Research Article

Molecular Detection and Antibiogram of Milk-Borne *Staphylococcus* spp., *Escherichia coli* and *Salmonella* spp. in Dhaka City, Bangladesh

Md. Nasim Hasan Mim[1], Md. Kamrul Hassan[1], Tayeaba Jannat[2], M. Rubaiyat Adnan[1], Md. Abir Hassan Sadi[1], Mithium Hussain Loveonnya[1], Mirza Synthia Sabrin[1], Md. Rashedul Islam[3], Nipu Sen[1] and Mahfuzul Islam[1]*

[1]Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh; [2]Department of Poultry Science, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh; [3]Department of Surgery and Theriogenology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

Abstract | Milk is commonly called a complete food for its high nutritional value. However, milk-borne pathogens like *Staphylococcus* spp., *Escherichia coli*, and *Salmonella* spp. pose significant public health risks. They often contaminate milk during milking, processing, or poor hygiene practices at the farm and market. Infected animals and fecal contamination may be the probable source of contatmination. The study aimed to isolate and identify milk-borne *Staphylococcus* spp., *Escherichia coli*, and *Salmonella* spp. from farm and market milk in Dhaka City, Bangladesh, based on a molecular basis and to investigate their antibiotic sensitivity patterns. 50 cow's milk samples, comprising 25 farm milk and 25 market milk, were collected from Dhaka City, Bangladesh, during the period from July to December 2023. A total of 46 isolates were found, in which the majority were *E. coli* (44%; n=22/50), followed by *Staphylococcus* spp. (28%; n=14/50) and *Salmonella* spp. (20%; n=10/50). Results of antibiotic sensitivity in this investigation showed almost all the pathogens showed the highest resistance against ampicillin and amoxicillin—*Staphylococcus* spp. (92.85%), *E. coli* (100%), and *Salmonella* spp. (100%) resistance. However, *Staphylococcus* spp. and *E. coli* showed the highest sensitivity to gentamicin (71.42% and 77.27%), while *Salmonella* spp. showed the highest sensitivity to meropenem (100%). Furthermore, 71.43% of *Staphylococcus*, 95.45% of *E. coli*, and 80.0% of *Salmonella* spp. showed multidrug resistance (MDR) properties. Diversified MDR patterns were reported among the isolated bacteria. Overall, it was concluded that milk can harbor pathogenic bacteria that may spread antibiotic resistance and pose a public health risk. Therefore, care should be taken to minimize microbial contamination of milk to ensure food safety.

Editor | Muhammad Abubakar, National Veterinary Laboratories, Park Road, Islamabad, Pakistan.
Received | September 10, 2025; **Accepted** | October 29, 2025; **Published** | December 18, 2025
***Correspondence** | Mahfuzul Islam, Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh; **Email:** mislam.mipa@sau.edu.bd
Citation | Mim, M.N.H., M.K. Hassan, T. Jannat, M.R. Adnan, M.A.H. Sadi, M.H. Loveonnya, M.S. Sabrin, M.R. Islam, N. Sen and M. Islam. 2026. Molecular detection and antibiogram of milk-borne *Staphylococcus* spp., *Escherichia coli* and *Salmonella* spp. in Dhaka city, Bangladesh. *Veterinary Sciences: Research and Reviews*, 12(1): 01-11.
DOI | https://dx.doi.org/10.17582/journal.vsrr/2026/12.1.01.11
Keywords | Cow's milk, *Staphylococcus* spp., *E. coli*, *Salmonella* spp., Antibiotic resistance, Multidrug resistance

Introduction

Milk is considered a valuable nutritional source as it fulfills the essential dietary requirements for children, pregnant women, and the elderly people (Javaid *et al*., 2009). However, fresh milk is prone to contamination with various pathogenic bacteria that poses a significant threat to its consumer's health (Torkar and Teger, 2008). Milk is a perfect growth medium for microorganisms because of its high water, protein, carbohydrate, and fat content (Griffiths, 2010). Various sources act as contamination source including milking equipment, animal feed, soil, manure, and the dairy environment itself (FAO and WHO, 2004). *Staphylococcus* spp., *E. coli*, and *Salmonella* spp. are the most significant pathogenic bacteria found in milk. They can cause serious foodborne illnesses (Oliver *et al*., 2005).

Staphylococcus spp., being one of the most pathogenic bacteria, when found in milk products, is prone to originate from infected udders and spread as a result of inappropriate handling of udders during milking (Radostits *et al*., 2006). Conversely, *E. coli*, which is a normal intestinal inhabitant of animals, when found in milk is assumed to be due to fecal contamination (Oliver *et al*., 2009). In addition, *Salmonella* species, particularly *Salmonella enterica*, may cause life threatening enteric infections upon consumption of contaminated raw milk (Capita *et al*., 2003).

The growing resistance of bacteria against antibiotics in milk has become a major global health challenge (Devriese *et al*., 1997). Antibiotic overuse in dairy farming has resulted in decreased treatment efficacy, which is the cause of the rising number of resistant strains (Pant *et al*., 2013). This resistance develops when sub-therapeutic antibiotic doses eliminate susceptible bacteria while allowing resistant variants to proliferate (Eichner and Gravitz, 2001). Majority of the prior studies on milk borne pathogens in Bangladesh primarily focused on identification of the pathogens based on cultural and biochemical tests. They often examined a single pathogen and mostly lacked advance molecular detection. The present study was taken into account to fill the critical research gap by combining advanced molecular detection along with the traditional method of identification for the three milk-borne pathogens, thus generating a contemporary antibiogram data and highlighting the urgency to intervene in Bangladesh's dairy sector to control contamination and resistance, which was previously under addressed.

Materials and Methods

Time frame, area of study and sample collection

This study was carried out in the span of six months, July-December 2023, in the Microbiology and Parasitology Laboratory of Sher-e-Bangla Agricultural University. From five randomly selected markets and five dairy farms of Dhaka City, Bangladesh, a total of fifty milk sample were collected aseptically and then transferred to the laboratory maintaining cool chain for further investigation.

Isolation and tentative identification of the isolates

The samples were first enriched in nutrient broth (HiMedia, India) to recover the likelihood of getting low number of bacteria and then cultured into selective media specific for the pathogens. Nutrient agar (HiMedia, India) plate was used first followed by mannitol salt (MS; HiMedia, India) agar for identification of *Staphylococcus* spp. For *E. coli* identification, eosin methylene blue (EMB; HiMedia, India) was complemented by McConkey (MC; HiMedia, India) agar media. Salmonella-Shigella (SS; HiMedia, India) agar, Xylose Lysine Deoxycholate agar (XLD; HiMedia, India) agar, and MC agar media were used for *Salmonella* spp. Gram staining procedure was also performed for morphological confirmation. By hanging drop technique, motility was checked and biochemical characters were ascertained by performing a series of biochemical tests like carbohydrate fermentation, catalase, Methyl Red and Voges-Proskauer (MR-VP), and indole tests (Cheesbrough, 2006).

DNA extraction

By using boiling method, total genomic DNA was extracted from bacterial cultures followed by centrifugation of 1ml of culture 14,000×g for 5 minutes. After getting the pellet, it was washed with sterile water and re-centrifuged. The cells were then re-suspended in 200 µL water, boiled for 10 minutes to lyse them, and immediately chilled on ice. Afterwards, it was centrifuged for one last time for collecting the DNA-containing supernatant followed by storage at -20°C for PCR analysis (Mthembu *et al*., 2019). This efficient protocol yielded high-quality DNA suitable for downstream applications.

Polymerase chain reaction (PCR) to identify Staphylococcus spp., E. coli, and Salmonella spp.

PCR was used to detect *Salmonella* (invA gene), *Staphylococcus*, and *E. coli* (16S rRNA) (Table 1). Each 25µL reaction containing master mix, primers, water and DNA template extracted from the serovars, they were allowed to thermal cycling conditions. It varied by target: *Staphylococcus* spp. (30 cycles: 94˚C/30s, 60˚C/30s, 72˚C/45s), *E. coli* (35 cycles: 94˚C/30s, 60˚C/30s, 72˚C/30s), and *Salmonella* spp. (30 cycles: 94˚C/1min, 64˚C/30s, 72˚C/30s) (Shome *et al*., 2018; Tsen *et al*., 1998; Kaushik *et al*., 2014). Afterwards, amplified DNA was analyzed on 1.5% agarose gels with DNA ladders. This method provided specific identification of all three pathogens.

Antibiotic sensitivity tests of isolated Staphylococcus spp., E. coli, and Salmonella spp.

The identified isolates were exposed to antibiotic sensitivity test by employing the Kirby-Bauer disk diffusion method against 10 widely used antibiotics (Bauer *et al*., 1966). Bacterial suspensions maintaining 0.5 McFarland standard, were spread on Mueller-Hinton agar, and antibiotic discs including ampicillin (AMP), 10 µg; amoxicillin (AMX), 30 µg; amoxicillin–clavulanic acid (AMC), 20/10 µg; ceftriaxone (CTR), 30 µg; ciprofloxacin (CIP), 5 µg; nalidixic acid (NA), 30 µg; erythromycin (E), 15 µg; gentamicin (GEN), 10 µg; tetracycline (TE), 30 µg; and meropenem (MEM), 10 µg were applied. After 24h incubation at 37˚C, inhibition zones were measured and interpreted using the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. Multidrug resistance was defined as resistance against at least one antibiotic from ≥3 antibiotic classes (Magiorakos *et al*., 2012).

Statistical analysis

Minitab 17 (Minitab Ltd., UK) was used to analyze the study's data. Pearson's Chi-square test was used to determine any significant differences between the variables.

Results and Discussion

Isolation and identification of milk borne Staphylococcus, E. coli, and Salmonella spp.

Figure 1 represented the cultural characteristics of isolated bacteria. Mannitol fermentation in MS agar makes it selective for *staphylococci* and differential for *S. aureus*, which ferments mannitol, producing a yellow color around the colonies; meanwhile

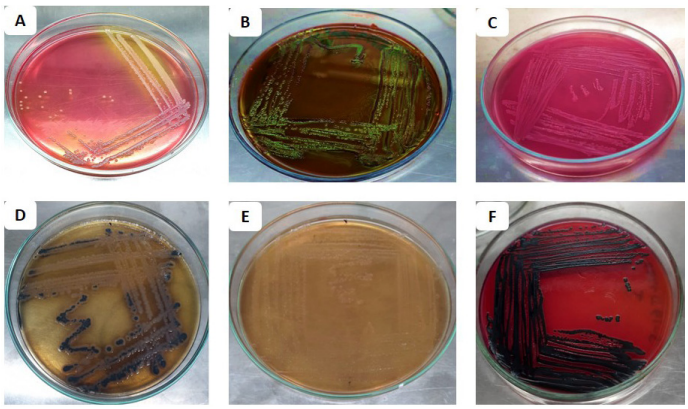

Figure 1: *Cultural properties of isolated milk-borne bacteria. (A) Staphylococcus spp. produced yellow fermented colonies on MS agar; (B) E. coli produced metallic sheen colonies on EMB agar; (C) E. coli produced pink fermented colonies on MC agar; (D) Salmonella spp. produced black centered colonies on SS agar; (E) Salmonella spp. produced colorless non-lactose fermenter colonies on MC agar; (F) Salmonella spp. produced black centered colonies on XLD agar.*

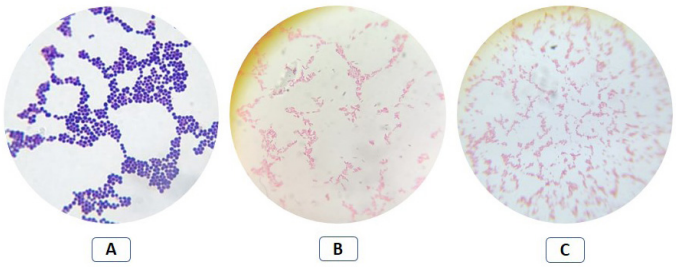

Figure 2: *Gram staining of isolated milk-borne bacteria. A: Staphylococcus spp. showed gram positive cocci with grapes like clusters on Gram's staining. B: Escherichia coli showed gram-negative rod-shaped bacteria on Gram's staining. C: Salmonella spp. showed gram negative coccobacilli on Gram's staining.*

in nutrient agar, they produce big yellow colonies (McLandsborough, 2005; Cheesbrough, 2006; Foster, 1996). On the other hand, *E. coli* exhibits distinct cultural characteristics on EMB agar, producing greenish colonies with a metallic sheen (Fesseha, 2020; Nayak *et al*., 2004) and in MC agar they produce bright pink, lactose-fermenting colonies. Conversely, *Salmonella* spp. exhibits black-centered colonies (H_2S production) in XLD agar, black colonies in (Salmonella-Shigella) SS, and non-lactose fermenter colonies on MC agar (Jones *et al*., 2008; Buxton and Fraser, 1977). *Staphylococcus* spp. showed gram positive cocci with grapes like clusters, *E. coli* showed gram-negative rod-shaped bacteria, while *Salmonella* spp. showed gram negative coccobacilli on Gram's staining (Figure 2). Table 2 represented the sugar fermentation patterns and

Table 1: *List of primers used in this study.*

Target pathogen	Primer sequence (5′→3′)	Amplicon size (bp)	Reference
Staphylococcus spp.	SG16P1(F): GTG ATC GGC CAC ACT GGA	842	Shome *et al.* (2018)
	SG16P1(R): CAACTTAATGATGGCAACTAAGC		
Escherichia coli	16E1(F): GGGAGTAAAGTTAATCCTTTGCTC	584	Tsen *et al.* (1998)
	16E2(R): TTCCCGAAGGCACATTCT		
Salmonella spp.	InvA(F): GTGAAATTATCGCCACGTTCGGGCAA	284	Kaushik *et al.* (2014)
	InvA(R): TCATCGCACCGTCAAAGGAACC		

bp= Base pair

Table 2: *Biochemical characteristics of milk-borne Staphylococcus spp., E. coli, and Salmonella spp.*

Parameters		*Staphylococcus* spp.	*E. coli*	*Salmonella* spp.
Catalase test		+ve	+ve	+ve
Sugar fermentation tests	Dextrose	A	AG	AG
	Lactose	A	AG	NF
	Sucrose	A	AG	NF
	Maltose	A	AG	AG
	Mannitol	A	AG	AG
Methyl red test		+ve	+ve	+ve
Voges-proskauer test		+ve	–ve	–ve
Indole test		–ve	+ve	–ve

+ve: positive; –ve: negative; A: only acid is produced after fermentation; AG: acid and gas is produced after fermentation; NF: no fermentation occurred.

biochemical characteristics of the isolated bacteria. *Staphylococcus* spp. fermented all five simple sugars with acid production (Cheesbrough, 2006; OIE, 2000; Freeman, 1985); *E. coli* demonstrated complete fermentation (Fesseha, 2020; Bedasa *et al.*, 2018; Asmelash, 2015; Giuida and Gohary, 2013); and *Salmonella* spp. gave positive result in dextrose, glucose, and mannitol fermentation, but were negative in lactose and sucrose fermentations, (Han *et al.*, 2011; Hossain, 2012). *Staphylococcus* spp. showed positive result in catalase and MR-VP but negative indole reactions (Cheesbrough, 2006; OIE, 2000; Freeman, 1985). *E. coli* isolates were catalase, MR, and indole positive but VP negative (Zinnah *et al.*, 2007). *Salmonella* spp. showed MR positive, VP negative, and indole negative results, in agreement with Buxton and Fraser (1977).

Molecular identification of milk-borne Staphylococcus spp., E. coli, and Salmonella spp.

DNA extracted from 14 *Staphylococcus* spp., 22 *E. coli* and 10 *Salmonella* spp. were subjected to PCR amplification, and identified the 842bp, 584bp, and 284bp DNA for *Staphylococcus* spp., *E. coli*, and *Salmonella* spp., respectively (Figures 3) which were in line with Shome *et al.* (2018), Tsen *et al.* (1998), and Kaushik *et al.* (2014), respectively.

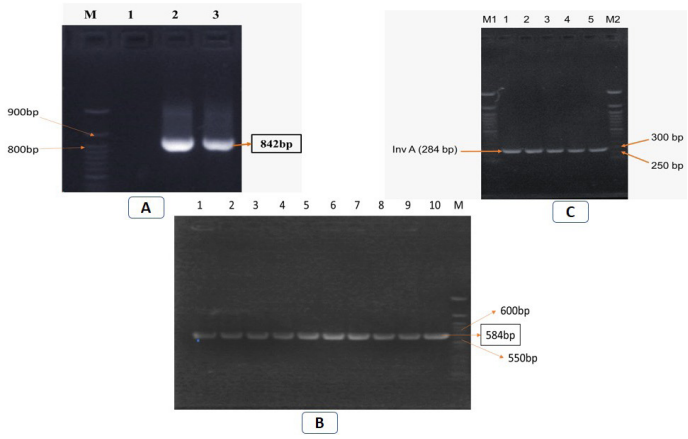

Figure 3: *Amplification of 842bp DNA of Staphylococcus spp., 584bp DNA of E. coli, and 284 bp DNA of Salmonella spp.*

Occurrence of Staphylococcus spp., E. coli and Salmonella spp. in farm milk and market milk

The occurrence of *Staphylococcus* spp. in milk samples (n=50) was 28%, with a higher prevalence in market milk (18%) compared to farm milk (10%) (P >0.05) (Table 3). The total occurrence of *E. coli* in the samples was 44%, with market milk exhibiting a higher prevalence (26%) than farm milk (18%) (P >0.05) (Table 3). The occurrence of *Salmonella* spp. was 20%, with market milk showing a slightly higher occurrence (12%) than farm milk (8%) (P >0.05) (Table 3). Although the prevalence of *Staphylococcus* spp. in this study is lower than that of another study carried out in Australia (60.3%) (Ramezanigardaloud *et al.*, 2025) and Ethiopia (39.3%) (Abebe *et al.*, 2024), it is comparable to that of Borena *et al.* (2023) (15.6%) in Ethiopia and Regasa *et al.* (2019) (16.6%) in Uganda. Similarly, *E. coli* prevalence (44%) is in agreement with Madani *et al.* (2022), Fesseha (2020), and Yasmin *et al.* (2015), while high levels were noted in the Czech Republic (Skočková *et al.*, 2018, 92.4%), Iran (Hassani *et al.*,

2022, 78%), and Indonesia (70.4%), while low levels were noted in Ethiopia (Demme and Abegaz, 2015, 9.9%). *Salmonella* spp. (20%) concurs with Garbaj *et al.* (2022), Hassani *et al.* (2022), and Marjan *et al.* (2014), but contrary to lower prevalence reported by Asefa *et al.* (2023), Gebeyehu *et al.* (2022), and Allied (2021). The dissimilarities may have been caused by the climatic conditions, hygiene maintenance and milk handling practices. Overall, these findings highlight the public health impact of drinking contaminated milk and require better hygiene and monitoring along the milk value chain.

Table 3: *Occurrence of Staphylococcus spp., E. coli and Salmonella spp. in farm milk and market milk.*

Name of Bacteria	No. of sample tested	Occurrence			Chi-square P value
		Overall No. (%)	Farm milk No. (%)	Market milk No. (%)	
Staphylococcus spp.	50	14 (28)	5 (10)	9 (18)	0.285
E. coli		22 (44)	9 (18)	13 (26)	0.394
Salmonella spp.		10 (20)	4 (8)	6 (12)	0.527
Chi-square *P* value	-	0.088	0.311	0.267	-

P value <0.05 was considered as significant differences.

Antimicrobial susceptibility of Staphylococcus spp., E. coli and Salmonella spp. in farm and market milk samples

Figure 4 shows a pattern of susceptibility to antibiotics for isolates of *Staphylococcus* spp. To ascertain the antibiotic sensitivity pattern, a total of 14 isolates of *Staphylococcus* spp. were obtained from farm and market milk samples. Out of 14 *Staphylococcus* spp. positive isolates the highest resistance was noticed against ampicillin and amoxicillin group which was 92.86% and the lowest resistance was found against gentamicin group i.e. 28.57%.

Figure 5 represents the results of susceptibility study and displayed that all the isolates of *E. coli* (n=22) were 100% resistant against amoxicillin and ampicillin and then followed by tetracycline (90.91%), nalidixic acid (72.73%), erythromycin (72.73%), ceftriaxone (63.64%), ciprofloxacin (63.64%), amoxicillin-clavulanic acid (45.45%), meropenem (36.36%), and gentamicin (13.64%), respectively. However, the highest sensitivity was found for gentamicin (77.27%) and the lowest susceptibility was documented for amoxicillin and ampicillin (0%).

The consequences of vulnerability investigation exposed that all the isolates of *Salmonella* spp. (n=10) were 100% susceptible to meropenem and then followed by ciprofloxacin (90%), gentamicin (80%), ceftriaxone (50%), amoxicillin-clavulanic acid (40%) and erythromycin (40%), nalidixic acid (30%) and tetracycline (10%) (Figure 6). Conversely, all the isolates of *Salmonella* spp. (n=10) were 100% resistant against amoxicillin and ampicillin followed by tetracycline (80%).

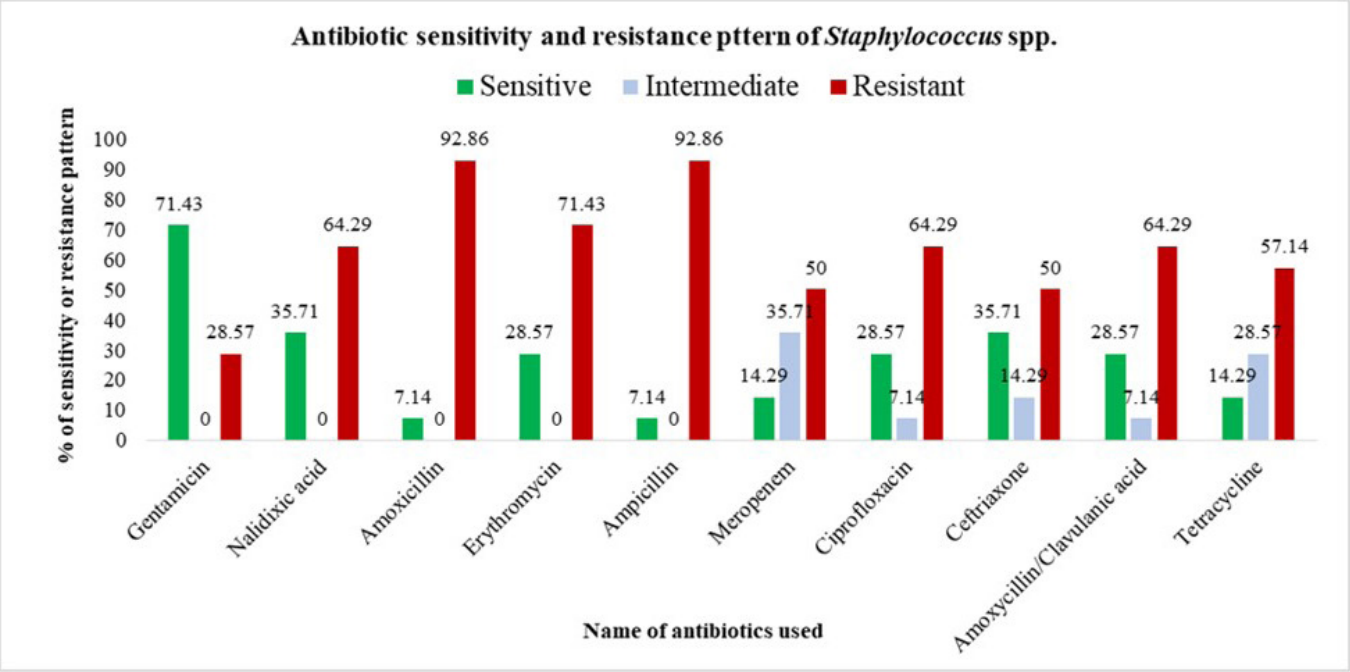

Figure 4: *Antibiotic sensitivity and resistance patterns of Staphylococcus spp. in cow milk.*

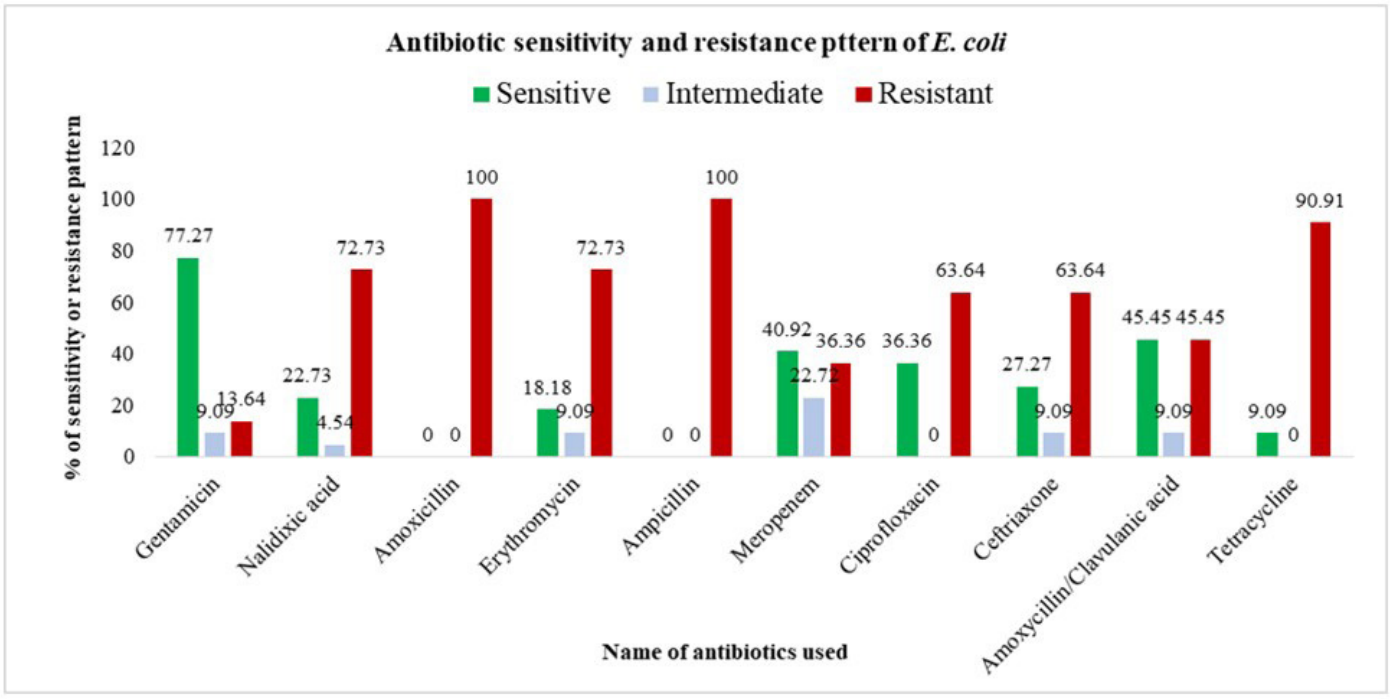

Figure 5: *Antibiotic sensitivity and resistance patterns of E. coli in cow milk.*

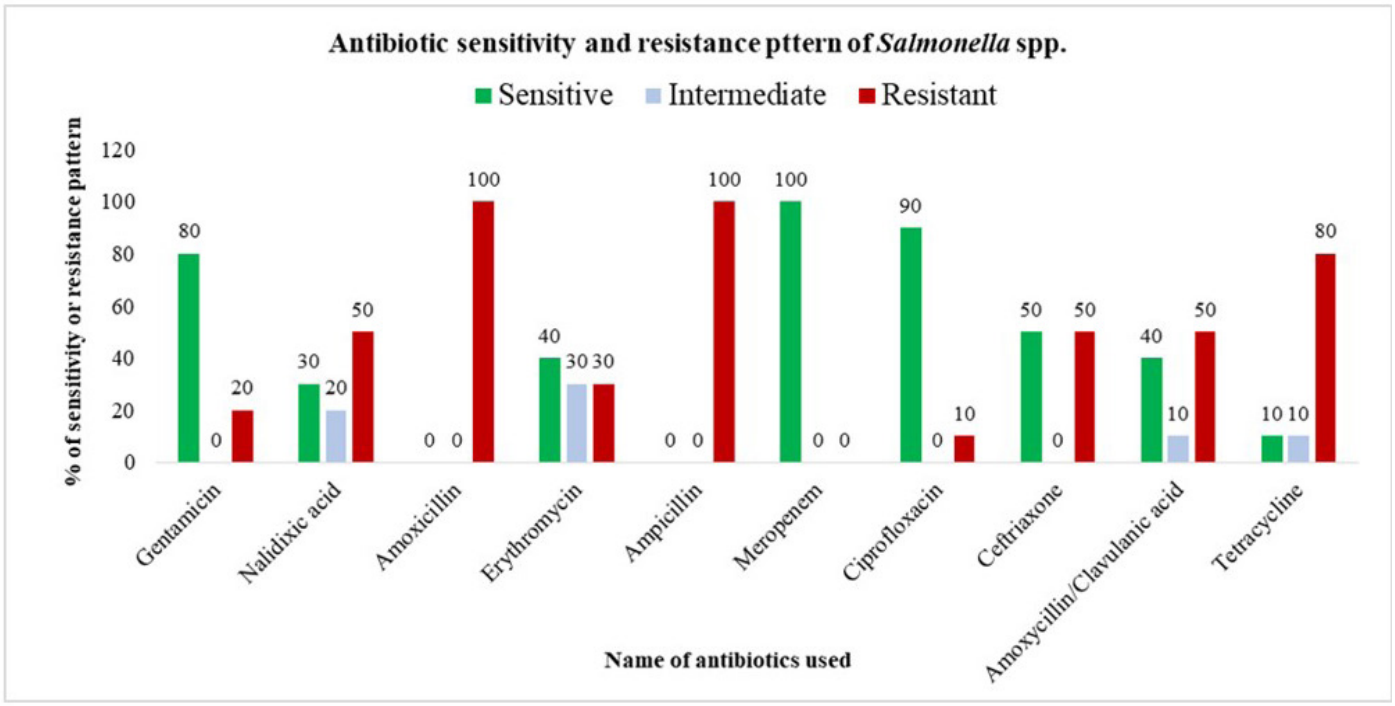

Figure 6: *Antibiotic sensitivity and resistance patterns of Salmonella spp. in cow milk.*

The present study indicates that ampicillin and amoxicillin are among the most commonly used antibiotics in the Bangladeshi dairy sector, and all three bacterial species examined showed high resistance against these drugs. Among *Staphylococcus* spp., the highest resistance was observed against ampicillin and amoxicillin, while the highest sensitivity to gentamicin. These findings are consistent with Borena *et al.* (2023), Gebremedhin *et al.* (2022), and Hassani *et al.* (2022), but contrast with Deddefo *et al.* (2022) and Al-Ashmawy *et al.* (2016), who reported lower resistance.

For *E. coli* isolates, resistance was highest against ampicillin and amoxicillin, followed by tetracycline, nalidixic acid, erythromycin, and ceftriaxone, with the highest sensitivity to gentamicin. Similar resistance levels to ampicillin and amoxicillin were reported by Rahman and Ahmed (2022), and Ranjbar *et al.* (2018), while Hassani *et al.* (2022)

Table 4: *Multidrug-resistant Staphylococcus spp., E. coli, and Salmonella spp.*

Name of Isolates	Antimicrobial compounds	Antibiotic classes	No. of MDR isolates (%)	Overall MDR (%)
Staphylococcus spp.	AMX-AMP-TE-AMC-CIP-NA-E-MEM	Pen-Tet-PenB-Flu-Qui-Mac-Carb	2 (20.0)	71.43% (n= 10/14)
	AMX-AMP-TE-AMC-CTR-CIP-NA-E-MEM	Pen-Tet-PenB-Cef-Flu-Qui-Mac-Carb	1 (10.0)	
	AMX-AMP-AMC-CTR-CIP-NA-E-GEN-MEM	Pen-PenB-Cef-Flu-Qui-Mac-Ami-Carb	3 (30.0)	
	AMX-AMP-TE-E	Pen-Tet-Mac	1 (10.0)	
	AMX-AMP-AMC-CTR-CIP-NA-E	Pen-PenB-Cef-Flu-Qui-Mac	2 (20.0)	
	AMX-AMP-AMC-CTR-CIP-NA-E-MEM	Pen-PenB-Cef-Flu-Qui-Mac-Carb	1 (10.0)	
	Total		10(100.0)	
E. coli	AMP-AMX-TE-AMC-CTR-CIP-NA-E-GEN	Pen-Tet-PenB-Cef-Flu-Qui-Mac-Ami	7 (33.33)	95.45% (n=21/22)
	AMP-AMX-CTR-CIP-NA-E-GEN	Pen-Cef-Flu-Qui-Mac-Ami	1 (4.76)	
	AMP-AMX-TE-MEM	Pen-Tet-Carb	2 (9.52)	
	AMP-AMX-AMC-MEM	Pen-PenB-Carb	1 (4.76)	
	AMP-AMX-TE-CTR-CIP-NA-E-GEN	Pen-Tet-Cef-Flu-Qui-Mac-Ami	4 (19.05)	
	AMP-AMX-TE-AMC-MEM	Pen-Tet-PenB-Carb	1 (4.76)	
	AMP-AMX-TE-AMC-NA-E-GEN	Pen-Tet-PenB-Qui-Mac-Ami	1 (4.76)	
	AMP-AMX-TE-CTR-CIP-NA-E-GEN-MEM	Pen-Tet-Cef-Flu-Qui-Mac-Ami-Carb	2 (9.52)	
	AMP-AMX-TE-GEN-MEM	Pen-Tet-Ami-Carb	1 (4.76)	
	AMP-AMX-TE-NA-E-GEN-MEM	Pen-Tet-Qui-Mac-Ami-Carb	1 (4.76)	
	Total		21 (100.0)	
Salmonella spp.	AMP-AMX-TE-CIP-NA	Pen-Tet-Flu-Qui	1 (12.50)	80.0% (n=8/10)
	AMP-AMX-AMC-CTR-E	Pen-PenB-Cef-Mac	1 (12.50)	
	AMP-AMX-TE-NA-E	Pen-Tet-Qui-Mac	1 (12.50)	
	AMP-AMX-TE-NA-GEN	Pen-Tet-Qui-Ami	1 (12.50)	
	AMP-AMX-TE-AMC-CTR-E	Pen-Tet-PenB-Cef-Mac	1 (12.50)	
	AMP-AMX-TE-AMC-CTR	Pen-Tet-PenB-Cef	1 (12.50)	
	AMP-AMX-TE-AMC-NA	Pen-Tet-PenB-Qui	1 (12.50)	
	AMP-AMX-TE-AMC-CTR-NA-GEN	Pen-Tet-PenB-Cef-Qui-Ami	1 (12.50)	
	Total		8 (100.0)	

AMP=Ampicillin, AMX=Amoxicillin, GEN=Gentamicin, TE=Tetracycline, NA=Nalidixic acid, AMC=Amoxycillin/Clavulanic acid, CIP=Ciprofloxacin, CTR=Ceftriaxone, MEM=Meropenem, E=Erythromycin, Pen=Penicillin, Ami=Aminoglycoside, Tet=Tetracycline, Qui=Quinolone, PenB=Penicillin-Beta lactamase inhibitor, Flu=Fluoroquinolone, Cef=Cephalosporins, Carb=Carbapenem, Mac=Macrolide, MDR=Multi-Drug Resistant.

documented lower resistance (70.51%). *Salmonella* spp. showed complete resistance to ampicillin and amoxicillin, but remained fully susceptible to meropenem, followed by ciprofloxacin and gentamicin. Comparable multidrug resistance (MDR) in *Salmonella* spp. has been reported by Van Kessel *et al.* (2013), Gebeyehu *et al.* (2022), Garbaj *et al.* (2022), and Rahman and Ahmed (2022), though Hassani *et al.* (2022) observed lower resistance levels.

Microorganisms that showed resistance to at least one agent in three or more antibiotic classes were considered MDR. Among the MDR isolates of this study, *Staphylococcus* spp. accounted for 71.43% (10/14), *E. coli* for 95.45% (21/22), and *Salmonella* spp. for 80.0% (8/10) isolate(s) (Table 4). The highest (30.0%) MDR pattern for *Staphylococcus* spp. was "Pen-PenB-Cef-Flu-Qui-Mac-Ami-Carb". Similarly, 33.33% *E. coli* showed MRD pattern of "Pen-Tet-PenB-Cef-Flu-Qui-Mac-Ami"; however, *Salmonella* spp. had eight different MDR pattern with equal percentage of 12.50. Several MDR patterns were observed among the isolates from milk samples indicates the haphazard and non-judicious used of them in animal

production and veterinary medicine (Bronzwaer *et al*., 2002). In Bangladesh, misuse is exacerbated by over-the-counter access, lack of veterinary guidance, and premature discontinuation of treatment courses. Such practices accelerate the development of resistant strains, posing risks to humans and animals. Continuous surveillance of antimicrobial susceptibility patterns is therefore essential for guiding rational antibiotic use and mitigating the threat of AMR.

Conclusion

The existence of pathogenic multidrug-resistant (MDR) bacteria such as *Staphylococcus* spp. and *E. coli* and *Salmonella* spp. in farm and market milk leads to a serious threat to public health. This study supports the need for improvement to hygienic milking, handling, and processing practices, should be accompanied with recommendations for improved farm management and pasteurization of farm milk and market milk. Preventing milk borne transmission of MDR pathogens for the sake of public health requires policy implications and raising awareness among dairy production stakeholders.

Acknowledgement

We acknowledged the Sher-e-Bnagla Agricultural University, Dhaka, Bangladesh.

Novelty Statement

This study detected MDR milk-borne bacteria from both the farm and the market with a molecular basis.

Author' Contribution

Md. Nasim Hasan Mim and Mahfuzul Islam contributed to the conception and design of the study. Md. Nasim Hasan Mim, M. Rubaiyat Adnan, Md. Abir Hassan Sadi, and Mithium Hussain Loveonnya conducted the field and laboratory work. Md. Nasim Hasan Mim, Md. Kamrul Hassan, Mithium Hussain Loveonnya, Mirza Synthia Sabrin, and Mahfuzul Islam examined the gathered data. Md. Nasim Hasan Mim, Tayeaba Jannat, Md. Rashedul Islam, Nipu Sen, and Mahfuzul Islam performed the data analyses. Md. Kamrul Hassan and Mahfuzul Islam supervised the research work. The manuscript was drafted with the assistance of Md. Nasim Hasan Mim, Tayeaba Jannat, and Mahfuzul Islam. The final manuscript has been revised and approved by all authors.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

References

Abebe, E., Gugsa, G., Ahmed, M., Awol, N., Tefera, Y. and Abegaz, S., 2024. Occurrence, associated risk factors, and antimicrobial resistance patterns of *Staphylococcus aureus* and methicillin-resistant *S. aureus* from foods of bovine origin in Dessie and Kombolcha towns, Ethiopia. Front. Sustain. Food Syst., 8: 1422850. https://doi.org/10.3389/fsufs.2024.1422850

Al-Ashmawy, M.A., Sallam, K.I., Abd-Elghany, S.M., Elhadidy, M. and Tamura, T., 2016. Occurrence, molecular characterization, and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolated from milk and dairy products. Foodborne Pathog. Dis., 13(3): 156-162. https://doi.org/10.1089/fpd.2015.2038

Allied, M.A.H., 2021. Multidrug-resistant *Salmonella* spp. and *Staphylococcus* spp. isolated from milk in Dhaka City. Ph.D. thesis, Brac Univ., Bangladesh.

Asefa, I., Legabo, E., Wolde, T. and Fesseha, H., 2023. Study on salmonella isolates from fresh milk of dairy cows in selected districts of Wolaita Zone, Southern Ethiopia. Int. J. Microbiol., 2023: 6837797. https://doi.org/10.1155/2023/6837797

Asmelash, T., 2015. Isolation, identification, antimicrobial profile and molecular characterization of enterohaemorrhagic *E. coli* O157: H7 isolated from ruminants slaughtered at De-bre Zeit elfora export abattoir and addis ababa abattoirs enterprise. M.S. thesis, Addis Ababa University, Ethiopia.

Bauer, H., Paronetto, F., Burns, W.A. and Einheber, A., 1966. The enhancing effect of the microbial flora on macrophage function and the immune response: A study in germfree mice. J. Exper. Med., 123(6): 1013-1024. https://doi.org/10.1084/jem.123.6.1013

Bedasa, S., Shifefarm, D., Abraha, A. and Moges,

T., 2018. Occurrence and antimicrobial susceptibility profile of *Escherichia coli* O157: H7 from food of animal origin in Bishoftu town, Central Ethiopia. Int. J. Food Contam., 5(1): 1-8. https://doi.org/10.1186/s40550-018-0064-3

Borena, B.M., Gurmessa, F.T., Gebremedhin, E.Z., Sarba, E.J. and Marami, L.M., 2023. *Staphylococcus aureus* in cow milk and milk products in Ambo and Bako towns, Oromia, Ethiopia: Occurrence, associated risk factors, hygienic quality, and antibiogram. Int. Microbiol., 26(3): 513–527. https://doi.org/10.1007/s10123-022-00317-x

Bronzwaer, S.L., Cars, O., Buchholz, U., Molstad, S., Goettsch, W., Veldhuijzen, I.K., Kool, J.L., Sprenger, M.J. and Degener, J.E., 2002. A European study on the relationship between antimicrobial use and antimicrobial resistance. Em. Infect. Dis., 8: 278-282. https://doi.org/10.3201/eid0803.010192

Buxton, A. and Fraser, G., 1977. *Escherichia coli*. In: Animal microbiology. Blackwell Scientific Publications, Vol. 1, Oxford, London, pp. 78-80.

Capita, R., Álvarez-Astorga, M., Alonso-Calleja, C., Moreno, B. and Camino García-Fernández, M., 2003. Occurrence of salmonellae in retail chicken carcasses and their products in Spain. Int. J. Food Microbiol., 81(2): 169-173. https://doi.org/10.1016/S0168-1605(02)00195-2

Cheesbrough, M., 2006. District laboratory practice in tropical countries (2nd Edition). London English Language Book Society. pp. 100-194. https://doi.org/10.1017/CBO9780511543470

CLSI, 2020. Performance standards for antimicrobial susceptibility testing; Clinical and Laboratory Standard Institute, United States of America.

Deddefo, A., Mamo, G., Leta, S. and Amenu, K., 2022. Occurrence and molecular characteristics of Staphylococcus aureus in Farm milk and milk products in Ethiopia: A systematic review and meta-analysis. Int. J. Food Contam., 9(1): 1-21. https://doi.org/10.1186/s40550-022-00094-5

Demme, B. and Abegaz, S., 2015. Isolation and identification of major bacterial pathogen from clinical mastitis cow Farm milk in Addis Ababa, Ethiopia. Acad. J. Anim. Dis., 4(1): 44-51.

Devriese, L., Haesebrouck, F., Hommez, J. and Vandermeersch, R., 1997. A 25-year survey of antibiotic susceptibility testing in Staphylococcus aureus from bovine mastitis in Belgium, with special reference to penicillinase. Vlaams. Diergeneeskundig. Tijdschriff. 66(4): 170-173.

Eichner, D. and Gravitz, B., 2001. The effects of under-usage of antibiotics on bacteria. 1: 1-7.

FAO and WHO, 2004. Codex alimentarius. In: Code of hygiene practices for milk and milk products. Vol. 57, Rome, Italy. pp. 181-232.

Fesseha, H., 2020. Isolation and Identification of Escherichia coli from Dairy Cow Farm milk in Bishoftu Town, Central Ethiopia. Arch. Vet. Anim. Sci. 1. 1-7.

Foster, T., 1996. Chapter 12: *Staphylococcus*. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston, Galveston, Texas.

Freeman, B.A., 1985. Burrows textbook of microbiology. 22nd ed., WB Saunders Company, Philadelphia, USA. pp. 464-475.

Garbaj, A.M., Gawella, T.B.B., Sherif, J.A., Naas, H.T., Eshamah, H.L., Azwai, S.M., Gammoudi, F.T., Abolghait, S.K., Moawad, A.A., Barbieri, I. and Eldaghayes, I.M., 2022. Occurrence and antibiogram of multidrug-resistant Salmonella enterica isolated from dairy products in Libya. Vet. World, 15(5): 1185–1190. https://doi.org/10.14202/vetworld.2022.1185-1190

Gebeyehu, A., Taye, M. and Abebe, R., 2022. Isolation, molecular detection and antimicrobial susceptibility profile of salmonella from farm cow milk collected from dairy farms and households in southern Ethiopia. BMC Microbiol., 22(1): 84. https://doi.org/10.1186/s12866-022-02504-2

Gebremedhin, E.Z., Ararso, A.B., Borana, B.M., Kelbesa, K.A., Tadese, N.D., Marami, L.M. and Sarba, E.J., 2022. Isolation and identification of *Staphylococcus aureus* from milk and milk products, associated factors for contamination, and their antibiogram in Holeta, Central Ethiopia. Vet. Med. Int., 2022: 6544705. https://doi.org/10.1155/2022/6544705

Griffiths, M.W., 2010. The microbiological safety of farm milk. In: Improving the safety and quality of milk. Vol. 2, Woodhead Publishing Limited. Cambridge, Cambridgeshire, United Kingdom. pp. 490-506. https://doi.org/10.1533/9781845699420.1.27

Gwida, M.M. and El-Gohary, F.A., 2013. Zoonotic

bacterial pathogens isolated from Farm milk with special reference to *Escherichia coli* and *Staphylococcus aureus* in Dakahlia Governorate, Egypt. Governorate. Egypt, 2(4): 705.

Han, J., David, J.D., Aaron, M.L., Pravin, K., Rajesh, N., Rossina, S. and Steven, L.F., 2011. Comparison of Salmonella enterica serovar Heidelberg isolates from human patients with those from animal and food sources. J. Clin. Microbiol., 49(3): 1130-1133. https://doi.org/10.1128/JCM.01931-10

Hassani, S., Moosavy, M.H., Gharajalar, S.N., Khatibi, S.A., Hajibemani, A. and Barabadi, Z., 2022. High Occurrence of antibiotic resistance in pathogenic foodborne bacteria isolated from bovine milk. Sci. Rep., 12(1): 3878. https://doi.org/10.1038/s41598-022-07845-6

Hossain, A., 2012. Presence and pattern of virulence genes in non-lactose fermenting *Escherichia coli* strains isolated from stools of children under 5 years in rural and urban Bangladesh. Int. J. Infect. Dis., 16(10): e395. https://doi.org/10.1016/j.ijid.2012.05.525

Javaid, S.B., Gadahi, J.A., Khaskeli, M., Bhutto, M.B., Kumbher, S. and Panhwar, A.H., 2009. Physical and chemical quality of market milk sold at Tandojam, Pakistan. Pakistan Vet. J., 29(1): 27-31.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. and Daszak, P., 2008. Global trends in emerging infectious diseases. Nature, 451: 990-993. https://doi.org/10.1038/nature06536

Kaushik, P., Anjay, Kumari, S.S., Bharti, S.K. and Dayal, S., 2014. Isolation and occurrence of salmonella from chicken meat and cattle milk collected from local markets of Patna, India. Vet. World, 7: 62-65. https://doi.org/10.14202/vetworld.2014.62-65

Madani, A., Esfandiari, Z., Shoaei, P. and Ataei, B., 2022. Evaluation of virulence factors, antibiotic resistance, and biofilm formation of *Escherichia coli* isolated from milk and dairy Products in Isfahan, Iran. Foods (Basel, Switzerland). 11(7): 960. https://doi.org/10.3390/foods11070960

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G. and Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect., 18(3): 268-281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Marjan, S., Kanta, D.K., Kishore, M.S. and Noor, R., 2014. Drug-resistant bacterial pathogens in milk and some milk products. Nutr. Food Sci., 44(3): 241-248. https://doi.org/10.1108/NFS-05-2013-0061

McLandsborough, L.A., 2005. Food microbiology laboratory - CRC series in contemporary food science, CRC Press LLC, New York, USA.

Mthembu, T.P., Zishiri, O.T. and El-Zowalaty, M.E., 2019. Detection and molecular identification of salmonella virulence genes in livestock production systems in South Africa. Pathogens, 8(3): 124. https://doi.org/10.3390/pathogens8030124

Nayak, R., Stewart, T., Wang, R.F., Lin, J., Cerniglia, C.E. and Kenney, P.B., 2004. Genetic diversity and virulence gene determinants of antibiotic-resistant salmonella isolated from preharvest turkey production sources. Int. J. Food Microbiol., 91: 51–62. https://doi.org/10.1016/S0168-1605(03)00330-1

Office International Des Epizootic (OIE), (2000). Manual of standards for diagnostic tests and vaccine (4th edition). pp. 401-413.

Oliver, S.P., Boor, K.J., Murphy, S.C. and Murinda, S.E., 2009. Food safety hazards associated with consumption of Farm milk. Foodborne Pathog. Dis., 6(7): 793-806. https://doi.org/10.1089/fpd.2009.0302

Oliver, S.P., Jayarao, B.M. and Almeida, R.A., 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog. Dis., 2(2): 115-129. https://doi.org/10.1089/fpd.2005.2.115

Pant, R., Nirwal, S. and Rai, N., 2013. Occurrence of antibiotic-resistant bacteria and analysis of microbial quality of Farm milk samples collected from different regions of Dehradun. Int. J. Pharmtech. Res., 5(2): 15.

Radostits, O.M., Gray, C.C., Hinchcliff, K. and Constable, P.D., 2006. Mastitis. In: Veterinary Medicine: A textbook of the disease of Cattle, horses, Sheep, Pigs, and Goats. 10th ed., Sounder, Spain. pp. 673-719.

Rahman, M.B. and Ahmed, M.S., 2022. Antibiogram of *E. coli* and *Salmonella* spp. isolated from chicken meat and frozen milk

in Barishal city, Bangladesh. Bangladesh J. Vet. Med., 20(1): 35-40. https://doi.org/10.33109/bjvmjj2022amrt1

Ramezanigardaloud, N., Loncaric, I., Mikuni-Mester, P., Alinaghi, M., Ehling-Schulz, M., Khol, J.L. and Grunert, T., 2025. Prevalence and diversity of *Staphylococcus aureus* in bulk tank milk from community-based alpine dairy pastures in Tyrol, Austria. Animals, 15(14): 2153. https://doi.org/10.3390/ani15142153

Ranjbar, R., Safarpoor Dehkordi, F., Sakhaei Shahreza, M.H. and Rahimi, E., 2018. Occurrence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing *Escherichia coli* strains isolated from farm milk and traditional dairy products. Antimicrob. Resist. Infect. Contr., 7: 53. https://doi.org/10.1186/s13756-018-0345-x

Regasa, S., Mengistu, S. and Abraha, A., 2019. Milk safety assessment, isolation, and antimicrobial susceptibility profile of *Staphylococcus aureus* in selected dairy farms of Mukaturi and Sululta town, Oromia region, Ethiopia. Vet. Med. Int., 11: 234-239. https://doi.org/10.1155/2019/3063185

Shome, B.R., Natesan, K., Mitra, S.D., Venugopal, N., Bhuvana, M.A.N.I., Ganaie, F., Shome, R. and Rahman, H., 2018. Development of simplex PCR assays for accurate identification of nine *staphylococcal* species at genus and species levels. J. Microbiol. Infect. Dis., 3: 120-127. https://doi.org/10.5799/jmid.458462

Skočková, A., Čížek, A., Vadlejch, J. and Barák, I., 2018. Detection of multi-drug resistant (MDR) Escherichia coli and tet gene. J. Adv. Vet. Anim. Res., 5(4): 388-396. https://doi.org/10.5455/javar.2018.e289

Torkar, K.G. and Teger, S.G., 2008. The microbiological quality of farm milk after introducing the two-day milk collecting system. Acta Agric. Slov., 92(1): 61-74. https://doi.org/10.14720/aas.2008.92.1.15090

Tsen, H.Y., Lin, C.K. and Chi, W.R., 1998. Development and use of *16S rRNA* gene targeted PCR primers for the identification of *Escherichia coli* cells in water. J. Appl. Microbiol., 85(3): 554–560. https://doi.org/10.1046/j.1365-2672.1998.853535.x

Van Kessel, J.S., Sonnier, J., Zhao, S. and Karns, J.S., 2013. Antimicrobial resistance of *Salmonella enterica* isolates from bulk tank milk and milk filters in the United States. J. Food Prot., 76(1): 18-25. https://doi.org/10.4315/0362-028X.JFP-12-263

Yasmin, S., Parveen, S., Munna, M.S. and Noor, R., 2015. Detection of *Salmonella* spp. and microbiological analysis of milk and milk-based products available within Dhaka Metropolis, Bangladesh. Br. Microbiol. Res. J., 5(6): 474-480. https://doi.org/10.9734/BMRJ/2015/11010

Zinnah, M.A., Bari, M.R., Islam, M.T., Hossain, M.T., Rahman, M.T., Haque, M.H. and Islam, M.A., 2007. Characterization of *Escherichia coli* isolated from samples of different biological and environmental sources. Bangladesh J. Vet. Med., 5(1, 2): 25-32. https://doi.org/10.3329/bjvm.v5i1.1305