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## Antibiogram assay of pathogenic verotoxic *E. coli* isolated from poultry cloacal swabs

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**Abstract**

The present study was conducted to isolate pathogenic *Escherichia coli* from cloacal swab of apparently healthy poultry and to determine their susceptibility to commonly used antibiotics. A total of 200 cloacal swab samples were collected aseptically and processed under required conditions for the isolation of *E. coli*. To confirm the isolates as *E. coli*, various biochemical tests like IMViC were performed. VTEC isolates were confirmed by using multiplex PCR. To assess the virulence of VTEC isolates, they were also subjected to Congo red dye assay. Antibiotic sensitivity pattern of pathogenic isolates was studied by Disc diffusion method. The percent positivity of *E. coli* was observed 80% (160/200) in cloacal swab samples. Based on multiplex PCR 23 isolates were found positive for VTEC. The percentage positivity on Congo red dye assay was 25.92%. The antibiogram of all 23 VTEC isolates against 15 antibiotics showed that majority of VTEC strains exhibited high level of sensitivity to Chloramphenicol, Imipenem and Amikacin. All isolates were 100% resistant to Clindamycin and Enrofloxacin.

**Keywords:** Poultry cloacal swab, *E. coli*, antibiogram

**Introduction**

*E. coli* are normal habitant of intestinal tract of various mammals, birds, reptiles and other animals. They are known as indicator of fecal contamination and their presence in food indicate the possible presence of other enteric pathogens. These can be transmitted in humans and animals through contaminated feed, water and direct contact. VTEC is one of the most pathogenic *E. coli* responsible for hemolytic uremic syndrome (HUS), hemorrhagic colitis (HC), thrombocytopenia and renal failure (Coia *et al.*, 2001) [6]. *E. coli* are found in the intestinal tract of poultry, and may contaminate poultry meat that may cause intestinal or extra-intestinal infections in humans (Allerberger, 2015) [1].

Indiscriminate use of antibiotic is most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Witte, 1998; Gunner *et al.*, 2004) [26, 7] also indiscriminate use of antibiotics in food producing animals has undesirable consequences on human health because of the presence of drug residue in foods, this decrease the effectiveness of the treatment of bacterial, fungal and parasitic infections (Cosgrove and Carmeh, 2003) [9].

In intensively reared food animals, antibiotics may be administered to whole flocks rather than individual animals, and antimicrobial agents may be continuously fed to food animals such as broilers and turkeys as antimicrobial growth promoters. Therefore the antibiotic resistance in bacterial poultry is high and as a result their faecal flora contains a relatively high proportion of resistant bacteria (Caudry and Stanisch, 1979) [5].

At the time of slaughter, these resistant strains from the gut may contaminate poultry carcasses thus poultry meats are often associated with multiresistant *E. coli* (Jayaratne *et al.*, 1990; Turtura *et al.*, 1990) [14, 24] likewise eggs become contaminated during laying (Lakhota and Stephens, 1973) [15]. It is also known that resistant faecal *E. coli* from poultry can infect humans both directly and through food, by colonizing the human intestinal tract and contributing resistant genes to human's endogenous flora (Bogaard *et al.*, 2001) [25]. However, the mechanism of spread of antibiotic resistance from food animals to humans remains controversial. Colonization of the intestinal tract with resistant *E. coli* from chicken has been shown in human volunteers (Linton *et al.*, 1977) [16] and there is historical evidence that animals are a reservoir for *E. coli* found in humans (Cooke *et al.*, 1971) [8].

**Material and methods**

A total 200 samples comprising of 50 cloacal swab samples each from turkey, quail, chabro and broiler species were collected from poultry farms of DUVASU, Mathura from July 2017

to April 2018. Isolation of *E. coli* was done as per the method described by Merchant and Packer (1967) [17] with slight modification. Cloacal swab samples were collected aseptically in capped tubes having Trypticase soy broth as transport media (autoclaved), then taken to the laboratory ice-cooled and processed within 3 hours of collection. Swab sticks were directly enriched in 9 ml Trypticase soya Broth containing acriflavin (10mg/lit) at 37 °C for 6 hours. Subculturing was done using Mac Conkey's Agar (MLA) plates (figure.1). Lactose fermenting, small, pink colonies were transferred to Eosin Methylene Blue (EMB) Agar plates for demonstration of characteristic "green metallic sheen" following incubation for 24 hrs at 37 °C. Different biochemical tests like indole, methyl red, voges- proskauer, citrate, nitrate reduction, oxidase and sugar fermentation were performed by kit (KB010Hi identification kit, Himedia, India).

#### Antimicrobial sensitivity of VTEC isolates

In-vitro antibiogram of all the pathogenic VTEC isolates was performed by Disc diffusion method (Bauer and Welch, 1996) on Muller Hinton agar. Isolates were tested against 15 commonly used antibiotics (table. 1) Amikacin (30µg), Cefotaxime (30µg), Cefotaxime/clavulanic acid (30/10µg), Chloramphenicol (30 µg), Ciprofloxacin (5µg), Clindamycin

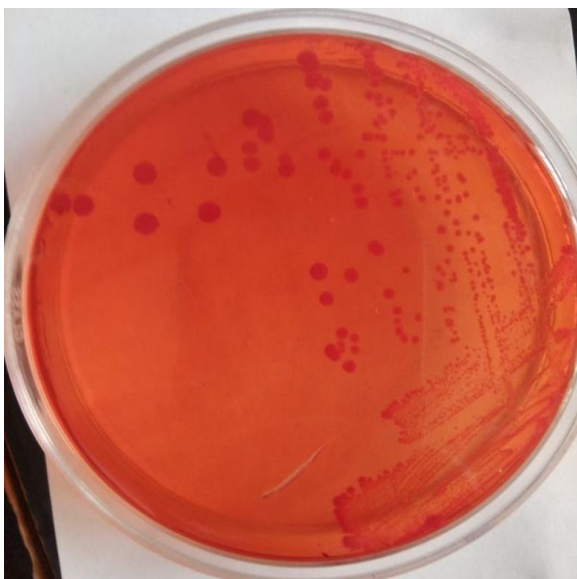
(2µg), Enrofloxacin (10 µg), Erythromycin (15µg), Gentamycin (10 µg), Imipenem (10µg), Kanamycin (30µg), Levofloxacin (5µg), Tetracycline (30µg), Trimethoprim (30µg), Streptomycin (10 µg) and. Zones of complete inhibition were measured according to Himedia Antimicrobial Susceptibility Testing (figure.2).

#### Results

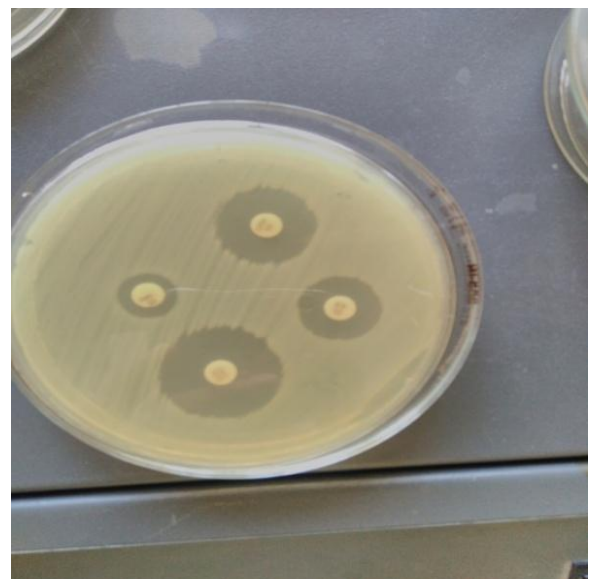
Out of 200 cloacal swab samples which were screened for isolation of *E. coli*, 160 samples (80%) were found positive for *E. coli* which were biochemically confirmed. VTEC strains were detected with the help of multiplex PCR as per the method described by Paton and Paton (1998) [19]. 23 isolates were found positive for VTEC strain having *stx1*, *stx2* and *hlyA* gene but no isolate was positive for O157 and O111 gene. A total of 7 (25.92%) isolates were positive on CR assay. All 23 pathogenic isolates were tested against 15 commonly used antibiotics. The majority of pathogenic strains exhibited a high level of sensitivity to Imipenem (100%), Chloramphenicol (100%), Levofloxacin (86.95%), Kanamycin (78.26%) and Amikcin (78.26%) and low level of sensitivity against Erythromycin and Cefotaxime/clavulanic acid. All the isolates showed 100% resistance to Clindamycin and Enrofloxacin.

**Table 1:** Antimicrobial susceptibility pattern of verotoxic *E. coli* isolates in poultry cloacal swab

Antimicrobial Disc	No. of isolates tested	No. of sensitive %	No. of Resistant %
Amikacin (AK)	23	78.26%	21.74%
Cefotaxime (CTX)	23	65.22%	34.78%
Cefotaxime/clavulanic acid (CEC)	23	13.04%	86.96%
Chloramphenicol (C)	23	100%	0%
Ciprofloxacin (CIP)	23	52.17%	47.83%
Clindamycin (CD)	23	0%	100%
Enrofloxacin (EX)	23	0%	100%
Erythromycin (E)	23	8.69%	91.31%
Gentamicin (GEN)	23	73.91%	26.09%
Imipenem (IPM)	23	100%	0%
Kanamycin (K)	23	78.26%	21.74%
Levofloxacin (LE)	23	86.95%	13.05%
Tetracycline (TE)	23	39.13%	60.86%
Trimethoprim (TR)	23	60.86%	39.14%
Streptomycin (S)	23	73.91%	26.09%



**Fig 1:** *E. coli* (VTEC) colonies with characteristics brick red colour on Congo red agar.



**Fig 2:** Antimicrobial sensitivity test for pathogenic *E. coli* (VTEC) isolates.

## Discussion

Large proportions of antibiotics (50%) of the total global consumption are administered to food producing animals for prophylaxis treatment and growth promotion purposes, but 80% of such total administration is unnecessary (Harrison and Ledderberg, 1998) <sup>[12]</sup>. In livestock industry antibiotics are used more frequently in poultry farms. Development of antibiotic resistant strains leads to lack of response to treatment in both animals and humans. There are reports of resistance of *E. coli* to antibiotics with associated treatment failure (Talan *et al.*, 2004; Blondeau, 2004) <sup>[23, 4]</sup>. The W.H.O. has recognized that antibiotic use in animals affects antibiotic resistance in human (Anonymous, 2000) <sup>[2]</sup>. In present study, out of 200 samples 160 (80%) were found positive for *E. coli*. This finding was similar to previous finding 80.2% by (Wani *et al.*, 2004) <sup>[26]</sup> and 88.8% by (Doregirae *et al.*, 2016) <sup>[10]</sup>. On the other hand lower prevalence was reported 65.22% by (Savita *et al.*, 2007) <sup>[20]</sup>. After determining by mPCR, a total of 23(11.5%) isolates were found positive, which is similar to finding of (Morabito *et al.*, 2001) <sup>[18]</sup>, who detected *stx* genes in 10.8% of the stool enrichment cultures collected from 649 feral pigeons in Italy.

In present study the isolates showed 100% resistance to clindamycin and enrofloxacin. Similar findings were also reported in previous study high resistance to erythromycin and clindamycin (96.15%), tetracycline (76.92%) by (Shecho *et al.*, 2017) <sup>[22]</sup> in cloacal swabs of healthy chickens. (Sharma *et al.*, 2017) <sup>[21]</sup> revealed high sensitivity of pathogenic *E. coli* in groundwater samples to imipenem (68.75%), gentamicin (93.75%), levofloxacin (68.75%); similar to our study. Resistance to tetracycline (65.93%), and streptomycin (61.54%) was also detected by (Dutta *et al.*, 2011) <sup>[11]</sup> in poultry. A study in domestic pigeons in Japan was conducted by (Ishiguro *et al.*, 1978) <sup>[13]</sup>, the isolated *E. coli* strains were resistant to tetracycline, streptomycin, sulfonamides and quinolones, they concluded that the high antimicrobial resistance occurrence may reflect the abusive use of antimicrobial substances in society.

## Conclusion

Poultry may be potential source of pathogenic *E. coli*. It is recommended that good management and hygienic practices shall be performed during and after their handling. Indiscriminate use of antibiotics in poultry farms should be avoided because it may lead to the development of drug-resistant strains of bacteria and their transfer into humans whom are difficult to treat. It is possible to prevent contamination of pathogenic *E. coli* by applying appropriate hygiene and sanitation methods in poultry farm and also by creating awareness.

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