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Short Communication

Congo red binding and Plasmid Profile of *E. coli* Isolates of Poultry Origin

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ABSTRACT

The Congo red binding ability is used as a phenotypic marker of colisepticaemic (invasive) and non-coliseptecaemic *E. coli* in poultry and also as an epidemiological marker for discrimination of pathogenic strains from the commensals. The Congo red binding ability and plasmid profile of 70 *E. coli* isolates from poultry was studied. A total of 65 (92.86 %) isolates showed a Congo red binding ability while 5 (7.14 %) isolates did not bind Congo red dye up to 72 hours post inoculation (PI). Out of 70 isolates tested, 37 (52.86 %) isolates showed the presence of plasmid, while 33 (47.14 %) isolates did not reveal any plasmid. The molecular mass of the plasmids was found to be more than 50 kb.

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Escherichia coli has been the focus of immense international research after its recognition as a major cause of large scale epidemics of gastrointestinal illnesses in animals and man (Deshmukh and Karpe,2006). Since, the poultry has been the widely accepted and readily available source of meat, the association of *E. coli* in poultry has a significance for human disease. Colibacillosis in poultry caused by *E. coli* is major infection recorded throughout the year (Jones et al., 2000, Chawdhury and Das, 2003). It has been established as one of the common causes of mortality in domesticated birds (Kumar et al., 2005).

Among important tests used to study the surface properties of bacteria, Congo red binding was commonly used as markers of hydrophobicity and has been linked directly to virulence and pathogenicity (Quadri et al., 1988). Congo red uptake has been considered a virulence factor responsible for pathogenicity in poultry, particularly in the avian pathogenic *E. coli* (APEC) (Ahmad et al., 2004). A positive correlation has been reported between Congo red binding and pathogenicity of *E. coli* and this character was used to confirm pathogenicity of *E. coli* serogroups (Ishiguro et al., 1985, Agarwal et al., 1999, Dubey et al., 2000) and was routinely used *in vitro* to assess the virulence of *E. coli* (Swaminathan et al., 2004).

Plasmid profile of the *E. coli* isolates was utilized extensively to establish their epidemiological relationship and virulence (Taylor et al., 1982). Presence of varying numbers of plasmids was reported in *E. coli* strains with a molecular weight ranging from 1.54 Kb to as large as 100 Kb (David et al., 1993, Doetkott et al., 1996).

The present study was undertaken to study the Congo red binding ability and plasmid profile of *E. coli* isolates of poultry origin.

Seventy *E. coli* isolates of poultry origin isolated from cases of colibacillosis in poultry and characterized by Vibha et al., (2008) were taken for the study. Congo red binding ability was tested as per the method described by Berkhoff and Vinal, (1986). The Congo red medium was prepared by adding 0.03 % of Congo red dye to the trypticase soya agar (TSA), the *E.coli* isolates were streaked onto the plates and plates were incubated at 37^0C for 24 to 72 hours. A positive reaction was indicated by appearance of intense orange or brick red coloured colonies after an incubation for 24, 48 and 72 hours, while pale or white colonies were considered as negative.

The Plasmid DNA was extracted using phenol: chloroform: isoamyl alcohol isolation method described by Birnboim and Doly (1979) and the Plasmid DNA was separated following gel electrophoresis using 0.7 agarose gel as per Meyer et al., (1976).

Table-1 Congo red binding of *E. coli* isolates.

Time in hrs (PI)	Mild or Negative	Positive	
		Moderate	Intense
24	43 (61.43)	16 (22.86)	11(15.71)
48	30 (69.77)	4 (9.30)	9 (20.93)
72	5 (16.67)	16 (53.33)	9 (30.00)

Out of 70 *E. coli* isolates studied, 65 (92.86 %) isolates showed a Congo red binding ability while 5 (7.14 %) isolates did not bind Congo red dye up to 72 hours post inoculation (PI). Out of sixty five Congo red positive isolates, 27 (38.57) % isolates showed positive reaction within 24 hours PI while 13 (27.08 %) isolates appeared positive after 48 hours PI and 25 (83.33 %) were found positive after 72 hours PI at 37^0C (Table-1). Among 27 isolates that were positive at 24 hour, 11 (15.71 %) appeared intense red coloured while 16 (22.86 %) showed moderate reaction while out of 13

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isolates, 9 (20.93 %) and 4 (19.30 %) isolates have shown intense red and moderate reaction respectively at 48 hours PI. Out of remaining 25 Congo red positive isolates, 9 (30 %) and 16 (33.33 %) isolates exhibited intense and moderate reactions respectively for uptake of Congo red dye after 72 hours PI. The ability to distinguish between pathogenic and non-pathogenic organism is an important parameter in monitoring virulence characters of bacteria in working cultures. Berkhoff and Vinal (1986) found a direct co-relation between the ability of clinical isolates of *E. coli* to bind Congo red dye, and their ability to cause infections in chickens. The *E. coli* isolates from poultry have been reported to bind Congo red dye and this ability was considered as a virulence marker for enteroinvasive *E. coli* (Corbett et al., 1987). Similar results were also reported by Dubey et al., (2000), Panigraphy and Ling (1990) and Raji et al., (2003).

Plasmid profile was recorded for these 70 isolates. A total of 37 (52.86 %) isolates showed presence of plasmid, while 33 (47.14 %) isolates did not reveal any plasmid. The molecular mass of the plasmids appeared to be more than 50 kb. In all the cases only one plasmid could be detected. Smith et al., (2003) also reported presence of plasmid in 47 % of the isolates of *E. coli* from animals. The molecular weight of the plasmids appeared to the more than 50 kb based on the migration of plasmid DNA. The plasmid size ranging from 30-80 MD with the commonest of the heavy plasmid of the 70 MD have been reported by Bebora et al., (1994). Similarly, Smith et al., (2003) also reported 56 kb plasmid in *E. coli* from animals.

The study reveals that the Congo red binding may be used as a marker for determining the virulence of the *E. coli* isolates *in vitro* as an alternate to the use of experimental animals.

REFERENCES

Agarwal RK, Kapoor KN, Verma JC, Bachhil VN, Singh BR, Kumar A, Sachan N, Singh DK, Malik SVS (1999). Isolation and characterization of *Salmonella* Stockhalm from beef. Ind. J. Comp. Microbiol. Immunol. Infect. Dis. 20(1):50 - 52.

Ahmad I, Ahmad S, Yadava JNS (2004). Pathogenic strains of *E.coli* and transferability of col plasmid to salmonella typhimurium. Ind. Vet. Med. J. 28:340 - 344.

Bebora LC, Yamomoto H, Sang WK, Oundo JO, Khamala J, Mwangudza A, Mukundi, PW (1994). Plasmid profile of *E.coli, salmonella* and *shigella* organisms isolated in Kenya. East Afr. Med. J. 74 (10): 619 - 623.

Berkhoff HA, Vinal AC (1986). Congo red medium to distinguish between invasiveness and non-invasiveness *E. coli* pathogenic for poultry. Avi. Dis. 30 : 117 - 121.

Birnboim HC, Doly J (1979). A rapid alkaline extraction procedure for screening of recombinant plasmid DNA. Nucleic Acids Res. 7: 1513 - 1523.

Chowdhury M, Das R (2003). Incidence of drug resistance in *E. coli*. Strains in West Bangal. Ind. Vet. J. 80: 81 - 82.

Corbett WT, Berkhoff HA, Vinal AC (1987). Study of bacterial cell wall and CR binding ability. Can. J. Vet. Res. 51 : 312.

David BP, Purushothaman V, Venkateson RA (1994). Comparison of molecular weight estimation techniques. Bacterial plasmid DNA. Ind. J. Anim. Sci. 63(11) :1146 - 1151

Deshmukh AS, Karpe AG (2006). In vitro transfer of plasmids in *E. coli*. Ind. J. Comp. Microbiol. Immunol. Infect. Dis. 27: 32 - 34.

Doetkott DM, Nolan LK, Gidding CW, Berryhill, DL (1996). Large plasmids of avian *E.coli* isolates. Avi. Dis. 40(4): 927 - 930

Dubey S, Sharda R, Chhabra D, Tiwari S, Pillai AGR (2000). Congo red colony pigmentation and haemolysin production by *E. coli* strains from diarrheic goats. Ind.. J. Comp. Microbiol. Infect. Dis. 21(1) : 157 - 158.

Ishiguro EE, Ainsworth T, Trust TJ, Kay WM (1985). Correlation between congo red binding and pathogenicity of *E. coli*. J. Bacteriol. 164 : 1233.

Jones MG, Bokshi KN, Mahanta B (2000). Isolation and Identification, Serotyping and antibiogram of *E.coli* Mahaboobnagar district. Ind. Vet. J. 77: 4 - 6.

Kumar N, Kashyap SK, Maharchandani S, Sharma, MM (2005). Characterization of *E. coli* strains associated with septicaemia in sheep. Ind. Vet. J. 82: 6 - 8.

Meyers JA, Sancez D, Elewell LP, Falklow S (1976). Simple agarose gel electrophoresis method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. 127 : 1529 - 1537.

Panigraphy P, Ling Y (1990) Differentiation of pathogenic and non-pathogenic *E. coli* isolated from poultry. Avi. Dis. 34 (4): 941 - 943.

Qadri F, Hossain SA, Ciznár I, Haider K, Ljungh A, Wadstrom V, Sack DA (1988). Congo red binding and salt aggregation as indicators of virulence in Shigella species. J. Clin. Microbiol. 26(7): 1343 - 1348.

Raji MA, Adekeye JO, Kwaga JKP, Bale JOO (2003). *In vitro* and *in vivo* pathogenecity studies of *Escherichia coli* isolated from poultry in Nigeria. Israel J. Vet. Med. 58 (1): 21 - 28.

Smith SI, Aboaba OO, Odeigha P, Shodipo K, Adeyeye JA, Ibrahim A, Adebiyi T, Onibokun H, Odunukwe NN (2003). Plasmid profile of *Escherichia coli* O157: H7 from apparently healthy animals. African J. Biotech. 2: 322 - 324.

Swaminathan TR, Chandran NDJ, Dorairajan N (2004). Virulence attributes of *E.coli* associated with colisepticaemic chickens. Ind. J. Anim. Sci. 74(3): 248 - 252

Taylor DN, Wachsmuth IK, Shangkaun YH, Schmidt RV, Barrett TU, Schrader JS, Scherach CS, McGee HB, Feldman RA, Bresner DU (1982). *Salmonellosis* associated with Marijuana: A multistate outbreak traced by plasmid finger printing. N. Engl. J. Med. 306 (21) : 1249 - 1253.

Yadav V, Joshi RK, Maurya, SK (2008). Isolation ,identification, serotyping and antibiogram of *Escherichia coli* from Diarrheic poultry. J. Vet. Pub. Hlth., 7(2): 145 - 147.

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