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Isolation, Identification, and Antimicrobial Susceptibility of Escherichia coli in Contaminated Milk and Milk Products

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ABSTRACT

Milk is regarded as a full and healthy sustenance for newborn mammals and humans; nonetheless, it serves as an effective medium for many bacteria. Milk consumption in raw form and its derivatives is prevalent, resulting in the spread of different illnesses. The research aimed to extract and identify Escherichia coli from raw milk and dairy products derived from cattle in selected dairy farms in Diyala. A total of 30 samples: 10 of raw milk, 10 of cheese, and 10 of kemeer was collected from cattle in selected dairy farms in Diyala. Culture media were prepared according to the manufacturer's instructions. Also, antimicrobial susceptibility test was done to isolated strains. The result showed that all samples were contaminated with E. coli according to the following percentage respectively (Milk 70%, Chesses 40%, Kemer 60%). The antimicrobial susceptibility test findings were generated from the isolated strains, with all isolates evaluated against a panel of 12 antimicrobials. E. coli was resistance to FOX, AMP, TM while it was sensitive to others.

Keywords: Contaminated milk, E. coli, Antimicrobial Susceptibility.

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INTRODUCTION

Milk is regarded as a full and healthy sustenance for newborn mammals and humans; nonetheless, it serves as an effective medium for many bacteria (Koirala *et al.*, 2024). Raw untreated milk continues to be used by several farming families and workers, as well as an increasing percentage of the overall population, who assert that. The milk is both safe and also has nutritional benefits that have been diminished by processing (Silveira *et al.*, 2022). The intake of both raw unprocessed milk and raw milk cheese has been frequently linked to food-borne illnesses (Saeed, 2024).. Developing nations are mostly impacted by food-borne diseases (Fagnani *et al.*, 2021). Due to the existing substandard food handling and sanitary protocols, insufficient regulatory frameworks for food safety, insufficient financial resources for the procurement of more secure equipment, and insufficient training for any individual handling food(Singh & Puniya, 2024; Food and Agriculture Organization and World Health Organization, 2004). Milk consumption in raw form and its derivatives is prevalent, resulting in the spread of different illnesses. Raw milk has the capacity to facilitate the proliferation of many pathogenic bacteria, which may result in product deterioration and consumer illnesses (Ntuli *et al.*, 2022).

Multiple epidemiological Research indicates that raw milk is often inhabited by many zoonotic food-borne pathogens, including Salmonella typhimurium, Listeria monocytogenes, S. aureus, Y. enterocolitica, Campylobacter jejuni, and enterohaemorrhagic *E. coli* (Ali & Alsayeqh, 2022). These infections have emerged from the agricultural environment, resulting from the amalgamation of contaminated milk with mastitic milk, manure, soil, and polluted water (Haldar et al., 2022; Gwida and EL-Gohary, 2013). *E. coli* is one of the primary infectious organisms linked to milk and some dairy related products (Saeed, 2024; Abebe *et al.*, 2014). Gram-negative, rod-

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shaped, facultatively anaerobic, and very motile, Escherichia coli is a constituent of the Enterobacteriaceae family of bacteria. Both people and animals' intestines naturally contain it (Basavaraju & Gunashree, 2022; Virpari *et al.*, 2013; Tchaptchet and Hansen, 2011) . Nonetheless, its recovery from food could pose a public health danger due to the potential presence of enteropathogenic and/or toxic strains that may induce a wide array of enteric and extraintestinal illnesses in animals.

Escherichia coli comprises many strains categorized into six classes of pathotypes according to their disease-causing mechanisms (Mohamed & Habib, 2023). Escherichia coli can be classified into several pathogenic types, including Enteropathogenic (EPEC), Attaching and Effacing (A/EEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enterohemorrhagic (EHEC), and Enteroaggregative (EAEC). Among these, certain strains are capable of producing Stx toxins, specifically Enterohaemorrhagic *E. coli* (EHEC), Shiga-toxigenic *E. coli* (STEC), and Vero Toxin-producing *E. coli* (VTEC) (Amin et al., 2022; Nataro and Kaper, 1998). Toxic strains of *E. Coli* O157:H7 may cause life-threatening symptoms, resulting in around 74,000 cases and 61 fatalities yearly in the USA due to outbreaks linked to the consumption of infected bovine products, particularly meat and raw milk (Saeed, 2024). Subsequently, outbreaks were linked to more dairy products, including cheese and yogurt (Doyle et al., 2006; Thani, 2023).

The intestines of healthy sheep, goats, deer, and cattle have also been shown to contain Escherichia coli O157:H7. It has been shown that cattle are a major source of *E. coli* O157. Since H7 was discovered to be the causal agent, major food poisoning outbreaks have been connected to the use of cattle-derived commodities, such as beef and dairy products (Acha and Szyfress, 2001).

Infections caused by *E. coli* O157:H7 can lead to severe complications, including haemorrhagic colitis and haemolytic uremic syndrome (HUS), which poses a significant threat, particularly to children and immunocompromised individuals (Lee *et al.*, 2021). Additionally, increasing antibiotic resistance among pathogenic strains further complicates treatment options (Nasrollahian *et al.*, 2024).

Differentiating pathogenic serotypes from the usual fecal flora including commensal *E. coli* strains can help one identify *E. coli* O157:H7(Battisti et al., 2006; Woynshet, 2014). Two unique biochemical indicators for *E. coli* O157:H7 include delayed fermentation of D-sorbitol and lack of β-D-glucuronidase activity, therefore enabling the phenotypic distinction of the bacterium.H7 isolates non-pathogenic *E. coli* strains. One of these markers (delayed sorbitol fermentation) helps various selective media (Sorbitol-MacConkey) to grow, therefore supporting the early detection of questionable colonies isolated from bloody stools(Woynshet, 2014). Although it lacks the specificity to identify STEC, *E. coli* O157:H7 must be isolated and enriched using selective and/or indicator media in order to be identified in food samples (Ji-Yeon *et al.*, 2005). Given the significant role of raw milk in bacterial transmission, this study aims to assess the prevalence of Escherichia coli O157:H7 in raw milk and its derivatives. Understanding its occurrence and potential risk factors can help in implementing better food safety strategies and public health policies to mitigate foodborne infections.

METHODS

Research Region:

The research aimed to extract and identify Escherichia coli from raw milk and dairy products derived from cattle in selected dairy farms in Diyala.

2.2 Study Design and Sampling Strategy

Cross-sectional research was undertaken from December 2023 to February 2024 to isolate and identify *E. coli* from raw cow milk and milk products from selected dairy farms in Diyala. The farms were chosen with basic random sampling methods. A total of 30 samples: 10 of raw milk, 10 of cheese, and 10 of kemeer.

Sample Collection

Samples were collected aseptically and placed in sterile screw-capped bottles, then transported to the microbiology laboratory for bacteriological investigation. The material was thereafter refrigerated at 4°C overnight and processed within 24 hours of collection.

Tools and media used:

Used equipment

(Tubes for transporting samples, loop, Petri dishes, Autoclaved, Sterile incubator, Berner, Beaker, Aluminum foil, Cotton).

The Media:

1-MacConkey Agar:

51.5 grams of the medium were measured and transferred to a glass beaker. The capacity was adjusted to 1 liter with distilled water following the manufacturer's specifications, thereafter sterilized in an autoclave at 121°C and 5.1 atmospheres of pressure for 15 minutes. Then leave the medium to cool. Then pour the medium into sterile plastic Petri dishes, then store it in a special refrigerator to preserve the dishes until use**.**

2-Muller Hinton Agar:

Thirty-eight grams of the medium were measured and transferred to a glass beaker. The volume was adjusted to 1 liter with distilled water as per the manufacturer's specifications, thereafter sterilized in an autoclave at 121°C and 5.1 atmospheres of pressure For 15 minutes. Then leave the medium to cool. Then pour the medium into sterile plastic Petri dishes, then store it in a special refrigerator to preserve the dishes until use.

3-: Nutrient Broth with Glycerin:

25 grams of the medium were weighed and placed inside a glass beaker. The volume was completed to 750ml distilled water. 25 ml of glycerin was added to it and mixed well according to the manufacturer's instructions. It was distributed in heat-resistant plastic Eppendorf tubes with a capacity of 1 ml for each tube, then sterilized with a sterilizer (autoclave). Then it was stored in a special refrigerator to store the dishes until use. It was used to activate and renew the isolates (long-term frozen preservation of bacterial cultures) and transfer them to other media.

Figure 1: Nutrient Broth preparation.

4-EMB (Eosin methylene blue)

Dissolve 36 grams of EMB Agar in 1000 milliliters of distilled water. Apply heat to fully dissolve the medium. Autoclave for 15 minutes at 15 pressure (121 °C) to administer and sterilize.

Method:

To isolate and identify *E. coli*, transfer one millilitre of incubated BPW to Eosin Methylene Blue (EMB) agar (Oxoid) and incubate at 37˚C for 24 hours. On sorbitol MacConkey agar (Oxoid), colonies exhibiting a metallic sheen were cultivated and incubated for 24 hours at 37˚C. For identification, the purified colonies were streaked over nutrient broth and cultivated for 18 to 24 hours at 37˚C.Simultaneously, another individual colony with similar features was selected from the agar plate and Gram stained. Bright-field microscopy was used to investigate the isolate's staining and morphological properties. E.Coli, which, when subcultured onto nutritional agar, showed a green metallic sheen on Eosin Methylene Blue and a pinkish hue on MacConkey agar, allowing colony features to be assessed. After being separated from EMB, pure colonies were placed on the non-selective medium nutrient agar. A susceptibility test was performed to determine the degree of the bacteria's resistance.

RESULTS

The results of isolating E. coli bacteria from 30 samples (10 each of milk, cheese, and kemeer) showed the presence of a percentage of E. coli bacteria, according to the table below.

The current study's findings indicated that, out of 30 samples, 13 were positive for *E. coli*. The isolates exhibited a brilliant pink coloration on plates of MacConkey agar (Fig. 2) and had a blue-green metallic sheen on EMB agar plates. (Fig. 3).

Antimicrobial susceptibility pattern

In order to prepare the bacterial inoculum according to the standard operational procedures, 25 ml of sterile nutrient broth was inoculated with *E. coli* bacteria. Then we used a sterile cotton swab to streak the surface of Mueller Hinton agar plates. The antimicrobial agents tested (filter paper disks)were (MRP Meropenem, CIP Ciprofloxacin, AK Amikacin, ATM Aztreonam, FOX Cefoxitin, TOB Tobramycin, CRO Ceftriaxone, CN Gentamicin, CTX Cefotaxime, AMP Ampicillin, FEP Cefepime, TM Trimethoprim). The *E. coli* antimicrobial susceptibility test findings were generated from the isolated strains, with all isolates evaluated against a panel of 12 antimicrobials (as detailed in Table 2). Figure 4,5: The different susceptibility and resistance patterns to the tested antibiotics against *E. coli*.

Table 1. The percentage of the presence of *E. coli*.

No.	Type sample	No. of sample	No. of positive sample	No. of negative sample	Percentage 100%
1	Milk	10	7	3	70%
2	Chesses	10	4	6	40%
3	Kemer	10	6	4	60%

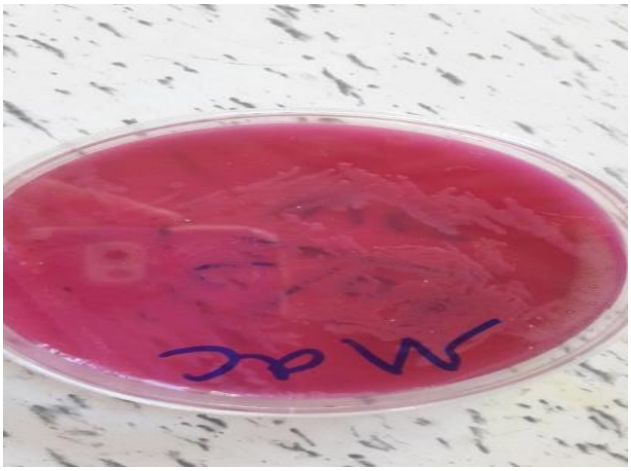

Figure 2: Bright pink color on MacConke.

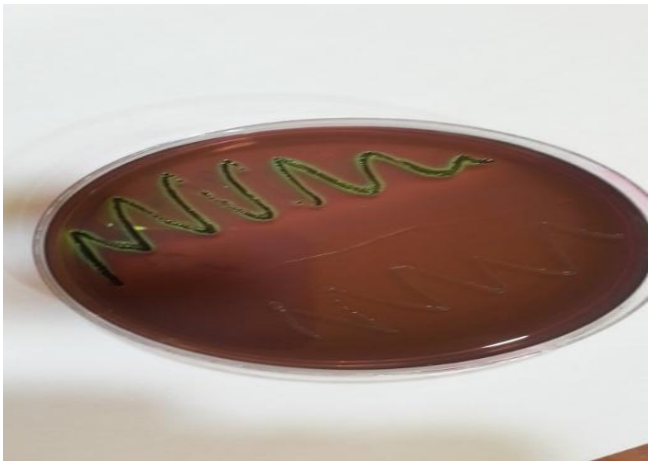

Figure 3: Blue-greenish metallic sheen on EMB.

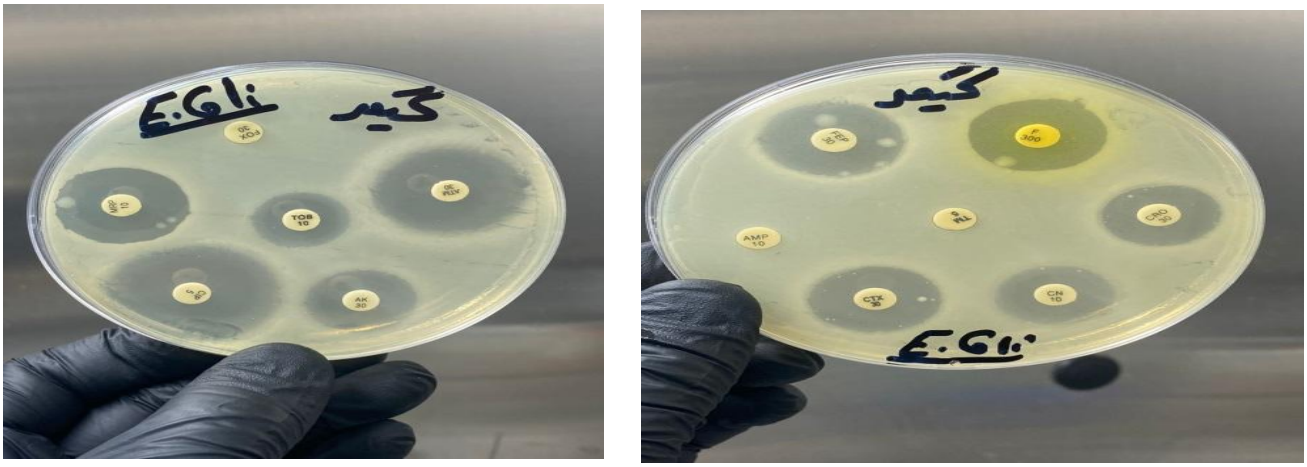

Figure 4,5: The different susceptibility and resistance patterns to the tested antibiotics against *E. coli.*

Table 2. Antimicrobial susceptibility test with 12 different antibiotics.

NO	Antimicrobial	sensitive	resistance
1	MRP	+	
2	CIP	+	
3	AK	+	
4	ATM	+	
5	FOX		+
6	TOB	+	
7	CRO	+	
8	CN	+	
9	CTX	+	
10	AMP		+
11	FEP	+	
12	TM		+

DISCUSSION

This research included the collection of thirty raw milk samples, which were subjected to bacteriological processing and biochemical assays to identify Escherichia coli. All E. coli isolates On MacConkey agar, produced brilliant pink colonies with metallic sheen; on EMB agar, pink coloring and unique metallic sheen.

In this investigation, 70% of raw milk, 40% of cheese, and 60% of kemeer were found to be contaminated with E. coli. These findings were similar to other previous studies (Kumar & Prasad, 2010; Soomro *et al.* ,2002).

CONCLUSION

According to findings of our research and in spite of the nutritional benefits of milk and milk products, the intake of raw unprocessed milk may cause many life threating diseases. More attention on the, washing of utensils, hygienic practices, regular sterilization of dairy equipment's

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