

Original Research Article

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Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) from Smoked Pork sold in Local Markets of Aizawl, Mizoram (India)

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ABSTRACT

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E. coli is recognised as a major pathogen of public health significance in developing countries and represents the leading etiological agent of diarrhoea. The presence of *E. coli* in food or water is an indication of uncleanliness and careless handling and also implies the presence of enteric pathogens. The Shiga Toxin-Producing *Escherichia coli* (STEC) are public health food pathogens. STEC is responsible for human diseases ranging from mild diarrhoea to hemorrhagic colitis (HC) and life-threatening hemolytic uremic syndrome (HUS), leading to kidney failure and death, both in outbreaks and intermittent events.

Introduction

Food protection has become a key issue in fresh products worldwide because of many factors, such as the import of fresh products from different countries, possible sources of increasing bacterial pathogens and poor domestic preparation (Wang *et al.*, 2020).

In the human gut, *Escherichia coli* is a part of the normal flora, but some strains they are pathogenic (Kim *et al.*, 2020). STEC is critical for public health, potentially causing diarrhoea, bloody diarrhoea (hemorrhagic colitis), hemolytic uremic syndrome (HUS)

and kidney injury (Castro *et al.*, 2019). STEC-produced toxins are categorised as type 1 (Stx1) and type 2 (Stx2) and several subtypes and variants of Stx1/Stx2 have been identified on the basis of receptor preference and toxin potency (Melton-Celsa, 2014).

The transmission of STEC can happen by various routes, through food (Frank *et al.*, 2011), fecal contamination of agri-food, the environment, or the water chain (Rothenburger *et al.*, 2017), person to person contact (Rowe *et al.*, 1993), animals such as cattle (Ferens and Hovde, 2011), swine (Gyles, 2007) etc.

Materials and Methods

A total of 200 smoked porks were collected from different local markets of Aizawl, Mizoram, India. All the swabs were processed for isolation and identification of *E.coli* (Ewing, 1986). All the isolates were further assessed by polymerase chain reaction (PCR) assay for the presence of putative virulence gene(s) (*stx₁* and *stx₂*) (Paton and Paton, 1998). All the isolates were then subjected to antimicrobial sensitivity assay by disc diffusion method using 14 commonly used antimicrobial agents (Bauer *et al.*, 1966).

Results and Discussion

A total of 30 *E. coli* were isolated, of which 10 (33.33%) isolates were found to be positive for at least one virulence gene (Table 1) of STEC. As depicted in Table 1, a total of 2 (6.66%) and 1(3.33%) isolates were positive for *stx₁* and *stx₂* genes and 2 (6.66%) isolates for *stx₁* and *stx₂* genes combination. Among the STEC isolates, *stx₂* (22.66%) was

predominant over *stx₁* (6.66%) which was in accordance to the findings of earlier workers (Omoruyi *et al.*, 2018; Kataria *et al.*, 2017). Detection of *stx₂* positive isolates from smoked pork may result in a public health concern as in a murine infection model, shiga toxin 2 is reportedly 400 times more toxic than Stx1 and shiga toxin 2 is more frequently related to complications of HUS than Stx1 (Shridhar *et al.*, 2017).

For *stx₂* and *eaeA*, *stx₂* and *hlyA*, *stx₂* and *eaeA* and *hlyA* combinations 1(3.33%), 2(6.66%) and 2(6.66%) isolates were positive respectively. A total of 7 (23.33%) isolates were carrying multiple genes. The key virulence factors of STEC are shiga toxins formed by the *stx₁* and *stx₂* genes, while the *eaeA* gene, which codes for intimate protein, mediates STEC's attachment to the enterocytes. HUS is mostly associated with STEC strains carrying the genes *stx₂* and *eaeA*, and this composition of the gene is known to be a high risk of developing HUS (Loconsole *et al.*, 2020).

Table.1 STEC virulence gene(s) profile in smoked pork from local market in Aizawl, Mizoram

Sl. no	Virulence (STEC)	Total (n=30)
1	<i>stx₁</i> only	2 (6.66%)
2	<i>stx₂</i> only	1(3.33%)
3	<i>stx₁</i> and <i>stx₂</i>	2 (6.66%)
4	<i>stx₂</i> and <i>eaeA</i>	1(3.33%)
5	<i>stx₂</i> and <i>hlyA</i>	2 (6.66%)
6	<i>stx₂</i> , <i>eaeA</i> and <i>hlyA</i>	2 (6.66%)

All the 10 STEC isolates showed 100% drug resistance to erythromycin, and amoxicillin. Imepenem, amoxyclov and ceftriaxone were the better antimicrobial agents in terms of sensitivity. Multiple drug resistance can result from propagation in STEC strains. The transmission of horizontal elements within and among bacterial species, such as plasmids,

prophages or transposons, is predominantly possible in environments like the gut microbiome (Amaechi *et al.*, 2015).

The key source of protein in Mizoram is pork and the development of high-proportion multi-drug-resistant STEC isolates may constitute a significant reservoir that poses a risk to public

health and may complicate potential therapeutic options.

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