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*

*$p < 0.05$.

Out of 160 isolates from the pork supply chain, the most prevalent were group B2 isolates (117) followed by those of groups A (22), D (13), and B1 (8). The most prevalent genes among the B2 isolates were *fimH* (89.7%, 105/117) followed by *iutA* (82.9%, 97/117), *kpsmII* (71.8%, 84/117), *papC* (61.5%, 72/117), *ireA* (60.6%, 71/117), *cnf* (57.3%, 67/117), *papA* (53.8%, 63/117), *hlyd* (30.7%, 36/117), *fyu* (21.4%, 25/117), and *vat* (9.4%, 11/117), whereas the most prevalent genes in B1 isolates were both *sfaS*, *focG* (87.5%, 7/8) and *afa* (37.5%, 3/8). The frequencies of the studied virulence genes in group B2 isolates from slaughterhouses in summer were higher than those in winter, wet market isolates in summer, supermarket isolates in summer, wet market isolates in winter, and supermarket isolates in winter, as shown in Figure 1.

The present study showed that *E. coli* isolates became more and more diverse along the PSCP, with group B2 being the most prevalent. Our results on the phylogenetic grouping of isolates are not in consistent with those in previous studies.[2,5,6] In one of the studies conducted on diseased pigs at our institute, most of the *E. coli* isolates belonged to group A followed by those in groups B1, D, and B2.[2] In the other study conducted on pigs and pork, most of the *E. coli* isolates belonged to group A, followed by those in groups B1 and B2.[6]

To further evaluate the zoonotic risk of *E. coli* isolates, 14 virulence genes were detected and compared with those in previous studies. Tan et al[2] tested 33 putative virulence genes in 315 ExPEC isolates from diseased pigs in China. The most prevalent phylogroups were groups A (30.8%) and B1 (29.2%), followed by groups D (22.5%) and B2 (17.5%). Among the B2 isolates from diseased pigs, the most prevalent virulence genes are *cvaC*, *fimH*, and *traT*. In another study,[6] the presence of eight ExPEC-related genes (*kpsmII*, *papA*, *papC*, *iutA*, *sfaS*, *focG*, *afa*, and *hlyD*) were tested. Among the B2 isolates, the most prevalent virulence genes are *kpsmII* (31.5%) and *iutA* (23.4%) followed by *papC*, *papA*, *sfaS*, and *hlyD*. The difference may be attributable to the different farming and pork retail systems used, as well as the different geographical regions covered.

It is worth noting that our isolates from the PSCP share many virulence genes with avian and human ExPEC isolates.[7,8] This suggests that porcine isolates share many virulence genes with human urinary tract infection isolates. The virulence genes of ExPEC such as *fim* and *sfa*, *pap* and *foc*, and *afa* have been reported to encode specific adhesins in human bladder, kidney, and uroepithelial cells, respectively.[9] It has been demonstrated that ExPEC isolates carrying a multiplex of four virulence genes—*vat*, *fyuA*, *chuA*, and *yfcV*—can efficiently colonize the urinary tract.[10] In our study, 59.3% (169/285) isolates belong to phylogroup B2, in which 100% (169/169) isolates carry *chuA*, 39.6% (67/169) carry *fyuA*, and 27.2% (46/169) carry *vat*. In conclusion, we isolated *E. coli* in every tonsil swab of clinically healthy pigs, and consumer-ready pork samples from retail markets. Phylogroup B2 is the most prevalent, followed by groups B1, A, and D. The most prevalent virulence genes in group B2 isolates were *chuA*, *kpsmII*, *iutA*, and *fimH*, whereas group B1 isolates were higher in *ireA*, *fyu*, and *vat*. Group B2 isolates share many virulence genes

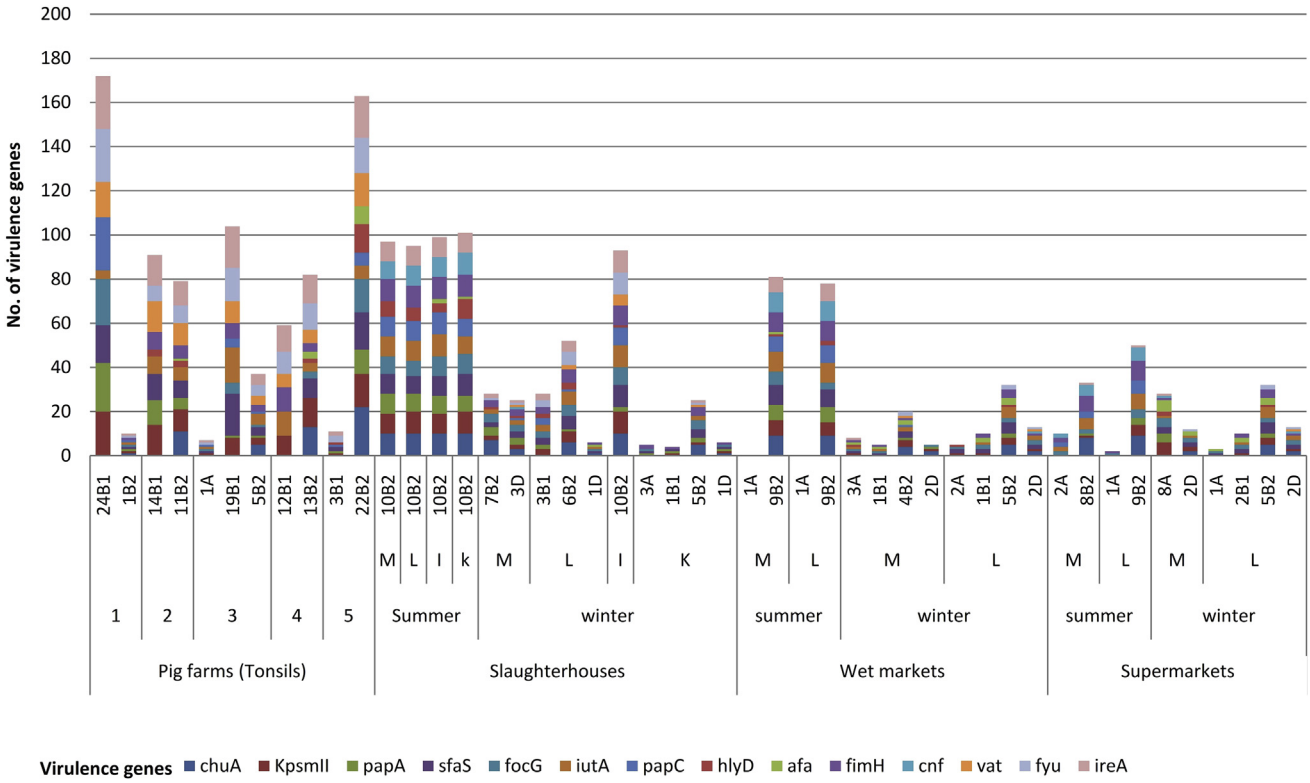

Figure 1. Distribution of virulence genes in different numbers of *Escherichia coli* strains belong to different phylogenetic groups isolated from tissues along PSCP. I = intestine; K = kidney; L = liver; M = meat; PSCP = production and supply chain of pork.

with human pathogenic isolates, suggesting a potential threat to public health.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

This study was supported by the International S & T Cooperation Program of China (ISTCP grant No. 2013DFG32360), and Special Fund for Agro-scientific Research in the Public Interest (201403054).

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Author contribution

GZ and YTC collected the samples. RX, SBK, BW, and LL carried out the experiments. SBK and RZ wrote the manuscript.