Journal of Microbiology, Immunology and Infection (2017) 50, 382–385

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BRIEF COMMUNICATION

Phylogenetic grouping and distribution of virulence genes in *Escherichia coli* along the production and supply chain of pork around Hubei, China

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Received 25 March 2015; received in revised form 17 February 2016; accepted 9 March 2016
Available online 31 March 2016

KEYWORDS
extraintestinal pathogenic *E. coli*;
phylogenetic grouping;
production;
supply chain;
virulence genes

Abstract *Escherichia coli* is an important foodborne zoonotic pathogen. A total of 285 strains of *E. coli* were isolated from the production and supply chain of pork in Hubei, China and characterized. Their phylogroups (A, B1, B2, and D) and virulence genes of public health importance become more and more diverse along the production and supply chain.

Escherichia coli are important causative agents of intestinal and extraintestinal diseases in humans and animals. *E. coli* are divided into four phylogenetic groups (A, B1, B2, and D). Extraintestinal pathogenic *E. coli* (ExPEC) belong mainly to group B2 and, to a lesser extent, to group D, whereas commensals belong to groups A and B1.[1] ExPEC are able to colonize and cause diseases in human such as urinary tract infection, septicemia, and meningitis in newborn babies. Molecularly, ExPEC can be defined as *E. coli* isolates that possess two or more virulence genes including *papA*, *papC*, *sfa/foc*, *afa/dra*, *kpsM II*, and *iutA*. These virulence factors assist in invasion and colonization of the host, disruption of host defense mechanisms, and induction of disease outside the intestine.[2–6]

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http://dx.doi.org/10.1016/j.jmii.2016.03.006

Little is known about the occurrence of ExPEC along the production and supply chain of pork (PSCP). Therefore, we carried out this study to probe the distribution of virulence genes in different phylogenetic groups of *E. coli* along the PSCP.

A total of 285 samples including 125 tonsil swabs from five intensive pig farms (4- to 6-week-old healthy pigs) and 160 tissue samples from different slaughterhouses (20 each for meat, livers, intestine, and kidneys), wet markets, and supermarkets (40 each for meat and livers) located in Hubei province were aseptically collected and transported to the laboratory under refrigeration temperature.

Prior to inoculation on MacConkey agar plate (Difco, Sparks, MD, USA), tonsil swabs were washed with phosphate-buffered saline, whereas tissue samples (50 g) were homogenized in brain—heart infusion broth (BHI; Difco, USA) and incubated at 37°C for 24 hours. Typical lactose fermenting, pink colonies (one colony/sample) on MacConkey agar plate were selected for further confirmation using an API 20E system (bioMerieux, Marcy-l'Etoile, France) as previously described.[2,7] Genomic DNA was extracted from the isolates using E.Z.Nce.A bacterial DNA kit (Omega Bio-Tek, Norcross, GA, USA).

All isolates were investigated for phylogenetic groups (A, B1, B2, and D) of *E. coli*, and 13 ExPEC-related virulence genes (*kpsM II*, *papA* and *papC*, *iutA*, *sfaS*, *focG*, *afa*, *hlyD*, *fimH*, c*nf*, *vat*, *fyuA*, and *ireA*) by multiplex polymerase chain reactions as described previously.[1—8]

The distribution of virulence genes were compared using Chi-square test by using SPSS statistics (Version 16.0; SPSS Inc., Chicago, IL, USA) program. A p value less than 0.05 was considered significant.

Among the 285 isolates, most were found in group B2 (169), followed by those in groups B1 (80), A (23), and D (13) as shown Table 1. It was observed that majority of isolates from pig farms belong to group B1 (72/125, 57.6%) and B2 (52/125, 41.6%). Meanwhile, most isolates from slaughterhouses belonged to group B2 (68/80, 85.0%), followed by groups D (5/80, 6.25%), B1 (4/80, 5.0%), and A (3/80, 3.75%). Except for those found in kidneys in winter, no group A isolates were obtained from slaughterhouses. In contrast, isolates from wet markets and supermarkets covered all four groups. The most prevalent was group B2 (49/80, 61.25%) followed by groups A (19/80, 23.75%), D (8/80, 10%), and B1 (4/80, 5%). The isolates of group B1 (72/125, 57.6%) and B2 (52/125, 41.6%) were more prevalent in tonsil swabs from pig farms, whereas group B2 isolates (117/160, 73.125%) were significantly higher in consumer-ready products from slaughterhouses to markets, from which group B2 isolates were more easily isolated in summer than in winter. Interestingly, all group D isolates were obtained from winter samples, and all intestine isolates (20/20) were observed in group B2.

As far as the distribution of virulence genes among the different phylogenetic groups along the PSCP is concerned, the most prevalent gene in group B2 isolates was *kpsmII* (74.5%) followed by *iutA* and *fimH* (70.4%), *papC* (47.3%), *cnf* (39.6%), and *hlyD* (31.9%). Among the group D isolates, the most prevalent virulence genes were *sfaS* & *focG* (76.9%), *fimH* (46.2%), *afa* (38.5%), and *cnf* (15.4%). Similarly, B1 isolates were higher in *ireA* (92.5%), *fyu* (77.5%), and *vat* (57.5%) genes.

Out of the 125 isolates from the tonsil swabs, the most prevalent were group B1 isolates (72) followed by those of groups B2 (52) and A (1). The most prevalent genes in group B2 isolates were *kpsmII* & *fyu* (80.7%, 42/52), *sfaS* (75.0%, 39/52), *vat* (67.3%, 35/52), *focG* (38.4%, 20/52), *hlyD* (34.6%, 18/52), and *afa* (23.8%, 12/52), whereas those of group B1 isolates were higher in *ireA* (98.6%, 71/72), *iutA* (54.1%, 39/72), *papA* (48.6%, 35/72), *papC* (40.2%, 29/72), and *fimH* (36.1%, 26/72).

Table 1 Distribution of virulence genes in different phylogenetic groups.

Virulence genes	Target class	Phylogenetic groups (%)				Total, $n = 285$ (%)	p
		A ($n = 23$)	B1 ($n = 80$)	B2 ($n = 169$)	D ($n = 13$)		
chuA	Heme transport	—	—	169 (100)	13 (100)	182 (63.8)	0.000*
kpsmII	Group 2 polysaccharide capsule	8 (34.8)	57 (71.3)	126 (74.5)	8 (61.5)	199 (69.8)	0.000*
papA	P fimbriae	5 (21.7)	38 (47.5)	81 (47.9)	5 (38.5)	129 (45.3)	0.110
sfaS	S fimbriae	8 (34.8)	57 (71.3)	123 (72.8)	10 (76.9)	198 (69.5)	0.000*
focG	F1C fimbriae	12 (52.2)	33 (41.3)	95 (56.2)	10 (76.9)	150 (52.6)	0.374
iutA	Iron acquisition system	5 (21.7)	45 (56.3)	119 (70.4)	6 (46.2)	175 (61.4)	0.000*
papC	P fimbriae	3 (13.1)	32 (40.0)	80 (47.3)	1 (7.7)	116 (40.7)	0.002*
hlyD	Cytolytic protein toxin	3 (13.1)	6 (7.5)	54 (31.9)	2 (15.4)	65 (22.8)	0.000*
afa	Afimbrial adhesion	6 (26.1)	3 (3.7)	21 (12.4)	5 (38.5)	35 (12.3)	0.000*
fimH	Type 1 fimbriae	4 (17.4)	33 (41.3)	119 (70.4)	6 (46.2)	162 (56.8)	0.021*
cnf	Cytotoxic necrotizing factor	2 (8.7)	—	67 (39.6)	2 (15.4)	71 (24.9)	0.000*
vat	Autotransporter serine protease toxin	—	46 (57.5)	46 (27.2)	2 (15.4)	94 (32.9)	0.000*
fyu	Yersiniabactin receptor	1 (4.4)	62 (77.5)	67 (39.6)	3 (23.1)	133 (46.6)	0.260
ireA	Iron-regulated outer membrane virulence protein	1 (4.4)	74 (92.5)	120 (71.0)	1 (7.7)	196 (68.7)	0.000*

*$p < 0.05$.