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Antibiotic resistance genes located in integrons isolated from Escherichia coli recovered from humans and animals

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Antibiotic Resistance Genes Located in Integrons Isolated from *Escherichia coli* Recovered from Humans and Animals

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ABSTRACT

Multi-drug resistant pathogens are the principal cause of failure in the treatment of bacterial infectious diseases. Accurate surveillance of antibiotic resistance genes in the community is essential to developing strategies for resistance control and prevention. In this study, a collection of 514 *Escherichia coli* strains from animal and human sources was examined for the presence of class 1, 2 and 3 integrons using a PCR-based screening method. A multiplex PCR was developed to simultaneously screen for *intI1*, *intI2* and *intI3* genes. This study characterised all gene cassettes including those that could not be PCR amplified using standard screening methods.

The frequency of class 1 and class 2 integrons detected in *E. coli* strains in this study was generally lower than that reported in previous studies. Class 1 integrons were detected in 81/514 *E. coli* strains sourced from animals and humans. Gene cassette arrays identified in class 1 integrons include *dfrA5, dfrA7, aadA1, aadA2, dfrA1/aadA1, dfrA17/aadA5* and *dfrA12/orfF/aadA2*. In addition, atypical integrons containing *dfrA5*-IS*26* and *dfrA15*-IS*26* elements were discovered. The *dfrA5*-IS*26* element, a unique class 1 integron with most of the integron 3′-conserved segment (CS) deleted by the insertion of IS*26,* was detected in 31/514 *E. coli* isolates. This novel integron-*dfrA5*-IS*26* element, which was widespread in *E. coli* isolates of bovine origin and also found in *E. coli* of human origin, may act as a conduit for the transfer of integron-related resistance genes to human pathogens. Utilisation of PCR targeting the integron-IS*26* element will allow the

characterisation cassette arrays in atypical class 1 integrons that remain undetected using currently available PCR-based screening strategies.

Seven of the 514 *E. coli* strains contained class 2 integrons and six of these harboured the gene cassette array analogous to that found in Tn*7*, *dfrA1-sat2-aadA1*. In the remaining *intI2* positive *E. coli* strain 80, in which the gene cassette region could not be PCR amplified using standard methods, the *intI2* gene was found to be located on a plasmid. The complete nucleotide sequence of this plasmid (pECTm80) was determined, revealing an intact *dfrA1-sat2* cassette array and a truncated *aadA1* gene cassette, due to the insertion of IS*1*. Open reading frames and Tn*7* transposition genes normally conserved at the 3′ end of Tn*7*-like class 2 integrons were not detected. This atypical class 2 integron is flanked by a Tn*3* family transposon or insertion sequence (IS) remnant and IS*1*. The plasmid pECTm80, of the incompatibility (Inc) group X, has the potential to facilitate the horizontal transfer of tightly-linked antibiotic resistance genes to diverse antimicrobial species. Features which contribute to the clinical relevance of this plasmid include its ability to be mobilised, the presence of genes to ensure the stable maintenance of the plasmid through successive bacterial cell divisions and the presence of a highly-regulated DNA replication system consisting of α, β and γ origins of replication.

This thesis provides a snapshot of the antibiotic resistance genes located in integrons in *E. coli* strains sourced from Australian animals and humans. The association of atypical integrons with IS elements suggests these DNA elements play an important role in the evolution of integrons.

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