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Bacteria causing omphalitis in newly hatched chicks from broiler and layer flocks and their antibiotic profiles

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ABSTRACT

The study was conducted for the isolation and identification of bacteria causing omphalitis in layer and broiler chicks as well as to assess their antibiogram profiles. Samples were collected from Phenix hatchery, Gazipur. The swab samples from unhealed navel were subjected to isolation and identification of bacteria by cultural, staining and biochemical tests. The isolates thus obtained were studied for their in vitro antibiotic sensitivity by disc diffusion method against 5 commonly used antibiotics. *Escherichia coli* (28%), *Salmonella* spp. (38%), *Staphylococcus* spp. (34%) from broiler chicks and *Escherichia coli* (32%), *Salmonella* spp. (36%), *Staphylococcus* spp. (32%) from layer chicks were identified. *Salmonella* spp. was more prevalent in day 1-3 of broiler (22%), layer (23%). All the bacterial isolates tested exhibited higher susceptibility to Ampicillin, Gentamicin and Kanamycin at day 1-3 and resistant to Kanamycin and Tetracyclin at day 4-7. Multidrug Resistant Organisms (MDROs) were also found which suggested that appropriate antibiotic should be provided before antibiotic therapy of chickens. Data of this study would be helpful for prevention and control of omphalitis in hatchery.

INTRODUCTION

Poultry industry in Bangladesh is developing rapidly since 1980. It plays an important role in poverty alleviation and economic development of the country. The current approximate poultry population is 300 million (30 cores) including 50 million (5 cores) ducks and 250 million (25 cores) chicken (DLS, October 2012-13) and it includes broiler, layer, and duck. There are about 1000 hatcheries in Government and private sector (Farm poultry and Livestock survey 2010). The number of day- old- chicks (DOC) produced in Government farms stands at about 125 lakhs (12.5 million) to 250 lakhs (25 million) numbers per month whereas that of private farms is at about 80 lace (8 million) to 1 core (10 million) per week.

Infectious diseases are a major cause of poultry loss worldwide and considered as the most leading causes of economic loss that discourage poultry rearing in this Bangladesh. Such pestilences are due to a growth of microorganisms including of bacteria, virus, fungus, mycoplasma, parasites and protozoa. The diseases that are most important in chicks include paratyphoid infection, omphalitis, fowl cholera, and salmonellosis etc.

Omphalitis may be defined technically as an inflammation of the navel. As commonly used, the term refers to improper closure of the navel with subsequent bacterial infection (navel ill; mushy chick disease). It occurs during the first few days of life, so it cannot be considered transmissible from bird to bird. It is transmitted from unsanitary equipment in the hatchery to newly hatched birds having unhealed navels. It has been reported that bacteria associated with omphalitis cause deterioration and decomposition of essential residual yolk sac (RYS) nutrients that should have been used as a source of energy in the post- hatch (Khan et al., 2004). Omphalitis has been reported in chicks in Bangladesh (Rahman et al., 2007).

Bacterial infection of navel area is one of the most common causes of mortality in chicks during the

first week after hatching (Pattison *et al*., 2008). Several bacteria such as *E. coli. Salmonella* spp. *Proteus* spp. *Enterobacter* spp. *Pseudomonas* spp. *Klebsiella* spp. *Staphylococcus* spp. *Streptococcus* spp. *Clostridium* spp. *Bacillus cereus* and Enterococcus have been isolated from the yolk sac infection of birds (Shane, 1999; Cortes *et al*., 2004). *E. coli* is the most common cause of omphalitis in chicks (Ahmed et al., 2009). The egg shells are known to be the major source of transmission of Salmonella infection in hatcheries (Cox *et al*., 1990). The recent nomenclature of *Salmonella pullorum* is Salmonella enterica sub species enterica serovar gallinarum bio var pullorum and the nomenclature of *Salmonella gallinarum* is Salmonella enterica sub species enterica serovar gallinarum biovar gallinarum Salmonella species can persist 4 to 5 years within the hatchery (Friend and Franson, 1999). The presence of Salmonella in the fertile eggs may result in embryonic deaths and abnormal hatching (Lecoanet, 1992). The infection of neonatal poultry results from insufficient immune defenses and inadequate macrophage phagocytosis (Henderson et al., 1999).

In this study we investigated the isolation and identification of bacterial agents responsible for omphalitis in newly hatched chicks from Broiler and Layer flocks in Gazipur.

MATERIALS AND METHODS

Sampling areas and period

A total 100 navel swabs were aseptically collected from broiler (n=50) and layer (n=50) chicks using sterile cotton swabs that were suffering from omphalitis about 1 to 7 days old chicks from the Phenix hatchery of Gazipur district in Bangladesh during the period from July 2014 to December 2014. After collection, samples were brought to the Bacteriological laboratory in the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh.

Isolation of bacterial agents

The collected Navel swab samples were enriched into nutrient broth by incubation at 37ºC for 24 hours. To isolate and to study the cultural properties of the bacterial agents enriched cultures were streaked onto different types of differential and selective culture media like Nutrient agar, EMB agar, Blood agar, SS agar, MS agar and XLD agar according to the method described by Cowan, 1985 where all of the media were brought from the Indian company, HiMedia.

Identification of bacterial agents

For identification of isolated *E. coli*, *Salmonella* spp. and *Staphylococcus* spp., Gram's staining; Motility test and biochemical tests were performed. Gram's staining was performed according to the method described by Merchant and Packer, 1967 where all of the reagents like crystal violet, Gram's iodine, safranin, acetone alcohol, immersion oil was brought from the German company, Merck. The motility test was performed to differentiate motile bacteria from non-motile one (Cheesbrough, 1984). Different types of biochemical tests like sugar fermentation test, Catalase test, Coagulase test, Indole test, MR-VP test was performed according to the procedure described by (Cowan, 1985), (Cheesbrough, 1984) and (Carter, 1979).

Antibiotic sensitivity test

Antimicrobial drug sensitivity test was performed on freshly prepared, dried up Mueller Hinton agar (Oxoid) against 5 commonly used antibiotics by disc diffusion method or Kirby-Bauer method (Bauer et al., 1966) according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS 2007) to assess the susceptibility and resistance pattern of the isolates. Antimicrobial discs were purchased commercially (Oxoid,UK). The selected antibiotics used were ampicillin (10 µg/disc), amoxicillin (10 µg/disc), tetracycline (30 µg/disc), kanamycin (30 µg/disc), gentamicin (10 µg/disc).

Maintenance of stock culture

Twenty parts of pure glycerine and 80 parts of PBS were mixed to make 20% sterile buffered glycerine. A loop full of thick bacterial culture was mixed with 20% sterile buffered glycerine in small vials and preserved at -20°C.

RESULTS AND DISCUSSION

Clinical signs

All the chicks processed for the study (Layer and Broiler birds) manifested the clinical signs of omphalitis characterized by poorly healed navels, reddish or bluish color of the abdominal muscles around the navel. The postmortem examination revealed an unabsorbed yolk sac in chicks. Theunabsorbed yolk sac was distended and hyperemic.

Bacteriological findings

Three genera of bacteria such as *E. coli, Salmonella* spp. and *Staphylococcus* spp.were isolated and identified from the suspected layer and broiler chicks with omphalitis. Cultural characteristics of *E. coli, Salmonella* spp. and *Staphylococcus* spp. on various media are summarized in Table 1.

Gram's staining and motility profiles

Isolated bacteria from different pure culture media were examined under compound microscope after Gram's staining (Figure 4, Figure 5, Figure 6) and motility profiles of bacteria was observed under microscope after hanging drop slide preparation

Table 1
Summary of cultural characteristics of bacteria isolated from yolk swab of broiler and layer chicks.

Bacterial isolates	Nutrient agar	EMB agar	Blood agar	Mannitol salt agar	SS agar
E. coli	Smooth, circular, white to grayish white colony	Smooth, large, circular, blue-black colonies with green metallic sheen	Colorless colonies without hemolysis	ND	Slight growth and pink to rose-red colony
Salmonella spp.	Circular, smooth opaque and translucent	Pink color, circular and smooth colony	Non-hemolytic colonies	ND	Black centered smooth, small, black colony
Staphylococcus spp.	Round, flat colonies of sticky, mucoid consistency	Slight growth with colorless colonies	Round, grayish and mucoid colony with musty odour and no hemolysis produced	Ferment mannitol and changes the color of the medium from pink to yellow due to acid by products and produce small gray white or yellowish colonies	ND

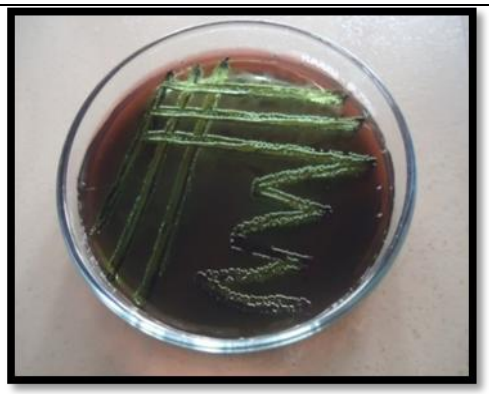


Figure 1
Blue-black colonies with green metallic sheen of *E. coli* on EMB agar (Broiler).

Figure 2
Black centered colony of *Salmonella* spp. On XLD agar (Broiler).

Figure 3
Colorless colonies of Staphylococcus spp.on Blood agar (Layer).

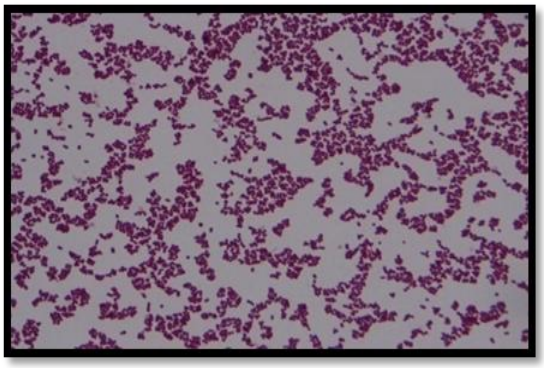


Figure 4
Gram positive cocci shaped bacteria arranged in grapes like cluster indicative of *Staphylococcus* spp. (400X).

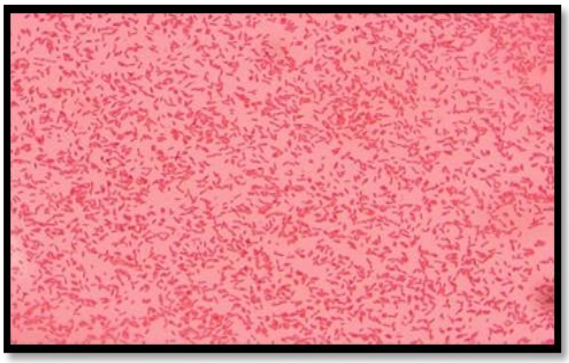


Figure 5
Gram negative single or paired short plump rods of *salmonella* spp. (400X).

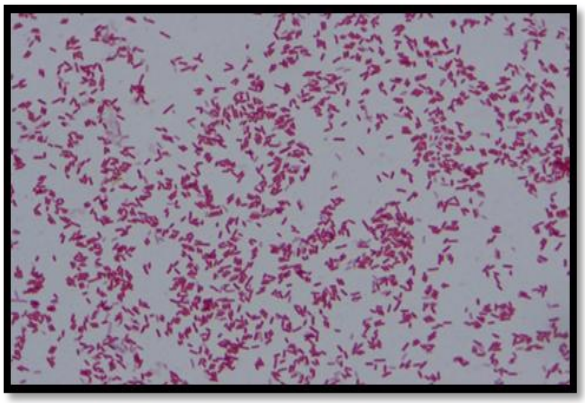


Figure 6
Gram negative or paired very short plump rods of *E. coli* (400X).

Biochemical properties of the bacterial isolates from Layer and Broiler chicks

Biochemical test

All five basic sugar such as: dextrose, maltose, lactose, sucrose and mannitol were fermented by *E. coli* with acid and gas production , *Salmonella* spp. gave positive results with dextrose, maltose and manitol with acid and gas production, *Staphylococcus* spp. fermented all five basic sugars with only acid.

Motility test

The results of motility test of bacterial isolates obtained from this study presented in Table 2.

Table 2
Motility test results of different bacterial species.

Growth in MIU medium	Results	Isolated bacteria
No turbidity and no changing of color of media	Non-motile	*Staphylococcus* spp.
Turbidity and changing of color of media	Motile	*Salmonella* spp.
Turbidity and changing of color of media	Motile	*E. coli*

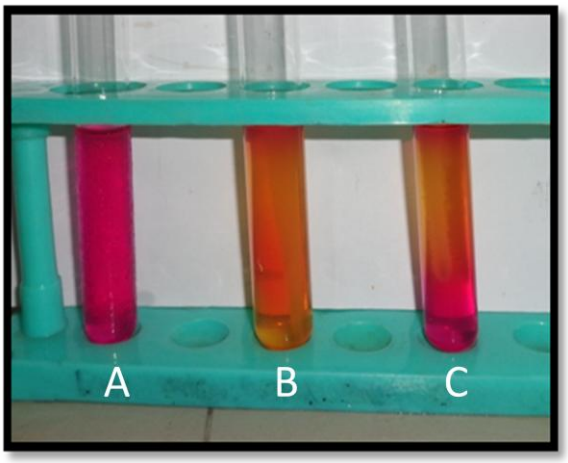


Figure 7
Motility test of *Staphylococcus* spp. (A) *Salmonella* spp. (B), *E. coli* (C).

Bacterial flora isolated from yolk of diseased Layer chicks

E. coli (32%), *Salmonella* spp. (36%) and *Staphylococcus* spp. (32%) were isolated from the 50 bird sample suffering from omphalitis. The summary of isolation of bacteria from Phenix hatchery is presented in Table 3. The prevalence of *E. coli*, *Salmonella spp.* and *Staphylococcus spp.* in omphalitis in Layer chicks in this study is shown in a pie chart (Figure 8).

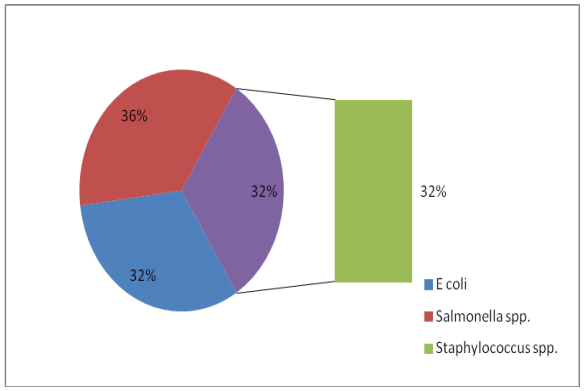


Figure 8
Number of bacteria isolated from the navel swab of Layer chicks suffering from omphalitis.

Table 3
Summary of results of *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. from navel swab of Layer chicks suffering from omphalitis in Phenix hatchery ltd.

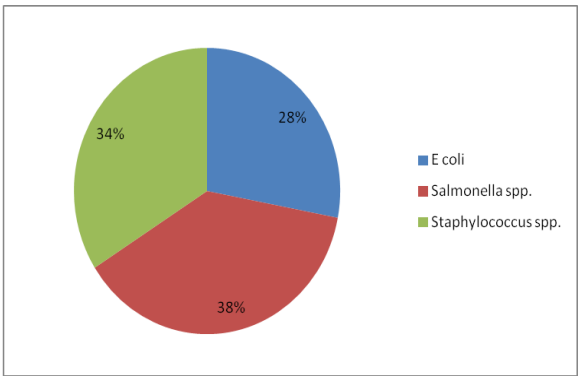
Chicks age	No of samples tested	No of positive samples		
		E. coli	*Salmonella* spp.	*Staphylococcus* spp.
1-3 days old	25	12	16	10
4-7 days old	25	11	10	13

Table 4
Summary of results of *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. from navel swab of Broiler chicks suffering from omphalitis in Phenix hatchery.

Chicks age	No of samples tested	No of positive samples		
		E. coli	*Salmonella* spp.	*Staphylococcus* spp.
1-3 days old	25	10	20	17
4-7 days old	25	15	18	11

Bacterial flora isolated from navel swab of diseased Broiler chicks

E. coli (28%), *Salmonella* spp. (38%) and *Staphylococcus* spp. (34%) were isolated from the 50 bird sample suffering from omphalitis. The summary of isolation of bacteria from Phenix hatchery ltd. are presented in Table 5. The prevalence of *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. in omphalitis in Broiler chicks in this study is shown in a pie chart (Figure 9).



Number of bacteria isolated from the navel swab of chicks (Broiler) suffering from omphalitis.

Results of antibiotic sensitivity tests

A total of five isolates of *Salmonella* spp. *Staphylococcus* spp. and *E. coli* were selected randomly from day 1-3 and day 4-7 from broiler and layer birds for antibiotic sensitivity assay. The results of antibiotic sensitivity assay are presented in Table 5, 6 and 7.

Figure 9

Table 5
Antimicrobial profile of *Staphylococcus* spp.

Antimicrobial agents	Symbol	Diameter of zone of inhibition (??)		Day 1-3	Day 4-7	Interpretation	
						Day 1-3	Day 4-7
		Day 1-3	Day 4-7				
Ampicillin	AMP	29	12	5(100%)	5(100%)	S	R
Amoxicillin	AMX	17	20	3(60%)	2(40%)	S	S
Gentamicin	GEN	20	19	4(80%)	1(20%)	S	S
Kanamycin	K	19	13	4(80%)	5(100)%	S	R
Tetracyclin	TE	11	15	5(100%)	3(60%)	R	S

Table 6
Antimicrobial profile of *E. coli.*

Antimicrobial agents	Symbol	Diameter of zone inhibition		Day 1-3	Day 4-7	Interpretation	
						Day 1-3	Day 4-7
		Day 1-3	Day 4-7				
Ampicillin	AMP	10	5	5(100%)	4(80%)	R	R
Amoxicillin	E	9	10	5(100%)	3(60%)	R	R
Gentamicin	GEN	20	16	4(80%)	2(40%)	S	S
Kanamycin	K	15	12	4(80%)	4(80%)	I	R
Tetracyclin	TE	12	18	2(40%)	3(60%)	I	S

Table 7
Antimicrobial profile of *Salmonella* spp.

Antimicrobial agents	Symbol	Diameter of zone		Day 1-3	Day 4-7	Interpretation	
						Day 1-3	Day 4-7
		Day 1-3	Day 4-7				
Ampicillin	AMP	11	5	5(100%)	5(100%)	R	R
Amoxicillin	E	12	14	5(100%)	2(40%)	S	I
Gentamicin	GEN	25	14	4(80%)	2(40%)	S	I
Kanamycin	K	20	11	5(100%)	4(80%)	S	R
Tetracyclin	CIP	28	15	5(100%)	3(60%)	S	R

In this study the characteristic clinical sign of omphalitis were observed in both broiler and layer chicks characterized by thickened hemorrhagic lesions in the navel area were found in case of omphalitis. Similar types of findings were also mentioned by Nasrin *et al*., 2012; Sarma *et al*., 1985; Zahdeh *et al*., 1987. After postmortem examination of both layer and broiler chicks it was found that chicks with navel buttons had larger yolk sacs than chicks with healed navels as reported earlier by Kawalilak*et al*., 2009. In the present study, omphalitis was observed both in layer and broiler birds in the age of 1-7 days and mortality due to omphalitis recorded in 3-5 days old layer and broiler chicks. Results of this study also in agreement with the findings of Cortes *et al*., 2004; Nasrin *et al*., 2012, which noticed peak mortality in 4-5 days old chicks and recorded declined mortality pattern thereafter until 7 days post hatch.

E. coli, Salmonella spp. And *Staphylococcus* spp. were identified as the major bacterial species responsible for omphalitis in broiler and layer chicks examined. These bacterial agents were also found to be associated with omphalitis in chicks earlier by Nasrin *et al.* (2012), Sato *et al.* (1961); Ijaz *et al.* (1994).

The highest 23% incidence of *Salmonella* spp.was recorded in layer of 1-3 days and in 4-7 days it was 14% and which are in case of broiler it was 22% and 12%. Glover *et al.*, 1944 reported highest incidence of *salmonella* spp. in case of omphalitis.

Characterization of the isolates based on the multiple antimicrobial resistance (MAR) was an important part of the study since the results indicated that chickens plays important role as reservoirs of multi-drug resistant bacteria. In case of broiler and layer chicks, it is important to determine the antibiotic sensitivity of *E. coli* isolates so that ineffective antibiotics can be avoided. In this study the highest resistance was found with Ampicillin, Amoxicillin, and Kanamycin at day 4-7. In this study, *E. coli* that isolated from the broiler chicks were 80% sensitive to Gentamicin at day 1-3 and 40% sensitive at day 4-7. Most *E. coli* isolates were resistant to Ampicillin and Amoxacillin. These findings were more or less similar to the Abadi *et al*., 2013, Akond *et al*., 2009. In this study, it was found that the *Salmonella* spp. isolated from these chicks almost all were sensitive to Kanamycin, Gentamicin and Amoxicillin and Tetracyclin at day 1-3 that were partly similar to Nasrin (2011). It was obseverd that coagulase-negative *Staphylococcus* spp. were 100% sensitive to Ampicillin, Amoxicillin, Gentamycin and Kanamycin at day 1-3.

Mixed bacterial infections were the predominant cases recorded due to *E. coli* together with any three species such as *Staphylococcus aureus*, *Proteus mirabilis* and rarely with *Bacillus cerus*. *E. coli* has been previously reported as one of the most frequently isolated organisms involved in the development of yolk sac infection or omphalitis (Rosario *et al*., 2004).

The variation in antibiogram profile might be due to indiscriminate use of antibiotic for treatment purposes in birds which results in enzymatic degradation, mutation at binding sites, down regulation of outer membrane proteins, efflux pumps and transduction of genes in bacterial isolates (Sharada RG 1999). The antibiotic resistance of *Salmonella* strains of avian origin is attributed to chromosomal mutation, gene transfer mechanisms like conjugation, transduction and transformation.

CONCLUSIONS

Present study was performed to isolate and characterize bacterial agents responsible for omphalitis in broiler and layer chicke. Total 50 chicks of layer and 50 chicks of broiler were collected from the Phenix hatchery ltd. And yolk sac swab samples were collected from those chicks. Omphalitis was more prevalent at the age of 1-3 days. *E. coli*, *Salmonella* spp. And *Staphylococcus* spp. was identified as the major bacterial species responsible for omphalitis in broiler and layer chicks examined. Many of the isolates tested were found to be multi drug resistant against three antibiotics such as ampicillin, amoxicillin and erythromycin. Antibiogram results might be helpful for the veterinarian in the study area to select appropriate antibiotic to treat omphalitis in chicks.

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