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ISOLATION AND ANTIMICROBIAL RESISTANCE PROFILE OF *Escherichia coli* IN RAW CAMEL MILK AND CAMEL MILK POWDER

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ABSTRACT

The present study was conducted to isolate *Escherichia coli* from raw camel milk and camel milk powder samples and identify antimicrobial pattern. A total of 200 samples consisting of raw camel milk (n=100) and camel milk powder (n=100) were collected from National Research Centre on Camels, Bikaner (Rajasthan). Several biochemical tests (imvic, lactose, glucose, sorbitol, sucrose, etc.) were performed to confirm *E. coli* isolates using Hi*E. coli*TM kits. Antibiotic resistance patterns in the *Escherichia coli* isolates were determined by the Kirby-Bauer disk diffusion method against 12 antibiotics. Twenty eight isolates of *Escherichia coli* were found in the raw camel milk samples and 5 isolates of *Escherichia coli* were present in camel milk powder samples. The *Escherichia coli* isolates were highly resistant to penicillin-G (100%) followed by chloramphenicol (87.87%), amoxicillin (81.81%) and erythromycin (63.63%). This was possibly due to poor personal hygiene of the milker, camel health, environment, poor equipment sanitation, storage and transport conditions. The existence of multi-drug resistance points to take strict measures to diminish its prevalence and combat antimicrobial resistance in food animals.

Key words: Antimicrobial resistance, camel milk powder, *Escherichia coli*, raw camel milk

Numerous microorganisms can contaminate milk and its by-products and contaminated raw milk and dairy products are the major sources of food-borne illnesses (Javaid *et al,* 2009, Zuleka *et al,* 2016). *Escherichia coli* stands out as one of the primary contaminants found in raw milk and it's presence consistently indicates faecal contamination and the potential existence of other enteric pathogens in raw milk, posing a significant public health risk to consumers (Soomro *et al,* 2002). The foodborne pathogens (including *Listeria monocytogenes,* enteropathogenic *E. coli*, *Staphylococcus aureus,* and *Salmonella* spp.) account for 65 and 72% of cases of foodborne illnesses and foodborne deaths, respectively (WHO, 2015). Milk and milk products are considered to be among the primary sources for these pathogens (Oliver *et al,* 2005). The growth behaviour of foodborne pathogens, i.e. *Staphylococcus aureus, Listeria monocytogenes*, *E. coli* O157:H7 and Salmonella spp. was studied in pasteurised camel milk and compared with pasteurised bovine milk at different incubation temperatures (Abusheliabi and Ayyash, 2017). Abera *et al* (2016) demonstrated bacterial contamination (88.7%) in raw camel milk samples. The overall mean Total Bacterial Counts and Coliform Counts of contaminated raw camel milk samples were 4.75 ± 0.17 and 4.03 ± 0.26 log CFU/ml, respectively. *E. coli* (31.5 %) was among the major bacterial microorganisms isolated. However, certain strains of *E. coli* pose risks to humans and are present in food animals. Among these strains, *E. coli* O157:H7 is the most widely recognised pathogenic variant (Oliver *et al,* 2009). In arid and semiarid regions, camel milk stands out as a crucial food source for pastoral communities. Recently, urban populations residing in resource-rich areas have shown a growing interest in consuming camel milk (Farah and Fischer, 2004).

The consumption of milk contaminated with antibiotics poses public health hazards, including allergic reactions, alterations in intestinal microflora and the proliferation of antibiotic-resistant pathogenic bacteria (Sheikh *et al,* 2013). The frequency of antibiotic-resistant bacteria could serve as a gauge for the extent of antimicrobial usage in livestock (Yang *et al,* 2015). Antimicrobial resistance is associated with the overutilisation of antimicrobial medications in food production, among animals and humans.

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Research indicates that the prevalence of multi-drug resistance in *E. coli* is a significant global issue. Nonetheless, there has been limited exploration of this phenomenon, especially in developing nations (Rahimi and Nayebpour, 2012).

Materials and Methods

Sample collection

A total of 200 samples consisting of raw camel milk (n=100) and camel milk powder (n=100) were collected aseptically in sterile glass bottles and milk powder in sterile plastic bags from National Research Centre on Camels, Bikaner (Rajasthan). These were taken to the laboratory and stored at 4°C.

Isolation and identification of E. coli

10 ml of sample was added to 90 ml MacConkey broth for raw camel milk and 10 gm of camel milk powder samples was added to 90 ml MacConkey broth and homogenised. The homogenate was incubated overnight at 37°C for enrichment of *E. coli*. The broth was streaked on a MacConkey agar (MCA) plate and incubated at 37°C for 24 hrs to obtain isolated colonies of bacteria. After 24-hour period of incubation, the individual isolated colonies were placed onto Eosin Methylene Blue agar (EMB) plates to isolate *Escherichia coli*. These were then further incubated for 24 hours at 37°C. Colonies showing dark centre with metallic sheen were considered as *E. coli*. All the *E. coli* isolates were further confirmed by gram staining, biochemical tests and preserved for additional bacterial identification.

Biochemical Characteristics

The isolates were subjected to a series of different biochemical tests using the HiEcoliTM Identification Kit (HiMedia, Mumbai) for *E. coli* confirmation.

Antibiotic susceptibility pattern of E. coli

The antimicrobial susceptibility testing for all *E. coli* isolates was performed using the Kirby-Bauer disk diffusion method against 12 antibiotics. These antibiotics included chloramphenicol, oxacillin, penicillin-G, erythromycin, azithromycin, tetracycline, streptomycin, gentamicin, ampicillin, ciprofloxacin, ceftriaxone and amoxicillin. For susceptibility testing, a pure culture of all identified *E. coli* cultures was taken from nutrient broth and a cotton swab was used to streak the bacteria around the surface of Muller-Hinton agar and wait for 3-5 minutes for the solution to dry. Antibiotic disks were then placed on the agar surface using clean sterile forceps and gently pressed to confirm their attachment. Following this, the plates were aerobically incubated at 37°C for 24 hrs. Finally, the diameters of the zone of inhibition around the discs were measured to the nearest millimetre using the meter scale and the isolates were classified as susceptible, intermediate and resistant to the drugs tested according to the chart provided by the manufacturer.

Results

Microbiological isolation and identification of E. coli

The findings of the current investigation showed that among the 200 samples analysed, 33 samples tested positive for *E. coli*. The isolates were identified as having a pinkish-red colour colony on MacConkey agar plates (Fig 1) and revealed a greenish metallic sheen on Eosin methylene blue agar plates (Fig 2). The Gram staining of the isolates revealed small rod- shaped organisms with a pink colour arranged singly or in pairs or short chains.

Biochemical characterisation of E. coli

The isolates that demonstrated positive results for the methyl red, indole, glucuronidase, nitrate reduction, ONPG, lysine utilisation, lactose, glucose, sucrose and sorbitol sugar tests, but tested negative for the Voges-Proskauer and citrate utilisation tests, were confirmed as *E. coli* (Fig 3).

Table 1. Biochemical characterisation of *E. coli*.

S. No.	Test	Reaction
1.	Methyl red	Positive
2.	Voges Proskauer's	Negative
3.	Citrate Utilisation	Negative
4.	Indole	Positive
5.	Glucuronidase	Positive
6.	Nitrate Reduction	Positive
7.	ONPG	Positive
8.	Lysine Utilisation	Positive
9.	Lactose	Positive
10.	Glucose	Positive
11.	Sucrose	Positive
12.	Sorbitol	Positive

Antimicrobial Resistance Pattern of E. coli

In the present investigation, a total of 12 distinct antibiotics were employed to assess the pattern of antibiotic resistance for 28 isolates of *Escherichia coli* isolated from raw camel milk, alongwith 5 isolates

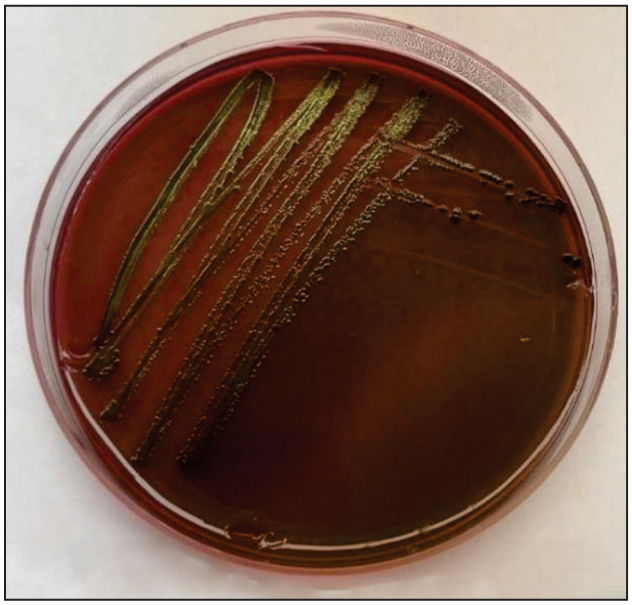

Fig 1. Isolation of *E. coli* on EMB Agar.

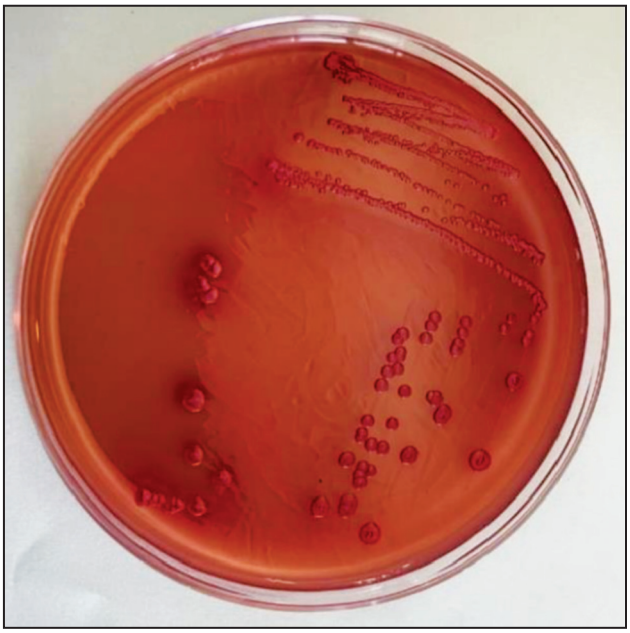

Fig 2. Isolation of *E. coli* on MacConkey Agar.

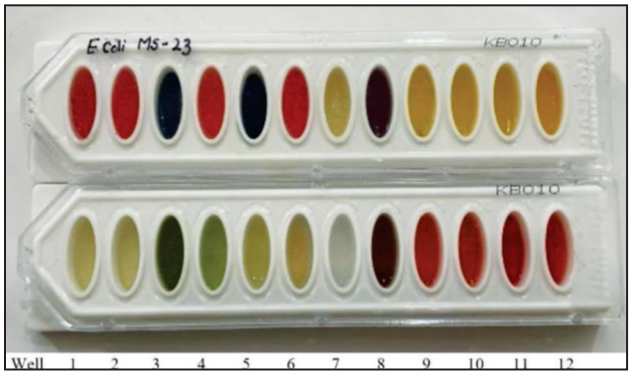

Fig 3. Biochemical test for *E. coli* isolates

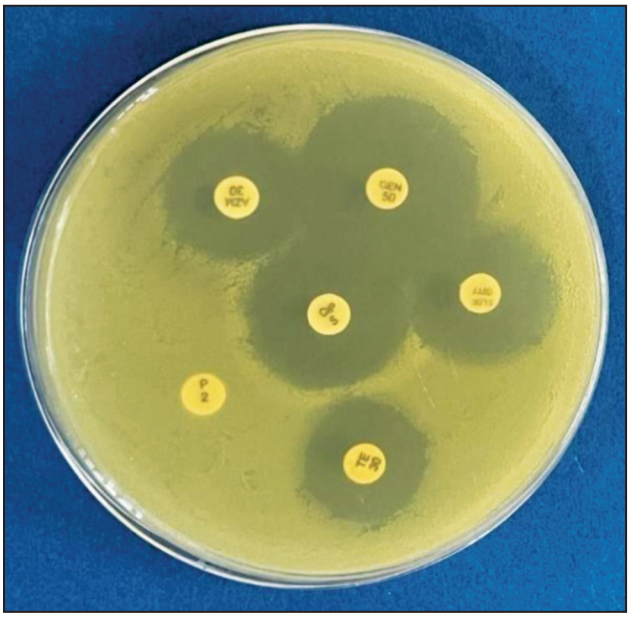

Fig 4. Antibiotic susceptibility pattern of *E. coli*.

of *Escherichia coli* retrieved from camel milk powder. Reactions of *E. coli* to antibiotics were classified into sensitive, intermediate and resistant categories. Each of the isolates demonstrated diverse levels of resistance and sensitivity to the antibiotics employed in this study. The antibiotic susceptibility pattern for *E. coli* isolated from raw camel milk and camel milk powder samples showed the highest resistance to penicillin-G (100 %) followed by chloramphenicol (87.87%), amoxicillin (81.81%) and erythromycin (63.63%).

The antibiotic susceptibility pattern for all the *E. coli* isolates in raw camel milk and camel milk powder samples for different antibiotic has been shown in Table 2.

Discussion

Out of 200 samples, 33 isolates (16.5%) were found positive for *E. coli* which exhibited distinctive features such as bright pinkish-red colonies on MacConkey agar, greenish metallic sheen colonies on EMB agar and pinkish-red coloured, small rod-shaped Gram-negative bacilli in Gram's staining. Isolates that demonstrated positive results for the methyl red, indole, glucuronidase, nitrate reduction, ONPG, lysine utilisation, lactose, glucose, sucrose and sorbitol sugar tests, but test negative for the Voges-Proskauer and citrate utilisation tests, were confirmed as *E. coli*. The disparities observed across various studies could stem from variations in sample size, sanitation practices related to milking equipment, diverse milking methods, geographical factors, environmental conditions, intervals of

Table 2. Results of antibiotic resistance profile of *Escherichia coli* isolates.

S. No.	Name of Antibiotics	Resistance	Intermediate	Sensitive
1.	Amoxicillin	27 (81.81%)	0 (0%)	6 (18.18%)
2.	Ampicillin	30 (90.90%)	0 (0%)	3 (9.09%)
3.	Azithromycin	0 (0%)	9 (27.27%)	24 (72.72%)
4.	Ceftriaxone	20 (60.60%)	1(3.03%)	12 (36.36%)
5.	Chloramphenicol	29 (87.87%)	0 (0%)	4 (12.12%)
6.	Ciprofloxacin	0 (0%)	0 (0%)	33 (100%)
7.	Erythromycin	21 (63.63%)	5 (15.15%)	7 (21.21%)
8.	Gentamicin	14 (42.42%)	0 (0%)	19 (57.57%)
9.	Oxacillin	19 (57.57%)	6 (18.18%)	8 (24.24%)
10.	Penicillin-G	33 (100%)	0 (0%)	0 (0%)
11.	Streptomycin	5 (15.15%)	8 (24.24%)	20 (60.60%)
12.	Tetracycline	18 (54.54%)	12 (36.36%)	3 (9.09%)

milk transportation and overall hygiene standards (Soomro *et al,* 2002). Furthermore, the presence of *E. coli* in milk does not invariably denote direct faecal contamination; rather, it implies insufficient hygiene practices and unsanitary procedures during milking and subsequent milk handling. Such circumstances pose a potential public health hazard to consumers (Meshref, 2013). Milk can possibly be contaminated from various sources, such as infected udders, milk handlers with inadequate personal hygiene, low-quality water and improperly cleaned or sanitised containers (Saeed *et al,* 2022). All of these factors contribute to the contamination of milk (Chye *et al,* 2004). In present study, the susceptibility of the *E. coli* isolates against twelve commonly used antimicrobials was tested and the isolates were characterised as susceptible, intermediate and resistant based on the size of the zone of inhibition. According to the test results most of the *E. coli* isolates were resistant to penicillin-G (100%), chloramphenicol (87.87%), amoxicillin (81.81%) and erythromycin (63.63%). (Table 3). Similar studies conducted by Mohammadi *et al* (2013) and Gezahegn *et al* (2023) observed comparable outcomes, indicating a 100% sensitivity to ciprofloxacin, mirroring the findings of the current study. Similarly, Alam *et al* (2017) demonstrated a 100% resistance to penicillin G, aligning closely with the results of the present investigation. Islam *et al* (2016) reported an 86.67% resistance to amoxicillin, a result closely resembling that of the present study. Dehkordi *et al* (2014) showed 84% resistance to tetracycline and also observed 36% resistance to streptomycin which is higher than the present investigations. Adzitey *et al* (2018) indicated a 61.8% resistance to erythromycin, which bears close relevance to the current study. The growth of antibiotic resistance among bacteria such as *E. coli* poses an important public health concern.

Conclusion

The frequency of contamination of *E. coli* was significantly higher in raw camel milk samples than in the camel milk powder samples. Elevated levels of *Escherichia coli* contamination were observed in raw milk samples, primarily due to inadequate hygiene practices. The *Escherichia coli* isolates were highly resistant to penicillin-G (100%) followed by chloramphenicol (87.87%), amoxicillin (81.81%) and erythromycin (63.63%). Additionally, the indiscriminate use of antimicrobial drugs in both humans and animals must be avoided to protect the public from ingesting antimicrobial resistant pathogens.

Conflicts of Interest

The authors declare no conflict of interest.

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