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Assessment of antibiotic residues and microbiological safety in Lebanese dairy products: Akkawi cheese, yogurt, and labneh, collected across Lebanese dairy industries

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Abstract

Antibiotic residues in dairy products and the resulting antibiotic-resistant bacteria represent a major global public health concern. This study evaluated the microbiological safety of 400 dairy samples collected over two consecutive years (2022 and 2023) from 16 dairy residue industries across four governorates in Lebanon. In addition, antibiotic residues were screened from 126 dairy product samples randomly chosen out of the 400. An optimized multi-class method for the identification and quantification of 30 antibiotics was developed using liquid chromatography-mass spectrometry. Results revealed that 48% of the analyzed samples were contaminated with at least one antibiotic residue, among which 33% showed co-occurrence of multiple antibiotic classes. Meanwhile, microbiological assessment of 400 dairy samples collected over 2 years from the same dairies revealed higher levels of contamination with total coliforms, *Escherichia coli*, and *Staphylococcus aureus* in *Akkawi* cheese samples collected during the first year (44%, 32%, and 15%, respectively) compared to those collected the second year (38.8%, 31%, and 0%). Moderate contamination levels were observed in *Labneh* (strained yogurt) samples collected during both seasons, with yeast (12%; 11%), molds (1.2%; 11%), total coliforms (6%; 11%), and *Escherichia coli* (3.7%; 11%). On the other hand, comparable levels of non-conformity were observed in yogurt samples across the two years, with contamination by yeast (7.5%; 6.2%), molds (3.75%; 6.2%), total coliforms (26%; 31.2%), *E. coli* (20%; 21%), and *Staphylococcus aureus* (1.25%; 0%). *Escherichia coli* isolates exhibited resistance to tetracycline while being 100% sensitive to ciprofloxacin and nalidixic acid. As for *S. aureus* isolates, they presented a high sensitivity to tetracyclines, doxycycline, and ciprofloxacin. These findings highlight the importance of implementing and maintaining good hygienic practices within dairy industries. Antimicrobial residues of oxytetracycline, tetracycline, ciprofloxacin, sulfamethazine, and doxycycline were detected at levels below the maximum residue limits.

Introduction

The Lebanese dairy sector has expanded and diversified over the years, highlighting its economic significance within the agro-industrial sector. The industry has evolved from a limited number of small-scale processing units into a heterogeneous system that includes semi-artisanal processors and modern well-established firms with advanced processing capacities.

Recent reports indicate the presence of 276 dairy-processing units (IFAD, 2017), and approximately 100 formal factories (USAID, 2017), with a concentration in the Bekaa Valley and Mount Lebanon. This growth reflects the sector's adaptability and its increasing contribution to rural livelihoods, local food security, and value-added dairy production.

However, many processing units operate without sanitary licenses from the Ministry of Agriculture, making it difficult to monitor and ensure the safety of dairy products in the market.

The Lebanese dairy industry comprises modern, semi-modern, and traditional operations, sourcing milk from local farmers, collection centers, and in some cases, their own farms, particularly in large-scale industries. Many dairies are semi-artisanal plants with limited control over milk quality and low innovation capacity in cheese and dairy product development. Moreover, a significant number of dairies do not maintain their own herds, relying primarily on milk sourced from local farmers. To improve milk sourcing and quality, the Ministry of Agriculture has supported the establishment of milk collection centers; currently, there are 40 centers in the Bekaa and Akkar regions, collecting 150-200 tons of milk per day from approximately 3000 farmers.

Milk collectors, commonly known as "Hallab," play a prominent role in the Lebanese dairy industry by linking milk producers with processors. In many cases, milk collectors are also producers themselves and manage the collection, transportation, and sale of milk on behalf of a network of farmers (Dib *et al.*, 2008).

The most commonly produced dairy products in the Lebanese market are cheese, labneh, and yogurt, with Akkawi cheese being the most widely manufactured due to its high demand in households and traditional dishes. Other cheeses, such as Halloumi, Baladi, and Shanklish, are produced on a smaller scale compared to Akkawi. Small-scale processors typically obtain milk either from local collectors or

directly from farmers, ensuring a consistent supply to meet production needs. These dairy products, valued for their rich nutrient content, can serve as reservoirs for a wide range of microbial pathogens such as coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, and *Listeria*, posing a potential risk for the transmission of foodborne diseases (Oliver *et al.*, 2005). Several foodborne outbreaks have been linked to the consumption of dairy products, with reported cases involving *E. coli* (Oliver *et al.*, 2005), *Staphylococcal enterotoxins* (Le Loir *et al.*, 2003), *Salmonella* (Olsen *et al.*, 2004), and *Listeriosis* (Carrique-Mas *et al.*, 2003).

In Lebanon, no officially reported outbreaks of foodborne disease have been associated with the consumption of dairy products or their potentially contaminating ingredients. This lack of reported cases is likely attributable to the absence of systematic registration and monitoring of foodborne incidents, rather than the absence of contamination, highlighting the need for improved monitoring and stricter hygiene controls within the Lebanese dairy sector.

Several studies have documented the presence of microbial pathogens in locally produced cheeses and other dairy items. Pathogens such as *Coliforms*, *Escherichia coli*, *Salmonella*, and *Listeria* have been detected in Akkawi, Baladi, and double cream cheeses, indicating potential food safety risks (Dib *et al.*, 2008).

Antibiotic treatment plays a crucial role in maintaining the health of dairy livestock (Quintanilla *et al.*, 2018). It is used for therapeutic purposes as well as for improving breeding efficiency. However, their use can result in residues remaining in treated animals, which may subsequently contaminate edible products such as milk and dairy items, thereby increasing human exposure to antibiotics (Bergwerff and Schloesser, 2003).

While assessing the use of antibiotics, exceptional application of such drugs is legally considered (European Parliament and the Council of the European Union, 2001). However, the risk of antibiotic residues in milk and dairy products may increase if the required withdrawal period is not well defined. Studies conducted on assessing antibiotic residues in dairy goats showed that the minimum seven-day withdrawal period established by legislation for off-label treatments is not always sufficient to ensure complete elimination of drug residues in milk (Ferrini *et al.*, 2010; Amer *et al.*, 2012).

Antibiotic residues in dairy products can have detrimental effects on consumer health by promoting microbial resistance, disrupting the intestinal flora, and triggering allergic reactions that may lead to anaphylaxis (Graham *et al.*, 2014). Beyond these health risks (European Food Safety Authority and European Centre for Disease Prevention and Control, 2018), such residues can also adversely affect the dairy industry by inhibiting the fermentation of milk into products such as cheese and yogurt (Cabizza *et al.*, 2017). Consequently, antibiotic screening represents an essential quality control measure in the production of milk and dairy products, as a lack of regulatory monitoring may result in the persistence of residues in cheese and other dairy products.

In Lebanon, few studies have investigated the occurrence of antibiotic residues in cow's milk and dairies. Among the limited available research, Joubrane *et al.* (2022) conducted a comprehensive investigation on microbiological contamination, antimicrobial residues, and antimicrobial resistance (AMR) in raw bovine milk collected from different regions of the country. Their findings revealed that a significant proportion of milk samples contained detectable levels of antibiotic residues, highlighting gaps in on-farm management practices and regulatory monitoring.

A study investigating the occurrence of gentamicin and streptomycin residues in raw milk reported that the mean concentrations of both antibiotics were below the maximum residue limit of 200 µg/L established by FAO/WHO guidelines (Zeina *et al.*, 2013). These findings suggest that, under the studied conditions, antibiotic residues in milk samples remained within internationally accepted safety thresholds, indicating compliance with food safety standards.

Similarly, (Kabrite *et al.*, 2019) assessed tetracycline and penicillin residues in 44 samples of milk (22), yogurt (10), and labneh (12) collected from Keserwan regions, showing that all estimated daily intake (EDI) values were below the acceptable daily intake (ADI) set for each antibiotic residue and did not exceed relevant toxicological reference values.

In a study conducted by El Kojok *et al.* (2022), fifty samples of traditional Lebanese cheeses were subjected to microbiological and chemical analyses, revealing that approximately 14% of the samples contained detectable levels of the antibiotic sulfamethazine.

Despite these studies, little is known about the microbiological profile and antibiotic residue levels in Akkawi cheese, labneh, and yogurt available in the Lebanese dairy sector. The main purpose of this study was to monitor the occurrence of antibiotic residues in dairy product samples collected from Lebanese farms over two seasons, providing data for subsequent exposure assessment. The study also aimed to evaluate the microbiological profiles and AMR patterns of pathogenic *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* isolated from the same dairy samples, and to assess the relationship between bacterial resistance and the detected antibiotic residues.

Materials and Methods

Study area and sample collection

In the present study, a total of 400 dairy product samples, including Akkawi cheese, labneh, and yogurt, were randomly collected from May to September over two consecutive years (2022-2023) from 16 dairy industries across four governorates of Lebanon: North Lebanon (NL), South Lebanon (SL), Bekaa (BL), and Mount Lebanon (ML).

The number of samples collected from each governorate was proportional to the volume of dairy production, with 52% of the visited industries located in the Bekaa Valley, the main dairy-producing region. Table 1 summarizes the sample sizes and sources for the years 2022 (a) and 2023 (b) of the collection periods. Two samples of yogurt and labneh from each dairy industry were subjected to antibiotic residue testing.

Collected samples (Akkawi cheese, labneh, and yoghurt) were placed in separate plastic zipper bags, properly labeled, and transported to the laboratory in ice-packed containers. Upon arrival, they were stored at refrigeration temperatures (2-4°C) until analysis.

Experiments were conducted at the Department of Biological Sciences, Faculty of Science, Lebanese University, in collaboration with the Faculty of Public Health, Lebanese University. These institutions provided the necessary facilities and expertise for the comprehensive evaluation of microbiological quality, antimicrobial residues, and resistance profiles in raw bovine milk samples collected across Lebanon.

Microbiological analysis

The microbiological quality of dairy samples, including Akkawi cheese, Labneh, and yogurt, was assessed according to International Organization for Standardization (ISO) guidelines. Analyses targeted total coliforms (ISO, 2006), *Escherichia coli* (ISO, 2001), *Staphylococcus aureus* (ISO, 1999), *Listeria monocytogenes* (ISO, 2017), and *Salmonella* (ISO, 2017). Yeasts and molds were additionally analyzed in Labneh and yogurt using standard mycological methods following FDA (2001) guidelines. Microbial analyses of the collected samples were conducted immediately, and all tests were performed in duplicate to ensure accuracy. Twenty-five grams (or 25 mL) of each dairy sample were aseptically transferred into a sterile plastic bag containing 225 mL of sterile saline solution for homogenization. Homogenization was carried out using a stomacher machine (Model 1605 BL Smart) for 3 minutes. These bags were incubated for 30 min at ambient temperature, followed by 24 h at 37°C to ensure pre-enrichment. Serial ten-fold dilutions were prepared from the homogenized samples using sterile diluent. Appropriate dilutions were selected for each microbiological analysis, and 1 mL or 0.1 mL aliquots were inoculated onto the corresponding selective media for enumeration. Table 2 presents the microbiological parameters investigated and the corresponding identification techniques based on ISO reference analytical methods.

For total coliform enumeration, 1 mL of each dilution was spread onto Violet Red Bile Agar (VRBA) and incubated at 37°C for 24 h. Colonies were counted and expressed as CFU/g.

For total coliform enumeration, 1 mL of each dilution was spread onto Violet Red Bile Agar (VRBA) and incubated at 37°C for 24 h. Colonies were counted using a colony counter and expressed as CFU/g.

Representative colonies were subcultured on nutrient agar slants under the same incubation conditions and subjected to biochemical identification, including catalase, citrate, indole, methyl red, Voges–Proskauer, and urease tests (ISO, 2006).

Enumeration of *E. coli* was performed using the chromogenic selective medium Tryptone Bile X-Glucuronide (TBX) for each 1 mL of the sample suspension and its first dilution. Following incubation at 44 °C for 24 h, green colonies, indicative of β -glucuronidase-positive *E. coli*, were counted (ISO, 2001).

For the enumeration of *S. aureus*, 0.1 mL of the sample suspension was spread on the surface of Baird–Parker agar and incubated aerobically at 37°C for 24–48 h according to ISO 6888-1:1999. Typical colonies appearing as black colonies surrounded by a clear halo were counted as presumptive staphylococci. Presumptive colonies were further subjected to confirmatory identification to distinguish *Staphylococcus aureus* from other staphylococcal species. In particular, isolates were tested for coagulase production using fresh rabbit plasma, and only coagulase-positive isolates were confirmed as *Staphylococcus aureus*. Coagulase-negative staphylococci were excluded from further analysis.

Detection of *Salmonella* spp. was carried out as follows: 25 mL of the sample was transferred to 225 mL of buffered peptone water for pre-enrichment and incubated at 37°C for 24 h. The pre-enrichment culture was then inoculated into Rappaport-Vassiliadis soy broth and Müller-Kauffmann tetrathionate broth, incubated at 41.5°C and 37°C, respectively. After 24 h, 0.1 mL of the enriched cultures was plated on Xylose Lysine Deoxycholate (XLD) and Salmonella–Shigella (SS) agar and incubated at 37°C for 24 h (ISO, 2017). Typical *Salmonella* colonies appeared as black-centered with light transparent areas on XLD agar, and as translucent or transparent colonies with generally black centers on SS agar. Presumptive *Salmonella* isolates were confirmed using biochemical tests, including the API 20E system (bioMérieux, Marcy l'Étoile, France), as well as urease and indole activity determination.

For *Listeria monocytogenes* isolation, samples were analyzed according to ISO 11290-1:2017 (ISO, 2017). Briefly, 25 mL (or 25 g) of each sample was aseptically transferred into 225 mL of Half Fraser broth and incubated at 30°C for 24 h for primary selective enrichment. Subsequently, 0.1 mL of the primary enrichment was transferred into Fraser broth and incubated at 37°C for 24–48 h for secondary enrichment. Following enrichment, cultures were streaked onto ALOA (Agar *Listeria* according to Ottaviani and Agosti) and incubated at 37°C for 24–48 h. Typical colonies appeared as green-blue colonies surrounded by an opaque halo and were considered presumptive *Listeria monocytogenes*.

Presumptive colonies were purified on TSA-YE (Tryptic Soy Agar with Yeast Extract) and incubated at 37°C for 24 h. Confirmation was performed using standard biochemical identification, including the CAMP test, and confirmed isolates were recorded as *Listeria monocytogenes*.

Suspected colonies of *E. coli*, *S. aureus*, and *Salmonella* spp. were isolated on Tryptone Casein Soy Agar (TSA) and incubated at 37°C for 24 h. Frozen stocks of the isolates were then prepared in cryovials containing nutrient broth with glycerol and stored at –20 °C for use in subsequent susceptibility tests.

Yeasts and molds were isolated and detected using Sabouraud Dextrose Agar (SDA, HiMedia). One milliliter of each diluted sample was spread evenly on the surface of dried SDA plates, which were incubated at 25±1°C for 5 days. Colonies were counted and recorded as CFU/g. This procedure follows the guidelines of the U.S. Food and Drug Administration (FDA, 2001).

Detection of antimicrobial residues

Sample extraction

Out of the 400 dairy samples collected in this study, 126 were selected for antimicrobial assessment as a proportional representation according to their respective distribution by regions. Thirty antimicrobials were selected among the 4 most frequently used classes in veterinary treatment in Lebanon according to the Lebanese Ministry of Agriculture (2010): β -lactams, tetracyclines, quinolones, and sulphonamides. The sample preparation procedure was based on a modified QuEChERS method (Anastassiades *et al.*, 2003). 2g of dairy product was weighed in a 20 mL glass centrifuge tube. 4mL of ultrapure water was added, then vortexed for 60 s and kept in the dark for 10 min. A multi-class method for the antimicrobial residues assessment using liquid chromatography-mass spectrometry was performed. The method was

optimized for detection of antibiotics in dairy products (European Parliament and Council of the European Union, 2002). 2g of dairy product was weighed in a 20 mL glass centrifuge tube. 4mL of ultrapure water was added, then vortexed for 60 s and kept in the dark for 10 min.

Then, 1 mL of EDTA was added as a chelating agent to compete with antibiotics such as tetracyclines. Ten milliliters of acetic acid (1%) in acetonitrile was added, and then the tubes were shaken vigorously for 1 min. Next, magnesium sulfate (MgSO_4 ; 4 g) and sodium chloride (NaCl ; 1 g) were added.

The tubes were subjected to centrifugation at 5000 rpm at 4°C for 5 min and then rested for 30 min.

6 mL of the supernatant was mixed with 50 mg of primary secondary amine (PSA), 150 mg of C18, and 900 mg of MgSO_4 . Another centrifugation was conducted at 5000 rpm at 4°C for 5 min. Four milliliters of the obtained supernatant were transferred to a new tube and evaporated under a gentle stream of nitrogen at 50 °C. Then the residue was re-dissolved in 1 mL solution with a mobile-phase water/acetonitrile ratio (70:30, v/v), and then subjected to filtration through a 0.22 μm polyvinylidene fluoride (PVDF) filter for further LC-MS/MS analysis under Multiple Reaction Monitoring (MRM) optimized conditions for each compound (Anastassiades *et al.*, 2003) (Table 3).

Antimicrobial residues were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) operated in MRM mode. Instrumental analysis was performed under optimized conditions for multi-class antimicrobial detection in dairy matrices. Optimized MRM parameters for each compound are presented in Table 3.

Quantification of antimicrobial residues by liquid chromatography-tandem mass spectrometry

LC–MS/MS analyses were performed on a triple quadrupole tandem Agilent 6430 LC/MS system (California, USA). The positive electrospray ion source (ESI+) was used with data acquisition in MRM mode (ion spray voltage: 4 kV, nitrogen for desolvation and dried gas: 11 L/min).

Quantification of the four antibiotic families in dairy product samples was performed by measuring peak areas in the MRM chromatogram and comparing them with the relevant matrix-matched calibration curves. A calibration curve ranging between 5 and 200 $\mu\text{g/L}$ was used to verify the linearity. The separation of the antibiotic residues was performed using a C18 analytical column (Zorbax 2.1 mm inner diameter I.D $\times$ 150 mm length, 3.5 μm particle size; California, USA). The separation of β -lactam was accomplished at 30°C. The flow rate and injection volume were 0.5 mL/min and 10 μL , respectively. The mobile phases used were (A) water and (B) acetonitrile. The gradient elution program started with 10% B for 1 min, increased to 65% for 6 min, then increased to 95% for 1 min and returned to the initial conditions in 1 min. The final run time of the method was 12 min.

The separation of sulfamides, tetracyclines, and quinolones was accomplished at 40°C. The flow rate and injection volume were 0.3 mL/min and 10 μL , respectively. The mobile phases used were (A) TFA (0.1%) in water and (B) acetonitrile. The gradient elution program was as follows: A (90%) (3 min), A (25%) (5 min), and A (90%) (1min); the final run time of the method was 15 min.

Performance of the analytical method

An in-house method was validated according to the criteria specified in Commission Decision 2002/657/EC. Regulation No 178/2002 and updated in Commission Implementing Regulation (EU) 2021/808 for the quantification of antimicrobial residues. Method verification was performed by spiking blank powdered milk at a concentration of 0.05 $\text{mg}\cdot\text{kg}^{-1}$. The selected level (0.05 $\text{mg}\cdot\text{kg}^{-1}$) falls within or close to the MRL range established for several antimicrobial compounds, thus providing a relevant indication of method performance. Powdered milk was selected as a representative matrix for method validation due to its compositional similarity to the investigated dairy products (cheese, yogurt, and labneh), which share the same primary constituents (proteins and lipids) derived from milk. The evaluated parameters included specificity, linear range, repeatability, and limit of quantification (LOQ), as detailed in *Supplementary Table 1*. Calibration curves were constructed using six matrix-matched standards per batch, covering a range of 0.005–0.02 $\text{mg}\cdot\text{kg}^{-1}$. The curves demonstrated excellent linearity, with correlation coefficients (R^2) exceeding 0.99. Accuracy was assessed based on the recovery of antimicrobial analytes at the 0.05 $\text{mg}\cdot\text{kg}^{-1}$ fortification level. Mean recoveries for three replicates

ranged from 62% for oxytetracycline to 98% for sulfamethazine, indicating acceptable method performance (*Supplementary Table 1*) (European Parliament and Council of the European Union, 2002). Precision was evaluated by calculating the relative standard deviation (RSD) for eight replicates at the lowest standard level. The RSD ranged from 5.58% for nalidixic acid to 14.72% for oxytetracycline, with all values below 20%, demonstrating very good repeatability. The limit of quantification (LOQ) was defined as the lowest concentration quantified with a signal-to-noise ratio of 10, in the presence of both transitions at the same retention time. The LOQ for all tested antimicrobials was determined to be 0.005 mg·kg⁻¹.

Antimicrobial resistance profile

Out of the contaminated dairy product samples collected, 62 bacterial strains (47 isolates of *E. coli* and 15 isolates of *S. aureus*) and 62 bacterial strains of *E. coli* were isolated from contaminated samples collected during 2022 and 2023, respectively.

The antibiotic susceptibility testing of *E. coli* was determined by the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2012) and the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol on Mueller-Hinton agar medium.

Antimicrobial discs, with known concentrations, tetracycline (30 µg), ciprofloxacin (5 µg), doxycycline (30 µg), and nalidixic acid (30 µg), were placed on the Agar plate and then incubated for 18 h at 35°C. To prepare the inoculum tube, loopful well-grown colonies were isolated from each plate and then transferred with a sterile loop into 1 mL of Nutrient agar. The tube is mixed by vortexing to create a smooth suspension. A sterile swab is dipped into the inoculum tube, and the dried surface of a Muller-Hinton agar plate by streaking the swab three times over the entire agar surface. Afterwards, appropriate antimicrobial-impregnated disks were placed on the surface of the agar and then incubated at 35°C for 24 hours. The diameters of the clear zone of inhibition produced by the inoculated bacteria were measured using a caliper. The zones of inhibition for each isolate were compared with standards classified as resistant (R), intermediate (I), or susceptible (S) according to the CLSI interpretive chart (CLSI, 2013).

Statistical analysis

Microbiological count data were entered, coded, and converted to log₁₀ cfu·mL⁻¹ in Microsoft Excel 2013. Descriptive statistics, including means and the percentages of non-conforming dairy samples from each region, were calculated. Data analysis was performed using IBM SPSS Statistics Version 23.0. Microbiological loads across the four regions were compared using one-way ANOVA at p<0.05, while the Chi-square test (χ²) was applied at p<0.05 or p<0.01.

Results and Discussion

Microbiological quality of dairy products

The non-conformity of dairy product samples was assessed according to the Lebanese Standards Institution (LIBNOR) norms specific to each dairy product type. The average microbiological profile of the dairy products: (a) Akkawi cheese, (b) Labneh, and (c) yoghurt samples collected over 2 years, 2022 and 2023, are presented in Table 4.

Differences among cheese samples from different regions were observed (Table 4), as well as between seasons one and two. Table 4 shows that cheese samples collected during the year 2022 exceeded LIBNOR norms for total coliforms, *E. coli*, and *S. aureus*, with contamination rates of 44%, 32%, and 15%, respectively. For samples collected during 2023, the corresponding contamination rates were 38.8%, 31%, and 0%. The decline in *S. aureus* contamination between 2022 and 2023 may reflect improved hygienic practices, as food contamination with *S. aureus* is often associated with improper handling by food workers, who can be carriers of the bacterium (Hatakka *et al.*, 2000). *Salmonella* and *Listeria monocytogenes* were not detected in any Akkawi cheese samples across both years.

Table 4 shows variability in the bacterial load of labneh samples among different regions, with samples from Bekaa exhibiting the highest levels of contamination with yeasts, molds, total coliforms, and *E.*

coli, exceeding LIBNOR standards. All analyzed samples were negative for *S. Aureus*, *Salmonella* spp., and *L. monocytogenes*.

Among the 80 samples, 5 and 9 Labneh samples, corresponding to the years 2022 and 2023, respectively, presented a microbiological load for total coliforms exceeding the Lebanese Microbiological standard, LIBNOR norm for Labneh (LIBNOR, 2012). This high level of contamination could be due to post-pasteurization contamination of the milk used to produce Labneh at one or more stages during processing (Serhan *et al.*, 2016). A study conducted by Al-Kadamany *et al.* (2002), monitoring the microbiological changes of Labneh during storage, has shown that the cloth-bag method used to produce Labneh could be a source of contamination at different stages of production.

Table 4 presents the mean concentrations of the bacterial load in yogurt samples collected over 2022 and 2023. According to the LIBNOR standard, the total coliform count in yogurt should not exceed 100 cfu/g (LIBNOR, 2012). The obtained results revealed that 26% and 31.2% of the analyzed samples, respectively, exceeded this limit and were therefore considered non-conforming. These values are notably higher than those reported by Maysoun and Nadine (2010), who found that only 14% of 42 yogurt samples collected from processing industries failed to meet the required standards.

The observed fecal contamination in the present samples may be attributed to several factors, including non-compliance with good farming practices (GFP), insufficient heat treatment of milk during processing, or temperature abuse during transportation and storage (Maysoun and Nadine, 2010). Furthermore, Table 4 shows that the percentage of non-conforming samples was 20% and 21% for the years 2022 and 2023, respectively, indicating a consistent level of contamination across both sampling years.

E. coli was detected in dairy products collected from 9 out of 16 farms (56%), highlighting a significant hygiene concern. The presence of *E. coli* in these dairy samples likely reflects poor sanitary conditions during milking and subsequent handling stages (EL-Ziney and AL-Turki, 2007).

Our study demonstrated that cheese samples were most contaminated by coliforms, with percentages of 44% and 39%, respectively for 2022 and 2023, respectively. In contrast, yogurt samples showed lower contamination rates of 26% and 31%, while labneh displayed the lowest levels at 6% and 11%. Overall, yogurt and labneh exhibited better microbial quality compared to cheese. This improved safety profile is likely due to strong acidification during fermentation, which inhibits the growth of various microorganisms, particularly coliforms (Robinson *et al.*, 2002). *S. aureus* contamination was observed at a very low rate in the dairy product samples analyzed in our study, being detected in only 5 out of the 16 industries tested. These results are consistent with the findings of Dib *et al.* (2008), who reported the absence of *S. aureus* in 69 cheese samples.

Assessment of antibiotic residues in dairy samples

The method described was applied to the determination of 30 antibiotics from four different chemical classes (sulfonamides, quinolones, tetracyclines, and β -lactams). The recovery percentage was determined at the maximum residue limit (MRL) for each antibiotic. A percentage range from 61% for penicillin G to 78% for sulfamethazine was calculated. In order to verify the results, an internal quality control was carried out on every batch of samples. Moreover, the retention time, quantification and confirmation transitions, and relative ion intensities of the detected ion in samples were compared to those of the corresponding calibration standards. Samples with concentrations above the MRL were defined as those exceeding the substance MRL according to EU Commission Regulations (European Commission, 2010). The antibiotic residue results showed that none of the 30 antibiotics were detected in 52% of samples (66 over 126 samples). However, only 15% of samples were contaminated by one antibiotic residue and 33% were multiresidue contaminated (Supplementary Table 2). The most antibiotic residues detected are oxytetracycline, sulfamethazine, and nalidixic acid. According to the EU maximum residue limit for veterinary drug residues, only 2% were above the MRL, with penicillin G and sulfamethazine levels. The tetracycline family has been detected in 30 samples (23%) below the quantification limit. Penicillin G is detected with a concentration above the MRL (5.89 $\mu\text{g/Kg}$) in one sample. The occurrence of tetracycline and β -lactam residues in dairy products has been reported from

various parts of the world (Alomirah *et al.*, 2007; Stolker *et al.*, 2008; Zanella *et al.*, 2010; Gaurav *et al.*, 2014; Pogurschi *et al.*, 2015).

Antibiotic resistance of the dairy product isolates

Based on the antibiotic residues detected in the dairy product samples, the antibiotic resistance testing was performed on the most common antibiotic family used in the veterinary sector in Lebanon. A total of 62 bacterial strains, including 47 *E. coli* and 15 *S. aureus* isolates, were evaluated. These strains, obtained from contaminated samples collected during 2022 and 2023, are presented in Table 5, which summarizes their resistance profiles.

It was observed that all *E. coli* isolates collected during seasons one and two were 100% sensitive to ciprofloxacin. These findings are consistent with the results reported by Khan *et al.* (2004), assessing the antibiotic sensitivity profiles of *E. coli* milk isolates, who showed a sensitivity of 93.3% against ciprofloxacin.

As shown in Table 5, the isolates collected during seasons one and two demonstrated high sensitivity to nalidixic acid, with percentages of 91% and 89%, respectively. These results align with previous studies indicating a high sensitivity of *E. coli* isolates to nalidixic acid (Adeyemo *et al.*, 1994).

Among the *E. coli* isolates from milk, resistance to tetracycline was the most prevalent, observed in 17% and 16% of isolates during seasons one and two, respectively. These results are consistent with earlier studies, including Srinivasan *et al.* (2007), San Martin *et al.* (2002), and Lanz *et al.* (2003), which reported tetracycline resistance in approximately 20% of *E. coli* isolates.

The bacterial isolates exhibited the highest resistance to the tetracycline family (tetracycline and doxycycline), followed by the quinolone family (nalidixic acid and ciprofloxacin). This pattern is likely attributable to the more frequent use of tetracyclines than quinolones as antimicrobial drugs in food-producing animals in Lebanon (Gaudin *et al.*, 2010).

Among the 47 *E. coli* isolates obtained from season one (Table 5), 3 isolates (6%) were monoresistant to two antibiotics, while 8 isolates (17%) were multiresistant to two antibiotics (tetracycline and doxycycline). Notably, all *E. coli* isolates carried one or more AMR genes in various combinations. These findings are consistent with the study of Srinivasan *et al.* (2007), which reported similar patterns of resistance among 135 *E. coli* isolates from milk.

In supplementary Table 3, the 15 *S. aureus* isolates exhibited high sensitivity to most tested antibiotics, with 87% sensitivity to ciprofloxacin and 80% sensitivity to both tetracycline and doxycycline. Resistance rates were 13%, 20%, and 20%, respectively. Among these isolates, 1 strain (7%) was monoresistant to two antibiotics, and 2 strains (13%) were multiresistant to three antibiotics. These observations align with the findings of Gündoğan *et al.* (2006), who reported that *S. aureus* strains isolated from 110 milk samples demonstrated resistance to one or more antibiotics.

These findings highlight the potential public health risk associated with the consumption of contaminated dairy products, as resistant bacteria can act as reservoirs for AMR genes. The results are in line with previous studies in Lebanon, which reported antibiotic-resistant *E. coli* to tetracycline (41%), doxycycline (39%), nalidixic acid (20%), and ciprofloxacin (3%), while *S. aureus* isolates were found resistant to doxycycline (63%) and tetracycline (51%) in raw milk (Joubrane *et al.*, 2022).

The presence of antibiotic-resistant bacteria in dairy products can be attributed to several factors: inappropriate use of veterinary antibiotics, failure to respect withdrawal periods, and poor hygiene practices during milking and processing. These findings underscore the need for strict monitoring of antibiotic usage in dairy farms, adherence to withdrawal periods, and regular surveillance of dairy products to minimize the risk of transmitting resistant bacteria to consumers.

Conclusions

This study demonstrated a high level of microbial contamination in Lebanese dairy products, with cheese exhibiting the highest contamination rates, followed by yogurt and labneh. Coliforms and *E. coli* were the most frequently detected bacteria, while *S. aureus* was found at relatively low levels. The results underscore the critical importance of implementing strict hygiene measures throughout the dairy

production chain, from milking to processing and storage. Improved Good Hygiene Practices (GHP), continuous training of personnel, and routine auditing of dairy facilities are essential to ensure the safety and quality of traditional dairy products. In addition, the antibiotic resistance profiles of isolates revealed significant resistance to commonly used veterinary antibiotics, particularly the tetracycline family, with some isolates exhibiting multi-drug resistance. While ciprofloxacin and nalidixic acid maintained high efficacy against *E. coli*, the presence of resistant strains emphasizes the need for prudent antibiotic use, strict adherence to withdrawal periods, and regular monitoring of both raw milk and finished products. Furthermore, the detection of antibiotic residues in several dairy samples highlights an additional public health concern, indicating possible misuse or overuse of veterinary antibiotics and insufficient compliance with recommended withdrawal periods. The presence of these residues may contribute to the development of AMR and poses potential health risks to consumers.

To strengthen food safety and public health, it is strongly recommended that large-scale, nationwide studies be conducted to assess raw milk quality, evaluate milking protocols, and optimize sanitation programs. Implementing a comprehensive surveillance system for microbial contamination, antibiotic-resistant bacteria, and antibiotic residues, coupled with educational programs for farmers and processors, will contribute to the production of safe, high-quality dairy products and reduce the risk of AMR in the Lebanese dairy sector.

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Table 1. Sampling of dairy products (Akkawi cheese, labneh, and yogurt) collected in 2022 and 2023.

Sampling in 2022					
Dairy product	North Lebanon (n=1)	South Lebanon (n=2)	Bekaa (n=10)	Mount Lebanon (n=3)	Total (n=80 samples)
Akkawi cheese	5	10	50	15	80
Labneh	5	10	35	10	60
Yogurt	5	10	35	15	65
Total samples	15	30	120	40	205
Sampling in 2023					
Dairy product	North Lebanon (n=1)	South Lebanon (n=2)	Bekaa (n=9)	Mount Lebanon (n=4)	Total (n=75 samples)
Akkawi cheese	5	10	40	20	75
Labneh	5	10	35	20	70
Yogurt	5	10	15	20	50
Total samples	15	30	90	60	195

n represents the number of dairy processing units (industries) visited in each governorate. The distribution of samples across governorates and dairy products was maintained throughout microbiological analyses, and results are reported accordingly in Table 4 to ensure consistency between sampling design and microbial outcomes.

Table 2. Microbiological parameters investigated and relative identification technique.

Microbiological parameters	ISO reference	Method principle	Selective medium	Incubation conditions
Total Coliforms	ISO 4832:2006	Colony count technique	Violet Red Bile Agar (VRBA)	37 °C for 24 h
<i>Escherichia coli</i>	ISO 16649-2:2001	β-glucuronidase-positive colony enumeration	Tryptone Bile X-glucuronide (TBX) agar	44 °C for 24 h
<i>Staphylococcus aureus</i>	ISO 6888-1:1999	Coagulase-positive staphylococci enumeration	Baird-Parker agar	37 °C for 24–48 h
<i>Salmonella</i> spp.	ISO 6579-1:2017	Detection (presence/absence)	Xylose Lysine Deoxycholate (XLD) agar; Salmonella-Shigella (SS) agar	37 °C for 24 h
<i>Listeria monocytogenes</i>	ISO 11290-1:2017	Detection (presence/absence)	ALOA (Agar Listeria according to Ottaviani and Agosti)	37 °C for 24–48 h

Table 3. Multiple reaction monitoring acquisition condition for each antibiotic used.

		Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)	Retention time (min)
Sulfonamides	Sulfacetamide	215	156, 92	75	4, 24	5.66
	Sulfapyridine	250.1	156, 92	110	16, 36	6.27
	Sulfamerazine	265.1	92, 156	110	32, 16	6.46
	Sulfamethazine	279.1	124, 186	110	24, 16	6.99
	Sulfadimidine	279.1	124, 186	105	24, 16	7.00
	Sulfameter	281.1	92, 156	115	32, 16	7.76
	Sulfamethoxypyridazine	281.1	116, 92	120	16, 32	8.05
	Sulfachloropyridazine	285	156, 92	100	12, 28	9.17
	Sulfadimethoxine	311.1	156, 92	125	20, 36	9.86
	Sulfadoxine	311.1	156, 92	115	16, 32	13.83
	Sulfamethoxazole	254.1	156, 92	90	12, 28	10.25
	Sulfabenzamide	277.1	92, 156	90	32, 8	13.15
	Sulfaquinoxaline	301.1	156, 92	120	16, 36	14.23
Quinolones	Nalidixic acid	233.1	215.1	85	12, 28	7.14
	Oxolinic acid	262.1	244, 202	100	16,36	7.50
	Ciprofloxacin	332.1	314, 231	125	20,40	4.62
	Norfloxacin	320.1	302, 198.5	125	20,44	4.50
	Ofloxacin	362.2	344, 318	135	20,20	4.50
	Flumequine	262.1	244, 202	110	16, 36	6.00
	Danofloxacin	358.2	340, 82	140	24, 52	4.78
	Enrofloxacin	360.2	342, 316	115	20, 20	5.04
	Lomefloxacin	352.2	334, 265	120	20, 24	4.76
	Sarafloxacin	386.1	368, 342	135	20, 16	5.56
Tetracyclines	Tetracycline	445.2	428, 154	110	16, 40	5.67
	Oxytetracycline	461.2	443, 426	130	10, 16	5.55
	Doxycycline	445.2	410, 154	125	16, 22	5.97
β-lactams	Amoxicillin	366.1	348.9, 114	98	4, 16	0.925
	Ampicillin	350.1	174, 106.1	98	12, 16	1.830
	Cloxacillin	436.1	160, 114.1	98	8, 44	6.10
	Penicillin G	335.1	176, 160	75	12, 8	5.38

Table 4. Mean concentration and non-conformity (%) of Akkawi cheese microbiological profile (2022-2023). Labneh microbiological profile (2022–2023). Yoghurt microbiological profile (2022-2023).

Product	Region	Year	Yeasts (cfu/g)	Molds (cfu/g)	Total Coliforms (cfu/g)	<i>E. coli</i> (cfu/g)	<i>Staphylococcus aureus</i> (cfu/g)	<i>Salmonella</i> (cfu/25 g)	<i>Listeria monocytogenes</i> (cfu/25 g)
Akkawi	North Lebanon	2022	-	-	5.84×10^4 (nc=0%)	1.96×10^3 (nc=0%)	<10 (nc=0%)	0 (nc=0%)	0 (nc=0%)
Akkawi	North Lebanon	2023	-	-	5.22×10^4 (nc=0%)	5.9×10^2 (nc=0%)	<10 (nc=0%)	0 (nc=0%)	0 (nc=0%)
Akkawi	South Lebanon	2022	-	-	7.9×10^3 (nc=0%)	4.78×10^4 (nc=0%)	4.8×10^2 (nc=0%)	0 (nc=0%)	0 (nc=0%)
Akkawi	South Lebanon	2023	-	-	8.64×10^4 (nc=30%)	2.08×10^3 (nc=40%)	0 (nc=0%)	0 (nc=0%)	0 (nc=0%)
Akkawi	Bekaa	2022	-	-	1.75×10^4 (nc=0%)	2.6×10^3 (nc=0%)	2.58×10^2 (nc=0%)	0 (nc=0%)	0 (nc=0%)
Akkawi	Bekaa	2023	-	-	1.21×10^5 (nc=40%)	1.63×10^3 (nc=0%)	0 (nc=0%)	0 (nc=0%)	0 (nc=0%)
Akkawi	Mount Lebanon	2022	-	-	4.70×10^4 (nc=0%)	4.27×10^2 (nc=0%)	<10 (nc=0%)	0 (nc=0%)	0 (nc=0%)
Akkawi	Mount Lebanon	2023	-	-	4.65×10^4 (nc=0%)	<10 (nc=0%)	<10 (nc=0%)	0 (nc=0%)	0 (nc=0%)
Labneh	North Lebanon	2022	3.34×10^3 (nc=20%)	20 (nc=0%)	500 (nc=20%)	121 (nc=80%)	<10 (nc=0%)	20 (nc=20%)	0
Labneh	North Lebanon	2023	70 (nc=0%)	70 (nc=0%)	121 (nc=0%)	<10 (nc=0%)	0	0	0
Labneh	South Lebanon	2022	98 (nc=0%)	3.1×10^3 (nc=30%)	<10 (nc=0%)	3.1×10^3 (nc=30%)	<10 (nc=0%)	<10 (nc=0%)	0
Labneh	Bekaa	2022	4.67×10^3 (nc=18%)	1.86×10^3 (nc=12%)	6.61×10^2 (nc=0%)	1.86×10^3 (nc=0%)	1.04×10^3 (nc=8%)	2.2×10^3 (nc=10%)	7.2×10^2 (nc=6%)
Labneh	Mount Lebanon	2022	2.4×10^2 (nc=0%)	3.15×10^2 (nc=0%)	15 (nc=0%)	3.15×10^2 (nc=0%)	<10 (nc=0%)	<10 (nc=0%)	0
Yoghurt	North Lebanon	2022	<10 (nc=0%)	<10 (nc=0%)	300 (nc=100%)	146 (nc=100%)	107.5 (nc=80%)	<10 (nc=20%)	0
Yoghurt	North Lebanon	2023	20 (nc=0%)	20 (nc=0%)	120 (nc=100%)	107.5 (nc=80%)	<10 (nc=0%)	0	0
Yoghurt	South Lebanon	2022	<10 (nc=0%)	36.7 (nc=0%)	170 (nc=30%)	82 (nc=40%)	75 (nc=20%)	<10 (nc=0%)	0
Yoghurt	Bekaa	2022	7.92×10^3 (nc=12%)	4.86×10^2 (nc=8%)	1.92×10^3 (nc=6%)	4.86×10^2 (nc=8%)	1.96×10^3 (nc=16%)	35.5 (nc=18%)	1.18×10^3 (nc=12%)
Yoghurt	Mount Lebanon	2022	1.84×10^2 (nc=0%)	2.24×10^2 (nc=6.7%)	1.45×10^2 (nc=0%)	2.24×10^2 (nc=6.7%)	5.80×10^2 (nc=33.3%)	1.67×10^2 (nc=46%)	66 (nc=20%)

Microbial counts are expressed as mean values (cfu/g for bacteria, yeasts, and molds; cfu/25 g for *Salmonella* spp. and *Listeria monocytogenes*). “<10” indicates values below the detection limit. Non-conformity (nc) was determined strictly according to LIBNOR standards for dairy products (LIBNOR 223:2002 for cheese; LIBNOR 2011 for Labneh and Yoghurt), and was assigned only when microbial loads exceeded the maximum permissible limits. Percentages of nc represent the proportion of non-compliant samples relative to the total number of samples analyzed per region and year. All analyses were performed in duplicate and results are presented as mean values.

Table 5 Antibiotic resistance of the *E.coli* isolates (47 for 2022 and 62 for 2023).

	Sensitive		Intermediate		Resistant		Sensitive		Intermediate		Resistant	
	Nb	%	Nb	%	Nb	%	Nb	%	Nb	%	Nb	%
Tetracycline	39	83	0	0	8	17	52	83.90	0	0	10	16
Doxycycline	32	68	5	11	10	21	56	90	3	4.8	3	4.80
Ciprofloxacin	47	100	0	0	0	0	62	100	0	0	0	0
Nalidixic acid	43	91	3	6	1	2	55	89	5	8	2	3.2
Antibiotics	47 isolates for <i>E.coli</i> from 2022						62 isolates for <i>E.coli</i> isolates from 2023					