Bacterial contamination assessment of local and Imported diary productions in Al-Diwaniya markets

تقييم التلوث البكتيري لمنتجات الألبان المحلية والمستوردة في أسواق مدينة الديوانية

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Abstract

The aim of this work was to evaluation of bacterial contamination indicators such as a total bacterial count, total coliform count and faecal coliform count for domestic and canned milk and its derivatives belonging to different origins (Iraqi, Iranian, Kuwaiti and Syrian), comparison with standards parameters, and authorized rates of expire date of this productions. then Isolation and Identification of contaminated bacteria. In the present study, the domestic productions were more contaminant samples, the result revealed that the domestic cow cheese has high number of total bacterial count (78×10^{11} cfu/gm) and domestic yogurt cow was less (37×10^7 cfu/gm), while domestic of cow cream had larger number of total coliform bacteria (90×10^7 cfu/gm) and less in domestic buffalo cream (22×10^2 cfu/gm). Also, the study shown that the high number of feacal coliform bacteria was in domestic cream of cow (47×10^6 cfu/gm) in comparison with domestic buffalo yogurt (2×10^2 cfu/gm). The bacterial tests showed that the all domestic productions samples were out of hygienic standard specifications, followed by Iranian and Iraqi canned cream with total bacteria count (3×10^7, 32×10^3 cfu/gm) respectively, whereas Iraqi canned cream samples were contained (24×10^2 cfu/gm) of total coliform bacteria, while the number of feacal coliform bacteria was (13×10^2 cfu/gm). The result revealed too, that the Iranian and Iraqi canned cheeses were out of standards specification, with total bacteria count (5×10^3 - 8×10^7 cfu/gm) respectively. While the total coliform bacteria in Iraqi canned cheese was (2×10^2 cfu/gm). The result revealed increase bacterial content in Iranian canned yogurt with number of total bacterial count (62×10^8 cfu/gm).

الخلاصة

هدفت هذه الدراسة إلى مقارنة مؤشرات التلوث البكتيري (العدد البكتيري الكلي و وبكتيريا القولون الكلية والقولون البرازية) لبعض نماذج الحليب المحلية والحليب المعلب ومنتجاتها من مناشىء مختلفة (العراقي, الإيراني, الكويتي والسوري), ومقارنتها مع المعايير القياسية وتحديد مدى صلاحية هذه المنتجات للاستهلاك, وعزل وتشخيص البكتيريا الملوثة لها. ومن خلال نتائج الدراسة الحالية, لوحظ إن المنتجات المحلية كانت أكثر العينات تلوثاً, إذ أظهر الجبن البقري المحلي محتوى عالي بكتيري بلغ(78×10^{11} خلية/غم), وكان اللبن البقري المحلي أقل النماذج من ناحية المحتوى البكتيري(37×10^7 خلية/غم), في حين كان أعلى محتوى من بكتيريا القولون الكلية في القشطة البقرية المحلية إذ بلغت(90×10^7 خلية/غم), واقلها في قشطة الجاموس المحلية(22×10^2 خلية/غم). كما بينت الدراسة ارتفاع محتوى القشطة البقرية المحلية ببكتيريا القولون البرازية(47×10^6 خلية/غم) مقارنة مع لبن الجاموس المحلية إذ بلغ المحتوى اقل عدد(2×10^2 خلية/غم). ومن خلال هذه المؤشرات فان كل نماذج الحليب ومنتجاته المحلية الإنتاج كانت خارج المواصفات القياسية الصحية, فيما جاءت القشطة المعلبة الإيرانية والعراقية من بين جميع النماذج في المرتبة الأولى من حيث المحتوى البكتيري, إذ بلغت(3×10^7 خلية/غم) و(32×10^3 خلية/غم), على التوالي, فيما بلغت بكتريا القولون الكلية في القشطة المعلبة العراقية(24×10^2 خلية/غم), أما بكتيريا القولون البرازية, فقد بلغت (13×10^2 خلية/غم). كما بينت النتائج أيضاً بان الجبن المعلب الإيراني والعراقي كان خارج الحدود المسموح بها إذ بلغ العدد الكلي للبكتيريا (5×10^3 خلية/غم) و(8×10^7 خلية/غم), على التوالي, وكانت بكتيريا القولون الكلية (2×10^2 خلية/غم) في الجبن العراقي. كما أوضحت النتائج ارتفاع المحتوى البكتيري في اللبن المعلب الإيراني إذ بلغ العدد الكلي للبكتريا الهوائية (62×10^8 خلية/غم).

Introduction

Milk plays a very important role in human life, primarily as a source of perfect food. Owing to their high availability and buffer properties, and have always occupied a significant position in the feeding style, but is also an efficient vehicle for transmission of diseases to humans. Pathogenic bacteria pose a serious threat to human health, and constitute about 90% of all dairy-related diseases[1].

The milk exposure to microbes contaminations from the cows until reach to consumers whether a raw milk or its products, and the natural contents of proteolytic, lipolytic and toxic produced bacteria, such as Pseudomonas, Alcaligenes, Micrococcus, Salmonella, Bacillus and Staphylococcus [2]. The components of milk and its physical and chemical properties provide a very favorable milieu for the multiplication of microorganisms [3]. The warm and rather humid climatic conditions that prevail in most tropical countries) would tend to support the survival and growth of many microorganisms in milk [4], and Iraq is one of them.

Recent studies have been established the emergence of new milk-borne bacterial pathogens such as Escherichia coli with more serious challenges for public health and the diary industry. E. coli is responsible for several outbreaks of diarrhoea in children and adults after ingestion of contaminated milk and dairy products [5]. Different studies show that 1-5% of food-borne infections were related to consumption of milk and dairy products, that 53% of cases of food-borne infections caused by contaminated cheese and that enteropathogenic E. coli is the causative agent of 18.33% of these cases [6]. The Staphylococcus spp. was the predominant organism isolated with the majority of them being Staph. aureus, the contamination presumably coming from the hands of the cheese-sellers. However, cows may excrete Staph. aureus from the udder, often without clinical evidence of mastitis [8].

In Iraq, milk, cheese and cream were two of the important dairy products, but little percentage (10%), were make in a hygienic conditions in large dairy factory, when the large percentage were make in unhygienic conditions whether in a dairy factory or houses or shops [9]. Yoghurt defects due to microbial contamination and the most frequent contaminants are yeasts and moulds [10]. The aim of this study was to determine bacterial contaminated of milk and its derivatives from different origins that circulation in AL-Diwaniya markets and investigation from its validity to consumption.

Samples collection

A total of 54 samples of milk and its derivatives were collected randomly from the Markets, (30) canned samples from different origins (Iraqi, Iranian, Syrian, and Kuwaiti) and (24) domestic samples during the period of October 2007 to January 2008: The samples were collected by taking 500 ml of milk and 250 gm of cream, cheese and yogurt in pre-sterilized container and transported in ice bucket to the laboratory for microbiological analysis during 3 hrs.

Each samples (milk, cream, cheese, yogurt) were processed as follows:

• In a 100 ml marked glass homogenizer, 10gm of (cream, cheese and yogurt) and 10ml of milk, were added to 90ml of sodium citrate solution.

• The martial thoroughly homogenized for 5 minute by Electric mixer to give a uniform mixture, then the volume completed to 100 ml by same solution to give stock solution which represent dilution 10^{-1}.

• The dilutions were prepared to 10^{-7} by using normal saline 0.85%.

• Total aerobic bacteria were determined by pour plate count by taking 1ml of all dilutions in Petri dishes and pour the nutrient agar media on it, then incubated at 22°C and 35°C for 24 hrs.

• Total and faecal coliform count determined by pour plate count by taking 1ml of all dilutions in Petri dishes and pour the then inoculated on violet red bile agar, later the first incubated at 37°C for 24 hrs and the second incubated at 44.5°C for 48hrs [11].

• The growth colonies were counted by colonies counter, number of bacteria exist by multiply of colonies number by dilution reverse and all microbiological tests were performed in triplicate.

• The growth bacteria was isolated, then examined and diagnosed according to biochemical, morphological characterizations as well as standard methylene blue reduction and resozarin tests were done [12, 13, 14, 15].

Results & Discussion

In present study, the results revealed that the total aerobic bacterial count obtained from Syrian canned milk accomplished $(54x10^3)$ cfu/ml, whereas quite empty from any total coliform and faecal coliform bacteria, therefore It was agreement with standard specifications which stipulate that not to increase $(5x10^5)$ cfu/ml [16]. While, total aerobic bacterial count of domestic milk samples for cows and buffalo were $(92x10^8)$&$(54x10^8)$ cfu/ml respectively, (Table 1 & 2). The results showed the increase of rates of total coliform bacteria count $(132x10^4)$&$(45x10^3)$ cfu/ml respectively, in domestic milk samples for cows and buffalo were more than the standard specifications of hygienic parameters that indicated not up 10 cfu/ml in milk [17] . This results were agreement with study that indicate to increase total aerobic bacterial count in raw milk [18].

The results found are manifested in the (Table, 2) revealed an increase in faecal coliform bacterial count with rates $(83x10^2)$ and $(32x10^2)$ cfu/ml in domestic milk samples for cows and buffalo respectively, This results were agreement with study that indicate to increase total coliform bacteria count in raw milk [18]. therefore they were disagreement with authorizations of world health organization [11].which indicate that empty the samples of milk from any bacterial cell, the presence of this bacteria may refers to faecal contamination. The cause of high bacterial contamination of domestic samples of milk may be return to infected animals with some diseases such as Mastitis and also owing to lack proper sanitation and absence of hygienic conditions during milking; they are also associated to storage temperature of milk without refrigeration, un pasteurization of milk, circulation and transportation [19].

Table (1): Total bacterial count, total and faecal coliform bacteria found in canned Milk and its product samples from different origins which circulation in AL-Diwaniya markets.

Canned samples	Origins	Total bacteria count	Total coliform bacteria	Faecal coliform bacteria
milk cfu/ml	Syrian	54 X 10 ^{3}	-	-
cream cfu/gm	Syrian	75 X 10^3	-	-
cream cfu/gm	Kuwaiti	-	-	-
cream cfu/gm	Iranian	3 X 10^7	-	-
cream cfu/gm	Iraqi	23 X 10^8	24 X 10^2	13 X 10^2
cheese cfu/gm	Syrian	-	-	-
cheese cfu/gm	Kuwaiti	-	-	-
cheese cfu/gm	Iranian	5 X 10^7	-	-
cheese cfu/gm	Iraqi	8 X 10^3	2 X 10^2	-
yogurt cfu/gm	Iranian	62 X 10^8	-	-

From tables 1 and 2 the result appeared that the Kuwaiti Cream was the best quality from another creams, it was a quit empty from any contamination, and hence, probable adherence to good manufacturing protocol, in addition to role of pasteurization of milk during manufacturing of cream in reduce number of bacteria[20]. While Syrian cream was accordance with standard specifications with rate $(75x10^3)$cfu/gm and heavy bacterial count were record in canned Iraqi and Iranian creams with rates $(23 x 10^8)$ and $(3 x 10^7)$ cfu/gm respectively, comparative with authorization of world health organization that is conditioned rates $(2x10^4$-$30x10^4)$ cfu/gm [11]. whereas American specifications was stipulated not increase total bacterial count about $(5X10^4)$cfu/gm in manufacturing cream from pasteurized milk [16]. According to this authorization the domestic cow and buffalo cream out of standard

specifications with rate (102×10^8) & (23×10^7) cfu/gm, respectively. The cause of heavy total bacterial content attributed to using milk with high bacterial contamination as well as raw material or water used in formulation or accidentally during use [20]. domestic Soft cheeses and creams both contains a high numbers of vegetative cells for multiple types of microbes, spores of aerobic and anaerobic bacteria, molds and yeasts during its production and manufacturing[21]. Heating treatments (pasteurization) of production cooking cheeses and pasteurized cream are enough to killing vegetative cells, but bacterial spores are resistance to this heat, therefore it can't get sterilized [22].

Table (2): Total bacterial count, total coliform bacteria and faecal coliform bacteria found in domestic Milk and its products samples which circulation in AL-Diwaniya markets.

Domestic samples	Total bacteria count	Total coliform bacteria	Faecal coliform bacteria
Cow milk cfu/ml	92×10^8	132×10^4	83×10^2
Buffalo milk cfu/ml	54×10^8	45×10^3	32×10^2
Cow cream cfu/gm	102×10^8	90×10^7	47×10^6
Buffalo cream cfu/gm	23×10^7	22×10^2	18×10^2
Cow cheese cfu/gm	78×10^{11}	74×10^6	50×10^5
Buffalo cheese cfu/gm	213×10^9	48×10^5	20×10^3
Yogurt Cow cfu/gm	37×10^7	48×10^6	28×10^5
Buffalo yogurt cfu/gm	15×10^8	39×10^3	2×10^2

While the samples of canned (Syrian, Kuwaiti and Iranian) creams were quite empty from any coliform bacterial cell, therefore they were agree with the hygienic specifications that recommended with 10 cells in milk and its produces [17]. whereas the world health organization recommended empty samples from any coliform bacterial cell. Hereby these specifications the canned Iraqi and domestic cow and buffalo creams were a hygienic rejected with rates of total coliform bacteria $(24 \times 10^2)(90 \times 10^7)(22 \times 10^2)$cfu/gm respectively, and feacal coliform bacteria accomplished (13×10^2) $(47 \times 10^6)(18 \times 10^2)$cfu/gm, respectively. These may be return to insufficient pasteurization of milk as well as the presence faecal contamination from human, animal or animal fertilizer [11].

The results were found are depicted in table(1) revealed that the Syrian and Kuwaiti canned cheeses origins were in the first stage comparative with another origins, they were empty from any contamination, while Iranian canned cheese was in the second stage with rate of total bacterial count (5×10^7)cfu/gm and empty from total and faceal coliform bacteria. While Iraqi canned cheese was out of world health organization authorization with rates of total bacteria count (8×10^3)cfu/mg, and total coliform count (2×10^2)cfu/gm, the cause attributed to the use of contaminant milk and insufficient of pasteurization, More over can be attributed to the practice of preparing large bulks in advanced of requirements, which are being held for long period at room temperature, It also, Indicator poor hygienic conditions and faults in manufacturing/handling during process of cheese productions [8]. While the domestic cheese of cow and buffalo witnessed a high contaminations Table(2). Therefore, they were disagree with standard specifications.

The present study, revealed that the total bacterial count in Iranian canned yogurt was (62×10^8)cfu/gm, the cause may be return to the presence of lactic acid bacteria, while the samples were empty from any faecal contamination comparative with domestic cow and buffalo yogurt which have high content of bacteria and they were out of American standards specifications.

On the other hand, this study included the detection of some genus and species of bacteria in milk and its canned and domestic produces (Table 3 and 4).

Table (3): Some of Isolated bacteria from canned milk and its products samples from different origins circulation in AL-Diwaniya markets.

Samples	origin	Bacillus sp.	E. coli	Ps. fluorescens	Enterobacter sp.	Ps. aeruginosa
Canned milk	Syrian	+	-	-	-	-
Canned cream	Syrian	+	-	-	-	-
Canned cheese	Iraqi	-	+	+	-	-
Canned cream	Iraqi	-	-	-	+	+

Entrobacter sp. was isolated and identified from domestic cow and buffalo milk, Iraqi canned cream and domestic cow and buffalo cream samples, and Bacillus spp. isolated from Syrian canned milk and cream, probably these bacteria may be responsible for cases of acute gastrointestinal infections, due to Enterotoxin [23]. Thus, the occurrence of these organisms may be introduced in to milk from the soil, cattle feed, and milking equipments, udder, and skin of humans and Bacillus spp. is able to produce spores, which can survive pasteurization [8]. Klebsiella pneumonia was isolated from buffalo domestic cream and cheese, this contamination attributed to internal and external sources because of it was resistance to dryness on skin and equipments so, its regard one of causatives cross-infection [24].

The results showed, that the Iraqi canned, domestic cow and buffalo cheese, and domestic cow and buffalo cream, were contaminated with E. coli bacteria. The occurrence of a high proportion of E. coli in cheese samples may be due to lack of proper sanitation and absence of pasteurization of milk used for cheese making. Therefore, stringent hygienic measures must be followed and pasteurization of milk should be imposed to prevent contamination of cheese with coliforms. Otherwise, these traditional cheeses should be collected from the market [19].

Table (4): Some of bacteria Isolated from domestic milk and its products samples circulation in AL-Diwaniya markets.

Domestic samples	Ps. aerogenosa	Ps. flourescens	Staph. aureus	Enterobacter sp.	E. coli	Strep. durans	K. pneumonia	Proteus sp.
Cow milk	+	-	+	+	-	-	-	-
Cow cream	-	-	-	+	+	-	-	-
Cow cheese	-	-	-	-	+	-	-	+
Buffalo milk	+	-	+	+	-	+	-	-
Buffalo cream	-	-	-	+	+	-	+	-
Buffalo cheese	-	-	-	-	+	-	+	-
Buffalo yogurt	-	+	-	-	-	+	-	-

Pseudomonas aeroginosa, was isolated from Iraqi canned cream, domestic cows and buffalo milk samples, Pseudomonas fluorescens also isolated from Iraqi canned cheese and buffalo yogurt.
Staphylococcus aureus was also, isolated from all samples of domestic milk. Most staphylococcal strains are able of producing one or more enterotoxins, which are the cause of the gastrointestinal symptoms that include vomiting with or without diarrhea, observed during intoxications[8].
The presence of this bacteria in milk samples and its produces may return to its a normal habitat in mouth, noise and skin [25]. As concern, Streptococcus durans isolated from all milk samples and domestic buffalo yogurt, these bacteria is an indicator to faecal contamination. Streptococcus bacteria are highly resistance to ambient conditions and aseptic [26]. While, domestic cheese cow was contain Proteus sp., it is an indicator of faecal contamination [27].

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