Investigating the amount of bacterial contamination in poultry hatcheries during hatching period

Israa L. AL□Jaryan

Department of Animal Production, College of Agriculture, Al-Qasim Green University

Abstract: -

The goal is to investigate the coliform bacterial serotype that exists in chicken hatcheries throughout the hatching period in Babylon. In order to detect *Escherichia coli* bacteria, 200 samples were taken from four hatcheries (Babylon, Al-Jaflawi, Asaad, and Al-Anwar). Cotton swabs were used to collect the samples, which were subsequently taken to the laboratory, where their serotypes were identified and their reactivity to antibiotics was examined. The investigation findings showed that 32 isolates of *Escherichia coli* were found, along with 108 *Klebsiella*, 28 *Proteus*, 8 *Pseudomonas*, 12 *Bacillus* and 12 isolates of *Staphylococcus*. According to the findings of the research, the enterohemorrhagic serotype of the *E. coli* bacterium was identified (EHEC). 16 isolates of the somatic serotype O114 (representing 80% of *E. coli* samples), 12 isolates of serotype O124 (representing 60%), and 4 isolates of type O142 (20%) were found. Aside from being resistant to Gentamicin, Cephalexin, Ampicillin, and Trimethoprim, the recovered *E. coli* also showed moderate sensitivity to Norfloxacin and Ciprofloxacin and high sensitivity to Imipenem, Amikacin, and Cefotaxime.

Introduction: -

One of the most significant pathogenic microorganisms responsible for contaminating hatching eggs, hatchery facilities, and equipment is *E. coli*. It is established that these bacteria may spread vertically (1). In this particular case, the egg shell gets contaminated by maternal feces as the egg crosses through the collector's aperture during laying. These germs can then penetrate the egg shell and contaminate its contents over time. Because they will contaminate the eggs that are ready to hatch and the hatching tools, these eggs are thought of as a source of infection within the hatchery. After that, breeding fields (2). When laying eggs inside nests of infected eggs, one may become contaminated with this particular bacterium. In this instance, the bacterium enters the egg's contents after a set amount of time. All medical interventions, such as sterilising and fumigating the eggs, are pointless in both contaminated and uncontaminated situations (3). Omphalitis, mushy chick illness, and navel sickness are the most significant local infections of colibacillosis in hatched chicks, which is known to be caused by the *E. coli*. When chicks with this ailment range in age from one day to seven days, some of them may die, while the afflicted chicks will exhibit symptoms of the sickness, such as refusing to eat or drink and experiencing diarrhoea. Examining ill or dead chicks reveals an enlarged abdomen, swelling around the umbilical cord, and an unpleasant smell coming from them (4). Thus, the aim of current investigation was to separate different types of bacteria like *Klebsiella, Proteus, Pseudomonas, Bacillus Staphylococcus* in addition to *E. coli* from poultry hatcheries in order to determine their serotypes and examine how sensitive they were to antibiotics.

Materials and Methods

Samples

After determining the origin and type of eggs, 200 randomly selected cotton swabs were collected from each of the four

ISSN 2072-3857

hatcheries in the Babylon Governorate (Babylon hatchery, Al-Jaflawi hatchery, Asaad hatchery, and Al-Anwar hatchery), as well as from the incubator's turning drawers, hatching boxes, and chick transport boxes. hatched and during various intervals of incubation, from the 2-3, 7-10, and 15-19 days, respectively, in addition to the hatched chicks prior to being moved to the breeding farms, depending on (5).

Sample Incubation

By using sterilised cotton swabs, samples were obtained by direct swabbing and then immediately put in MacConky and Nutrient broth, which are known to be carrier mediums for bacteria. After that, they were placed in chilled containers and left for no more than three hours. After that, the samples were incubated in a British-made incubator (Gallen Hamp) for 24 hours at 37°C to allow the bacteria to proliferate until the isolation and classification processes are finished, depending on (6).

Bacterial Culture and identification

To find out if there were any bacteria present, 200 Petri dish filled with nutrient agar were streaked and incubated for 24 hours at 37°C (7). The morphology of bacterial colonies on solid nutritional medium was examined as part of the procedure for diagnosing *E. coli* bacteria, and the bacteria were stained with the Gramme stain to help distinguish positive from negative bacteria (8). Biochemical tests were used to confirm the diagnosis of bacteria, which included:

1-Culturing:

Since MacConkey agar medium is a selective culture medium for Gram-negative bacteria, the resultant negative bacteria were cultivated on it in 176 petri dishes. The dishes were then incubated in an incubator at a temperature of 37°C for 24 hours to determine whether the bacteria under study were lactose fermenting bacteria. Following the first diagnosis of the bacteria, they were isolated once more on the mentioned medium in order to accumulate a pure single isolate of the bacteria necessary for a complete diagnosis. Subsequently, the resultant bacteria were cultivated on Blood agar for 24 hours at 37°C in the incubator to test their capacity to analyse blood.

2-Kligler iron agar slant:

After that, the bacteria were cultured on Kligler agar for 24 hours at 37°C in order to measure their capacity to ferment carbohydrates and release H2S gas.

Identification of the E. coli serotype

One drop of each of the four kinds of Antiserum Escherichia coli was placed on a clean glass slide to conduct a slide agglutination test for the bacterium, which were Trivalent Ⅰ [Enterotoxigenic (O111+O55+O26)], Trivalent Ⅱ [Enteroinvasive (O86+O119+O127)], Trivalent Ⅲ [Enteropathogenic (O125+O126+O128)], Trivalent Ⅳ [Enterohaemorrhagic (O114+O124+O142)]. Each drop was mixed with a swab of isolated and diagnosed bacteria using a wooden stick. The amount of mixing was observed, as each mixed drop indicated a different serotype.

***E. coli* bacterial susceptibility test**

To guarantee that the bacteria propagated throughout the culture medium in all directions, the isolated and identified bacteria were cultured on Molar Hinton agar using a spreading technique. The culture media was mixed with antibiotic tablets (Imipenem (IPM 10), Cefotaxim (CTX 30), Amikacin (AK 30), Gentamycin (CN 10), Ciproflaxin (CIP 5), Cephaloxine (CL 30), Noroflaxin (NOR 10), Ampicillin (AM 10), Trimethprim (TMP 5)) and incubated for 24

ISSN 2072-3857

hours at 37°C. The diameter of the ensuing inhibition zone for each kind of antibiotic was then measured to determine the inhibition zone.

Results and Discussion

Bacterial isolates

In addition to *E. coli*, other isolates were found in the investigation of the bacterial isolation process. These included Gram-positive bacteria like *Bacillus* and Gram-negative bacteria including *Klebsiella, Pseudomonas, Proteus,* and Staphylococci. Table 1 showed that 32 isolates of *E. coli* were collected, representing 16% of the total. *Klebsiella* accounted for 54%, *Proteus* for 14%, and *Pseudomonas* for 4%. Furthermore, the number of isolates of Gram-positive bacteria, specifically *Bacillus* and *Staphylococcus*, was 12 per kind, or 6% of the total.

Table (1) bacteria types and their proportion

Bacteria	Gram stain	Cultured number	Percentage (%)
Klebsiella	G-	108	54
Escherichia coli	G-	32	16
Proteus	G-	28	14
Pseudomonas	G-	8	4
Bacillus	G+	12	6
Staphylococcus	G+	12	6

Because it contributes to microbial contamination of hatching eggs, either from the mothers producing the hatching eggs or through contamination of egg shell throughout laying eggs on the nests or bringing them from the breeding fields to the hatcheries, *E. coli* bacteria is considered as one of the most notable and dangerous of these bacterial species (9). The hatching procedure and the hatching equipment used in the hatcheries may be contaminated with bacteria (10). Thus, despite the possible existence of several diseases in the chicks, the Escherichia coli bacteria will be responsible for a decline in the hatching rate and the development of pathological conditions in the chicks, such as omphalitis, yolk sac, colic septicemia, and others. Gram-positive bacteria, such as bacilli and staphylococci, may have a role in the decrease in hatching rate and embryo mortality, despite the fact that no systemic illnesses specific to these bacteria are accountable for such disorders (11). *E. coli* was observed as gram-negative bacilli when they were stained with Gramme stain (12), which caused the culture to become pink. The MacConkey agar medium used to cultivate the bacteria was also found to alter in colour, which indicates that the bacteria had the ability to ferment lactose (13), as well as, when cultured in Kligler iron agar slant medium showed that the glucose bacteria by fermentation produced a gas through the separation of the agar from the bottom due to the formation of this gas (14)

Regarding the serotyping of the Escherichia coli, the bacteria demonstrated that they belonged to the enterohemorrhagic serovar type by agglutination on the glass slide (haemorrhages *E. coli*, or EHEC) The

ISSN 2072-3857

autosomal serotypes of this bacterium are shown in Table 2. Serotype o114 by 16 isolates, 4 isolates of serotype 142, and 12 isolates of O 124. Consequently, the studied bacteria's serotype was O114+O124+O142, indicating that it belonged to type IV (142 Type □).

Table (2) Serotypes of *E. coli* isolates

***E. coli* Serotype**	**Isolates number**	**Percentage (%)**
O114	16	80
O124	12	60
O142	4	20

The results showed that the predominant serotype (O) of *E. coli* bacteria isolated from hatching eggs of chickens in hatcheries is enterohaemorrhagic E. coli, or EHEC (15). This finding is in line with findings made by (16), who reported that *E. coli* comprises six serotypes.

Numerous coliform bacterial serotypes, including 2O2:H, 25O2:H, and 15H2O, were identified by researchers (10), and they played an important role in penetrating and invading the intestinal cells of children, causing diarrhoea (17). The World Health Organization (WHO) also indicated that there are multiple types of serotypes of *E. coli*, which are 26 O, 128 O, 127 O, 125 O, 119 O, 114 O, 111 O, 86 O, 55 O, and 158 O. O and 214 O. Here it was noted that these strains were classified by (5) as being enteropathogenic to humans. As for the presence of *E. coli* in birds, which is the focus of the current research, the researcher (18) indicated the presence of serotype O2 of the coli bacteria and classified it as a type (Avian Pathogenic *Escherichia coli*, APEC) that only infects birds, that is, it is exclusively pathogenic for birds. It has been observed that the synergistic action of the enterohemorrhagic serovar strain of *E. coli* with *Mycoplasma gallisepticum* on chicken chicks causes severe cellular necrosis and sloughing of the mucous membrane of the bronchioles of the lungs, in addition to swelling of the lungs, and the spread of this swelling even reaches the liver in many bird species (9).

Table (3) *E. coli* antibiotic susceptibility

Antimicrobial Agent	**Symbol**	**Disc contents (mcg)**	**Inhibition zone (mm)**
Imipenem	**IPM**	**10**	**19**
Amikacin	**AK**	**30**	16
Cefotaxime	**CTX**	30	10
Norfloxacin	**NOR**	10	4
Ciprofloxacin	**CIP**	5	2.2
Gentamycin	**GN**	10	---
Cephalexin	**CL**	30	---
Ampicillin	**AM**	10	---
Trimethoprim	**TMP**	5	---

ISSN 2072-3857

The sensitivity test showed in table (3) high sensitivity to each of Imipenem, where the diameter of the zone of inhibition was 19 mm, to Amikacin, the diameter of which was zone of inhibition was 16 mm, and to Cefotaxime, the diameter of which was zone of inhibition was 10 mm. It was also noted that *E. coli* was moderately sensitive, to Norfloxacin with a diameter of 4 mm in the inhibition zone and to Ciprofloxacin with a diameter of 2.2 mm in the zone of inhibition, the bacteria also showed high resistance to Gentamycin, Trimethoprim, Ampicillin and Cephalexin.

Table (4) Antibiotic resistance among isolates

Antimicrobial Agent	Sensitive isolates	Intermediate isolates	Resistant isolates
Imipenem	++++	—	—
Amikacin	+++	—	—
Cefotaxime	++	—	—
Norfloxacin	—	+	—
Ciprofloxacin	—	+	—
Gentamycin	—	—	+++
Cephalexin	—	—	+++
Ampicillin	—	—	+++
Trimethoprim	—	—	+++

Table (4) indicates the degree of sensitivity of *E. coli* isolates to antibiotics. Highly sensitive isolates were ++++, sensitive isolates were +++ and ++, moderately sensitive isolates were +, and resistant isolates were indicated (—). The results of the research showed that the bacteria under study were its high sensitivity to: Imipenem, because it is a bacterial inhibitor, as it inhibits the synthesis of the cell wall and has a destructive effect on the Penicillin Binding Protein (PBS) bond that makes up the bacterial wall. Imipenem inhibits transpeptidase enzyme responsible for the synthesis of peptidoglycan, which is the main component of the bacterial cell wall (19). As for Amikacin and Cefotaxime, their effectiveness as antibiotics depends on inhibiting the synthesis of the bacterial cell wall protein. Therefore, it works to penetrate the cell wall and eliminate bacteria (20). The above two antibiotics are considered modern and rarely used antibiotics due to the high cost of their application in programs to prevent and control diseases in poultry.

In addition, the results indicated that the *E. coli* under study were sensitive to the antibiotics ciprofloxacin and norfloxacin, albeit less so than to the antibiotics previously mentioned. This is in line with the findings of (21), who also reported that the antibiotic norfloxacin was moderately sensitive to these. At a dosage of 25 mcg, the bacteria were 67% sensitive to norfloxacin; in contrast, the bacteria were 83% sensitive to Nitrofurantoin (31 mcg), 81% sensitive to Ofloxacin (30 mcg), and 43% sensitive to Amoxicillin (16 mcg), in addition to the antibiotic Nalidixic acid at a 40% (15 mcg) dosage.

As for the antibiotic Ampicillin, *E. coli* bacteria were resistant to it because the

ISSN 2072-3857

bacteria break down ampicillin and therefore it does not affect the bacteria. Therefore, it strengthens the bond between the penicillin protein (PBPs) and trimethoprim, and therefore the bacteria are resistant to this antibiotic as well, and the other is 29B, since at the beginning of the infant's life, all *E. coli* microbes were of the A29 type and were resistant to ampicillin, but not immediately, while as the infant's age increases, the strain 29B, which is sensitive to ampicillin simultaneously, meaning that it is resistant to this antibiotic (1), multiplies, and from this we conclude that: Strain 29B is more resistant to ampicillin than 29A.

The results of the current study show that bacteria are resistant to both Gentamycin and Cephalexin. Cephalexin Studies have varied on the effectiveness of the antibiotic gentamycin, as it was noted that when it is combined with ampicillin, bacteria are sensitive to it, but the sensitivity is moderate and also varies from one country to another and also according to the area of infection. For example, Escherichia coli, which infects the respiratory system of birds, is more resistant to these antibiotics. (Gentamycin and Cephalexin) among other types (22). As for the screening rates, they were very close to what the researchers found (8), as they were moderately sensitive to norfloxacin and resistant to both ampicillin and trimethoprim, but they were sensitive to gentamicin by 21% and to ciprofloxacin by 12%, and this is the opposite of what was found. The results of the research showed that the bacteria were moderately sensitive to ciprofloxacin and resistant to gentamycin.

References

1.Swelum, A. A., Elbestawy, A. R., El-Saadony, M. T., Hussein, E. O., Alhotan, R., Suliman, G. M., ... & Abd El-Hack, M. E. (2021). Ways to minimize bacterial infections, with special reference to Escherichia coli, to cope with the first-week mortality in chicks: an updated overview. Poultry science, 100(5), 101039.

2. Kang, K. I., El-Gazzar, M., Sellers, H. S., Dorea, F., Williams, S. M., Kim, T., ... & Mundt, E. (2012). Investigation into the aetiology of rutting and stunting syndrome in chickens. Avian Pathology, 41(1), 41-50.

3. Pitcovski, J., & Goyal, S. M. (2020). Avian reovirus infections. Diseases of poultry, 382-400.

4. Talaiekhozani, A. (2013). Guidelines for quick application of biochemical tests to identify unknown bacteria. Account of Biotechnology Research (2013).

5. Ievy, S., Islam, M. S., Sobur, M. A., Talukder, M., Rahman, M. B., Khan, M. F. R., & Rahman, M. T. (2020). Molecular detection of avian pathogenic Escherichia coli (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. Microorganisms, 8(7), 1021.

6. Harrigan, W. F., & McCance, M. E. (2014). Laboratory methods in microbiology. Academic press.

7. Rai, B. K. (2016). Basic Practical Manual on Industrial Microbiology. Lulu. com.

8. Mrug, V. M., Rymsha, O. V., & Trofimenko, Y. Y. (2018). Approaches to optimization of mastering of the discipline "Microbiology, virology and immunology". Reports of Vinnytsia National Medical University, 22(2), 377-380

9. Bajwa, N. Z.; Siddique, M.; Javed M. T. 1992. Pathogenesis of *Escherichia coli* in previously *Mycoplasma gallisepticum* infected layer chicks. journal of Islamic academy of sciences, Pakistan.5(2):123- 126

ISSN 2072-3857

10. Albert, M.J.; Ansaruzzaman, M. and Bhuiyan, N. A.1993. Epithelial cell invasiveness of enteropathogenic serotypes of *Escherichia coli* J. Diarrhoeal Dis. Res. 11(2):101-104.
11. Janssen, T.C.; Schwarz, P.; Preikschat, M.; Voss, M.; Philipp, H.C. and L. H. Wieler.
2001. Virulence – associated genes in avian pathogenic *Escherichia coli* (APEC) isolated from internal organs of poultry. Int. J. Med.Microbiol.291:371-378.
12. McFadden J F. 1979. Biochemical test for identification of Medical Bacteria. Waverly.
Press Inc.
13. Brook GF, Bute J S, Morse S A. Jawetz, Melnick and Adelbergs, 2001. Medical Microbiology 22nd. Ed. Lage Medical Books McGraw- Hill New York Chicago. San Francisco.
14. Morello, J. A.; Mizer, H. E. and Granato, P.A. 2006. Laboratory Manual and Work
Book in Microbiology. 8th.ed. The Mc Grow-Hill companies.
15. Adhikari, S. D. 2005. The impact of organic acids and pH on the virulence factor expression of *E. coli* O157: H7. M.Sc. North Carolina State University p: 9- 12.
16. Mulla, Z.; B A and M S P H.1999.E. coli: Serotypes other thanO157:H7. DOH, Regional Epidemiologist.
17. Malek, A. 2005. Inactivation of *Escherichia coli* and *Salmonella enteritidis* in liquid
egg products using pulsed electric field. Ph. D. Dissertation. McGill
university, Montreal, Quebec.
18. Skyberg, J.A.; Johnson, T. J.; Johnson, J. R.; Clabots, C.; Logue, C.M. and Lisa K. Nolan. 2006. Acquisition of Avian Pathogenic *Escherichia coli* Plasmids by a commensal E. coli Isolate Enhances Its Abilities to Kill Chicken Embryos, Grow in Human Urinemic and Colonize the murine Kidney. American Society for Microbiology, Infection and Immunity, Nov.74(11):6287-6292.
19. Sharp, M. and D. Corp, 2010. Primaxin I. M. (Imipenem and cilastatin for Injectable
Suspension). Merck & CO, INC., white house station, NJ 08889, USA.Pp:1-10
20. Susan, K.; Micota,D V M ; Donald C. P. and D. Pharm.2003.Amikacin Sulfate. Elephant
care International. http:// www.elephantcare.org/ World Health Organization. 1987. program for control of diarrhael diseases (CDD /83.3 Review .1). In. Manual for laboratory investigations of acute enteric infections. Geneva: World Health Organization.27. Cited by Albert, M. J; Ansaruzzaman, M. and Bhuiyan, N. A.1993.Epithelial cell invasiveness of non enteropathogenic serotypes of Escherichia coli. J. Darrhoeal Dis. Res.11(2):101-104.
21. Karki, A.; Tiwari B R and S B Pradhan. 2004. Study of bacteria isolated from urinary
tract infection and their sensitivity pattern. Journal of Nepal Medical
Association. July-August .43:200-203.
22. Mortazavi F and Shahin N.2009.Changing patterns in sensitivity of bacterial uropathogens
to antibiotics in children. Pak. J. Med. Sci.25(5):801-805.

ISSN 2072-3857

ISSN 2072-3857