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Characterization of bacterial pathogens associated with milk microbiota in Egypt

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Milk is a substantial source of nutrients needed by all humans across lifespan development. Given its nutritional composition, milk is considered a vehicle for various microbes including beneficial and pathogenic bacteria. In this study, 270 milk samples comprising raw cow and buffalo milk and pasteurized milk with different shelf-life durations were tested along with pasteurized organic milk for the presence of *Staphylococcus aureus and Escherichia coli.* Collectively, 21 *E. coli* and 14 *S. aureus* isolates were cultivated and identified from total milk samples. All *E. coli* and *S. aureus* isolates exhibited resistance to erythromycin and penicillin, respectively. Serogroups O26, O128, and O111 were the most frequently identified amongst *E. coli* isolates, whereas staphylococcal enterotoxins (SEs) were inconsistently produced across *S. aureus* isolates. The molecular profile showed clustering of 6 isolates of *E. coli* by harboring *stx1, stx2, eaeA* genes, and 5 isolates of *S. aureus* by *mecA* gene. Findings revealed the bacteriological quality of popularly consumed milk in Egypt, including raw and pasteurized milk with preference to pasteurized organic milk and 7-day shelf life (7DSL) pasteurized milk. However, raw milk and 3MSL pasteurized milk were the major sources of *E. coli* and *S. aureus*, posing a serious public health issue.

Key words: Raw milk, pasteurization, *Staphylococcus aureus and Escherichia coli,* shelf-life.

INTRODUCTION

Milk and dairy products are substantial sources of macro- and micronutrients needed by humans that make them prone to contamination with microbial pathogens. Simultaneously, milk nutrients support the growth of specific beneficial microbes (e.g. lactobacilli and bifidobacteria) that promote human health and fitness

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(Fernandes, 2009). Though the ingestion of contaminated milk either raw or pasteurized is the major cause of serious food-poisoning outbreaks, potentially result from microbial toxins production (Dhanashekar et al., 2012). Contaminated milk may harbor harmful microbes that lead to either milk spoilage (e.g. *Pseudomonas* and thermoduric microbes such as *Clostridium* and *Bacillus*) or the emergence of public health issues (e.g. *Listeria, Salmonella, E. coli,* and *S. aureus*) (Bennett et al., 2013; Quigley et al., 2013b). Milk is sterile at secretion in udder but is contaminated with extraneous microbes before leaving the animal udder (Elgadi et al., 2008). In developing countries especially rural areas, raw milk is directly used for either consumption or local dairy production (FAO, 2011; Zeinhom and Abdel-Latef, 2014). Raw milk has a short shelf life that could be extended by heating. However, in the dairy industry, the shelf life of pasteurized milk is greatly influenced by the microbiological quality of the used raw milk (Murphy et al., 2016). In general, spoilage of commercialized milk and dairy products is attributable to various contamination sources including; pre-pasteurization psychrotophic growth, the degradable activity of heat-resistant microbial enzymes, and contamination after pasteurization process which is the most probable source (Sarkar, 2015). Gram-negative rods are the major psychrotrophic bacteria inhabiting raw milk (e.g., Enterobacteriaceae family including coliform bacteria that encompasses 5 to 33% of milk psychrotrophic bacteria) and proliferate during storage with the production of thermoresistant degradative enzymes (De Oliveira et al., 2015; Barbano et al., 2006; Mallet et al., 2012; Lewis and Gilmour, 1987). In addition, some Gram-positive bacteria contaminate raw milk with less frequent existence compared to Gram-negative psychrotrophs such as *Staphylococcus* species (Vithanage et al., 2016).

In general, pasteurization and ultra-high temperature (UHT) sterilization are the most commonly used techniques in the dairy industry for proper preservation and prolonged usability periods (Rais et al., 2013). Pasteurization meant to destroy common pathogens inhabiting raw milk microbiotas, especially those responsible for milk spoilage and influencing the shelf-life duration. Furthermore, pasteurization inactivates microbial enzymes that catalyze the breakage of milk macro-nutrients (e.g., lipids and proteins) and result in spoilage and invalidity of dairy products for consumption (Sarkar, 2015). In UHT treatment, heating is applied in the range of 135 to 150°C for up to 4 s for safe commercial dairy products combined with prolongation of the milk shelf-life duration (up to 12 months) (Vranješ et al., 2015).Though, aseptic packaging is crucial in both techniques that assure safety and extended usability of final dairy products (Deeth, 2017).

There is a considerable number of published studies that have been conducted on the prevalence of *E. coli* and *S. aureus* in milk (Kandil et al., 2018; Vahedi et al., 2013). Milk and dairy products are one of the major causes of the transmission of pathogenic *E. coli* strains into the human (Ombarak et al., 2019; Momtaz et al., 2012). With the advent of the high throughput sequencing technology, *Escherichia coli* was reported as a dominant inhabitant of the healthy human gut microbiome (Desmarchelier and Fegan, 2016). However, some *E. coli* strains exhibited virulence traits that enabled them to infect different body organs and cause illness (Awadallah et al., 2016; Zeinhom and Abdel-Latef, 2014). Noteworthy, diarrheagenic *E. coli* strains increasingly become the leading cause of pediatric diarrhea. The most important diarrheagenic *E. coli* that threaten human health worldwide are enteropathogenic *E. coli* (EPEC) (the etiological agent of watery diarrhea in infants), enterohemorrhagic *E. coli* (EHEC) (leads to hemorrhagic colitis and hemolytic-uremic syndrome), entero-aggregative *E. coli* (EAEC) (causes persistent diarrhea), and enterotoxigenic *E. coli* (ETEC) (known to cause traveler's diarrhea) (Nataro and Kaper, 1998). The pathogenicity of diarrheagenic *E. coli* is attributed to possessing genetically encoded virulence traits. For instance, enterohemorrhagic *E. coli* (EHEC) causes illness through the expression of intimin outer membrane protein encoded by *eae* gene and required for tissue colonization along with the production of Shiga toxins (ST) (e.g., *Stx1, Stx2* or *Stx2* variants) (Kaper et al., 2004). However, Enteropathogenic *E. coli* (EPEC) lacks *ST* genes, but exhibits its pathogenicity through the formation of A/E lesions on the intestinal cells, and is identified as *eae*-harboring diarrheagenic *E. coli* (Aidar-Ugrinovich et al., 2007).

S. aureus is a facultative anaerobic Gram-positive coccus and one of the world top pathogens that causes food-poisoning (Tirado and Schmidt, 2001; Hennekinne et al., 2012). Globally, enterotoxigenic *S. aureus* is implicated in udder infection of dairy cows combined with improper handling and poor storage conditions that result in frequent contamination of milk and dairy products. *S. aureus* produces several toxins including classical staphylococcal enterotoxins (SE) (SEA to SEE), in addition to other new types (SEG to SElU2) (Argudín et al., 2010). *S. aureus* could be inactivated by pasteurization however, thermostable SEs were found to retain their biological activity after the thermal treatment (Asao et al., 2003). Furthermore, more recent evidence suggests that SEA is the leading cause of staphylococcal food poisoning worldwide (Argudín et al., 2010). In order to verify the prevalence of genes encoding SE in *S. aureus* isolated from milk and dairy products, the phenotypic/serotypic assays of SE production should be conducted (Morandi et al., 2007). Of the classical techniques used for SE serotyping analysis, the gel-

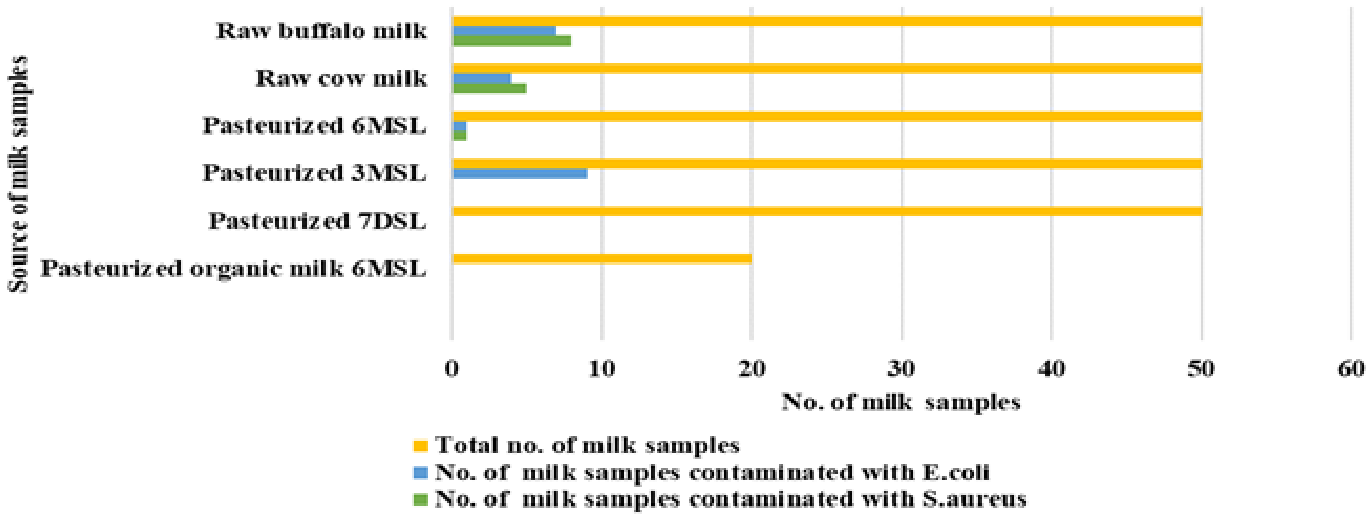

Figure 1. Prevalence of isolated and identified *E. coli* and *S. aureus* contaminants across milk samples collected from different sources. 3MSL: 3-month shelf life; 6MSL: 6-month shelf life and 7DSL: 7-day shelf life.

diffusion test, agglutination test, and reverse passive latex agglutination (RPLA) test kits (Wu et al., 2016). When compared to molecular techniques, the serological tests have limited sensitivity and specificity for SEs detection and cannot be used for total quantification of SE (Wu et al., 2016).

So far, culture-dependent methods are still used as a routine protocol for the microbial assessment of raw and pasteurized milk. However, the detection of bacterial species that exist at subdominant levels is needed since the conventional laboratory methods are not enough to support the *in vitro* growth of milk-associated microbiota (Quigley et al., 2013a). Nowadays, culture-based foodborne pathogen detection methods have been developed to reduce the inspection time and improve product quality. One of the most informative and cost-effective molecular-based detection techniques is the multiplex PCR, which enables the screening of multiple target genes within a single reaction (Postollec et al., 2011).

In developing countries, consumption of raw milk is not prohibited and the advanced pasteurization techniques are still neither regulated nor implemented. Given the nutritional importance of milk and its widespread consumption particularly, among women and children, the study aimed to investigate the bacteriological quality of popularly consumed milk in the Delta area, Egypt for the presence of *E. coli* and *S. aureus* as major milk contaminants. The identified isolates were subjected to further testing for their potential pathogenicity through serotypic characterization and molecular profiling along with their antibiotic susceptibility profile.

MATERIALS AND METHODS

Samples collection

Two hundred and seventy milk samples (10 ml each) were randomly collected (from January to June 2017) from local grocery stores and farmer vendors in El-Beheira governorate that represents Delta area in Egypt as street vendors are coming from different villages of neighbor Delta governorates. The milk samples included 100 samples of raw milk (50 samples of cow milk and 50 samples of buffalo milk), and 170 samples of pasteurized milk (50 samples of 6-month shelf life (6MSL), 50 samples of 3-month shelf life (3MSL), 50 samples of 7-day shelf life (7DSL) and 20 samples of pasteurized organic milk (6MSL)) (Figure 1 and Table S1A). All milk samples were collected in an icebox and brought to the laboratory to assess them for the presence of *E. coli* and *S. aureus* contaminants.

Isolation and identification of *E. coli*

Under aseptic conditions, 1 ml of each milk sample was drawn, homogenized with 10 ml of nutrient broth and incubated overnight at 37°C. Next day, 100 µl of the cultivated broth were streaked on MacConkey agar plate and incubated overnight for selection of enteric Gram-negative (Gm-ve) bacteria. Every lactose-fermenting (LF) colony was picked up using sterile toothpicks and streaked on Eosin methylene blue (EMB) agar plate, then incubated overnight at 37°C for further purification. Colonies exhibited blue-black color with a metallic green sheen were isolated and examined under a light microscope for gram stain. *E. coli* candidates were biochemically confirmed using indole, methyl Red, Voges Proskauer, citrate, triple sugar iron, and urease tests (Table S1B) according to Kreig and Holt (1984) and Miller (1992).

Isolation and identification of *S. aureus*

For isolation of *S. aureus*, 100 µl of overnight cultivated milk samples were streaked on Mannitol salt agar (MSA) plate and incubated overnight for bacterial growth. A yellow colony grown on a red/pink (MSA) medium was picked up and then streaked on a Baired parker (BP) agar plate for further purification. Every unique single colony was gram stained and visualized under the light microscope. The identification of *S. aureus* isolates was confirmed by performing a specific scheme of biochemical tests including coagulase, oxidase and DNase tests (Table S1C) according to MacFaddin (2000) and Lachica et al. (1971). 50% glycerol stocks of all identified bacterial isolates under this study were prepared and stored at -80°C freezer for further experiments.

Antibiotic susceptibility testing

The susceptibility of *E. coli* and *S. aureus* isolates to antibiotics were tested using the agar disk diffusion method. 11 antibiotics including ampicillin 10 µg (AML), amoxicillin/clavulanic 30 µg (AMC), imipenem 10 µg (IPM), cefipime 30 µg (CPM), cefotaxime 30 µg (CTX), gentamicin 10 µg (CN), azithromycin 15 µg (AZM), chloramphenicol 30 µg (C), tetracycline 30 µg (TE), sulphamethoxazole/trimethoprim 1.25/23.75 (SXT) and ciprofloxacin 5 µg (CIP) were used for the screening of *E. coli* isolates. With respect to testing *S. aureus* isolates, 9 antibiotics including penicillin 10U (P), cefoxitin 30 µg (CX), vancomycin 30 µg (VA), gentamicin 10 µg (CN), erythromycin 15 µg (E), chloramphenicol 30 µg (C), tetracycline 30 µg (TE), sulphamethoxazole/trimethoprim 1.25/23.75 (SXT) and ciprofloxacin 5 µg (CIP) were used. Following 16 to 18 h of aerobic incubation at 37°C, the plates were examined for bacterial growth and the diameter of inhibition zones surrounding antibiotic disks were scored in millimeter (mm). The zone diameters were interpreted as resistant (R), intermediate (I) or susceptible (S) according to (CLSI, 2017).

Serotyping of *E. coli* isolates

Serotyping of *E. coli* isolates was performed using rapid diagnostic *E. coli* antisera sets (Denka Seiken Co, Japan) for lab diagnosis of Enteropathogenic serotypes according to the manufacturer's instructions. All antisera were obtained and absorbed with the corresponding cross-reacting antigens to remove the non-specific agglutinins.

Staphylococcal enterotoxins (SE) production test using SET-RPLA assay

S. aureus isolates were tested for enterotoxin production (SEA to SED) using SET-RPLA assay (SET-RPLA; Denka Seiken Co. Ltd., Tokyo, Japan) (Park and Szabo, 1986). The serotypic assay was performed according to the manufacturer's instruction

Genomic DNA purification

DNA was purified from *E. coli* and *S. aureus* isolates along with used reference strains using a genomic DNA purification QIAamp kit (Qiagen, Germany) according to the manufacturer's recommendations. The used reference strains for *E. coli* were: *E. coli* O157:H7 Sakai (positive for *stx1, stx2, eaeA,* and *hylA* genes) and *E. coli* K12 DH5α (a non-pathogenic negative control strain). Whereas enterotoxigenic *S. aureus* strains ATCC 13565 (positive for *sea* gene), ATCC 14458 (positive for *seb* gene), ATCC 19095 (positive for *sec* gene), ATCC 23235 (positive for *sed* gene), 95-S-739 (positive for *mecA* gene) were used as positive controls for *S. aureus* molecular profiling, and *S. xylosus* ATCC 29971 was used as a negative control.

Molecular shiga toxin profiling and *eaeA* gene in *E. coli* isolates

The multiplexed-PCR technique was used for molecular profiling of *E. coli* isolates through amplification of shiga toxin-encoding genes; *stx1*, *stx2* along with intimin-encoding gene (*eaeA*). The PCR reaction was performed using primers listed in (Table 1) in a Thermal Cycler (Master Cycler, Eppendorf, Hamburg, Germany). Approximately 50 ng of bacterial DNA was added to 12.5 µl DreamTaq Green PCR Master Mix (2X) (Thermo), 0.5 µl (5 pmol) of each primer and the final volume was adjusted to 25 µl by adding sterile ultrapure water. The amplification conditions started by initial denaturation for 3 min at 95°C followed by 35 cycles of 95°C for 20 s, 58°C for 40 s, and 72°C for 90 s. The final cycle was followed by 72°C final extension for 5 min. The amplified DNA fragments were separated by 1.5% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer and captured as well as visualized on a UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine each amplicon size and strains; *E. coli* O157:H7 Sakai and *E. coli* K12 DH5-α were used as a positive and negative control, respectively.

Molecular enterotoxin profiling and *mecA* gene in *S. aureus*

The genotypic profile of *S. aureus* isolates was generated based on the presence of *sea*, *seb*, *sec* and *sed* SE-encoding genes using multiplexed PCR along with conventional PCR for *mecA* gene amplification. PCR conditions used in *E. coli* molecular profiling were adapted by changing the annealing temperature to 50°C for 1 min and 56°C for 30 s for multiplexed and conventional PCR, respectively. *S. aureus* strains ATCC 13565, ATCC 14458, ATCC 19095, ATCC 23235 and 95-S-739 were used as positive controls for *sea, seb, sec, sed* and *mecA* genes, respectively and *S. xylosus* ATCC 29971 was used as a negative control. Sequences of the used primers are listed in (Table 2).

RESULTS

Prevalence of *E. coli* and *S. aureus* contaminants across milk samples

In the current study, a total of 21 (7.8%) *E. coli* isolates were identified in particular, from raw and pasteurized 3MSL milk samples (Figure 1 and Table S1B). At the other side, raw and pasteurized 6MSL milk samples were the main sources of *S. aureus* isolates (14 isolates, accounting for 5.2% of the total milk samples) (Table S1C). Interestingly, pasteurized 7DSL and organic 6MSL samples exhibited negative bacterial growth (Figure 1).

Antibiotic susceptibility testing

Findings revealed the resistance of all *E. coli* isolates to

Table 1. Primers used for molecular profiling of *E. coli* isolates.

Primer	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
stx1 (F)	5′ACACTGGATGATCTCAGTGG′3	614	Olowe et al. (2014)
stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3		
stx2 (F)	5′CCATGACAACGGACAGCAGTT′3	779	
stx2 (R)	5′CCTGTCAACTGAGCAGCACTTTG′3		
eaeA (F)	5′ GTGGCGAATACTGGCGAGACT ′3	890	Kargar and Homayoon (2015)
eaeA (R)	5′ CCCCATTCTTTTTCACCGTCG ′3		

Table 2. Primers used for molecular profiling of *S. aureus* isolates.

Primer	Oligonucleotide sequence (5' → 3′)	Product size (bp)	Reference
sea (F)	5′ TTGGAAACGGTTAAAACGAA′3	120	Rall et al. (2008)
sea (R)	5′ GAACCTTCCCATCAAAAACA ′3		
seb (F)	5′ TCGCATCAAACTGACAAACG ′3	478	
seb (R)	5′ GCGGTACTCTATAAGTGCC ′3		
sec (F)	5′ GACATAAAAGCTAGGAATTT ′3	257	
sec (R)	5′ AAATCGGATTAACATTATCC ′3		
sed (F)	5′ CTAGTTTGGTAATATCTCCT ′3	317	
sed (R)	5′ TAATGCTATATCTTATAGGG ′3		
mecA (F)	5′ TAGAAATGACTGAAC GTCCG ′3	533	
mecA (R)	5′ TTGCGATCA ATGTTACCGTAG ′3		

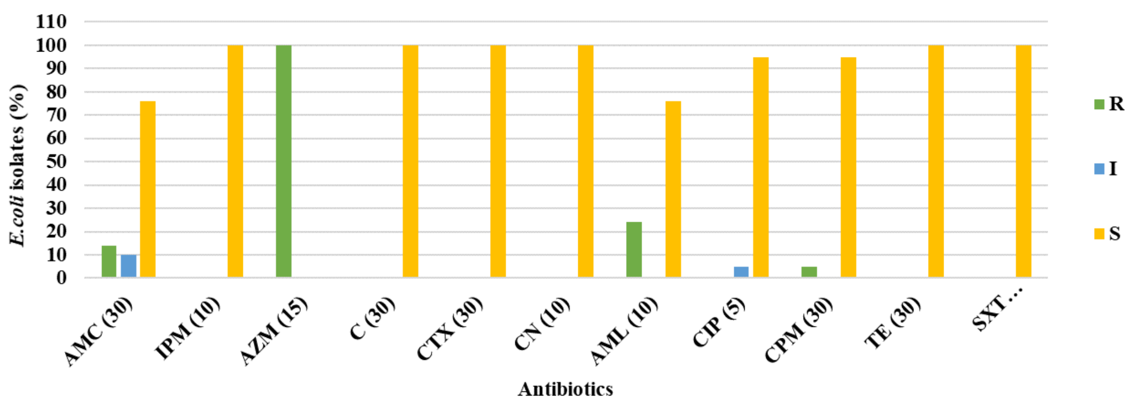

Figure 2. Antibiotic susceptibility patterns of *E. coli* isolates. R: Resistant; I: Intermediate; S: sensitive.

erythromycin (100%) whereas 24 and 14% of the total *E. coli* isolates exhibited resistance to amoxicillin and amoxicillin/clavulanic acid, respectively. Of note, all *E. coli* isolates were susceptible to imipenem, chloramphenicol, gentamicin, cefotaxime, tetracycline, and sulfamethoxazole (Figure 2). Similarly, all *S. aureus* isolates showed resistance to penicillin followed by far behind cefoxitin (50%) and sulfamethoxazole (29%). Meanwhile, vancomycin and ciprofloxacin inhibited the growth of all *S. aureus* isolates (Figure 3).

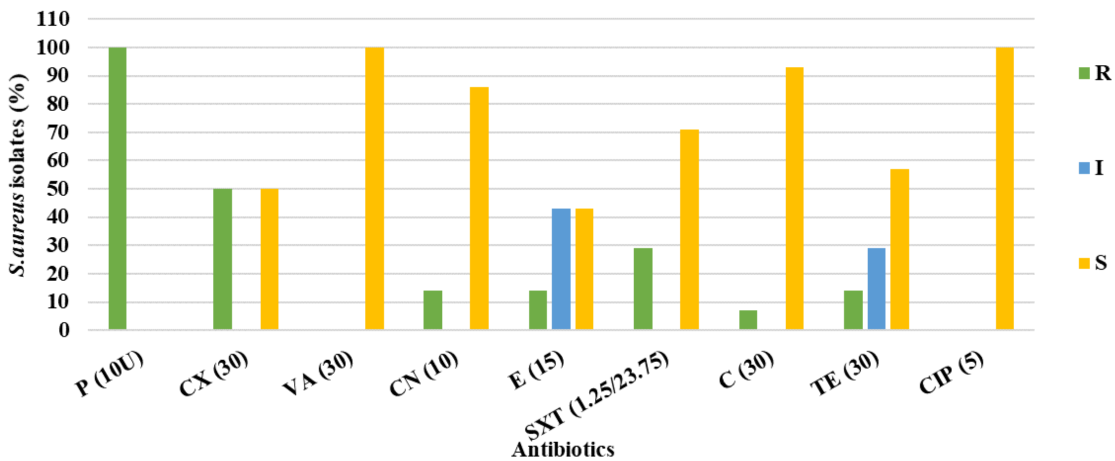

Figure 3. Antibiotic susceptibility patterns of *S. aureus* isolates. R: Resistant; I: Intermediate; S: sensitive.

Serotyping of *E. coli* and *S. aureus* isolates

The serological typing of *E. coli* isolates showed that EHEC was the most dominant pathotype accounting for 62% (13 out of 21 isolates), followed by far behind ETEC (19%, 4 isolates), EPEC (14%, 3 isolates), and EIEC (5%, 1 isolate) (Table 3). Interestingly, O26, O128, and O111 were the most prevalent serogroups identified in 29, 19 and 14% of the isolates, respectively. With respect to staphylococcal enterotoxin production, RPLA assay showed that 3 out of 14 isolates (21.4%) produced different SE listed in (Table 4).

Molecular profiling of *E. coli* and *S. aureus* isolates

The molecular profiling of *E. coli* isolates showed positive results for the presence of *stx1, stx2, eaeA* genes accounting for 90.5% (19 out of 21) of total *E. coli* isolates, and spanning different sources of milk samples (Table). However, *stx1*, *stx2*, *eaeA* genes were amplified altogether in 31.6% (6 out of 19) of *E. coli* isolates. Of note, these 6 isolates were purified from raw milk and 3MSL pasteurized milk (Figure 4 (A and B) and Table 3). Interestingly, 35.7% (5 out of 14) of *S. aureus* isolates exhibited positive PCR products for *mecA* gene, exclusively collected from raw milk (Figure 4) (C and D) and (Table 4). Only 3 *S. aureus* isolates showed positive results for the tested SE-encoding genes with an exception for *sed* gene (Table 3).

DISCUSSION

Bacterial contamination of milk may originate from diverse sources mainly; infected udders and unhygienic practices during the milking process. Of the major bacterial contaminants of milk; *E. coli* and *S. aureus* that are responsible for serious food-poisoning outbreaks worldwide (Vahedi et al., 2013). In the current study, 270 milk samples including raw and pasteurized milk of different shelf life durations (Figure 1) were tested for the presence of *E. coli* and *S. aureus* contaminants. Interestingly, 11% (11 out of 100) of raw milk samples were the source of approximately half of the identified *E. coli* isolates (11 out of 21 *E. coli* isolates). This percentage was significantly lower than previously published reports from Iran and Egypt, where *E. coli* was identified from 42% (Vahedi et al., 2013), 33% (Hassan et al., 2015) and 60% (Kandil et al.,2018) of tested milk samples. 36.4% (4 out of 11 isolates) of identified *E. coli* isolates from raw milk originated from 8% (4 out of 50 samples) of raw cow milk (Figure 1). Similarly, cultivated raw buffalo milk samples resulted in the isolation of 14% (7 out of 50) of *E. coli* isolates which is a lower rate compared to previously published studies (Ranjbar et al., 2018). These findings indicated a relatively good bacteriological quality of raw milk in El-Beheira area when compared to previous studies (Bali et al., 2013; Garedew et al., 2012; Disassa et al., 2017; Reta et al., 2016). With regard to pasteurized milk, 5.9 % of tested samples resulted in the isolation of 10 *E. coli* isolates (9 out of 50 samples (18%) from 3MSL, and 1 out of 50 samples (2%) from 6MSL milk samples). Contrarily, in other published work (Kandil et al., 2018; Hassan et al., 2015; Garedew et al., 2012), none of the pasteurized/sterile milk samples was reported for in vitro bacterial growth of *E. coli.*

The incidence of *S. aureus* in milk is increasingly ubiquitous as a result of the widespread of various

Table 3. Summary of the serological identification and molecular profiling along with the antibiotic resistance patterns of *E. coli* isolates.

Sample source	Sample code	Serotyping characterization	Serodiagnosis	Molecular profiling	Antibiotic failed to inhibit bacterial growth
Raw buffalo milk (n = 7)	EB15	EIEC	O124	-	E, AMC, AML
	EB16	EHEC	O121:H7	*stx2*	E
	EB23	EHEC	O111:H2	*stx1,eaeA*	E, AML, CPM
	EB25	EHEC	O26:H11	*stx1,stx2*	E
	EB26	EHEC	O26:H11	*stx1,stx2,eaeA*	E
	EB24	EPEC	O146:H21	*stx2*	E
	EB39	EPEC	O15:H2	*stx2*	E, AML
Raw cow milk (n = 4)	EC27	EHEC	O121:H7	*stx2*	E
	EC29	EHEC	O111:H2	*stx1,stx2,eaeA*	E
	EC30	ETEC	O128:H2	*stx1*	E
	EC32	ETEC	O128:H2	*stx1*	E, AML
Pasteurized 3MSL milk (n = 9)	ET2	EHEC	O91:H21	*stx1, stx2*	E
	ET4	ETEC	O128:H2	*stx1*	E
	ET5	EPEC	O119:H6	-	E
	ET6	EHEC	O26:H11	*stx1,stx2,eaeA*	E
	ET7	EHEC	O111:H2	*stx1,stx2,eaeA*	E
	ET33	EHEC	O26:H11	*stx1,stx2,eaeA*	E
	ET35	EHEC	O91:H21	*stx1, stx2*	E, AMC, AML
	ET37	EHEC	O26:H11	*stx2,eaeA*	E
	ET38	EHEC	O26:H11	*stx1,stx2,eaeA*	E
Pasteurized 6MSL milk	ES41	ETEC	O128:H2	*stx1*	E

EHEC: Enterohaemorrhagic *E. coli*, ETEC: Enerotoxigenic *E. coli*; EPEC: Enteropathogenic *E. coli*; EIEC: Enteroinvasive *E. coli.*

Table 4. Summary of the serological identification and molecular profiling along with the antibiotic resistance patterns of *S. aureus* isolates.

Sample source	Sample code	Serotyping characterization	Molecular profiling	Antibiotic failed to inhibit bacterial growth
Raw cow milk	SC93	SEC	*sec*	P, CX, SXT, C
	SC118	SEA	*sea, mecA*	P, E, SXT, TE
	SC95	-	-	P, CX, SXT, TE
	SC75	-	-	P, CN
	SC55	-	-	P
Raw buffalo milk	SB57	SEA, SEB	*sea, seb, mecA*	P, CX
	SB119	-	-	P, CX, SXT
	SB61	-	-	P, CX, CN
	SB81	-	*mecA*	P, CX
	SB113	-	*mecA*	P, CX
	SB48	-	-	P
	SB67	-	*mecA*	P
	SB68	-	-	P
Pasteurized 6 MSL milk	SS94	-	-	P, E

SEA: Staphylococcal enterotoxin A; SEB: Staphylococcal enterotoxin B; SEC: Staphylococcal enterotoxin C.

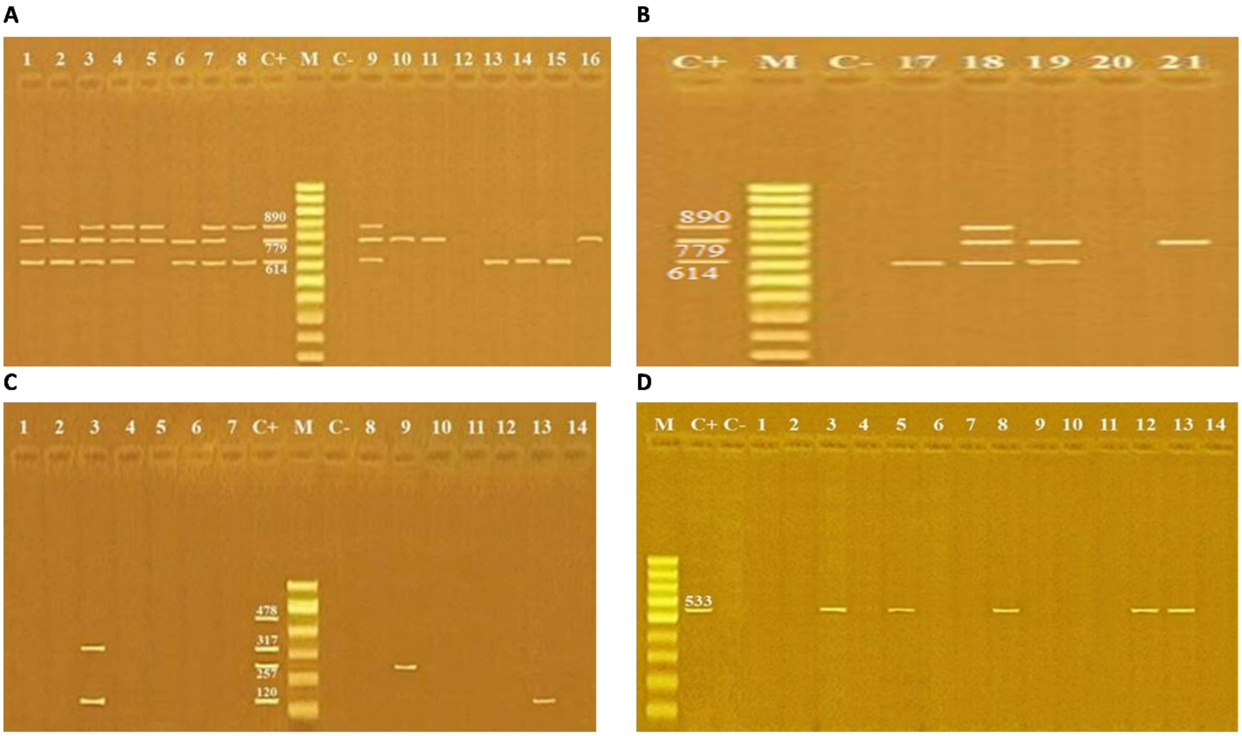

Figure 4. Molecular profiles of bacterial contaminants associated with tested milk samples: panel A and B show the multiplexed PCR profile of identified *E. coli* isolates for the presence of *stx1*(614 bp), *stx2* (779 bp) and *eaeA* (890 bp) genes, panel C shows the multiplexed PCR profile of identified *S. aureus* isolates for the presence of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp) and *sed* (317 bp) genes, and panel D shows the PCR profile of identified *S. aureus* isolates for the presence of *mecA* gene (533 bp).

pathogenicity factors including; toxin-mediated virulence, invasiveness, and antibiotic resistance (Kadariya et al., 2014). 14 *S. aureus* isolates from all tested milk samples were biochemically identified. Approximately, 93% (13 out of 14 isolates) of *S. aureus* isolates were cultivated from raw milk (100 samples) accounting for 13% of the tested samples (Figure 1). The results came in accordance with those obtained by Zeinhom et al. (2015) and Mansour et al. (2017) that reported 12 and 16.3% of tested raw milk samples were contaminated with *S. aureus*, respectively. However, moderate and high contamination levels were also reported worldwide indicating the crucial importance of livestock health combined with the hygienic practices of milking on the safety of the dairy industry. For instance, a study from Egypt recorded the highest contamination incidence rates of raw milk with *S. aureus* accounting for 80% of the tested samples (Kandil et al., 2018). Interestingly, 10% (5 out of 50 samples) of the raw cow milk samples were contaminated with *S. aureus* that is comparatively lower than a previous report (24.2%) from Reta et al. (2016) in Ethiopia. However, only 0.6% (1 out of 170 samples) of pasteurized milk (6MSL milk) was contaminated with *S. aureus*. This result is consistent with a report published by Kandil et al. (2018) where *S. aureus* had zero existence in pasteurized milk samples in Egypt. In contrast, a higher contamination rate (14.92%) had been reported in Algeria (Matallah et al., 2019).

Globally, the unsupervised use of antimicrobial agents in the treatment of animal and human infections have been contributed to the emergence of antimicrobial resistance (Van Boeckel et al., 2015). The antimicrobial resistance mainly originates from the transfer of resistance genes across microbes enabling them to survive in the presence of antimicrobial agents that eventually resulted in failure of antibiotic therapeutic protocols (Blair et al., 2015). Furthermore, the overuse of antibiotics in animal husbandry as growth promoters could be a potential source of bacterial resistance through dissemination of resistant microbes from intestinal microbiotas of livestock that contaminate the surrounding environment and enhance the transmission of resistant genes to autochthonous bacteria (resident microbes) of the surface water systems (McEwen and

Collignon, 2018). In this study, all *E. coli* isolates exhibited susceptibility to tetracycline, ciprofloxacin, sulfamethoxazole and chloramphenicol (except for one isolate that was resistant to ciprofloxacin) (Figure 2) which disagreed with reports published by Nobili et al. (2016), Schroeder et al. (2002), Mora et al. (2005), Abebe et al. (2014) and Ranjbar et al. (2018). However, the results reported by Tadesse et al. (2018) were relatively similar to our study where the *in vitro* growth *E. coli* was restrained by gentamicin, ciprofloxacin, and tetracycline. Of note, erythromycin inhibited the growth of all *E. coli* isolates, whereas Tadesse et al. (2018) reported a considerably moderate percentage (60%) of erythromycin resistance. Interestingly, only 14% of *E. coli* isolates were resistant to amoxicillin-clavulanic acid, while Nobili et al. (2016) reported a significantly higher percentage (100%). Furthermore, all *E. coli* isolates exhibited sensitivity to tested sulfa-drug antibiotic that disagreed with reports from Tadesse et al. (2018) and Nobili et al. (2016) where the susceptibility levels were 40 and 50%, respectively. Regarding the antibiotic resistance patterns of *S. aureus*, the isolates exhibited resistant to penicillin, cefoxitin, sulfamethoxazole, tetracycline, gentamicin, and erythromycin (Figure 3) which concurred with the findings published by Hoque et al. (2018) and Reta et al. (2016). Interestingly, 29% of *S. aureus* isolates showed resistance to sulphamethoxazole-trimethoprim that completely agreed with Hoque et al. (2018), and spiking high when compared to those reported by Reta et al. (2016) and Umaru et al. (2013). Despite previous studies, Umaru et al. (2013) and Reta et al. (2016) reported variable sensitivity rates (44.3 and 6.9%, respectively) of *S. aureus* isolates to vancomycin, findings showed absolute susceptibility of all tested isolates to it. Similarly, all *S. aureus* isolates were susceptible to ciprofloxacin that disagreed with findings reported by Hoque et al. (2018) and Zeinhom et al. (2015).

Enterohemorrhagic *Escherichia coli* (EHEC) strains comprise a subgroup of Shiga-toxin (ST)-producing *E. coli* (STEC) and are the most frequently implicated in severe clinical illness worldwide (Vendramin et al., 2014). In this study, we found that 62% of *E. coli* isolates were serologically identified as EHEC (Table 3), known to cause outbreaks of bloody diarrhea. This percentage is higher than Vanitha et al. (2018), Vendramin et al. (2014), Momtaz et al. (2012) and Ranjbar et al. (2018). Interestingly, the molecular profiling showed that 90% (19 out of 21 isolates) of *E. coli* isolates were positive for *stx* genes, whereas 42.8% (9 out of 21 isolates) of them were positive for both *stx1* and *stx2* genes (Figure 4 and Table 3). However, these results were higher than that reported in previous studies (Tabaran et al., 2017; Nobili et al., 2016; Neher et al., 2015; Virpari et al., 2013) (Figure 4 and Table 3). Furthermore, 38% (8 out of 21 isolates) of *E. coli* isolates harbored *eaeA* gene and serotypically characterized as EHEC including O26 and O111 serogroup (Figure 4 and Table 3). These results were congruent with previously published studies (Momtaz et al., 2012; and Vanitha et al., 2018) where 33.33 and 36% of identified *E. coli* isolates were positive for *eaeA* gene, respectively. Contrarily, in a study conducted by Nobili et al. (2016), all STEC isolates exhibited negative results for *eaeA* gene.

S. aureus isolates are able to produce enterotoxins posing a public health threat. This means that the detection of SE in milk is very crucial for the bacteriological assessment of milk and dairy products (Wu et al., 2016). In the current study, the molecular detection of SE-encoding genes was greatly helpful for proper characterization of SE-producing *S. aureus*. In general, multiplex PCR detection could infer the presence of genes but does not consider their expression. Therefore, RPLA technique is needed to emphasize the SE production (van Belkum, 2003). Here, SET-RPLA assay showed that 21.4% (3 out of 14 isolates) of *S. aureus* isolates produced classic enterotoxins (SEA, SEC, SED) (Figure 4 and Table 4), which is in line with results reported by Fagundes et al. (2010). Interestingly, the molecular profiling of *S. aureus* isolates for SE-encoding genes confirmed the results of SET-RPLA technique (Figure 4 and Table 4) and agreed with previously published reports (Mansour et al., 2017). In contrast, in a study performed by Rall et al. (2008), a higher prevalence rate of *S. aureus* was reported, whereas 68.4% of the *S. aureus* isolates were positive for one or more enterotoxins-encoding-genes. Of note, Arcuri et al. (2010) detected SE genes in 13.6% of mastitic cow milk and 41.7% of a bulk milk tank. In general, methicillin-resistant *S. aureus* (MRSA) strains have the ability to express multiple antibiotic resistance genes that pose a global threat to animal and human health (Shah et al., 2019). In this study, *mecA* gene was detected in approximately 36% of the total *S. aureus* isolates that indicated the potential emergence of MRSA outbreaks from consumption of contaminated raw milk in particular, in traditional societies (Figure 4 and Table 4). Noteworthy, similar percentages (22.2 and 20%) of MRSA detection in milk were reported by Umaru et al. (2013) and Hoque et al. (2018), respectively.

Conclusion

To conclude, findings revealed that raw and 3MSL pasteurized milk are most prone to be contaminated by the pathogenic *E. coli* and *S. aureus* isolates, that poses serious health issues upon direct consumption of milk from these sources. Noteworthy, pasteurized organic milk and 7DSL milk were found to be of the highest bacteriological quality when tested for the presence of *E. coli* and *S. aureus*. Eventually, our findings implicitly highlighted the importance of constituting strict regulations with regard to milk handling in local farms and dairy plants to minimize the chance of milk contamination and the transmission of bacterial pathogens along with their antimicrobial resistance from dairy animals to humans.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abebe M, Hailelule A, Abrha B, Nigus A, Birhanu M, Adane H, Genene T, Daniel H, Getachew G, Merga G, Haftay A (2014). Antibiogram of *Escherichia coli* strains isolated from food of bovine origin in selected Woredas of Tigray, Ethiopia. Journal of Bacteriology Research 6(3):17-22.

Aidar-Ugrinovich L, Blanco J, Blanco M, Blanco JE, Leomil L, Dahbi G, Mora A, Onuma DL, Silveira WD, Pestana de Castro AF (2007). Serotypes, virulence genes, and intimin types of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolated from calves in São Paulo, Brazil. International Journal of Food Microbiology 115(3):297-306.

Arcuri EF, Angelo FF, Guimarães MFM, Talon R, Borges MF, Leroy S, Loiseau G, Lange CC, Andrade NJ, Montet D (2010). Toxigenic status of *Staphylococcus aureus* isolated from bovine raw milk and Minas frescal cheese in Brazil. Journal of Food Protection 73(12):2225-2231.

Argudín MÁ, Mendoza MC, Rodicio MR (2010). Food Poisoning and *Staphylococcus aureus* Enterotoxins. Toxins 2(7):1751-1773.

Asao T, Kumeda Y, Kawai T, Shibata T, Oda H, Haruki K, Nakazawa H, Kozaki S (2003). An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. Epidemiology and Infection 130(1):33-40.

Awadallah MA, Ahmed HA, Merwad AM, Selim MA (2016). Occurrence, genotyping, shiga toxin genes and associated risk factors of *E. coli* isolated from dairy farms, handlers and milk consumers. The Veterinary Journal 217:83-88.

Bali OS, Lajnef R, Felfoul I, Attia H, Ayadi MA (2013). Detection of *Escherichia coli* in unpasteurized raw milk. International Journal of Agriculture and Food Science 3(2):53-55.

Barbano DM, Ma Y, Santos MV (2006). Influence of raw milk quality on fluid milk shelf life. Journal of Dairy Science 89:E15-E19.

Bennett SD, Walsh KA, Gould LH (2013). Foodborne Disease Outbreaks Caused by *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus*-United States, 1998-2008. Clinical Infectious Diseases 57(3):425-433.

Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ (2015). Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology 13(1):42-51.

Clinical and Laboratory Standards Institute (CLSI) (2017). Performance standards for antimicrobial disk susceptibility tests; approved standard-12th ed. M02-A13. Clinical and Laboratory Standards Institute, Wayne, PA.

De Oliveira GB, Favarin L, Luchese RH, McIntosh D (2015). Psychrotrophic bacteria in milk: How much do we really know? Brazilian Journal of Microbiology 46(2):313-321.

Deeth H (2017). Optimum Thermal Processing for Extended Shelf-Life (ESL) Milk. Foods (Basel, Switzerland) 6(11).

Desmarchelier P, Fegan N (2016). Pathogens in Milk: *Escherichia coli*. Reference Module in Food Science. https://doi.org/10.1016/B978-0-08-100596-5.00989-6

Dhanashekar R, Akkinepalli S, Nellutla A (2012). Milk-borne infections. An analysis of their potential effect on the milk industry. Germs 2(3):101-109.

Disassa N, Sibhat B, Mengistu S, Muktar Y, Belina D (2017). Prevalence and Antimicrobial Susceptibility Pattern of *E. coli O157:H7* Isolated from Traditionally Marketed Raw Cow Milk in and around Asosa Town, Western Ethiopia. Veterinary Medicine International. https://doi.org/10.1155/2017/7581531

Elgadi ZAM, Abdel Gadir WS, Dirar HA (2008). Isolation and Identification of Lactic Acid Bacteria and Yeast from Raw Milk in Khartoum State (Sudan). Research Journal of Microbiology 3(3)163-168.

Fagundes H, Barchesi L, Filho AN, Ferreira LM, Oliveira CAF (2010). Occurrence of *Staphylococcus aureus* in raw milk produced in dairy farms in São Paulo state, Brazil. Brazilian Journal of Microbiology 41(2):376-380.

Food and Agriculture Organization (FAO) (2011). World Livestock. Livestock in Food Security, Rome, Italy.

Fernandes R (2009). Liquid milk products. In: Fernandes R., Microbiology handbook dairy products. Leatherhead Food International and RSC Publishing pp. 1-19.

Garedew L, Berhanu A, Mengesha D, Tsegay G (2012). Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. BMC Public Health 12:950.

Hennekinne JA, De Buyser ML, Dragacci S (2012). *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiology Reviews 36(4):815-836.

Hoque MN, Das ZC, Rahman ANMA, Haider MG, Islam MA (2018). Molecular characterization of *Staphylococcus aureus* strains in bovine mastitis milk in Bangladesh. International Journal of Veterinary Science and Medicine *6*(1):53-60.

Kadariya J, Smith TC, Thapaliya D (2014). *Staphylococcus aureus* and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. BioMed Research International pp. 1-9.

Kandil AA, Elhadidy M, El-Gamal A, Al-Ashmawy MA (2018). Identification of *S. aureus* and *E. coli* from Dairy Products Intended for Human Consumption. Advances in Animal and Veterinary Sciences 6(11):509-513.

Kaper JB, Nataro JP, Mobley HLT (2004). Pathogenic *Escherichia coli*, Nature reviews, Microbiology 2(2):123-40.

Kargar M, Homayoon M (2015). Prevalence of shiga toxins (stx1, stx2), eaeA and hly genes of *Escherichia coli O157:H7* strains among children with acute gastroenteritis in southern of Iran. Asian Pacific Journal of Tropical Medicine 8(1):24-28.

Kreig N, Holt J (1984). Bergey's Manual of systemic bacteriology (1) Williams and Wilkins, Baltimore M.D.21202, USA.

Lachica RVF, Genigeorgis C, Hoeprich PD (1971). Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Applied Microbiology 21(4):585-587.

Lewis SJ, Gilmour A (1987). Microflora associated with the internal surfaces of rubber and stainless steel milk transfer pipeline. Journal of Applied Bacteriology 62(4):327-333.

Hassan GM, Meshref AMS, Gomaa SM (2015). Microbiological Quality and Safety of Fluid Milk Marketed in Cairo and Giza Governorates. Current Research in Dairy Sciences 7(1):18-25.

MacFaddin JF (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd Edition, Lippincott Williams & Wilkins, Philadelphia.

Mallet A, Guéguen M, Kauffmann F, Chesneau C, Sesboué A, Desmasures N (2012). Quantitative and qualitative microbial analysis of raw milk reveals substantial diversity influenced by herd management practices. International Dairy Journal 27(1-2):13-21.

Mansour AS, Wagih GE, Morgan SD, Elhariri M, El-shabrawy MA, Abuelnaga ASM, Elgabry EA (2017). Detection of Staphylococcus aureus enterotoxigenic strains in bovine raw milk by reversed passive latex agglutination and multiplex polymerase chain reaction. Veterinary World 10(8):843-847.

Matallah AM, Bouayad L, Boudjellaba S, Mebkhout F, Hamdi TM, Ramdani-Bouguessa N (2019). *Staphylococcus aureus* isolated from selected dairies of Algeria: Prevalence and susceptibility to antibiotics. Laboratory of Medical Biology 12(2):205-210.

McEwen SA, Collignon PJ (2018). Antimicrobial Resistance: a One Health Perspective. Microbiology Spectrum 6(2).

Miller JH (1992). A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for *Escherichia coli* and related bacteria. Plainview, NY: Cold Spring Harbor Laboratory Press; 1992.

Momtaz H, Farzan R, Rahimi E, Safarpoor Dehkordi F, Souod N (2012). Molecular characterization of Shiga toxin-producing *Escherichia coli* isolated from ruminant and donkey raw milk samples and traditional dairy products in Iran. The Scientific World Journal 2012:231342.

Mora A, Blanco JE, Blanco M, Alonso MP, Dhabi G, Echeita A, González EA, Bernárdez MI, Blanco J (2005). Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Research in Microbiology 156(7):793-806.

Morandi S, Brasca M, Lodi R, Cremonesi P, Castiglioni B (2007). Detection of classical enterotoxins and identification of enterotoxin genes in *Staphylococcus aureus* from milk and dairy products. Veterinary Microbiology 124(1-2):66-72.

Murphy SC, Martin NH, Barbano DM, Wiedmann M (2016). Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? Journal of Dairy Science 99(12):10128-10149.

Nataro JP, Kaper JB (1998). Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews 11(1):142-201.

Neher S, Hazarika AK, Sharma RK, Barkalita LM, Bora M, Deka P (2015). Detection of Shiga-Toxigenic *Escherichia Coli* in Milk Samples of Cattle by PCR. Journal of Agriculture and Veterinary Science 8(5):75-78.

Nobili G, Franconieri I, Basanisi MG, La Bella G, Tozzoli R, Caprioli A, La Salandra G (2016). Short communication : Isolation of Shiga toxin-producing *Escherichia coli* in raw milk and mozzarella cheese in southern Italy. Journal of Dairy Science 99(10):7877-7880.

Olowe OA, Aboderin BW, Idris OO, Mabayoje VO, Opaleye OO, Adekunle OC, Olowe RA, Akinduti PA, Ojurongbe O (2014). Genotypes and phenotypes of Shiga toxin-producing *Escherichia coli* (STEC) in Abeokuta, Southwestern Nigeria. Infection and Drug Resistance 7:253-259.

Ombarak RA, Zayda MG, Hinenoya A, Yamasaki S (2019). Serotypes, pathogenic potential and antimicrobial resistance of *Escherichia coli* isolated from subclinical bovine mastitis milk samples in Egypt. Japanese Journal of Infectious Diseases 72(5):337-339.

Park CE, Szabo R (1986). Evaluation of the reversed passive latex agglutination (RPLA) test kits for detection of staphylococcal enterotoxins A, B, C, and D in foods. Canadian Journal of Microbiology 32(9):723-727.

Postollec F, Falentin H, Pavan S, Combrisson J, Sohier D (2011). Recent advances in quantitative PCR (qPCR) applications in food microbiology. Food Microbiology 28(5):848-861.

Quigley L, McCarthy R, O'Sullivan O, Beresford TP, Fitzgerald GF, Ross RP, Stanton C, Cotter PD (2013a). The microbial content of raw and pasteurized cow milk as determined by molecular approaches. Journal of Dairy Science 96(8):4928-4937.

Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF, Cotter PD (2013b). The complex microbiota of raw milk. FEMS Microbiology Reviews 37(5):664-698.

Rais M, Acharya S, Sharma N (2013). Food processing industry in India: S&T capability, skills and employment opportunities. Journal of Rural Development 4(9):1-13.

Rall VLM, Vieira FP, Rall R, Vieitis RL, Fernandes A, Candeias JMG, Cardoso KF, Araújo JP (2008). PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. Veterinary Microbiology 132(3-4):408-413.

Ranjbar R, Dehkordi FS, Shahreza MHS, Rahimi E (2018). Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of shiga-toxin producing *Escherichia coli* strains isolated from raw milk and traditional dairy products. Antimicrobial resistance and infection control 7(53).

Reta MA, Bereda TW, Alemu AN (2016). Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. International Journal of Food Contamination 3(1):4.

Sarkar S (2015). Microbiological Considerations: Pasteurized Milk. International Journal of Dairy Science 10:206-218.

Schroeder CM, Meng J, Zhao S, Debroy C, Torcolini J, Zhao C, McDermott PF, Wagner DD, Walker RD, White DG (2002). Antimicrobial resistance of *Escherichia coli* O26, O103, O111, O128, and O145 from animals and humans. Emerging infectious diseases 8(12):1409-1414.

Shah MS, Qureshi S, Kashoo Z, Farooq S, Wani SA, Hussain MI, Banday MS, Khan AA, Gull B, Habib A, Khan SM, Dar BA (2019). Methicillin resistance genes and in vitro biofilm formation among *Staphylococcus aureus* isolates from bovine mastitis in India. Comparative Immunology, Microbiology and Infectious Diseases 64:117-124.

Tabaran A, Mihaiu M, Flaviu T, Colobatiu L, Reget O, Borzan MM, Dan SD (2017). First study on characterization of virulence and antibiotic resistance genes in verotoxigenic and enterotoxigenic *E . coli* isolated from raw milk and unpasteurized traditional cheeses in Romania. Folia Microbiologica 62(2):145-150.

Tadesse HA, Gidey NB, Workelule K, Hailu H, Gidey S, Bsrat A, Taddele H (2018). Antimicrobial Resistance Profile of *E . coli* Isolated from Raw Cow Milk and Fresh Fruit Juice in Mekelle, Tigray, Ethiopia. Vetrinary Medicine International. https://doi.org/10.1155/2018/8903142

Tirado C, Schmidt K (2001). WHO Surveillance Programme for Control of Foodborne Infections and Intoxications: Preliminary Results and Trends Across Greater Europe. Journal of Infection 43(1):80-84.

Umaru AG, Kabir J, Umoh VJ, Bello M, Kwaga JKP (2013). Methicillin-resistant *staphylococcus aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. International Journal of Drug Research and Technology 3(3):67-75.

Vahedi M, Nasrolahei M, Sharif M, Mirabi AM (2013). Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011. Journal of preventive medicine and hygiene 54(2):120-123.

Van Belkum A (2003). Molecular diagnostics in medical microbiology: yesterday, today and tomorrow. Current Opinion in Pharmacology 3(5):497-501.

Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R (2015). Global trends in antimicrobial use in food animals. Proceedings of the National Academy of Sciences of the United States of America 112(18):5649-5654.

Vanitha HD, Sethulekshmi C, Latha C (2018). An epidemiological investigation on occurrence of enterohemorrhagic *Escherichia coli* in raw milk. Veterinary World 11(8):1164-1170.

Vendramin T, Kich DM, Molina RD, Souza CFVde, Salvatori RU, Pozzobon A, Bustamante-filho IC (2014). Molecular screening of bovine raw milk for the presence of Shiga toxin-producing *Escherichia coli* (STEC) on dairy farms. Food Science and

Technology (Campinas) 34(3):604-608.

Virpari PK, Nayak JB, Brahmbhatt MN, Thaker HC (2013). Study on isolation, molecular detection of virulence gene and antibiotic sensitivity pattern of *Escherichia coli* isolated from milk and milk products. Veterinary World 6(8):541-545.

Vithanage NR, Dissanayake M, Bolge G, Palombo EA, Yeager TR, Datta N (2016). Biodiversity of culturable psychrotrophic microbiota in raw milk attributable to refrigeration conditions, seasonality and their spoilage potential. International Dairy Journal 57:80-90.

Vranješ AP, Popović M, Jevtić M (2015). Raw milk consumption and health. Srpski Arhiv Za Celokupno Lekarstvo 143(1-2):87-92.

Wu S, Duan N, Gu H, Hao L, Ye H, Gong W, Wang Z (2016). A Review of the Methods for Detection of *Staphylococcus aureus* Enterotoxins. Toxins 8(7).

Zeinhom MMA, Abdel-Latef GK (2014). Public health risk of some milk borne pathogens. Beni-Suef University Journal of Basic and Applied Sciences 3(3):209-215.

Zeinhom MMA, Abdel-Latef GK, Jordan K (2015). The Use of Multiplex PCR to Determine the Prevalence of Enterotoxigenic *Staphylococcus aureus* isolated from Raw Milk, Feta Cheese, and Hand Swabs. Journal of Food Science 80(12):M2932-M2936.

Table S1A. Primary microbiological testing of milk samples for the presence of *E.coli and S.aureus*

Sample code	Sample source	Pasteurization technique	*E.coli*		*S.aureus*	
			Macconkey	EMB	Mannitol salt	BP
B1	Buffalo milk	Raw (Non pasteurized)	LF	+	+	-
B2	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B3	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B4	Buffalo milk	Raw (Non pasteurized)	LNF	-	+	+
B5	Buffalo milk	Raw (Non pasteurized)	LNF	-	+	+
B6	Buffalo milk	Raw (Non pasteurized)	LNF	-	+	-
B7	Buffalo milk	Raw (Non pasteurized)	LF	+	+	-
B8	Buffalo milk	Raw (Non pasteurized)	LF	+	+	-
B9	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B10	Buffalo milk	Raw (Non pasteurized)	LF	+	+	-
B11	Buffalo milk	Raw (Non pasteurized)	LF	+	+	-
B12	Buffalo milk	Raw (Non pasteurized)	-	-	+	+
B13	Buffalo milk	Raw (Non pasteurized)	LF	+	+	+
B14	Buffalo milk	Raw (Non pasteurized)	LF	+	+	+
B15	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B16	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B17	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B18	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B19	Buffalo milk	Raw (Non pasteurized)	LF	-	-	ND
B20	Buffalo milk	Raw (Non pasteurized)	LF	+	+	+
B21	Buffalo milk	Raw (Non pasteurized)	-	-	-	ND
B22	Buffalo milk	Raw (Non pasteurized)	LF	+	+	-
B23	Buffalo milk	Raw (Non pasteurized)	LNF	-	NA	-
B24	Buffalo milk	Raw (Non pasteurized)	LF	+	+	+
B25	Buffalo milk	Raw (Non pasteurized)	LF	-	NG	ND
B26	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B27	Buffalo milk	Raw (Non pasteurized)	LF	-	NA	-
B28	Buffalo milk	Raw (Non pasteurized)	LF	-	+	-
B29	Buffalo milk	Raw (Non pasteurized)	LF	-	+	-
B30	Buffalo milk	Raw (Non pasteurized)	LF	-	NA	-
B31	Buffalo milk	Raw (Non pasteurized)	LF	+	+	-
B32	Buffalo milk	Raw (Non pasteurized)	LF	-	NG	ND

Table S1A. contd.

B33	Buffalo milk	Raw (Non pasteurized)	LF	-	-	-
B34	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B35	Buffalo milk	Raw (Non pasteurized)	LF	-	-	-
B36	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B37	Buffalo milk	Raw (Non pasteurized)	LF	-	+	+
B38	Buffalo milk	Raw (Non pasteurized)	LF	-	+	-
B39	Buffalo milk	Raw (Non pasteurized)	LF	-	NA	-
B40	Buffalo milk	Raw (Non pasteurized)	LF	-	-	-
B41	Buffalo milk	Raw (Non pasteurized)	LF	+	+	+
B42	Buffalo milk	Raw (Non pasteurized)	LF	+	+	+
B43	Buffalo milk	Raw (Non pasteurized)	LNF	-	-	-
B44	Buffalo milk	Raw (Non pasteurized)	LNF	-	NA	-
B45	Buffalo milk	Raw (Non pasteurized)	LNF	-	-	-
B46	Buffalo milk	Raw (Non pasteurized)	LNF	-	+	+
B47	Buffalo milk	Raw (Non pasteurized)	LF	+	+	+
B48	Buffalo milk	Raw (Non pasteurized)	LF	+	-	-
B49	Buffalo milk	Raw (Non pasteurized)	LF	-	NA	-
B50	Buffalo milk	Raw (Non pasteurized)	LF	-	-	-
C1	Cow milk	Raw (Non pasteurized)	LNF	-	+	-
C2	Cow milk	Raw (Non pasteurized)	LNF	-	NA	-
C3	Cow milk	Raw (Non pasteurized)	LNF	-	-	-
C4	Cow milk	Raw (Non pasteurized)	-	-	+	-
C5	Cow milk	Raw (Non pasteurized)	LNF	-	-	-
C6	Cow milk	Raw (Non pasteurized)	LNF	-	NA	-
C7	Cow milk	Raw (Non pasteurized)	-	-	+	+
C8	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C9	Cow milk	Raw (Non pasteurized)	-	-	+	-
C10	Cow milk	Raw (Non pasteurized)	LF	+	+	-
C11	Cow milk	Raw (Non pasteurized)	LF	-	+	-
C12	Cow milk	Raw (Non pasteurized)	-	-	NA	-
C13	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C14	Cow milk	Raw (Non pasteurized)	LF	+	+	-
C15	Cow milk	Raw (Non pasteurized)	LF	+	+	-
C16	Cow milk	Raw (Non pasteurized)	LNF	-	+	-

Table S1A. contd.

C17	Cow milk	Raw (Non pasteurized)	LF	+	+	-
C18	Cow milk	Raw (Non pasteurized)	LNF	-	+	-
C19	Cow milk	Raw (Non pasteurized)	LNF	-	+	-
C20	Cow milk	Raw (Non pasteurized)	-	-	+	-
C21	Cow milk	Raw (Non pasteurized)	LF	+	+	-
C22	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C23	Cow milk	Raw (Non pasteurized)	LF	+	+	-
C24	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C25	Cow milk	Raw (Non pasteurized)	-	-	+	-
C26	Cow milk	Raw (Non pasteurized)	-	-	+	-
C27	Cow milk	Raw (Non pasteurized)	LNF	-	+	-
C28	Cow milk	Raw (Non pasteurized)	LNF	-	+	-
C29	Cow milk	Raw (Non pasteurized)	-	-	+	-
C30	Cow milk	Raw (Non pasteurized)	-	-	NA	-
C31	Cow milk	Raw (Non pasteurized)	LNF	-	NG	ND
C32	Cow milk	Raw (Non pasteurized)	LNF	-	NG	ND
C33	Cow milk	Raw (Non pasteurized)	LNF	-	NA	-
C34	Cow milk	Raw (Non pasteurized)	LNF	-	NG	ND
C35	Cow milk	Raw (Non pasteurized)	LNF	-	NA	-
C36	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C37	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C38	Cow milk	Raw (Non pasteurized)	-	-	+	+
C39	Cow milk	Raw (Non pasteurized)	-	-	+	+
C40	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C41	Cow milk	Raw (Non pasteurized)	LF	+	+	+
C42	Cow milk	Raw (Non pasteurized)	LNF	-	+	-
C43	Cow milk	Raw (Non pasteurized)	-	-	+	-
C44	Cow milk	Raw (Non pasteurized)	-	-	+	-
C45	Cow milk	Raw (Non pasteurized)	LNF	-	+	-
C46	Cow milk	Raw (Non pasteurized)	LF	+	+	-
C47	Cow milk	Raw (Non pasteurized)	LF	+	+	+
C48	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C49	Cow milk	Raw (Non pasteurized)	LNF	-	+	+
C50	Cow milk	Raw (Non pasteurized)	LNF	-	+	+

Table S1A. contd.

S1	Pasteurized 6MSL	UHT	LF	-	NA	-
S2	Pasteurized 6MSL	UHT	LF	-	NA	-
S3	Pasteurized 6MSL	UHT	LF	-	+	-
S4	Pasteurized 6MSL	UHT	LF	-	+	-
S5	Pasteurized 6MSL	UHT	LF	-	+	-
S6	Pasteurized 6MSL	UHT	LF	-	+	-
S7	Pasteurized 6MSL	UHT	NG	ND	+	-
S8	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S9	Pasteurized 6MSL	UHT	NG	ND	NA	-
S10	Pasteurized 6MSL	UHT	NG	ND	+	-
S11	Pasteurized 6MSL	UHT	LF	-	NG	ND
S12	Pasteurized 6MSL	UHT	LF	+	+	-
S13	Pasteurized 6MSL	UHT	LF	+	+	-
S14	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S15	Pasteurized 6MSL	UHT	NG	ND	+	+
S16	Pasteurized 6MSL	UHT	LF	-	+	+
S17	Pasteurized 6MSL	UHT	LF	-	NG	ND
S18	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S19	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S20	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S21	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S22	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S23	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S24	Pasteurized 6MSL	UHT	LNF	-	NG	ND
S25	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S26	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S27	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S28	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S29	Pasteurized 6MSL	UHT	LNF	-	NG	ND
S30	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S31	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S32	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S33	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S34	Pasteurized 6MSL	UHT	NG	ND	NG	ND

Table S1A. contd.

S35	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S36	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S37	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S38	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S39	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S40	Pasteurized 6MSL	UHT	LNF	-	NG	ND
S41	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S42	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S43	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S44	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S45	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S46	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S47	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S48	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S49	Pasteurized 6MSL	UHT	NG	ND	NG	ND
S50	Pasteurized 6MSL	UHT	NG	ND	NG	ND
T1	Pasteurized 3MSL	Sterilized	LF	+	NG	ND
T2	Pasteurized 3MSL	Sterilized	LNF	-	NG	ND
T3	Pasteurized 3MSL	Sterilized	LF	+	+	+
T4	Pasteurized 3MSL	Sterilized	NG	ND	+	+
T5	Pasteurized 3MSL	Sterilized	LF	+	NA	-
T6	Pasteurized 3MSL	Sterilized	LF	+	NG	ND
T7	Pasteurized 3MSL	Sterilized	LF	+	NA	-
T8	Pasteurized 3MSL	Sterilized	NG	ND	+	-
T9	Pasteurized 3MSL	Sterilized	NG	ND	+	-
T10	Pasteurized 3MSL	Sterilized	NG	ND	+	-
T11	Pasteurized 3MSL	Sterilized	LF	+	+	-
T12	Pasteurized 3MSL	Sterilized	LNF	-	NA	-
T13	Pasteurized 3MSL	Sterilized	NG	ND	+	-
T14	Pasteurized 3MSL	Sterilized	NG	ND	+	-
T15	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T16	Pasteurized 3MSL	Sterilized	NG	ND	+	-
T17	Pasteurized 3MSL	Sterilized	NG	ND	+	-
T18	Pasteurized 3MSL	Sterilized	LF	+	+	+

Table S1A. contd.

T19	Pasteurized 3MSL	Sterilized	LF	+	NA	-
T20	Pasteurized 3MSL	Sterilized	LF	+	NG	ND
T21	Pasteurized 3MSL	Sterilized	LF	+	+	-
T22	Pasteurized 3MSL	Sterilized	LF	+	NG	ND
T23	Pasteurized 3MSL	Sterilized	LF	+	NG	ND
T24	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T25	Pasteurized 3MSL	Sterilized	NG	ND	+	-
T26	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T27	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T28	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T29	Pasteurized 3MSL	Sterilized	LF	+	NA	-
T30	Pasteurized 3MSL	Sterilized	NG	ND	NA	-
T31	Pasteurized 3MSL	Sterilized	NG	ND	NA	-
T32	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T33	Pasteurized 3MSL	Sterilized	NG	ND	NA	-
T34	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T35	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T36	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T37	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T38	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T39	Pasteurized 3MSL	Sterilized	NG	ND	NA	-
T40	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T41	Pasteurized 3MSL	Sterilized	LNF	-	NG	ND
T42	Pasteurized 3MSL	Sterilized	LNF	-	NG	ND
T43	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T44	Pasteurized 3MSL	Sterilized	NG	ND	NA	-
T45	Pasteurized 3MSL	Sterilized	LNF	-	NA	-
T46	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T47	Pasteurized 3MSL	Sterilized	LNF	-	NG	ND
T48	Pasteurized 3MSL	Sterilized	NG	ND	NG	ND
T49	Pasteurized 3MSL	Sterilized	LNF	-	NG	ND
T50	Pasteurized 3MSL	Sterilized	LNF	-	NG	ND
D1	Pasteurized 7DSL	ultra processed	NG	ND	+	-
D2	Pasteurized 7DSL	ultra processed	NG	ND	NA	-

Table S1A. contd.

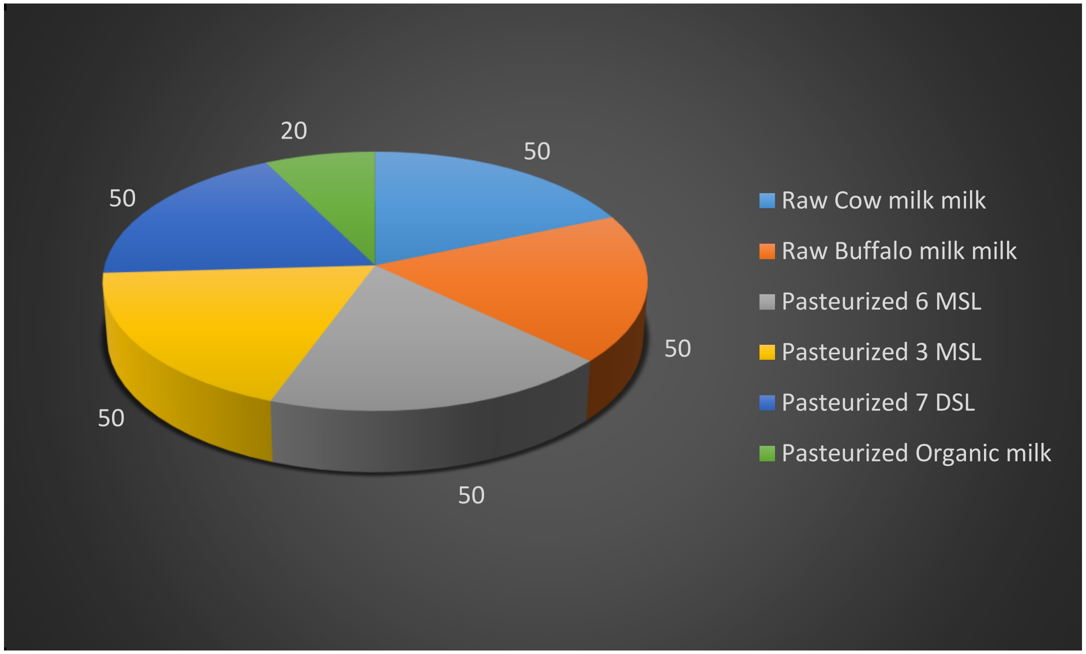
D3	Pasteurized 7DSL	ultra processed	NG	ND	NA	-
D4	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D5	Pasteurized 7DSL	ultra processed	NG	ND	NA	-
D6	Pasteurized 7DSL	ultra processed	NG	ND	NA	-
D7	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D8	Pasteurized 7DSL	ultra processed	NG	ND	NA	-
D9	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D10	Pasteurized 7DSL	ultra processed	LNF	-	NG	ND
D11	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D12	Pasteurized 7DSL	ultra processed	LNF	-	NG	ND
D13	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D14	Pasteurized 7DSL	ultra processed	LNF	-	NG	ND
D15	Pasteurized 7DSL	ultra processed	LNF	-	NG	ND
D16	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D17	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D18	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D19	Pasteurized 7DSL	ultra processed	NG	ND	NA	-
D20	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D21	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D22	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D23	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D24	Pasteurized 7DSL	ultra processed	NG	ND	NA	-
D25	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D26	Pasteurized 7DSL	ultra processed	NG	ND	NA	-
D27	Pasteurized 7DSL	ultra processed	NG	ND	NA	-
D28	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D29	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D30	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D31	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D32	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D33	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D34	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D35	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D36	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND

Table S1A. contd.

D37	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D38	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D39	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D40	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D41	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D42	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D43	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D44	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D45	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D46	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D47	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D48	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D49	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
D50	Pasteurized 7DSL	ultra processed	NG	ND	NG	ND
O1	Pasteurized Organic	UHT	NG	ND	NA	-
O2	Pasteurized Organic	UHT	NG	ND	NA	ND
O3	Pasteurized Organic	UHT	NG	ND	NG	ND
O4	Pasteurized Organic	UHT	NG	ND	NA	-
O5	Pasteurized Organic	UHT	LNF	-	NG	ND
O6	Pasteurized Organic	UHT	LNF	-	NG	ND
O7	Pasteurized Organic	UHT	NG	ND	NG	ND
O8	Pasteurized Organic	UHT	NG	ND	NG	ND
O9	Pasteurized Organic	UHT	NG	ND	NA	-
O10	Pasteurized Organic	UHT	NG	ND	NA	-
O11	Pasteurized Organic	UHT	NG	ND	NG	ND
O12	Pasteurized Organic	UHT	NG	ND	NG	ND
O13	Pasteurized Organic	UHT	NG	ND	NG	ND
O14	Pasteurized Organic	UHT	NG	ND	NG	ND
O15	Pasteurized Organic	UHT	NG	ND	NG	ND
O16	Pasteurized Organic	UHT	NG	ND	NG	ND
O17	Pasteurized Organic	UHT	NG	ND	NG	ND
O18	Pasteurized Organic	UHT	NG	ND	NG	ND
O19	Pasteurized Organic	UHT	NG	ND	NG	ND
O20	Pasteurized Organic	UHT	NG	ND	NG	ND
Total	**270**			**40 predicted *E. coli***		**67 predicted *S. aureus***

Table S1A. contd.

LF	**Lactose fermenter**
LNF	**Lactose non-fermenter**
NG	**No growth**
NA	**Non aureus**
ND	**Not determined**
Macconkey	
LF	Red non mucoid colonies
LNF	White/colorless colonies
EMB	
+	Purple coloured colonies with green metallic sheen
-	Purple mucoid colonies
Mannitol	
+	Yellow colonies with yellow halo.
-	Pink colonies with pink halos
Baired parker (BP)	
+	Grey-black shiny convex 1-1.5 mm diameter (18 hours) up to 3 mm (48 hours) narrow white entire margin surrounded by zone of clearing 2-5mm
-	Brown colonies (Colonies which do not form the black pigmentation)



Sample source		**Number of collected samples**
Raw	Raw Cow milk milk	50
	Raw Buffalo milk milk	50
Pateurized	Pasteurized 6 MSL	50
	Pasteurized 3 MSL	50
	Pasteurized 7 DSL	50
	Pasteurized Organic milk	20
Total number of collected samples		270
MSL	Month Shelf life	
DSL	Day Shelf life	

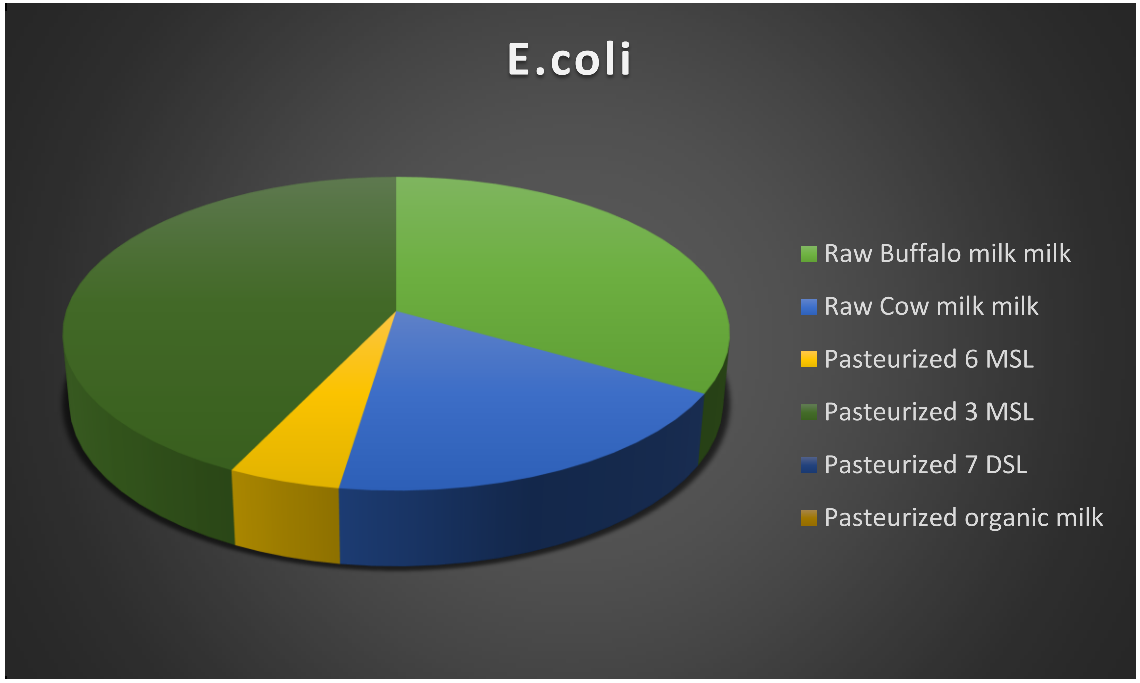
Table S1B. Biochemical identification of purified ***E. coli*** isolates.

Sample code	Source	Indole test	MR test	VP test	Citrate test	TSI test	isolate code	Interpretation
B1	Buffalo milk	+	+	-	-	+	15	*E. coli*
B7	Buffalo milk	+	+	-	-	+	23	*E. coli*
B8	Buffalo milk	-	-	+	+	-	12	*Enterobacter aerogens*
B10	Buffalo milk	+	+	-	-		E16	*E. coli*
B11	Buffalo milk	-	-	+	+	+	21	Not *E. coli*
B13	Buffalo milk	-	-	+	+	+	13	Not *E. coli*
B14	Buffalo milk	-	-	+	+	+	14	Not *E. coli*
B20	Buffalo milk	-	+	+	+	+	11	Not *E. coli*
B22	Buffalo milk	-	-	+	+	+	9	Not *E. coli*
B24	Buffalo milk	-	+	+	+	+	40	Mixed
B31	Buffalo milk	+	-	+	+	+	22	Not *E. coli*
B41	Buffalo milk	+	+	-	-	+	E25	*E. coli*
B42	Buffalo milk	+	+	-	-	+	E26	*E. coli*
B47	Buffalo milk	+	+	-	-	+	E24	*E. coli*
B48	Buffalo milk	+	+	-	-	+	E39	*E. coli*
C10	Cow milk	-	+	+	+	-	17	Hafnia species
C14	Cow milk	+	-	+	+	+	19	Not *E. coli*
C15	Cow milk	-	+	+	+	-	31	Mixed
C17	Cow milk	+	-	+	+	+	8	Not *E. coli*
C21	Cow milk	+	-	+	+	-	18	Not *E. coli*
C23	Cow milk	+	+	-	-	+	E32	*E. coli*
C41	Cow milk	+	+	-	-	+	E30	*E. coli*
C46	Cow milk	+	+	-	-	+	E27	*E. coli*
C47	Cow milk	+	+	-	-	+	E29	*E. coli*
C48	Cow milk	+	-	+	+	-	28	Not *E. coli*
S12	Pasteurized 6MSL	-	-	+	+	+	1	*Enterobacter agglomerans*
S13	Pasteurized 6MSL	+	+	-	-	+	E41	*E. coli*
T1	Pasteurized 3MSL	-	-	+	+	-	3	mixed
T3	Pasteurized 3MSL	+	-	+	+	-	34	Not *E. coli*
T5	Pasteurized 3MSL	+	+	-	-	+	E35	*E. coli*
T6	Pasteurized 3MSL	+	+	-	-	+	E2	*E. coli*
T7	Pasteurized 3MSL	+	+	-	-	+	E37	*E. coli*
T11	Pasteurized 3MSL	+	+	-	-	+	E7	*E. coli*

Table S1B. contd.

T18	Pasteurized 3MSL	+	+	-	-	+	E5	*E. coli*
T19	Pasteurized 3MSL	+	+	-	-	+	E38	*E. coli*
T20	Pasteurized 3MSL	+	+	-	-	+	E6	*E. coli*
T21	Pasteurized 3MSL	+	+	-	-	+	E4	*E. coli*
T22	Pasteurized 3MSL	+	-	+	+	-	20	Not *E. coli*
T23	Pasteurized 3MSL	-	-	+	+	+	36	*Enterobacter aerogens*
T29	Pasteurized 3MSL	+	+	-	-	+	E33	*E. coli*
Total	**40**							***21 E. coli***

Triple sugar iron (TSI) test	Butt	Slope	H2S
+	Acid/Gas	Acid	-
Indole test			
+	red color change		
-	no color change		
Methyl Red (MR)			
+	stable red color		
-	yellow color		
Voges-Proskauer (VP) test			
+	pink-red color on the surface of the medium 15 minutes to one hour after the addition of the reagents.		
-	yellow color on the surface of the medium		
Citrate test			
+	colour change of medium to blue		
-	no colour change of medium		



Types of milk samples	***E. coli***
Raw Buffalo milk milk	7
Raw Cow milk milk	4
Pasteurized 6 MSL	1
Pasteurized 3 MSL	9
Pasteurized 7 DSL	0
Pasteurized organic milk	0
Total	21

Table S1C. Biochemical identification of purified *S.aureus* isolates.

Sample code	Source	Coagulase test	DNase test	Isolate code	Interpretation
B1	Buffalo milk	-	-	46	Not *S. aureus*
B2	Buffalo milk	+	+	S67	*S. aureus*
B3	Buffalo milk	-	-	78	Not *S. aureus*
B4	Buffalo milk	-	-	65	Not *S. aureus*
B5	Buffalo milk	-	-	104	Not *S. aureus*
B6	Buffalo milk	-	-	49	Not *S. aureus*
B7	Buffalo milk	-	-	79	Not *S. aureus*
B8	Buffalo milk	-	-	69	Not *S. aureus*
B9	Buffalo milk	+	+	S57	*S.aureus*
B10	Buffalo milk	-	-	72	Not *S. aureus*
B11	Buffalo milk	-	-	45	Not *S. aureus*
B12	Buffalo milk	-	-	44	Not *S. aureus*
B13	Buffalo milk	-	-	54	Not *S. aureus*
B14	Buffalo milk	-	-	42	Not *S. aureus*
B15	Buffalo milk	-	-	70	Not *S. aureus*
B16	Buffalo milk	+	+	S48	*S.aureus*
B17	Buffalo milk	+	+	S68	*S.aureus*
B18	Buffalo milk	+	+	S61	*S.aureus*
B20	Buffalo milk	-	-	71	Not *S. aureus*
B22	Buffalo milk	-	-	47	Not *S. aureus*
B24	Buffalo milk	+	+	S81	*S.aureus*
B26	Buffalo milk	-	-	58	Not *S. aureus*
B28	Buffalo milk	-	-	88	Not *S. aureus*
B29	Buffalo milk	-	-	83	Not *S. aureus*
B31	Buffalo milk	-	-	43	Not *S. aureus*
B34	Buffalo milk	-	-	62	Not *S. aureus*
B36	Buffalo milk	-	-	112	Not *S. aureus*
B37	Buffalo milk	+	+	S119	*S.aureus*
B41	Buffalo milk	-	-	116	Not *S. aureus*
B42	Buffalo milk	+	+	S113	*S.aureus*
B46	Buffalo milk	-	-	117	Not *S. aureus*
B47	Buffalo milk	-	-	115	Not *S. aureus*

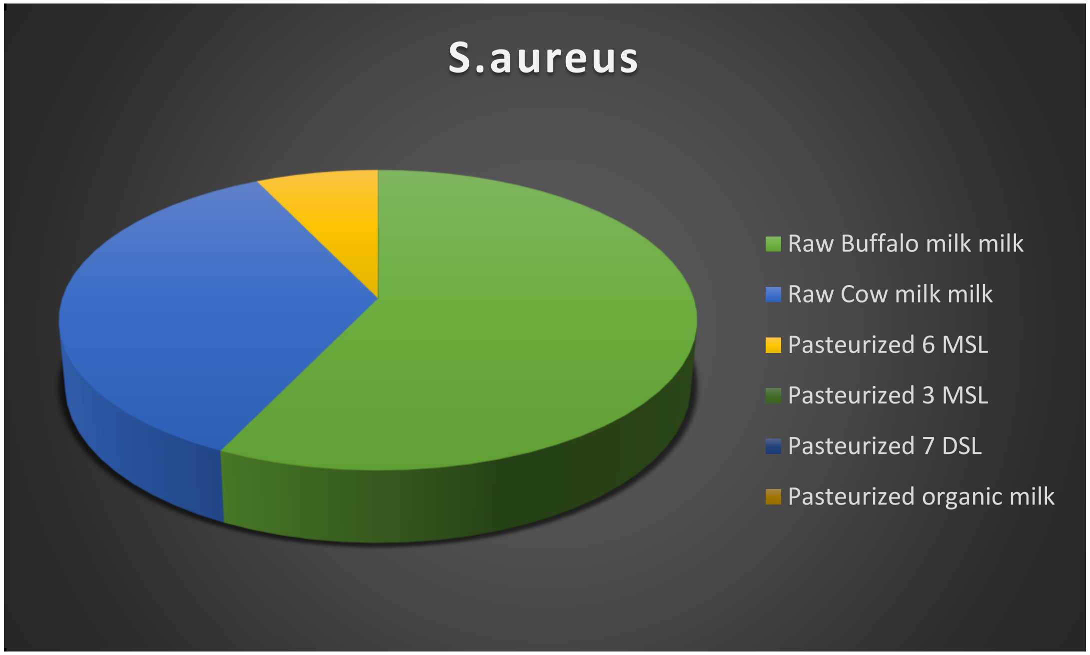
Table S1C. Contd.

C4	Cow milk	-	-	56	Not *S. aureus*
C7	Cow milk	+	+	S55	Not *S. aureus*
C8	Cow milk	-	-	85	Not *S. aureus*
C9	Cow milk	-	-	99	Not *S. aureus*
C11	Cow milk	-	-	110	Not *S. aureus*
C13	Cow milk	+	+	S93	*S.aureus*
C14	Cow milk	-	-	96	Not *S. aureus*
C15	Cow milk	-	-	63	Not *S. aureus*
C16	Cow milk	-	-	108	Not *S. aureus*
C17	Cow milk	-	-	77	Not *S. aureus*
C18	Cow milk	-	-	50	Not *S. aureus*
C19	Cow milk	-	-	97	Not *S. aureus*
C20	Cow milk	-	-	73	Not *S. aureus*
C21	Cow milk	-	-	132	Not *S. aureus*
C22	Cow milk	+	+	S95	*S.aureus*
C23	Cow milk	-	-	82	Not *S. aureus*
C24	Cow milk	+	+	S75	*S.aureus*
C25	Cow milk	-	-	122	Not *S. aureus*
C26	Cow milk	-	-	74	Not *S. aureus*
C28	Cow milk	-	-	123	Not *S. aureus*
C29	Cow milk	-	-	124	Not *S. aureus*
C39	Cow milk	-	-	120	Not *S. aureus*
C40	Cow milk	-	-	121	Not *S. aureus*
C48	Cow milk	+	+	S118	*S.aureus*
S16	Pasteurized 6MSL	+	+	S94	*S.aureus*
T3	Pasteurized 3MSL	-	-	64	Not *S. aureus*
T4	Pasteurized 3MSL	-	-	66	Not *S. aureus*
T8	Pasteurized 3MSL	-	-	91	Not *S. aureus*
T9	Pasteurized 3MSL	-	-	84	Not *S. aureus*
T10	Pasteurized 3MSL	-	-	102	Not *S. aureus*
T11	Pasteurized 3MSL	-	-	105	Not *S. aureus*
T16	Pasteurized 3MSL	-	-	111	Not *S. aureus*
T17	Pasteurized 3MSL	-	-	51	Not *S. aureus*

Table S1C. Contd.

T18	Pasteurized 3MSL	-	-	53	Not *S. aureus*
T25	Pasteurized 3MSL	-	-	103	Not *S. aureus*
D1	Pasteurized Organic	-	-	80	Not *S. aureus*
Total	**67**	**14**	**14**		

Coagulase	
+	Clumping
-	No clumping
DNase	
+	Clear zone precipitate around the test organism
-	No Clear zone around the test organism



Types of milk samples	***S. aureus***
Raw Buffalo milk milk	8
Raw Cow milk milk	5
Pasteurized 6 MSL	1
Pasteurized 3 MSL	0
Pasteurized 7 DSL	0
Pasteurized organic milk	0
Total	14