**Short Paper**

# Comparative possession of Shiga toxin, intimin, enterohaemolysin and major extended spectrum beta lactamase (ESBL) genes in *Escherichia coli* isolated from backyard and farmed poultry

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## Summary

The present work was conducted to compare the occurrence of *Escherichia coli* possessing virulence and ESBL genes in backyard and farmed poultry. Three hundred and sixty samples from the poultry kept in backyard system and 120 samples from the farmed birds were collected from West Bengal, India. Among the *E. coli* isolates of backyard poultry (O2, O10, O25, O55, O60, O106, UT), none of them possessed any of the Shiga toxin genes and eight *E. coli* isolates (8/272; 2.9%) harboured *eaeA* gene alone. Whereas among the *E. coli* isolated from the farmed poultry (O17, O20, O22, O102, O114, O119, rough, UT), four isolates (4/78, 5.1%) harboured $stx_1$/$stx_2$ gene and 11 isolates (11/78, 14.1%) possessed *eaeA* gene. None of the *E. coli* isolates from the backyard poultry harboured any studied ESBL gene. Whereas 29.4% of *E. coli* isolates from the farmed poultry were found to possess the ESBL genes.

**Key words:** Backyard, ESBL, Poultry, STEC, West Bengal

## Introduction

Intestinal pathotypes of *Escherichia coli* may cause diarrhoea in human and animals (Samanta, 2013). Among them, food associated outbreaks in human was observed with Shiga toxin-producing *E. coli* (STEC) and Enteropathogenic *E. coli* (EPEC) (Newell *et al*., 2010).

Further, transmission of antimicrobial resistant *E. coli* such as extended-spectrum β-lactamase (ESBL) producers in the human food chain from the chicken is a well recognized phenomenon (Hammerum and Heuer, 2009).

The poultry ranks first as a source of food borne infection in human (Batz *et al*., 2011). There are reports of STEC prevalence in farmed poultry (Schouten *et al*., 2005; Dipineto *et al*., 2006) and such information from backyard poultry is scanty. Hence the purpose of the present work was to compare the occurrence of STEC and EPEC and possession of major ESBL genes in backyard and farmed poultry.

## Materials and Methods

Three hundred and sixty samples (cloacal swabs, feed, drinking water, utensil swabs, litter, soil, dried manure, eggs) for bacteriological analysis from the backyard poultry were collected from four agro-climatic zones of West Bengal, India (Terai, New Alluvial, Coastal, Red Laterite) during the period of July-September, 2011. The majority of the birds were young (1-4 weeks), female and apparently healthy. Further, 30 cloacal swabs were collected from the birds kept in organized poultry farm in the same agro climatic zone (n=120). The majority of the birds were young (1-4 weeks), female and apparently healthy.

The standard technique was followed for preparation of the samples and isolation of *E. coli* as described earlier (Samanta *et al*., 2014a).

The DNA from all *E. coli* isolates was extracted as per the earlier method (Samanta *et al*., 2014a). All isolates including positive control were subjected to m-PCR for detection of $stx_1$, $stx_2$, *eae* and *ehxA* genes (Paton and Paton, 1998). The m-PCR was performed in a gradient thermocycler (Eppendorf ProS, Germany). All the reagents and oligo nucleotide primers were procured from Genetix Biotechnology Asia Private Limited, India. The STEC strain possessing all the four genes supplied by CAU was used as positive control and sterile distilled water was used as negative control.

All the *E. coli* isolates having the Shiga toxin/intimin/enterohaemolysin genes were sent for O-serogrouping to Central Research Institute, HP, India.

All the *E. coli* strains including controls were subjected to PCR for detection of major ESBL ($bla_{CTX-M}$, $bla_{TEM}$, $bla_{SHV}$) genes (Samanta *et al*., 2014b). The *E. coli* strain (CAU, India) possessing ESBL genes and the sterile distilled water was used as positive and negative control, respectively.

## Results

Among the 272 *E. coli* isolates from the backyard poultry examined by m-PCR, none (0/272) of them possessed any of the Shiga toxin ($stx_1$/$stx_2$) or enterohaemolysin genes (*ehxA*). However, eight *E. coli* isolates (8/272; 2.9%) were found to harbour *eaeA* gene alone (Table 1) and they belonged to O2, O10, O25, O55, O60, O106, and untypeable (UT) serogroups (Table 1).

**Table 1:** Characterization of EPEC isolated from backyard birds in different agro-climatic zones in West Bengal, India

| Agro-climatic zone | Serogroup | Genotype | | | |
|---|---|---|---|---|---|
| | | $stx_1$ | $stx_2$ | *eaeA* | *ehxA* |
| Terai (3) | O2 | - | - | + | - |
| | O10 | - | - | + | - |
| New alluvial (2) | O25 | - | - | + | - |
| | O55 | - | - | + | - |
| Coastal (1) | O60 | - | - | + | - |
| | O106 | - | - | + | - |
| Red laterite (2) | UT | - | - | + | - |

Number in parenthesis indicates the number of positive serogroups

Among the 78 *E. coli* isolated from the commercial farmed poultry four isolates (4/78, 5.1%) were found to harbour $stx_1$/$stx_2$ gene (Table 2, Fig. 1). Further, 14.1% occurrence of EPEC was detected in healthy farmed poultry (Table 2, Fig. 1). The STEC and EPEC isolates belonged to O17, O20, O22, O102, O114, O119, rough, and untypeable serogroups.

None of the *E. coli* isolates from the backyard poultry harboured any studied ESBL gene in PCR. Whereas 29.4% of *E. coli* isolates from the farmed poultry were found to possess none of the ESBL genes in terai and new alluvial zones (Table 3, Figs. 2, 3, 4). In both zones, isolation of $bla_{CTX-M}$ possessing *E. coli* was highest (43.4%) which was followed by isolation of $bla_{SHV}$ (34.7%) and $bla_{TEM}$ (21.7%) possessing *E. coli*, respectively (Table 3). None of the ESBL-possessing isolates harboured any gene for Shiga toxins or intimin.

## Discussion

The present study could not detect any *E. coli* isolates possessing Shiga toxin genes from the backyard poultry. Similar finding was observed in free range chicken in Spain (Esteban *et al*., 2008). No study was apparently available regarding the isolation of EPEC from backyard

**Table 2:** Characterization of STEC/EPEC isolated from commercial poultry in different agro-climatic zones in West Bengal, India

| Agro-climatic zone | Serogroup | Genotype | | | |
|---|---|---|---|---|---|
| | | $stx_1$ | $stx_2$ | *eaeA* | *ehxA* |
| Terai (7) | O17 | + | - | - | - |
| | UT | + | - | - | - |
| | O20 | - | - | + | - |
| | O22 (2) | - | - | + | - |
| New alluvial (4) | UT | - | + | - | - |
| | Rough | - | + | - | - |
| | O119 | - | - | + | - |
| | O114 | - | - | + | - |
| | UT | - | - | + | - |
| Coastal (3) | O102 | - | - | + | - |
| | UT | - | - | + | - |
| Red laterite (1) | O20 (2) | - | - | + | - |
| | Rough | - | - | + | - |

Number in parenthesis indicates the number of positive serogroups

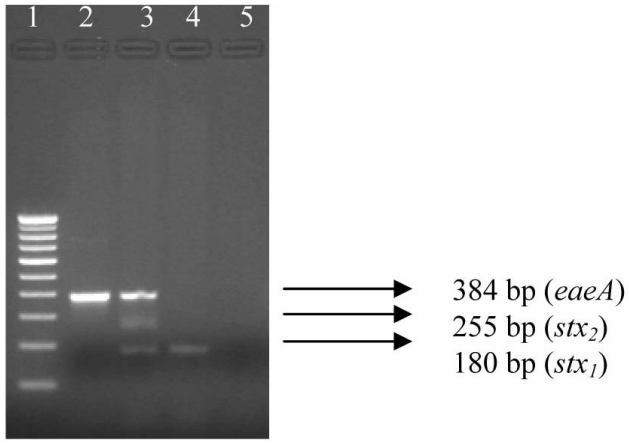


**Fig. 1:** PCR gel documentation showing amplification of different virulence genes of STEC isolated from farmed poultry. Lane 1: 100 bp DNA ladder, Lane 2: Representative isolate possessing *eaeA* gene alone, Lane 3: A mixed PCR product showing the presence of $stx_1$, $stx_2$, *eaeA* genes together, Lane 4: Representative isolate possessing $stx_1$ gene only, and Lane 5: Negative control

**Table 3:** Distribution of major ESBL genes in *E. coli* isolated from farmed poultry in different agro-climatic zones of West Bengal, India

| Agro-climatic zones | No. of cloacal swabs collected | No. of *E. coli* isolated | Distribution of studied ESBL genes among the *E. coli* isolates | | |
|---|---|---|---|---|---|
| | | | $bla_{TEM}$ | $bla_{SHV}$ | $bla_{CTX-M}$ |
| Terai | 30 | 19 (63%) | 1 | 2 | 3 |
| New alluvial | 30 | 23 (76%) | 4 | 6 | 7 |
| Coastal | 30 | 17 (56%) | 0 | 0 | 0 |
| Red laterite | 30 | 19 (63%) | 0 | 0 | 0 |
| | | | 5 (21.7%) | 8 (34.7%) | 10 (43.4%) |
| Total | 120 | 78 (65%) | | 23 (29.4%) | |

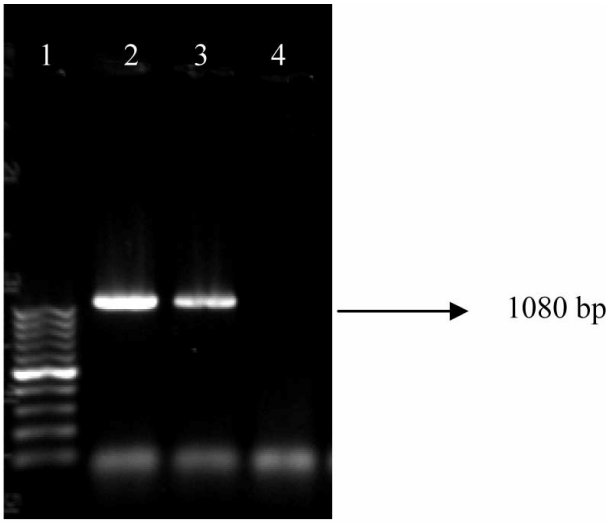


**Fig. 2:** PCR gel documentation showing amplification of *bla*$_{TEM}$ in *E. coli* isolates from farmed poultry. Lane 1: 100 bp ladder, Lane 2: Representative sample showing positive *bla*$_{TEM}$ gene, Lane 3: Positive control, and Lane 4: Negative control

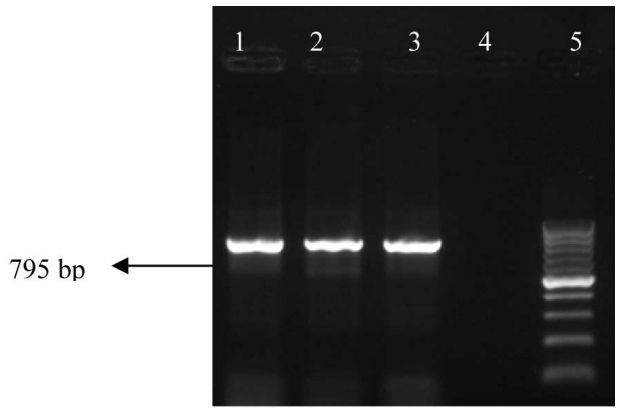


**Fig. 3:** PCR gel documentation photo showing amplification of *bla*$_{SHV}$ in *E. coli* isolates from farmed poultry. Lane 1, 2: Representative sample showing positive *bla*$_{SHV}$ gene, Lane 3: Positive control, Lane 4: Negative control, and Lane 5: 100 bp ladder

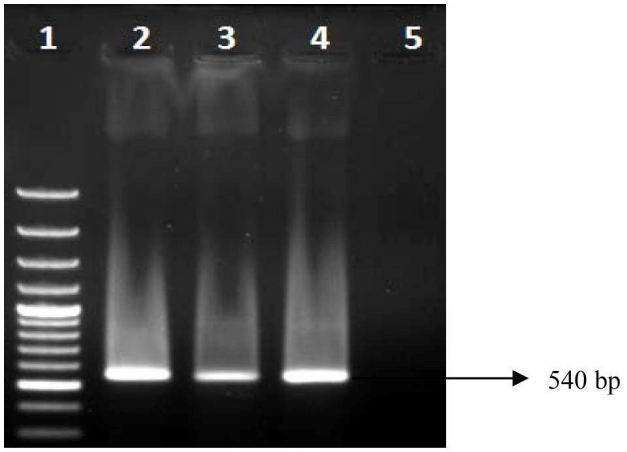


**Fig. 4:** PCR gel documentation showing amplification of *bla*$_{CTX-M}$ in *E. coli* isolates from farmed poultry. Lane 1: 100 bp ladder, Lane 2, 3: Representative sample showing positive *bla*$_{CTX-M}$ gene, Lane 4: Positive control, and Lane 5: Negative control

poultry to compare the present finding. The serogroups such as O2, O55 and UT were detected as EPEC serogroup from the broiler birds in India earlier (Wani *et al*., 2004).

The present study detected Shiga toxin producing-*E. coli* isolates (4/78, 5.1%) from the farmed poultry. Similarly, previous works isolated STEC (O157:H7) from live poultry in other countries (Schouten *et al*., 2005; Dipineto *et al*., 2006), as well as from carcass of the broilers in Iran (Salehi, 2012). The occurrence of EPEC (14.1%) in the farmed poultry in the present study was consistent with earlier findings in India (Farooq *et al*., 2009).

The serogroups O17, O20, O22, O78, O102, rough and UT, isolated in the present study as STEC, were also earlier isolated as STEC from pigeon in Northern India (Farooq *et al*., 2009) and chicken meat in Western India (Zende *et al*., 2013). The serogroups O114 and O119 werealso previously reported as EPEC serogroup from chicken in Brazil (Gonzalez *et al*., 2000). Comparing the STEC/EPEC isolation status, it seems that the backyard rearing conditions might have an advantageous effect on diminishing STEC shedding as detected earlier (Esteban *et al*., 2008).

The present study could not detect any ESBL gene in the *E. coli* isolates from the backyard poultry probably due to lack of antibiotic use, specially those associated with the studied ESBL genes. Whereas 29.4% of *E. coli* isolates from the farmed poultry were found to possess none of the studied ESBL genes. Similarly, 33.3% *E. coli* isolates from the broiler meat was detected to carry *bla*$_{CTX-M}$ in the UK (Warren *et al*., 2008). The possible use of antibiotics in farmed poultry (cephalosporin, ampicillin) coupled with the greater density of birds in intensive farming system might explain such high levels of resistance in the studied zones. A similar kind of comparative study revealed that the *E. coli* isolates from the backyard poultry were free of phenotypical resistance to tetracycline, streptomycin and sulphonamide in comparison to the *E. coli* isolates from farmed poultry in Nigeria (Ojeniyi, 1985). Another study revealed the presence of ESBL in *E. coli* (3/59, 5.08%) in very low proportion in free range birds than the commercial broilers (15/58, 25.8%). The presence of ESBL in free range layer was due to vertical transfer of the resistance gene from the breeder (Obeng *et al*., 2012). Further, the STEC/EPEC isolates of the farmed and backyard poultry did not possess any ESBL gene, which is supported by earlier findings except in one study where ESBL-possessing STEC was detected from farmed poultry (Roset *et al*., 2007).

With respect to STEC shedding and antimicrobial resistance genes the backyard poultry or their products seem to be safer for human consumption than the farmed poultry in the studied zones in India.

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