



Detection of Common Bacterial Contaminants of Locally Produced Al-Arab Soft Cheese in Diyala Province

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Abstract

One of the common types of cheese manufactured in Iraq is Al-arab cheese. The reason for this is due to high proportion of protein, texture and price. However, the conventional way that used to produce and sell it affords many opportunities for microbial contamination. To determine the hygiene quality of Al-arab cheese production in Diyala, 75 samples were collected randomly from (Baqubah, Dali Abbas, Khalis, Jalawlaa, Khan bani saad) towns. All samples were cultured on nutrient agar and positive culture was subjected to antibiotics susceptibility test using (Trimethoprim, Tetracycline, Vancomycin, Cloxacilin, and Amoxicillin). *Staphylococcus aureus* recovered from Baqubah, Khalis, Khan bani saad, and Jalawlaa respectively (80%, 60%, 40% and 20%). *Escherichia coli* was the predominant in samples gathered from Dali abbas, Baqubah, Khalis, and Jalawlaa respectively (80%, 60%, 60% and 20%). *Pseudomonas* spp. Recovered from Baqubah, Khalis, and Dali abbas respectively (40%, 10% and 40%). *Salmonella* spp. was recorded the lowest percentage of contamination (10%) in the samples were collected from Al-Khalis.

Trimethoprim was effective against *S. aureus* and *Klebsiella* with mean inhibitory zone diameter 34mm and 36.7mm respectively while the inhibitory zone diameter for *E. coli* was (30.3) toward tetracycline. Amoxicillin, Cloxacilin, and Vancomycin were not effective against *Staphylococcus aureus*, *E. coli*, *Klebsiella* spp.

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The high frequency of isolation for *S.aureus* , *E. coli*, *Klebsiella*, *Salmonella* spp and *pseudomonas* spp from Al-arab cheese indicated that both heat treatment and storage temperature not sufficient to ensure consumer safety. Adequate heat treatment for superior raw milk quality and low initial contamination level with bacteria during Al-arab cheese processing and storage are regarded as the important steps for reduction contamination in locally produced products.

Keywords: Soft cheese, *Staph aureus*, *E. coli*, *Klebsiella* sp.

الكشف عن الملوثات الجرثومية الشائعة لجبن العرب الطري المنتج محلياً في محافظة ديالى

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الخلاصة

جبن العرب شائع الاستخدام في العراق وقد يعزى السبب في ذلك محتواه العالي من البروتين والسعر المناسب. الطريقة التقليدية المستخدمة في إنتاج وبيع جبن العرب تتيح فرصاً عديدة للتلوث الميكروبي ولتحديد جودة النظافة الى الجبن الأبيض الطري في ديالى تم جمع 75 عينة عشوائياً من المنتج لعدد من مناطق محافظة ديالى (بعقوبة، دالي عباس، الخالص، جلولا، و خان بني سعد). تمت زراعة جميع العينات المختارة على الأجار المغذي وأظهرت النتائج أن بكتريا *Staphylococcus aureus* التي تم الحصول عليها من بعقوبة، خالص، خان بني سعد وجلولا (80٪، 60٪، 40٪، 20٪ على التوالي)، بينما كانت الإشريكية القولونية. البكتيريا السائدة (80٪، 60٪، 60٪، 20٪) في العينات التي جمعت من دالي عباس، بعقوبة، الخالص وجلولا. وأظهرت النتائج أن الزوائف التي تم الحصول عليها من بعقوبة، خالص ودالي عباس (40٪، 10٪، 40٪ على التوالي)، بينما *Salmonella* spp سجلت أقل نسبة تلوث (10٪) في العينات التي جمعت من الخالص. استخدمت خمسة أنواع مختلفة من المضادات الحيوية (*Cloxacilin* , *Tetracyclin* , *Vancomycin* , *Trimethiprim* , *Amoxicillin*) لتحديد الحساسية ضد المكورات العنقودية و الايكولاي والكلبيسيلا. وقد أظهرت أن *Trimethiprim* كان الأكثر فعالية ضد *Staphylococcus aureus* و *Klebsiella* بمتوسط قطر المنطقة المثبطة (7، 34 و 37) ملم على التوالي بينما كان أكبر قطر لمنطقة التثبيط لجرثومة الإشريكية القولونية (30.3) ملم تجاه التتراسيكلين و بناءً على النتائج فإن *Amoxicillin*, *Cloxacilin*, *Vancomycin* كانت غير فعالة ضد المكورات العنقودية الذهبية والإشريكية القولونية

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والكلبيسيلا. أشارت النسبة العالية للبكتيريا التي تم الحصول عليها من هذه الدراسة إلى أن كلا من المعاملة الحرارية ودرجة حرارة التخزين لهذا المنتج غير كافيين لضمان سلامة المستهلك، تعتبر المعالجة الحرارية الكافية للحصول على جودة عالية للحليب الخام ومستوى التلوث الأولي المنخفض بالبكتيريا أثناء تصنيع الجبن الطري وتخزينه خطوات مهمة للحد من التلوث في المنتجات المصنعة محليًا.

الكلمات المفتاحية: الجبن الطري، المكورات العنقودية الذهبية، الايشيريكية الكولونية و كليبسيلا.

Introduction

Generally, the milk resulting from cows recognize is main source for human consumption, while the manufacturing of dairy products resulting from milk belong to animal species like goat, buffalo, and sheep. Milk produced is consume with several ways about halve of this produced utilized as boiled or fresh, one sixth as curd or yoghurt and residual is use for manufacturing of several kinds of dairy products such as cheese, butter, ice cream, milk powder and yoghurt which are commonly available [1].The soft cheeses are rich in vitamins, minerals, moisture, and neutral which make them as a suitable medium for microorganisms growth [2]. The nutritional and economical values of the soft cheese made it accepted in different countries [3].Cheese is the earliest food and the first dairy product that was discovered in the Middle East and spread to the different countries in Europe. The production is regarded many methods of preserving of the milk during the manufacturing with a rennet which is derived from an animal stomach [4].

Consumption of contaminated food causes food borne disease, it can be divided in to two types, infectious resulting from toxic agent and food poisoning [5]. Several conditions are required to induce food poisoning outbreaks especially with Staphylococci such as source of contamination by enterotoxin-producing Staphylococci, raw food, transporting materials, dirty preparation and equipment with poor hygienic practices. Food with a good physicochemical characteristics for *Staph. aureus* growth and toxinogenes can provide a favorable circumstance for the bacterial growth and toxin production. Ingestion of food containing sufficient amounts of toxin can

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induce symptoms and most outbreaks occur from the poor hygienic practices [6]. Food safety is an important issue throughout the world, and is one of the WHO's strategic objectives, the impact of food-related illness has recently been published in the USA reports, the US Food and Drug Administration (FDA) has stepped up efforts to improve the traceability of contaminated products [7]. Many bacterial pathogens that can contaminate the soft cheeses - pathogenic *Escherichia coli* serotype O157:H7, *Salmonella* spp., *Listeria* spp and *Staphylococcus aureus*. Milk contaminated by any spoilage or pathogenic bacteria through the different stages of milking or cheese production[4]. In the storage and distribution, the bacteria will be present in the last dairy products and could cause health hazard to the costumers [8]. Cremonesi *et al.* (2007) tested 33 samples of raw milk cheese and found all the samples were positive for *S.aureus* contamination. *E. coli* was isolated from 76 samples out of 77 random samples, and 19.48% of isolates belonged to EPEC serogroup in Kerman, Iran [9]. Abbar and Kaddar (1991), reported that 40.5% of soft cheese samples were contaminated by Coliform bacteria, *E. coli*, *S. aureus*, and mold-yeast counts were detected in some dairy products in Iraq[10].

Rajeev *et al.*, 2011 found in most isolates, as they found that *S. aureus* is the most prevalent 52 (27.37%); followed by coagulase negative *Staphylococcus* spp. 24 (12.63%); *E. coli* 17(8.95%); *Pseudomonas* spp. 15 (7.89%); *Streptococcus* spp.11 (5.79%); *Klebsiella* spp. 3 (1.57%) and *Bacillus* spp. 1(0.52%) isolates[11]. Syed *et al.*, 2014 Milk from a healthy udder considered sterile, then the bacterial isolates observed are suspected to contaminate the sample from various sources upon milking, which could be due to, the environment, utensils used, the state of hygiene of the animal from which the milk was collected and the sanitary conditions of the milk collectors [12]. The microbes multiplication in the cheese depends on numerous factors such as water activity storage temperature, the availability of nutrients and pH. Cheeses are good medium to the microbial spoilage because of high moisture content, low concentration of salt and neutral pH [13].

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Materials and Methods

A total of 75 Al-arab soft cheese samples were collected in a sterile polyethylene bags from local market of (Baqubah, Dali Abbas, Khalis, Jalawlaa, Khan bani saad) towns from September to December 2019. All samples were directly kept in a cool box and transported to the laboratory of veterinary public health at Diyala university. The microbiological tests were done to isolate most common bacteria and to determine antibacterial activities of selected antibiotics. A total of 10 grams of cheese were added to 90ml of sodium citrate solution and thoroughly homogenized for 5 minutes to give a uniform mixture, after that the volume completed to 100 ml by same solution to give stock solution which represent dilution 10^{-1} . Only 1ml of dilutions was add to prepared culture media (MacConkey, Mannitol salt agar, EMB, SS agar), then incubated at 37°C for 24 hrs. The growth of bacteria was monitored and diagnosed according to biochemical and morphological features due to limitation of resource in the public health laboratory of veterinary college [14].

Antibiotic susceptibility test

A disc diffusion process, known as Kirby- Bauer method [15] was used to determine the susceptibility of the bacterial isolates against selected antibiotic agents. Five different antibiotic discs (Trimethiprim, Vancomycin ,Cloxacilin ,Amoxicillin, Tetracycline) were obtained from commercial sources (Oxoid Ltd, Baring-stoke, Hampshire, England).The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute [16].

Results

Isolation and identification of bacteria

In current study, all samples which give a green metallic sheen on Eosin methylene blue agar(EMB agar), figure 1 a characteristic feature for *E. coli* was confirmed by biochemical identification using (IMViC) tests as per [17], *E. coli* give positive indole production.

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All sample which appeared as yellow colonies on mannitol salt agar as in figure 2 was initially identified as *S. aureus* and subjected to gram staining, biochemical tests coagulase and catalase tests. Samples showed mucoid colonies, opaque, and pinkish color on MacConkey agar and exhibited urease activity, a characteristic feature for *Klebseilla* figure 3 were positive for citrate utilization test. Black colony growth on SS agar identified as salmonella as in figure 4, finally figure 5 show Oxidase test (dark purple at first 10 second) to confirm *Pseudomonas* bacteria.

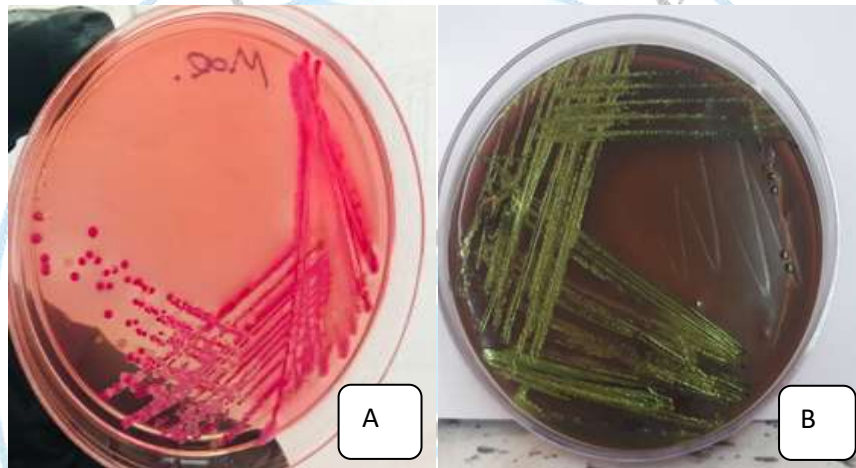


Figure 1: A: Growth of *E. coli* on MacConkey agar (A) EMB agar (B)

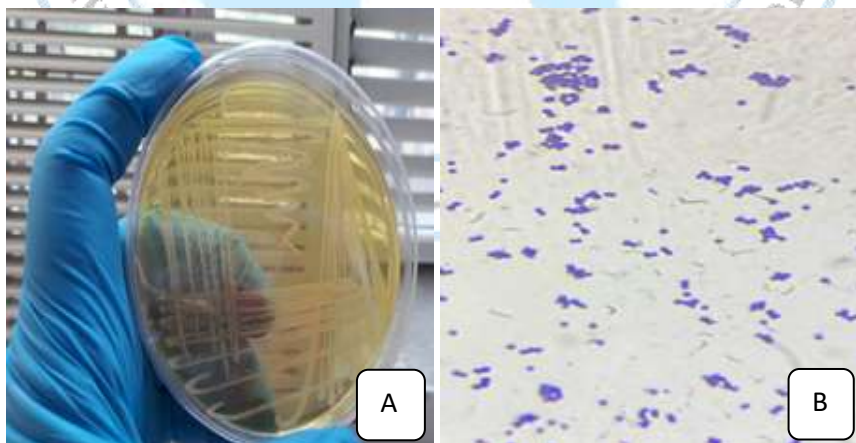


Figure 2: A: growth of *S.aureus* on mannitol salt agar (Golden yellow in color), (B) grape like cluster under microscope

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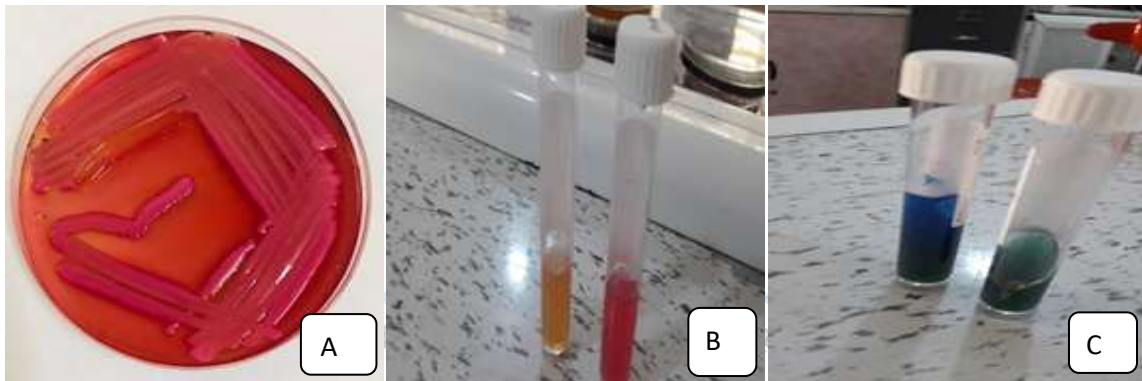


Figure 3: A: growth of *Klebsiella* on MacConkey agar (mucoid colonies), B- positive Urease test (pink color) for *Klebsiella*, C-citrate test (Blue color +) for *Klebsiella*



Figure 4: Black colonies of *Salmonella* spp on SS agar

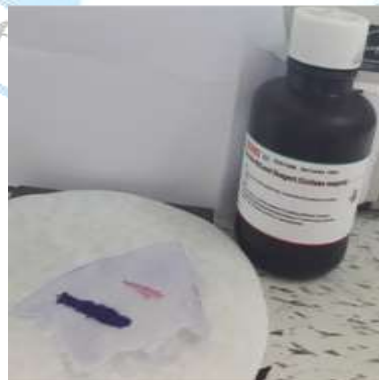


Figure 5: Oxidase test (+) for *Pseudomonas* (dark purple)

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Table 1: Bacterial species isolated from Al-Arab Soft Cheese

Name of town	No. of sample	Bacterial species	Frequency	Percentage
Baqubah	15	<i>S. aureus</i>	12	80%
		<i>Klebsiella</i> spp	12	80%
		<i>E.coli</i>	9	60%
		<i>Pseudomonas</i> spp	6	40%
Khalis	15	<i>S. aureus</i>	9	60%
		<i>E.coli</i>	12	80%
		<i>Salmonella</i> spp	3	20%
		<i>Pseudomonas</i> spp	3	20%
		<i>Klebsiella</i> spp	9	60%
Khan bani saad	15	<i>S. aureus</i>	6	40%
		<i>Klebsiella</i> spp	3	20%
Jalawlaa	15	<i>S. aureus</i>	3	20%
		<i>Klebsiella</i> spp	3	20%
		<i>E.coli</i>	3	20%
Dalii abbas	15	<i>S. aureus</i>	0	0%
		<i>E.coli</i>	12	80%
		<i>Klebsiella</i> spp	6	40%
		<i>Pseudomonas</i>	6	40%

Antibiotic Susceptibility against *S. aureus*, *E. coli*, and *Klebsiella*

The average diameters of the inhibition zones are presented in table 2

Table 2: Antimicrobial susceptibility test against *S. aureus*, *E. coli* and *Klebsiella*

Bacterial spp	Antibiotic	Diameter of inhibition zone (mm)			Mean(mm)
<i>S. aureus</i>	Trimethoprim (TMP)	34	34	36	34.7
	Vancomycin (VA)	0	0	0	0
	Cloxacilin (CX)	0	0	0	0
	Amoxicillin (AX)	0	0	0	0
	Tetracycline (TE)	30	30	31	30.3
<i>E.coli</i>	Trimethoprim (TMP)	24	25	24	24.3
	Vancomycin (VA)	0	0	0	0
	Cloxacilin (CX)	0	0	0	0
	Amoxicillin (AX)	0	0	0	0
	Tetracycline (TE)	30	30	32	30.3
<i>Klebsiella</i>	Trimethoprim (TMP)	34	40	34	36

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Vancomycin (VA)	0	0	0	0
Cloxacilin (CX)	0	0	0	0
Amoxicillin (AX)	0	0	0	0
Tetracycline (TE)	26	24	26	25.3

Discussion

Cheese is a ready- to- eat dairy product, which is a wealthy supply of vitamins, protein, calcium, and phosphorus. Microbial contamination of cheese can occur from a variety of sources including packaging material, handler ,and environment [18, 19]. The majority general spoilage microorganisms of milk and dairy products are gram-positive and Gram-negative bacteria which include spore-forming bacteria, Bacillus, clostridium spp, lactic acid producing bacteria ,*Streptococcus* spp , *Pseudomonas* spp., and *Coliforms*. [20, 21]. The contamination of the samples can occur since of the health and hygiene of the cow, the procedures used in cleaning and sanitizing the milking , storage equipment ,the environment in which the cow is housed and milked ,and temperature and length of storage all influence microbial numbers in raw milk [22].Previous study conducted by [23],indicating that the curd-cutting knife is source of contamination in cheese production.

A study done by [24] documented several pathogens resulting to milk-borne zoonotic diseases including brucellosis, tuberculosis and enterotoxaemia. The risk of infection by milk-borne zoonotic diseases is one of the reasons for public health regulations, which discourages the informal milk markets and consumption of raw or unpasteurized milk [25, 26]. Another study carried by [27] appeared that the microorganisms can attack the udder tissues then multiply in mammary tissues and later discharge in milk. A significant source of contamination is the infected water which used for washing milking equipment [28]. Cheeses are food products since they do not suffer any additional treatment to ensure their protection before utilization. In addition, contamination of cheeses may occur at several stages in the production chain. Therefore, all the information about bacterial characteristics and susceptibility is necessary to prevent contamination of dairy products with pathogens [29]

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Previous study conducted by [30] showed that the outbreaks occur due to cheese made from unpasteurized milk are often caused by *Salmonella* spp. (34%), *Campylobacter* (26%), *Brucella* (13%), Shiga toxin-producing and *E.coli* (11%). Pasteurization has the potential to kill Listeria and it is rare outbreak this strain of bacteria to be associated with pasteurized dairy products, including cheese [31]. In present study, our results in table 1, revealed that the *S.aureus* that obtained from Baqubah, Khalis, Khan bani saad, and Jalawlaa (80%, 60%, 40%, 10% respectively), this were in agreement with [32], Who reported that the *Staphylococcus* spp. was the predominant organism isolated with the majority of them being *S.aureus*, the contamination presumably coming from the hands of the cheese-sellers. Cows may excrete *S.aureus* from the udder, often without clinical evidence of mastitis. Current result in this study documented that the percentage contamination of soft cheese with *E.coli* were 80%, 60%, 60%, 20% which obtained from Dalii abbas, Khalis, Baqubah, and Jalawlaa, respectively, while the proportion of contamination with Klebsiella were 80%, 60%, 40%, 20%, 10% obtained from Baqubah, Khalis, Dali abbas, Jalawlaa, Khan banisaad respectively this were in agreement with study conducted by [33] who revealed that high level of contamination with coliforms bacteria in the soft cheese is regarded as indicator for the unsanitary practices during the cheese making process. Soft cheese is characterized by many of gas holes, with spongy texture. *Pseudomonas* is aerobic bacteria, Gram-negative, non-spore forming, have high inherited variety and poor nutritional requirements permit them to survive in diverse environments, such as vegetation, water, atmospheric dust, and soil. moreover, these personality let them to stay alive on the apparatus used in the dairy production chain, for example bulk tanks, milking pipelines and apparatus [34].

In this study, *Salmonella* spp. (10%) was isolated from soft cheese which prepared at al Khalis city, this result similarity with studies carried out by [35] showed that 3-6% of raw milk samples and 19% of milk filters were positive for *Salmonella* spp. and soft cheeses made from unpasteurized or insufficiently pasteurized milk may also be contaminated with *Salmonella* spp

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(40%, 40%, 10%) isolated from Baqubah, Dali abbas, Al khalis respectively. Similar results reported by [36] who isolated *Pseudomonas* spp. from 54.5% of the cheeses which manufacturing from raw milk. [37] reported that the presence of *Pseudomonas* spp. in food is usually an pointer of post- pasteurization contamination. Results of a study done by [38] confirm the several cases of food poisoning outbreaks as a result of utilization of cheese contaminated by *P.aeruginosa*. Bacterial antimicrobial resistance has become a worldwide difficulty in both the agricultural and medical and fields. Antibiotic resistant strains of bacteria are an growing threat to human and animal health, (McDermott et al., 2002). Himi *et al.* (2015) found E. coli were sensitive to Levofloxacin and Ciprofloxacin. The E. coli should be considered as unsafe to health and advocate the preventing risk factors. However, in the present study Tetracycline and Trimethoprim were proved to be the best antibiotics to treat E. coli infection and mastitis in cattle since they were highly effective. Mushfia *et al.*, (2015) showed that *S. aureus* isolates were establish to be resistant against many different antibiotics and sensitive to gentamicin, ciprofloxacin, and imipenem. This dissimilarity in the results of different studies indicates the antibiotic resistance pattern of *S. aureus* changing.

In Iraq, milk, cheese and cream were two of the important dairy products the important dairy products, but little proportion (10%), were made in a sanitary conditions in large dairy factory, while the large percentage were create in unhygienic conditions whether in a dairy factory or houses or shops [39].

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