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Prevalence of Shiga toxin- and enterotoxin-producing *Escherichia coli* in patients and animals in Guizhou, China

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Abstract: [Objective]: To assess the public health risk, we studied the prevalence of enterotoxigenic *Escherichia coli* (ETEC) and Shiga toxin-producing *E. coli* (STEC) among pig, cattle and human in Guizhou Province. **[Methods]:** *E. coli* isolates from fecal samples were investigated for their virulence markers by polymerase chain reaction (PCR) assays. **[Results]:** Of 333 *E. coli* isolates, ETEC was predominant and detected in 73 of 112 isolates from patients, 82 of 106 isolates from pigs, and 18 of 115 isolates from cattle. The distribution of genes *st*, *lt*, and *st/lt* was equivalent in ETEC isolates. The detection rate of STEC from pig isolates was higher than that from patient and cattle isolates, most of which carried genes for *st* or *lt* or both. Furthermore, we analyzed the presence of the *fedA* gene encoding the major subunit of F18 fimbriae in *E. coli* isolates. Although most isolates were negative in the PCR, the presence of F18 fimbriae in the *E. coli* isolates was always associated with enterotoxin genes. In 25 *stx*-positive STEC isolates, however, only 4 STEC from pigs with diarrhea detected *fedA*. **[Conclusion]:** These results indicate that ETEC, coexisting with F18 fimbriae, is common in patients, cattle, and pigs, while STEC is dominant in pigs in Guizhou Province, China.

Keywords: prevalence; Shiga toxin-producing *E. coli* (STEC); enterotoxigenic *Escherichia coli* (ETEC); *fedA*; toxin gene
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Five classes of *Escherichia coli* have been well associated with diarrhea in several epidemiological studies[1]: enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and Shiga toxin-producing *E. coli* (STEC). Of these *E. coli*, STEC and ETEC are important in endemic and epidemic diarrhea worldwide, especially in developing countries. STEC has been implicated as the cause of hemorrhagic colitis and hemolytic-uremic syndrome in humans [2, 3], diarrhea in calves [4], and edema disease in swine [5]. STEC is considered to be highly virulent and life-threatening to animals and humans. *E. coli* O157: H7 is by far the most prevalent serotype of STEC associated with large outbreaks in humans from many countries [6−8]. STEC may carry either Shiga toxin subtype Stx1 and/or Stx2 (with several variants such as: Stx2c, Stx2d, Stx2e, Stx2f) [2]. Another group of *E. coli* that is a leading cause of diarrhea worldwide is ETEC. ETEC strains result in diarrhea by producing heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), or both [9]. Human is infected with ETEC and STEC by exposure to the pathogen in contaminated food or water, or direct contact with animal feces [10]. Swine, cattle, and sheep are the major reservoirs of ETEC and STEC infection. Birds may also be potential carriers as shown in experimentally infected animals [11, 12]. The aim of this study was to determine the distribution of STEC and ETEC in patients and animals with diarrhea in Guizhou Province of China.

1 MATERIALS AND METHODS

1.1 Sample collection

Fecal specimens of patients with diarrhea were collected from two hospitals (People's Hospital of

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Guizhou Province and Huaxi Hospital) in Guiyang, China. The diarrhea samples of pigs were collected from pig farms in the cities of Guiyang, Zunyi, Bijie, and Anshun. Stool samples of cattle were collected from scattered cattle. All procedures performed were reviewed and approved by the Animal Care and Use Committee of Guizhou University before initiation of the study.

1.2 Isolation of *E. coli*

A total of 333 stool samples were collected between 2004 and 2006 in Guizhou Province. Samples were plated on Mac-Conkey agar, incubated overnight at 37°C, and a loopful of growth from the first inoculation streak was suspended in 0.5 ml of distilled water and boiled for 10 min. After centrifugation of the lysate, the supernate was used in PCR.

1.3 Detection of virulence genes by PCR

All primers used in this study are listed in Table 1. Template DNA was prepared as described above. The presence of the toxin genes (*lt*, *st*, *stx*) and adhesion gene (F18) was identified by a PCR with the primers described in previous studies [13].

Table 1 Primers used in this study

Primer	Sequence (5′→3′)	Gene	Fragments /bp
stF	AACATGACGGGAGGTAAC	*st*	234
stR	ATAACATGGAGCACAGGC		
ltBF	GCTCCCCAGACTATTACAG	*ltB*	312
ltBR	CTAGTTTTTCATACTGATTGC		
stxF	TCCATGACAACGGACAGCAG	*stx*	192
stxR	CGTAAGGCTTCTGCTGTGAC		
fedAF	ATGAAAAGACTAGTGTTTATTTC	*fedA*	513
fedAR	CTTGTAAGTAACCGCGTAAGC		

2 RESULTS

2.1 Isolation and Detection of ETEC and STEC

A total of 333 *E. coli* isolates were obtained from patients, pigs, and cattle with diarrhea. *E. coli* isolates were initially examined for their morphological character in MacConkey agar and virulence markers by PCR assays (Fig. 1). The prevalence of

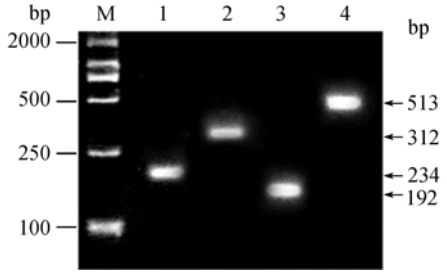

Fig. 1 Detection of toxins and adhesion genes in *E. coli* isolates by PCR. Lanes: M. DL2000 DNA marker; 1. *st* (234 bp); 2. *ltB* (312 bp); 3. *stx* (192 bp); 4. *fedA* (513 bp).

ETEC and STEC is shown in Table 2. ETEC was predominant among the 333 *E. coli* isolates, with a total of 173 isolates (52.0%) from stool samples of human (n=73), pigs (n=82), and cattle (n=18). A total of 38 STEC isolates (11.4%) were obtained from pigs (n=25), cattle (n=7) and patients (n=6). The most prevalence of STEC was detected from pig samples. Thirteen STEC (3.9%) were tested genes of both Shiga toxin and enterotoxin.

Table 2 Distributions of ETEC and STEC in human and animals

	Humans N= 112	Pigs N=106	Cattle N=115	Total *E. coli* N= 333
ETEC	73	82	18	173 (52.0%)
STEC	6	25	7	38 (11.4%)
ETEC/STEC	4	9	0	13 (3.9%)

2.2 Detection of toxin genes

The distribution of toxin genes in *E. coli* isolates from patients, pigs, and cattle is shown in Table 3. Sixty percent of the isolates were positive for one or more toxin genes, including the following six combinations: *lt*, *st*, *st/lt*, *stx*, *stx/lt*, and *stx/st*. The most prevalent toxins among all patient isolates were those encoded by the *lt* (34 of 112 isolates; 30%), *st* (18 of 112 isolates; 16%) and *st/lt* (17 of 112 isolates; 15%) genes. Among the 106 pig isolates, 77% isolates (n=82) carried *st* or *lt*, or both genes (Fig. 1; Table 3). Comparatively, the detection rate of the toxin genes among cattle isolates was lower than that from patients and pigs, and the dominant prevalence was the *st* gene. The *stx* gene was detected in 25 isolates from pigs, 6 from patients, and 7 from cattle, 34.2% of which carried *st* or *lt* genes. The detection rate of the *stx* gene from pig isolates was higher than that from patient or cattle isolates.

Table 3 Virulence genes of *E. coli* isolated from human and animals with diarrhea

Virulence genes	Humans (N=112)	Pigs (N=106)	Cattle (N=115)	Total
lt	34	19	2	55
st	18	19	15	52
lt/st	17	35	1	53
stx	2	16	7	25
stx/lt	3	6	0	9
stx/st	1	3	0	4

2.3 Relationship of fedA and toxin genes

The presence of the *fedA* gene encoding the major subunit of F18 fimbriae was analyzed in *E. coli* isolated from 112 patients, 106 pigs, and 115 cattle with diarrhea (Fig.1). As shown in Table 4, most isolates were negative in the PCR, showing no F18 fimbrial genes. The presence of F18 fimbriae in those *E. coli* isolates was always

associated with the presence of the toxin genes. F18 fimbriae were exclusively expressed by the *lt*+ (11 isolates), or *st*+ (13 isolates), or both (15 isolates). *fedA* genes were also found in *lt*+*stx*, and *st*+*stx* positive isolates. In 38 *stx*+ STEC isolates, however, only two STEC from patient with diarrhea produced *fedA*.

Table 4 Detection of *fedA* in ETEC and STEC isolates in Guizhou Province

Isolates	*FedA* alone	ETEC			ETEC/STEC		Total
		fedA/*lt*	*fedA*/*st*	*fedA*/*st*/*lt*	*fedA*/lt/*stx*	*fedA*/st/*stx*	
Total	17	11	13	15	3	1	60
Humans (N=112)	6	6	3	5	1	1	22
Pigs (N=106)	5	5	3	10	2	0	25
Cattle (N=115)	6	0	7	0	0	0	13

3 DISCUSSION

In this study, two classes of diarrheagenic *E. coli* were detected in patients and domestic animals with endemic diarrhea in Guizhou Province of China. ETEC was the dominant category in patients, pigs, and cattle. Genes encoding for *lt* in patients, *st* in cattle, and both *lt* and *st* in pigs was the most prevalent in the Guizhou Province of China. The results showed a higher proportion of ETEC in patient isolates with diarrhea than that reported in previous studies [14, 15]. The difference between our results and those previously reported may be due to the sanitation conditions.

The detection rate of STEC was 5.4% in patients, 23.6% in pigs, and 6.1% in cattle isolates. STEC and the *stx* gene were the most prevalent in pig isolates. The proportion of STEC in pigs was higher than that in cattle. In other reports, the detection rate of STEC from cattle is much higher [16, 17]. In the outbreaks of STEC O157:H7 in the United States from 2002 to 2003, STEC isolates from cattle were 11.4% and 1.2% in pigs [16]. In New Zealand, 27.3% (51/187) of healthy cattle were positive for STEC [17]. The difference between our data and previous reports might be due to the source of samples in the present study, which were collected from the scattered cattle. Another reason might be the difference in cattle breeds [18]. Both of the STEC detection rates of cattle fecal samples from Guizhou and Changchun (1.7%) in China were lower than other countries [16, 17]. The cattle in Guizhou are local breeds with yellow fair and dwarfish body, weighing about 300 kilograms. However, it could be confirmed that cattle are still one of the asymptomatic reservoirs of STEC, as well as a source of human infection from meat consumption or water contaminated by the feces of livestock.

Notably, the percentage of STEC in patients is 5.4% (6/112) although it is not as high as the percentage documented in the gastrointestinal outbreak in Spain (17.5%, 14/80) [19]. STEC has been shown to be prevalent in developed and developing countries. Numerous outbreaks have been reported and patients have developed life-threatening complications, such as hemolytic-uremic syndrome (HUS) and hemorrhagic colitis (HC), which may be fatal up to 5% of cases [2]. Furthermore, most of the STEC detected in the present study carried genes for *st* or *lt*, which has been extensively studied in a previous study [20]. Therefore, it is reasonable that the vaccine targeting both Stx and enterotoxin might be much more effective in preventing disease caused by STEC in humans and domestic animals.

A low prevalence of *fedA*-positive *E. coli* isolates (18%) from patients and animals with diarrhea was found. These results were lower than the previous cases [21], in which the detection rate of F18 gene was 26.3%. The presence of F18 fimbriae in the *E. coli* isolates from patients, pigs, and cattle with diarrhea was always associated with the presence of the toxin genes. By mediating adhesion to the microvilli of epithelial cells in the small intestine, fimbrial adhesion is one of the virulence factors of ETEC and STEC [22]. Obviously, vaccines targeting on adhesion to block the colonization of either human or animal reservoirs and together with toxins (Stx and enterotoxin) would be benefit for controlling STEC or ETEC infection.

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产志贺样毒素和肠毒素大肠杆菌分子流行病学

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摘要: 【目的】人和动物腹泻的主要病原菌为大肠杆菌，本文主要研究贵州省致腹泻大肠杆菌毒力因子的分布类型。【方法】采用 PCR 技术对各毒力因子的基因分布进行研究。【结果】共分离到 333 株大肠杆菌，其中产肠毒素大肠杆菌（ETEC）在腹泻的人、猪、牛群中占优势，分别为：人群 73（n=112），猪群 82（n=106），牛群 18（n=115）。在 ETEC 菌株中检测到热敏肠毒素（*lt*）和不耐热肠毒素（*st*）基因，还存在 *lt*/*st* 并存现象。从人、猪、牛群中还检测到产志贺样毒素大肠杆菌（STEC），其中源自猪的 STEC 的检出率最高。大部分 STEC 同时携带 *lt*、*st* 或 *lt* 和 *st* 同时并存。编码 F18 菌毛的主亚基由 *fedA* 基因编码。对所分离大肠杆菌 F18 菌毛进行的研究结果表明，*fedA* 基因主要与肠毒素基因共存，与 *stx* 基因并存的类型较少，25 份猪源 STEC 菌株中仅有 4 份检测到 *fedA* 基因。【结论】贵州省人群、猪群和牛群致腹泻病原菌中以带 F18 菌毛的 ETEC 为主，STEC 主要分布在腹泻的猪群中。

关键词: 流行病学；产志贺样毒素大肠杆菌（STEC）；产肠毒素大肠杆菌（ETEC）；*fedA* 基因；毒素基因

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