Study on occurrence and antibiogram pattern of *Escherichia coli* from raw milk samples in Anand, Gujarat, India

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Abstract

Aim: The study was carried out with aim to isolate *Escherichia coli* from raw milk samples and determine antibiogram pattern of *E. coli* isolates.

Materials and Methods: During 6 months duration of study a total of 100 raw milk samples were collected from different places in and around Anand city such as individual household, cattle farms, milk collection centres of Co-operative milk dairies and milk vendors. All raw milk samples were enriched in peptone water and inoculated on selective media and various biochemical tests were performed for confirmation of isolates. Antibiogram pattern of *E.coli* to antimicrobial agents was evaluated by disk diffusion method. *E. coli* isolates were sent to National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh for serotyping.

Results: The result of present study revealed that out of 100 samples, 38 samples were found contaminated with *E.coli*. Antibiogram pattern revealed high resistance against ampicillin (100 %), whereas moderate resistance was observed for streptomycin (57.89 %), oxytetracycline (47.37 %) and amoxy-clav (42.11 %). Also lesser percentage of resistance was observed for co-trimoxazole (13.16 %) and chloramphenicol (5.26 %). Serotypes detected were O24 (7 isolates), followed by O36 (1 isolate), O89 (1 isolate), O91 (1 isolate) and O153(1 isolate).

Conclusions: Results suggested a possibility of potential public health threat of *E.coli* originating from raw milk sources.

Key words: Antibiogram pattern, *E.coli*, Enteropathogenic, Raw milk, Serotypes.

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Introduction

Food safety and quality is a topic of public concern throughout the world. Well publicized and widespread food borne disease outbreaks have created an awareness of potential threats to human health from food products. Markets and consumers for raw milk and their products have existed in many parts of the world. Raw or processed milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product or infections/ intoxications in consumers [1, 2].

Generally, bacteria in the milk can occur through colonization of the teat canal or an infected udder (clinical and subclinical mastitis) or gets contaminated at various stages be it from the animal, milker (manual as well as automated), extraneous dirt or unclean water [3-7]. Many microorganisms can get access to milk and milk products, among these are *E. coli*. Coliforms and *E. coli* are often used as marker organisms [8, 9, 10]. Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard [11]. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man [12]. The milk sold in raw forms and because of possibilities of contamination with *E. coli* posses a great hazard to public health [13].

In addition to the presence of *E. coli* denoting fecal contamination, the presence of virulence – related genes in *E. coli* strains refer to the pathogenicity of the isolates. Previous studies documented the presence of some *E. coli* isolates from raw milk and products for virulence markers [14-17].

Thus, the objective of this study was to investigate the occurrence of the opportunistic pathogen *E. coli* in raw milk, determine antibiogram pattern and serotyping of *E. coli isolates.*

Materials and Methods

Sample collection: From February 2011 to July

Table-1. Morphological and culture characteristics of bacteria

Isolated bacteria	Gram staining	Culture characteristics on selective media
Escherichia coli	Gram negative short rods	Mac Conkey Agar: Pink coloured colonies. Eosin Methylene Blue agar: Metallic sheen.

Table-3. Antibiogram pattern of 38 *E.coli* isolates from raw milk

Antimicrobials	No. of resistance	% resistance
Ampicillin	38	100.00
Streptomycin	22	57.89
Oxytetracycline	18	47.37
Amoxy-clav	16	42.11
Co-trimoxazole	5	13.16
Chloramphenicol	2	5.26

Table-2. Biochemical characterisation of *E. coli*

Biochemical test	Reaction
Catalase	+
Indole	+
Methyl red	+
Voges-Proskauer	-
Citrate utilization	-
Nitrate reduction	+
Urease	-

Table-4. Serotyping results obtained from Central Research Institute, Kasauli

Sr. no.	Serotypes	No. of isolates
1	O24	7
2	O36	1
3	O89	1
4	O91	1
5	O153	1
6	Untypable	3
7	Dead during transit	24

2011, a total of 100 raw milk samples were randomly collected in sterilized screw capped bottles from different localities in and around Anand city such as individual household, cattle farms, milk collection centres of Co-operative milk dairies and milk vendors. The milk samples were transported in a cool box to the laboratory of the Post Graduate Department of Veterinary Public Health, College of Veterinary Science & Animal Husbandry, AAU, Anand and tested within 24 hrs for the isolation and identification of *E .coli*.

Isolation and identification of *Escherichia coli:* Isolation of *E.coli* was attempted according to Singh and Prakash [18] with slight modification. A part of each sample (10 ml) was enriched in peptone water (HiMedia Pvt. Ltd.) (90 ml) and was incubated at 37 ºC for 24 hours. Enriched samples were inoculated on Mac Conkey Agar (MCA) (HiMedia Pvt. Ltd.) a dual purpose (selective and differential) medium, by four flame technique and plates were incubated at 37 ºC for 24 hours. Pink coloured colonies appeared were considered as presumptive of *E.coli*.

A single isolated colony was picked and streaked on Eosin Methylene Blue Agar (EMB) medium (HiMedia Pvt. Ltd.) and incubated at 37 ºC for 24 hours. *E. coli* produces metallic sheen on EMB agar. Such colonies were taken into nutrient broth for further biochemical examination.

Biochemical examination: Biochemical tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Citrate utilization and Urease production.

Antibiogram pattern of the isolated *E.coli* to some antimicrobial agents: The susceptibility of isolates to different antimicrobial agents was done by disk diffusion method using commercial disks [19] procured from HiMedia Pvt. Ltd. and almost all antimicrobial agents were having expiry date after 5-6 months of completion of research.

The antimicrobial agents tested were the following: ampicillin (10 µg), amoxy-clav (30 µg), chloramphenicol (20 µg), co-trimoxazole (30 µg), oxytetracycline (30 µg), streptomycin (10 µg).

Serotyping of *E. coli* isolates: Isolates were sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh.

Results

In the present study, it was revealed that 38 samples (38.00%) out of 100 raw milk samples were found positive for *Escherichia coli* according to morphological and cultural characteristics (Table 1) and biochemical tests (Table 2).

The antibiogram pattern revealed that 38 *E.coli* isolates showed high resistance against ampicillin (100 %), whereas moderate resistance was observed for streptomycin(57.89 %), oxytetracycline(47.37 %) and amoxy-clav(42.11 %). Also lesser percentage of resistance was observed for co-trimoxazole(13.16 %) and chloramphenicol(5.26 %) (Table 3).

The serotyping report received from National Salmonella and Escherichia Centre, Central Research Institute, Kasauli revealed that serotypes detected were O24 (7 isolates), followed by O36 (1 isolate), O89 (1 isolate), O91 (1 isolate) and O153(1 isolate) (Table 4).

Discussion

E.coli is not only regarded as an indicator of

fecal contamination but more likely as an indicator of poor hygiene and sanitary practices during milking and further handling. Higher prevalence of *E.coli* was reported by Ali and Abdelgadir [11] who found 63 % prevalence and Lingathurai and Vellathurai [20] who found 70 % prevalence. The incidence of the species of *E. coli* itself in milk and milk products, as a possible cause of food born disease, is not significant if *E. coli* is normally an ubiquitous organism [21], yet the pathogenic strains if present could be harmful to consumers.

Coliform bacteria can be carried into milk duct of the cow during milking by suction of the milking machine and then flushed out during subsequent milking without causing clinical symptoms of infection. Previous studies provided evidence that *Escherichia coli* are frequently occurring organism in milk. The methods of production, transportation, handling and sale of milk are entirely unhygienic [11]. High incidence of *E. coli* was found in different types of milk by many researchers [22-26]. In India, the raw milk and products were heavily contaminated by *E.coli* [9]. In South Africa, Lues *et al.* [27] detected a higher percentage of *E.coli* in raw milk. In Malaysia, Chye *et al.* [28] indicated that 90% of the examined raw milk were contaminated by coliform bacteria and 65% were *E.coli* positive. In Saudi Arabia, Salji et al. [29] recorded coliforms as the main contaminants of raw milk. Moreover in Saudi Arabia, Al-Kanhal et al. [30] reported that coliforms count exceeded the American standards in most of the dairy plant studied. Antibiotic resistance development among the bacteria poses a problem of concern. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous.

Out of 5 different serotypes found in this study, O24, O36 and O89 are not associated with clinical conditions, whereas serotypes O91 and O153 are associated with bloody diarrhea (Haemolytic uremic syndrome) in humans.

Serotypes like O157, O26, O8, O55, O86, O126 and O128 have been found to be associated with infantile diarrhoea among neonates and adult human patients suffering from gastroenteritis as reported by Beutin *et al*. [31] and Nishikawa *et al*.[32]. Thus, these serotypes may be of zoonotic importance. In present study, none of the above serotypes were found but still the virulence of *E. coli* should not be considered less.

Conclusion

Results clearly indicated that microbial quality and safety of raw milk produced by local farmers and distributors was unsafe. The presence of fecal indicator organism not only indicates the poor hygiene but also itself may be pathogenic. The pathogenic bacteria such as *E.coli* may pass to the milk; this suggests that raw milk should be considered a vehicle for the transmission of potentially pathogenic bacteria. For this, hygienic milk production techniques should be followed such as regular sterilization of dairy equipment, washing of utensils, milker's hands, udder, , etc. As well as pasteurization or boiling of milk prior to consumption is required. Frequent use of antibiotics should be stopped as antibiotic resistant strains are continuously increasing. This study highlights the need for continuous surveillance of antibiotic sensitivity pattern of *E.coli* with a view to selecting appropriate therapy.

Author's contribution

HC Thaker, MN Brahmbhatt and J B Nayak implemented the study design and analyze samples. HC Thaker analyzed the data. HC Thaker, MN Brahmbhatt and JB Nayak drafted and revised the manuscript. All the authors read and approved final manuscript

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Competing interest

Authors declares that they have no competing interest.

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