



AEROBIC BACTERIA ISOLATED FROM FRESH AND FROZEN CHICKEN'S CARCASSES IN KHARTOUM STATE, SUDAN

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ABSTRACT

In this investigation which lasted in 6 months of the year 2018, a total of 30 bacterial isolates were obtained from swabs of 30 fresh chickens' carcasses. According to cultural characteristic and biochemical tests, the identified bacteria were 10 *Staphylococcus* spp. (33.3%), 9 *Salmonella* spp. (30.0%), 6 *Escherichia coli* (20.0%), 3 *Klebsiella pneumoniae* (10.0%) and 2 *Listeria monocytogenes* (6.7%). Staphylococci isolated were: 6 *S. aureus* (20.0%), 2 *S. epidermidis* (6.7%) and 2 *S. xylosum* (6.7%). Gram negative bacteria represented the predominant bacteria isolated from fresh chickens' carcasses (60.0%), compared to gram positive bacteria (40.0%). In this investigation a total of 30 bacterial isolates were obtained from 26 frozen chickens' carcasses' swabs. The identified bacteria were: 10 *Escherichia coli*

(38.4%), 10 Staphylococci (38.4%), 3 *Salmonella* spp. (11.5%), 7 *Klebsiella pneumoniae* (7.8%) and 1 *Listeria monocytogenes* (3.8%). Staphylococci isolated were: 8 *S. aureus* (30.6%) and 2 *S. epidermidis* (7.8%). Gram negative bacteria represented the predominant bacteria isolated from frozen chickens' carcasses (57.8%), compared to gram positive bacteria (42.2%). The study revealed that Chickens' carcasses are more susceptible for contamination during processing than storage.

I. INTRODUCTION

Contaminated poultry products are among the most important sources of food-borne outbreaks in humans. Microbial contaminants are reported more often from poultry and poultry products compared with other animal species.^[1] Food-borne diseases associated with the consumption of poultry meat and its processed products are the cause of big public health problem worldwide.^[2] Broiler meat consumption acts as one of the main sources of food-borne illness in humans.^[3] The most frequent source of primary micro flora of poultry meat are skin and feathers, in addition to respiratory system and intestinal tract, therefore, type of microorganism of processed poultry carcasses mostly depends on a healthy condition and external micro flora of a bird and the hygienic conditions during slaughtering and processing.^[4] A significant number of poultry meat produced from the birds slaughtered in traditional sector where chickens are being slaughtered and dressed in contaminated condition, ends to significant contamination rates of market chicken products, and the majority of the consumers purchase meat from this sector.^[5] Although processing is generally decreases microbial contamination of broiler's carcass, cross-contamination between carcasses, processing water, and tools may actually increase the level of carcass contamination during some processing steps.^[6]

Bacterial contamination of broiler meat is mainly found on the skin and/ or visceral cavity which occurs during washing, plucking and evisceration processing.^[7] isolated *E. coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus warnerii* and *Staphylococcus sciuri*, *Kocuria kristinae* and *Lactococcus garvieae* from frozen carcasses in Khartoum State. Poultry meat can easily contaminated with microorganisms, thus modern processing needs high rate of through put to meat consumers demand. However, many healthy broilers entering poultry slaughterhouse might be highly contaminated with microorganisms and act as healthy carriers of these microorganisms They might be carrying food-borne diseases or deteriorating microorganisms such as *Salmonella* species, *Campylobacter* species, *Clostridium perfringes*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and other bacteria.^[8] Heavy microbial contamination decreases the shelf life of carcasses; furthermore some of these contaminants may be potential or harmful pathogens to the consumers.^[9] Skin of carcasses can be analyzed in order to indicate the microbial type, the level of hygiene in production and handling and the correct maintenance of cold food chain.^[10] The quality of poultry meat during slaughtering, packaging, hygienic status of slaughter house and monitoring of all steps of process aiming the food safety of final

product (HACCP) in poultry industry is extremely important and safety program to serve both internal and external market.^{[11][12]}

This study was aiming to determine the source of broiler chickens' carcass bacterial contamination.

II. MATERIALS AND METHODS

Area of Study

This study was conducted out in Khartoum North Khartoum state during the year 2018. The study covered 6 broilers farms including open and closed breeding systems.

Source of samples

In this study, 60 swabs were collected from fresh and frozen broilers' carcasses. Samples were collected from Khartoum North of Khartoum State.

Sampling procedure

Swabs from the skin of the broilers' carcasses were collected using sterile swabs then placed into sterile container and then in ice box containing ice and transported to the laboratory. The sample preserved in a deep-freezer at -20°C until analysed. On the next day samples were removed from the deep freezer and left on the bench to thaw. Samples were then subjected to bacteriological analysis (isolation, identification and characterization of the isolated bacteria).

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 37°C 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 4°C.

Biological and biochemical identification

The purified isolates were identified as previously described.^[13] and^[14] 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, coagulase 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 chickens' carcasses

In this investigation a total of 56 bacterial isolates were obtained from 60 chickens' carcasses' swabs. According to cultural characteristic and biochemical tests (Table 1), the identified bacteria were 20 Staphylococci (35.7%), 16 *Escherichia coli* (28.6%). 12 *Salmonella* spp. (21.4%), 5 *Klebsiella pneumonia* (8.9%) and 3 *Listeria monocytogenes* (5.4%) (Figure 1). The isolated Staphylococci were: 14 *S. aureus* (25.0%), 4 *S. epidermidis* (7.1%) and 2 *S. xylosus* (3.6%) (Figure 2). Gram negative bacteria represented the predominant bacteria isolated from frozen chickens' carcasses (57.8%), compared to gram positive bacteria (42.2%).

Bacteria isolated from fresh chickens' carcasses

In this investigation which lasted in 6 months of the year 2018, a total of 30 bacterial isolates were obtained from swabs of 30 fresh chickens' carcasses. According to cultural characteristic and biochemical tests (Table 1), the identified bacteria were 10 *Staphylococcus* spp. (33.3%), 9 *Salmonella* spp. (30.0%), 6 *Escherichia coli* (20.0%), 3 *Klebsiella pneumoniae* (10.0%) and 2 *Listeria monocytogenes* (6.7%) (Figure 3). Staphylococci isolated were: 6 *S. aureus* (20.0%), 2 *S. epidermidis* (6.7%) and 2 *S. xylosus* (6.7%). Gram negative bacteria represented the predominant bacteria isolated from fresh chickens' carcasses (60.0%), compared to gram positive bacteria (40.0%).

Bacteria isolated from frozen chickens' carcasses

In this investigation a total of 30 bacterial isolates were obtained from 26 frozen chickens' carcasses' swabs. According to cultural characteristics and biochemical tests (Table 1), the identified bacteria were: 10 *Escherichia coli* (38.4%), 10 Staphylococci (38.4%), 3 *Salmonella* spp. (11.5%), 7 *Klebsiella pneumonia* (7.8%) and 1 *Listeria monocytogenes* (3.8%) (Figure 4). Staphylococci isolated were: 8 *S. aureus* (30.6%) and 2 *S. epidermidis* (7.8%).

Gram negative bacteria represented the predominant bacteria isolated from frozen chickens' carcasses (57.8%), compared to gram positive bacteria (42.2%).

Table (1): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	<i>E. coli</i>	<i>Salmonella spp.</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. xyloso</i>	<i>Klebsiella pneumoniae</i>	<i>Listeria monocytogenes</i>
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	+	+	+	-	+	+	-
Coagulase	-	-	+	-	-	-	-

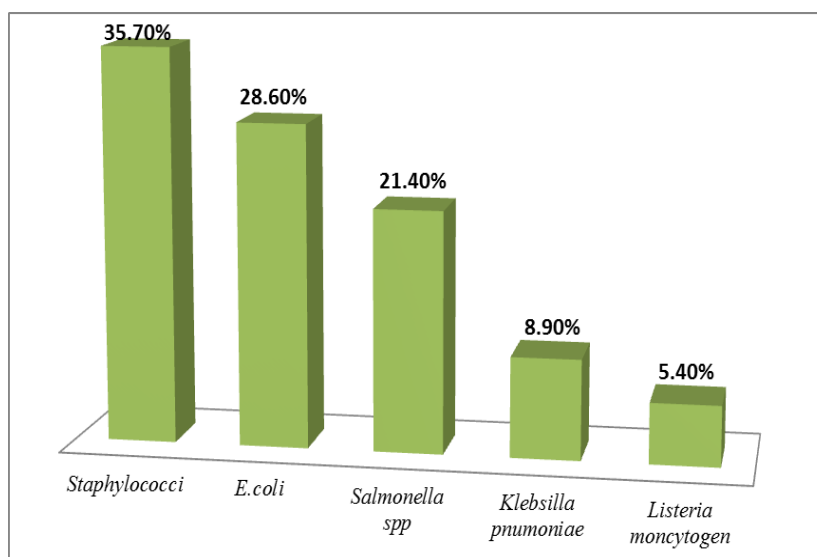


Fig. (1): Bacteria isolated from chickens' carcasses.

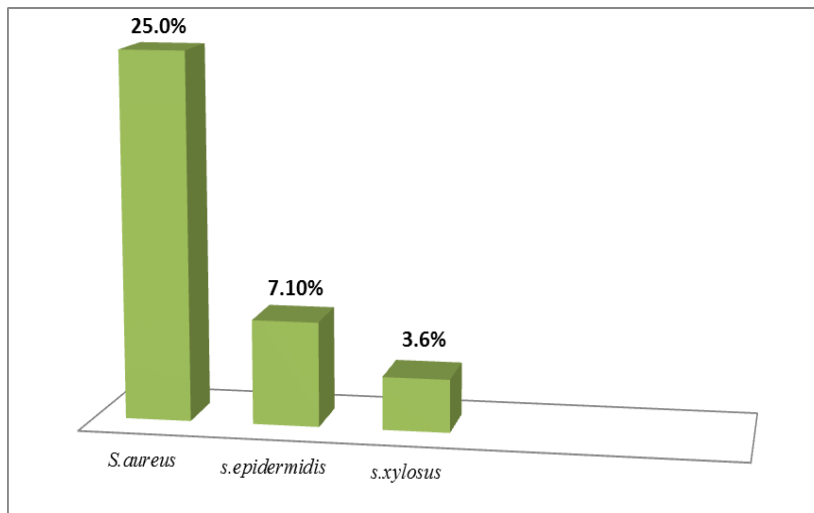


Fig. (2): Staphylococci isolated from chickens' carcasses.

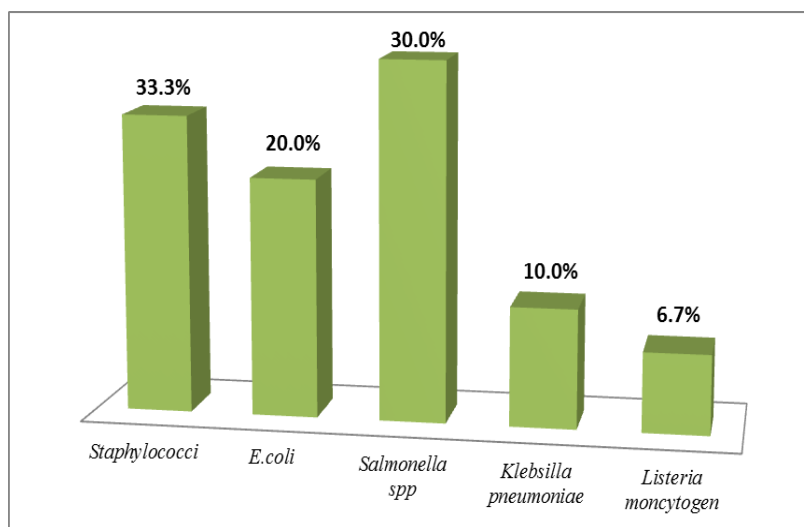


Fig. (3): Bacteria isolated from fresh chickens' carcasses.

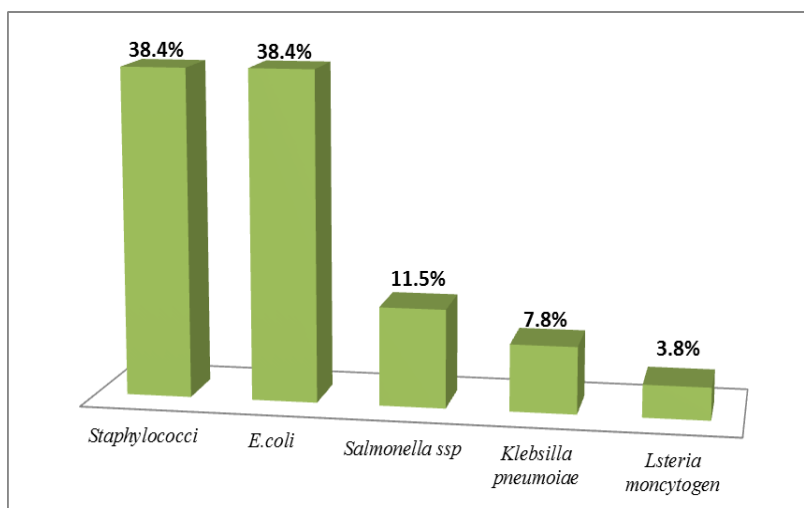


Fig. (4): Bacteria isolated from frozen chickens' carcasses.

IV. DISCUSSION

Poultry meat can be contaminated with a variety of microorganisms. While heavy contamination shortens the shelf- life of carcasses, some of these contaminants may be harmful or potential pathogens to the consumers. Microbial contamination of poultry carcasses is a natural result of different procedures necessary to produce retailed products from living birds. Most of bacterial contaminants are non-pathogenic; however, poultry are known to harbour a large number of bacteria that are pathogenic to human being.^[15] In this investigation a total of 56 bacterial isolates were obtained from 60 chickens' carcasses' swabs. According to cultural characteristic and biochemical tests, the identified bacteria were 20 Staphylococci (35.7%, 16 *Escherichia coli* (28.6%), 12 *Salmonella* spp. (21.4%), 5 *Klebsiella pneumonia* (8.9%) and 3 *Listeria monocytogenes* (5.4%). The isolated Staphylococci were: 14 *S. aureus* (25.0%), 4 *S. epidermidis* (7.1%) and 2 *S. xylosus* (3.6%). Gram negative bacteria represented the predominant bacteria isolated from frozen chickens' carcasses (57.8%), compared to gram positive bacteria (42.2%).^[16] isolated *E. coli*, *Staphylococcus aureus*, *Salmonella* spp., *L. monocytogenes* and *klebsiella* spp. from broilers' meat.^[17] reported that *S. aureus* represented 62% of the total bacteria isolated from frozen chickens' carcasses. In this study Staphylococcus spp. represented 35.7% from fresh carcasses and 30.6% from frozen ones of the total bacteria isolated. This result was in line with^[15] who reported that Staphylococcus spp. are the dominant bacteria isolated from broiler chickens. The enterotoxin produced by Staphylococcus spp. at favorable temperature is the common cause of human foodborne illnesses throughout the world.^[18] The percentage of isolated Staphylococcus spp. in this study, is considered low compared with the percentage of 90.63% reported by^[19] in chicken meat in Bangladesh. Staphylococci spp. was not recovered from chicken meat in study done by.^[8] ^{[20][21]} reported low percentages of *S. aureus* 1.75% and 8.6% contrary in Sudan. In this study *S. epidermidis* represented 7.8% of the total bacteria. ^[7] reported that *S. epidermidis* represented 4.9% of the total bacterial isolates. In this study *E. coli* represented 28.6% of the total isolates.^{[21][22][20]}, isolated *E. coli* in low percentage (2.0%), (1.0%) and (5.26%) respectively from chickens' carcasses. Higher percentage (40%) and (65%) was obtained by^[23] and^[7] respectively. Previous studies in Sudan^[20] and^[24] reported the isolation of *E. coli* with the percentage of 63%. The presence of salmonellae in chicken meat may be attributed to the health status of the living bird which carries salmonellae, Bad hygienic conditions during slaughtering, cross contamination either from other birds, instruments, machines, workers, scalding tanks, defeathering machines, crop removal, manual evisceration, during slaughter and intestinal contents can spill and

contaminate the muscle and organs of the chicken, which is the important source of *Salmonella* in meat and chilling tanks.^[25] In this study *Salmonella* spp. represented 21.4% of the total isolates. This result was in different line with the finding of^[26] who reported the percentage of 1.6%.^[27] reported the percentage of 2.08%,^[20] reported the percentage of 5.26%,^[27] reported the percentage of 12.3%,^[28] reported the percentage of (1.1%) and^[30] reported the percentage of 7.46%. Higher percentages of *Salmonella* spp. were reported by^[29] (57%).^[31] reported the percentage of 62.79%,^[32] reported the percentage of 46.2% and^[33] reported the percentage of 67.5%. In this study *Klebsiella pneumonia* was isolated from fresh carcass and could be originated from the intestinal content of birds being processed according to.^[11] In this study *Listeria monocytogenes* represented 5.4%. (6.7% from fresh carcasses and 3.8% from frozen ones).^[30] and^[28] reported the percentage of 3.0% and^[30] reported the percentage of 4.5 %. Lower percentage (0.5%) was reported by^[26].^[34] isolated *Listeria spp.* (27.8%) from fresh chicken samples.

V. CONCLUSION

This study revealed that many bacteria contaminated poultry carcasses and that may contribute to food borne diseases. The identified bacteria from chickens' carcasses were *Staphylococci*, *Escherichia coli*, *Salmonella* spp., *Klebsiella pneumonia* and *Listeria monocytogenes*. Gram negative bacteria represented the predominant bacteria isolated from chickens' carcasses (58.9%), compared to gram positive bacteria (40.1%). Almost the same bacterial species were isolated from fresh and frozen carcasses. Chickens' carcasses are more susceptible for contamination during processing than storage.

VI. ACKNOWLEDGEMENT

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