Some Bacteriological and Immunological Studies on Camel's Milk

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Key words	ABSTRACT:
Camel's Milk; bacteria; isolation; identification; immunological parameters; insulin level.	The aim of the present study was to investigate the correlation between the isolated bacteria from camel's milk with special milk constituents that have immunogenic and therapeutic importance to human consumers. Milk samples were obtained from 90 she-camels from 3 Egyptian Governorates. The result showed that coagulase negative staphylococci (CNS) were the most frequently isolated bacteria either as a single isolate (20%) or as mixed infections (27.8%). *Staphylococcus aureus* (*S. aureus*) was isolated either as single (3.33%) and mixed infection (27.8%). *Escherichia coli* (*E. coli*), *Yersinia enterocolotica* (*Y. enterocolotica*), *Proteus vulgaris* and environmental streptococci were also isolated in single and/or mixed forms. Total protein, albumin and globulin levels were not significantly affected by different isolates. Nitric oxide (NO) and lysozyme were significantly stimulated by presence of *S. aureus*, CNS and *E. coli*. Insulin level was significantly reduced by *S. aureus* and CNS. Superoxide dismutase (SOD) was not significantly affected, while glutathione peroxidase (GPx) level was significantly stimulated by *S. aureus* and CNS infection. Control camel's whey samples without phytohemagglutinin (PHA) had the higher stimulant effect on lymphocytes proliferation as compared with control samples with PHA. Conclusively, the isolated bacteria not only constitute health hazards for consumers but also they made changes in the bioactive ingredients of camel's milk that may deteriorate the therapeutic and immunogenic benefits of camel's milk. So, there is a great need for strict hygienic measures during the production and handling of camel's milk.

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1. INTRODUCTION

Camel is of significant socio-economic importance in many arid and semi-arid parts of the world. Its milk constituents represent an important component of human diets (Yagil, 1982). Dromedary camels are known to produce high amount of milk for longer period than any other dairy animal held under the same harsh conditions. Camel's milk not only contains more nutrients compared to cow's milk (Arrowal *et al*., 2005), but also it has therapeutic and antimicrobial effect (El-Agamy *et al*., 1992). The use of camel's milk as therapeutic agent in liver impaired patients was early reported in the Hadith of the prophet 'Mohamed', in the 'Kanon' book of Ibn Sina and by Abu-Bakr Al-Razi (El-Badry, 2007). Camel's milk has been used in different regions in the world to treat number of diseases such as dropsy, jaundice, tuberculosis and asthma (Agrawal *et al*., 2007) and recently for treatment of autism (Wernery *et al*., 2012) and cancer (Cock, 2014). Moreover, the diabetics drinking camel's milk is a widely spread phenomenon, as it was found that relatively large concentrations of insulin are present in camel's milk (Singh, 2001) that does not form coagulum in acidic environment (Wangoh, 1993), so it will pass rapidly through the stomach and remains available for absorption in intestine. Furthermore, camel's milk can act as essential antibiotic due to its high content of lactoferrin, immunoglobulins, lysozymes and lactoperoxidase (El-Agamy *et al*., 1992; Al Haj and Al Kanhal, 2010).

In a view of its health benefits, there is a fast growing demand for raw camel's milk around the world and it is introduced recently as a new functional food in the European market (Faye and Bonnet, 2012). As camel's milk is usually consumed in its raw state, it may be contaminated with different types of microorganisms and it is an excellent culture medium for the growth and multiplication of these microorganisms. The ability of microorganisms to cause spoilage and disease depends upon the type present, the initial load of contamination of the milk and handling conditions (Omer and Eltinay, 2008). In general, camel's milk at farm has less microbial contamination. However, with commercialization, there is need to address handling procedures to reduce further contamination and possibility of occurrence of pathogens. This could contribute to food security and nutrition of the consumers (Matofari *et al*., 2013). So, the present study aimed to investigate the main bacterial contaminants in the camel's milk that may constitute health-hazards to human consumers and to find the correlation between each of these bacterial isolates with the milk constituents, which are known to have immunogenic and therapeutic benefits, with highlights on the immune-stimulant activities of

camel's milk using the lymphocyte transformation assay.

2. MATERIALS and METHODS

2.1. Milk Samples:

Milk samples were collected from 90 apparently healthy she camels from Mattrouh, Behira and Giza Governorates. Teats were washed thoroughly, dried with a separate towel and disinfected with 70% alcohol before sampling. The first three streams of milk from each teat were discarded and about 50 ml of milk were collected from each animal in sterile bottles and transported to the laboratory on ice. Each milk sample was divided into 3 parts; the 1st part of milk sample was fresh milk for bacteriological examination. The 2nd part was directed for whey preparation (using cooling centrifuge) for detection of Tuberculosis antibodies, also for lactoferrin, lysozyme, antioxidants, nitric oxide (NO) and whey proteins estimation. The 3rd part was skimmed for estimation of insulin level.

2.2. Bacteriological Examination:

Bacteriological examination was performed according to Quinn *et al*. (2011). Milk samples were pre-incubated at 37°C /12h then 50 µl of the incubated sample was streaked onto blood agar (with 7% sheep blood), MacConkey agar for coliform bacteria, CIN (Cefsulodin-Irgasan-Novobiocin) agar for *Y. enterocolotica*, Mannitol salt agar for *Staphylococcus species* and Edward's media for isolation of *Streptococcus species*. Colonies were identified for their morphological characteristic appearance and hemolytic activity, followed by Gram's staining and motility tests before being transferred into semisolid agar to be subjected for further identification. Pure cultures of the isolates were identified using biochemical tests; Catalase, tube coagulase test, CAMP test, carbohydrate fermentation tests, indole production, methyl red, urease production, citrate utilization and triple sugar iron agar (TSI) were performed as required. In cases where no growth was detected, plates were re-incubated at 37°C for an additional 24 h. *Y. enterocolitica* strains were tested for virulence factor using the auto-agglutination test (37°C) in Methyl Red-Voges Proskauer broth (Fukushima *et al*., 2011).

2.3. Detection of *Mycobacterium tuberculosis* antibodies (T.B.) by ELISA:

An indirect ELISA was carried out to estimate *Mycobacterium tuberculosis* antibodies in milk whey samples according to Muyldermans *et al*. (1994) with some modification. Briefly, wells of microtitre plates (96 wells) were coated with 100 µL of 0.005 % protein purified derivative (PPD,

Veterinary Serum and Vaccine Research Institute, Abassiah, Cairo, Egypt) in sodium carbonate buffer and incubated for 1 h at 37 °C, then overnight at 4°C. The plates were washed 3 times with PBS (pH 7.2) containing 0.05% Tween 20 (PBS-T, 200 µL/well). To minimize the non-specific binding of the antibody, the plates were then incubated for 2 h at 37°C with 200 µL/well PBS-T and 2% bovine serum albumin. Subsequently the plates were washed three times with PBS-T before adding 100 µL of milk whey (diluted 1:20 in PBS) to each well in a duplicated manner and incubated for 1 h at 37 °C. After three washes with PBS-T, 100 µL of protein A- peroxidase from *S.aureus* (1:80,000 in PBS-T, Sigma, Germany) were added to each well and the plates were incubated for 1 h at 37°C then washed a further 3 times in PBS-T, then 100 µL/well of o-phenylenediamine substrate indicator mixture was added and incubated for 25 min in a dark place. The reaction was stopped by adding 50 µL of stopping buffer in each well. Optical densities were recorded using ELISA reader at 492 nm. There were 5 wells used as control negative to detect the cutoff point. The ELISA reading that ≥double figure of the reading of control negative was considered as positive according to Bassiri *et al*. (1993).

2.4. Immunological Examinations:

2.4.1. Nitric oxide (NO) level in milk whey: was measured according to the assay described by Rajaraman *et al*. (1998) using ELISA reader at 570 nm.

2.4.2. Lysozyme activity in milk whey: was estimated according to Schultz (1987) as the diameter of the clear zone ring of lysis developed in the translucent agarose gel after diffusion of lysozymes through the agarose gel containing a suspension of *Micrococcus lysodeikticus*.

2.4.3. Estimation of globulin level: Total whey proteins were estimated according to Biuret-taratrate method described by Henery (1974). Albumin estimation was performed according to Dumas *et al*. (1971). Total whey globulin level was obtained by subtraction of estimated albumin from the total protein.

2.4.4. Estimation of lactoferrin concentration: by electrophoresis according to Laemmli (1970). Whey samples were separated with the aid of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the presence of 1.1% (w/v) SDS and 5% (v/v) 2-mercaptoethanol. Electrophoresis was performed at 4.9% staking and 15% resolving polyacrylamide gels, running in 0.125M Tris–HCl pH 6.8 and 0.38M Tris–HCl pH

8.8 buffers, respectively. Whey samples and pure bovine lactoferrin (5mg/ml) used as standard (Sigma, St. Louis) were loaded in the gel. Proteins were stained for 30 min by 0.1% Coomassie blue R250 in a mixture of 50% ethanol and 10% acetic acid. Lactoferrin concentration was estimated from pure band at molecular weight 72 KDa in each sample in comparison to the band of the injected known concentration of lactoferrin.

2.5. Skimmed Milk Insulin Level: was estimated according to Insulin enzyme immunoassay test kit REF according to Sacks (1994).

2.6. Antioxidant Activity: Superoxide dismutase (SOD) (Nishikimi *et al*., 1972) and Glutathione peroxidase (GPx) (Paglia and Valentine, 1967) activities were estimated by using Bio diagnostic colorimetric kits**.**

2.7. Measurement of Lymphocyte Proliferation:
To evaluate lymphocyte proliferative response, peripheral bovine blood samples were obtained for lymphocyte separation by centrifugation through a ficoll-hypaque gradient at 400xg. The number of lymphocyte was concentrated as $1x\ 10^6$cells/ml and cultured in 96 well tissue culture plate with 10% fetal calf serum at 37°C and 5% CO_2 for 72 h. The proliferation of lymphocytes in response to both mitogen and camel's milk whey was estimated using MTT reduction assay according to Rai-Elbalhaa *et al*. (1985).

2.8. Statistical Analysis
Data were subjected to statistical analysis according to Sendecor and Cochran (1989) by Two-way ANOVA employing a completely randomized design at P< 0.05.

3. RESULTS
It was noticed that by using ELISA, all the examined milk samples (n= 90) were negative for *Mycobacterium tuberculosis* antibodies.

As shown in table 1, only 11 samples out of 90 (12.22%) were bacteriologically negative. The coagulase negative staphylococci (CNS) were the most frequently isolated bacteria either as a single isolate (20%) or as mixed forms (27.78%). *S. aureus* was isolated either as single (3.33%) and/or mixed forms (27.78%). *E. coli, Y. enterocolotica, Proteus vulgaris* and environmental streptococci were also isolated in single and/or mixed forms.

As presented in table 2, the total protein, globulin and albumin levels were non-significantly affected by the presence of any of the bacterial isolates in camel's milk. Except for milk samples contained *Y. enterocolotica*, samples harboring other bacteria had significantly (P ≤ 0.05) higher levels of nitric oxide and lysozymes when compared to the control ones. On the other hand, samples containing *E. coli* and *S. aureus* in mixed forms had significantly (P ≤ 0.05) lower levels of lactoferrin when compared to the control milk samples.

As depicted in Fig.1 from right to left; the 1st lane is the marker which range from 240 to 3.5 KDa, the lanes from 3-9 show different bands of the camel's milk whey samples. The most common band present in all samples was that at 72KDa which correlated with the 2nd lane that carry lactoferrin standard band. The protein profile obtained by electrophoretic analysis (SDS-PAGE) showed that camel's milk whey contained several types of proteins. The more pronounced one was lactoferrin which detected at molecular weight of 72 kD.

Table (1): Incidence of isolated bacteria from camel's milk.

Isolated bacteria		No. of infected samples (%)		Total no. (n= 90)
Single infection	CNS	18	20%	
	S. aureus	3	3.33%	29
	E. coli	3	3.33%	(32.22%)
	Y. enterocolotica	5	5.56%	
CNS mixed infection	CNS +environmental streptococci	10	11.11%	
	CNS+ *Proteus vulgaris*	4	4.44%	
	CNS+ environmental streptococci +*E.coli*	7	7.78%	25
	CNS+environmental streptococci+*Proteus vulgaris*	3	3.33%	(27.78%)
	CNS+ environmental streptococci + *Y.enterocolotica*	1	1.11%	
S.aureus mixed infection	*S.aureus*+environmental streptococci	9	10%	
	S.aureus +*E.coli*	8	8.89%	
	S.aureus+*Y.enterocolotica*	2	2.22%	25
	S.aureus+environmental streptococci+ *E.coli*	4	4.44%	(27.78%)
	S.aureus+environmental streptococci +*proteus vulgaris*	2	2.22%	
Bacteriologically negative milk samples		11	12.22%	11 (12.22%)

The bacteriologically negative milk samples were taken as control samples for the other following results.

Table (2): The effect of isolated bacteria on some biochemical constituents of camel's milk whey (Mean ± SE).

Isolated bacteria	Total protein (g/dl)	Total Globulin (g/dl)	Total albumin (g/dl)	Nitric oxide (µM/ml)	Lysozyme (µg/ml)	Lactoferrin (mg/ml)
S.aureus mixed	2.84±0.27 [a]	1.23±0.24 [a]	1.61±0.09 [a]	46.3±11.3 [ab]	164.2±7.1 [a]	2.25±0.12 [b]
CNS	3.45±0.78 [a]	1.58±0.67 [a]	1.87±0.35 [a]	73.4±7.6 [a]	166.9±6.3 [a]	3.43±0.64 [a]
CNS mixed	2.69±0.09 [a]	1.05±0.28 [a]	1.64±0.08 [a]	42.2±8.5 [ab]	147.8±6.04 [ab]	3.17±0.60 [a]
E.coli mixed	3.3±0.15 [a]	1.52±0.31 [a]	1.78±0.06 [a]	73.9±11.6 [a]	160.3±4.5 [a]	2.03±0.07 [b]
Y.enterocolotica	2.79±0.08 [a]	1.25±0.05 [a]	1.53±0.03 [a]	10.21±2.01 [b]	122.7±11.8 [ab]	4.05±0.017 [a]
Control	2.87±0.41 [a]	1.04±0.12 [a]	1.83±0.28 [a]	9.5±1.01 [b]	95.2±5.5 [b]	4.09±0.21 [a]

Within columns, means with different alphabetical superscripts are significantly different at least at P < 0.05.
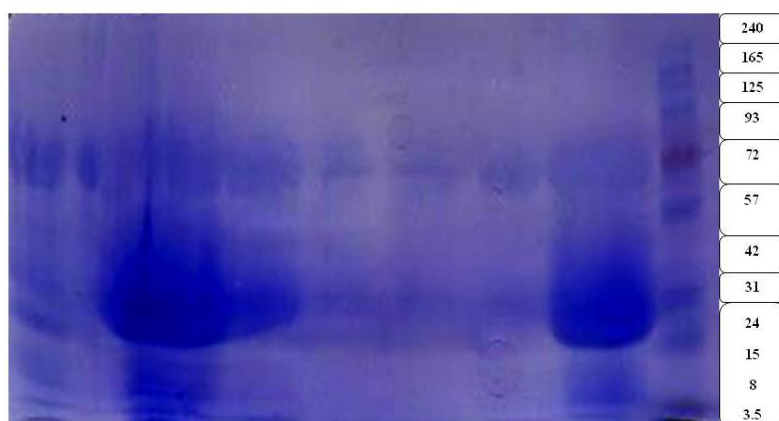

Fig (1): Protein profile of different milk whey samples with the standard protein marker (from up to down; 240, 165, 125, 93, 72, 57, 42, 31, 24, 15, 8, 3.5 KDa).

Table (3): The effect of isolated bacteria on insulin level in camel's skimmed milk (Mean ± SE).

Isolated bacteria	Insulin (U/ml)
S. aureus mixed infection	40.1±7.5 [c]
CNS	101.8±6.06 [b]
CNS mixed infection	158.6±5.98 [ab]
E. coli mixed infection	136.4±9.3 [ab]
Y. enterocolotica	Below the detectable level
Control	173.13±6.87 [a]

Means with different alphabetical superscripts are significantly different at least at P < 0.05.

Table (4): The effect of isolated bacteria on some antioxidant activities in camel's milk whey (Mean ± SE).

Isolated bacteria	SOD (U/ml)	GPx (U/ml)
S.aureus mixed infection	339.0 ± 8.60 [a]	264.3 ± 15.8 [a]
CNS	336.0 ± 11.40 [a]	213.9 ± 10.4 [a]
CNS mixed infection	311.0 ± 5.60 [a]	239.8 ± 23.4 [a]
E.coli mixed infection	269.3 ± 8.30 [b]	48.6 ± 5.6 [c]
Y.enterocolotica	352.0 ± 6.41 [a]	106.7 ± 25.7 [b]
Control	353.0 ± 5.09 [a]	110.2 ± 23.3 [b]

Within columns, means with different alphabetical superscripts are significantly different at least at P < 0.05.

Data regarding the insulin level among milk samples was presented in table 3. The presence of bacterial contaminants resulted in deleterious effect on insulin level in milk samples in different degrees and this effect is more pronounced in case of *S. aureus* mixed infection, if compared with control samples. The insulin was not detected in milk samples contained *Y. enterocolotica*.

As shown in table 4, *E. coli* mixed contamination resulted in the significantly lowest levels of SOD and GPx among the tested samples. *S. aureus* mixed infection as well as CNS single and mixed infections resulted in significantly higher GPx levels but not SOD levels.

Table (5): The effect of camel's milk whey on Lymphocyte transformation in response to phytohemagglutinin in comparison to control (with and without mitogen).

Isolated bacteria	Lymphocyte transformation in OD.
S.aureus mixed infection	0.353±0.167 b
CNS	1.50 ± 0.54 ab
CNS mixed infection	2.630 ± 0.82 a
E.coli mixed infection	0.239 ± 0.01 b
Y.enterocolotica	0.269 ± 0.07 b
Control without mitogen	2.34 ± 0.50 a
Cultured lymphocytes with mitogen	1.11 ± 0.04 b

Means with different alphabetical superscripts are significantly different at least at P < 0.05.

As shown in table 5, except for milk samples contaminated with CNS, control samples without mitogen (PHA) had the highest proliferation of lymphocytes as compared with milk containing bacterial isolates and control samples with of PHA while there was significant depression in case of *S. aureus, E. coli* and *Y. enterocolotica* infection.

4. DISCUSSION

The present study was directed with the main interest to declare the bacterial eminence of Egyptian camel's milk collected from three different ecological areas (Mattroh, Behira and Giza Governorates) and to find at which extent these bacteria could make changes in milk composition that may have an impact on the well-known therapeutic and immunogenic benefits of camel's milk, as the production of camel's milk is gradually increasing due to an increased interest by consumers in recent years (Bhavbhuti *et al*., 2015).

Using ELISA, examination of the 90 raw camel's milk samples here revealed the absence of *Mycobacterium tuberculosis* antibodies. This result was coincided with the concern of Fowler (2010) that Camelids were not considered highly susceptible to tuberculosis. Tuberculosis was rare among camels kept under nomadic conditions. The disease occurred more frequently when camels were kept in close quarters with other camels or in close contact with cattle (García-Bocanegra *et al*., 2010).

In the present study, only 11 samples out of 90 (12.2%) yielded no growth of bacteria and there was high bacterial contamination rate (87.8%). Similarly, sixteen (18.6%) out of 86 milk samples from clinically healthy camels managed by Bedouin nomads in Palestine were reported to be bacteriologically negative while 81.4% of samples contained bacteria (Guliye *et al*., 2002). The subclinical mastitis was found to be more common in camels (Aljumaah *et al*., 2011 and Wanjohi *et al*., 2013). One explanation of this high bacterial isolation rate was given by El-Ziney and Al-Turki (2007) who reported that approximately 50% of the examined raw camel's milk samples were produced and handled under poor hygienic conditions with high health risk to the consumers.

Our results revealed that coagulase negative staphylococci (CNS) and *S. aureus* were the most frequently isolated bacteria (47.8% and 31.11%, respectively) in single and mixed forms followed by *E. coli* (24.44%; single and mixed forms). *Y. enterocolotica, Proteus vulgaris* and environmental streptococci were also isolated in single and/or mixed forms. *S. aureus* was the most frequently isolated bacteria by other workers investigated in camels; in Egypt (Aly and Abo-Al-Yazeed, 2003), UAE (Al-Juboori *et al*., 2013), Iraq (Al-Tofaily and Al-Rodhan, 2011), Saudi Arabia (Bakhsh *et al*., 2012), Bahrain (Abubakr *et al*., 2012), Nigeria (Kalla *et al*., 2008) and India (Lenin Bhatt *et al*., 2004). However, Eyassu and Bekele (2010) from Ethiopia found that the chief isolated bacteria in their study area were found to be CNS. While El-Haj *et al*. (2014) demonstrated high *E. coli* count for Ethiopian camel's milk. Al-Majali *et al*. (2007) considered camel's milk samples from which bacteria were isolated as subclinical mastitic milk even though somatic cell count was lower than 200,000 cells/ml. Based on our results, the isolation of environmental streptococcus species at high rates revealed the cross contamination of raw camel's milk either from the animal itself or from the milkers. Likewise, the existence of coliform bacteria (*E. coli, Proteus vulgaris* and *Y. enterocolotica*) may not necessary indicate a direct fecal contamination of milk, but precisely as an indicator for poor sanitary practices during milking and further handling processes. Similarly, El-Ziney and Al-Turki (2007) reported that raw camel's milk was produced and handled under poor hygienic conditions. Also kotb *et al*. (2010) concluded that the contamination of camel's milk and consequently the milk quality were influenced by post-milking environmental contaminants rather than by camel infections. So, there is a need for strict hygienic measures during the production and handling of

camel's milk to reduce public health hazards.

In the current study, the total protein, albumin and total globulin levels were high and not affected by presence of any of the isolated bacteria as compared with the bacteriologically negative milk samples. In these concerns, El-Agamy *et al.* (1992) and El-Hatmi *et al.* (2007) illustrated that, camel's milk contained more whey protein than cow's milk. This variation is primarily due to the higher content of albumin in camel's milk which was the major whey protein. Camel's whey contained a higher content of anti-microbial factors such as lysozyme, lactoferrin and immunoglobulins if compared to the bovine species that closely related to our results. In contrary, Kato *et al.* (1989) and Amena *et al.* (2011) reported that the total protein level was increased significantly in milk samples from clinical and subclinical mastitis in compare with those of healthy she camels.

Regarding the relation between bacterial contents and NO levels in the examined camel's milk whey, it was noticed that the level of NO was significantly increased (P<0.05) in all types of isolated bacteria in comparison to the control samples (except in case of *Y. enterocolotica*). Also, in cow's milk, Bastan *et al.* (2013) and Hanaa and Inas (2013) found elevated NO concentrations in *S. aureus* and CNS contaminated samples as compared with samples had no bacterial growth. Moreover, Osman *et al.* (2010) suggested the promising use of whey NO concentration variabilities as prognostic parameters on the degree of bacterial contamination of milk samples. One explanation of these variations between samples in NO content was due to change in xanthine oxidase activity, as camel's milk can generate NO (Hashad, *et al.*, 2006) through its content of xanthine oxidase.

In the current study, the bacterial presence in milk samples significantly (P < 0.05) increased the lysozyme content in milk samples as compared to the bacteria-free samples. Similarly, Barbour *et al.* (1984) reported more than two-fold increase in lysozyme content in camel's milk samples that showed growth inhibition of one or more organisms in filter-paper disk assay. In general, camel's milk is reported to have a stronger inhibitory system than that of cow's milk (El-Agamy *et al.*, 1992). In particular; the levels of lysozyme and lactoferrin were reported to be two and three times higher than those of cow's milk, respectively (Barbour *et al.*, 1984 and Kappeler, 1999). Benkerroum (2008) stated that the antibacterial activity of milk lysozyme is part of the unspecific innate defense mechanism which acts either independently by lysing sensitive bacteria or as a component of complex immunological reactions to enhance the phagocytosis of bacteria by macrophages.

One of the major findings of camel's milk was the presence of lactoferrin which was used as anti-cancer and antioxidant to protect the body against viral and bacterial infections and could prevent DNA damage by binding catalytic iron (Benkerroum, 2008 and Cock, 2014). In the current work, a clear band with apparent MW of 72 KDa was identified as camel lactoferrin. Also, Yamina *et al.* (2013) reported the presence of 72 KDa lactoferrin in camel's milk samples. While, Ochirkhuyag *et al.* (1998) and Redwan and Tabll (2007) identified camel lactoferrin at a MW of 78 and 80 kDa, respectively. Due to the unavailability of anti-camel lactoferrin, the lactoferrin was quantified in our study according to the results of electrophoresis, not by ELISA or immunodiffusion assays, and that is because camel lactoferrin was shown by El-Agamy *et al.* (1996) to have no antigenic relationship with bovine lactoferrin when anti-camel lactoferrin was used in immunodiffusion analysis. Our findings came in accordance with Al-Majali *et al.* (2007) in that camel's milk contaminated with *E. coli* showed the lowest concentration of lactoferrin when compared to milk contaminated with other bacterial isolates. In contrary to our finding that *S. aureus* resulted in marked reduction in lactoferrin level, Al-Majali *et al.* (2007) reported that, the log lactoferrin concentrations in camel's milk contaminated with *S.aureus* was significantly higher than those for other bacterial isolates.

Recent randomized human studies revealed that regular consumption of camel's milk by diabetics resulted in a substantial reduction in the mean dose of insulin needed to obtain glycemic control and improvement of fasting blood sugar and glucosylated hemoglobin [HbA1c] (Agrawal *et al.*, 2011 and Ajamaluddin *et al.*, 2012). Such beneficial effects of camel's milk might be due to the presence of insulin in milk at higher level than the milk from other animals (150 U/ml; Zagorski *et al.*, 1998) and due to the lack of coagulation of camel's milk in the human stomach (Agrawal *et al.*, 2003). Higher levels of insulin (173.13 U/ml) were recorded in the bacteria-free milk samples of the present study. The presence of bacterial contaminants reduced the insulin level in milk samples and this effect was more pronounced in case of *S.aureus* mixed contamination. Moreover, the insulin was below the detectable level in samples containing *Y. enterocolotica*, this may be attributed to that the

isolated *Y. enterocolotica* were virulent strains. There was no available data to compare these deleterious effects of bacterial contaminants on insulin level. These results open the door for evaluation of the safety of raw camel's milk for diabetic patients as the high isolation rates of infectious bacteria in our study may have both health hazards for diabetic patients as well as they may badly influence the insulin content of camel's milk. So, it is highly recommended to use bacteria-free camel's milk for these patients.

The glutathione peroxidase (GPx) level in our control milk samples (mean= 110.2 U/ml) was higher than that recorded for human (38.8 U/ml) and bovine milk (20.3 U/ml) by Hojo (1982). In the current study, it was found that there was a marked increase in GPx level in the *S. aureus* and CNS contaminated samples as compared with the control milk samples. Similarly, in cow's milk samples, Andrei *et al*. (2011) reported a significant increase in GPx activity in subclinically mastitic samples as compared to normal milk. This increased GPx activity in milk may occur as a result of adaptation mechanisms of some pathogens; as the resistance of bacteria to oxidative stress was found to be dependent on the accumulation of glutathione (Janowiak and Griffith, 2005). In the present study, *E.coli* contamination resulted in the significantly lowest levels of SOD and GPx among the tested samples. On the other hand, in rats, camel's milk supplementation decreased the oxidative stress and normalized antioxidants biomarkers that were ameliorated by *E. coli* injection (Yassin *et al*., 2015).

Except for milk samples contaminated with CNS, control whey samples without mitogen (PHA) had the higher lymphocytes proliferation as compared with control samples with PHA. This came in accordance with the findings of Badr *et al*. (2011) that supplementation of mice with whey protein enhance cytoskeletal rearrangement and chemotaxis in B and T cells, and subsequently improving the immune response. Similarly, Ismael *et al*. (2013) found that camel lactoferrin increased lymphocyte transformations mean values in a dose dependant manner, but the lactoferrin decreased lymphocyte transformations mean values in a dose dependant manner when combined with PHA. Also Ebaid (2014) shown that there was a strong, positive correlation between camel's why protein and immune function during diabetes, therefore, served to activate the proliferation of B lymphocytes in diabetic rats both in *vivo* and in *vitro*.

In conclusion, the present study revealed relatively high incidence of bacterial contaminants in camel's milk in Egypt, presumably because of poor application of basic hygienic standards by camel herders. Moreover, the isolated bacteria not only constitute health hazards for consumers but also they make changes in composition of the bioactive ingredients of camel's milk that may deteriorate the therapeutic and immunogenic benefits of camel's milk. Based on these findings, it is strongly recommended that strict hygienic measures during the production and handling of camel's milk must be followed, in addition to strict education of the camel owners about the importance of hygienic milking practices.

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