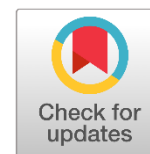




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Isolation and Identification of Gram-Positive and Gram-Negative Bacteria from Different Types of Cheese in Baghdad's Local Markets

Ali Nayyef Umayra^a, Jabbar S. Hassan^{*b}, Hala Faez Abdul-Hadi^c

^a Medical Microbiology, Al-Qasim Green University, Baghdad – Iraq

^b Medical Microbiology, College of Medicine, Al-Nahrain University, Babylon – Iraq

^c Department of Biology, College of Science, University of Babylon, Babylon – Iraq

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ABSTRACT

The current study was undertaken to determine the occurrence of different types of bacteria in cheese samples. A total of 100 samples of white cheese were collected from different locally supermarkets. Plate Count Agar was used to determine the total Counts of the samples. Ten milliliters of the pre-enrichment, sample were incubated in Petri dishes from Violet Red Bile Agar (VRBA), Mannitol Salt agar, and Xylose Lysine Deoxycholate agar (XLD agar) mixing vigorously to ensure homogeneity. The agar was put upside-down in the laboratory incubator that was set to 37°C overnight. Plates with 20 to 300 colonies were counted using Manual Colony Counter with a 100mm magnifying glass. The results of current survey based on microbial plate counts showed that there are different types of bacteria present in different kinds of cheese samples. The most predominant bacterial species isolated from cheese were *E. coli* and *Staphylococcus aureus* in which a considerable number of contaminations with these bacteria were detected. The current study demonstrated a high number of bacterial isolates particularly *E. coli* and *S. aureus* in the cheese samples as a result of poor cheese manufacturing and storage conditions, inadequate refrigeration, and a lack of quality control measures throughout transportation.

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1. Introduction

The term "cheese" refers to a broad range of dairy-based food items and cheese is one of the most popular milk products. It is made all over the world in a diverse range of flavors, textures, and designs (Khalaf, 2018). Due to its high

protein, fat, calcium, iron, and phosphorus contents as well as its good source of vitamins and vital amino acids, cheese is a valuable food for both young and old individuals (Ferrari, et al., 2019). Due to the contents, Cheese is an excellent medium since it contains nutrients such as proteins, lipids, minerals, and vitamins. Processed cheese may be contaminated with different types of microorganisms, including various fungi and pathogenic bacteria species like *Staphylococcus aureus* and *Salmonella* species that may be grown in it, so the time of preservation should not last longer than a few days (Imre, et al., 2022).

Foodborne infections have been identified as an important public health and economic problem in developed as well as developing nations. Hence, microbial food safety has emerged as a significant global issue for the consumer, industry, researcher, and regulatory bodies. Microbial contamination of milk and dairy products is a universal

***Corresponding author:** Jabbar S. Hassan, Medical Microbiology, College of Medicine, Al-Nahrain University, Babylon – Iraq

E-mail: jabbarsalman30@yahoo.com

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problem. Foodborne microbial diseases account for twenty million cases annually in the world. The microbial load and incidence of bacterial pathogens in foods are indicators of food quality (Abdulghani & Kareem, 2019). The primitive methods used for manufacturing cheese from unpasteurized raw milk with the absence of standard hygienic measures afford the pathway for its contamination with different foodborne pathogens and subsequently hazard to consumers (Mohamed, et al., 2021). The aim of the current study was to isolate and determine the occurrence of different types of bacteria in different cheese samples.

2. Materials and Methods

Sample Collection

A total of 100 samples of white cheese were collected from different supermarkets in Baghdad province at Al-Kadhimiya, Al-Shu'ala, Al-Hurriya, and Al-Taji; some of these cheeses included in the study are imported and others are locally made. Those samples were microbiologically quantified in the period between (October to November) 2022. All samples were placed in sterile and cold storing conditions by crushed ice and examined for microbial plate counts within 4-6 h. Each sample was macerated well according to Duncan et al. (2004) with a sterile spatula and the needed amount of each sample was weighted carefully in sterile conditions (Duncan, et al., 2004).

Basic dilution (10^{-1}) was obtained by mixing 25 g of each sample with 225 ml of warmed (40 to 45 °C), sterile, 2% sodium citrate solution ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) (Laird et al., 2004), and then followed by homogenization of the sample twice for 2 minutes and left for 30 min before the completion of the work. Serial dilution was prepared for each sample of buffered peptone water (Laird, et al., 2004).

Identification and Isolation of Bacteria

Total Microbial Count

Plate Count Agar was used to determine the total Counts in the samples. Ten milliliters of the pre-enrichment sample were incubated in Petri dishes from Violet Red Bile Agar (VRBA), Mannitol Salt agar, and Xylose Lysine Deoxycholate agar (XLD agar) mixing vigorously to ensure homogeneity. The agar was put upside-down in a Laboratory incubator that was set to 37°C overnight. Plates with 20 to 300 colonies were counted using Manual Colony Counter with a 100mm magnifying glass (Zimbro, 2003) (Zimbro, et al., 2003).

Coliform Bacteria

Coliform bacteria count in cheese samples was done on MacConkey and Violet Red Bile Agar (VRBA). Dilutions of 10^{-3} and 10^{-4} were used to determine Coliform Bacteria. Petri dishes were cultivated upside-down in an incubator at 32 °C for 24 h. colonies with a violet color and 1-2 mm in diameter were counted (Davidson, et al., 2004).

Staphylococcus Aureus

Blood agar and Mannitol salt agar were used to count it. The plates were left for 15 minutes and then incubated upside down at 32 °C for 48 h. Yellow colonies with yellow zones were counted.

Salmonellae

According to Abdulghani (2019), all cheese samples were tested for the presence of Salmonella species. Cheese sample equal to 25 ml was pre- enriched in 225 ml of buffered peptone water, then incubated at 37 °C for 24 hours, following that, 10 ml of this sample was re-incubated for 24 hours at 37 °C in 100 ml of tetrathionate broth base. After an incubation time enrichments samples were streaking onto XLD Agar, the media were incubated at 37°C for 24 hr. Salmonella species were found to have red colonies with black centers (Abdulghani & Kareem, 2019).

3. Results and Discussion

The results of the current survey based on microbial plate counts showed that there are different types of bacteria present in different kinds of cheese samples, especially in levels of *E. Coli* and *Staphylococcus aureus* during the study. The most predominant bacterial species isolated from cheese were *E. Coli* and *Staphylococcus aureus* in which a considerable number of contaminations with these bacteria was detected. however, a large number of *E. coli* number (2×10^{10}) was reported in Kanoon cheese made in Turkey. While; Vonk cheese made in Hungary reported (3×10^{10}) *S. aureus* isolates. Furthermore; Salmonella species was isolated from Salty Arab and Sweet Arab cheeses (Iraqi homes made); in addition to that, *Pseudomonas* species was identified in Salty Arab cheese/Homemade; as shown in table (1). Cheese can get microbial contaminated by a number of factors, including the packaging, the handler, and the environment (Pal, et al., 2014; Alshaibani, 2020).

In the current study, different Gram-Negative and Gram-positive Bacteria were isolated from imported, local, and homemade cheese. *E. Coli* and *Staphylococcus* species counts were generally high. These results surpass the Iraqi Quality Standards (Microbiological Limits in Food IQS: 2270/5), which allow for a maximum of 10^2 CFU/g of coliform, and *Staphylococcus aureus* and Salmonella should also not be present.

Table 1

Different kinds of bacteria obtained from different samples of cheese

Almost the types of cheese included in present study being imported and manufactured outside Iraq, the isolation of different types of bacteria in these cheeses is remarkable, such findings make such products unacceptable and reflected unhygienic conditions used during production and storage. Numerous authors have noted that the presence of coliforms such as *E. coli* in food samples typically denotes either direct or indirect fecal contamination of milk or its product during handling, processing, and distribution, and consequently the potential for having pathogenic bacteria, viruses, or protozoa of fecal origin in the food (Pal, et al., 2016; Pal, 2014). While in the case of *Staphylococcus aureus*; the cheese vendors are probably to blame for the *S. aureus* contamination in addition to that cow may nevertheless, expel *S. aureus* from the udder, frequently without showing any signs of mastitis (Jaber, 2011).

Any point in the food chain that is susceptible to food contamination such events can lead to food spoilage which refers to any alteration that makes it unfit for ingestion by humans. Numerous factors affect the food's microbiological safety when it is being stored; starting from the final phase of production and consumption by humans for instance, the process of transporting and storing products over long

distances and when accompanying by instability of temperatures can lead to propagation of bacteria and other microbes in such food (Vrdoljak, et al., 2016).

Two types of homemade Iraqi cheese called (Gibin Al-Arab) were enrolled in the current study which is Salty Arab cheese and Sweet Arab cheese; interestingly *Salmonella* species and *E. coli* were isolated from the two types, while *Pseudomonas* species isolated from Salty Arab cheese only. Human error and unsanitary environments are the main causes of milk and milk-derived products contamination. At locations where milk is collected, several microorganisms frequently contaminate the milk; The farmers use a conventional process to make soft cheese, paying no attention to the quality of the raw milk or the products' hygiene standards. Many germs can reach milk products in these circumstances (Saab Khudhir, et al., 2013). *Salmonella* contamination was predicted because this sort of cheese is made from unpasteurized milk, and it is also more likely to occur because of the poor hygiene practices used in its production, processing, and distribution in our local markets (Nazal, 2013).

Raw milk is the source of many of the microbial risks connected to dairy products including butter, cheese, and yogurt. The dairy sector continues to face challenges in preventing post-process contamination by spoiling bacteria and slowing the growth of surviving organisms. Therefore, it is important to give the microbiological analysis of milk and dairy products more attention. It is important to note that when making dairy products, proper hygiene procedures should be taken and milk should be thoroughly pasteurized. Additionally, from the perspective of food safety, it is crucial to teach food handlers about personal cleanliness (Pal, et al., 2016).

4. Conclusion

In the current work, the high number of bacterial isolates particularly *E. Coli* and *S. aureus* in the studied cheese samples may be due to the poor cheese manufacturing and storage conditions, inadequate refrigeration, and a lack of quality control measures throughout transportation. The quality of the raw materials, as well as the environmental and hygienic conditions during preparation and serving, should be under control. The research's findings showed that the local cheese made by Abu-Garib factories never contaminated with any type of bacteria. While the homemade cheese from Iraq, known as "Gibin Al- Arab," had poor hygienic quality and insufficient assurance for the general public's health. It is important to take precautions to control the quality of the raw materials, the environment, and the hygienic conditions during preparation and serving. Markets and processing facilities should also undergo routine expert inspections.

Solutions for Such Problems

- Activate the correct role of health control
- Follow the international ISO system that monitors the product from the factory to the consumer, which is applicable in most countries of the world.
- Upon the product's arrival at the country's borders, the importing merchant's refrigerated warehouses must be there and ready to store the product safely and keep it cool while the importation paperwork is processed.

- The product is not permitted to cross the border until the laboratory examination is complete and its suitability for human consumption is confirmed.

Competing Interests

The authors had no competing interests.

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