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Assessment of bacterial content in raw and packaged cow milk in Saladin Governorate, Iraq

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Abstract

Background: Cow's milk is the most consumed product worldwide. However, due to bacterial contamination, milk can be risky for consumer's health. Despite pasteurization and techniques applied to date, they have not demonstrated efficacy in eliminating contaminants. It is important to know the content of bacteria in raw and packaged cow milk to avoid food-borne diseases. **Objective:** The study was designed to assess the bacterial prevalence of raw cow's milk in Saladin Governorate, Iraq, and to compare it with the bacterial prevalence of imported packaged cow milk. **Method:** The study involved ninety milk samples, thirty samples of each pasteurized raw domestic milk, imported cow milk, and domestic cow milk, and analyzed the morphological properties of the colony, gram stain, and biochemical tests. **Results:** The study findings indicated that raw milk was contaminated with *Staph. aureus*, *Staph. epidermidis*, *Staph. saprophyticus*, *E. coli*, *P. aeruginosa*, *E. aerogenes*, and *P. mirabilis*. The prevalence percentages of bacterial species in raw milk samples were 21%, 12%, 6%, 9%, 3%, 6%, and 6%, respectively. In comparison, imported packaging milk had a lower percentage of bacteria than raw milk.

Conclusion: Our study reveals that raw home milk in Saladin Governorate is contaminated with various bacteria. The contamination arises from inadequate hygiene practices during milk handling. While imported milk is less contaminated, it still contains bacteria. This can be attributed to contamination that occurs after the production process. Should subject raw domestic and imported milk to pasteurization before consumption to decrease the risk of foodborne illness.

Keywords: Raw milk; Cow milk; Packaged milk; *Staphylococcus aureus*; Saladin governorate.

Introduction

Milk is exceptionally nutritious. It contains protein, fat, and minerals such as calcium, phosphorus, iron, and vitamins. These crucial components make it a significant nutritional resource for infants, neonates, and individuals of all ages [1]. Milk is a highly nutritious food that is an excellent growth medium for various microorganisms. In healthy udder cells, milk is deemed free from microorganisms [2]. Still, subsequent contamination can occur from numerous sources, such as the teat apex, milking tools, feed, grass, dirt, surrounding air, waste, water or moisture content, and other sets [3]. Handlers inadvertently contaminating food can result in various bacterial

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strains, such as Staphylococcus aureus, in raw milk or its byproducts. *Staphylococcus aureus*, responsible for food poisoning, can also be transmitted through subclinical mastitis [4]. *E. coli* is a type of bacteria that can cause infections in the intestines. It is also a potential source of public health concern as it can contaminate milk worldwide [5-7]. The phrase "total coliform" encompasses a diverse range of gram-negative rod-shaped bacteria, including thermotolerant coliforms and bacteria of fecal origin found in the environment. Coliforms are microorganisms that can cause various illnesses when given the opportunity, while many others are naturally present in the intestines [8]. The presence of these organisms in milk and milk products suggests that the milk and milk utensils were not appropriately handled or produced in unhygienic conditions [9]. Fecal coliforms constitute a minor proportion of the overall coliform population. *E. coli* is widely recognized as the main coliform bacteria, indicative of the presence of fecal matter. Another study demonstrated that a limited number of *Ruminococcus*, *Bifidobacterium*, and *Peptostreptococcaceae* bacteria were found in the same animals. While these findings do not definitively prove the theory that intestinal bacteria are transferred into mammary secretions in cows, they do provide evidence for the presence of a natural conduit inside the cow's body for some bacterial components to travel from the intestines to the mammary glands during lactation [10, 11]. Raw milk can become contaminated either internally or endogenously. Internal contamination originates when an animal becomes infected with pathogens that are subsequently transported to the bloodstream (systemic infection) or infect the udder, resulting in the transfer of these microbes into the raw milk [12]. Several bacteria can cause udder infection and mastitis, but most cases were caused by *Streptococcus* spp. or *Staphylococcus* spp. Directing programs toward the most commonly occurring pathogens enhances efficiency in mastitis control, which is reflected in milk hygiene [13]. Exogenous or external contamination refers to milk contamination during or after collection. Feces can cause this contamination, as well as the outer surface of the udder, teats, skin, and other sources of environmental contamination [14]. Various factors can influence the contamination of raw milk by harmful bacteria, both those that cause spoiling and those that are pathogenic. These factors include the dairy animals' health, the milking process's cleanliness, the circumstances in which the milk is stored, the environment, the procedures followed in managing the farm, and the variations in location and season [15]. In line with our study, numerous investigations were conducted to examine the prevalence of bacteria in raw milk. One of these studies found that the highest percentage of *Pseudomonas aeruginosa* isolates, reaching 80%, was obtained from raw milk in Diyala Province [16]. In Baghdad city, the isolation percentages of total coliform, fecal coliform, *Escherichia coli,* and *Staphylococcus aureus* in raw milk were 82%, 69%, 54%, and 42%, respectively [17]. Another study in Poland exhibited the presence of seven bacterial species (*Enterobacteriaceae*, *Enterococcus*, *Escherichia coli*, *Staphylococcus*, *Salmonella*, and *Listeria monocytogenes*) in unpasteurized domestic raw milk [18]. Previous studies have shown a lack of detection of bacterial contamination in raw milk in Saladin Governorate. The current paper assesses the microbial quality of both raw and packaged cow's milk. More specifically, this study aims to determine the prevalence of some of the usual bacterial strains that are isolated from milk and their respective patterns of resistance to antimicrobial agents. The research attempted to evaluate factors that make microbes contaminate milk and the consequent dangers for consumers.

Material and methods

Sample collection

Ninety milk samples were obtained from Saladin province markets, representing 30 samples of local raw milk, 30 samples of imported packaging cow milk (nada), and 30 samples of imported packaging cow milk (kalleh) from various regions. The samples were stored in a sterile plastic bag and a secure freeze

box. They were promptly transported to the biology department laboratories at the College of Education, University of Kirkuk. The samples were handled hygienically and inspected quickly without any delays. Subjected to examination upon arrival at the laboratory for bacteriological study, following the procedure outlined by Islam et al. [19].

Bacterial analysis

At first, 25 ml of each local raw milk, local packaging milk, and imported packaging milk samples were poured into a sterile flask containing 225 ml of 0.1% peptone water. Subsequently, the mixture was thoroughly blended. Each sample was diluted in 0.1% peptone water several times to make future serial decimal dilutions [20].

Culturing the sample's swabs

The swabs were cultured by inoculating them into a nutrient broth and then incubating them at 37^{o} C for 5 hours. A small amount of the incubated broth was evenly spread across the surface of MacConkey agar using a loop. The agar plate was then placed in an incubator at 37^{o} C for 24 hours, following the protocol described by Stromberg et al. [21].

Characterization and identification of the colony

The colony isolates were defined and identified based on an initial morphological analysis of the colonies observed on the plate. Bacteria are identified and classified using the gram staining method and biochemical testing, as previously described by Bergey's Manual [22].

Statistical analysis

The IBM SPSS statistical software version 20 package was employed to determine how each element affected the study's parameters and to find out whether there were any statistically significant differences among means, mean, standard error, and LSD [23].

Results

The study results (as shown in Table 1) indicated the presence of various bacterial species in the three types of milk. The domestic raw cow milk and imported packing milk from Kalleh showed contamination with *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staph saprophyticus*, *E. coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Proteus mirabilis*. At the same time, the imported packaged milk (nada) was contaminated with *Staphylococcus aureus*, *Staphy. epidermidis*, and *E. coli*.

The results of the bacterial isolates in each milk sample indicated that the highest number of bacterial isolates was found in raw domestic milk and imported packaging milk from the brand "Kalleh," particularly in comparison to the imported packaging milk from the brand "Nada". The predominant bacterial isolate was *Staph. aureus*. At the same time, the least common one was *P. aeruginosa* among all contaminated milk samples, as shown in Table (2) and Figure (1, 2, 3, 4, 5, 6).

Table (1): Bacterial identification in various milk sources.

Bacterial type	Domestic raw cow milk	imported packaging milk from the brand "Nada."	imported packaging milk from the brand "Kalleh."
Staphylococcus aureus	+	+	+
Staphylococcus epidermidis	+	+	+
Staph saprophyticus	+	-	+
Escherichia coli	+	+	+
Pseudomonas aeruginosa	+	-	+
Enterobacter aerogenes	+	-	+
Proteus mirabilis	+	-	+
+: signified existence; -: absence			

Table (2): Percentage of isolates of bacteria present in milk samples.

Bacterial type	Mean ± SE [a]			
	imported packaging milk from the brand "Nada."	imported packaging milk from the brand "Kalleh."	Domestic raw cow milk	LSD [b]
Staphylococcus aureus	**A** 9 ± 1.155 **b**	**A** 12 ± 1.155 **b**	**A** 21 ± 0.577 **a**	**6.92**
Staphylococcus epidermidis	**B** 3 ± 0.577 **b**	**B** 6 ± 1.732 **ab**	**B** 12 ± 1.155 **a**	**8.64**
Staph saprophyticus	**B** 0 ± 0.00 **b**	**CB** 3 ± 0.577 **ab**	**BC** 6 ± 0.577 **a**	**3.26**
Escherichia coli	**B** 3 ± 0.577 **b**	**B** 6 ± 0.577 **ab**	**BC** 9 ± 1.155 **a**	**5.66**
Pseudomonas aeruginosa	**B** 0 ± 0.00 **a**	**CB** 3 ± 0.577 **a**	**C** 3 ± 0.577 **a**	**N. S**
Enterobacter aerogenes	**B** 0 ± 0.00 **b**	**CB** 3 ± 0.577 **ab**	**BC** 6 ± 1.732 **a**	**3.35**
Proteus mirabilis	**B** 0 ± 0.00 **b**	**CB** 3 ± 0.577 **ab**	**BC** 6 ± 0.577 **a**	**3.26**
LSD[b]	**3.24**	**5.62**	**6.06**	**-**

a: Standard error, b: Least Significant Difference, Unique capital letters indicate statistically significant differences (P<0.05) between the means of each column. When there are statistically significant differences (P<0.05), the means of the respective row are indicated by unique lowercase letters. N=30

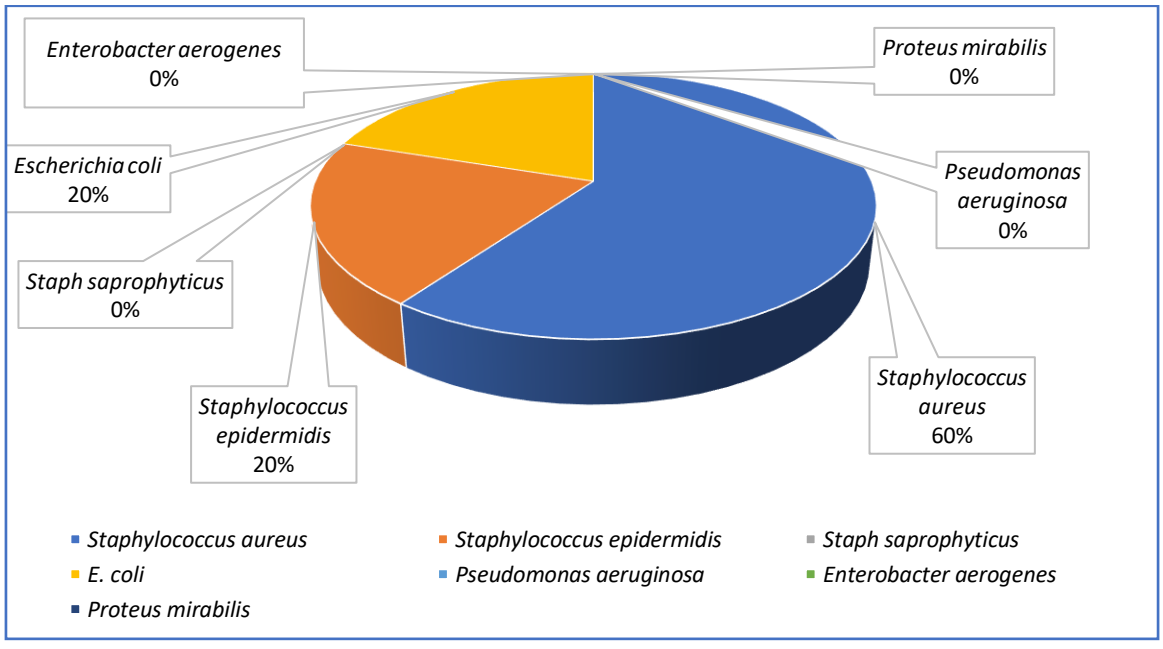

Figure (1): The proportion of bacterial isolates in the sample of imported packaging milk from the brand "Nada"

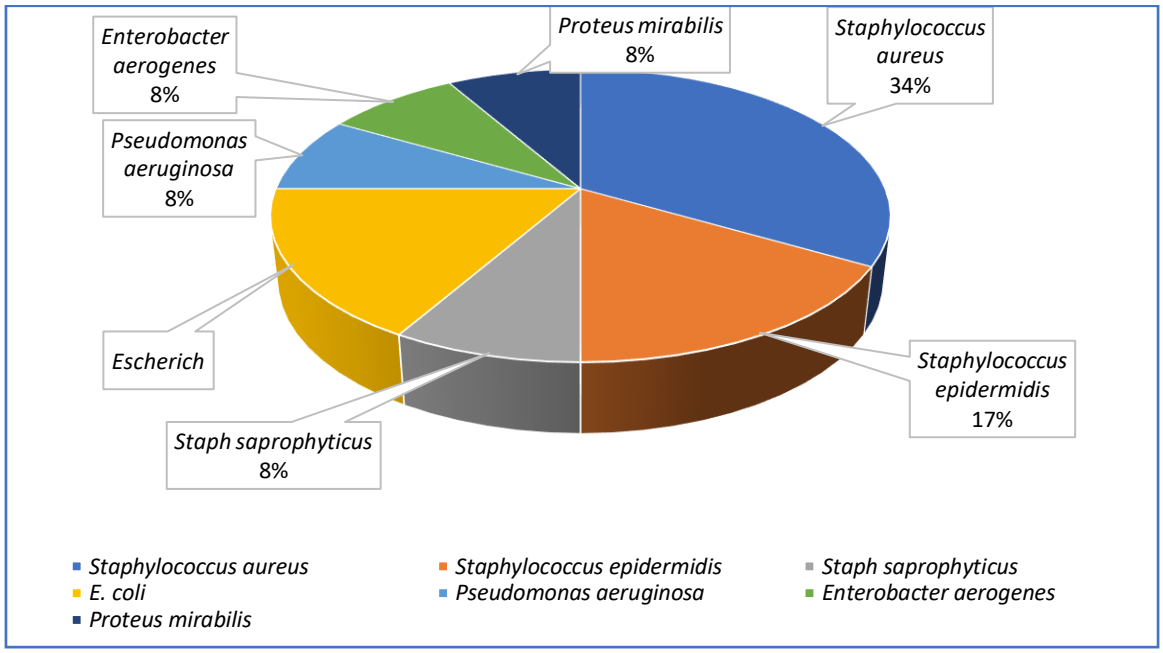

Figure (2): The proportion of bacterial isolates in the sample of imported packaging milk from the brand "Kalleh"

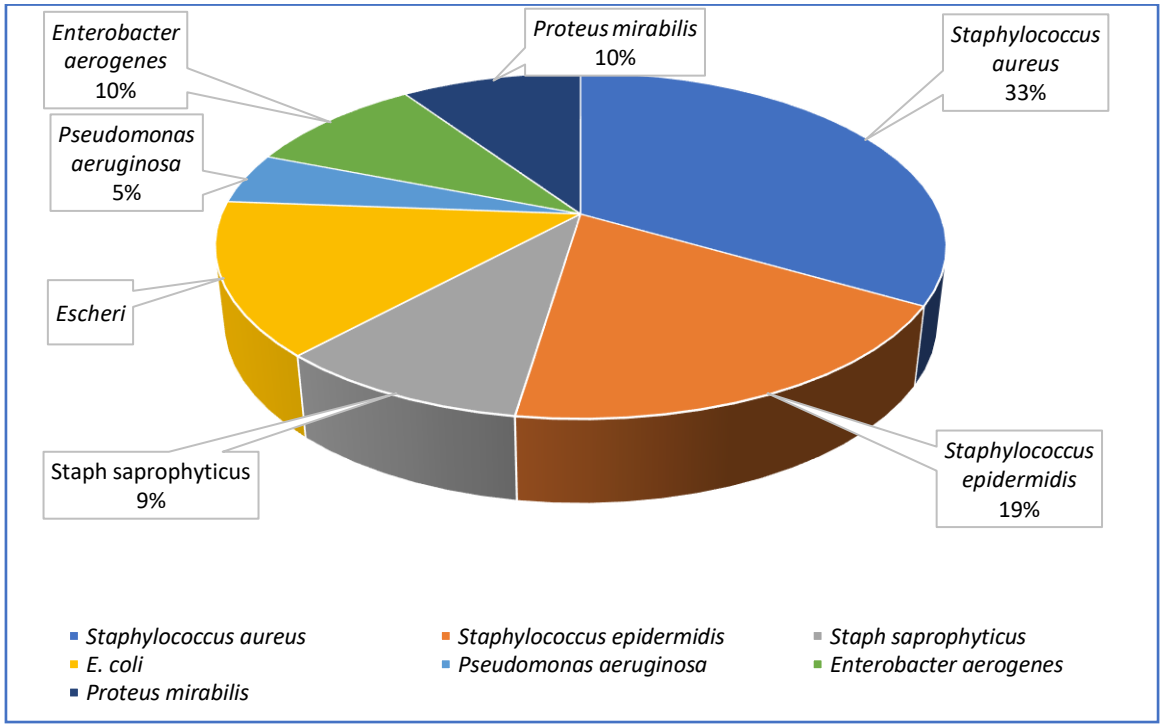

Figure (3): The proportion of bacterial isolates in the domestic raw cow milk sample.

Figure (4): cultured bacteria obtained from a raw milk sample on MacConkey agar medium.

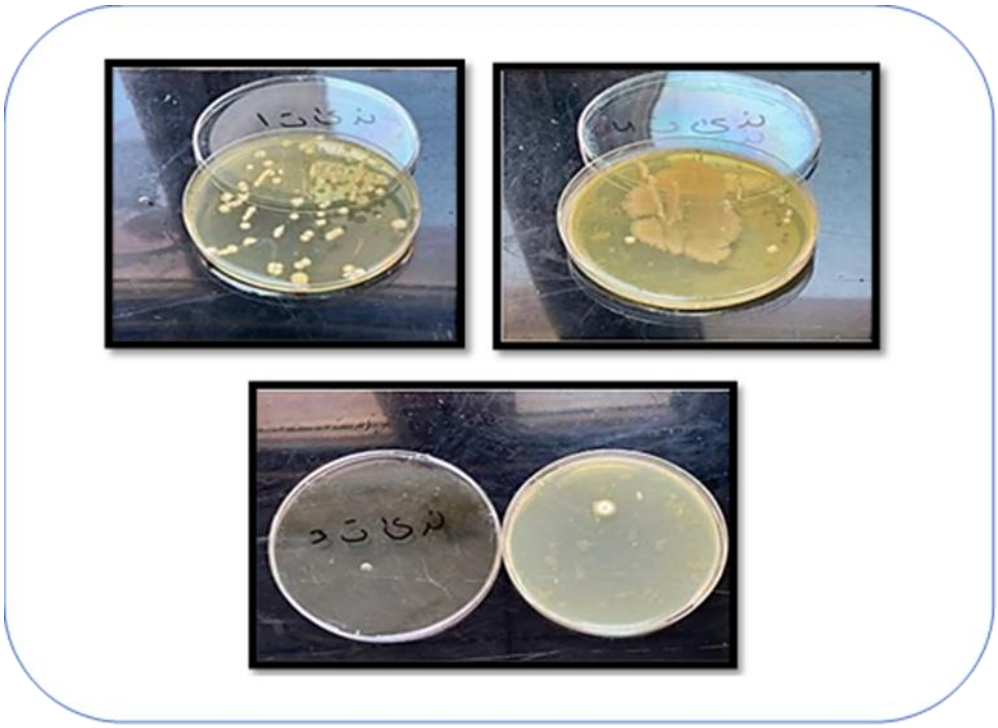

Figure (5): cultured bacteria obtained from imported packaging milk from the brand "Nada" sample on nutrient agar medium

Figure (6): cultured bacteria obtained from imported packaging milk from the brand " Kalleh" sample on nutrient agar medium.

Discussion

The study revealed that raw milk was contaminated with miscellaneous species of bacteria, the most common of which was Staph. aureus, with a prevalence of 21%. Our results come in agreement with Papadopoulos et al. [24]. Whose reported that found a prevalence of 24.14% of *Staph. aureus* in Ethiopian raw milk. Another study in northern Greece exhibited a prevalence of 21.1% [25]. While the contamination with *Staph. epidermidis* in unpasteurized milk from domestic sources was found to be 12%, another study in Ukraine reported a percentage of *Staph. epidermidis* at 9% [26]. A detailed study in Iran reported a prevalence rate of 15.7%. In the present study, it was demonstrated that the occurrence of *E. coli* in unpasteurized household milk was 9%. This finding aligns with earlier research that has documented a prevalence rate of 10.9% in India and 10.4% in Iraq, Diyala City [27]. In our study, the prevalence of *Pseudomonas aeruginosa* in raw milk was 3%. While a study conducted in China documented a prevalence of 2%. Another study exhibited a prevalence of 7% [28].

Our study found that the prevalence of *Enterobacter aerogenes* in raw milk was 6% in residents. A detailed study conducted in Egypt reported a rate of 13% [29]. Additionally, another study in Egypt found a prevalence of 17.5% [30]. The prevalence of *Proteus mirabilis* in our study was 6%; in a study in India, it was mentioned there was a prevalence of 7.5% [31].

Our analysis revealed a diverse spectrum of bacterial occurrences in imported milk samples. Inadequate hygienic standards during milking may lead to contaminated raw milk with bacteria, increasing the chances of intermammary infection by bacteria. The bacterial contamination of post-milking liners, detected after milking most cows, arises from the healthy skin of cows' teats and teat canals. The udder of sick cows is the main source of infection, as it transmits germs through many means, such as the milker's hands, utensils, towels, and the floor of the cow's housing environment. low hygiene criteria, dirty manufacturing units and machinery, bad sanitation practices by farm staff, poor quality materials employed, and contaminated water utilized for utensil washing. Likewise, the adjacent surroundings, such as bedding, air, grass, and collection vessels, may contribute to milk products' heightened bacterial contamination throughout and following the manufacturing process. Furthermore, individuals working in dairy farms played a substantial role in the heightened bacterial contamination. Consequently, milkers must thoroughly sanitize their hands before milking cows. The variability among these criteria can explain the differences in the percentage of bacterial prevalence in various cities [32 - 36].

A limitation of our investigation was that we only collected specimens from one town, Saladin Governorate, rather than from many cities. That was done to focus on the contamination of raw milk with bacteria in that specific region. Collecting and storing milk samples from several towns in Iraq proved challenging.

Conclusions

Our study found that raw domestic milk in Saladin Governorate cities is contaminated with *Staph. aureus*, *Staph. epidermidis*, *Staph. saprophyticus*, *E. coli*, *P. aeruginosa*, *E. aerogenes*, and *P. mirabilis*. Each type of bacteria has a different prevalence percentage. This contamination is due to inadequate hygienic standards during milk handling. Imported milk also showed bacterial contamination but was less prevalent than domestic raw milk. This may be due to post-manufacturing contamination. Based on the results, it is recommended that milk consumers pasteurize both raw domestic and imported milk before consumption to minimize the risk of foodborne illnesses.

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