Occurrence of some pathogenic microorganisms in kareish cheese and their public health significance

G. M. Hassan¹, Samia I. Afify²

¹Department of Food Hygiene, Faculty of Veterinary Medicine, Bani-Suef University, Egypt and ²Department of Microbiology, Animal health research Institute, Bani-Suef, Egypt.

Fifty random samples of Kareish cheese were collected from different localities in Bani-suef Governorate. All samples were examined chemically for acidity, salt and moisture percent and bacteriologicaly for the presence of *Escherichia coli, Staphylococcus aureus*, Enterococci, *Bacillus cereus, Clostridium perfringens, Yersinia enterocolitica*, Salmonella and Shigella species. The obtained results revealed that the mean values of acidity, salt and moisture % were 1.63 ± 0.095 , 3.55 ± 0.299 and 58.54 ± 0.599 in the examined kareish cheese samples, respectively.

Escherichia coli, Staphylococcus aureus, Enterococci, Bacillus cereus, Clostridium perfringens were recovered from 16 (32%), 12(24%), 46 (92%), 25 (50%) and 3 (6%) with a mean value of $4.86 \times 10^2 \pm 4.21 \times 10^2$, $4.84 \times 10^5 \pm 2.91 \times 10^5$, $3.74 \times 10^6 \pm 1.55 \times 10^6$, $7.08 \times 10^4 \pm 2.61 \times 10^4$ and $9.5 \times 10^1 \pm 7.37 \times 10^{-1}$ of the examined samples , respectively. Yersinia enterocolitica could be isolated from 12% of the examined samples. Salmonella and Shigella species could not be detected in any of the examined samples. The isolated *Escherichia coli* were examined for serological identification, Enterotoxigenicity and the susceptibility of the isolated serovars to various chemotherapeutic agents. The public health significance and economical importance of the isolated organisms and the recommendations to be followed in the processing, handling and storage of such dairy product were discussed

Kareish cheese is a kind of soft cheese which is manufactured from raw buffaloe's and cow's skimmed milk in farmer's houses. The increasing demand for it by the Egyptian consumers is mainly attributed to its high protein content and low price. Raw milk is considered as a good medium for growth of different pathogenic microorganisms. Robinson (1990) showed that the main sources of pathogenic bacteria in cheese are contaminated raw milk, food handlers, dust, utensils and insects. Van Netten et al., (1990) investigated three outbreaks of B. cereus food poisoning in Spain and Netherlands. Wieneke et al., (1993) stated that milk and milk products were the vehicle in 8% of 359 outbreaks and sporadic cases of staphylococcal food poisoning in the United Kingdom between 1969 and 1990; Djuretic, et al., (1997) mentioned that verocytotoxin producing E.coli was responsible for 3 outbreaks associated with consumption of milk and milk products in England and Wales. Enterococci affects on the microbiological, the physicochemical and the sensory characteristics of cheese as it positively affected the counts of non-starter lactic acid bacteria (NSLAB), micrococci and Coliforms (Sarantinopoulos et al., 2002). The presence of Clostridium perfringens in dairy products is indicator of faecal or soil contamination and usually associated with spoilage problems as late blowing in cheese Robinson (1990). The aim of the present work was determination of acidity, salt, moisture % as well as occurrence of some pathogenic bacteria in kareish cheese, studying the Enterotoxigencity and resistance of isolated *E.coli* strains to some chemotherapeutic agents in vitro and discussion of the public health significance of the isolated organisms.

Material and methods

Sampling. A total of 50 random samples of kareish cheese were collected in sterile bags from different local markets in Bani-Suef Governorate during spring and summer of 2007 and transported directly to the laboratory in ice box to be examined chemically and bacteriologically.

Chemical examination. The moisture content, sodium chloride and titratable acidity % of kareish cheese samples were determined according to the methods recommended by A.P.H.A. (1992).

Bcteriological examination. Each sample was thoroughly mashed in a sterile mortar, eleven grams of prepared cheese sample were

Parameter	No. of examined samples	Minimum	Maximum	Mean ± SEM
Acidity %	50	0.300	3.63	1.63 ± 0.095
Salt %	50	0.293	8.78	3.55 ± 0.299
Moisture %	50	47.800	69.00	58.54 ± 0.599

Table (1): Statistical analytical results of chemical examination of the examined kareish cheese samples.

Table (2): Statistical analytical results of Escherichia coli, Staphylococcus aureus, Enterococci, Bacillus cereus and Clostridium perfringens counts in the examined kareish cheese samples.

Bacterial counts	Positive samples		Minimum	Maximum	Mean ± SEM
	No.	%	-		
Escherichia coli	16	32	<3	2.1x104	$4.86 \times 102 \pm 4.21 \times 102$
Staphylococcus aureus	12	24	<100	1.1x107	$4.84 \times 105 \pm 2.91 \times 105$
Enterococci	46	92	<100	5.5x107	$3.74 \times 106 \pm 1.55 \times 106$
Bacillus cereus	25	50	<100	9.1x105	$7.08 \times 104 \pm 2.61 \times 104$
Clostridium perfringens	3	6	<100	3.6x103	$9.5 \times 101 \pm 7.37 \times 101$

Table (3): Frequency distribution of Escherichia coli (MPN) count/g in the examined kareish cheese samples.

Intervals	Escherichia coli (1	MPN)
	No. of samples	%
<3	34	68
3-9	4	8
$10 - <10^2$	5	10
$10^2 - <10^3$	5	10
$10^3 - < 10^4$	1	2
$3-910 -<102102-<103103-<104\geq 10^{4}$	1	2
Total	50	100

aseptically homogenized with 99 ml of sterile 2% sodium citrate solution at 40°C, serial dilutions of the homogenates were prepared. Isolation and identification of *E.coli*, Salmonella spp., Shigella spp., *Staph. aureus*, *B. cereus*, *C. perfringens*, *Y. enterocolitica* and Enterococci were done according to (A.P.H.A. 1992; Collee *et al.*, 1996).

Serological typing of *E. coli.* Agar slants containing generous growth of *E. coli* isolates were submitted for agglutination tests using polyvalent and monovalent O *E. coli* antisera.

Detection of *E. coli* enterotoxin. It was performed according to (Guarino *et al.*, 1987) Swiss albino suckling white mice aged 2-4 days old were used to detect enterotoxigenicity of *E. coli*. These mice separated from their mothers immediately before use, 22 tested suckling mice were used. Each isolated strain was inoculated into Brain heart infusion broth and incubated at 37° C for 18 hrs then centrifuged at 3000 r.p.m. for 20 minutes, The supernatant fluid was filtrated and tested for toxigenic activity by

orogastric inoculation of 0.1 ml of supernatant in each of two infant mice. All mice were kept at room temperature for four hours, after which they were anaesthetized with ether, the abdomen was opened and the entire intestine was removed, the weight of the gut and the remaining carcases of two mice were taken, the ratio of the gut weight was calculated for each mouse and the results averaged, the ratio below 0.083 was considered negative while ratio of 0.083 or above was considered positive result for enterotoxigenicty

Susceptibility of *E.coli* isolates to various chemotherapeutic agents. The disk diffusion technique was adopted according to (Koneman *et al.*,1992). The following antibiotic discs were used; ceftiofur (30 mg), chloramphenicol (30 mg), lincomycin (15mg), spectinomycin (200mg), amoxicillin (25 mg), norfloxacin (10 mg), neomycin (30 mg), gentamycin (30mg) and doxycycline (30 mg).

Results and Discussion

Intervals	Staphylo aure		Entero	Enterococci Bacillus cereus		Clostridium perfringens		
	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
<10 ²	38	76	4	8	25	50	47	94
$10^2 - <10^3$	4	8	10	20	4	8	2	4
$10^{3} - < 10^{4}$	2	4	9	18	7	14	1	2
$10^4 - < 10^5$	2	4	9	18	6	12		
$10^{5} - < 10^{6}$	1	2	6	12	8	16		
10⁶-<10 ⁷	2	4	7	14				
≥ 10 ⁷	1	2	5	10				
Total	50	100	50	100	50	100	50	100

Table (4): Frequency distribution of Staphylococcus aureus, Enterococci, Bacillus cereus and Clostridium perfringens counts/gm (SPC) in the examined kareish cheese samples.

Table (5): Serological identification of Escherichia coli isolated from the examined kareish cheese samples.

Escherichia coli serogroups	NO.	%
O ₁₁₁	4	25
O_8	3	18.75
O ₁₂₅	3	18.75
0 ₂₉	1	6.25
Untypable	5	31.25

Table (6): Enterotoxigenicity of isolated Escherichia coli serogroups from the examined kareish cheese samples.

Escherichia coli serogroups	Enterotoxigenic activity						
	Negative	%	Positive	%			
O ₁₁₁ (4)	1	25	3	75			
O_8 (3)	2	66.67	1	33.33			
O_{125} (3)	1	33.33	2	66.67			
O_{29} (1)	1	100	0	0			

Table(7): The susceptibility of isolated Escherichia coli serogroups from the examined kareish cheese samples to various chemotherapeutic agents.

Chemotherapeutic		O ₁₁₁			O_8			O ₁₂₅				O ₂₉				
agents]	R S		S	R		S R		S		R		S			
	No.	%	No.	%	No.	%	N0.	%	No.	%	No.	%	No.	%	No.	%
1-Ceftiofur	0	0	4	100	0	0	3	100	0	0	3	100	0	0	1	100
2-chloramephenicol	3	75	1	25	3	100	0	0	2	66.67	1	33.33	1	100	0	0
3-Lincomycin + Spectinomycin	0	0	4	100	0	0	3	100	0	0	3	100	0	0	1	100
4-Amoxicillin	4	100	0	0	1	33.33	2	66.67	3	100	0	0	0	0	1	100
5-Norfloxacin	1	25	3	75	0	0	3	100	0	0	3	100	0	0	1	100
6-Neomycin	2	50	2	50	2	66.67	1	33.33	2	66.67	1	33.33	0	0	1	100
7-Gentamycin	0	0	4	100	1	33.33	2	66.67	0	0	3	100	0	0	1	100
8-Doxycycline	2	50	2	50	2	66.67	1	33.33	3	100	0	0	0	0	1	100

Isolates	No. of examined samples	Positive samples		
	_	No.	%	
Yersinia enterocolitica	50	1	2	
Salmonella	50	0	0	
Shigella	50	0	0	

Table (8): Incidence of *Yersinia enterocolitica*, Salmonella spp. and Shigella spp, in the examined kareish cheese samples.

The results in Table (1) revealed that the acidity percentage of the examined kareish cheese samples ranged from 0.3 % to 3.63 % with a mean value of 1.63 ± 0.095 . Nearly similar results were reported by Mahmoud (1993); Abd El-Shaheed (1996); Khair Allah (2000), comparatively lower results were reported by Saleh(1989); Nawar (2001); Bahout and Moustafa(2006) while higher results were reported by El-Mossalami (1999).

The reported high value of acidity % may be attributed to the method of kareish cheese manufacture, in which, skimmed milk is placed at warm place for about 24-48 hours giving the chance for lactic acid producing microorganisms to grow and multiply with the production of acid as well as contamination of raw milk by these organisms.

The results in Table (1) indicated that the salt percentage of the examined kareish cheese samples ranged from 0.293 % to 8.78 % with a mean value of 3.55 ± 0.299 . Nearly similar results were reported by Abd El-Shaheed(1996); Khair Allah (2000); Nawar (2001), while higher results were reported by El-Leboudy (1989); Saleh(1989); Mahmoud (1993); Bahout and Moustafa (2006). Lower values were reported by El-Mossalami (1999) Aly *et al.*, (2007).

Salt is added to kareish cheese to prevent or at least to retard the growth of microorganisms leading to prolongation of the shelf life and permission of suitable acid fermentation.

The results in Table (1) revealed that the moisture content of the examined kareish cheese samples varried from 47.8 % to 69.0 % with a mean value of 58.54 ± 0.599 .

Nearly similar results were reported by Bahout and Moustafa(2006); Aly, *et al.*,(2007).

The wide range of variation in salt % and moisture content may be attributed to the different sources of samples or the differences in marketing times where the prolonged aging causes declining in the moisture content of the kareish cheese leading to the increase in the sodium chloride concentration. The results in Table (2) revealed that Escherichia coli was present in 16 (32%) of the samples and Escherichia coli counts (MPN/g) ranged from <3 to 2.1×10^4 with a mean value of $4.86 \times 10^2 \pm 4.21 \times 10^2$ /g in the examined kareish cheese samples. 68% of the sample contained <3 Escherichia coli/g (Table 3).

Nearly similar results for incidence were reported by Aman (1994); EL-Kholy, *et al.*, (1995), but higher incidence was reported by Ahmed, *et al.*, (1987); Nazem (1991) while lower incidence was reported by Halawa and El-Mossalami (1998). Similar results for count were reported by Ahmed, *et al.*, (1987); Bahout and Moustafa (2006)

Five main types of Escherichia coli have been associated with food borne diseases. The first, enterotoxigenic Escherichia coli which produce heat labile toxin type I is implicated in most cases of foodborne outbreaks Mayer. et al... (1991) and causes traveler's diarrhoea in human especially during visits to warmer countries al., (1998). (Hau, et The second. enteropathogenic Escherichia coli which causes infantile gastroenteritis associated with fever and bloody diarrhoea (Hoeprich. et al., (1994). The third, enterohemorrhagic Escherichia coli, which is responsible for hemorrhagic colitis and haemolytic uraemic syndrome (Griffin, 1991). The fourth, enteroinvasive Escherichia coli, which produces an illness very similar to dysentery. The last type, enteroaggregative Escherichia coli which has been implicated as a cause of prolonged diarrhoea in developing (Troller, 1993). countries Moreover the organisms were found to be responsible for cases of gastroenteritis, cystitis, pylitis, pyelonephritis as well as appendicitis and peritonitis Pyatki(1967); Marier, et al., (1973); Singh and Ranganattan (1974); Mossel (1975).

The most important serogroups among EHEC are O_{26} , O_{111} , O_{157} and of which O_{157} :H7 being the most relevant serotype in food borne outbreaks Gonzalez (2002).

Regarding to E.coli isolated from the examined samples and as recorded in Table (5):

5(31.25%) out of 16 isolates were untypable, and the remaining isolates 11(68.75%) were serologically typed as follows 4(25%) O111, 3(18.75%) O₈, 3(18.75%) O₁₂₅ and 1(6.25%) O₂₉. similar results were recoded by Gomez, *et al.*, (2002); Gonzalez and Blanco (1989) recorded that E. Coli untypable for O antingen represented 47%, of the verotoxigenic strains , Moussa, *et al.*, (2005) isolated 8 strains of E. Coli from milk samples, 3 (37.5%) of them were O₁₁₁.

The results recorded in Table (6) revealed that out of 16 E. Coli strains 6 (37.5%) were enterotoxgenic, similar results were recorded by Krogh (1983); Poh1, *et al.*, (1989).

The results tabulated in Table (7) showed that Enterotoxigenic strains isolated from kareish Cheese were highly resistant to Chloramphenicol, and Amoxicillin, Moderately resistant to and Doxycyclinne and senstive to Ceftiofur, Lincomycin + Spectinomycin, Norflooxacin and Gentamycin. These results nearly resemble that recorded by Ahmed, *et al.*, (1994); Mellata, *et al.*, (1998).

The results in Table (2) proved that Staphy lococcus aureus could be isolated from 12(24%) of the samples with a range of <100 to $1.1x10^7$ and a mean count of $4.84x10^5\pm2.91x10^5/g$ of the examined kareish cheese samples. 84% of the examined samples contained $<10^2-<10^3$ Staphylococcus aureus/g (Table 4). Similar results were reported by Araujo, *et al.*, (2002); Bahout and Moustafa (2006), while higher values were reported by Ahmed (1980); de Almeida and Nader(2001).

The presence and growth of Staphylococcus aureus in dairy products is a potential public health hazard ICSMF (1978) since many strains of Staphylococcus aureus under favourable environmental conditions have the ability togrow and multiply with the production of thermostable enterotoxins, which cause food intoxication within 2-4 hrs after consumption of contaminated food Newsome (1988).

The enterotoxins of S.aureus are antigenically different types and include $(A,B,C_1,C_2,E$ and TST "Toxic Shock Toxin") – All these types of enterotoxins except TST were involved in foodborne illness. On the other hand, postpasteurization of the toxin contaminated milk as well as dairy products will not make it safe for consumption Robinson (1990).

Among the reasons of food examination for Staphylococcus aureus are to confirm that these organisms may be the causative agent of food borne illness, to determine whether the product is the potential source of Staphylococcus aureus food poisoning and to demonstrate postprocessing contamination, which mainly due to defect in the personal hygiene or exposure the food to inadequately sanitized food processing surfaces.

Staphylococcus aureus are wide spread in nature and it is a good indicator of the personal hygiene especially the workers with respiratory infections and suppuration Harvey and Gilmour (1990); Kamat, *et al.*, (1991). Also inadequately cleaned utensils or equipment may also be a source of contamination.

Hence, presence of large number of Staphylococcus aureus in the product is considered a good indication that sanitation and temperature control have somewhere been inadequate ICMSF (1980).

The coordinator of the French surveillance system revealed that 69 documented outbreaks for milk and milk products were confirmed as the vehicle by the isolation of the etiologic agent. The food vehicles were distributed as follows: milk, 10%; cheese, 87%; others, 3%. S. aureus was by far the most frequent pathogen associated with 85.5% of the outbreaks, De Buyser, *et al.*, (2001).

Inspection of Table (2) revealed that Enterococci could be detected in 46(92%) of the samples with a range from <100 to 5.5×10^7 , and a mean count of $3.74 \times 10^6 \pm 1.55 \times 10^6$ /g of the examined kareish cheese samples. The highest frequency distribution (38%) lies within the range from $10^2 - <10^4$ Enterococci/g (Table 4). Similar results for incidence were reported by Ahmed, et al., (1987); Aman (1994), but higher results were reported by Mahmoud (1993) and Bahout and Moustafa (2006). Also similar results for counts were reported by El-Barbary (1992); Bahout and Moustafa(2006), while lower values were reported by Ahmed, et al., (1987) and higher values were reported by Mahmoud (1993); Aman (1994).

Enterococci being normal inhabitants in alimentary tract of both man and animals, thus their presence in milk and its products is considered a definite index of faecal contamination Brooks (1974); ICMSF (1982), also they are found in the soil, on plants and in the intestine of the insects and birds Gelsomino, *et al.*, (2002). Enterococci being comparatively heat resistant, salt tolerant and can grow at wide range of temperature, low pH and more resistant to drying, detergents, freezing and disinfectants. Enterococci may help in assessing the hygiene in factories Slanetz, et al., (1983); Colman and Ball (1984); Rao, et al., (1986); Harrigan (1998). Therefore, they are frequently responsible for producing unfavourable changes as bitterness, proteolysis and other defects in milk products Harrigan and Micance, (1966). They are widely distributed in nature and gain entry into milk and milk products through the contaminated water supply, equipment and unhygienic conditions of production and handling. They have been incriminated as direct or indirect cause of disease and food poisoning because of their ability to produce extracellular toxic metabolites Garg and Mital, (1991); Roushdy, et al., (1998).

The obtained high values of enterococci reflect the poor sanitary practices during manufacturing, handling, storage and distribution and the high numbers may at time constitute a public health hazard and may induce food poisoning

Bacillus cereus. The results in Table (2) showed that Bacillus cereus could be isolated from 25(50%) of the samples with a range of<100 to 9.1×10^5 and a mean count of $7.08\times10^4\pm2.61\times10^4/g$ from the examined kareish cheese samples. Most of samples (58%) of the examined samples contained $<10^2-<10^3$ Bacillus cereus /g (Table 4). Nearly similar results were reported by Halawa and Moawad (1999); Bahout and Moustafa(2006)

Bacillus cereus is a food poisoning microorganism, produces one emesis-causing toxin and three enterotoxins that elicit diarrhea. There are two types of food poisoning syndromes caused by B. cereus. The first was diarrhoeal type syndrome while the second was vomiting type Ehling-schulz, *et al.*, (2004). It has been recognized that high number of B. cereus ranged from $10^6-10^8/\text{ml}$ is needed to elicit symptoms of food poisoning, Mossel, (1982). However, in compromised consumers a much smaller dose of $1.2 \times 10^3/\text{ml}$ may cause illness, Gianella and Brasilla, (1979). Neither two forms of illness should be considered life-threating to normal healthy individual.

The contamination of milk and milk products by such organisms may be attributed to the fact that B. cereus is widely distributed in nature and usually contaminate milk during milking or storage on the farm, then gain entrance to dairy products from which they are prepared. However, the extent of B. cereus contamination depends on the effectiveness of hygienic measures applied during processing, handling and distribution of milk products.

Inspection of Table (2) revealed that Clostridium perfringens could be detected in 3(6%) with a range from <100 to 3.6×10^3 , and a mean count of $9.5\times10^1\pm7.37\times10^1/g$ of the examined kareish cheese samples. The highest frequency distribution 47(94%) was in the range $<10^2$ Clostridium perfringens /g (Table 4). Higher results reported by Amer, *et al.*, (1996); Bahout and Moustafa (2006)

Clostridium perfringens is a classical agent of food-borne disease but because of the mildness and self-limiting nature of the illness, many cases are undiagnosed Sanz, *et al.*, (2002).

The results in Table (8) showed that Yersinia enterocolitica could be isolated from 1(2%) out of the examined samples. Similar results were obtained by Abdel Hady (1993), however lower results were recorded by Northolt (1983). The lower incidence of Yersinia enterocolitica in cheese samples can be attributed to the high contamination of the cheese with other competitors and the difficulty in detection of small numbers of Yersinia enterocolitica among large numbers, Bottone (1977).

Yersinia enterocolitica is considered a causative agent of gastroenteritis, Morse, *et al.*, (1984), arthritis, Archer (1985) and pseudoappendicular syndrome, deGroote, *et al.*, (1982); Cover and Aber (1989).

A Norwegian study of 458 hospitalized patients over 10 years indicates that acute and chronic disease of the liver, pancreas and gastrointestinal tract can result from Yersiniosis, Saebo and Lassen(1991, 1992a,b,c). On the other hand Yersinia enterocolitica could be isolated from 73/333 (21.9%) of patients, mostly women over 50 years with erythema nodosum (Winblad, 1975).

Salmonella and Shigella could not be detected in any of the examined samples. Similar results were reported by De Centorbi, *et al.*, (1989); El-Kholy(1989); Maifreni, *et al.*, (1993); Windrantz and Arias (2000).while higher results were reported by Wouafo, *et al.*, (1996); Kruy, *et al.*, (2001). Therefore, to safeguard the consumer from being infected and to save the products from contamination the following suggestions are to be considered. Educational programs should be imposed for producers and handlers. Production, handling and distribution should be done under strict hygienic measures. Continuous refrigeration of milk from time of milking till used in manufacturing of Kareish cheese. Prevention of recontamination of manufactured Kareish cheese. The consumers should take in consideration the cleanliness of sales persons. Efficient cleaning of all utensils and equipment. Timely notification of food borne diseases must be encouraged. The final retail containers used are preferred to be dispensable and efficiently closed or covered.

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مدى تواجد بعض الميكروبات الممرضة في الجبن القريش وأهميتها الصحية