ENTEROHAEMORRHAGIC ESCHERICHIA COLI ON BEEF PROCESSING SURFACES*

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Abstract

Enterohaemorrhagic *Escherichia coli* (EHEC) is primarily an emerging foodborne bacterial pathogen. It is associated with severe complications such as haemorrhagic colitis and haemolytic uraemic syndrome in humans. Biofilm formation by EHEC on various food contact surfaces in processing plants is an important reason for cross-contamination. The present study was undertaken to know the occurrence of EHEC in beef processing surfaces, since ruminants are the reservoir host for this organism. For the study, 30 surface swabs were collected in 0.1 percent peptone water from three different cattle slaughter houses in Kerala. The selective enrichment was carried out in EC O157: H7 selective broth at 37^0 C for 24 h and plated on to Eosin Methylene Blue agar by incubating at 37^0 C for 24 h. The *E. coli* positive colonies were streaked on to Cefixime Tellurite- Sorbitol Mac Conkey agar and incubated at 37^0 C for 48 h. For further confirmation, suspected colonies were transferred on to 4- Methyl umbelliferyl beta- D-Glucuronide agar plates and incubated at 37 ^{0}C for 24 to 48 h. Positive isolates were subjected to biochemical tests. Out of the 30 samples tested for EHEC, two samples were found to be positive. This study shows that EHEC on processing surfaces can act as an important source of infection to beef consumers and it envisages the necessity for effective disinfection procedures in beef processing surfaces.

Keywords: *Enterohaemorrhagic Escherichia coli, beef processing*

Enterohaemorrhagic *Escherichia coli* (EHEC) is a food-borne zoonotic agent associated with outbreaks worldwide. Its significance as a public health problem was recognized in 1982, following an outbreak in the United States of America. It is associated with severe complications such as haemorrhagic colitis and haemolytic uraemic syndrome in humans (Orth *et al*., 2006). *E. coli* O157:H7 is the most important EHEC serotype in relation to public health; however, other serotypes have frequently been involved in sporadic cases and outbreaks. In the year 2011, fifty cases of death were reported from Germany due to EHEC infection (WHO, 2011). Recently in 2014, twelve cases were reported from four states of USA

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and epidemiologic investigation revealed that contaminated ground beef produced by Wolverine Packing Company was the source of this outbreak. After the identification of source of infection that company recalled approximately 1.8 million pounds of ground beef products that was contaminated with *E. coli* O157:H7. (CDC, 2014).

Ruminants are the natural reservoirs for EHEC and adult ruminants usually remaining as asymptomatic for EHEC infection. Currently, no treatment is available for EHEC infections and it is reported that antibiotics exacerbates the toxin release and the severity of infection (Goldwater and Bettelheim, 2012). Biofilm formation by EHEC on various food contact surfaces in processing plants is also an important reason for cross-contamination. Hence, the present study was undertaken to know the occurrence of EHEC in beef processing surfaces which may result in serious outbreak among beef consumers.

Material and Methods

For the study, 30 surface swabs were collected from beef processing surfaces (floor and tabletops) of three different slaughter houses (SH1, SH2 and SH3) in Kerala. All the samples were collected in 0.1 percent peptone water aseptically. The selective enrichment was carried out in EC O157: H7 selective broth at 37^0 C for 24 h. Enriched samples were plated on to Eosin Methylene Blue agar (EMB) and incubated at 37^0C for 24 h. A loop full of colonies having greenish metallic shean with black centers were streaked on to Cefixime Tellurite-Sorbitol Mac Conkey agar (CT- SMAC) and incubated at 37^0C for 48 h (Meng *et al*., 2001). For further confirmation, suspected colonies were transferred on to 4- Methyl umbelliferyl beta- D- Glucuronide agar (MUG) plates and incubated at 37 ^{0}C for 24 to 48 h (Fujisawa *et al*., 2000). Positive isolates were subjected to gram staining. Further confirmation was done by various biochemical tests (Barrow and Feltham, 1993).

Results and Discussion

In the present study 13 *E. coli* isolates and two EHEC isolates were confirmed by culture and biochemical methods. The overall prevalence of *E. coli* and EHEC were 43.3 percent and 6.67 percent, respectively (Table 1). Two EHEC isolates, one each from SH1 and SH2 were identified by its characteristic non-sorbitol fermenting transparent neutral grey colonies with smoky centre having a diameter of 1- 2 mm. These positive isolates appeared non fluorescent on MUG EC O157 agar under UV illumination which is characteristic of EHEC. The Positive isolates appeared as pink short rods on gram staining. On performing biochemical tests, EHEC isolates were found to be positive for Catalase, Methyl Red, Indole production and Sorbitol utilization .Isolates were negative for Voges Proskauer and Citrate utilization.

In the present study, even though there is lower prevalence of the organism, low infectious dose and no treatment envisages the importance of EHEC. Particular attention should be paid to maintain the cleanliness of animals and prevention of spread of faecal material, which is the primary source of contamination. All abattoirs or establishments where animals are slaughtered should have in place a system of good hygienic practice and an effective HACCP plan covering all stages in the production process, from the time animals arrive until the carcasses or meat products leave the establishment. In handling carcasses, particular care should be taken during the dehiding stage, removal of hoofs, evisceration, and cutting to minimize cross contamination.

Table 1. Occurrence of *E. coli* and EHEC in different slaughter houses

	SHI	SH2	SH3
Total samples collected	7	15	8
***E. coli* positive isolates**	2	8	3
EHEC positive isolates	1	1	0

In addition, care should be taken to minimize spillage of intestinal tract contents (Reilly, 1998).

This study shows that presence of EHEC on processing surfaces can act as an important source of infection to beef consumers and it necessitates the need for effective disinfection procedures in beef processing surfaces. By examining the sources and distribution of this bacteria, and how they cause disease, we will be in a better position to prevent and treat the inevitable future cases of sporadic disease and victims of common source outbreaks.

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