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Pathotyping of *E. coli* from Poultry

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ABSTRACT: Colibacillosis, caused by pathogenic *Escherichia coli*, is one of the main causes of economic losses among the poultry, worldwide. Losses occur at all ages, due to increase in morbidity and mortality, thus directly influencing the management and treatment costs. The characteristics of the disease and its aetiological agent have not yet been well defined. Thirty seven isolates of *E. coli* were collected from different age groups of chicks, evincing pathogenic lesions and their pathogenicity was confirmed by chick lethality test and congored binding assay. Positive correlation existed between the two tests in identifying the pathogenic isolates. This will give new insights in the early diagnosis of pathogenic *E. coli* in poultry to formulate the control strategies.

Keywords: *E. coli*, Pathotyping, Congored binding, Chick letahality.

INTRODUCTION

Theodore Escherich, a German pediatrician discovered *Escherichia coli* and described as *Bactrium coli commune* from the stools of breast-fed children. Castellani and Chalmers in 1999 assigned the present name of *Escherichia coli* and it was previously named as *Bacterium (Bacillus) commune* (Ewing, 1986).

Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *Escherichia coli* (APEC), including colisepticaemia, coligranuloma (Hjarre's disease), air sac disease, coliform cellulitis, swollen head syndrome, coliform peritonitis, coliform salpingitis, coliform osteomyelitis / synovitis, coliform panophthalmitis and coliform omphalitis/yolk sac infection (John Barnes *et al.*, 2002). *E. coli* infection is mainly a 'production disease', which cause million of dollars loss annually and 40% of the loss directly or indirectly attributed by *E. coli* infection.

Antibiotic resistance is a major public health threat in India. A high burden of infectious diseases, unregulated sale of antibiotics, financial incentives for healthcare providers to prescribe antibiotics, patient expectations, rising incomes, and limited public health response have helped drive the emergence of resistance (Kakkar *et al.*, 2017).

Berkhoff and Vinal (1986) reported that there was direct correlation between the ability of clinical isolates of *E. coli* to bind congored dye and their ability to cause septicaemic infections in chickens. Congored medium could identify a virulent form of *E. coli*, which caused air sacculitis and colisepticaemia in poultry (Corbett *et al.*, 1987). Wooley *et al*. (1993) assessed the isolates of *E. coli* from clinical cases of colibacillosis and from intestinal tracts of normal broilers at slaughter by the embryo lethality tests to determine their virulence. Srinivasan *et al*. (2003) studied the pathological and immunosuppressive effects of *E. coli* alone and IBDV - *E. coli* coinfection experimentally in chicken. Hussain *et al*. (2017) investigated the epidemiology of ESBL-producing *E. coli* of poultry origins, which may be directly linked to public health. This shows the need to early diagnosis of pathogenic *E. coli* to avoid mortality and production loss and severe economic lossess to poultry farmer. So this study was undertaken to diagnose the pathogenic *Eoli* at farm level for effective control.

MATERIAL AND METHODS

Collection of clinical samples. A total of 120 heart blood swabs were collected in sterile method (Ewing, 1986) from diseased and dead birds from poultry farms in and around Namakkal. The collected heart blood swabs were subjected bacteriological analysis.

Bacteriological examination. The collected clinical samples were inoculated in Nutrient broth and incubated for 18 h at 37°C. Then, a loopful from the previously inoculated broth was streaked onto MacConkey agar (Hi media) plates and incubated for 24 h at 37°C. Pink colored colonies were taken up and inoculated onto Eosin Methylene Blue (Hi media) and incubated at 37°C for 24 h. determination of *E. coli* isolates were performed based on the colony and biochemical tests as per Ewing (1986).

Pathogenicity Testing.

1. Congo red binding assay. Congo red binding assay of *E. coli* isolates were carried out and interpreted as per Berkhoff and Vinal (1986).

2. Chick lethality test

• **Caudal air sac route.** Pathogenicity of the *E. coli* isolates were ascertained by inoculating 10^8 CFU / ml of *E. coli* through caudal air sac route in day-old chicks as per Panigrahy and Yushen (1990).

• **Intravenous route of inoculation.** Pathogenicity of *E. coli* isolates were ascertained by inoculating 10^8 CFU / ml of *E. coli* organisms through intravenous route among four-week-old chicks as per Arp and Jensen (1979).

3. Histopathology. Histopathological studies were conducted at Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal.

RESULTS AND DISCUSSION

Among thirty seven *E. coli* isolates, nineteen isolates produced characteristic wrinkled red coloured colonies on congo red agar plates (Plate – 1C), The remaining isolates produced smooth white/red colonies (Plate – 1D).

All the nineteen *E. coli* isolates, which produced characteristic wrinkled red coloured colonies in CR binding assay, caused death of day-old chicks within 2-5 days p.i. inoculated through caudal air sac route

Pathogenic *E. coli* isolates that caused death of day–old chicks, when administered via caudal air sac route of inoculation, were also administered via intravenous route of inoculation in four-week–old chicks. All the isolates caused death of chicks within 1-4 days of p.i.

In acute cases of deaths, (24 hrs p.i.), colisepticaemic lesions with air sacculitis, and congestion of heart and liver were observed among birds inoculated, intravenously. Chicks died, two to four days of p.i. evinced marked lesions of air sacculitis, pericarditis and perihepatitis during inoculation by caudal air sac and intravenous route of inoculations. The pathognomonic lesions of fibrinous pericarditis and perihepatitis were more severe at about 5^{th} day p.i. The macroscopic lesion scores of the experimental birds inoculated through caudal airsac and intravenous routes are presented in the Table 1, and the gross lesions of experimental birds are depicted in Plate – 2A.

On microscopical examination, liver showed congestion (Plate – 2B), fibrinous thrombi in sinusoidal space (Plate – 2C) and hepatocytes with vascular degeneration (Plate – 2D). The heart showed heterophilic, mononuclear infiltration with myocardial haemorrhages.

The positive correlation between congored binding ability and pathogenicity of *E. coli* isolates correlate with the findings of Sivakumar (1996). *In vitro* pathogenicity of *E. coli* isolates was assessed by using CR binding assay and the similar results were recorded by Raji *et al.* (2003). The CR positive *E. coli* isolates produced characteristic wrinkled red colour colonies on congo red agar plates. Congo red binding has also been proposed as a potential virulence marker by Raja Swaminathan (1997), which closely relates the results of the present study. But Mellata *et al.* (2001) failed to record similar results.

The *in vivo* pathogenicity of *E. coli* isolates was assessed by chick lethality by various routes of inoculations. Similarly Raji *et al.* (2003) compared the *in vivo* pathogenicity of all the serotypes. Gibbs *et al.* (2004) reported that the embryo lethality assay (ELA), intravenous and subcutaneous (subQ) challenge models similarly demonstrate the ability to discriminate between virulent and avirulent avian *E. coli* isolates.

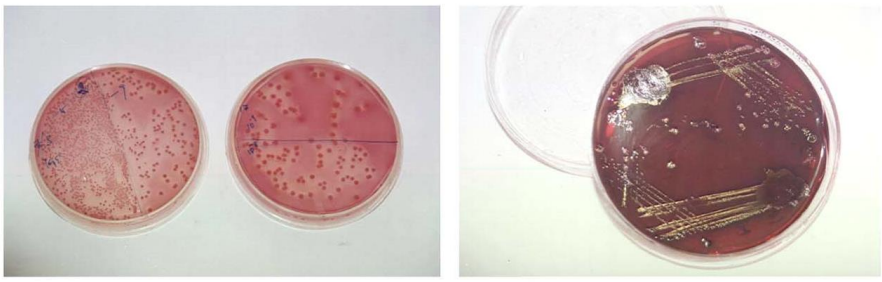
The gross lesions of chicks inoculated with *E. coli* correlates well with the findings of Panigrahy and Yushen (1990); Srinivasan *et al.* (2003); Gibbs *et al.* (2004).

On microscopical examination, the liver evinced congestion, fibrinous thrombi in sinusoidal space and hepatocytes with vascular degeneration. These findings were in accordance with the observations of Nakamura *et al.* (1992); Baliar Singh *et al.* (1993); John Barnes *et al.* (2002).

Table 1: Chick Lethality Test (Macroscopic Lesion Score).

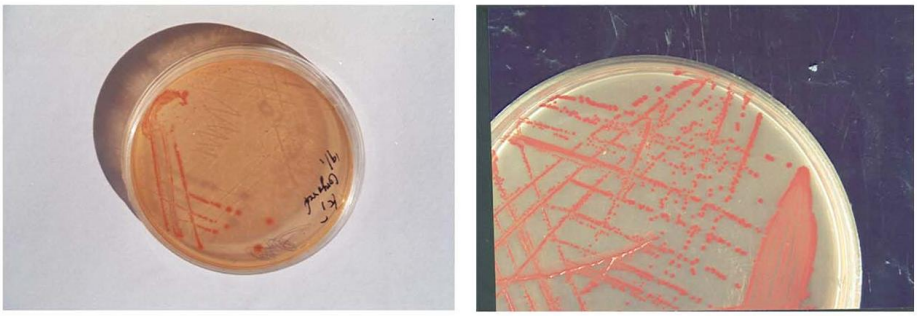
Sr. No.	Route of inoculation	Airsacculitis				Pericarditis				Perihepatitis				Mortality	Mortality (%)
		+ + +	+ +	+	0	+ + +	+ +	+	0	+ + +	+ +	+	0		
1.	Caudal airsac	31	7	-	-	30	8	-	-	33	5	-	-	38	100
2.	Intravenous	33	5	-	-	36	2	-	-	30	8	-	-	38	100

Macroscopic lesion score (+) indicates the intensity of post mortem lesions considering '+ + + +' as the maximum.



1A MacConkey agar plate-plink coloured colonies - *E. coli* organism.

1B EMB agar plate-plink characteristic metallic Sheened greenish black coloured colonies - *E. coli* organism.

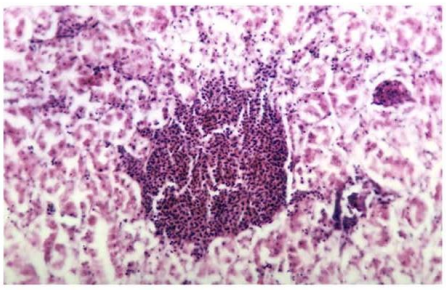


1C Congo red agar plate - wrinkled reddish colonies - virulent *E. coli.*

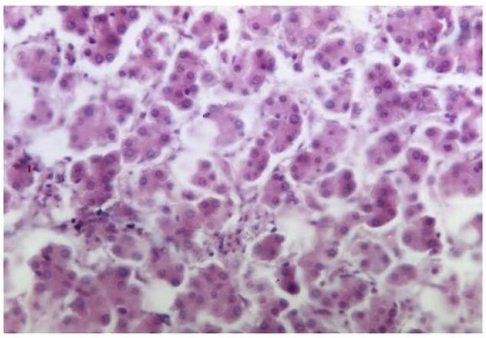
1D Congo red agar plate - smooth reddish colonies - avirulent *E. coli.*

PLATE 1: Cultural Characters of *E. coli.*

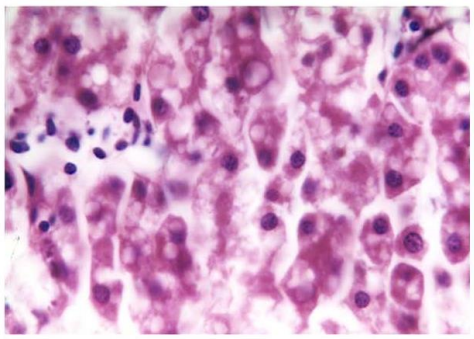
2A Gross pathology – pathogenic *E. coli* infected bird – pericarditis, perihepatitis and airsacculitis.



2B Histopatholgy – liver congestion H & E 100 X



2C Histopatholgy – liver fibrinous thrombi in sinusoidal space – H & E 400 X



2D Histopatholgy – liver hepatocytes with vascular degeneration – H & E 1000 X

PLATE 2: Gross and histopathology.

SUMMARY

Congo red binding assay confirmed the pathogenicity of the isolates, which could be further confirmed by chick lethality tests by pathognomonic postmortem and histopathological lesions.

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Conflicts of Interest. None.

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