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RESEARCH ARTICLE

Chemical and Microbiological Evaluation of Raw Buffalo Milk Locally Produced in Sharkia Governorate

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Abstract

A total of 100 samples of raw Buffalo milk including (50 from dairy shops and 50 from dairy farms) were collected randomly at Sharkia Governorate for chemical and microbiological evaluation. Chemical assessment of the milk samples collected from dairy shops revealed that the mean values of Fat, Solid Not Fat (SNF), Protein, Lactose and Salts percentages were 6.06±0.36, 9.08±0.23, 3.54±0.09, 4.73±0.14 and 0.74±0.02 respectively, while dairy farm samples were 6.18±0.31, 9.53±0.44, 3.89±0.09, 5.12±0.15 and 0.78±0.03; correspondingly. Microbiological examination revealed that the mean values of faecal coliforms were $2.03\times10^6 \pm 0.75\times10^6$ and $1.8\times10^6 \pm 0.59\times10^6$ in dairy shops and farms, respectively. The identified species of isolated coliform organisms in both types of milk were *Citrobacter diversus* (11.3% vs 11.1%) *Citrobacter freundii* (9.6% vs 9.6%), *Enterobacter aerogenes* (12.1% vs 9.6%), *Enterobacter agglomerans* (11.3 vs 10.4%), *Enterobacter cloacae* (13% vs 11.1%), *Klebsila oxytoca* (9.6% vs 11.9%), *Klebsila pneumoniae* (9.6% vs 10.4%) and *E.coli* (23.5% vs 25.9%); respectively. Mean values of total staphylococci were $4.29\times10^6 \pm 0.21\times10^6$ and $8.08\times10^6 \pm 2.27\times10^6$ in milk samples of shops and farms respectively. The identified species in both types were *S. aureus, S. epidermidis, S. saprophyticus, S. capitis and S. intermedius* with percentages of 28% vs 35%, 48% vs 41%, 10% vs 12%, 8% vs 7% and 6% vs 5%; respectively. It was exposed that 8 strains (28.57%) and 10 strains (28.57%) were identified as methicillin-resistant *S. aureus* that containing *mecA* gene. In conclusion, high prevalence of different udder pathogens among dairy animals may attributed to the lack of sanitary conditions that adapted in dairy farm. So, restriction to application of hygienic measures in dairy farms as well as quality control and quality assurance programs should be adopted to get safe and good quality raw milk.

Keywords: Raw milk, Chemical composition, Coliforms, *S. aureus*, MRSA.

Introduction

Milk is a white liquid produced by the mammary glands of mammals and is considered as one of the most valuable and regularly consumed foods [1]. Milk contains all the essential nutrients for all physiological function of the body system. The main constituents of milk are; water, fat, protein, lactose and ash. Milk is also a good source of phosphorus, calcium, fat and water-soluble vitamins, so it is considered as the most natural nearly complete food [2]. Buffalo milk has turned into a research subject and got utmost attentions in many countries due to its richness of fats, protein, lactose, total dry matter, vitamins and minerals [3, 4]. Raw milk is a suitable medium for nourishment and development of microorganisms because of its high water contents, nearly neutral pH, in addition to presence of variety of available fundamental supplements that renders it as a well-known amongst the best media for microbial development and multiplication [5]. The bacterial contamination of milk diminishes wholesome quality and the utilization of such milk threatens the public health. Microorganisms may contaminate milk at different phases of delivering, handling and distribution. The poor health of dairy animal

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and its conditions, improperly cleaned and disinfected milk-handling equipment as well as workers do not observe the basic rules of personal hygiene could serve as potential sources of microbial contamination [6].

Coliform bacteria are one of the natural flora of human and animal intestinal tract. Their detection is commonly used as an index for judging the hygienic quality of foods, as their presence indicates the possibility of environmental and/or faecal contamination [7]. Coliform bacteria commonly contaminate raw milk via several environmental sources, particularly water, soil and bedding and they proliferate on insufficiently cleaned surfaces. Typical coliform species of a food-safety concern include *Escherichia coli (E. coli), Enterobacter, Citrobacter, Klebsiella and Serratia* spp. In this sense, *E. coli* is a well-known contaminant of raw milk and processed milk products [8]. Milk can be contaminated by *Staphylococcus aureus* through infection of mammary glands or through bad hygienic habits, as coughing or sneezing and neglecting the cleanliness [9]. *Staphylococcus aureus* is an opportunistic pathogen that forms some portion of the ordinary commensal flora of humans and domesticated animals, colonizing approximately 30%–50% of the human population, and it is considered as the most clinically significant species [10]. *S. aureus* is considered as one of the most rapidly evolving bacteria, being able to develop a resistance towards a wide variety of antibiotics. It had acquired resistance to penicillin by producing a β-lactamase enzyme that rendered penicillin inactive [11]. Infections caused by *S. aureus* extend from minor superficial skin to lethal deep seated infections [12]. Its presence in foods represents a risk to human health, causing a public health problem as foodborne intoxication [13]. *S. aureus* infections are difficult to control and are well known to cause subclinical, clinical, and chronic mastitis, while treatment approaches are frequently compromised [14]. This pathogenicity is due to various genetic capabilities of these microorganisms, the important among them is methicillin-resistant *S. aureus* (MRSA) strains which enhance the pathogenesis of *S. aureus* in mastitis and evade the immune response of the host [15,16]. Methicillin-resistant *Staphylococcus aureus* produces penicillin-binding proteins (PBPs) that reduce the activity of the β-lactam antibiotics [17].

The low binding affinity of this PBP2a encoded by the *mecA* gene to β-lactam antibiotics permits the sustained synthesis of the peptidoglycan cell wall in MRSA regardless of the presence of lethal concentrations of methicillin. From the preceding data about the importance of hygienic quality of raw buffalo milk and its subsequent public health importance, the objectives of this study were designed for chemical evaluation (using milk scan, lacto - scan) as well as microbiological evaluation (Enumeration and identification of coliforms, staphylococci and molecular identification of methicillin resistant *Staphylococcus aureus* (MRSA) using PCR assay targeting *mecA* gene specific for MRSA) of raw buffalo milk samples collected from Sharkia province, Egypt.

Materials and Methods

Collection of samples

One hundred random samples of raw buffalo milk were collected from different dairy shops and farms (50 samples, each) in Sharkia Governorate, Egypt during the period from August to December of 2018. Approximately 500 mL of the samples were transferred directly to the laboratory of Food Control Department, Faculty of Veterinary Medicine, Zagazig University in an insulated ice box at 4°C with a minimum of delay to be examined chemically and microbiologically.

Preparation of samples

On arrival to the laboratory, each sample was perfectly mixed and then divided into two portions to be examined chemically and microbiologically [18].

Chemical examination

Determination of milk constituents

The percentages of fat, protein, solid not fat, lactose and salts were determined by using ultrasonic portable milk analyzer (milkotester model- Master Mini 9949).

Milk samples should be at a temperature range of 5-35˚C and mixed well before examination.

Microbiological examination

Preparation of serial dilution

Eleven milliliter of well mixed milk samples were aseptically transferred into sterile bottle containing 99 ml of sterile peptone water solution 1% and thoroughly mixed to make a dilution of 1/10 from which decimal serial dilution were prepared [18].

Enumeration and identification of coliforms

One milliliter from each of the previously prepared dilution was transferred into a sterile labeled petri plate. Ten millimeter of tempered melted Violet Red Bile Agar (VRBA) (cooled at 44- 46°C) were poured onto the surface of the inoculated plate, then thoroughly and uniformly mixed with the inoculum. The plates were then left to stand at room temperature for about 15-30 minutes to solidify. After solidification of the media, an additional 3 to 4 mL of plating medium were distributed as an overlay, completely covered the surface of the solidified medium to inhibit surface colony formation. The inoculated plates were incubated in an inverted position for 24±2 h at 32±1°C. Suspected colonies showed a dark purplish-red colonies surrounded by a red zone of precipitated bile acid on uncrowded plates were counted (15-150 coliforms colonies) and the results were recorded [18].

Identification of coliforms

The isolated coliforms were identified microscopically by Gram staining [19]. Suspected coliforms (evenly stained Gram negative, non-spore forming, short rods or cocci) were subjected to biochemical identification (indole test, Voges-Proskauer test, methyl red test, citrate utilization test, triple sugar iron test (TSI) test, gelatin hydrolysis test , urease test, nitrate reduction test, arginine dihydrolase test, sugar fermentation (lactose, sucrose, dulcitol, salicin, arabinose, inositol and xylose), lysine decarboxylase, ornithine decarboxylase and O-nitrophenyl-beta-D-galactopyranoside (ONPG) test) according to Cruickshank *et al*. [20].

Enumeration and identification of staphylococci

Isolated staphylococci duplicate Baired Parker agar plates that were inoculated with each dilution of the samples by spreading 0.1 ml evenly onto the surface of each plate with sterile glass spreading rod. The plates were incubated under aerobic conditions at 37°C for 24 to 48 h [21]. Gray to black colonies with lecithinase positive as well as negative activity were chosen for further identification of species by biochemical tests (Colonies of *Staph. aureus* are typically circular, smooth, convex, moist, 2 to 3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone. Colonies have buttery to gummy consistency when touched with inoculating needle.

Suspected colonies were identified microscopically by using Gram's stain to see Gram +ve cocci arranged in grapes. They were submitted to biochemical identification by using certain biochemical tests as oxygen requirements, coagulase, clumping factor, heat-stable nuclease (thermonuclease), hemolysins, catalase, oxidase, alkaline phosphatase, urease, ornithine decarboxylase, pyrrolidonyl arylamidase, b-galactosidase, acetoin production, nitrate reduction, esculin hydrolysis, aerobic acid production from a variety of carbohydrates including d-trehalose, d-mannitol, d-mannose, d-turanose, d-xylose, d-cellobiose, l-arabinose, maltose, lactose, sucrose, and raffinose, and intrinsic resistance to novobiocin and polymyxin B according to Kloss and Bannerman [19].

Determination of methicillin-resistant S. aureus (MRSA)

Primer sequences [nuc F. 5′ GCGATTGATGGTGATACGGTT ′3 and nuc R. 5′ AGCCAAGCCTTGACGAACTAAAGC ′3] specific for nuc coding genes were used according to Brakstad [22] for confirmation of *S. aureus*. However, mecA (For**)** 5′ TAGAAATGACTGAAC GTCCG ′3 and mecA (Rev) 5′ TTGCGATCA ATGTTACCGTAG according to Louie *et al.* [23].

DNA extraction

DNA was extracted according to QIAamp DNA Mini Kit (Catalogue no.51304) instructions

DNA amplification

Multiplex PCR was performed for the detection of *mecA* and *nucA* which is responsible for the production of thermostable nuclease and was included in the multiplex PCR to confirm that the isolates were indeed *S. aureus* and not other staphylococcal species.

Extracted DNA by using QIAamp DNA Mini Kit by boiling for 10 min in 100 µL of Triton X-100 lysis buffer (100 mM NaCl, 10 mM Tris-HCl (pH 8), 1 mM EDTA (pH 9), and 1% Triton X-100) [24]. The suspension was cooled at room temperature for 5 min and centrifuged at 14,000 rpm for 1 min. Next, 1 µL of the supernatant was used as the template. PCR was performed in a 25- µL volume, with 1× PCR buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl, 200 µM concentrations of each deoxynucleoside triphosphate, 2.5 U of*Taq* polymerase, and 0.2 µM concentrations of each primer. Thermocycling conditions in a GeneAmp 9600 thermocycler (PE Biosystems, Mississauga, Ontario, Canada) were as follows: 94°C for 2 min, followed by 30 cycles of 94°C for 1 s and 55°C for 15 s, with a final 10-min extension at 72°C.

The PCR products were electrophoresed on 1.5% agarose gel (Applichem, Germany, GmbH) and visualized by ethidium bromide staining on UV transilluminator. A 100 bp DNA Ladder was used as a molecular weight standard. (Qiagen, Germany, GmbH)

Statistical analysis

All data were statistically analysed using SPSS software (IBM SPSS Statistics Version 23: IBM release 2015).

Results and Discussion

Buffaloes are significant sources of milk for human consumption in various parts of the world because it is regarded as by higher solids contents for being richer source of lipids, protein, lactose and minerals. Buffalo milk is valued by its significant chemical composition [26]. Milk composition depends on multiple factors as breed, health of lactating dairy animals, lactation period, type of nutrition(feeding on roughage or concentrates), season of the year, method of milking (manual or automatic), age and number of lactation, and on the animal itself (body mass, moving, etc.) [27]. This study was carried out to chemically and micrbiologically evaluate raw buffalo milk that is produced and marketed in Sharkia Governorate.

Table 1: Milk scan profile of raw buffalo milk from dairy shops and farms (50 samples, each) in Sharkia Governorate, Egypt (August-December 2018).

Source of milk	Parameters	Minimum	Maximum	Mean ± SE
Dairy shops	**Fat** [a]	3.57	8.88	6.06 ± 0.36
	SNF [b]	6.5	11.72	9.08 ± 0.23
	Protein	2.53	4.56	3.54 ± 0.09
	Lactose	3.1	6.18	4.73 ± 0.14
	Salt	0.53	0.96	0.74 ± 0.02
Dairy farms	**Fat** [a]	3.34	8.99	6.18 ± 0.31
	SNF [b]	3.38	11.28	9.53 ± 0.44
	Protein	2.31	4.35	3.89 ± 0.09
	Lactose	2.78	5.88	5.12 ± 0.15
	Salt	0.27	0.91	0.78 ± 0.03

[a] Fat percent in 32 buffalo milk samples from dairy shops (64%) and 38 from dairy farms (76%) were compatible with the Egyptian standard of fat (ES, 154/1/2005) that is not less than 5.5. [b] Solid not fat percent in 36 buffalo milk samples from dairy shops (72%) and 42 from dairy farms (84%) were compatible with the Egyptian standard of fat (ES, 154/1/2005) that is not less than 8.75.

The results were reported in Table (1) showed the chemical composition of raw buffalo milk samples collected from dairy shops where there was no significant difference between them as the mean values of fat %, SNF %, protein%, Lactose % and salts % were 6.06±0.36, 9.08±0.23, 3.54±0.09, 4.73±0.14 and 0.74±0.02, respectively, while in dairy farms, the respective mean values were 6.18±0.31, 9.53±0.44, 3.89±0.09, 5.12±0.15 and 0.78±0.03. These results were in agreement with those reported by Lingathurai *et al*. [28] and El-Leboudy *et al*. [29], while higher results were declared by Hussain *et al*. [30] and Zeki *et al.* [31]. However, lower results were recorded by Enb *et al*. [32] and Hashmi *et al*. [33]. Region, climatic conditions and lactation periods are the primary occasional changes which have impacts on the milk composition predominantly milk fat because of the negative relationship between ecological temperature and the measure of milk fat and protein content as when temperature increases, the solid fat decrease. Moreover, the light-to-dark proportion can prompt obvious changes in milk yield and composition [34].

Comparison between the obtained results of the chemical constituents of examined samples and the Egyptian standards (2005) revealed that 64% and 76% of raw buffalo milk from dairy shops and dairy farms, respectively were compatible with Egyptian standards (2005) of fat that is not be less than 5.5. Solid not fat percent was also compatible with the Egyptian standard (Not less than 8.75%) in 72% of dairy shops milk and 84% of dairy farm milk. The more prominent variation in milk fat was due to outdoor grazing in summer, bar feeding and adulteration by partial skimming by farmers**,** genetic variation, and animal health.

All examined samples were contaminated with coliforms with a mean value of $2.03 \times 10^6 \pm 0.75 \times 10^6$ in the examined samples from dairy shops and all samples from dairy farms were also contaminated with coliforms with a mean value of $1.8 \times 10^6 \pm 0.59 \times 10^6$. Both of them were not significantly different. These findings confirmed those reported by Hadrya *et al*] and Soomro *et al* [35, 36]. However, lower values were obtained by Hashmi *et al* and El-Leboudy *et al* [29, 33]. On the other hand higher results were recorded by Bayoumi and Tahoun [37, 38]. The presence of coliform organisms in milk indicates unsanitary conditions during production, processing and storage. Hence their presence in large number in dairy products gave an indication about the presence of potentially hazard in consumers' health. Pathogenic *E. coli* most recently has constituted a public health hazard ranging from diarrhea to potentially faecal hemolytic urarnic syndromes.

Table 2: Total coliforms count and the occurrence rate of the identified coliforms in raw buffalo milk from dairy shops and farms (50 samples, each) in Sharkia Governorate (August-December 2018).

Isolated Coliforms	Source of raw buffalo milk			
	Dairy shops		Dairy farms	
	*No	%	No	%
Citrobacter diversus	13	11.3	15	11.1
Citrobacter Freundii	11	9.6	13	9.6
Enterobacter aerogenes	14	12.1	13	9.6
Enterobacter agglomerans	13	11.3	14	10.4
Enterobacter cloacae	15	13	15	11.1
Klebsiella oxytoca	11	9.6	16	11.9
Klebsiella pneumoniae	11	9.6	14	10.4
Escherichia coli	27	23.5	35	25.9
Total	115	100%	135	100%

*The percentage was calculated according to the number of coliforms isolates. The total coliforms count ranged from 3.6×10^3 - 1.4×10^7 (Mean ± SE of $2.03 \times 10^6 \pm 0.75 \times 10^6$) in raw buffalo milk from dairy shops and 5×10^3 - 1.12×10^7 (Mean ± SE of $1.8 \times 10^6 \pm 0.59 \times 10^6$) in raw buffalo milk from dairy farms with no significant difference ($p > 0.05$) between both sources based on Independent sample T- test.

The summarized results in Table (2), showed that the percentages of isolation of *Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloacae, Klebsiella oxytoca, Klebsiella pneumoniae* and *E.coli* in raw milk from dairy shops versus those in from dairy farms were (11.3% vs 11.1%), (9.6% vs 9.6%), (12.1% vs 9.6%), (11.3% vs 10.4%), (13% vs 11.1%), (9.6% vs 11.9%), (9.6% vs 10.4%) and (23.5% vs 25.9%) that were calculated as a percentage from the total coliforms isolates recorded in each source (n=50). Similar strains were isolated in previous studies by Donkor *et al*. and El-Mossalami *et al*. [39, 40]. Higher results of isolated coliforms organisms were obtained by Lingathurai *et al.* [28].

Certain quantities of *Citrobacter* had been suspected to cause enteric infection [41]. *C. freundii* had been established amongst urinary and other pyogenic contaminations in humans. Certain uncommon strains of *C. freundii* have been correlated with entrepreneurial nosocomial contaminations of the respiratory tract, urinary tract, blood and various other typically sterile sites in immune compromised patients [42]. *Klebsiell*a organisms are responsible for food-borne outbreaks. *K. pneumoniae* constituted a part of the flora of the mouth and intestinal tract of man and animal. It is responsible for pneumonia and upper respiratory tract infection as well as meningitis, pyemia, cystitis, septicemia and urinary tract infection [43]. *Enterobacter* spp. mainly *E. aerogenes* were found in soil, water, and intestinal tract are implicated in urinary tract infection and septicemia.

E. coli is considered as a reliable indicator of fecal contamination and revealed a possible presence of enteropathogenic and/or toxigenic E. coli, which comprise a public health hazard. Milking udder with sub-clinical mastitis and wet environment initiates contamination of bulk tank milk and subsequently raw milk reaches the consumers with raised coliforms count [44].

Table 3: Total staphylococcal count /ml and occurrence rate of the identified staphylococcus species in the examined raw buffalo milk from dairy shops and farms (50 samples, each) in Sharkia Governorate (August-December 2018).

Samples examined	Positive samples		Minimum	Maximum	Mean ± SE
	No	%			
Dairy shops raw milk	50	100	3.2×10^4	4.4×10^7	$4.29 \times 10^6 \pm 1.21 \times 10^6$ [a]
Dairy farms raw milk	50	100	5.7×10^3	4.9×10^7	$8.08 \times 10^6 \pm 2.27 \times 10^6$ [a]

Means within the same column carrying same superscripts are not significantly different at ($p > 0.05$) based on Independent sample T- test.

It is evident from the obtained results (Table 3) that all examined raw milk samples were contaminated by staphylococci, with levels of contamination with a mean value of $4.29 \times 10^6 \pm 0.21 \times 10^6$ and $8.08 \times 10^6 \pm 2.27 \times 10^6$ in examined raw buffalo milk samples from dairy shops and farms, respectively. These findings revealed that there was no significant difference between them and they substantiated results reported by Bayoumi [37]. Lower results were obtained by Amer *et al*. [45] and El-Mossalami *et al*. [40], while higher results achieved by Eraky [46] and Alnakip [47]. *Staphylococcus aureus, S. epidermidis, S. saprophyticus, S. capitis and S. intermedius* could be identified in positive staphylococci raw buffalo milk samples from dairy shops and farms in percentages of 28% vs 35%, 48% vs 41%, 10% vs 12%, 8% vs 7% and 6% vs 5%, respectively. Similar results were obtained by Suelam *et al*. [48], and Saadat *et al.* [49], lower results were obtained by Ben Hassen *et al*. [50] and Alnakip [47]. However, higher results were achieved by Andre *et al*. [51] and Tarekgne *et al*. [52]. The presence of staphylococci in high counts is a potential health hazard as it potentiates the presence of enterotoxigenic strains. *S.*

epidermidis colonizes the skin and mucous membranes and is considered the principle bacterium in the normal human microbiota [53]. *S. epidermidis* represents the fundamental pathogen in catheter-related blood stream contaminations and early-beginning neonatal sepsis and is likewise a successive reason of joint diseases, valve endocarditis, and other biomedical device-related contaminations [54]. *S. saprophyticus* is a coagulase-negative spp. related fundamentally to community-acquired lower urinary tract diseases (UTI) in youthful and moderately aged ladies [55]. Complications of *S. saprophyticus* infection such as recurrent infection, acute pyelonephritis, nephrolithiasis, septicemia and endocarditis have been recorded but are rare [56]. Milk can be contaminated with *S. aureus* through infection of mammary glands or through bad hygienic habits, as coughing or sneezing and neglecting of cleanliness. *S. aureus* possesses a public health hazard due to production of thermostable enterotoxin that is responsible for food poisoning, Leucocidin Enterotoxin (A to E) and toxic shock syndrome toxin (TSST) and all were produced by S. *aureus* [41]. Additionally, *S. aureus* is the most important and predominant mastitis pathogen; being existed in several peracute, acute, subacute, and chronic forms of intra-mammary infections [47].

S. aureus is one of the major bacterial pathogens that generally causes superficial skin and soft tissue contaminations, surgical wound infections, and occasionally- lethal circulatory system contaminations and pneumonia. The proceeding with development of drug-resistant pathogens, particularly multiple-drug-resistant isolates and methicillin-resistant *S.aureus.* (MRSA) is a reason for serious worries in the public health because of the restricted selection of anti-microbials for powerful treatment of MRSA contaminations. Among *S. aureus,* Methicillin-resistant strains (MRSA), have lately developed as a serious life-threaten infective agent which does not respond to a lot of antimicrobial treatments. MRSA synthesizes a penicillin binding protein (PBP2a), encoded by the *mecA* gene on a mobile genetic element (Staphylococcal cassette chromosome mec SCCmec), which has a role of counteracting the inhibitory impact of Beta-lactam (b-lactam) anti-infection agents by keeping them from adequately binding to cell wall proteins [57].

Table 4: Occurrence rate of the isolated Methicillin Resistant *S. aureus* in the examined raw buffalo milk samples from dairy shops and farms (50 samples, each) at Sharkia Governorate (August-December, 2018).

	positive staphylococci		positive *S. aureus*		positive *S. aureus* contain both nuc and mecA genes	
	No	**%**	**No**	**%**	**No**	**%**
Dairy shops raw milk	100	100.00	28	28.00	8	28.57
Dairy farms raw milk	100	100.00	35	35.00	10	28.57

Percentage (%) was calculated according to the number of each examined samples.

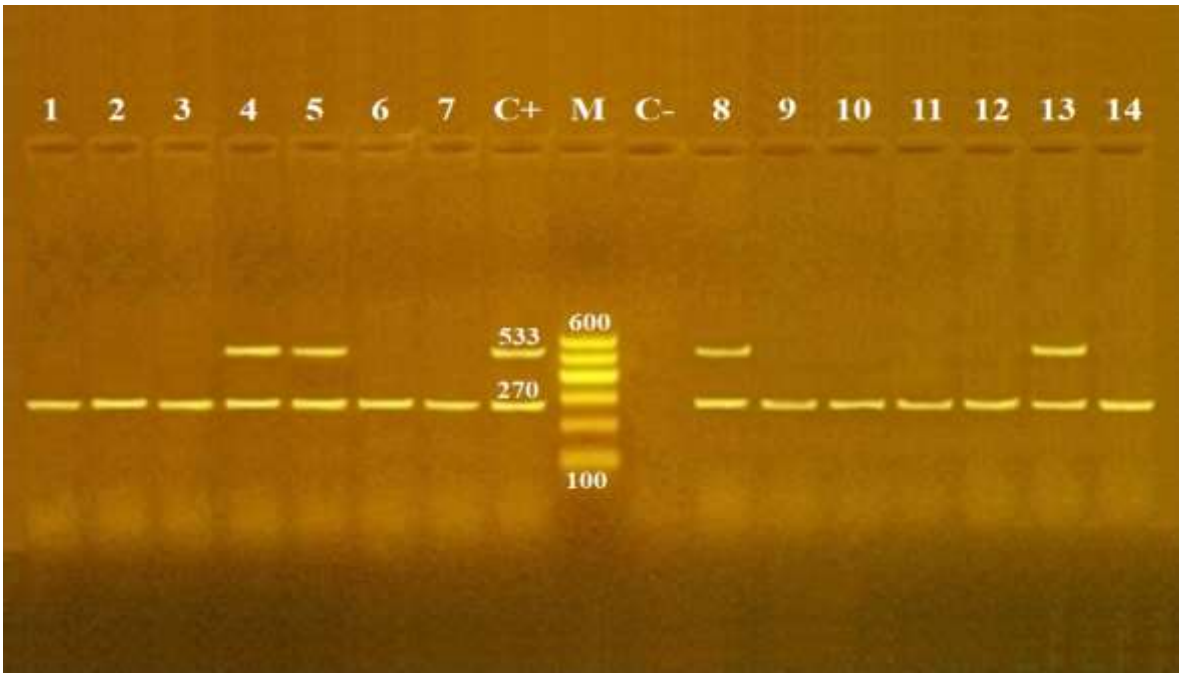

Figure 1: Agarose gel electrophoresis of multiplex PCR of *nuc* (270bp) and *mec*A (533bp) virulence genes of *S. aureus.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for *nuc* and *mec*A genes. Lane C-: Control negative. Lanes from 1 to 14: Positive *S. aureus* strains for *nuc* gene. Lanes 4, 5, 8 & 13: Positive *S. aureus* strains for *nuc* and *mec*A genes.

Table (4) showed that *S. aureus* constituted 28 and 35% out of the total isolated staphylococcus species (n=100, each) from raw buffalo milk from dairy shops and farms, respectively. Eight *S. aureus* isolates (28.57%) from dairy shops' raw milk and 10 (28.57%) from dairy farms' raw milk were identified as methicillin-resistant *S. aureus* containing both *nuc* (270bp) and *mecA* (533bp) genes as showed in Figure (1). Similar results were obtained by Huimin *et al*. [42] who detected *S. aureus* in 54 (27.7%) samples out of 195 milk samples examined, 16 isolates of them were identified as methicillin-resistant *S. aureus*. Higher results were obtained by Aqib *et al.* [58] who stated that the prevalence of MRSA was 38% in buffalo milk, however, lower results were achieved by Ismail [59], who stated that the prevalence of MRSA was 18.2% out of *S. aureus* isolates (22.4%) obtained from cows with acute mastitis.

Conclusion

It is clear from the microbiological results that milk contamination and subsequently the milk quality were affected by the poor hygienic conditions during milking and handling in addition to post-milking environmental contaminants. The existing situation must be improved and this can be achieved by regular training of milk producers to raise awareness regarding good hygienic practices (GHP) .Strict hygienic measures should be applied during milking collection and transportation. Milk must be heat treated before consumption or manufacture to dairy products. HACCP programs must be applied at the farm level and milk production area. Finally, it seems necessary that concerned authorities should impose regulations and bacteriological standards to govern raw milk and its products.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي

التقييم الكيميائى والميكروبيولوجى لحليب الجاموس الخام المنتج محليا بمحافظة الشرقية

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أجريت هذه الدراسة على مائة عينة عشوائية من لبن الجاموس الخام (خمسون عينة من متاجر الألبان وخمسون عينة من مزارع الألبان) تم تجميعها من اماكن مختلفة من مدينة الزقازيق بمحافظة الشرقية. وتضمنت هذه الدراسة فحص هذه العينات كيميائياً وميكروبيولوجيا لتقرير حالتها الصحيه ومدى تلوثها بالميكروبات الممرضة وكذلك المسببة لفساد اللبن مما قد يترتب عليه خسارة اقتصادية كبيرة. أوضحت الفحوص الكيميائية لعينات اللبن الخام الموجودة فى متاجر الألبان أن متوسط نسبة الدهن ، المواد الصلبة غير الدهنية ، البروتين ، اللاكتوز ، الأملاح (٦,٠٦±٠,٣٦) ، (٩,٠٨±٠,٢٣) ، (٣,٥٤±٠,٠٩) ، (٤,٧٣±٠,١٤) ، (٠,٧٤±٠,٠٢) على الترتيب بينما كانت فى العينات المأخوذة من مزارع الألبان على النحو التالى (٦,١٨±٠,٣١) ، (٩,٥٣±٠,٤٤) ، (٣,٨٩±٠,٠٩) ، (٥,١٢±٠,١٥) ، (٠,٧٨±٠,٠٣) على الترتيب. أوضحت الفحوص الميكروبيولوجيه تواجد ميكروبات الكوليفورم فى كل عينات لبن الجاموس الخام الموجوده فى متاجر الألبان ومزارع الألبان وكان متوسط عدد ميكروبات الكوليفورم فى هذه العينات على النحو التالى (٢,٠٣ × ١٠^{٦} ± ٠,٧٥ × ١٠^{٦} و ١,٨ × ١٠^{٦} ± ٠,٥٩ × ١٠^{٦}) على الترتيب. تم عزل وتصنيف عترات ستروباكتر دايفيرساس وستروباكتر فريوندى وانتيروباكتر ايروجينس وانتيروباكتر اجلوميرانز وانتيروباكتر كلواكى وكليبسيلا أوكستوكا وكليبسيلا نيمونى وايشيريشا كولاى من عينات لبن الجاموس الخام الموجوده فى متاجر ومزارع الألبان بنسب (١١,٣٪ مقابل ١١,١٪ ، ٩.٦٪ مقابل ٩.٦٪ ، ١٢.١٪ مقابل ٩.٦٪ ، ١١,٣٪ مقابل ١٠.٤٪ ، ١٣٪ مقابل ١١,١٪ ، ٩.٦٪ مقابل ١١.٩٪ ، ٩.٦٪ مقابل ١٠.٤٪ ، ٢٣,٥٪ مقابل ٢٥.٩٪) على الترتيب. اظهرت النتائج ان متوسط العدد الكلى للمكورات العنقودية فى عينات لبن الجاموس الخام الموجود فى متاجر ومزارع الألبان (٤,٢٩ × ١٠^{٦} ± ٠,٢١ × ١٠^{٦} و ٨,٠٨ × ١٠^{٦}) على الترتيب. تم عزل عترات المكورات العنقودية الذهبية والمكورات العنقودية الجلدية والمكورات العنقودية المترممة والراسيه والمتوسطه من عينات لبن الجاموس الخام الموجوده فى متاجر ومزارع الألبان بالنسب ٢٨٪ مقابل ٣٥٪ ، ٤٨٪ مقابل ٤١٪ ، ١٠٪ مقابل ١٢٪ ، ٨٪ مقابل ٧٪ ، ٦٪ مقابل ٥٪ على الترتيب. تم عزل ٨ عترات (بنسبة ٢٨,٥٧٪) من ميكروب العنقود الذهبى المقاوم للميثيسيللين من اصل ٢٨ عتره ميكروب عنقودى ذهبى من لبن الجاموس المتواجد فى المتاجر وعزل ١٠ عترات (بنسبة ٢٨,٥٧٪) من ميكروب العنقود الذهبى المقاوم للميثيسيللين من اصل ٣٥ عتره ميكروب عنقودى ذهبى من لبن الجاموس المتواجد فى المزارع.

الخلاصه: يتعرض اللبن الخام إلي التلوث بالميكروبات من مصادر مختلفة أثناء إنتاجه وتداوله حيث يمثل اللبن بيئة خصبة لنمو وتكاثر الميكروبات مما قد يسبب خطورة علي صحة المستهلك, فضلا عما قد تحدثه من عيوب تؤدي إلى فساد المنتج والتأثير السلبى على جودته نتيجة للتغيرات الغير مرغوبة مما يجعله غير صالح للاستهلاك الآدمي.

هذا وقد تمت مناقشة أهمية الميكروبات المعزولة من الناحيتين الصحية والإقتصادية بالإضافة إلي التوصيات الواجب إتباعها لتحسين جودة المنتج وحفاظا علي صحة المستهلك.