COMPARISON BETWEEN THE PETRIFILM™ AND THE CONVENTIONAL METHODS FOR ENUMERATING AEROBIC BACTERIA AND *E.COLI* IN LOCALLY PRODUCED SOFT CHEESE IN BAGHDAD

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ABSTRACT

The main objectives of the present study were to compare the petrifilm ^{TM} Aerobic count plates (ACP) with conventional standard plate count (SPC) for enumerating aerobic bacteria and secondly to compare the petrifilm ^{TM} coliform count plates (CCP) with conventional coliform plate count (CPC) method for isolation and enumeration of *E.coli* in locally produced soft white cheese samples .A total of 60 samples of soft cheese (30 samples to each petrifilm^{TM} and conventional methods) have been collected randomly at weekly intervals from different retail markets in Baghdad province and its surroundings during the period of 6 months from the December 2011 till the May 2012.All results of cultural characteristics and biochemical reactions of *E.coli* isolates were in accordance with the main features described in Bergeys Manual of determinative bacteriology .The laboratory studies of the cultural isolation revealed that 20 (66.6%) isolates of *E.coli* were isolated from 30 soft white cheese samples by the conventional direct plating (CPC) method while 24 (80%) isolates of *E.coli* were isolated from another 30soft cheese samples by using a new petrifilm ^{TM} technique. The detection limit for aerobic bacteria by the petrifilm ^{TM} technique versus the conventional direct plating were $16x10^9$ cfu/g and $5x10^8$ cfu/g respectively while the detection limit of *E.coli* by the petrifilm ^{TM} technique versus the conventional coliform plate count (CPC) were $22X10^6$ cfu/g and $12x10^5$ cfu/g respectively. Results obtained in this study revealed that the petrifilm ^{TM} technique has been recognized to be significantly ($P<0.05$) more efficient in its sensitivity for enumeration of both *E .coli* and aerobic bacteria than the conventional direct plating method.

INTRODUCTION

The conventional techniques for the enumeration of microorganisms in food are laborious and material-intensive .Petrifilm^{TM} is a ready-to-use culture medium system contains standard nutrients ,a cold-water-soluble jelling agent and a tetrazolium indicator that eases and speeds colony enumeration . Petrifilm^{TM} (ACP) plates are

ready to receive 1.0 ml of sample with the benefits of saving time , labour and the space that an incubator requires. Petrifilm™ has been validated as an alternative for aerobic count by the international American Public Health Association (1) .Some workers have found differences in the count between the conventional and petrifilm™ methods in some fermented foods (1). Milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms. Therefore in the processing of milk, some of them may produce undesirable effects and some micro-organisms produce food infections which can either carry the pathogens that will increase the likelihood of infection of the consumer`s food. Contamination of milk and milk products is largely due to human factor and unhygienic conditions. Usually milk is contaminated with different kinds of microorganisms at milk collecting places .Milk is a major part of human food and plays a prominent role in the diet. Approximately 50 percent of the milk produced, is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as Ice cream and Butter (2). The manufacture of soft cheese by the farmer is based on traditional method without any regard to the quality of raw milk used and/ or the hygienic quality of the products. Under such conditions many microorganisms can find access to the milk products. Among all micro-organisms *Escherichia coli* is frequently contaminating organism, and is reliable indicator of fecal pollution generally in insanitary conditions of milk production and other dairy products (3). Milk products like cheese and curd are widely consumed and market for them has existed in many parts of the world for many generations. There is an increase demand by the consumer for high quality natural food, free from artificial preservatives, and contaminating microorganisms. Contamination of milk and milk products, with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions (4,5) . Presence of *E. coli* in milk products indicates the presence of Enteropathogenic microorganisms, which constitute a public health hazard. Enteropathogenic *E. coli* can cause severe Diarrhea and vomiting in infants, and young children (6). The aim of this work was to compare the Petrifilm™ with the conventional method for enumerating aerobic bacteria and *E.coli* in locally produced soft white cheese samples.

MATERIALS AND METHODS

1-Collection of samples:

A total of 60 soft white cheese samples (30 samples for each conventional and Petrifilm™) were collected randomly at weekly intervals from different retail markets in Baghdad province and its surrounding during the period of 6 months from (December 2011 till May 2012) . Each sample was kept in a sterile pouches and transported to the laboratory inside ice box until analysis was conducted.

2-Microbial analysis:

Isolation and enumeration of *E.coli* from soft cheese by Petrifilm™ method :

Representative 11 gram portion from the surface and the core of each soft cheese sample (30 samples) were extracted aseptically and added to 99 ml of sterile 0.1%

Pepton water versus aqueous 2% Sodium citrate (Dual - purpose medium) enrichment medium which pre warmed to 40 °C (7 ,8)and homogenized for 5min in a stomacher and Then serial 10- fold dilutions were prepared and plated in duplicate (9). Inoculated Petrifilm ™ plates were incubated at 32C° for 48 h for aerobic bacteria count and 42 C° for 24 h for *E.coli* count .

Isolation of *E.coli* and enumeration aerobic bacteria from soft cheese (30 samples) by conventional cultural methods:

Conventional methods were based on cultural and biochemical properties of *E.coli* and aerobic bacteria from soft cheese (9) . For the isolation and identification of *E. coli*, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37 °C for 24 hr . Morphologically typical colonies (at least 4 / plate) producing metallic sheen were taken into nutrient broth for further identification. (10) . Four to five suspected colonies from each bacterial plate were picked, cultured and then identified by the various biochemical tests. Biochemical tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, test, Urease production, Simon citrate agar, and sugar fermentation test . Aerobic bacteria growing in typical white colonies on Aerobic Count Plates agar. (Table 2).

Statistical analysis: All the experiments were conducted three times each in duplicate. The statistical analysis of the data was performed by F test analysis (one-way ANOVA) then the Least Significant Differences (LSD) were conducted to find the significant differences between the means values and the significant differences were determined at ($P<0.05$). Chi square used to determine the differences between observed and expected data (11).

RESULTS

The prevalence of *E.coli* in locally produced soft cheese samples by using both conventional and Petrifilm™ (CCP) methods is shown in table 1.The laboratory studies of cultural isolation revealed that twenty isolates (66.6%) of *E.coli* were isolated by conventional method from thirty soft white cheese samples while twenty four isolates (80%)of *E.coli* isolated from another thirty soft cheese samples by Petrifilm™ coliform count plates(CCP) method. In current study the results revealed that Petrifilm™ (CCP) method has been demonstrated to be more sensitive in detecting *E.coli* than the direct conventional plating method. Results obtained in this study revealed that out of 60 soft white cheese samples forty four isolates(73.3%) were confirmed as *E.coli* as shown in Table 1.

Table (1) : prevalence of *Escherichia coli* in locally produced soft cheese samples by conventional and petrifilm™ methods :

Methods	Number of soft cheese samples	Number of positive isolates	Isolation percentage%
Conventional method for isolation *E.coli*	30	20	66.6
Petrifilm™ *E .coli*	30	24	80
Total	60	44	73.3

X^2 =3.158 df=3

Colonies of *E.coli* were isolated from soft white cheese samples and their identification were confirmed on the basis of morphological , cultural and biochemical characteristics that are shown in tables 2 and 3 .*E.coli* colonies growing on Petrifilm™ plates appeared as red owing to the reduction of the indicator dye and identified by the gas bubbles trapped around each colony .*E.coli* colonies on VRB agar were appeared as dark red while on nutrient agar while on nutrient agar were appeared as yellowish white colonies and on EMB agar showed the occurrence of green-metalic sheen on the surface of the colonies .*E.coli* was gram negative rods and lactose fermentation while negative for both citrate and urease (Table3).

Table (2): Culture characteristics of *E.coli* and aerobic bacteria on different media:

Medium	Result and Descriptions
Petrifilm™ ***E .coli*** plate	Red colonies associated with entrapped gas on the Petrifilm EC plate (within approximately one colony diameter (reddish- pink with gas)
Violet Red bile agar (VRBA)	Small, circular pink colonies.
Eosine Methylene blue agar (EMB).	Metallic green sheen
Nutrient Agar	Colorless and yellowish white, circular, smooth colonies with entire edge.
Petrifilm™ **aerobic count plate** (ACP)	A red indicator dye in the plate colours all colonies red.
Aerobic plate count agar (SPC) Agar	White colonies .
MacConkey agar	Smooth circular pink colonies

Table (3): Biochemical reactions of *E.coli* bacteria isolated by conventional method:

NO of samples for conventional method	NO. of Isolates	Biochemical test	Reaction
30	20	Gram staining	Gram Negative
		Lactose fermentation	+
		Catalase	+
		Simmon's Citrate	–
		Indole Production	+
		Urease	–

The aerobic bacteria and *E.coli* counts(cfu/g) in soft cheese samples using the Petrifilm^{TM} (ACP) and (CCP) versus the conventional pour plating (SPC and CPC) were shown in Table 4.The detection limits (number of bacteria) for aerobic bacteria by the Petrifilm^{TM} (ACP) versus the conventional pour plating were $16x10^9$cfu/g and $5x10^8$ cfu/g respectively while the detection level of *E.coli* by the Petrifilm^{TM} (CCP) versus the conventional Coliform plate count (CPC)were $22x10^6$ cfu/g and $12x10^5$ cfu/g respectively results obtained in this study revealed that the Petrifilm^{TM} technique has been recognized to be significantly ($P<0.05$) more efficient for enumeration of both *E.coli* and aerobic bacteria than conventional pour plating method as shown in Table 4.

Table(4) Microorganism counts on the petrifilm and conventional media :

Microorganism	Methods	
	Petrifilm^{TM} Counting cfu/g Mean± SE	**Conventional methods Counting cfu/g Mean± SE**
Aerobic bacteria	$16x10^9 \pm 4041^a$ A	$5x10^8 \pm 3000^b$ B
E.coli	$22x\ 10^6 \pm 502^a$ A	$12x10^5 \pm 320^b$ B

The small different letters in the same row denoted that significantly differences between bacteria.

The capital different letters in the same row denoted that significantly differences between methods .

± SE (Standard Error).

DISCUSSION

Microbiological analysis of foods is based on the detection of microorganisms by visual ,biochemical ,immunological , or genetic means ,either before enrichment

(quantitative or enumerative methods) or after enrichment (qualitative methods, also known as presence /absence tests). Traditional culture methods for detecting microorganisms in food are based on the incorporation of the food sample into a nutrient medium in which the microorganisms can multiply , thus providing visual confirmation of their growth , these conventional test methods are simple , easily adaptable, very practical , and generally inexpensive .Although not lacking in sensitivity , they can be laborious and depend on the growth of the microorganism in different culture media (pre-enrichment , selective enrichment , selective plating identification) which may require several days before results are known (13) .Products that are minimally processed have an inherently short shelf life , which prevents the use of many of these conventional methods. Therefore, extensive research has been carried out over the years to reduce assay and reduce the amount of manual labor by alternative methods whenever possible (14). In order to meet the increasing demand for food quality and safety , the control of pathogenic microorganisms from " farm to fork " is continuous challenge . This challenge has become more important due to changes in animal production , product processing and distribution , new food habits higher numbers of consumers at risk for infection and increased awareness (15). The Petrifilm^{TM} Aerobic Count plate (ACP) developed by 3M laboratories , is a ready-to-use culture medium system ,useful for the enumeration of aerobic count by international associations such as American Public Health Association .Conventional techniques for the enumeration of microorganisms in food are laborious and material intensive while The Petrifilm^{TM} Aerobic Count plate (ACP) and Petrifilm™ *E .coli* system contains standard nutrients ,a cold-water-soluble jelling agent and a tetrazolium indicator that eases and speeds colony enumeration (16) . The Petrifilm^{TM} plates are ready to receive 1.0 ml of sample with benefits of saving time, labour and the space that an incubator requires. Petrifilm^{TM} (ACP) was compared with the standard method for the enumeration of aerobic flora in several different food products with satisfactory results .However ,some workers have found differences in the count between both methods in some fermented foods (16,17).Also the Petrifilm^{TM} system has the advantage of being easy to use , traditional media preparation is unnecessary and save both labour and time .On other hand , the only disadvantage seen in this work was that on overcrowded plates the center didn't show visible colonies and many small colonies were seen on the edges therefore further dilution of sample was required .In conclusion the process of selecting an appropriate method must consider the main criteria of the sensitivity of analysis , the time detection and the specificity of the test. The cornerstone of any method is accuracy .This consists of the sensitivity and specificity .The intent in developing a rapid assay is to reduce the time required to obtain an accurate results (18). The two methods studied in this work were able to detect of organisms, complying with the conventional standards methods; however, the Petrifilm AC plates detected the organisms in less time than the other method. The Petrifilm ^{TM} plates gave reproducible results compared with the conventional methods and are compatible with the demanded rapid industrial requirements for milk quality control.

مقارنة بين البيتريفلم والطرق التقليدية في عد البكتريا الهوائية والايشير يشيا القولونية في الجبن الطري المصنع محليا في بغداد

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الخلاصة

الهدف الرئيس للدراسة الحالية هو مقارنة البتريفلم لعد البكتريا الهوائية بالطرائق التقليدية بالإطباق,ثانيا عزل وتعداد الايشيريشيا القولونية باستخدام الطرائق التقليدية وطريقة البتريفلم من عينات الجبن الأبيض الطري المصنع محليا .جمعت 60 عينة من الجبن الطري 30 عينة لكل من الطرائق التقليدية وطريقة البيتريفلم عشوائيا وعلى فترات أسبوعية من الأسواق المحلية في محافظة بغداد والمناطق المحيطة بها خلال مدة 6 أشهر (كانون الاول 2011 وحتى ايار 2012).كانت جميع نتائج الخصائص الزراعية والتفاعلات الكيموحيوية للعزلات البكترية مطابقة للميزات الرئيسة التي ذكرت في دليل Bergeys لعلم البكتريا . حددت الدراسات المختبرية للعزلات البكترية الى 20 عزلة (66.6%) من الايشيريشيا القولونية من عينات الجبن الابيض الطري بالطرائق التقليدية ,بينما حددت اربعة وعشرون عزلة(80%) من الايشيريشيا القولونية من 30 عينة جبن طري اخرى وباستعمال تقنية البتريفلم .كانت حدود الكشف للبكتريا الهوائية $X5\ 10^8$ وحدة تكوين المستعمرة / غم و$X16\ 10^9$ وحدة تكوين المستعمرة / غم من عينات الجبن وعلى التوالي . في حين كانت حدود الكشف للايشيريشيا القولونية $X\ 12\ 10^5$ وحدة تكوين المستعمرة / غم و$22\ x\ 10^6$وحدة تكوين المستعمرة / غم من عينات الجبن الطري وعلى التوالي . اشارت النتائج ان طريقة البيتريفلم هي الاكثر كفاءة وحساسية في عد كل من الايشيريشيا القولونية والبكتريا الهوائية وبمستوى معنوية ($P<0.05$) من الطرائق التقليدية للعزل المباشر من عينات الجبن الطري .

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