

Isolation and Diagnosis of Pathogenic Bacteria from the Bowels of Local Chicken and Identifying Some of the Virulence Factors

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Abstract

This study was conducted to the isolation and identification of bacteria from chicken intestine and livers in Mosul city. A total of 35 samples from intestine and 35 liver samples from local chicken were collected during a period from September 2018 to March 2019, the bacteria were diagnosed according to morphological, cultural and biochemical characteristics. The results showed (100%) positive to bacterial isolation for each samples of intestine and liver, (12) types of bacteria from (71) isolates for intestine, while (10) types from (36) isolates for liver. *E. coli* formed the highest percentage of intestinal isolates (28.16%), while *Corynebacterium spp* formed the highest percentage in liver isolates (33.33%). The bacterial types were isolated from intestine included: *E. coli* (28.16%), *Corynebacterium spp* (25.35%), *Enterococcus faecalis* (15.49%), *Klebsiella pneumonia* (8.45%), *Bacillus spp* (7.04%), *Proteus spp* (5.63%), *Staphylococcus aureus* (4.22%), *Lactobacillus spp* (2.81%), *Arcanobacterium pyogenes* (1.4%), *Citrobacter spp* (1.4%). The bacterial types were isolated from liver included: *Corynebacterium spp* (33.33%), *E.coli* (19.44%), *Staphylococcus aureus* (16.6%), *Bacillus spp* (11.11%), *Enterococcus faecalis* (5.55%), *Klebsiella pneumonia* (5.55%), *Pseudomonas aeruginosa* (2.77%), *Listeria monocytogenes* (2.77%), *Arcanobacterium pyogenes* (2.77%). The bacterial types isolated from both intestine and liver were, *E.coli*, *Corynebacterium spp*, *Staphylococcus aureus*, *Bacillus spp*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Proteus spp* and *Arcanobacterium pyogenes*. The virulence factors tests were used for some liver isolates which included, protease, lecithinase, lipase, urease, coagulase and haemolysin.

Keywords: Isolation, Diagnosis, Bacteria, Chicken guts, Virulence factors

عزل وتشخيص الجراثيم المرضية من احشاء الدجاج المحلي وتحديد بعض عوامل الضراوة

الخلاصة

تضمنت الدراسة الحالية عزل وتشخيص الجراثيم من امعاء وكبد الدجاج المحلي في مدينة الموصل , تم جمع 35 عينة امعاء و35 عينة كبد من الدجاج المحلي المذبوح حديثا في مدينة الموصل وذلك للفترة من ايلول 2018 ولغاية اذار 2019. شخّصت الجراثيم المعزولة اعتمادا على الصفات الشكلية والزرعية والكيموحيوية, أظهرت النتائج (100%) إيجابية للعزلة الجرثومية لكل من عينات الأمعاء والكبد , (12) نوعا بكتيريا من (71) عزلة من الأمعاء , بينما (10) أنواع من (36) عزلة للكبد. شكلت جراثيم الايشيريكيا القولونية *E.coli* النسبة الاعلى من عزلات الامعاء (28.16%)، بينما شكلت جراثيم الوتديات *Corynebacterium spp* اعلى نسبة في عزلات الكبد (33.33%). تضمنت الانواع الجرثومية المعزولة من الامعاء *E.coli*: بنسبة (28.16 %)، *Corynebacterium spp* بنسبة (25.35%)، السبحيات المعوية *Enterococcus faecalis* (15.49%)، *Klebsiella pneumonia* (8.45%)، العصيات *Bacillus spp* (7.04%)، المتقلبات *Proteus spp* (5.63%)، المكورات العنقودية *Staphylococcus aureus* (4.22%)، العصيات اللبنية *Lactobacillus spp* (2.81%)، *Citrobacter spp* (1.4%)، *Arcanobacterium pyogenes* (1.4%). أما الانواع الجرثومية التي عزلت من الكبد فقد تضمنت: *Corynebacterium spp* (33.33%)، *E.coli* (19.44%)، *Staphylococcus aureus* (16.6%)، *Bacillus spp* (11.11%)، *Enterococcus faecalis* (5.55%)، *Klebsiella pneumonia* (5.55%)، *Pseudomonas aeruginosa* (2.77%)، *Listeria monocytogenes* (2.77%)، *Arcanobacterium pyogenes* (2.77%). وقد ظهرت العديد من العزلات المشتركة من العينات المأخوذة من الامعاء مع العزلات المأخوذة من عينات الكبد والتي شملت جراثيم *E.coli*, *Corynebacterium spp*, *Bacillus spp*, *Enterococcus faecalis* و بكتيريا *Arcanobacterium pyogenes*. تم اجراء اختبارات فحص الضراوة لقسم من الجراثيم المرضية المعزولة من الكبد والتي تضمنت فحص البروتينيز، الليستينيز، اللايباز، الكوايوليز اضافة الى الحال الدموي

Introduction

Chicken meat is one of the most consumed types of meat at the present time, because of its high nutritional value of proteins, amino acids and vitamins which are necessary for humans to contain a few calories, and it considered good meat consumed by individuals who want to determine their weight and avoid obesity, as well as it is easy to digest and is suitable for all ages (1).

Uninfected chicken meat is free from bacteria, but contamination occurs quickly during slaughtering, processing and marketing due to lack of attention to the health side or the result of an external sources as contaminated knives, workers' hands or from the ground (2).

Poultry meat is one of the important sources which is responsible for infecting humans with many bacteria, including *Salmonella*, *Staphylococcus* and *Escherichia coli*, because of the high rate of contamination with infected chicken feces during the operations of slaughtering in slaughterhouses or butchers shops (3,4,5,6). The normal flora which is found in chicken's intestines and skin have the ability to analyze the chicken protein due to its enzymatic activity, which is activated after slaughter when the pH of the chicken protein becomes 6.2 -6.4. As a result of inappropriate conditions and other reasons due to bacterial infections, opportunistic bacteria were activated and biological processes are affected within the body, intestines and liver are the most affected organs where the liver performs a variety functions in digestive system and the metabolism of proteins, lipid and carbohydrates. Bacteria in chicken intestine play important role in pollution of the environment through mixing of the intestinal content with meat during unhealthy slaughters, these bacteria are transfer to humans and lead to intestinal infections by toxins which produced (7). The risk of bacterial pathogens represented in two important things: the first concerns with public

health, the most cases of intestinal poisoning in humans due to *Staphylococcus aureus*, *E. coli*, *Salmonella spp* and *Enterococcus faecalis* which is produced intestinal toxins (8,9,10) and other bacterial causes, due to poultry meat consumption (11). The second is the economic importance of poultry diseases, which is a wide world problem, and causes significant economic losses due to a decrease in the rate of fertilization and hatching in flocks, maternal weight loss, reduced egg production and carcass contamination in the slaughterhouse (12).

The aim of this study to identify some of the pathogenic bacteria present in the internal organs of newly slaughtered chickens in slaughter shops located in different areas of Mosul city.

Materials and methods

1- Samples collection

Seventy samples of the internal guts from newly slaughtered chickens were collected from different areas in Mosul city, in a period from September 2018 to March 2019. (35) samples for both intestine and liver. The samples were transported with sterile glass containers under cooling conditions to the microbiology laboratory at the College of Veterinary Medicine, Mosul University for the purpose of laboratory tests and diagnosis.

2- Samples culturing

A piece of samples was taken from the internal organs, which included both the intestines and the liver, with a length of 1 cm from the small intestine, and a small piece of liver weighing 10 g. The growth models were placed individually in Nutrient broth and incubated at 37 °c for 24 h. A loop inoculation of bacterial growth was transplanted and cultured on culture media from Himedia India company, included Blood agar, MacConkey agar, Nutrient agar. The petri dishes were placed in the incubator at 37°C for 24 hours. the pure and single colonies were selected to

know the staining reactions in Gram's stain and then grown on selective media which included Mannitol salt agar, Eosin methylene blue agar, Edward agar. Salmonella and Shigella agar, Blood agar with potassium tolerate and MacConkey agar.

3- Microscopic examinations

Microscopic examinations were carried out on the isolates for studying the shape, arrangement and reaction of the staining, which included Gram's stain, capsule stain, and the motility test of the isolated bacteria after subculture on the medium of the Nutrient broth at 37°C for 4 hours (13).

4- Biochemical tests

Biochemical tests were applied on bacterial isolates, which included Catalase, Oxidase, Indole production, Methyl red (MR), Voges-proskauer (VP), Hydrogen sulphide production (H₂S), Urease and Fermentation of sugars, including glucose, xylose, ramnose and mannitol (14).

5-Virulence factors tests

Some virulence factors of the isolated bacteria were detected by using test for lecithinase, protease, lipase, coagulase, and blood hemolysis (15).

Results and Discussion

1-Bacterial isolation

Through bacteriological examination of 70 samples, were taken from the local chicken intestine and liver in Mosul city. (71) and (36) bacterial isolates were obtained from the intestine and liver respectively, as shown in Table (1).

The study showed that *E. coli* bacteria formed the highest percentage of intestinal isolates (28.16%). These bacteria are part of the normal flora of the intestine as they are shedding with feces and

contaminate the environment therefor, they cause many infections to other birds and humans (7,9,10,16), while *E. coli* formed (19.44%) isolates from the liver, this bacterium was considered as a serious indicator on the poultry industry as it causes many diseases. The most common Colibacillosis disease as well as Colisepticaemia, Acute airsacculitis and Coligranuloma of liver (17,18,19).

2- virulence factors test

Through virulence factors test were noticed the presence of several isolates of *E. coli* were hemolytic the blood as shown in Table (2) this result was agreement with (20) who found that (92.6%) of *E.coli* bacteria isolated from chicken liver were hemolytic. While Al Hayley *et.al* were isolated *E.coli* at ratio (55%) from the experimental infected chicken(21), and (22,23) isolated *E.coli* from chicken guts. The variation in proportion is attributed to differences in geographical distribution and environmental conditions, especially temperature differences (24).

Corynebacterium spp was the second type of the intestinal bacterial were isolated in (25.35%), while the liver isolates were formed the (33.3%), highest incidence. These bacteria is naturally found in the environment, and therefore can contaminate chicken meat and organs when the health conditions of slaughter are not available(25). *Enterococcus faecalis* formed (15.49%) and (55.5%) from the intestinal and liver isolates respectively, which is hemolytic bacteria, endemic in chicken guts and shedding with feces, it is an opportunistic pathogen causing disease when predisposing factors were available, which lead to purulent infections after a secondary invasion (25,26).

Klebsiella pneumonia, *Proteus spp* and *Citrobacter spp* were also isolated from intestine in (8.45%, 5.63%, 1.4%) respectively. *Klebsiella pneumonia* formed (5.55%) from the liver

isolates. These bacteria are referring to Enterobacteriaceae which are widespread in the nature and endemic the gastrointestinal tract of humans and animals, saprophytic and cause many diseases when predisposed factors were available (27,28 29).

Staphylococcus aureus was formed (4.22%) for the intestines and (16.6%) for the liver isolates from the total isolates. This study has been agreed with Alfalogy *et al* (30) who isolated it with percentage (16.2%) of the domestic chicken meat in Syria. These bacteria are found naturally on the skin, mucous membranes, intestinal tract, in addition to the external environment, It is one of the most opportunistic pathogens when conditions are predisposed to infection. It causes various diseases in chickens, including septicemia, yolk sac infection, arthritis, and bone marrow (4,29). This is confirmed by the virulence tests of the isolates under study as shown in Table (2). *Bacillus spp* was formed (7.4%) and (11.11%) for both intestinal and liver isolates respectively. It is one of the most widespread bacteria in nature, because it has high resistance spores to environmental conditions (8,26). *Arcanobacterium pyogenes* formed (1.4%) and (2.77%) of the total intestinal and liver isolates respectively. It is one of the opportunistic pathogens that cause osteoarthritis in poultry because it has many virulence factors such as protease, dnase , neuraminidase, and hemolysin (Table 2) in addition to adhesion factors (23,31). *Pseudomonas aeruginosa* constituted (2.77%) of liver isolates, this percentage disagreement with (21) who isolated it by (22.5%). It is a pathogenic bacterium that causes septicaemia of poultry, because it has many virulence factors such as hemolysin, protease enzyme and endotoxins (29,23). *Listeria monocytogenes*, constituted (2.77%) of chicken liver isolates. This result is comparable to Mustafa and Nasal, who obtained 5% *L. monocytogenes* of frozen chicken liver (34). These bacteria are part of the gastrointestinal tract, they are excreted with feces

and contaminate the environment, and then transmitted to humans and animals. It can penetrate many types of cells, which facilitates the process of transmission and access to different organs of the body, such as the liver and brain through the bloodstream and lead to septicaemia and systemic diseases such as encephalitis, meningitis and abortion in pregnant women (31,35) because it has many virulence factors as shown in Table (2). *Lactobacillus spp* was isolated in (2.81%) from the intestines. These bacteria are considered as a part of the normal flora of the intestines in humans and animals (30).

3-Mixed isolates

The study showed that several isolates of the intestine samples were similar to those isolated from the liver in addition to the presence of common isolates in the same sample as shown in Table (3,4). As a result of the possibility of transmission the bacteria from the intestine to the liver or may reach the liver by extension from adjacent air sacs or from less frequently, by extension up the biliary tree (36). Contamination of the liver and meat can occur during the process of slaughter and eviscerated (30) ,the poultry intestines are a source of contamination of the environment with bacteria in them and through the mixing of infected intestinal content with meat during unhealthy slaughter, these bacteria may transmitted to humans and lead to intestinal infections from the toxins producing by them(7) therein lies the danger of transmitting many diseases from poultry and its organs to the consumer (16).

Table (1) Number and percentage of bacterial isolates from chicken intestine and liver in Mosul city.

Bacterial name	Intestine		Liver	
	No of Isolates	%	No of Isolates	%
<i>E.coli</i>	20	28.16	7	19.44
<i>Corynebacterium spp.</i>	18	25.35	12	33.33
<i>Enterococcus faecalis</i>	11	15.49	2	5.55
<i>Klebsiella pneumonia</i>	6	8.45	2	5.55
<i>Bacillus spp</i>	5	7.04	4	11.11
<i>Proteus spp.</i>	4	5.63	0	0
<i>Staphylococcus aureus</i>	3	4.22	6	16.6
<i>Lactobacillus spp.</i>	2	2.81	0	0
<i>Arcanobacterium pyogenes</i>	1	1.4	1	2.77
<i>Pseudomonas aeruginosa</i>	0	0	1	2.77
<i>Citrobacter spp.</i>	1	1.4	0	0
<i>Listeria monocytogenes</i>	0	0	1	2.77
Total	71	%100	36	%100

Table (2) virulence factors of Some Bacteria Isolated from Local Chicken Liver in Mosul city

Bacteria Test	Heamolysin	Protease	Lecithenase	Lipase	Coagulase
<i>E.coli</i>	V	-	-	-	-
<i>Staphylococcus aureus</i>	+	+	+	+	+
<i>Enterococcus faecalis</i>	+	+	+	-	-
<i>Listeria monocytogenes</i>	+	+	+	+	-
<i>Arcanobacterium pyogenes</i>	+	+	+	-	-
<i>Pseudomonas aeruginosa</i>	+	+	+	+	-

Table (3) Number and percentage of bacterial isolates (pure and mixed) from the local chicken intestine in Mosul city.

Bacterial name	No .isolates	%
<i>E.coli</i>	9	25.71
<i>Corynebacterium spp.</i> + <i>E.coli</i>	6	17.14
<i>Enterococcus faecalis</i> + <i>E.coli</i> + <i>Corynebacterium spp</i>	5	14.28
<i>Klebsiella pneumonia</i>	3	8.57
<i>Klebsiella pneumonia</i> + <i>Enterococcus faecalis</i> + <i>Corynebacterium spp.</i>	3	8.57
<i>Proteusspp.</i> + <i>Enterococcus faecalis</i> + <i>Corynebacterium spp.</i>	2	5.71
<i>Proteus spp.</i> + <i>Corynebacterium spp.</i> + <i>Bacillus spp.</i>	2	5.71
<i>Staphylococcus aureus</i> + <i>Bacillus spp.</i>	2	5.71
<i>Citroacter spp.</i> + <i>Arcanobacterium pyogenes</i> + <i>Bacillus spp.</i>	1	2.85
<i>Enterococcus faecalis</i> + <i>Lactobacillus spp.</i>	1	2.85
<i>Staphylococcus aureus</i> + <i>Lactobacillus.</i>	1	2.85
Total	35	100%

Table (4) Numbers and percentages of bacterial isolates (pure and mixed) from local chicken liver in Mosul city.

Bacterial name	No. isolates	%
<i>Corynebacterium spp.</i>	6	27.27
<i>E.coli</i>	2	9.09
<i>Staphylococcus aureus</i>	2	9.09
<i>Corynebacterium spp</i> + <i>E.coli</i> +	3	13.63
<i>Corynebacterium spp</i> + <i>E.coli</i> + <i>Staphylococcus aureus</i>	2	9.09
<i>Corynebacterium spp</i> + <i>Bacillus spp.</i>	1	4.54
<i>Klebsiella pneumonia</i> + <i>Bacillus spp</i> + <i>Staphylococcus aureus</i>	2	9.09
<i>Listeria monocytogenes</i> + <i>Enterococcus faecalis</i>	1	4.54
<i>Enterococcus faecalis</i> + <i>Bacillus spp.</i>	1	4.54
<i>Arcanobacterium pyogenes</i>	1	4.54
<i>Pseudomonas aeruginosa</i>	1	4.45
Total	22	100%

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