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Studies of Virulence Factors of Avian Pathogenic *Escherichia coli* in Avian Colibacillosis by *In vitro* Method and PCR

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ABSTRACT

Background: Avian colibacillosis is considered as major cause of morbidity and mortality in poultry. It is a common bacterial disease of poultry and many virulence factors of *E. coli* are associated with the disease. The current study was aimed to investigate the presence of some virulence factors of *E. coli* isolated from the cases of colibacillosis.

Methods: In present study, total 150 samples (liver, heart, lungs, air sacs and feaces) of chicken exhibiting pathological conditions of colibacillosis were collected from various poultry farms (organized and backyard) situated in and around Mhow and Indore cities. *E.coli* was isolated and identified from the samples on the basis of cultural characteristics and biochemical test. All *E. coli* isolates were further subjected to evaluate the presence of virulence factors such as biofilm production, haemolysis, invasiveness and molecular detection of *fim*H and *stx*1 gene.

Result: Out of these 51.33% of incidence of *E. coli* was recorded. *E. coli* O84 and O149 serotypes were found most prevalent. Out of 77 isolates, 46 (59.7%) and 45 (58.4%) were positive for biofilm formation by tube method and modified CRA method, respectively. All *E. coli* isolates were showing invasiveness in congo red binding assay while none of the isolates was found haemolytic. Molecular detection revealed the presence of *fim*H (508bp) gene in 33.3% of tested samples while *stx*1 gene could not be detected in any isolates.

Key words: Avian pathogenic, Biofilm, Congo red binding assay, Colibacillosis, *E. coli*, *fimH*, PCR.

INTRODUCTION

Poultry is an important source of egg and meat worldwide and the consumption of poultry products is continuously increasing due to rising human population. India ranks 3rd in egg production and 4th in chicken production in the world (Singh, 2018a). The high consumption of poultry products also necessitates great care in safeguarding the industry against intimidating factors in terms of public health also. There are many bacterial and viral diseases that affect the poultry production and leads to great economic loss in industry. Among them, avian colibacillosis is a common bacterial disease of poultry characterized by air sacculitis, pericarditis, perihepatitis, peritonitis, osteomylitis or yolksac infection. The clinical signs of disease are reported as depression loss of appetite, dropped wing closed eyes, weight loss, decrease egg production, *etc.*

Escherichia coli is the most important pathogen causing primary and secondary bacterial infection such as colisepticaemia, coligranuloma, omphalitis and Hjarre's disaese. Although the majority of *E.coli* strains remain commensal in avian gastrointestinal tract and other surface mucosa but certain strains are capable of causing disease and these are designated as avian pathogenic *E. coli* (APEC). Colisepticemia is a serious life-threatening condition, which is associated with acute onset of very generalized clinical signs of sickness, such as listlessness, depression, weakness, loss of appetite, and sudden death of birds (Kuznetsova *et al.,* 2020).

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Serotyping is useful tool for epidemiological studies. Immune response in poultry against APEC is directly related to "O" antigens (Nolan *et al.,* 2013). Several virulence factors have been associated with the pathogenicty of APEC including haemolysin, adhesin (*fim*), toxins, invasins, capsule as well as lipopolysaccharide complex (Ewers *et al.,* 2003; Saha *et al.,* 2020). However, several studies have demonstrated that all virulence factors are rarely present in same isolate. Keeping these views, this study was carried out to investigate the presence of some virulence factors such as haemolysin, adhesin, invasiveness, fimbrial adhesin (*fim*) and heat stable toxins (*stx*) of *E. coli* isolated from poultry with confirmed cases of colibacillosis.

MATERIALS AND METHODS

The work was carried out in the Department of Veterinary Microbiology, College of Veterinary Sciences and A.H., Mhow during May 2018 to June 2019.

Sample collection

A total number of 150 samples (liver, heart, lungs, air sacs and feaces) were collected from chickens exhibiting different pathological conditions like perihepatitis, pericarditis, enteritis and air sacculitis from various organized poultry farms and backyard poultry situated in and around Mhow and Indore cities (Fig 1). All samples were collected aseptically in sterile containers and kept at 4°C till further use.

Isolation and identification of *E. coli*

Samples were enriched in brain heart infusion (BHI) broth with incubation at 37°C for 24 hrs followed by subculture on nutrient agar, MacConkey agar and selective media eosin methylene blue (EMB) agar. The organisms were confirmed on the basis of bacterial morphology, cultural characteristics and biochemical tests (Cheesbrough, 1994).

Serotyping of *E. coli*

The identified *E. coli* isolates were sent to National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli (H.P.) for serotyping of somatic (O) antigen.

Haemolysis

Haemolytic activity of *E. coli* was demonstrated on 5% defibrinated sheep blood agar plate as per the method of Shiva Shankar *et al.* (2010). The plates were exhibiting "greening" or clearing of the agar around areas of bacterial growth as an indication of alpha or beta hemolytic activity (Rodriguez-siek *et al.,* 2005).

Biofilm formation
Tube method

Tube method of biofilm formation was followed as per Dhanawade *et al.* (2010). All the samples were inoculated in tryptose soya broth (TSB) and after 12 hrs incubation 50% media were replaced with the TSB containing 0.25% sucrose followed by staining with 0.1% safranin. A visible film lined the inner wall and bottom of the tube was considered positive for biofilm formation. The amount of biofilm formation was scored as weak, moderate and strong.

Modified congo red agar method

Congo red agar was prepared by using blood agar, glucose and congo-red dye as described by Nachammai *et al.* (2016). Black colonies with a dry crystalline consistency indicated biofilm production; non-biofilm producers usually remained pink.

Congo red dye binding assay

The test was performed to determine invasiveness of pathogen as per method described by Yadav *et al.* (2014). The isolates were streaked on tryptone soya agar media containing 0.03% congo red dye, and incubated for 24-72 hrs at both 37°C and 25°C. Appearance of intense orange or red colonies was recorded as a positive reaction. Negative colonies did not bind the dye and remained white or grey.

Molecular detection of Virulence genes *(fimH and Stx1)*
DNA extraction

DNA was extracted from 18 randomly selected *E. coli* isolates. Bacterial pellet was formed from 1.5 ml of an overnight bacterial culture of each isolate and DNA was extracted from each pellet as per manufacturer instructions supplied with the kit (HipurA Bacterial genomic DNA purification kit, Hi Media). DNA was stored at -20°C till further use.

Polymerase chain reaction for FimH and Stx1

The PCR amplification was carried out for *fimH* gene and *stx1* gene of *E. coli* with specific published primers (Fernandes *et al.,* 2011; Eid *et al.,* 2016). The nucleotide sequences of the forward and reverse primers for amplification of 508 bp product of *fimH* gene were as - forward (5′-TGCAGAACGGATAAGCCGTGG-3′) and reverse (5′- GCAGTCACCTGCCCTCCGGTA-3′) (Table1). The nucleotide sequences of the forward and reverse primers were *stx1* F (5′- CAGTTAATGTGGTGGCGAAG-3′) and *stx1* R (5'- GCAGTCACCTGCCCTCCGGTA-3') for amplification of 180 bp product of *stx1* gene. The PCR reaction was optimized for both amplification by adding 2.5 µl of 10X PCR buffer (without $MgCl_2$), 2 µl of 25mM $Mgcl_2$, 1.0 µl of forward and reverse primer (20 pm/µl) each, 1.0 µl of dNTPs mix (10mM each), 0.2 µl Taq DNA polymerase (5 units/ µl), 5 µl of extracted DNA (150-200ng) and the reaction was made up to 25 µl using nuclease free water. The amplification cycles for *fimH* gene were initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 45 sec, annealing at 60°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 10 min. Annealing temperature for *stx1* gene was 51°C for 30 sec and rest of the cycles were same as *fimH* gene The negative control consisted of sterile water instead of DNA template. The amplified products were electrophoresed in 1.5% agarose gel and visualized in gel documentation system (Syngene, USA). A 508 bp amplified product of *fim H* gene was sequenced commercially. The presence of *fimH* gene of *E. coli* was confirmed through NCBI blast GenBank database.

RESULTS AND DISCUSSION

Avian colibacillosis contiues to be a major problem of morbidity and mortality in poultry and chickens. *E.coli* is very diverse species of bacteria found naturally in the intestinal tract of poultry. Several structures and products of *E.coli*

Table 1: Incidence of *E. coli* in chicken.

Type of Farm	No. of samples		Incidence %
	Tested	Positive	
Organized	90	40	44.44%
Backyard Poultry	60	37	61.66%
Total	150	77	51.33%

have been found to contribute in virulence of the strain, such as capsule, pilli, haemolysin etc. This investigation was associated with the determination of many virulence factors of *E.coli* strains isolated from colibacillosis cases of poultry.

In present study, out of 150 samples, 77 (51.33%) *E. coli* isolates were recovered from colibacillosis cases on the morphological and cultural characteristics of *E. coli* were confirmed on the basis of previous observations recommended by Edwards and Ewing (1972). The biochemical behaviour of the isolates in accordance of Edwards and Ewing (1972) and Sahoo *et al.* (2012). All *E. coli* isolates were exhibited purple-black colonies with dark centre metallic sheen on EMB agar (Fig 2). The incidence of *E. coli* was found 44.44% and 61.66% in organized farms and backyard poultry, respectively (Table 1). Several studies also recorded higher rates of *E.coli* in poultry (Peer *et al.,* 2013; Ammar *et al.,* 2014). However, in a study 24% of incidence of *E.coli* were revealed from diarrhoic and non-diarrhoeic poultry samples (Debbarma *et al.,* 2019). Young aged broilers have high affinity to disease in comparison to older chickens (Radwan *et al.,* 2014). Kumar and Gupta (2019) also recovered 106 *E.coli* isolates from broilers suffering from colibacillosis. There are many different predisposing factors which may increase the affinity of poultry to colibacillosis such as immunosupression due to respiratory viral infections of chickens, production stress and close confinement. In present study, backyard poultry highly affected than organized poultry farm. It may be because of bad hygenic measusres and poor management of poultry.

All isolates were serotyped from National Salmonella and *Escherichia* Centre, Central Research Institute, Kasauli, (H.P.). Out of 77 isolates 69 could be typed in different serogroups such as O84 and O149 serogroups were found most prevalent (15.58% each) followed by O120 (11.68%), O9 and O145 (10.38%), O20 (9.09%), O83 (7.79%), and O2 and O119 (2.59%, each) while remain 8 isolates could not be typed (Fig 3). In this study 10 different serotypes were identified from 69 *E.coli* isolates. *E.coli* O2 is reported most prevalent serotype associated with colibacillosis worldwide. In accordance to many studies, serotype O2 also recovered from poultry colibacillosis (Eid *et al.* 2016, Kumar *et al.* 2019 and Ibrahim *et al.* 2019). Our results revealed lower percentage of *E. coli* O2 serotype which is similar to findings of Eid *et al.* (2016), Shiva Shankar *et al.* (2010). The serogroup O11 was recorded by Wani *et al.* (2004) and Serogroup 09 was also found in the reports of Panda *et al.* (2010) and Sahoo *et al.* (2012). Similar to our findings serogroups O2, O9, O11, O20, O83, O119, O120 were reported by Rodriguez-siek *et al.* (2005). According to Ewers *et al.* (2007) the occurrence of a specific serotype and its role in disease production depends upon the health status of the birds, climatic conditions, geographical situation and management strategies. The variations are found in serotype prevalence from time to time and from region to region. However, serogroups do not reflect the virulence of the strains and therefore serotyping cannot be used as a useful diagnostic tool. It may be used only to characterize strains.

E. coli produce many virulence factors that may contribute to its pathogenicity in extra-intestinal infections (Sharma *et al.,* 2007). These virulence factors enable some members of the normal flora to elicit an infection by overcoming the host defence mechanisms (Emody *et al.,* 2003). In the present investigation, none of the 77 *E. coli* isolates produced haemolysis on 5% defibrinated sheep blood agar. Various studies reported the avian *E. coli* as non-hemolytic and independent of haemolytic activity (Eid *et al.,* 2016), which corresponds with our findings. However, some reports suggested that *E.coli* has the ability to produce haemolysin (Zahid *et al.,* 2016). The production of different types of hemolysin has been frequently contributed to *E.*

Fig 1: Pericarditis in poultry.

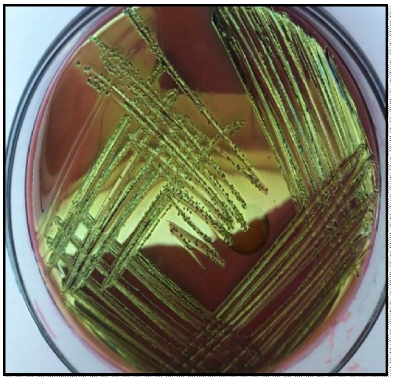

Fig 2: *E. coli* on EMB agar showing metallic sheen**.**

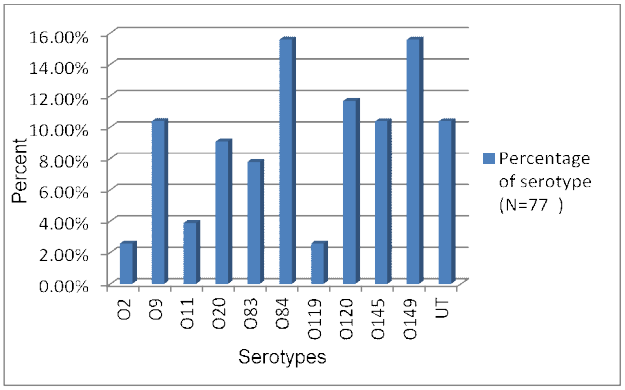

Fig 3: Frequency of 'O' serotypes of *E. coli* isolates.

coli from intestinal and extraintestinal diseases. It causes the release of the Ferro from cells, providing iron for the bacteria (Salehi *et al.,* 2007). But Sharada *et al.* (1999) also reported that avian *E. coli* to be pathogenic need not to be hemolytic. Shiva Shankar *et al.* (2010) reported only 1.52% *E.coli* isolates positive by qualitative test for hemolysis.

Biofilms are the surface bound colonized bacteria surrounded by exopolisaccharide matrix (Donlan and Costerton, 2002). Besides having zoonotic potential, avian pathogenic *E. coli* isolates are difficult to eradicate because of constant faecal excretion by the host and the ability of bacteria to form biofilms (Bauchart *et al.,* 2010). Biofilms are of particular interest in the poultry industry and public health as these films can harbour pathogenic microorganisms. It confers bacteria with greater resistance to surface cleaning and disinfection since antimicrobial entry and diffusion into the biofilm matrix then become difficult (Skyberg *et al.,* 2007). In the present study, a total 59.7% and 58.4% of *E. coli* isolates were found positive for biofilm formation by tube method and modified congo red agar method. These results were in agreement with the Skyberg *et al.* (2007) and Rodrigues *et al.* (2018) who reported biofilm formation in 55.8 and 55.2 percent *E. coli* isolates, respectively. In present study, the percentages of biofilm formation by two methods were close. However, contrast to our findings, lower percentage of isolates positive for biofilm production by CRA was reported by Deka (2014) and Dhanawade *et al.* (2010). Singh (2018b) reported a very high percentage of isolates positive for biofilm production by CRA (70.37%) than by tube (33.33%) method. Several factors have been implicated in the development of a model biofilm system, such as media composition, temperature, presence of antimicrobial agents, the causal organism, quantity of inoculum, hydrodynamics forces and characteristics of the substrata (Donlan and Costerton, 2002).

The Congo red binding ability is used as a phenotypic marker of colisepticemic (invasive) and non-colisepticemic *E. coli* in poultry and also as an epidemiological marker for discrimination of pathogenic strains from the commensals (Yadav *et al.,* 2014). In present study, all 77 *E. coli* isolates were found positive for congo red dye binding assay. Several studies also reported 91.7% to 100% of *E. coli* positive for this assay (Shiva Shankar *et al.,* 2010; Yadav *et al.,* 2014; Eid *et al.,* 2016), which is in agreement with present study. Among many tests, Congo red binding is commonly used as markers of hydrophobicity and has been linked directly to virulence and pathogenicity (Qadri *et al.,* 1988).

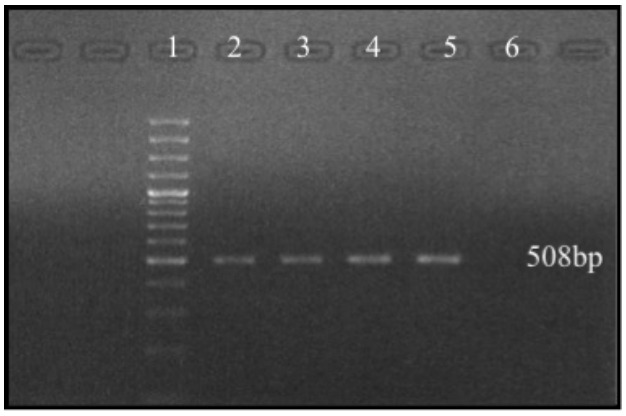

Fig 4: Results of PCR showing *fimH* gene**.**

Lane 1: 100 bp ladder

Lane 2: Positive control

Lane 3-5: Positive sample

Lane 6: Negative control

Infection ability of *E. coli* strains is facilitated by a broad variety of virulence factors coded by their virulence-associated genes (VAG). Among them host tissue colonization is thought to be initiated by fimbrial adhesins. Type 1 fimbriae are most commonly encountered in avian pathogenic *E. coli*. These are encoded by a *fim* cluster gene which required for its biosynthesis (Pusz *et al.,* 2014). *FimH* are claimed to be responsible for the first step in the colonization and this indicates that *fimH* have important role in the pathogenesis of avian colibacillosis (Roussan *et al.,* 2014). Out of 18 tested samples, 33.3% isolates were exhibit 508 bp of amplified product of *fimH* gene while all tested samples found negative for *stx1* gene (Fig 4). Serotype O83, O120, O149, O11 and UT (Untyped) were revealed the presence of *fimH* gene. Amplified *fimH* gene sequence was confirmed by blasting in NCBI GenBank.

In this study 33.3% of type 1 fimbrial adhesion gene (*fimH*) were reported in tested samples, which is similar to the findings of Mbanga and Nyarari (2015). Other workers have reported higher occurrences of the *fimH* gene (Van der westhuizen and Bragg, 2012). In present study, *stx*1 gene could not be detected, which agree with reports of Janben *et al.* (2001) and Wani *et al.* (2004), but disagree with findings of Eid *et al.* (2016) who detected *stx1* gene in 100% of *E. coli* isolates. These finding revealed that *stx1* is not the responsible factor for virulence of *E. coli* in tested samples.

This study conclude that avian colibacillosis prevalent in Mhow and Indore region. Congo red binding activities, biofilm production and presence of *fim H* gene were found responsible virulence factors associated with the colibacillosis in this study. Haemolysis is not the essential virulence factor for pathogenecity of *E.coli*. Multiple virulence factors of APEC can be responsible for colibacillosis but these all are not present in same isolate.

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REFERENCES

Ammar, A.M.A.E.H., Eid, S.E.A. and Eloksh, A.M. (2014). Studies on virulence genes of *E. coli* from different sources and their relation to antibiotic resistance pattern. Zagazig Veterinary Journal. 42: 183-196.

Bauchart, P., Germon, P., Bree, A, Oswald, E., Hacker, J. and Dobrindt, U. (2010). Pathogenomic comparison of human extraintestinal and avian pathogenic *Escherichia coli* search for factors involved in host specificity or zoonotic potential. Microbial Pathogenesis. 49: 105-115.

Cheesbrough, M. (1994). Medical Laboratory Manual for Tropical Countries. 1st edn. Vol.II, E.L.B.S. and Butterworth and Co. Ltd., Kent. pp 401-404.

Debbarma, S., Bora, D.P., Hazarika, R.A., Tamuly, S., Barua, A.G., Das, P.,Goswami, C., Joute, J.R. and Sinha, S. (2020). Molecular characterization of *Escherichia Coli* Isolates from food animals. Indian Journal of Animal Research. 54(8): 985-993

Deka, N. (2014). Comparison of tissue culture plate method, tube method and congo red agar method for the detection of biofilm formation by coagulase negative staphylococcus isolated from non clinical isolates. International Journal of Current Microbiology and Applied Sciences. 3: 810-815.

Dhanawade, N.B., Kalorey, D.R., Srinivasan, R., Barbuddhe, S.B., and Kurkure N.V. (2010). Detection of intercellular adhesion genes and biofilm production in *Staphylococcus aureus* isolated from bovine subclinical mastitis. Veterinary Research Communications. 34: 81-89.

Donlan, R.M. and Costerton J.W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews. 15: 167-193.

Edwards, R. and Ewing, W.N. (1972). Identification of Enterobacteriaceae. 3rd edn., Burgess Publishing Co., Minnesota. 211p.

Eid, H.I., Algammal, A.M., Nasef, S.A., Elfeil, W.K. and Mansour G.H. (2016). Genetic variation among avian pathogenic *E. coli* strains isolated from broiler chickens. Asian Journal of Animal and Veterinary Advances. 11: 350-356.

Emody, L., Kerenyia, M. and Nagy, G. (2003). Virulence factors of uropathogenic *Escherichia coli*. International Journal of Antimicorobial Agents. 22: 29-33.

Ewers, C., Janssen, T. And Wieler, I.H. (2003). Avian Pathogenic *E. coli* (APEC): A Review. Berliner und Munchener tierarztliche Wochenschrift. 116: 381-395.

Ewers, C., Wilking, H., Kiessling, S., Alt, K., Antao, E.M., Laturnus, C., Diehl, I., Glodde, G., Homeier, T., Bohnke, U., Steinruck, H., Philipp, H.C. and Wieler L.H. (2007). Avian pathogenic, uropathogenic, and newborn meningitis causing *Escherichia coli*: how closely related are they? International Journal of Medical Microbiology. 297: 163-176.

Fernandes, J.B.C., Zanardo, L.G., Galvao, N.N., Carvalho, I.A., Nero, L.A. and Moreira, M. A. S. (2011). *Escherichia coli* from clinical mastitis serotypes and virulence factors. Journal of Veterinary Diagnostic Investigation. 23:1146-1152.

Ibrahim, R.A., Cryer T.L., Lafi, S.Q., Basha, E.A., Good, L. and Tarazi Y.H. (2019). Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Veterinary Research. 15: 159.

Janben, T., Schwarz, C., Preikschat, P., Voss, M., Philipp H.C. and Wieler L.H. (2001). Virulence-assocated genes in avian pathogenic *Escherichia coli* (APEC) isolated from internal organs of poultry having died from colibacillosis. International Journal of Medical Microbiology. 291: 371-378.

Kumar, S. and Gupta, R. (2019). Risk factors for antimicrobial resistance in *Escherichia coli* isolates from poultry in Haryana. Indian Journal of Animal Research. 53(7): 918-925.

Kumar, S., Gupta, R., Jindal, N. and Bangar, Y.C. (2019). Phylogenectic group and serotype of *E.coli* isolates are important risk factors affecting intensity of colibacillosis in broilers. Indian Journal of Animal Research. 53(6): 814-820.

Kuznetsova, M.V., Gizatullina, J.S., Nesterova, L.Y. and Erjavec, M.S. (2020). *Escherichia coli* isolated from cases of colibacillosis in Russian Poultry Farms (Perm Krai): sensitivity to sntibiotics and bacteriocins. Microorganisms. 8: 741. doi: 10. 3390/ microorganisms8050741.

Mbanga, J. and Nyarari, Y.O. (2015). Virulence gene profiles of avian pathogenic *Escherichia coli* isolated from chickens with colibacillosis in Bulawayo, Zimbabwe. Onderstepoort Journal of Veterinary Research. 82: 850-858.

Nachammai, S.M., Jayakumar, K. and Aravazhi, A.N. (2016). The effectiveness of antibiotics against a major uropathogen-*Escherichia coli* and its biofilm assay by phenotypic methods. International Journal of Research in Medical Sciences. 4: 4820-4828.

Nolan, L.K., Barnes, J.H., Vaillancourt, J.P., Aziz, T.A., Logue, C.M. (2013). Colibacillosis. In: Diseases of Poultry Swayne, [D.E.**,** Glisson, J.R., *et al* (Eds.)], 13th ed., Wiley-Blackwell Publishing, Ames, IA, pp: 751–805.

Panda, B.K., Padhi, M.K., Sahoo, S. K., Panda, S.K. and Rath, S.K. (2010). Incidence of colibacillosis in ducks. Indian Veterinary Journal. 87(5): 443-444.

Peer, F.U., Ansari, M.M., Gani, I.A. and Willayat, M.M. (2013). Serotyping and antibiotic sensitivity patterns of *Escherichia coli* isolates obtained from broiler chicks in Kashmir Valley, India. Advances in Animal and Veterinary Sciences. 1:75-76.

Pusz, P., Bok, E., Mazurek, J., Stosik, M. and Baldy-Chudzik, K. (2014). Type 1 fimbriae in commensal *Escherichia coli* derived from healthy humans. Acta Biochimica Polonica. 61: 389-392.

Qadri, F., Hossain, S.A., Ciznar, I., Haider, K., Ljungh, A., Wadstrom, V. and Sack, D.A. (1988). Congo red binding and salt aggregation as indicators of virulence in *Shigella* species. Journal of Clinical Microbiology. 26: 1343-1348.

Radwan, I.A.E.H., Salam, H.S.H., Abd-Alwanis, S.A.E.A. and Al-Sayed, M.A.Y. (2014). Frequency of some virulence associated genes among multidrug-resistant *Escherichia coli* isolated from septicemic broiler chicken. International Journal of Advanced Research. 2: 867-874.

Rodrigues, S.V., Laviniki, V., Borges, K.A., Furian, T. Q., Moraes, H.L. S., Nascimento, V.P. Salle, C.T.P. (2018). Biofilm formation by avian pathogenic *Escherichia coli* is not related to *in vivo* pathogenicity. Current Microbiology. 76: 194-199.

Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Johnson, T.J. and Nolan, L.K. (2005). Characterizing the APEC pathotype. Veterinary Research. 36: 241-256.

Roussan, D.A., Zakaria, H., Khawaldeh, G. and Shaheen, I. (2014). Differentiation of avian pathogenic *Escheria coli* strains from broiler chickens by multiplex polymerase chain reaction (PCR) and random amplified polymorphic (RAPD) DNA. Open Journal of Veterinary Medicine. 4: 211-219.

Sahoo, T.K., Sahoo, L., Sarangi, L.N. and Panda, S.K. (2012). Prevalence, isolation, characterization and antibiogram study of pathogenic *Escherichia coli* from different poultry farms of Odisha. Journal of Advanced Veterinary and Animal Research. 2: 169-172.

Saha, O., M. Hoque, N., Islam, O.K., Rahaman, M., Sultana M. and Hossain, M.A. (2020). Multidrug-resistant avian pathogenic *Escherichia coli* strains and association of their virulence genes in Bangladesh. *Microorganisms* 2020, *8*: 1135. doi: 10. 3390/microorganisms8081135.

Salehi, Z.T., Safarchi, A. Peighambari, S.M., Mahzounieh, M. and Khorasgani, R.M. (2007). Detection of *stx1, stx2, eae, espB* and *hly* genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction. Journal of Veterinary Research. 62: 37-42.

Sharada, R., Ruban, S. and Thiyageeswaran, M. (2008). Antibiotic resistance pattern of *Escherichia coli* isolated from poultry in Bangalore. International Journal of Microbiology. 7: 15.

Sharma, S., Bhat, G.K. and Shenoy, S. (2007). Virulence factors and drug resistance in *E. coli* isolated from extraintestinal infections. Indian Journal of Medical Microbiology. 25: 369-373.

Shiva Shankar, T.V., Sharma, A. and Grover Y.P. (2010). Studies on different virulence factors of avian pathogenic *Escherichia coli*. Haryana Veternarian. 49: 45-47.

Singh, A. (2018a). Current Livestock Production Statistics of India. http: //www. vetextensio.com/current-livestock-animal-husbandry-statistics-india.

Singh, A. (2018b). Isolation and characterization of *E. coli* causing mastitis in buffaloes. M.V.Sc. thesis (Microbiology), Nanaji Deshmukh Veterinary Science University, Jabalpur.

Skyberg, J.A., Siek, K.E., Doetkott, C. and Nolan, L.K. (2007). Biofilm formation by avian *Escherichia coli* in relation to media, source and phylogeny. Current Microbiology. 102: 548-54.

Van Der Westhuizen, A.W. and Bragg, R.R. (2012). Multiplex polymerase chain reaction for screening avian pathogenic *Escherichia coli* for virulence genes. Avian Pathology. 41: 33-40.

Wani, S.A., Samanta, I., Bhat, M.A. and Nishikawa, Y. (2004). Investigation of shiga toxin-producing *Escherichia coli* in avian species in India. Letters in Applied Microbiology. 39: 389–394.

Yadav, V., Joshi, R.K. Joshi, N. and Diwakar, R.P. (2014). Congo red binding and plasmid profile of *E. coli* isolates of poultry origin. Journal of Animal Health and Production. 2: 31-32.

Zahid, A.A.H., Al-Mossawei, M.T.M. and Mahmood, A.B. (2016). *In vitro* and *In vivo* pathogenicity tests of local isolates APEC from naturally infected broiler in Baghdad. International Journal of Advanced Research and Biological Sciences. 3: 89-100.