antigen serotyping scheme and a high throughput detection assay to simultaneously detect all types of these O-AGCs.

MATERIALS AND METHODS

Bacterial Strains and Genomic DNA Preparation

Fifty-two strains were selected in the current study: type strain LMG20976 (Huys et al., 2003); one strain from the stool of a diarrheal patient resident in Shanghai in 2013; and 50 strains isolated from multiple sources in Zizong city of Sichuan province between 2014 and 2015 (Table 1). Thirty of these were also used in our previous study (Wang H. et al., 2016). An additional 12 strains were isolated from Luzhou city of Sichuan province in 2016 and used in an agglutination test and the development of the high throughput xTAG Luminex detection assay (Table 1). Strains were cultured on Luria-Bertani (LB) plates (Oxoid, UK) and genomic DNA was extracted using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). Seven housekeeping genes were used for multilocus sequence typing (MLST) analysis according to the E. coli MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). E. coli O3 and O181 antisera were purchased from Statens Serum Institut (SSI, Copenhagen, Denmark) for the agglutination test. All strains were verified to be E. albertii based on the combination of 16S rDNA sequencing, diagnostic multiplex PCR, and MLST analysis as described in our previous study (Wang H. et al., 2016).

Sequencing and Bioinformatics Analysis

Forty-two strains were chosen for whole genome sequencing (WGS) based on the MLST results. For each strain, a library was constructed (500–2,000 bp) and then sequenced on an Illumina Hiseq 4,000 system (Illumina, San Diego, CA, USA) to produce 150 bp paired-end reads, which were then assembled into scaffolds using the program SOAP de novo (Release 1.04, http://soap.genomics.org.cn/soapdenovo.html). Open reading frames (ORFs) were identified and annotated using the Artemis program (www.sanger.ac.uk) and homology searches against several databases including GenBank (www.ncbi.nlm.nih.gov/GenBank), the Clusters of Orthologous Groups (COG; www.ncbi.nlm.nih.gov/COG/), and Pfam (pfam.sanger.ac.uk) protein motif databases (Altschul et al., 1997; Tatsumori et al., 2001; Bateman et al., 2002). Each O-AGC between the galF and gnd genes was extracted from the draft genome sequence. Based on these O-AGC sequences, another 10 O-AGCs were sequenced by primer walking PCR. The TMHMM (v2.0) analysis program (http://www.cbs.dtu.dk/services/TMHMM/) was used to identify potential transmembrane segments from the amino acid sequences. The Artemis comparison tool (ACT) (Carver et al., 2005) was used to visualize the data.

Preparation of Specific Antisera

Based on the typing result of strains SP140089, SP150020, SP140724, D140513, T150248, T150072, and ZG141049 were initially used as standard antigen strains to produce antisera. Three New Zealand white rabbits (female, 1.5 to 2 kg body weight) were immunized intravenously with heat-killed (100°C, 2 h) cells four times with the same doses (2.5 × 10^10 CFU) for each strain. The second immunization booster was performed 14 days after the first immunization. The third and fourth immunization boosters were performed 5 days and 10 days after the second immunization, respectively. Serum was obtained 5 days after the last immunization. Prepared serum was used to test all E. albertii strains in this study by slide agglutination and the strain was heat-killed in 100°C for an hour before the agglutination test. Visual agglutination apparent within 20 s was recorded as a positive result. The antisera that agglutinated all corresponding serotype strains but did not agglutinate any other strains from other serotype groups was referred to as specific antisera for the corresponding serotype.
### TABLE 1 | E. albertii strains used in this study.

| Strain | Source | Geography | Isolation time | Serotype | MLST sequence type (ST) | Accession number of O-AGCs | References |
|--------|--------|-----------|----------------|----------|-------------------------|-----------------------------|------------|
| LMG20976 | Faeces of diarrhoeal child | Dhaka, Bangladesh | 90's | O7 | ST383 | KY574555 | Wang H. et al., 2016 |
| SP140128 | Mutton | Zigong city, Sichuan province | 2014 | O1.1 | ST4479 | KY574559 | Wang H. et al., 2016 |
| SP140150 | Chicken intestines | Zigong city, Sichuan province | 2014 | O1.2 | ST4479 | KY574562 | Wang H. et al., 2016 |
| SP140152 | Chicken intestines | Zigong city, Sichuan province | 2014 | O2 | ST3762 | KY574563 | Wang H. et al., 2016 |
| SP140089 | Chicken intestines | Zigong city, Sichuan province | 2014 | O1.3 | ST4479 | KY574565 | Wang H. et al., 2016 |
| SP140047 | Chicken intestines | Zigong city, Sichuan province | 2014 | O1.4 | ST4479 | KY574566 | Wang H. et al., 2016 |
| SP140084 | Chicken meat | Zigong city, Sichuan province | 2014 | O3 | ST4633 | KY574567 | Wang H. et al., 2016 |
| SP140602 | Chicken intestines | Zigong city, Sichuan province | 2014 | O4 | ST4638 | KY574564 | Wang H. et al., 2016 |
| SP140610 | Chicken intestines | Zigong city, Sichuan province | 2014 | O5 | ST4635 | KY574565 | Wang H. et al., 2016 |
| SP140618 | Chicken intestines | Zigong city, Sichuan province | 2014 | O6 | ST4636 | KY574564 | Wang H. et al., 2016 |
| SP140619 | Chicken intestines | Zigong city, Sichuan province | 2014 | O6 | ST4633 | KY574565 | Wang H. et al., 2016 |
| SP140637 | Chicken intestines | Zigong city, Sichuan province | 2014 | O4 | ST4634 | KY574565 | Wang H. et al., 2016 |
| SP140638 | Chicken intestines | Zigong city, Sichuan province | 2014 | O4 | ST4635 | KY574566 | Wang H. et al., 2016 |
| SP140645 | Chicken intestines | Zigong city, Sichuan province | 2014 | O3 | ST4633 | KY574566 | Wang H. et al., 2016 |
| SP140674 | Chicken intestines | Zigong city, Sichuan province | 2014 | O6 | ST4637 | KY574567 | Wang H. et al., 2016 |
| SP140692 | Duck meat | Zigong city, Sichuan province | 2014 | O2 | ST3762 | KY574572 | Wang H. et al., 2016 |
| SP140701 | Chicken meat | Zigong city, Sichuan province | 2014 | O4 | ST4638 | KY574573 | Wang H. et al., 2016 |
| SP140724 | Chicken intestines | Zigong city, Sichuan province | 2014 | O4 | ST4633 | KY574574 | Wang H. et al., 2016 |
| SP140733 | Duck intestines | Zigong city, Sichuan province | 2014 | O4 | ST4634 | KY574575 | Wang H. et al., 2016 |
| SP140748 | Duck intestines | Zigong city, Sichuan province | 2014 | O1.5 | ST4479 | KY574566 | Wang H. et al., 2016 |
| SP140749 | Duck intestines | Zigong city, Sichuan province | 2014 | O1.6 | ST4479 | KY574567 | Wang H. et al., 2016 |
| SP140753 | Duck intestines | Zigong city, Sichuan province | 2014 | O1.7 | ST4479 | KY574568 | Wang H. et al., 2016 |
| SP140754 | Duck intestines | Zigong city, Sichuan province | 2014 | O1.4 | ST4479 | KY574569 | Wang H. et al., 2016 |
| SP140771 | Chicken intestines | Zigong city, Sichuan province | 2014 | O3 | ST4633 | KY574570 | Wang H. et al., 2016 |
| SP140791 | Pork meat | Zigong city, Sichuan province | 2014 | O6 | ST4619 | KY574580 | Wang H. et al., 2016 |
| SP140807 | Chicken intestines | Zigong city, Sichuan province | 2014 | O4 | ST4636 | KY574581 | Wang H. et al., 2016 |
| SP140813 | Chicken intestines | Zigong city, Sichuan province | 2014 | O3 | ST4633 | KY574582 | Wang H. et al., 2016 |
| SP140837 | Duck intestines | Zigong city, Sichuan province | 2014 | O2 | ST4639 | KY574583 | Wang H. et al., 2016 |
| SP140839 | Duck meat | Zigong city, Sichuan province | 2014 | O2 | ST4639 | KY574584 | Wang H. et al., 2016 |
| ZG141049 | Diarrhoea patient | Shanghai | 2014 | O4 | ST4947 | KY574562 | This study |
| D140513 | Faeces of egret | Zigong city, Sichuan province | 2014 | O7 | ST4947 | KY574563 | This study |
| SP150020 | Duck intestines | Zigong city, Sichuan province | 2015 | O2 | ST3762 | KY574585 | This study |
| SP150021 | Duck intestines | Zigong city, Sichuan province | 2015 | O6 | ST3762 | KY574586 | This study |
| SP150027 | Duck intestines | Zigong city, Sichuan province | 2015 | O4 | ST3762 | KY574587 | This study |
| SP150036 | Duck intestines | Zigong city, Sichuan province | 2015 | O5 | ST1996 | KY574588 | This study |
| SP150104 | Duck intestines | Zigong city, Sichuan province | 2015 | O1.1 | ST4479 | KY574589 | This study |
| T150072 | Faeces of healthy butcher | Zigong city, Sichuan province | 2015 | O6 | ST4619 | KY574598 | This study |
| T150248 | Faeces of healthy butcher | Zigong city, Sichuan province | 2015 | O6 | ST4619 | KY574599 | This study |
| T150298 | Faeces of healthy butcher | Zigong city, Sichuan province | 2015 | O6 | ST4637 | KY574600 | This study |
| SH13EC413 | Faeces of diarrhoeal patient | Shanghai | 2013 | O6 | ST4488 | KY596024 | This study |
| SP150175 | Duck intestines | Zigong city, Sichuan province | 2015 | O1.1 | ST4606 | KY574590 | This study |
| SP150183 | Duck intestines | Zigong city, Sichuan province | 2015 | O1.1 | ST4606 | KY574591 | This study |

(Continued)
Development of the High Throughput xTAG Luminex Detection Assay

MagPlex –xTAG Microspheres (superparamagnetic beads in 6.5 microns diameter) precoupled with a 24-base oligonucleotide “anti-TAG” sequence were used in the assay. Sequences and working concentration of the primers used to amplify E. albertii specific gene hysP (Hyman et al., 2005) and the serotype-specific wzy gene were listed in Table 2. The primers were designed based on the principles described in previous study (Bai et al., 2015). Briefly, the lengths of the primers were between 22 and 26 oligonucleotides, their melting temperatures were between 49 and 52°C, and the amplification size ranged between 100 and 500 base pairs. In order to conjugate with MagPlex –xTAG Microspheres, a corresponding 24-base oligonucleotide “TAG” sequence was added at the 5′ terminus of each upstream primer. To facilitate the interaction between MagPlex –xTAG Microspheres and amplified productions, a spacer was made by incorporating a 12-carbon amine containing group between “TAG” sequence and primer. Each downstream primer was biotinylated at the 5′ terminus. The products were amplified using cycling parameters at 94°C for 5 min; 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, followed by a final elongation step at 72°C for 10 min. The threshold of the detection limit was determined by using serially diluted DNA from a representative strain of each serotype. Pure genomic templates from 113 strains were used to determine the specificity of the assay in the study. Two independent experiments were performed to establish the sensitivity and specificity of the system.

Ethics Statement

This study was reviewed and approved by the ethics committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The rights and the welfare of the rabbits used in the study were adequately protected. All necessary steps were taken to minimize suffering and distress to the rabbits in these studies.

RESULTS

Grouping and General Features of the O-AGCs

On the basis of sequences and genetic structures of the entire O-AGC regions, the O-AGCs from 52 strains were placed into seven groups (denoted O1–O7) where O1 (n = 17) was the most prevalent, followed by O4 (n = 9), O2 (n = 8), O3 (n = 6), O5 (n = 5), O6 (n = 4), and O7 (n = 3). In our previous studies, we dissected the chemical structures of the O-specific polysaccharides (OPSs) of E. albertii O1, O3, O4, O6, and O7 (Naumenko et al., 2017; Zheng et al., 2017). The predicted gene functions of O-AGCs were consistent with their OPS structures. The data indicated that O-AGCs extracted are responsible for the O-antigen synthesis of E. albertii.

All O-AGCs carried the wzx and wzy genes. The DNA sequence identities of the wzy and wzx genes were >99.9% within the same serotype group and <8% among different serotype groups. Genes coding for enzymes involved in the synthesis of sugars forming the O subunit and glycosyltransferases were found in each serotype group.

Significant differences among the seven groups were also observed where the size ranged from 7.2 kb (O5, including 7
genes) to 16.4 kb (O4, including 16 genes) and the G+C content of seven O-AGCs ranged from 29.2% (O5) to 38.9% (O4). Simple insertions of transposase genes were found in E. albertii O1, O6, and O7 without any gene disruption (Figure 1). Genetic heterogeneity was only found within E. albertii O1-AGC. Within the six other O-AGC groups, we observed high sequence conservation (>99% DNA sequence identity).

Within E. albertii O1-AGC, all strains harbored a transposase gene in the 5′ region. The tandem repeat number of “CTCTG” in the transposase gene was different between the strains (eight types of tandem repeat were found from 11 copies to 73 copies). Meanwhile, two types of transposase gene were found in central regions of E. albertii O1-AGC. Nineteen E. albertii O1 strains were assigned into 10 subtypes based on the variable sequence and organization of transposase genes, named E. albertii O1.1-AGC to O1.10-AGC. E. albertii O1.1-AGC (n = 5) was the dominant subtype (Table 1).

**MLST of E. albertii**

Thirteen sequence types (STs) were found in 52 strains. Eleven of these were reported previously (Wang H. et al., 2016). E. albertii O1, O2, O4, O5, and O7 contained multiple STs (Table 1). All strains with identical STs were clustered into the same serotype group.

**Comparison of O-AGCs between E. albertii and Other Species**

In our previous studies, we have found that the O-antigen of E. albertii O1, O3, O4, O6, and O7 is structurally and genetically related to the O-antigens of other species (Naumenko et al., 2017; Zheng et al., 2017). In the study, we compared the O-AGCs of E. albertii O2 and O5 to those of other species.

| Serotype | No. of beads coupled with “anti-TAG” sequences | Sequence (5′–3′) | Working concentration (µM) | Sensitivity (pg) | PCR product size (bp) |
|----------|---------------------------------------------|-----------------|-----------------------------|-----------------|----------------------|
| O1       | 39                                         | ACAAAATCTAACTACTACAAAA−12C−TCCAGCTTCTTTTCCGGAATTTT | 0.3             | 0.5             | 107                 |
| O2       | 42                                         | *AGTTTCTGCGGTGAAAATAC | 0.3 | 1 | 232 |
| O3       | 43                                         | CACTACATTTTCACATAAGAC−12C−ATAGCGGCGATTTTCTTTTTACTGAA | 0.3 | 1 | 275 |
| O4       | 44                                         | *TAAACCTGAAATACCCGACGAA | 0.3 | 0.5 | 102 |
| O5       | 45                                         | GTAGTCTTCCTTTTACATTACATTAC−12C−TTACTGCGGTTGAGAAGTGG | 0.3 | 10 | 271 |
| O6       | 46                                         | CACTACATTTTCACATAAGAC−12C−ATAGCGGCGATTTTCTTTTTACTGAA | 0.3 | 1 | 212 |
| O7       | 47                                         | TTGATTTTACACATTCAACATAA−12C−AGATATAACCGGCTGTGAATTG | 0.3 | 10 | 250 |
| All (lysP) | 48                                     | AATCAACACACAAATAACCTTATA−12C−GGGCGCTTTCATTTTCTTTCTT | 0.1 | 1 | 252 |

*TAG sequences and the 12-carbon amine containing group are indicated by underline and bold text, respectively. *Indicates reverse primer is biotinylated at the 5′ terminus.

| xTAG sequences and the 12-carbon amine containing group are indicated by underline and bold text, respectively. *Indicates reverse primer is biotinylated at the 5′ terminus. | Working concentration (µM) | Sensitivity (pg) | PCR product size (bp) |
|---------------------------------------------------------------|-----------------------------|-----------------|----------------------|
| O1 (lysP)                                                     | 0.3                         | 0.5             | 107                 |
| O2 (lysP)                                                     | 0.3                         | 1               | 232                 |
| O3 (lysP)                                                     | 0.3                         | 1               | 275                 |
| O4 (lysP)                                                     | 0.3                         | 0.5             | 102                 |
| O5 (lysP)                                                     | 0.3                         | 10              | 271                 |
| O6 (lysP)                                                     | 0.3                         | 1               | 212                 |
| O7 (lysP)                                                     | 0.3                         | 10              | 250                 |
| All (lysP)                                                    | 0.1                         | 1               | 252                 |
strains, *E. albertii* O7 (*n* = 4) was the most prevalent amongst these, followed by O2 (*n* = 3), O1 (*n* = 2), O3 (*n* = 2), O4 (*n* = 2), and O5 (*n* = 2) (Table S1). Another 16 published genomes harbored novel O-AGCs, which were placed into 13 groups. All 16 O-AGCs possessed similar characteristics to those of *E. albertii* O1-7 in that: (i) located in a fixed region between *galF* and *gnd*; (ii) carried the *wzx* and *wzy* genes. It is noteworthy that *wzy* was serotype-specific gene of 13 O-AGCs.

The size ranged from 8.1 kb (CB9791, including 8 genes) to 16.4 kb (NIAH_Bird_23, including 14 genes) and the G+C content ranged from 31.5% (K7394) to 39.1% (CB9791). Thirteen of them were found homologies in O-AGCs of *E. coli*, including O41, O49, O58, O65, O115, O128, O130, O152, O182, and O184 (Table S1). Three of them were not found homologies in any O-AGCs of other species.

Establishment of *E. albertii* O Serogroups

Seven antisera were ultimately selected for the current *E. albertii* serotyping scheme, and all 52 tested *E. albertii* strains were clearly assigned to one of these seven serotypes. The agglutination results of 52 strains were completely consistent with their O-AGCs grouping results. All antisera were specific for their homologous strains. In general, homologous titers were high, varying from 640 to 1280 (Table 3). An additional 12 strains isolated in 2016 were also typed using the seven antisera. Nine of these were typed as *E. albertii* O1, while the others were typed as *E. albertii* O2 (2) and *E. albertii* O4 (1), respectively (Table 1).

Development and Evaluation of a High Throughput xTAG Luminex Assay to Simultaneously Detect Seven O-AGCs

Specific detection was based on the unique sequence of *wzy* for each O-AGC (Table 2). The *wzy* gene was amplified in a multiplex PCR format. The detection limit for the seven O-AGCs varied from 0.5 to 10 pg of purified DNA per reaction.

The performance of this system was tested on 64 strains used in the study. Cross- and non-specific amplification between sequences was not observed. All 64 strains were correctly designated to corresponding serotype groups, which were completely consistent with their agglutination test results.
TABLE 3 | Agglutination of *E. albertii* antisera.

| Antiserum type | Agglutination titer to type strains |
|---------------|-----------------------------------|
| O1 (SP140089) | 1,280                             |
| O2 (SP150020) | 1,280                             |
| O3 (SP140724) | 1,280                             |
| O4 (D140513)  | 1,280                             |
| O5 (T150248)  | 1,280                             |
| O6 (T150072)  | 1,280                             |
| O7 (ZG141049) | 1,280                             |

*Agglutination titers lower than 10 are not shown.*

**DISCUSSION**

Serotyping remains the “gold standard” for identifying and monitoring organisms. The chemical composition and structure of the O antigen show high levels of variation even within a single species revealing it to be serologically diverse. Prior to the present study, little was known about the distribution and diversity of O-AGCs in *E. albertii*. Combination of chemical structures of the O-specific polysaccharides (OPSs) of *E. albertii* (Naumenko et al., 2017; Zheng et al., 2017) and sequence analysis, serotyping scheme in the present study, we defined the characteristics of O-AGCs in *E. albertii*: (i) Similar to *E. coli*, all O-AGCs of *E. albertii* were located in a fixed region of the genome between *galF* and *gnd*. (ii) High diversity among different O-AGCs group was observed. (iii) O-antigens of *E. albertii* were synthesized by the Wzx/Wzy-dependent pathway. The Wzx/Wzy-dependent assembly pathway is conserved in a wide range of both Gram-negative and Gram-positive bacteria, and is encoded in dedicated gene clusters. Within 185 well identified O-AGCs in *E. coli*, 174 of these were synthesized by the Wzx/Wzy pathway (Iguchi et al., 2015). Within 90 serotypes of *Streptococcus pneumoniae*, 88 of these were synthesized by the Wzx/Wzy pathway (Bentley et al., 2006).

In this study, we found and named seven serotypes as *E. albertii* O1–O7 in Chinese strains. Amongst these, the O1 serotype comprised approximately 40% (26/64) of all the strains, which was the most dominant serotype. Even though *E. albertii* O1 was the dominant serotype identified in this study, it was not found in strains from diarrheal patients in the current and previous studies (Fiedoruk et al., 2014; Ooka et al., 2015) (Table 1 and Table S1). Further studies are needed to understand the relationship between serotype and pathogenic potential. Different from Chinese strains, 19 types of O-AGCs were found in 31 public genomes of *E. albertii*, which were composed of 6 serotypes reported in the present study and 13 different O-AGCs. This may suggest that host specificity and ecological environment may contribute to the serotype diversity of strains between China and other countries.

To date, 20 O-AGCs of *E. albertii* were identified. It is relatively lower compared to 185 O serogroups of *E. coli* (Iguchi et al., 2015), 54 serotypes of *Shigella* spp. (Muthirulandi Sethuvel et al., 2017) and more than 200 serotypes of *Vibrio cholerae* (Stine and Morris, 2014). We cannot rule out the possibility that more serotypes may be found with additional testing of *E. albertii* strains.

In this study, we performed an O-antigen serotyping scheme for *E. albertii* based on specific antisera against seven O-antigens, but the conventional serotyping method using the agglutination test with serotype-specific antisera is laborious, time-consuming and expensive. High-throughput molecular serotyping methods allow for simultaneous detection of multiple nucleic acid sequences in a single reaction, and can greatly reduce the time, cost, and work. These technologies have become attractive alternatives to conventional serotyping methods. mPCR coupled to Luminex xTAG technology-based detection provides a clear and attractive approach for multiplex analysis. The low conservation between *wzy* genes of different serotypes means *wzy* gene is an excellent molecular marker for molecular serotyping. In present study, a high throughput xTAG Luminex assay using unique sequence of *wzy* for each serotype to simultaneously detect seven O-AGCs was developed. All the tested strains were accurately typed into seven O-AGCs which were completely consistent with their seroagglutination results. The detection system can be completed in 40 min post-PCR amplification. The limitation of the system is that only seven serotypes revealed in the study can be detected. Thirteen O-AGCs present in public genome of *E. albertii* (Table S1) were not added in the system for lacking strains to evaluate the system. However, the system has great potential to increase the multiplicity in a single reaction.

Comparing to whole genome, the pretty low G+C content of 20 O-AGCs suggest that they may have originated from a different species. *E. albertii* has recently been recognized as a close relative of *E. coli* (Ooka et al., 2015). It is noteworthy that 12 O-AGCs of *E. albertii* were found homologies in O-AGCs of *E. coli*. Additionally, many genes present in O-AGCs of *E. albertii* were also widely distributed in O-AGCs of *E. coli*. Moreover, all strains of *E. albertii* O3 and O6 were agglutinated with *E. coli* O181 and O3 serum, respectively. It is noteworthy that identical O-AGCs among different species have also been reported in previous studies (Sugiyama et al., 1997; Cheng et al., 2006; Feng et al., 2007). Meanwhile, the *E. albertii* strains with identical O-antigens were isolated from diverse sources and belonged to different sequence types. The finding suggested the O-AGCs
can also readily spread among *E. albertii* strains, even among *Enterobacteriaceae*. Further studies are needed to understand the ability of this organism to spread and cause disease.

In conclusion, our data revealed the highly genetic diversity of O-AGCs in *E. albertii* and that *E. albertii* O1 was the dominant serotype. Our study provided valuable serotyping methods for the epidemiological study of this newly emerging enteric pathogen.

**AUTHOR CONTRIBUTIONS**

HZ, HJ, JX, and YX designed the project; HW, QL, XL, LZ, NZ, GY, and ZZ carried out the sampling work; HW, HZ, XYu, JW, PD, and XqL carried out the experiments and generated data; HZ and YX analyzed data and drafted the manuscript. All authors have read and approved the final version of the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2017.01857/full#supplementary-material
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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