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Research Article

Isolation, characterization, and antibiotic sensitivity testing of *Escherichia coli* strains from poultry eggs in and around Patna, India

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Abstract

The objective of the study was to isolate *Escherichia coli* along with antibiotic sensitivity profiling of isolates from eggshell and egg content. The study was conducted by taking three hundred samples (300) from one hundred fifty (150) eggs collected from organized and unorganized poultry farms as well as local markets in and around Patna. The morphological examination of E. coli was performed by microscopic examination of a Gram-stained smear of *E. coli*. The colonies of presumptive *E. coli* from EMB agar were confirmed by the microscopic morphological examination, motility test, and a battery of biochemical tests like IMViC tests, nitrate reduction test, triple sugar iron agar test, oxidase test, catalase test and urease test. The antibiotic susceptibility pattern was performed by the disc diffusion method. Incidence of *E. coli* infestation was 30.00% in eggshells and 22.00% of egg content of eggs collected from organized poultry farms, whereas the incidences of 38.00% *E. coli* in eggshells and 28.00% in egg content were recorded in eggs from unorganized poultry farms. Similarly, the incidence of 46.00% in eggshells and 36.00% in egg content of market eggs. Egg shells had a comparatively higher incidence of contamination with *E. coli* than egg content, with the highest incidence in market eggs followed by eggs from unorganized poultry farms and organized poultry farms. *E. coli* isolates had a resistance of 13.00 to 76.00% to antibiotics, with maximum resistance of 76.00% to penicillin and the least resistance of 13.00% to amikacin. The isolates had maximum sensitivity of 81% to amikacin, whereas they were least sensitive to penicillin and oxytetracycline (24% each).

Keywords: Amikacin, Egg, *Escherichia coli*, Penicillin, Poultry farm

Highlights

- The poultry eggs can get contamination either horizontally or vertically and could serve as a potential source of food-borne diseases.
- The microscopic examination of Grams-stained *E. coli* was performed for morphological examination.
- The colonies of presumptive *E. coli* from EMB agar were confirmed by the microscopic morphological examination, motility test, and a battery of biochemical tests like IMViC tests, nitrate reduction test, triple sugar iron agar test, oxidase test, catalase test and urease test.
- The antibiotic sensitivity was performed by the disc diffusion method and *E. coli* showed maximum resistance (76.00%) to penicillin, whereas minimum resistance (24%) to amoxicillin+clavulanic acid.
- Minimum incidence of *E. coli* infestation is 30.00% in eggshells and 22.00% in egg content of eggs collected from organized poultry farms, whereas the maximum incidence of 46.00% in eggshells and 36.00% in egg content of market eggs was recorded.

INTRODUCTION

Egg consumption is increasing day by day in India. Eggs are used in diets of people of all ages because these are highly digestible and provide a unique source of nutrients. Eggs are the primary source of protein in the human diet and have been used in a number of traditional Indian dishes for decades (Réhault-Godbert *et al*., 2019). Since the egg is an excellent source of nutrients, it also serves as a nice medium for the growth of several microbes. The common microbes that infect eggs include bacteria, viruses and fungi. Microbes affect egg contents by penetrating through the shell and inner membranes and multiplying to unacceptable levels causing egg rotting (Gautron *et al*., 2021). Bacterial spoilage caused by gram-negative proteolytic bacteria may produce mustiness and rotting (Roto *et al*., 2016).

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Enteric bacteria like *E. coli*, *Salmonella*, *Listeria,* etc., could contaminate eggs and may cause egg-borne diseases (Maka *et al.*, 2015; Elisha *et al.*, 2017). *Escherichia coli* is quite important from a zoonotic point of view, because of its vast host–spectrum, complex pathogenesis, and the complicated epidemiological chain involving man, animals, and their environment. *E. coli* population can be used as a measure of food quality and sanitary processing conditions. Human illness resulting in the consumption of poultry eggs contaminated by *E. coli* can be expensive in terms of the total annual cost of medical care (Stromberg *et al.*, 2017). Hence, it is mandatory to know the microbial quality of poultry eggs.

For the control of disease and improved performance, antimicrobials are widely used. Due to the indiscriminate use of antimicrobials, there is a continuous emergence of drug-resistant strains of microbes (Adebowale *et al.*, 2022). Bacterial drug resistance poses a great threat to public health because these are capable of transferring resistance genes to other pathogens through the episomal transfer of resistance factors. Food poisoning associated with egg-borne pathogens may cause severe morbidity or mortality with diarrhoea, vomiting, nausea, and abdominal cramps (Mitchell, 2005). So, the present study was designed for the isolation of *Escherichia coli* along with antibiotic sensitivity profiling of isolates from eggshell and egg content.

MATERIALS AND METHODS

A total of three hundred egg samples (300) were used from one hundred and fifty (150) eggs collected from organized (50) and unorganized poultry farms (50) as well as local markets in and around Patna (50) during the period from July 2015 to July 2016. The eggshell and egg contents of each egg were used. The samples were processed in the laboratory of the Department of Veterinary Microbiology, Bihar Veterinary College, Patna. Freshly laid indigenous as well as exotic eggs were used within 3 days of laying. The whole egg was collected aseptically as a sample in a sterile sample bag, properly labelled with sample details. The sample bags were placed in containers with ice cubes and transported to the laboratory. The processing of the sample was initiated within 1 hr. of collection.

The egg sample was processed by wiping the eggshell at the place of an air sac with 70% alcohol, the eggshell membrane was separated by the use of sterile tweezers (Zhu *et al.*, 2021). Inoculums were prepared by separation of the egg contents (both albumin and yolk) from the eggshell. Approximately 1 gm of triturated eggshell and 1 mL of mixed egg content were inoculated with 10 mL of pre-sterilized MacConkey broth in front of the gas flame and incubated for 24 hr at 37^{o}C (Cox *et al.*, 1982). Eosin methylene blue agar was used as selective media for the isolation of *E. coli* from all collected samples. A loop full of MacConkey broth grown overnight and showed turbidity or change in colour was streaked on the EMB agar and incubated at 37^{o}C for 24 hr. The characteristic colonies of *E. coli* on EMB agar i.e., purple with a black centre and green metallic sheen colonies were selected for studies (Fig. 1). The colonies of presumptive *E. coli* from EMB agar were confirmed by the microscopic morphological examination, motility test, and a battery of biochemical tests like IMViC tests, nitrate reduction test, triple sugar iron agar test, oxidase test, catalase test and urease test.

The morphological examination of *E. coli* was performed by microscopic examination of a Grams-stained smear of the characteristic colonies of *E. coli.* The antibiotic susceptibility pattern was performed by the disc diffusion method (Hudzicki, 2009). Co-trimaxazole (COT25), gentamycin (GEN 10), ciprofloxacin (CIP5), penicillin (P10), chloramphenicol (C30), oxytetracycline (Q30), doxycycline (DO30), ceftriaxone (CTR30) and amoxicillin+clavulanic acid (AMC30) discs were used. All antibiotic disc-containing plates were incubated for 18-24 h at 37^{o}C. The zone of inhibition of growth was measured by the scale, and results were interpreted according to the guidelines of CLSI, 2014 (Omoya and Ajayi, 2016).

RESULTS

The present study showed that the incidence of *E. coli* infestation is 30.00% in eggshells and 22.00% in egg content of eggs collected from organized poultry farms, whereas the incidence of 38.00% *E. coli* in eggshells and 28.00% in egg content were recorded in eggs from unorganized poultry farms. Similarly, the incidence of 46.00% in eggshells and 36.00% in egg content of market eggs was recorded (Table 1 and Fig. 2).

The morphological stains appeared as pink rod-shaped bacteria on Gram's staining. The selective streaking on EMB agar showed characteristic purple colonies with a black center and green metallic sheen. The isolates *E. Coli* were positive for indole, methyl red, glucose, lactose, sorbitol, mannitol, and rhamnose, whereas negative for Voges-Proskauer's, citrate utilization, and sucrose fermentation. the isolates had a resistance of 76.00% to penicillin, 72.00% to oxytetracycline, 63.00% to co-trimoxazole, 59.00% to gentamicin, 38.00% to doxycycline, 37.00% to ceftriaxone, 35.00% to chloramphenicol, 24.00% to

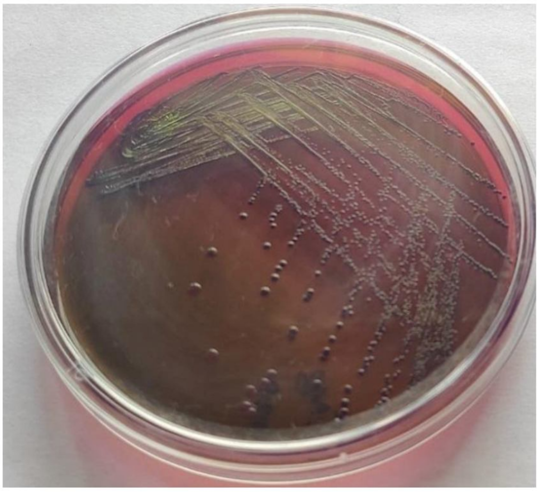

Fig. 1. ***E. coli*** **colony on EMB agar plate**

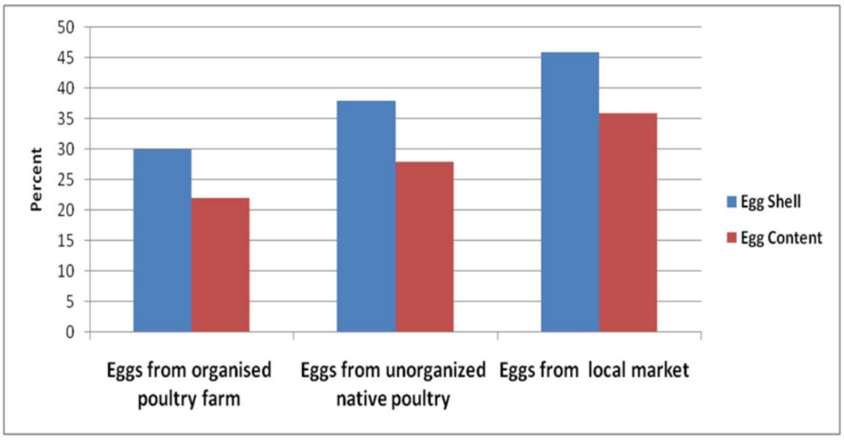

Fig. 2. Incidence of ***E. coli*** **in egg shell and egg content**

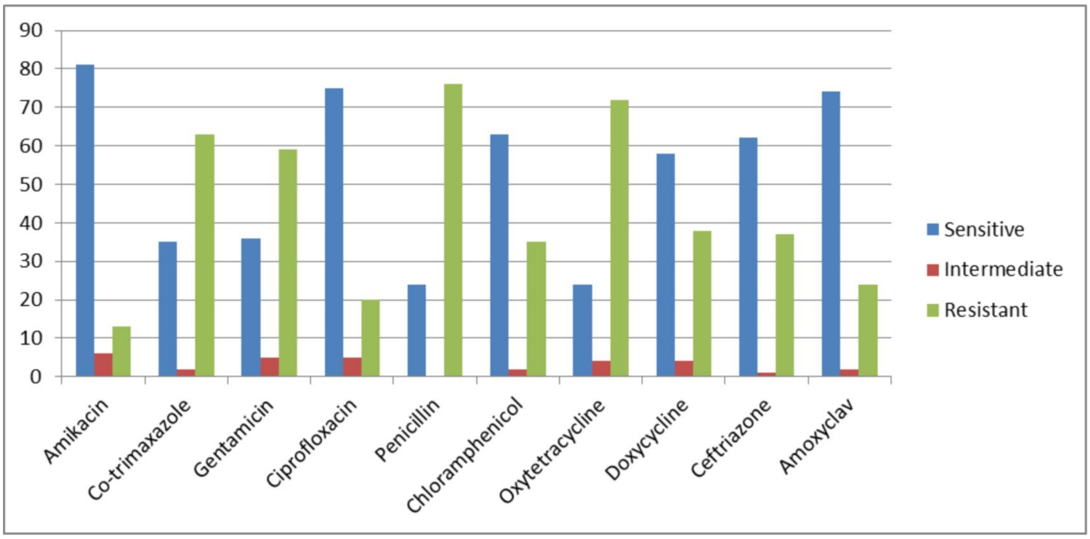

Fig. 3. Antibiogram of isolated ***E. coli***

Table 1. Incidence of *E. coli* in egg shell and egg content

Sl. no.	Sample source	Sample type	Total sample screened	Growth in Mac Conkey broth	EMB agar	Biochemical test
1	Eggs from an organized poultry farm	Eggshell	50	40 (80%)	28 (56%)	15 (30%)
		Egg content	50	26 (52%)	18 (36%)	11(22%)
Total			100	66	46	26
2	Eggs from an unorganized native poultry farm	Eggshell	50	46 (92%)	36 (72%)	19 (38%)
		Egg content	50	38 (76%)	22 (44%)	14 (28%)
Total			100	74	58	33
3	Eggs from the local market in and around Patna	Eggshell	50	47 (94%)	36 (72%)	23 (46%)
		Egg content	50	39 (78%)	28(56%)	18 (36%)
Total			100	86	64	41

Table 2. Antibiotic sensitivity testing of *E. coli* isolates (n=100)

Sl. no.	Antibiotic discs used	Sensitive %	Intermediate %	Resistance %
1	Amikacin (AK30)	81	06	13
2	Co-trimaxazole (COT25)	35	02	63
3	Gentamycin (GEN 10)	36	05	59
4	Ciprofloxacin (CIP5)	75	05	20
5	Penicillin (P10)	24	00	76
6	Chloramphenicol (C30)	63	02	35
7	Oxytetracycline (Q30)	24	04	72
8	Doxycycline (DO30)	58	04	38
9	Ceftrioazone (CTR30)	62	01	37
10	Amoxicillin+Clavulanic acid (AMC30)	74	02	24

amoxicillin+clavulanic acid, 20.00% to ciprofloxacin and the least resistance of 13.00% to amikacin. The isolates also showed a sensitivity of 24.00% to penicillin and oxytetracycline, 35.00% to co-trimoxazole, 36.00% to gentamicin, 58.00% to doxycycline, 62.00% to ceftriaxone, 63.00% to chloramphenicol, 74.00% to amoxicillin+clavulanic acid, 75.00% to ciprofloxacin and the maximum sensitivity of 81% to amikacin. However, some isolates also showed an intermediate susceptibility of 01.00% to ceftriaxone, 02.00% to co-trimoxazole, amoxicillin+clavulanic acid, and chloramphenicol, 04.00% to oxytetracycline and doxycycline and 05.00% to gentamicin and ciprofloxacin while 06.00 % to amikacin (Table 2 and Fig. 3).

DISCUSSION

The present study showed an incidence of 33.33% (100/300) of *E. coli* isolates from egg shells and egg content which was in concordance with the finding of Khan *et al*. (2015), who reported a slightly higher incidence (37.00%) of *E. coli* in table eggs. This finding is also in agreement with another study conducted by Elisha *et al*. (2017) in Trinidad, who reported that 38.6% of table eggs were positive for enteric microbes including *E. coli* and *Salmonella* spp. However, Stepien-Pysniak (2010) reported a higher incidence of 40.30% bacterial contamination in table eggs with *E. coli* as the most dominant contaminant. The present study showed the highest contamination of *E. coli* in eggs collected from the local markets (eggshell, 46%; egg content, 36%), followed by unorganized (eggshell, 38%; egg content, 28%) and organized poultry farms (eggshell, 30%; egg content, 22%). The variation can be attributed to better management practices at organized poultry farms. The contamination was higher in eggshells in comparison to the egg contents because the eggshell is more exposed to the possible contamination factors. However, Elisha *et al*. (2017) reported 36.3 to 69.6% contamination from different points like supermarkets, malls and farms. These differences might be due to differences in management, handling, and hygienic conditions used at farm and or sale outlets.

The poultry eggs can get contamination either horizontally (through the shell) or vertically (trans-ovarian) and could serve as a potential source of pathogens participating in the etiology of food-borne diseases (Stepien-Pysniak, 2010). Persad and LeJeune (2018) reported trans-ovarian transmission of bacterial pathogens like *Salmonella* spp. in table eggs collected from commercial poultry farms in Trinidad. Although egg yolk contains maternal immunoglobulin IgG (also called IgY), its level could be influenced by various factors like the functional quality of the immunological system and or antibiotics exposure to fowl (Tokarzewski, 2002). Moreover, the quantitative contamination of eggs depends upon bacterial load in the environment where eggs are laid and or handled (Sterpien-Pysniak, 2010). The results of the present study indicated the incidence of *E. coli* as 38.00% and 28.67% in eggshell and egg content, respectively. In concordance with the present study, Sterpien-Pysniak (2010) also reported a high contamination level of eggshells as compared to other internal contents. This is probably due to the exposure of eggshells to the environment like bedding material, faecal contamination, water, handlers, etc. In contrast to the finding of the present study, a slightly lower incidence was reported by Elisha *et al*. (2017), who reported a contamination level of 19% and 13% in eggshell and egg contents, respectively. It has been suggested that temperature and or storage conditions provided to the eggs at farm and retail outlets significantly impact the bacterial load of eggs without affecting the bacterial prevalence (Rachtanapun *et al.*, 2022; El Ftouhy *et al.*, 2023).

Antibiotic sensitivity study of *E. coli* isolates was performed by Disc Diffusion Method using 10 antibiotic discs (Himedia Laboratories, Private limited) including penicillin, oxytetracycline, co-trimoxazole, gentamicin, doxycycline, ceftriaxone, chloramphenicol, amoxicillin+clavulanic acid, ciprofloxacin, and amikacin. The present finding revealed that 13.00 to 76.00 isolates were resistant to antibiotics. The results were more or less in line with the findings reported by previous studies. Akond *et al*. (2009) isolated and identified *E. coli* from poultry sources of different poultry markets, and sensitivity to antimicrobials was recorded as 86, 80, 60, 36, 30, and 26% to norfloxacin, gentamicin and chloramphenicol, neomycin, tetracycline, streptomycin and ampicillin, respectively. Raji *et al*. (2007) observed ciprofloxacin as a highly active (85-100%) antibiotic against *E. coli* isolates. Antimicrobial agents by Disc Diffusion Method and tested on 131 bacterial isolates of *E. coli* showed that 125 (95.4%) isolates were resistant to one or more antimicrobial agents with high resistance against streptomycin (90.1%), tetracycline (51.9%) and kanamycin (30.5%). Musgrove *et al*. (2006) and Ansari *et al.* (2014) reported that *E. coli* had 29.9, 6.2, and 3.1% resistance to tetracycline, streptomycin and gentamicin, respectively.

Egg shells are more contaminated with *E. coli* than egg content, and a higher incidence of *E. coli* was recorded in market eggs, followed by eggs from unorganized poultry farms and organized poultry farms. *E. Coli* isolates have a resistance of 13.00 to 76.00% to antibiotics with maximum resistance to penicillin and maximum susceptibility to amikacin. Adopting significant hygienic measures in handling eggs and not consuming inadequately cooked eggs or egg products is advised.

Conflict of interest: The authors have declared that no competing interests exist. There is absolutely no conflict of interest between the authors and the pharmaceutical company. Also, the research was not funded by the pharmaceutical company; rather, it was funded under the research grant of the institute.

Author's contribution: P: Carried out the research; SK, SK: Helped with the research work and are also responsible for writing the manuscripts; A: Helped in conducting antibiotic sensitivity test of samples collected from the eggs; PK: Helped in the collection of samples from different sources; RK: Helped in data evaluation and manuscript writing.

Ethical statement: The study was conducted according to norms of institutional ethical guidelines.

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