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Biotyping of Avian Pathogenic *E. coli*

Thangavel Kandasamy*
Assistant Professor, Department of Veterinary Microbiology,
Veterinary College and Research Institute, Namakkal (Tamil Nadu), India.

(Corresponding author: Thangavel Kandasamy)*
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ABSTRACT : Colibacillosis is one of the devastating diseases, which leads to a profound economic loss to the poultry entrepreneurs and causes morbidity and mortality. The mortality rate may range from 5 - 50% in poultry. *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. In the present study, all the isolates evinced cent percent positive reactions to arabinose, fructose, galactose, maltose, mannitol, mannose, melibiose, xylose, decarboxylation and IMViC tests and negative reactions to adonitol, inulin, cellobiose, inositol, thus correlating with biotyping results of the standard *E. coli* organism. Pathogenic *E. coli* isolates in the present study, evinced negative reaction to adonitol and fermentation of dulcitol by 68.4%. This evidenced the variable biotyping pattern of pathogenic *E. coli.*

Keywords: *E. coli*, Biotyping, Pathogenic.

INTRODUCTION

The Indian poultry industry is on the threshold of a major leap with increase in poultry population throughout the country, as a result of newer and better technologies adopted with every passing day. Hence, an effective dissemination of the corresponding knowledge from the 'lab (oratory) to land, is absolutely required to maintain this tempo.

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).

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). 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. 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 (Wani *et al.*, 2020). Bettelheim and Taylor (1969) biotyped strains of *E. coli* on the basis of decarboxylase tests and fermentation of sixteen carbohydrates. Crichton *et al.* (1979) re-examined the biotyping of *E. coli* in an attempt to establish tests that were easy to perform, reproducible and highly discriminatory. They also evaluated the usefulness of the tests in differentiating between strains of *E. coli* isolated from patients.

Characterization of *E. coli* is very important to formulate early diagnosis of colibacillosis. Biotyping is one of the important tool to easily diagnose the *E.coli* infection. Molecular diagnostic tests are very expensive and need to be expertise. Hence this study is formulated to characterize the *E. coli* based on their biochemical characters.

MATERIALS AND METHODS

Collection of clinical samples. A total of 54 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 as per Edwards (1986).

Confirmatory Biochemical Tests. The isolates were identified by growth on various selective media and IMViC method, and then were inoculated on to TSI slants as per Cowan (1993).

Biotyping. The sugar fermentation and amino acid decarboxylation tests were carried out as per Cowan (1993), and the results were interpreted as per Bergy (1993).

RESULTS AND DISCUSSION

Out of fifty four samples, thirty seven samples evinced discrete pink colonies, thus indicating positive reaction to lactose fermentation on MacConkey agar plates (Plate 1A). All the isolates exhibited discrete, metallic sheened greenish black colonies (Plate 1B), when cultured on EMB agar plates, thus confirming *E. coli* colonization. They also evinced positive reactions to catalase test (Plate 2A), indole production at 44ºC (Plate 2B), MR test (Plate 2C), nitrate reduction test and negative reactions to VP test (Plate 2C), citrate utilization and oxidase tests (Plate 2D). Acid butt and slant in TSI agar slants also could be observed (Plate 2E). These results confirmed the field samples as *E. coli.* The incidences of colibacillosis in different age group of birds are furnished in Table 1.

Incidence of colibacillosis, among birds, below twenty five weeks age group was 54.0% and that among birds, above twenty five weeks age group was 45.9% (Table 1).

Sugar fermentation reactions were observed upto a maximum of seven days for acid and gas production. The colour changes in the phenol red broth from purple to yellow indicated acid production by the *E. coli* organisms. Gas production was indicated by accumulation of gas inside the Durham's tube and subsequent lifting up of the same (Plate 3A, 3B, 3C). Strains, which were genotypically non-fermenting, often, produced late positive results due to selection and growth of fermenting mutants. It was possible to identify a definitive time of reading (24 -72 hrs), which gave optimal separation of the genotypically fermenting types from the non-fermenting types for each substrate. The decarboxylase tests were read as positive, when the medium became alkaline (purple) at 48 hrs and the amino acid free medium remained acid (yellow).

All the isolates evinced cent percent positive reactions to arabinose, fructose, galactose, maltose, mannitol, mannose, melibiose and xylose and negative reactions to adonitol, inulin, cellobiose and inositol.

Variations in sugar fermentation and amino acid decarboxylation (Plate – 3D) reactions were taken into consideration for grouping of *E.coli* isolates, by which 16 biotyping patterns could be obtained. Among the variable biotyping patterns, the S.no. 7 occurred more frequently (6 times) than the others as presented in Table 2.

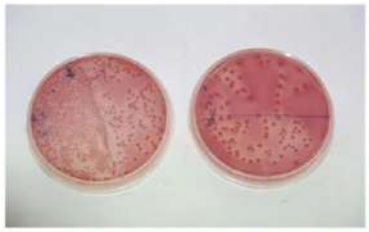
Among sugars employed for fermentation reactions, rhamnose was fermented by 86.48% of isolates, sucrose by 86.48%, and dulcitol by 68.4% of the isolates. Only 35.13% of isolates evinced positive results to decarboxylation reaction to L-Arginine hydrochloride, when compared to L-Lysine hydrochloride (64.86%), and L-Ornithine hydrochloride (64.86%).

Table 1: Incidence of Colibacillosis in Different Age Group of Birds.

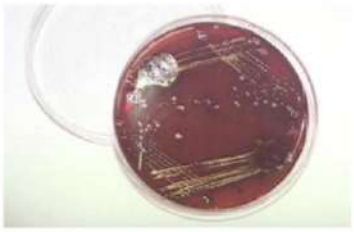
Sr. No.	Age in weeks	No. of samples	No. of samples positive for *E. coli* infection	Percentage
1.	< 25 weeks	25	20	54.0
2.	> 25 weeks	29	17	45.9
3.	**Total**	**54**	**37**	100

Table 2: Biotyping Patterns Based on Variable Sugar Fermentation and Amino Acid Decarboxylation Results.

Sr. No.	Biotyping patterns	Isolate identification No.	Total No. of isolates
1.	Rh, Su, Ly, Or	1,6,33,34	4
2.	Du, Rh, Su, Ar, Ly	2,17,25,26	4
3.	Du, Rh, Su, Or	3,4,5,20	4
4.	Rh, Su, Ar, Ly	7, 18, 19	3
5.	Du, Rh, Ar, Or	14, 8	2
6.	Du, Su, Ly, Or	9	1
7.	Du, Rh, Su, Ly, Or	10, 21, 22, 23, 27, 28	6
8.	Rh, Su, Or	11, 24	2
9.	Du, Su, Or	12	1
10.	Du, Su, Ar, Or	13	1
11.	Rh, Ar, Ly	15, 16	2
12.	Du, Rh, Or	29	1
13.	Du, Rh, Ly	30, 31	2
14.	Rh, Su, Ar	32	1
15.	Du, Su, Ly	35	1
16.	Du, Rh, L, Or	36, 37	2



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

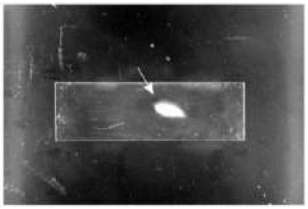


1B EMB agar plate-plink characteristic metallic Sheened greenish black coloured colonies - *E. coli* organism.

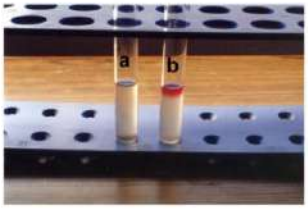
1C Congo red agar plate - wrinkled reddish colonies - virulent *E. coli.*

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

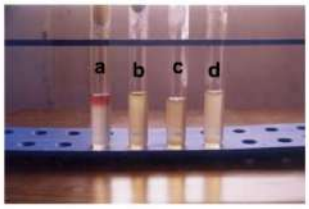
PLATE 1: Cultural Characters of *E coli.*



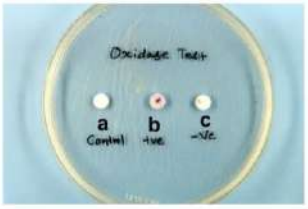
2A. Catalase test - effervescence (arrow) - positive (*E. coli*)



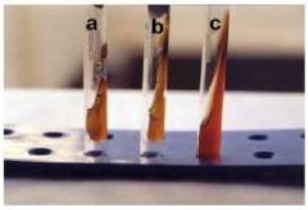
2B. Indole test - a. Control
b. Positive (*E. coli*)



2C. MR-VP test - a. Positive (MR - *E. coli*)
b. Negative (VP - *E. coli*)
c. Negative (Uninoculated control)
d. Negative (Inoculated control)



2D. Oxidase test -
a. Control
b. Positive (*Pseudomonos aeruginosa*)
c. Negative (*E. coli*)



2E. E. coli on TSI Slant
a. Acid and gas production (Y/Y/+/-)
b. Acid and gas production (Y/Y/+/-)
c. Uninoculated control

PLATE 2: Identification of confirmation of *E coli.*

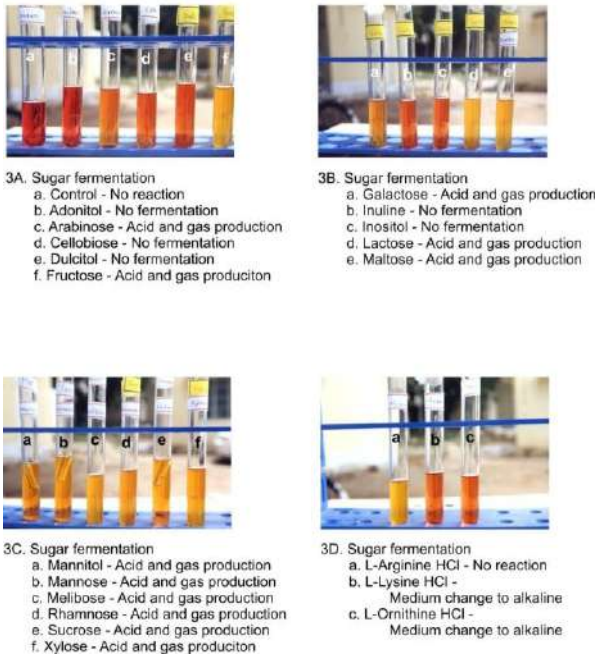


PLATE 3: Biotyping of *E. coli* in Phenolred broth base by Sugar fermentation and Amino acids decrboxylation tests.

Enteric diseases are important concern to the poultry industry due to loss of productivity, increased mortality and the associated contamination of poultry products for human consumption. Among all the diseases of poultry, *E. coli* infection attracts a major concern of management, because damage by *E. coli* is both directly as colisepticaemic disease as such and indirectly as coinfection predisposing to many other diseases without showing any symptom.

E. coli isolates produced positive reaction to lactose fermentation on MacConkey agar plates, metallic sheened greenish black colonies, on EMB agar plates and they also evinced positive reactions to MR test, indole production at 44ºC, acid butt and slant in TSI agar slants, nitrate reduction test and catalase test and negative to VP test, citrate utilization and oxidase tests. These findings correlated well with the presentations of Cowan (1993).

The prevalence of colibacillosis in the present study revealed that, the birds at the age of less than twenty five weeks are more susceptible to *E. coli* infection, which accorded well with the observations of Bisgaard (1995); Gross (1994).

During sugar fermentation tests, using sixteen discs of respective sugars, the *E. coli* evinced variable results. In the present study, all the isolates evinced cent percent positive reactions to arabinose, fructose, galactose, maltose, mannitol, mannose, melibiose, xylose, decarboxylation and IMViC tests. and negative reactions to adonitol, inulin, cellobiose, inositol, thus correlating with biotyping results of the standard *E. coli* organism observed by Cowan, (1993); Krishnamohan Reddy *et al.* (1994); Sivakumar (1996); Parimal *et al.* (2004).

Pathogenic *E. coli* isolates in the present study, evinced negative reaction to adonitol, as against 20% of the pathogenic *E. coli* isolates from poultry in and around Madras city, being reported to be fermenting adonitol by Premkumar *et al*. (1991).

These findings also corresponded with the specific biochemical characters for *Escherichia coli* as previously suggested by other workers (Islam *et al*., 2014).

SUMMARY

Colibacillosis is one of the devastating diseases, which leads to a profound economic loss to the poultry entrepreneurs. Avian colibacillosis is one of the important causes of morbidity and mortality.

Thirty seven isolates of avian *E. coli* were obtained by examining fifty four necropsy specimens collected from dead / ailing birds, suspicious of *E. coli* infection. The isolated *E. coli* organisms were identified and confirmed by inoculating into various basic and selective media and subjecting them to selective biochemical tests, based on previous (most of the) studies, thus enabling efficient comparison of their results.

To obtain biotyping patterns of *E.* coli isolates, biochemical reactions of sixteen sugars and three amino acids were employed. It is evident that the biochemical characterization and biotyping is the most valuable test to early and easy identification of pathogenic *E. coli.*

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Conflicts of Interest. None.

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