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

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**ABSTRACT**

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

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

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## INTRODUCTION

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

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

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## MATERIALS AND METHODS

### Bacteria

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

### Polymerase chain reaction (PCR)

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

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

### Determination of LPS core type by PCR

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

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

PCR was performed in a 25µl of reaction mixture containing: 2.5µl of 10X Mg-free PCR buffer, 1.5µl of 50mM $MgCl_2$, 25pM of each primer, 0.2µl of Taq DNA polymerase, 0.2µl of deoxynucleotide triphosphate mix (dNTPs) (10mM), 13.1µl of ultra pure water, 2.5µl of template DNA. The PCR amplification profiles were: initial denaturation at 94°C for 4 minutes, followed by 35 cycles of, 94°C (denaturation) for 20 seconds, 50°C for 30 seconds (annealing), and 72°C for 135 seconds (polymerization). On completion of 35 cycles final polymerization was carried out at 72°C for 120 seconds. PCR products were separated by horizontal gel electrophoresis in 1% agarose gel and DNA visualized with ethidium bromide under ultraviolet illumination.

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

Sequences of the primers specific for each gene are shown in Table 1 Amplification of each gene carried out separately in a 25µl reaction mixture containing: 2.5µl of 10x Mg-free PCR buffer, 2.5µl of 50mM $MgCl_2$, 50pM of each primer, 0.2µl of Taq DNA polymerase, 0.5µl of dNTP mixture (10mM), 11.8µl of ultra pure water and 2.5µl of template DNA. The PCR conditions were: 5 minutes at 95°C , nine cycles of 1 minute at 95°C, 30 seconds at 55°C, 1 minute at 72°C and 28 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C and followed by 7 minutes at 72°C. PCR products were separated by horizontal gel

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

**Phylogenetic analysis**

Two step triplex PCR, described by Clermont *et al.* (2000) was used for phylogenetic analysis. Primers (Table 1) specific for the genes *chu A, yja A* and DNA fragment, TSPE4.C2 (20pM each) used in 20µl of reaction mixture containing: 2µl of 10x buffer (containing $MgCl_2$), 2µl of PCR enhancer (supplied with Taq polymerase), 0.5µl of Taq DNA polymerase, 1µl of dNTP mixture, 6.5µl of ultra pure water and 2.5µl template DNA. The conditions used for PCR were denaturation at 94°C for 4 minutes, 30 cycles of 94°C for 5 seconds 59°C for 10 seconds and final extension at 72°C for 5 minutes.

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

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

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

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

**Serum resistance**

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

**Bio statistics**

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

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

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

## RESULTS

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

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

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

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

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

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

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Three virulence determining genes were found to be present in *E. coli* isolates representing all core types. However, *iss* and *tsh* genes were less commonly detected in *E. coli* isolates with R1 core types and represented 24% and 15% respectively. Among the four isolates with R2 core types, the *tsh* gene was detected in three and the *iss* gene was not detected in any of those isolates. Of the clinical isolates with the R3 core type, 67.7% possessed *tsh* and or *iss*. The three commensal isolates with the *tsh* gene also had the R3 core type. The majority of *E. coli* isolates with the R4 LPS core type had *tsh* (76%) and *iss* (58%) genes. The virulence genes *tsh* and *iss* were not equally distributed in the *E. coli* isolates with different core types and were significantly associated with R4 LPS core (*tsh* $P<0.001$ and *iss* $P<0.03412$).

The presence of *cvaC* gene was common amongst isolates with R1 (32%) and R3 (30%) LPS core types. Lipopolysaccharide core type representing R4 had only 4 isolates (19%) with *cvaC* gene. However, the *cvaC* gene did not exhibit significant association with any of those LPS core types ($P = 0.2166$).

**Serum bactericidal activity**

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

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

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

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

**DISCUSSION**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

| Gene | Primer sequences | Amplicon size | Reference |
|---|---|---|---|
| **LPS core** | | | |
| R1 | F- GGGATGCGAACAGAATTAGT<br>R- TTCCTGGCAAGAGAGATAAG | 551bp | Amor *et al*. (2000) |
| R2 | F- GATCGACGCCGGAATTTTTT<br>R- AGCTCCATCATCAAGTGAGA | 1141bp | |
| R3 | F-GGCCAAAACACTATCTCTCA<br>R- GTGCCTAGTTTATACTTGAA | 1785bp | |
| R4 | F-TGCCATACTTTATTCATCA<br>R-TGGAATGATGTGGCGTTTAT | 699bp | |
| K12 | F- TTCGCCATTTCGTGCTACTT<br>R- TAATGATAATTGGAATGCTGC | 916bp | |
| **Complement resistant related traits** | | | |
| *tsh* | F- GGGAAATGACCTGAATGCTGG<br>R- CCGCTCATCAGTCAGTACCAC | 420bp | Maurer *et al*. (1998) |
| *iss* | F- TGGCGAAAACTAGTAAAACAGC<br>R- CGCCTCGGGGTGGATAA | 760bp | Horne *et al.* (2000) |
| cvaC | F- CACACACAAACGGGAGCTGTT<br>R-CTTCCGCAGCATAGTTCCAT | 679bp | Johnson and Stell (2000) |
| **Phylogenetic grouping** | | | |
| *ChuA* | F- GACGAACCAACGGTCAGGAT<br>R- TGTGAAGTGTCAGGAGACGCTG | 279bp | Clermont *et al*.(2000) |
| *YjaA* | F- CCGCCAGTACCAGGGACA<br>R- ATGGAGAATGCGTTCCTCAA | 211bp | |
| TSPE 4.C2 | F- GAGTAATGTCGGGGCATTCA<br>R- CGCGCCAACAAAGTATTACG | 152bp | |

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

| **LPS Core** | **Origin** | **No. of isolates** | **Phylogenetic group** | | | | **No. of serum resistant isolates** | **No. of isolates with APEC associated genes** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **A** | **B1** | **B2** | **D** | | ***tsh*** | ***iss*** | ***cvaC*** |
| 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 |

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