Vet.Med.J.,Giza. Vol.54, No.4. (2006) :769-776.

STUDIES ON STAPHYLOCOCCUS AUREUS IN MILK AND YOGHURT TREATED BY BIFIDOBACTERIA

ABDEL- FATTAH, S. A.

Agriculture Research Center; Animal Health Research Institute, Dokki, Egypt.

Received: 8. 8. 2006.
Accepted: 19.11. 2006.

SUMMARY

Milk samples and lab-made yoghurt were used to investigate the antibacterial effect of *bifidobacteria* bifidum on *Staphylococcus aureus* pathogen.All samples were analyzed microbiologically for presence of *S. aureus* when fresh and after 2 and 5 days of storage at refrigeration temperature for milk and yoghurt respectively proven to be contaminated with *S. aureus* from infected, non treated dairy cows and expermintally milk inoculated with *S. aureus* (5x 10^4 cfu/ml).The obtained results indicated that there were adequate difference in *S.aureus* count in the examined samples treated with *Bifidobacteria bifidum* from zero time till the 2nd day of storage in bifudus milk. The reduction of S.aureus were 100% at 2nd, 3rd, 4th and 5th days of storage in bifudus yoghurt manufactured from inoculated milk with *S.aureus*. The current study indicates that Bifidobacteria may restrict and /or inhibit *S. aureus* in milk and yoghurt.

INTRODUCTION

In recent years probiotic bacteria have increasingly been incorporated into foods as dietary adjuncts for many reasons: Inhibition of pathogenic species; anticarcinogenic activity; control of serum cholesterol levels and increasing the immune response (Shin, et al. 2000). Milk is extremely perishable and many means have been developed to preserve it, the earliest one which has been used for many thousands of years is fermentation. Milk can be fermented by inoculating fresh milk with the appropriate bacteria, as the bacteria grow, it convert milk sugar (lactose) to lactic acid which preserve the milk by lowering the pH which prevents the growth of putrefactive and/or pathogenic bacteria who do not grow well in acid

conditions. Casein consider the predominant protein in milk soluble at a neutral pH but insoluble in acid thus when milk sours, casein precipitates and thickens of the product occur.

The high prevalence of *S. aureus* may be attributed to the wide distribution of the organism inside the mammary gland and skin of the teats and udder. S.aureus establishe a mild subclinical infection over a long duration and they usually shed in the milk, which serve as a source of infection for milk consumer, also *Staphylococci* are present as major mastitis pathogens in the dairy industry worldwide (Leslie & Schukken, 1993 and Radostits, et al., 2000). The uses of milk and milk products with *Bifidobacteria* inhibit *S. aureus* pathogens besides its production of lactic acid and acetic acid, these organisms have become popular in various fermented dairy products because of their potential health benefits. Dahi (1986); Robenson & Tamime, (1990); Hughes & Hoover (1995); Nayra & hosney (2003) and Barkema, et al.(2006).So the purpose of this work to find a solve for protection of the consumer by Bifidobacteria against *S.aureus* to inhibit this pathogen in milk and yoghurt during refrigeration.

MATERIALS AND METHODS

- Group I, raw milk collected from non treated dairy cows proved to be free from *S. aureus* sample was sterilized in the autoclave at 121°C for 5 minutes, after cooling it divided into two portions. The 1st used as a control bifidus and non bifudus milk each into100 ml sterile glass bottles, while the 2nd one used as a control bifidus and non bifudus yoghurt.
- Group II, raw milk sample collected from non treated dairy cows proved to be free from *S. aureus* was sterilized in the autoclave at 121°C for 5 minutes, after cooling and expermintally inoculated with *S. aureus* at a rate of 5×10^4 cfu/ml (the same *S. aureus* isolates and count in proven contaminated milk), the inoculated milk were divided into two portions. The 1st used in bifidus and non bifudus milk each into100 ml sterile glass bottles. While the 2nd one used in manuafactured bifidus and non bifudus yoghurt.
- Group III, raw milk collected from non treated dairy cows and proven to be contaminated with *S. aureus* were divided into 1st part as non bifudus milk and bifidus milk each into100 ml sterile glass bottles, the 2nd part for manufacturing yoghurt which in turn subdivided into bifidus and non bifudus yoghurt.
- Lyophilized *Bifidobactereria bifidum* (obtained kindly from Fac.of Sci. Zagazig Univ.) was suspended and incubated at 45°C for 6 hours to give a viable count of 1×10^7 cfu/ml (Robenson and Tamime, 1990).
- Purified strains of *Strept. thermophilus* were suspended and incubated at 36°C for 8 hours to be used in manufacturing of yoghurt.
- pH measurement by using electric pH meter (3310 pH meter, UK) were done daily all over the study period.

- All milk and yoghurt samples were incubated at 40°C for 4 hrs followed by storage at refrigerator temperature, milk only 2 days and 5 days for yoghurt each experiment was replicated three times.
- Microbiological eumeration of *S.aureus* according to ICMSF(1986) while isolation and identification were carried out according to Buchanan and Gibbons (1975).
- Statistical analysis: the obtained data from the present study were analysed statistically with SPSS for windows version 9.0 software.

RESULTS

Table (1): Boiled milk and manufactured yoghurt from the same boiled milk free from *S. aureus* (negative control) .

Storage Time (days)	Control negative of milk						Control negative of yoghurt					
	Non bifidus milk			Bifidus milk			Non bifidus yoghurt			Bifidus yoghurt		
	S.aureus count	reduction%	pH	*S.aureus* count	reduction %	pH	*S.aureus* count	reduction%	pH	*S.aureus* count	reduction%	pH
0 time	0	0	6.5	0	0	5.0	0	0	4.6	0	0	4.5
1st day	0	0	6.5	0	0	4.7	0	0	4.5	0	0	4.5
2nd day	0	0	6.4	0	0	4.5	0	0	4.5	0	0	4.2
3rd day	–	–	–	–	–	–	0	0	4.5	0	0	4.2
4th day	–	–	–	–	–	–	0	0	4.4	0	0	4.0
5th day	–	–	–	–	–	–	0	0	4.4	0	0	3.8

Table (2): Survival and mean values of *S. aureus* log 10 CFU / ml in milk samples.

Storage Time (days)	Milk proven to be contamnated with *S. aureus*						Milk experimentally inoculated with *S. aureus*					
	Non bifidus milk			Bifidus milk			Non bifidus milk			Bifidus milk		
	S.aureus count	reduction%	pH	*S.aureus* count	Reduction %	pH	*S.aureus* count	reduction%	pH	*S.aureus* count	Reduction %	pH
0 time	4. 69	0.00	6.5	4. 69	0.00	5.0	4. 69	0.00	6.5	4. 69	0.00	5.0
1st day	4.60	1.92	6.5	2.69	42.64	4.7	4.60	1.92	6.5	2.47	47.33	4.8
2nd day	4.47	4.69	6.4	2.47	47.33	4.5	4.40	6.18	6.4	2	57.36	4.5

Table (3): Survival and mean values of *S. aureus* log 10 CFU / g in yoghurt samples.

Storage Time (days)	Yoghurt manuafactured from milk proven to be contamnated with *S. aureus*						Yoghurt experimentally inoculated with *S. aureus*					
	Non bifidus yoghurt			Bifidus yoghurt			Non bifidus yoghurt			Bifidus yoghurt		
	S.aureus count	reduction%	pH	*S.aureus* count	reduction%	pH	*S.aureus* count	reduction%	pH	*S.aureus* count	reduction%	pH
0 time	4. 69	0.00	4.6	4. 60	1.92	4.5	4. 69	0.00	4.6	4. 60	1.92	4.5
1st day	3.84	18.12	4.5	2	57.36	4.5	3.77	19.62	4.5	2	57.36	4.5
2nd day	3.30	29.64	4.5	0	100.00	4.3	3.30	29.64	4.5	0	100.00	4.2
3rd day	2.69	42.64	4.4	0	100.00	4.2	2.69	42.64	4.4	0	100.00	4.2
4th day	2	57.36	4.4	0	100.00	4.0	2	57.36	4.4	0	100.00	4.0
5th day	2	57.36	4.4	0	100.00	3.9	2	57.36	4.4	0	100.00	3.8

DISCCUSION

Milk and milk products may be produced by individual owners with lack of sanitary conditions, thus milk and dairy products harber a natural microbial flora and/ or other microorganisms.So, consumption of milk and yoghurt constitutes a major source of food borne diseases (Montasser & Abdel-wahab 2003). Presence of S.aureus in milk and its products is usually taken as an index for contamination especially in cases of mastitis. The proportion of *S.aureus* isolated from milk with an isolation rate of 39.40% to 48.7%. as reported by Kayihura, et al. (1987); Vandal & Biasizzo (2000) and Kirkan, et al. (2005).

As shown in table (1), pH value of bifidus milk and bifidus yoghurt neither contaminated nor experimentally inoculated with *S. aureus* reach to 4.5 in bifudus milk and 3.8 in bifidus yoghurt during storage. this could be due to the production of more acetic acid by *Bifidobacteria* during storage. Similar results were obtained by Lim, et al.(1995); Samona, et al. (1996), and Shin , et al (2000) Montasser & Abdel-wahab (2003) who evaluated milk and yoghurt containing *Bifidobacteria* for viability of *Bifidobacteria* during refrigerated storage and found that viability of *Bifidobacteria* and lactic acid bacteria in milk and yoghurt remained high.

Table (2) reveals the survival of *S. aureus* could survive. The whole storage time in contamination and experimental inocculated bifidus milk at pH of 4.5 with reduction rate of 57.36% and 47.33% respectively. This is an important consideration

because *Bifidobactereria* produce appreciable quantities of acetic acid from the fermentation of lactose besides their pH- reducing effect on either *S.aureus* or other organisms which may present in milk. Some degree of similarity was observed between the results of our study and previous ones as reported by Kurman and Rasic (1991) and Baron & Vuillemard (2000). Also Lankaputhra, et al. (1996) and Nremark, et al., (2002) concluded that fermented milk with probiotic organisms inhibite the growth of *S. aureus.*

Table (3) showed that in bifidus yoghurt manufactured from milk proven to becontamnated and experimentally inoculated milk with *S. aureus showed* a noticable reduction 100% and S.aureus dispeared at 2nd , 3th, 4th and 5th days. Generally, these results agree with those given by Rybka (1994) and Ashutosh, et al. (2002) who observed that *Shigella dysentriae* showed maximum inhibition followed by *S. aureus*, it may be attributed to the *Bifidobacterium bifidum* numbers in bifidus milk and bifidus yoghurt were nearly constant, also its acetic acid production has the highest inhibitory effect against pathogenic organisms than another acids.

Murad, et al. (2000) stated that the bactericidal action of *Bifidobacteria* may be attributed to the production of some kind of antimicrobial agent (bacteroicins) which was relatively stable at acid conditions in addition to their pH- reducing effect which inhibit the food borne pathogens such *S.aureus, E.coli, Bacillus cereus*, and *Pseudomonas flouresences.*

As *Bifidobacteria* are probiotic organisms that improve the microbial balance in the human gut it can be incorporated as a live cultures in fermented dairy foods, *Bifidobacteria* have the ability to survive as a normal inhabitant microoeganism in the upper gastrointestinal tract of man and animal as human fecal flora playing a role in colon carcinogenesis prevention; Bartram , et al.(1994); Lankaputhra & Shah (1995); Adhikari, et al.((2000); Amer and El-ham (2000); Xiao , et al. (2003) and Pochart, et al.(2004).

The results of the current study indicate that the use of *Bifidobacteria* in milk and yoghurt restricts and /or inhibits the growth of the pathogen examined in this study. Therefore, it can be concluded from the observations presented here that *Bifidobacteria* have a bactericidal effect against *S. aureus* in milk and yoghurt.

REFERENCES

Adhikari, K.; Mustapha, A.; Grun, I. and Fernando, L. (2000): Viability of microencapsulated *Bifidobacteria* in set yoghurt during refrigerated storage. J.Dairy Sci., 83: 1946 - 1951.

Amer, K.D. and El-ham, I. E. (2000):Probiotic dairy products processing. Arab. Agric. Res. J., 4 (2): 333 - 347.

Ashutosh, D.N.; Bajad, C.D.; Khedkar, M.; Patil, R. and Sarode, A.R.(2002): Studies on Antibacterial Properties

of Acido-Bifido-Yoghurt. J. Dairy Sci. , 85 (5):1058-69.

Barkema, H. W.; Schukken, Y. H. and Zadoks, R. N (2006): Invited Review: The Role of Cow Pathogen and Treatment Regimen in the Therapeutic Success of Bovine *Staphylococcus aureus* Mastitis. J. Dairy Sci., 89:1877-1895.

Baron, M. D. and Vuillemard, J.C. (2000): Biochemical characteristics of fermented milk produced by mixed cultures of yoghurt cultures and *Bifidobacteria*. Lait. 80: 465 - 478.

Bartram, H.; Scheppach, W .; Gerlach, S .; Ruckdeschel, G .; Kelber, E . and Kasper, H .(1994): Does yoghurt enriched with *Bifidobacterium longum* affect colonic microbiology and fecal metabolites in health subjects?. Am. J. Clin Nutr. , 59 (2):428-32.

Buchanan, R.E. and Gibbons, N.E. (1975): Bergy,s Manual of Determinative Bacteriology. 8th .Ed., The Williams and Wilikins Company/ Baltimore.

Dahi, J.(1986): *Staphylococcus* in raw milk. Damsk Veterinertidss Krigt, 69 (9) 553. Dairy Sci. Abst., 48,599.

Hughes, D.B. and Hoover, D.G. (1995):Viability and enzymatin activety of *Bifidobacteria* in milk. J. Dairy Sci., 78: 288 - 276.

ICMSF (1986): Microorganisms in food. I. Their significance and methods of enumeration. 2th ed . Univ. Toronto press, Toronto, Buffalo and London.

Kayihura,M.; Kaburia, H.; Arimi, S. and Lindqvist, K. (1987): Staphylococcal enterotoxin "A" in raw and pasteurized milk. East-African-Medical-Journal., 64 (3): 177-182.

Kirkan, P.; KSOY, G. and Osman, K. (2005): Identification and Antimicrobial Susceptibility of *Staphylococcus aureus* and Coagulase Negative *Staphylococci* from Bovine Mastitis in the Aydyn Region of Turkey. Turk. J Vet. Anim. Sci., 29 :791- 796.

Kurman, J.A. and Rasic, J.L. (1991): The health potential of products containting *Bfidobactereria*. R.K. Robenson, ed. Elsevier Appl. Food Sci., London, United Kingdom.

Lankaputhra, W. E. and Shah, N. P. (1995): Survival of Lactobacillus acidophilus and *Bifidobacterium* spp. in the presence of bile acid salts. Cultured Dairy Products J. 30 (3).

Lankaputhra, W. E.; Shah, N.P. and Britz, M. (1996): Survival of *Bifidobactereria* during refrigerated storage in the presence of acid hydrogen peroxide. Milchwissenschaft, 51: 65 - 70.

Leslie, K.E. and Schukken, Y.H. (1993): Herd Programs for Elimination and Preventing *Staphylococcus aureus* Mastitis. In: National Mastitis Council Annual Meeting. Kansas City, 36-47.

Lim, K.S.; Huh, C.S.; Back, Y.J . and Kim, H.U . (1995): A selective enumeration medium for *Bifidobacteria* in fermented dairy products. J. Dairy Sci. , 78 (10):2108-12.

Montasser, A. M. and Abdel-wahab, M. M. (2003): Effect of *Bifidobacteria* on milk and milk products infected with Brucella. 7th Sci. Cong. Egyptian Society for Cattle disease, Assiut, Egypt.

Murad, H. A.; Fatma, A. F. and zeinab, I. S. (2000): Bacteriocinogenic effect of *Bifidobacterium* bifidum and some strains of Lactic acid bacteria on growth of *Staphylococcus aureus*. Minufiya J. Agric. Res., 25(3): 637 - 647.

Nayra, S.M. and hosney, I. M. (2003): Functional fermented milk with date. Arab. Univ. J. Agric. Sci., 11 (1): 247- 259.

Nremark, M.; Nilsson, L. and Waller, K. (2002): The effect of fermented milk products on growth of udder pathogenic bacteria. Svensk Veterinartidning, 54 (6): 305-310.

Pochart, P .; Marteau, P .; Bouhnik, Y .; Bourlioux, P . and Rambaud, J. (2004): Survival of *Bifidobacteria* ingested via fermented milk during their passage through the human small intestine: an in vivo study using intestinal perfusion. Am. J. Clin. Nutr. , 55 (1):78-80.

Radostits, O. M.; Blood, D. C.; Gay, C. C.and Hinchcliff, K. W. (2000): Mastitis in veterinary medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses., 9th ed. W.B. Saunders, London.

Robenson, R. K. and Tamime, A. Y. (1990): Microbiology of fermented milks. In dairy microbiology, Ed. R.K. Robenson. Elsevier App. Sci. London.

Rybka, S. (1994): The enumeration of Lactobaccillus, Streptococcus and *Bifidobacterium* in yogurt. B.Sc. Dissertation University of NSW, Sydney.

Samona, A.; Robinson, R. and Marakis, S. (1996): Acid production by *Bifidobacteria* and yoghurt bacteria during fermentation and storage of milk. Food Microbiology, 13 (4): 275-280.

Shin, H.S .; Lee, J. H .; Pestka, J. J . and Ustunol, Z . (2000): Viability of *Bifidobacteria* in commercial dairy products during refrigerated storage. J. Food Prot. 63 (3): 327-31.

Vandal, M. and Biasizzo, M. (2000): Microbiological irrevocability of milk samples examined from January 1996 to May 1999. Mljekarstvo, 50 (4): 325 - 332.

Xiao, J. ; Kondo, S .; Takahashi, N .; Kokubo, S .; Hosono, A. (2003): Effects of milk products fermented by Bifidobacterium longum on blood lipids in rats and healthy adult male volunteers. J. Dairy Sci., 86 (7): 452-61.