

Original Research Article

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Bacteriological Quality Assessment and Molecular Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) from Milk and Meat Based Fast Foods from Mizoram (India)

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ABSTRACT

Keywords

Foodborne disease (FBD), *Escherichia coli*, STEC

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Diseases that result from foods are one of the major health problems in developing and developed countries. The determination of the microbiological quality of foods needs to be established by analytical protocols to detect indicator and pathogenic organisms. Commonly approved indices of microbial quality include the Total Viable Count (TVC) and Coliform count. Shiga toxin-producing *Escherichia coli* (STEC), food-borne pathogens of public health concern and responsible for both outbreaks and sporadic cases of human diseases, ranging from mild diarrhea to hemorrhagic colitis (HC) and life-threatening hemolytic uremic syndrome (HUS) leading to kidney failure and death.

Introduction

Foodborne disease (FBD) is a global public health concern, and foods from animal sources have been associated with outbreaks of food-related illness (Jaja *et al.*, 2018).

Shiga toxin-producing *Escherichia coli* (STEC) are a group of food and water-borne pathogens associated with a wide spectrum of human diseases, ranging from mild diarrhea to hemorrhagic colitis (HC), thrombocytopenia, hemolytic uremic syndrome (HUS), and can also lead to people death (Baranzoni *et al.*,

2016). Humans most frequently become infected with STEC by ingestion of contaminated food (meat, especially undercooked beef hamburgers; raw milk; cheese; ready-to-eat sausages; sprouts) or water or by direct contact with animals (Gyles, 2007).

Materials and Methods

A total of 205 numbers of milk (73 nos) and meat (132 nos) based fast food were collected from four different districts (Aizawl, Kolasib, Champhai and Siaha) in Mizoram. All the

samples were collected aseptically and were brought to the laboratory under cold chain. The samples were processed immediately upon arrival for bacteriological quality and isolation of *E. coli*. Total viable count (TVC) and coli form count were determined according to the procedure recommended by American Public Health Association (1976). Isolation and identification of *E. coli* was done as per Cowan and Steel (1993). 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 fast food samples (205 nos) were examined for TVC and coliform count. The TVC of the entire sample ranged between 4.51 and 5.74 log₁₀ cfu/g with a mean value 4.82±0.01 log₁₀ cfu/g (Table 1).

The mean values of milk based fast food from Aizawl, Kolasib, Champhai and Siaha districts were 4.82±0.06, 4.72±0.06, 4.83±0.06, 4.82±0.02 and the mean values of meat based fast food from Aizawl, Kolasib, Champhai and Siaha districts were 4.81±0.05, 4.85±0.05, 4.83±0.04, 4.86±0.02 which was in accordance to the findings of earlier workers (Mahmoudi *et al.*, 2014; Hassan, 1986)

147 (71.70%) samples were found to be positive for coliform organism. The coliform count of the entire sample ranged from 4.00 and 4.71 with a mean value of 4.57±0.01 log₁₀ cfu/g. The mean value of coliform count was 4.53±0.01, 4.59±0.01, 4.58±0.01, 4.61±0.01 log₁₀ cfu/g respectively for Aizawl, Kolasib, Champhai and Siaha districts. No significance difference was observed between Aizawl, Champhai, Kolasib and Siaha districts in case of milk based products however, in case of meat based fast food, significance difference was found between Aizawl and Kolasib at P≤0.05, Aizawl and Champhai and Aizawl and Siaha districts at P≤0.01. Significance difference was also found between Kolasib and Siaha district at P≤0.05 (Table 1). Similar findings were also recorded by Mahmoudi *et al.*, (2014) and Datta *et al.*, (2012). As 71.70% of the samples were positive for coliform organism and exceeded the acceptable limit set by ICMSF (1986). This could be due to poor hygiene practices followed by food handlers and inadequate handling practices at the selling point. The higher incidence of coliform was also in agreement with Kuljinder and Kahlon (2014) and also with Rath and Patra (2012) who reported coliform organism in 86.66% of the fast food samples tested.

Table.1 Statistical analysis of Total Viable Count (TVC) and coliform count of milk and meat based fast foods in four different districts of Mizoram

District	Total Viable Count (TVC)		Coliform Count	
	Milk based fast food (log ₁₀ cfu/g) ± S.E)	Meat based fast food (log ₁₀ cfu/g) ± S.E)	Milk based fast food (log ₁₀ cfu/g) ± S.E)	Meat based fast food (log ₁₀ cfu/g) ± S.E)
Aizawl	4.82±0.06	4.81± 0.05	4.47± 0.04 ^b	4.56±0.01 ^c
Kolasib	4.72±0.06	4.85 ±0.05	4.57±0.01 ^a	4.60±0.01 ^b
Champhai	4.83±0.06	4.83±0.04	4.53±0.01 ^{ab}	4.61±0.01 ^{ab}
Siaha	4.82±0.02	4.86±0.02	4.58±0.01 ^a	4.63±0.01 ^a
F value	0.707 ^{NS}	0.213 ^{NS}	4.140*	6.323**

Note: Values bearing different superscripts within the column differs significantly from each other; * = single asterisk denotes significantly different at ≤ 0.05 and ** = double asterisk denote significantly different at ≤ 0.01

A total of 46 *E. coli* isolates (30 isolates were from meat based and 16 were from milk based fast food) were isolated from 205 fast food samples, two isolates (4.34%) were found to be positive for *stx₂* gene (both from milk based products) and none were found to be positive for *stx₁* gene. Low prevalence of the *stx₂* genes were also observed by other workers (Gupta *et al.*, 2013; Zende *et al.*, 2013 and Kalliopi *et al.*, 2012). *Stx₂* is considered to be the most important virulence factor associated with human diseases. It is about 400 fold more toxic to mice than *Stx₁* and has also shown to induce foetoplacental reabsorption, intrauterine haematoma, fibrin deposition and neutrophil infiltration (Tesh *et al.*, 1993). Though low in prevalence, detection of virulence genes indicates distribution of potentially pathogenic *E. coli* in milk and meat based fast food in Mizoram. Therefore, presence of virulence genes in the fast food is of great importance from public health point of view as it may serve as a vehicle for transmission of *E. coli* infection to the human being through consumption.

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