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ISOLATION AND IDENTIFICATION OF AEROBIC BACTERIA FROM FRESH AND STORED TABLE EGGS IN KHARTOUM STATE, SUDAN

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ABSTRACT

In this investigation which lasted in 6 months from January to June of the years 2016 and 2017, a total of 160 bacterial isolates were obtained from 77 egg samples collected from different localities of Khartoum State. According to the cultural characteristics, bacterial morphology and biochemical reactions results, the identified bacteria were: Staphylococci 40.0%, *Bacillus Cereus* 28.1%, *Escherichia coli* 24.4%, *Salmonella spp.* 5.0% and *Listeria monocytogenes* 2.5%. The isolated Staphylococci were identified as: *Staphylococcus aureus* 25.0%, *Staphylococcus epidermidis* 11.3%, *Staphylococcus xylosus* 3.7%. According to frequency of isolation, *Bacillus Cereus* came in second place to Staphylococci as causes of eggs contamination followed by *Escherichia coli*, *Salmonella spp.* and *Listeria monocytogenes*. Gram positive bacteria represented the predominant isolated bacteria (70.6%), compared to Gram negative bacteria (29.4%). Bacteria isolated from market egg samples represented 60 (37.5%) of the total

bacterial isolates while bacteria isolated from store egg samples represented 54 (33.8%) and bacteria isolated from farm egg samples represented 46 (28.7%).

KEYWORDS: *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus xylosus.*

I. INTRODUCTION

Foodborne disease is a major public health problem and the main cause of diarrhoeal diseases affecting all developed and developing countries.[1] The world consumption of poultry products, namely meat and eggs, is remarkably increasing with increase in number of people, and this is because of good quality and effective price. The wide spread of human consumption of poultry meat and eggs necessitates the control of microbial contamination. Chicken eggs are familiar, versatile, nutritious, economical and an easy to prepare food, as they provide a well balanced source of nutrients for man world-wide.[2][3] Moreover, their high quality protein, low caloric value and ease of digestibility make eggs value**.** Freshly laid eggs are generally devoid of organisms. However, following exposure to environmental conditions (for example, soil, dust and dirty nesting materials), eggs become contaminated with different types of microorganisms.[4] [5] Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdrawal through pores of the shells[6], and also through the transovarian route.[7] Other factors such as environmental temperature and humidity influence the bacterial penetration and thus, enhance the infection and spoilage.[8] More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus*, Salmonella, *Clostridium perfringens, Campylobacter, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus,* and Entero-pathogenic *Escherichia coli.* These bacteria are commonly found on many raw foods. Normally a large number of food-poisoning bacteria must be present to cause illness.[9]

This study was aiming to isolate and identify important bacteria encountered in egg shell and egg contents, determine which is safe the fresh or stored eggs and determine factors associated with egg contamination.

II. MATERIALS AND METHODS

Area of Study

This study was conducted out in Khartoum State during the years 2016 and 2017.

Source of samples

A total of 77 egg samples were collected from farms and stores in three localities of Khartoum State. 25 samples were collected from Omdurman, 26 from Khartoum locality and 26 from Bahri and East Nile locality (Table 1).

Sampling procedure

The surface of the intact egg was swabbed aseptically with sterile cotton swab. The swab was streaked onto the surface of the media. For culturing of bacteria from egg contents, eggs were first dipped in 75% ethanol for 5 min and allowed to air dry. The upper end of the egg were flamed for 5–10 sec and then holed with sterilized implement. The whole egg contents were mixed in sterile container and then cultured on the surface of the media. The swabs were cultured onto blood agar, MacConkey agar and nutrient agar. The inoculated plates were incubated aerobically at 37 C^{o}.

Preparation of culture

Samples were enriched in nutrient broth at 37°C for 24 hours.

Isolation, identification and characterization of bacterial isolates

All media (Oxoid media) were prepared and sterilized according to the manufacturer instructions. For the primary isolation of bacteria, a loop full of the enriched broth streaked onto blood agar, McConkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37oC for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar. Whereas, broth media were checked for turbidity, change in colour, accumulation of gases in CHO media and for sediment formation. One half colony from each plate was used for performing gram staining. Purification was based on the characteristics of colonial morphology and smear. This was obtained by sub culturing of a typical discrete colony on blood agar plate. Pure cultures were preserved on slants of blood agar and egg media at 4C^{o}.

Biological and biochemical identification

The purified isolates were identified as previously described[10] and[11] The identification include: Gram's reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, coaggulase test, oxidation

fermentation test, motility test, glucose breakdown test, fermentation of carbohydrates, urease activity, citrate utilization, gelatine hydrolysis test, nitrate reduction test).

III- RESULTS

Bacteria isolated from egg samples

In this investigation a total of 160 bacterial isolates were obtained from 77 egg samples collected from Khartoum State (Table 2). According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 3) the identified bacteria were: Staphylococci 40.0%, *Bacillus cereus* 28.1%, *Escherichia coli* 24.4%, *Salmonella spp.* 5.0% and *Listeria monocytogenes* 2.5%. The isolated staphylococci were: *Staphylococcus aureus* 25.0%, *Staphylococcus epidermidis* 11.3%, *Staphylococcus xylosus* 3.7% (Figure 1). Gram positive bacteria represented the predominant isolated bacteria (70.6%), compared to gram negative bacteria (29.4%) (Figure 2). Bacteria isolated from market egg samples represented 37.5% of the total bacterial isolates, bacteria isolated from store egg samples represented 33.8% and bacteria isolated from farm egg samples represented 28.7% (Table 4). Bacteria isolated from egg shells represented 90.6% of the total bacteria isolated from egg samples and egg contents bacteria represented 9.4% (Figure 3). Bacteria isolated from egg shells were: *Staphylococcus aureus* 21.9%, *Staphylococcus epidermidis* 11.2%, *Staphylococcus xylosus* 3.8%, *Bacillus Cereus* 24.4%, *Escherichia coli* 21.9%, *Salmonella spp.* 5.0% and *Listeria monocytogenes* 2.5%. Bacteria isolated from egg contents were: *Staphylococcus aureus* 3.1%, *Bacillus Cereus* 3.8% and *Escherichia coli* 2.5% (Table 5). The highest percentage of bacteria isolated from egg samples was in Bahri and East Nile localities 40.6% then Khartoum locality 34.4% and Omdurman locality 25.0% (Table 6).

Table (1): Source of egg samples.

Locality	Number of eggs
Khartoum	**26**
Omdurman	**25**
Bahri and east Nile	**26**
Total	**77**

Table (2): Types of bacteria isolated from egg samples collected from Khartoum State.

Isolated bacteria	Number	Percentage%
Bacillus cereus	45	28.1%
Staphylococcus aureus	40	25.0%
Escherichia coli	39	24.4%
Staphylococcus epidermidis	18	11.3%
Salmonella spp.	8	5.0%
Staphylococcus xylosus	6	3.7%
Listeria monocytogenes	4	2.5%
Total	160	100%

Table (3): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	*E. coli*	*Salmonella spp.*	*S. aureus*	*S. epidermidis*	*S. xylosus*	*Bacillus cereus*	*Listeria monocytogenes*
Aerobic growth	+	+	+	+	+	+	+
Colonies on MacConkey	Bright pink	Pink	Pink	Pink	Pink	Pink	Pink
Haemolysis on blood agar	+	-	+	-	-	+	+
Gram reaction	-	-	+	+	+	+	+
Shape	Rods	Rods	Cocci	Cocci	Cocci	Rods	Rods
Spore	-	-	-	-	-	+	-
Motility	+	+	-	-	-	+	+
Catalase	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	+	-
Indole	+	+	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+
VP	-	-	-	+	-	+	+
Citrate	-	-	-	-	-	+	-
O/F	+	+	+	+	+	-	+
Glucose	+	-	+	+	+	-	+
Lactose	+	-	+	+	+	+	-
Mannitol	+	+	+	-	+	+	-
Coaggulase	-	-	+	-	-	-	-

Table (4): Number and percentage of bacteria isolated from different sources of egg samples collected from Khartoum State.

Source	No. of samples	No. of bacterial isolates
Farm	25	46 (28.7%)
Store	27	54 (33.8%)
Market	25	60 (37.5%)
Total	77	160 (100%)

Table (5): Bacteria isolated from egg shells and contents collected from Khartoum State.

Bacteria	Egg contents' bacteria (No./ %)	Egg shells' bacteria (No./ %)
Bacillus cereus	6 (3.8%)	39 (24.4%)
Staphylococcus aureus	5 (3.1%)	35 (21.9%)
Escherichia coli	4 (2.5%)	35 (21.9%)
Staphylococcus epidermidis	-	18 (11.2%)
Salmonella spp.	-	8 (5.0%)
Staphylococcus xylosus	-	6 (3.8%)
Listeria monocytogenes	-	4 (2.5%)
Total	15 (9.4%)	145 (90.6%)

Table (6): Number and percentage of bacteria isolated from egg samples collected from different localities of Khartoum State.

Locality	No. of samples	Bacterial isolates (No./ %)
Khartoum	25	55 (34.4%)
Omdurman	26	40 (25.0%)
Bahri and east Nile	26	65 (40.6%)
Total	77	160 (100%)

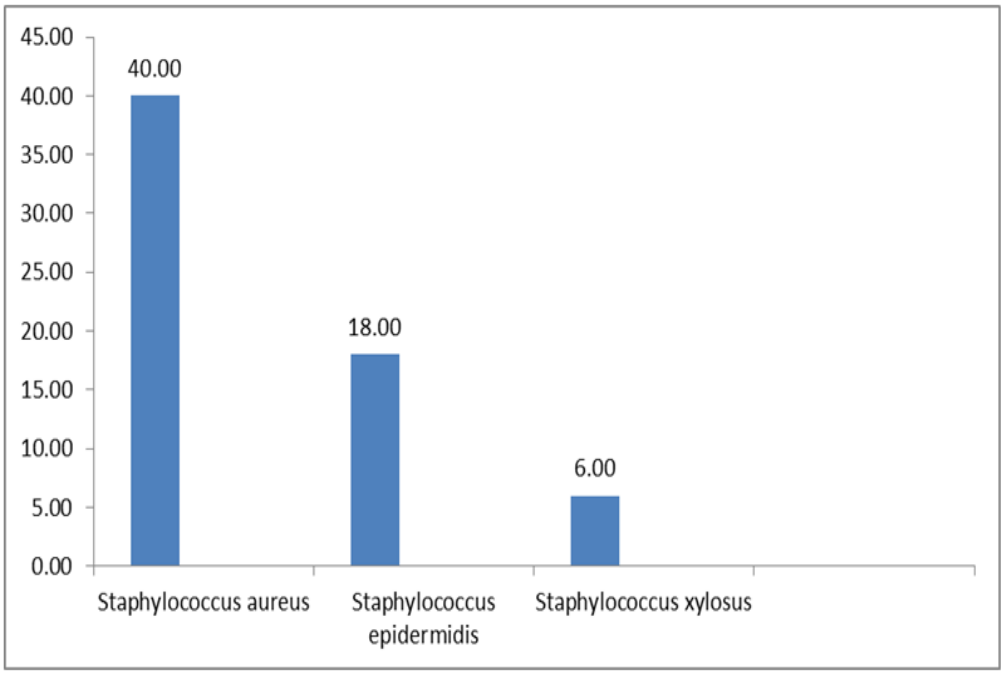

Fig. (1): Staphylococci isolated from egg samples collected from Khartoum State.

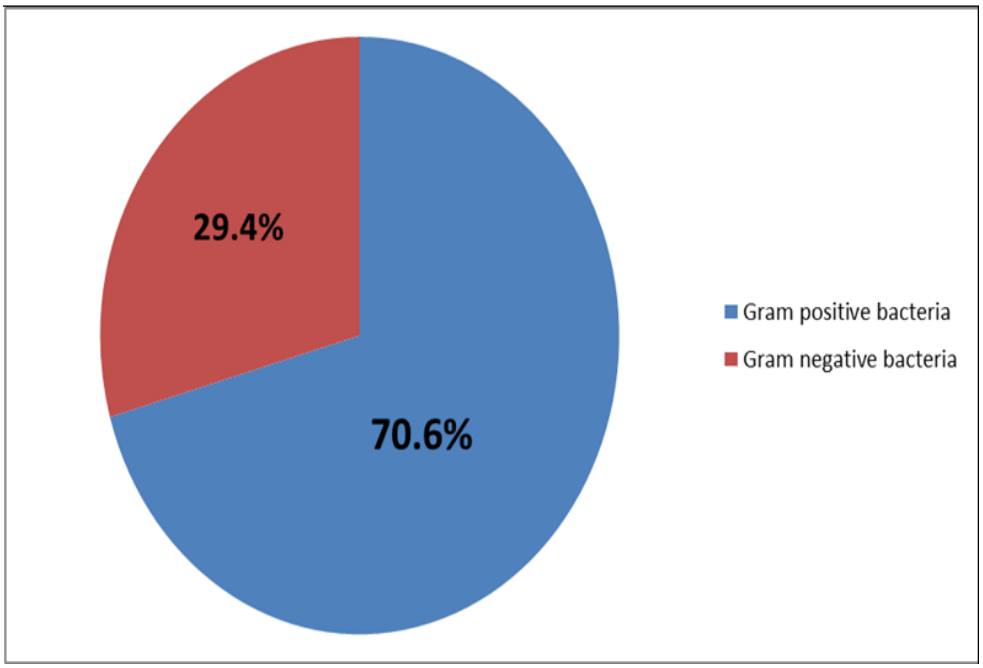

Fig. (2): Types of isolates from Khartoum State according to gram reaction.

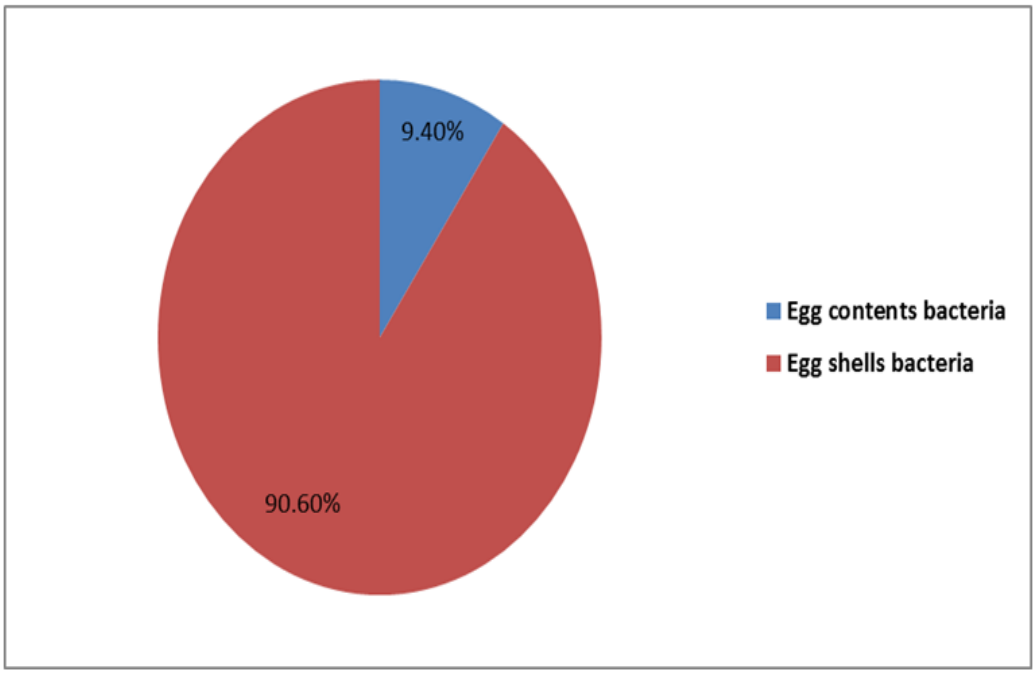

Fig. (3): Bacteria isolated from egg shells and contents collected from Khartoum State.

IV. DISCUSSION

The findings of this study is similar to Kbar and Anal[1], who reported that the most common food borne pathogens associated with food of animal origin are *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157.[12] found that the majority acute food poisoning was caused by bacteria such as Salmonella, Shigella, *Escherichia coli* and Campylobacter.[13] reported that eggs are considered to be a medium to low risk food for food borne illness which can become contaminated with bacteria, like *Salmonella* and other enteric pathogens. Staphylococci represented the highest percentage of bacterial isolates (40.0%). *Staphylococcus aureus* represented the highest percentage among staphylococci (25.0%). *S. aureus* has been demonstrated to be a common and wide spread food poisoning organism.[14] *Bacillus cereus* represented 28.1% of the total isolates.[15] reported that the diarrhoeal syndrome is caused by diarrhoeal toxins produced during growth *Bacillus cereus* in the small intestine. *Escherichia coli* represented 24.4% of the total isolates.[16] reported that the human disease syndromes resulting from ingestion of enteropathogenic *E. coli* have been divided into two main groups, the first enerotoxigenic groups responsible for infantile dirrhoeal disease and traveller's diarrhea, and the second invasive serotype responsible for invasive illness. *Salmonella spp.* represented 5% of isolated bacteria.[17] reported that the enteric fevers (including typhoid and paratyphoid fevers) is caused primarily by *S. typhimurium* and *S. enteritidis*; it occurs following ingestion of

contaminated or on fingers and other objects. *Listeria monocytogenes* represented 2.5% of isolated bacteria.[18] mentioned that Listeria is now recognized as an important food borne pathogen.[19] reported that bacterial contamination can happen at three main parts of egg (egg yolk, albumen and shell membrane/ egg shell). Bacteria isolated from egg shells represented 70.6% and from egg contents bacteria represented 29.4% of the total bacterial isolates. The findings is similar to[20] who mentioned that egg can be contaminated at both egg shell and egg contents by a variety of microbes with a wide range of pathogens such as *Campylobacter jejuni*, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica* and especially *salmonella spp*.[21] reported that Staphylococci are most common bacteria contaminating egg shells and microorganisms found in egg shells are capable of breaching the shell's microbial barriers. Also the findings agrees with[24] who reported that once the shell's microbial barriers have been broken, Gram negative bacteria are more capable of withstanding the antimicrobials present in the albumen. Therefore, the internal contaminants of eggs are commonly gram negative organisms such as *Alcaligenes* spp., *Achromobacter spp*., *Pseudomonas fluorescens*, *Salmonella* spp., and *Escherichia spp*.[25] reported that Contamination is more likely linked with cracked egg, dirty shells and storage in contaminated surroundings. It can be contaminated during formation and laying process. In this study Bacteria isolated from markets egg samples represented 37.5% of the total bacterial isolates, while bacteria isolated from stores egg samples represented 33.8% and bacteria isolated from farms egg samples represented 28.7%. These findings agrees with[26] who reported that stored or aged eggs have more possibility to become contaminated than fresh eggs due to the degradation of natural defense mechanisms in egg over time.[27] mentioned that the majority of eggs produced by healthy hens are thought to be clean at the time of lay, but eggs are contaminated to some extent when they come in contact with environmental debris and bacteria after being laid.[28] mentioned that environmental factors such as temperature, humidity, and the presence of CO2 are also of prime importance to maintain of egg quality. However, eggs remained fairly acceptable sensorial up to 10 days of storage at ambient condition. And naturally occurring microorganisms on the egg shell surface and in egg contents got markedly increased during storage.[29] found that microbial contamination of table eggs in the process of production, handling and marketing has been therefore, of a major public health concern.

V. CONCLUSION

In this investigation a total of 160 bacterial isolates were obtained from 77 egg samples collected from different localities of Khartoum State. The recovered bacteria were: Staphylococci 40.0%, *Bacillus Cereus* 28.1%, *Escherichia coli* 24.4%, *Salmonella spp.* 5.0% and *Listeria monocytogenes* 2.5%. The isolated staphylococci were: *Staphylococcus aureus* 25.0%, *Staphylococcus epidermidis* 11.3%, *Staphylococcus xylosus* 3.7%. Gram positive bacteria represented the predominant isolated bacteria (70.6%), compared to gram negative bacteria (29.4%) in all localities of Khartoum state. Staphylococci represented the highest percentage of bacterial isolates (40.0%) of the total bacterial isolates from egg samples in Khartoum state. followed by *Bacillus cereus, Escherichia coli, Salmonella spp.* and *Listeria monocytogenes*. Bacteria isolated from egg shells was greater in number than that from egg contents. Bacteria isolated from market egg samples represented 60 (37.5%) of the total bacterial isolates while bacteria isolated from store egg samples represented 54 (33.8%) and bacteria isolated from farm egg samples represented 46 (28.7%).

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