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# Antimicrobial sensitivity of bacterial pathogens isolated from day old broiler

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## ABSTRACT

The intention of this study was to determine the bacterial pathogens from the liver and cloacal swab of day old chicks (DOC) with their characterization. For this purpose a total of 50 (Fifty) DOC were collected from 05 (Five) hatcheries. Samples from vital organ like liver and cloacal swabs were examined bacteriologically for the presence or absence of bacteria. For the identification and characterization of bacteria morphology, staining, cultural, biochemical properties, molecular characterization and antibiogram studies were accomplished. The highest load was found *Escherichia coli* (52%), *Salmonella spp.* (38%) and *Staphylococcus spp.* (36%). Antibiogram profiles revealed that *E. coli* and *Salmonella* was strongly sensitive to Colistin sulphate and Gentamycin, moderately sensitive to Cefixim, Ciprofloxacin and Neomycin and less sensitive to Doxycycline, Norfloxacin, Levofloxacin and Tetracyclin.

**Keywords:** Day old chicks, *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., antibiogram.

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## INTRODUCTION

Bacterial load in Day Old Chicks (DOC) mainly depends on breeder performance, such as location and sanitary condition of the parent's stock farm, cleanliness of feeder and drinker, quality of feed, condition of litter used, ventilation, bio-security system, prevention and control measures of mycoplasma and bacterial diseases with antibiotic and proper vaccination program. The types and quantity of bacteria in DOC also depends on collection of eggs, hygienic condition of nest boxes, egg-belt, egg-cleaning systems, egg sanitation, egg fumigation, eggs storage systems, egg storage periods and hatchery conditions.

Among the infectious disease colibacillosis, avian salmonellosis and staphylococcosis are more common and newly hatched chicks and/or Day-old-chicks carry causative organisms of those diseases. Avian colibacillosis is an infectious disease of birds caused by *Escherichia coli*, which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions, either as primary pathogen or as a secondary pathogen. It causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, cellulitis and salpingitis and peri-hepatitis (Calnek et al., 1997). Staphylococcosis, an infectious disease caused by *Staphylococci* that affects all bird species. The disease is generally transmitted in feed, litter, and water; it may also be transmitted transovarially, that is, by way of the egg (Shareef et al., 2009). Acute avian staphylococcosis in chickens is marked by diarrhea, depression, and inflammation of the joints; the birds die in two to

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six days. The chronic form of the disease is characterized by lameness, anorexia, severe thirst, and cessation of egg laying; affected birds die in 10 to 14 days (Kassaye et al., 2009). Fowl typhoid and Pullorum disease are septicaemic diseases primarily of chickens and turkeys. The diseases are caused by Gram-negative bacteria, *Salmonella gallinarum* and *Salmonella pullorum* respectively. Pullorum disease is usually confined to the first 2-3 weeks of age occasionally occurs in adults (Kassaye et al., 2009).

Most of these bacteria have the antimicrobial resistance because of repeated exposure due to unhygienic condition and lack of proper knowledge of usages of antibiotics to poultry industry. As a result, the bacteria are growing resistant to antibiotic owing to improper use. It is, therefore, important that sensitivity of different bacteria isolated from breeder and commercial poultry farm needs to be studied from time to time in order to formulate appropriate therapeutic measures (Kaura et al., 1988).

Subsequently more powerful and /or new antibiotic is being needed to deal with the altered bacterial population. In this way, the bacteria become more and more resistant to antibiotics and new generation of antibiotics are being developed. Considering the above points of consideration the present study was undertaken to evaluate antimicrobial sensitivity of bacterial pathogens isolated from day old broiler chicks.

## MATERIALS AND METHODS

### Experimental birds

Day old broiler chicks were collected from five (05) hatcheries of Bangladesh (Kazi, CP, Aftab, Paragon, and NI) which were carried to the laboratory of Department of Microbiology Hajee Mohammad Danesh Science and Technology University, Dinajpur Bangladesh, during the period of July 2014 to December 2014.

### Sample collection

All samples (liver and cloacal swab) were collected from post mortem birds. Sterilized apparatus were used and samples were collected aseptically and transferred immediately into Petridishes and tubes containing peptone water. Then it was inoculated into Nutrient broth and incubated at 370C for 24 hours. After 24 hours it was streaked into Nutrient agar and MacConkey agar medium by inoculating loop.

### The experiment

After collection of samples, these were inoculated into nutrient both and then it was incubated at 37°C for 24 hrs. After 24 hours the incubated broth were streaked onto different bacteriological media (Brilliant green agar, Nutrient agar, Blood agar, MacConkey agar, Salmonella-Shigella agar, Eosin Methylene Blue agar) using inoculating loop.

Gram's staining, motility test and biochemical test were performed to characterized the organism. For biochemical test, stock nutrient broth was taken for sugar fermentation test in five basic sugars like dextrose, lactose, sucrose, maltose and mannitol to observe the production of acid and gas. Other biochemical test performed were indole test, MR-test and VP-test. Lastly antibiotic sensitivity test was conducted to know their drug sensitivity and resistance pattern.

### Isolation and identification of bacteria

Samples were cultured into different bacteriological media at 37ºC for 24 hours as mentioned above. The culture plates were examined for the colony characteristics produced by specific bacterial group. The morphological characteristics (shape, Size, surface texture, edge elevation, colour, opacity, etc) developed after 24 hours of incubation were carefully studied as described by Marchant and Packer (1967) and recorded. The representative bacterial colonies were characterized morphologically using Gram's stain according to the method described by Merchant and Packer (1967). The motility test was performed to differentiate motile bacteria from the non-motile one according to the method described by Cowan, 1985.

Various biochemical tests were performed for species identification. For this study isolated organisms with supporting growth characteristics of *E.coli* on EMB and Salmonella on SS and BGA

were subjected to various biochemical tests named carbohydrate fermentation tests, TSI agar slant reaction, MR-VP, MIU, Indole reaction and Citrate utilization test were carried out for identification of suspected *Salmonella* and *E. coli* (Cheesbrough, 1985).

**Characterization of bacteria**

Individually isolated colonies of the same morphology were selected from appropriate agar plates, cloned and checked for purity of growth prior to characterization of the respective genera and species. Characterization into respective genera and species were done on the basis of morphological cultural and biochemical and serological reaction. The classification and specification of organisms was based on the scheme presented in Bergey's Manual of Systematic Bacteriology (Holt and Wilkins 1986).

**Maintenance of stock culture**

The stock culture was maintained by Agar slant method and 20% sterile buffered glycerin method) following the procedures describe in paper published by (Buxton and Fraser (1977).

**Antibacterial sensitivity pattern of the isolated *Salmonella* spp. and *E. coli***

Susceptibility of the isolated *Salmonella* spp. and *E. coli* to different antimicrobial agents was performed to determine the drug sensitivity pattern and to interpret their diseases potential. Antibacterial disc were applied aseptically to the surface of the plate at an appropriate arrangement with the help of sterile forceps and incubated at 37ºC for 24 hours, aerobically (Carter, 1979). Antibiotic sensitivity pattern of isolated *E. coli* and Salmonella were performed against 14 commonly used antibiotics belonging to different groups. After incubation plates were examined and diameters of the zones of inhibitors for individual antibacterial agents were measured with compus scale and inputed the mm (diameter) on the antimicrobial sensitivity testing software (ABIS Online Encyclopedia, 2013) and then designated as highly sensitive ≤24, moderately sensitive ≥20, less sensitive ≤15 and resistant ≥10.

**Statistical analysis**

Data were entered into a database (spreadsheet of Microsoft Excel) and was analyzed with P value by using Graph pad software.

## RESULTS AND DISCUSSION

**Isolation and identification of bacteria**

***Gram's stain examination***

In Gram's staining, observation under compound light microscope the organism revealed Gram-negative character with red small bacilli shaped arranged in single or paired organism which are the characteristics of *E. coli*. Gram negative very short plump rod shape organism which are characteristics of Salmonella . Gram positive character with violate colour as well as the morphology of Cocci in change or pair which is the characteristics of *Staphylococcus* sp (Table 1).

Table 1
Characterization of isolated bacterial pathogens by Gram's staining technique.

| Gram's Staining | | | |
|---|---|---|---|
| Shape | Arrangement | Gram's staining reaction (+/-) | Identification |
| Short plump rods | Single, paired or in short chain | Gram negative | *E. coli* |
| Very short plump rods | Single | Gram negative | *Salmonella* spp. |
| Cocci arranged | grape- like clusters | Gram-positive | *Staphylococcus* spp. |

Table 2
Characterization of isolated bacterial pathogens by cultural properties.

| Name of Culture media used | *E. coli* | *Salmonella* spp. | *Staphyloccous* spp. |
|---|---|---|---|
| Nutrient agar | Smooth, circular, white to grayish colony with peculiar fetid odour | Small, round and smooth Colony | growth of circular, small smooth, convex, and golden yellowish colonies |
| Staphyloccous agar n110 | No growth (-) | No growth (-) | Golden yellowish color colony |
| Mac Conkey agar | Rose pink lactose fermenter colony | Colourless, pale, translucent colony. | No growth (-) |
| Eosin-Methylene Blue (EMB) agar | Moist circular colonies with dark centers yellow green metallic sheen | No growth (-) | No growth (-) |
| Salmonella-Shigella (SS) agar | Pink colour colony | Translucent colourless smooth Colony | No growth (-) |
| Briliant Green agar | - | Pinkish colony with pink background | |
| Simmons Citrate agar | - | Turn to bluish color | |

***Cultural examination***

The individual cultural characteristics of bacterial isolates are presented in Table-2.

***Biochemical tests***

For identification biochemical tests especially for *E. coli*, *Salmonella* and *Staphylococcus* were performed (Table 3).

Carbohydrate fermentation was positive in dextrose, lactose and maltose for *E. coli*, *Salmonella*, *Staphylococcus* spp. Some isolates produced a good amount of acid and gas while the other produced small amount which was exhibited by colour change and gas production in Durhams tube. *E. coli* produced acid and gas and colour change from reddish to yellow and gas production noted as gas bubble in inverted Durhams tube. *Salmonella* produced no acid or gas.

All types of the isolates (*E. coli*, *Salmonella and Staphylococcus* spp.) were MR positive and VP negative. *E. coli* was indole positive and *Salmonella* spp. were indole negative. In MIU test, all organisms showed positive. In TSI test all organisms showed positive but-yellow slant yellow except *Salmonella* spp. but –yellow slant-red and production of Hydrogen sulphate indicate black color.

***Motility test***

The straight forward movement of *E. coli* due to flagella present on one side acts as tail, for individual swirling of organism around the focus indicate *Salmonella* under microscope. On MSRV (Semi-solid Rappaport Vassiliadis) medium, migration of bacterial pathogen by forming a round circles around the inoculation point. In this case *E. coli* has no motility whereas *Salmonella* has motility capacity on this media.

Table 3
Characterization by biochemical reactions of *E. coli*, *Salmonella* spp. and *Staphylococcus* spp.

| Isolated organism | Indole production test | Methyl-red test | Voges-Poskauer reaction | Citrate utilization test | MIU test | TSI Test | Hydrogen sulphide |
|---|---|---|---|---|---|---|---|
| *E. coli* | + | + | _ | _ | + | Butt-Y Slant-Y | - |
| *Salmonella* spp. | _ | + | _ | _ | + | Butt-Y Slant-R | + |
| *Staphylococcus* spp. | _ | + | _ | _ | _ | Butt-Y Slant-Y | + |

Note : D = Differential biochemical types; + = Positive reaction; - = Negative reaction
MIU=Motility Indole Urease, TSI=Triple Sugar iron, Y= Yeloow, R= Red

Table 4
Percentage of *E. coli*, *Salmonella* and *Staphylococcus* on the basis of Isolated Organs of DOC.

| Isolated Organs | No. of chicks | No. of affected chicks | | | Percentage (%) | | |
|---|---|---|---|---|---|---|---|
| | | *E. coli* | *Salmonella* | *Staphylococcus* | *E. coli* | *Salmonella* | *Staphylococcus* |
| Liver | 50 | 21 | 19 | 16 | 42 | 38 | 32 |
| Cloacal swab | 50 | 26 | 16 | 12 | 52 | 32 | 24 |

Table 5
Percentage of *E. coli*, *Salmonella* and *Staphylococcus* on the basis of Hatcheries of DOC.

| Name of Hatchery | No. of chicks | No. of affected chicks | | | Percentage (%) | | |
|---|---|---|---|---|---|---|---|
| | | *E. coli* | *Salmonella* | *Staphylococcus* | *E. coli* | *Salmonella* | *Staphylococcus* |
| Kazi | 10 | 06 | 05 | 04 | 60 | 50 | 40 |
| Aftab | 10 | 04 | 03 | 03 | 40 | 30 | 30 |
| Paragon | 10 | 05 | 04 | 03 | 50 | 40 | 30 |
| Nilsagar | 10 | 07 | 05 | 04 | 70 | 50 | 60 |
| Cp | 10 | 04 | 02 | 02 | 40 | 20 | 20 |
| Total | 50 | 26 | 19 | 16 | 52 | 38 | 36 |

**Isolation of bacteria from different organs of day old chicks**

Different types of bacteria were isolated from the liver and cloacal swab of day old chicks which are present in table 4.

**Antimicrobial sensitivity test**

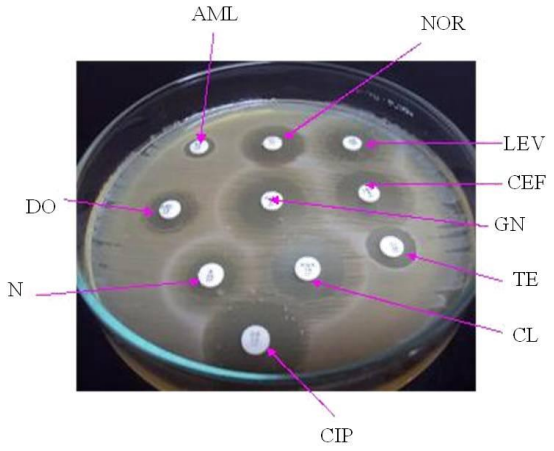
Isolated *E coli*. and *Salmonella* spp. were tested for antibiotic sensitivity against commonly used antibiotics. Zone of inhibition varies from 10-30 millimeter were characterized into resistant (-), weakly sensitive (+), moderately sensitive (++) and strongly sensitive (+++).

Table 6
Result of antibiotic sensitivity test of *E. coli* and *Salmonella* spp. day old chicks.

| Organism | Antibiotic disc use | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Do | AML | LEV | CIP | TE | NOR | N | CL | CEF | CN |
| *E. coli* | + | + | + | ++ | + | + | ++ | +++ | ++ | +++ |
| *Salmonella* | + | - | + | ++ | + | + | ++ | +++ | ++ | ++ |

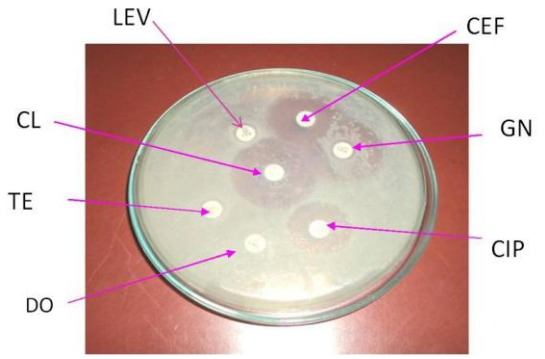
CIP = Ciprofloxacin; CN = Gentamycin; DO = Doxycycline; AML = Amoxycillin; TE = Tetracyclin; N = Neomycin; CL = Colistin Sulphate; LEV = Levoflxacin; NOR = Norfloxacin; CEF = Cefixim;
- = Resistance; + = Less sensitive; ++ = moderately sensitive; +++ = Highly sensitive

Both *E. coli* and *salmonella* spp. were found to be strongly sensitive to Colistin sulphate and Gentamycin was moderate sensitive to Cefixim, Ciprofloxacin and Neomycin and less sensitive to Doxycycline, Norfloxacin, Levofloxacin and Tetracyclin (Figure 1, 2)




GN = Gentamicin; CIP = Ciprofloxacin; AML = Amoxycillin; DO = Doxycycline; N = Neomycin; NOR = Norfloxacin; TE = Tetracyclin; LEV = Levofloxacin; CEF = Cefexin; CL = Colistin sulphate

Figure 1
Antibiogram of *E. coli.*




Legends: GN = Gentamicin; CIP = Ciprofloxacin; DO = Doxycycline CL = Colistin sulphate; TE = Tetracyclin; LEV = Levofloxacin; CEF = Cefexin

Figure 2
Antibiogram of *Salmonella* spp.

Isolation and identification of the microorganisms were confirmed by their morphology on different cultural media, staining characteristics and biochemical tests. Several selected culture media were used simultaneously in this study. The bacteriological media employed in this study were selected considering the experience of by Buxton and Fraser (1977), and Nazir *et al.* (2005). A total of 50 samples were examined for the isolation of bacteria of which 26 (52.00%) samples were positive for *E. coli*, 19 (38.00%) samples were positive for *Salmonella sp,* 18 (36.00samples were positive for *Staphylococcus* sp. The frequency of distribution of bacterial isolates in different samples was found variable. Result of the present study indicated that all the four different types of bacteria were not present in the same sample collected from the day old chicken just after hatching. The incidence of different types of bacteria isolated from collected sample of day old chicken, co-related with the findings of Shareef et al. (2009) and Kassaye et al. (2009) with slight variation.

In this study, colony characteristics of *E. coli* observed in NA, EMB, MacConkey agar/broth, BG, SDA, MS and SS agar were similar to the findings of Buxton and Fraser (1977), and Nazir et al. (2005). In Gram's staining, the morphology of the isolated bacteria exhibited Gram positive cocci in chains or pairs and Gram negative character with short rod arranged in single or paired and

motile which was supported by several authors Merchant and Packer (1967), Buxton and Fraser (1977), Freeman (1979).

In this study, simultaneously several different selective media were used to culture the organism because all of them are not equally suitable for all the serovers of *Salmonella* (OIE manual, 2006). In the present study, specific enriched media and biochemical tests were used for the isolation and identification of *Salmonella*, which was also used by a number of researchers (Lee *et al.,* 2003; Dhruba *et al.,* 1999; Cheesbrough 1985; Rahman 1977; Buxton and Fraser, 1977). In this study, colony characteristics of *Salmonella* spp. on MC agar, SS agar and BG agar were similar to the findings of other authors (Buxton and Fraser, 1977; Rahman 1977 and Hossain 2002). In Gram's staining, the morphology of the isolated *Salmonella* from chicken and rat exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope which was supported by other researchers (Cheesbrough, 1985; Freeman, 1985). In motility test, the isolates did not show swinging movement which differentiates the motile bacteria from non-motile and also from *E. coli*, so the isolated *Salmonella* were non-motile. Motility test was fundamental basis for the detection of motile and non-motile *Salmonella* organisms (Buxton and Fraser, 1977; Freeman, 1985; Hossain, 2002).

Antibiotic sensitivity pattern of isolated *E. coli* and Salmonella was performed after isolation against seven commonly used antibiotics belonging to different groups. From the antibiotic sensitivity study it was observed that *E. coli* was strongly sensitive to colistin sulphate, moderately sensitive to ciprofloxacin and these findings were almost similar to the reports of Nazir *et al.,* (2005). Less sensitive was to amoxicillin, ampicillin, oxytetracyclin and resistant to cotrimoxazole, Gentamycin and these findings almost similar to the report of Okoli *et al*., (2006). Rahma *et al*., (2004) reported *E. coli* was highly sensitive to ciprofloxacin. They stated that it might be due to fact that it had been recently introduced, had broad spectrum of action and limited used so far by the poultry farmers.

The antibiogram study revealed that most of the *E. coli* and *Salmonella* spp. were less sensitive to Doxycycline, Norfloxacin, Amoxicillin, Levofloxacin and Tetracycline. However, most of the *E. coli,* and *Salmonella sp.* were susceptible to Cefixim, Gentamycin and Colistine sulphate this is to indicate that the use of these antimicrobial may have the preference to be choice in clinical control of *Salmonella*.

The presence of bacterial pathogen suggested that strict hygienic and sanitary measures during and after hatching of day old chicks as well as the hygienic management of the breeder farms and proper control and prevention program against bacterial diseases were followed. From the antibiotic sensitivity study, it was observed that *E. coli and Salmonella* were sensitive enough to Cephalexin, Neomycin, Gentamycin and Ciprofloxacin suggesting that these antibiotics could be used for therapeutic purpose if there is occurrence of colibacillosis.

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