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Prevalence and characterization of Shiga toxin-producing *Escherichia coli* (STEC) isolated from Chinese beef processing plants

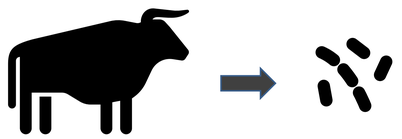
Xueqing JIANG[1], Xin LUO[1], George-John NYCHAS[2], Yimin ZHANG[1], Pengcheng DONG[1*]

[1] *Lab of Beef Processing and Quality Control, Shandong Agricultural University, China*
[2] *Agricultural University of Athens, Greece*
**Corresponding author e-mail: pcdong@sdau.edu.cn*

Introduction

Besides *E.coli* O157:H7, Shiga toxin-producing *Escherichia coli* (STEC) has emerged as a great public health concern due to the increasingly apparent risk of causing serious disease. Beef cattle are considered as the main reservoir of STEC and the consummation of contaminated beef can pose significant health risks to humans. As opposed to the past, where the consumption of beef in China was limited only included fully cooked meat, various raw and processed beef products such as steaks have been increasingly consumed at the national level in recent years. However, information about the prevalence and characteristics of STEC during slaughter of beef cattle in China is limited.

The aim of the study was to elucidate the prevalence, cross-contamination and biological characteristics of Shiga toxin-producing *Escherichia coli* isolated from two beef plants with a processing capacity of 60-70 animals per day. The results of this study is anticipated to useful for the development of food safety objectives and the improvement of control measures for beef safety control.



Materials and Methods

- During the period of 2021.4–2021.11, 435 swabs or meat samples were collected from two beef processing lines with a processing capacity of 60-70 animals per day. Eight points were selected for sampling including feces, slaughter fence, hide, pre-evisceration carcasses, post-washing carcasses, chilled carcasses, meat and environment samples.
- Immunomagnetic separation (**IMS**) was carried out using immunomagnetic beads coated with an anti-*E. coli* O157:H7 anti-body. After separation, detection of $flic_{h7}$ and *rfbE* by PCR, secondary screening and isolation of the PCR positive samples using CHROMagar™ O157.
- For non-O157 STEC samples, after removal of impurities by centrifugation, three genes stx_1, stx_2 were detected by multiplex PCR, the *stx*-positive samples were selected for a secondary screening and characterization by CHROMagar™ STEC.
- Each STEC isolates were characterized for O serogroups and virulence gene by **PCR**.
- The **Kirby Bauer** method used for drug resistance testing.
- Traceability analysis of these isolates was applied with the **multi locus sequence typing (MLST)** method.

Results

Among the 62 broths were found as stx_1 and/or stx_2-positive, only 17 broths (27.4%) were further confirmed by CHROMagar™ STEC. A total of 45 strains were isolated.

Table1 Prevalence of *stx* genes in different slaughter stages in two beef processing plants.

Plant	Method	Feces(%)	Slaughter fence(%)	Hides(%)	Pre-evisceration carcasses(%)	Post-washing carcasses(%)	Chilled carcasses(%)	Meat(%)	Environment samples(%)	Total(%)
A	PCR	8.00^{b}	24.00^{ab}	32^{a}	12^{ab}	12^{ab}	0^{b}	4^{b}	0^{b}	10.22^{A}
	95% CI (%)	0-19.4	6-42	12.3-51.7	0-25.7	0-25.7	-	0-12.3	-	6.2-14.2
B	PCR	13.33^{bc}	46.67^{a}	23.33^{ab}	16.67^{bc}	-	3.33^{c}	20^{b}	6.67^{bc}	18.57^{B}
	95% CI (%)	0.4-26.2	27.7-65.6	7.3-39.4	2.5-30.8	-	0-10.2	4.8-35.2	0-16.1	13.3-23.9
Total	PCR	10.90^{b}	36.36^{a}	27.27^{ab}	14.55^{b}	12^{b}	1.81^{c}	12.72^{b}	2.5^{c}	14.25
	95% CI (%)	2.4-19.4	23.2-49.5	15.1-39.4	4.9-24.2	0-25.7	0-5.5	3.6-21.8	0-6	11-17.6

$^{a-c}$ Different letters indicate significant differences between sampling points
$^{A-B}$ Different letters indicate significant differences between plants
\- No spraying process in this plant

- The prevalence of STEC in **slaughter fence** samples was the highest, followed by **hides**.
- All of the O157 isolates were typed as **ST-11**.
- The gene types of the isolates from **hides** were more diverse.
- Traceability analysis of strains by combining MLST with virulence genes and serotypes revealed a serious cross-contamination situation of **hides-feces** and **hides-hides** in pre-slaughter animals.

✓ MLST

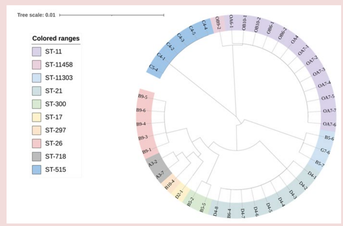


Fig.1 Phylogenetic tree of Shiga toxigenic *Escherichia coli* isolates

✓ Serotype

- Two different serotypes (**O157** and **O121**) were identified among the isolated 45 strains, 33.33% (15/45) of the strains were O157 and 42.22% (19/45) of the strains were O121. There are also 11 strains can't be serotyped by the used PCR method.

✓ Virulence gene

- The highest subtype of stx_2 was $\boldsymbol{stx_{2a}}$ (19/29; 65.51%), followed by $\boldsymbol{stx_{2b}}$ (6/29; 20.69%) and $\boldsymbol{stx_{2d}}$ (3/29; 10.34%).
- All the stx_1 gene belong to the subtype $\boldsymbol{stx_{1a}}$.

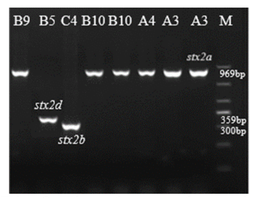


Fig.2 The electrophoresis of virulence

✓ Antimicrobial resistance

- The highest resistance rate to **tetracycline** was 44.44% followed by **streptomycin** resistance rate of 40.00%.
- **16** strains (35.55%) showed multidrug resistance (Table 2).

Table 2 Antimicrobial resistance spectrum characteristics of STEC

Number of antimicrobials classes	Resistant phenotype	Number of strains
1	TE	2
3	TE-S-NA	1
	TE-S-C	1
4	TE-S-NA-AM	2
5	TE-S-C-GM-CXM-AMX-AT-CF-SXT-AM	9
6	TE-S-C-GM-CXM-AMX-CIP-CF-SXT-AM	1
	TE-S-C-GM-CXM-AMX-AT-CIP- CF-SXT-AM	4

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