

PREVALENCE AND CHARACTERIZATION OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* DURING PROCESSING IN CHINESE BEEF PROCESSING PLANTS

Tongtong Xiao, Pengcheng Dong, Yimin Zhang, Lixian Zhu* and Xin Luo*

College of Food Science and Engineering, Shandong Agricultural University, Taian, Shandong, 271018, PR China;

*Corresponding authors email: luoxin@sdaa.edu.cn; zhly@sdaa.edu.cn.

Abstract – China is the 4th-largest beef-producing country, but little is known of the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in beef processing plants. In this study, 600 samples were collected from two beef cattle plants at six different processing points. All samples were preliminary selected by multiple PCR and further confirmed by selective medium and serotype identification. A total of 26 positive samples were detected, most of which (18) were isolated from feces, with O39 and O76 as the primary serotypes. Antimicrobial susceptibility test showed that the STEC strains isolated from beef cattle plants in China were resistance to many antibiotics, and multi-drug resistance were also found.

Key Words –STECC, serotypes, detection.

I. INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is an important food-borne pathogen and its clinical outcomes ranging from diarrhoea to haemorrhagic colitis (HC) and a life-threatening complication known as haemolytic uremic syndrome (HUS) posing a great threat to public food hygiene and human life safety [1, 2]. Because of the lack of Shiga toxin receptors, ruminants, especially cattle, can carry STEC without clinical symptoms and became a potential contamination reservoir [3]. People were often infected by such pathogen via eating food and drinking water contaminated with bovine excrement.

China is the 4th-largest beef-producing nation, but to the best of our knowledge, there is no published data on whether, and with which frequency, STEC is present in plants during beef processing. Adequate microbiological data is needed to describe the prevalence, antibiotics susceptibility, serotype, genotype and other profiles of those foodborne pathogens in China.

II. MATERIALS AND METHODS

A total of 600 samples were collected from two beef-processing plants (designated plants A and B) during 2016. Both commercial plants included in this study were equipped with a production line with a design capacity of 30-50 animals per hour. Six points in the processing line were selected for sampling including hide, post-dehiding, post-washing, feces, chilled carcasses and raw meat. The sampling procedure were conducted as previously described [4]. The wiped gauze, raw meat and feces were cultured with TSB broth at 37°C for 24 h. After the removing of impurities by centrifugation, DNA was extracted by a commercial extraction kit and two target gene *stx*₁ and *stx*₂ were detected by multiplex PCR. Positive samples were subjected to secondary screening with STEC chromogenic medium, and the positive strains were cultured and maintained for serotyping. The Kirby-Bauer method was used to determine the antibiotic resistance.

III. RESULTS AND DISCUSSION

Overall, the prevalence of STEC in two plants was 4.33%. The prevalence on the hide, in post-dehiding, post-washing, feces, chilled carcasses and raw meat were 4%, 1%, 2%, 1.4%, 18%, 1%, and 0%, respectively. The overall prevalence of STEC in feces samples was significantly greater than other points ($P < 0.05$), this result was also find in plant A and B respectively (Table 1). Hide samples has a second highest positive rate and significantly higher than that of raw meat samples in the overall prevalence. This result indicates the feces and hide are the main reservoir of STEC in cattle. No positive samples was detected in raw meat (Table 1). The prevalence of STEC at six different processing points had no difference between the two plants including feces samples (Table 1).

Table 1 Prevalence of STEC contaminated in two beef cattle processing plants in China

		hide	post dehiding	post washing	feces	chilled carcasses	raw meat	total
A	samples	50	50	50	50	50	50	300
	detections	2(4.0%) ^b	0(0.0%) ^b	1(2.0%) ^b	10(20.0%) ^{a_x}	0(0.0%) ^b	0(0.0%) ^b	13(4.33%) _x
B	samples	50	50	50	50	50	50	300
	detections	2(4.0%) ^b	1(2.0%) ^b	1(2.0%) ^b	8(16.0%) ^{a_x}	1(2.0%) ^b	0(0.0%) ^b	13(4.33%) _x
T	Samples	100	100	100	100	100	100	600
	detections	4(4.0%) ^b	1(1.0%) ^{bc}	2(2.0%) ^{bc}	18(18.0%) ^a	1(1.0%) ^{bc}	0(0.0%) ^c	26(4.33%)

^{a, b} Different letters indicate significant differences between sampling points in the same row ($p < 0.05$).

_{x, y} Different letters indicate significant differences between plants in the same column ($p < 0.05$).

As to the seasonal factor, autumn have a significantly higher prevalence of STEC in this study (8.67%), the detection rate in spring and winter were a little lower (Figure 1). Except two strains that can't be serotyped, all of the rest 24 ones were classed into 16 different serotypes. O39 and O76 were the main serotypes having 6 and 4 strains respectively (Table 2). Of the 26 STEC strains 30.77%, 26.92%, 23.08% 23.08% and 19.23% displayed resistance to tetracycline, ampicillin, cephalosporin, cefuroxime and ampicillin. Six strains were found resistant to more than three different antibiotic at the same time. Most strains (96.15% and 80.77%) were sensitive to fosfomycin and ciprofloxacin.

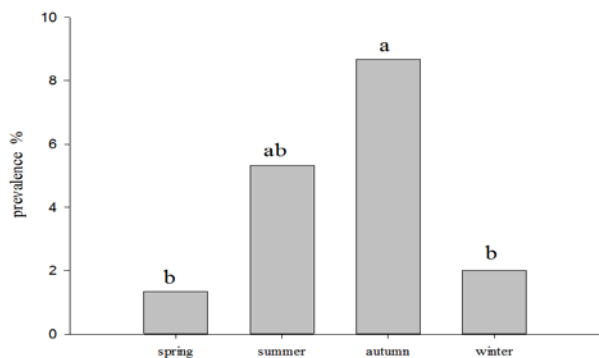


Figure 1 Prevalence of STEC in different seasons

Table 2 Serological identification of STEC strains

processing points	Serotypes
hide	O15、O33、O79
post-dehiding	O39
post-washing	O73、O39
feces	O39、O39、O76、O146、O76、O76、O119、O76、O87、O92、O40、O68、O157、O39、O116、O39、O142
chilled carcasses	O59
raw meat	-

IV. CONCLUSION

The overall prevalence of STEC in feces and hide samples was greater than other points indicating the feces and hide are the main reservoir of STEC in cattle. Seasonal factor may have an influence on the prevalence of the STEC. In the current study, O39 and O76 were found the dominant serotype in Chinese beef plants. High resistance to many antibiotics were found and multi-drug resistant strains were found.

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REFERENCES

1. Bandyopadhyay S, Biswas T K, Sasmal D, (2009). Virulence gene and antibiotic resistance profile of Shiga-toxin-producing *Escherichia coli* prevalent in captive yaks (*Poephagus grunniens*). *Veterinary microbiology*, 138(3): 403-404.
2. Daniel S L, Hung K F, Janezic K J, (2013) et al. Phenotypic and genotypic characterization of *Escherichia coli* isolated from untreated surface waters[J]. *The open microbiology journal*, 7.
3. Blanco M, Blanco J E, Mora A, et al (2004). Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-ξ). *Journal of clinical microbiology*, 42(2): 645-651.
4. Dong, P., Zhu, L., Mao, Y., Liang, R., Niu, L., Zhang, Y., & Luo, X. (2015). Prevalence and characterization of *Escherichia coli* O157:H7 from samples along the production line in Chinese beef-processing plants. *Food Control* 54:39-46.