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Prevalence of some pathogenic bacteria in dairy products

Hemmat, E. El-Toukhy [1,*], Hamdi M. Abdelsamei [2], Hend A. El-Barbary [2], [1]Marionette, Z. Nassif [1]

[1] *Department of Food Hygiene, AHRI, ARC, Egypt*

[2] *Milk Hygiene, Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Egypt*

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ABSTRACT

The study was planned to bacteriologically and molecularly recording the prevalence of *Escherichia coli* (*E. coli*), *Bacillus cereus* (*B. cereus*), and *Staphylococcus aureus* (*S. aureus*) in milk and milk products (raw milk, Kareish cheese and packed feta cheese, plain yoghurt and raw cream). Samples were taken from different supermarkets, dairy shops and street vendors at Qalubiya Governorate, Egypt. Results illustrated that the incidence of *E. coli*, *B. cereus* and *S. aureus* in the total of the examined samples were 47.5, 3.3 and 70.83%, respectively. In addition, the examined *iss* and *fim*H genes of *E. coli*, *ica*D and *hlg* genes of *S. aureus*, *nhe* and *cyt*K genes of *B. cereus* were detected in all of the examined isolates indicating pathogenicity and virulence of the isolated strains.

1. INTRODUCTION

Milk is a highly proteinaceous and easily digested food; which is also rich in minerals, energy, hormones and growth factors (Anema, 2020). Milk and its products act as a good environment for microbial growth which constitutes biological hazards for consumers (Eman et al., 2011). Bacterial pollution in milk can occur at any step during production, manufacturing and distribution. Bacterial pollution could occur because of the animal, parlor, milk utensils, additives used in production and workers (Garedew et al., 2012). Milk with high microbial load has short shelf life, the sources of contamination include internal causes as nutrients, water activity, pH, temperature, or external causes occurred during production stages, processing and packaging (Hosny et al., 2011). Yoghurt is healthy dairy products with valuable health benefits (Serafeimidou et al., 2012). Fermentation processes enhance proliferation of beneficial microflora; in addition of lactic acid bacteria (LAB) so milk has been believed to have biological preservatives (Tesfaye et al., 2011). The microbial load in fresh cream depends upon the hygiene during production conditions and storage temperature (Anand, 2019). The count of pathogenic and spoilage bacteria in milk and its products depend on various factors like the animal health, the dairy farm environment, production conditions, storage facilities and technologies, farm management, geographical area and season (Muehlhoff et al., 2013). Therefore, in order to decrease the risks associated with dairy products, must apply preventive measures like good source of animal feeding, good manufacturing and hygiene practices, consumer's safety awareness, and proper application of control points throughout the dairy chain (Kenny, 2013). Entero-pathogenic *E. coli* is a food–borne pathogen that causes severe lethal diarrhea for children, which was recorded in poor countries (Alunso et al., 2011). *E. coli* resist production environments because of the continuous source of contamination. Shiga toxin-producing *E. coli* (STEC) are most common as it habituated in the colon of healthy animal and man, so it is easy to be found in the environment. It can be transmitted to humans via consumption of contaminated food. The incidence of Shiga-like toxin produced by *E. coli* (STEC) in raw dairy products is depending on application of effective control (Valente et al., 2019). *Bacillus cereus* involved in two types of foodborne illness – emetic (vomiting) and diarrheal type. The emetic syndrome result from consumption of food containing toxin, it has a short incubation period (1–5 hours) and recovery time (6–24 hours), the symptoms are nausea, vomiting and abdominal cramping. Diarrheal type caused by ingesting food contaminated with high *B.cereus* spores count (Senesi and Ghelardi, 2010). *Staphylococcus aureus* is a common pathogenic bacterium contaminating milk-related environments where it can cause gastrointestinal diseases (Lukas *et al.*, 2013). *S. aureus* is the most pathogen able to produce thermostable enterotoxins, but at temperature in between 10 to 25ºC didn't able to produce amounts of staphylococcus enterotoxin C (SEC) that would be sufficient to cause food poisoning (Valihrach et al., 2013). Therefore, the main target of the current research was to evaluate the prevalence of *E. coli*, *B. cereus* and *S. aureus* in milk and some dairy products collected from Qalubiya Governorate markets.

2. MATERIAL AND METHODS

2.1. Collection of samples

One hundred and twenty random samples of raw milk, raw cream, cheese (Kareish and Packed feta cheese) and yoghurt (30 sample each).

Correspondence to: hemmateltokhy@gmail.com

Table (1): Specific oligonucleotide primer sequence used in this study

Bacteria	Gene	Primer sequence(5'-3')	Amplified product	Reference
E. coli	iss	F-ATGTTATTTTCTGCCGCTCTG R-CTATTGTGAGCAATATACCC	266 bp	Yaguchi et al., 2007
	fimH	F-TGCAGAACGGATAAGCCGTGG R-GCAGTCACCTGCCCTCCGGTA	508 bp	Ghanbarpour and Salehi, 2010
S. aureus	icaD	F-AAA CGTAAG AGA GGT GG R-GGC AAT ATG ATC AAGATA	381 bp	Ciftci et al., 2009
	hlg	F-GCCAATCCGTTATTAGAAAATGC R-CCATAGACGTAGCAACGGAT	937 bp	Kumar et al., 2009
B. cereus	Nhe	F-AAG CIG CTC TTC GIA TTC R-ITI GTT GAA ATA AGC TGT GG	766 bp	Ehling-Schulz et al., 2006
	cytK	F-ACA GAT ATC GGI CAA AAT GC R-CAA GTI ACT TGA CCI GTT GC	421 bp	

F: forward primer, R: reverse primer

They were collected from supermarkets, dairy markets and street salesman at Qalubiya Governorate, Egypt. All samples were kept in tank containing ice and delivered without delay to the laboratory

2.2. Preparation of samples according to APHA, (2004)

2.2.1. Raw milk samples

Raw cow milk samples were collected in clean, dry and sterile sampling bottles (200 ml). 11 ml from each milk samples were added to 99 ml of 1% peptone water solution in a sterile bottle and thoroughly mixed to make a dilution of 1/10 from which tenth fold serial dilution was prepared.

2.2.2. Cheese (Kareish and Packed feta)

Two hundred fifty grams from the thirty random samples (15 for each Kareish and Packed feta were collected in sterile air tight sampling jars. 11 grams of each cheese sample thoroughly mashed with 99 ml of 2% sterile solution of sodium citrate for 1 min solution in a sterile electric mixer. Then, tenth fold serial dilution was prepared.

2.2.3. Raw cream

Thirty random raw cream samples were collected from supermarkets, dairy shops and street vendors in sterile air tight sampling jars (250 gm). 11 grams of each cream sample was thoroughly mashed with 99 ml of 1% peptone water solution in a sterile electric mixer, then, tenth fold serial dilution was prepared.

2.2.4. Yoghurt

Seventy five grams yoghurt cups samples were thoroughly mashed with 99 ml of 1% peptone water solution in a sterile electric mixer, then, tenth fold serial dilution was prepared

2.3. Isolation and Identification of E. coli according to ISO, (2001)

One ml from the serial dilution was cultured in TBX agar by pour plating method, and incubated at 44º C for 24 hours. Morphologically typical colonies (blue colony) were taken into nutrient agar slope and incubated at 37 ˚C for 24 hrs for further identification (Microscopically examination, Biochemical tests and PCR examination).

2.4. Isolation and Identification of Bacillus cereus following FDA, (2001)

The volume of 0.1 ml from the serial dilution was cultured over of *Bacillus cereus* agar medium, and then was incubated at 37 °C for 24 hrs. Typical *B. cereus* colonies show rosette shape with greenish to bluish color and change the media color to bluish color, were taken into nutrient agar slope and incubated at 37˚C for 24 hrs for further identification (Microscopically examination, Biochemical tests and PCR examination).

2.5. Isolation and identification of S. aureus according to ISO, (2003)

The volume of 0.1 ml from the previously prepared serial dilution was spread over Baird-Parker agar plates, and incubated at 35±2ºC for 24-48 hours. suspected colonies are, circular, smooth, convex, moist 2-3 mm in diameter, gray to jet black, with light colored (off –white) margin, surrounded by opaque zone with an outer zone and had gummy consistency when touched with inoculating needle, were picked up onto nutrient agar slant and incubated at 37 ºC for 24hrs for further identification (Microscopical, Biochemical tests and PCR examination).

2.6. Molecular detection of some virulent genes of some isolated strains

In the present work, nine pairs of primers (metabion, Germany and Biobasic, Canada) were used. Their special sequences are displayed in Table (1). Bacterial DNA was extracted following QIAamp DNA Mini Kit (Catalogue no.51304). Preparation of master mix and thermal profile was adopted according to the manufacturer instructions (Emerald Amp GT PCR master mix (Takara) Code No. RR310A).

3. RESULTS

As recorded in Table (2); *E. coli* was detected in 57 samples representing 47.5% of the total examined samples. In detail, raw milk and cream samples were the prominent contaminated with *E. coli*; followed by yoghurt and kareish cheese samples, respectively, while *E. coli* was not detected in packed feta cheese samples.

Table (2): The prevalence of *E. coli* in the examined dairy products

Dairy products	Number of Samples	Positive samples	
		No.	%
Raw milk	30	23	76.7%
Raw cream	30	23	76.7%
Retail kareish cheese	15	4	13.3%
Packed feta cheese	15	ND	-
Yoghurt	30	7	23.3%
Total	120	57	47.5%

ND: not detected

On the other hand, *B. cereus* was only detected in raw milk sample with the prevalence of 13.3%, while could not be detect in other examined samples (Table, 3).

Table (3): The prevalence of *B. cereus* in examined dairy products

Dairy products	Number of Samples	Positive samples	
		No.	%
Raw milk	30	4	13.3%
Raw cream	30	ND	-
Retail kareish cheese	15	ND	-
Packed feta cheese	15	ND	-
Yoghurt	30	ND	-
Total	120	4	3.33%

ND: not detected

While in Table (4), results revealed detection of *S. aureus* in a total of 70.83% of the examined samples; where it was detected in all of the examined kareish cheese samples (100%). The lowest prevalence was recorded in yoghurt samples (50%).

Table (4): The prevalence of *S. aureus* in examined dairy products

Dairy products	Number of Samples	Positive samples	
		No.	%
Raw milk	30	22	73.3%
Raw cream	30	23	73.7%
Retail kareish cheese	15	15	100%
Packed feta cheese	15	10	66.6%
Yoghurt	30	15	50%
Total	120	85	70.83%

Referring to the obtained results of molecular detection of some virulent genes of the examined isolates; *iss* and *fim*H genes of *E. coli*, *nhe* and *cyt*K genes of *B. cereus*, *hlg* and *ica*D genes of *S. aureus* were detected in all of the examined isolates (figs 1 to 3).

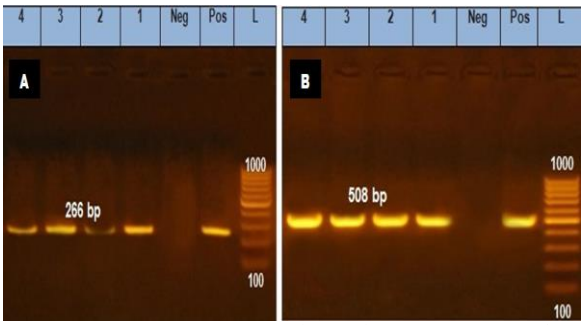

Fig. (1): Agarose gel electrophoresis of PCR of *iss* (266 bp) (A), and *fim*H (508 bp) (B) virulence genes of *E. coli*. Lane L: 100 bp ladder as molecular size DNA marker. Lane Pos.: Control positive *E. coli* for *iss*, and *fim*h genes.Lane Neg.: Control negative. Lanes 1, 2, 3 and 4: Positive *E. coli* for *iss* and *fim*h genes

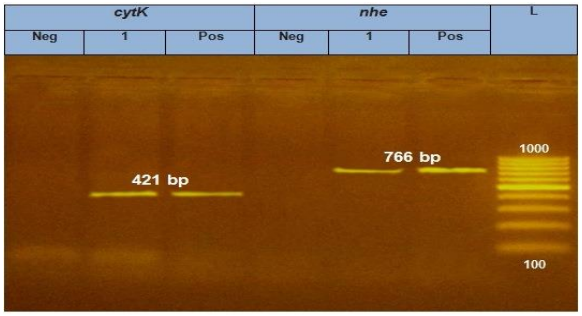

Fig (2) Agarose gel electrophoresis of PCR of *nhe* (766 bp), and *cyt*K (421 bp) virulence genes of *B. cereus*.Lane L: 100 bp ladder as molecular size DNA marker. Lane Pos.: Control positive *B. cereus* for *nhe*, and *cyt*K genes.Lane Neg.: Control negative. Lanes 1: Positive *B. cereus* for *nhe* and *cyt*K genes

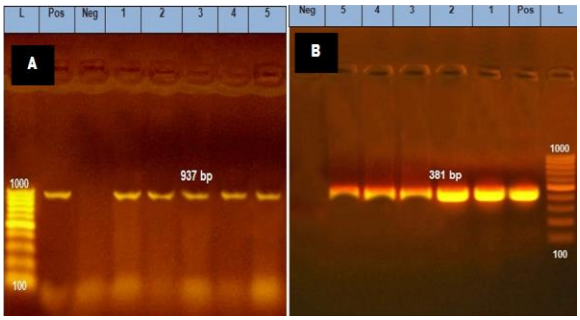

Fig. (3): Agarose gel electrophoresis of uPCR of *hlg* (937 bp) (A), and *ica*D (381 bp) (B) virulence genes of *E. coli*.Lane L: 100 bp ladder as molecular size DNA marker.Lane Pos.: Control positive *S. aureus* for *hlg*, and *ica*D genes.Lane Neg.: Control negative.Lanes 1, 2, 3 and 4: Positive *S. aureus* for *hlg* and *ica*D genes.

4. DISCUSSION

Milk and its products are very important sources of nutrition elements, which needed for the human health; so, they must be produced under complete aseptic conditions to be free from any biological, physical or chemical hazard. Detection of foodborne bacteria in milk and its products is mainly referred to direct or indirect contact between healthy dairy animals and/or their milk with different contamination sources starting with the farms to the consumer (Owusu-Kwarteng et al., 2020). Referring to the obtained results in comparison with previous studies, *E. coli* was detected in 76.7% of raw milk samples and raw cream samples which were higher than those found by Metwally et al. (2015) and El-Bastawesy et al. (2016), but they were less than the results of Nagah et al. (2012) and Bonyadian et al. (2014). In Kareish cheese, *E. coli* was recorded in 13.3% of tested samples which were higher than records of Brooks et al. (2012) and Jang et al. (2018), and lower than Metwally et al. and (2015) Gundogan and Avci (2014); but *E. coli* couldn't be detected in feta cheese samples. Furthermore, *E. coli* was found in 23.3% of yoghurt samples which was nearly similar to the recorded results of El-Bastawesy et al. (2016), and higher than those of Metwally et al. (2015). Presence of *E. coli* in dairy products is used an index for the sanitation of the manufacturing environment, water quality employed in the handling and processing of milk products and personal hygiene of food handlers (Metz et al., 2020). Concerning with heat treated dairy products, the process of pasteurization can easily kill *E. coli*; therefore, the finding of *E. coli* in heat treated dairy products indicates some level of contamination has occurred after pasteurization during manufacturing and/or packaging (Vahedi *et al.*, 2013). All isolated *E. coli* identified by PCR all were positive for *iss* and *fim*H primers. Bacillus cereus was detected in raw milk samples only with 13.3% which was lower than the recorded results of Yobouet et al. (2014); while was not be detected in other tested samples. *Bacillus cereus* is wide spread in the environment and can speculate in different media as earth and plants, but it is also can grow in the digestive tract of insects, animals and in food such as raw milk and cheese (Arnesen et al., 2008). All isolated *B. cereus* identified by PCR all are positive for *nhe* and *cyt*K primers. *Staphylococcus aureus* was detected in 76.7% of raw milk samples and raw cream samples, that were higher than which recorded by Al-Gamal et al. (2019), and lower than those of Kateřina *et al.* (2014). In kareish cheese, *S.aureus* was recorded in 100% of the examined kareish cheese samples, while in 66.6% of examined feta cheese. These results were lower than those reported by Rahimi (2013), Gundogan and Avci (2014) and Jang et al. (2018). *Staphylococcus aureus* present in raw milk and its products and mastitic udder which is considered as a source of toxic strains in raw milk, high storage temperature of raw milk before separation enhance growth of *S. aureus* and can encourage the production toxins (Helena et al., 2010).All isolated *S. aureus* identified by PCR were positive for *hlg and ica*D primers. Variations in the prevalence of the detected organisms could be due to occurrence of different types of sources of contamination among different environmental settings. Thus, exposure of raw milk to these sources could vary among different countries and farms within a country. Using raw milk contain foodborne pathogens in the production line which were resist in biofilms considered as an important source of post heat treatment contamination which is health hazard for the consumer.

5. CONCULOSION

It could be concluded that the non-heat treated milk and its products have risky role in transmission of pathogenic bacteria such as *E. coli, B. cereus and S. aureus* to humans where the milk become contaminated with foodborne pathogens either through direct or indirect contact with contamination reservoirs in the farm or from the mastitic udder. Detection of foodborne bacteria using PCR technique is an important method of accurate, rapid and precise detection of milk and dairy product contamination with food poisoning bacteria. For all the previous, Hazard Analysis and Critical Control Points system (HACCP) must be applied at all production steps for providing safe and healthy dairy products (Oliver et al., 2009).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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