# Prevalence, isolation and antibiogram study of pathogenic *E. coli* from poultry farms in and around Patna

## Apoorva Vatsa, Sanjiv Kumar\*, Kaushal Kumar and Sanjay Kumar¹

Department of Veterinary Pathology, Bihar Veterinary College, BASU, Patna, India, <sup>1</sup>Department of Livestock Production and Management

#### Address for Correspondence

Sanjiv Kumar, Department of Veterinary Pathology, Bihar Veterinary College, BASU, Patna, E-mail: mrsanvet@rediffmail.com

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#### **ABSTRACT**

Escherichia coli (E. coli), a member of the Enterobacteriaceae family, is a Gram-negative, rod-shaped, facultatively anaerobic bacterium commonly found in the intestines of birds and animals. Although it is part of the normal gut flora, certain enteropathogenic and toxigenic strains can cause a range of enteric and extra-intestinal infections in poultry. While previous studies have investigated the prevalence, isolation and antibiotic susceptibility profiles of E. coli, its characteristics are known to vary across regions and time periods. This study was undertaken to assess the prevalence of E. coli infections contributing to poultry diseases in and around Patna, with a specific focus on the antimicrobial resistance patterns of isolated strains. A total of 500 birds underwent necropsy, from which 200 suspected cases of colibacillosis on the basis of history and gross lesions were selected for further microbiological analysis and confirmation. Escherichia coli was successfully isolated from 120 of these samples, representing 60.0%. Of these, 113 isolates (56.5%) were biochemically confirmed as E. coli. Infections were more prevalent among younger chickens during the monsoon season. Antimicrobial susceptibility testing was conducted on all biochemically confirmed isolates using the standard disc diffusion method. The results indicated that tetracycline exhibited the highest resistance, followed by ciprofloxacin. Based on the findings, E. coli was identified as a predominant gastrointestinal pathogen in poultry and cephalexin was suggested as an effective treatment option against the prevailing strains.

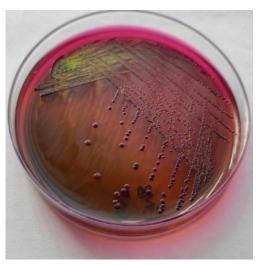
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During the last few decades, systems of poultry husbandry have transformed significantly due to an importance on intensive rearing which has made the birds susceptible for the incidence of various infectious diseases. Poultry business suffers great losses due to these infections. Despite adoption of modern managemental practices, preventive precautions and medications the poultry is plagued with a number of bacterial infections in recent years. Bacterial infections is the most common challenge for the gastro-intestinal tract of bird. In poultry, infections from Escherichia coli (E. coli) are most common among all other pathogenic bacteria that are associated with poultry production. E. coli are normal inhabitant of the intestines of birds and animals1, but is of the concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to wide variety of enteric and extra-intestinal diseases in birds. Avian pathogenic E. coli (APEC) causes a syndromic poultry infection known as colibacillosis which can affect birds of all ages and different types of poultry and has zoonotic importance because these organisms are transmitted from raw poultry meat to the human consumers and the workers handling the poultry or poultry products<sup>2,3</sup>. E. coli alone accounts for more than 14.0% of the infectious diseases4. The above facts have necessitated an in-depth study of prevalence of E. coli infection in causation of poultry sufferings in and around Patna. The purpose of this study also aimed to investigate the antimicrobial resistance profile of *E. coli* isolated from poultry samples.

The samples were collected from dead birds of different age groups from different farms of Patna, Bihar and its vicinity. A total of 500 birds were necropsied and based on the gross lesions like fibrinous pericarditis and perihepatitis, opaque air sacs, whitish adherent pericardial sac, congestion and haemorrhagic lesions in the intestinal tract, a total of 200 samples suspected for collibacillosis infection

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were collected and processed for isolation, identification and their biochemical characterization. Single colony of *E. coli* was picked from agar plate and stained with Gram's stain and their morphological characteristics were examined using microscopy. Isolated colonies were subjected to different biochemical tests like indole test, methyl red test, Voges-Proskauer test, citrate utilization test, TSI etc. using strip tests of standard company. Biochemically confirmed isolates were further tested for antibiotic sensitivity test by disc diffusion method using a set of antibiotics. The antibiotics in study were selected after questionnaire



**Fig. 1.** Showing the characteristic colony of *E. coli* (metallic sheen appearance) in EMB Culture media.

from the poultry owner and practitioners. The zone of inhibition was measured using standard method and results were interpreted as per guidelines and recommendations of CLSI.

All the data collected were statistically analysed using one-way ANOVA and Duncan's multiple range test with the help of software IBM SPSS version 20-bit 32 as per method of<sup>5</sup>.

All the 200 samples suspected for colibacillosis were allowed for enrichment in MacConkey broth with an overnight incubation at 37°C followed by streaking on the eosin methylene blue (EMB) agar plates. By conventional methods of enrichment and selective plating on EMB, the characteristic colony of *E. coli* (metallic sheen appearance) was obtained from 120 samples (60.0%), after incubation at 37°C for 24 hours (Fig. 1). In the present study, out of 120 isolates of *E. coli*, only 113 (56.5%) of *E. coli* isolates showed the confirm biochemical characteristic reactions (Fig. 2).

The area wise isolation and cultural study of *E. coli* revealed that out of 200 samples analysed for the

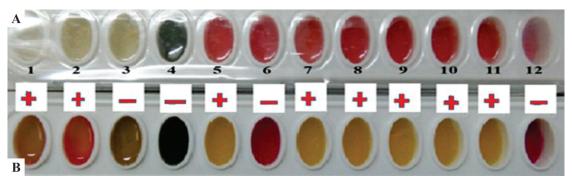
presence of *E. coli*, 120 (60%) samples harboured *E. coli* that belongs to maximum occurrence in Masaudhi (76%) followed Patna (70.58%), Naubatpur (63.15%), Danapur (58.97%), Bihta (53.84%), Sonpur (50%), Muzaffarpur (45.45%), Hajipur (37.5%) and minimum occurrence at Jahanabad (22.22%).

Previous studies and observation for the isolation of *E. coli* from poultry samples also detailed the varying percentage of prevalence<sup>6</sup> made an effort for isolation of *E. coli* from poultry samples (chicken meat, n = 228 and eggs, n = 24) in Patna and revealed 27.1% of prevalence. Prevalence of 89.4% *E. coli* by processing different poultry samples in Rajasthan<sup>7</sup>. These findings conclude the variation of prevalence in different areas and the present study also signifies it with a range around 22% to 68% prevalence.

The present study also emphasized on the age-wise study of incidence of colibacillosis. Birds of 0-2 weeks of age were found to suffer most with an incidence of 30.57% followed by 3-5 weeks of birds (18.96%), 6-10 weeks of age (8.45%), 11-20 weeks of age (4.87%) and least in >20 weeks of age (4.34%). These findings indicates that there was decrease in the incidence of colibacillosis with the aging of birds (Fig. 3).

However, a number of studies conducted worldwide have also reported a varying percentage in the incidence of colibacillosis. Highest mortality was found in 11-15 days old chicks (93%) as compared to 6-10 days (83.33%) and 1-5 days old chicks (21.42%) reported<sup>8</sup>.

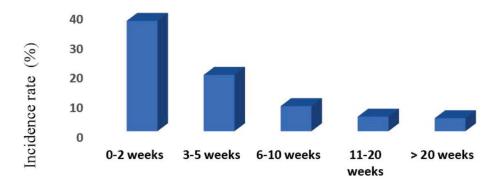
The season-wise incidence of colibacillosis in present study revealed that the highest incidence was seen in the monsoon season (34.39%) followed by autumn (26.43%), winter (17.64%), spring season (11.36%) and least in summer season (10.75%). Few attempts had been done to understand the seasonal prevalence of colibacillosis like reported the total prevalence of colibacillosis in broiler flocks was 12.50%. The season wise prevalence showed the highest in spring season (17.86%), followed by winter 14.47%, summer 9.62% and autumn 7.14%. The



**Fig. 2.** Showing biochemical characterization of *E. coli* by HiMViC biochemical strip kit. 1: Indole, 2: Methyl red, 3: Voges Proskauer's, 4: Citrate utilization, 5: Glucose, 6: Adonitol, 7: Lactose, 8: Sorbitol, 9: Mannitol, 10: Rhamnose, 11: Sucrose. A: Un-inoculated test kit, B: Test kit inoculated with sample.

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### Age wise incidence rate of colibacillosis in birds

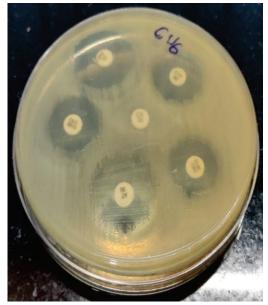


Age (in weeks)

Fig. 3. Bar-diagram showing age-wise incidence of colibacillosis in poultry.

maximum prevalence of colibacillosis in monsoon season and minimum in winter season reported by<sup>10</sup>.

Antibiotics are indiscriminately used in poultry rearing as growth stimulants or to treat infectious diseases. The overuse and misuse of antibiotics play a significant role in the emergence and spread of antibiotic-resistant *E. coli*<sup>11</sup>. In this present study, all the biochemically confirmed isolates of *E. coli* were screened for *in-vitro* antibiotic sensitivity profiling by standard disc diffusion assay using the disc of antibiotics *viz.*, tetracycline (30 µg), gentamicin (10 µg), amoxiclav (20/10 µg), cephalexin (30 µg), cefpodoxime (10 µg), ciprofloxacin (5 µg) and ceftriaxone (30 µg) (Fig. 4). The *E. coli* isolates showing zone of inhibition  $\leq$  11 mm for tetracycline (30 µg),  $\leq$  12 mm for gentamicin (10 µg),  $\leq$ 



**Fig. 4.** Antibiotics sensitivity testing of *E. coli* on Mueller Hinton Agar.

13 mm for amoxiclav (20/10  $\mu$ g),  $\leq$  18 mm for cephalexin (30  $\mu$ g),  $\leq$  17 mm for cefpodoxime (10  $\mu$ g),  $\leq$  15 mm for ciprofloxacin (5  $\mu$ g) and  $\leq$  13 mm for ceftriaxone (30  $\mu$ g) were considered to be resistant whereas zone of inhibition  $\geq$  19 mm,  $\geq$  15 mm,  $\geq$  21 mm for respective antibiotics were considered to be susceptible. The range lies in between these were considered as intermediate.

The present study revealed that the tetracycline showed the maximum resistant (83.33%) towards E. coli followed by ciprofloxacin (76.1%), amoxiclav (52.21%), gentamicin (50.44%), cefpodoxime (43.36%), ceftriaxone (31.85%) and cephalexin (29.02%) showed the least (Fig. 5). However, a number of studies and analysis had reported different resistance of antibiotics against E. coli. E. coli showed the highest resistance<sup>12</sup> to sulfamethoxazoletrimethoprim (71%), tetracycline (63%), ampicillin (62%), where gentamicin (23%) showed the lowest resistance, followed by ceftriaxone (26%). Reports of showed the maximum resistance of the isolates against cefuroxime (89.1%) and penicillin (89.4%), followed by ampicillin (80.43%), vancomycin (74.1%), co-trimoxazole (73.1%), cephalothin (60.8%), ceftriaxone (28.2%), tetracycline (17.4%), gentamicin (13%), amikacin (13.04%), ofloxacin (13%) and ciprofloxacin (6.5%).

In a study<sup>13</sup> on 30 isolates (94%) showed resistance to more than one antibiotics with percentage resistance were tetracycline 81%, sulphamethoxazole 67%, streptomycin 56%, trimethoprim 47%, ciprofloxacin 42%, ampicillin 36%, spectinomycin 28%, nalidixic acid 25%, chloramphenicol 22%, neomycin 14%, gentamicin 8%, amoxicillin-clavulanate, ceftiofur, cefotaxime, colistin, florfenicol and apramycin 0%.

#### CONCLUSION

# Antibiotic sensitivity of different antibiotics

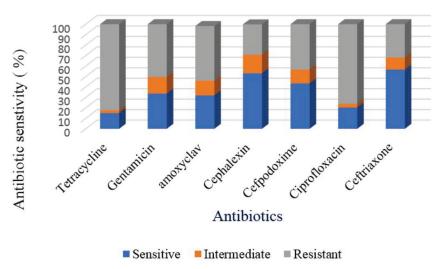


Fig. 5. Showing antibiogram profile of different antibiotics.

From the research findings it could be concluded that the pathology of enteric disease in poultry can be varied as gastro-intestinal tract is being constantly exposed to a wide variety of potentially harmful substances. E. coli is the prominent and commonest bacteria affecting GIT with various manifestations and cephalexin can be drug of choice against prevailing strain of E. coli. The findings of the present study suggest that being the most exposed surface for the pathogens, gastrointestinal system demands a major emphasis from the poultry farm owners considering for their shake of higher production and economic stabilization. Among one of the major poultry diseases, colibacillosis is of major concern so disease control authority should prioritize the disease burden and plan their efficient control strategies for the poultry farm owners to orchestrate their disease management protocol. As antibiotic resistance potentiates the pathogens to create serious animal as well as human health implications so it is of major concern.

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