Journal of Animal and Veterinary Advances 12 (14): 1233-1236, 2013
ISSN: 1680-5593

Prevalence and Characterization of Serotype and out Membrane Protein Diversity of *Escherichia coli* Isolated from Southern China

Rui-Ai Chen, Bing Li, Xianhao Zhang, Haopeng Liu and Dongsheng He
Key Laboratory of Biotechnology and Bioproducts Development for Animal Epidemic Prevention, Ministry of Agriculture, South China Agricultural University, 510642 Wushan, Guangzhou, China

Abstract: Seventy four pathogenic swine *Escherichia coli* strains were isolated from large-scale pig farms in 5 provinces in Southern China. Fourty seven kinds of single-factor O-antigen serum were used to classify the serotypes of isolates. The serotypes of 66 of 74 swine *Escherichia coli* strains had been identified which belonged to 14 serotypes. O_{107}, O_{101}, O_9, O_{26} and O_{60} were the dominant serotypes. There were 8 strains failed to be identified, accounting for 10.8% of the total strains. Eighteen swine *Escherichia coli* strains were selected from the dominant serotypes to pathogenicity test in mice. The results showed that 7 of 18 representative strains were virulent strains. Further, researchers analyzed the Outer Membrane Protein (OMP) of 7 virulent strains, the result showed that OMP of 7 strains, respectively from 1-3 protein bands which belonged to 3 OMP type (I~III). This is first time in Southern China to investigate systematically on the predominant serotypes of pathogenic swine *Escherichia coli* and laid a foundation on the prevention and control of porcine colibacillosis as well as the research of new OMP subunit vaccine.

Key words: *Escherichia coli*, serotype, outer membrane protein, mice, pathogenic swine

INTRODUCTION

Escherichia coli (*E. coli*) belong to Gram-negative bacillus which can lead to colibacillosis in pigs of all age especially for piglets. According to the age and clinical symptoms can be divided into yellow dysentery of newborn piglets, white dysentery of postweaning piglets and Edema disease (Pluske, 2013; Russo and Johnson, 2003; Griffin and Tauxe, 1991; Karmali, 1989; Miniats and Roe, 1968). The disease is epidemic in many countries and regions which cause high morbility, mortality and huge economic losses (Ding *et al*., 2012). It is one of the important diseases affecting pig production which takes difficulty to the prevention and control. The classification of *E. coli* was mainly based on the O-antigen (Moredo *et al*., 2012; Evans *et al*., 1980; Osman *et al*., 2012; Zhang *et al*., 2012; Glantz and Jacks, 1969; Cai *et al*., 2005). Presently, 173 kinds of O-antigen have been identified. The O_8, O_9, O_{20}, O_{60}, O_{101}, O_{141}, O_{149}, O_{157} and others may cause porcine colibacillosis. The prevention of this disease mainly adopts vaccine approach. Due to low cross protective immunity of vaccines between the numerous serotypes *E. coli*, the colibacillosis prevention should base on full investigation of the predominant pathogenic serotypes. Correspondingly, the vaccine can only be used in small range (Johnson *et al*., 1996). OMP of *E. coli* has good immunogenicity (Ruiz *et al*., 2001; Bhattacharjee *et al*., 1996; Ruiz *et al*., 2001; Liechti and Goldberg, 2012) which may have cross protection to different strains. Development of OMP subunit vaccine may be much effective. One hundred and thirty four samples were collected from 38 large-scale pig farms in 5 provinces in Southern China to investigate the epidemic situation and dominant serotype distribution of porcine colibacillosis. At the same time, comparative analysis on the OMP of the *E. coli* virulent strains were studied. It provides the theoretical basis for the prevention and control of the disease and lays a foundation to the research of *E. coli* OMP subunit vaccine.

MATERIALS AND METHODS

Samples collection, bacterial culture, biochemical tests and serotypes classify: Porcine samples (including liver, spleen, mesenteric lymph nodes and other organ) were collected from 38 large-scale pig farms in 5 provinces in South China (Table 1). The samples were cultured on MocConkey medium for the initial isolation of the bacteria for 18 h at 37°C. The single pink colonies were picked and seeded on eosin methylene blue medium which was used to isolate the colonies with metallic luster. Smear staining

Corresponding Author: Dongsheng He, Key Laboratory of Biotechnology and Bioproducts Development for Animal Epidemic Prevention, Ministry of Agriculture, South China Agricultural University, 510642 Wushan, Guangzhou, China

to detect whether the bacteria were Gram-negative bacteria by their morphology. The biochemical properties were observed and recorded including glucose, maltose, nitrates, lactose, indole, MR, sucrose, gelatin and VP. Seventy four swine *E. coli* strains have been defined by isolation and culture, morphological characteristics and biochemical tests. Forty seven kinds of *E. coli* O-antigen single-factor serum were used to glass agglutination test (Wilson and Svendsen, 1972) to serotype the *E. coli* strains (Evans *et al*., 1980; Glantz and Jacks, 1969; Cai *et al*., 2005).

Pathogenicity test in mice: Analyzed the glass agglutination test result, 18 swine *E. coli* strains were selected as the dominant serotypes and inoculated to Kunming SPF mouse. Each groups including 4 mice and each mice received 10^9 CFU mL^{-1} bacteria in 0.2 mL through intraperitoneal injection route. Four SPF mice were kept as control and each of them received 0.2 mL nutrient broth through the same route. The morbidity and mortality of mice were recorded. Liver of dead mice was selected sterilely and cultured on MocConkey medium for 18 h at 37°C, the isolates were re-identified by conventional method. Bacterial virulence assessment standards: Strains can lead to 3 or 4 mice died are virulent strains, 2 died were medi-virulent strains, 1 died were low strains, not lethal to avirulent strains.

Table 1: Source of 74 isolates of *E. coli* from large-scale pig farms in Southern China

Districts	No. of isolates	Organ sources
Guangdong		
Huayang	3	Liver, mesenteric lymph nodes and spleen
Ruhu	3	Liver, mesenteric lymph nodes, kidney and heart
Hengli	5	Liver, mesenteric lymph nodes, kidney and spleen
Huizhou	3	Liver, kidney, spleen and heart
Conghua	2	Liver, kidney and spleen
Enping	7	Liver, mesenteric lymph nodes, kidney, spleen and heart
Guangxi		
Bobai	4	Liver, kidney and spleen
Xingye	2	Liver
Pingguo	3	Liver, mesenteric lymph nodes and kidney
Shaoping	5	Liver, mesenteric lymph nodes, kidney, spleen and heart
Luchuan	2	Liver and spleen
Beihai	5	Liver, kidney and spleen
Fujian		
Zhuzhou	4	Liver, kidney and spleen
Yongxing	2	Liver and kidney
Chenxi	3	Liver, kidney and spleen
Hunan		
Zhuzhou	6	Liver, mesenteric lymph nodes, kidney and spleen
Yongxing	4	Liver, heart and spleen
Chenxi	3	Liver, kidney and spleen
Jiangxi		
JiAn	4	Liver, mesenteric lymph nodes, kidney and spleen
Fuzhou	3	Liver, kidney and spleen
Shangrao	1	Liver

The outer membrane protein of dominant *E. coli* serotypes preparation: Seven *E. coli* virulent strains were selected and cultured on nutrient agar plates. Single colonies were inoculated into 6 mL nutrient broth for 18 h at 37°C. The cells were harvested by centrifugation at 6,000 rpm min^{-1} for 10 min at 4°C. The precipitation was re-suspended in 5 mL HEPES (10 mmol L^{-1}, pH 7.4) and decomposed by ultrasonic cell fragmentation instrument for 10 min at 78 W. The precipitation were collected by centrifuged at 6,000 rpm min^{-1} for 10 min at 4°C then mixed them with 2% N-lauroyl sarcosine (5/1 V/V) and put at room temperature for 30 min. The precipitation was collected after ultracentrifugation and was suspended in 4 mL HEPES (10 mmol L^{-1}, pH 7.4). The production was analyzed by SDS-PAGE.

RESULTS

Seventy four swine *E. coli* strains were isolated from the samples collected in Southern China. Cultured on MocConkey medium for 18 h at 37°C, the colonies are pink, orbicular, flattened, neat edge, smooth surface. Cultured on eosin methylene blue medium for 18 h at 37°C, the colonies are purple with metallic luster. Staining and observing under microscope, these colonies blunt ends Gram-negative bacteria. Biochemical test results suggested that 74 isolates are all *E. coli* (Fig. 1).

The glass agglutination test suggested that 66 of 74 swine *E. coli* strains belonged to 14 different serotypes. The dominant serotypes were O_{107}, O_{101}, O_9, O_{60}, O_{26}, accounting for 70.3% of the total. Another 8 strains remain undefined, accounting for 10.8% (Fig. 2). Eighteen representative strains were selected from the dominant serotypes O_{107}, O_{101}, O_9, O_{60} and O_{26} to pathogenicity test in mice. According to the results there were 7 virulent strains among the 18 strains, 7 medi-virulent strains, 2 low virulent strains and 2 avirulent strains (Fig. 3).

Serotyped in O_{107}, O_{101}, O_9, O_{60} and O_{26}, respectively the OMP of 7 swine *E. coli* virulent strains were extracted

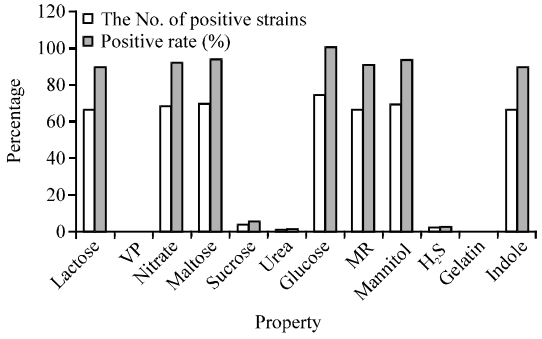

Fig. 1: Biochemical identification of the isolated strains

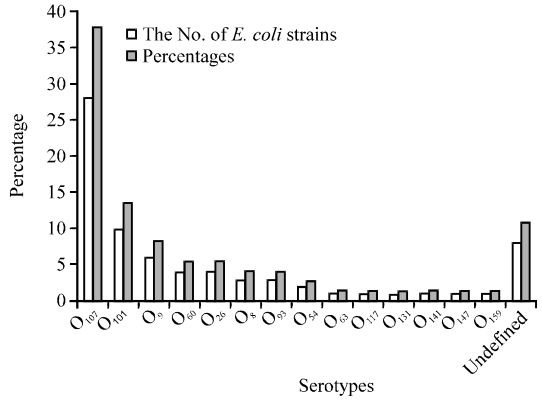

Fig. 2: Serotype classification of *E. coli* isolated from Southern China

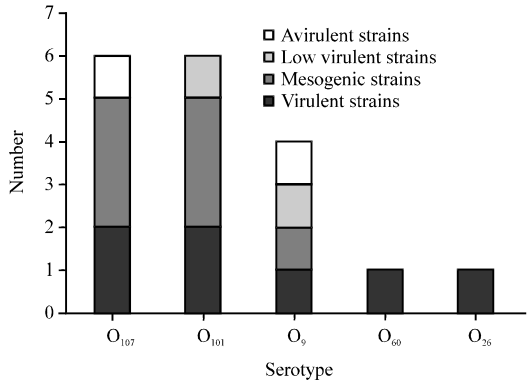

Fig. 3: Pathogenicity test of serotyped swine *E. coli*

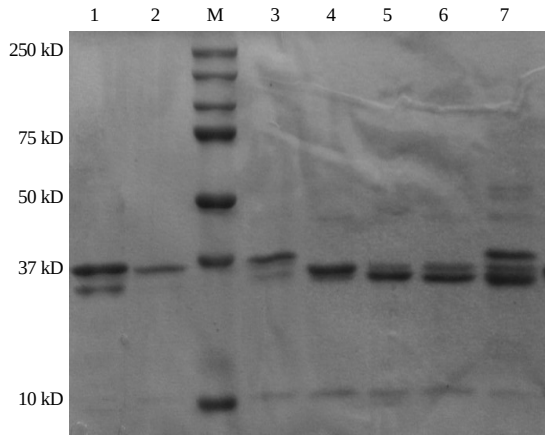

Fig. 4: Results OMP of 7 virulent strain SDS-PAGE electrophoresis. M = Marker; 1 = GX-1 belongs to O_{101} serotype; 2 = GX-2 belongs to O_{101} serotype; 3 = FJ-1 belongs to O_{60} serotype; 4 = GD-1 belongs to O_{107} serotype; 5 = GD-2 belongs to O_{107} serotype; 6 = GD-3 belongs to O_{26} serotype; 7 = GD-4 belongs to O_9 serotype

and analyzed by SDS-PAGE. The protein bands were between 31~40 kD, mainly in 37 kD that coincided with the reported molecular mass of OMP extracted from poultry *E. coli*. The result suggested that the OMP proteins were extracted successfully (Fig. 4). Presently there are no standards to distinguish the type of the OMP protein. The OMP protein were classified by the number of the protein bands between 31~40 kD. Each *E. coli* strains had 1~3 main OMP bands located between 31~40 kD. Base on this result, researchers classified the virulent *E. coli* strains into 3 types, OMP-I, OMP-II and OMP-III. The main OMP of GX-2 and GD-1 *E. coli* strains were similar and composed of 1 protein band, classified as OMP-I. GX-1, FJ-1, GD-2 and GD-3 *E. coli* strains were similar and composed of 2 protein bands, classified as OMP-II. GD-4 *E. coli* strain composed of 3 protein bands, classified as OMP-III.

DISCUSSION

In this study, the 74 swine *E. coli* strains were isolated and serotyped in large-scale pig farms in Southern China firstly. When serotyping the swine *E. coli*, the O-antigen of certain bacteria could react with several single-factor sera. Some *E. coli* strains reacted with several single-factor sera due to the cross-reaction of O-antigen. These strains may share the same epitopes or have several surface antigens. Presently, serological method is widely used for the typing of *E. coli*, further studies on common antigen of *E. coli* need to be developed.

The prevalence serotypes of swine pathogenic *E. coli* will change in different regions and time (Cai *et al*., 2005; Johnson *et al*., 1996). Even, in the same area may change when time passed. In 2005, Shanxi Province, the predominant serotypes of swine *E. coli* were O_{139}, O_{101}, O_{149}, O_{141} and O_{60}. In 2008, the results of *E. coli* investigation of some farms in East China suggested that O_{149}, O_{107}, O_{139}, O_{93} and O_{91} were the dominant serotypes. In 2009, the dominant *E. coli* serotypes of Guangdong Province were O_{65}, O_{131}, O_8 and O_{158}. In 2010, the dominant serotypes were O_{138} and O_9. In the research, 74 swine *E. coli* strains belonged to 14 specific serotypes and 8 strains were unclassified. The dominant *E. coli* serotypes were O_{107}, O_{109} and O_9. Whether the 8 strains belonging to the serotypes in addition to the 47 major types or the new epidemic serotypes, needed to further research. In the provinces in Southern China, the *E. coli* serotypes were diversity distribution. In the same large pig farm several serotypes strains existed at the same time. Therefore, the investigation of *E. coli* in large pig farms must be conducted periodically. Prevention and control of porcine colibacillosis is to develop effective vaccine.

Generally, enzyme electrophoresis method or determination of OMP type was used to study the genetic correlation of *E. coli*. OMP type determination is mainly through determination of OMP and comparison of different strains to be classified. The classification of *E. coli* OMP has no uniform standards. In this study, the types were classified based on the number of OMP bands. SDS-PAGE result suggested that 8 *E. coli* strains of 5 serotypes were divided into 3 subtypes of OMP. Strains in O_{26}, O_{101} and O_{107} belonged to the same OMP type. But the same serotype isolations such as O_{107}, belonged to different OMP type. To sum up, the same serotype isolations might belong to different OMP types. The different serotype isolations might have the same OMP type.

In recent years, vaccine immunization was used to control porcine colibacillosis. But the polyvalent vaccine can not meet the requirements of protection. Based on the OMP type, the immunodominant antigen as subunit vaccine has broad prospects.

CONCLUSION

In this research, researchers made a systematic investigation on the predominant serotypes of swine pathogenic *E. coli* in Southern China. Sixty six swine *E. coli* isolates belonged to 5 specific serotypes. The higher virulent strains could be found in each 5 dominant serotypes and OMP of virulence strain was preliminarily studied. The research laid a foundation for the disease control and the OMP subunit vaccine.

REFERENCES

Bhattacharjee, A.K., S.M. Opal, R. Taylor, R. Naso and M. Semenuk *et al*., 1996. A noncovalent complex vaccine prepared with detoxified *Escherichia coli* J5 (Rc chemotype) lipopolysaccharide and *Neisseria meningitidis* Group B outer membrane protein produces protective antibodies against Gram-negative bacteremia. J. Infect. Dis., 173: 1157-1163.

Cai, X., H. Chen, P.J. Blackall, Z. Yin, L. Wang, Z. Liu and M. Jin, 2005. Serological characterization of *Haemophilus parasuis* isolates from China. Vet. Microbiol., 111: 231-236.

Ding, Y., X. Tang, P. Lu, B. Wu and Z. Xu *et al*., 2012. Clonal analysis and virulent traits of pathogenic extraintestinal *Escherichia coli* isolates from swine in China. BMC Vet. Res., Vol. 8. 10.1186/1746-6148-8-140.

Evans, D.J., D.G. Evans, L.S. Young and J. Pitt, 1980. Hemagglutination typing of *Escherichia coli*: Definition of seven hemagglutination types. J. Clin. Microbiol., 12: 235-242.

Glantz, P.J. and T.M. Jacks, 1969. A bacteriological and serological study of experimental *Escherichia coli* infection of calves. Can. J. Comp. Med., 33: 128-133.

Griffin, P.M. and R.V. Tauxe, 1991. The epidemiology of infections caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli* and the associated hemolytic uremic syndrome. Epidemiol. Rev., 13: 60-98.

Johnson, R.P., W.C. Cray and S.T. Johnson, 1996. Serum antibody responses of cattle following experimental infection with *Escherichia coli* O157: H7. Infect. Immun., 64: 1879-1883.

Karmali, M.A., 1989. Infection by verocytotoxin-producing *Escherichia coli*. Clin. Microbiol. Rev., 2: 15-38.

Liechti, G. and J.B. Goldberg, 2012. Outer membrane biogenesis in *Escherichia coli*, *Neisseria meningitidis* and *Helicobacter pylori*: Paradigm deviations in *H. pylori*. Front. Cell. Infect. Microbiol., Vol. 2. 10.3389/fcimb.2012.00029.

Miniats, O.P. and C.K. Roe, 1968. *Escherichia coli* infections in early weaned pigs. Can. Vet. J., 9: 210-217.

Moredo, F.A., J.A. Cappuccio, L. Insarralde, C.J. Perfumo, M.A. Quiroga and G.A. Leotta, 2012. Genotypic characterization of toxigenic *Escherichia coli* isolated from pigs with postweaning diarrhea (PWD) and edema disease (ED). Revista Argentina Microbiologia, 44: 85-88.

Osman, K.M., A.M. Mustafa, M. Elhariri and G.S. AbdElhamed, 2012. Identification of serotypes and virulence markers of *Escherichia coli* isolated from human stool and urine samples in Egypt. Indian J. Med. Microbiol., 30: 308-313.

Pluske, J.R., 2013. Feed-and feed additives-related aspects of gut health and development in weanling pigs. J. Anim. Sci. Biotechnol., Vol. 4. 10.1186/2049-1891-4-1.

Ruiz, A., S. Oliveira, M. Torremorell and C. Pijoan, 2001. Outer membrane proteins and DNA profiles in strains of *Haemophilus parasuis* recovered from systemic and respiratory sites. J. Clin. Microbiol., 39: 1757-1762.

Russo, T. and J.R. Johnson, 2003. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: Focus on an increasingly important endemic problem. Microbes Infect, 5: 449-456.

Wilson, M.R. and J. Svendsen, 1972. Immunity to *Escherichia coli* in pigs: Serum gamma globulin levels, indirect hemagglutinating antibody titres and bactericidal activity against *E. coli* in pigs up to five weeks of age. Can. J. Comp. Med., 36: 38-43.

Zhang, J., C. Xu, L. Guo, H. Shen and X. Deng *et al*., 2012. Prevalence and characterization of genotypic diversity of *Haemophilus parasuis* isolates from southern China. Can. J. Vet. Res., 76: 224-229.