

Prevalence and Susceptibility Profile of E. Coli O157:H7 Isolated from Raw Milk in Kohat, Pakistan

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ABSTRACT

Diseases development due to food is a major threat to consumers; especially in countries where sanitation facilities are poor. E. coli O157:H7 strain causes major outbreaks among foodborne diseases. The current work investigated the prevalence of E. coli O157:H7 and their antibiogram profile in raw milk samples. A total of 180 samples were collected from milk shops in Kohat, Pakistan. The isolates were initially identified on colony morphology on different Media and later through biochemical tests. Isolates were also screened to differentiate E. coli O157:H7 from other fecal E. coli via sorbitol MacConkey agar. Out of 180 samples 134 (74.4%) milk samples were positive for E. coli and 46 (25.6%) milk samples were negative. Antibiotic susceptibility test was performed by using antibiotics of different classes. Most of the isolates showed susceptibility to ciprofloxacin (82.5%) (80.4%), gentamicin (82.5%) (80.5%) and Oxytetracycline (85.5%) (85.6%) respectively and slightly resistant to Tetracycline (55.8%) (48.2%), Imepenam (52.6%) (42.8%), Ceftriaxone (45.5%) (40.6%), Trimethoprim (54.4%) (36.6%), Chloramphenicol (38.6%) (30.5%), and shows resistance to Amikacin (25.5%) (18.4%) and Cloxacillin (10.5%) (4.5%) respectively. The high frequency of E. coli O157:H7 in milk samples indicate poor hygiene during milking, transportation and milk storage. The awareness against spreading of this pathogen is necessary among stakeholders at different levels of milk handling.

Keywords: E. coli O157:H7; Antibiotic resistance, Milk

INTRODUCTION

Microorganisms in food have either positive effect (food fermentation) or negative effect (food spoilage and foodborne diseases) ¹. The presence of E. coli in food is the indicator of enteropathogenic or toxigenic bacteria which causes gastrointestinal diseases. When pathogenic bacteria gain access to milk, they serve as an indicator of contamination from manure, soil, or contaminated water ^{2,3}. Pathogenic E. coli can cause wide range of diseases that target gastrointestinal tract, bloodstream, urinary tract, and CNS. The pathogenic strains of E. coli sero group O157 have been reported worldwide from different countries including Asia, United States, Australia Canada, Africa countries and Europe by different sources and cause death ⁴. E. coli O157:H7 are foodborne pathogens worldwide and classified into six such as Enterotoxigenic enterohemorrhagic, enteropathogenic enteroinvasive, enteroaggregative and diffusely adherents E. coli are the different pathogenic classifications based on pathogenesis ⁵. E. coli O157:H7 strains are considerably active food pathogens linked with bloody diarrhea outbreaks around the world ⁶. The hemolytic uremic syndrome and hemorrhagic colitis (HC) in humans cause by E. coli O157:H7 is the main serotype associated with outbreaks due to food in United States ^{7,8}. Infections with E. coli O157:H7 were responsible for 73,000 illnesses in the United States in 2005, with over 2000 hospitalizations and 60 deaths ⁹. E. coli O157:H7 also cause outbreak in southern Africa and Swaziland in 1992 and affect thousands of peoples due to surface water contamination with dung and carcasses of animals. The E. coli O157:H7 outbreaks occurs among children's that consumed raw milk and milk products ⁹.

Antibiotic against E. coli O157:H7 strain is controversial due to increased Shiga toxin synthesis and secretion. However, in recent years, there has been a surge in antibiotic resistance ¹⁰. For diagnose of E. coli O157:H7, currently there are a lot of options of selective and differential media for the isolation and presumptive identification including Sorbitol MacConkey Agar, Cefixime-Tellurite Sorbitol MacConkey Agar, CHROM agar TM O157, Tellurite CHROM agar TM O157, Vancomycin Cefixime Cefsulodin CHROM agar TM O157. Many authors claimed these media to be

effective in isolation and identification by performing studies on food, or on feedlots samples ^{11,12}. Raw milk and its derivatives are dangerous to consumers' health since they can spread a variety of diseases ¹³. The milk has the ability to support pathogenic bacteria growth that leads to intoxications, infections in consumer and cause spoilage of milk and milk product ¹⁴. The most commonly pathogens in milk are Bacillus cereus, Listeria monocytogenes, Yersinia enterocolitica, Salmonella spp., and Escherichia coli ¹⁴. Foodborne diseases occurs in low income countries due to poor sanitation, inadequate food safety laws, lack of regulatory systems, less economic resources for equipment and illiteracy in food handlers. The poor sanitation account for 60% of disease burden reported by National Hygiene and Sanitation Strategy program ¹⁵.

Milk is an important nutrition and use as dietary supplement for humans and as well as a source of income for producers. On milk safety and quality limited bacteriological assessment have been available in developing countries particularly in Pakistan. Therefore objectives of the study were to determine the prevalence of E. coli and E. coli O157:H7 and antimicrobial susceptibility pattern to evaluate milk quality that are a critical points for consumer specially children, patient and older people.

MATERIALS AND METHODS

Sample collections: A total of 180 samples of raw milk were obtained in pre sterilized containers (gamma radiations) (screw caps bottles) and give temporary ID, kept the bottles icebox, and transported to Microbiology laboratory of Kohat University of Science and Technology. The samples were analyzed within 5 hours after collection or kept at 4°C for further analysis. The samples were examined for bacterial isolation, and various biochemical analyses were carried out in accordance with the previous study ¹⁶.

Culture and screening of sample: 10ml of raw milk samples were homogenized by shaker for at least 10 mins. As differential media for E. coli a sterile loop was used to streak samples over MacConkey agar and incubated for 18-24 hrs at 37°C temperature. The presumptive colonies on MacConkey agar were sub cultured on Eosin Methylene Blue selective media and incubated for 18-24

hrs at 37°C. After incubation presumptive colonies were noted. To get pure E. coli colonies, a well-isolated colony was chosen and sub cultured on nutrient agar for biochemical tests. Isolates were also screened to find out E. coli O157:H7 by culturing isolates on sorbitol MacConkey agar.

Biochemical tests: Single pure colonies from nutrient agar plates were used for biochemical test. Tests such as Oxidase, Catalase, triple sugar iron test and IMViC were used for E. coli presence in samples. The colonies negative for oxidase, catalase, citrate and Voges-Proskauer while positive for indole production, Methyl red and TSI shows Yellow slant and butt and gas productions and black precipitate absence¹⁶. Moreover Gram staining was performed on clean glass slide as defined previously¹⁷.

Antibiotic Susceptibility Test: The susceptibility test for antibiotic against isolates was performed according by the CLSI-2010. Transfer pure colonies from nutrient agar plates to 5ml saline water tube and emulsified to achieved 0.5 turbidity standards. A pre sterilized swab was dipped in 5ml saline containing bacteria were swabbed uniformly over Muller-Hinton agar plate surface in a safety cabinet and allow at room temperature to dry. The plates were incubated for 18–24 hours at 37°C with antibiotic discs of defined concentration. The ten different antibiotic discs were used

like, Ciprofloxacin CIP, Gentamicin GN, Oxytetracycline OT, Tetracycline TE, Imepenam IMO, Ceftriaxone CTX, Trimethoprim TR, Chloramphenicol C, Amikacin AK, Cloxacillin OB. After 24 hours incubation the zones of inhibition were measured in mm.

RESULTS

Milk samples collected randomly from different area of Kohat were streaked onto MacConkey agar plates. The presumed typical colonies on MacConkey agar were again sub cultured on Eosin Methylene Blue selective media and incubated for 24hrs at 37°C. Five to eight presumptive colonies (a metallic green sheen) on EMB agar were observed. All the isolates were identified E. coli and E. coli O157:H7 by IMViC pattern. To confirm the E. coli O157:H7 all isolates were cultured on sorbitol MacConkey agar to find colorless colonies. While the other fecal flora E. coli ferment sorbitol and gives pink colonies. Of 180 raw milks samples 134 (74.4%) were found positive for Pathogenic E. coli O157:H7 76 (42.2%) and E. coli nonpathogenic 58 (32.2%) and the remaining samples 46 (25.5%) were found negative (Table 1). The occurrences of potentially pathogenic isolates were further subject to antimicrobial resistance.

Table 1: Frequency of pathogenic and non pathogenic E.coli in milk samples

Sample	No. of samples Collected (%)	No. of samples positive for E. coli (%)	No. of samples positive for E. coli O157:H7 (%)	No. of Negative samples (%)
Raw Milk	180 (100%)	58 (32.2%)	76 (42.2%)	46 (25.6%)

A total of both pathogenic E. coli O157:H7 and nonpathogenic E.coli isolates were tested against ten different antibiotics class by disc diffusion technique. All isolates were susceptible to Ciprofloxacin E. coli (82.5%), E. coli O157:H7 (80.4%) Gentamycin (82.5%) (80.5), and Oxytetracycline (85.5%) (85.6%). There was lower susceptibility to Tetracycline (55.8%)

(48.2%), Imepenam (52.6%) (42.8%), Ceftriaxone (45.5%) (40.6%), Trimethoprim (54.4) (36.6%), and Chloramphenicol (36.6%) (30.5%) and However, a different resistance percentage was detected to Amikacin (30.5%), (25.4%) and Cloxacillin (10.5%) (4.5%) respectively (Table 2).

Table 2: Antibiogram profile of pathogenic and non pathogenic E.coli in milk samples

Isolates	CIP	GN	OT	TE	IMP	CTX	TR	C	AK	OB
E. coli	82.5%	82.5%	85.5%	55.8%	52.6%	45.5%	54.4%	38.6%	25.5%	10.5%
E. coli O157:H7	80.4%	80.5%	85.6%	48.2%	42.8%	40.6%	36.6%	30.5%	18.4%	4.5%

Ciprofloxacin CIP, Gentamicin GN, Oxytetracycline OT, Tetracycline TE, Imepenam IMO, Ceftriaxone CTX, Trimethoprim TR, Chloramphenicol C, Amikacin AK, Cloxacillin OB.

DISCUSSION

The presence of viable bacteria in food increases the likelihood of spoilage and increase the risk of foodborne outbreaks¹⁸. The raw milk quality directly reflected the sanitary, transportation without control temperature and bulk storage, and it's processing¹⁹. The current investigation aim to check the quality of raw milk stored in milk shop and find out the root cause of pathogenic E. coli O157:H7 spreading which leads to sever gastrointestinal infections in human populations. In the current study, out of 180 samples 134 (74.4%) milk samples were positive for E. coli and 46 (25.6%) milk samples were negative. In accordance with our study, a previous study also reported E. coli in milk²⁰. The current study not only isolates nonpathogenic E. coli but isolate the pathogenic strain of E. coli O157:H7 from raw milk similar to previous study²¹. In New Zealand pathogenic strain of E. coli O157:H7 was not observed in raw milk²². It may due to quality sanitation in New Zealand, educated and well trained farmer, milk obtained by automated machines proper, transportation of raw milk in control temperature sterile container and proper handling of manure. New Zealand have a multinational standard in dairy product that's why they are using suitable hygienic technique while dealing milk and milk product. Similar study also conducted in America found very low prevalence of E. coli O157:H7¹⁴. The high frequency might be due to poor hygienic condition of animals, inadequate safety practices of milk handler, and bulk storage tank in markets.

The raw milk or and pasteurized milk is used in manufacturing of child's candies in a small factory and stored for

3 to 6 months for desired flavor development which might be a cause of sever gastro intestinal infection in children. The E. coli in milk and water is an indicator of fecal contamination, if E. coli is nonpathogenic general it is not consequently crucial. However, if E. coli O157:H7 strains are present they could be potentially harmful for consumers. In this study E. coli (32.2%) E. coli O157:H7 (42.2%) were investigated in raw milk. In support of our study [22] conduct investigation on milk and dairy products and isolate a potentially pathogenic E. coli that possess many virulence genes using molecular techniques. In recent years, antibiotic resistance has become more common among foodborne bacteria and bacteria transfer antibiotic resistance determinants from one bacterial species to another, posing a serious hazard to general public²³. The recent work also explores resistance in food borne pathogens. In present work 10 difference classes of antibiotics were used for susceptibility pattern to find out resistance in pathogenic and nonpathogenic E. coli strains isolated from milk samples and results were as as E. coli and E. coli O157:H7 isolates shows high susceptibility to ciprofloxacin (82.5%) (80.4%), gentamicin (82.5%) (80.5%) and Oxytetracycline (85.5%) (85.6%) respectively and slightly resistant to Tetracycline (55.8%) (48.2%), Imepenam (52.6%) (42.8%), Ceftriaxone (45.5%) (40.6%), Trimethoprim (54.4%) (36.6%), Chloramphenicol (38.6%) (30.5%), and shows resistance to Amikacin (25.5%) (18.4%) and Cloxacillin (10.5%) (4.5%) respectively. To find out the resistance different investigation was previously done to find out resistance in milk isolates^{3 24}. In comparable to the current investigation on resistance, another study reported [24] reported that use of

Oxytetracycline, Ciprofloxacin, and Gentamycin gives highest susceptibility. The recent study exposed that all isolates of *E. coli* were extremely resistant to Amikicin and Cloxacillin of 75.5%, 81.6% respectively. The present study is also supported by other works on resistance profile^{25 24} reported resistance against Amikicin (86.88%) and cloxacillin (54.91%) from usually reported diarrheic patients have little difference from the current finding²¹ The current study isolates shown slightly a high resistance as compared to a previous study²⁴. The difference might be due origin of study conducted. The isolated pathogen is frequently associated with food and linked to a number of human intestinal diseases the current work shows that this pathogenic organisms is going to become fully resistant to antibiotics a near future.

CONCLUSION

The prevalence of food borne diseases is increasing day by day. In this study, the presence of *E. coli* as an indicator organisms and *E. coli* O157:H7 as pathogenic organisms showed that milk is of poor microbiological quality. The consumption of raw milk and dairy product manufactured from raw milk plays a vital role in spreading of these pathogens. The most common contamination of raw milk in this investigation was *E. coli* O157:H7. Because of its zoonotic potential, the occurrence of *E. coli* in milk is a public health problem. The high frequency of *E. coli* O157:H7 in milk samples indicate poor hygiene during milking, transportation and milk storage. The awareness against spreading of this pathogen is necessary among stakeholders at different levels of milk handling.

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