

University of Wollongong - Research Online

Thesis Collection

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

Author: Fay Ellen Dawes

Year: 2009

Repository DOI:

Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

Research Online is the open access repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

2009

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

Fay Ellen Dawes
University of Wollongong

Follow this and additional works at: <https://ro.uow.edu.au/theses>

University of Wollongong

Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

Recommended Citation

Dawes, Fay Ellen, Antibiotic resistance genes located in integrons isolated from *Escherichia coli* recovered from humans and animals, Doctor of Philosophy thesis, School of Biological Sciences, University of Wollongong, 2009. <https://ro.uow.edu.au/theses/3105>

NOTE

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

UNIVERSITY OF WOLLONGONG

COPYRIGHT WARNING

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

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

A thesis submitted in fulfillment of the requirements for the award of the degree

Doctor of Philosophy

from

University of Wollongong, Australia

By

Fay Ellen Dawes, B. Biotechnology (Hons class 1)

School of Biological Sciences

2009

TABLE OF CONTENTS

CHAPTER 1 – INTRODUCTION	1
1.1 – Antibiotic resistance.....	1
1.2 – Response from the pharmaceutical industry	1
1.3 – Mechanisms of antibiotic resistance	3
1.4 – Antibiotic resistance gene stability, amplification and transmission.....	4
1.5 – Monitoring the movement of antibiotic resistance genes	6
1.6 – Acquisition and emergence of resistance genes.....	7
1.6.1 – Mutation	7
1.6.2 – Horizontal transfer	8
1.6.3 – Insertion sequence (IS) elements	10
1.7 – Integrons.....	11
1.7.1 – Gene cassette integration and excision	12
1.7.1.1 – Multi gene cassette arrays	13
1.7.2 – Integron classes	14
1.7.2.1 – Class 1 integrons	15
1.7.2.2 – Class 2 integrons	17
1.7.2.2.1 – Mechanism of Tn7 transposition.....	19
1.7.2.3 – Class 3 integrons	20
1.7.2.4 – Class 4 integrons	22
1.7.2.5 – Other integron classes	25
1.8 – <i>Escherichia coli</i>	26
1.8.1 – Pathogenic <i>E. coli</i> strains and associated diseases.....	28
1.8.1.1 – Diarrhoeal diseases.....	28
1.8.1.2 – Hemolytic uremic syndrome	29
1.8.1.3 – Septicemia	29
1.8.1.4 – Urinary tract infections	30
1.8.1.5 – Meningitis	31
1.8.2 – Gene cassettes detected in <i>E. coli</i>	32
1.9 – Aims	32
CHAPTER 2 - MATERIALS AND METHODS	34
2.1 – Media and bacterial strains	34
2.2 – <i>E. coli</i> strains.....	35

2.2.1 – Strain selection	35
2.3 – Characterisation of <i>E. coli</i> strains	38
2.4 – Antimicrobial testing	39
2.5 – DNA manipulations	40
2.5.1 – Agarose gel electrophoresis	40
2.5.2 – Restriction endonuclease analysis of DNA	40
2.5.3 – Extraction of DNA from agarose gels	40
2.5.4 – Crude DNA extraction	41
2.5.5 – Plasmid DNA extraction	41
2.5.6 – Chromosomal DNA extraction	43
2.6 – Polymerase chain reaction	43
2.6.1 – General PCR parameters	44
2.6.2 – Class 1 integron-related PCR	45
2.6.3 – Class 2 integron-related PCR	46
2.6.4 – Expand Long Template PCR system	46
2.6.5 – Second-step PCR	47
2.6.5.1 – Core-sample PCR	47
2.7 – Restriction fragment length polymorphism	48
2.8 – DNA sequence determination and analysis	48
2.8.1 – Cycle-sequencing conditions	48
2.8.2 – Purification of sequencing extension products	49
2.8.3 – Analysis of DNA sequence data	49
2.8.4 – DNA sequencing of plasmids from <i>E. coli</i> strains D22 and 80	50
2.9 – Cloning	51
2.9.1 – Digestion of vector and insert DNA	51
2.9.2 – Ligation reaction	51
2.10 – Southern hybridisation	52
2.10.1 – DNA transfer	52
2.10.2 – Labelling the probe	53
2.10.3 – Hybridisation and detection	54
2.10.4 – Stripping membrane for re-probing	56
2.11 – Microbiological techniques	56
2.11.1 – Preparation of chemically competent cells	56
2.11.1.1 – Chemical transformation	56
2.11.2 – Preparation of electro-competent cells	57

2.11.2.1 – Electroporation	57
2.11.3 – Bacterial conjugation	58
CHAPTER 3 – DEVELOPMENT OF A MULTIPLEX POLYMERASE CHAIN REACTION AND SCREENING FOR CLASS 1, 2 AND 3 INTEGRONS	59
3.1 – Introduction	59
3.2 – Stages in the development of the multiplex PCR	61
3.2.1 – Touchdown PCR	63
3.2.2 – Long-range temperature gradient	63
3.2.3 – Redesigning primers and optimising conditions for multiplex PCR	64
3.3 – Multiplex PCR screening results for <i>intI1</i> , <i>intI2</i> and <i>intI3</i>	66
3.4 – PCR amplification of gene cassettes	69
3.4.1 – Investigation of “non-amplifiable” class 1 gene cassette arrays	69
3.5 – Discussion	72
3.6 – Conclusion	76
CHAPTER 4 – MOLECULAR CHARACTERISATION OF CLASS 1 INTEGRONS: WIDESPREAD DETECTION OF A UNIQUE CLASS 1 INTEGRON-IS26 ELEMENT IN <i>ESCHERICHIA COLI</i> ISOLATED FROM CATTLE AND HUMANS	78
4.1 – Introduction	78
4.2 – Results	80
4.2.1 – PCR detection of the <i>intI1</i> gene	80
4.2.2 – Characterisation of gene cassette arrays	81
4.2.3 – Sequence of a class 1 integron containing a “non-amplifiable” gene cassette array	87
4.2.4 – Distribution of class 1 integron- <i>dfrA5</i> -IS26 elements and Tn21 transposition genes	90
4.2.5 – Analysis of other class 1 integrons containing “non-amplifiable” gene cassette arrays	94
4.2.6 – Serotypes and resistance phenotypes	96
4.3 – Discussion	97
4.4 – Conclusion	101
CHAPTER 5 – MOLECULAR CHARACTERISATION OF CLASS 2 INTEGRONS ISOLATED FROM <i>ESCHERICHIA COLI</i>	103
5.1 – Introduction	103
5.2 – Results	105
5.2.1 – PCR detection of class 2 integrons	105
5.2.2 – Serotypes and resistance phenotypes	106
5.2.3 – Molecular characterisation of class 2 integrons	109

5.2.4 – Determination of class 2 integron (Tn7) insertion sites	110
5.2.5 – DNA Sequence analysis of pECTm80 containing a unique class 2 integron gene cassette array.....	113
5.3 – Discussion	120
5.4 - Conclusion	126
CHAPTER 6 – CONCLUSIONS	127
6.1 – Introduction	127
6.2 – Main results and future directions.....	127
6.3 – Final remarks.....	130
APPENDICES	131
Appendix I: Media and solutions	131
Appendix II: PCR primers and cycling parameters	135
Appendix III: <i>E. coli</i> isolates screened for class 1, 2 and 3 integrons	139
Appendix IV: Oral presentation and manuscripts based on this thesis.....	164
REFERENCES.....	165

LIST OF TABLES

Table 1.1. Mutation of chromosomal genes inducing antibiotic resistance.....	8
Table 1.2. Characteristics of mobile DNA elements.....	9
Table 1.3. Features of IS26, IS1 and IS91.....	11
Table 1.4. The major categories of pathogenic <i>E. coli</i> strains.....	28
Table 2.1. Bacterial strains and plasmids used in this study.....	35
Table 2.2. <i>E. coli</i> strains examined for class 1 integron carriage.....	38
Table 2.3. Concentration of reagents used in a typical PCR amplification.....	45
Table 2.4. PCR cycling conditions used to develop specific PCR parameters.....	45
Table 2.5. Concentration of reagents used in a typical sequencing reaction.....	49
Table 2.6. Standard cycling parameters used for the sequencing reaction.....	49
Table 2.7. Concentrations of reagents used for the ligation reaction.....	52
Table 2.8. Reagents used in PCR for the synthesis of the DIG-labelled probe.....	54
Table 2.9. PCR cycling parameters for synthesis of the DIG-labelled probe.....	54
Table 3.1. Integrase m-PCR conditions described by B. Dillon and J. Iredell.....	62
Table 3.2. Cycling parameters for long-range m-PCR.....	64
Table 3.3. Cycling parameters for the integrase/ <i>uspA</i> m-PCR.....	65
Table 3.4. M-PCR screening of <i>E. coli</i> isolates for <i>intI1</i> , <i>intI2</i> , and <i>intI3</i> genes.....	68
Table 3.5. PCR amplification of gene cassette regions.....	70
Table 3.6. The frequency of class 1 and 2 integrons.....	74
Table 4.1. Gene cassette arrays detected in class 1 integrons.....	83
Table 4.2. Structure of atypical class 1 integrons	91
Table 5.1. Description of class 2 integron positive <i>E. coli</i> strains.....	108
Table 5.2. Identification of CDS in the nucleotide sequence of pECTm80.....	115
Table II.1. Oligonucleotides used for PCR amplification in this study.....	135
Table II.2. PCR cycling parameters used in this study.....	137
Table III.1. <i>E. coli</i> strains screened for class 1, 2 and 3 integrase.....	139

LIST OF FIGURES

Figure 1.1. The integration and excision of a gene cassette in a class 1 integron.....	13
Figure 1.2. Basic structure of a class 1 integron.....	16
Figure 1.3. Variation in cassette genes carried by class 1 integrons.....	16
Figure 1.4. Genetic organisation of Tn7 and Tn7-like class 2 integrons.....	18
Figure 1.5. Class 2 integrons associated with the insertion sequence IS1.....	19
Figure 1.6. Variation in gene cassettes carried by class 3 integrons.....	22
Figure 1.7. Recruitment of genes from <i>V. cholerae</i> by a class 1 integron.....	25
Figure 1.8. Comparison of a class 1 integron with environmental integrons.....	26
Figure 3.1. Agarose gel: amplification products generated using m-PCR conditions described by J. Iredell and B. Dillon.....	62
Figure 3.2. Agarose gel: amplification products generated using preliminary conditions developed for m-PCR.....	64
Figure 3.3. Agarose gel: amplification products generated using optimum conditions for m-PCR screening	66
Figure 3.4. Agarose gel: m-PCR screening of representative <i>E. coli</i> strains.....	67
Figure 3.5. PCR primers used to investigate “non-amplifiable” gene cassette arrays...	71
Figure 4.1. RFLP analysis and genetic structure of gene cassette arrays.....	82
Figure 4.2. Genetic structure of an atypical class 1 integron	88
Figure 4.3. DNA sequence and features of the integron- <i>dfrA5</i> -IS26 element.....	89
Figure 4.4. Genetic structure of atypical class 1 integrons.....	95
Figure 5.1. Tn7 genetic map and agarose gels showing PCR amplification of <i>intI2</i> , <i>tnsC</i> and gene cassette arrays and RFLP analysis.....	107
Figure 5.2. Genetic map showing Tn7 insertion in the <i>E. coli</i> chromosome. Southern hybridisation and agarose gels investigating Tn7 insertion.....	111
Figure 5.3. Diagram showing variation in Tn7 insertion in the <i>E. coli</i> chromosome..	112
Figure 5.4. Plasmid map of pECTm80 isolated from <i>E. coli</i> strain 80.....	114
Figure 5.5. DNA sequence comparison of the plasmids pECTm80 and pOU1114.....	119

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*-IS26 and *dfrA15*-IS26 elements were discovered. The *dfrA5*-IS26 element, a unique class 1 integron with most of the integron 3'-conserved segment (CS) deleted by the insertion of IS26, was detected in 31/514 *E. coli* isolates. This novel integron-*dfrA5*-IS26 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-IS26 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 Tn7, *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 *IS1*. Open reading frames and Tn7 transposition genes normally conserved at the 3' end of Tn7-like class 2 integrons were not detected. This atypical class 2 integron is flanked by a Tn3 family transposon or insertion sequence (IS) remnant and *IS1*. 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.

ACKNOWLEDGMENTS

I would like to thank Prof. Mark J. Walker and Assoc. Prof. Steven P. Djordjevic for giving me the opportunity to do my PhD and for supervising this project. I thank Mark for his mentoring and guidance; for his seemingly endless patience, his analytical approach to solving problems and his dependable calm manner. I thank Steve for his enthusiasm for antibiotic resistance research.

I thank and acknowledge the assistance of our collaborators in this project: Dr. Karl A. Bettelheim, Dr Michael A. Hornitzky, Prof. G. F. Browning and Dr. D. M. Gordon for supplying *E. coli* strains; Dr. Karl Bettelheim and Alexander Kuzevski for performing serotyping and resistance profiles on *E. coli* strains; and Dr. Dieter M. Bulach for his assistance with sequence annotation.

I thank all the members of lab 105 at Wollongong University for their friendliness and practical help, which was always freely given and for just making the lab a good place to be. I want to especially thank Vidiya for her friendship.

I am indebted to my amazing family and friends for their constant encouragement and support. I want to give a special thank you to my husband Chris for his loving support throughout my many years of study.

I dedicate this thesis to my three wonderful sons, Matthew, Brett and Andrew.