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Research Article

Identification of Bacteria Associated With Chicken Omphalitis and Their Antibiotic Profiles

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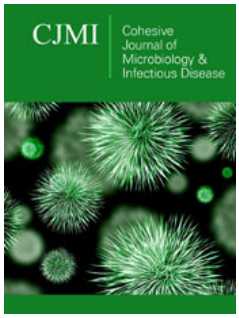

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Abstract

Omphalitis is a non-contagious infection, which affects young poultry's navel or yolk sac. It's more likely to happen in an unclean environment, where opportunistic bacterial infection is more common. A study was conducted on the properties of bacteria associated with chicken omphalitis and their antibiotic profile. In this research, we took 55 samples from sick chickens on five separate farms in Dinajpur Sador; Satabganj; Fulbari; and Basherhat; and evaluated them using bacteriological; biochemical; molecular; and antibiotic sensitivity tests. Three bacteria genera (*Escherichia coli*; *Staphylococci aureus*; and *Salmonella spp*.) were isolated from yolk swab samples in omphalitis-infected chicks in this investigation. In both farms, *E. coli* consumed the maximum percentage of positive cases (94.11%; 80.00%; 83.33%; 71.42%; and 88.88%; respectively); *Staphylococcus spp*. came in second for the percentage of positive cases (58.82%; 50.00%; 66.66%; 57.14%; and 55.55% respectively); and the percentage of positive cases is the 3rd highest prevalence in *Salmonella spp*. (32.29 %; 40.00 %; 50.00 %; 42.65 %; and 33.33% respectively).

E. coli was the frequent bacteria (46%); subsequently *Staphylococcus aureus* (32%); and *Salmonella spp*. (22%). 576 bp DNA fragments were used to identify *E. coli*, confirming its identification. Isolates E. coli were sensitive to chloramphenicol; gentamycin; levofloxacin; azithromycin; and ciprofloxacin on the other hand resistant to erythromycin; amoxicillin; tetracycline; ampicillin; and Cefixime; according to the drug study. *Salmonella spp*. was resistant to erythromycin; azithromycin; ampicillin; and tetracycline but sensitive to gentamycin; Cefixime; chloramphenicol; levofloxacin; and ciprofloxacin. Chloramphenicol; levofloxacin; Cefixime; and ciprofloxacin were all effective against *Staphylococcus aureus*; however, gentamycin; tetracycline; ampicillin; erythromycin; and amoxicillin were not. The outcome of the antibiogram study; has found that all isolates were unaffected against most of the antibiotics; which is not respectable at all. To those antibiotics, which the isolates were susceptible; should be applied in recommended dose for treatment purposes and also need to maintain proper cleaning on environment.

Keywords: Antibiotic sensitivity; Bacteria isolation rate; Chicken; Omphalitis; Yolk sac infection

Abbreviation: EMB: Eosine Methylene Blue; SS: Salmonella Shigella; NA: Nutrient Agar; BGA: Brilliant Green Agar; MSA: Manitol Salt Agar; MAC: MacConckey Agar; XLD: Xylose Deoxycholate Agar; TSI: Triple Sugar Iron

Introduction

Omphalitis is a non-contagious disease that affects young poultry's navel or yolk sac. It's almost certain in a messy climate where bacteria can spread quickly. In the first fourteen days after hatching, it causes navel discomfort; anorexia; sadness; decreased weight gain; and increased mortality. Omphalitis; also known as yolk sac contamination; most common causes of death in newborn chicks [1]. It arises because of filthy hatchery equipment. Gloomy expressions; hanging heads; and crouching near to the hot source are all symptoms of the affected chicks [2]. The poultry industry in Bangladesh is growing quickly starting around 1980. It undertakes an important part in the destitution lightening and economic improvement of the country. The current rough poultry general population is 300 million

(30 cores) including 50 million (5 cores) ducks and 250 million (25 cores) chickens (DLS; October 2012-13); and it incorporates broiler; layer; and duck. There are around 1000 hatcheries facilities in Government and private areas (farm poultry and Livestock survey 2010). The quantity of Day-Old Chicks (DOC) created in Government farms remains at around 125 lakhs to 250 lakhs number each month whereas that of private farms is at around 8 million to 1 core each week [3]. Most frequent causes of death in infants in beginning thereafter brewing is a bacterial infection of the umbilical region [4]. *E. coli* is the most well-known contaminant in chickenpox; and about 70% of omphalitis-infected chicks have such bacteria in their yolk sac. In contrast, a tiny quantity of *E. coli* is commonly improved from the normal yolk sac [5].

Open navels contact with the contaminated surfaces is the primary cause of chicken omphalitis. Bacteria can move the patent yolk stem upwards and infect the yolk sac if they meet a contaminated environment before their navel is completely closed. Mixed infections are prevalent; and opportunistic microbes are frequently involved. There is virtually little information on omphalitis in the northern portion of Bangladesh. Even though this problem has become a persistent concern to our chicken sector due to these frequent occurrences at the farmer level; there is no reported study that analyzes the treatments isolation; identification; molecular characterization; and control. The current investigation was done with the isolation and characterization of etiological agents of omphalitis by cultural; biochemical; and molecular approaches with antibiogram profiles; which is a new effort in this region. The goal of this study was to define the bacteria associated with omphalitis in freshly hatched chicks from five hatcheries; as well as to determine which antibiotics are most vulnerable and resistant to omphalitis; which varies from place to location and nation to country.

Materials and Methods

Ethical approval

The methodology of this research was approved by ethical committee of Hajee Mohammad Danesh Science and Technology University [approval number: (HSTU)/IRT/84].

Study design

This study was complemented at the bacteriology research laboratory of the Dept. of Microbiology at Hajee Mohammad Danesh Science and Technology University in Basherhat; Dinajpur; Bangladesh. This study was conducted in January to June 2020. Overall; 55 swab samples were obtained from chicks aged 1 to 7 days from five separate farms. Different bacteriological tests were performed for the characterization of suspected isolates.

Isolation and characterization of bacteria

All swab samples were cultured on NA plates and incubated at 37 °C for 24 hours. Confirmation of microbiological growth some selective media were used for bacterial cultures, such as *Staphylococcus* agar no. 110; MacConkey agar; Xylose Lysine Deoxycholate agar; EMB agar; Blood agar; SS agar; MSA agar and BGA agar (HI Media; India). All culture plates were sub-cultured and then incubated overnight at 37 °C. By using previously published protocol pure cultures were maintained in a bacteriological laboratory [6]. In this work; several morphological; and biochemical examinations were applied. Gram stain was utilized to assess the morphology and staining properties of microorganisms [6]. Different biochemical tests like Coagulase; catalase; Oxidase; Methyl Red (MR); TSI; Voges-Proskauer (VP); Indole; and Simon's citrate measures were likewise performed using conventional procedures [7].

Antibiotics susceptibility test

The antimicrobial susceptibility profile of the isolates was assessed using the standard Kirby-Bauer disk diffusion method [8] in accordance with the National Committee for Clinical Laboratory Standards guidelines [9]. Antibiotic sensitivity was investigated using different types of commercial antibiotic discs on a Muller-Hinton agar plate (HI Media). Using sterile forceps; antibiotic disks were administered. Plates were then incubated at 37 °C for 24 hours. After overnight incubation the zone was measured by millimeter scale, according to manufacturer guidelines. 10 commercially available antibiotics was used for the antibiotic sensitivity test (Table 1).

Table 1: Antibiotics with their disc concentration.

Legends: µg: Microgram; SI: Serial; No: Number

SI. No.	Antibiotics Name	Concentration of Disc (µg/disc)
01	Gentamycin (GEN)	10
02	Chloramphenicol (C)	30
03	Azithromycin (AZM)	30
04	Levofloxacin (LE)	05
05	Amoxicillin	30
06	Ampicillin (AMP)	25
07	Cefixime	05
08	Tetracycline (TE)	30
09	Erythromycin (E)	15
10	Bacitracin (B)	10
11	Ciprofloxacin	30

Molecular detection

DNA extraction: *E. coli* genomic DNA isolated since liquid culture 1ml of Nutrient broth overnight growth used in the work. DNA was extracted with a Robotic DNA extractor (Maxwell-16; Source: Promega-USA) as per the maker's guidance. The genomic DNA was tested for concentration and purity using Nano-Drop Spectrophotometer (ND-2000; Source: Thermo Scientific-USA). The PCR primer: marks gene and PCR cycling summary are showed in Table 2.

Table 2: Oligonucleotide primers structures, mark genes, and cycling situations.

Mark gene	Primer sequence (5′-3′)	Seg. (bp)	Pre Heat	Amplification- 35 cycles			Final exten.	Ref.
				Denat.	Annealing	Extension		
tetA	GGTTCACTCGAACGACGTCA	576	95 °C for 2 min	95 °C for 30 sec	52 °C for 30 sec	72 °C for 50 sec	72 °C for 5 min	[10]
	CTGTCCGACAAGTTGCATGA							

Analysis of PCR products: PCR examination was finished by victimization microorganism by specific primer targeting gene (tetracycline-resistant gene) of *E. coli.* PCR item was divided by gel electrophoresis utilizing 1.5% of agarose gel marked with ethidium bromide solution and imagined below ultraviolet light by a gel documentation framework.

Data analysis: Data from different farm were entered in excel sheets (Microsoft Excel) and analyzed by using R version 3.6.0. Descriptive analysis summarizing the results. Chi-square test was also used to determine the significance difference in different farm.

Results

Clinical signs

All the chicks handled for the study showed the clinical sign of omphalitis characterized by inflammation of navels, reddish or bluish color of the abdominal muscles around the navel. The postmortem examinations observe an unabsorbed yolk sac in checks.

Identification of bacteria

In this research, 55 samples were collected from infected chickens from five different farms. *E. coli*; *Staphylococcus* spp.; and *Salmonella* spp. were identified from omphalitis infected chicks' yolk swab samples. Highest prevalence was observed in *E. coli* 47 (85%); followed by *Staphylococcus* spp.; 32 (58%) and *Salmonella* spp. 22 (40%) respectively. Out of 55 samples a total of 101 isolates were isolated from different farms. The results were more or less like [10-12]. *E. coli* showed the highest % of positive cases in both farm (94.11%; 80.00%; 83.33%; 71.42% and 88.88%) respectively whereas *Staphylococcus* spp. showed in five farms (58.82%; 50.00%; 66.66%; 57.14%; and 55.55%). The % of positive cases third-ranked in *Salmonella* spp. in five farms; the percentage is (32.29%; 40.00%; 50.00%; 42.65%; and 33.33% respectively). The significance difference ($P<0.05$) was observed in nizam and brother poultry farm with highest predominant bacterial isolates (Table 3).

Table 3: Bacteria were detected from a suspected case of omphalitis.

Farm	Total no. of chicks	No. Examined	% of positive cases			x^2	p-value
			E. coli	*Salmonella spp.*	*Staphylococcus spp.*		
Nizam & brother poultry farm	750	17	16 (94.11%)	10 (58.82%)	6 (35.29%)	14.971	0.0005612
Satota traders	480	10	8 (80.00%)	5 (50.00%)	4 (40.00%)	3.5294	0.1712
Boishakhee poultry farm	530	12	10 (83.33%)	8 (66.66%)	6 (50.00%)	3	0.2231
Panchabari agro farm	350	7	5 (71.42%)	4 (57.14%)	3 (42.65%)	1.1667	0.558
Emon poultry farm	490	9	8 (88.88%)	5 (55.55%)	3 (33.33%)	5.8295	0.05422
Overall % of Positive cases		55	47 (85%)	32 (58%)	22 (40%)	24.25	0.000005423

Cultural and morphological finding

The cultural appearances of *E. coli*; *Staphylococcus* spp. and *Salmonella* spp. on different selective media; are observed with different characteristics. *E. coli* shows green metallic sheen colony on EMB agar; produce whitish colony on XLD agar. *Staphylococcus* spp. shows Small whitish or yellowish colony on MSA agar while produce small white colony on Staphylococci media no. 110. In XLD agar *Salmonella* spp. shows red; black center colony. The morphological characteristics of *E. coli* gives pink colored; rod in shaped; singular arrangement; pair or short chain under microscopy while *Staphylococcus* spp. shows cluster; cocci shape with purple color. *Salmonella* spp. appears under microscopy with small rod shape with pink color (Figure 1).

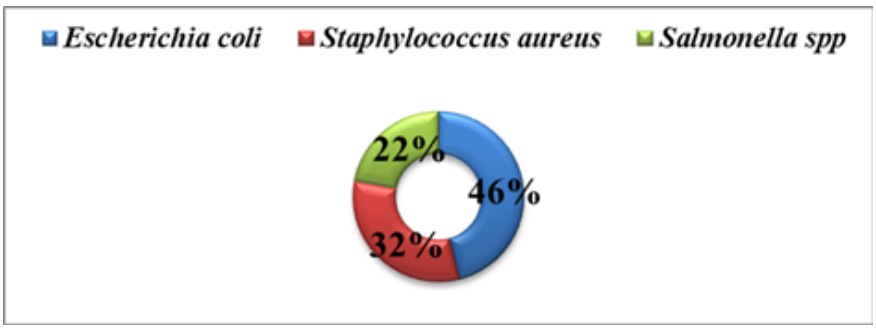

Figure 1: Prevalence of three identify organisms in chicks with clinical signs of omphalitis.

Antibiotic sensitivity test

The isolated *Staphylococcus* spp. E. coli: and Salmonella spp. were subjected to an antibiotic test to define the sensitivity and resistance pattern against the commonly used antibiotic disc. The results of antibiotic sensitivity tests are presented in Table 4.

Table 4: Antibiogram results of E. coli, *Staphylococcus spp.* and *Salmonella spp.*

Sl. No.	Name of Antibiotics	Disc code (µg/disc)	*E. coli* N=6			*Staphylococcus spp.* N=6			*Salmonella spp.* N=6		
			S	I	R	S	I	R	S	I	R
1	Gentamycin (GEN)	10	4	2	0	0	3	3	5	1	0
2	Chloramphenicol (C)	30	5	1	0	4	2	0	4	2	0
3	Azithromycin (AZM)	30	1	5	0	0	4	2	0	3	3
4	Levofloxacin (LE)	5	4	2	0	3	3	0	4	2	0
5	Tetracycline (TE)	30	0	0	6	0	2	4	0	2	4
6	Ampicillin (AMP)	25	0	2	4	0	2	4	0	4	2
7	Ciprofloxacin (CIP)	5	2	4	0	5	1	0	4	2	0
8	Erythromycin (E)	15	0	3	3	0	3	3	0	2	4
9	Amoxicillin (AMX)	30	0	3	3	0	4	2	1	4	1
10	Cefixime (CFM)	5	0	2	4	2	4	0	5	1	0

S: sensitive; I: intermediate; R: resistant

Detection of tetracycline-resistant gene of *Escherichia coli*

In this study; *E. coli* in 47 positive samples by cultural; morphological; and biochemical test. Then 5 representative samples were subjected to characterization by PCR; on the PCR technique all *E. coli* are produce 576 bp band by gel electrophoresis. PCR primers targeting gene (tetracycline-resistant gene) of *E. coli* amplified 576 bp fragments of DNA confirmed the identity of *E. coli*. The result of PCR for *E. coli* is shown in Figure 2.

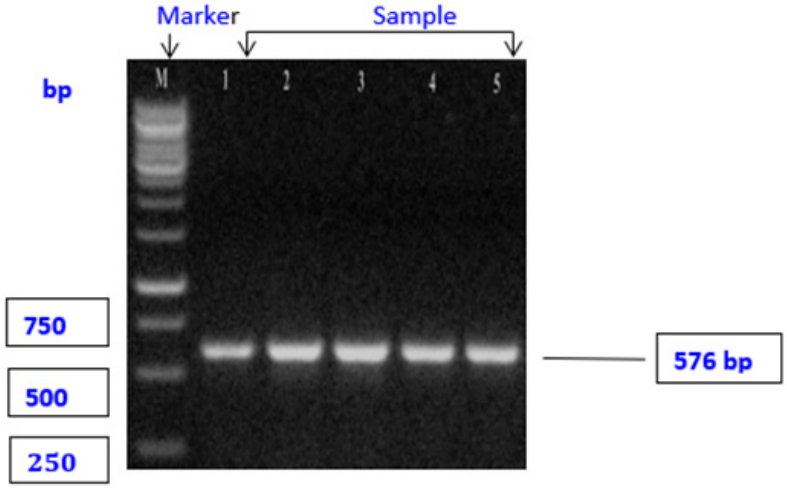

Figure 2: Profiles of F1 and R1 primers, *E. coli* showing positive band at 576bp. M: denotes 1kb DNA ladder (Marker). Seg. (bp)= amplified segment.

Discussion

Omphalitis is a non-contagious illness that affects young poultry's navel or yolk sac. It's more likely to happen in an unclean environment, where opportunistic bacterial infection is more common. Omphalitis is one of the most serious poultry diseases, causing significant losses. It causes poorer hatchability; greater mortality; and a higher culling rate in afflicted flocks owing to a change in the formation of immunoglobulin proteins followed by bacterial infection; culminating in immunodeficiency [13]. The current study; which involved the isolation of bacterial agents linked to omphalitis in five separate hatcheries in Dinajpur; is the first to indicate the relevance of omphalitis as a source of excessive fatality in chicks at the start of their lives in the research location. *E. coli* is found in 47 cases: *Staphylococcus* spp. in 32 and *Salmonella* spp. in 22 cases (Table 3). This might be related to

the increased immunological state of chicks; improved hatchery hygiene conditions; and improved environmental conditions to reduce omphalitis infection in chicks. Several previous studies that showed the common relationship such bacterial isolates with yolk sac infection; agree to findings three primary bacterial isolates in our study from omphalitis infection [2,14,15].

The isolated organism was described using Gram's staining method and morphological evaluation. Gram staining indicated that the isolated organism was *E. coli*; which was Gram-negative; rod-shaped; and grouped in single; pair; or short chains. Gram staining revealed Gram-negative; single; or paired short plump rods in *Salmonella* spp. and Gram-positive cocci-shaped bacteria arranged in the cluster (grape-like) in *Staphylococcus* spp. with the motility profile of bacteria observed under the microscope after hanging drop slide preparation. Our findings were near closed to [16]. In our current research, morphologically and culturally identified isolates were then using different biochemical tests (MR; VP; Indole; Catalase test; TSI; Coagulase test; etc). This observation also revealed those isolated organisms are *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. with their biochemical feedbacks. The catalase test was positive (presence of bubble) for *Staphylococcus* spp.; *Salmonella* spp.; and negative (absence of bubble) for *E. coli*. Coagulase test was positive (presence of bubble) *Staphylococcus* spp. and negative (absence of bubble) for *E. coli*; S*almonella* spp. Oxidase test was positive (produce deep purple color) *Staphylococcus* spp. and negative (no color change) for *E. coli*; *Salmonella* spp. Indole tests were also positive (presence of a pink; red-colored circle on the surface of media) for *E. coil*. Whereas negative (absence of a pink; red-colored circle on the surface of media) for *Staphylococcus* spp. and *Salmonella* spp. MR reactions were positive (presence of a red color ring on the surface of media) for *E. coil*; *Staphylococcus* spp.; *Salmonella* spp. VP tests were positive (presence of the red color ring on the surface of the media) for *Staphylococcus* spp. and negative (absence of a red color ring on the surface of the media) for *E. coli* and S*almonella* spp. MIU tests were positive (diffuse; hazy growth; slightly opaque media) for *E. coli* and *Salmonella* spp. (absence of color) for *Staphylococcus* spp. These findings were also supported by other researchers [2].

In this study, all the suspected isolates obtained from morphological; cultural; and Biochemical studies were subjected to a culture PCR approach. Among three (*E. coli*; *Staphylococcus* spp.; and *Salmonella* spp.) bacterial isolates; only one *E. coli*; isolates were used for culture PCR for molecular characterization using DNA amplification. In our present study, it was isolated *E. coli* characterized by using tet A primer sets for the molecular identification and characterization of *E. coli* amplified at 576 base pairs. All examined *E. coli* isolated for tetA gene using PCR were totally positive with an incidence of 100% (Figure 2). These findings were supported by [10]. Antibiogram research was performed on the identified isolates in the current study. The findings demonstrated that the field isolates were responsive to a variety of antibiotics utilized in the study to variable degrees. Amoxicillin 50%; ampicillin 67%; Cefixime 67%; Erythromycin 50%; and tetracycline 100% resistance was found in all *E. coli* isolates. Azithromycin 50%; ampicillin 33%; erythromycin 67%; amoxicillin 17% and tetracycline 67% resistance were found in *Salmonella* spp. Gentamycin 50%; erythromycin 50%; amoxicillin 33%; tetracycline 67%; azithromycin 33% and ampicillin 67% resistance was found in all *Staphylococcus* spp. which is related to [2]. Therefore, we strongly suggest using ciprofloxacin; chloramphenicol and levofloxacin against chicken omphalitis.

Conclusion

The researchers wanted to isolate and describe the bacteria that causes yolk sac infection in chicks. *E. coli*: *Salmonella* spp. and *Staphylococcus* spp. were cardinal isolates that causes omphalitis in chicks and prevalence percentages were 85%; followed by 58% and 40% respectively. Our study indicates that ciprofloxacin; chloramphenicol and levofloxacin are more effective treatment against omphalitis. The discovery of antibiotic resistant isolates of *E. coli*; *Salmonella* spp. and *Staphylococcus* spp. against omphalitis in chicks is concerning; resist it could expand to germs affecting human's livestock. More research is needed in Bangladesh to find policies for the prohibition and dominate of microbial yolk sac infection in chicks.

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Authors Contribution

Supervised and designed experiment: MRA and FA.

Performed laboratory work: MM.

Wrote the manuscript: MHH and MHR.

Data analysis: MHH; RR and MSR.

Plagiarism and grammar check: MM; MHH.

All authors read the manuscript before final submission.

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