



Incidence of some pathogenic microorganisms in bulk tank milk in some farms of Gharbia governorate.

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ABSTRACT

Milk and its products can harbor a variety of microorganisms and can be important sources of food borne pathogens. The presence of food borne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. The foodborne pathogens can reach humans by direct contact, ingestion of raw contaminated milk or cheese, or contamination during the processing of milk products. Isolation of bacterial pathogens with similar biotypes from dairy farms and from outbreaks of human disease substantiates this hypothesis. This study was conducted to determine the incidence of some pathogenic micro organisms in bulk tank milk from 3 dairy farms in Gharbia governorate, Egypt. *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* were detected with percentages of 37.5, 6.25 and 12.5% in examined bulk milk samples collected from farm I, 25, 12.5 and 25% from farm II and 12.5, zero, and 12.5% from farm III., respectively. The presence of these pathogenic microorganisms in bulk tank milk contribute a potential risk to public health, these findings underscore the need to control them and to limit bacterial multiplication in bulk tank milk.

Keywords: Bulk tank milk, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*.

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1. INTRODUCTION

Milk is an excellent medium for the growth of numerous microbes which produce consequential spoilage of the milk and various milk products or food borne pathogens to consumers (Oliver, et al., 2005). According to the procedure of milk production, the microbial content of milk is a major feature in determining its quality (Torkar and Teger, 2008). The existence of food borne pathogens in raw milk may increase the threat of transmission of food borne pathogens and ingestion of harmful toxins. Huge numbers of microbes can get access to milk and various milk products including, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella Spp.*,

Shigella spp., *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Brucella abortus*, *Campylobacter jejuni*, *Bacillus cereus*, and *Listeria monocytogenes* (Garbutt, 1997). At the farm level, microbial contamination of bulk tank milk occurs via 3 main sources, bacterial contamination from the external surface of the udder and teats, from the surface of milking equipments, and from mastitis organisms within the udder (Murphy and Boor, 2000). *Staphylococcus aureus* is one of the most common agents causing food poisoning. It is involved in intramammary infections in bovine causing economic losses and milk safety problems (Taverna et al., 2007). It produces a number of proteins,

extracellular virulence factors and toxins that one of the most important of them is enterotoxin that cause food poisoning (Orwin *et al.*, 2003). Eleven major antigenic types of SES have been recognized (SEA to SEJ) (Monday and Bohach, 1999 and Tamarapu *et al.*, 2001). It is known that about 95% of staphylococcal food poisoning outbreaks are caused by Staphylococcal enterotoxins (SE), SEA to SEE types (Bergdoll *et al.*, 1983). The remaining 5% of outbreaks may be associated with other newly identified SEs. Actually, SEB is the most important enterotoxin that causes gastroenteritis, nausea, vomiting, abdominal cramps and diarrhea (Rosec and Gigaud, 2002 and Letertre *et al.*, 2003). *Streptococcus agalactiae* is an important bovine pathogen, as it can cause both clinical and sub-clinical mastitis in dairy cows (Keefe, 1997). Mastitis constitutes a source of economic loss for the dairy industry due to its effects on milk quality. It decreases milk yield and lowers the quality of cheese and other manufactured milk products (Politis and Ng-Kawai-Hang, 1988). It also reduces milk quality and nutritive value of milk due to the great changes in its composition mainly lactose and casein Milk yield of a cow with an infected quarter may drop by as much as 40% while the cow does not show any apparent clinical signs of mastitis. *Streptococcus agalactiae* is considered a major cause of elevated somatic cell count as related to standards in bulk tank milk, as somatic cell count rise because of mastitis. A reduction in milk quality ultimately leads to loss of income of the dairy farmer as milk prices are related to milk composition and premiums are lost when somatic cell counts and bacteria counts increase (Karima *et al.*, 2007). In humans, *Streptococcus agalactiae* has been described as one of the most common factors of invasive infections in neonates and it causes invasive and non-invasive infections in adults (Schuchat 2001). *Streptococcus agalactiae* also causes significant morbidity and mortality in

humans, both infants and adults, all over the world (Blumberg *et al.*, 1992). *Escherichia coli* are the most common contaminant of raw and processed milk, amongst the coliforms (Quinn *et al.*, 2002). *E. coli* is often used as an indicator of faecal contamination in milk (Singh and Parakash, 2008). Enterotoxigenic *Escherichia coli* (ETEC) have been implicated in sporadic and epidemic outbreaks of diarrhea in both infants and adults in many parts of the world. ETEC produces one or both of two plasmid-mediated enterotoxins: a heat-stable enterotoxin (ST) and a heat-labile enterotoxin (LT) (Gyles *et al.*, 1974 and Smith and Halls, 1968). LT and ST toxin genes are the main pathogenic elements of ETEC strains. This strain is an intestinal *E. coli* causing diarrhea in infected individuals, also can cause urinary hemolytic syndrome which often happens after an intestinal infection (Johnson *et al.*, 2002). The most important cause of food borne diseases is shiga toxin producing *E. coli* (STEC), which is one of the other seropathotypes of *E. coli* (Beutin and Stephan, 2006). Humans infected with STEC show symptoms, such as abdominal pain and watery diarrhea, and a number of patients develop a life-threatening disease, such as hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) (Verweyen *et al.*, 2000 and Brett *et al.*, 2003).

2. MATERIAL AND METHODS

2.1. Samples

A total of 48 bulk milk samples collected from 3 dairy farms in Gharbia governorate (16 samples from each farm) were subjected to bacteriological examination for detection of some pathogenic microorganisms including enumeration of staphylococci, streptococci and coliform counts and isolation and identification of *Staphylococcus aureus*, *Streptococcus agalactiae* and coliform true faecal type.

2.2. Bacteriological examination

2.2.1. Preparation of serial dilutions (APHA, 1992)

Eleven milliliters of a well prepared milk samples was added to 99 ml of sterile saline solution and mixed to make a dilution of 10-1 from which tenfold serial decimal dilutions were prepared.

2.2.2. Detection of total Staphylococci count cfu/ml (Thatcher and Clark, 1978)

A 0.1 ml of the previously prepared dilution was spread evenly on to the dry surface of Baird-parker agar plate with a sterile bent glass rod until the surface appeared dry. The inoculated plates were incubated at 37°C for 48 hours. The Staphylococci count/ml of milk samples was calculated and recorded.

2.2.2.1. Identification of *Staph. aureus* (Mac Faddin, 1985)

The purified *Staph. aureus* isolates were identified through different biochemical tests [catalase test, coagulase test].

2.2.3. Enumeration of total Streptococci count cfu/ml (Sawant et al., 2002)

A 0.1 ml of previously prepared dilution was spread onto, the dry surface of modified Edwards agar supported with colistin sulphate and oxolinic acid. The inoculated plates were incubated at 37°C for 48 hours. The streptococci/ml of milk sample was calculated and recorded.

2.2.3.1. Identification of *Streptococcus agalactiae* (Murray et al., 2003)

Hemolysis was helpful in identifying *Streptococcus agalactiae* which appear as small grayish white colonies which surrounded by small area of complete beta hemolysis. Identification of *Streptococcus agalactiae* occur through different biochemical tests such as potassium hydroxide 3%, catalase test, Esculin

hydrolysis test, growth at 45°C 10°C, growth at pH 9.6, CAMP test and growth at 6.5 sodium chloride.

2.2.4. Enumeration of Total Coliform (MPN/g) (ICMSF, 1978)

Estimation of coliforms was done by using Most Probable Number technique with MacConkey's broth tubes. A series of 3 fermentation tubes containing MacConkey's broth and inverted Durham's tubes were inoculated with 1 ml from the previously prepared 10th fold serial dilutions. After thorough mixing the inoculated and control tubes were incubated at 37 °C 24-48 hours. Tubes showing acid and gas were considered as positive for the test. From the laboratory records, the Most Probable Number (MPN) of coliforms/g. was calculated by matching with MPN table.

2.2.4.1. Identification of *E.coli*

Positive MacConkey tubes were recultured on Eosin Methylene Blue agar (EMB). The inoculated plates were incubated at 37°C for 24 hours. Typically strong lactose fermenting colonies, notably *E. coli*, strong lactose fermenting colonies, notably *E. coli*, produce colonies that are green-black with a metallic sheen. Suspected isolates were biochemically identified using the biochemical reactions which were applied according to Koneman et al. (1994).

The biochemical tests include indol production test (Kovacs, 1928), citrate utilization test (Bailly and Scott, 1998), Methylene red test (Cowan and Steel, 1965), voges proskauer test (Cowan and Steel, 1965) and Eijkman's test (Growth at 44 °C) (Cruickshank et al., 1973).

3. RESULTS

In the present study, table (1) presents the enumeration results for total staphylococci count, total streptococci count and total coliform counts giving an idea about the

Table (1): Statistical analysis of total staphylococci counts, total streptococci counts and total coliform counts in BTM samples in the examined farms.

	Total Staphylococci count Mean \pm SE	Total streptococci count Mean \pm SE	Total coliform count Mean \pm SE
Farm I	$37 \times 10^3 \pm 7.4 \times 10^3$	$28.65 \times 10^3 \pm 5.75 \times 10^3$	$5.81 \times 10^3 \pm 1.77 \times 10^3$
Farm II	$33 \times 10^3 \pm 9.6 \times 10^3$	$22 \times 10^3 \pm 5.7 \times 10^3$	$13.3 \times 10^3 \pm 10.15 \times 10^3$
Farm III	$27 \times 10^3 \pm 7.9 \times 10^3$	$27.1 \times 10^3 \pm 14.2 \times 10^3$	$4.7 \times 10^3 \pm 1.7 \times 10^3$

Table (2): Incidence of some pathogenic microorganisms isolated from examined BTM samples of examined 3 dairy farms.

	Farm I		Farm II		Farm III	
	No.	%	No.	%	No.	%
<i>Staph. aureus</i>	6	37.5	4	25	2	12.5
<i>Str. agalactiae</i>	1	6.25	2	12.5	-	-
<i>E. coli</i>	2	12.5	4	25	2	12.5

levels of these pathogenic microorganisms in the 3 dairy farms. The mean values of total staphylococci count for farms I, II and III were $37 \times 10^3 \pm 7.4 \times 10^3$, $33 \times 10^3 \pm 9.6 \times 10^3$ and $27 \times 10^3 \pm 7.9 \times 10^3$ cfu/ml respectively. The mean values of total streptococci counts for farms I, II and III were $28.65 \times 10^3 \pm 5.75 \times 10^3$, $22 \times 10^3 \pm 5.7 \times 10^3$ and $27.1 \times 10^3 \pm 14.2 \times 10^3$ cfu/ml respectively. The mean values of total coliform counts for farms I, II and III were $5.81 \times 10^3 \pm 1.77 \times 10^3$, $13.3 \times 10^3 \pm 10.15 \times 10^3$ and $4.7 \times 10^3 \pm 1.7 \times 10^3$ cfu/ml respectively.

The incidence rate of *Staph. aureus*, *Streptococcus agalactiae* and *E.coli* was observed in table (2). The incidence rates of *Staph. aureus* in farms I, II and III were 37.5, 25 and 12.5 % respectively, while for *Streptococcus agalactiae* in farms I, II and III were 6.25, 12.5 and Zero % respectively and incidence rate of *E. coli* of farms I, II and III were 12.5, 25 and 12.5 % respectively.

4. DISCUSSION

The safety of milk is an important attribute of consumers of milk and dairy products. Milk pasteurization safeguards consumers from many potential food borne hazards in milk

and milk products. Despite the pasteurization process, the quality and safety of raw milk are important in reducing the risk of food borne diseases associated with milk because raw milk is the starting point of the milk production-consumption chain. The presence of food poisoning organisms in raw milk generally comes from cows with mastitis, handlers or deficient hygiene. Their presence in foods constitutes a public health problem, as the bacteria produces toxins that can cause toxic food infections. In the present study total of 48 BTM samples 16 of each were collected from 3 dairy farms in Gharbia governorate, Egypt. These samples were investigated bacteriologically to detect occurrence of *Staph. aureus*, *Str. agalactiae* and *E. coli*. The mean values of total *Staph. aureus* count for farms I, II and III were $37 \times 10^3 \pm 7.4 \times 10^3$, $33 \times 10^3 \pm 9.6 \times 10^3$ and $27 \times 10^3 \pm 7.9 \times 10^3$ cfu/ml respectively, observed in Table (1). Nearly similar results were obtained by Gillespie et al. (2012). *Staph. aureus* is one of the causative agents of mastitis in dairy herds (Barkema et al., 2006). This disease involves inflammation of the mammary glands and a resultant sporadic shedding of *Staph. aureus* cells into the raw milk (Barkema et al., 2006). Therefore, the

presence of large concentrations of *Staph. aureus* is indicative of mastitis in a dairy herd. From a food safety perspective, it is recognised that *Staph. aureus* is an enterotoxin-producing pathogen but that the concentration needs to exceed 10^5 cfu/ml for sufficient toxin to be produced to cause human illness (Hill, 1983). None of the raw milk samples in this study contained numbers of *Staph. aureus* that were close to this count.

Incidence rate of *Staph. aureus* was (37.5), (25) and (12.5) % in the three farms respectively, nearly similar results showed by Stephan et al. (2001) showed only 32.4% *Staph. aureus* of examined raw milk samples and Khudor et al. (2012) where *Staph. aureus* isolated from raw milk by percentage of 28.5% . Rahimi and Alian (2013) could isolate *Staph. aureus* from raw milk by percentage of 17.5%. Higher results were reported by Rall et al. (2008) whom isolated *Staph. aureus* from raw milk by percentage of 68% and 70.4% respectively. On the other hand lower results were reported by Kivaria et al. (2006). The mean values of total streptococci count for farms I, II and III were $28.65 \times 10^3 \pm 5.75 \times 10^3$, $22 \times 10^3 \pm 5.7 \times 10^3$ and $27.1 \times 10^3 \pm 14.2 \times 10^3$, respectively, (Table 1). The incidence rate of *Streptococcus agalactiae* in farms I, II and III were 6.25, 12.5 and 0% respectively. Higher incidence rate of *Streptococcus agalactiae* was reported by Zadoks et al. (2004) 31% of 48 examined bulk tank samples and Cheng et al. (2010), 27% of examined 100 raw milk samples nearly similar incidence rate was reported by Moawad and Osman (2005) who found incidence rate of *Streptococcus agalactiae* was 9.687%. Lower incidence rate (1.4%) of examined raw milk samples was reported by Karima et al. (2007). Table (1) illustrated that the mean total *Coliform counts* for farms I, II and III were $5.81 \times 10^3 \pm 1.77 \times 10^3$, $13.3 \times 10^3 \pm 10.15 \times 10^3$ and $4.7 \times 10^3 \pm 1.7 \times 10^3$ cfu/ml respectively. Our results were lower than those reported by Gihan

(1997) and Jayarao and Wang (1999) and nearly similar to those reported by Hassan and Al-Sanjary (1999) but lower results were reported by Al-Hawary (2005) and Firstenberg-Edem et al. (2004). Coliform counts of raw bulk tank milk should be routinely performed to identify the hygienic status under which milk is produced and handled and sanitary status of udder and milking machines (Boor et al., 1998). Results in Table (2) showed the incidence of *E. coli* in the samples comprising of BTM was (12.5%), (25%) and (12.5%), higher incidence of *E. coli* (52%) was observed by Virpari et al. (2013) and in Soomro et al. (2002) 57%, while nearly similar results (26.4%) was reported by Bandyopadhyay et al. (2011) and (30.2%) by Farzan et al. (2012) lower incidence rate 2.4% was reported by Jayarao et al. (2006).

Conclusion: Bulk tank milk can harbor a variety of pathogenic microorganisms such as *Staph. aureus*, *Streptococcus agalactiae* and *E. coli*. The presence of these foodborne pathogens in milk is either due to un efficient cleaned and sanitized equipments and udder and / or dairy cows with clinical or sub clinical mastitis. These pathogenic microorganisms in bulk tank milk contribute a potential risk to public health. These findings underscore the need to control them and to limit the bacterial multiplication in bulk tank milk by application of strict hygienic measures at farm level.

5. REFERENCES

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