Isolation, Antibiogram and Molecular Characterization of Escherichia coli in Broiler Flocks

Pradhnya Choudhari1*, Dinesh J. Ghodasara1, Digjay V. Kabariya1, Bharat B. Bhanderi2, Sohilabbas G. Momin1

ABSTRACT

The present study was conducted on 30 broiler flocks (organs of 5 birds per flock homogenized as composite or pooled one sample) for cultural isolation, identification, biochemical characterization and antibiogram profile of Escherichia coli (E. coli). All the 30 composite samples (100%) revealed colonies with typical morphology and biochemical characteristics of E. coli. Antibiogram study was conducted by disc diffusion method and results revealed the highest 100% sensitivity to antibiotic colistin followed by amikacin (83.33%) ceftriaxone (80.00%), chloramphenicol (60.00%), cefotaxime (60.00%) gentamicin (53.33%) and norfloxacin (6.66%), while the organisms were highly resistant to norfloxacin (83.33%) followed by gentamicin (46.66%). In addition, all the 30 E. coli isolates were tested for presence of virulence associated genes, i.e., iss, papC, tsh and vat by PCR using gene specific primers. Result showed the highest prevalence of iss gene 26/30 (86.66%) followed by vat 24/30 (80.00%), tsh 16/30 (53.33%) and papC 9/30 (30.00%) in the flocks tested.

Keywords: Escherichia coli, Prevalence, Antibiogram, Broiler, Virulence genes.

INTRODUCTION

Escherichia coli is considered as the fundamental cause of morbidity and mortality in birds. Normally, E. coli is present in intestinal tract and environment of poultry, but certain strains designated as Avian Pathogenic Escherichia coli (APEC) possess specific virulence factors and are able to cause disease. There are various manifestations of E. coli infection among which the most severe is colisepticemia, which is characterized by multiple organ lesions like airsacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis or yolk sac infection. Now a day, there is increase in incidence and severity of colisepticemia in broilers as well as in layers and thus impose a major threat on the poultry industry. APEC strains impose severe problems on aviculture industries. A number of potential virulence associated genes (iss, papC, tsh, vat, irp-2, iucD, hlyF, iron, cva/cvi, and astA) have been identified in APEC, including their virulence-associated bacterial properties (Dho-Moulin and Fairbrother, 1999). This study was therefore aimed to isolate and study the antibiogram and molecular characterization of Escherichia coli in broiler flocks of middle Gujarat.

MATERIALS AND METHODS

The study was carried out in thirty broiler flocks (5 birds per flock) received for post-mortem diagnosis at Department of Pathology, Veterinary College, Anand. For isolation, identification, antibiogram profile and molecular characterization of E. coli, a total of thirty composite samples (consisting of homogenized mixture of heart, liver, lung and spleen from all 5 birds of a flock) were collected during post-mortem examination and stored in cryo-vials at -20°C.

Isolation and Identification of E. coli

Thirty composite samples collected from 30 farms were streaked on MacConkey agar plates. After 24 hours of incubation the pink colored colonies were identified and subcultured on Eosin Methylene Blue (EMB) agar. After incubation, bacterial colonies were investigated on the basis of staining, colony morphology, cultural and biochemical characters of pure isolates.

Antibiogram of E. coli Isolates

All the thirty isolates of E. coli obtained were subjected
to *in vitro* antimicrobial sensitivity test against seven commonly used antimicrobials, viz., amikacin, ceftriaxone, chloramphenicol, colistin, cefotaxime, norfloxacin, and gentamicin. The test was carried out by disc diffusion technique of Bauer *et al.* (1966). The code and concentrations of antimicrobial discs used are mentioned in Table 1.

**Molecular Characterization of *E. coli* isolates**

The DNA extraction was done from all the thirty isolates of *E. coli* obtained during the study. The suspension of the organisms from each isolate was prepared by picking up a typical colony in a 200 μl PCR tube in 100 μl of Milli-Q water. The prepared suspension was heated at 95°C for 15 minutes and all the cell debris were removed by centrifugation at 10,000 rpm for 1 minute and 3 μl of the supernatant was used as a template DNA. For the molecular characterization of *E. coli*, extracted DNA from each isolate was subjected to PCR amplification of *E. coli* virulence associated genes. The PCR amplification of virulence associated genes was carried out using primers as in Table 2. The steps and conditions of thermal cycling for different primers of virulence genes used are given in Table 3.

**RESULTS AND DISCUSSION**

**Isolation and Identification of *E. coli***

Cultural isolation and identification of *E. coli* was done on MacConkey agar (MCA) plate. Lactose fermentation by bacteria on MCA plates resulted in appearance of pink coloured colonies (Fig. 1). These colonies were subcultured on Eosin Methylene Blue (EMB) agar plate. Colonies with metallic greenish sheen on EMB agar plates were tentatively considered to be positive for *E. coli* (Fig. 2). For confirmation, a loopful colony was selected for Gram staining which revealed pink coloured Gram negative bacilli (Fig. 3). Based on cultural characteristics all the thirty pooled/composite samples were found positive for *E. coli*.

**Biochemical Characterization of *E. coli* Isolates**

All the thirty *E. coli* isolates obtained were characterized by biochemical tests, *i.e.*, indol production, methyl red (MR), Voges-Proskaur (VP) and citrate utilization test and the results were interpreted according to the Bacteriological Analytical Manual (US FDA: BAM, 2002). All the isolates revealed similar IMViC pattern of + + - - (Fig. 4).

---

**Table 1:** Details of antimicrobial agents and concentration of discs used for antimicrobial susceptibility of *E. coli*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the antimicrobial disc</th>
<th>Code</th>
<th>Conc. (in mcg)</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>1</td>
<td>Amikacin</td>
<td>AK</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Gentamicin</td>
<td>GEN</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Norfloxacin</td>
<td>NK</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Chloramphenicol</td>
<td>C</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Ceftriaxone</td>
<td>CTR</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>Colistin</td>
<td>CL</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Cefotaxime</td>
<td>CTX</td>
<td>30</td>
<td>22</td>
</tr>
</tbody>
</table>

**Table 2:** Primer sequence used for the detection of virulence associated genes in *E. coli* isolates

<table>
<thead>
<tr>
<th>Name of Primers</th>
<th>Sequences (5'→3')</th>
<th>Expected product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>iss</td>
<td>Forward</td>
<td>CCCCCAATTGGACAGAGAAAA</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>ATCGATGGGCCCTATTGTGAG</td>
<td></td>
</tr>
<tr>
<td>papC</td>
<td>Forward</td>
<td>AAATAAAAACGTGCGGACTG</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AGCGAGTAAAGCAGAATCGT</td>
<td></td>
</tr>
<tr>
<td>tsh</td>
<td>Forward</td>
<td>TCTCAATGCGCTGTAACG</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CCTTCAGATGAACGTCAGCAA</td>
<td></td>
</tr>
<tr>
<td>vat</td>
<td>Forward</td>
<td>CAGCCTACTGTAATGCGTCTGAA</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TGCGCAGTATGTTGTTGAA</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Steps and conditions of thermal cycling for detection of virulence associated genes of *E. coli*

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Initial Denaturation</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>iss, papC</td>
<td>94°C, 5 min</td>
<td>94°C, 30 sec</td>
<td>56°C, 30 sec</td>
<td>72°C, 30 sec</td>
<td></td>
</tr>
<tr>
<td>tsh, vat</td>
<td>94°C, 3 min</td>
<td>94°C, 30 sec</td>
<td>58°C, 30 sec</td>
<td>72°C, 30 sec</td>
<td>72°C, 5 min</td>
</tr>
</tbody>
</table>
Isolation, Antibiogram and Molecular Characterization of E. coli in Broilers

The in vitro antimicrobial drug sensitivity pattern of all the thirty E. coli isolates tested against seven commonly used antimicrobials (Fig. 5) revealed that the E. coli isolates were highly sensitive to antibiotic colistin, i.e., methane sulphonate 30/30 (100.00%) followed by amikacin 25/30 (83.33%), ceftriaxone 24/30 (80.00%), chloramphenicol 18/30 (60.00%), cefotaxime 18/30 (60.00%) gentamicin 16/30 (53.33%) and norfloxacin 2/30 (6.66%), whereas they were highly resistant to norfloxacin 25/30 (83.33%) followed by gentamicin 14/30 (46.66%) and chloramphenicol 11/30 (36.66%) as shown in Fig. 6.

Chaudhari (2017) and Halfaoui et al. (2017) also showed highest sensitivity of E. coli to colistin (100 and 93.46 %). On the contrary, Mohamed et al. (2014), Jahantigh and Dizaji (2015), Subedi et al. (2018) and Awad et al. (2020) showed lower sensitivity.

The present sensitivity pattern of E. coli isolates to Amikacin (83.33%) was well supported by Sahoo et al. (2012) Subedi et al. (2018) and Sarba et al. (2019), while Soufi et al. (2009) reported 100 % sensitive of E. coli isolates to amikacin. Similarly, sensitivity to ceftriaxone (80.00%) observed during the present study was also well supported by Sahoo et al. (2012) and Chaudhari (2017).

The sensitivity of E. coli to chloramphenicol (60.00%), cefotaxime (60.00%) and Gentamicin (53.33%) observed during the present study was well in accordance with the reports of Soufi et al. (2009), Sahoo et al. (2012), Halfaoui et al. (2017), Subedi et al. (2018) and Awad et al. (2020).

Further, the highest resistance (83.33%) of E. coli isolates was observed against norfloxacin in the present study. Mohamed et al. (2014) and Jahantigh and Dizaji (2015) also showed comparable 96.00 and 88.00 % resistance to norfloxacin.

The pattern of antibiotic sensitivity using different antibiotic discs against E. coli isolates in poultry has been reported time to time by previous workers. The sensitivity/resistance pattern of a specific antibiotic was found to be variable by different workers. The antibiotic sensitivity pattern of the present study showed colistin to be most sensitive antibiotic followed by amikacin, ceftriaxone, cefotaxime and chloramphenicol, while gentamicin and norfloxacin were found less sensitive. It should be noted that, the antibiotic resistance to E. coli in poultry is becoming a major issue and unnecessary use of antibiotics should be avoided. When necessary, specific antibiotics should be used after the sensitivity test.

Molecular Characterization of E. coli Isolates

During the present study, PCR based identification of all thirty E. coli samples for the iss, papC, tsh and vat genes was targeted by using gene specific primers, which revealed the highest (26/30 ,86.66%) prevalence of iss gene followed by vat (1
Isolation, Antibiogram and Molecular Characterization of E. coli in Broilers

24/30, 80.00%, tsh (16/30, 53.33%) and papC (9/30, 30.00%) (Fig. 7, Fig. 8). Similar findings for presence of virulence gene iss of E. coli in broiler chickens have been reported by Ewers et al. (2004), Mohamed et al. (2014), Deshmukh (2016) and Chaudhari (2017). Similarly, Subedi et al. (2018) and Awad et al. (2020) also identified the presence of iss gene in the highest number of E. coli isolates. In contrast, Delicato et al. (2003) and Won et al. (2009) reported lower frequency of this gene.

Nine (30.00%) isolates yielded an amplified product of 201 bp of papC gene (Fig. 7). Similarly, Delicato et al. (2003), Ewers et al. (2004), Won et al. (2009), Deshmukh (2016) and Chaudhari (2017) detected lower frequencies of papC at the rate of 18.50, 22.70, 14.40, 27.50 and 33.33%, respectively, whereas, Roussan et al. (2014) and Mohamed et al. (2014) found this gene at the rate of 50.00 and 44.40%, respectively, on E. coli isolates in poultry.

Sixteen (53.33%) E. coli isolates yielded an amplified product of 153 bp of tsh gene (Fig. 7). This was in line with observations of Ewers et al. (2004), Won et al. (2009), Deshmukh (2016) and Chaudhari (2017), whereas, Roussan et al. (2014) and Subedi et al. (2018) found higher occurrence of this gene at the rate of 66.00 and 62.20%, respectively. In contrast, Delicato et al. (2003) reported lower occurrence of tsh gene in strains of E. coli isolated from chickens with colibacillosis.

Twenty four (80.00%) E. coli isolates yielded an amplified product of 168 bp of vat gene (Fig. 7), which concurred well with the reports of Roussan et al. (2014) and Chaudhari (2017), respectively. Whereas, Won et al. (2009) and Deshmukh (2016) found lower prevalence of this gene.

On the basis of results obtained during the present study, it can be concluded that a combination of more than one virulence associated genes may be responsible for pathogenicity of E. coli organism and are capable of causing colisepticemia. All the thirty E. coli isolates revealed presence of multiple virulence associated genes; 19 were found carrying two out of four virulence associated genes. Seven samples were positive for three genes, while four had all the four virulence associated genes in E. coli genome.

ACKNOWLEDGEMENT

Authors are grateful to Dean, Veterinary College, AAU, Anand for facilities provided and poultry farmers for their cooperation and support to carry out this study.

REFERENCES


Isolation, Antibiogram and Molecular Characterization of *E. coli* in Broilers

The Indian Journal of Veterinary Sciences and Biotechnology, Volume 16 Issue 2, 3 & 4 (October-December 2020)

poultry with colibacillosis. *Veterinary Microbiology*, 94(2), 97-103.


Rousson, D.A., Zakaria, H., Khawaldeh, G., & Shaheen, I. (2014). Differentiation of avian pathogenic *Escherichia coli* strains from broiler chickens by multiplex polymerase chain reaction (PCR) and random amplified polymorphic (RAPD) DNA. *Open Journal of Veterinary Medicine*, 4(10), 211.


