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Isolation and identification of bacterial pathogens causing infection in commercial layers

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Abstract
The success of poultry industry is being hampered by various pathogenic bacteria causing remarkable morbidity and mortality losses. Hence a study was designed to isolate and identify the bacterial pathogens causing infection in layer birds. A total of 279 samples were collected from ailing layer birds of which 252 samples gave 341 isolates. 272 of the isolates were Gram negative bacteria and the remaining 69 isolates were Gram positive bacteria. Based on colony characteristics and biochemical tests, four different types of bacteria *E. coli*, *Pasterurella* spp *Salmonella* spp and *Staphylococcus* spp were identified.

Keywords: birds, bacterial infection, bacterial culture, bacterial pathogens

Introduction
Poultry have been found in different agro-climatic regions of India and have evolved over many generations through natural selection and domestication and adaptation to varied environmental conditions. India, with its huge human population, has a huge demand for food; in particular, there is an ever increasing demand for egg and meat products. The total poultry population in the country has increased by 12.39 per cent over the previous census and the total poultry in the country is 729.2 million (Report, 2013) [1]. But the advancement of poultry industry is being disturbed by various pathogenic bacterial infections causing remarkable mortality in the layer as well as broiler flocks. Various pathogenic microbes, such as *Escherichia coli*, *Salmonella* spp., *Bacillus* spp., *Streptococcus* spp. *and Staphylococcus* spp., have been implicated to reduce the growth of poultry (Duke, 1986) [2].

Escherichia coli is a common pathogen for commercial poultry causing colibacillosis all over the world. It is the most common bacterial infection of all ages and have large economic impact (Johnston, 2007) [3]. It is a major cause of respiratory and septicemic diseases in broiler chicken causing mortality less than 5 per cent and morbidity over 50 per cent but in layer it affects the reproductive tract resulting failure of egg productivity and fertility (Barens and Gross, 1997) [4]. Avian Salmonella infection occur in poultry either in acute or chronic form by one or more member of the genus Salmonella, under the family Enterobacteriaceae (Hofstad *et al*., 1984) [5]. Increased mortality, anorexia, sudden drop in egg production and white or yellow diarrhea are the characteristic clinical signs of this disease. Fowl cholera (FC), which is also known as avian cholera, avian pasteurellosis or avian hemorrhagic septicemia, is an infectious disease affecting domesticated and wild birds (Rimler and Glisson, 1997) [6]. Fowl Cholera can affect birds of any age, but it rarely occurs in commercial poultry of less than 8 weeks of age (Rimler, 1994) [7]. Death of birds may be the first sign of Fowl Cholera while other signs are depression, diarrhea, ruffled feathers, increased respiratory rate, and cyanosis (Calnek *et al*., 1997) [8].

At the field level in our country, most of the poultry diseases are diagnosed on the basis of symptoms and gross lesions which aid to diagnose a disease but laboratory diagnosis is necessary for confirmation of the diseases (Banda, 2002) [9]. Therefore, this study was designed to isolate and identify the associated bacteria prevalent in ailing layer birds. The knowledge on the prevalence of bacterial pathogens will be helpful for choosing the apt antibiotic and also to take necessary actions for the prevention and control of diseases caused by bacterial pathogens in poultry.

Materials and Methods

Sample population

The study was carried out for the isolation and identification of the causative agents in birds suspected to be suffering with bacterial infection in commercial layer farms in West Godavari district in Andhra Pradesh.

Collection of samples

A total of 279 samples were collected aseptically from infected chicken which showed clinical symptoms such as serous or mucoid discharge, lacrymation, sneezing, conjunctivitis, diarrhoea and facial swelling. The samples included purulent lachrymal discharge from ailing birds and tracheal swabs collected from pharyngeal region by opening the mouth using sterile cotton swab. All samples were collected from farms where the flocks had been vaccinated as per schedule.

Bacterial culture

Samples collected aseptically were inoculated in nutrient broth followed by inoculation in differential and selective medium and incubated at 37˚C for 24 hours for isolation and identification of the bacterial pathogens. Biochemical tests like Catalase test, Oxidase test, IMViC test, Oxidation fermentation test (o/f), Sugar fermentation test, Urease test, Hydrogen sulphide production and Motility test were further done to identify the isolates as per Quinn *et al*. (2002) [10].

Results and Discussion

Prevalence of bacterial flora

A total of 279 samples were collected from infected flocks of which 27 samples did not show any bacterial growth. The remaining 252 samples gave 341 isolates in which 272 of them were Gram negative bacteria and the remaining 69 isolates were Gram positive bacteria. Four different types of bacteria were isolated namely *E.coli*, *Salmonella* spp, *Pasteurella* spp and *Staphylococcus* spp (Fig.1-4).

In this study, *E.coli* was isolated from 146 (42.81%) cases. This correlates with the findings of Hasan *et al*. (2010) [11]; Poppy *et al*. (2011) [12] and Hossain *et al*. (2013) [13]. *E.coli* was reported as a widespread microflora in raw feeding materials and poultry feeds (DaCosta *et al.*, 2007) [14]. Clinical signs of *E.coli* were diarrhoea, depression, soiling of cloaca with semisolid cheesy material, respiratory distress (coughing, sneezing), reduced egg production, loss of condition and death. Similar findings were recorded by Vegad and Katiyar (2003) [15]. The present study showed a high percentage of samples positive for *E.coli*. The occurrence of *E.coli* could be attributed from contaminating source such as feces during their laying period from the infected birds. Zahida (2004) [16] stated respiratory tract as the primary route of entry of *E.coli*. Nighot (2002) [17] reported that faulty management and lack of routine vaccination against some viral diseases may lead to bacterial infection by altering the normal flora of bacteria thus resulting in disturbances in natural immunity and natural defense mechanism. *Pasteurella* spp were also isolated in 52 (15.25%) cases. The common clinical signs of *Pasteurella* spp were nasal and ocular discharge, darkened head and combs, diarrhoea, ruffled feathers, increased respiratory rate, swollen wattles, high temperatures and lameness which agreed with the findings of Shivachandra *et al.* (2005) [18].

In the present study 74 (21.70%) cases were diagnosed as Salmonellosis. Most common clinical signs of salmonellosis were drowsiness, huddling, poor growth, chalky white diarrhoea with pasted vent, dehydration and reduced egg production which agreed with the findings of Calnek *et al.* (1997) [8a]. Staphylococcus spp were isolated in 69 (20.24%) cases which had been reported earlier by Linzitto *et al.* (1988) [19].

Identification of bacterial agents causing infection in layers

In this study, colony characteristics of *E.coli* observed in EMB and Brilliant green agar were similar to the findings of Nazir *et al.* (2005) [20]. The colony characteristics of *Pasteurella* species examined in blood agar and nutrient agar was in accordance with Woo *et al.* (2006) [21]. Biochemical tests done to identify the bacterial colonies revealed a complete fermentation of 5 basic sugars by producing both acid and gas by the *E.coli* isolates. The isolates also revealed positive reaction in MR test and Indole test but negative in VP test (Honda *et al.*, 1982) [22]. *Pasteurella* spp revealed a complete fermentation of dextrose, sucrose and mannitol completely and production acid without gas but no fermentation was recorded in case of maltose and lactose. These biochemical properties closely correlated with the findings of Calnek *et al.* (1997) [8b]. Isolates of *Staphylococcus* spp., revealed a complete fermentation of 5 basic sugars and production of acid which was supported by Beutin *et al*. (1991) [23] and OIE manual (2000) [24]. Isolated *Staphylococcus* spp were coagulase negative and they were non pathogenic.

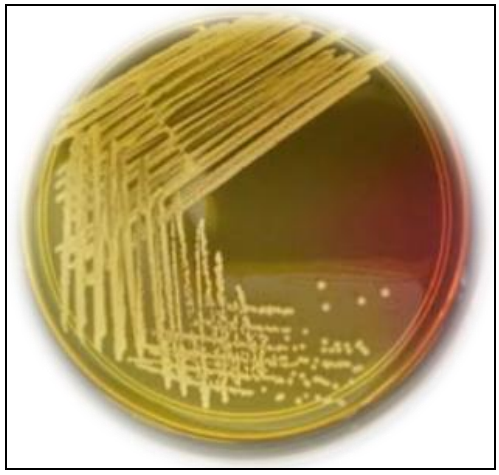

Fig 1: *Staphylococcus* colonies on Mannitol Salt agar

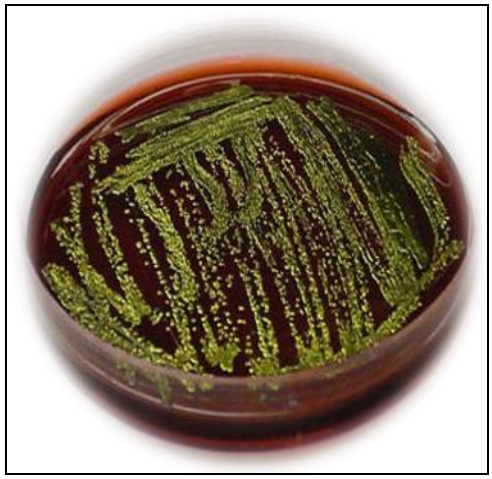

Fig 2: *E. coli* colonies on EMB agar

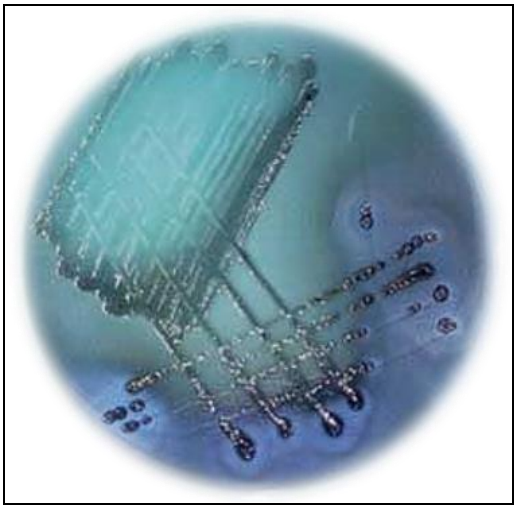

Fig 3: *Salmonella* colonies on Bismuth sulphite agar

Fig 4: *Pasteurella* colonies on Blood agar

Conclusion

The present study revealed *E.coli*, *Salmonella* spp, *Pasteurella* spp, and *Staphylococcus* spp.
as the predominant bacterial pathogens causing infection in layer birds and hence recommended adoption of strict hygienic measures in poultry farms to thwart such bacterial infections causing morbidity and mortality losses in poultry industry.

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