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ORIGINAL RESEARCH

Isolation of pathogenic *Escherichia coli* from buffalo meat sold in Parbhani city, Maharashtra, India

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Abstract

Aim: Isolation, characterization, in-vitro pathogenicity and antibiogram study of *E.coli* from buffalo meat sold in Parbhani city.

Materials and Methods: Meat samples were collected from buffalo immediately after slaughter. Isolation, identification and enumeration of *E. coli* were done by following standard methods and protocols. Hemolysin test and Congo red binding assay were used to study *in-vitro* pathogenicity of *E. coli* isolates. Disc diffusion method was used to study antibiogram of pathogenic *E. coli* isolates.

Results: A total of 250 buffalo meat samples were collected and processed. A total of 22 (8.80 percent) *E. coli* isolates were isolated with average differential count of 1.231 ± 0.136 $\log_{10}$ cfu/g on EMB agar. All the *E. coli* isolates were confirmed by Grams staining, biochemical reactions and sugar fermentation and motility tests. A total of 9 (3.6 percent) *E. coli* isolates were found to be pathogenic by *in-vitro* pathogenicity testing. Antibiogram studies of pathogenic *E. coli* isolates showed that all 9 isolates were sensitive to gentamycin (20 ± 1.49 mm) while 7 isolate showed resistance to enrofloxacin (18.22 ± 3.58 mm) and tetracycline (11.44 ± 2.04 mm).

Conclusion: Buffalo meat sold in Parbhani city is an important source of *E. coli* infection to human population. A total of 9 pathogenic *E. coli* were isolated from buffalo meat immediately after slaughter. All isolates were characterized and confirmed pathogenic by *in-vitro* pathogenicity tests. Antibiogram studies of all isolates revealed sensitivity to gentamicin and resistance to tetracycline and enrofloxacin.

Keywords: antibiogram, buffalo meat, *E. coli*, pathogenicity

Introduction

India is a leading exporter of buffalo (*Bubalus bubalis*) meat earning foreign exchange. The production of buffalo meat (beef) is increasing by 4.5 percent per annum [1]. According to Animal Husbandary statistical database of Department of Animal Husbandary, Dairying and Fisheries (DADF), there are a total of 5,520 recognized and 4,707 unrecognized slaughter houses in the country. The buffalo carcass has less fat, less bone and higher proportion of muscle than cattle [2]. Sanitary indices refer to certain organisms or group of organisms which indicates presence of potentially pathogenic or spoilage organisms in food. Coliform organisms in food indicate poor sanitary practices and presence of other hazardous organisms. This group includes *E. coli* and enumeration of *E. coli* counts is important to determine sanitary indices [2]. Presence of *E. coli* in food is an indicator of poor sanitary conditions during processing. Isolation of *E. coli* from meat and meat products is a common phenomenon [3, 4]. Studies showed that normal intestinal microflora specially commmensal *E .coli* strains under specific condition, might serve as reservoir of resistance genes that could be acquired by pathogenic bacteria [5]. Since commensal bacterial strains are exposed to the same selective pressure as pathogenic strains, they might be used as an indicator of trends in antimicrobial resistance [6]. Also *E. coli* being a member of *Enterobacteriacae* is being exposed to various antibiotics and antimicrobial agents in maintenance of hygiene in animal husbandry practices as well as during processing of meat. This results into development of drug resistant condition. Antibiotic resistance to beef borne *E. coli* is well documented [8,9].

The growing problem of antibiotic resistant has become a significant public health concern world wide. All uses of antimicrobial in human medicine and animal husbandry create selective pressure that favors emergence of antimicrobial resistance among micro-organism [7].

Keeping in view the public health importance and hazards caused by pathogenic *E. coli*, the present study was undertaken to isolate and identify these pathogenic and drug resistant organisms from buffalo meat sold in Parbhani city.

Materials and Methods

Sample collection and processing: Buffalo beef samples were collected from Parbhani Municipal Council Abattoir. A total of 250 meat samples approximately weighing 50 gm were collected from neck site of slaughtered animal in sterile polyethylene schachets for the period from December 2011 to May 2012. These meat samples collected were brought to the laboratory on ice within one hour and processed immediately.

Isolation, identification and enumeration of *E. coli*: Isolation of *E. coli* was done as per the method

Table-1. Results of sensitivity pattern of *E. coli* isolates

Antibiotics	S	I	R	Average zone of Inhibition in mm
Gentamycin	9	0	0	20.00 ± 1.49
Enrofloxacin	2	0	7	18.22 ± 3.58
Tetracycline	1	1	7	11.44 ± 2.04

S - Sensitive, I - Intermediate sensitive, R - Resistant

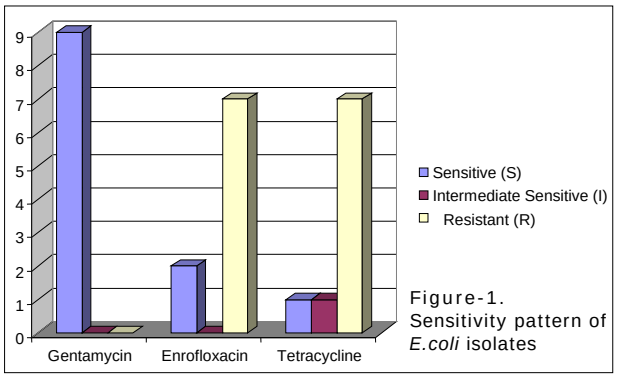

Figure-1. Sensitivity pattern of *E.coli* isolates

described in BAM [10]. A quantity of 10gm of beef sample was minced with the help of sterile scissors and mixed in 90 ml normal saline solution (pH 7.2) in screw cap bottle. Ten fold serial dilutions were made up to 10^{-5} dilution in normal saline solution (pH 7.2). A quantity of 0.1 ml inoculam from 10^{-3} and 10^{-4} dilutions was used by spread plate technique on Eosin Metheline Blue agar (EMB) (Himedia Laboratories, Mumbai). Incubation was done at 37^{0}C for 24 hrs and colony counts were taken. Typical characteristics colony of *E. coli* on EMB agar as greenish metallic sheen was enumerated and isolated. Identification of *E. coli* was done by biochemical test i.e IMViC, catalase, oxidase & nitrate reduction and sugar fermentation and motility tests. [11].

In-vitro pathogenicity test of *E. coli*: *In-vitro* pathogenicity test of confirmed *E. coli* isolates was done by heamolysis and Congo red binding assay [12,13]. Colonies showing hemolytic zone are considered as heamolytic positive. While isolates, producing intense orange or brick red colour on Congo red medium were considered as positive and those producing grayish white colonies recorded as negative.

Antibiogram of *E. coli:* Antibiotic sensitivity of all pathogenic *E. coli* isolates was done by disc diffusion method [14] on Muller-Hinkton agar. Three antimicrobial discs were used with different concentrations viz, Gentamycin (10 mcg), Tetracycline (30mcg) and Enrofloxacin (10mcg). Zones of complete inhibition were measured in millimeter with rule.

Results

250 buffalo meat samples were screened for isolation of *E. coli* on EMB agar. A total of 22 *E. coli* isolates were obtained. The isolates were identified based upon colony characteristics comprising of bluish green metallic sheen. The percentage of *E. coli* isolation observed was 8.80 percent. Having average differential count of 22 isolates of *E. coli* on EMB agar observed was 1.231 ± 0.136 $\log_{10}$ cfu/ gm. A total of 22 *E. coli* isolates were subjected to identification based upon Grams staining, biochemical characters, sugar fermentation and motility tests. All the 22 isolates were subjected to *in-vitro* pathogenecity test that is hemolysin test and Congo red binding assay. A total of 9 *E. coli* isolates were found positive for pathogenecity in both the *in-vitro* pathogenecity tests. The percent positivity of hemolysin test and Congo red binding assay observed was 3.60 percent. All 9 pathogenic *E. coli* isolates were subjected to antibiotic sensitivity test against gentamycin, tetracycline and enrofloxacin. The zones of inhibition of antibiotic were recorded. The average zone of inhibition seen were gentamicin (20.00 ±1.49mm), enrofloxacin (18.22±3.58mm) and tetracycline (11.44±2.04 mm). All 9 isolates were sensitive to gentamicin while 7 isolates showed resistance to enrofloxacin and Tetracycline. A complete sensitivity of all 9 isolates (100 percent) was observed against gentamicin.

Discussion

In present study, very low percentage(08.80 percent) of *E. coli* was isolated. The findings of present study are in agreement with earlier reports [3,15]. The *E. coli* counts are in the of range 6.85 to 7.40 $\log_{10}$ cfu/g [16]. Scanning of available literature reveals that percentage of *E. coli* isolation and identification varies considerably [17,18]. The variation in percentage of *E. coli* isolation and identification may be due to difference in hygienic conditions at different slaughter houses.

All the 22 *E. coli* isolates shown typical Grams staining reaction, biochemical reactions, motility and sugar fermentation reactions described earlier [11]. Many workers successfully used staining characters, biochemical reactions, sugar fermentation reactions and motility patterns for confirmation of *E. coli* isolated from meat [19].

Hemolysis of sheep RBC is an important criterion for identification of pathogenic strains of *E. coli*. *In-vitro* pathogenecity studies of hemolytic *E. coli* were done by using 5 percent sheep blood agar [20]. Many workers successfully used Congo red binding assay in tryptose soya agar for identification of pathogenic *E. coli* [20-22]. The observations are in agreement with earlier workers [20,21].

Antimicrobial sensitivity testing of the pathogenic *E. coli* isolated from beef samples confirms that all 9 pathogenic isolates were highly sensitive to Gentamycin. Out of nine isolates 7 isolates shown resistance to Enrofloxacin and Tetracycline. Similar reports in previous studies showed resistance of meat borne *E. coli* to Gentamycin and Enrofloxacin [23], Tetracycline and Ciprofloxacin [24,25]. In present study sensitivity of *E. coli* to Tetracyclin was observed

in 2 isolates but at low level.

The resistance of pathogenic *E. coli* to the antibiotics may be due to indiscriminate use of antibiotics in animal husbandry practices. Pathogenic *E. coli* of meat origin are always having grater potential to enter into food chain. Buffalo meat, beef and other types of meat are cheap source of human infection [4,26].

Conclusion

Buffalo meat sold in Parbhani city is an important source of *E. coli* infection to human population. All isolates were characterized and confirmed pathogenic by *in-vitro* pathogenicity tests. Antibiogram studies of all isolates revealed sensitivity to gentamicin and resistance to tetracycline and enrofloxacin.

Authors' contribution

The present work was carried out by CSS under the guidance of VV with the technical support of RW. RW drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

Authors declares that they have no competing interest.

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