

Full Length Research Paper

Clinical isolating outer membrane protein pattern from Avian *Escherichia coli* of China

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The outer membrane protein (OMP) were extracted from 38 strains of avian *Escherichia coli* which were isolated from the dead chickens of chicken breeding farms in 3 areas of Baoding, Qinhuangdao and Beijing by using ultrasonic cleaving and N-Lauroyl sarcosine sodium and OMP typing was done by SDS-PAGE to understand the genetic relationship of isolated strains from Avian pathogenic *E. coli*. There were 3 OMP types in these 38 strains of *E. coli*, in which, type OMP-1 was in common for 6 serotypes of isolated strains of O₇₈, O₈₈, O₂, O₁₈, O₉₃ and O₇₆, type OMP- α belonged to isolated strain of O₇₆, OMP- β was found from all of the isolated strains of O₉₃, O₇₈, O₈₈, O₂, this indicated the identical serotype strain might belong to quite different OMP patterns and among isolated strains without any relationship in serotype could have the same OMP type. Among isolated strains with the same serotype strains might have genetic differentiation. And among the isolated strains of different serotypes, there might be the genetic relationship with different degree.

Key words: Avian *Escherichia coli*, outer membrane protein, O serotypes.

INTRODUCTION

Outer membrane protein (OMP) is the main structure and half of cell wall of gram-negative bacteria. OMP is consisted of major OMP and trace of protein. The molecular weight of sub-units of major OMP is about 30 - 40 kD and the descending order of molecular weight is as follows: microporous protein, K protein, outer membrane protein A and Plasmid coded protein (PCP). Microporous protein contains OMPF and OMPC which both have good penetration for most of water-soluble antibiotic drugs (Nikaidoh et al., 1992). Therefore, the OMP of *E. coli* plays an important role in drug-resistant process (Nikaidoh et al., 1983; Lu et al., 2002). The components of OMP are encoded by chromosome except PCP. The difference between major OMP and its mobility can indirectly reveals the difference of OMP encoding gene in chromosome of isolated strains. The results showed that *E. coli* OMP achieve their pathogenic effects mainly by helping bacteria escape the immune defense and promote adsorption of host cells. Fantinatti et al. (1994) found that the 40700 and 28800 of major OMP were absent in non-pathogenic strains through analyzing

the OMP types with SDS-PAGE, these 2 proteins could play a role in the pathogenic process of sepsis caused by avian *E. coli*. *E. coli* OMP antibodies also have a passive immune protection effect. Gao et al. (2001) used purified OMP as coating antigen and detected OMP antibodies produced by chicken suffered from colibacillosis with enzyme-linked immunosorbent assay (Gao et al., 2001). Yu et al. (2008) amplified *pilA* gene and OMP C gene of avian pathogenic *E. coli* strain, the expressed fimbriae and OMP C were transformed into vaccine and the protective immune response was proved after the mice were immunized (Yu et al., 2008). Chen et al. (2005) analyzed the OMP of *E. coli* O₂, O₇₈ and resistant fusion strain isolated from poultry through SDS-PAGE, the results showed that the major OMP bands were the same and had a cross-protective immunity. Through protoplast fusion of avian *E. coli* to construct *E. coli* OMP fusion strains by both parents OMP antigen and O antigen, laid the foundation for the development of new *E. coli* bivalent vaccine (Ding et al., 2004). These studies show that the OMP plays an important role in occurrence and immune protection of avian colibacillosis. It is significant in immune treatment of avian colibacillosis to understand the genetic relationship of isolated strains from avian pathogenic *E. coli* in each area and screen out the dominant OMP strains. We have isolated 38 strains of avian *E. coli*

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from the dead chickens of chicken breeding farms in 3 areas of Baoding, Qinhuangdao and Beijing and extracted the OMP by using ultrasonic cleaving and N-Lauroyl sarcosine sodium and OMP typing was done by SDS-PAGE.

MATERIALS AND METHODS

Experimental strains and serum

A total of 38 strains of Avian *E. coli* were collected from the dead chickens of chicken breeding farms in 3 areas of Baoding, Qinhuangdao and Beijing and identified as pathogenic *E. coli* by conventional method. There were 13 strains from Baoding, 13 strains from Qinhuangdao and 12 strains from Beijing and numbered as follows: BD1, BD2, BD3, - BD13; QHD1, QHD2, - , QHD13; BJ1, BJ2 - BJ12. The diagnostic serum of *E. coli* O factor was purchased from Chinese veterinary medicine monitor.

Main reagents and instruments

The 0.1 mol/l Tris-HCl (pH 6.8) buffer containing 4% SDS, 20% glycerol, 5% mercaptoethanol and 0.002% bromophenol blue; tryptone and yeast extract (LB medium) were purchased from oxid corporation; Acrylamide (Acr), N-N-methylene-Bis-Acrylamide(Bis), tris-aminomethane(Tris), sodium dodecyl sulfate (SDS), over samine (APS), tetramethylethylenediamine (TEMED), coomassie brilliant blue R250, HEPES and N-Lauroyl sarcosine sodium were purchased from Sigma-Aldrich Corp.(St. Louis, USA); low molecular weight standard proteins were supplied by Chinese academy of sciences Shanghai institute of Biochemistry (Shanghai, China). Low-temperature high-speed centrifuge (6K15), stable voltage and current electrophoresis apparatus (DYY- β 2), electrophoresis tank (DYY- β 28A), ultrasonic cell crushing instrument (FS300).

Identification of *E. coli* O serotype

The identifications of O serotype were carried out according to the instructions.

Extraction of OMP

The preserved pure culture of *E. coli* was inoculated into 2 ml LB broth and incubated at 37°C with shaking for 6 - 7 h. Then the 1 ml culture fluid was inoculated into 100 ml LB culture medium and incubated at 37°C with shaking overnight. Then they were centrifuged at 4000 r/min for 5 min and the precipitate was collected and suspended in 3 ml 10 mmol/l HEPES, after these cells were crushed by low-temperature ultrasonic, they were centrifuged at 7,000 r/min for 15 min at 4°C. The supernatant were collected and the 0.75 ml 2% lauroyl sarcosine sodium were added, after 20 min at room temperature, they were centrifuged at 10,000 r/min for 1 h at 4°C. The precipitate was dissolved with 3 ml 10 mmol/l HEPES and equal volume of 2% Lauroyl sarcosine sodium solution, then the above step was repeated again. The precipitate was dissolved with an appropriate amount of 10 mmol/l HEPES and stored at -20°C.

Analysis of OMP

The analysis of OMP was conducted by SDS-PAGE method (4.8%

concentrated gel and 10% separation gel). The 10 μ l OMP samples of *E. coli* were added in the 10 μ l 2 \times Tris-HCl buffer and heated in boiling water for 5 min and then cooled to room temperature. The low molecular weight standard proteins and above OMP samples were infused to the sample tanks by microsyringe. Then the electrophoresis was conducted with 20 mA constant current and stopped it until the indicator reached at a distance of 1 cm from the silicone rubber frame bottom. After electrophoresis, the gels were immersed in 0.25% coomassie brilliant blue staining for 1 h. Then staining fluids were discarded and the gels were rinsed thoroughly with distilled water several times. Finally, these gels were kept bleaching by bleaching solution with shaking until the protein bands clearly appeared, then the results were recorded.

RESULTS AND ANALYSIS

OMP types of 38 strains of Avian *E. coli*

After isolated 38 strains of Avian *E. coli* were analyzed by SDS-PAGE and coomassie brilliant blue staining, a few of protein bands appeared and the main protein band at 30 - 43 kD. OMP-type was determined according to the amount of protein bands in 30 - 40 kD lanes of SDS-PAGE. OMP-I type appeared 2 bands, 3 bands belonged to OMP- α type, there was one band in the lane leaded to OMP- β type.

OMP types of 13 BD strains of Avian *E. coli*

The results as shown in Figure 1, there were 11 BD strains appeared 2 bands with similar molecular weight in 1st, 2nd, 3rd, 4, 8, 9, 10, 11, 12, 13 and 14th lanes, respectively, therefore their OMP type belonged to OMP-I type; the OMP type of BD strains in 5 and 6th lanes consisted of one band and belonged to OMP- β type.

OMP types of 13 QHD strains of Avian *E. coli*

The results as shown in Figure 2, the OMP type of 9 QHD strains consisted of 2 bands with similar molecular weight in 1st, 2nd, 3rd, 5, 10, 11, 12, 13 and 14th lanes belonged to OMP-I type; the OMP type of three QHD strains consisted of 3 bands in 4, 8 and 9th lanes belonged to OMP- α type; the OMP type of one QHD strain consisted of one band in 6th lane belonged to OMP- β type.

OMP types of 12 BJ strains of Avian *E. coli*

The results as shown in Figure 3, the OMP type of nine BJ strains consisted of 2 bands with similar molecular weight in 2nd, 3rd, 5th, 6, 8, 9, 10, 12 and 13th lane belonged to OMP-I type; the OMP type of one BJ strain consisted of one band in 1st, 4th and 11th lane belonged to OMP- β type.

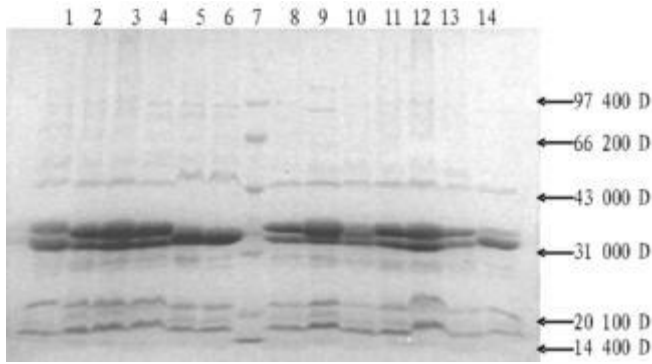


Figure 1. OMP type of *E. coli* isolates from chicken in Baoding Lane 1 - 6. BD1 - BD6; Lane 7. Marker protein with low molecular weight; Lane 8 - 14. BD7 - BD 13.

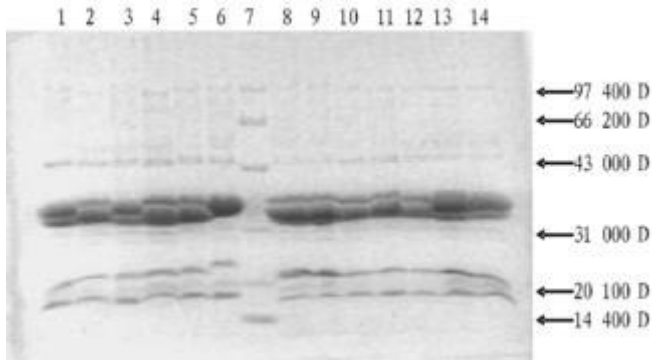


Figure 2. OMP type of *E. coli* isolates from chicken in Qinhuangdao. Lane 1 - 6. QHD 1c - QHD 6; Lane 7. Marker protein with low molecular weight; Lane 8 - 14. QHD 7 - QHD 13.

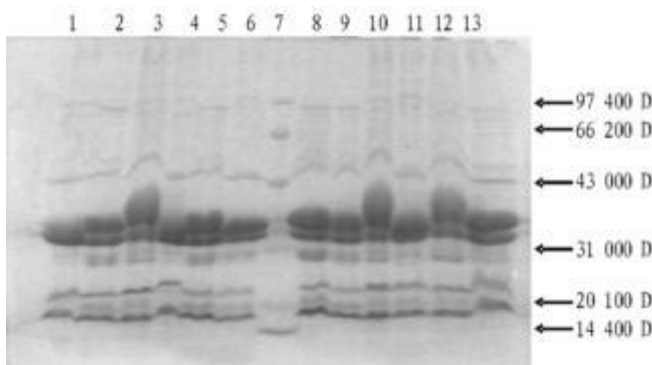


Figure 3. OMP type of *E. coli* isolates from chicken in Beijing. Lane 1-6. BJ 1 - BJ 6; Lane 7. Marker protein with low molecular weight; Lane 8 -13. BJ 7 - BJ 12.

Comparison of OMP type and serotype

As shown in Table 1, there were 3 OMP types in these 38 strains of *E.coli*. Among different serotypes could have

the same OMP type, while among the same serotypes have different OMP types.

DISCUSSION AND CONCLUSIONS

At present, there is no unified standard on division of OMP type of *E. coli*, which needs further study and develop a unified standard. Dassouli et al. (1988). divided the 21 strains of *O*₇₈ *E. coli* included 3 Avian *E. coli* strains isolated from septic animals into OMP-A, B1, B2, C, D-type. The difference of OMP1–OMP6 type divided by Achtmanm and Dho (Achtmanm et al., 1986; Dhomm et al., 1990) indicates that the OMP type is divided on author own. We determine the OMP type of *E. coli* according to the order of protein band appearing in the SDS-PAGE and this does not affect the study on the genetic relationship of isolated strains from *E. coli*.

The study on OMP type extracted from 38 strains of Avian *E. coli* from 3 areas of Baoding, Qinhuangdao and Beijing has shown that there were 3 OMP types in these 38 strains of *E. coli*, in which, type OMP-1 was in common for 6 serotype of isolated strains of *O*₇₈, *O*₈₈, *O*₂, *O*₁₈, *O*₉₃ and *O*₇₆, type OMP- α belonged to isolated strain of *O*₇₆, OMP- β was found from all of the isolated strains of *O*₉₃, *O*₇₈, *O*₈₈, *O*₂ (Table 1). This indicated that the identical serotype strain might belong to quite different OMP patterns and among isolated strains without any relationship in serotype could have the same OMP type. This also verified that among isolated strains with the same serotype strains might have genetic differentiation and among the isolated strains of different serotypes might have the genetic relationship with different degree. Sequenced and analyzed three OMPA genes of Avian *E. coli*, and found that they had exactly the same nucleotide sequence (Cao et al., 2004), CONFIRMED *O*₂, *O*₇₈ have the same outer membrane protein antigen on the genetic level. Yu et al determined the outer membrane patterns of 130 isolates ducks pathogenic *E. coli* of 34 serotypes, the results showed there were 4 different OMP patterns among all strains tested, OMPA genes of 8 strains height virulence and 2 strains medium virulence were cloned and the nucleotide sequences had high homology with an identity ranging from 95.8% to 100% among 10 pathogenic *E. coli* strains from ducks (Yu et al., 2009).

Ding et al. (2003) also identified the OMP types of 6 advantage serotypes isolated strains of *O*₇₈, *O*₈₈, *O*₂, *O*₄₅, *O*₅₃, and *O*₁₄₅ and found that 6 isolated strains of *O*₇₈ contained 3 OMP types, three isolated strains of *O*₈₈, 3 isolated strains of *O*₂ and 2 isolated strains of *O*₁₄₅ appeared two OMP types, respectively, but there were only one OMP type found in two isolated strains of *O*₄₅ and *O*₅₃. Thus, the identical serotype strains might belong to different bacteria cloning and have different origins, and the strains without any relationship in serotype could belong to the same bacteria cloning and have common origins. OMP type can be used as genetic marker of Avian pathogenic *E. coli*, while the conventional

Table 1. Comparison of serotypes and OMP type of 38 strains of *E. coli* isolates from chicken.

No. of strains	OMP type	Serotype	No. of strains	OMP type	Serotype	No. of strains	OMP type	Serotype
BD1	OMP-I	O ₂	QHD1	OMP-I	O ₁₈	BJ1	OMP-β	O ₈₈
BD2	OMP-I	O ₁₈	QHD2	OMP-I	O ₁₈	BJ2	OMP-I	O ₈₈
BD3	OMP-I	O ₁₈	QHD3	OMP-I	O ₁₈	BJ3	OMP-I	O ₈₈
BD4	OMP-I	O ₉₃	QHD4	OMP-α	O ₇₆	BJ4	OMP-β	O ₂
BD5	OMP-β	O ₉₃	QHD5	OMP-I	O ₇₈	BJ5	OMP-I	O ₇₆
BD6	OMP-β	Unidentified	QHD6	OMP-β	O ₇₈	BJ6	OMP-I	unidentified
BD7	OMP-I	O ₁₈	QHD7	OMP-α	O ₇₆	BJ7	OMP-I	unidentified
BD8	OMP-I	Unidentified	QHD8	OMP-α	O ₇₆	BJ8	OMP-I	O ₈₈
BD9	OMP-I	O ₁₈	QHD9	OMP-I	O ₈₈	BJ9	OMP-I	O ₈₈
BD10	OMP-I	O ₁₈	QHD10	OMP-I	O ₇₆	BJ10	OMP-β	O ₈₈
BD11	OMP-I	O ₁₈	QHD11	OMP-I	O ₁₈	BJ11	OMP-I	O ₂
BD12	OMP-I	O ₁₈	QHD12	OMP-I	unidentified	BJ12	OMP-I	O ₇₆
BD13	OMP-I	O ₁₈	QHD13	OMP-I	O ₇₆			

O serotyping technique can not fully reflect the genetic relationship among strains and can not be used as the cloning marker of isolated strains. Achtman et al. (1986) also pointed out that the serotypes did not reflect the cloning relationship of *E. coli* because there no universal rules on difference between serotype and cloning.

In recent years, vaccine immunization has become an important means of controlling the Avian colibacillosis, the inactivated vaccine of *E. coli* has been developed based on the O serotyping (multivalent vaccine made of representative strains with different serotypes). Because the serotypes do not reflect the genetic relationship of isolated strains of Avian *E. coli* and multivalent vaccines do not meet the both requirements of protective force and antigen content, and monovalent vaccines only have protections in homologous strains, thereby it is difficult to achieve the perfect immunizing effects with selecting the vaccine strains base on the O serotyping. Therefore, it requires further research whether the multivalent has better immunizing effects based on the OMP types or not.

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