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Pathology of Colibacillosis and its Molecular Detection from Recent Outbreaks in Chicks

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Abstract

Colibacillosis is caused by avian pathogenic Escherichia coli (APEC) that affects poultry. This disease causes great economic losses. Prevention of colibacillosis is becoming challenging due to the emergence of virulent and multi drug resistant strain. Early detection is essential to prevent colibacillosis. For this reason, this study was designed to investigate the pathology and to detect causal agent of colibacillosis. Dead birds and cloacal swabs from sick live birds were collected and enriched in tetrathionate broth. Then the bacteria were characterized by different bacteriological media and biochemical test, and confirmed by PCR and histological studies. The organisms showed metallic sheen on EMB agar. It revealed pink colored, short rod to coccoid shaped, single or paired on Gram's staining. Escherichia coli was confirmed through biochemical test. Postmortem findings showed congested and consolidated lungs and liver. The intestine showed hemorrhage with mucus. Histologically, sections of heart showed pericarditis with thickening of pericardium. The livers exhibited coagulation type of focal necrosis with infiltration of heterophils and lymphocytes densely located in the portal area. These findings will help field veterinarians and also poultry farmers for effective management. However, further studies are warranted to identify the genes responsible for emergences of E. coli infection.

Keywords: Colibacillosis, Chicks, e. Coli, Pathology, PCR

Chickens are the most popular amongst different poultry species around the globe. Owing to their relatively low fat and cholesterol content, chicken meat is considered a healthy protein of animal origin for all age group of people irrespective of cultural belief. Chicken ranks to be the cheapest among the meats consumed globally [1]. Poultry is an important sub-sector of livestock and contributes 1.6% in national GDP, and approximately 6 million people work in this sector to earn livelihood in Bangladesh [2]. The chicken accounts for almost 36% of the whole animal protein sources in Bangladesh [3]. However, poultry industry is facing challenges with flock health issues associated with infectious diseases including colibacillosis, mycoplasmosis, salmonellosis, mycotoxicosis and Newcastle disease, and hindering expansion of poultry farming in Bangladesh [3]. Colibacillosis, the most common bacterial disease in chickens, possesses major health problem in chicken industry worldwide including Bangladesh [4]. Avian pathogenic E. coli (APEC) strains belong to the phylogenetic group linked with extra-intestinal pathogenic E. coli (ExPEC) and show a wide serological diversity among strains [5]. The mortality of chicks by APEC is less than 10% for non-virulent strains, 10-29% for intermediate strain and greater than 29% for virulent strains [4]. Poor navel health, damage of mucosa due to viral infections and immunosuppression are thought to be some predisposing factors

for colibacillosis in chickens. It causes economic losses both in broiler and layer in terms of morbidity, mortality, decreased egg productivity and chick quality [6]. APEC produces different types of lesions in chicks including colisepticemia, coligranuloma, peritonitis, osteomyelitis, omphalitis, swollen head syndrome, yolk sac infection, arthritis and synovitis [7]. Prevention and controlling of colibacillosis is becoming challenging due to the emergence of virulent and multidrug resistant strains [7]. E. coli is identified by cultural and staining properties, biochemical tests, gene detection and sequencing. However, works on characterization of colibacillosis from field outbreak is rare in Gazipur district, a poultry rearing hub in Bangladesh. Therefore, this study aimed to characterize pathology, cultural, staining and biochemical properties and molecular detection of E. coli from natural outbreak of colibacillosis in Gazipur district.

Materials and Methods

Ethics Statement

The experiments described in this manuscript were conducted in accordance with the principles of care and handling of experimental animals approved by the ethical committee of the Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU/ AREC/ 2023/26).

Collection of Samples

A total of 30 dead chicks (20 broilers and 10 layers) died of suspected colibacillosis were dissected, gross lesions were recorded and tissue samples from liver, lungs, spleen, intestine, heart and kidney were collected aseptically for culture and in 10% neutral buffered formalin for histopathology. Cloacal swabs from 20 clinically sick chicks (10 broilers and 10 layers), were collected aseptically in tetrathionate broth (TB) from the same flock [8].

Isolation and Identification of Bacteria

The bacterial population were increased by incubating the samples collected in TB at 37°C for 24 hr. The cultural characteristics such as shape, size, surface texture, edge and elevation and color, opacity of bacteria on different media were recorded and were followed by standard procedure [8].

Staining Character of Bacteria

Gram's staining was used to determine the size, shape and arrangement of bacteria as described previously [9].

Bacterial Motility Test

Bacterial motility test was performed by hanging drop slide technique [10].

Biochemical Tests

Biochemical test was performed by adding 0.2 ml of nutrient broth culture of pure isolated colonies into the test tube containing sugars namely glucose, sucrose, lactose, mannitol and maltose, and incubated at 37°C for 24 hours. Color change from red to yellow was indicated the acid production and bubbles in the inverted Durham's tube indicated gas accumulation [11].

Polymerase Chain Reaction (PCR)

DNA was extracted from E. coli isolate using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) and extracted DNA was quantified by using spectrophotometer (DU-640, Beckman, Germany) on the basis of optical density ratio at 260: 280 nm [10]. The primers were 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) used for the amplification 16S rRNA gene.

Agarose Gel Electrophoresis

Amplified products were analyzed by electrophoreses on 1.5% agarose gel containing 5 µg per ml ethidium bromide with a 100 bp ladder as molecular weight marker [10]. Bands were observed under UV light on a transilluminator (Labortechnic, Germany).

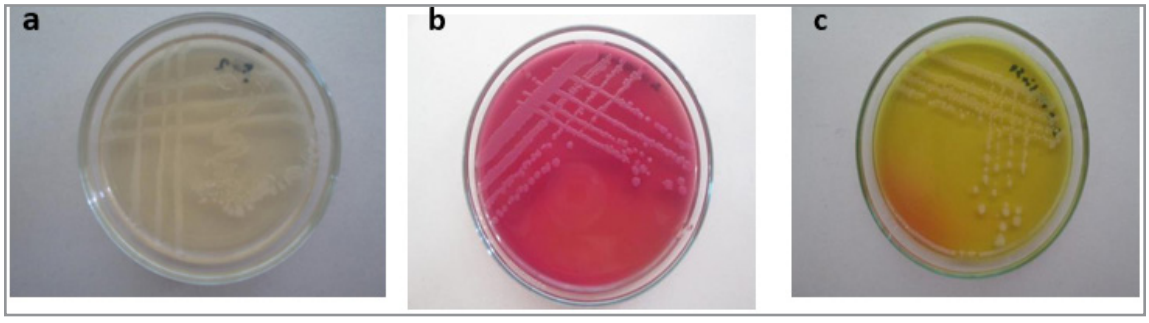
Histopathological Examination

The formalin fixed tissues were trimmed and re-fixed in 10% neutral buffered formalin (pH 7.4) for overnight. Tissues were placed in 70% ethanol, dehydrated through a series of graded ethanol, cleared in xylene and embedded in paraffin. Tissues sectioned at a thickness 5 µm were stained routinely with hematoxylin and eosin (HE) and mounted with Canada Balsam (Haider et al. 2003). Images were grabbed by using Photographic Microscope System (ZEISS AxioCam ERc 5s).

Results and Discussion

Cultural, Biochemical and Staining Properties

The organisms were shown different colony characters in different media (Fig. 1). Staining of bacteria showed short rod to coccoid shaped, single or paired Gram-negative bacilli (Fig. 2). The organisms fermented basic sugars such as dextrose, maltose, mannitol, arabinose, sucrose, lactose and dulcitol with production of acid and gas. Mannitol and dulcitol were fermented, and Inositol was not fermented by isolated E. coli. Acid production was indicated by the accumulation of bubbles in the inverted Durham's tube and color changes from reddish to yellow on TSI slant culture. The isolated bacteria did not ferment inositol. Active motility was observed on hanging drop slide prepared with broth culture.



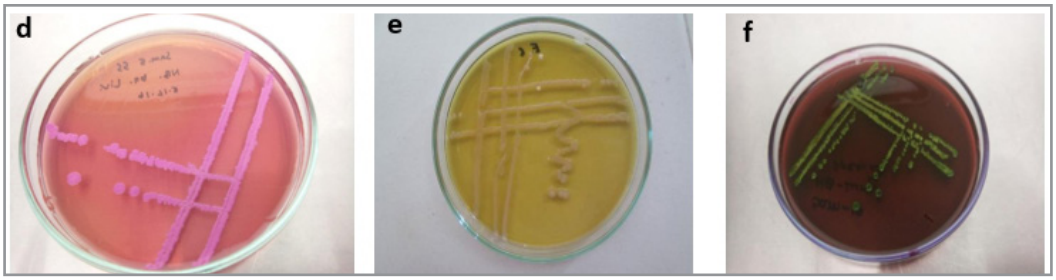


Figure 1: The Organisms Showed Round and White Colonies on Nutrient Agar (a), Bright Pink Smooth and Raised Colonies on Macconkey Agar (b), Yellowish Green Colonies Surrounded by an Intense Yellow Green Zone on Brilliant Green Agar (c), Pink Colonies on Ss Agar (d), Transparent Colonies on Tsi Agar (e) And Greenish Colonies with Metallic Sheen on Emb Agar (f) After Overnight Incubation.

The study was conducted for isolation, identification and characterization of the causal agent of colibacillosis and characterization of its pathology in naturally infected broiler and layer chicks. All isolates of E. coli produced pink colonies on MacConkey agar and greenish colonies with metallic sheen on EMB agar. Similar results were reported by different authors [10, 12]. Morphology of E. coli as evidenced by Gram's staining were pink colored, short rod to coccoid shaped, single or paired Gram-negative bacilli and on hanging drop slide all isolates were found to be motile [13]. In the present study all of the isolates of E. coli fermented dextrose, maltose, mannitol, arabinose, sucrose, lactose and dulcitol which are in agreement with the findings reported earlier [12].

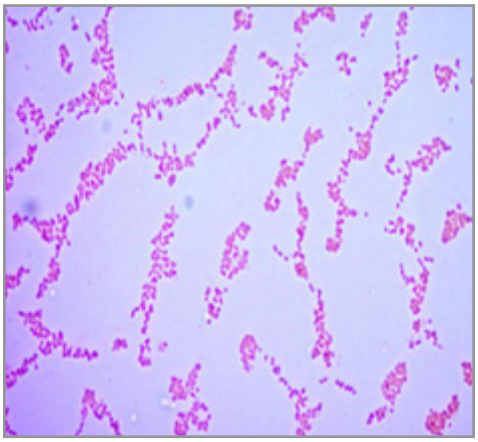


Figure 2: The Isolates of E. Coli were Pink Color Short Rod to Coccoid in Shape with Gram's Stain (100X).

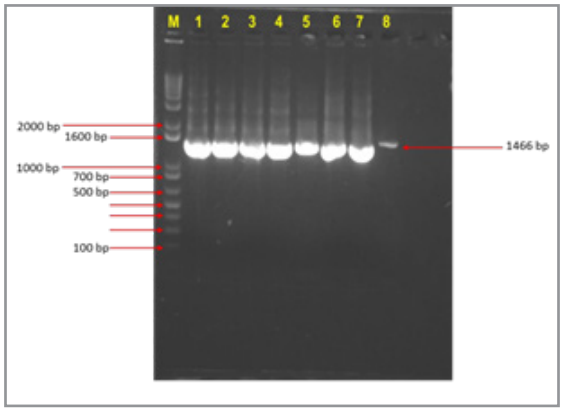


Figure 3: Amplified DNA Showed an Amplicon Size of 1466 bp on Agarose Gel Electrophoresis.

Polymerase Chain Reaction (PCR)

The extracted DNAs were amplified by using 27F and 1492R primer sets targeting 16S rRNA, and found 1466 bp products comparing with 100 bp ladder as evidenced by agarose gel electrophoresis (Fig. 3) which was supported by [14].

Gross Lesions

The necropsy examination revealed congestion and consolidation of lungs. The intestine showed profuse mucus, congestion and hemorrhagic enteritis, hemorrhage was also evident in the cecal tonsil (Fig. 5c). The air sacs were cloudy and opaque, and the livers were with whitish thick capsule indicating air saculitis and perihepatitis, respectively. In some cases, the livers showed necrotic foci and greenish hue (Fig. 5b). The spleens were enlarged and showed severe congestion with thickening of capsule (Fig. 5c). The pericardium was cloudy with moderate thickening indicative of pericarditis. Gross appearances showed in the lungs and intestines were congestions, consolidations and hemorrhages which were also extended to cecal tonsil. Presence of these lesions were variable in all dead birds and these changes could be due to the vascular damage caused by E. coli endotoxin which were corresponded with others [15].

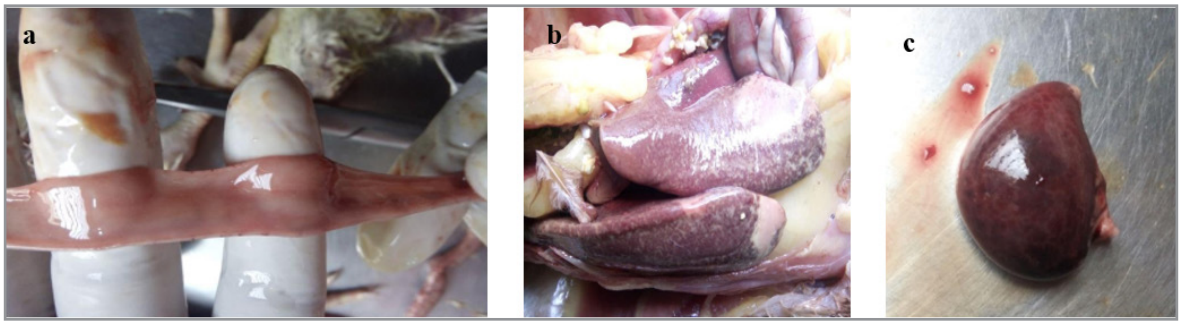


Figure 5: Gross Lesions of Colibacillosis: Intestine Showed Mucus and Congestion (A), Liver Exposed Multifocal Necrosis and Perihepatitis (B), And Hemorrhage and Necrotic Foci Were Seen in Spleen (c).

Microscopic lesions

Histopathological examinations of different organs were shown different types of lesions (Fig. 6). Histopathological evaluation exhibited intense damage of the pericardium and moderately inflammation of duodenum. The observed inflammatory and necrotic lesions in the liver, lungs and spleen of the affected birds were due to vascular injury by endotoxin released from E. coli [16]. The coagulative necrosis of liver with focal lesions could be due to tissue hypoxia associated with vascular damage [17]. Comparable focal necrosis and cellular infiltrations were documented earlier in the liver and heart of birds that died of colibacillosis [15]. Similarly, congestions of central veins and sinusoids with cellular infiltrations around the periportal area were described in the liver of broiler chicks suffering from colibacillosis [18].

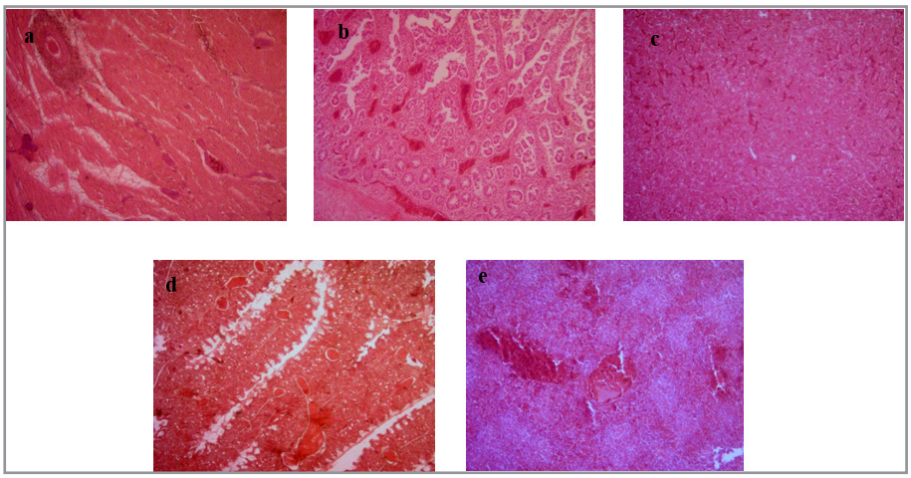


Figure 6: Section of Heart Showed Pericarditis with Infiltration of Heterophils (a). Necrosis and Infiltration of Heterophils and Lymphocytes in the Mucosa, Submucosa and Lamina Propya of the Intestine (b). Section of Liver Showed Necrosis of Hepatocytes and Infiltration of Heterophils (c). Section of Lung Showed Congestion in Blood Vessels and Sero-Fibrinous Exudation in Lungs (d). Spleen Showed Focal Necrosis and Severe Congestion, and Proliferation of Re Cell (e).

Conclusions

Colibacillosis is considered one of the leading causes of chick mortality in poultry industry in Bangladesh. Early diagnosis of colibacillosis based on the cultural and staining properties, post-mortem findings and confirmation PCR could be the choice for effective diagnostic methods. This study will boost up the poultry industry a rapid and reliable diagnosis and strategic management. Findings of this work will assist field veterinarian and practitioner to suggest the poultry farmers for the management of their farms in a better way.

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