

ENTEROPATHOGENIC E. COLI IN RAW AND COOKED BEEF

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SUMMARY

Two hundred samples, 100 each of raw and cooked beef were collected from different restaurants in Benha.

Samples were examined for rapid screening of *E. coli* by a fluorogenic assay.

E. coli was detected in 7% of fluorocult lauryl sulphate enrichment broth which emits a blue fluorescence under UV light.

The incidences of *E. coli* were 9% and 5% in examined raw and cooked beef respectively.

O26: K60 (B6) and O111 : K58 (B4) were only the enteropathogenic serotypes isolated from the examined samples. The public health significance of isolated pathogens was discussed. Moreover, suggestive measures for the improvement of the microbial quality of meat were mentioned.

INTRODUCTION

Escherichia coli is the classical indicator of the possible presence of enteric pathogens in meat.

Thus high number of *E. coli* in cooked meat suggests careless cleanliness in production, handling and storage of cooked meat (ICMSF, 1978 and 1988).

Therefore, the necessity of rapid detection of *E. coli* arises for the safety of consumers. Fluorocult media supplemented with 4- methyl-umbelliferyl-B- D- glucuronide (MUG) is useful for the direct detection of *E. coli* within 24 hours.

Moberg et al., 1988 stated that the enzyme B-glucuronidase present in *E. coli* is responsible for emitting a blue fluorescence under UV light due to hydrolysis of 4- methyl-umbelliferyl-B- D- glucuronide (M- U- G-) in the fluorocult media and production of methyl umbelliferone.

Many investigators indicate the usefulness of this method to detect *E. coli* in a variety of foods (Damare et al., 1985; Moberg, 1985; Moberg et al., 1988 and Ibrahim et al., 1995).

The present investigation is therefore carried out to detect *E. coli* in raw and cooked beef by fluorogenic assay.

MATERIAL AND METHODS

Samples:

Two hundred samples, 100 each of raw and cooked beef were collected from different restaurants in Benha. The samples were collected in sterile polyethylene bags and transferred to the laboratory for examination of *E. coli* using Fluorocult media for rapid detection of *E. coli*.

Technique:

One ml of meat homogenate (prepared according to ICMSF, 1978) was added to 9 ml of fluorocult lauryl sulphate broth (Merck, 12588) and incubated at 37°C for 24 hours, *E. coli* positive tubes emit a blue fluorescence under the light of UV lamp (4 w/366 nm-Merck, 13203).

Isolation and identification of *E. coli*:

A loopful from each of the positive fluorescence tubes was streaked onto Eosin Methylene (EMB) agar plates. The inoculated plates were incubated at 37°C for 24 hours. Typical colonies (greenish metallic with dark purple center) were picked and purified by streaking onto plate nutrient agar for further identification using Enterotube II, Roche.

Serodiagnosis of *E. coli*:

The isolates were identified serologically using diagnostic sera "Welcome *E. coli* agglutination sera" for diagnosis of Enteropathogenic serotypes.

RESULTS

Table (1): Incidence of *E. coli* in examined beef samples using Fluorogenic Assay.

Samples	No. of examined samples	F.L.S. tubes fluorescence	<i>E. coli</i> positive samples	
			No.	%
Raw beef	100	22	9	40.9
Cooked beef	100	10	5	50
Total	200	32	14	43.75

F.L.S. Fluorocult Lauryl Sulphate broth tubes.

Table (2): Enterobacteriaceae with glucuronidase activity isolated from examined beef samples.

Isolates	Raw beef	Cooked beef	Total
<i>Escherichia coli</i>	9	5	14
<i>Citrobacter freundii</i>	3	1	4
<i>Enterobacter cloacae</i>	4	2	6
<i>Serratia liquefaciens</i>	2	1	3
<i>Klebsiella pneumonia</i>	3	1	4
<i>Shigella boydii</i>	1	0	1
Total	22	10	32

Table (3): Serotypes of enteropathogenic *E. coli* isolated from beef.

Sample	No. of isolates	<i>E. coli</i> serotype
Raw beef	2	O ₂₆ : K ₆₀ (B ₆)
	2	O ₁₁₁ : K ₅₈ (B ₄)
Cooked beef	3	O ₂₆ : K ₆₀ (B ₆)

DISCUSSION

Raw and cooked meat may constitute public health hazard either due to the presence of spoilage bacteria responsible for deteriorative changes or pathogenic microorganisms leading to infection or intoxication. Presence of *E. coli* in meat is frequently considered as reliable index of low standard of hygiene (Harrijan and McCance, 1982). There are pathogenic strains that cause distinct syndromes of diarrhoeal disease and that have been associated with food-borne illness. These food pathogens are grouped into categories based on distinct virulence properties, different interactions with the intestinal mucosa, distinct clinical syndromes, differences in epidemiology

and distinct O: H serogroup. These categories are the following:-

Enterotoxigenic *E. coli* (ETEC) and Enterohaemorrhagic *E. coli* (EHEC) (Doyle, 1990 and Elmer et al., 1992).

The ordinary technique for detection of *E. coli* in foods is based on their properties of acid and gas production from lactose fermentation. It involves two steps, the detection of coliforms, followed by confirmation of the presence of *E. coli* at an elevated temperature (44.5±0°C). This technique was found to be laborious, time consuming (complete diagnosis requires 5 days). In addition

cultivation of an aerogenic and non-lactose fermenting strains representing about 5% of *E. coli* population (Husson et al., 1981) or the presence of lactose fermenting non coliform may interfere with *E. coli* detection.

On the contrary Feng and Hartmam (1982) reported that the Fluorogenic assay is a rapid and sensitive technique which allows the presumptive detection of *E. coli* within 24 hours.

The obtained results (table 1) reveal the presence of Enterobacteriaceae microorganisms with glucuronidase activity in 32 samples out of 200 samples. Typical *E. coli* was detected only in 14 samples presenting a percentage of 7% . The rest of isolates were identified as *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia liquefaciens*, *Klebsiella pneumonia* and *Shigella boydii* (Table 2).

Robison (1984) mentioned that the B-D-glucuronidase activity is a special property of *E. coli*, 94% of all *E. coli* strains have this enzyme. In contrast, only 1% of enterobacter, 2% of klebsiella, 5% of citrobacter, 8% of serratia (Hartman, 1987), 20% of salmonellae (Feng and Hartman, 1982) 44% of shigella (Kilian and Bulow, 1976) have been able to show this enzyme activity.

E. coli was detected in 9% of raw beef. Higher incidence was reported by El-Daly (1983), Al-Cherif (1985) and Nassar (1988), while in cooked beef. *E. coli* was found in 5% of examined samples. This finding nearly simulates what has been reported by Ahmed (1989) and Hassan

(1991). The data presented in table (3) denote that out of 14 isolates of *E. coli* from examined raw and cooked beef samples, only 7 isolates (50%) were enteropathogenic. Isolated serotypes were O₂₆: K₆₀ (B6) 5 strains and O₁₁₁: K₅₈ (B4) strains.

Similar serotypes were isoalted by (Niazi and Refai, 1988). Zaki (1990) from sausage and from meat and meat contact surfaces (Yassien, 1992), and from minced meat, kofla, steak burger, oriental sausage and beef burger (Ibrahim et al., (1995).

These enteropathogenic serotypes have been implicated in cases of gastroenetritis, epidemic diarrhoea in infants, sporadic summer diarrhoea in children as well as in cases of food poisoning (Abd El-Aziz, 1979; Edelman and Levine , 1983 and Marzouk, 1985). In this respect, an outbreak of disease caused by enterohaemorrhagic Escherichia coli serotype O111 associated with the consumption of Mettwurst was recorded. The declared outbreak affected three children with haemolytic uraemic syndrome (HUS) had been reported to the Communicable Disease Center (CDC) of the South Australian Health Commission (Cameron, 1995).

Suggestive measures for reduction of microbial hazards and to provide safe foods free from pathogenic food-borne infections and intoxications were recommended in international code of practice general principles of food hygiene (CAC, 1979). They include hygiene requirements for food establishments (design and facilities), maintenance of hygiene and health

requirements for food handlers and hygiene processing requirements.

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