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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.

Keywords: Avian pathogenic *E. coli*; Core oligosaccharide; Lipopolysaccharide

1. Introduction

The lipopolysaccharide (LPS) of *Escherichia coli* contains a common form of architecture that consists of three major components: (i) hydrophobic lipid A,

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(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.

2. Materials and methods

2.1. 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.

2.2. 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 100 μl 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.

2.3. 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 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 25 μl of reaction mixture containing: 2.5 μl of 10× Mg-free PCR buffer, 1.5 μl of 50 mM $MgCl_2$, 25 pM of each primer, 0.2 μl of Taq DNA polymerase, 0.2 μl of deoxynucleotide triphosphate mix (dNTPs) (10 mM), 13.1 μl of ultra pure water, 2.5 μl of template DNA. The PCR amplification profiles were: initial denaturation at 94 °C for 4 min, followed by 35 cycles of, 94 °C (denaturation) for 20 s, 50 °C for 30 s (annealing), and 72 °C for 135 s (polymerization). On completion of 35 cycles final polymerization was carried out at 72 °C for 120 s.