Isolation of Sorbitol non Fermenting *Escherichia coli* from Beef Samples in Coimbatore and its Antibiogram

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E.coli **is a normal intestinal flora in animals and human beings. Diarrhoea causing *E.coli* is classified as Enterotoxigenic *E.coli*, Enteroinvasive *E.coli*, Enteropathogenic *E.coli,* and Enterohaemorrhagic *E.coli* (EHEC). EHEC is an emerging pathogen causing haemorrhagic colitis and Haemolytic Uremic Syndrome.The most common EHEC is *E.coli* O157:H7.In this study, *E.coli* is isolated from 100 faecal samples of cattle in Coimbatore. Among the *E.coli* isolates, 32 were sorbitol non fermentors. These sorbitol non fermentors were subjected to drug susceptibility to 12 different antibiotics. Multiple drug resistance was observed.**

Key words: Drug resistance, Sorbitol non fermenting *E.coli*, *E.coli* biovars.

Enterohaemorrhagic *Escherichia coli* (EHEC) 0157:H7 is a disease of public health importance. It is contracted when people get in contact with infected cattle, a popular vehicle of transmission (Robinson *et al.,*2004) It is also acquired when one eats undercooked ground beef, cider, vegetables or other products. EHEC 0157:H7 persists for long periods of time (up to 21 days) in manure, and even in soil. Person-to-person transmission has also been documented (Chapman *et al.,* 2001)

Awareness of the consequences of the meat borne pathogen *Escherichia coli* O157:H7 has increased in the general public opinion making this organism a household name in the 21st century (Ransom *et al.*, 2003). Recently, the preliminary Food Net data on the incidence of infection with pathogens transmitted through food in the USA showed a decline in infections caused by *Campylobacter*, *Listeria*, *Shigella* and *Yersinia*; however, those caused by Shiga toxin-producing *Escherichia coli* O157 and *Salmonella* did not decrease significantly, while *Vibrio* infections increased (Centers for Disease Control and Prevention (CDC, 2007).

Modern life conditions, with some of them related to or being the result of globalization, ensure that factors responsible for disease emergence are more strongly prevalent than ever. Even when the categorization of these factors is somewhat arbitrary, some of the main ones are ecological changes, intensive agriculture, anomalies in the climate, human demographical changes and behavior, travel and commerce, technology and industry, microbial adaptation and change and the breakdown of public health

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measures. Further on, concerning pathogens, their most striking feature is their diversity and selection for drug resistance, suggesting that infections will continue to emerge and will probably increase, which emphasizes the urgent need for effective surveillance and control (Morse, 2007). In India few reports are available on the occurrence of Sorbitol non fermenting *E.coli* The objective of this study was to determine the prevalence of Sorbitol non fermenting *E.coli* in raw beef samples in Coimbatore, as this has not been investigated previously.

MATERIAL AND METHODS

Collection and storage of fecal samples

In the periods from December 2009 to February 2010, fecal samples from adult cattle were collected at the slaughterhouses in different regions of the City. Immediately after slaughter, samples of the rectal contents were collected aseptically by rectal palpation and were kept at 4 to 8°C. The adult cattle sampled were randomly selected. The collected fecal samples were transported immediately to the laboratory, where the microbiological examination was started within 20 h.

Isolation of sorbitol non-fermenting *E. coli* by selective plating

A 20-g portion of each fecal sample was added to 180 ml of modified *E. coli* broth containing novobiocin (20 mg/liter). After homogenization for 1 min, the samples were incubated for 18 to 20 h at 37°C on a rotary shaker (100 rpm). Each enrichment culture was serially diluted 10-fold to 10^{-6} in 0.1% peptone water. One hundred-microliter volumes of the 10^{-5} and 10^{-6} dilutions were spread plated onto sorbitol MacConkey agar (SMAC). The plates were incubated at 42°C for 18 to 20 h. Sorbitol-nonfermenting colonies (up to 5 per sample) were selected for confirmation using standard biochemical methods. (Heuvelink *et al*., 1997).

Susceptibility Testing

Sorbitol non fermenting *E.coli* (32) strains were subjected to drug sensitivity assay. Susceptibility to 24 antibiotics namely amikacin (AK-30 mcg), aztreonam (AO-30 mcg), cefazolin (CZ-30 mcg), ceftazidime (CA-30 mcg), cephotaxime (CE-30 mcg), ciprofloxacin (CF-5 mcg), gentamycin (G-5 mcg), kanamycin (K-30 mcg), nalidixic acid (NA-30 mcg), trimethoprim (TR-5 mcg), cefixime (CFX-5 mcg) and tetracycline (T-30 mcg), were tested. Zones of inhibition were determined in accordance with procedures of the National Committee for Clinical Laboratory Standard (NCCLS, 1999).

RESULTS AND DISCUSSION

Isolation of *E. coli*

Sorbitol non fermenting *E.coli* strains were isolated from 32% of the fecal samples. This shows a higher incidence of these strains. The prevalence of STEC in beef samples reported from countries such as Belgium, New Zealand, India and USA has ranged from 1.8% to 50% (Pie'rard *et al.,* 1997; Brooks *et al*., 2001; Dutta *et al.,* 2002 and Samadpour *et al.,* 2002). Other studies reported prevalence of *E. coli* O157 in beef which ranged from 1.1% to 13.4% (Chapman *et al.,* 2001). When compared to these studies, the recovery of sorbitol non-fermenting *E.coli* is higher in our study.

Sorbitol non fermenting *E.coli* with the inability to produce glucuronidase is the major identification characteristic of *E.coli* O157:H7 (Raji *et al*., 2008). But this needs further confirmation using PCR. Cattle are considered to be the principal natural reservoir of this pathogen, but strains of this pathogen are also prevalent in the gastrointestinal tracts of other domestic animals, particularly ruminants. Consumption of foods of bovine origin, particularly raw or undercooked ground beef products and raw milk contaminated with bovine feces, has been associated with large food poisoning outbreaks in which this organism was identified as the etiologic agent (WHO, 1997). To our knowledge this is the first survey of the prevalence of sorbitol non fermenting *E.coli* in raw beef in Coimbatore.

Antibiotic resistance

All the *E.coli* isolates were resistant to one or more antibiotics. 90 per cent of the isolates were resistant to cefixime. 80 per cent of the isolates were resistant to cefazolin. The lowest resistance rates were observed in ciprofloxacin and trimethoprim. The drug resistance patterns of these isolates are shown in Fig. 1.

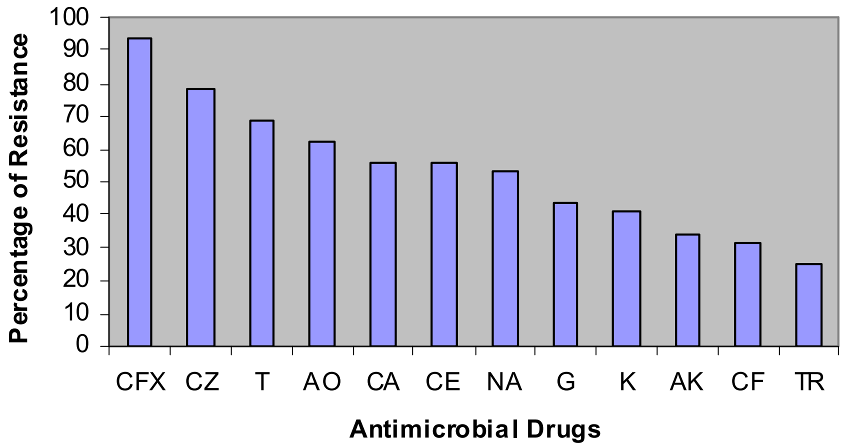

Fig. 1. Antimicrobial resistance patterns in 32 *Escherichia coli* strains taken from beef samples in coimbatore

Aztreonam is an extended spectrum beta-lactam antibiotic. 70 per cent of the *E.coli* isolates are resistant to this antibiotic. Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations.

The antimicrobial susceptibility results of the *E.coli* isolates are a cause for concern as more than 60% of the isolates were resistant to three or more drugs. Similar incidences of resistance have been reported for isolates obtained elsewhere (Maidhof *et al.,* 2002; Khan *et al.,* 2002a).

The high incidence in this study may be due in part to selective pressure resulting from incorporation of antibiotics into animal feeds. Expansion of this study to include genes encoding putative accessory virulence factors, such as intimin or the plasmid-encoded hemolysin, is necessary to further evaluate significance of these strains in human disease. Antimicrobial resistant bacteria from animals may colonize human population via the food chain; it is therefore possible that resistant bacteria may be readily transferred to humans from animals used as food sources (Van den Bogaard & Stobberingh, 2000).

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