

RESEARCH ARTICLE

Studies of water borne *E.coli* from the Congo red medium differentiation between pathogenic & non pathogenic in the broiler farms in Madhya Pradesh

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ABSTRACT

To aim of this study was to isolate and to identify the pathogenicity of *E. coli* from the water in poultry farms. To conduct this study, water samples were collected from the poultry farms in the vicinity of Jabalpur. Around 200 water samples were collected from the 60 poultry farms, during the period of September 2011 to January 2014. All the water samples were cultured on Mac-Conkey's and EMB agar plates. The positive samples were examined for coliform. confirmation of *E. coli* was based on the morphology of colonies, gram staining and biochemical characters. *In vitro* pathogenicity test for *E. coli* was carried out through the Congo-red binding activity. Out of 200 water samples processed for coliforms, 150 produced growth on Mac-Conkey's agar. Biochemical characteristics identified 66 of these as *E. coli*. Out of 66 isolates of *E. coli*, 40 resulted in the growth of brick red colonies shown the pathogenic. While remaining 26 samples produced colorless colonies after 72 hours of incubation at room temperature and were confirmed as non pathogenic.

Keywords: Water, *E. coli*, Congo Red, Broilers poultry farms, Madhya Pradesh.

INTRODUCTION

Water is regarded as the most essential factor for life, it is not possible to say its exact requirements. Chicken generally take double amount of water compare the amount of feed consumed on a weight basis. During summer season, water requirements increased upto four times. The presence of microorganisms is typically a result of surface contamination by organic materials and can result in poor performance. (Blake ,Hess: 2001). Through the fecal matter, drinking water is contaminated with coliform bacteria (including *E.coli*, *klebsiella*, *Enterobacter*, *Proteus*). As per standard bacterial contaminants should not be present in drinking water and levels maintains should be zero. (<http://www.2ca.uky.edu/>).

An insufficient amount of water resulting depletion in growth and egg production. Microbes free drinking water has important role in the broiler farming. Contaminated water affects the growth of birds and increases the economic losses to poultry farmer. Contaminated water is the main source of disease spread among the chicken birds. If drinking water is contaminated it can cause different bacterial infections including *Colibacillosis*, *salmonellosis*, *staphylococcosis*. So that hygiene of water is utmost important for gaining the good profit by the production and health management in a poultry flock.

E.coli is pathogen causing various diseases in poultry viz: CRD, CCRD, salpingitis, yolk sac infection, air sac disease, perihepatitis, enteritis, omphalitis, colibacillosis etc. (Vegad, 2007).

The objective of present study was carried out to check the presence and pathogenicity of *E. coli* in drinking water at different poultry farms in Jabalpur. Around 200 water samples were collected from the 60 poultry farms, during the period of September 2011 to January 2014.

MATERIALS AND METHODS

To know the presence of *E.coli* in drinking water, we collected 200 water samples from 60 broiler poultry farms in and around the Jabalpur city. Out of these, 125 samples collected directly from the water bore well and 75 from the drinkers and channels placed in poultry shed. The sample of water was not treated with any sanitizers and acidifiers. A water sample of 200 ml was collected directly in sterile screw capped glass bottles after running the tap few minutes. Taken 5 ml water sample was mixed d an aliquot of 50 ml was cultured on Mac-Conkey's broth. These Mac-Conkey's broth samples were streaked on agar plates

of on EMB, Mc-Conkey's agar, Tergitol -7 agar, purified lactose fermenting colonies were counted. Picked up for further morphological and biochemical characterization including, Indole, MR, VP, Citrate utilization, Urease activity, oxidase, catalase, motility. (Cowan and Steel,1975; Ewing 1986).

Fermentation of carbohydrates:

Isolate of *E.coli* were confirmed by the carbohydrates fermentation activity: maltose, lactose, sucrose, dulcitol, adonitol, dextrose, xylose, mannitol. Peptone water with phenol red broth used as fermentation indicator. Isolates of *E. coli* were inoculated into tubes in the carbohydrates and kept at 37°C for 24hrs. The positive results shown from red to yellow, while the negative results remained red.

Congo red Binding Assay:

E.coli. positive samples streaked on congo red agar plates to know the pathogenicity of isolates. Described by Berkhoff and Vinal (1986). Rosenberger *et al.* (1985), Kalorey *et al.* (2002), Parul & Bist *et al.* (2014) Panigrahy *et al.* (1990) Sharma, *et al.* (2006). Each isolate were streaked on separate plate and kept at 37°C for 24hrs. After 24hrs incubation, the cultures were kept at room temperature for 48 hours. Pathogenic *E. coli* were identified by Congo red positive isolates produced brick red colonies. The non-pathogenic isolates appeared as colourless after 48 hours in room temperature.

RESULTS AND DISCUSSION

Out of 200 water samples processed for Coliform count, 150 produced growth on Mac Conkey's agar and were confirms as Coliform bacteria. The results of the Congo red binding test indicate that 53.3% of samples of *E.coli* produced Congo red positive (Table 1).

Table 1: Classification of the water samples on the basis of bacterial counts

S. No.	Water samples divide on the basis of Coliform count (CFU/ml)	No. of Total samples	No. of Lactose Fermenters Colonies
1	5-49	38	22
2	50-99	46	28
3	100-250	35	16
4	More than 250	31	11
	Total	150	77

In between period of this experiments, we also found other bacterial species in the water samples viz: *Pseudomonas spp.*, *Salmonella spp.*, *Proteus spp.*, *Enterobacter*, *Klebsiella*, *Edwardsiella spp.*, *Citrobacter* and *Serratia* etc. This study focused on the percentage of *E. coli* in poultry water samples, hence other coliform was neglected. (see Table-1). 45% bind the Congo red dye out of seventy seven samples, and were considered as entero-invasive *E. coli*. This finding is according the result of other workers. Those recommended the use of Congo red dye with the aim of differentiation between pathogenic and non-pathogenic microorganisms (Berkhoff and Vinal, 1986; Stebbins *et al.* 1992).

CONCLUSION

Out of 77 samples 45% bind the Congo red dye and were grouped as pathogenic *E. coli*. It is confers that the occurrence of coliform has main role in farm sanitation and disease management. If drinking water is contaminated it increases the chance of infection of pathogenic *E. coli*. In the presence of other member of *Enterobacteriaceae* is insignificant as they are considered to be introduced mainly from soil and sewage source. Also it considered *E. coli* is present in environment.

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