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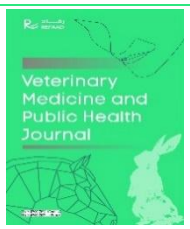

Occurrence of Coliform Bacteria in Mudaffara Cheese from Producers and Supermarkets in Khartoum, Sudan, 2018

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Review Article

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Abstract:

Objectives: This study aimed to investigate the prevalence of coliform bacteria in Mudaffara cheese from local markets in Khartoum City.

Methods: This is a descriptive cross–sectional study designed to isolate coliform bacteria from Mudaffara cheese in the markets of Khartoum. The study was designed to investigate the presence of coliform bacteria in Mudaffara cheese obtained from the producers and supermarkets of the Khartoum Locality.

Results: The results of coliform bacterial isolation have revealed the presence of 3 species of bacteria including 6 (14.3%) isolates of E. coli, 4 (9.5%) isolates of Salmonella spp, 2 (4.8%) isolates of Shigella spp. This study concluded that Mudaffara cheese is produced and marketed in Khartoum by traditional procedures, which might subject the product to deterioration in quality. Moreover, if the contaminated cheese is consumed it can be a source of potential pathogens.

Conclusion: Therefore, it is necessary that cheese must be produced and marketed according to the standards and regulations.

Keywords: *Mudaffara cheese; E. coli; Salmonella spp.; Shigella spp.; local markets; Khartoum City; Sudan.*

1 Introduction

Cheese is a dairy product that has played a key role in human nutrition for centuries and it is the most popular dairy product in Sudan (Mohammed Salih et al., 2011, Abdalla and Gamer Eldin, 2018, Farah and El Zubeir, 2020). Cheese provides a useful service in extending the shelf-life of milk (Farah and El Zubeir, 2019). Moreover, the quality of cheese depends on a variety of factors such as raw milk composition, technological parameters, bacteria species, storage, transportation and delivery conditions (El Owni and Osman, 2009, Idris and Alhassan, 2010, Elkhider et al., 2012, Mohamed and El Zubeir, 2018).

Mudaffara cheese is a pickled semi-hard cheese braided into a characteristic shape, with added spices such as black cumin for flavor (El gabali et al., 2022). Mudaffara cheese production in Sudan is a small business, and a standard procedure is adopted by the different producers for its production (Mohammed Salih et al., 2011, Abdalla and Gamer Eldin, 2018, Farah and El Zubeir, 2020). The production of Mudaffara cheese includes a high percentage of salt. Meanwhile the rennet that are added to the milk to obtain a firm coagulum (El gabali et al., 2022).

Inadequate sanitary practices during milking and on farms lead to the prevalence of coliform bacteria in raw milk. Furthermore, coliform bacteria may be used as an indicator of the sanitation of dairy processing facilities (Faja et al., 2023). The coliform bacteria can cause serious trouble in cheese making, they liberate large amounts of gas resulting in fermented cheese with a bitter unclean taste (Brandão et al., 2013). During cheese-making, ripening and storage, direct microbial contamination might occur as well as during processing or in retail market (Possas et al., 2021). Moreover, *Brucella* spp., *E. coli*, and *L. monocytogenes* can survive the production, ripening and storage of cheeses (Verraes et al., 2015). *Salmonella* spp. and *Shigella* spp. count in Kareish cheese in Egypt was in a range of 2.45 to 3.38 log cfu/gram, while *E. coli* was ranging from 2.38 to 5.47 log cfu/gram (Abd-Alla et al., 2024). It is important to notice that detecting pathogenic bacteria in cheese samples brings attention to public health concerns (Faja et al., 2023). Food-borne bacteria that often contaminate dairy products especially cheese in developing countries are *E. coli* and *Salmonella* spp. (Hatta et al., 2013).

Because Mudaffara is the second most commonly consumed cheese in Sudan, this study is conducted to investigate the occurrence of coliform bacteria produced and available at local markets in Khartoum.

2 Materials and Methods

2.1 Study Area

The study was conducted in Khartoum Locality, Khartoum State during the period from May to September 2018.

2.2 Study Design

This is a descriptive cross–sectional study designed to isolate coliform bacteria from Mudaffara cheese in the markets of Khartoum. The study was designed to investigate the presence of coliform bacteria in Mudaffara cheese obtained from the producers and supermarkets of the Khartoum Locality.

2.3 Sample Size

The sample size was calculated according to the formula:

n = N/1+ (N–1) d2

Where:

n = sample size

N= total population

d= 0.05

Total population (Total number of supermarkets in Khartoum locality = 23 and the total number of cheese producers in Khartoum locality =19) = 42

n = 42/1+ (42–1) ×0.0025 = 38

Then the sample size of supermarkets in Khartoum locality = 23/42×38 = 21

The sample size of cheese producers in Khartoum locality

= 19/42×38= 17

2.4 Source and Collection of Samples

A total of 42 samples were randomly collected from individual local markets (n= 23) and cheese producers (n= 19) disturbed widely in Khartoum Locality. All samples were collected in sterile containers, that were labeled and kept in an ice box and transported to the laboratory of the Department of Food Hygiene and Safety, Faculty of Public and Environmental Health, University of Khartoum to carry out the bacteriological examination.

2.5 Examination of Cheese Samples

2.5.1 Sterilization

Glasses such as slides, cover slips, needles and scalpels were sterilized by flaming. The loop wire and spatulas were sterilized by holding them on a Bunsen burner flame until became red. A hot air oven was used to sterilize test tubes, pipettes, flasks and forceps for one hour at 180°C. Steaming at 100°C was used for sterilizing sugars and media that could not be autoclaved. Disinfectants with 70% alcohol were used for the workplace.

The culture media were prepared following the manufacturers' instructions, then sterilized in the autoclave at 15 pounds per square inch for 15 minutes at 121°C or 115 °C for 20 minutes according to its nature (Barrow and Feltham, 1993).

2.5.2 Preparation of samples

All samples were prepared by taking 10 grams from he cheese samples; separately; and mixed well in previously prepared peptone water (100 ml) and incubated at 37°C for 18–24 hours.

2.5.3 Cultural methods

All samples that were prepared in the peptone water were cultured by streaking a full wire loop into MacConkey agar (HIMEDIA, M008–500G) and incubated aerobically for 24 hours at 37°C. Negative results were re–incubated for 48 hours before they were discarded as negative. Also, a full loop from the nutrient agar (S.d Fine Chem. Ltd 74056) cultures were re–cultured on MacConkey agar and incubated aerobically for 24 hours at 37°C. Again the negative results were re–incubated for a further 48 hours before they were discarded as negative.

2.5.4 Examination of cultures

After the incubation period, the characteristics of colonies were observed by the naked eye for growth and colonial morphology. Smears were made from all fermenting red, pink or non–fermenting colonies, then dried by air, fixed by heating, stained by Gram's stain and examined under the light microscope for cell morphology and staining reaction (Barrow and Feltham, 1993).

2.5.5 Purification of the isolates

Pure cultures were obtained by several sub–culturing from a single well–separated colony. The rustling growth was checked for purity by examining smears stained by Gram's methods, pure cultures were stored in the refrigerator and then subjected for different tests (Barrow and Feltham, 1993).

2.5.6 Identification of bacteria

After the purification, the isolates were identified according to Barrow and Feltham, 1993), based on reaction to Gram stain, the organism morphology on the selective media, growth condition and colony characteristics in the different media. Finally, the isolates were subjected to biochemical tests. The biochemical tests were performed according to the standard methods (Barrow and Feltham, 1993). For the identification of Salmonella and Shigella, a full loop of each sample was cultured by streaking on SSA medium (Hi Media M108) and incubated for 24 hours at 37° C. After incubation, the colonies were morphologically tested and then subjected to Gram stain and further identification, using biochemical tests, was conducted for the confirmation. Meanwhile, for isolation and identification of *E. coli*, a full loop of each sample was cultured by streaking on EMB agar medium (Conda Cat no. 1039) and incubated at 37° C for 24 hours. After examining the typical colonies (metallic sheen green colonies). The isolates were subjected to Gram stain examination and the identification tests were performed for the confirmation.

The biochemical confirmatory tests used include Oxidation fermentation (OF), Catalase, Oxidase, Motility, Indole, Methyl red (MR), Voges-Proskuer (V.P), Citrate, Urease. Also, Kligr iron agar (KIA) for detecting fermentation of glucose and lactose and H_2S production.

2.6 Data Analysis

The analysis was conducted using Statistical Package for Social Science, SPSS, version 16 (SPSS, 2008).

3 Results

A total of 42 samples, 19 samples of Mudaffara cheese obtained from producers and 23 samples from supermarkets in Khartoum Locality were tested for the presence of bacteria. The obtained data showed that the frequency of Gram-negative bacteria was significantly ($P \leq 0.05$) lower than that of Gram-positive bacteria (Table 1 and Figure 1).

Table 1: Frequency (%) of isolated bacteria from Mudaffara cheese in local markets in Khartoum.

Item	Source		X^2 P–value
	Supermarkets	Producers	
Gram stain			
Gram-positive (%)	56.5	89.5	P< 0.05
Gram-negative (%)	43.5	10.5	
Total (%)	100	100	

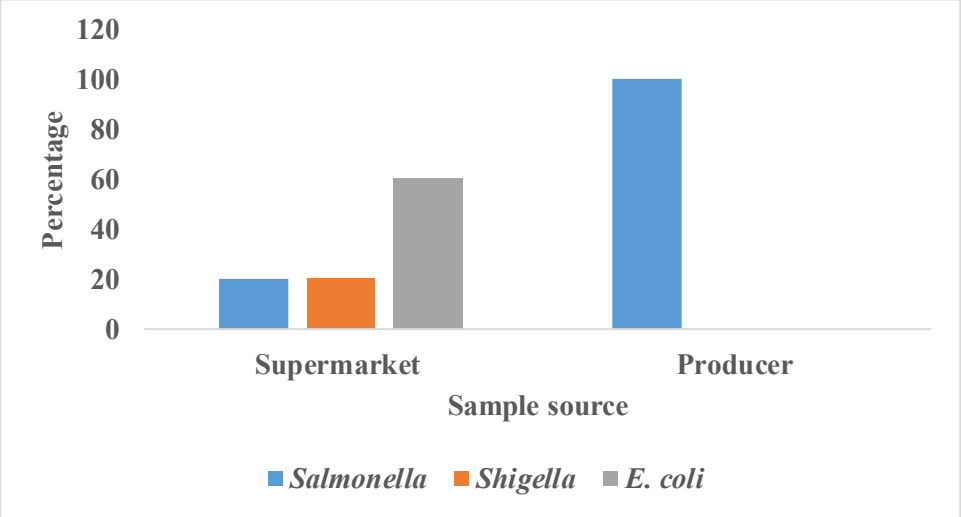

Figure 1: Frequency (%) of coliform bacteria isolated from Mudaffara cheese at local markets in Khartoum.

Table 2 shows the biochemical tests performed to confirm the coliform bacteria isolated from Mudaffara cheese at local markets in Khartoum.

Table 2: Biochemical tests used for the identification of coliform bacteria isolated from Mudaffara cheese at local markets in Khartoum.

Tests	*E. coli*	*Salmonella* spp.	*Shigella* spp.
Gram stain/ Shape	–ve Rod	–ve Rod	–ve Rod
Motility	+ve	+ve	–ve
Oxidase	–ve	–ve	–ve
Catalase	+ve	+ve	+ve
Urease	–ve	–ve	–ve
Citrate	–ve	–ve	–ve
Indole	+ve	+ve	+ve
H_2S production	+ve	+ve	–ve
OF	+ve	+ve	–ve
Gas production	+ve	+ve	–/ve
VP	–ve	–ve	–ve
MR	+ve	+ve	–ve

The results of coliform isolation showed that 12 (28.5%) samples were positive for isolation, two of them were found in the samples obtained from the producers and belonged to *Salmonella* spp. However, the remaining 10 (23.8%) samples were positive for the presence of *E. coli* (6, 50%), *Salmonella* spp. (2, 16.6%) and *Shigella* spp. (2, 16.6%) in the cheese obtained from supermarkets in Khartoum (Table 3).

Table 3: Source and type of coliform bacteria isolated from Mudaffara cheese from local markets in Khartoum.

Source	Type of isolated coliform bacteria		
	E. coli	*Salmonella* spp.	*Shigella* spp.
Supermarkets (n= 10)	6 (50%)	2 (16.6%)	2 (16.6%)
Producers (n= 2)	0	2 (16.6%)	0
Total	6 (50%)	4 (33.3%)	2 (16.6%)

4 Discussion

Significant (P≤0.05) variation was found in the occurrence of Gram-negative and Gram-positive bacteria in the cheese samples collected from producers and the retailers' supermarkets (Table 1 and Figure 1). Moreover, the high coliform bacteria in this study was obtained in cheese samples collected from the producers (Table 3 and Figure 1). Similarly, the traditional mudaffara cheese sold in Khartoum revealed a coliform count of log 2.0-2.80 cfu/g in 89% of the examined samples (Abdalla et al., 2013). The reason could be the low quality of the milk used in cheese making, poor processing conditions and/ or post-processing contamination (Nour El Diam and El Zubeir, 2006). Also, the cheese samples collected from different producers in rural areas of eastern Sudan showed that the level of hygiene and production methods, source of raw milk and its handling could be the main factors attributed to their high loads, which might affect the quality of cheese (Elkhider et al., 2011). Moreover, in Sudan, a high coliform count was reported in the imported and locally produced processed cheese (Hussein et al., 2011). Also, a high count of coliform bacteria was reported in the Sudanese white cheese that revealed 5.5×10 cfu/ml (Salih *et al*., 2012). However, Abdalla et al. (2012) found that the cheese samples kept in plastic lined with polyethylene bags containers were significantly (P≤0.05) lower in coliform compared to those packed into metal tins, plastic non-lined containers and metal gallons during the storage. The safety of cheese depends upon a variety of factors influencing the growth, survival, and inactivation of microorganisms, such as the microbiological quality of the raw milk, the rate and degree of acidification during production, and the water activity of the final product (Primavilla et al., 2023). Moreover, cheese like other types of food is exposed to contamination with microorganisms during the production process and white cheese may be subjected to spoilage by coliform (Ali, 2022).

This study showed that *E. coli* was detected in 6 samples of the cheese from the producers (Table 2 and 3). Moreover positive isolation of *E. coli* was reported in Sudanese white cheese (Warsama et al., 2006, Mohamed and El Zubeir, 2018). These results also agree with the study conducted in Brazil, which found that *E. coli* was detected in 50.0% of the cheese samples (Yamanaka et al., 2016). Moreover, in Egypt, fecal coliform in cheese samples revealed a range of 2.3×10^2 to 4.1×10^2 cfu/g and that the contamination with coliform and fecal *E. coli* indicates a need to improve efficient cleaning and sanitation programs in cheese chain processing (Lotfy et al., 2018). However, significant (P≤0.05) reduction of coliform bateria and *E. coli* were found during the storage period of Sudanese white cheese (El Owni and Hamid, 2008). Furthermore, *E. coli* was isolated from cheese in different countries. The *E. coli* O157:H7 was detected in the cheese samples obtained from traditional dairy factories in Egypt (Lotfy et al., 2018). *Escherichia coli* was found in 73% of the examined Indonesian traditional cheese (Hatta et al., 2013). The *E. coli* count was unsatisfactory in 12 out of 61 cheese samples in Italy (Primavilla et al., 2023). Also, pathogenic *E. coli* was found in 3.1% of the raw cheese samples examined in Portugal (Mendonça et al., 2024).

The isolation of *E. coli* from cheese obtained from the producers (Table 3 and Figure 1) should be considered a risk factor. One of the major significant of *E. coli* bacteria is its negative effect on both human and animal species in addition to its deteriorating the milk particularly raw milk and other milk products as a result of poor hygienic measures (Garbaj et al., 2016, Lara et al., 2016). Moreover, the workers and equipment of cheese processing might become risk factors for the contamination of cheese by *E. coli* as the cheese produced by the farmers potentially causes health problems for consumers if it is eaten raw (Hatta et al., 2013). The *E. coli* O157:H7 might survive and contaminate the final product (Bintsis and Papademas, 2002).

The prevalence of *Salmonella* spp. was detected in 4 cheese samples, the occurrence of *Salmonella* spp. in the cheese obtained from producers and retailers (Table 3 and Figure 1) could be attributed to the poor hygienic conditions during cheese production and marketing. Moreover, *Salmonella* spp. was detected in fresh Sudanese white cheese that was purchased from El Dueim City in White Nile State and then it disappeared after 60 days of storage (Abdalla et al., 2012). *Salmonella typhi* and *Salmonella paratyphi* were isolated from Sudanese white cheese (Warsama et al., 2006). Moreover, The isolated *Salmonella paratyphi* (6%) were equally identified as *paratyphi* A and *paratyphi* B in Sudanese white cheese collected from Khartoum State, Sudan (Yagoub et al., 2006). On the other hand, a high count (3.5×10 cfu/ml) for *Salmonella*

was found in the Sudanese white cheese (Salih et al., 2012). Food from animal origin, particularly milk and milk products is considered a primary source of human salmonellosis (Younis et al., 2018).

Similar to the present study, *Salmonella* spp. was found in some samples of types of cheese obtained from traditional dairy factories in Egypt (Lotfy et al., 2018). The presence of 14 (34.2%) isolates of *Salmonella* spp. in the cheese samples were reported in Iraq (Faja et al., 2023). Moreover, the samples of local fresh unsalted white cheese that containing different *Salmonella* genotypes could have originated from different contamination sources (Faja et al., 2023). Also, Salmonella was found positive in 25 grams in three cheeses samples of soft white cheese produced traditionally in Jordan (Haddad and Yamani, 2017). *Salmonella* spp. was found in 7% (2/30) of Dangke Indonesian traditional cheese (Hatta et al., 2013). Similarly, *Salmonella* spp. was detected in 15 out of 373 cheese samples collected from Italy (Primavilla et al., 2023). *Salmonella* spp. was detected in 1.0% of the raw cheese samples in Portugal (Mendonça et al., 2024) and in 6.3% of cheese samples in Brazil Yamanaka et al., 2016).

The contamination by *Shigella* in 2 samples of cheese obtained from supermarkets (Table 3 and Figure 1) could be due to unhygienic handling, cutting the cheese into small pieces and packing it into small retailer bags. Similarly, *Shigella* spp. were found in some samples of cheeses obtained from traditional dairy factories in Egypt (Lotfy et al., 2018). Moreover, Kareish cheese in Egypt was contaminated with *S. dysenteriae, S. flexneri, S. sonnei and S. boydii* (Abd-Elhamed and Thabet, 2020). The occurrence of *Shigella* spp. was higher in Kareish cheese (13%) obtained from markets than in raw cow milk (8%) obtained from dairy farms (Elkenany et al., 2022). Therefore, the presence of *Shigella* is a good indicator of the workers' hygiene. Moreover, foodborne outbreaks caused by *Shigella* mainly occur while foods are subjected to preparation or processing by hand, exposed to insufficient thermal processing and delivery or when served raw to the consumers (Younis et al., 2018). The reason could be that *Shigella* spp. can survive in acidic and salty conditions and thus be transmitted to the human host to cause diseases (Baker and The, 2018). Fresh vegetables, deli meats, and unpasteurized milk are more susceptible to contamination with *Shigella* spp., which represents the third most reported bacterial foodborne pathogen. Foods are mainly contaminated with these pathogens by infected food handlers with poor personal hygiene (Younis et al., 2018). *Shigella spp.*, as the causative agent of bacillary dysentery, has been involved in several foodborne and waterborne outbreaks (Kotloff et al., 2018).

The presence of *E. coli*, *Shigella* spp, *Salmonella* spp. in the present study could be due to their survival during ripening and storage of cheeses. The levels of coliform bacteria, *E. coli* in the traditional short and long-ripened cheeses made with raw and pasteurized cow's milk indicated insufficient hygiene procedures during the production and that the cheeses did not comply with the criteria for food safety and hygiene (Pyz-Łukasik et al., 2018). Hence, the virulent strains of Salmonella and Shigella species that have public health hazards should be overcome by proper hygienic measures during milking, handling and manufacturing of dairy products and effective training and education should be done targeting the farmers to minimize the occurrence of milk borne zoonosis (Younis et al., 2018). This is also because *Salmonella* and *Shigella* species revealed resistance to commonly prescribed antibiotics, which can be considered a real threat to children and the general community (Diriba et al., 2020). Also, consuming uncooked food and not washing hands were significantly associated with the prevalence of *Salmonella* and *Shigella* (Abera et al., 2021).

The main limitations of this study is the small sample size as few producers are located in Khartoum Locality. Also, the season is not considered during this study. The full bacterial identification could not be achieved due to Corona pandemic.

5 Conclusion

This study concluded that Mudaffara cheese, which is produced and offered for sale in the local markets in Khartoum might be a source of potential coliform pathogens such as *E. coli*, *Salmonella* spp, *Shigella* spp. that were detected in some of the investigated samples. Therefore, hygienic handling during milking, cheese processing, storage (packaging and preservation) and marketing should be improved and controlled. It is also necessary for all cheeses to be produced and marketed according to the standards and regulations to safeguard consumers. Therefore, awareness training programs are needed at producers and handlers of

the cheese. Also, regulation and motioning should be adopted in the country.

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