

Prevalence of Bacterial Contamination of Table Eggs from Retails Markets, Homes and Farms in Khartoum State, Sudan

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Abstract

This study was carried out to investigate and identify the bacteria associated with egg samples vended for sale in Khartoum State, Sudan. A total of 60 samples were collected randomly from Khartoum State (farms, retail markets and homes) twenty from each and delivered to the food microbiology lab to be tested. The contents of eggs were cultured for the bacteria and standard microbiological tests performed to identify the isolated organism. Experimental examination revealed that all samples of eggs collected from farms were sterile, 75% of samples collected from retail stores were contaminated and all samples of eggs collected from homes were highly contaminated. The isolates from retail stores showed that 66.7% were *Bacillus* and 33.3% were *Staphylococcus*. The isolates from homes egg showed that 60% were *Staphylococcus* and 40% were *Bacillus*. These findings highlighted and reflected the magnitude of bacterial contaminants of eggs in Khartoum state specially most of them have public health impact.

Keywords

Bacterial Contamination, Table Eggs, *Staphylococcus*, *Bacillus*

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1. Introduction

Eggs considered as a good mixture of nutrient, provide all the essential amino acid for human and supply several vitamins and minerals [1]. At the same time, many nutrient substances present in egg generate an excellent environment for the development of different microflora, including pathogenic bacteria [2]. Three types of poultry keeping are distinguished in Sudan: Household poultry, traditional open house poultry and modern intensive poultry keeping [3].

Freshly laid eggs are generally free from organisms. However, following exposure to environmental conditions for example, soil, faces and dirty nesting materials, eggs become contaminated with different types of bacteria [4].

Contamination of eggs with bacteria can affect egg quality, which may lead to spoilage and pathogen transmission. This may induce food borne infection or intoxication to consumers [5]. Furthermore, these bacteria may contaminate the egg contents either by penetration or withdrawal through pores of the shells [6].

In spite of the antibacterial factors, eggs can be infected with different bacteria such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Escherichia coli* and *Staphylococcus aureus*. Many investigations around the world reported the outbreak, contamination of egg by the *Salmonella* spp., *C. jejuni*, *L. monocytogenes* and *E. coli* [7, 8]. Good hygiene practices were rarely implemented in the retail points and homes in Khartoum State, Sudan. Therefore,

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the aim of the present study was: to isolate and identify different types of bacteria from eggs collected from farms, retail stores and homes in Khartoum State, Sudan.

2. Material and Methods

2.1. Collection of Sample

A total of sixty (60) samples were collected randomly from Khartoum state (farms, retail stores and homes) twenty from each. Fresh laid eggs approximately one day were collected from farms. Samples of eggs collected from retail stores and homes the period of storage was unknown. Every 4 eggs were placed in a sterile plastic bag and transferred to the laboratory without delay for microbiological examination.

2.2. Bacteriological Study of the Specimen

2.2.1. Microscopic Examination

Gram- stain: one colony was picked carefully with sterile loop, emulsified in a drop of normal saline, placed on a clean slide and spread evenly to make a thin film. The slide was allowed to dry in air, then the smear was fixed. The slide was flooded with 0.5% methyl violet for 3 min, rinsed with water. Lugol's iodine was applied for 1 min, then washed. The smear was decolorized with acetone for 10 seconds and counter-stained with dilute carbol fuchsin for 1 min, rinsed with water, blotted with filter paper and examined under oil immersion objective lens. Bacteria taking the red colour were recorded as gram-negative, while those taking violet colour were recorded as gram-positive organisms [9].

2.2.2. Motility Test

The hanging drop technique was applied to differentiate between motile and non-motile organisms [9].

A loop full of an 18 h broth cultures was transferred using a wire loop to a dry clean cover-slip, the edges of which were covered with a thin layer of paraffin wax. The cavity slide was pressed gently on the cover-slip and quickly inverted. The drop of culture that was hanging on the cavity center was examined under high power ($\times 40$) objective lens while the iris diaphragm was partially closed to avoid excessive illumination which render the bacteria invisible.

2.2.3. Spore Stain

It was done according to [10]. The fixed smear was flooded with strong carbol fuchsin and heated till the steam came out. The slide was washed after 5 min, with distilled water, decolorized with ethanol till no red colour came out and washed thoroughly with water. The smear was counter-stained with Loeffler's methyl blue for 1-2 min, washed and blotted to dry. Vegetative cell stained blue, while spores stained red.

2.3. Cultural Methods

Blood agar was used to isolate both gram negative and positive as well as Nutrient agar [10]. All the plates were incubated at 37°C for 24hrs.

2.4. Biochemical Tests

2.4.1. Coagulase test

The test was performed as described by [9]. To 0.5ml of 1:10 dilution of human plasma in saline, 0.1ml of 18-24 h old culture of the tested organism was added, then incubated at 37°C and examined after 6-24 h for coagulation. Definite clot formation indicated positive result.

2.4.2. Catalase Test

The test was carried out as described by [9]. A drop of 3% H₂O₂ was placed on clean slide and then colonies of tested culture on nutrient agar was picked by glass rod and added to the drop of 3% H₂O₂. A positive reaction was indicated by production of air bubbles.

2.4.3. Oxidase Test

The method of [9] was used. Strip of filter paper was soaked in 1% solution of tetramethyl-p-phenylenediamine dihydrochloride and dried in hot air oven and then placed on clean glass slide by sterile forceps. A fresh young tested culture on nutrient agar was picked off with sterile glass rod and rubbed on the filter paper. If a purple color developed within 5-10 seconds, the reaction was considered positive.

2.5. Experimental Examination

All eggs were candled to locate the air cell. Cracked eggs were rejected. ethanol (95%) was sprayed on to the shell to sterilize the surface. A small hole was drilled through the shell above the air cell.

Sterile wire loop used to take a sample from the egg white and cultured it in blood agar plates containing 10% defibrinated horse blood. Sterile wire loop used to take a sample from the egg yolk and cultured in the blood agar plates and incubated at 37°C for 24 hours. Well isolated colonies of different types of bacteria were subcultured on fresh nutrient agar and again for purification orange, yellow, large and small white colonies were examined microscopically.

3. Results and Discussion

3.1. Isolation of Bacteria from Eggs Collected from Farms in Khartoum State

Consumption of raw and under cooked eggs especially by

infants and babies in the Sudan stimulated the interest for microbial investigation of fresh eggs. Sixty samples were collected from farms, retail stores and homes, 20 from each. From farm fresh laid eggs approximately one day were collected. The egg yolk and egg white were cultured separately both are found to be sterile. Freshly laid eggs are generally free from organisms. However, following exposure to environmental conditions for example, soil, faces and dirty nesting materials, eggs become contaminated with different types of micro-organisms [11]. Table 1 shows isolation of bacteria from eggs collected from farms in Khartoum State. In farms the shade of the hens were cleaned every day. The cages where the eggs were laid were clean and eggs were collected immediately in the morning. This helps in avoiding the contamination with fecal matter from hens and reduce the chance for microorganisms to contaminate the surface of shell eggs and enter inside the egg white or egg yolk. Also eggs were collected in a proper way in clean baskets and distributed immediately to the markets in rakes made of cartoon.

Table 1. Isolation of bacteria from eggs collected farms.

No of Samples	Result
1	-
2	-
3	-
4	-
5	-
6	-
7	-
8	-
9	-

No of Samples	Result
10	-
11	-
12	-
13	-
14	-
15	-
16	-
17	-
18	-
19	-
20	-

-: negative sample

3.2. Bacterial Contamination of Eggs Collected from Retail Stores in Khartoum State

The samples that were collected from retail stores revealed that five samples showed no growth (sterile) while fifteen samples were found to be contaminated with different types of bacteria. Table 2 shows the classification and identification of bacteria isolated from eggs collected from retail stores. Figure 1 shows that 66.7% of the isolates were Bacillus and 33.3% of the isolates were Staphylococcus. High percentage of contamination of eggs collected from retail stores may be attributed to the fact that the eggs were stored under room temperature for many days until purchase. Thus improper way of storage give the chance for contaminants (air, dust and worker hands) to increase and multiply of microorganisms on the surface of the shell-egg and then enter inside the eggs.

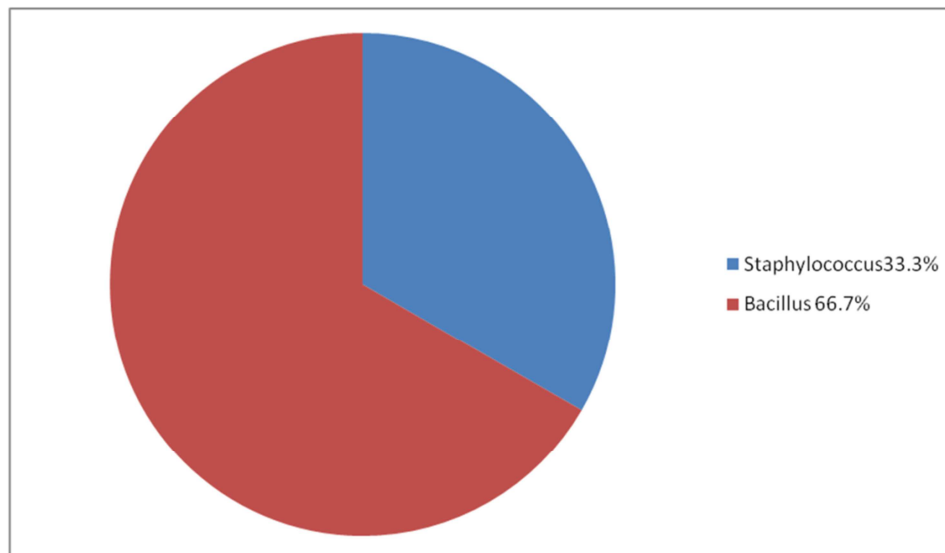


Figure 1. The percentage of Bacillus and Staphylococcus in eggs collected from retail stores.

Table 2. Isolation and Identification of bacteria from eggs collected from retail stores.

Sample No	Gram stain	Shape	Isolation from		Growth in air	Motility	Catalase	Coagulase	Oxidase	Spore
			Egg yolk	Egg white						
1	+	R	+	-	+	+	+	ND	+	+
2	+	R	+	-	+	+	+	ND	+	-

Sample No	Gram stain	Shape	Isolation from		Growth in air	Motility	Catalase	Coagulase	Oxidase	Spore
			Egg yolk	Egg white						
3	+	R	+	-	+	-	+	ND	-	-
4	+	R	+	-	+	-	+	ND	-	-
5	+	R	+	-	+	+	+	ND	-	-
6	+	R	+	-	+	+	+	ND	+	+
7	+	R	+	-	+	-	+	ND	+	+
8	+	S	+	-	+	ND	+	+	-	ND
9	+	R	+	-	+	+	+	ND	-	+
10	+	R	+	-	+	-	+	ND	-	+
11	+	S	+	-	+	ND	+	+	-	ND
12	+	S	+	-	+	ND	+	+	-	ND
13	+	S	+	-	+	ND	+	+	-	ND
14	+	S	+	-	+	ND	+	+	-	ND
15	+	R	+	-	+	+	+	ND	+	+

+: positive sample; -: negative sample; ND: not found; S: sphere; R: Rod

3.3. Isolation and Identification of Bacteria from Eggs Collected Homes in Khartoum State

Bacterial investigation of eggs collected from homes showed that all samples were highly contaminated. Table 3 shows the classification and identification of bacteria that isolated from eggs collected from homes. Figure 2 shows that 60% of the isolates were Staphylococcus and 40% of the isolates were Bacillus. In eggs collected from homes the bacterial growth were found to be in egg yolk and egg white. In spite of the natural microbial inhibitors that found in white such as lysozyme which decomposes the cell wall of certain gram – positive cocci and bacilli. This fact reflect that eggs collected

from homes were highly contaminated due to dirtiness of shades and cages where the eggs were laid, irregular collection of the eggs, improper storage of eggs and contamination of shell-egg by fecal matter from hens, hens feet or feather and droppings. Also feed of hens and poor handling of the eggs may be sources of contamination in accordance to [12, 13]. [3] reported very low prevalence (0.4%) of Staphylococcus aureus in poultry products in Khartoum State. Whereas [14] found a very high percentage of 33.33%. [15] stated that Salmonella spp. represented one of the important contaminants of poultry in Khartoum state, Sudan with prevalence of 10.4% (meat 11.8%; egg 5.8%).

Table 3. Isolation and identification of bacteria from eggs collected homes.

Sample No	Gram stain	shape	Isolation from		Growth in air	Motility	Catalase	Coagulase	Oxidase	Spore
			Egg yolk	Egg white						
1	+	S	+	+	+	ND	+	+	-	ND
2	+	S	+	+	+	ND	+	+	-	ND
3	+	S	+	+	+	ND	+	+	-	ND
4	+	S	+	+	+	ND	+	+	-	ND
5	+	R	+	+	+	-	+	ND	-	+
6	+	R	+	-	+	-	+	ND	-	+
7	+	R	+	-	+	+	+	ND	+	+
8	+	R	+	-	+	+	+	ND	+	+
9	+	R	+	-	+	+	+	ND	+	+
10	+	S	+	+	+	ND	+	+	-	ND
11	+	S	+	+	+	ND	+	+	-	ND
12	+	S	+	+	+	ND	+	+	-	ND
13	+	R	+	-	+	-	+	ND	+	-
14	+	R	+	+	+	-	+	ND	+	+
15	+	S	+	-	+	ND	+	+	-	ND
16	+	R	+	-	+	+	+	ND	-	+
17	+	S	+	-	+	ND	+	+	-	ND
18	+	S	+	-	+	ND	+	+	-	ND
19	+	S	+	+	+	ND	+	+	-	ND
20	+	S	+	+	+	ND	+	+	-	ND

+: positive sample; -: negative sample; ND: not found; S: sphere; R: Rod

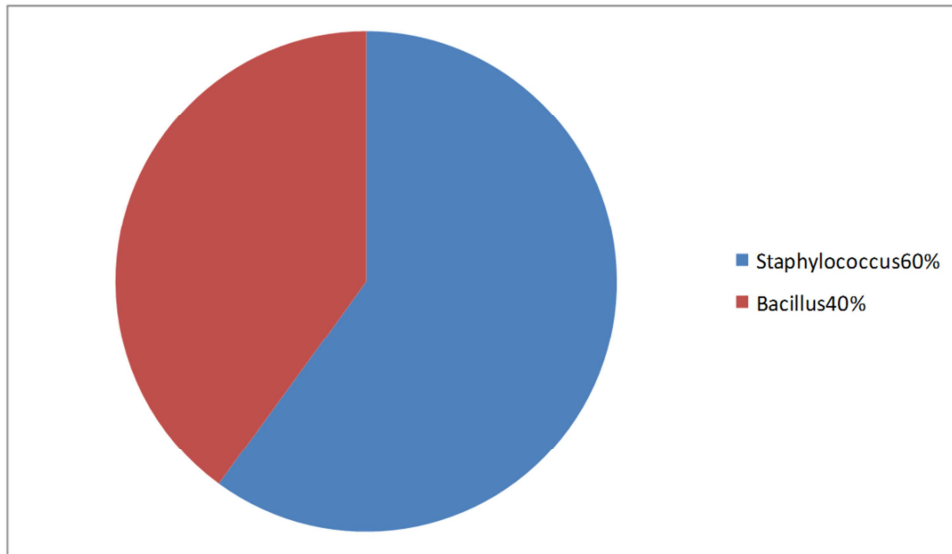


Figure 2. The percentage of Staphylococcus and Bacillus in eggs collected from homes.

3.4. Level of Contamination of Eggs Collected from Farms, Retail Stores and Homes in Khartoum State

Figure 3 shows the level of contamination of eggs collected from farms, retail stores and homes, 100% of eggs collected

from homes showed the growth of bacteria, the isolation include egg-white and egg-yolk, compared with retail stored eggs the contamination was very high in homes eggs. On the other hand 100% of the fresh laid eggs collected from farms showed no growth of bacteria in other words the eggs sterile when they are fresh.

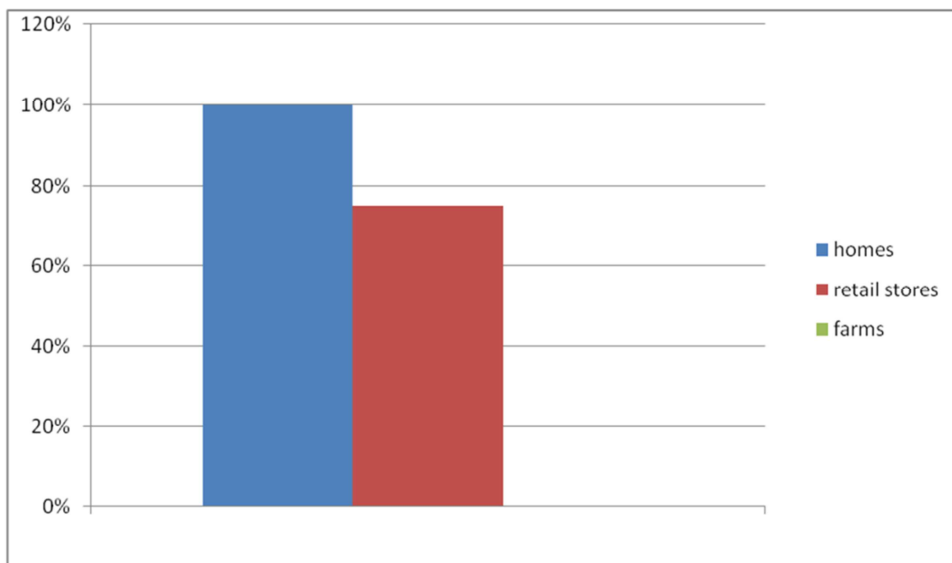


Figure 3. Level of contamination of eggs collected from farms, retail stores and homes.

4. Conclusion

From the above mentioned results it could be conclude that table eggs from homes and retail stores were highly contaminated with bacteria which render them unsafe for consumption than the farm eggs. The isolates from retail stores showed that 66.7% were Bacillus and 33.3% were Staphylococcus. The isolates from homes egg showed that 60% were Staphylococcus and 40% were Bacillus. It was

concluded that good hygiene practice and proper cleaning of the egg before storage and less storage timing can minimize the risk of contamination in eggs.

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