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°C 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: isooamyl 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 I Congo red binding of E. coli isolates.

<table>
<thead>
<tr>
<th>Time in hrs (PI)</th>
<th>Mild or Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>Intense</td>
</tr>
<tr>
<td>24</td>
<td>43 (61.43)</td>
<td>16 (22.86)</td>
</tr>
<tr>
<td>48</td>
<td>30 (69.77)</td>
<td>4 (9.30)</td>
</tr>
<tr>
<td>72</td>
<td>5 (16.67)</td>
<td>16 (33.33)</td>
</tr>
</tbody>
</table>

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 (20.93 %) isolates appeared positive after 48 hours PI and 25 (33.33 %) were found positive after 72 hours PI at 37°C (Table-I). 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
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 correlation 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