

COMPARATIVE ANALYSIS OF DIFFERENT PATHOGENIC MICROBE COLLECTED FROM MAMMALIAN MILK

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Abstract: Milk comprises proteins, fats, minerals, carbohydrates, multiple vitamins, and various special substances dispersed in water. Different types of pathogenic microbes present in unpasteurized raw milk, cause gastrointestinal tract infections and food-borne infections. The current study was based on evaluating pathogenic microbe's frequencies among the mammal (buffalo, cow, camel, goat and sheep) milk and assessing their nutritional parameters along with the screening of antibiogram of these pathogens in the district Peshawar, Pakistan. A total of 25 milk samples (05 from each mammals) were collected. Among the analyzed milk samples, *S. aureus* and *Shigella* spp. were observed n= 04 (16%), while *E. coli*, *Salmonella* spp., and *P. aeruginosa* were n= 03 (12%). The *S. aureus* was found highly resistant (100%) to Meropenem, Gentamicin, Amoxicillin, Aztreonam, and Doxycycline, while found highly sensitive (50%) to Amikacin, and Levofloxacin. *E. coli* showed high resistant (100%) to Meropenem, Ciprofloxacin, Amoxicillin, Ampicillin, and Doxycycline, while found to be highly sensitive (50%) to Levofloxacin, and Cefotaxime. The *Salmonella* spp. was found highly resistant (100%) to Amikacin, Meropenem, Amoxicillin, Cefotaxime, Ampicillin, Aztreonam, and Doxycycline, while found highly sensitive (50%) to Ciprofloxacin, and Gentamicin. The *Shigella* spp. was found highly resistant (100%) to Meropenem, Amoxicillin, Ampicillin, and Doxycycline, while found highly sensitive (25%) to Amikacin, Levofloxacin, Ciprofloxacin, Gentamicin, Cefotaxime, and Aztreonam. The *P. aeruginosa* was highly resistant (100%) to Amikacin, Levofloxacin, Ciprofloxacin, Gentamicin, Cefotaxime, Amoxicillin, Aztreonam, Doxycycline, while found highly sensitive (25%) to Meropenem, and Ampicillin. The high ESBL-producing bacterial pathogen was found *E. coli* (66.66%), followed by *Shigella* spp. (50%), *Salmonella* spp., and *P. aeruginosa* (33.33%), respectively. The nutritional analysis of mammal milk samples significantly ($p < 0.05$) with different parameters. Among these parameters, the fat Mean \pm SD (3.36 ± 0.11), protein content Mean \pm SD (2.91 ± 0.04), lactose content Mean \pm SD (4.31 ± 0.10), and total solid content Mean \pm SD (11.61 ± 0.27) were observed. In conclusion, of the current study, pathogenic microbes are present in unpasteurized milk, which shows high resistance to various antibiotics, and the nutritional values of milk were also low.

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Introduction

Milk is a significant source of macro and micronutrients. It comprises vitamins, amino acids, lipids, proteins, carbs, and minerals, among other vital components (Moosavy *et al.*, 2019). The taste and pleasure of dairy products depend on the composition of the milk. The composition varies with the milking strategy, lactation, diet, feeding device, environment, variety, season, and high diversity among the species (Kittivachra *et al.*, 2007; Medhammar *et al.*, 2012). According to new research, camel and donkey milk is another option for infants allergic to bovine milk.

Clinical investigations on animal models have shown that donkey milk has anti-diabetic, antioxidant, anti-cancer, and anti-inflammatory properties. Vitamins (A, B, and E) trace elements, including phosphorus, calcium, potassium, and magnesium, are abundant in cow, sheep and goat milk. Numerous proteins in milk, such as lactoferrin, lysozyme, and lactoperoxidase, are linked to its inhibitory effect. A specific antibacterial pattern emerges from the interaction of these chemicals (Ebrahimi *et al.*, 2021). Among the milk ingredients, proteins are the most important part,

which helps in human weight loss and helps to improve the diet naturally. The main types of milk protein are casein and whey, which are classified as the quality of high proteins with rapid absorption, utilization, and high digestibility rate (97-98%). In particular, casein is an environmentally friendly nutrient due to the slow and continuous release of amino acids into the blood (Jahan-Mihan *et al.*, 2011). The high quality and feasible attentiveness of modern BCAAs (Branched chain amino acids) in milk proteins are essential to protect tissue growth and restore and prevent catabolic movement during exercise. In addition, cysteine amino acids can increase glutathione levels, exhibit antioxidant activity and help improve the immune system against various diseases (Pereira, 2014).

Milk is a nutrient-dense food derived from a wide range of animals, including goats, cows, buffalo, sheep, and humans. So, the abundance of nutrients in these kinds of milk creates an excellent environment for growing numerous bacteria. Although the debate rages over whether or not pasteurized milk contains lactic acid bacteria (LAB), a bacterium group that ferments lactose into lactate is a major component of raw milk before pasteurization. *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, and *Leuconostoc* are some of the most prevalent LAB genera found in milk. *Acinetobacter* and *Pseudomonas* species are typically found in psychrotrophic communities, which form during cold storage and contribute significantly. Milk contains a variety of moulds, and yeasts, as well as strains from genera other than LAB. In another word, human milk is often dominated by *Staphylococcus*, *Streptococcus*, *Bifidobacterium* species, and *Lactobacillus*. The microbial composition of milk directly impacts dairy products development (Quigley *et al.*, 2011). Different pathogenic microbes present in unpasteurized raw milk, including; *Escherichia coli*, *Staphylococcus aureus* *Mycobacterium tuberculosis*, *Salmonella* species, *Campylobacter*, *Klebsiella*, *Proteus*, *Coxiella burnetii*, etc. (Karshima *et al.*, 2013; Zeryehun *et al.*, 2017; Bano *et al.*, 2020). Among these microbes, the most dominant pathogens are *S. aureus* and *E. coli* in milk. They cause gastrointestinal tract infections and food-borne infections. *S. aureus* also produces enterotoxins, which cause serious human complications (Kumar and Prasad, 2010). Beta-lactam antibiotics, the first-line treatment in veterinary medicine, have potential public health consequences due to the increased risk of antibiotic residues in milk and the emergence of multidrug-resistant bacteria, which could be transmitted to human consumers via raw milk and its derivative products. Over the last five decades, methicillin-resistant *Staphylococcus aureus* (MRSA) has lately emerged as a community-acquired and livestock-associated pathogen. The expression of the *mecA* gene, or its homolog *mecC*, located on the *Staphylococcal* cassette chromosome *mec*, creates an

altered penicillin-binding protein with a very low affinity for lactam antibiotics (Oliver and Murinda, 2012). Raw milk production and its microbial pathogenicity will also have a significant social and economic impact on many stakeholders. Still, in Pakistan, research and information on these serious infectious pathogens are needed. Therefore, it is necessary to in-depth study the value of milk and non-milk dairy products, especially the value of proteins, peptides, amino acids, and their microbiological quality to improve milk quality. The current research study aimed at a comparative analysis of pathogenic microbes collected from mammalian milk.

Material and methods

Sample Collection

A total of 25 milk samples (05 from each mammal, buffalo, cow, camel, goat and sheep) were collected from markets in sterilized bottles from the district Peshawar, Pakistan. All the milk samples were centrifuged at 3000 rpm for 30 minutes. After centrifugation, the deposits of milk samples were processed for total bacterial count using a standardized plate count method (Zeinhom and Latef, 2014).

Isolation of microorganisms

The samples were streaked through the sterilized loop on different culture media, including; Shigella Salmonella (SS) Agar, Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB), MacConkey Agar, and *Pseudomonas* Cetrimide agar (PCA) were used for the isolation of *Salmonella*, *S. aureus*, *E. coli*, *Klebsiella* and *P. aeruginosa*, respectively. The media were incubated for 24 hours at 37°C (Zeinhom and Latef, 2014).

Identification of microorganism

The microorganisms were identified by their characteristics of motility, Grams reaction, Indole production, carbohydrate fermentation, Oxidase reaction, and gas production (Zeinhom and Latef, 2014).

Biochemical tests

a) Indole test

Peptone water was made and autoclaved in a conical flask. Each sterile tube was filled with 5ml of media and allowed to cool at room temperature. The test tube was incubated at 37°C for 20-28 hours. After incubation, a few drops of Kovac's reagent were added to each test tube, then a red-colored ring was seen in the test tube.

b) Oxidase test

A sterile filter paper was placed in a petri dish, and a few drops of newly produced oxidase reagent were applied. A bacterial culture colony was taken with a toothpick and spotted on filter paper containing oxidase reagent. Within 10-30 seconds, the color of the infected patch of paper changed to deep blue or purple.

c) Citrate test

Simmons citrate was added to distilled water in a sterile conical flask. After the autoclave procedure,

allow the medium to cool for 30 minutes at room temperature. A bacterial culture colony was collected and streaked at 37°C for 24-48 hours. The results were analyzed when the incubation period was completed.

d) Catalase test

Using a sterile wire loop, a colony from a growing culture was extracted and combined with a drop of Hydrogen Peroxide put on a glass slide. The appearance of bubbles indicated a positive catalase test.

e) Antibiotics Sensitivity

The "Muller Hinton Agar" tested antimicrobial susceptibility by disc diffusion method. The Amikacin (AK) 30µg, Meropenem (MEM) 10µg, Levofloxacin (LEV) 5µg, Ciprofloxacin (CIP) 5µg, Gentamicin (CN) 10µg, Cefotaxime (CTX) 30µg, Ceftriaxone (CRO) 30µg, Amoxicillin (AMC) 30µg, Ampicillin (AMP) 30µg, Aztreonam (ATM) 30µg, and Doxycycline (DOX) 30µg, antibiotics were used for the assessment of antibiogram (Preethirani et al., 2015). The McFarland 0.5 standard was used to examine the overnight growth of the tested microorganisms in 2 mL of Muller Hinton broth. Bacteria from the broth were struck on the surface of Muller Hinton agar with a sterilized cotton swab. The antibiotic discs were placed on the agar surface, and the plates were incubated for 24 hours at 37 degrees Celsius. The inhibitory zone was measured millimetres (mm) after incubation (Preethirani et al., 2015).

f) Detection of Extended spectrum β-lactamase (ESBL)

The isolated bacteria were seen out for possible production of ESBL by performing different tests such as extended spectrum beta-lactamase (EBSLs) screening test using ceftriaxone (CTR)

ceftazidime (CAZ) disc furthermore double disc energy test (DDST) was used for the bacterial isolates which show a positive result for ESBL production and Double disc diffusion test (DDDT) was used to confirm positive double disc synergy test according to Pamuk et al., (2019).

g) Nutritional analysis

Nutritional analysis of raw milk was performed using the Association of Official Analytical Chemists (AOAC 2012). The experiment was done at the livestock Research and Development department, Veterinary Research Institute Peshawar (VRI). This method measured different other components such as crude fat, dry matter, crude protein and fibers (Sobczak et al., 2020).

Statistical analysis

The obtained results were analyzed and organized using Microsoft Excel and Word. The obtained nutritional values were analyzed by two-way analysis of variance test using Statistical Packages of Social Sciences (SPSS) 23.0 version.

Result

Samples (25) of mammal’s milk were collected from the district Peshawar, and isolates were analyzed, and characterized by biochemical tests (Figure 1). Among the isolates, *S. aureus* and *Shigella* spp. were observed n= 04 (16%), while *E. coli*, *Salmonella* spp., and *P. aeruginosa* were n= 03 (12%). The occurrence of pathogenic bacteria among different mammals, buffalo, cow, camel, goat and sheep, were observed, as shown in table 1. Collectively, the high microbial contamination was found in Buffalo, Cow, Sheep n= 04 (16%), Goat n= 03 (12%), and Camel n=02 (08%), respectively. The area-wise occurrence is presented in table 2.



Figure 1: Biochemical characteristics of bacterial pathogens, A) Indole test, B) Citrate test, C) Oxidase test, D) Catalase test

Table .1: Occurrence of pathogenic bacteria among different mammal’s samples

Mammals	<i>S. aureus</i> n (%)	<i>E. coli</i> n (%)	<i>Salmonella</i> spp. n (%)	<i>Shigella</i> spp. n (%)	<i>P. aeruginosa</i> n (%)
Buffalo	2 (08%)	1 (04%)	0 (0%)	1 (04%)	0 (0%)
Cow	1 (04%)	1 (04%)	0 (0%)	1 (04%)	1 (04%)
Camel	0 (0%)	0 (0%)	1 (04%)	0 (0%)	1 (04%)
Goat	1 (04%)	0 (0%)	1 (04%)	1 (04%)	0 (0%)
Sheep	0 (0%)	1 (04%)	1 (04%)	1 (04%)	1 (04%)

Total = 25	4 (16%)	3 (12%)	3 (12%)	4 (16%)	3 (12%)
Mammals	<i>S. aureus</i>	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>P. aeruginosa</i>
	n (%)	n (%)	n (%)	n (%)	n (%)
Buffalo	2 (08%)	1 (04%)	0 (0%)	1 (04%)	0 (0%)
Cow	1 (04%)	1 (04%)	0 (0%)	1 (04%)	1 (04%)
Camel	0 (0%)	0 (0%)	1 (04%)	0 (0%)	1 (04%)
Goat	1 (04%)	0 (0%)	1 (04%)	1 (04%)	0 (0%)
Sheep	0 (0%)	1 (04%)	1 (04%)	1 (04%)	1 (04%)
Total = 25	4 (16%)	3 (12%)	3 (12%)	4 (16%)	3 (12%)

Table 2: Area wise occurrence of pathogenic bacteria among different mammal's samples

Pathogens	Area				
	Chargano chawk	Saddar Bazar	University town	Board bazar	Karkhano Bazar
<i>S. aureus</i>	+	+	-	+	+
<i>E. coli</i>	+	-	+	+	-
<i>Salmonella</i> spp.	-	+	-	+	+
<i>Shigella</i> spp.	+	-	+	+	+
<i>P. aeruginosa</i>	-	+	-	+	+

Antibiotics sensitivity pattern

Among the isolated pathogens, *S. aureus* was found highly resistant (100%) to Meropenem, Gentamicin, Amoxicillin, Aztreonam, and Doxycycline, while sensitive (50%) to Amikacin, and Levofloxacin. The *E. coli* was found highly resistant (100%) to Meropenem, Ciprofloxacin, Amoxicillin, Ampicillin, and Doxycycline, while sensitive (50%) to Levofloxacin, and Cefotaxime. The *Salmonella* spp. was found highly resistant (100%) to Amikacin, Meropenem, Cefotaxime, Amoxicillin, Ampicillin, Aztreonam, and Doxycycline, while found highly

sensitive to Ciprofloxacin, and Gentamicin. The *Shigella* spp. was highly resistant (100%) to Meropenem, Amoxicillin, Ampicillin, and Doxycycline, while 25% sensitive to Amikacin, Levofloxacin, Ciprofloxacin, Gentamicin, Cefotaxime, and Aztreonam, respectively. The *P. aeruginosa* was highly resistant (100%) to Amikacin, Levofloxacin, Ciprofloxacin, Gentamicin, Cefotaxime, Amoxicillin, Aztreonam, and Doxycycline, while 25% sensitive (25%) to Meropenem, and Ampicillin (Table 3).

Table 3: Antibiotics sensitivity profile of pathogenic microbes isolated from milk samples

Antibiotics		<i>S. aureus</i> n (%)	<i>E. coli</i> n (%)	<i>Salmonella</i> spp. n (%)	<i>Shigella</i> spp. n (%)	<i>P. aeruginosa</i> n (%)
Amikacin (AK) 30µg	R	2 (50%)	3 (75%)	4 (100%)	3 (75%)	4 (100%)
	S	2 (50%)	1 (25%)	0 (0%)	1 (25%)	0 (0%)
Meropenem (MEM) 10µg	R	4 (100%)	4 (100%)	4 (100%)	4 (100%)	3 (75%)
	S	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)
Levofloxacin (LEV) 5µg	R	2 (50%)	2 (50%)	3 (75%)	3 (75%)	4 (100%)
	S	2 (50%)	2 (50%)	1 (25%)	1 (25%)	0 (0%)
Ciprofloxacin (CIP) 5µg	R	3 (75%)	4 (100%)	2 (50%)	3 (75%)	4 (100%)
	S	1 (25%)	0 (0%)	2 (50%)	1 (25%)	0 (0%)
Gentamicin (CN) 10µg	R	4 (100%)	3 (75%)	2 (50%)	3 (75%)	4 (100%)
	S	0 (0%)	1 (25%)	2 (50%)	1 (25%)	0 (0%)
Cefotaxime (CTX) 30µg	R	3 (75%)	2 (50%)	4 (100%)	3 (75%)	4 (100%)
	S	1 (25%)	2 (50%)	0 (0%)	1 (25%)	0 (0%)
Amoxicillin (AMC) 30µg	R	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)
	S	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ampicillin (AMP) 30µg	R	3 (75%)	4 (100%)	4 (100%)	4 (100%)	3 (75%)
	S	1 (25%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)
Aztreonam (ATM) 30µg	R	4 (100%)	4 (100%)	4 (100%)	3 (75%)	4 (100%)
	S	0 (0%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)
Doxycycline (DOX) 30µg	R	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)
	S	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

R= Resistance, S= Sensitive

ESBL producing microbes

Among the isolated pathogenic microbes, the high ESBL-producing bacterial pathogen was found *E. coli*

(66.66%), followed by *Shigella* spp. (50%), *Salmonella* spp., and *P. aeruginosa* (33.33%). Besides these results, no ESBL-producing *S. aureus* was found, as shown in figures 2 and 3.

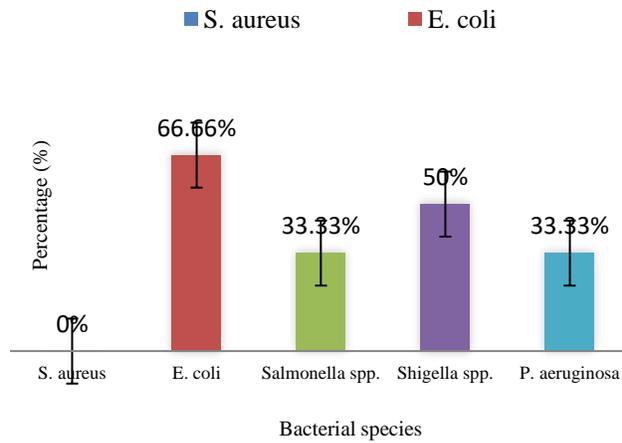


Figure 2: Frequencies of ESBL-producing bacteria among the milk samples



Figure 3: ESBL-producing bacteria among the milk samples

Nutritional analysis of Milk samples

The nutritional analysis of mammal milk samples differs significantly ($p < 0.05$) with different parameters. Among these parameters, the fat content was high (3.67%) in Goat milk samples, followed by Sheep (3.56%), Camel (3.32%), Buffalo (3.29%), and Cow (2.99%), respectively, with overall Mean \pm SD (3.36 \pm 0.11). The protein content was high (3.03%) in Camel milk samples, followed by Goat (2.98%), Buffalo (2.91%), Sheep (2.89%), and Cow (2.78%), respectively, with overall Mean \pm SD (2.91 \pm 0.04). The lactose content was high (4.56%) in Camel milk samples, followed by Goat (4.53%), Cow (4.32%), Sheep (4.13%), and Buffalo (4.01%), respectively, with overall Mean \pm SD (4.31 \pm 0.10). The total solid content was high (12.56%) in Camel milk samples, followed by Goat (11.94%), Cow (11.34%), Sheep (11.16%), and Buffalo (11.09%), respectively, with overall Mean \pm SD (11.61 \pm 0.27) (Table 4).

Table 4: Nutritional analysis of mammal milk samples

Mammals	Fat (%)	Protein (%)	Lactose (%)	Total Solid (%)
Buffalo	3.29	2.91	4.01	11.09
Cow	2.99	2.78	4.32	11.34
Camel	3.32	3.03	4.56	12.56
Goat	3.67	2.98	4.53	11.94
Sheep	3.56	2.89	4.13	11.16
Mean \pm SD	3.36 \pm 0.11	2.91 \pm 0.04	4.31 \pm 0.10	11.61 \pm 0.27

Buffalo	3.29	2.91	4.01	11.09
Cow	2.99	2.78	4.32	11.34
Camel	3.32	3.03	4.56	12.56
Goat	3.67	2.98	4.53	11.94
Sheep	3.56	2.89	4.13	11.16
Mean \pm SD	3.36 \pm 0.11	2.91 \pm 0.04	4.31 \pm 0.10	11.61 \pm 0.27

Discussion

Milk is often dominated by *Staphylococcus*, *Streptococcus*, *Bifidobacterium* species, and *Lactobacillus* and directly impacts the development of dairy products. Microorganisms produce lactate during milk fermentation, which has several effects on the sensory, texture, flavor, and organoleptic aspects of the final product. When psychro-tolerant bacteria proliferate in milk under refrigeration, they produce extracellular lipases and proteases, which cause spoiling. Raw milk tainted with pathogens, can cause serious sickness if consumed. Therefore, the microbial composition of the milk has health consequences as well. On the other hand, other raw milk bacteria are said to benefit health by easing digestion or reducing the frequency of allergies, such as asthma and atopic illnesses, in children who drink raw milk early in life (Quigley et al., 2013). The present study results showed that findings in agreement with the previous studies; they reported that different types of pathogenic microbes present in unpasteurized raw milk, including; *Escherichia coli*, *Staphylococcus aureus* *Mycobacterium tuberculosis*, *Salmonella* species, *Campylobacter*, *Klebsiella*, *Proteus*, *Coxiella burnetii*, etc. (Karshima et al., 2013; Zeryehun et al., 2017; Bano et al., 2020). Extended-spectrum beta-lactamase (ESBL) bacteria isolates have grown common in food-producing and companion animals worldwide. They constitute a fast-growing group of enzymes that confer resistance to most beta-lactams used in humans and animals. While ESBL-producing *K. pneumoniae* was found in Italy, the UK, and most recently in Tunisia due to the increasing prevalence of highly resistant *Enterobacteriaceae* (primarily *E. coli*), recent studies have revealed an increase in these bacteria in other countries, raising concerns for veterinary and public health on a global scale (Klibi et al., 2019). In the present study, we found the high ESBL-producing bacterial pathogen in milk samples was *E. coli* (66.6%), followed by *Shigella* spp. (50%), *Salmonella* spp., and *P. aeruginosa* (33.33%), respectively. Besides these results, no ESBL-producing *S. aureus* was found. These findings are similar to the previously reported studies of Klibi et al. (2019), Pamuk et al. (2019), and Geser et al. (2012).

In the current study, the antibiotics sensitivity profile of isolated bacteria showed that *S. aureus* was found highly resistant to Meropenem, Gentamicin, Amoxicillin, Aztreonam, and Doxycycline, while highly sensitive to Amikacin, and Levofloxacin. The *E. coli* was found to be highly resistant to

Meropenem, Ciprofloxacin, Amoxicillin, Ampicillin, and Doxycycline, while sensitive to Levofloxacin, and Cefotaxime. The *Salmonella* spp. was found highly resistant to Amikacin, Meropenem, Cefotaxime, Amoxicillin, Ampicillin, Aztreonam, and Doxycycline, while found highly sensitive to Ciprofloxacin, and Gentamicin. The *Shigella* spp. was found highly resistant to Meropenem, Amoxicillin, Ampicillin, and Doxycycline, while sensitive to Amikacin, Levofloxacin, Ciprofloxacin, Gentamicin, Cefotaxime, and Aztreonam. The *P. aeruginosa* was highly resistant to Amikacin, Levofloxacin, Ciprofloxacin, Gentamicin, Cefotaxime, Amoxicillin, Aztreonam, and Doxycycline, while found sensitive to Meropenem, and Ampicillin. Overall these findings show a high resistance pattern to various antibiotics, similar to Pamuk et al.'s (2019); they also reported high resistance of milk pathogenic microbes. Milk is a nutrient-dense food derived from a wide range of animals, including goats, cows, buffalo, sheep, and humans. In the current study we found the nutritional parameters of mammals milk samples; the fat content was high (3.67%) in Goat milk samples, followed by Sheep (3.56%), Camel (3.32%), Buffalo (3.29%), and Cow (2.99%), with overall Mean \pm SD (3.36 \pm 0.11). In support of our findings, the Sudharani et al. (2021) study showed that the levels of every component were higher than that of cow and sheep milk for all species of buffalo and goat's milk. Specific gravity, terrible acidity, ash and protein content was higher than cow's milk, but lactose levels were lower than that of goat and cow's milk. They concluded that all parameters tested in buffalo and cat milk were identical, except for the higher lactose content of goat milk.

Conclusion

The study concludes that among the milk samples *S. aureus* and *Shigella* spp., *E. coli*, *Salmonella* spp., and *P. aeruginosa* were observed. The Study shows that among mammals, milk *Shigella* and *S. aureus* are highly reported as compared to other species. Based on the research study, camel milk has fewer pathogens than the others. The study proposes that the milk of board bazar is more pathogenic than to the other selective area. All isolates show high resistance to various commercially available antibiotics. The study concludes that *E. coli* is a high ESBL-producing bacteria. Among Mammals milk, camel milk is a high nutritional value compared to the others.

Conflict of interest

The authors declared an absence of conflict of interest.

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