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Microbial Contamination Of White Soft Local Chesse In AL-Refaee City

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Abstract: *Seventy specimens of white cheese, presented for sale in local market at the center and in some towns of AL-Refaee city from November 2015 to May 2016.The study showed that the contamination rate was (100%)among them, mix growth was found in 21 specimens (30%), where as 48 (68.57%) were single growth resulting 91 isolates, most of them were related to Enterobacteriaceae which represent (58/91).Escherichia coliwas the most common isolated bacteria 19(20.6%), followed by Staphylococcus aureus15(16.3%) and other included: Salmonella spp. 12(13.1%),Klebsiella spp. 10(10.9%), Shigella spp. 9(9.8%), Staphylococcus epidermidis 9(9.8%), Proteus spp. 8(8.7%), Brucellamelitensis 5(5.4%),Listeria mocnocytogenes 4(4.3%).The ability of some pathogenic bacteria transmitted through contaminatedcheese to produce enzymes asHaemolysin, Protease, Lecithinase and Lipase.The results showed the ability of Staphylococcus aureusand L. Monocytogenesto produceHaemolysin,Lecithinase,Lipase and Protease. B. Melitensisisolates were unable to produce them.*

Key Wards: *White Cheese, Contamination, Pathogenic bacteria, Al-Refaee City.*

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I. Introduction

Milk and milk products are among the important food substances for every age group due to the nutrients they contain (Torres-Vitela*et al*., 2012). The milk, which is milked from the teats of a healthy animal, contains very low levels of microorganisms. Since the milked milk is a medium, where the microorganisms that are contaminated during milking, transporting and presenting for sale can develop easily, the foods, which are produced and marketed under unsuitable hygienic and sanitation conditions, cause infections or intoxications in human beings (Shresthaet *al*., 2011). The microorganisms, which infect through various sources, exhibit a very rapid growth in cheese, a nutrient that is quite rich in protein and calcium (Evrendilek*et al*., 2008). Today a considerable amount of milk produced in our country is produced in dairies that are deprived of technical information and modern instruments and by village women. Although classical methods are used generally in transforming milk into cheese, there are some application differences. The primary differences are pasteurization, coagulation (yeast addition) and maturation periods. Coliform bacteria constitute the most dangerous group among the microorganisms that infect cheeses through various sources (Giammanco*et al*., 2011).

II. Material Method:

Sampling:
70 sample white cheese samplewerecathered from different places of Al-Refaee city and srrounded area from November 2015 to May 2016

Isolation:
1 gram of white chesse sample was cut into small peice by kniefe then transported to container contain 10 ml of sodium citrate (2%) mixed gently (Harrigan and McCance.,1976) full loop of this solution was cultured on different media which include:

1. blood agar.
2. Oxford agar.
3. Brucella agar
4. PLCAM selective Agar.
5. Maconky agar.

(lovett and Hitchins.,1987;Beumer*et al*.,1997;Curtis *et al*.,1989 and VanNettene*t al*.,1989)

Identification:

Cultural characteristics :-

The shape ,elevation and color of the colonies in addition to the type of haemolysis observed after plating the samples on blood and nutrient agar.

MicroscopicExamination:

The suspected cultures were studied using gram stain and their shapes,coloursand arrangement were observed under microscope (Holt *et al.* 1994),(Cowan,2002), (Cheesbrough.,2000) ,(MacFaddin.,2000) and (Collee *et al*.,1996)

Biochemical Tests:

1. Oxidase Test:

A filter paper was soaked with several drops of freshly prepared oxidase reagent, then a small portion of the colony was picked up and placed on the filter paper.Positive oxidase was indicted by the production of a Purple or blue colour within30 seconds(Baron *et al*., 1994).

2. Catalase Test:

Few drops of 3% H_2O_2 were dropped on a clean dry slide,and a small portion of the culture transported to be tested by mixing it . The formation of bubbles is a positive result (Baron *et al*., 1994).

3. Indol Test:

Tubes containing peptone water were inoculated with the colony of tested bacteria and incubated at 37˚Cfor 48 hours. Then several drops of kovac's reagent were added to the broth medium with gentle mixing. The appearance of red ring on the surface liquid medium was a positive result (Cruickshank *et al*., 1976).

4. Methyl Red Test:

Tubes containing10ml of MR.VP broth were inoculated with the young culture of tested bacteria and incubated at 37˚Cfor 48 hours. Then 5 drops of methyl red reagent were added to the broth medium with gentle mixing . A bright red in color was Positive result (Colle *et al*., 1996).

5. VogusProskaur Test:

The tubes of (MR-VP broth) were inoculated with the bacterial culture and incubated at 37˚C for 48 hrs., then 0.6 ml of reagent A and 0.2 ml of reagent B .Appearance of red colour after 15 minutes,and this a positive result referring to a partial analysis of glucose (MacFaddin., 2000).

6. Citrate utilization test :

The test was done by inoculating the simmon's citrate slant medium with colony of the tested bacteria and incubated at 37 ˚C for 1–3 days. A change in colour of the medium from green to blue refers to the Positive result (MacFaddin., 2000).

7. Growth on Kligler's Iron Agar:

According to the instructions sheet of the manufacturer ,the gram negative bacteria was inoculated on to medium by stabbing the buttom of the tube and streaking the surface of slant. Fermentation was detected by a change in the indicator phenol red to yellow. The pH changes in the buttom and the slant of medium were recorded after 18–24 hours of incubation. Gas formation is usually visualized as bubbles in the medium caused by the gas formed in the agar. The black precipitate on the buttom indicates the production of H2S.

8. Growth on mannitol salt agar:

This medium used for the selective isolation,cultivation and enumeration of *Staphylococci*.Mannitol-utilizing organisms turn the medium yellow (Benson.,2002).

9. Coagulase Test (Tube method):

A colony of *Staph. aureus* was inoculated in nutrient broth over night at 37˚C, 0.5ml of this culture was added to 0.5ml of plasma in a test tube.The tubes were incubated at 37˚C. The tubes were examined after 1, 2, and 4 hours to see any clot formation. The negative tubes were left at room temperature overnight and examined (Colleeet *al*., 1996).

10. Carbohydrate Fermentation Test:

The solution of the carbohydrates added to the basal medium, the final concentration of each was 1%.The bacterial colonies were inoculated in carbohydrate fermentation broth and incubated at 37˚C for 1-3 days. The yellow color media with or without gas production indicates positive test (MacFaddin., 2000).

11. Motility Test:

Tubes containing Semi solid medium were inoculated with a young agar culture by stabbing once at the center with inoculating needle, then incubated at 37 ˚C for 24hours. The motile bacteria spread out from the line of stabbing (Cheesbrough.,2000).

12- Deoxyribonuclease (DNase) Test:

The DNA-ase plate was divided into the required number of strips by marking the underside of the plate. A colony of bacterial isolates was inoculated with a sterile loop on to medium plate. The plate Incubated aerobically at 37°C over night. After incubation, the surface of the plate was covered with 1N hydrochloric acid solution to precipitate unhydrolysed DNA. The plate was examined for looking for clearing around the colonies within 5 minutes of adding the acid. Colonies that producing DNA-ase are surrounded by clear zone (Cheesbrough.,2000).

13-Urease Production Test :

This was carried out by inoculating the urea slopes with colonies of the organism and incubated at 37˚C.The slopes were examined after four hours and after overnight incubation. , no tubes were reported negative until after 4 days of incubation. A change in colour of the medium from yellow to pink was a positive result(Colleee*t al*., 1996).

Qualitative detection ofsome virulence factors:

1-Hemolysin production Test :

Blood agar medium was streaked with the bacterial culture and incubated at 37˚C for 24-48 hours. The appearance of a clear zone around the colony indicates ß–hemolysis while the presence of green-color indicates α–hemolysis (Baron *et al.*, 1994).

2- Protease production Test:

A single colony of each isolate was inoculated in 5 ml nutrient broth, incubated at 37˚C over night. 20(µl) of each culturing was transported to the prepared wells in the skimmed milk agar plates .The plates were incubated at 37˚C for 24 – 48 hours (Harrigan and MaCcance.,1976).

3-Lecithinase and Lipase production Test:

The colonies of the test organisim were inculated onto Egg-yolk agar plate by sterile stick and incubated at 37˚C over night. Lecithinase-producing colonies were surrounded by zones. The plate was flooded with a saturated aqueous solution of copper sulphate, after 20 minutes, the excess solution was drained off and the plate dried for a short time in the incubater. The greenish-blue colour of copper soaps of fatty acids referring to the lipase activity (Collee *et al*., 1996).

III. Result:

Identification of Bacterial Isolates:

All bacterial isolates were characterized by cultural , morphological and biochemical characteristics according to Bergey's manual (Holt *et al*,. 1994) as well as, other features reported by (Baron, *et al*., 1994); (Collee *et al*., 1996) and (MacFaddin.,2000).

Identification of *Listeria monocytogenes*:

The identification of *Listeria monocytogenes* was done according to morphological and biochemical tests according to(Holt et al., 1994; Koneman et al., 1997) after 24 hours on Oxford selective media in 37^{o} C*Listeria monocytogenes* gave brownish green colony and trun the media to black color as a result to Hydrolysis of Esculin, on sheep blood agar the colony were small white to grey in color and gave complete zone of haemolysisarround colony, under microscope the bacteria appear gram positive rod single or pair some time as short chain (Marth and Steel.,2001), many biochemical tests were done for confirm identifications Table(1).

Table (1) results of biochemical test of *Listeria monocytogenes*:

Tests	Result
Motility at 25 C	+
β Hemolytic activity	+
Catalase	+
Oxidase	-
Indole	-
Methyl red	+
Voges-proskauer	+
Citrate test	-
Urease production	-
Fermentation of glucose	+
CAMP test	+
Hydrolysis of Esculin	+
H_2S production	-

Identification of *Brucellamelitensis*:

Brucellamelitensis were identified according to cultured microscopical and biochemical tests (Konema*et al*., 1997 ; Holt *et al*., 1994) ,on brucella agar after incubation for 2-5 days in 37 C colony appear small,smoth and pale yellow in color,under microscope gram negative coccobacilli single (0.6-1.5)micrometer ,maney biochemical tests was done for confirm diagnosis table (2).

Table (2) results of biochemical test for *Brucellamelitensis*

Tests	Result
Catalase	+
Oxidase	+
Motility at 25 C	-
H_2S production	-
CO_2 requirement	
Indole	-
Methyl red	-
Voges-proskauer	-
Citrate test	-
Growth in presence of fuchsin	+
Growth in presence of thionin	+
Urease production	-
Gelatin lequification	-
Fermentation of glucose	-

Isolation:

Soft white cheese is widely consumed by the population AL-Refaee city. However. Truzyan (2003) stated that improper milking hygiene without subsequent pasteurization of milk and the lack of general food–hygiene-related knowledge and infrastructure of marketing could be the sources and causes of such contamination.

A total of 70 white chesse sample were subject to culturing on different types of culture media,they were collected from different region of AL-Refaee city. The results revealed that all samples gave positive bacterial culture, among them, mix growth was found in 21 specimens (30%), where as 48 (68.57%) were single growth resulting 91 isolates,.

In the current study, it was shown that gram negative bacteria constitute (63/91) (69.23 %) from the total isolates and were considered the predominant bacteria that cause contaminate white chesse compared to gram positive bacteria which constituted (28/91) (30.76 %) as shown in table (3) .

Table (3) Percentages of gram positive bacteria and gram negative bacteria.

Type of Bacteria	No of isolates	Ratio(%)
Gram negative bateria	63	69.23%
Gram positive bateria	28	30.76%
Total	91	100

Table no(4)shows the predominant organism isolated in the present study was *Escherichia coli* 19(20.6%) followed by*S.aureus* 15(16.3%) This is in accordance with the studies of Kareem (2003) who found 42isolate of *Escherichia coli*in 70 sample and 40 isolate of*Staph.aureus* from 70, the result of current study was

disagree with Al-Ubaidy and Sharif 2008 who there results showed that*S.aureus*isolate with percentage (14.16) as predomenant organism followed by *Escherichia coli*with percentage (12.5%) as seconed most common bacteria isolated from white chesse.

The third most common organism in this studywas *Salmonella spp.* 12(13.1%) In this study, the presence of E. coli,Salmonellaspp. and*S. aureus*in the examined cheeses seems to be related withthe use of raw milk and non-hygienic production processes. Furthermore, it was reported by various researchers that low microbiological quality of white cheese is referred to the poor hygienic conditions in small primitive production establishments (Bostan*et al*., 1992; Tekinsen*et al*., 1993).

Other type of bacteria as follow:

Klebsiella spp. 10(10.9%),*Proteus spp.* 8(8.7%) , 9 isolate (9.8%) *Staphylococcus epidermidis* were isolated , although these bacteria consider as oppertunisiticorganisium cause disease to immunisuperssed people it could produce some virulance factor such as endotoxins(Forbes *et al*., 2002).

The *Shigella spp.* contaminated milk from humen sources because these bacteria did not found in nature,in our study *Shigella spp.* 9(9.8%) found in chesse sample.

*Brucellamelitensis*isolated 5(5.4) contamination of chesse with tese bacteria belong to many reason such screation of bacteria in milk from infected animal , the hand chesse maker during proceeing the chesse (Shaarbaf and Yahya, 1988).Human brucellosis is the most common bacterial zoonosis. Syria, Mongolia, Kyrgyzstan, Iraq and Turkey have the highest incidence world-wide (Pappas *et al*., 2005).

Listeria monocytogenes 4 isolate with percentage (4.3%) this agreed with Al-Ubaidy and Sharif (2008) who isolate this bacteria with percentage (1.7%). From wihtechesse samples.

Table (4) type and number of bacteria isolated from white chesse in study

Bacteria	No.	%
Escherichia coli	**19**	**20.6%**
Staphylococcus aureus	**15**	**16.3%**
Salmonella spp.	**12**	**13.1%**
Klebsiella spp.	**10**	**10.9%**
Shigella spp.	**9**	**9.8%**
Staphylococcus epidermidis	**9**	**9.8%**
Proteus spp.	**8**	**8.7%**
Brucellamelitensis	**5**	**5.4%**
Listeria monocytogenes	**4**	**4.3%**
	91	**100%**

production of Virulance Factor by bacterial isolates:

Table (5) explains the Production of virulence factors by the clinical isolates of CSOM by number and ratio.

Table (5)Production of virulence factors by some clinical isolates of current study.(H)Haemolysin, (Pro)Protease, (Li)Lipase(Le)Lecithinase.

BacterialIsolates	No. of isolates	(H) No(%)	(Pro) No(%)	(Li) No(%)	(Le) No(%)
Staph.aureus	**15**	**15(100)**	**15(100)**	**15(100)**	**15(100)**
Brucellamelitensis	**5**	**0(0)**	**0(0)**	**0(0)**	**0(0)**
Listeria monocytogenes	**4**	**4(100)**	**4(100)**	**4(100)**	**4(100)**

1- Production of Haemolysin:

This factor causes membrane damging toxins that lyse eukaryotic cell membrane (Murray *et al.*, 2003).This study showed thatisolates of *Staph.aureus* 24(100%) were able to produce Haemolysin,and isolates of *Proteus mirabilis* 4(80%) .These results agreed with (Hussein, 2010) who found in his studythat isolates of *Staph.aureus* 43 (100%) were able to produce Haemolysin,and isolatesof *Proteus mirabilis* 28 (100%) . Whereas the isolates of*Staph.epidermidis* 2 (15.38%) produced Haemolysin.allisolte of *Listeria monocytogenes*are able to produce haemolysin .Other bacterial isolates did not show any haemolysis,the results showed that *K. pneumoniae*isolate could not produce hemolysin .This result agreed with (Al-wae'li.,2008) who showed that all *K. pneumoniae*isolates could not produce hemolysinextracellulary when cultured on blood agar.

2-Protease Production:

Some bacteria particularly pathogenic can use proteins as their source of carbon and energy. They secrete protease enzymes that hydrolyze proteins and polypeptides to amino acids, which are transported into the cell and catabolized. (Prescott *et al*., 2008). This study showedthatisolates of *Staph.aureus* 15(100%)and all isolate 4(100%) *Listeria monocytogenes*have the ability to produce protease enzyme,this study agreed with (Al-wae'li.,2008), (Al-Hadethi,2009) and (Mahmood, 2010) that *Staph. aureus* have the abilityto produce this enzyme, while all isolates of *Brucellamelitensis* 5(100%) did not produce protease enzyme.

3-Lecithinase and Lipase Production:

The results of this study showed that all isolates of*Staph. aureus*15(100%) were able to produce lecithinase and lipase, *and the Staph. epidermidis* isolates produced only lipase enzyme .These results agreed with (Mahmood, 2010) and (Al-Hadethi,2009).also isolate of *Listeria monocytogenes*4(100%) showed the ability to produce both these enzyme these result ageed with Feresu and Jones (1988). while all isolates of *Brucellamelitensis* 5(100%) did not produce lipase enzyme .

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