International Journal of Sciences & Applied Research

www.ijsar.in

Study of pathogenic E*scherichia coli* based on molecular profiling of virulence genes and antibiotic resistance

Amit G. Singh*, A. K. Upadhyay, Maansi, Ipshita, Anil Kumar, and P. K. Singh

Department of Veterinary Public Health & Epidemiology, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar-263145.

Correspondence Address: *Amit G. Singh, Department of Veterinary Public Health & Epidemiology, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar-263145.

Abstract

Escherichia coli is a bacterium which is responsible for various diseases not only in human but also in domestic animals and poultry. The present study is done with the objective of Isolation of *Escherichia coli* from postmortem samples of chickens, infants' faecal samples and calves diarrhoea samples, characterization of *Escherichia coli* through biochemical tests, pathogenicity test using Swiss albino mice, revelation of antibiotic sensitivity of *E. coli* isolates and identification of virulence genes by PCR along with *in silico* study.

Keywords: *Escherichia coli*, virulence genes, faecal samples

Introduction

Escherichia coli is a bacterium which is responsible for various diseases not only in human but also in domestic animals and poultry. The present study is done with the objective of Isolation of *Escherichia coli* from postmortem samples of chickens, infants' faecal samples and calves diarrhoea samples, characterization of *Escherichia coli* through biochemical tests, pathogenicity test using Swiss albino mice, revelation of antibiotic sensitivity of *E. coli* isolates and identification of virulence genes by PCR along with *in silico* study.

Materials and methods

Glassware, plasticware and other disposables required for the study were purchased from M/S Axygen Scientific (USA) and M/S Tarson Product Pvt. Ltd. (India). Sterilization of the glasswares was done as per the standard protocols. All bacteriological media, chemicals and reagents used in the study ware purchased from Hi- media (India) and Difco (USA). Experimental Swiss albino mice (weaned) were procured from Mukteshwar, IVRI as per rules and regulations of Animal Ethical Committee for testing pathogenicity of *Escherichia coli* isolates. In the present study of molecular typing of pathogenic isolates of *Escherichia coli* based on virulence factors, primers synthesized by Metabion international AG were used. The primers for pTJ100 related genes include *iss* (increased serum survival) (Binns *et al*., 1979; Chuba *et al*., 1989 and Pfaffl *et al*., 2000), *cvaC* (structural gene for colicin V operon) (Vidal *et al*., 2004) and *iucD* gene (gene for aerobactin operon encoding

membrane bound enzyme synthesizing N^6-hydroxylysin) (Marta *et al.*, 1988) were used. However, primers for iron related genes include *ireA* (encodes an iron responsive element) (Russo *et al.*, 2001) and *irp2* (Iron-repressible gene associated with yersiniabactin synthesis) (Schubert *et al*., 1998). Genes encoding certain adhesions included *papC* (encoding parts of P pilus) (Kylie *et al*., 2005), *intimin* (virulence factor of EPEC adhesin) and *stx* (Shiga toxic gene) both (Estelle *et al*., 2006).

Isolation of bacteria was done according to the standard protocol and biochemical test was done using Biochemical test kit (KB010: HiMVic). Samples were sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (Himachal Pradesh) for serotyping. Congo red dye test and haemolytic test were performed as the protocol described by Catana *et al*., (2009) and Joon and Kaura (1993). Animal inoculation test was done by the protocol described by Coburn *et al.* (1954). For isolation of genomic DNA the procedure followed with slight modification was in agreement with that of Wilson and Ochman (1987). PCR reaction was carried out for observing the presence of *iss, irp2, iucD, cvaC, papC, ireA* and *eae* genes as the protocol described by Shannon *et al.* (2009) and Christa *et al*. (2005). For sequencing the PCR products thus obtained were sent to SciGenom Labs Private Ltd., Kerla. The sequences thus obtained were subjected for homology search using Bioinformatic tool BLAST (Basic Local Allignment Search Tool) and phylogenetic relationship is determined using ClustalW software.

Results

Out of 120 samples, 35 samples belonging to 7 different 'O' serogroups (O-2, O-8, O-11, O-25, O-55, O-60 and O-90) while four isolates were found to be untypable and one was rough isolate were obtained. Haemolytic activity test showed 8 colonies whereas congo red dye reduction test showed 10 colonies positive. Animal inoculation test in swiss albino mice caused death of 13 (37.14%) mice inoculated with nine different serotype. MTCC443 isolate was used as positive control for the same. Study for the presence of seven virulence genes (*iss, irp2, iucD, cvaC, papC, ireA* and *eae*) in *Escherichia coli* using PCR revealed presence of only five as *iss, irp2, iucD, cvaC* and *papC* in isolates acquired from poultry, human and calves samples. Most of the isolates contained *iss* (65.71%) gene followed by *irp2*, *cvaC*, *papC* gene each 54.28% whereas only 45.71% isolates exhibited presence of *iucD* gene. The sequencings of different genes were obtained in FASTA format. The BLASTn analysis of sequence of *iss* gene (309 bp) for 297 bases, and of *irp2* gene (413 bp) for 396 bases showed 99% identity with the sequences retrieved. The *iucD* gene (714 bp) showed identity of 99% with the nine sequences and 96% with one sequences for 693 bases. The *cvaC* gene (679 bp) also had maximum identity of 99% with the nine and 98% with one sequences for 672 baces. The *papC* gene (205 bp) showed 100% identity with nine sequences and 99% with one for 205 bases as observed by Larkin *et al.* (2007). Antibiotic sensitivity test was executed for 35 isolates of *Escherichia coli* (21 of poultry, 06 of calves and 08 of human) and ATCC 25922 reference strains intended for common antibiotics. The result showed that Ceftriaxone was most effective (37.14%) drug among all whereas Nalidixic acid and Amoxyclave both were resistant (62.85%). Kanamycin and Streptomycin was most sensitive drug (42.8%) whereas Vancomycin and Amoxyclave was resistant drug (61.9%) against poultry isolates. Ceftriaxone was most sensitive drug (50%) whereas Streptomycin, Nalidixic acid and Kanamycin were resistant (75%) against isolates of human diarrhea samples. Vancomycin and Amoxyclave were most sensitive but Nalidixic acid and Gentamicin

showed 100% resistant for calves' diarrhea samples.

Discussion

Prevalence of *Escherichia coli* was in agreement with that of Kumar *et al*. (2009). However, Hussein and Bollinger (2005) reported higher prevalence i.e. 28% in case of poultry isolates 70% in case of calf diarrhea sample and 55% in human infant stool sample. Lower prevalence was reported by Sharma *et al*. (2004) in well supervised farms. Serotype O-11 and O-60 were found common in human diarrhoea as well as calf diarrhea sample whereas O-55 was reported in poultry as well as calf diarrhorea sample. The finding of haemolytic test and congo red dye test was in accordance with that of Boro *et al.* (1983) and Catana *et al*. (2009). Blanco *et al*. (2004) and Mansfield *et al.* (2001) obtained the similar result as revealed in this study. Histopathological lesions shown by the mice died of E. coli infection was in accordance with that of Thomson and Schiffler (1996). The result of antimicrobial resistance test was corroborative with that of Panda and Panda (1987) and Sutariya (1993). All *E. coli* isolates deemed potentially pathogenic possesed the gene *iss*, *iucD* and *cvaC*. Similar genes were also reported in the study of Johnson *et al*. (2006) on identification of minimal predictors of avian pathogenic *E. coli* virulence for the use of a rapid diagnostic tool. The effect of *cvaC* gene association with *iss* in enhancing the virulence of avian pathogenic *E. coli* was detected by Delicato *et al.* (2003). The result of *in silico* study obtained was in agreement with that of Winarsih *et al.* (2011).

References

Binns, M. M., Davies, D. L. and Hardy, K. G. 1979. Cloned fragments of the plasmid ColV, I-K94 specifying virulence and serum resistance. Nature, 279: 778–781.

Blanco, J. E., Blanco, M., Alonso, M. P., Mora, A., Dahbi, G., Coira, M. A. and Blanco, J. 2004. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing Escherichia coli isolates from human patients: prevalence in Lugo, Spain, from 1992 through 1999. J. Clin. Microbiol., 42: 311–319.

Boro, B. R., Sarma, A. K. and Sarma, G. 1983. Studies on the strains of E. coli isolated from various clinical conditions in animals. Ind. Vet. J., 60: 245-249.

Catana, S., Virgilia, P., Popa, A. and Ionica F. 2009. Binding of congo red, phenotypical marker for discrimination of apec strains .lucr. stiinł. Med. Veter. 102: 46- 50.

Christa, S., Alexander, H., Treusch, S., Leininger, A., Kletzin, S. C., Schuster, H. and Peter, K. 2005. Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling Environ. Microbiol., 7: 1985–1995.

Chuba, P. J., Leon, M. A., Banerjee, A. and Palchaudhuri, S. 1989. Cloning and DNA sequence of plasmid determinant iss, coding for increased serum survival and surface exclusion, which has homology with lambda DNA. Mol. Gen. Gen., 216: 287–292.

Coburn, H., Evans J. and Jean N. 1954. Studies on the pathogenicity of streptococcus Pyogeates I. The experimental induction of Respiratory tract infections in swiss albino Mice with group a haemolytic streptococcus. The Brit. J. of Expe., 3: 270-278

Delicato, E. R., Brito B. G., Gaziri, L. C. and Vidotto, M. C. 2003. Virulence associated genes in Escherichia coli isolates from poultry with Colibacillosis. Vet. Microbiol., 94: 97- 103

Estelle, L., Monique K., Lothar B.. Eric, O. and Hubert B. 2006. Characterization of Shiga Toxin Gene (stx)-Positive and Intimin Gene (eae)-Positive Escherichia

coli Isolates from Wastewater of Slaughterhouses in France. Am. Soc. for Microbiol., 72: 3245–3251.

Hussein, H. S. and Sakuma, T. 2005. Prevalence of Shiga toxin producinf Escherichia coli in dairy cattle and their products. J. Dairy Sci., 88: 450- 465.

Johnson, T. J., Siek, K. E., Johnson, S. J. and Nolan, L. K. 2006. DNA sequence of a ColV plasmid and prevalence of selected plasmid- encoded virulence genes among avian Escherichia coli strains. Journal Bacteriology. 188: 745-758.

Joon, D. S. and Kaura, Y. K. 1993. Isolation and characterization of some of enterotobacteria from diarrhoeic and non-diarrhoeic calves. Ind. J. Anim. Sc., 63: 373-383.

Kumar, R., Surendran, P. K. and Thampuran, N. 2009. Evaluation of culture, ELISA and PCR assays for the detection of Salmonella in seafood. Lett. Appl. Microbiol., 46: 221–226.

Kylie, E., Rodriguez, S., Catherine, W., Giddings, C., Doetkott, T., Johnson, J., Mohamed, K. and Nolan, L. K. 2005. Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian Colibacillosis. Microbiol. 151: 2097–2110.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, W. F., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D.G. 2007. Sequence analysis Clustal W and Clustal X version 2.0 Bioinf. Appl. Note, 23: 2947–2948.

Mansfield, K. G., Lin, K. C., Newman, J., Schauer, D., MacKey, J., Lackner, A. A. and Carville, A. 2001. Identification of enteropathogenic Escherichia coli in simian immunodeficiency virus-infected infant and adult rhesus macaques. J. Clin. Microbiol. 39: 971–976.

Marta, H., Victor, L., and Neilands, J. B. 1988. Nucleotide Sequence of the iucD Gene of the pColV-K30 Aerobactin Operon and Topology of Its Product Studied with phoA and lacZ Gene Fusions. J. of Bacteriol., 21: 56-64.

Panda, N. N. and Panda, S. N. 1987. Studies on calf diarrhea in Orissa with special reference to colibacillosis. Indian J. Anim. Hlth., 26: 109-112.

Pfaffl, M. S., Horne, S. M., Giddings, C. W., Ebert, J. O., Doetkott, C., Smith, M. H. and Nolan, L. K. 2000. Complement resistance-related traits among Escherichia coli isolates from apparently healthy birds and birds with colibacillosis. Av. Dis., 44: 23–33.

Russo, T. A., Carlino, U. B. and Johnson, J. R. 2001. Identification of a new iron-regulated virulence gene, ireA, in an extraintestinal pathogenic isolate of Escherichia coli. Infect Immun. 69: 6209–6216.

Schubert, S., Rakin, A., Karch, H., Carniel, E. and Heesemann, J. 1998. Prevalence of the 'high-pathogenicity island' of Yersinia species among Escherichia coli strains that are pathogenic to humans. Infect. Immun., 66: 480–485.

Shannon, D., Manning, R., Madera, T., William S., Stephen, E., Walid, K., William, B., Thomas, S., Whittam, P., James, T. and Rudrik, J. 2009. Surveillance for Shiga Toxin– producing Escherichia coli, Michigan, 2001–2005. Emerg. Infect. Dis. 13: 36- 40.

Sharma, D. K., Soni, S. S., Kashyap, S. K. and Shringi, B. N. 2004. Seroprevalence, antibiotic sensitivity pattern and transfer of plasmid coded characters of E. coli. Indian Vet. J., 81: 6-8.

Sutariya, P.H. 1993. Studies on biochemical characters, drug resistance, colicinogeny and virulence associated characters of E. coli isolated from clinical samples. M.V.Sc. Thesis submitted to the Gujarat Agricultural University, Anand Campus, Anand.

Thomson, J. A. and Scheffler, J. J. 1996. Hemorrhagic typhlocolitis associated with attaching and effacing Escherichia

coli in common marmosets. Lab. Anim. Sci., 46: 275–279.

Vidal, R. M., Lagos, R., Levine, M. and Prado, V. 2004. Multiplex PCR for diagnosis of enteric infections associated with diarrheagenic Escherichia coli. J. clin. Microbial. 42: 1787- 1789.

Wilson, A. C. and Ochman, H.1987. Evolutionary history of enteric bacteria. Escherichia coli and salmonella typhimurium. Cellul. and Mol. biol. pp. 1649- 1654.

Winarsih, S., Wardani, A. K., Risandiansyah, R. and Santoso, S. 2011. The similarity and Epitope Prediction of Adhesin OMP among Salmonella species based on multiple sequence alignment. Canad. J. Biomed. Engin. and Technolo., 2: 18-26.