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Isolation and Molecular Detection of Bacteria Causing Omphalitis in Poultry with Look on Antibiotic and Disinfectant Resistance

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ABSTRACT

Antimicrobial resistance is an international concern and creates critical health issues for both humans and animals. This research aimed to ascertain the prevalence of isolates, antibiotic and disinfectant resistance patterns from omphalitis suspected chicks in Dinajpur, Bangladesh. A total of 24 yolk swab samples were collected from different hatcheries for microbiological analysis and antibiotic sensitivity tests. The PCR employing *16s* and *23s RNA* genes was conducted to detect *Escherichia coli* and *invA* genes were used to detect *Salmonella* spp. and 23S rRNA genes were applied for *Staphylococcus* spp. A total of 35 isolates were identified where 14 (40%) *E. coli*, 10 (28.58%) *Salmonella* spp., and 11 (31.42%) *Staphylococcus* spp. from day 1 -8, respectively. The largest amount of bacteria on day 5, including day 7 (20%), followed by days 3, 4, and 6, including 6 (17.14%) observed, respectively. The PCR band of *E. coli* was detected at 232 bp, *Salmonella* spp. 284 bp and *Staphylococcus* spp. 1267 bp respectively. *E. coli* was highly resistant to Amoxicillin (100%), followed by Tetracycline (83.33%), whereas highly sensitive to cefotaxime (100%), Gentamicin and Cotrimoxazole (83.33%). *Salmonella* spp. indicates high susceptibility to Cefotaxime, Co-trimoxazole, and Ceftazidime (83.33%), followed by Gentamicin (66.67%) respectively, whereas *Staphylococcus* spp. was found to be highly resistant to Methicillin (100%) and Ampicillin (100%) followed by Gentamicin and Tetracycline (83.33%) respectively. The MAR index calculation of isolated *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. from different sources of poultry hatcheries was measured at 0.77, 0.79, and 0.77, which affect the newly hatched chicks in poultry industries. Therefore, an urgent surveillance program is needed to fight antimicrobial resistance in poultry production sectors in Bangladesh.

Keywords: Molecular detection, Bacteria, Omphalitis, Poultry, Antibiotic, and Disinfectant resistance.

INTRODUCTION:

Omphalitis is an infectious and non- contagious condition of yolk sac accompanied by unhealed navels in chicks. According to Abdel-Tawab *et al.* (2016), exaggerated chicks appear normal, right up to a few hours before they pass away. Yolk sac infection caused chick mortality during the first week of the post-hatching (Abdel-Tawab *et al.,* 2016). Chicken is the most economical source of animal protein in the comparison with red meat, particularly in developing countries, global broiler production exceeded 100.5 million tons in 2021 and is expected to further increase by 2% in 2022 (Saad *et al.,* 2023). Pathogenic bacteria are the primary causes of poultry diseases worldwide; they are responsible for the most significant economic losses

resulting in a huge annual financial loss exceeding 50 billion USD (Rahman *et al*., 2019; Saad *et al.,* 2023).

The poultry industry is the important economic sector in Bangladesh and the largest food supplier to the global population. The development of the poultry industry in Bangladesh has increased and is driven by the high market demand for poultry commodities. Many bacterial isolates are responsible for navel illness including *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus cereus* and *Enterococcus* spp. were isolated from yolk sac in chicks in different locations all over the world (Abdel-Tawab *et al.,* 2016). In previous research several researchers identified *Escherichia coli* (*Eaea*) virulence gene, *Salmonella* invasion (*invA)* gene and *Staphylococcus* (*MRSA)* gene (Abdel-Tawab *et al.,* 2016. All enterprises involved in animal farming, especially chicken farming, are frequently vulnerable to disease. As such, data and understanding regarding the prevalence of diseases as well as initiatives to stop, manage, and completely eradicate them are crucial (Shahen *et al*., 2019; Wibisono *et al.,* 2022).

Antimicrobial resistance, especially MDR, is a difficult problem to overcome when treating infectious diseases (Wibisono *et al.,* 2022). The *qacED1* gene was found to be present in isolated *E. coli* with a 100% incidence rate in the previous investigation that looked for disinfectant-resistant genes in unhatched chicken eggs in Egypt (Ibrahim *et al.,* 2019). The study explains why antibiotics fail to treat *E. coli* infections in poultry and increase the infection rate in the first week of life. Antibiotic resistance is common in poultry farms and the environment and can spread to humans via food or water. Very few researches were conducted to identify disinfectant and antibiotic resistance genes of different bacterial isolates from omphalitis infected chicks in Bangladesh. These antibiotic resistance genes are very harmful for broiler chickens as well as human health. Drug resistance is becoming a growing threat to public health, and the environment, it also induced therapeutic failure and economic losses in animal production. Therefore, the aim of this research was to investigate the incidence, investigate antibiotic and disinfectant resistance isolates from yolk sack of suspected chicken omphalitis, as well as detect specific resistance gene by molecular detection method (PCR).

MATERIALS AND METHODS:

Study area and yolk swab sampling

In this study, 24 yolk swab samples were collected from broiler chicks of different hatcheries including 1 to 8 days under Dinajpur District of Bangladesh and aseptically transported with ice box to the bacteriology laboratory, Hajee Mohammad Danesh Science and Technology University for bacteriological analysis. The full research period was July - December 2023.

Phenotypic and biochemical characterization of isolates

According to Azam *et al.* (2023), bacterial isolates were primarily identified by cultural tests such as Nutrient agar, MacConkey agar, Eosin Methylene agar, Salmonella-Shigella agar, Mannitol salt agar were usually applied for the detection of pure isolates of suspected bacteria from yolk by microscopic (gram staining) and standard biochemical tests such as MR-VP, Indole, Oxidase, Catalase, TSI, Citrate utilization test were used for identification. In this research purpose, all microbiological media were purchased from Hi Media Private Ltd. India.

Molecular identification (DNA extraction and purification)

According to Riffon *et al.* (2001), genomic DNA was extracted from selected isolates. Forward primer (5' ATCAACCGAGATTCCCCCAGT 3') and Reverse primer (5' TCACTATCGGTCAGTCAGGAG 3') were used for detection of *16s* rRNA gene of *E. coli* which in a fragment with about 232 bp length. According to Rahn *et al.* (1992) Forward primer- (5' GTGAAATTATCGCCACGTTCGGGCAA 3') and Reverse primer- (5' TCATCGCACCGTCAAAGGAACC 3') were used for detection of *invA* gene of *Salmonella* spp. amplified by PCR techniques and final band measure about 284 bp length. According to Riffon *et al.,* (2001), Forward primer Sau-234 and Reverse primer - 1501 were used to detect DNA fragment of *Staphylococcus* spp. which result in 1267 bp length. According to manufacturer (Maxwell-16, source: Promega-USA) guideline, a robotic DNA extractor was applied to extract DNA from *E. coli*. The purity of *E. coli, Salmonella* spp., and *Staphylococcus* spp., DNA was measured by a Nanodrop spectrophotometer (ND-200, source: Thermo Scientific -USA). 1.5% agar gel electrophoresis was

used for visualization of PCR band with specific base pair whereas UV-trans illuminator detects photograph of band.

Table 1: PCR Condition for *Escherichia coli* (*Eco* 223 and *Eco* 455).

Step	Temperature (°C)	Duration	Cycles
1. Initial denaturation	95	2 min	01
2. Denaturation	95	1 min	28
3. Annealing	56	40 sec	
4. Extension	72	1 min	
5. Final Extension	72	5 min	01
6. Holding	4	Until Analysis	-

Table 2: PCR Condition for *Salmonella* spp. (*invA* 139 F and *invA* 141 R).

Step	Temperature (°C)	Duration	Cycles
1. Initial denaturation	95	2 min	01
2. Denaturation	95	1 min	30
3. Annealing	55	1 min	
4. Extension	72	1 min	
5. Final Extension	72	5 min	01
6. Holding	4	Until Analysis	-

Table 3: Condition of PCR for *Staphylococcus* spp. (*Sau*-234 F and *Sau*-1501 R).

Step	Temperature	Duration	Cycle
Initial Denaturation	94°C	2 min	
Denaturation	94°C	45 sec	
Annealing	58°C	45 sec	
Extension	72°C	2 min	35 × Step 2
Final extension	72°C	6 min	
Holding	4°C	∞	

Evaluating the Antibiotic Susceptibility

According to Clinical and Laboratory Standards Institute (CLSI) procedure, agar disc diffusion techniques were used to determine the antibiotic sensitivity patterns of isolates on Muller-Hinton agar (CLSI, 2021). A total of 12 commercially available antibiotic discs (Oxoid Limited, UK) such as, Amoxicillin (30 µg), Ampicillin (30 µg), Gentamicin (10 µg), Tetracycline (30 µg), Levofloxacin (5 µg), Co-trimoxazole (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Methicillin (5 µg), Amikacin (30 µg), Azithromycin (30 µg) and Vancomycin (30 µg) were applied for antimicrobial sensitivity test. A pure bacterial colony (0.1 ml) were spread on Mueller-Hinton agar plate and antibiotic discs were placed on the plates. Then the plates were incubated at 37°C for 16 to 18 hours in standard incubator (BIOBASE, china). After overnight incubation the zones of inhibition was measured by millimeters scale according to (CLSI, 2021) guidelines. All tests were done 3 times for result accuracy.

A. Multidrug Resistance (MDR) Strains Identification

Multidrug resistance bacterial isolates were determined by using disc diffusion method according to (Ezekiel *et al.,* 2011). Multidrug resistance (MDR) was taken as resistant to four or more antibiotics tested.

B. Multiple Antibiotic Resistance (MAR) index determination

According to Saad *et al.,* (2023) the Multiple Antibiotic Resistance (MAR) index for each strain was determined in which,

MAR index = No. of resistance isolates / Total number of tested antibiotics

MAR index = A/B Where,
A=No. of resistance isolates;
B=Total number of tested antibiotics;

C. Disinfectant

Four commercially available disinfectants are used such as, Lysol (Reckitt Benckiser, USA), Virocid (Cid lines, Belgium), FAM 30 (Renata, Bangladesh)

and EMSEN (SK+F Eskayef, Bangladesh) were used at 1% concentrations as per recommended dilution. The zone of inhibition was measured in mm on Mueller-Hinton agar with disinfectant against selected isolates.

Statistical Analysis

Statistical analysis was done based on data variables of isolates. Excel version 13 was applied for data analysis as well as standard deviation and standard error measurement.

Frequency of bacterial isolates among chicks based on different categories

The results of incidence of bacteria in chicks are presented in **Table 4**. From **Table 4**, out of 24 examined chicks, 17 (70.83%) were found to be positive cases from two different poultry hatcheries with age 1-8 days. The highest number of positive cases were found in day 4, 7 and 8 (100%) followed by 1, 5 and 5 (66.67%) respectively. The positive cases and isolated bacteria were competatively different from two hatcheries with different ages.

RESULTS:

Table 4: Incidence of bacteria in chicks from day 1-8 days.

Hatcheries	AGE/Day	Examined chicks	Positive case	Prevalence (%)
1	1	3	2	66.67
	2	3	1	33.33
	3	3	1	33.33
	4	3	3	100.00
2	5	3	2	66.67
	6	3	2	66.67
	7	3	3	100.00
	8	3	3	100.00
Total		24	17	70.83

Primary screening of isolates

A total of 35 bacterial isolates were morphologically and biochemically identified through EMB argar, SS agar, MSA agar and a group of biochemical tests which are presented in **Table 5**.

Table 5: Results of Biochemical Tests.

Name of isolates	OX	CT	IN	MR	VP	SC	TSI		MIU		
							slant	Butt	M	I	U
E. coli	-	+	+	+	-	-	A(yellow)	A(yellow)	+	+	+
Salmonella spp.	+	+	+	-	-	+	K(pink)	A(yellow)	+	+	+
Staphylococcus spp.	-	+	+	+	+	+	A(yellow)	AG	-	-	-

Legends: + = positive, - = negative, A=acid, K= alkaline, G= gas, NC= no color change OX= oxidase, CT=catalase, IN= indole, MR = methyl-red, VP = voges-proskauer, SC = simmon's citrate, TSI = triple sugar iron, MIU = motility indole urease.

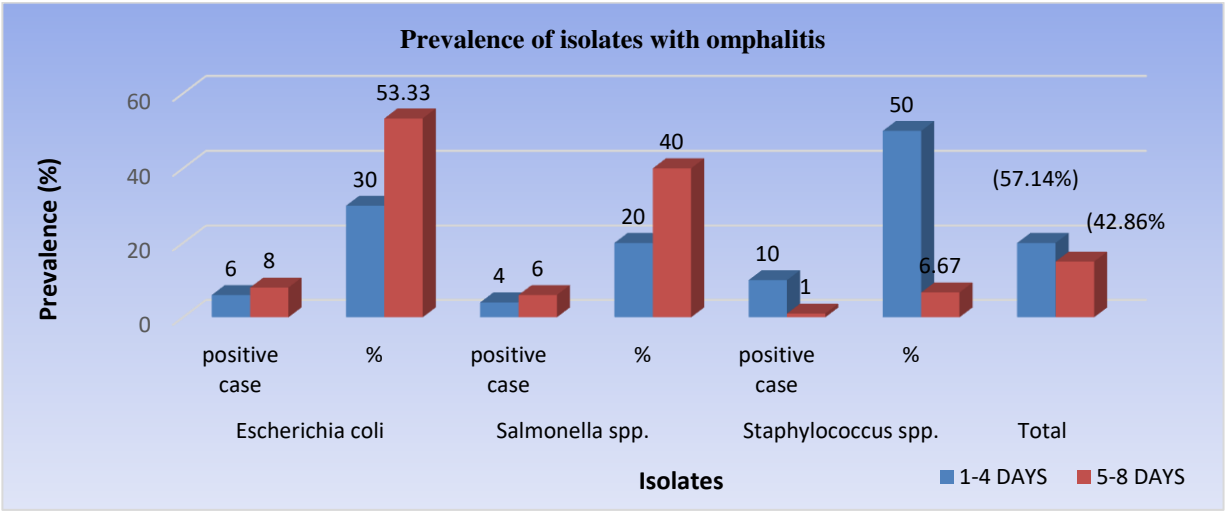

Fig. 1: Prevalence of isolates (*E. coli*, *Salmonella* spp. and *Staphylococcus* spp.) in 1-4 days and 5-8 days old clinically suspected chicks.

Prevalence of isolates

In this current research three bacterial isolates including *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. was isolated from yolk swab samples from suspected omphalitis chicks from different hatcheries in Dinajpur, Bangladesh. From Hatchery 1, 20 isolates were identified including *E. coli* 6 (30%), *Salmonella* spp. 4 (20%) and *Staphylococcus* spp. 10(50%) whereas 8(53.33%) *E. coli*, 6 (40%) *Salmonell*a spp. and 1 (6.67%) *Staphylococcus* spp. was found in hatchery 2. Out of 35 bacterial isolates 20 (57.14%) were isolated from hatcheries 1 and 15 (42.86%) were identified from hatchery 2 respectively. The prevalence of bacteria associated with omphalitis is presented in **Fig. 1**.

Table 6 represents the correlation bacteria and age group of broiler chicks. The bacterial isolates obtained in our study were 14 (40%) *E. coli*, 10 (28.58%) *Salmonella* spp. and 11 (31.42%) *Staphylococcus* spp. from day 1-8 respectively. The highest number of bacteria we isolated from day 5 including 7 (20%), followed by day 3, 4 and 6 including 6 (17.14%) respectively (**Table 6**). The highest prevalence of isolates is showed in **Fig. 2** with standard deviation and standard error value of day 5 omphalitis suspected chicks.

Table 6: Correlation between mortality due to yolk sac infection based on age.

Isolates	Day 1 (n= 3)	Day 2 (n= 3)	Day 3 (n= 3)	Day 4 (n= 3)	Day 5 (n= 3)	Day 6 (n= 3)	Day 7 (n= 3)	Day 8 (n= 3)	Total (%)
E. coli	1 33.33%	1 33.33%	2 66.67%	2 66.67%	3 100%	3 100%	1 33.33%	1 33.33%	14 40%
Salmonella spp.	0 0.0%	2 66.67%	1 33.33%	1 33.33%	3 100%	3 100%	0 0.0%	0 0.0%	10 28.58%
Staphylococcus spp.	1 33.33%	3 100%	3 100%	3 100%	1 33.33%	0 0.0%	0 0.0%	0 0.0%	11 31.42%
Total	2 5.71%	6 17.14%	6 17.14%	6 17.14%	7 20%	6 17.14%	1 2.86%	1 2.86%	**35** **100%**

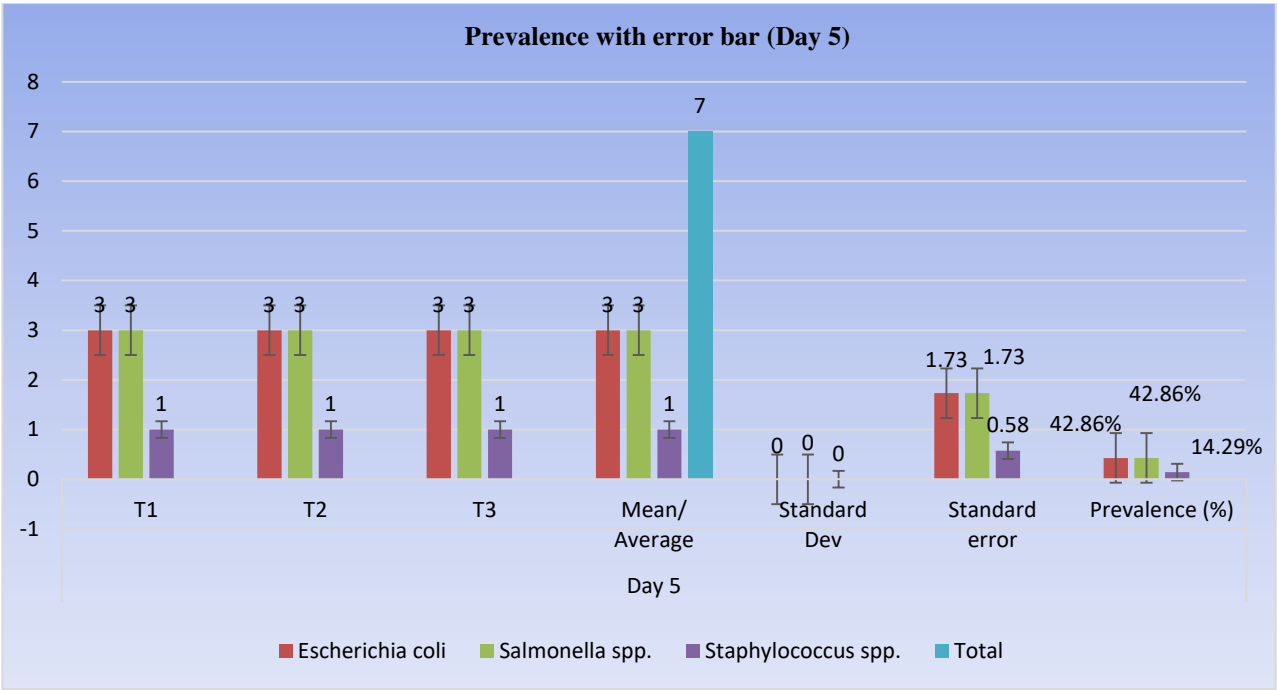

Fig. 2: Prevalence of infection in Day 5 with error bar.

Molecular detection of isolates by PCR

After confirmation by cultural and biochemical test, *E. coli*, *Salmonella* spp., and *Staphylococcus* spp., were subjected to molecular confirmation. After PCR amplification with specific primers and specific gene *E. coli* band were detected 232 bp, *Salmonella* spp., 284 bp and *Staphylococcus* spp., 1267 bp respectively. The PCR bands are represented in **Fig. 3, 4**, and **5**.

A. Molecular confirmation of isolated *E. coli* by PCR technique using *Eco*-223 and *Eco*-455 primers

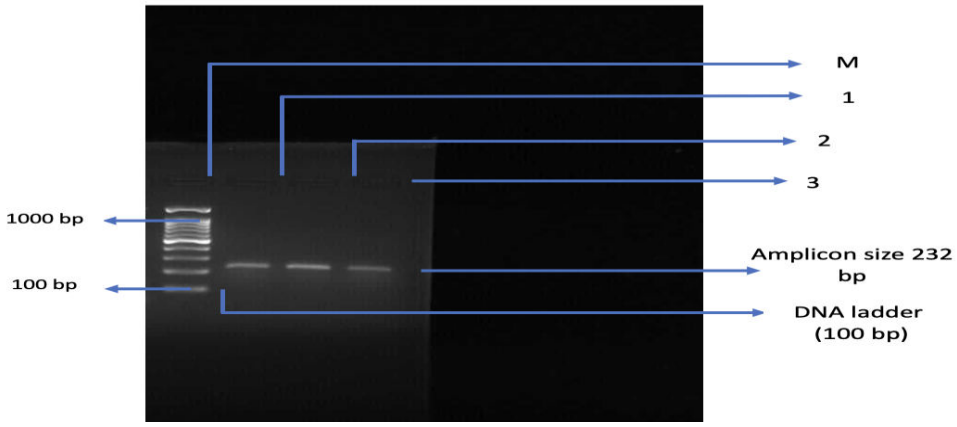

Fig. 3: *16s* and *23s RNA* genes of *E. coli* detected by *Eco*-223 and *Eco*-455 primers design confirming 232 bp. M: 100 bp DNA Ladder, NC: negative control, Lanes 1-3 field samples.

B. Molecular confirmation of *invA* gene of *Salmonella* spp.

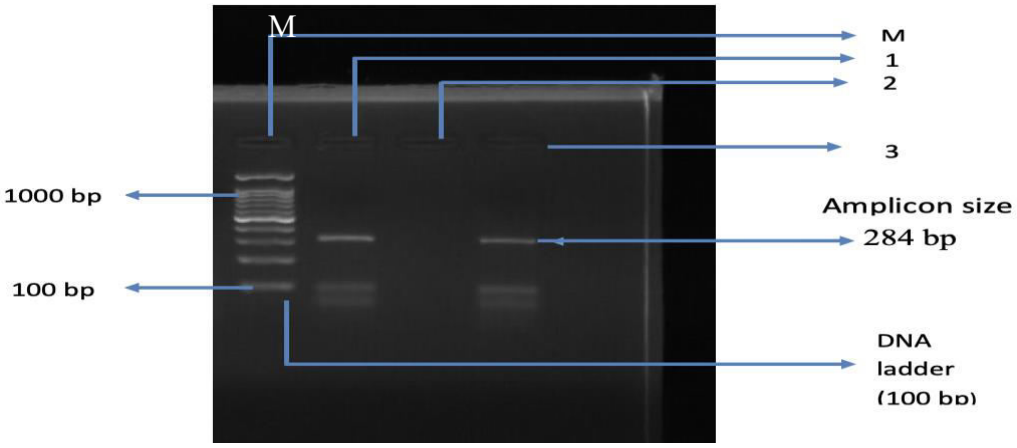

Fig. 4: *invA* genes of *Salmonella* spp. detected by *invA*-139 and *invA*-141 primers design confirming 284 bp. M: 100 bp DNA Ladder.

C. Molecular confirmation of *Sau-234* gene and *Sau-1501* gene of *Staphylococcus* spp.

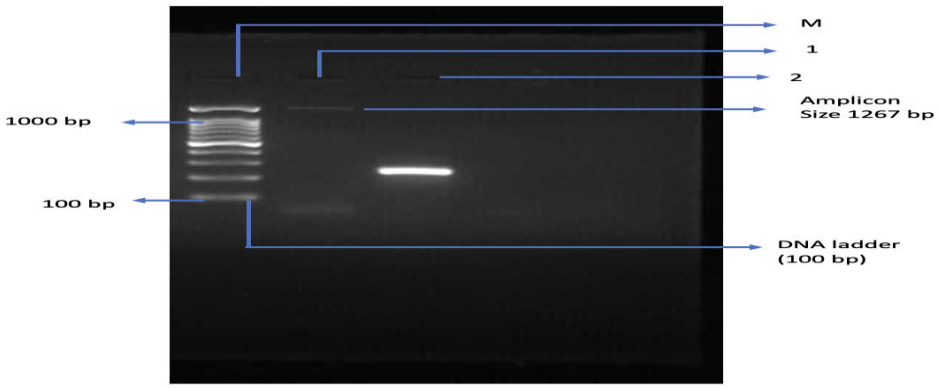

Fig. 5: 23S rRNA genes of *Staphylococcus aureus* detected by *Sau* – 234 (F) and *Sau* – 1501 (R) primer design confirming 1267 bp bands, L: Ladder.

Result of antibiotic sensitivity tests

A. Antibiotic sensitivity test by disc diffusion method on Mueller-Hinton agar

Table 7 showed that, *E. coli* was found to be highly resistant to Amoxicillin (100%), followed by Tetracycline (83.33%), Ampicillin (66.67%) whereas highly sensitive to Cefotaxime (100%), Gentamicin and Co-trimoxazole (83.33%) respectively. The analysis of *Salmonella* spp. sensitivity indicates high susceptibility to, Cefotaxime (83.33), Co-trimoxa-

zole (83.33) and Ceftazidime (83.33%), followed by Gentamicin (66.67%) respectively. However very high resistance of *Salmonella* spp. was detected to Amoxicillin (100%) and Ampicillin (100%) and to Tetracycline (66.67%) which is presented in **Fig. 6**. The resistance profile of *Staphylococcus* spp. was found to be highly resistance to Methicillin, Ampicillin (100%) followed by Gentamicin and Tetracycline (83.33%) whereas highly sensitive to Amikacin (100%) which are mentioned in **Table 8** respectively.

Table 7: Antibiotic resistance profile of *E. coli.*

Antibiotics name	*E. coli* Number of tested isolates, n=6			
	S		R	
	No.	%	No.	%
Amoxicillin	0	0	6	100.00
Gentamicin	5	83.33	0	0.00
Ceftazidime	4	66.67	0	0.00
Tetracycline	0	0.00	5	83.33
Levofloxacin	1	16.67	2	33.33
Ampicillin	2	33.33	4	66.67
Co-trimoxazole	5	83.33	1	16.67
Cefotaxime	6	100	0	0.00

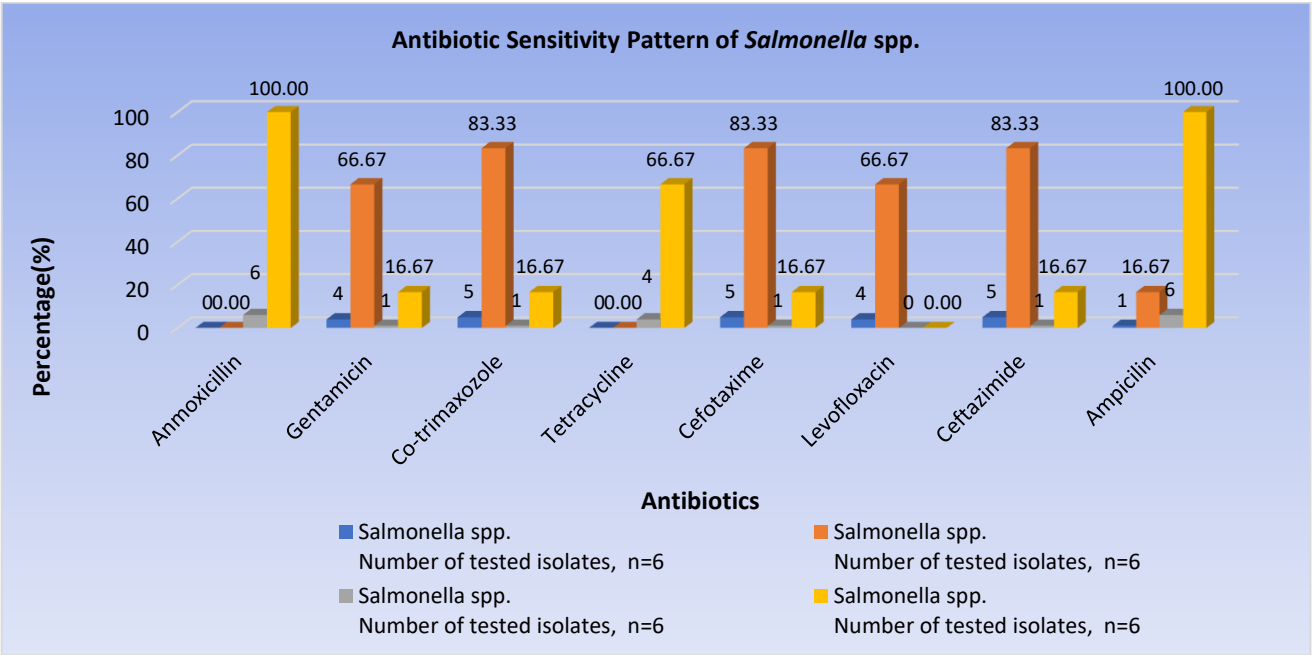

Fig. 6: Antibiotic resistance profile of *Salmonella* spp.

Table 8: Antibiotic resistance profile of *Staphylococcus* spp.

Antibiotics name	*Staphylococcus* spp. Number of tested isolates, n=6			
	S		R	
	No.	%	No.	%
Methicillin	0	0.00	6	100.00
Gentamicin	0	0.00	5	83.33
Azithromycin	4	66.67	0	0.00
Tetracycline	0	0.00	5	83.33
Amikacin	5	83.33	0	0.00
Levofloxacin	3	50.00	0	0.00
Ampicillin	0	0.00	6	100.00
Vancomycin	4	66.67	0	0.00

B. Multidrug resistance and MAR index calculation of isolates

Fig. 7, 8 and **9** represented the MAR index calculation of isolated *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. from different source of poultry hatcheries. In **Fig. 7**, the MAR Index ranged begin from 0.5 to 1.00 and the average MAR index being 0.77 in six isolates. In **Fig. 8**, the MAR Index ranged begin from 0.625 to 1.00 and the average MAR index being 0.79 in six isolates. In **Fig. 9**, the MAR Index ranged begin from 0.5 to 1.00 and the average MAR index being 0.77 in six isolates.

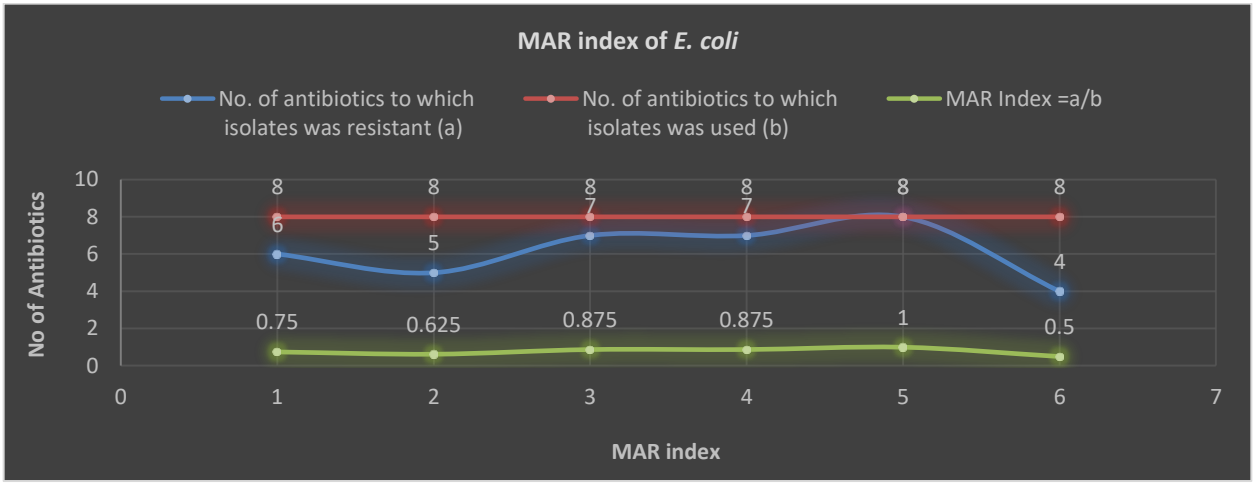

Fig. 7: MAR index calculation of *E. coli.*

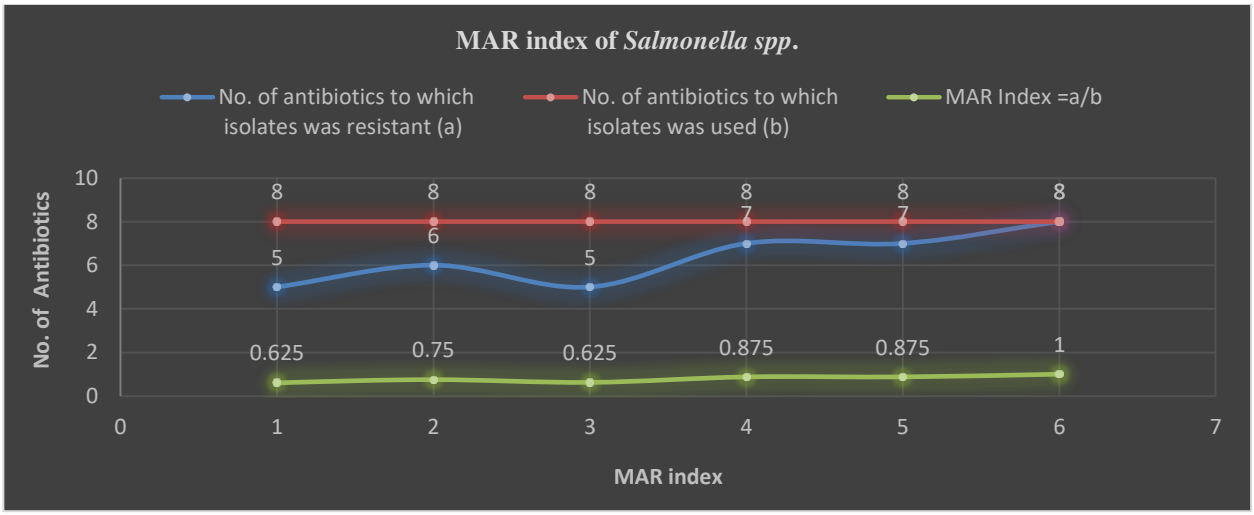

Fig. 8: MAR index calculation of *Salmonella* spp.

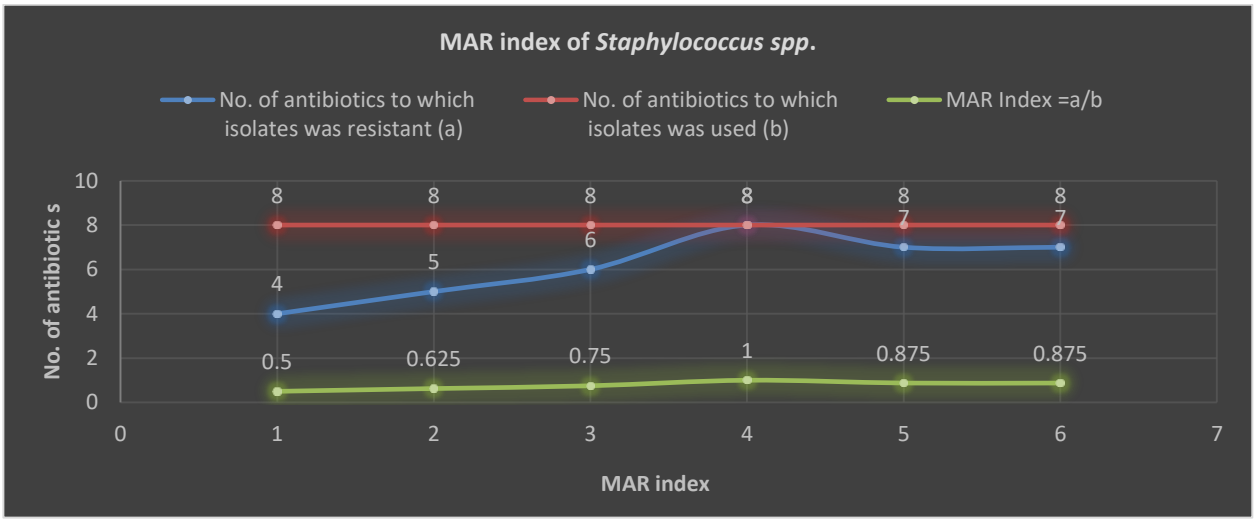

Fig. 9: MAR index calculation of *Staphylococcus* spp.

C. Disinfectant Resistance Profile of Isolates

Four commercially available disinfectants such as Lysol, Virocid, FAM 30 and EMSEN were used at 1% concentrations as per recommended dilution. The zone of inhibition was measured in mm on Mueller-Hinton agar with disinfectant against selected isolates. **Table 9** indicated that 1% virocid demonstrated the largest inhibition zone (36 mm) among *E. coli* followed by, *Salmonella* spp. 27 mm and *Staphylococcus* spp. 24 mm respectively. *E. coli* showed 28 mm zone with 1% Lysol and *Staphylococcus* spp. showed 22 mm. In 1 % EMSEN disinfectant, 22 mm zone were measured in *E. coli*.

Table 9: Zone of inhibition of disinfectant measured in mm on Mueller-Hinton agar.

Isolates	Zone of Inhibition (mm)			
	Lysol (cons)	FAM 30/ Iodine	Virocid	EMSEN
E. coli	28	19	36	22
Salmonella spp.	15	17	27	17
Staphylococcus spp.	22	12	24	16

DISCUSSION:

It is important to note that bacterial agents play a significant role in hatcheries because they reduce the hatchability rate and impact the health of newly hatched chicks and their future performance. There is a lack of knowledge on the co-resistance of antibiotics and disinfectants in bacteria and antimicrobial resistance.

In the current study, a bacteriological examination of 24 different yolk swab samples from different hatcheries revealed that the recovered isolates *E. coli* 14 (40%), *Salmonella* spp. 10 (28.58%) and *Staphylococcus* spp. 11(31.42%), respectively. Our findings agree with the results of many scientists (Al-Khalaf *et al.,* 2010; Kirunda *et al.,* 2010; Azmy, 2010) who could identify similar isolates from omphalitis-suspected chicks. Moreover, Saad *et al.* (2023) identified bacterial isolates that contaminated hatchery chicks, includes *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp. Similarly, Alfifi *et al.* (2022) recorded the highest prevalence of *E. coli,* 45.8%, which is more or less similar to our findings. Due to climate and geographic differences, heavy contamination of the eggs, and improper handling and storage of hatching eggs, the isolation rate of bacteria may be different.

According to the results, Multi drug resistant *E. coli* was found to be highly resistant to Amoxicillin (100%) whereas highly sensitive to Cefotaxime (100%), Gentamicin, and Co-trimoxazole (83.33%); these results agreed with Ahmed, (2016) and Abdel-Tawab *et al.,* (2016). *Salmonella* spp. were sensitive to Ceftazidime, Co-trimoxazole, and Cefotaxime (83.33%). In comparison, it is highly resistant to Ampicillin and Amoxicillin (100%), and the findings agreed with Ahmed *et al.,* (2011) and Ashraf *et al.,* (2016) - consequently, *Staphylococcus* spp. Obtained resistance to Methicillin and Ampicillin (100%) whereas sensitive to Amikacin (83.33%), these results were similar to Eid *et al.* (2015) who observed in their research that *Staphylococcus* spp. were sensitive to Ciprofloxacin (73.30%). Similarly, Ahmed, (2016) and Abdel-Tawab *et al.* (2016) obtained *Staphylococcus* spp. were sensitive to Gentamicin 80%, whereas our findings were 100% resistant.

According to Adenaike *et al.* (2016), a MAR index of 0.2 or higher indicates a high-risk source of contamination, and a MAR index of 0.4 or higher is associated with human fecal sources of contamination. In this study, 40% of *E. coli* having a MAR index of 0.77, and 28.58% of *Salmonella* spp. having a MAR index of 0.79, while *Staphylococcus* spp. having 0.77. Regarding the molecular detection of virulence genes, PCR techniques were applied to detect and amplify *Eco-223*, *Eco-445* gene, *invA* gene, and *23S rRNA* genes. The PCR band of *E. coli* was 232 bp, *Salmonella* spp. 284 bp and *Staphylococcus* spp. 1167 bp was found by earlier researchers by Shahat *et al.* (2019). According to Naem *et al.,* (2023), several disinfectants were applied to detect disinfectant resistance isolates, including *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. However, our study demonstrated that *E. coli* was highly sensitive to 1% virocid with an inhibition zone (36 mm), followed by *Salmonella* spp. 27 mm and *Staphylococcus* spp. 24 mm, respectively.

Multi-drug resistance bacterial isolates from newly hatched poultry chicks are dangerous to public health and animal health. Therefore, it is most

important to remove these bacterial contaminants from poultry hatcheries by improving the level of hygiene in chick production in the hatchery's environment. This study suggests effective disinfectants instead of commercial resistance antibiotics to prevent omphalitis in newly hatched chicks.

CONCLUSION:

The occurrence of multi-drug resistance isolates (*E. coli*, *Salmonella* spp., *Staphylococcus* spp.) in broiler chicks with omphalitis may cause alarming issues for humans and animals due to the spread of causal agents from infected chicks. Effective disinfectants and antibiotics will be the best treatment for chicks' omphalitis. Antibiotics such as Cefotaxime, Co-trimoxazole, Amikacin and Gentamicin will be the best choice for omphalitis treatment, while disinfectants viroid and Lysol play a crucial role in preventing and controlling the spread of infection in poultry hatcheries. Additionally, poultry farmers should be aware of the use of antibiotics and their activity against pathogens that are responsible for chick's omphalitis. If poultry owners know pathogens and maintain proper hatchery guidelines, the chick's production loss will be reduced. Identifying the specific resistance genes that are responsible for omphalitis is a great challenge for poultry farmers. Therefore, more and more research on chick's omphalitis will be needed in the future for Bangladesh to reduce production loss and improve the chick's quality.

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CONFLICTS OF INTEREST:

The authors have no conflict of interests.

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