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Occurance of Enterobacteriaceae in Sudanese White Cheese in Restaurants of Khartoum State (Sudan)*

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Abstract: In the present survey a total of thirty one Enterobacteriaceae were found in twenty samples (66.7%) out of thirty samples of Sudanese white cheese. The total isolated Enterobacteriaceae include *Citrobacter freundii* (14, 46.7%), *E. coli* (6, 20%), *Enterobacter aerogenes* (2, 6.6%), *Pseudomonas aeruginosa* (1, 3%), *P. mirabilis* (5, 16.7%) and *Sallmonella* ssp. (3, 10%). The restaurants located at Khartoum North revealed 4 (44.4%), 1 (11.11%), 1 (11.11%) and 2 (22.2%) isolate of *C. freundii*, *E. coli*, *Pseudomonas aeruginosa* and *P. mirabilis*, respectively. Similarly the isolates from restaurants located in Khartoum were 5 (55.5%) isolates of *C. freundii*, 3 (33.3%) isolates of *E. coli*, 2 (22.22%) isolates of *Enterobacter aerogenes*, 1 (11.11%) isolate of *P. mirabilis* and 2 (22.2%) isolates of *Salmonella paratyphi*. The restaurants located in Omdurman revealed 5 (41.7%) isolates of *C. freundii*, 2 (16.7%) isolates of *E. coli*, 2 (16.7%) isolates of *P. mirabilis* and 1 (8.3%) isolate of *Salmonella typhi*. The mean, minimum and maximum counts of Enterobacteriaceae were found to be $2\times10^4\pm3.1\times10^4$, 0 and 8×10^4; $2.6\times10^4\pm1.8\times10^4$, 0 and 5×10^4 and $1.33\times10^4\pm1.96\times10^4$, 0 and 6.0×10^4, respectively, for the cheese samples collected from restaurants of Khartoum North, Khartoum and Omdurman. The total bacterial counts were found to be higher in samples with higher count of Enterobacteriaceae and this was mainly noticed in restaurants at Khartoum, which revealed $5.6\times10^8\pm3.7\times10^8$, 2×10^7 and 9×10^8, respectively. However the bacterial count were found to be $2.9\times10^8\pm2.4\times10^8$, 2×10^7 and 6×10^8 and $2.7\times10^8\pm2.1\times10^8$, 2×10^7 and 7×10^8 in Khartoum north and Omdurman restaurants, respectively. There was a significant difference ($p<0.05$) for the total bacterial count between Khartoum and the other studied locations, as the samples collected from Khartoum revealed the higher counts compared to other two areas. However non significant variation were recorded for Enterobacteriaceae counts.

Key words: Enterobacteriaceae, Sudanese white cheese, restaurants, Khartoum State, Sudan

INTRODUCTION

Cheese is a solid curd with some or all of the whey drained off and it is either used in its form or when dried on further maturation during storage (Payne, 1993). Sudanese white cheese falls into family of a soft and semi soft picheld cheese of east European countries, the east Mediterranean region and

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North Africa (Abdalla, 1992). He also added that the Sudanese white cheese in general has higher acidity and lower fat content than the Egyption Domiati cheese.

Enterobacteriaceae strains posses proteolytic systems active on all casein fractions under cheese manufacturing and ripening conditions, the effects on caseins were similar for strains belonging to the same genus (Morales *et al*., 2003). Kosikowski (1982) reported that coliform can grow well in cold or warm cheese milk and in cheese curd and their numbers may reach 100 to 500 millions per gram if their initial level in the milk was high and curd acid devolpment was slow. Moreover the presence of Enterobacteriaceae in cheeses may affect proteolysis during ripening (Morales *et al*., 2003). Kosikowski (1982) added that coliforms produce gas in the cheese curd causing slite eye. According to Banwat (1981) the gas-forming organisms produce off flavours and gas holes in the cheese, which is made from raw milk.

It is in dispotable that some outbreaks of food borne illness have been clearly linked with the consumption of cheese, the majority of those reported being associated with cheese made from un-pasteurized milk (Professional Food Microbiological Group, 1998). Whilst pathogens can gain access to cheese after curd formation, it is clear that many food borne pathogens are fecal in origin. Moreover, pathogens may also be excreated into the milk directly from the udder (Rampling, 1996). However correctly, controlled milk pasteurization kills such bacteria (Professional Food Microbiological Group, 1998).

The first confirmed outbreak of *E. coli* O157:H7 infection in Canada associated with raw milk hard cheese was reported by Honish *et al*. (2005) who concluded that a review of federal legislation vis-a-vis raw milk hard cheese may be in order. Maher *et al*. (2001) observed that during manufacturing and ripening of smear ripered cheese there was growth of *E. coli* O157:H7 to a level that permitted survival during an extended storage of the cheese. However, Ansay and Kasper (1997) suggested that *E. coli* sero type O157:H7 was not prevalent in dairy product ingredients and processing environment. Similary, Spano *et al*. (2003) showed that curd at 8°C, for 5 min resulted in the loose of culture ability of *E. coli* O157:H7 during the production of mozzarella cheese. Moreover at 4°C *Eschechichia coli* O157:H7 did not grow in pasteurized and unpasteurized whey; however, the pathogen persisted longer in pasteurized samples (Marek *et al*., 2004). Their result indicated the potential risk of persistence of *E. coli* O157:H7 in whey in the event of contamination with this pathogen.

Coveney *et al*. (1994) found that the incidence of coliforms were higher in soft, semi soft and semi hard Irish cheeses than in hard types. Messa *et al*. (1992) reported that high concentration of fecal coliform was observed in 41 samples of Mozzarella cheese. They identified three-isolates as *Klebsiella pneumonia*, 2 isolates as *Klebsiella oxytoca* and one isolate for each *Enterobacter aerogenosa* and *E. coli*.

Allagabo (1986) found standard plate count of 4×10^5 per gram, average yeast and mould count of 2.9×10^3 per gram and salt tolerant bacteria of 2×10^2 per gram in Sudanes white cheese. Moreover in study included contamination of Sudanes white soft cheese from Khartoum North (Sudan) by Warsama (2003), she found high total bacterial and coliform counts in all cheese samples positive isolates for *E. coli* and *Salmonella* ssp.

MATERIALS AND METHODS

Source and Collection of Cheese Samples

Thirty random samples of Sudanese white cheese were collected from restaurants from 10 major markets of Khartoum State during the period of April to June 2005. Three samples from each selected

restaurants in Khartoum North, Khartoum and Omdurman. Omdurman were collected into sterile container and transported in an ice bag to the laboratory of Dairy Microbiology, Department of Dairy Production, University of Khartoum, where the analysis was done.

Microbiological Examination

The samples were enumerated for counting total bacteria (standard plate count) and Enterobacteriaceae on standard plate medium and MacConkey agar, respectively. All media were obtained in dehydrated forms and prepared according to the manufactures' instructions. Serial dilution of the cheese was done according to Richardson (1985). Purification was done by subculturing different colonies on nutrient agar medium to identify the harmful bacteria isolated from cheese using the primary and secondary biochemical test as described by Barrow and Feltham (1993).

Statistical Analysis

The data of the present study were analyzed statistically using complete randomized design. ANOVA test and the least significant difference were used to determine the difference between means. The analysis was carried out using SSPS (10).

RESULTS

Frequencies of Bacteria Isolated from Sudanese White Cheese

In the present survey seven different types of Enterobacteriaceae (*E. coli*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *P. mirabilis*, *S. typhi* and *S. paratyphi*) were found in Sudanese white cheese (Table 1).

Enterobacteriaceae Isolated from Sudanese White Cheese

A total of thirty one Enterobacteriaceae were found in twenty samples (66.7%) out of thirty samples of Sudanese white cheese. Of these 14 (46.7%) isolates were *Citrobacter freundii*, 6 (20%) isolates of *E. coli*, 2 (6.6%) isolates of *Enterobacter aerogenes*, 1 (3%) isolate of *Pseudomonas aeruginosa*, 5 (16.7%) isolates of *P. mirabilis* and 3 (10%) isolates of *Sallmonella* ssp. (Table 1). In restaurants located at Khartoum North 4 (44.4%), 1 (11.11%), 1 (11.11%) and 2 (22.2%) isolate of *C. freundii*, *E. coli*, *Pseudomonas aeruginosa* and *P. mirabilis*, respectively were isolated. Similarly from restaurants in Khartoum 5 (55.5%) isolates were identified for *C. freundii*, 3 (33.3%) isolates of *E. coli*, 2 (22.22%) isolates of *Enterobacter aerogenes*, 1 (11.11%) isolate of *P. mirabilis* and 2 (22.2%) isolates of *Salmonella paratyphi*. Restaurants in Omdurman revealed 5 (41.7%) isolates of *C. freundii*, 2 (16.7%) isolates of *E. coli*, 2 (16.7%) isolates of *P. mirabilis* and 1 (8.3%) isolate of *Salmonella typhi* (Table 1).

The counts of Enterobacteriaceae were found to be $2\times10^{4}\pm3.1\times10^{4}$, 0 and 8×10 for mean±standard deviation, minimum and maximum values, respectively (Table 2) for the cheese samples

Table 1: The incidence of some pathogens in Sudanese white cheese collected from restaurants of Khartoum State

	Number of the isolated pathogens						
Major markets	*C. freumdii*	*E. coli*	*E. aerogence*	*P. Aeruginosa*	*P. mirabili*	*S. typhi*	*S. paratyphi*
Khartoum North	4	1	0	1	2	0	0
Khartoum	5	3	2	0	1	0	2
Omdurman	5	2	0	0	2	1	0
Total number	14	6	2	1	5	1	2

Table 2: Frequencies of Enterobacteriacae and total bacteria counts of the Sudanese white cheese in Khartoumm State

		Enterobacteriacae counts (cfu g^{-1})			Total bacteria counts (cfu g^{-1})		
Area	Market	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum
Khartoum	Hilat Kuku	$8.0\times10^{3}\pm1.9\times10^{4}$	0	2×10^{4}	$3.7\times10^{8}\pm2.9\times10^{8}$	1.3×10^{8}	6×10^{8}
North	Bahri (Elmahta Elwasta)	0.0	0	0	$2.7\times10^{8}\pm15.7\times10^{7}$	6.0×10^{7}	3×10^{8}
	El Markazy	$5.0\times10^{4}\pm3.0\times10^{4}$	12×10^{3}	8×10^{4}	$3.3\times10^{8}\pm2.2\times10^{8}$	2.0×10^{7}	5×10^{8}
	Sub total samples	$2.0\times10^{4}\pm3.1\times10^{4}$	0	8×10^{4}	$2.9\times10^{8}\pm2.4\times10^{8}$	2.0×10^{7}	6×10^{8}
Khartoum	El Arabi market	$1.0\times10^{4}\pm9.4\times10^{3}$	1.5×10^{3}	2×10^{4}	$7.3\times10^{8}\pm11.9\times10^{7}$	6.0×10^{8}	9×10^{8}
	El Shaaby Khartoum	$2.0\times10^{4}\pm13\times10^{3}$	8×10^{3}	3×10^{4}	$4.3\times10^{8}\pm3.4\times10^{8}$	2.0×10^{8}	7×10^{8}
	El Markazy Khartoum	$3.3\times10^{4}\pm2.6\times10^{4}$	0	5×10^{4}	$4.0\times10^{8}\pm3.4\times10^{4}$	2.0×10^{7}	6×10^{8}
	Sub total samples	$2.6\times10^{4}\pm1.88\times10^{4}$	0	5×10^{4}	$5.6\times10^{8}\pm3.7\times10^{8}$	2.0×10^{7}	9×10^{8}
Omdurman	El Shaaby Omdurman	$2.3\times10^{4}\pm3.4\times10^{4}$	0	6×10^{4}	$3.0\times10^{8}\pm3.4\times10^{8}$	1.2×10^{8}	7×10^{8}
	Omdurman	$1.5\times10^{4}\pm1.38\times10^{4}$	0	2×10^{4}	$3.7\times10^{8}\pm1.85\times10^{8}$	1.7×10^{8}	5×10^{8}
	El Shegla	$1.9\times10^{4}\pm1.77\times10^{4}$	0	3×10^{4}	$3.3\times10^{8}\pm2.7\times10^{8}$	9.0×10^{7}	6×10^{8}
	Libya	$5.0\times10^{2}\pm8\times10^{2}$	0	1.5×10^{3}	$8.7\times10^{7}\pm2.7\times10^{7}$	2.0×10^{7}	1.4×10^{8}
	Sub total samples	$1.33\times10^{3}\pm1.96\times10^{3}$	0	6×10^{4}	$2.7\times10^{8}\pm2.1\times10^{8}$	2.0×10^{7}	7×10^{8}

Table 3: Comparison of the counts Enterobacteriacae and total bacteria from Sudanese white cheese in Khartoum State using Duncan Multiple Range Test

Area	Total bacteria count (cfu g^{-1})	Enterobacteriacae count (cfu g^{-1})
Omdurman	2.84×10^{8a}	1.33×10^{4a}
Khartoum North	2.97×10^{8a}	2.00×10^{4a}
Khartoum	5.46×10^{8b}	2.56×10^{4a}

Mean within same column bearing the differed superscripts are significantly different ($p<0.05$)

collected from restaurants in Khartoum North. Similarly the cheese samples collected from Khartoum showed counts of $2.6\times10^{4}\pm1.8\times10^{4}$, 0 and 5×10^{4} for mean±standard deviation, minimum and maximum values, respectively. However the counts of Enterobacteriaceae were found to be $1.33\times10^{4}\pm1.96\times10^{4}$, 0 and 6.0×10^{4} respectively, for cheese samples collected from Omdurman restaurants (Table 2).

Total Bacterial Count (SPC)

The total bacterial counts were found to be higher in the samples with higher counts of Enterobacteriaceae and this was mainly noticed in restaurants at Khartoum, which revealed $5.6\times10^{8}\pm3.7\times10^{8}$, 2×10^{7} and 9×10^{8} for mean±standard deviation, minimum and maximum values, respectively (Table 2). However the bacterial count were found to be $2.9\times10^{8}\pm2.4\times10^{8}$, 2×10^{7} and 6×10^{8} in Khartoum north (Table 2), respectively. Similarly the total bacterial counts were found to be $2.7\times10^{8}\pm2.1\times10^{8}$, 2×10^{7} and 7×10^{8} in Omdurman restaurants for mean±standard deviation, minimum and maximum values, respectively (Table 2).

There was a significant difference ($p<0.05$) between in the total bacterial count between Khartoum and the other studied locations, as the samples collected from Khartoum revealed higher counts compared to other two areas (Table 3). However non significant variations were recorded for Enterobacteriaceae counts (Table 3).

DISCUSSION

The most common organism isolated was *Citrobacter freundii* (46.7%) followed by *E. coli* (20%) and *Enterobacter aerogence* (6.6%). This high load of coliforms agreed with Kosikowski (1982), Massa *et al*. (1992), Coveney *et al*. (1994) and Warsama (2003). Moreover Jay (1986) reported that *Citrobacter freundii* can be found in milk due to post contamination during ripening of cheese from soil, water, bad hygienic, because it is found in the intestinal tract of man and animals. Similarly *E. coli* can

contaminate cheese and the sources of it are soil, feaces and its presence in large number in foods and is generally taken to indicate fecal contamination, also *Enterobacter aerogence* is found in water, soil and human bowel (Jay, 1986).

Salmomella ssp. (10%) that were isolated during this study, agreed with Kosikowski (1982), Massa, *et al*. (1992), Conveny *et al*. (1994) and Warsana (2003). According to Asperger (1994) raw milk contamination usually takes place by salmonella from external sources (feaces, the farmer, water pollution and dust). Similarly the isolation of *Pseudomonas aeruginosa* (3%) supported Speck (1972) who reported that pathogenic bacteria could be controlled by the use of starter culture, such as *Lactobacilli*, which produce antibodies that could suppress the growth of *Pseudomonas*. Also the isolation of *Proteus* ssp. (16.7%) in this study agreed with Kosikowski (1982), Massa *et al*. (1992), Coveney *et al*. (1994) and Ahmed (1997).

The counts of Enterobacteriaceae of cheese samples in the three studied areas showed non significant differences (Table 3). The minimum counts are 0 and that was explained by the suggestion that some samples collected from some restaurants do not contaminated by Enterobacteriaceae. Moreover when minimum and maximum values are 0, it indicates that theses restaurants are free from Enterobacteriaceae (Table 2). This result agreed with Kosikowski (1982), Massa *et al*. (1992), Coveney *et al*. (1994) and Warsama (2003). Moreover, Kosikowski (1982) reported that coliforms bacteria grow well in cold or warm cheese causing slit eyes. He also reported that coliforms do not survive in pasteurized cheese milk but may be present as a result of post pasteurization contamination. Similarly, Massa *et al*. (1992) reported that higher concentration of fecal coliforms were observed in mozzarella cheese. Coveney *et al*. (1994) also found that the incidence of coliforms were higher in soft cheese, semi soft and hard cheeses. Moreover Araujo *et al*. (2002) showed that 95.5% of cheese samples had high levels of faecal coliforms. Also positive isolation of *Salmonella* ssp. in the restaurants suggested that large number of people are at high risk for subjecting to those foods borne diseases that might be due to mishandling or improper hygiene (Warsma, 2003). According to Asperger *et al*. (1994) raw milk contamination usually takes place by *Salmonella* from external sources (feaces, the farmer, water pollution and dust). Similarly, Carson and Dewitt (2002) reported that Salmonellae food poisoning can occur when some one drinks unpasteurized milk or eat any food contaminated during preparation, poor hygiene can also allow such carrier to spread infection to other. However the present result disagreed with Ahmad (1997) who found that *Salmonella* ssp. was absent in the Sudanese white cheese. Hence the present study supported Warsma (2003) who isolated *Salmonella* ssp. from Sudanese white cheese.

There were significant different between the three areas. The counts of total bacterial count in Khartoum were higher than that of Omdurman and Khartoum North (Table 2). The results indicated the presence of potential pathogenic bacteria (Salmonella and colifofim) as shown in Table 1 during the present survey. This disagreed with Ahmed (1997) who demonstrated the absence of coliform and salmonella in Sudanese white soft cheese. However it supported Warsama (2003) who isolated *Salmonella* ssp., *E. coli* and other coliforms bacteria from Sudanese white cheese. Moreover, the high counts of Enterobacterriaceae in cheese samples in the present survey were indication of contamination by the isolated potential food borne pathogens and/or spoilage organisms (*Salmonella*, *E. coli*, *Proteus* ssp., *Pseudomonas* and coliform). Salmonellosis are one are the main causes of food borne infections in industrialised countries. In France, the incidence of human salmonellosis recorded by the National Reference Centre for Salmonella and Shigella (CNRSS) in 2001 was 21 cases per 100,000 inhabitants and *Salmonella serotype enteritidis* represented 39% of cases (Haeghebaert *et al*., 2003).

The present study concluded that the high bacterial loads were found to be associated with cheese sample collected from some restaurants. This might indicated that the level of hygienic and storage of the product and its handling in these restaurants were poor. Moreover, contamination of the cheese might be because the exposure to air and this was facilities by cutting it into small pieces (Warsma,

2003). The high Enterobacteriaceae (environmental bacteria) supported this suggestion. Hence we recommended the processing of high quality milk that produced from healthy animals in hygienic manner using hygienic utensils and prevention of post processing contamination of dairy products particularly the Sudanese white cheese that was use by large population in Sudan. Periodic check out for all food-distributing centers and ensurance that healthy people are handling food are urgently needed. Further studies are also needed to demonstrate the vehicle and source of contamination of cheeses and molecular characterization of food borne pathogens related to cheeses.

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