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Isolation and biochemical characterization of avian pathogenic *Escherichia coli* from different organs in colibacillosis affected broiler chicken

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Abstract
The present study conducted in division of Veterinary Pathology, SKUAST Kashmir was aimed to isolate and biochemically characterize *Escherichia coli* from different organs of Colibacillosis affected broiler chicken. The affected live birds showed clinical signs ranging from lameness, retarded growth, head swelling, respiratory distress and ruffled feathers, whilst the dead birds were selected for the study. The dead birds on necropsy revealed lesions typical to Colibacillosis *viz*; Pericarditis, perihepatitis, spleenitis and peritonitis. For isolation of *Escherichia coli*, samples from heart, lung, liver and spleen were collected. Identification of isolates as *Escherichia coli* were carried out using standard cultural and biochemical tests like IMVIC. Spleen, heart and lung were the common samples found positive for *Escherichia coli* followed by liver. Furthermore the techniques used in this study *viz*; standard cultural and biochemical tests like IMVIC was found to be very efficient, accurate and certainly quicker to detect the *Escherichia coli* in Colibacillosis affected broiler chicken. This study was conducted in Ganderbal district of Kashmir Valley.

Keywords: Biochemical tests, broiler chicken, colibacillosis, *Escherichia coli*, samples

Introduction
Poultry farming in India, in spite of several constraints has progressed considerably during the last decade and represents a major success story. Poultry production has been rising at the rate of around 8 percent per annum as compared to agricultural production which has been found to be around 2 percent per annum over the past two to three decades (Mehta and Nambiar, 2010) [1]. The exploitation of avian genetic resources in recent years by selective breeding has led to evolution of superior strains of broiler chickens. However, the broiler production is constrained by many factors *viz*; sudden climatic changes, poor nutrition, poor management, absence of biosecurity that have imposed stress on poultry birds making them more vulnerable to various diseases (Calnek *et al*., 1997) [2]. Losses have also been attributed to limited housing, disease outbreaks and veterinary care services. However, the most important factors affecting poultry industry is diseases. Among diseases, avian colibacillosis caused by *Escherichia coli* (*E. coli*), is considered as one of the principal causes of morbidity and mortality either as primary pathogen or as a secondary pathogen (LutfulKabir, 2010) [3]. These infections occur in chickens of all age groups but broiler chickens of 4-6 weeks of age are found to be more vulnerable and severely affected with considerable mortality (Leitner and Heller, 1992) [4]. This bacterium *E. coli* is a gram-negative, motile, pleomorphic rod, facultative anaerobe from the family *Enterobacteriaceae* and has been found to grow rapidly in standard media and on some selective media as McConkey agar (Bergey's, 1994) [5]. Avian pathogenic *Escherichia coli* (APEC) bring about a variety of diseases in broiler chickens like air sacculitis, pericarditis, peritonitis and yolk sac infections. However, the most common form is respiratory tract infection followed by septicemia. The disease is mainly caused by the predominant serotypes O1:K1, O2:K1 and O78:K80 (Barnes and Gross, 1997) [6]. However, in Kashmir, the most prevalent serogroup is O2 followed by O1, O8 and O76 (Shah, 2017) [7]. This bacterium *Escherichia coli* has created havoc in commercial broiler industry interms of morbidity, mortality, condemnations and cost associated with treatment and prophylaxis, therefore the study related to isolation and biochemical characterization of this gram negative

organism is of prime importance interms of disease diagnosis in commercial broiler industry.

Materials and Methods

The present study conducted in division of Veterinary Pathology, SKUAST Kashmir involves the thorough and systematic examination of dead birds for examining and recording of lesions true to colibacillosis which included perihepatitis**,** pericarditis, peritonitis, airsacculitis and cellulitis. However, the history regarding clinical signs of affected birds was also recorded.

The isolation and identification of *E. coli* was carried as per the standard microbiological procedure (Buchanan and Gibbon, 1994) [8]. Representative samples from (heart, spleen, lung and liver) were inoculated in nutrient broth and incubated at 37 ºC for 24 hours following re-inoculation on MacConkey agar and again incubated at 37 ºC for 24 hours. The lactose fermenting colonies were re-inoculated on Eosin Methylene Blue agar and colonies producing metallic sheen were transferred to nutrient agar slants and incubated at 37 ºC for 24 hours and stored at 4°C for further identification. IMVIC reactions, a set of four chemical reactions (Indole test, Methyl red test, Voges Proskauer test and Citrate utilization test) were employed for the identification of *E.*coli.

Result and Discussion

In this study, the affected birds revealed inappetence, ruffled feathers and were unable to move and few of them were sitting on their hocks (Fig.1). Head swelling and respiratory signs like laboured breathing, gasping and respiratory rales were observed in certain affected birds. The results in this study were in concurrence to the findings of Kaura *et al*., (1988) [9] and Nakamura *et al*., (1985) [10] who also reported of closure of eyes, reduced feed and water intake, ruffled feathers in outbreaks of Colibacillosis. Swollen head syndrome characterized by oedematous swelling, cellulitis over eyes of broilers was also observed by O'Brien, (1985) [11] and he was able to isolate *E.coli* from the affected birds.

On postmortem examination of dead birds, lesions typical to Colibacillosis ranging from pericarditis and perihepatitis were observed (Fig.2). The liver of the affected birds was covered by thick layer of fibrin giving a characteristic bread and butter appearance to it. Kumar *et al*., (2013) [12] reported deposition of fibrinous exudate on liver surface besides other changes like congestion and necrotic areas. Renu *et al*., (2012) [13] also reported thick fibrinous layer on all visceral organs in avian colibacillosis. Grossly, heart in most of the affected birds revealed congestion but in few birds it was covered with thick layer of fibrin. Nakamura *et al*., (1985) [14] and Gangane *et al*., (2006) [15] also revealed similar type of lesions in colibacillosis outbreak. Spleen was found to be enlarged and congested and in some birds necrotic foci were present on spleenic surface. These results are in agreement with the findings of earlier workers (Cheville and Arp, 1978; Truscott *et al*., 1973; Nakamura *et al*., 1985) [16, 17, 18]. Grossly, the lungs revealed congestion, oedema and consolidation. Similar type of lesions have been observed by Gangane *et al*., (2006) [19] and Kumar *et al*., (2013) [20] which correspond to congestion, oedema and pneumonic foci. Pathogenesis of *E.*coli started with initial step of colonization in the respiratory tract, followed by crossing and penetration into the mucosa of air sacs, and then multiplication in the blood stream and internal organ such as liver, heart and spleen. Production of deleterious effect from cells and tissues leading to lesions developed followed with clinical signs (Levine *et al*., 1983) [21].

In the present study, the bacteria *Escherichia coli* was isolated from the samples *viz*; liver, lung, heart and spleen collected from the infected broiler birds using McConkey agar and EMB agar. The *E.coli* colonies appeared pink coloured when allowed to grow on McConkey agar plates (Fig.3) and showed typical metallic sheen on EMB agar (Fig.4). The pink colour of *E. coli* colonies and its appearance as metallic sheen on EMB are attributed to its lactose fermenting ability and due to the formation of amide linkage between eosin and Methylene respectively as earlier explained by Horvath and Ropp, (1974) [22]. These results corresponded with the characteristics of *Escherichia coli* as previously suggested by other workers (Islam *et al*., 2014) [23]. On Gram's staining, the rose pink bacterial colonies isolated on nutrient agar were typically Gram-negative rods (Fig.5) and this has been found in concordance with the findings of several authors (Buxton and Fraser, 1977) [24].

In this study, specific biochemical methods like IMVIC reactions were employed for the detection of *Escherichia coli*. All the bacterial isolates in this study were positive for Indole test, methyl red test and negative for Voges Proskauer reaction and citrate utilization test (Fig.6). The indole production by the bacteria is attributed to the enzyme typtophanase which acts on amino acid tryptophan to produce it. In contrast to Indole test, Methyl Red test is used to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. These findings also corresponded with the specific biochemical characters for *Escherichia coli* as previously suggested by other workers (Buxton and Fraser, 1977; Islam *et al*., 2014) [25, 26].

Fig 1: Colibacillosis affected broiler chicken showing ruffled feathers, inability to move and sitting on hocks

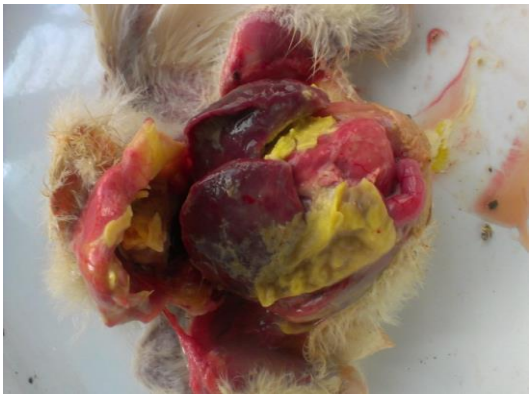

Fig 2: Carcass of Colibacillosis affected broiler chicken showing characteristic pericarditis and perihepatitis

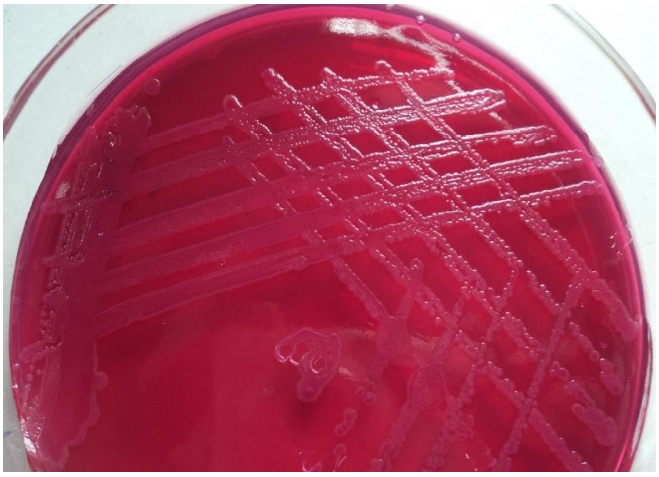

Fig 3: Rose pink colonies of *Escherichia coli* on MacConkey agar.

Fig 4: Typical metallic sheen of *Escherichia coli* on EMB Agar.

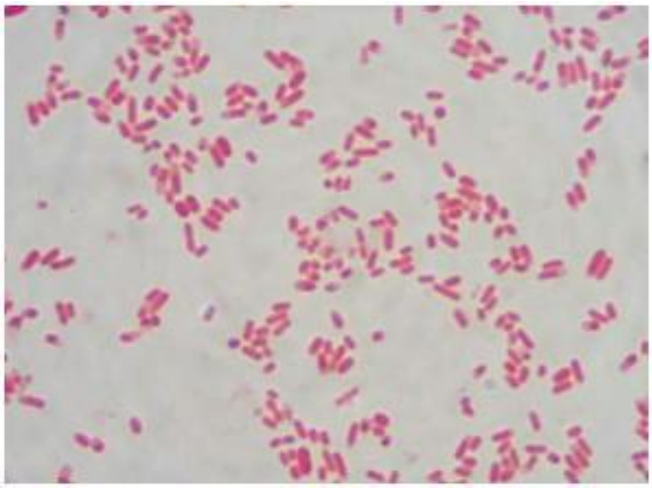

Fig 5: Gram negative, pink, short rod shaped bacteria. Gram's x 1000

Fig 6: *Escherichia coli* isolates showing positive Indole and Methyl Red test and negative Voges Proskauer and Citrate Utilization test

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