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Authors: D.R.A. Dissanayake, T.G. Wijewardana, G.A. Gunawardena, I.R. Poxton



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1 **Distribution of Lipopolysaccharide Core Types among Avian Pathogenic**
2 ***Escherichia coli* in Relation to the Major Phylogenetic Groups**

3

4 **D R A Dissanayake¹, T G Wijewardana¹, G A Gunawardena² and I R Poxton³**

5

6 ¹Department of Pathobiology

7 Faculty of Veterinary Medicine and Animal Science

8 University of Peradeniya,

9 Peradeniya

10 Sri Lanka.

11

12 ²Division of Microbiology

13 Veterinary Research Institute

14 Gannoruwa

15 Sri Lanka

16 ³Professor Ian R Poxton

17 Medical Microbiology

18 Centre for Infectious Diseases

19 University of Edinburgh College of Medicine and Veterinary Medicine

20 The Chancellor's Building

21 49 Little France Crescent

22 Edinburgh, EH16 4SB

23 Tel and Fax +44 (0)131 242 9122

Email: i.r.poxton@ed.ac.uk

24 **ABSTRACT**

25 Five distinct lipopolysaccharide (LPS) core types, namely R1 - R4 and K12 have been
26 identified in *Escherichia coli*. The aims of this study were to determine, primarily by means
27 of PCR, the distribution of those oligosaccharide core types among avian pathogenic *E. coli*
28 and their relationship to phylogenetic groups. To identify putative avian pathogenic *E. coli*,
29 serum resistance and the presence of three virulence genes encoding temperature sensitive
30 haemagglutinin (*tsh*), increased serum survival (*iss*) and colicin V (*cvaC*) were determined.
31 Of the 143 clinical isolates examined 62% possessed the R1 core, 22% were R3, 13% were
32 R4 and 3% were R2. Fifty commensal isolates consisted of 58% with R1 core, 38% with R3
33 core, 4% with R4 core, and none with R2. None of the isolates were of K12 core type. The
34 distribution of core oligosaccharide types in clinical and commensal isolates were not
35 statistically significant ($P = 0.51$). Three genes, *tsh*, *iss* and *cvaC* were found in *E. coli* of all
36 four core types. The genes *tsh* ($P < 0.001$) and *iss* ($P = 0.03412$) were significantly associated
37 with the R4 core oligosaccharide type. The isolates containing R4 core type LPS were mainly
38 confined to phylogenetic group D. The widespread R1 core type showed less ability to
39 possess virulence genes and 83% were in the phylogenetic group A. Results of this study
40 indicated that *E. coli* with R1, R2, R3 and R4 were important in causing infections in
41 chickens and further, the *E. coli* with R4 core type were less common among commensals,
42 possessed more virulence genes and were related to phylogenetic groups pathogenic for
43 poultry.

44

45 **Key words: avian pathogenic *E. coli*, core oligosaccharide, lipopolysaccharide**

46 **INTRODUCTION**

47 The lipopolysaccharide of *Escherichia coli* contains a common form of architecture that
48 consists of three major components; (i) hydrophobic lipid A (ii) an oligosaccharide core
49 which is divided into inner and outer regions and (iii) O-polysaccharide (Westphal, 1975;
50 Galanos and Luderitz, 1984). Unlike the highly variable O-polysaccharide chains, the LPS
51 core exhibits little variation, with the inner core being much more conserved among the
52 members of Enterobacteriaceae while the outer core consists of ubiquitous hexoses that vary
53 in composition and arrangement. These differences led to the identification of five different
54 core types in *E. coli*, which are denoted as R1, R2, R3, R4 and K12 (Gibb *et al.*, 1992;
55 Poxton, 1995; Heinrichs *et al.*, 1998). This limited variability of LPS core has attracted much
56 attention as a potential vaccine candidate (Bennett-Guerrero *et al.*, 2000; Erridge *et al.*, 2002).
57 In such attempts, knowledge on the distribution of LPS core types in that particular subset of
58 *E. coli* and its association with the disease of interest is a prerequisite.

59

60 This study was carried out to determine the LPS core types associated with avian pathogenic
61 *E. coli* (APEC) and their distribution in relation to phylogeny. However, the standard
62 procedure of identifying APEC is still unresolved. It has been proposed that this virulence
63 capacity is likely to be acquired by horizontal transmission of certain genes located on
64 plasmids, bacteriophages or particular regions of DNA called pathogenicity islands. Genes
65 responsible for increased serum survival (*iss*), temperature sensitive haemagglutinin (*tsh*) and
66 Colicin V (*cvaC*) have been recognized and considered as APEC-associated genes (Delicato
67 *et al.*, 2002; Skyberg *et al.*, 2003). Therefore, the presence of *tsh*, *iss* and *cvaC* genes and
68 serum resistance of the isolates were determined to identify APEC and association with
69 particular LPS core type with those strains.

70 MATERIALS AND METHODS

71 Bacteria

72 *E. coli* strains used in this study were isolated from chickens (broiler and layer) in intensively
73 managed commercial chicken farms in Kandy, Kurunegala, Gampola, Kaluthara and
74 Colombo Districts in Sri Lanka between the period 2001 and 2006. Clinical isolates were
75 from at least 57 farms and only one isolate was selected from one particular outbreak. Heart
76 blood, liver and spleen were collected from birds showing signs of septicaemia, pericarditis,
77 perihepatitis and salpingitis. Yolk sac contents of chicks were sampled from suspected cases
78 of yolk sac infections. Fifty cloacal swabs were collected from apparently healthy
79 commercial chickens (broiler and layer), from 50 different cages in 18 different farms.

80

81 Polymerase chain reaction (PCR)

82 PCR reactions were carried out using a TC-412 thermal cycler (Techine) and unless
83 otherwise stated primers were obtained from VH Bio Ltd., UK while Taq DNA Polymerase
84 was from Moltaq, Molyzyme, UK.

85

86 All strains were sub-cultured on nutrient agar (Oxoid UK) and one isolated colony was
87 transferred to 100µl of pyrogen-free water in a screw-capped micro-centrifuge tube. Bacterial
88 suspensions were heated for 10 min at 100°C to ensure complete lysis of all cells and the
89 resultant lysate was used as DNA template.

90

91 Determination of LPS core type by PCR

92 To determine LPS core types, primers specific for genes encoding specific enzymes for
93 biosynthesis of the R1, R2, R3, R4 and K12 oligosaccharides (primer sequences are listed in

94 Table 1) were purchased from MWG Biotech, Germany using the published primer
95 sequences (Amor et al., 2000). All strains were initially screened for R1 and strains which
96 did not produce the specific bands were re-screened for R2 and then for R3, R4 and K12 until
97 all strains were assigned to a core type.

98

99 PCR was performed in a 25 μ l of reaction mixture containing: 2.5 μ l of 10X Mg-free PCR
100 buffer, 1.5 μ l of 50mM MgCl₂, 25pM of each primer, 0.2 μ l of Taq DNA polymerase, 0.2 μ l
101 of deoxynucleotide triphosphate mix (dNTPs) (10mM), 13.1 μ l of ultra pure water, 2.5 μ l of
102 template DNA. The PCR amplification profiles were: initial denaturation at 94°C for 4
103 minutes, followed by 35 cycles of, 94°C (denaturation) for 20 seconds, 50°C for 30 seconds
104 (annealing), and 72°C for 135 seconds (polymerization). On completion of 35 cycles final
105 polymerization was carried out at 72°C for 120 seconds. PCR products were separated by
106 horizontal gel electrophoresis in 1% agarose gel and DNA visualized with ethidium bromide
107 under ultraviolet illumination.

108

109 **Determination of presence of *tsh*, *iss* and *cvaC* genes**

110 Sequences of the primers specific for each gene are shown in Table 1 Amplification of each
111 gene carried out separately in a 25 μ l reaction mixture containing: 2.5 μ l of 10x Mg-free PCR
112 buffer, 2.5 μ l of 50mM MgCl₂, 50pM of each primer, 0.2 μ l of Taq DNA polymerase, 0.5 μ l of
113 dNTP mixture (10mM), 11.8 μ l of ultra pure water and 2.5 μ l of template DNA. The PCR
114 conditions were: 5 minutes at 95°C, nine cycles of 1 minute at 95°C, 30 seconds at 55°C, 1
115 minute at 72°C and 28 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at
116 72°C and followed by 7 minutes at 72°C. PCR products were separated by horizontal gel

117 electrophoresis in 1% agarose gel and DNA was visualized with ethidium bromide under
118 ultraviolet illumination.

119

120 **Phylogenetic analysis**

121 Two step triplex PCR, described by Clermont *et al.* (2000) was used for phylogenetic
122 analysis. Primers (Table 1) specific for the genes *chu A*, *yja A* and DNA fragment, TSPE4.C2
123 (20pM each) used in 20µl of reaction mixture containing: 2µl of 10x buffer (containing
124 MgCl₂), 2µl of PCR enhancer (supplied with Taq polymerase), 0.5µl of Taq DNA
125 polymerase, 1µl of dNTP mixture, 6.5µl of ultra pure water and 2.5µl template DNA. The
126 conditions used for PCR were denaturation at 94°C for 4 minutes, 30 cycles of 94°C for 5
127 seconds 59°C for 10 seconds and final extension at 72°C for 5 minutes.

128

129 The phylogenetic group of each strain was determined as follows; Chu A (+), Yja A (+),
130 TSPE4. C4 (+/-) was assigned to group B2; Chu A (+), Yja A (-), TSPE4.C4 (+/-) was
131 assigned to group D; Chu A (-), Yja A (+/-), TSPE4.C2 (+) was assigned to group B1; and
132 chu A (-), Yja A (+/-), TSPE4. C2 (-) was assigned to group A.

133

134 **Determination of LPS core type by dot blot analysis**

135 A well-isolated bacterial colony was suspended in 100µl of pyrogen free water and 2 µl of
136 the suspension was spotted onto four separate nitrocellulose grids. After drying and washing
137 in Tris buffered saline (TBS) the nitrocellulose papers were blocked with 3% fish gelatin in
138 TBS and incubated separately for 3 hours with our monoclonal antibodies specific for R1
139 (VN2.423.10), R2 (H4 361.23), R3 (U4 434.07) LPS core types, and monoclonal antibody
140 WN1 222.05 which reacts with all *E. coli* core types as described by Gibb *et al.* (1992).

141 Monoclonal antibodies were diluted at 1:50 in 1% fish gelatin in TBS prior to use. After
142 washing in Tween-Tris buffered saline (TTBS) the nitrocellulose grids were incubated for 1
143 hour with horseradish peroxidase (HRP) conjugated rabbit anti-mouse IgG antibodies (Sigma)
144 at a 1: 2000 dilution in 1% fish gelatin in TBS. After washing twice in TBS, the colour was
145 developed using a freshly prepared solution containing methanol (20ml), 4-chloro-1-naphthol
146 (60mg) (BioRad), hydrogen peroxide (60 μ l) and TBS (100ml). Reactions were stopped by
147 washing in pyrogen free water.

148

149 **Serum resistance**

150 Bacterial isolates were grown on Luria-Bertani broth at 37°C aerobically with agitation to an
151 optical density of 0.2 at 600nm were collected by centrifugation and washed once with
152 complement fixation test buffer (Oxoid, Basingstoke, UK) to an optical density of 0.4 at
153 600nm. Bacterial cell suspensions (10%) were mixed with pooled serum samples (90%)
154 obtained from 14 days old, healthy broiler chickens. Cells incubated with heat-inactivated
155 chicken serum (incubated at 56°C for 1hour) were used as controls. Test samples and
156 controls were diluted appropriately and plated on nutrient agar plates to determine the
157 number of viable cells at 0 and 3 hour. Bacterial strains which showed more than 10 fold, a
158 0-10 fold increase and a decrease in number after 3 hours were subsequently categorised as
159 resistant, intermediately resistant and sensitive.

160

161 **Bio statistics**

162 Results were analyzed by 'R-work' software, version 2.5.1 (R-work web reference). Fisher's
163 exact test was used to determine the significance of distribution of LPS core types among

164 clinical and commensal *E. coli* isolates and significance of association virulence marker
165 genes (*tsh*, *iss* and *cvaC*) with LPS core types.

166

167 Significance of association between *iss*, *tsh* and *cvaC* genes among clinical and commensal *E.*
168 *coli* isolates was determined by Pearson's Chi-squared test with Yates continuity correction.
169 Differences were considered statistically significant if $P \leq 0.05$.

170

171 RESULTS

172 Distribution of LPS core types among clinical and commensal avian *E. coli* isolates

173 One hundred and ninety three avian *E. coli* isolates, of which 143 were from clinical cases
174 and 50 were from cloacae of apparently healthy birds, were screened by PCR to determine
175 LPS core types. The PCR amplified products representing genes encoding enzymes specific
176 for R1, R2, R3 and R4 LPS cores represented 551bp, 1141bp, 1785bp and 699bp bands
177 respectively. Distribution of LPS core types among clinical and commensal *E. coli* isolates
178 are shown in Table 2. Of the 193 *E. coli* isolates examined, 118 (61%) had the R1 core type
179 and R3, R4 and R2 represented 50 (26%), 21 (11%) and 4 (2%) respectively. None of the
180 isolates belonged to the K12 core type. The majority of clinical and commensal isolates had
181 the R1 core type which was almost equally distributed amongst the two categories (clinical,
182 62% and commensal, 58%). Of the remaining core types, R3 was the second most common
183 core type representing 22% in clinical and 38% in commensal *E. coli*. The LPS core type R4
184 was detected in 13% clinical isolates and 4% commensal isolates and only four clinical
185 isolates had R2 LPS core type. However, the distribution of LPS core types amongst clinical
186 and commensal avian *E. coli* isolates was not statistically significant ($P=0.051$). The core
187 types R1, R2 and R3 were also determined using monoclonal antibodies. Out of 118 strains

188 with R1 core type, only 81 (80.9%) were correctly identified by dot blot analysis but it was
189 more accurate in identifying R2 (100%) and R3 (93%) core types.

190

191 **Distribution of LPS core types among APEC strains**

192 The *E. coli* strains used in this study were categorized as clinical and commensal depending
193 on the site of isolation. If an organism was isolated from heart blood, liver, bone marrow or
194 the yolk sac of a dead chicken with lesions suggestive of an *E. coli* infection, it was simply
195 categorized as clinical. But this did not necessarily mean all organisms coming under the
196 'clinical' category were pathogenic. There may be some other primary causes (e.g. viral
197 infection) and *E. coli* may be secondary or the commensal *E. coli* strains may invade the
198 tissues after death. In order to identify APEC and determine the association of any particular
199 LPS core type (s) with those strains, the presence of *tsh*, *iss* and *cvaC* and serum resistance of
200 the isolates were determined.

201

202 The distribution of the APEC associated genes and resistant to serum bactericidal activity
203 among clinical and commensal *E. coli* with different LPS core types are shown in Table 2.
204 Among all 193 *E. coli* strains tested, 50 (47/143 clinical and 3/50 commensal) had the *tsh*
205 gene, 61 (55 clinical and 6 commensal) had the *iss* gene and 57 (46 clinical and 11
206 commensal) had the *cvaC* gene. The genes *tsh* and *iss* were significantly associated with
207 clinical isolates (*tsh*: $P < 0.0001$ and *iss*: $P < 0.0012$) and helpful in identifying APEC strains.
208 However, the association of *cvaC* gene with clinical isolates was not statistically significant
209 ($P = 0.2696$).

210

211 Three virulence determining genes were found to be present in *E. coli* isolates representing
212 all core types. However, *iss* and *tsh* genes were less commonly detected in *E. coli* isolates
213 with R1 core types and represented 24% and 15% respectively. Among the four isolates with
214 R2 core types, the *tsh* gene was detected in three and the *iss* gene was not detected in any of
215 those isolates. Of the clinical isolates with the R3 core type, 67.7% possessed *tsh* and or *iss*.
216 The three commensal isolates with the *tsh* gene also had the R3 core type. The majority of *E.*
217 *coli* isolates with the R4 LPS core type had *tsh* (76%) and *iss* (58%) genes. The virulence
218 genes *tsh* and *iss* were not equally distributed in the *E. coli* isolates with different core types
219 and were significantly associated with R4 LPS core (*tsh* $P < 0.001$ and *iss* $P < 0.03412$).
220 The presence of *cvaC* gene was common amongst isolates with R1 (32%) and R3 (30%) LPS
221 core types. Lipopolysaccharide core type representing R4 had only 4 isolates (19%) with
222 *cvaC* gene. However, the *cvaC* gene did not exhibit significant association with any of those
223 LPS core types ($P = 0.2166$).

224

225 **Serum bactericidal activity**

226 Statistically significant differences were observed between the clinical and commensal
227 groups in resistance to the bactericidal activity of chicken serum ($P < 0.001$). Of the 143
228 clinical isolates examined, 109 were resistant to the serum bactericidal activity and only nine
229 out of 50 commensal isolates demonstrated this ability. However, 34 clinical isolates were
230 also sensitive to the bactericidal action of serum. A majority of the isolates (91%) possessing
231 *tsh* and *iss* were serum resistant and demonstrated more than 100-fold increase of viable
232 bacterial count after 3 hours.

233

234 **Distribution of LPS core types in relation to phylogenetic groups**

235 All four LPS core types occurred in phylogenetic groups A, B1 and D. The distribution of *E.*
236 *coli* isolates in each phylogenetic group was as follows; group A, 138 (71%); group B1, 8
237 (4.1%); group B2, 11(7.9%) and group D, 36 (18.65%). A significantly greater proportion of
238 *E. coli* with R1 and R3 core types were associated with phylogenetic group A. Thirty seven
239 of the 50 *E. coli* isolates (74%) with R3 core type and 98 of the 118 (83%) with R1 core type
240 belonged to phylogenetic group A.

241

242 It may be important to note that the majority of the commensal isolates (86%) were also in
243 the phylogenetic group A. The R4 core type was less common in group A and mainly
244 confined to group D. Fifteen of the 19 clinical isolates with R4 core type were in the
245 phylogenetic group D. Only limited numbers of isolates belonged to the phylogenetic group
246 B2 which is reputed to contain the extra-intestinal *E. coli* (ExPEC) and it is interesting to
247 note that none of the commensal isolates occurred in this group. *E. coli* isolates representing
248 R1, R3 and R4 core types occurred in group B2. The distribution of LPS core types and
249 virulence genes in relation to the phylogenetic groups are summarized in Table 2.

250

251 **DISCUSSION**

252 The distribution of LPS core types among septicaemic and commensal isolates of *E. coli* in
253 both human and bovine along with the *Escherichia coli* reference collection (ECOR) has
254 been described previously (Gibb et al., 1992; Appelmelk et al., 1994; Currie and Poxton,
255 1999; Amor et al., 2000; Gibbs et al., 2004). All these studies clearly indicated the high
256 prevalence of the R1 core type. Assessment of LPS core types of 72 isolates of ECOR
257 collection revealed 69.4% of the isolates carrying R1 core type (Amor et al., 2000). A similar

258 study carried out with *E. coli* isolates obtained from bacteraemic patients reported the
259 presence of 68% of R1 LPS core (Appelmelk et al., 1994). Bovine and human commensal *E.*
260 *coli* isolates also possessed 63% and 49% of R1 core respectively. The present study carried
261 out to determine the distribution of LPS core types among avian *E. coli* isolates is also in
262 agreement with the previous findings representing 61% R1 core among clinical and 58%
263 among commensal isolates.

264

265 The proportion of R3 core types detected in this study among avian clinical and commensal
266 isolates was comparatively higher than that of humans and bovine commensal isolates and
267 bacteraemic patients' isolates. The R3 LPS core type is reported to have a strong association
268 with verotoxin producing *E. coli* isolates. The studies carried out by Currie and Poxton (1999)
269 and Amor and colleagues (2000) reported that all of the O157:H7 and non-O157 verotoxin
270 producing *E. coli* possessed the R3 core type.

271

272 Of the four isolates with R2 core type, three demonstrated the presence of *tsh* gene and the
273 ability to resist bactericidal activity of serum supporting a capacity to potentially cause
274 infections.

275

276 The avian *E. coli* isolates with an R4 core exhibited a significant association with APEC.
277 Seventeen of the 19 (89.4%) clinical isolates of R4 core had *tsh* and/or *iss* and a majority of
278 the R4 isolates (16/21) belonged to the phylogenetic group D. Interestingly, this sub-set of
279 APEC is highly resistant to the bactericidal action of chicken serum. To exclude the
280 possibility of the same strain with R4 LPS core being repeatedly isolated, LPSs extracted
281 from those strains were electrophoresed on PAGE which confirmed that the LPS patterns were

282 different (data not shown). However, it is important to analyze more samples from different
283 geographical locations to determine whether the R4 core type has strong association with
284 APEC. Of the four isolates with R2 core type, three demonstrated the presence of *tsh* gene
285 and the ability to resist bactericidal activity of serum supporting a capacity to potentially
286 cause infections.

287

288 It is interesting to note the absence of K12 core type among the avian *E. coli* isolates studied.
289 However, a pilot study carried out in Edinburgh detected the K12 core type in faecal *E. coli*
290 isolates of healthy chickens (Leesa Cornet and I.R Poxton, unpublished data) indicating the
291 variation in distribution of LPS core types in different geographical locations.

292

293 In the present study LPS core types were determined by both PCR and core specific
294 monoclonal antibodies. The lack of monoclonal antibodies specific for R4 and K12 core
295 types hampered the identification of those core types. Identification of R2 and R3 core types
296 using monoclonal antibodies was more accurate than identifying R1 LPS core. Nearly 20%
297 of R1 core types were not identified by the monoclonal antibodies and certain isolates with
298 the R4 core types were erroneously identified as R1. This incorrect identification can be
299 explained by the very close structural similarity of the outer core regions of R1 and R4 core
300 types. The R1 and R4 core types differ from each other only by a single sugar residue in the
301 outer core region. Appelmek et al. (1994) identified LPS core types using rabbit anti-sera
302 raised against rough strains expressing respective core and reported that certain isolates
303 reacted with both R1 and R4 core types. The PCR based core typing of those isolates
304 revealed that those isolates actually possessed R1 core type (Amor et al., 2000).

305

306 No attempts were made to serotype the isolates used in this study as previous studies clearly
307 indicated the diversity of avian *E. coli* serogroups and presence of high proportion of
308 untypable strains (Dozois et al., 1992; Blanco et al., 1998 Silveira et al., 2002; Ngeleka et al.,
309 2002). Rodriguez-Siek and colleagues (2005) indicated that the 524 avian *E. coli* isolates
310 studied belonged to 60 different O-serogroups while 30% was untypable. In addition, there
311 are no relationships between the O-serogroup and core oligosaccharide type. Isolates of the
312 same serogroup can have different LPS core types (Gibbs et al., 2004; Amor et al., 2000).

313

314 Previous studies have demonstrated the high association of *tsh*, *iss* and *cvaC* genes with
315 APEC but not with avian faecal *E. coli* isolates (Rodriguez-Siek et al., 2005). The results of
316 this study also proved the strong association of *tsh* and *iss* with disease-causing avian *E. coli*.
317 However, the presence of the *cvaC* gene did not appear to be a significant virulence trait of
318 clinical avian *E. coli* as previously suggested.

319

320 Delicato and colleagues (2002) studied the occurrence of *tsh* gene and reported a 39.5%
321 occurrence and is in agreement with our results. High serum resistance of the isolates which
322 possessed both *tsh* and *iss* genes was an important indication of the link of those genes to
323 serum resistance. The presence of virulence genes and the capacity to resist the serum
324 bactericidal activity was demonstrated by the *E. coli* strains of all four core types.

325

326 Nearly, half of the isolates of the clinical category lacked the virulence determinants tested.
327 There is a possibility that those strains possess some other virulence mechanism or the
328 pathogenic origin of those isolates is questionable and may be opportunistic. The capability
329 of causing infections by non-virulent organisms is overlooked in most instances and

330 especially the avian *E. coli* infections which are largely dependent on environmental or host
331 factors. Therefore, the possibility of opportunistic infections and tissue invasion of
332 commensal organism after death needs to be considered and perhaps is the reason for fewer
333 occurrences of these genes and the presence of serum sensitive strains amongst clinical
334 isolates.

335

336 The relationship between phylogenetic groups and ExPEC has been demonstrated by several
337 study groups and it has been proposed that *E. coli* causing extra-intestinal disease mainly
338 belong to group B2 and to a lesser extent group D (Johnson et al., 2001). The studies carried
339 out on UPEC isolates are in agreement with this, but Rodriguez-Siek et al. (2005) studied
340 both UPEC and APEC isolates and showed that more APEC strains were assigned to group
341 D than B2. They reported that 38% were in the phylogenetic group A, 15.5% were in the
342 group B1, 18.5% group B2 and 28.1% group D. The results of the present study are also
343 consistent with those findings with the vast majority of isolates possessing virulence traits
344 being found firstly in the group A and secondly in the group D.

345

346 It is important to note that most of the virulence determinants could be acquired by horizontal
347 transmission without disrupting the clonal lineage. Therefore the most likely reason for the
348 diversity in distribution of these virulence determinants in different phylogenetic groups is
349 horizontal gene acquisition. However, it is still not known whether the mere acquisition of
350 these genes is enough to make an organism virulent or if a specific genetic background is
351 required for the transfer and expression of these genes (Escobar-Pa'ramo et al., 2004). Amor
352 et al. (2000) studied the ECOR collection and reported the high prevalence of R1 core type in
353 all four major phylogenetic groups and highlighted the strong association of UPEC

354 possessing R1 core type with phylogenetic groups B2 and D. This observation raised the
355 question of whether the structural organization of LPS in the R1 core type possesses any
356 selective advantage, which facilitates acquisition of virulence genes. However, the high
357 prevalence of R1 LPS core type is not only reported in the disease causing *E. coli*, but also in
358 the commensal population (Gibbs et al., 2004). It is quite obvious that the LPS with R1 core
359 type confers some advantage over other core types to facilitate its wide spread nature, but to
360 what extent it supports the acquisition of virulence genes, colonisation in different organs and
361 ability to survive and multiply in the circulatory system is a subject which needs further
362 studies. It is important to determine whether the different core types are important in causing
363 infections in different host species or whether the LPS core types play any role in acquisition
364 of virulence genes.

365

366

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372

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465 **Table 1 Sequences of primers, amplicon size and the references for the primers used for**466 **PCR**

467	Gene	Primer sequences	Amplicon size	Reference
468				
469				
470	LPS core			
471	R1	F- GGGATGCGAACAGAATTAGT	551bp	Amor <i>et al.</i> (2000)
472		R- TTCCTGGCAAGAGAGATAAG		
473				
474	R2	F- GATCGACGCCGGAATTTTTT	1141bp	
475		R- AGCTCCATCATCAAGTGAGA		
476				
477	R3	F-GGCCAAAACACTATCTCTCA	1785bp	
478		R- GTGCCTAGTTTATACTTGAA		
479				
480	R4	F-TGCCATACTTTATTCATCA	699bp	
481		R-TGGAATGATGTGGCGTTTAT		
482				
483	K12	F- TTCGCCATTTCTGTGCTACTT	916bp	
484		R- TAATGATAATTGGAATGCTGC		
485				
486	Complement resistant related traits			
487				
488	<i>tsh</i>	F- GGGAAATGACCTGAATGCTGG	420bp	Maurer <i>et al.</i> (1998)
489		R- CCGCTCATCAGTCAGTACCAC		
490				
491	<i>iss</i>	F- TGGCGAAAACACTAGTAAAACAGC	760bp	Horne <i>et al.</i> (2000)
492		R- CGCCTCGGGGTGGATAA		
493				
494	<i>cvaC</i>	F- CACACACAAACGGGAGCTGTT	679bp	Johnson and
495		R-CTTCCGCAGCATAGTTCCAT		Stell (2000)
496				
497				
498	Phylogenetic grouping			
499	<i>ChuA</i>	F- GACGAACCAACGGTCAGGAT	279bp	Clermont <i>et al.</i> (2000)
500		R- TGTGAAGTGTCAGGAGACGCTG		
501				
502	<i>YjaA</i>	F- CCGCCAGTACCAGGGACA	211bp	
503		R- ATGGAGAATGCGTTCCTCAA		
504				
505	TSPE	F- GAGTAATGTCGGGGCATTCA	152bp	
506	4.C2	R- CGCGCCAACAAAGTATTACG		
507				

508 **Table 2** Distribution of chicken *E. coli* isolates of clinical and commensal origin in relation
 509 to the LPS core types, phylogenetic groups, serum resistance and APEC associated genes (*tsh*,
 510 *iss* and *cvaC*)

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LPS Core	Origin	No. of isolates	Phylogenetic group				No. of serum resistant isolates	No. of isolates with APEC associated genes		
			A	B1	B2	D		<i>tsh</i>	<i>iss</i>	<i>cvaC</i>
R1	clinical	89	71	03	06	09	56	18	26	35
	commensal	29	25	01	0	03	16	0	05	04
R2	clinical	04	02	01	0	01	02	03	0	0
	commensal	0	0	0	0	0	0	0	0	0
R3	clinical	31	20	01	03	07	25	10	18	09
	commensal	19	16	01	0	02	11	03	01	06
R4	clinical	19	01	01	02	15	17	16	11	03
	commensal	02	01	0	0	01	02	0	0	0
K12	clinical	0								
	commensal	0								
		193	136	08	11	38	129	50	61	57