ความหลากหลายทางพันธุกรรม และการป้องกันเชื้อ *แคมไพโลแบคเตอร์* ในไก่พันธุ์เนื้อ และไก่เนื้อ

นายทศพล ธำรงสุวรรณกิจ

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย
The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository (CUIR)
are the thesis authors' files submitted through the University Graduate School.

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาอายุรศาสตร์สัตวแพทย์ ภาควิชาอายุรศาสตร์
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2559

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

GENETIC DIVERSITY AND PREVENTION OF *CAMPYLOBACTER* SPP. IN BROILER BREEDERS AND BROILERS

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Mr. Thotsapol Thomrongsuwannakij

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Veterinary Medicine
Department of Veterinary Medicine
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2016

Copyright of Chulalongkorn University

Thesis Title	GENETIC DIVERSITY AND PREVENTION OF *CAMPYLOBACTER* SPP. IN BROILER BREEDERS AND BROILERS
By	Mr. Thotsapol Thomrongsuwannakij
Field of Study	Veterinary Medicine
Thesis Advisor	Associate Professor Niwat Chansiripornchai, D.V.M., M.Sc., Ph.D., DTBVM
Thesis Co-Advisor	Professor Jiroj Sasipreeyajan, D.V.M., Ph.D., DTBVM

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

....Dean of the Faculty of Veterinary Science

(Professor Roongroje Thanawongnuwech, D.V.M., M.Sc., Ph.D., DTBVP)

THESIS COMMITTEE

....Chairman

(Associate Professor Achara Tawatsin, B.Sc., M.Sc.)

....Thesis Advisor

(Associate Professor Niwat Chansiripornchai, D.V.M., M.Sc., Ph.D., DTBVM)

....Thesis Co-Advisor

(Professor Jiroj Sasipreeyajan, D.V.M., Ph.D., DTBVM)

....Examiner

(Associate Professor Somsak Pakpinyo, D.V.M., Ph.D., DTBVM)

....Examiner

(Associate Professor Rungtip Chuanchuen, D.V.M., M.Sc., Ph.D.)

....External Examiner

(Associate Professor Patrick Joseph Blackall, B.Sc., Ph.D.)

ทศพล ธำรงสุวรรณกิจ : ความหลากหลายทางพันธุกรรม และการป้องกันเชื้อ *แคมไพโลแบคเตอร์* ในไก่พันธุ์เนื้อและไก่เนื้อ (GENETIC DIVERSITY AND PREVENTION OF *CAMPYLOBACTER* SPP. IN BROILER BREEDERS AND BROILERS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.นิวัตร จันทร์ศิริพรชัย, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.จิโรจ ศศิปรียจันทร์, 129 หน้า.

เนื้อสัตว์ปีกที่มีการปนเปื้อนนับเป็นสาเหตุสำคัญของโรคแคมไพโลแบคทีโอซิสในมนุษย์ การศึกษาเกี่ยวกับเรื่องนี้ในประเทศไทยมีเพียงจำนวนน้อย ดังนั้นวัตถุประสงค์ของการศึกษานี้ประกอบด้วย 1) เพื่อระบุหาความสัมพันธ์ของเชื้อ*แคมไพโลแบคเตอร์*ที่แยกได้จากไก่พันธุ์เนื้อ โรงฟัก ไก่เนื้อ และสิ่งแวดล้อมในโรงเรือนเหล่านั้น 2) เพื่อประเมินระดับการดื้อยาต้านจุลชีพของเชื้อ*แคมไพโลแบคเตอร์* 3) เพื่อประเมินประสิทธิภาพการป้องกันเชื้อของ competitive exclusion ภายหลังการให้เชื้อพิษทับของ *แคมไพโลแบคเตอร์ เจจูไน* ในไก่เนื้อ เพื่อให้บรรลุวัตถุประสงค์ดังกล่าว การศึกษาฉบับนี้จึงประกอบด้วย 2 ส่วนย่อย ได้แก่ 1) เก็บตัวอย่างจากฟาร์มไก่พันธุ์ จำนวน 5 ฟาร์ม โรงฟัก จำนวน 2 โรง และฟาร์มไก่เนื้อจำนวน 5 ฟาร์ม จำนวนไอโซเลตของเชื้อ *C. jejuni* และ *C. coli* ที่แยกได้จากกระบวนการผลิตไก่เนื้อ 2 ห่วงโซ่อุปทานเท่ากับ 36 และ 94 ไอโซเลตตามลำดับ นำมาทดสอบหาค่าความไวรับต่อยาต้านจุลชีพด้วยวิธี two-fold agar dilution เชื้อ*แคมไพโลแบคเตอร์*ส่วนใหญ่มีรูปแบบการดื้อต่อยาหลายชนิด และดื้อต่อยาเอนโรฟลอกซาซิน ในสัดส่วน *C. jejuni* เท่ากับ 100% และ *C. coli* เท่ากับ 98.9% สุ่มเลือกเชื้อ *C. jejuni* และ *C. coli* อย่างละ 24 ไอโซเลต เพื่อวิเคราะห์หาการกลายพันธุ์ที่ quinolone resistance determining region (QRDR) ของยีน *gyrA* ด้วยวิธีการวิเคราะห์ลำดับนิวคลีโอไทด์ พบว่าทุกไอโซเลตสามารถตรวจพบการเปลี่ยนแปลงที่ตำแหน่ง Thr-86-Ile (ACA-ATA สำหรับ *C. jejuni* หรือ ACT-ATT สำหรับ *C. coli*) การศึกษาฉบับนี้พบว่าที่มาของการติดเชื้อ*แคมไพโลแบคเตอร์* เกิดจากการติดเชื้อแบบ horizontal เป็นหลัก ดังที่เห็นได้จากเมื่อนำเชื้อ*แคมไพโลแบคเตอร์*ทุกไอโซเลตมาจำแนกด้วยเทคนิค *fla*A-RFLP สามารถแยกกลุ่มของเชื้อได้เป็น 10 กลุ่มที่แตกต่างกัน 2) เก็บตัวอย่างมูลไก่จากไก่พื้นบ้านและไก่ไข่ที่เลี้ยงแบบชีวภาพจากฟาร์มที่ไม่มีประวัติการใช้ยาต้านจุลชีพจำนวน 60 ตัวอย่าง นำมาจำแนกหา competitive exclusion (CE) โดยตรวจพบเชื้อ *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* แล้วจึงนำไปทดสอบความไวรับต่อยาต้านจุลชีพ เพื่อป้องกันการส่งผ่านการดื้อยาตามคำแนะนำของ EFSA และทดสอบการทนกรดและน้ำดีในห้องปฏิบัติการ พบว่า *Lactobacillus acidophilus* 1/4, *Bacillus subtilis* 206/1 and *Enterococcus faecium* 122 มีประสิทธิภาพสูงในการทนกรดและน้ำดี จึงถูกคัดเลือกมาใช้เป็น CE เพื่อป้อนปากแก่ลูกไก่เนื้อที่อายุ 1-3 วัน ภายหลังการให้เชื้อพิษทับด้วย *C. jejuni* แก่ไก่เนื้อที่อายุ 14 วัน พบว่าจำนวนเชื้อ *C. jejuni* และอัตราการแลกเนื้อที่อายุ 41 วันไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ โดยสรุปแล้ว การศึกษาฉบับนี้ชี้ให้เห็นถึงอุบัติการณ์เพิ่มขึ้นอย่างรวดเร็วของการดื้อต่อยาต้านจุลชีพหลายชนิด ซึ่งเป็นสิ่งที่น่ากังวล ดังนั้นการใช้ยาต้านจุลชีพที่ฟาร์ม ควรใช้อย่างรอบคอบและมีการตรวจเฝ้าระวังการดื้อยา เพื่อช่วยลดความเสี่ยงทางด้านสาธารณสุข

ภาควิชา	อายุรศาสตร์	ลายมือชื่อนิสิต	....
สาขาวิชา	อายุรศาสตร์สัตวแพทย์	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
ปีการศึกษา	2559	ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

5375964031 : MAJOR VETERINARY MEDICINE
KEYWORDS: ANTIMICROBIAL RESISTANCE, CAMPYLOBACTER JEJUNI, CAMPYLOBACTER COLI, COMPETITIVE EXCLUSION, GYRA MUTATION,

THOTSAPOL THOMRONGSUWANNAKIJ: GENETIC DIVERSITY AND PREVENTION OF *CAMPYLOBACTER* SPP. IN BROILER BREEDERS AND BROILERS. ADVISOR: ASSOC. PROF. NIWAT CHANSIRIPORNCHAI, D.V.M., M.Sc., Ph.D., DTBVM, CO-ADVISOR: PROF. JIROJ SASIPREEYAJAN, D.V.M., Ph.D., DTBVM, 129 pp.

Contaminated poultry meat is regarded as the main source of human campylobacteriosis. In Thailand, there is only a few publications studied in poultry farms, so the aims of this study were 1) to identify the relationship of *Campylobacter* isolates among broiler breeders, broilers and their environments, 2) to examine antimicrobial resistance profiles of *Campylobacter* spp., and 3) to examine the protection of competitive exclusion after challenging *Campylobacter jejuni* in broilers. To complete the objectives, there were 2 parts in this study. First, five commercial breeder flocks, 2 hatcheries, and 5 broiler flocks were sampled. Thirty-six *C. jejuni* and 94 *C. coli* isolates collected through two broiler production chains were tested by two-fold agar dilution for their susceptibility to antimicrobial agents. Most *Campylobacter* isolates were multidrug resistance (MDR) (*C. jejuni*: 100%; *C. coli*: 98.9%), and exhibited high resistance to enrofloxacin (*C. jejuni*: 100%; *C. coli*: 98.9%). A selected subset of 24 *C. jejuni* and 24 *C. coli* were characterized for their mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene by nucleotide sequence analysis. The Thr-86-Ile substitution (ACA-ATA in *C. jejuni* or ACT-ATT in *C. coli*) was found in all isolates. Horizontal transmission was the major route of *Campylobacter* transmission in this study, as all *Campylobacter* isolates were typed and ten distinct clusters were recognized by *fla*A-RFLP typing. Second, competitive exclusion (CE) were identified from 60 adult chicken feces of native chickens and organic layers raised under non-antimicrobial usage farms. *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* were identified and tested their antimicrobial susceptibilities for preventing transmissible antimicrobial resistance, as recommended by EFSA. Furthermore, those isolates were *in vitro* tested for acid and bile tolerances. *Lactobacillus acidophilus* 1/4, *Bacillus subtilis* 206/1 and *Enterococcus faecium* 122 demonstrated their powerful *in vitro* activities and were, therefore, used as CE during oral gavage of 1-day-old broilers for 3 days consecutively. After *C. jejuni* challenges at 14 days in broilers, the treatment groups had no significant differences in *C. jejuni* re-isolations or feed conversion ratio at 41 days. In conclusions, the emergence of MDR and high resistance rates to several antimicrobials are major concerns identified in this study. The prudent use of these agents and active surveillance of resistance at the farm level are essential steps to reduce the public health risks identified in this work.

Department:	Veterinary Medicine	Student's Signature	....
Field of Study:	Veterinary Medicine	Advisor's Signature	....
Academic Year:	2016	Co-Advisor's Signature	....

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my advisor, Associate Professor Dr. Niwat Chansiripornchai and my co-advisor, Professor Dr. Jiroj Sasipreeyajan for their supports, valuable advices and encouragements. I have learned so much from them including the concept of avian medicine and practice in the fields.

I would like to thank my committee members, Associate Professor Achara Tawatsin, Associate Professor Dr. Somsak Pakpinyo and Associate Professor Dr. Rungthip Chuanchuen their important suggestions and helpfulness. I would like to give a special thank to Associate Professor Dr. Patrick J. Blackall (Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia) for his willingness to share knowledge and give me the opportunity to practice at his laboratory in Australia.

I would like to thank Dr. Conny Turni (Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia), Dr. Wechsiri Wannaprasat, Pheempat Bengtong Taveesak Janetanakit, and graduate students of Avian Health Research Unit for good cooperation in many ways that support me in performing this work. I am grateful to all staffs at the Department of Veterinary Medicine and the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University. I would to thank all farmers for their cooperation in sample collections

Finally and Importantly, I would like to thank the Graduate School of Veterinary Science Faculty, the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) for financial support. I also thank Thailand Research Fund (The Royal Golden Jubilee Ph.D. Program) for research funding.

Finally, I wish to express my most sincere thank to my family for their supporting, understanding and encouragement given during my whole life.

CONTENTS

Page

THAI ABSTRACT ... iv

ENGLISH ABSTRACT ... v

ACKNOWLEDGEMENTS ... vi

CONTENTS ... vii

LIST OF TABLES ... 11

LIST OF FIGURES ... 12

LIST OF ABBREVIATIONS ... 13

CHAPTER I ... 14

INTRODUCTION ... 14

CHAPTER II ... 18

LITERATURE REVIEW ... 18

2.1 Taxonomy and characteristics of *Campylobacter* spp. ... 18

2.3 Isolation and identification of *Campylobacter* spp. ... 20

2.4 Public Health Significance ... 23

2.5 Prevalence ... 24

2.6 Mode of transmission of *Campylobacter* in poultry ... 25

2.6.1 Horizontal transmission ... 25

2.6.2 Vertical transmission ... 26

2.7 Molecular techniques for genetic characterization of *Campylobacter* ... 26

2.7.1 Flagellin gene typing ... 27

2.8 Antimicrobial resistance in *Campylobacter* spp ... 28

2.9 Fluoroquinolones resistance mechanism in *Campylobacter* spp. ... 30

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Page

2.10 Intervention strategies 31
2.10.1 Biosecurity 32
2.10.2 Vaccination 33
2.10.3 Competitive exclusion 33
CHAPTER III 35
Materials and Methods 35
3.1 Antimicrobial resistance, the characterization of *gyrA* mutation, and genetic diversity by *fla*A-RFLP of *Campylobacter jejuni* and *Campylobacter coli* collected through commercial broiler production chains in Thailand 37
3.1.1 Farm description 37
3.1.2 Sample collection 40
3.1.3 *Campylobacter* isolation and identification 43
3.1.4 Antimicrobial susceptibility test 45
3.1.5 Detection of mutation(s) in the quinolone resistance determining region (QRDR) of the *gyrA* and mismatch amplification mutation assay by PCR (MAMA-PCR) detecting the Thr-86-Ile mutations 46
3.1.6 *fla*A-RFLP Typing 49
3.2 Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers 51
3.2.1 Sample collections from native chickens and layers 51
3.2.2 Isolation of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* 51
3.2.3 Identification of genus and species 52
3.2.4 Antimicrobial susceptibility test 54

Page

3.2.5 Acid and Bile tolerance tests....54

3.2.6 *Campylobacter jejuni* challenges....55

3.2.7 *Campylobacter* isolation, identification and enumeration....58

3.2.8 Statistical analysis....58

CHAPTER IV....59

RESULTS....59

4.1 Antimicrobial resistance, the characterization of *gyrA* mutation, and genetic diversity by *fla*A-RFLP of *Campylobacter jejuni* and *Campylobacter coli* collected through commercial broiler production chains in Thailand....59

4.1.1 Prevalence of *Campylobacter* from 5 chicken production flocks....59

4.1.2 Phenotypic antimicrobial resistance....61

4.1.3 Genetic characterization of *gyrA* mutation and MAMA-PCR....64

4.1.4 Genotypic diversity by *fla*A-RFLP....66

4.2 Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers....70

4.2.1 Antimicrobial resistance phenotypes....70

4.2.2 Survival rate of acid and bile tolerance tests....72

4.2.3 *C. jejuni* challenged against CE application....74

CHAPTER V....76

DISCUSSIONS....76

5.1 Antimicrobial resistance, the characterization of *gyrA* mutation, and genetic diversity by *fla*A-RFLP of *Campylobacter jejuni* and *Campylobacter coli* collected through commercial broiler production chains in Thailand....76

Page

5.2 Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers 86

CONCLUSIONS AND SUGGESTIONS 89

REFERENCES 91

APPENDIX 106

VITA 129

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

LIST OF TABLES

Table 1: Characteristics of *C. jejuni* and *C. coli* (ISO 10272-1, 2006)........................22

Table 2: Amino acid substitutions in the QRDR of gyrase A......................................31

Table 3: Number of samples collected in this study 41

Table 4: Sample collection procedures used in this study 42

Table 5: Sets of primers for *Campylobacter* identification (Wang et al., 2002).....44

Table 6: Set of primers for QRDRs of the *gyrA* genes of *Campylobacter* spp. 48

Table 7: Set of primers detecting Thr-86-Ile mutation of the *gyrA* genes of *Campylobacter* spp. (Zirnstein et al., 1999; Zirnstein et al., 2000)...........................48

Table 8: A set of primers for *flaA* gene amplification (Nachamkin et al., 1993)....50

Table 9: Oligonucleotide primers used in this study 53

Table 10: CE application programs in broilers during 1-3 days of age 57

Table 11: Antimicrobial resistance pattern of *C. jejuni* (n = 36) and *C. coli* (n = 94)...63

Table 12: The *gyrA* mutations and the MIC for enrofloxacin of the selected *Campylobacter* isolates and the two control strains..65

Table 13: MIC data of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium*...71

Table 14: Number of bacteria in each level of percent of survival rate of acid and bile tolerance tests...73

Table 15: Average number of *C. jejuni*, FCR and body weight..................................75

LIST OF FIGURES

Figure 1: The flow chart of experiments..36

Figure 2: Diagram of sample collection from breeder flocks, hatcheries, and broiler flocks. 38

Figure 3: Geographic location of sampling sites in Lopburi province. A = broiler flocks 3, 4, and 5, B= breeder flocks 3, 4, and 5, C= breeder flocks 1 and 2, D= broiler flocks 1 and 2, E = hatchery A and F= hatchery B. 39

Figure 4: Prevalence percentage of *C. jejuni* and *C. coli* isolated from chicken-related samples and environmental samples...60

Figure 5: Frequency of resistance to 9 antimicrobial agents in *C. jejuni* (n = 36) and *C. coli* (n = 94). AMX, amoxicillin; BAC, bacitracin; COL, colistin; DOX, doxycycline; ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; MDR, multidrug resistance.....62

Figure 6: Phylogenetic analysis representing *flaA*-RFLP of *C. jejuni* and *C. coli* isolated from breeders and broilers in Thailand at the 72% cut-off genetic similarity. The similarity matrix was computed using Dice similarity coefficient and clustering by the Unweighted Paired Group Method with Arithmetic Mean Values (UPGMA). Band position tolerance and the optimization coefficient were both set to 2% using Bionumerics...69

LIST OF ABBREVIATIONS

^{o}C	degree Celcius
C.	*Campylobacter*
CBA	Columbia blood agar
CFU	colony forming unit
CLSI	Clinical and Laboratory Standards Institute
flaA	*flagellin A gene*
gyrA	gyrase A gene
mCCDA	modified Charcoal Cefoperazone Deoxycholate Agar
MIC(s)	Minimum Inhibitory Concentration(s)
ml	milliliter
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
µl	microlitre
spp.	species

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER I

INTRODUCTION

Campylobacter has considered to be a major cause of foodborne bacterial gastro-infectious disease in both developed and developing countries for several years (Jay-Russell et al., 2012; Sahin et al., 2012). The pathogen causes mild to severe symptoms including bloody diarrhea (Blaser et al., 1979). Invasive infections infrequently result in reactive arthritis, meningitis, pneumonia and a severe form of Guillain-Barré syndrome involving neuromuscular paralysis of the extremities (Blaser et al., 1986).

The organism is a cytochrome oxidase positive, microaerophilic, curved gram-negative rod, 0.2 to 0.8 μm wide and 0.5 to 5 μm long exhibiting cockscrew motility. Cells in old cultures may form coccoid bodies which are considered degenerative forms rather than a dormant stages of the organism. There are currently 29 recognized species and 4 subspecies of *Campylobacter* (Takamiya et al., 2011). The human pathogenic species of *Campylobacter* comprise 4 species including *C. jejuni, C. coli, C. lari* and *C. upsaliensis* which are often referred as the thermophilic *Campylobacter* species. The term thermotolerant means that those *Campylobacter* species are capable of growing at 41-43°C. The species most frequently associated with human campylobacteriosis is *C. jejuni* with only 10% due to *C. coli* and less than 1% from *C. lari* (Nachamkin et al., 1998). Even though campylobacteriosis is generally self-limiting,

incapacity may last a few weeks and up to 10% of reported cases may require medical treatment (Arcila-Londono and Lewis, 2012). More serious systemic infections are well demonstrated and occur most commonly in the immunocompromised person. In addition, an association of campylobacteriosis with post-infectious neuropathies such as Guillain-Barre syndrome has been identified; however, neurological consequences of infection are rare, they might be extremely serious (Arcila-Londono and Lewis, 2012).

Campylobacter jejuni and *C. coli* live in the avian gut as commensal bacteria. These organisms are not only be isolated from domestic chickens, but they are also recovered from all other domestic poultry including turkeys, ducks, quails and pigeons, as well as from wild birds and even ostriches (Yogasundram et al., 1989; Borck and Pedersen, 2005). Only in the ostrich this colonization is associated with clinical disease (Verwoerd, 2000). The different pathogenesis of *Campylobacter* in human and poultry is not clearly understood.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

There are many risk pathways for human exposure to *Campylobacter*. *Campylobacter* spp. are found widespread in the environment and in farmed animals (Evans and Wegener, 2003). Despite the fact that multiple sources of *Campylobacter* have been recognized with different importance in relation to human illness, the handling and consumption of undercooked poultry is an important source of human campylobacteriosis (Ridley et al., 2011; Thakur et al., 2013). *Campylobacter* contamination of broiler carcasses during slaughter is primarily due to cross-contamination during the feather removal and evisceration process (Hue et al., 2010).

Formerly, *Campylobacter* was normally susceptible to various antimicrobials, but recent reports showed that *Campylobacter* isolates derived from animals and humans increased resistance to several antibiotics including fluoroquinolones, macrolides and tetracyclines (Price et al., 2005; Han et al., 2007). Based on laboratory diagnosis and farm studied, treatment of infected chickens with fluoroquinolones resulted in fluoroquinolones-resistant *Campylobacter* mutants passing through human eating contaminated meat (Padungtod et al., 2006). In Thailand, there is also an increasing trend of multiple antimicrobial resistances in both animal and human origins which the cause might be an inappropriate use of antimicrobial drugs in animals (Boonmar et al., 2005; Padungtod and Kaneene, 2005).

The aims of this study had three reasons. First, the relationship of *Campylobacter* isolates among broiler breeders, broilers and their environments was identified. Second, antimicrobial resistance profiles of *Campylobacter* spp. were updated. Third, the protection of competitive exclusion after challenging *Campylobacter jejuni* in broilers was demonstrated.

CHULALONGKORN UNIVERSITY

Objectives of Study

1. To identify the relationship of *Campylobacter* isolates among broiler breeders, broilers and their environments.
2. To examine antimicrobial resistance profile of *Campylobacter* spp.

3. To examine the protection of competitive exclusion after challenging *Campylobacter jejuni* in broilers.

Keywords (Thai): การดื้อยาต้านจุลชีพ, ไก่เนื้อ, ไก่พันธุ์เนื้อ, *แคมไพโลแบคเตอร์*, คอมเพททิทีพเอ็กคลูชั่น, ความหลากหลายทางพันธุกรรม

Keywords (English): antimicrobial resistance, broiler, broiler breeder, *Campylobacter* spp., competitive exclusion, genetic diversity

Research questions

1. Is there any relationship of *Campylobacter* isolates among broiler breeders, broilers and their environments?
2. What is the MIC value of *Campylobacter* spp. isolated from broiler breeders and broilers?
3. Can competitive exclusion reduce numbers of *Campylobacter jejuni* infection?

CHAPTER II

LITERATURE REVIEW

2.1 Taxonomy and characteristics of *Campylobacter* spp.

The genus *Campylobacter* belongs to the *Epsilon-proteobacteria*, in the family *Campylobacteraceae* (Vandamme et al., 1991), which includes the genera *Campylobacter*, *Arcobacter, Dehalospirillum* and *Sulfurospirillum*. Available genomes of the genus *Campylobacter* currently comprises 29 species and 4 subspecies (Takamiya et al., 2011). *Campylobacter* is a spiral rod shape with one or two flagella at the pole and is motile. *Campylobacter* grows at optimum temperature of 42°C, with a range between 30.5°C and 45°C, optimum growth at 10% carbon dioxide, 5-6% oxygen and 85% nitrogen (Blaser et al., 1986). *Campylobacter* cells in old cultures may form coccoid bodies which are considered as degenerative forms (Hazeleger et al., 1995). Biochemical characteristics of several species generally require fumarate with formate or hydrogen for a microaerobic growth. This genus can reduce nitrate to nitrite. Tyrosine, casein, gelatin and starch are not hydrolyzed. There is no lipase or lecithinase activity but oxidase activity is presented in every species except *C. gralcilis*. Most enteropathogenic *Campylobacters* are catalase-positive. *Campylobacter* is generally sensitive to oxygen, desiccation, osmotic stress, low pH and high temperature (>60°C) (Park, 2002).

2.2 Incubation period, clinical signs and pathological lesions

Although chickens can be easily infected with *Campylobacter* naturally or experimentally, they normally do not show clinical illness and *Campylobacter*-associated diarrhea in poultry is quite not happened. Colonization could experimentally occur as early as one day after inoculation (Knudsen et al., 2006). Incubation period is between 1 and 3 days (Sanyal et al., 1984; Welkos, 1984).

Campylobacter is rarely detected in birds that are younger than 2-3 weeks of age. The reasons may be related to multiple factors including the presence of maternally-derived antibodies (Sahin et al., 2003) or environmental or host-related factors differences. Once a flock is infected, *Campylobacter* spreads thoroughly within the flock causing colonization of most of birds within a few days (Evans and Sayers, 2000).

The members of thermophilic *Campylobacter*, primarily *C. jejuni* and *C. coli*, are frequently isolated from avian species, especially domestic poultry including chickens, turkeys, ducks and geese (Sahin et al., 2002). *C. jejuni* and *C. coli* well adapt to the avian host and habit in the intestinal tract of birds. Even though poultry have extensive colonization, *Campylobacter* infections produce little or no clinical diseases in them (Corry and Atabay, 2001). On the other hand, there was a published report indicating that infectious hepatitis associated with *C. coli* and *C. jejuni* in ostriches caused a high morbidity and mortality (Stephens et al., 1998). There was an experiment inoculated with a high dose of *C. jejuni* to 3 days old chickens resulting diarrhea within

Chulalongkorn University

72 hours which lasted for 10 days, weight losses as well as a mortality of 32% (Ruiz-Palacios et al., 1981). In experimentally infected chicks, gross pathological lesions associated with *Campylobacter* infection were minimal and mainly found only in the gastrointestinal tract. Blood and mucus in the small intestinal tract and petechial hemorrhages in the gizzard mucosa of chicks can be seen sometimes (Welkos, 1984).

2.3 Isolation and identification of *Campylobacter* spp.

Thermophilic *Campylobacter* are fastidious and slow-growing requiring a microaerobic atmosphere (containing 5% O_2, 10% CO_2 and 85% N_2) and showing optimal growth at 42°C. Culture-based isolation and detection methods can be divided into 2 groups including passive filtration and using selective media. Firstly, passive filtration is a method developed by Steele and McDermott (Steele and McDermott, 1984). According to the characteristic of the organism, *Campylobacter* has a cockscrew shape that can pass through 0.45 or 0.65 μm filter paper onto a Columbia blood agar (CBA). CBA, an enriched non-selective medium, is created for the isolation, quantitation and partial identification of a variety of microorganism. Passive filtration is very useful for the isolation of antimicrobial-sensitive *Campylobacter* spp. This method does not use expensive media, so it may be useful for laboratories with limited resources. CBA is also suitable for use in antibiotic differential disk examination and spot biochemical testing (ISO10272-1, 2006). Secondly, selective media is widely used for the recovery of *Campylobacter* spp. because the use of selective media for *Campylobacter*

isolation showed higher prevalence than using the filtration method (Bolton and Robertson, 1982). Many selective media can be used for isolation of *Campylobacter* spp. There are 2 types of selective media for *Campylobacter* isolation including charcoal-based solid media and blood-containing solid media. The examples of charcoal-based solid media composed of modified charcoal-cefoperazone-deoxycholate agar (mCCDA), which is the recommend medium, Karmali agar and CAT agar (cefoperazone, amphotericin and teicoplanin) (Corry et al., 1995). The latter medium was developed for facilitating growth of *C. upsaliensis* (Aspinall et al., 1993). The examples of blood-containing solid media composed of Preston, Skirrow, Butzler and Campy-cefex agar which are considered alternative choices (Skirrow, 1977; Bolton and Robertson, 1982). The major characteristics of *C. jejuni* and *C. coli* are shown in Table 1.

Formerly, hippurate hydrolysis could be used to differentiate of *C. jejuni* and *C. coli* because the hippuricase gene was only found in *C. jejuni* (Rautelin et al., 1999). Even though some *C. jejuni* isolates are hippuricase-negative, they are unable to differentiate *C. coli* from hippuricase-negative *C. jejuni* by using purely biochemical tests (Fields and Swerdlow, 1999).

CHULALONGKORN UNIVERSITY

Table 1: Characteristics of *C. jejuni* and *C. coli* (ISO 10272-1, 2006)

Characteristic	*C. jejuni*	*C. coli*
Morphology	small curved bacilli	
Motility	corkscrew motility	
Microaereobic growth at 25 C	-	
Aerobic growth at 41.5 C	-	
Oxidase	+	
Catalase	+	+
Nalidixic acid	R/S*	R/S
Cephalothin	R	R
Hydrolysis of hippurate	+/-	-
Indoxyl acetate	+	+

*R = resistant, S= sensitive

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Polymerase chain reaction (PCR) was also recognized as a method for culture confirmation or for direct detection of *Campylobacter* from environmental or poultry samples. PCR primers can be designed for genus detection or differentiation of species or strains (Wang et al., 2002). There are a variety of PCR assays targeting genus or species-specific sequences developed to detect and identify *Campylobacter* from poultry feces and environmental samples (Waegel and Nachamkin, 1996; Chuma et al., 1997; Wang et al., 2002).

2.4 Public Health Significance

Campylobacter has now emerged as an important bacterial agent of foodborne gastroenteritis worldwide (Zenner and Gillespie, 2011). In 2007, the Foodborne Diseases Active Surveillance Network reported 5,818 *Campylobacter*'s cases per 100,000 enteric cases (FoodNet, 2007). Regarding Thai human campylobacteriosis, Thailand do not have national surveillance programs for this pathogen, so incidence values in terms of number of cases for a population do not exist (Coker et al., 2002). Most estimates of incidence in developing countries are from laboratory surveillance of pathogens responsible for diarrhea (Coker et al., 2002). Echeverria et al. (1989) reported that isolation rate from diarrhea specimens from <5-year-old children in Thailand was about 13%. Most of *Campylobacter*-related illnesses in humans are sporadic and characterized by self-limiting watery and/or bloody diarrhea, abdominal cramp and possible fever. Severe conditions may occur in immunocompromised patients requiring an effective antibiotic treatment (Mead et al., 1999). In addition, it can be associated with Guillain-Barré syndrome (GBS), a post-infection autoimmune disease characterized by acute and progressive neuromuscular paralysis (Nachamkin et al., 1998). Human campylobacteriosis generally presents 3-5 days of acute watery or bloody diarrhea, usually with severe abdominal pain. Most cases of infection are due to *C. jejuni*, with only 10% due to *C. coli* and less than 1% from *C. lari* (Nachamkin et al., 1998).

A high prevalence of *Campylobacter* has been detected both in the intestinal tract of poultry at a farm level and in poultry carcasses at the processing plants.

Chulalongkorn University

Consequently, poultry products at retail are often contaminated by *Campylobacter* (Jeffrey et al., 2001). A significant risk factor for human campylobacteriosis is a handling or eating undercooked poultry meat (Friedman et al., 2004).

2.5 Prevalence

Many types of poultry such as broilers, layers, turkeys, ducks, fowl, quails and ostriches can become colonized with *Campylobacter* (Sahin et al., 2002). The numbers of *Campylobacter* positive poultry flocks are commonly high (Sahin et al., 2002). The proportion of *C. jejuni* was higher than *C. coli*, but it was vary by regions, seasons and the production types (conventional, free-range and organic farms) (Heuer et al., 2001). Focusing on broiler breeder, there were 30-90% prevalence rate (Colles et al., 2011; Perez-Boto et al., 2012). From the prevalence studies conducted in Europe and the United States reported *Campylobacter* positive in broiler flocks ranging from 50-97% (Heuer et al., 2001; Bouwknegt et al., 2004; Barrios et al., 2006). From the data collected in Thailand, Chansiripornchai and Sasipreeyajan (2009) found that the prevalence of *C. jejuni* was between 55 and 80% (average at 65%) from samples of seven broiler flocks. Likewise, the prevalence of *Campylobacter* in chickens at the farm, slaughterhouse and market in northern Thailand were 64, 38 and 47%, respectively (Padungtod and Kaneene, 2005). Interestingly, several longitudinal studies indicated that broilers were not detected *Campylobacter* at day-old chicks, but intensively

reared flocks became detectably positive normally at 2 to 3 weeks of age (Berndtson et al., 1996; Evans and Sayers, 2000).

2.6 Mode of transmission of *Campylobacter* in poultry

2.6.1 Horizontal transmission

According to farm-based studies, horizontal transmission from the environment to poultry houses is the most usual mode of *Campylobacter* transmission on poultry farms (Sahin et al., 2002). Key sources of infection come from mainly through fecal contact old litter, other farm animals, domestic pets, wildlife species, house flies, insects and farm personnel via their boots (Sahin et al., 2002). Poultry feed does not play an important role in the spread of *Campylobacter* because it is very sensitive to oxygen and temperature (Humphrey et al., 1993). *Campylobacters* are not able to grow outside an animal host and either die or enter a viable but non-culturable (VBNC) state (Buswell et al., 1998). However, they can be isolated from fecal contaminated environmental sources, such as soil and surface water (Sahin et al., 2002) .The presence of *Campylobacters* is the result from recent fecal contamination, because they are unable to multiply outside of host animals (Jones, 2001).

Chulalongkorn University

2.6.2 Vertical transmission

Although there were some reports showing that vertical transmission of *Campylobacter* can be occurred, this finding was still unclear (Doyle, 1984; Petersen et al., 2001). Some studies showed that *C. jejuni* had been isolated from the reproductive tract of healthy hens (Camarda et al., 2000; Buhr et al., 2002) and from semen of commercial broiler breeder roosters (Cox et al., 2002), but another side of this argument had several reasons also. Firstly, although breeders were infected with *Campylobacter*, young broiler chickens hatched from their eggs frequently lack of *Campylobacter* before 2 or 3 weeks of age (Bull et al., 2006). Secondly, the infected strains found in broiler flocks were usually different from their breeder flocks (Chuma et al., 1997). Finally, isolation of *Campylobacter* from eggs has been rarely found, and no studies have reported isolation of live *Campylobacter* cells from hatcheries or young chickens (Hiett et al., 2002).

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

2.7 Molecular techniques for genetic characterization of *Campylobacter*

Epidemiological investigations are important to discover the source of broiler house contamination and the mechanisms by which *Campylobacter* spp. spreads between the chickens in order to develop appropriate strategies for reducing the risk of this foodborne zoonosis. To know the epidemiological relationship between bacteria, molecular genotyping is the best way to explore and establish them because the reported genetic instability in *Campylobacter* spp. limited their molecular

epidemiological interpretation (Dingle et al., 2008). Nowadays, several genotyping methods were developed to demonstrate genetic diversity of *Campylobacter* spp. such as multilocus enzyme electrophoresis (MLEE), repetitive element sequence-based PCR (rep-PCR), amplified fragment length polymorphism (AFLP), PCR-restriction fragment length polymorphism analysis of the *flaA* gene (*flaA*-RFLP), sequencing of the short variable region of the *flaA* gene (*flaA*-SVR sequencing), pulse-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) (Charununtakorn et al., 2015; Duffy et al., 2015). These techniques have both advantages and disadvantages. For example, MLST, a technique of typing by sequencing seven house-keeping genes, is considered the gold standard, but typing of large strain sets can be costly and time consuming. Recently, a study found that *flaA*-RFLP is a suitable preliminary typing method due to the ease of operation, equipment availability and cost (O'Reilly et al., 2006).

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

2.7.1 Flagellin gene typing

The characteristic motility of *Campylobacter* spp. is due to the fact that they have a single unsheathed polar flagellum at one end or both ends of the cell (Lastovica et al., 2014). The flagellar filaments are composed of repeats of a flagellin subunit which is encoded by a *fla* gene (Lastovica et al., 2014). *C. jejuni and C. coli* have two flagellin genes, designated *fla*A and *fla*B (Lastovica et al., 2014). Each gene is approximately 1.7 kb and is separated by an intergenic space region of 170 bp

(Nachamkin et al., 1993). These genes encode repeats of flagellin subunits that form the flagellum found on one or both ends of *Campylobacter* cells. The 5’ and 3’ regions of each gene are highly conserved, with considerable sequence variation in the region in between (Nachamkin et al., 1993). The achievable genotyping methods for *Campylobacter* spp. is *fla*A-RFLP due to the fact that it is a simple and reliable method with a good discriminatory power (Petersen and Newell, 2001). In addition, *fla*A-RFLP was the best method associated with epidemiology in the Walkerton outbreak in 2000 (Clark et al., 2005). The concept of *fla*A-RFLP is to amplify *fla*A gene which has highly conserved with considerable sequence variation followed by restriction fragment length polymorphism to exhibit the variability within an amplicon.

2.8 Antimicrobial resistance in *Campylobacter* spp.

C. jejuni and *C. coli* are increasingly resistant to antimicrobials which have become a major concern for public health because antimicrobials used in poultry production may lead to the rise of antimicrobial-resistant bacteria in humans (Smith et al., 2002). Although *Campylobacter* infections in humans are mild, self-limiting and usually resolve without antimicrobial therapy, antimicrobial treatment is required for some severe, prolonged infections or for infections in immunocompromised patients (Gibreel and Taylor, 2006). Macrolides are considered the drugs of choice, but fluoroquinolones (FQs) are also frequently used because of their broad spectrum of activity against enteric pathogens (Engberg et al., 2001). This pathogenic organism is

increasingly resistant to antimicrobials used in humans. In addition, FQ-resistant *Campylobacter* spp. was rapidly emerged among poultry flocks (Hein et al., 2003) and antimicrobial-resistant *Campylobacter* were increasingly found in developing countries using unrestricted antibiotics for humans and animals. Increasingly, antimicrobial use in food animal production is a global concern as antimicrobial-resistant zoonotic pathogens can develop at the farm level and lead to human exposure and infection via various pathways, including meat and poultry products (Quinn et al., 2007). Importantly, the use of FQs such as enrofloxacin in poultry production might lead to an increasing of FQ-resistant *Campylobacter* populations (Gupta et al., 2004). In 2002, a United States FDA quantitative risk assessment estimated that fluroquinolone use in chickens and turkeys caused >10,000 human infection with FQ-resistant *Campylobacter* in people seeking medical care and subsequent antimicrobial treatment (USFDA, 2000). In 2005, FQs were banned from poultry production in U.S., but the proportion of FQ-resistant *Campylobacter* isolates following this withdrawal was not statistically different to that before withdrawal (Price et al., 2007). From chicken samples, there were some reports found high proportions of *Campylobacter* resistant to nalidixic acid and ciprofloxacin in Thailand (Padungtod et al., 2003; Padungtod et al., 2006). Sukhapesna et al. (2005) also reported that they the proportions of *C. jejuni* isolated from retail markets in Nakhon Pathom province which was resistant to tetracycline, sulfamethoxazole, nalidixic acid and norfloxacin,

respectively and nearly 97% of the isolates were multiple resistance to more than 4 antimicrobial agents tested.

2.9 Fluoroquinolones resistance mechanism in *Campylobacter* spp.

FQs belong to a family of broad-spectrum synthetic antimicrobial agents. The FQs are used for the treatment of gastrointestinal tract infection of both animal and human diseases (Van Bambeke et al., 2005; Martinez et al., 2006). Generally, the FQs target two important microbial enzymes, DNA gyrase and DNA topoisomerase IV (Smith and Fratamico, 2010). In *Campylobacter*, the resistance to FQs is mainly mediated by point mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase A (*gyrA*) (Payot et al., 2006). No mutations in DNA gyrase B (*gyrB*) have been associated with FQ resistance in *Campylobacter* (Piddock et al., 2003). Although the genes encoding topoisomerase IV (*parC/parE*) are involved in FQ resistance in gram-negative bacteria, these genes are absent in *Campylobacter* meaning that *parC/parE* mutations are not implicated in *Campylobacter* resistance to FQ antimicrobials (Piddock et al., 2003; Payot et al., 2006). Several studies have shown that mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene of *Campylobacter* confers high-level resistance to FQs, with the mutation at position threonine-86-isoleucine (ACA - ATA in *C. jejuni* or ACT – ATT in *C. coli*) being most common (Zirnstein et al., 1999; Zirnstein et al., 2000). Mismatch amplification mutation assay by PCR (MAMA-PCR) is a rapid method applied to detect the Thr-86-Ile mutation (Zirnstein et al., 1999; Zirnstein

et al., 2000). Mismatch primers were created to fully complement to the template strand except for one nucleotide at the 3' end. These primers are used to detect the mutants. Thus, wild types and mutants can be differentiated by this technique. In addition, a list of amino acid substitutions conferring resistance to FQs is given in Table 2 (Smith and Fratamico, 2010).

Table 2: Amino acid substitutions in the QRDR of gyrase A conferring resistance to FQs

Gyrase subunit	Amino acid substitution
	Ala-70-Ser
	Thr-86-Ile
A	Thr-86-Ala
	Asp-90-Tyr
	Asp-90-Asn
	Gly-119-Ser

*Ala, alanine; Ser, serine; Thr, threonine; Ile, isoleucine; Asp, aspartic acid; Tyr, tyrosine; Asn, asparagine; Gly, glycine

2.10 Intervention strategies

It is widely recognized that the handling and consumption of undercooked chickens is a major source of human campylobacteriosis, so many researchers have

จุฬาลงกรณ์มหาวิทยาลัย CHULALONGKORN UNIVERSITY

tried to reduce its population at the farm level. One of the most challenging tasks is to control the flock colonization by *Campylobacter* because poultry houses can be contaminated by *Campylobacter* in many different ways from various environmental sources. Nowadays, there is no single protocol to completely control *Campylobacter* infections on poultry farms. For controlling *Campylobacter*, on-farm control strategies can be divided into two approaches: (1) biosecurity-based interventions, and (2) non-biosecurity based measured such as vaccination and competitive exclusion.

2.10.1 Biosecurity

There are many reports found a correlation between decreased *Campylobacter* infection in broiler flocks and the use of strict biosecurity measures and hygienic practices on farms (Humphrey et al., 1993; Berndtson et al., 1996; Evans and Sayers, 2000). Most of these studies showed that the use of strict biosecurity measures could either reduce the level or delayed the onset time of *Campylobacter* colonization, but they were not totally successful in preventing introduction of *Campylobacter* into broiler flocks (Humphrey et al., 1993; Berndtson et al., 1996; van de Giessen et al., 1998). Considering on-farm biosecurity measures, they showed different results in controlling of *Campylobacter* in Europe. In northern European countries such as Norway, Sweden, and Finland, these measures are successful in reducing *Campylobacter* incidence in broilers. On the other hand, they have met limited success in UK, the Netherlands and Denmark (EFSA, 2011).

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

2.10.2 Vaccination

Nowadays, there are no commercial vaccines available for controlling *Campylobacter* in poultry (de Zoete et al., 2007). It is a critical challenge for developing effective vaccines of *Campylobacter* conferring broad-spectrum protection because the commensal nature of this organism in poultry and the antigenic diversity among different *Campylobacter* strains. Formerly, Some researchers developed *Campylobacter* vaccines using different methods such as killed whole cells, live-attenuated vaccines, flagellin-based subunit vaccines, genetically engineered live vectors expressing *Campylobacter*-specific antigens, and chitosan-encapsulated DNA vaccine which most of them demonstrated some protection in chickens (Wyszynska et al., 2004; de Zoete et al., 2007; Lin, 2009; Buckley et al., 2010). However, more attempts are needed to develop vaccines that induce protective immune responses and can be done on poultry farm in a practical.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

2.10.3 Competitive exclusion

Competitive exclusion (CE) was first discovered by Nurmi and Rantala (1973). The definition of CE is the oral administration of non-pathogenic intestinal bacteria which can establish and colonize the intestinal tract and maintain or increase the natural flora to prevent or decline colonization of pathogenic organisms (Nurmi and Rantala, 1973; Vanbelle et al., 1990). The CE cultures can be categorized into 2 groups: defined and undefined cultures. The defined CE cultures are microbial isolates

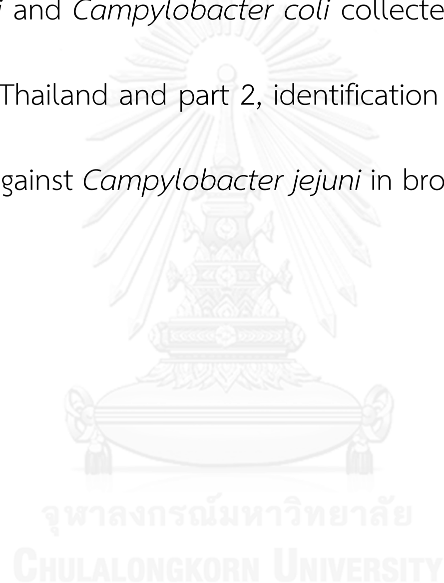
identified and characterized for their properties such as antimicrobial sensitivity and acid and bile tolerance, while the latter are incompletely characterized (Zhang et al., 2007). Ideally, CE should use defined cultures instead of crude intestinal mucus suspension. Importantly, CE or probiotics used in poultry should not have antimicrobial resistance phenotypes because they can increase the risk of transferrable drug resistant genes to other gut bacteria (Schwarz et al., 2001). For defined CE cultures, *Lactobacillus* spp. *Bacillus* spp. and *Enterococcus faecium* are focused for studying on their CE properties against pathogenic bacteria (Morishita et al., 1997; Thomrongsuwannakij et al., 2016). The treatment of broilers with pure cultures of *Lactobacillus acidophilus* and *Streptococcus faecium* from day 1 to day 3 demonstrated a 70% reduction in the frequency of *C. jejuni* shedding in colonized chickens (Morishita et al., 1997). A recent report has shown that *Enterococcus faecalis* MB 5259 showed an *in vitro* inhibitory effect to *C. jejuni* MB 4185, but there was no inhibition in the *in vivo* experiment (Robyn et al., 2013) leading to raise a question to find the appropriate non-pathogenic bacteria that can reduce *Campylobacter* colonization in chickens in the future. Nevertheless, the effect of defined CE on *Campylobacter* was variable and inconsistent (Schoeni and Wong, 1994; Mead et al., 1996).

CHULALONGKORN UNIVERSITY

CHAPTER III

Materials and Methods

The experiment was divided into 2 parts, including part 1, antimicrobial resistance, the characterization of *gyrA* mutation, and genetic diversity by *fla*A-RFLP of *Campylobacter jejuni* and *Campylobacter coli* collected through commercial broiler production chains in Thailand and part 2, identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers (Figure 1).


จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Genetic diversity and prevention of *Campylobacter* spp.

in broiler breeders and broilers

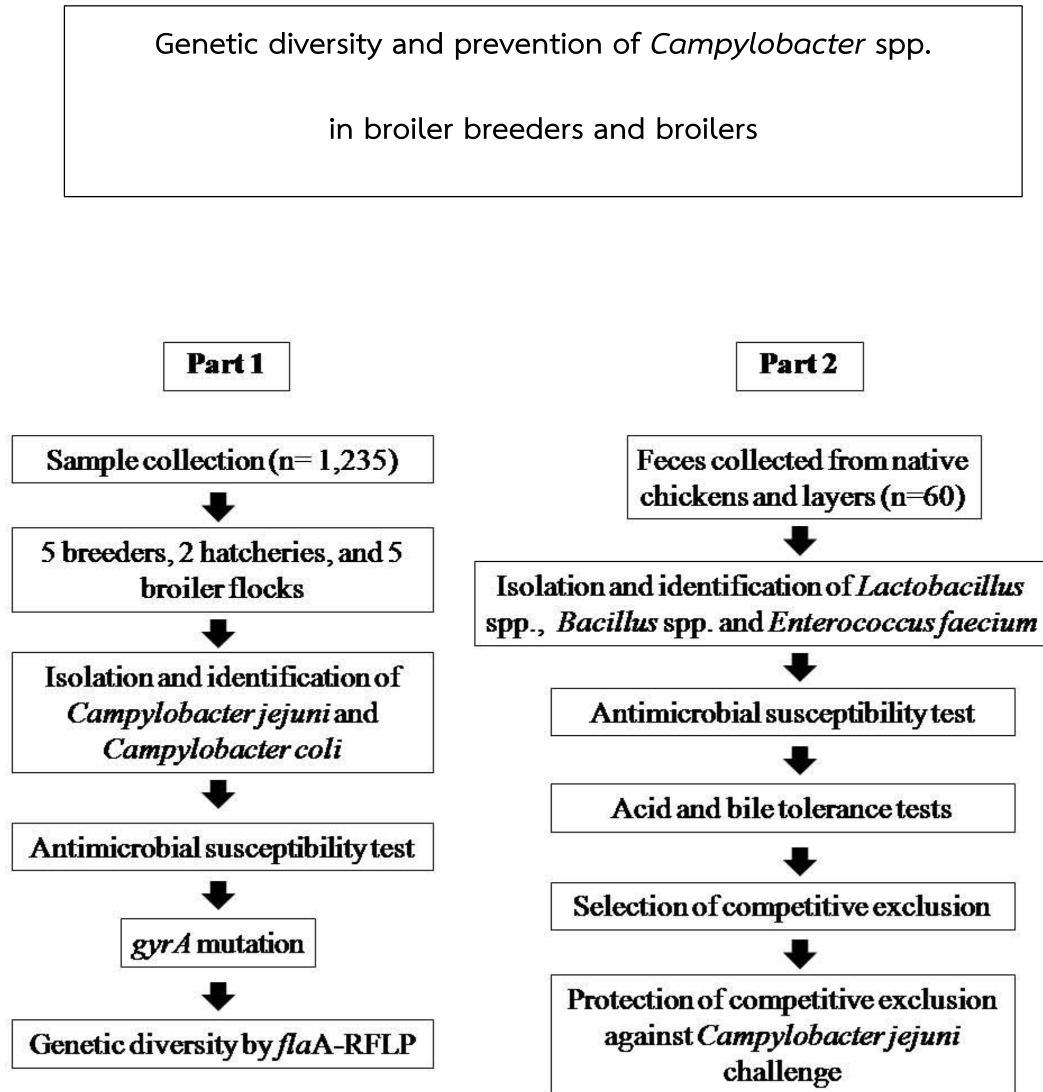


Figure 1: The flow chart of experiments

3.1 Antimicrobial resistance, the characterization of *gyrA* mutation, and genetic diversity by *fla*A-RFLP of *Campylobacter jejuni* and *Campylobacter coli* collected through commercial broiler production chains in Thailand

3.1.1 Farm description

During the period between September 2014 and February 2015, two chicken production chains from one integrated chicken company located in Lopburi province, a central area of Thailand were chronologically sampled from broiler breeder farms, hatcheries, and the broiler farms. This province has intensive poultry production facilities and other companies are also facilitated in the same province. Five commercial breeder flocks (breeder 1, 2, 3, 4, and 5), 2 hatcheries (hatchery A and B), and 5 broiler flocks (broiler 1, 2, 3, 4, and 5) were sampled in this study (Figure 2). Cobb chickens were raised in all flocks except that breeder flock 4 and broiler flock 4 were Hubbard chickens. The number of birds in each of breeder and broiler flocks was approximately 8,000 and 18,000 birds, respectively. Breeder flocks 1 and 2 were located on the same farm while breeder flocks 3, 4, and 5 were on another farm. Likewise, broiler flocks 1 and 2 were raised on the same farm while broiler flocks 3, 4, and 5 were raised on another farm. Fertile eggs from breeder flocks 1 and 2 were sent to hatchery A, while breeder flocks 3, 4, and 5 supplied eggs to hatchery B (Figure 2). All farms were located within a radius of around 50 km (Figure 3). Birds were raised under standard, controlled environment sheds that used evaporative cooling systems.

Chulalongkorn University

The "all in-all out" by flock system was used on all broiler farms. Hence, one-day-old birds were placed in one day for each broiler flock, grown in the same house and all birds were sent to the processing plant on the same day. Broilers from flocks 1 and 2 were reared until 35 days of age whereas broilers from flock number 3, 4, and 5 were reared until 43 days of age. This difference reflected market demands during the time of the study. The houses were cleaned and disinfected, and litter was totally removed after each production cycle. All cleaned houses always have a 21-day-downtime period before starting a new production cycle. No other livestock is raised on these farms. All visitors must sign their names, and their vehicles must be disinfected before entering the farms.

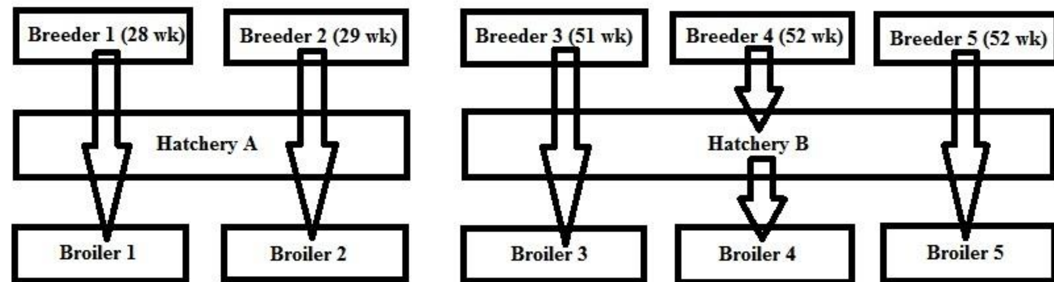


Figure 2: Diagram of sample collection from breeder flocks, hatcheries, and broiler flocks.

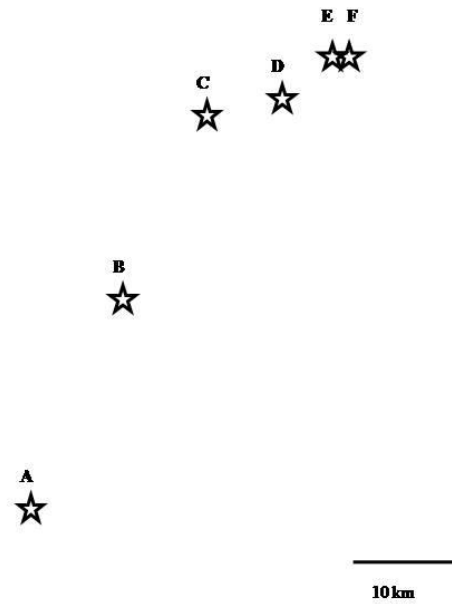


Figure 3: Geographic location of sampling sites in Lopburi province. A = broiler flocks 3, 4, and 5, B= breeder flocks 3, 4, and 5, C= breeder flocks 1 and 2, D= broiler flocks 1 and 2, E = hatchery A and F= hatchery B.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

3.1.2 Sample collection

In total, 1,235 samples were collected from the breeder and broiler farms and the hatcheries (Table 3). *Campylobacter* colonization of both breeder and broiler flocks was determined from cloacal swabs and environmental samples (e.g., pan feeders, footwear, darkling beetles, flies, feed and water). The eggs from the breeder flocks were followed to hatcheries. At the hatcheries, early embryonic deaths were investigated. Samples from the egg tray, egg shells, hatcher and water were investigated as well. Boot swabs, house equipment, and environmental samples (pan feeders, footwear) from the cleaned broiler houses were obtained before the chicks arrived. One-day-old broilers were sampled by cloacal swabbing. After that, cloacal swabs, boot swabs and environmental samples (e.g., pan feeders, footwear, darkling beetles, flies, feed and water) were taken at days 14 and 28 for all farms and at day 35 (broiler flock 1 and 2) or day 43 (broiler flock 3, 4 and 5). The number and type of samples taken in this study are shown in Tables 3 and 4, respectively. All samples were kept on ice and transported to the laboratory, where they were then processed for bacterial isolation within 6 hr from the time of collection.

Table 3: Number of samples collected in this study

	Number of samples from each flock				
	Flock 1	Flock 2	Flock 3	Flock 4	Flock 5
Breeder flock					
- Cloacal swabs	30	30	30	30	30
- Boot swabs	4	4	4	4	4
- House equipment and environmental samples	19	19	19	19	19
(e.g., pan feeders, footwear, beetles, flies, feed, and water)					
Hatchery					
- Equipment and environmental samples	8	8	8	8	8
(e.g., egg tray, egg shell, hatcher and water)					
- Early embryonic deaths	5	5	5	5	5
Broiler flock					
Before rearing period					
- Boot swabs	4	4	4	4	4
- House equipment and environmental samples (e.g., pan feeders, footwear)	9	9	9	9	9
During rearing period					
- Cloacal swabs					
Day 1	20	20	20	20	20
Day 14	20	20	20	20	20
Day 28	20	20	20	20	20
Day 35	20	20	-	-	-
Day 43	-	-	20	20	20
- Boot swabs	16	16	16	16	16
- House equipment and environmental samples	72	72	72	72	72
(e.g., pan feeders, footwear, beetles, files, feed and water)					
Total	247	247	247	247	247

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Table 4: Sample collection procedures used in this study

Type of sample	Sample collection procedures
Farm samples	
Cloacal swab	Moistened cotton swab was inserted into cloaca and then put into Cary-Blair transport medium
Animal feed	Approximately 500 grams of animal feed were collected from silo and 3 areas of pan feeder (front, middle and back of the house).
Boot swab	The man whose footwear covered by boot swabs walked inside or around the house. After that, they were kept in sterile plastic bags.
Pan feeder	Pan feeder was sampled using a moistened cotton swab. Three pan feeders from front, middle and last areas of the house were grouped into 1 sample.
Water	
- Nipple water	Nipple water was collected from 3 areas of the house including front, middle and back of the house. Each sterile bottle contained around 500 ml of water.
-Water inlet	Water from the main water inlet to each house was collected (approximately 500 ml) in a sterile glass bottle.
Beetle	Approximately 10 g of beetles were collected from litter in the house and kept in a sterile plastic bag.
Flies	Approximately 10 g of flies were collected from each house and kept in a sterile plastic bag.
Footwear	Footwear used inside or outside the house were sampled
Hatchery samples	
Hatcher	The hatcher walls were swabbed by moistened gauze swab on 3 inner sides and the swab kept in a sterile plastic bag.
Egg shell	Approximately 25 g of egg shell were collected from each egg tray. Three egg trays were sampled per flock.
Early embryonic death	Five samples of early embryonic death were collected from each flock. Their ceca were cut using a sterile technique in the laboratory.
Water	Approximately 500 ml of water used for egg's incubator, hatcher, and general use in the hatchery was sampled and kept in a sterile glass bottle.

Chulalongkorn University

3.1.3 *Campylobacter* isolation and identification

Campylobacter isolation was performed as described in ISO 10272-1 (ISO10272-1, 2006). All samples were inoculated into Bolton broth (OXOID, Basingstoke, Hampshire, England) with a ratio 1:10 (v/v) of sample: enrichment medium. The inoculated broths were incubated at 37°C for 4-6 hr, and then at 41.5°C for 40-48 hr under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) using a gas pack jar system (Mitsubishi Chemicals, Tokyo, Japan). One loopful (1 µl) of enrichment broths was streaked onto modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubated in a microaerobic atmosphere at 41.5°C for 40-48 hr. Suspected *Campylobacter*-like colonies were subcultured onto Columbia blood agar (CBA) (OXOID, Basingstoke, Hampshire, England) and incubated in a microaerobic atmosphere 41.5°C for 24-48 hr. Genomic DNA was extracted from fresh cultures by using a Presto™ mini gDNA bacterial kit (Geneaid Biotech, Taiwan) following the manufacturer's instructions. Presumptive *Campylobacter* colonies were confirmed by multiplex PCR as previously described (Wang et al., 2002). Sets of primers for *Campylobacter* identification were shown in Table 5. The 25 µl of PCR mixture contained 2.5 µl of 10x PCR buffer; 1 µl of 10 mM deoxynucleoside triphosphate; 1 µl of 10 µM primers 23S, *C. jejuni,* and *C. coli*; 2.5 µl (100 ng) of DNA template; and 0.2 µl of a 5 U/ µl *Taq* DNA Polymerase (Roche Diagnostics, GmbH, Germany). Two reference strains, *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559, were used as positive controls. PCR mixtures were amplified in a thermal cycler (Life express, BIOER®). The PCR cycles included an initial

denaturation step at 95°C for 6 min followed by 30 cycles of denaturation at 95°C for 0.5 min, amplification at 59°C for 0.5 min and extension at 72°C for 0.5 min, ending with a final extension at 72°C for 7 min. PCR products were examined on 1% agarose gel in 1xTris-borate-EDTA (TBE) buffer at 80 V for 30 min. All gels were stained with ethidium bromide and visualized by ultraviolet transilluminator (E-BOX VX2, Vilber-Lourmat®). Confirmed isolates were stored in tryptone soya broth (TSB) (OXOID, Basingstoke, Hampshire, England) with 15% glycerol at -80°C for further study.

Table 5: Sets of primers for *Campylobacter* identification (Wang et al., 2002)

Primer	Sequence (5'-3')	size (bp)	Target gene
23SF	TAT ACC GGT AAG GAG TGC TGG AG	650	*Campylobacter*
23SR	ATC AAT TAA CCT TCG AGC ACC G		23S rRNA
CJF	ACT TCT TTA TTG CTT GCT GC	323	*C. jejuni hipO*
CJR	GCC ACA ACA AGT AAA GAA GC		
CCF	GTA AAA CCA AAG CTT ATC GTG	126	*C. coli glyA*
CCR	TCC AGC AAT GTG TGC AAT G		

3.1.4 Antimicrobial susceptibility test

Minimum inhibitory concentrations (MICs) tests were done using Muller Hinton agar (MHA) supplemented with 5% defibrinated sheep blood and a two-fold agar dilution technique (CLSI, 2013). Briefly, the *Campylobacter* isolates were grown on MHA supplemented with 5% defibrinated sheep blood (Difco®, MD, USA) at 42°C overnight under microaerobic condition. Single colonies were picked and transferred to 0.85% normal saline. The turbidity of bacterial suspension was adjusted to 0.5 McFarland (~ 10^8 CFU/ml). The suspension was then decimally diluted to 10^7 CFU/ml in normal saline and inoculated onto MHA supplemented with 5% defibrinated sheep blood containing appropriate concentrations of antimicrobials using multipoint inoculators (~ 10^4 CFU/ml). After incubation at 42°C for 24 hr under microaerobic condition, the MIC value, the lowest concentration of an antimicrobial agent that completely inhibits visible growth of bacteria, was recorded. The control organisms for antimicrobial susceptibility determination were *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559. The nine antimicrobials used and the breakpoints for determining resistance were as follows: amoxicillin (AMX, 32 µg/ml), bacitracin (BAC, 8 µg/ml), colistin (COL, 8 µg/ml), doxycycline (DOX, 8µg/ml), enrofloxacin (ENR, 4 µg/ml), erythromycin (ERY, 32 µg/ml), gentamicin (GEN, 16 µg/ml), tetracycline (TET, 16 µg/ml) and trimethoprim-sulfamethoxazole (SXT, 4/76 µg/ml). Susceptibility categorization for DOX, ERY, GEN, and TET were based on the Clinical and Laboratory Standards Institute (CLSI) guidelines for *C. jejuni* and *C. coli* (CLSI, 2013). For ENR, the breakpoint for

ciprofloxacin was used according to CLSI guidelines for *C. jejuni* and *C. coli* (CLSI, 2013). For AMX and SXT, the interpretation criteria were the CLSI recommendations for enteric bacteria in the family *Enterobacteriaceae* (CLSI, 2013) since there are no available specific breakpoints for *Campylobacter* spp. for these drugs. For BAC and COL breakpoints, the breakpoints recommended by CLSI for other non-*Enterobacteriaceae* isolates was used for the same reason (CLSI, 2011). All antimicrobials were obtained from Sigma-Aldrich (St Louis, MO).

3.1.5 Detection of mutation(s) in the quinolone resistance determining region (QRDR) of the *gyrA* and mismatch amplification mutation assay by PCR (MAMA-PCR) detecting the Thr-86-Ile mutations

A total of 24 *C. jejuni* and 24 *C. coli* isolates, which were resistant to enrofloxacin, were subjected to nucleotide sequence analysis of the QRDR of the *gyrA* gene using the forward and reverse primers as previously described (Zirnstein et al., 1999; Zirnstein et al., 2000). The QRDRs of the *gyrA* genes of the *Campylobacter* isolates were amplified by PCR. PCR primers used in this protocol were shown in Table 6. The 50 µl of PCR mixture contained 5 µl of 10x PCR buffer; 2 µl of 10 mM deoxynucleoside triphosphate; 2.5 µl of 10 µM of forward and reverse primers; 5 µl (100 ng) of DNA template; and 0.4 µl of a 5 U/ µl *Taq* DNA Polymerase (Roche Diagnostics, GmbH, Germany). PCR mixtures were amplified in a thermal cycler (Life

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

express, BIOER®). The PCR cycles included an initial denaturation step at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, amplification at 50°C for 1 min and extension at 72°C for 1 min, ending with a final extension at 72°C for 5 min. *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 served as control strains and were also sequenced. Five microliters of each PCR mixture were loaded onto agarose gels and stained with ethidium bromide for analysis after electrophoresis. The remaining *gyrA* PCR products were purified using the GenepHlow™ gel/pcr kit (Geneaid Biotech, Taiwan) and were submitted for nucleotide sequencing at First Base Laboratories (Seri Kembangan, Selangor, Malaysia). The DNA sequences obtained were compared and aligned with that of *C. jejuni* UA580 (Genbank accession number L04566). In addition, all 50 isolates were examined, as previously described (Zirnstein et al., 1999; Zirnstein et al., 2000), by a MAMA-PCR for the single point mutation (Thr-86-Ile) in the QRDR of the *gyrA* gene. This mutation is known to be a cause of high-level resistance to fluoroquinolones (Zirnstein et al., 1999). The MAMA-PCR protocols (25 µl each) were the same as above except the PCR cycling conditions were as follows: an initial denaturation step at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 0.5 min, amplification at 50°C for 0.5 min and extension at 72°C for 0.3 min. Primers used in this protocol were shown in Table 7. PCR products were examined on 1% agarose gel in 1xTris-borate-EDTA (TBE) buffer at 80 V for 30 min. All gels were stained with ethidium bromide and visualized by ultraviolet transilluminator (E-BOX VX2, Vilber-Lourmat®).

CHULALONGKORN UNIVERSITY

Table 6: Set of primers for QRDRs of the *gyrA* genes of *Campylobacter* spp. (Zirnstein et al., 1999; Zirnstein et al., 2000)

Primer	Sequence (5'-3')	Purpose	size (bp)
GZ*gyrA5*	ATT TTT AGC AAA GAT TCT GAT	*C. jejuni gyrA* forward primer	673
GZ*gyrA6*	CCA TAA ATT ATT CCA CCT GT	*C. jejuni gyrA* reverse primer	
GZ*gyrA*Ccoli3F	TAT GAG CGT TAT TAT CGG TC	*C. coli gyrA* forward primer	505
GZ*gyrA*Ccoli4R	GTC CAT CTA CAA GCT CGT TA	*C. coli gyrA* reverse primer	

Table 7: Set of primers detecting Thr-86-Ile mutation of the *gyrA* genes of *Campylobacter* spp. (Zirnstein et al., 1999; Zirnstein et al., 2000)

Primer	Sequence (5'-3')	Purpose	size (bp)
CampyMAMA*gryA1*	TTT TTA GCA AAG ATT CTG AT	*C. jejuni* Thr-86-Ile (ACA-ATA) mutation detection forward primer	265
CampyMAMA*gyrA5*	CAA AGC ATC ATA AAC TGC AA	*C. jejuni* Thr-86-Ile (ACA-ATA) mutation detection reverse primer	
GZ*gyrA*Ccoli3F	TAT GAG CGT TAT TAT CGG TC	*C. coli* Thr-86-Ile (ACT-ATT) mutation detection forward primer	192
CampyMAMA*gyrA8*	TAA GGC ATC GTA AAC AGC CA	*C. coli* Thr-86-Ile (ACT-ATT) mutation detection reverse primer	

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.1.6 *fla*A-RFLP Typing

flaA-RFLP typing was conducted by following the procedure of Nachamkin et al. (Nachamkin et al., 1993). For *fla*A amplification (Table 8), the 50 µl of PCR mixture contained 5 µl of 10x PCR buffer; 2 µl of 10 mM deoxynucleoside triphosphate; 2.5 µl of 10 µM of forward and reverse primers; 5 µl (100 ng) of DNA template; and 0.4 µl of a 5 U/ µl *Taq* DNA Polymerase (Roche Diagnostics, GmbH, Germany). Two reference strains, *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559, were used as positive controls. PCR mixtures were amplified in a thermal cycler (Life express, BIOER®). The PCR cycles included an initial denaturation step at 94°C for 1 min followed by 35 cycles of denaturation at 92°C for 0.5 min, amplification at 55°C for 1.5 min and extension at 72°C for 2.5 min, ending with a final extension at 72°C for 5 min. Ten microlitres of PCR product were brought to examine on 1% agarose gel in 1xTris-borate-EDTA (TBE) buffer at 80 V for 30 min. All gels were stained with ethidium bromide and visualized by ultraviolet transilluminator. For RFLP analysis, each PCR product was prepared a reaction mix containing 3.0 µl H_2O, 1.5 µl 10x H buffer (New England Biolabs, Massachusetts, USA), 0.5 µl *Dde*I (10 units/µl) (New England Biolabs, Massachusetts, USA) and added 10 µl PCR product. All reactions were incubated at 37°C for 3 hours. The whole reaction was run in 2% DNA grade agarose (Progen) gel in 1xTris-borate-EDTA (TBE) buffer at 70 V for 100 min. All gels were stained with ethidium bromide and visualized by ultraviolet transilluminator (E-BOX VX2, Vilber-Lourmat®). TIFF image

files were imported into Bionumerics 6.5 (Applied MathsSint-Martens-Latem, Belgium). The similarity matrix was computed using Dice similarity coefficient and clustering by the Unweighted Paired Group Method with Arithmetic Mean Values (UPGMA). Band position tolerance and the optimization coefficient were both set to 2%. Clusters were defined at the 72% similarity level using Bionumerics.

Table 8: A set of primers for *flaA* gene amplification (Nachamkin et al., 1993)

Primer	Sequence (5'-3')	size (bp)	Target gene
flaA-F	GGA TTT CGT ATT AAC ACA AAT GGT GC	1700	*flaA*
flaA-R	CTG TAG TAA TCT TAA AAC ATT TTG		

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.2 Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers

3.2.1 Sample collections from native chickens and layers

Four native chicken farms and 1 commercial layer farm in the Central area of Thailand which age of birds were in a range of 30-40 weeks for native chicken farms and 90 weeks for the commercial layer farm were selected based on their non-use antimicrobial history. The native chicken farms had no record of *Campylobacter* spp. prevalence while the commercial layer farm had 35% of *Campylobacter jejuni* prevalence. Feces were collected from 10, 10, 10, 10 and 20 birds at each farm, respectively. Fecal samples were kept at 4°C and transported to the laboratory where they were then processed for bacterial isolation within 24 hr.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.2.2 Isolation of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium*

Isolation of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* was performed following ISO 15214, ISO 7932 and European Community Project SMT4 CT98-2235 standards, respectively (ISO-7932, 1993; ISO-15214, 1998; European_commission, 2003). Briefly, a single 1 g from each fecal sample was dissolved in 9 ml of 0.85% normal saline. Using 1 loopful, the samples were streaked onto selective agar including de Mans Rogosa and Sharpe (MRS) agar, Manitol Egg Yolk

Polymyxin-B agar and SF-streptococcus agar for *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus* spp., respectively. The inoculated plates were incubated at 37°C for 24-48 hr. Suspected colonies were primarily identified by Gram staining and biochemical tests. All bacterial isolates were kept as 15% glycerol stock at -80°C

3.2.3 Identification of genus and species

Oligonucleotide primers used in this study are listed in Table 9. DNA template was extracted by heating method (Kwon et al., 2004). In brief, single colonies of each strain on an agar plate were suspended in distilled water and heated at 100°C for 10 min. They were then centrifuged at 12,000 rpm for 5 min. The supernatants were collected for use as the DNA template of polymerase chain reactions (PCRs). Multiplex PCR assay was done to verify genus and species of *Lactobacillus* and *Enterococcus faecium* (Ke et al., 1999; Dubernet et al., 2002; Jackson et al., 2004; Kwon et al., 2004). Amplified ribosomal DNA restriction analysis (ARDRA) was demonstrated for *Bacillus* identification (Wu et al., 2006). All PCRs were performed using KAPA® master mix (KapaBiosystems, Wilmington, USA) as described in the manufacturer’s instructions. Reference strains obtained from the Thailand Institute of Scientific and Technological Research (TISTR) were as follows: L. *acidophilus* TISTR 1034, *L. casei* subsp. *rhamnosus* TISTR 108, *L. plantarum* TISTR 1265, *L. delbrueckii* subsp. *bulgaricus* TISTR 892, *B.*

subtilis TISTR 1460, *B. licheniformis* TISTR 1109, *E. faecium* TISTR 2058 and *E. faecalis* TISTR 379.

Table 9: Oligonucleotide primers used in this study

Primers	Sequence (5'-3')	PCR type	PCR products (bp)	References
Lactobacillus				
R16-1	CTT GTA CAC ACC GCC CGT CA	Genus-specificity	250	(Dubernet et al., 2002)
LbLMA1-rev	CTC AAA ACT AAA CAA AGT TTC			
IDL03R	CCA CCT TCC TCC GGT TTG TCA	All *lactobacillus*	-	(Kwon et al., 2004)
IDL04F	AGG GTG AAG TCG TAA CAA GTA GCC	All *lactobacillus*	-	(Kwon et al., 2004)
IDL11F	TGG TCG GCA GAG TAA CTG TTG TCG	*L. casei* group	727	(Kwon et al., 2004)
IDL22R	AAC TAT CGC TTA CGC TAC CAC TTT GC	*L. acidophilius*	606	(Kwon et al., 2004)
IDL31F	CTG TGC TAC ACC TAG AGA TAG GTG G	*L. delbrueckii*	184	(Kwon et al., 2004)
IDL42R	ATT TCA AGT TGA GTC TCT CTC TC	*L. gasseri*	272	(Kwon et al., 2004)
IDL52F	ACC TGA TTG ACG ATG GAT CAC CAG T	*L. reuteri*	1105	(Kwon et al., 2004)
IDL62R	CTA GTG GTA ACA GTT GAT TAA AAC TGC	*L. plantarum*	428	(Kwon et al., 2004)
IDL73R	GCC AAC AAG CTA TGT GTT CGC TTG C	*L. rhamnosus*	448	(Kwon et al., 2004)
Bacillus				
B-K1F	TCA CCA AGG CRA CGA TGC G	All *Bacillus*	1,114	(Wu et al., 2006)
B-K1R	CGT ATT CAC CGC GGC ATG			
Enterococcus				
Ent1	TAC TGA CAA ACC ATT CAT GAT G	Genus-specificty	112	(Ke et al., 1999)
Ent2	AAC TTC GTC ACC AAC GCG AAC			
FM1	GAA AAA ACA ATA GAA GAA TTA T	*E. faecium*	215	(Jackson et al., 2004)
FM2	TGC TTT TTT GAA TTC TTC TTT A			

3.2.4 Antimicrobial susceptibility test

Antimicrobial susceptibilities to ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, chloramphenicol and tylosin were evaluated by determining the minimum inhibitory concentrations (MICs). According to Clinical and Laboratory Standards Institute guidelines (CLSI) (CLSI, 2013), MICs were done in Muller Hinton agar (MHA) using a two-fold agar dilution technique which is almost the same as mentioned in 3.1.4 except that the bacterial isolates were grown in MHA at 37°C overnight and the MIC value was recorded after incubation at 37°C for 18-24 hr. The choice of antimicrobials and breakpoints for clarifying *Lactobacilllus, Bacillus* and *Enterococcus faecium* as resistant were suggested by the European Food Safety Authority (EFSA) (EFSA, 2012). *Escherichia coli* ATCC 25922 was used as a control organism. All antimicrobials were bought from Sigma-Aldrich (St Louis, MO).

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

3.2.5 Acid and Bile tolerance tests

Acid and bile tolerance tests were performed to strains that qualified the acceptable range of MIC values according to the protocols of Hyronimus et al. (2000) with some modifications. For the acid tolerance test, the stock bacteria kept at -80°C were grown in MRS broth at 37°C for 24 hr; they were then pipetted into another MRS broth with pH value adjusted to 2.5 using 5M HCl (Merck) and sampled for counting

colony numbers at 0 and 3 h of incubation time onto MRS agar by a pour plate technique. Survival rates were calculated by using the below formula. Like the bile tolerance test, it has a similar protocol with the acid test, but it was changed from MRS broth (pH 2.5) to oxgall bile 0.3% (Difco) (Gilliland et al., 1984). Counting colony numbers for bile tolerance test were conducted at 0 and 24 hours of incubation time.

$$\text{Survival rates (\%)} = \frac{\log N}{\log No} \times 100$$

where log N equals the log number of the presenting colony at the end of the test

and log N_0 equals the log number of presenting colony at the start of the test.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.2.6 *Campylobacter jejuni* challenges

Two hundred and ten 1-day-old non-vaccinated female Cobb broilers from a commercial hatchery were divided into ten groups. As shown in Table 10, groups 1-7 were orally gavaged with 0.5 ml of the top-three qualified CE bacteria that passed MICs and acid and bile tolerance criteria as a single, double or triple strain at 1-3 days of age. Group 8 was gavaged by a commercial product (AVIPROB™, Diasham Resources, Singapore) at 1-3 days of age. Group 9 and 10 served as positive control and negative control groups, respectively. At 11 days of age, the feces of all broilers were collected

to produce cultures to confirm *Campylobacter* spp. free status before challenges. All *Campylobacter*-negative broilers except the negative control group were orally inoculated with Thai field strain number CU11 of *Campylobacter jejuni* with an approximate concentration at 10^6 CFU/ml, 1 ml/each at 14 days. Fifteen fecal samples of each group were collected to count *Campylobacter* colonies at 17, 21, 28 and 35 days of age, respectively. At 41 days of age, all broilers were euthanized and ceca were collected to count *Campylobacter* colonies. All broilers were weighed at 1, 14 and 41 days to calculate their feed conversion ratio (FCR) and body weight. Birds were provided feed and water *ad lib* and raised under an ethical approval for animal experimentation approved by Chulalongkorn University Animal Care and Use Committee no. 13310021.

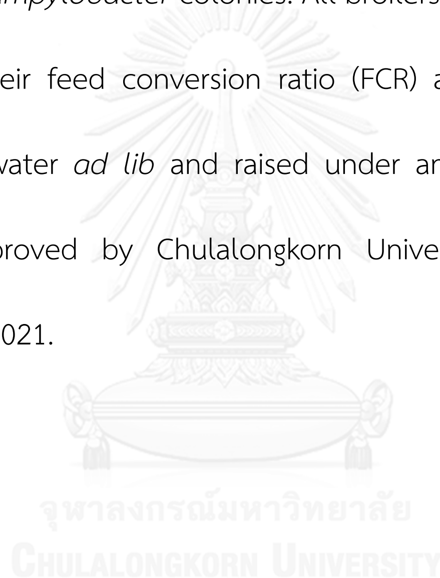
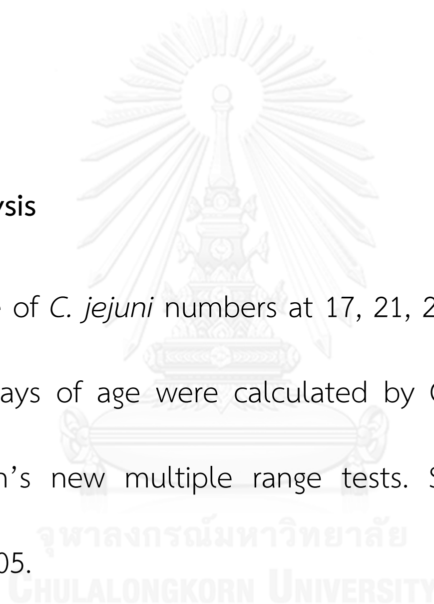

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Table 10: CE application programs in broilers during 1-3 days of age

Group	Selected CE or products	Total Conc. (CFU/ml)	Challenge *C.jejuni* at 14 days
1	*Lactobacillus acidophilus* 1/4	2×10^8	+
2	*Bacillus subtilis* 206/1	2×10^8	+
3	*Enterococcus faecium* 122	2×10^8	+
4	*Lactobacillus acidophilus* 1/4 + *Bacillus subtilis* 206/1	4×10^8	+
5	*Lactobacillus acidophilus* 1/4 + *Enterococcus faecium* 122	4×10^8	+
6	*Bacillus subtilis* 206/1 + *Enterococcus faecium*122	4×10^8	+
7	*Lactobacillus acidophilus* 1/4 + *Bacillus subtilis* 206/1+ *Enterococcus faecium* 122	6×10^8	+
8	Commercial product (AVIPROB™)	2×10^8	+
9	positive control	0.85% NSS	+
10	negative control	0.85% NSS	-

3.2.7 *Campylobacter* isolation, identification and enumeration

One gram fecal samples were added to 9 ml of 0.85% normal saline. The suspension was decimally diluted, and 0.1 ml of each diluted suspension was spread onto mCCDA in duplicate for *Campylobacter* enumeration. The inoculated plates were incubated at 42°C for 48-72 hr under microaerobic conditions using a gas pack jar system. Presumptive *Campylobacter* colonies were confirmed by multiplex PCR as mentioned in 3.1.3.


จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.2.8 Statistical analysis

The difference of *C. jejuni* numbers at 17, 21, 28, 35 and 41 days of age and body weight at 41 days of age were calculated by One-way Analysis of Variance (ANOVA) and Duncan's new multiple range tests. Significance was tested at a probability level of 0.05.

CHAPTER IV

RESULTS

4.1 Antimicrobial resistance, the characterization of *gyrA* mutation, and genetic diversity by *fla*A-RFLP of *Campylobacter jejuni* and *Campylobacter coli* collected through commercial broiler production chains in Thailand

4.1.1 Prevalence of *Campylobacter* from 5 chicken production flocks

Overall of 1,235 samples, 130 samples were positive for *Campylobacter* spp. (10.5%) which was composed of 36 (2.9%) *C. jejuni* and 94 (7.6%) *C. coli* isolates. In this study, *Campylobacter* isolates were categorized into 2 groups -*Campylobacter* isolated from chicken-related samples and those isolated from environmental samples. In breeder flocks, the prevalence of *Campylobacter* spp. was between 53.3% and 86.7% (Figure 4). Birds from breeder flock 2 showed the highest prevalence of *Campylobacter* spp. at 86.7%. *C. coli* were isolated from environmental samples (e.g. boot swabs inside and outside the houses, darkling beetles and pan feeder) of all breeder flocks at a range between 4.3% and 13% whereas *C. jejuni* was only isolated from boot swabs of breeder flock 1 at 8.7% prevalence. No *Campylobacter* was detected in either hatchery, with the samples being from early embryonic deaths and

the environment. In broiler flocks, *Campylobacter* spp. was not detected until 35 days of age. Only *C. coli* was recovered from broiler flock 1 and 2 at 12.5% and 2.5% prevalence, respectively. Broiler flocks 3, 4, and 5 were *Campylobacter* negative throughout this study. *Campylobacter* spp. was not isolated from environmental samples taken in any broiler flock.

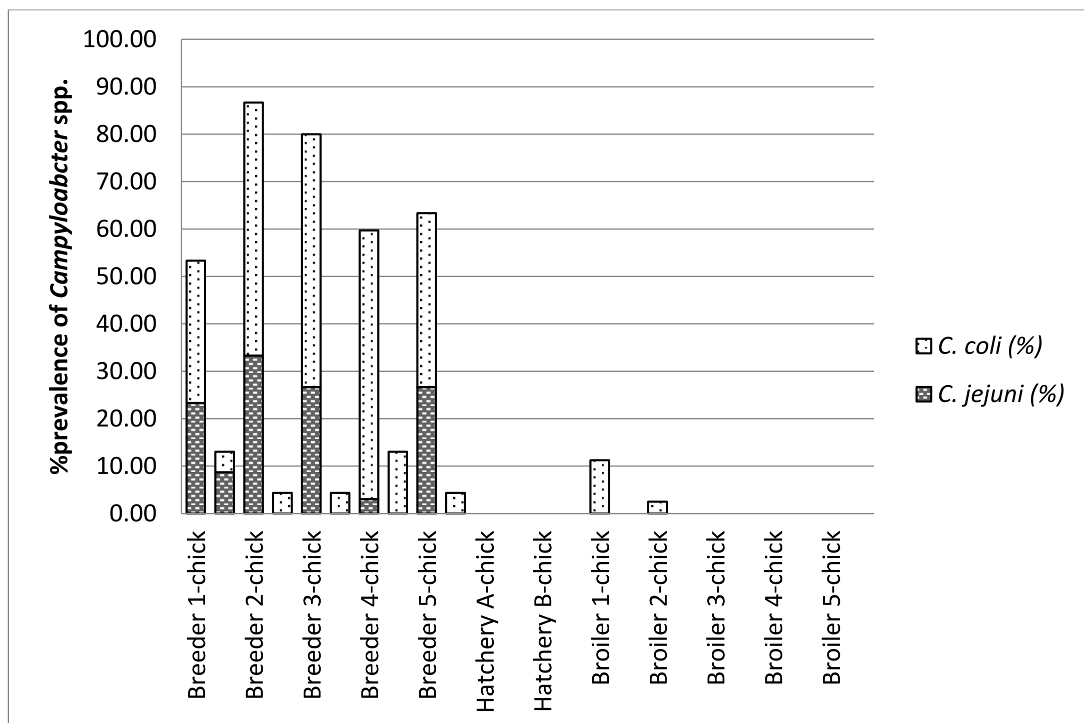


Figure 4: Prevalence percentage of *C. jejuni* and *C. coli* isolated from chicken-related samples and environmental samples.

4.1.2 Phenotypic antimicrobial resistance

According to CLSI protocols (CLSI, 2013), *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 was used as control organisms and all MIC values were within the MIC quality control ranges in all batches. Overall, Thai *Campylobacter* isolates were commonly resistant to various classes of antimicrobial agents except erythromycin and gentamicin**.** Sixty-six percent of *C. jejuni* isolates and 97.9% of *C. coli* isolates were multidrug resistance (MDR), defined as being resistant to three or more antimicrobial classes. As well, all *C. jejuni* and *C. coli* isolates were resistant to amoxicillin and bacitracin (Figure 5). Moreover, a high frequency resistance was shown against enrofloxacin (100%), colistin (66.7%), tetracycline (55.6%), doxycycline (50%), and trimethoprim-sulfamethoxazole (36.1%) for *C. jejuni.* For *C. coli,* there was a high level of resistance to enrofloxacin (98.9%), tetracycline (97.9%), trimethoprim-sulfamethoxazole (81.9%), and doxycycline (79.8%). However, only 16% of *C. coli* isolates were resistant to colistin. All Thai *Campylobacter* isolates were susceptible to erythromycin and gentamicin. The most resistance patterns found in this study were AMX-BAC-COL-ENR for *C. jejuni* and AMX-BAC-DOX-ENR-TET-SXT for *C. coli* as shown in Table 11.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

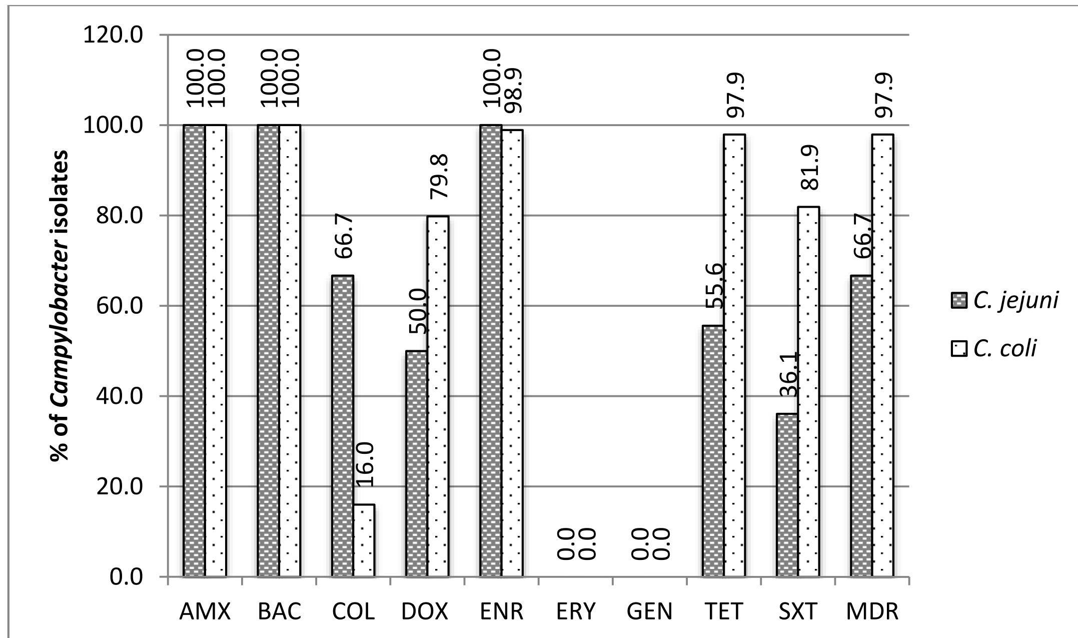


Figure 5: Frequency of resistance to 9 antimicrobial agents in *C. jejuni* (n = 36) and *C. coli* (n = 94). AMX, amoxicillin; BAC, bacitracin; COL, colistin; DOX, doxycycline; ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; MDR, multidrug resistance.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Table 11: Antimicrobial resistance pattern of *C. jejuni* (n = 36) and *C. coli* (n = 94).

species	Antimicrobial resistance pattern[a]	No. of isolates (%)
C.jejuni	AMX-BAC-COL-ENR	9 (25.0)
	AMX-BAC-COL-DOX-ENR-TET	5 (13.9)
	AMX-BAC-DOX-ENR-TET-SXT	5 (13.9)
	AMX-BAC-COL-DOX-ENR-TET-SXT	5 (13.9)
C. coli	AMX-BAC-ENR-TET	5 (5.3)
	AMX-BAC-DOX-ENR-TET	9 (9.6)
	AMX-BAC-ENR-TET-SXT	10 (10.6)
	AMX-BAC-DOX-ENR-TET-SXT	55 (58.5)
	AMX-BAC-COL-DOX-ENR-TET-SXT	11 (11.7)

[a]Only the antimicrobial resistance patterns represented by at least five isolates are shown. AMX, amoxicillin; BAC, bacitracin; COL, colistin; DOX, doxycycline; ENR, enrofloxacin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole;

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

4.1.3 Genetic characterization of *gyrA* mutation and MAMA-PCR

The DNA sequences of the *gyrA* gene of *C. jejuni* ATCC 33560, the 24 Thai *C. jejuni* isolates, *C. coli* ATCC 33559 and the 24 Thai *C. coli* isolates were submitted to Genbank and run in numerical order from KX982317 to KX982366. The MIC values for enrofloxacin of the selected 48 Thai *Campylobacter* isolates ranged between 4 and 16 μg/ml. All enrofloxacin resistant *C. jejuni* isolates had Thr-86-Ile non-synonymous mutation (ACA-ATA). In addition, some isolates had additional non-synonymous mutations such as Arg-6-Ser (AGC-AGG), Gln-7-Lys (AAA-CAA), Ser-22-Gly (AGT-GGT), Asn-203-Ser (AAT-AGT) and Ala-206-Val (GCA-GTA). In contrast, only the Thr-86-Ile non-synonymous mutation (ACT-ATT) was found in the 24 selected *C. coli* isolates (Table 12). In addition, all selected Thai *Campylobacter* isolates were positive to MAMA-PCR detecting *gyrA* mutation at a position of Thr-86-Ile.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Table 12: The *gyrA* mutations and the MIC for enrofloxacin of the selected *Campylobacter* isolates and the two control

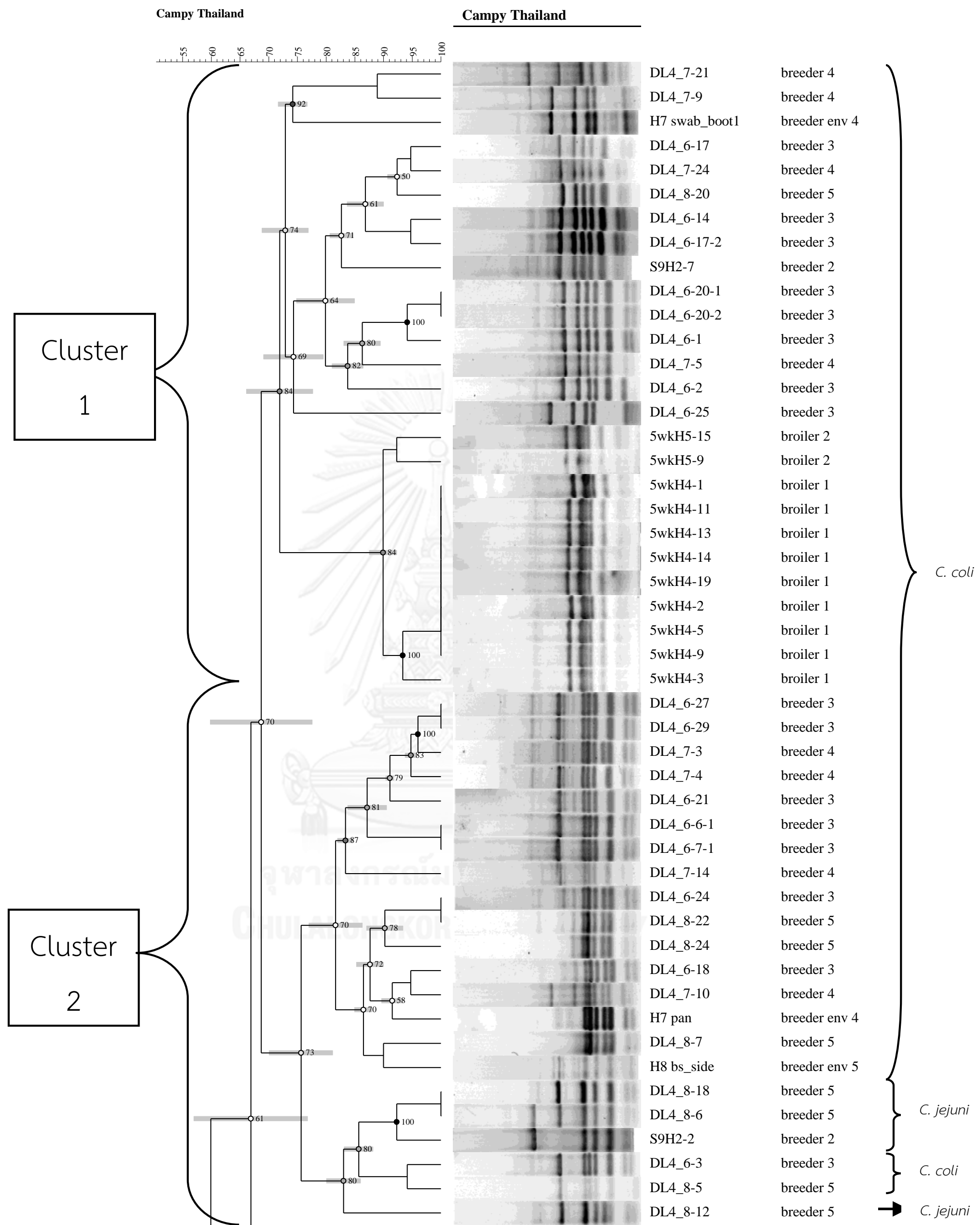
		Enrofloxacin	Nucleic acid codons and corresponding amino acid of *Campylobacter* QRDR of *gyrA*[b]											
		MIC range		Amino		Amino		Amino		Amino		Amino		Amino
Pattern[a]	n	(µg/ml)	Codon	acid	Codon	acid	Codon	acid	Codon	acid	Codon	acid	Codon	acid
C. jejuni														
ATCC 33560-Enr^S	1	0.125	AGG	Arg-6	CAA	Gln-7	AGT	Ser-22	ACA	Thr-86	AAT	Asn-203	GCA	Ala-206
UA 580-$Cip^{S,d}$	1	<0.5	--C	Ser-6	A--	Lys-7	---	-	---	-	---	-	---	-
Enr^R-1	3	4-16	--C	Ser-6	A--	Lys-7	G--	Gly-22	-T-	Ile-86	-G-	Ser-203	---	-
Enr^R-2	3	8-16	---	-	---	-	G--	Gly-22	-T-	Ile-86	-G-	Ser-203	-T-	Val-206
Enr^R-3	17	4-16	---	-	---	-	G--	Gly-22	-T-	Ile-86	-G-	Ser-203	---	-
Enr^R-4	1	8	---	-	---	-	---	-	-T-	Ile-86	---	-	---	-
C. coli														
ATCC 33559-Enr^S	1	0.25	n/a[c]	n/a	n/a	n/a	n/a	n/a	ACT	Thr-86	n/a	n/a	n/a	n/a
Enr^R-1	24	2-16	n/a	n/a	n/a	n/a	n/a	n/a	-T-	Ile-86	n/a	n/a	n/a	n/a

[a]Enr^S, enrofloxacin susceptible (MIC ≤ 0.25 µg/ml); Enr^R, enrofloxacin resistant (MIC ≥ 2 µg/ml); Cip^S, ciprofloxacin susceptible (MIC ≤ 1 µg/ml)

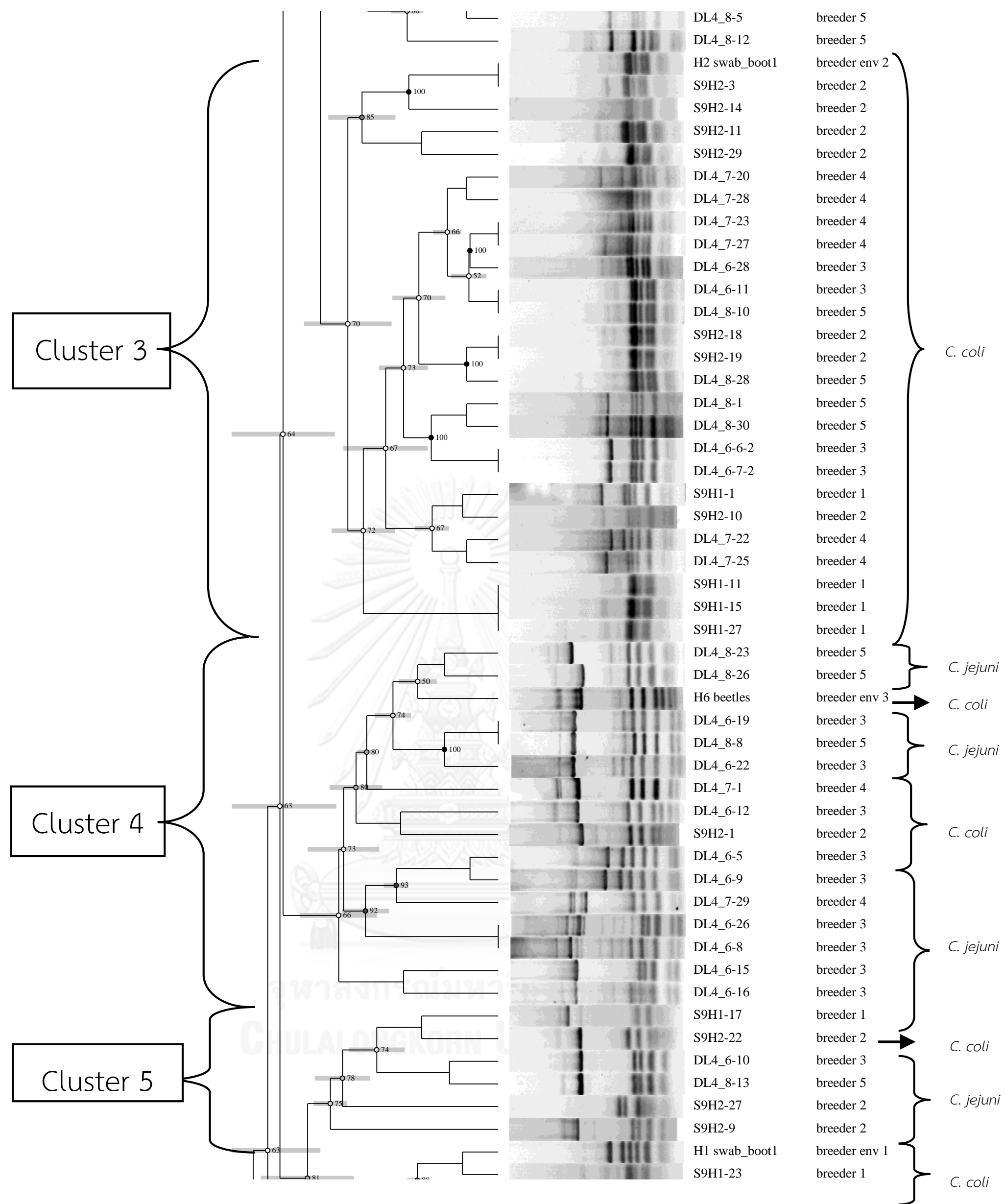
[b]-, no change compared to enrofloxacin-susceptible isolates, [c]n/a, not available due to no amplified based at that position, [d]*C. jejuni* UA 580

4.1.4 Genotypic diversity by *fla*A-RFLP

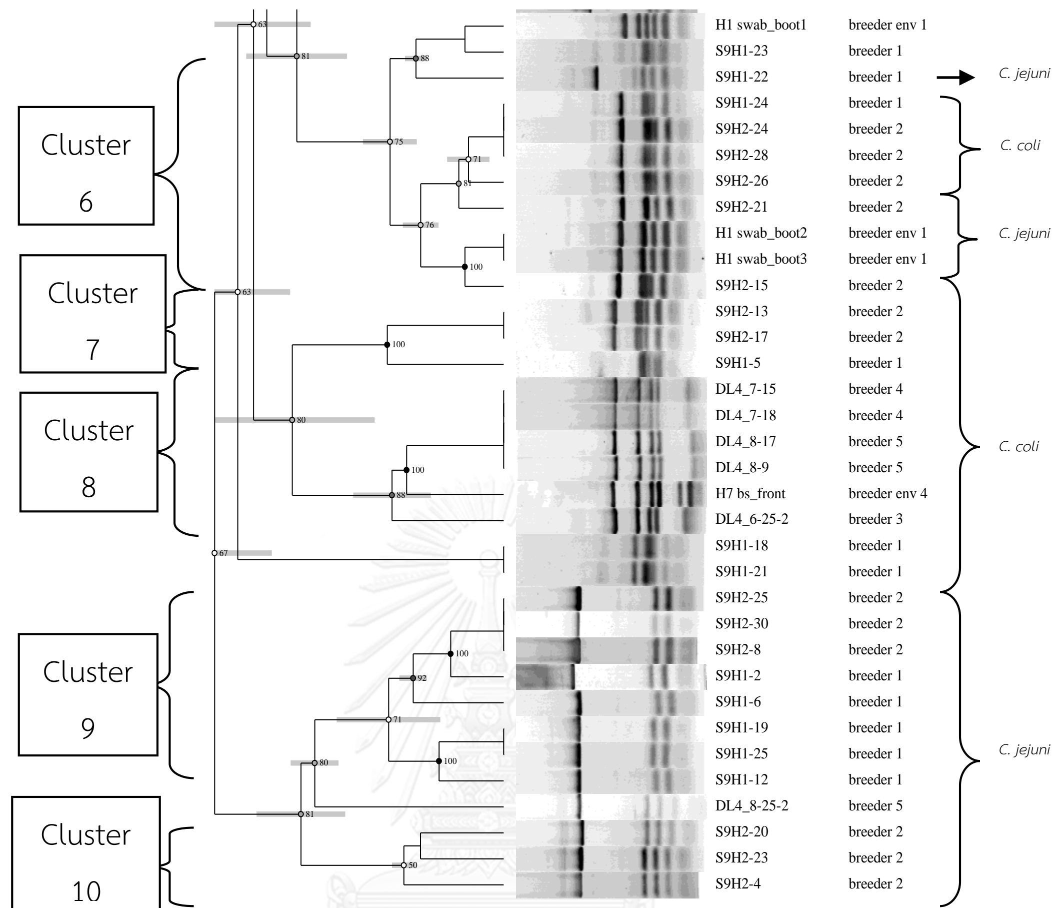
All of the 130 *Campylobacter* isolates (36 *C. jejuni* and 94 *C. coli*) were genotyped by *fla*A-RFLP. Ten distinct genotypic clusters were obtained at 72% similarity (Figure 6). Breeder flocks 1 and 2 were co-located on the same farm while breeder flocks 3 to 5 were co-located on another farm. Broiler flocks 1 and 2 were co-located on another farm (Fig. 2). Cluster 1-5 contained *Campylobacter* isolated from a variety of breeder flocks, whereas cluster 6, 7, and 9 contained isolates from breeder flocks 1 and 2 and cluster 8 contained isolates from breeder flocks 3, 4, and 5. In addition, cluster 10 was composed of a unique of isolates from breeder flock 2. For environmental samples from breeder flocks, cluster 1-4 contained isolates from environment of those breeder flocks. For broiler samples, these isolates were genotyped in cluster 1 only which also contained isolates from breeder flock 2, 3, 4, and 5. The largest clusters were clusters 1 and 3 which contained 26 strains of *C. coli* each. Thai *C. jejuni* and *C. coli* isolates cannot be separately genotyped as a different cluster by *fla*A-RFLP as seen in cluster 2, 4, 5, and 6.



Campy Thailand
Campy Thailand
55
60
65
70
75
80
85
90
95
100
Cluster 1
Cluster 2
DL4_7-21 breeder 4
DL4_7-9 breeder 4
H7 swab_boot1 breeder env 4
DL4_6-17 breeder 3
DL4_7-24 breeder 4
DL4_8-20 breeder 5
DL4_6-14 breeder 3
DL4_6-17-2 breeder 3
S9H2-7 breeder 2
DL4_6-20-1 breeder 3
DL4_6-20-2 breeder 3
DL4_6-1 breeder 3
DL4_7-5 breeder 4
DL4_6-2 breeder 3
DL4_6-25 breeder 3
5wkH5-15 broiler 2
5wkH5-9 broiler 2
5wkH4-1 broiler 1
5wkH4-11 broiler 1
5wkH4-13 broiler 1
5wkH4-14 broiler 1
5wkH4-19 broiler 1
5wkH4-2 broiler 1
5wkH4-5 broiler 1
5wkH4-9 broiler 1
5wkH4-3 broiler 1
DL4_6-27 breeder 3
DL4_6-29 breeder 3
DL4_7-3 breeder 4
DL4_7-4 breeder 4
DL4_6-21 breeder 3
DL4_6-6-1 breeder 3
DL4_6-7-1 breeder 3
DL4_7-14 breeder 4
DL4_6-24 breeder 3
DL4_8-22 breeder 5
DL4_8-24 breeder 5
DL4_6-18 breeder 3
DL4_7-10 breeder 4
H7 pan breeder env 4
DL4_8-7 breeder 5
H8 bs_side breeder env 5
DL4_8-18 breeder 5
DL4_8-6 breeder 5
S9H2-2 breeder 2
DL4_6-3 breeder 3
DL4_8-5 breeder 5
DL4_8-12 breeder 5
C. coli
C. jejuni
C. coli
C. jejuni



Cluster 3
Cluster 4
Cluster 5
DL4_8-5 breeder 5
DL4_8-12 breeder 5
H2 swab_boot1 breeder env 2
S9H2-3 breeder 2
S9H2-14 breeder 2
S9H2-11 breeder 2
S9H2-29 breeder 2
DL4_7-20 breeder 4
DL4_7-28 breeder 4
DL4_7-23 breeder 4
DL4_7-27 breeder 4
DL4_6-28 breeder 3
DL4_6-11 breeder 3
DL4_8-10 breeder 5
S9H2-18 breeder 2
S9H2-19 breeder 2
DL4_8-28 breeder 5
DL4_8-1 breeder 5
DL4_8-30 breeder 5
DL4_6-6-2 breeder 3
DL4_6-7-2 breeder 3
S9H1-1 breeder 1
S9H2-10 breeder 2
DL4_7-22 breeder 4
DL4_7-25 breeder 4
S9H1-11 breeder 1
S9H1-15 breeder 1
S9H1-27 breeder 1
DL4_8-23 breeder 5
DL4_8-26 breeder 5
H6 beetles breeder env 3
DL4_6-19 breeder 3
DL4_8-8 breeder 5
DL4_6-22 breeder 3
DL4_7-1 breeder 4
DL4_6-12 breeder 3
S9H2-1 breeder 2
DL4_6-5 breeder 3
DL4_6-9 breeder 3
DL4_7-29 breeder 4
DL4_6-26 breeder 3
DL4_6-8 breeder 3
DL4_6-15 breeder 3
DL4_6-16 breeder 3
S9H1-17 breeder 1
S9H2-22 breeder 2
DL4_6-10 breeder 3
DL4_8-13 breeder 5
S9H2-27 breeder 2
S9H2-9 breeder 2
H1 swab_boot1 breeder env 1
S9H1-23 breeder 1
C. coli
C. jejuni
C. coli
C. jejuni
C. coli
C. jejuni
C. coli
C. jejuni
C. coli



จุฬาลงกรณ์มหาวิทยาลัย

Figure 6: Phylogenetic analysis representing *flaA*-RFLP of *C. jejuni* and *C. coli* isolated from breeders and broilers in Thailand at the 72% cut-off genetic similarity. The similarity matrix was computed using Dice similarity coefficient and clustering by the Unweighted Paired Group Method with Arithmetic Mean Values (UPGMA). Band position tolerance and the optimization coefficient were both set to 2% using Bionumerics.

4.2 Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers

4.2.1 Antimicrobial resistance phenotypes

The numbers of all isolates, totally 346 strains, identified as *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* equaled 195, 93 and 58 strains, respectively. All strains were tested for antimicrobial susceptibility. The frequencies of antimicrobial resistance and their MIC ranges are shown in Table 13. Those strains which had lowered or equaled cut-off MIC values proposed by EFSA totaled 51 strains, which included 27, 15 and 9 strains of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium*, respectively. Most qualified strains had quite low MIC data compared to the breakpoints in each recommended antimicrobial agent.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table 13: MIC data of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium*

Strain (n)	MIC range (µg/ml)									
	AMP ()[a]	CHP	CLI	ERY	GEN	KAN	STR	TET	TYL	VAN
Lactobacillus spp. (27)	0.125-1 (4)	0.5-2 (8)	<0.125-0.5 (2)	<0.125-0.5 (1)	0.5-4 (32)	1-8 (64)	1-8 (64)	0.5-2 (32)	n.r.[b]	0.25-1 (2)
Bacillus spp. (15)	n.r.	<1-4 (8)	0.5-2 (4)	<0.5-2 (4)	0.25-1 (4)	<0.5-4 (8)	<2-4 (8)	0.5-2 (8)	n.r.	0.5-2 (4)
E. faecium (9)	0.5-2 (2)	<1-4 (16)	0.5-2 (4)	<0.5-2 (4)	4-16 (32)	64-256 (1024)	32-64 (128)	0.5-2 (4)	0.5-1 (4)	0.5-4 (4)

n= number of isolates in each row, [a]microbiological cut-off values (µg/ml) is indicated in brackets, AMP, ampicillin;

CHP, chloramphenicol; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; STR, streptommycin;

TET, tetracycline; TYL, tylosin; VAN, vancomycin., [b]n.r. = not required

4.2.2 Survival rate of acid and bile tolerance tests

A total of 27 *Lactobacillus*, 15 *Bacillus* and 9 *Enterococcus faecium* were tested for acid and bile tolerance. Survival rate of those strains are summarized in Table 14. For acid tolerance, 3 *Bacillus* strains had the highest survival rates, with a range 100-110% more than *Lactobacillus* spp. and *Enterococcus faecium.* All strains had a wide range in survival rates for the bile tolerance test. *Enterococcus faecium* showed a quite low ability to tolerate bile acid compared to *Lactobacillus* spp. and *Bacillus* spp. According to the results of MIC values as well as acid and bile tolerance, the best performance of *Lactobacillus* spp., *Bacillus* spp. and *Enterococcus faecium* were selected, and their species level was identified using the PCR method. The top performance of selected CE was *Lactobacillus acidophilus* 1/4, *Bacillus subtilis* 206/1 and *Enterococcus faecium* 122 which had 96.85, 101.47 and 99.39% survival rates of acid tolerance and 113.93, 130.97 and 90.10% survival rates of bile tolerance, respectively. These CE were preferred to use for the challenge experiment in broilers.

CHULALONGKORN UNIVERSITY

Table 14: Number of bacteria in each level of percent of survival rate of acid and bile tolerance tests

Genus	Strains	Survival rates (%)		Strains	Survival rates (%)	
		acid tolerance	bile tolerance		acid tolerance	bile tolerance
Lactobacillus spp.	L 22/2	98.74	95.95	L 58/3	93.19	90.28
	L 31/3	97.13	86.03	L 44/4	93.04	95.25
	L 1/4	96.85	113.93	L 44/1	91.41	101.61
	L 31/4	96.78	95.87	L 28/1	91.4	96.28
	L 48/1	95.96	89.87	L 27/2	91.16	98.74
	L 23/1	95.41	88.48	L 27/1	90.87	92.78
	L 1/3	94.6	94.45	L 49/4	90.46	86.22
	L 40/1	94.27	95.31	L 55/4	90.38	99.36
	L 10/2	93.98	102.08	L 38/1	90.02	92.82
	L 8/1	93.82	87.12	L 19/2	89.69	105.69
	L 5/3	93.58	93.91	L 8/2	88.11	88.54
	L 35/2	93.58	101.56	L 17/1	84.37	89.36
	L 50/1	93.39	88.25	L 19/1	82.27	78.55
	L 14/4	93.36	98.15			
Bacillus spp.	B 201/1	101.57	94.72	B 205/2	92.19	89.34
	B 206/1	101.47	130.97	B 227/2	92.15	102.57
	B 214/2	100.77	93.9	B 220/1	91.43	123.13
	B 230/1	97.3	118.16	B 206/2	90.04	91.3
	B 235/1	95.77	99.18	B 224/1	89.23	103.32
	B 239/1	95.67	102.75	B 230/2	86.16	96.47
	B 204/1	94.36	103.21	B 210/1	84.99	99.27
	B 217/2	93.54	100.87			
Enterococcus faecium	E 122	99.39	90.1	E 172	88.61	75.07
	E 135	98.45	75.06	E 144	88.34	61.09
	E 110	95.36	71.66	E 107	86.74	82.63
	E 130	92.25	82.58	E 118	77.03	78.07
	E 114	91.69	76.72			

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

4.2.3 *C. jejuni* challenged against CE application

At 11 days of age, all birds tested negative for *Campylobacter* spp. At 14 days of age, the birds in all groups except for the negative control group were challenged with Thai field strain number CU11 of *C. jejuni*. At 17, 21, 28 and 35 days of age, fecal samples of 15 birds were collected for *C. jejuni* counting; at 41 days of age, the cecal content of all broilers was counted for *C. jejuni* colonies. No statistically significant difference in the *C. jejuni* numbers from both fecal and cecal samples was observed between the positive control and treatment groups (Table 15). FCR was recorded at 14 and 41 days and body weight was calculated at 41 days. At 41 days of age, the body weight of broilers in group 8 given a commercial CE was significantly higher than that of broilers in group 1, 2 and 10 given *Lactobacillus acidophilus* 1/4, *Bacillus subtilis* 206/1 and negative control, respectively.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table 15: Average number of *C. jejuni*, FCR and body weight

Group	Average number of *C. jejuni* (log CFU/g) (mean±SD)					FCR at 14 days	FCR at 41 days	Body weight at 41 days
	17 days	21 days	28 days	35 days	41 days			
1	5.80 ± 0.86	6.17 ± 0.64	6.18 ± 0.88	6.24 ± 0.55	6.48 ± 0.99	1.14	1.82	1843.50±348.96^{a}
2	5.94 ± 0.89	6.23 ± 0.66	6.38 ± 0.51	6.53 ± 0.71	6.75 ± 1.00	1.19	1.84	1856.32±323.86^{a}
3	6.29 ± 0.81	6.39 ± 0.73	6.39 ± 0.59	6.09 ± 0.64	6.97 ± 1.03	1.16	1.77	1937.25±240.43^{ab}
4	6.43 ± 0.63	6.53 ± 0.41	6.31 ± 0.67	5.59 ± 0.45	6.01 ± 0.89	1.13	1.82	1894.75±204.07^{ab}
5	6.53 ± 0.59	6.50 ± 0.57	6.56 ± 0.53	6.10 ± 0.80	6.15 ± 1.19	1.17	1.74	1935.00±217.64^{ab}
6	6.22 ± 0.61	6.46 ± 0.94	6.26 ± 0.71	5.54 ± 0.37	6.02 ± 0.89	1.15	1.78	1896.90±137.67^{ab}
7	5.92 ± 0.47	6.55 ± 0.62	5.81 ± 0.47	5.82 ± 0.64	5.81 ± 0.65	1.14	1.68	1992.62±106.79^{ab}
8	6.78 ± 0.45	6.93 ± 0.76	6.74 ± 0.40	6.20 ± 1.02	6.52 ± 0.90	1.15	1.72	2061.94±149.02^{b}
9	6.46 ± 0.52	6.86 ± 0.40	6.74 ± 0.82	6.79 ± 0.49	6.74 ± 1.03	1.14	1.72	1991.05±178.60^{ab}
10	n.d.	n.d.	n.d.	n.d.	n.d.	1.18	1.79	1820.52±307.63^{a}

n.d: not detected (detection limit = 2 log CFU/g), ^{a,b}The different superscript in each column means statistically significant difference (p<0.05), Broilers in all groups received different CE application program during 1-3 days of age. Gr. 1: *Lactobacillus acidophilus* 1/4 2 x 10^{8} CFU/ml, Gr. 2: *Bacillus subtilis* 206/1 2 x 10^{8} CFU/ml, Gr. 3: *Enterococcus faecium* 122 2 x 10^{8} CFU/ml, Gr. 4: *Lactobacillus acidophilus*1/4 + *Bacillus subtilis*206/1 4 x 10^{8} CFU/ml, Gr. 5: *Lactobacillus acidophilus* 1/4 + *Enterococcus faecium* 122 4 x 10^{8} CFU/ml, Gr. 6: *Bacillus subtilis*206/1 + *Enterococcus faecium*1224 x 10^{8} CFU/ml, Gr. 7: *Lactobacillus acidophilus 1/4 + Bacillus subtilis 206/1+ Enterococcus faecium* 122 4 x 10^{8} CFU/ml,Gr. 8: Commercial product (AVIPROB™) 2 x 10^{8} CFU/ml, Gr. 8: Commercial product (AVIPROB™) 2 x 10^{8} CFU/ml, Gr. 9: positive control, Gr. 10: negative control

CHAPTER V

DISCUSSIONS

5.1 Antimicrobial resistance, the characterization of *gyrA* mutation, and genetic diversity by *fla*A-RFLP of *Campylobacter jejuni* and *Campylobacter coli* collected through commercial broiler production chains in Thailand

This study was conducted to investigate the prevalence, antimicrobial resistance pattern and genetic diversity of *C. jejuni* and *C. coli* isolates through broiler production chains in Thailand. Furthermore, the presence of mutations in the QRDR of the *gyrA* was assessed also. Although, antimicrobial agents are not used to treat *Campylobacter* spp. infection in chickens, the antimicrobial agents used in the current study were selected to represent several classes, including those commonly used on chicken farms in Thailand for prevention or treatment of other pathogens.

CHULALONGKORN UNIVERSITY

The major *Campylobacter* found in this study was *C. coli* (94/130, 72.31%). While most studies report a predominance of *C. jejuni* when examining broiler chicken feces (Giacomelli et al., 2014; Prachantasena et al., 2016), there are reports of a predominance of *C. coli* in some studies. For example, a previous study in Thailand that reported 51.5% of the isolates from chicken feces were *C. coli* (Padungtod et al., 2006). As well, a study conducted in China (Ma et al., 2014) found that 56% of the isolates from broiler ceca were *C. coli*. It should be noted that the majority of

Campylobacter isolates in this study came from breeder samples and that *C. coli* has been demonstrated as the predominant species in breeders in a previous study (O'Mahony et al., 2011). Another reason that might explain this was a difference of energy sources between *C. jejuni* and *C. coli.* Although the principal energy sources for *C. jejuni* are amino acids, citric acid cycle intermediates and short chain fatty acids, *C. jejuni* is unable to metabolise propionic acid while *C. coli* is able to metabolise this carbon source (Wagley et al., 2014). The presence of propanoate-CoA ligase and 2-methyl-synthase genes were demonstrated in *C. coli* and their absence in *C. jejuni* (Wagley et al., 2014). Levels of propionate have been reported to be high in the gastrointestinal tract of chickens and their litters (Chi et al., 2005). Competition for nutrients in the gut is violent, so the ability to use a potentially toxic metabolic waste product as an energy source may confer *C. coli* with a selective advantage when it colonises in the chicken gastrointestinal tract (Wagley et al., 2014).

Chulalongkorn University

The proportion of *Campylobacter* strains resistant to antimicrobial agents, particularly FQs has rapidly surged in many countries worldwide and is a major public health concern (Engberg et al., 2001; O'Mahony et al., 2011). Contaminated poultry meat products are considered as the major source of human campylobacteriosis, meaning that resistant *Campylobacter* strains can be transmitted through the food chain (Quinn et al., 2007). For this reason, updates on the AMR profiles, using both phenotypic and genotypic susceptibility testing methods, of *Campylobacter* strains

from chicken are important to help prevent the risk of the transfer resistant *Campylobacter* to humans. Most isolates in the current study were resistant to the various antimicrobial agents tested except erythromycin and gentamicin. Macrolides are considered the drugs of choice for the treatment of severe human campylobacteriosis (Luangtongkum et al., 2009). In the present study, all *Campylobacter* isolates were sensitive to erythromycin, a similar result to previous studies conducted in Thailand that showed a low percentage of erythromycin resistance at 0.69% and 5.8%, respectively (Padungtod et al., 2006; Charununtakorn et al., 2015). In contrast, 11.1% of *C. jejuni* and 87.5% of *C. coli* isolated from broilers in Italy were resistant to erythromycin (Giacomelli et al., 2014). Likewise, 18.8% of *C. jejuni* and 92% of *C. coli* isolated from broilers in China were resistant to erythromycin (Ma et al., 2014).

Remarkably, 66.7% of *C. jejuni* isolates and 97.9% of *C. coli* isolates were classified as MDR. All isolates were susceptible to erythromycin and gentamicin. The absence of resistance to gentamicin was similar to previous studies conducted in Thailand (Chokboonmongkol et al., 2013; Charununtakorn et al., 2015) showing that all tested *C. jejuni* isolates were susceptible to gentamicin. In contrast, 71% of *C. jejuni* isolated from broiler ceca in China were resistant to gentamicin (Ma et al., 2014). Beta-lactam antimicrobial agents such as amoxicillin had been widely used for disease prevention in Thai chicken industry (Chongsuvivatwong and Kitikoon, 2007). In this

study, all *Campylobacter* isolates were resistant to amoxicillin. In addition, *Campylobacter* spp. are intrinsically resistant to bacitracin (Luangtongkum et al., 2009), a polypeptide antimicrobial agent, explaining our finding that all isolates were resistant to bacitracin.

Regarding resistance to tetracyclines, we found that the *C. coli* were more resistant (97.9% for tetracycline and 79.8% for doxycycline) than the *C. jejuni* isolates (55.6% for tetracycline and 50% for doxycycline). This result was in agreement with previous studies conducted in Thailand (Padungtod et al., 2006; Chokboonmongkol et al., 2013; Charununtakorn et al., 2015). Moreover, *Campylobacter* spp. isolated from broiler ceca in China demonstrated 100% tetracycline resistance (Ma et al., 2014). Colistin, also known as polymyxin E, is an important drug in humans, being the drug of last choice if the use of a Beta-lactam, aminoglycoside, or quinolone is ineffective (Livermore, 2002). To our knowledge, no data on colistin resistance in *Campylobacter* isolated from chickens in Thailand have ever been published. In this study, we found higher resistance rate of colistin from *C. jejuni* (66.7%) compared to *C. coli* (16%). A previous study showed a similar result that 67% of all *C. jejuni* subsp. *jejuni* isolates from domestic geese in Turkey were resistant to colistin sulphate (Aydin et al., 2001). Interestingly, there is a recent report on the emergence of a plasmid-mediated colistin resistance mechanism, termed MCR-1, in animals and human beings in China that has caused global concern (Liu et al., 2016). Few data are available on the topic of

Campylobacter resistance to sulphonamides and trimethoprim. Nevertheless, high levels of resistance have been published in poultry isolates in different countries (El-Adawy et al., 2012; Giacomelli et al., 2014), similar to the findings of the current study. In addition, resistance to trimethoprim has been considered intrinsic in *C. jejuni* and *C. coli* (Taylor and Courvalin, 1988).

Resistance to enrofloxacin was present in 100% of *C. jejuni* isolates and 98.9% of *C. coli* isolates in the current study. A previous Thai study (Padungtod et al., 2006) showed a high percentage resistance of ciprofloxacin and nalidixic acid, drugs in the group of FQs, with 87% of *C. coli* and 71.4% of *C. jejuni* isolates being resistant to ciprofloxacin and 89.1% of *C. coli* and 69.6% of *C. jejuni* isolates being resistant to nalidixic acid. According to the antimicrobial usage data from the flocks examined in the current study, enrofloxacin was used to treat breeders, on a case by case only. Despite this restricted use, there was a very high prevalence of enrofloxacin resistance. A previous report (Price et al., 2007) indicated that FQ-resistant *Campylobacter* were isolated from poultry products, even though the on-farm use of FQ drugs had been stopped for a year, indicating that the resistance may persist for long periods.

Campylobacter resistance to FQs is generally associated with a mutation in the QRDR of the *gyrA* gene. The current study found apparently novel *gyrA* mutations including Ser-6-Arg, Gln-7-Lys, Ser-22-Gly, Asn-203-Ser and Ala-206-Val that have, to our best knowledge, not been previously reported in *C. jejuni*. All of these isolates also

had the well known Thr-86-Ile mutation that has already been documented in *C. jejuni* (Zirnstein et al., 1999). The MIC values of enrofloxacin of the isolates with these novel mutations ranged from 4-16 μg/ml. We also identified one isolate that detected that had only Thr-86-Ileamino acid substitution – with that isolate having an MIC of 8 μg/ml MIC for enrofloxacin, Hence, while we found additional mutations, our results confirm that the Thr-86-Ile amino acid substitution is still a key target detecting for genotypic tests for FQ resistance as in a previous report (Jesse et al., 2006). In contrast, the only *gyrA* mutation we found in the *C. coli* isolates was the Thr-86-Ile position, with these isolates having MIC values for enrofloxacin that ranged from 4-16 μg/ml. In the current study, the QRDR of the *gyrA* gene in all strains were compared with *gyrA* gene of *C. jejuni* ATCC 33560 (Genbank no. KX982317) and *C. jejuni* UA580 (Genbank no. L04566). A previous study (McIver et al., 2004) selected *C. jejuni* UA580 as an enrofloxacin-susceptible strain, but we found that the 6th and 7th amino acids of this strain were different from strain ATCC 33560. Hence, *C. jejuni* ATCC 33560, in our view, is a better enrofloxacin-susceptible reference strain. The MAMA-PCR detecting Thr-86-Ile in the QRDR of the *gyrA* gene was positive in all isolates, which correlated with the DNA sequencing of the *gyrA* gene of both *C. jejuni* and *C. coli*. This correlation has been previously reported (Zirnstein et al., 1999; Zirnstein et al., 2000). Therefore, the MAMA-PCR technique, a simple and rapid tool as compared with DNA sequencing, can be used as a screening tool or routine detection of the *gyrA* mutation.

CHULALONGKORN UNIVERSITY

The results of the current study provide several lines of evidence that suggest that vertical transmission is not a major route of transmission for *Campylobacter* transmission in the Thai broiler production chain. Firstly, all breeder flocks were found to be positive for both *C. jejuni* and *C. coli.* In contrast, no broiler flock was positive for *C. jejuni* – the only positive broiler flocks, flocks 1 and 2, yielded *C. coli*. Secondly, *Campylobacter* was not isolated from the hatchery and early embryonic death samples. Thirdly, while broiler flock 1 and the connected breeder flock 1, all yielded *C. coli*, the *fla*A- RFLP showed that the isolates belonged to different clusters – clusters 1 and 3, respectively. Another study conducted in Thailand (Prachantasena et al., 2016) also suggested that vertical transmission might not be the major route of *Campylobacter* transmission in Thai broiler production chain because all breeder flocks were colonized with *Campylobacter* whereas the organism was not recovered from hatchery samples or tray liners of 1-day-old chicks.

CHULALONGKORN UNIVERSITY

Interestingly, *Campylobacter* spp. was not recovered from broiler flocks 3, 4, and 5. As well, broiler flocks 1 and 2 had a very low level *Campylobacter* prevalence. These findings might be due to the fact that the risk of *Campylobacter* infection has a correlation with bird age and the biosecurity level of poultry farms (Evans and Sayers, 2000). Evans and Sayers (2000) reported that 50 out of 100 broiler flocks in Great Britain housed in a sound, well functioning buildings were free of *Campylobacter* infection at 35 days of age and 50% and 32% of flocks sent to slaughterhouse at 28-35 and 36-42

days were *Campylobacter* negative, respectively. After Thailand experienced outbreaks of highly pathogenic avian influenza (HPAI) of the H5N1 subtype during 2004-2005 that had serious consequences on poultry production (Tiensin et al., 2007), high standards of biosecurity are strictly used in integrated poultry farms in Thailand. For example, disinfectant solutions in boot dips are always replaced immediately if there has been an increase of organic matter or the solution has been diluted. As well, all-in-all-out production systems are used on all broiler farms. It is possible that this excellent biosecurity, combined with a short bird life, has reduced the risk of infection with *Campylobacter* greatly. Another reason might be the fact that broiler flocks 3, 4 and 5 were located on a farm which was shut down for around 6 months before this study. Reports of broiler flocks that are negative for *Campylobacter* at first pick up do exist. In a study comparing litter management strategies, Chinivasagam et al. (2016) reported that, in six sequential flocks raised on new bedding, one farm was negative for *Campylobacter* in five flocks while a second farm was negative in three flocks. Another study conducted in Thailand also reported a 0.8% prevalence of *Campylobacter* spp. in a large-scale broiler flock (Prachantasena et al., 2016).

Typing of bacterial isolates from different sources delivers epidemiological information that is crucial for infection control and contributes to risk assessment of *Campylobacter* transmission. For molecular typing methods, *fla*A-RFLP has been described as a stable and highly discriminatory analysis of *Campylobacter* isolates

(Nachamkin et al., 1993; Duffy et al., 2015). One-hundred and thirty *Campylobacter* isolates were analyzed by *fla*A-RFLP. All the isolates were successfully typed by this technique, similar to the previous study (Nachamkin et al., 1993). This finding was different to the result of other researchers who reported that some *Campylobacter* isolates were not typeable because DNA was not amplified (Wittwer et al., 2005; Behringer et al., 2011). Amongst 10 clusters recognized in the current study (Fig. 5), clusters 1, 3, 7, and 8 contained only *C. coli* isolates while clusters 9 and 10 contained only *C. jejuni* isolates. In contrast, both species were present in clusters 2, 4, 5, and 6.

Interestingly, all of samples from broiler flock 1 were grouped into cluster 1 but none of the isolates from the connected breeder flock, flock 1, was present in this cluster. This lack of a connection between the genetic types present in the breeder flock and the connected broiler flock supports the absence of vertical transmission as a major transmission route. In a similar manner, an earlier Thai study found that the genotypes of *Campylobacter,* as identified by flagellin A short variable region sequencing and MLST, in breeders and their offspring were different (O'Reilly et al., 2006). Interestingly, eight of the 9 isolates from broiler flock 1 showed the same genetic pattern, meaning that the infection might come from the same source. Cluster 3 was composed of *C. coli* from all breeder flocks. Importantly, all sampled flocks (broiler and breeder) were located in the same province within a radius of around 50 km. As well, the trucks for feed or bird delivery used the same main road. The high

density of farms and the shared transport avenues might explain the occurrence of cluster 3 across all breeder flocks.

5.2 Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers

In this study, *in vitro* for characterizing CE and *in vivo C. jejuni* challenges were performed, as CE has been known to prevent pathogenic bacteria in poultry for decades. Nurmi and Rantala (1973) showed how newly hatched chickens treated with intestinal contents from adult chickens have increased resistance to infection by *Salmonella* spp. CE bacteria composed of 2 groups, defined and undefined CE. The defined CE cultures are more acceptable because the microbial isolates are identified and characterized for their properties such as antimicrobial susceptibility and acid and bile tolerance (Zhang et al., 2007). Normally, CE bacteria should be isolated and used in the same hosts because of their host specificity (Fuller, 1975). In this study, samples were collected from feces different from previous studies that used samples isolated from intestinal organs (Garriga et al., 1998; Ehrmann et al., 2002). Although *Lactobacillus acidophilus*, *Bacillus subtilis* and *Enterococcus faecium* are considered Generally Recognize as Safe (GRAS), their antimicrobial susceptibility needed to be clarified. CE bacteria may serve as hosts for antibiotic resistance genes that are probably transferred to commensal and pathogenic bacteria in the gut leading to a concern of antimicrobial resistance in humans. All selected strains were sensitive to several antimicrobials, including some of the many drugs used in poultry farms such as amoxicillin, tylosin and erythromycin, none of which will lead to the spread of

resistant properties against these antimicrobials to bacterial hosts (Schwarz et al., 2001).

CE has to survive passage through the gastrointestinal tracts of broilers. From *in vitro* experiments, *Lactobacillus acidophilus* 1/4, *Bacillus subtilis* 206/1 and *Enterococcus faecium* 122 demonstrated good survival rates after 3 and 24 h incubation time for acid and bile tolerance tests. These results indicate that 3 CE bacteria might be able to survive the transit and reach the broiler ceca environment since a total movement through the broiler gastrointestinal tract takes around 4 to 9 h, depending on the feed and age of the broilers (Sundu, 2009). *Bacillus* spp. was quite more tolerable to acid and bile tests compared *Lactobacillus* and *Enterococcus* spp., because *Bacillus* spp. can produce endospores structured by a complex protein coat under stressful environmental conditions (McPherson et al., 2005).

Timmerman et al. (2004) revealed a mixture of different strains, rather than only one strain, would be successful for use as CE bacteria, but this study's results showed no significant difference of *C. jejuni* numbers between treatment and control groups, which is in agreement with the study of Robyn and colleagues (2013) . Although some CE bacteria preparations can decrease the level of colonization in chickens (Mead et al., 1996; Zhang et al., 2007), other studies did not observe the protective effect of CE (Shanker et al., 1988; Stern et al., 2001). The reason why the results were inconsistent remains unclear, but it might reflect the variable nature of the CE agent

and susceptibility of *Campylobacter* strain. In this study, the results showed that these CE cannot be able to compete against *C. jejuni* challenges in broilers which might be the result of pathogenesis of *C. jejuni* that it primarily colonized in the mucosal layer in gastrointestinal tract of chickens (Young et al., 2007). This is different from *C. jejuni* pathogenesis in humans that it can move into the intestinal epithelial layer leading to an inflammation and diarrhea.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CONCLUSIONS AND SUGGESTIONS

Campylobacteriosis is a major bacterial gastroenteric disease in humans. Poultry are considered the main reservoir of *Campylobacter* spp. to human due to the consumption of undercooked poultry meats. Most epidemiological data of *Campylobacter* spp. conducted in developed countries, but this study also provided genetic diversity, antimicrobial resistance, *gyrA* mutation profiles of *Campylobacter* isolated in Thailand. Moreover, CEs were identified to compete with *Campylobacter jejuni* challenges.

In conclusion, the genetic diversity of *Campylobacter* isolated from broiler production chains in Thailand demonstrated the spread of this organism is complex. Horizontal transmission is the major route of infection from bird to bird, meaning that prevention methods have to focus on preventing the first entry of *Campylobacter* into a broiler shed. Both the prevalence of isolates that show MDR and the overall high antimicrobial resistance rates are issues of concern. In particular, the resistance to FQs is a major issue. Programs to ensure prudent use of antimicrobial agents and active surveillance at the farm level are essential to monitor the prevalence, and prevention the spread of, antimicrobial resistant *Campylobacter* in Thai chickens.

CE isolated from fecal samples exhibited non-resistant antibiotic profiles and great survival rates for acid and bile tolerance. Although they could not reduce

significantly *C. jejuni when* compared to positive control broilers, these CE bacteria should be further evaluated as protection against other foodborne bacteria found in broilers such as *Salmonella* and *E. coli* in further studies.

REFERENCES

Arcila-Londono X and Lewis RA 2012. Guillain-Barre syndrome. Semin Neurol. 32(3): 179-186.

Aspinall ST, Wareing DR, Hayward PG and Hutchinson DN 1993. Selective medium for thermophilic *Campylobacters* including *Campylobacter upsaliensis*. J Clin Pathol. 46(9): 829-831.

Aydin F, Atabay HI and Akan M 2001. The isolation and characterization of *Campylobacter jejuni* subsp. *jejuni* from domestic geese (*Anser anser*). J Appl Microbiol. 90(4): 637-642.

Barrios PR, Reiersen J, Lowman R, Bisaillon JR, Michel P, Fridriksdottir V, Gunnarsson E, Stern N, Berke O, McEwen S and Martin W 2006. Risk factors for *Campylobacter* spp. colonization in broiler flocks in Iceland. Prev Vet Med. 74(4): 264-278.

Behringer M, Miller WG and Oyarzabal OA 2011. Typing of *Campylobacter jejuni* and *Campylobacter coli* isolated from live broilers and retail broiler meat by *flaA*-RFLP, MLST, PFGE and REP-PCR. J Microbiol Methods. 84(2): 194-201.

Berndtson E, Emanuelson U, Engvall A and Danielsson-Tham M-L 1996. A 1-year epidemiological study of *Campylobacters* in 18 Swedish chicken farms. Prev Vet Med. 26(3): 167-185.

Blaser MJ, Berkowitz ID, LaForce FM, Cravens J, Reller LB and Wang WL 1979. *Campylobacter* enteritis: clinical and epidemiologic features. Ann Intern Med. 91(2): 179-185.

Blaser MJ, Perez GP, Smith PF, Patton C, Tenover FC, Lastovica AJ and Wang WI 1986. Extraintestinal *Campylobacter jejuni* and *Campylobacter coli* infections: host factors and strain characteristics. J Infect Dis. 153(3): 552-559.

Bolton FJ and Robertson L 1982. A selective medium for isolating *Campylobacter jejuni/coli*. J Clin Pathol. 35(4): 462-467.

Boonmar S, Sangsuk L, Suthivarakom K, Padungtod P and Morita Y 2005. Serotypes and antimicrobial resistance of *Campylobacter jejuni* isolated from humans

and animals in Thailand. Southeast Asian J Trop Med Public Health. 36(1): 130-134.

Borck B and Pedersen K 2005. Pulsed-field gel electrophoresis types of *Campylobacter* spp. in Danish turkeys before and after slaughter. Int J Food Microbiol. 101(1): 63-72.

Bouwknegt M, van de Giessen AW, Dam-Deisz WD, Havelaar AH, Nagelkerke NJ and Henken AM 2004. Risk factors for the presence of *Campylobacter* spp. in Dutch broiler flocks. Prev Vet Med. 62(1): 35-49.

Buckley AM, Wang J, Hudson DL, Grant AJ, Jones MA, Maskell DJ and Stevens MP 2010. Evaluation of live-attenuated *Salmonella* vaccines expressing *Campylobacter* antigens for control of *C. jejuni* in poultry. Vaccine. 28(4): 1094-1105.

Buhr RJ, Cox NA, Stern NJ, Musgrove MT, Wilson JL and Hiett KL 2002. Recovery of *Campylobacter* from segments of the reproductive tract of broiler breeder hens. Avian Dis. 46(4): 919-924.

Bull SA, Allen VM, Domingue G, Jorgensen F, Frost JA, Ure R, Whyte R, Tinker D, Corry JE, Gillard-King J and Humphrey TJ 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. Appl Environ Microbiol. 72(1): 645-652.

Buswell CM, Herlihy YM, Lawrence LM, McGuiggan JT, Marsh PD, Keevil CW and Leach SA 1998. Extended survival and persistence of *Campylobacter* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. Appl Environ Microbiol. 64(2): 733-741.

Camarda A, Newell DG, Nasti R and Di Modugnoa G 2000. Genotyping *Campylobacter jejuni* strains isolated from the gut and oviduct of laying hens. Avian Dis. 44(4): 907-912.

Chansiripornchai N and Sasipreeyajan J 2009. PCR detection of four virulence-associated genes of *Campylobacter jejuni* isolates from Thai broilers and their abilities of adhesion to and invasion of INT-407 cells. J Vet Med Sci. 71(6): 839-844.

Charununtakorn P, Prachantasena S and Luangtongkum T 2015. Antimicrobial resistance patterns and *flaA* genotypes of *Campylobacter jejuni* isolated from contracted broiler farms in eastern Thailand. Thai J Vet Med. 45(2): 283-287.

Chi FH, Lin PH and Leu MH 2005. Quick determination of malodor-causing fatty acids in manure by capillary electrophoresis. Chemosphere. 60(9): 1262-1269.

Chinivasagam HN, Estella W, Rodrigues H, Mayer DG, Weyand C, Tran T, Onysk A and Diallo I 2016. On-farm *Campylobacter* and *Escherichia coli* in commercial broiler chickens: Re-used bedding does not influence *Campylobacter* emergence and levels across sequential farming cycles. Poult Sci. 95(5): 1105-1115.

Chokboonmongkol C, Patchanee P, Golz G, Zessin KH and Alter T 2013. Prevalence, quantitative load, and antimicrobial resistance of *Campylobacter* spp. from broiler ceca and broiler skin samples in Thailand. Poult Sci. 92(2): 462-467.

Chongsuvivatwong V and Kitikoon V 2007. Pattern and determinant of antibiotics used on broiler farms in Songkhla province, southern Thailand. Trop Anim Health Prod. 39(5): 355-361.

Chuma T, Makino K, Okamoto K and Yugi H 1997. Analysis of distribution of *Campylobacter jejuni* and *Campylobacter coli* in broilers by using restriction fragment length polymorphism of flagellin gene. J Vet Med Sci. 59(11): 1011-1015.

Clark CG, Bryden L, Cuff WR, Johnson PL, Jamieson F, Ciebin B and Wang G 2005. Use of the oxford multilocus sequence typing protocol and sequencing of the flagellin short variable region to characterize isolates from a large outbreak of waterborne *Campylobacter* sp. strains in Walkerton, Ontario, Canada. J Clin Microbiol. 43(5): 2080-2091.

CLSI 2011. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. Vol. 31

CLSI 2013. Performance standards for Antimicrobial Disk and Dilution Susceptibility tests for Bacteria Isolated from animals. Page 128 *in* CLSI Document VET01-A4. 4th ed. Wayne, PA.

Chulalongkorn University

Coker AO, Isokpehi RD, Thomas BN, Amisu KO and Obi CL 2002. Human campylobacteriosis in developing countries. Emerg Infect Dis. 8(3): 237-244.

Colles FM, McCarthy ND, Layton R and Maiden MC 2011. The prevalence of *Campylobacter* amongst a free-range broiler breeder flock was primarily affected by flock age. PLoS ONE. 6(12): e22825.

Corry JE and Atabay HI 2001. Poultry as a source of *Campylobacter* and related organisms. J Appl Microbiol. (30): 96S-114S.

Corry JE, Post DE, Colin P and Laisney MJ 1995. Culture media for the isolation of *Campylobacters*. Int J Food Microbiol. 26(1): 43-76.

Cox NA, Stern NJ, Wilson JL, Musgrove MT, Buhr RJ and Hiett KL 2002. Isolation of *Campylobacter* spp. from semen samples of commercial broiler breeder roosters. Avian Dis. 46(3): 717-720.

de Zoete MR, van Putten JP and Wagenaar JA 2007. Vaccination of chickens against *Campylobacter*. Vaccine. 25(30): 5548-5557.

Dingle KE, McCarthy ND, Cody AJ, Peto TE and Maiden MC 2008. Extended sequence typing of *Campylobacter* spp., United Kingdom. Emerg Infect Dis. 14(10): 1620-1622.

Doyle MP 1984. Association of *Campylobacter jejuni* with laying hens and eggs. Appl Environ Microbiol. 47(3): 533-536.

Dubernet S, Desmasures N and Gueguen M 2002. A PCR-based method for identification of lactobacilli at the genus level. FEMS Microbiol Lett. 214(2): 271-275.

Duffy LL, Blackall PJ, Cobbold RN and Fegan N 2015. Mapping the carriage of *flaA*-restriction fragment length polymorphism *Campylobacter* genotypes on poultry carcasses through the processing chain and comparison to clinical isolates. Food microbiology. 48: 116-122.

Echeverria P, Taylor DN, Lexsomboon U, Bhaibulaya M, Blacklow NR, Tamura K and Sakazaki R 1989. Case-control study of endemic diarrheal disease in Thai children. J Infect Dis. 159(3): 543-548.

EFSA 2011. Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA Journal. 9(4): 2015.

EFSA 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal. 10: 2740-2750.

Ehrmann MA, Kurzak P, Bauer J and Vogel RF 2002. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. J Appl Microbiol. 92(5): 966-975.

El-Adawy H, Hotzel H, Dupre S, Tomaso H, Neubauer H and Hafez HM 2012. Determination of antimicrobial sensitivities of *Campylobacter jejuni* isolated from commercial turkey farms in Germany. Avian Dis. 56(4): 685-692.

Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P and Nachamkin I 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. Emerg Infect Dis. 7(1): 24-34.

European_commission 2003. On a generic approach to the safety assessment of micro-organisms used in feed/food and feed/food production: a working paper open for comment. Health and consumer protection directorate-general, Directorate C-Scientific opinions, C2-Management of scientific committees; scientific co-operation and networks. 1-55.

Evans MC and Wegener HC 2003. Antimicrobial growth promoters and *Salmonella* spp., *Campylobacter* spp. in poultry and swine, Denmark. Emerg Infect Dis. 9(4): 489-492.

Evans SJ and Sayers AR 2000. A longitudinal study of *Campylobacter* infection of broiler flocks in Great Britain. Prev Vet Med. 46(3): 209-223.

Fields PI and Swerdlow DL 1999. *Campylobacter jejuni*. Clin Lab Med. 19(3): 489-504, v.

FoodNet 2007. Surveillance report. Page 48.

Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender J, Shiferaw B, Reddy S, Ahuja SD, Helfrick DL, Hardnett F, Carter M, Anderson B and Tauxe RV 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin Infect Dis. 38 Suppl 3: S285-296.

Fuller R 1975. Nature of the determinant responsible for the adhesion of lactobacilli to chicken crop epithelial cells. J Gen Microbiol. 87(2): 245-250.

Garriga M, Pascual M, Monfort JM and Hugas M 1998. Selection of lactobacilli for chicken probiotic adjuncts. J Appl Microbiol. 84(1): 125-132.

Giacomelli M, Salata C, Martini M, Montesissa C and Piccirillo A 2014. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* from poultry in Italy. Microb Drug Resist. 20(2): 181-188.

Gibreel A and Taylor DE 2006. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob Agents Chemother. 58(2): 243-255.

Gilliland SE, Staley TE and Bush LJ 1984. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. J Dairy Sci. 67(12): 3045-3051.

Gupta A, Nelson JM, Barrett TJ, Tauxe RV, Rossiter SP, Friedman CR, Joyce KW, Smith KE, Jones TF, Hawkins MA, Shiferaw B, Beebe JL, Vugia DJ, Rabatsky-Ehr T, Benson JA, Root TP and Angulo FJ 2004. Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. Emerg Infect Dis. 10(6): 1102-1109.

Han K, Jang SS, Choo E, Heu S and Ryu S 2007. Prevalence, genetic diversity, and antibiotic resistance patterns of *Campylobacter jejuni* from retail raw chickens in Korea. Int J Food Microbiol. 114(1): 50-59.

Hazeleger WC, Janse JD, Koenraad PM, Beumer RR, Rombouts FM and Abee T 1995. Temperature-dependent membrane fatty acid and cell physiology changes in coccoid forms of *Campylobacter jejuni*. Appl Environ Microbiol. 61(7): 2713-2719.

Hein I, Schneck C, Knogler M, Feierl G, Plesss P, Kofer J, Achmann R and Wagner M 2003. *Campylobacter jejuni* isolated from poultry and humans in Styria, Austria: epidemiology and ciprofloxacin resistance. Epidemiol Infect. 130(3): 377-386.

Heuer OE, Pedersen K, Andersen JS and Madsen M 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. Lett Appl Microbiol. 33(4): 269-274.

Hiett KL, Cox NA and Stern NJ 2002. Direct polymerase chain reaction detection of *Campylobacter* spp. in poultry hatchery samples. Avian Dis. 46(1): 219-223.

Hue O, Le Bouquin S, Laisney MJ, Allain V, Lalande F, Petetin I, Rouxel S, Quesne S, Gloaguen PY, Picherot M, Santolini J, Salvat G, Bougeard S and Chemaly M 2010. Prevalence of and risk factors for *Campylobacter* spp. contamination of broiler chicken carcasses at the slaughterhouse. Food microbiology. 27(8): 992-999.

Humphrey TJ, Henley A and Lanning DG 1993. The colonization of broiler chickens with *Campylobacter jejuni*: some epidemiological investigations. Epidemiol Infect. 110(3): 601-607.

Hyronimus B, Le Marrec C, Sassi AH and Deschamps A 2000. Acid and bile tolerance of spore-forming lactic acid bacteria. Int J Food Microbiol. 61(2-3): 193-197.

ISO10272-1 2006. Microbiology of food animal feeding stuffs - Horizonal method for detection and enumeration of *Camyloabacter* spp. Page 16 *in* Part 1: Detection method.

ISO-7932 1993. Microbiology general guidance for the enumeration of *Bacillus cereus* Colony-count Technique at 30 C. 1-10.

ISO-15214 1998. Microbiology food and animal feeding stuffs-Horizontal method for the enumeration of mesophilic lacid acid bacteria Colony-count Technique at 30 C. 1-16.

Jackson CR, Fedorka-Cray PJ and Barrett JB 2004. Use of a genus- and species-specific multiplex PCR for identification of enterococci. J Clin Microbiol. 42(8): 3558-3565.

Jay-Russell MT, Mandrell RE, Yuan J, Bates A, Manalac R, Mohle-Boetani J, Kimura A, Lidgard J and Miller WG 2012. Using MOMP typing as an epidemiologic tool to investigate milkborne *Campylobacter jejuni* outbreaks in California. J Clin Microbiol. JCM. 01845-01812.

Jeffrey JS, Tonooka KH and Lozanot J 2001. Prevalence of *Campylobacter* spp. from skin, crop, and intestine of commercial broiler chicken carcasses at processing. Poult Sci. 80(9): 1390-1392.

Chulalongkorn University

Jesse TW, Englen MD, Pittenger-Alley LG and Fedorka-Cray PJ 2006. Two distinct mutations in *gyrA* lead to ciprofloxacin and nalidixic acid resistance in *Campylobacter coli* and *Campylobacter jejuni* isolated from chickens and beef cattle. J Appl Microbiol. 100(4): 682-688.

Jones K 2001. *Campylobacters* in water, sewage and the environment. J Appl Microbiol. (30): 68S-79S.

Ke D, Picard FJ, Martineau F, Menard C, Roy PH, Ouellette M and Bergeron MG 1999. Development of a PCR assay for rapid detection of enterococci. J Clin Microbiol. 37(11): 3497-3503.

Knudsen KN, Bang DD, Andresen LO and Madsen M 2006. *Campylobacter jejuni* strains of human and chicken origin are invasive in chickens after oral challenge. Avian Dis. 50(1): 10-14.

Kwon HS, Yang EH, Yeon SW, Kang BH and Kim TY 2004. Rapid identification of probiotic *Lactobacillus* species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA. FEMS Microbiol Lett. 239(2): 267-275.

Lastovica AJ, On SL and Zhang L 2014. The Family *Campylobacteraceae*. In: the Prokaryotes. Springer. 307-335.

Lin J 2009. Novel approaches for *Campylobacter* control in poultry. Foodborne Pathog Dis. 6(7): 755-765.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH and Shen J 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 16(2): 161-168.

Livermore DM 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin Infect Dis. 34(5): 634-640.

Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM and Zhang Q 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol. 4(2): 189-200.

Ma L, Wang Y, Shen J, Zhang Q and Wu C 2014. Tracking *Campylobacter* contamination along a broiler chicken production chain from the farm level to retail in China. Int J Food Microbiol. 181: 77-84.

Martinez M, McDermott P and Walker R 2006. Pharmacology of the fluoroquinolones: a perspective for the use in domestic animals. Vet J. 172(1): 10-28.

McIver C, Hogan T, White P and Tapsall J 2004. Patterns of quinolone susceptibility in *Campylobacter jejuni* associated with different *gyrA* mutations. Pathology. 36(2): 166-169.

McPherson DC, Kim H, Hahn M, Wang R, Grabowski P, Eichenberger P and Driks A 2005. Characterization of the *Bacillus subtilis* spore morphogenetic coat protein CotO. J Bacteriol. 187(24): 8278-8290.

Mead GC, Scott MJ, Humphrey TJ and McAlpine K 1996. Observations on the control of *Campylobacter jejuni* infection of poultry by 'competitive exclusion'. Avian Pathol. 25(1): 69-79.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM and Tauxe RV 1999. Food-related illness and death in the United States. Emerg Infect Dis. 5(5): 607-625.

Morishita TY, Aye PP, Harr BS, Cobb CW and Clifford JR 1997. Evaluation of an avian-specific probiotic to reduce the colonization and shedding of *Campylobacter jejuni* in broilers. Avian Dis. 41(4): 850-855.

Nachamkin I, Allos BM and Ho T 1998. *Campylobacter* species and Guillain-Barre syndrome. Clin Microbiol Rev. 11(3): 555-567.

Nachamkin I, Bohachick K and Patton CM 1993. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. J Clin Microbiol. 31(6): 1531-1536.

Nurmi E and Rantala M 1973. New aspects of *Salmonella* infection in broiler production. Nature. 241(5386): 210-211.

O'Mahony E, Buckley JF, Bolton D, Whyte P and Fanning S 2011. Molecular epidemiology of *Campylobacter* isolates from poultry production units in southern Ireland. PLoS ONE. 6(12): e28490.

O'Reilly LC, Inglis TJ and Unicomb L 2006. Australian multicentre comparison of subtyping methods for the investigation of *Campylobacter* infection. Epidemiol Infect. 134(4): 768-779.

Padungtod P and Kaneene JB 2005. *Campylobacter* in food animals and humans in northern Thailand. J Food Prot. 68(12): 2519-2526.

Padungtod P, Kaneene JB, Hanson R, Morita Y and Boonmar S 2006. Antimicrobial resistance in *Campylobacter* isolated from food animals and humans in northern Thailand. FEMS Immunol Med Microbiol. 47(2): 217-225.

Padungtod P, Kaneene JB, Wilson DL, Bell J and Linz JE 2003. Determination of ciprofloxacin and nalidixic acid resistance in *Campylobacter jejuni* with a fluorogenic polymerase chain reaction assay. J Food Prot. 66(2): 319-323.

Park SF 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. Int J Food Microbiol. 74(3): 177-188.

Payot S, Bolla JM, Corcoran D, Fanning S, Megraud F and Zhang Q 2006. Mechanisms of fluoroquinolone and macrolide resistance in *Campylobacter* spp. Microbes Infect. 8(7): 1967-1971.

Perez-Boto D, Garcia-Pena FJ, Abad-Moreno JC and Echeita MA 2012. Dynamics of populations of *Campylobacter jejuni* in two grandparent broiler breeder farms: persistent vs. transient strains. Vet Microbiol. 159(1-2): 204-211.

Petersen L and Newell DG 2001. The ability of *Fla*-typing schemes to discriminate between strains of *Campylobacter jejuni*. J Appl Microbiol. 91(2): 217-224.

Petersen L, Nielsen EM and On SL 2001. Serotype and genotype diversity and hatchery transmission of *Campylobacter jejuni* in commercial poultry flocks. Vet Microbiol. 82(2): 141-154.

Piddock LJ, Ricci V, Pumbwe L, Everett MJ and Griggs DJ 2003. Fluoroquinolone resistance in *Campylobacter* species from man and animals: detection of mutations in topoisomerase genes. J Antimicrob Chemother. 51(1): 19-26.

Prachantasena S, Charununtakorn P, Muangnoicharoen S, Hankla L, Techawal N, Chaveerach P, Tuitemwong P, Chokesajjawatee N, Williams N, Humphrey T and Luangtongkum T 2016. Distribution and genetic profiles of

Campylobacter in commercial broiler production from breeder to slaughter in Thailand. PLoS ONE. 11(2): e0149585.

Price LB, Johnson E, Vailes R and Silbergeld E 2005. Fluoroquinolone-resistant *Campylobacter* isolates from conventional and antibiotic-free chicken products. Environ Health Perspect. 113(5): 557-560.

Price LB, Lackey LG, Vailes R and Silbergeld E 2007. The persistence of fluoroquinolone-resistant *Campylobacter* in poultry production. Environ Health Perspect. 115(7): 1035-1039.

Quinn T, Bolla JM, Pages JM and Fanning S 2007. Antibiotic-resistant *Campylobacter*: could efflux pump inhibitors control infection? J Antimicrob Chemother. 59(6): 1230-1236.

Rautelin H, Jusufovic J and Hanninen ML 1999. Identification of hippurate-negative thermophilic *Campylobacters*. Diagn Microbiol Infect Dis. 35(1): 9-12.

Ridley A, Morris V, Gittins J, Cawthraw S, Harris J, Edge S and Allen V 2011. Potential sources of *Campylobacter* infection on chicken farms: contamination and control of broiler-harvesting equipment, vehicles and personnel. J Appl Microbiol. 111(1): 233-244.

Robyn J, Rasschaert G, Hermans D, Pasmans F and Heyndrickx M 2013. In vivo broiler experiments to assess anti-*Campylobacter jejuni* activity of a live *Enterococcus faecalis* strain. Poult Sci. 92(1): 265-271.

Ruiz-Palacios GM, Escamilla E and Torres N 1981. Experimental *Campylobacter* diarrhea in chickens. Infect Immun. 34(1): 250-255.

Sahin O, Fitzgerald C, Stroika S, Zhao S, Sippy RJ, Kwan P, Plummer PJ, Han J, Yaeger MJ and Zhang Q 2012. Molecular evidence for zoonotic transmission of an emergent, highly pathogenic *Campylobacter jejuni* clone in the United States. J Clin Microbiol. 50(3): 680-687.

Sahin O, Luo N, Huang S and Zhang Q 2003. Effect of *Campylobacter*-specific maternal antibodies on *Campylobacter jejuni* colonization in young chickens. Appl Environ Microbiol. 69(9): 5372-5379.

Sahin O, Morishita TY and Zhang Q 2002. *Campylobacter* colonization in poultry: sources of infection and modes of transmission. Anim Health Res Rev. 3(2): 95-105.

Sanyal SC, Islam KM, Neogy PK, Islam M, Speelman P and Huq MI 1984. *Campylobacter jejuni* diarrhea model in infant chickens. Infect Immun. 43(3): 931-936.

Schoeni JL and Wong A 1994. Inhibition of *Campylobacter jejuni* colonization in chicks by defined competitive exclusion bacteria. Appl Environ Microbiol. 60(4): 1191-1197.

Schwarz S, Kehrenberg C and Walsh TR 2001. Use of antimicrobial agents in veterinary medicine and food animal production. Int J Antimicrob Agents. 17(6): 431-437.

Shanker S, Lee A and Sorrell TC 1988. Experimental colonization of broiler chicks with *Campylobacter jejuni*. Epidemiol Infect. 100(1): 27-34.

Skirrow MB 1977. *Campylobacter* enteritis: a "new" disease. Br Med J. 2(6078): 9-11.

Smith DL, Harris AD, Johnson JA, Silbergeld EK and Morris JG, Jr. 2002. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc Natl Acad Sci U S A. 99(9): 6434-6439.

Smith JL and Fratamico PM 2010. Fluoroquinolone resistance in *Campylobacter*. J Food Prot. 73(6): 1141-1152.

Steele TW and McDermott SN 1984. The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from feces. Pathology. 16(3): 263-265.

Stephens CP, On SL and Gibson JA 1998. An outbreak of infectious hepatitis in commercially reared ostriches associated with *Campylobacter coli* and *Campylobacter jejuni*. Vet Microbiol. 61(3): 183-190.

Stern NJ, Cox NA, Bailey JS, Berrang ME and Musgrove MT 2001. Comparison of mucosal competitive exclusion and competitive exclusion treatment to reduce *Salmonella* and *Campylobacter* spp. colonization in broiler chickens. Poult Sci. 80(2): 156-160.

Sukhapesna J, Amavisit P, Wajjwalku W, Thamchaipenet A and Sukpuaram T 2005. Antimicrobial resistance of *Campylobacter jejuni* isolated from chicken in Nakhon Pathom province, Thailand. KASETSART JOURNAL. 240.

Sundu B 2009. Gastro-intestinal response and passage time of pelleted diets in digestive tract of broilers. International Journal of Poultry Science. 8(10): 976-979.

Takamiya M, Ozen A, Rasmussen M, Alter T, Gilbert T, Ussery DW and Knochel S 2011. Genome Sequence of *Campylobacter jejuni* strain 327, a strain isolated from a turkey slaughterhouse. Standards in genomic sciences. 4(2): 113-122.

Taylor DE and Courvalin P 1988. Mechanisms of antibiotic resistance in *Campylobacter* species. Antimicrob Agents Chemother. 32(8): 1107-1112.

Thakur S, Brake J, Keelara S, Zou M and Susick E 2013. Farm and environmental distribution of *Campylobacter* and *Salmonella* in broiler flocks. Res Vet Sci. 94(1): 33-42.

Thomrongsuwannakij T, Chuanchuen R and Chansiripornchai N 2016. Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers. The Thai Journal of Veterinary Medicine. 46(2): 279-286.

Tiensin T, Nielen M, Songserm T, Kalpravidh W, Chaitaweesub P, Amonsin A, Chotiprasatintara S, Chaisingh A, Damrongwatanapokin S, Wongkasemjit S, Antarasena C, Songkitti V, Chanachai K, Thanapongtham W and Stegeman JA 2007. Geographic and temporal distribution of highly pathogenic avian influenza A virus (H5N1) in Thailand, 2004-2005: an overview. Avian Dis. 51(1 Suppl): 182-188.

Timmerman HM, Koning CJ, Mulder L, Rombouts FM and Beynen AC 2004. Monostrain, multistrain and multispecies probiotics--A comparison of functionality and efficacy. Int J Food Microbiol. 96(3): 219-233.

USFDA 2000. Human health impact of fluoroquinolone-resistant *Campylobacter* attributed to the consumption of chicken. 18 Oct 2000 ed.

Van Bambeke F, Michot JM, Van Eldere J and Tulkens PM 2005. Quinolones in 2005: an update. Clin Microbiol Infect. 11(4): 256-280.

van de Giessen AW, Tilburg JJ, Ritmeester WS and van der Plas J 1998. Reduction of *Campylobacter* infections in broiler flocks by application of hygiene measures. Epidemiol Infect. 121(1): 57-66.

Vanbelle M, Teller E and Focant M 1990. Probiotics in animal nutrition: a review. Arch Tierernahr. 40(7): 543-567.

Vandamme P, Falsen E, Rossau R, Hoste B, Segers P, Tytgat R and De Ley J 1991. Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int J Syst Bacteriol. 41(1): 88-103.

Verwoerd DJ 2000. Ostrich diseases. Revue scientifique et technique. 19(2): 638-661.

Waegel A and Nachamkin I 1996. Detection and molecular typing of *Campylobacter jejuni* in fecal samples by polymerase chain reaction. Mol Cell Probes. 10(2): 75-80.

Wagley S, Newcombe J, Laing E, Yusuf E, Sambles CM, Studholme DJ, La Ragione RM, Titball RW and Champion OL 2014. Differences in carbon source utilisation distinguish *Campylobacter jejuni* from *Campylobacter coli*. BMC Microbiol. 14: 262.

Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward DL and Rodgers FG 2002. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. J Clin Microbiol. 40(12): 4744-4747.

Welkos SL 1984. Experimental gastroenteritis in newly-hatched chicks infected with *Campylobacter jejuni*. J Med Microbiol. 18(2): 233-248.

Wittwer M, Keller J, Wassenaar TM, Stephan R, Howald D, Regula G and Bissig-Choisat B 2005. Genetic diversity and antibiotic resistance patterns in a *Campylobacter* population isolated from poultry farms in Switzerland. Appl Environ Microbiol. 71(6): 2840-2847.

Wu XY, Walker MJ, Hornitzky M and Chin J 2006. Development of a group-specific PCR combined with ARDRA for the identification of *Bacillus* species of environmental significance. J Microbiol Methods. 64(1): 107-119.

Wyszynska A, Raczko A, Lis M and Jagusztyn-Krynicka EK 2004. Oral immunization of chickens with avirulent *Salmonella* vaccine strain carrying *C. jejuni* 72Dz/92 *cjaA* gene elicits specific humoral immune response associated with protection against challenge with wild-type *Campylobacter*. Vaccine. 22(11-12): 1379-1389.

Yogasundram K, Shane SM and Harrington KS 1989. Prevalence of *Campylobacter jejuni* in selected domestic and wild birds in Louisiana. Avian Dis. 33(4): 664-667.

Young KT, Davis LM and Dirita VJ 2007. *Campylobacter jejuni*: molecular biology and pathogenesis. Nat Rev Microbiol. 5(9): 665-679.

Zenner D and Gillespie I 2011. Travel-associated *Salmonella* and *Campylobacter* gastroenteritis in England: estimation of under-ascertainment through national laboratory surveillance. J Travel Med. 18(6): 414-417.

Zhang G, Ma L and Doyle MP 2007. Potential competitive exclusion bacteria from poultry inhibitory to *Campylobacter jejuni* and *Salmonella*. J Food Prot. 70(4): 867-873.

Zirnstein G, Helsel L, Li Y, Swaminathan B and Besser J 2000. Characterization of *gyrA* mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. FEMS Microbiol Lett. 190(1): 1-7.

Zirnstein G, Li Y, Swaminathan B and Angulo F 1999. Ciprofloxacin resistance in *Campylobacter jejuni* isolates: detection of *gyrA* resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. J Clin Microbiol. 37(10): 3276-3280.

APPENDIX

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Appendix A: Culture Media

1. de Mans Rogosa and Sharpe Agar (MRS Agar) (Difco^{TM}, MD, USA)

• Proteose peptone	10.0 g
• Meat extract	8.0 g
• Yeast extract	5.0 g
• Tri-ammonium citrate	2.0 g
• Sodium acetate	0.5 g
• $Mg_2SO_4.7H_2O$	0.1 g
• K_2HPO_4	2.0 g
• Glucose	20.0 g
• Tween-80	1.0 g
• Tryptone	5.0 g
• Agar	15.0 g
• Distilled water	1,000 ml

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

2. Manitol Egg Yolk Polymyxin-B Agar (MYP Agar) (Difco^{TM}, MD, USA)

• Beef extract	1.0 g
• Peptone	10.0 g
• D-Mannitol	10.0 g
• NaCl	10.0 g

- Agar 15.0 g
- Phenol Red 0.2% aqueous solution 15.0 ml
- Distilled water 1,000 ml

3. SF-Streptococcus Agar (SF Agar) (Difco™, MD, USA)

- Tryptone 20.0 g
- Dextrose 5.0 g
- K_2PO_4 4.0 g
- NaCl 5.0 g
- Sodium Azide 0.5 g
- Agar 20.0 g
- Bromocresol purple 0.2% aqueous solution 32 mg
- Distilled water 1,000 ml

4. Muller Hinton Agar (MHA) (Difco™, MD, USA)

- Beef Extract Powder 2.0 g
- Acid Digest of Casein 17.5 g
- Starch 1.5 g
- Agar 17.0 g
- Distilled water 1,000 ml

5. Modified charcoal cefoperazone desoxycholate agar (mCCD agar) (Oxoid™, England)

- Meat extract 10.0 g
- Enzymatic digest of animal tissues 10.0 g
- Sodium chloride 5.0 g
- Charcoal 4.0 g
- Enzymatic digest of casein 3.0 g
- Sodium desoxycholate 1.0 g
- Iron (II) sulfate 0.25 g
- Sodium pyruvate 0.25 g
- Agar 12.0 g
- Distilled water 1,000 ml

Antibiotics

1. Antibiotic solution for mCCD agar 1 litre

- Cefoperazone 0.032 g
- Amphotericin B 0.01 g
- Water 5 ml

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Appendix B: Alignment of partial *gyrA* gene of 24 C. jejuni, 24 C. coli and control strains

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 1
21 µÿ³"§¡ 2016 14:15

```
                                                         10                                      20
L04566          Met Glu Asn Ile Phe Ser Lys Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                ATG GAG AAT ATT TTT AGC AAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014CJ0001   -   -   -   -  Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- --- TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL6008   -   -   -   -  Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- --- TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL6009   -   -   -   -  Phe Ser Lys Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- --- TTT AGC AAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL6010   -   -   -   -   -   -  Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- --- --- --- CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL6015   -   -   -   -  Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- --- TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL6019   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL7029   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL8006   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL8012   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL8013   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL8018   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL8023   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014DL8100   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS1002   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS1006   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 2
21 µÿ³"§¡ 2016 14:15

```
AHRU2014SS1007   -   -   -  Ile Phe Ser Lys Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGC AAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS1019   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS1022   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS1025   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS2002   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS2004   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS2008   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS2009   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS2021  ??? ??? ??? Ile Phe Ser Lys Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --T ATT TTT AGC AAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
AHRU2014SS2027   -   -   -  Ile Phe Arg Gln Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                --- --- --- ATT TTT AGG CAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
```

```
                                                         30                                      40
L04566          Lys Ser Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
                AAA AGT AGT TAT TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014CJ0001  Lys Ser Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
                AAA AGT AGT TAT TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL6008  Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
                AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL6009  Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
                AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL6010  Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
                AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL6015  Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
```

```
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL6019 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL7029 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL8006 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL8012 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL8013 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL8018 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL8023 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014DL8100 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS1002 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS1006 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS1007 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS1019 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS1022 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS1025 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS2002 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS2004 Lys Ser Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA AGT AGT TAT TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS2008 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
```



```
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS2009 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS2021 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA
AHRU2014SS2027 Lys Gly Ser Tyr Leu Asp Tyr Ser Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
             AAA GGT AGT TAC TTA GAC TAT TCT ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA

                                                   50                                      60
L04566         Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014CJ0001 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL6008 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL6009 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL6010 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL6015 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL6019 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL7029 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL8006 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL8012 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL8013 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL8018 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
             AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 5
21 µÿ³ª§¡ 2016 14:15

```
AHRU2014DL8023 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014DL8100 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS1002 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS1006 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS1007 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS1019 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS1022 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS1025 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS2002 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS2004 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS2008 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS2009 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS2021 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
AHRU2014SS2027 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA

                                                           70                                      80
L04566         Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014CJ0001 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 6
21 µÿ³ª§¡ 2016 14:15

```
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL6008 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL6009 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL6010 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL6015 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL6019 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL7029 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL8006 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL8012 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL8013 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL8018 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL8023 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014DL8100 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS1002 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS1006 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS1007 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS1019 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS1022 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 7
21 µÿ³"§¡ 2016 14:15

```
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS1025 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS2002 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS2004 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS2008 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS2009 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS2021 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
AHRU2014SS2027 Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
```

```
                                                    90                                     100
L04566         His Pro His Gly Asp Thr Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAC CCA CAT GGA GAT ACA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014CJ0001 His Pro His Gly Asp Thr Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAC CCA CAT GGA GAT ACA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL6008 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL6009 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL6010 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL6015 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL6019 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL7029 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 8
21 µÿ³"§¡ 2016 14:15

```
AHRU2014DL8006 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL8012 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL8013 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL8018 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL8023 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014DL8100 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS1002 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS1006 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS1007 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS1019 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS1022 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS1025 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS2002 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS2004 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAC CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS2008 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS2009 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
AHRU2014SS2021 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 9
21 µÿ³"§¡ 2016 14:15

```
AHRU2014SS2027 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGA GAT ATA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT

                                                110                                     120
L04566         Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGT GCC
AHRU2014CJ0001 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGT GCC
AHRU2014DL6008 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL6009 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL6010 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGT AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL6015 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL6019 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL7029 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL8006 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL8012 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL8013 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGT AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL8018 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL8023 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014DL8100 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS1002 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 10
21 µÿ³"§¡ 2016 14:15

```
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS1006 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS1007 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS1019 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS1022 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS1025 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS2002 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS2004 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGT GCC
AHRU2014SS2008 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS2009 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGT AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS2021 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT
AHRU2014SS2027 Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGC GCT

                                                130                                     140
L04566         Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014CJ0001 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL6008 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL6009 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 11
21 µÿ³"§¡ 2016 14:15

```
AHRU2014DL6010 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL6015 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL6019 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL7029 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL8006 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL8012 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL8013 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL8018 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL8023 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014DL8100 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS1002 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS1006 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS1007 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS1019 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS1022 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS1025 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS2002 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 12
21 µÿ³"§¡ 2016 14:15

```
AHRU2014SS2004 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS2008 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS2009 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS2021 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
AHRU2014SS2027 Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
               GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
```

```
                                                         150                                 160
L04566         Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGC GAA CCT GAT
AHRU2014CJ0001 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGC GAA CCT GAT
AHRU2014DL6008 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL6009 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL6010 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL6015 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL6019 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL7029 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL8006 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL8012 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL8013 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 13
21 µÿ³"§¡ 2016 14:15

```
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL8018 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL8023 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014DL8100 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS1002 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS1006 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS1007 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS1019 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS1022 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS1025 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS2002 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS2004 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGC GAA CCT GAT
AHRU2014SS2008 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS2009 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS2021 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT
AHRU2014SS2027 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGT GAA CCT GAT

                                                170                                     180
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 14
21 µÿ³"§¡ 2016 14:15

```
L04566         Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014CJ0001 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL6008 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL6009 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL6010 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL6015 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL6019 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL7029 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL8006 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL8012 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL8013 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL8018 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL8023 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014DL8100 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS1002 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS1006 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS1007 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 15
21 µÿ³"§¡ 2016 14:15

```
AHRU2014SS1019 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS1022 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS1025 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS2002 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS2004 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS2008 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS2009 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS2021 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
AHRU2014SS2027 Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTC TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT

                                                         190                                    200
L04566         Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014CJ0001 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL6008 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL6009 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL6010 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL6015 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL6019 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
```

Alignment Report of 26 C.jejuni 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 16
21 µÿ³"§¡ 2016 14:15

```
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL7029 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL8006 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL8012 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL8013 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL8018 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL8023 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014DL8100 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS1002 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS1006 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS1007 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS1019 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS1022 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS1025 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS2002 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS2004 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS2008 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS2009 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
```

```
                ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS2021  Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
                ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
AHRU2014SS2027  Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
                ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
```

```
                                              210                                     220
L04566          Leu Asp Asn Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AAT AAA GAT GCA AGC CTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014CJ0001  Leu Asp Asn Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AAT AAA GAT GCA AGC CTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL6008  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL6009  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL6010  Leu Asp Ser Lys Asp Val Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GTA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL6015  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL6019  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL7029  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL8006  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL8012  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL8013  Leu Asp Ser Lys Asp Val Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GTA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL8018  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014DL8023  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
```



```
AHRU2014DL8100  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS1002  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS1006  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS1007  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS1019  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS1022  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS1025  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS2002  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS2004  Leu Asp Asn Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AAT AAA GAT GCA AGC CTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS2008  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS2009  Leu Asp Ser Lys Asp Val Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GTA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS2021  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
AHRU2014SS2027  Leu Asp Ser Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
                CTT GAT AGT AAA GAT GCA AGC TTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
```

```
                                              230                                     240
L04566          Pro Thr Gly Gly Ile Ile Tyr Gly Lys Lys Gly Ile Ile Glu Ala Tyr Arg Thr Gly Arg
                CCA ACA GGT GGA ATA ATT TAT GGT AAA AAA GGT ATT ATA GAA GCT TAT CGC ACA GGG CGT
AHRU2014CJ0001  Pro Thr Gly Gly
                CCA ACA GGT GGA
AHRU2014DL6008  Pro Thr Gly Gly
```

```
             CCA ACA GGT GGA
AHRU2014DL6009 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014DL6010 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014DL6015 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014DL6019 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014DL7029 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014DL8006 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014DL8012 Pro Thr Gly Gly Asn
             CCA ACA GGT GGA A
AHRU2014DL8013 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014DL8018 Pro Thr Gly Gly Asn
             CCA ACA GGT GGA A
AHRU2014DL8023 Pro Thr Gly Gly Asn
             CCA ACA GGT GGA A
AHRU2014DL8100 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014SS1002 Pro Thr Gly Gly Asn
             CCA ACA GGT GGA A
AHRU2014SS1006 Pro Thr Gly Gly Asn
             CCA ACA GGT GGA A
AHRU2014SS1007 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014SS1019 Pro Thr Gly Gly Asn
             CCA ACA GGT GGA A
AHRU2014SS1022 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014SS1025 Pro Thr Gly Gly
```

```
             CCA ACA GGT GGA
AHRU2014SS2002 Pro Thr Gly Gly Asn
             CCA ACA GGT GGA A
AHRU2014SS2004 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014SS2008 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014SS2009 Pro Thr Gly Gly
             CCA ACA GGT GGA
AHRU2014SS2021 Pro Thr Gly
             CCA ACA GGT GGA
AHRU2014SS2027 Pro Thr Gly Gly
             CCA ACA GGT GGA

                                            250                                         260
L04566       Gly Arg Val Lys Val Arg Ala Lys Thr His Ile Glu Lys Lys Thr Asn Lys Asp Val Ile
             GGT CGC GTG AAA GTG CGA GCT AAA ACT CAT ATT GAA AAA AAG ACA AAT AAA GAT GTT ATT
AHRU2014CJ0001

AHRU2014DL6008

AHRU2014DL6009

AHRU2014DL6010

AHRU2014DL6015

AHRU2014DL6019

AHRU2014DL7029

AHRU2014DL8006
```

```
                                                    10                                                  20
L04566          Met Glu Asn Ile Phe Ser Lys Asp Ser Asp Ile Glu Leu Val Asp Ile Glu Asn Ser Ile
                ATG GAG AAT ATT TTT AGC AAA GAT TCT GAT ATT GAA CTT GTA GAT ATA GAA AAT TCT ATA
U63413          ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014CC0001  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL6001  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL6002   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL6003  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL6005  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL6011   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL6014  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL6017  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL6024  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL7003  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL7014  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL7024  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014DL7025  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014GL4009  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

```
AHRU2014GL5009  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS1001  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS1005  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS1015  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS1021  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS2001  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS2005  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS2010  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS2013  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS2022  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014SS2026  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                    30                                      40
L04566          Lys Ser Ser Tyr Leu Asp Tyr Ser|Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala|
                AAA AGT AGT TAT TTA GAC TAT TCT|ATG AGT GTT ATT ATA GGT CGT GCT TTG CCT GAC GCA|
U63413          ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
                --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014CC0001  ??? ??? ??? ??? ??? ??? ??? ???|Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala|
                --- --- --- --- --- --- --- --T|ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAT GCT|
AHRU2014DL6001  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???|Ile Ile Gly Arg Ala Leu Pro Asp Ala|
                --- --- --- --- --- --- --- --- --- --- --T|ATT ATC GGT CGT GCA CTT CCT GAC GCT|
AHRU2014DL6002   -   -   -   -   -   -   -   - |Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala|
```

```
              --- --- --- --- --- --- --- --- ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL6003 ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --- --- --- -TT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL6005 ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --- --- --- --T ATT ATC GGT CGT GCA CTT CCT GAT GCT
AHRU2014DL6011  -   -   -   -   -   -   -   -  Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --- ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL6014 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL6017 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL6024 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL7003 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL7014 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL7024 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014DL7025 ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --- --- --- --T ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014GL4009 ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --- --- --- --T ATT ATC GGT CGT GCA CTT CCT GAT GCT
AHRU2014GL5009 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAT GCT
AHRU2014SS1001 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS1005 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS1015 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS1021 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS2001 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
```



```
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS2005 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS2010 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS2013 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS2022 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT
AHRU2014SS2026 ??? ??? ??? ??? ??? ??? ??? ??? Met Ser Val Ile Ile Gly Arg Ala Leu Pro Asp Ala
              --- --- --- --- --- --- --- --T ATG AGC GTT ATT ATC GGT CGT GCA CTT CCT GAC GCT

                                                   50                                      60
L04566         Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Gln Asn Asp Glu Ala
               AGA GAT GGT TTA AAG CCT GTT CAT AGA AGA ATT TTA TAT GCT ATG CAA AAT GAT GAG GCA
U63413         ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
               --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AHRU2014CC0001 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL6001 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL6002 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL6003 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL6005 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL6011 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL6014 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL6017 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
```

```
AHRU2014DL6024 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL7003 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL7014 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL7024 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014DL7025 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014GL4009 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014GL5009 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS1001 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS1005 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS1015 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS1021 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS2001 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS2005 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS2010 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS2013 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS2022 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
AHRU2014SS2026 Arg Asp Gly Leu Lys Pro Val His Arg Arg Ile Leu Tyr Ala Met Asn Asp Leu Gly Val
               AGA GAT GGC TTA AAG CCT GTT CAC AGA AGA ATA CTT TAT GCT ATG AAT GAT CTT GGC GTA
```



```
                                                        70                                      80
L04566         Lys Ser Arg Thr Asp Phe Val Lys Ser Ala Arg Ile Val Gly Ala Val Ile Gly Arg Tyr
               AAA AGT AGA ACA GAT TTT GTC AAA TCA GCC CGT ATA GTG GGT GCT GTT ATA GGT CGT TAT
U63413         ??? ??? ??? ??? Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               --- --- --- -GT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014CC0001 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL6001 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL6002 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL6003 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL6005 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL6011 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL6014 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL6017 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL6024 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL7003 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL7014 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL7024 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014DL7025 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014GL4009 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
```

Alignment Report of 1 C.jejuni+26 C.coli 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 7
21 µÿ³"§¡ 2016 14:42

```
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014GL5009 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS1001 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS1005 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS1015 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS1021 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS2001 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS2005 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS2010 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS2013 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS2022 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
AHRU2014SS2026 Gly Ser Arg Ser Ala Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Ile Gly Lys Tyr
               GGA AGT AGA AGT GCA TAT AAA AAA TCT GCT CGT ATA GTA GGG GAT GTT ATC GGT AAG TAT
```

```
                                                   90                                     100
L04566         His Pro His Gly Asp Thr Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAC CCA CAT GGA GAT ACA GCA GTT TAT GAT GCT TTG GTT AGA ATG GCT CAA GAT TTT TCT
U63413         His Pro His Gly Asp Thr Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ACT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTT TCT
AHRU2014CC0001 His Pro His Gly Asp Thr Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ACT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTT TCT
AHRU2014DL6001 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
```

Alignment Report of 1 C.jejuni+26 C.coli 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 8
21 µÿ³"§¡ 2016 14:42

```
AHRU2014DL6002 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL6003 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL6005 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTT TCT
AHRU2014DL6011 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL6014 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL6017 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL6024 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL7003 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL7014 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL7024 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014DL7025 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014GL4009 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTT TCT
AHRU2014GL5009 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTT TCT
AHRU2014SS1001 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014SS1005 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014SS1015 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014SS1021 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
```

```
AHRU2014SS2001 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014SS2005 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014SS2010 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014SS2013 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014SS2022 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT
AHRU2014SS2026 His Pro His Gly Asp Ile Ala Val Tyr Asp Ala Leu Val Arg Met Ala Gln Asp Phe Ser
               CAT CCA CAT GGC GAT ATT GCT GTT TAC GAT GCC TTA GTA AGA ATG GCA CAA GAT TTC TCT

                                                    110                                     120
L04566         Met Arg Tyr Pro Ser Ile Thr Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Ser Ala
               ATG AGA TAT CCA AGT ATT ACA GGA CAA GGC AAC TTT GGA TCT ATA GAT GGT GAT AGT GCC
U63413         Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014CC0001 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL6001 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL6002 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL6003 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL6005 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL6011 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL6014 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL6017 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
```



```
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL6024 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL7003 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL7014 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL7024 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014DL7025 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014GL4009 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014GL5009 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS1001 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS1005 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS1015 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS1021 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS2001 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS2005 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS2010 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS2013 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS2022 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
               ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT
AHRU2014SS2026 Met Arg Tyr Pro Ser Ile Asp Gly Gln Gly Asn Phe Gly Ser Ile Asp Gly Asp Gly Ala
```

```
                 ATG CGT TAT CCA AGT ATC GAT GGA CAA GGA AAC TTT GGT TCT ATC GAT GGT GAT GGC GCT

                                                   130                                     140
L04566           Ala Ala Met Arg Tyr Thr Glu Ala Lys Met Ser Lys Leu Ser His Glu Leu Leu Lys Asp
                 GCT GCG ATG CGT TAT ACT GAA GCA AAA ATG AGT AAA CTT TCT CAT GAG CTT TTA AAA GAT
U63413           Ala Ala Met
                 GCT GCG ATG CG
AHRU2014CC0001   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL6001   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL6002   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL6003   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL6005   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL6011   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL6014   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL6017   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL6024   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL7003   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL7014   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL7024   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014DL7025   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
```



```
AHRU2014GL4009   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014GL5009   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS1001   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS1005   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS1015   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS1021   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS2001   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS2005   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS2010   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS2013   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS2022   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT
AHRU2014SS2026   Ala Ala Met Arg Tyr Thr Glu Ala Arg Met Thr Ile Leu Ala Glu Glu Leu Leu Arg Asp
                 GCT GCA ATG CGT TAT ACT GAA GCT AGA ATG ACA ATT TTA GCA GAA GAG CTT TTA CGC GAT

                                                   150                                     160
L04566           Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Gly Ser Glu Ser Glu Pro Asp
                 ATA GAT AAA GAT ACG GTC GAT TTT GTT CCA AAT TAT GAT GGT TCA GAA AGC GAA CCT GAT
U63413

AHRU2014CC0001   Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
                 ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGC GAG CCC GAT
AHRU2014DL6001   Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
```

Alignment Report of 1 C.jejuni+26 C.coli 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 13
21 µÿ³"§¡ 2016 14:42

```
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL6002 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL6003 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL6005 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL6011 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL6014 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL6017 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL6024 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL7003 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL7014 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL7024 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014DL7025 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014GL4009 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014GL5009 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS1001 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS1005 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS1015 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS1021 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
```

Alignment Report of 1 C.jejuni+26 C.coli 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 14
21 µÿ³"§¡ 2016 14:42

```
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS2001 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS2005 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS2010 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS2013 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS2022 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT
AHRU2014SS2026 Ile Asp Lys Asp Thr Val Asp Phe Val Pro Asn Tyr Asp Asp Ser Met Ser Glu Pro Asp
               ATA GAT AAA GAT ACG GTA GAT TTT GTT CCA AAC TAC GAT GAT TCT ATG AGT GAG CCC GAT

                                                170                                     180
L04566         Val Leu Pro Ser Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT TCT AGG GTT CCA AAT TTA TTA TTA AAT GGT TCA AGT GGT ATA GCT GTA GGT
U63413

AHRU2014CC0001 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL6001 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL6002 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL6003 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL6005 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL6011 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL6014 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
```

Alignment Report of 1 C.jejuni+26 C.coli 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 15
21 µÿ³"§¡ 2016 14:42

```
AHRU2014DL6017 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL6024 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL7003 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL7014 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL7024 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014DL7025 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014GL4009 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014GL5009 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS1001 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS1005 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS1015 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS1021 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS2001 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS2005 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS2010 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS2013 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
AHRU2014SS2022 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT
```

Alignment Report of 1 C.jejuni+26 C.coli 101016.meg ClustalW (Slow/Accurate, Gonnet) Page 16
21 µÿ³"§¡ 2016 14:42

```
AHRU2014SS2026 Val Leu Pro Ala Arg Val Pro Asn Leu Leu Leu Asn Gly Ser Ser Gly Ile Ala Val Gly
               GTT TTA CCT GCT AGG GTG CCA AAT TTA TTA TTA AAT GGC TCT AGT GGT ATT GCT GTA GGT

                                                  190                                     200

L04566         Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Ile Asp Gly Leu Leu Tyr Leu
               ATG GCG ACA AAC ATC CCA CCT CAT AGT TTA AAT GAG TTG ATA GAT GGA CTT TTA TAT TTG
U63413

AHRU2014CC0001 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014DL6001 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014DL6002 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Asp
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014DL6003 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014DL6005 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014DL6011 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Asp
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014DL6014 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014DL6017 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014DL6024 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014DL7003 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014DL7014 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014DL7024 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014DL7025 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu
```

```
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014GL4009 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014GL5009 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014SS1001 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014SS1005 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014SS1015 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014SS1021 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014SS2001 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014SS2005 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014SS2010 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT G
AHRU2014SS2013 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014SS2022 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C
AHRU2014SS2026 Met Ala Thr Asn Ile Pro Pro His Ser Leu Asn Glu Leu Val Asp Gly
               ATG GCG ACA AAT ATT CCT CCG CAT AGT CTT AAC GAG CTT GTA GAT GGA C

                                                210                                     220
L04566         Leu Asp Asn Lys Asp Ala Ser Leu Glu Glu Ile Met Gln Phe Ile Lys Gly Pro Asp Phe
               CTT GAT AAT AAA GAT GCA AGC CTA GAA GAG ATT ATG CAG TTT ATC AAA GGT CCA GAT TTT
U63413

AHRU2014CC0001
```

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

VITA

Mr. Thotsapol Thomrongsuwannakij was born on August 14, 1984 in Bangkok, Thailand. He got the degree of Doctor of Veterinary Medicine (1st Class Honors) from the Faculty of Veterinary Science, Chulalongkorn University, Thailand in March 2008. After that, he became a sale representative at Fort Dodge animal health (Thailand). In October 2010, he enrolled as a PhD student in the Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University.