STUDY OF AEROTOLERANT *CAMPYLOBACTER JEJUNI* IN BROILER PRODUCTION PROCESS

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การศึกษา *แคมไพโลแบคเตอร์ เจจูไน* ชนิดทนต่อออกซิเจนในกระบวนการผลิตเนื้อไก่

น.ส.ณิชนันทน์ จารุศิริเวช

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ณิชนันทน์ จารุศิริเวช : การศึกษา *แคมไพโลแบคเตอร์ เจจูไน* ชนิดทนต่อออกซิเจนในกระบวนการผลิตเนื้อไก่. (STUDY OF AEROTOLERANT *CAMPYLOBACTER JEJUNI* IN BROILER PRODUCTION PROCESS) อ.ที่ปรึกษาหลัก : ผศ. น.สพ.ดร.ธราดล เหลืองทองคำ

แคมไพโลแบคเตอร์ เจจูไน เป็นแบคทีเรียที่สำคัญในการก่อให้เกิดโรคกระเพาะอาหารและลำไส้อักเสบในคนทั่วโลก แม้ว่าแคมไพโลแบคเตอร์ จะเป็นแบคทีเรียที่อาศัยออกซิเจนในปริมาณเพียงเล็กน้อยเพื่อการเจริญและมีความไวต่อการสัมผัสกับออกซิเจน แต่ก็มีการรายงานการพบแคมไพโลแบคเตอร์ชนิดทนต่อออกซิเจนในหลายประเทศ อย่างไรก็ตามข้อมูลเกี่ยวกับแคมไพโลแบคเตอร์ชนิดทนต่อออกซิเจนในประเทศไทยยังมีอยู่อย่างจำกัด ดังนั้นการศึกษาครั้งนี้จึงมีจุดประสงค์เพื่อ 1) ศึกษาความชุกของแคมไพโลแบคเตอร์ เจจูไน ชนิดทนต่อออกซิเจนในฟาร์ม โรงเชือด และซูเปอร์มาเก็ต และเปรียบเทียบความชุกของแคมไพโลแบคเตอร์ เจจูไน ชนิดทนต่อออกซิเจนระหว่างตัวอย่างจากฟาร์ม ได้แก่ อุจจาระจากทวารร่วมและมูลไก่ และตัวอย่างจากโรงเชือด ได้แก่ อุจจาระจากลำไส้ใหญ่และน้ำล้างซาก และ 2) เพื่อวิเคราะห์ลักษณะทางพันธุกรรมของแคมไพโลแบคเตอร์ เจจูไน ที่แยกได้จากแต่ละขั้นตอนในกระบวนการผลิตเนื้อไก่ ในการศึกษานี้ แคมไพโลแบคเตอร์ เจจูไน จำนวน 275 ไอโซเลตที่แยกได้จากตัวอย่างชนิดต่างๆ ถูกนำมาทดสอบความทนต่อออกซิเจน โดยการเพาะเลี้ยงในอาหารเลี้ยงเชื้อชนิดเหลวด้วยการสั่นที่ความเร็ว 150 รอบต่อนาที ภายใต้สภาวะที่มีออกซิเจน ที่ 42 องศาเซลเซียส เป็นเวลา 24 ชั่วโมง นอกจากนี้ แคมไพโลแบคเตอร์ เจจูไน ที่พบยังถูกนำไปวิเคราะห์ลักษณะทางพันธุกรรมเบื้องต้นด้วยวิธี *flaA* SVR sequencing technique และวิธี MLST จากการศึกษาพบว่า ไอโซเลตที่แยกได้จากตัวอย่างที่โรงเชือดมีสัดส่วนของแคมไพโลแบคเตอร์ เจจูไน ที่ทนต่อออกซิเจนสูงที่สุด (56.0%) ตามด้วยไอโซเลตจากตัวอย่างที่ซูเปอร์มาเก็ต (51.0%) และไอโซเลตจากตัวอย่างที่ฟาร์ม (41.3%) ในการเปรียบเทียบสัดส่วนของแคมไพโลแบคเตอร์ เจจูไน ชนิดทนต่อออกซิเจนระหว่างตัวอย่างอุจจาระจากทวารร่วมและมูลไก่ และ ระหว่างตัวอย่างน้ำล้างซากกับอุจจาระจากลำไส้ใหญ่ ของการศึกษานี้ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ และไม่พบแคมไพโลแบคเตอร์ เจจูไน ชนิดทนต่ออากาศเป็นพิเศษ (hyper-aerotolerant strain) เนื่องจากไม่มีไอโซเลตที่สามารถทนต่อการทดสอบได้นานถึง 24 ชั่วโมง โดยการศึกษาครั้งนี้พบว่าแคมไพโลแบคเตอร์ เจจูไน สามารถทนต่อออกซิเจนได้นานสูงสุด 18 ชั่วโมง ซึ่งที่ระยะเวลาดังกล่าวมีความเข้มข้นของเชื้อเท่ากับ 2.59 log CFU/ml, 3.53 log CFU/ml และ 3.06 log CFU/ml ในไอโซเลตจากฟาร์ม โรงเชือด และซูเปอร์มาร์เก็ต ตามลำดับ การศึกษาลักษณะทางพันธุกรรมสามารถระบุเชื้อที่ทนและไม่ทนต่อออกซิเจนด้วยวิธี *flaA* SVR allele type ได้ทั้งหมด 41 แบบ และด้วยวิธี MLST 17 แบบ โดย allele type หลักที่พบในเชื้อชนิดที่ไม่ทนและทนต่อออกซิเจน คือ *flaA* SVR allele type 142 และ 783 ตามลำดับ โดยส่วนใหญ่ลักษณะทางพันธุกรรมที่พบในฟาร์มและโรงเชือดมีลักษณะคล้ายคลึงกัน ผล MLST พบว่า sequence type 460 พบในเชื้อชนิดที่ไม่ทนต่ออากาศเป็นหลัก ในขณะที่ sequence type 1232 พบในเชื้อชนิดที่ทนต่ออากาศเป็นหลัก ในการศึกษานี้ clonal complexes ที่พบมากที่สุด ได้แก่ CC-353 รองลงมาคือ CC-45 โดยส่วนใหญ่ sequence types ที่พบในการศึกษาครั้งนี้ (เช่น ST-1075 และ ST-1232) เคยพบในไก่และผู้ป่วยด้วยโรคกระเพาะอาหารและลำไส้อักเสบของประเทศไทย ซึ่งแสดงให้เห็นว่าแคมไพโลแบคเตอร์ เจจูไน เป็นหนึ่งในสาเหตุสำคัญในการก่อโรคกระเพาะอาหารและลำไส้อักเสบในคน และชี้ให้เห็นถึงความสำคัญของการลดการปนเปื้อนของแคมไพโลแบคเตอร์ในเนื้อไก่ โดยการปฏิบัติตามหลักสุขอนามัยและความปลอดภัยทางชีวภาพ ซึ่งจะช่วยลดการถ่ายทอดเชื้อมาสู่คนและส่งผลให้สามารถลดจำนวนของผู้ป่วยโรคอาหารเป็นพิษจากแคมไพโลแบคเตอร์ได้ในที่สุด

สาขาวิชา	สัตวแพทยสาธารณสุข	ลายมือชื่อนิสิต .....
ปีการศึกษา	2564	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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Nichanan Jarusirivait : STUDY OF AEROTOLERANT *CAMPYLOBACTER JEJUNI* IN BROILER PRODUCTION PROCESS. Advisor: Asst. Prof. TARADON LUANGTONGKUM, D.V.M., Ph.D.

Campylobacter jejuni is the leading cause of human gastroenteritis worldwide. Although they are microaerophile and sensitive to oxygen, aerotolerant *C. jejuni* has been reported in many countries. Currently, limited information on aerotolerant *C. jejuni* in Thailand has been reported. Therefore, the objectives of this study were 1) to examine the occurrence of aerotolerant *C. jejuni* in farms, slaughterhouses and retail markets and compare the level of aerotolerant strain between different sample types at the farm (i.e., cloacal swabs VS manures) and slaughterhouse level (i.e., cecal contents VS carcass rinses) and 2) to identify common genotypes of aerotolerant and aerosensitive *C. jejuni* strains in each poultry production stage. A total of 275 *C. jejuni* strains isolated from cloacal swabs, manures, cecal contents, carcass rinses and raw chicken products were tested for aerotolerance by aerobic shaking at 150 rpm under aerobic condition at 42°C for 24 hours. Representatives of *C. jejuni* isolates were primarily genotyped by *flaA* SVR sequencing technique and further subtyped by MLST. The highest number of aerotolerant *C. jejuni* isolates were found at the slaughterhouse stage (56.0%), followed by the retail market stage (51.0%) and the farm stage (41.3%). The level of aerotolerant *C. jejuni* strain between different sample types at the farm and slaughterhouse level were not significantly different. Hyper-aerotolerant strain was not detected in this study as no bacterial strains survived 24 h of aerobic shaking. The longest viability of *C. jejuni* strains under aerobic culture was 18 h with the viable counts of 2.59 log CFU/ml, 3.53 log CFU/ml and 3.06 log CFU/ml at the farms, slaughterhouses and retail markets, respectively. Forty-one *flaA* SVR allele types and 17 sequence types (STs) were identified among aerotolerant and aerosensitive *C. jejuni* strains. The predominant allele types in aerosensitive and aerotolerant strains was *flaA* SVR allele number 142 and 783, respectively. Mostly, genetic similarity was found in isolates from farms and slaughterhouses. For MLST, the results revealed that the most frequently detected ST in aerosensitive strains was ST-460, while ST-1232 was predominant in aerotolerant strains. The main clonal complexes (CCs) observed in this study were CC- 353, followed by CC-45. Most of the STs (e.g., ST-1075 and 1232) found in this study were previously identified in chickens and gastroenteritis patients in Thailand, which highlight the significance of broilers as one of the most important sources of human campylobacteriosis. Our findings suggested that the reduction of *Campylobacter* contamination in the final products by implementation of good hygienic practices and strict biosecurity in poultry production steps is essential and would help decrease the transmission of *Campylobacter* to human, which eventually reduce the number of foodborne campylobacteriosis cases.

Field of Study:	Veterinary Public Health	Student's Signature .....
Academic Year:	2021	Advisor's Signature .....

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LIST OF ABBREVIATIONS

Bp	base pair
℃	degree (s) Celcius
CFU	colony forming unit
C. jejuni	*Campylobacter jejuni*
flaA SVR	*flaA* short variable region
h	hour (s)
ISO	International Organization for Standardization
mCCDA	Modified Charcoal-Cefoperazone-Deoxycholate
MLST	multilocus sequence typing
PCR	polymerase chain reaction
SE	standard deviation

CHAPTER I
INTRODUCTION

Campylobacter jejuni is the most common cause of gastroenteritis among young children in Thailand (Samosornsuk et al., 2015) and bacterial gastroenteritis caused by *Campylobacter* spp. is trending to increase every year (WHO, 2016). *C. jejuni* and *C. coli* are zoonotic bacteria that can be found in the intestinal tract of various animals especially poultry (Murphy et al., 2006; Bronowski et al., 2014). These organisms are an important cause of Campylobacteriosis. The symptoms of foodborne Campylobacteriosis range from mild to bloody diarrhea and may be followed by post-infection complications such as acute flaccid paralysis known as Guillain-Barré Syndrome (GBS) (Coker et al., 2002).

Campylobacter spp. are gram negative bacteria that require small amount of oxygen for growth (Davis and DiRita, 2008). Despite their microaerophilic nature, recent studies show that they have ability to grow in atmospheric oxygen which means that they are aerotolerant (Kaakoush et al., 2007; Oh et al., 2015). There is a high prevalence of aerotolerant *C. jejuni* in raw chicken meat. In addition, genetic classification of aerotolerant *C. jejuni* strains found in Canada revealed that most of the strains were similar to those identified in gastroenteritis patients (Oh et al., 2015). Moreover, the study in Canada also reported that aerotolerant *C. jejuni* can survive in raw chicken meat for 7 to 14 days, whereas aerosensitive *C. jejuni* have only 3 days viability (Oh et al., 2017). The aerotolerant feature of the bacteria can increase their viability and consequently increase the opportunity in transmission to human.

Contaminated poultry products are the main source of foodborne Campylobacteriosis (Suzuki and Yamamoto, 2009). Poultry products become contaminated with *Campylobacter* during slaughtering of *Campylobacter* positive broiler flocks (van Gerwe et al., 2010). Aerotolerant bacteria can survive longer in

aerobic condition along the production line and this can increase the chance of infection in human (O'Kane and Connerton, 2017) Some strains of aerotolerant *C. jejuni* were found to be invasive and harbor more virulence genes than aerosensitive strains. This may be the reason why aerotolerant *Campylobacter* are often involved in human infection (Sails et al., 2003; Oh et al., 2017).

In Thailand, a high prevalence of *Campylobacter* spp. in broiler production chain starting from breeder flocks to slaughterhouses was reported (Prachantasena et al., 2016). Crop and cecal content of broilers contain as high as 10^5 and 10^9 CFU/g of *Campylobacter* spp., respectively (van Gerwe et al., 2010). The high number of *Campylobacter* in broiler flocks can lead to poultry products contamination, especially during an evisceration step. Although several studies in Thailand reported the distribution of *Campylobacter* spp. throughout poultry production system (Chokboonmongkol et al., 2013; Prachantasena et al., 2016), there is currently no information on aerotolerant *Campylobacter* spp. in Thai broiler farms or slaughterhouses.

Poultry products contaminated with aerotolerant *Campylobacter* can potentially increase the chance of bacterial transmission to humans. The impact that aerotolerant *Campylobacter* have on human health and wellness is an obvious public health concern. Therefore, this study was conducted to examine the presence of aerotolerant *C. jejuni* in bacterial collection obtained from broiler farms, slaughterhouses and retail markets in Thailand and identified sequence types of aerotolerant *C. jejuni* strains commonly found in each poultry production stage.

Objectives of study

1. To examine the presence of aerotolerant *C. jejuni* in bacterial collection obtained from broiler farms, slaughterhouses and retail markets
2. To identify genotypes of aerotolerant *C. jejuni* commonly found in isolates from each chicken production stage

Research questions

1. What is the proportion of aerotolerant *C. jejuni* in bacterial collection obtained from broiler farms, slaughterhouses and retail markets?
2. What are the genotypes of aerotolerant *C. jejuni* commonly found in isolates from each broiler production stage?

CHAPTER II
LITERATURE REVIEW

2.1 Biology of *Campylobacter*

Campylobacter are Gram-negative bacteria that belong to *Campylobacteriaceae* family. *Campylobacter* are spirally curved bacteria with corkscrew-like motility, but they can change into coccoid form when they expose to unfavorable condition (Park, 2002). *Campylobacter* grow optimally under 5% O_2, 10% CO_2 and 85% N_2 at 37 - 42°C (Bronowski et al., 2014). *Campylobacter* utilize amino acids as the main source of energy because they cannot oxidize or ferment carbohydrates (Line et al., 2010). *Campylobacter* have one or two polar flagella for motility. These flagella are important for intestinal colonization (Hermans et al., 2011). They regarded as commensal bacteria in various animals, especially poultry (Hermans et al., 2011; Kim et al., 2015). Despite their ubiquity, *Campylobacter* are fastidious and fragile organisms. They are sensitive to many environmental stresses such as high oxygen tension, desiccation and unable to multiply outside their hosts (Murphy et al., 2006; Bronowski et al., 2014). Despite their thermophilic nature, *C. jejuni* still retain metabolic function and remain motile in temperature as low as 4°C (Hazeleger et al., 1998).

2.2 Survival mechanisms of *Campylobacter* under unfavorable conditions

Campylobacter harbor several stress defense mechanisms to survive under harsh environments encountered during food processing steps or transmission to human. The bacteria enter the viable but non culturable (VBNC) state in response to unfavorable conditions such as starvation or oxygen exposure. In this state, *Campylobacter* change their form from spiral to coccoid and decrease in cell size

(Bronowski et al., 2014). The bacteria still remain viable but do not form colonies on growth media. However, it has been reported that the bacteria have potential to recover from this stage and retain the ability to cause infection. Moreover, expression of genes involving adhesion and invasion was found and ability to adhere to chicken carcasses was also reported during VBNC state (Jang et al., 2007; Patrone et al., 2013). Another survival mechanism of *Campylobacter* under aerobic condition or starvation is biofilm formation (Reeser et al., 2007; Reuter et al., 2010). Biofilm provides microenvironment for *Campylobacter* to remain viable under aerobic condition. Notably, the formation of biofilm varies among strains of the bacteria (Joshua et al., 2006). The study of Mouftah et al. (2021) reported that the ability to form biofilm was associated with aerotolerance status of *Campylobacter*. Hyper-aerotolerant *C. jejuni* were able to form more biofilm than aerotolerant and aerosensitive strains (Mouftah et al., 2021).

2.3 Aerotolerance of *Campylobacter*

Oxygen exposure results in accumulation of reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) in bacterial cells which causes oxidative stress (Lushchak, 2011). ROS are toxic substances commonly generated from i) metabolism of bacterial cells, ii) exposure to atmospheric oxygen and iii) cytotoxic and phagocytic cells in host immune system. These substances can result in bacterial cell death or damages of cellular DNA, protein and lipid (Flint et al., 2016). *Campylobacter* are sensitive to oxygen; however, many studies show that the bacteria employ oxidative stress defense which is essential to aerotolerance. Unlike other bacteria, *Campylobacter* spp. have fewer genes that encode for enzymes that function in eliminating ROS (Flint et al., 2016). Three key major enzymes including superoxide dismutase (SOD), catalase and alkyl hydroperoxide reductase (AhpC) have been found to play an important role in oxidative stress response in *Campylobacter* (Oh et al.,

2015; O'Kane and Connerton, 2017). Mutation of genes that encode for these enzymes result in decrease in ability to survive in aerobic condition and colonization fitness (Palyada et al., 2009; Flint et al., 2012). In addition, complementary mechanism also found to be involved in oxidative stress defense. Methionine sulphoxide reductases (MsrA and MsrB) are enzymes encoded by *msrA* and *msrB*, respectively. These enzymes protect *Campylobacter* from oxidative stress by either scavenging substances related to ROS production or repairing damaged cells caused by ROS (Kim et al., 2015). Similarly, mutation of *msrA* and *msrB* also show increase sensitivity of *Campylobacte*r to oxidative stress (Atack and Kelly, 2008). Other proteins associated with oxidative stress response in *Campylobacter* are cytochrome c peroxidase, quinone reductase and several DNA repairing proteins (Atack and Kelly, 2008).

2.4 Prevalence of aerotolerant *Campylobacter* in poultry production system

Campylobacter have long been recognized as microaerophilic bacteria that are sensitive to oxygen but recently, several studies showed that the bacteria have aerotolerance feature (Kaakoush et al., 2007; Rodrigues et al., 2015; Oh et al., 2017). Although aerotolerance of *Campylobacter* has been studied in many aspects, study of the bacteria in poultry production system has not been widely investigated. In Canada, the study of Oh et al. (2015) showed that there was a high prevalence of hyper-aerotolerant and aerotolerant *C. jejuni* in raw chicken meats purchased from supermarkets. However, data of the prevalence of aerotolerant *Campylobacter* in farms and slaughterhouses are still not available. In Thailand, the data of aerotolerant *Campylobacter* in poultry production chain has not been reported as well.

2.5 Genetic characterization of *Campylobacter*

Many genotyping methods are now available for *Campylobacter* genetic characterization such as pulsed-field gel electrophoresis (PFGE) (Yan et al., 1991), DNA sequencing of the flagellin gene short variable region (*flaA* SVR) (Meinersmann et al., 1997), multilocus sequence typing (MLST) (Dingle et al., 2001), *flaA* restriction fragment length polymorphism of the flagellin gene (*flaA*-RFLP) (Nebola and Steinhauserova, 2006) and comparative genomic fingerprint (CGF) (Taboada et al., 2012). These methods have been proven to be useful in molecular subtyping of *Campylobacter* spp. PFGE is considered as a gold standard for genotyping most bacterial species, including *Campylobacter*. Although PFGE provides high discriminatory power, it is expensive and time consuming (Tabit, 2016). MLST is also widely used for molecular typing in many bacteria. This technique is based on sequencing of fairly conserved seven housekeeping genes and is considered as a suitable technique for *Campylobacter* genotyping because of its high discriminatory power and reproducibility (Patchanee, 2012). However, this method is expensive and labor-intensive. Similary, *flaA* SVR technique is based on sequencing of amplified short variable region of flagellin A gene. This technique provides sufficient discriminatory power with cost-effectiveness (Wassenaar and Newell, 2000). Sequence-based methods, such as *flaA* SVR and MLST, are useful for tracking the sources of outbreak. Moreover, the results from these schemes can be compared globally via electronic database (pubMLST.org/campylobacter) (Dingle et al., 2001), whereas interlaboratory comparison of other methods, such as PFGE or CGF, is not feasible (Noormohamed and Fakhr, 2014). However, relying on the result of single DNA sequencing subtyping method is a concern due to frequent genetic recombination among *Campylobacter* population which consequently results in genetic instability (Schouls et al., 2003). In order to obtain reliable results, many studies suggest the combination of molecular

typing techniques for outbreak investigation (Wassenaar and Newell, 2000; Sails et al., 2003). The combination of *flaA* SVR and MLST provides reliable level of discrimination for both short- and long-term study of *Campylobacter* population (Price et al., 2006).

2.6 Sequencing of *flaA* SVR in *Campylobacter*

flaA SVR technique is one of many molecular techniques that are proven to be useful in the study of genetic distribution of *Campylobacter*. Many studies have been successfully applied this technique to track the sources of *Campylobacter* contamination in poultry production system (Wassenaar et al., 2009; Singh and Kwon, 2013; Zhang et al., 2018). Several studies revealed that allele types present within poultry flocks were genetically diverse (El-Adawy et al., 2013; Marotta et al., 2015). Generally, allele types identified within the flocks were usually present in the final processed poultry products indicating that poultry flocks are the major source of carcass contamination (Hiett et al., 2007; Marotta et al., 2015; Prachantasena et al., 2016).

2.7 MLST subtyping of *Campylobacter*

The studies of molecular epidemiology in *Campylobacter* by using MLST are increasingly common among countries as interlaboratory results can be readily compared via online database (pubMLST.org/campylobacter) (Sheppard et al., 2009). Oh et al. (2015) revealed that most of aerotolerant *Campylobacter* strains isolated from raw chicken meats belonged to ST-21 and ST-45 complex. These two clonal complexes are considered as the most dominant groups identified in many regions around the world such as America, Australia, Canada and Europe (pubMLST.org/campylobacter). According to *Campylobacter* MLST database, ST-21

and ST-45 complex are highly prevalent in *Campylobacter* isolates from human origins and are found to be associated with isolates from gastroenteritis patients (Nielsen et al., 2010; Colles and Maiden, 2012). Interestingly, ST-45 complex is considered as environmentally adapted strains which can survive well outside hosts and in harsh environmental conditions (Sopwith et al., 2008). In Thailand, MLST analysis of *Campylobacter* strains isolated from broiler flocks and slaughterhouses revealed that the dominant clonal complexes were ST-45, ST-353, ST-354 and ST-574 complex (Prachantasena et al., 2016). Unlike other studies, ST-21 complex is rarely detected in Thailand (Griekspoor et al., 2010; Prachantasena et al., 2016). ST-45 and ST-353 complex are commonly found in isolates of human and poultry origins (Habib et al., 2009; Sheppard et al., 2009). Likewise, ST-354 and ST-574 complex are dominant clonal complexes mainly found in human and poultry in Thailand (pubMLST.org/campylobacter). In addition, the study of Wongbundit et al. (2016) revealed that ST-574 was commonly identified in *C. jejuni* isolated from both Thai children with diarrhea and broilers. Moreover, MLST analysis suggested that *Campylobacter* isolates from chickens are often associated with human Campylobacteriosis (Sheppard et al., 2009). These data support the evidence that contaminated chicken meat and products are the main source of human infection.

CHAPTER III
MATERIALS AND METHODS

This study consists of 4 phases. The first phase is sample collection and selection of *C. jejuni* isolates, the second phase is *Campylobacter* isolation and confirmation, the third phase is aerotolerance test and the last phase is molecular subtyping of aerotolerant *C. jejuni* isolates. Research outline of this study is shown in Figure 1.

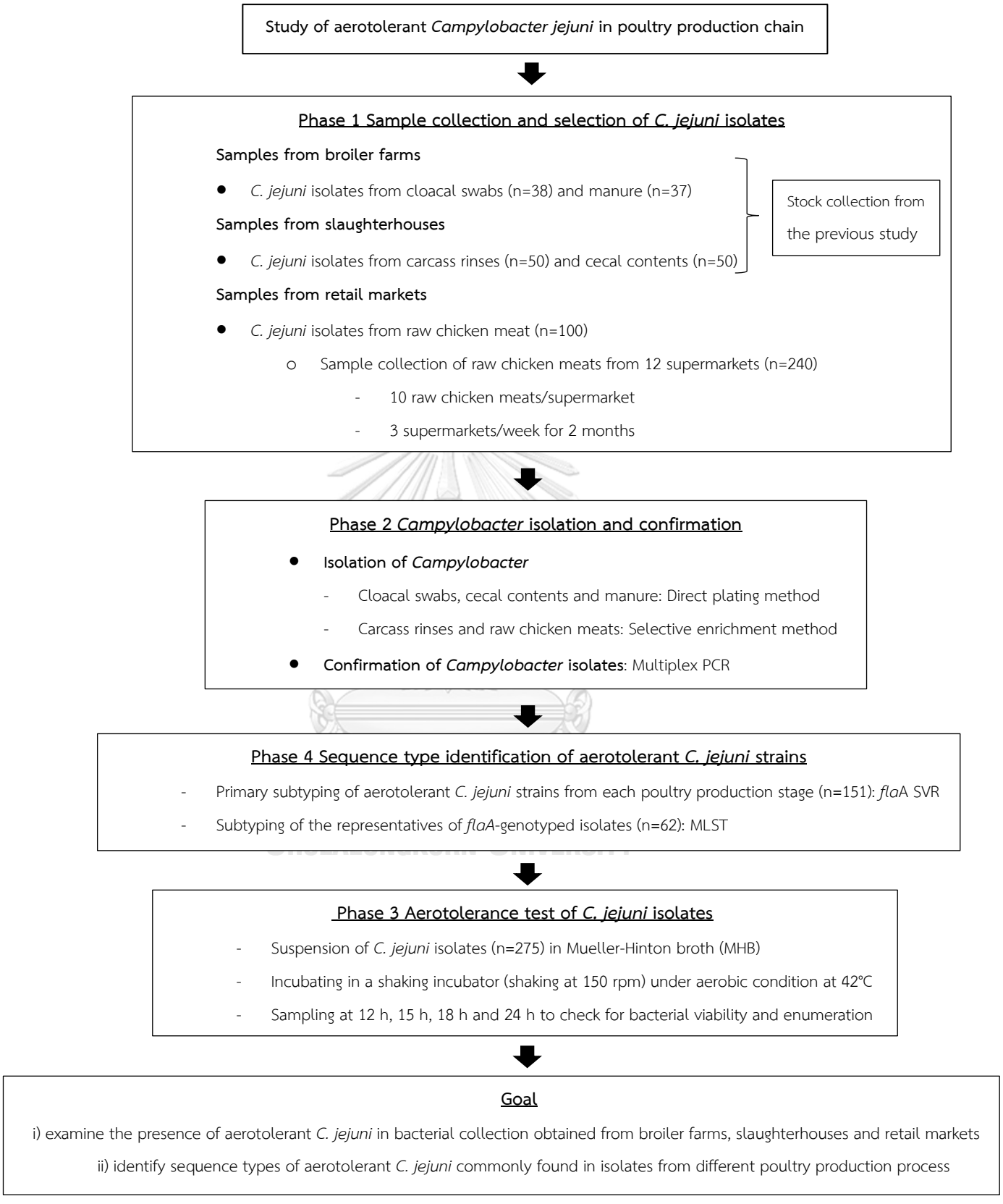

Figure 1 Research outline of this study

Phase 1 Sample collection and selection of *C. jejuni* isolates

In this study, *C. jejuni* isolates were obtained from the previous study and from new sample collection of raw poultry products. Five sample types from different poultry production stages which are broiler farms, slaughterhouses and retail markets were included in this study. Criteria for selection of sample types are based on our hypothesis that each sample type may be exposed to different levels of oxygen for different duration which consequently result in different levels of aerotolerance.

1.1 Samples from broiler farms

Samples from broiler farms consist of cloacal swabs and manure as we hypothesize that manure may be exposed to higher level of oxygen for longer duration than cloacal swabs. *Campylobacter* from cloacal swab and manure samples were recovered from the stock collection of the previous study. Briefly, the cloacal swab and manure samples were collected from 12 large-scale broiler farms located in central region of Thailand where the highest density of broilers population has been reported over the last five years by the Department of Livestock Development, Ministry of Agriculture and Cooperative, Thailand.

1.2 Samples from slaughterhouses

Samples from slaughterhouses comprised carcass rinses and cecal contents as we speculate that carcass rinses may be exposed to higher level of oxygen for longer duration than cecal contents. *C. jejuni* isolated from carcass rinses and cecal contents were recovered from the stock collection of the previous study. *Campylobacter* strains were isolated from carcass rinse and cecal content samples obtained from a slaughterhouse where broilers described in 1.1 were sent for processing. This slaughterhouse belongs to one of Thailand's leading integrated agriculture and food businesses located in Lopburi province.

1.3 Samples from retail markets

Samples from retail markets were raw chicken products. According to sample size calculation in 1.4, around 200 raw chicken products were collected from 12 supermarkets in Bangkok, which belong to two major supermarket chains in Thailand. Raw chicken products (n=10) were collected from each supermarket. Three different supermarkets were visited weekly. Each supermarket was visited twice during 2 months period of sample collection.

1.4 Sample size calculation

The number of isolates used for aerotolerance test in this study was calculated based on the prevalence of *Campylobacter* in Thai poultry production system reported in the previous studies by using 95% confidence interval, 10% desired precision, 20%, 40% and 50% expected prevalence for broiler farms, slaughterhouses and retail markets, respectively (Padungtod and Kaneene, 2005; Vindigni et al., 2007; Prachantasena et al., 2016). Approximately 270 target *C. jejuni* isolates which is the sum of *C. jejuni* isolates from broiler farms (n=75), slaughterhouses (n=100) and retail markets (n=100) were tested for aerotolerance. *C. jejuni* isolates from broiler farms and slaughterhouses were obtained from the previous study, whereas *C. jejuni* in retail market stage were isolated from raw chicken meat samples. Around 200 raw chicken products were collected in order to obtain approximately 100 target *C. jejuni* isolates.

Phase 2 *Campylobacter* isolation and confirmation

C. jejuni isolates from farms and slaughterhouses were obtained from the previous study. *Campylobacter* were isolated from cloacal swab, manure and cecal content samples by using direct plating method, while selective enrichment method was used for isolation of *Campylobacter* from carcass rinse samples. To isolate *Campylobacter* from raw chicken samples, selective enrichment method were

performed according to the ISO 10272-1: 2017 (ISO, 2017). Briefly, 10 g of raw chicken portion was added to 90 ml of Preston broth (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) to obtain a 1:10 dilution ratio and then the mixture was homogenized in stomacher machine for 1 minute. The mixture of samples and broth were incubated at 42°C under microaerobic condition for 24 h ± 2 h. After 24 h of incubation, a loopful of enrichment broth was aseptically streaked on to modified Charcoal-Cefoperazone-Deoxycholate Agar (mCCDA) (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and the inoculated plates were incubated at 42°C for 44 h ± 4 h under microaerobic condition.

Presumptive colonies of *Campylobacter* were selected and confirmed by multiplex PCR with specific primers according to the previously published protocol with some modifications (Wang et al., 2002). DNA templates were prepared by whole cell boiling method. DNA amplification were performed in a total volume of 25 µl PCR reaction mixture containing 12.5 µl of Kapa Taq ReadyMix DNA polymerase (Kapa biosystem, Wilmington, MA, USA), 1.25 µl of each 0.5 µM *C. jejuni* forward and reverse primer, 2.5 µl of each 1 µM *C. coli* forward and reverse primers and 0.5 µl of each 0.2 µM 23S rRNA forward and reverse primers and 2.5 µl of DNA template. The final volume was adjusted to 25 µl by adding sterile distilled water. PCR thermocycling conditions were performed as follows: an initial denaturation at 95°C for 6 minutes; followed by 30 cycles of amplification: denaturation at 95°C for 45 seconds, annealing at 58°C for 45 seconds, extension at 72°C for 45 seconds; and final extension at 72°C for 7 minutes. PCR amplification products were analyzed in 1.2% agarose gel electrophoresis. The primers previously published by Wang et al. (2002) were used for confirmation of *Campylobacter* species in this study. These primers are shown in Table 1.

Table 1 Primers for *Campylobacter* multiplex PCR (Wang et al., 2002)

Primer	Sequence (5' to 3')	Target gene	Size
23SF	TATACCGGTAAGGAGTGCTGGAG	23S rRNA	650 bp
23SR	ATCAATTAACCTTCGAGCACCG		
CCF	GTAAAACCAAAGCTTATCGTG	*glyA*	126 bp
CCR	TCCAGCAATGTGTGCAATG		
CJF	ACTTCTTTATTGCTTGCTGC	*hipO*	323 bp
CJR	GCCACAACAAGTAAAGAAGC		

Phase 3 Aerotolerance test

Confirmed *C. jejuni* isolates from each poultry production stage were tested for aerotolerance. The experiment was carried out by resuspending overnight culture of *C. jejuni* isolates on Mueller-Hinton agar (MH agar) (Difco™, MD, USA) into MH broth (Difco™, MD, USA) and diluted to an OD_{600} of 0.2 which is equivalent to approximate cell density of 10^8 CFU/ml. Bacterial suspension was incubated at 42°C under aerobic condition with shaking at 150 rpm in a shaking incubator. Samples were taken at 12, 15, 18, and 24 hours to check for the number of viable bacteria. Bacterial counts were examined on MHA according to the previously published paper (O'Kane and Connerton, 2017). The aerotolerance test was performed in duplicate. *C. jejuni* NCTC11168 was used as a control strain. The bacterial strains were classified into aerosensitive, aerotolerant and hyper-aerotolerant group based on the level of aerotolerance (Oh et al., 2015). Aerosensitive group means the bacteria lose their viability before 12 h of aerobic culture, whereas aerotolerant and hyper-aerotolerant

groups mean that the bacterial strains can survive between 12 h to approximately 24 h or remain viable after 24 h of aerobic shaking, respectively.

Phase 4 Sequence type identification of aerotolerant *C. jejuni* in each production stage

flaA SVR sequencing technique was used for primary screening of genotypes in aerotolerant *C. jejuni* strains (n=151). Additionally, representative *flaA* SVR subtyped strains were selected for further sequence type identification by MLST (n=62).

4.1 Sequencing of *flaA* short variable region (*flaA* SVR)

The *flaA* gene amplification was performed according to Meinersmann et al. (1997). DNA templates were prepared by whole cell boiling method. PCR reaction was performed in 25 µl mixture consisted of 1X PCR buffer (Kapabiosis, Boston, MA, USA), 0.4 mM of dNTP and 1.25 U of KapaTaq polymerase (Kapabiosis, Boston, MA, USA). DNA amplification was performed as follows: initial denaturation at 94°C for 1 minute followed by denaturation, annealing and extension at 92°C for 30 seconds, 55°C for 90 seconds, and at 72°C for 2.5 minutes, respectively for 35 cycles with a final extension at 72°C for 5 minutes. The 425 bp DNA amplicons products were purified by NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel and Düren, Germany). The purified products were submitted for sequencing at 1st BASE Pte Ltd. (Selangor Darul Ehsan, Malaysia). Nucleotide sequences were compared to the published sequences of *flaA* SVR alleles via online database (http://pubmlst.org/campylobacter/flaA/). Primers for *flaA* SVR identification and sequencing are shown in Table 2.

Table 2 Oligonucleotide primers for *flaA* SVR identification (Meinersmann et al., 1997)

Primer	Sequence (5' to 3')	Size
FLA242FU	CTA TGG ATG AGC AAT T(AT)A AAA T	425 bp
FLA625RU	CAA G(AT)C CTG TTC C(AT)A CTG AAG	

4.2 Multilocus sequence typing (MLST)

The aerotolerant *C. jejuni* strains primarily subtyped by *flaA* SVR were selected for further subtyping by MLST (n=62) according to the protocol previously described by Dingle et al. (2001). Seven house-keeping genes comprise of *aspA* (aspartase A), *glnA* (glutamine synthetase), *gltA* (citrate synthase), *glyA* (serine hydroxymethyltransferase), *pgm* (phosphoglucomutase), *tkt* (transketolase) and *uncA* (ATP synthase α subunit) were amplified by PCR using primers shown in Table 3. Each 25 µl of PCR reaction consisted of 5 µl of DNA template, 10 pmol of the gene-specific primers, 1XPCR buffer, 1.5mM of Mg^{2+}, 0.4 mM of dNTP and 1.25 U of KapaTaq DNA polymerase (Kapabiosis, Boston, MA, USA). The PCR reaction was performed as follows: denaturation at 94°C for 2 minutes, annealing at 50°C for 1 minute and extension at 72°C for 1 minute for a total of 35 cycles. The size of amplified products were visualized by gel electrophoresis and the PCR products were purified using NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel and Düren, Germany). The purified products were submitted for sequencing at 1st BASE Pte Ltd. (Selangor Darul Ehsan, Malaysia). The sequence type of the isolates was identified by submitting the sequences of genes into the *C. jejuni* MLST database (http://pubmlst.org/campylobacter/MLST).

Table 3 Oligonucleotide primers for *C. jejuni* MLST (Dingle et al., 2001)

Locus	Function	Primer sequences		Amplicon size (bp)
		Forward primer	Reverse primer	
aspA	Amplification	5'-AGT ACT AAT GAT GCT TAT CC-3'	5'-ATT TCA TCA ATT TGT TCT TTG C-3'	899
	Sequencing	5'-CCA ACT GCA AGA TGC TGT ACC-3'	5'-TTA ATT TGC GGT AAT ACC ATC-3'	
glnA	Amplification	5'-TAG GAA CTT GGC ATC ATA TTA CC-3'	5'-TTG GAC GAG CTT CTA CTG GC-3'	1,262
	Sequencing	5'-CAT GCA ATC AAT GAA GAA AC-3'	5'-TTC CAT AAG CTC ATA TGA AC-3'	
gltA	Amplification	5'-GGG CTT GAC TTC TAC AGC TAC TTG-3'	5'-CCA AAT AAA GTT GTC TTG GAC GG-3'	1,012
	Sequencing	5'-GTG GCT ATC CTA TAG AGT GGC-3'	5'-CCA AAG CGC ACC AAT ACC TG-3'	
glyA	Amplification	5'-GAG TTA GAG CGT CAA TGT GAA GG-3'	5'-AAA CCT CTG GCA GTA AGG GC-3'	816
	Sequencing	5'-AGC TAA TCA AGG TGT TTA TGC GG-3'	5'-AGG TGA TTA TCC GTT CCA TCG C-3'	
pgm	Amplification	5'-TAC TAA TAA TAT CTT AGT AGG-3'	5'-CAC AAC ATT TTT CAT TTC TTT TTC-3'	1,150
	Sequencing	5'-GGT TTT AGA TGT GGC TCA TG-3'	5'-TCC AGA ATA GCG AAA TAA GG-3'	
tkt	Amplification	5'-GCA AAC TCA GGA CAC CCA GG-3'	5'-AAA GCA TTG TTA ATG GCT GC-3'	1,102
	Sequencing	5'-GCT TAG CAG ATA TTT TAA GTG-3'	5'-ACT TCT TCA CCC AAA GGT GCG-3'	
uncA	Amplification	5'-ATG GAC TTA AGA ATA TTA TGG C-3'	5'-GCT AAG CGG AGA ATA AGG TGG-3'	1,120
	Sequencing	5'-TGT TGC AAT TGG TCA AAA GC-3'	5'-TGC CTC ATC TAA ATC ACT AGC-3'	

Data analysis

Descriptive statistic was used to describe different levels of aerotolerant *C. jejuni* in bacterial collection obtained from each poultry production stage and sample type. Chi-square was used for comparing the proportion of aerotolerant *C. jejuni* strains in broiler farms, slaughterhouses and retail markets ($p<0.05$). In addition, the proportion of aerotolerant *C. jejuni* strains between sample types in each poultry production stage was compared using chi-square. IBM SPSS program version 22.0 was used for chi-square statistical analysis ($p<0.05$).

CHAPTER IV
RESULTS

4.1 Proportion of aerotolerant *C. jejuni* in different poultry production stages

A total of 275 *C. jejuni* isolates including 75 isolates from farms, 100 isolates from slaughterhouses and 100 isolates from retail markets were tested for aerotolerance. According to the criteria described in 3.3.1, *C. jejuni* isolates that can survive for 12 h or longer but less than 24 h under aerobic condition were considered as aerotolerant strain, whereas isolates that lost viability before 12 h were classified as aerosensitive strain. In addition, isolates that remain viable after 24 h of aerobic culture were considered hyper-aerotolerant strain. In this study, the highest proportion of aerotolerant *C. jejuni* was found among isolates from slaughterhouses (56/100; 56%), followed by isolates from retail markets (51/100; 51%) and isolates from farms (31/75; 41.33%), respectively (Figure 2). No hyper-aerotolerant *C. jejuni* strain was detected in this study. Despite our hypothesis that *C. jejuni* isolates from retail markets may expose to higher level of oxygen for longer duration than samples from other stages, the proportion of aerotolerant *C. jejuni* among production stages was not significantly different (p=0.15).

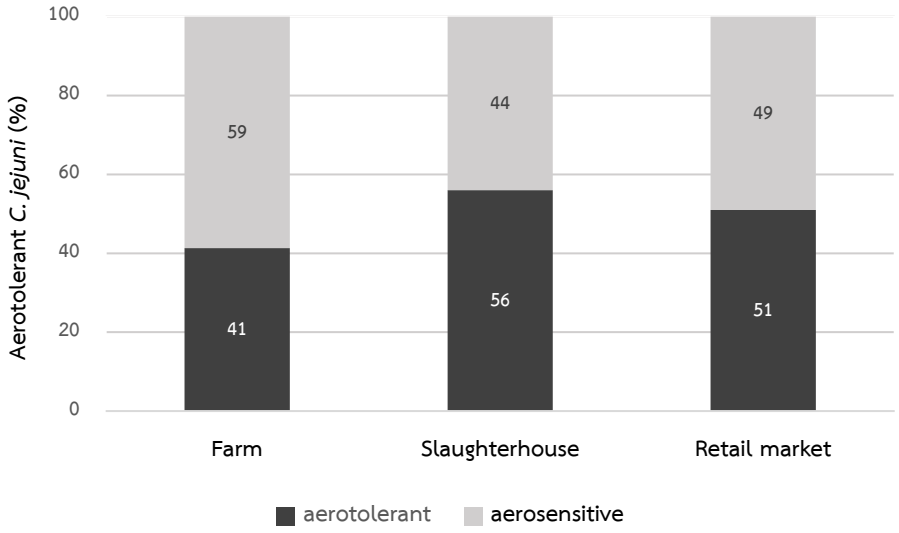

Figure 2 Proportion of aerosensitive and aerotolerant *C. jejuni* strains in each production stage

Additionally, a comparison of aerotolerant *C. jejuni* level among different types of samples was conducted. According to our hypothesis, various sample types may expose to different level of oxygen which may subsequently result in different proportion of aerotolerant *C. jejuni.* For example, at the farm level, manures may expose to oxygen for longer period than cloacal swabs, while at the slaughterhouse level, carcass rinses may expose to higher level of oxygen than cecal contents. The results showed that 43% (16/37) and 39% (15/38) of the isolates from manures and cloacal swabs were aerotolerant *C. jejuni,* respectively (Figure 3). Surprisingly, the isolates from cecal contents (29/50; 58%) had higher proportion of aerotolerant strains than the isolates from carcass rinses (27/50; 54%) (Figure 3). However, the difference in proportion of aerotolerant *C. jejuni* between isolates from cloacal swabs and manures (p=0.74) and between carcass rinses and cecal contents (p=0.68) was not statistically significant.

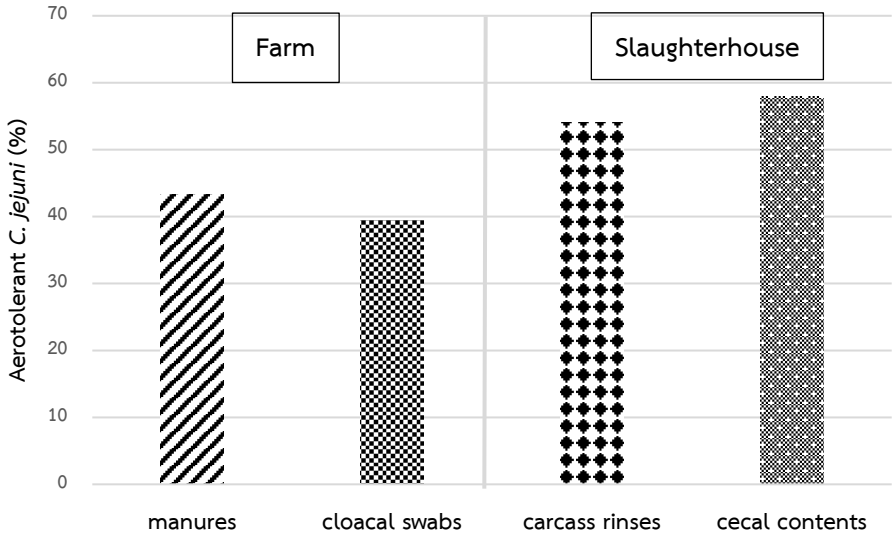

Figure 3 Proportion of aerotolerant *C. jejuni* in different sample types

The longest viability of the aerotolerant *C. jejuni* strains in this study was around 18 h under aerobic culture. Most of the bacterial strains could be recovered at 12 h but fewer strains survived at 15 and 18 h of aerobic shaking. At 12 h, 31, 56 and 51 isolates were recovered from farms, slaughterhouses, and retail markets, respectively while 22 isolates from farms, 24 isolates from slaughterhouses and 18 isolates from retail markets survived the aerobic shaking for 15 hours. At 18 h under aerobic condition, only a few aerotolerant strains could be recovered from the entire production process and none of the bacterial isolates survived the aerobic culture for 24 hours (Figure 4).

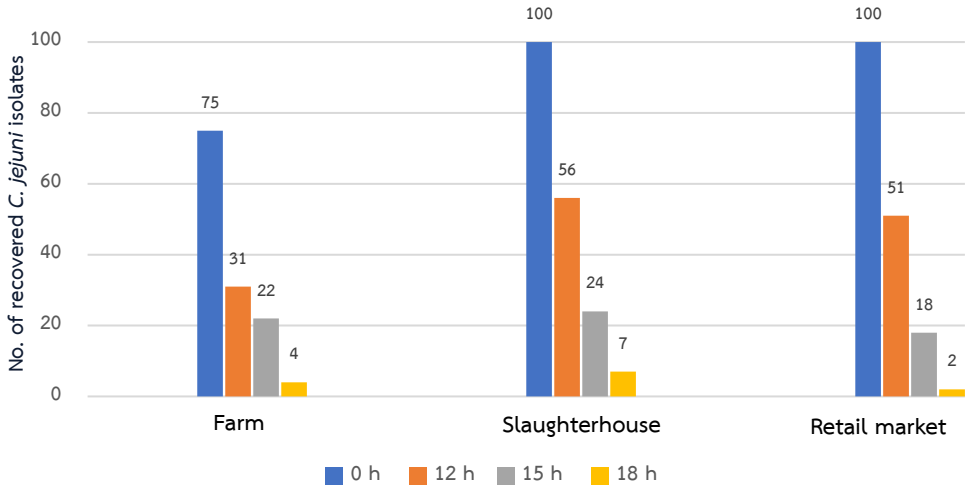

Figure 4 Number of aerotolerant *C. jejuni* isolates recovered at 12, 15 and 18 h of aerobic shaking

4.2 Mean CFU counts of aerotolerant *C. jejuni* in different poultry production stages

Samples from *C. jejuni* suspension were recovered at 12, 15, 18 and 24 h of aerobic cultivation to quantitatively evaluate the viable bacteria. The mean bacterial counts among aerotolerant strains at the farm level at 12, 15 and 18 h of aerobic shaking were 5.60 log CFU/ml (3.41-7.19 log CFU/ml; SE=0.16), 4.16 log CFU/ml (1.95-5.69 log CFU/ml; SE=0.22) and 2.59 log CFU/ml (2-3.14 log CFU/ml; SE=0.29), respectively. Likewise, the mean viable counts at the slaughterhouse stage at the same time period were 5.14 log CFU/ml (1.90-7.62 log CFU/ml; SE=0.17), 4.30 log CFU/ml (1.90-6.47 log CFU/ml; SE=0.26) and 3.53 log CFU/ml (2.20-5.44 log CFU/ml; SE=0.55), respectively. At the retail market stage, the mean bacterial counts were 5.39 log CFU/ml (2.34-7.39 log CFU/ml; SE=0.16), 3.91 log CFU/ml (2.11-5.55 log CFU/ml; SE=0.27) and 3.06 log CFU/ml (2.46-3.67 log CFU/ml; SE=0.60) at 12, 15 and 18 h of aerobic culture, respectively. Notably, isolates from farms had around 1.5 log CFU/ml reduction of bacterial counts between each time period, whereas approximately 0.8

log CFU/ml reduction was found among isolates from slaughterhouses. Bacterial counts among isolates from retail markets decreased by 1.5 and 1 log CFU/ml from 12 to 15 h and 15 to 18 h of aerobic culture, respectively (Figure 5).

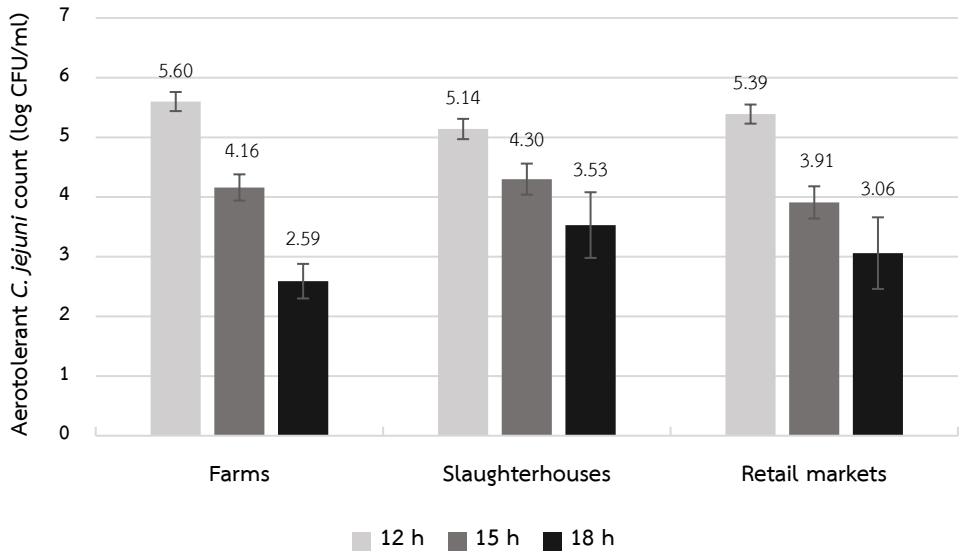

Figure 5 Mean CFU count (log CFU/ml) of aerotolerant *C. jejuni* strains from farms, slaughterhouses and retail markets at 12, 15 and 18 h of aerobic shaking

4.3 Genotypic identification of *C. jejuni* strains

4.3.1 *flaA* SVR genotyping

Of all 275 isolates tested for aerotolerance, at least 40% of aerotolerant isolates and 30% of aerosensitive isolates from each production stage were selected for *flaA* SVR genotyping. Overall, 151 representative isolates including 59 aerosensitive and 92 aerotolerant strains were selected for primary subtyping by *flaA* SVR sequencing technique. In total, 41 *flaA* SVR allele types were identified in this study. In aerosensitive strains, 28 *flaA* SVR allele types were found. Similarly, 28 *flaA* SVR allele types were detected in aerotolerant strains and 15 *flaA* SVR allele types were observed in both aerosensitive and aerotolerant strains (Figure 6).

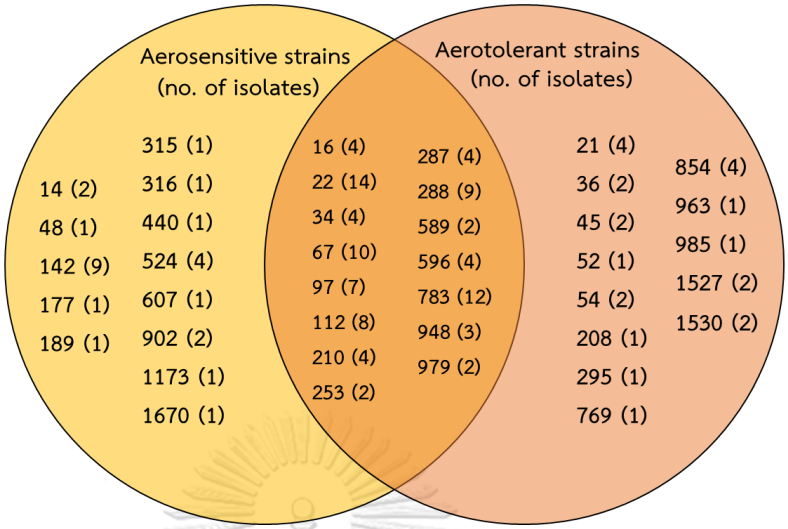

Figure 6 *flaA* SVR allele types of aerosensitive and aerotolerant *C. jejuni* identified in this study

In this study, *C. jejuni* isolates were recovered from broiler samples collected from 10 broiler farms at both farm and slaughterhouse levels. Overall, multiple *flaA* SVR allele types were detected in each farm. Mostly, *flaA* SVR allele types that found on the farms were also detected in the slaughterhouses regardless of the aerotolerant status of *C. jejuni* isolates.

At the farm level, 22 aerosensitive and 14 aerotolerant *C. jejuni* isolates were selected for *flaA* SVR typing. Overall, 15 *flaA* SVR allele types were identified. *flaA* SVR allele number 34, 67, 142, 288, 524, 607, 948, 1173 and 1670 were found only in aerosensitive strains, while *flaA* SVR allele number 21, 52, 783 and 854 were detected only in aerotolerant strains. Additionally, *flaA* SVR allele number 97 and 112 were observed in both aerosensitive and aerotolerant strains. The most common allele type in aerosensitive strains was *flaA* SVR allele number 142, whereas *flaA* SVR allele number 783 was the most prevalent allele type in aerotolerant strains (Figure 7).

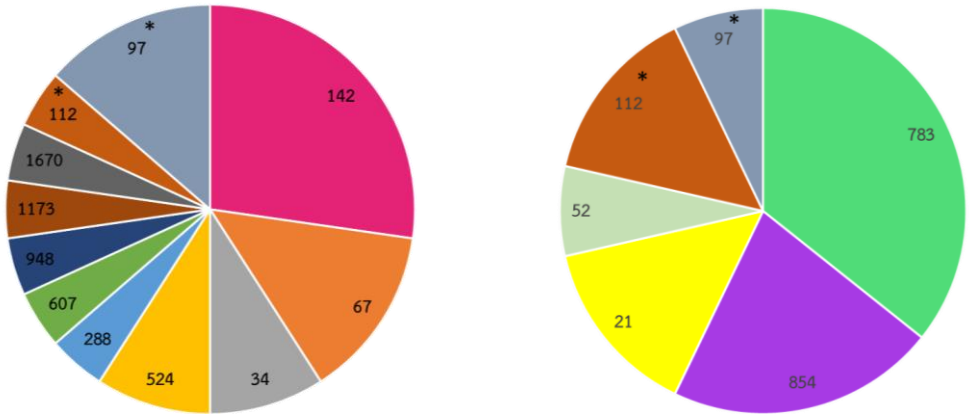

Figure 7 *flaA* SVR allele types identified in aerosensitive and aerotolerant strains at the farm level. Numbers represent *flaA* SVR allele types. Asterisk (*) defined as *flaA* SVR allele types that found in both aerosensitive and aerotolerant strains.

At the slaughterhouse level, 23 *flaA* SVR allele types were identified among 48 selected isolates. Twelve and 17 *flaA* SVR allele types were found among 15 aerosensitive strains and 33 aerotolerant strains, respectively. Additionally, 6 *flaA* SVR allele types including 16, 67, 97, 112, 589 and 948 were detected in both aerosensitive and aerotolerant strains. Similar to the farm level, predominant *flaA* SVR allele types in aerosensitive strains was *flaA* SVR allele number 142, whereas *flaA* SVR allele number 67 was the most common allele type found in aerotolerant strains (Figure 8).

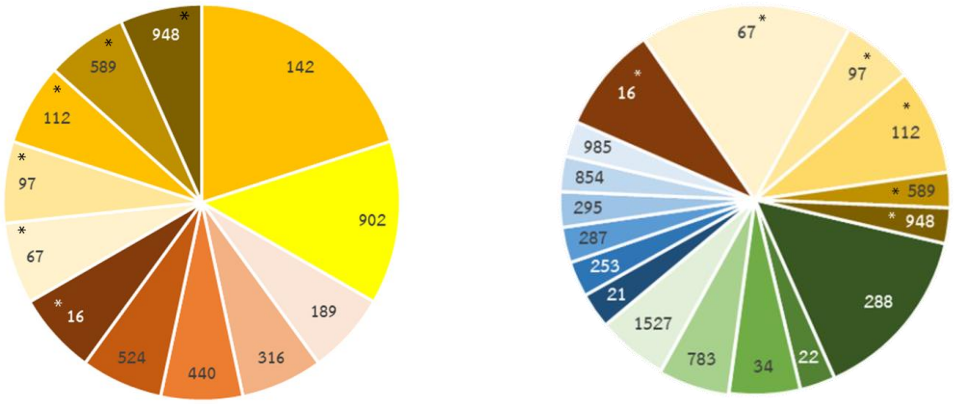

Figure 8 *flaA* SVR allele types identified in aerosensitive and aerotolerant strains at the slaughterhouse level. Numbers represent *flaA* SVR allele types. Asterisk (*) defined as *flaA* SVR allele types that found in both aerosensitive and aerotolerant strains.

To determine genetic similarity between *C. jejuni* isolates from farms and slaughterhouses, *flaA* SVR allele types of isolates from both production sites were compared. Mostly, genotypes found at the farm level were also detected at the slaughterhouse level. For example, *flaA* SVR allele number 142 was the dominant allele type found at farm and slaughterhouse levels of farm A and all isolates were identified as aerosensitive strain. Similarly, only *flaA* SVR allele number 34 was detected at both production sites of farm B and these isolates were classified as aerosensitive and aerotolerant strains. Two most frequently detected *flaA* SVR allele types (i.e., *flaA* SVR allele number 97 and 783) were observed at both farm and slaughterhouse levels of farm C. Interestingly, *flaA* SVR allele number 97 was described as both aerosensitive and aerotolerant strains, whereas *flaA* SVR allele number 783 was identified as aerotolerant strain. Similar to *flaA* SVR allele number 783, *flaA* SVR allele number 21, which was one of the most common genotypes detected in F, was all classified as aerotolerant strain and this genotype was detected at both farm and

slaughterhouse levels. *flaA* SVR allele number 112 and 948 were the predominant genotype of farm G and I, respectively. These *flaA* SVR allele types were found at both production stages and were identified as both aerosensitive and aerotolerant strains. Interestingly, *flaA* SVR allele number 67 was not only the predominant genotype of farm D but also the predominant genotype of farm E. This *flaA* SVR allele type was detected at both production sites and was classified as both aerosensitive and aerotolerant strains. Likewise, *flaA* SVR allele number 783 found in farm C and D was all classified as aerotolerant strain. Although, majority of genotypes that detected in farm were also presented in slaughterhouses, certain *flaA* SVR allele types sometimes observed only at the farm or slaughterhouse level. For example, *flaA* SVR allele number 16 and 589 were the predominant genotypes of farm F and J, respectively. Both genotypes were found only in slaughterhouses. Unlike other farms, no common *flaA* SVR allele types were shared among *C. jejuni* isolates in both farm and slaughterhouse stages of farm H. One genotype (*flaA* SVR allele number 524) was found at the farm level which was all identified as aerosensitive strain, while two genotypes (*flaA* SVR allele number 288 and 440) were detected at the slaughterhouse level. *flaA* SVR allele number 288 was described as aerotolerant strain, while *flaA* SVR allele number 440 was classified as aerosensitive strain (Table 4).

Table 4 *flaA* SVR allele types of aerosensitive and aerotolerant *C. jejuni* strains identified in farms and slaughterhouses

Farm	***flaA* SVR genotype (no. of isolates)**			
	Aerotolerant strain		Aerosensitive strain	
	Farm	Slaughterhouse	Farm	Slaughterhouse
A	N/A	22 (1)	142 (6) [a]	142 (3)
B	N/A	34 (2) [b]	34 (2) [a]	N/A
C	97 (1) 783 (5)	97 (1) 287 (1) 288 (3) 783 (1)	97 (3)	97 (1)
D	854 (2)	67 (3) 253 (1) 783 (1) 1527 (1)	67 (1) 112 (1) 1670 (1)	902 (1)
E	112 (1)	67 (2) 295 (1)	67 (2)	67 (1) 524 (1) 902 (1)
F [c]	21 (2) 52 (1)	16 (3) 21 (1)	N/A	16 (1)
G	112 (1)	112 (3)	288 (1) 607 (1) 1173 (1)	112 (1) 189 (1)
H	N/A	288 (2)	524 (2) [a]	440 (1)
I	854 (1)	948 (1) 985 (1)	948 (1)	948 (1)
J [d]	N/A	67 (1) 589 (1) 854 (1) 1527 (1)	N/A	316 (1) 589 (1)

[a] - All isolates from farm were tested as aerosensitive strains.

[b] - All isolates from slaughterhouse were tested as aerotolerant strains.

[c] - All isolates from farm were tested as aerotolerant strains.

[d] - No isolates from farm were tested since *C. jejuni* isolates from farm J could not be recovered from the stock collection.

In retail markets, aerotolerance tested isolates were obtained from 2 supermarket chains located in Bangkok and vicinity. Fifty-five isolates composed of 22 isolates from aerosensitive strains and 33 isolates from aerotolerant strains were selected for *flaA* SVR typing. In total, 23 *flaA* SVR allele types were detected. Six *flaA* SVR allele types including 14, 48, 177, 253, 315 and 524 were only detected in aerosensitive strains, whereas 10 *flaA* SVR allele numbers 21, 36, 45, 54, 97, 112, 208, 769, 963 and 1530 were only found in aerotolerant strains. In addition, 7 *flaA* SVR allele types 22, 210, 287, 288, 596, 783 and 979 were found in both aerosensitive and aerotolerant strains. The most predominant *flaA* SVR allele type identified in both aerosensitive and aerotolerant strains was *flaA* SVR allele number 22 which was accounted for 23.6 percent of *flaA* SVR genotyped isolates at retail market stage (Figure 9)

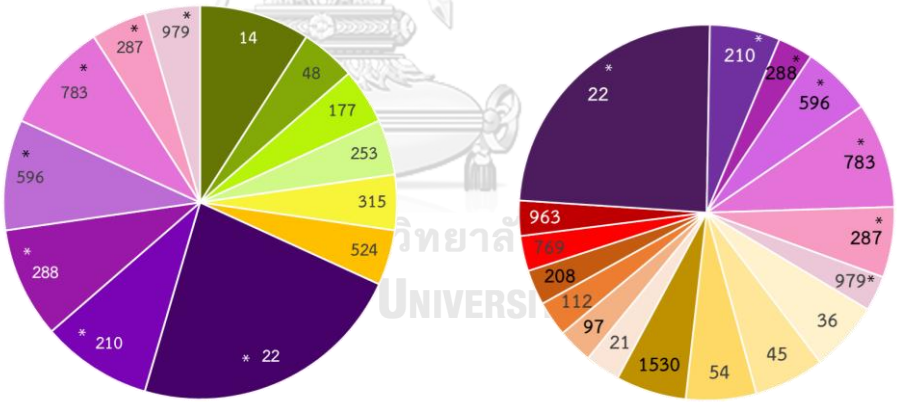

Figure 9 *flaA* SVR allele types identified in aerosensitive and aerotolerant strains at the retail market level. Numbers represent *flaA* SVR allele types. Asterisk (*) defined as *flaA* SVR allele types that found in both aerosensitive and aerotolerant strains.

4.3.2 MLST identification

Approximately, 40% of *C. jejuni* isolates that were primarily genotyped by *flaA* SVR technique in each production stage were selected for further genetic identification by MLST. Overall, 62 isolates comprising 22 aerosensitive and 40 aerotolerant strains were selected. Seventeen isolates were from farms, 20 isolates were from slaughterhouses and 25 isolates were from retail markets.

Among 62 isolates genotyped by MLST, 17 sequence types (STs) were detected. Ten STs (ST-137, 354, 583, 1075, 1232, 1898, 2274, 2325, 6455, and 6736) were found in both aerosensitive and aerotolerant strains. Four STs (ST-45, 460, 2281 and 8835) were only detected in aerosensitive strains and three STs (ST-51, 464 and 1919) were found only in aerotolerant strains. From 17 identified STs, 12 STs can be clustered into 7 clonal complexes (CCs), whereas 5 STs (ST-2274, 2325, 6455, 6736 and 8835) cannot be grouped into any available CCs (Table 5).

Table 5 Clonal complexes and sequence types identified in aerosensitive and aerotolerant *C. jejuni* strains in this study

MLST		
Clonal complex	**Sequence types** (no. of isolates)	
	Aerotolerant strain	**Aerosensitive strain**
45	137 (3) 583 (3)	45 (1) 137 (2) 583 (2)
52	1919 (1)	N/A
353	1075 (5) 1232 (10) 1898 (2)	1075 (1) 1232 (2) 1898 (2) 2281 (1)
354	354 (1)	354 (1)
443	51 (4)	N/A
460	N/A	460 (4)
464	464 (1)	N/A
Not assigned	2274 (4) 2325 (1) 6455 (1) 6736 (4)	2274 (2) 2325 (1) 6455 (1) 6736 (1) 8835 (1)

The most common CCs identified in this study were CC-353, followed by CC-45 (Figure 10). These 2 CCs were distributed throughout the production processes in both aerosensitive and aerotolerant strains. At the farm and slaughterhouse stages, CC-443 were found only in aerotolerant strains, while CC-460 were detected only in aerosensitive strains. In addition, CC-52 was identified only in aerotolerant *C. jejuni*

strain from slaughterhouses. At the retail market stage, CC-354 was observed in both aerosensitive and aerotolerant *C. jejuni* strains, whereas CC-464 was only detected in aerotolerant strain (Figure 11).

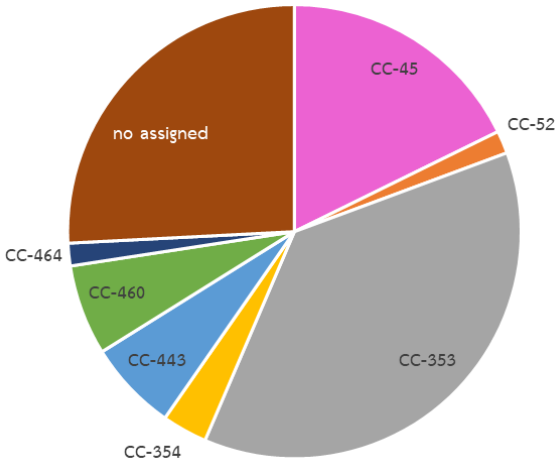

Figure 10 proportion of clonal complexes (CCs) identified in aerosensitive and aerotolerant *C. jejuni* in this study

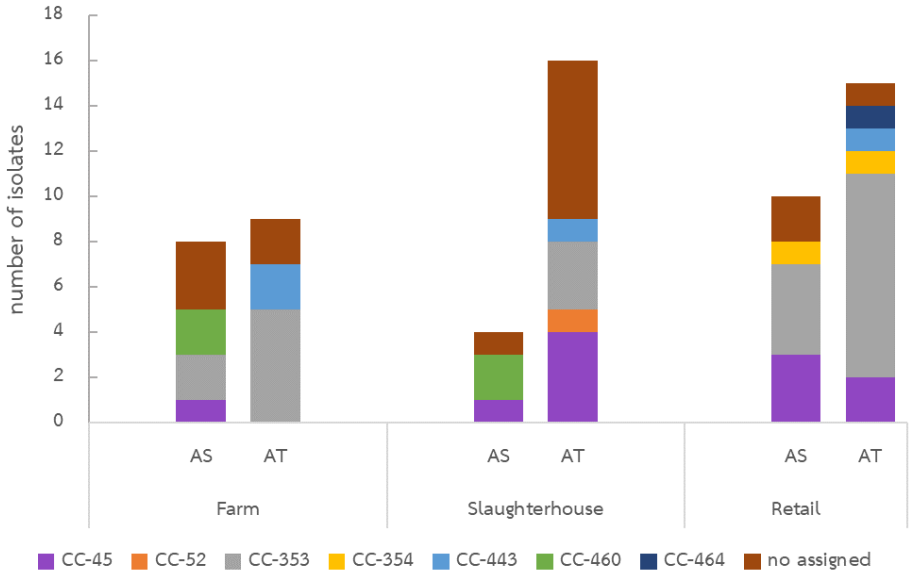

Figure 11 Distribution of clonal complexes of aerosensitive (AS) and aerotolerant (AT) strains of *C. jejuni* in different poultry production processes

In this study, multiple sequence types were detected in each production stage. Predominant sequence type in aerosensitive strains was ST- 460, while the most frequently detected sequence type in aerotolerant strains was ST-1232.

At the farm level, 8 aerosensitive *C. jejuni* isolates and 9 aerotolerant *C. jejuni* isolates were selected for MLST. Overall, these *C. jejuni* isolates were assigned into 8 sequence types. ST-137, 460, 1898, 2274, 6455 and 6736 were detected in aerosensitive strains, while 4 sequence types (ST-51, 1232, 1898 and 2274) were found in aerotolerant strains. Among these identified STs, two sequence types (ST-1898 and 2274) were observed in both aerosensitive and aerotolerant strains (Figure 12).

At the slaughterhouse level, 10 sequence types (ST-51, 137, 460, 583, 1232, 1898, 1919, 2274, 6455 and 6736) were identified among 20 *C. jejuni* isolates. In aerosensitive strains, 3 sequence types (ST-137, 460 and 2274) were found, whereas 9 sequence types (ST-51, 137, 583, 1232, 1898, 1919, 2274, 6455 and 6736) were

observed among 16 aerotolerant *C. jejuni* isolates. Additionally, ST-137 and 2274 were detected in both aerosensitive and aerotolerant strains (Figure 12).

At the retail market level, 11 sequence types (ST-45, 51, 137, 354, 464, 583, 1075, 1232, 2281, 2325, 8835) were noticed among 25 *C. jejuni* isolates. In aerosensitive strains, 8 sequence types (ST-45, 354, 583, 1075, 1232, 2281, 2325 and 8835) were identified from 10 *C. jejuni* isolates Similarly, 8 sequence types (ST-51, 137, 354, 464, 583, 1075, 1232 and 2325) were found among 15 aerotolerant *C. jejuni* isolates. Common sequence types observed in both aerosensitive and aerotolerant strains were ST-354, 583, 1075, 1232 and 2325 (Figure 12).

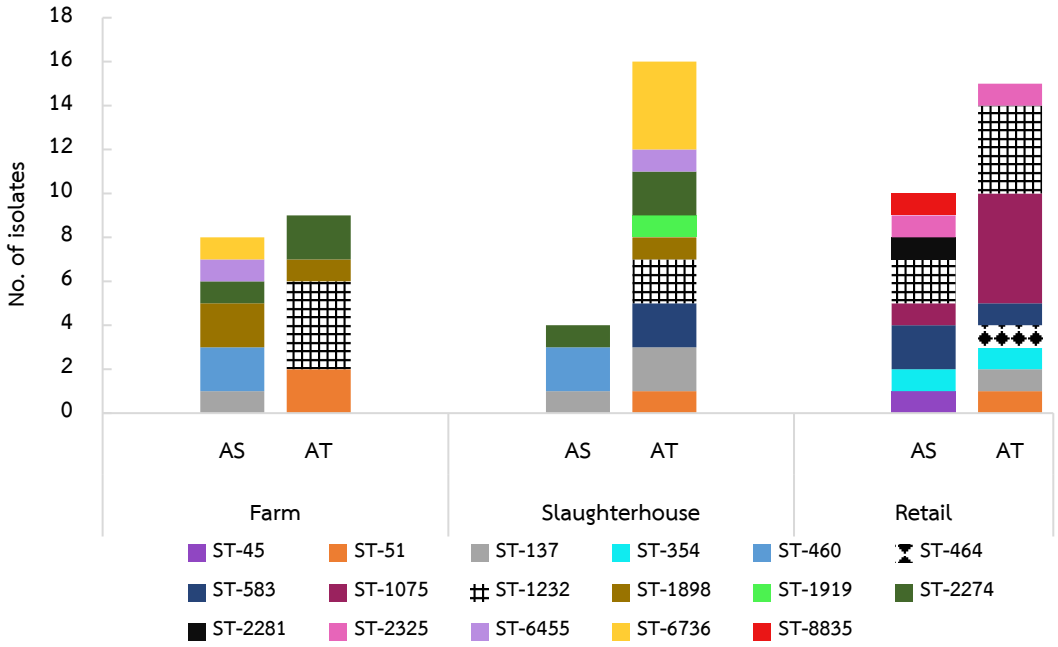

Figure 12 Distribution of sequence types of aerosensitive (AS) and aerotolerant (AT) strains of *C. jejuni* in different poultry production processes

A neighbor-joining tree was constructed based on the MLST results to analyze phylogenetic relationship among *C. jejuni* strains. The isolates were clustered into 5 subgroups. Isolates from all of the production processes with both aerosensitive and aerotolerant strains were distributed in subgroups I, III, IV and V. In subgroup II, no isolates from retail market were found. Interestingly, CC-353 was distributed in several subgroups (i.e., subgroup I, III and V). In this study, the highest number of CCs was found in subgroup I (i.e., CC-353, 354, 443 and 460), while subgroup II, IV and V comprised only one CC which was CC-52, 45 and 353, respectively. Two CCs (CC-353 and 464) were found in subgroup III. In addition, two STs (ST-6455 and 6736) and three STs (ST-2274, 2325 and 8835) that were unassigned to any available CCs were observed in subgroup II and V, respectively (Figure 13). The number of aerotolerant strain was higher than aerosensitive strain in all subgroups. The highest proportion of aerotolerant strain was found among isolates in subgroup II, whereas isolates in subgroup IV had the highest proportion of aerosensitive strain. (Figure 14).

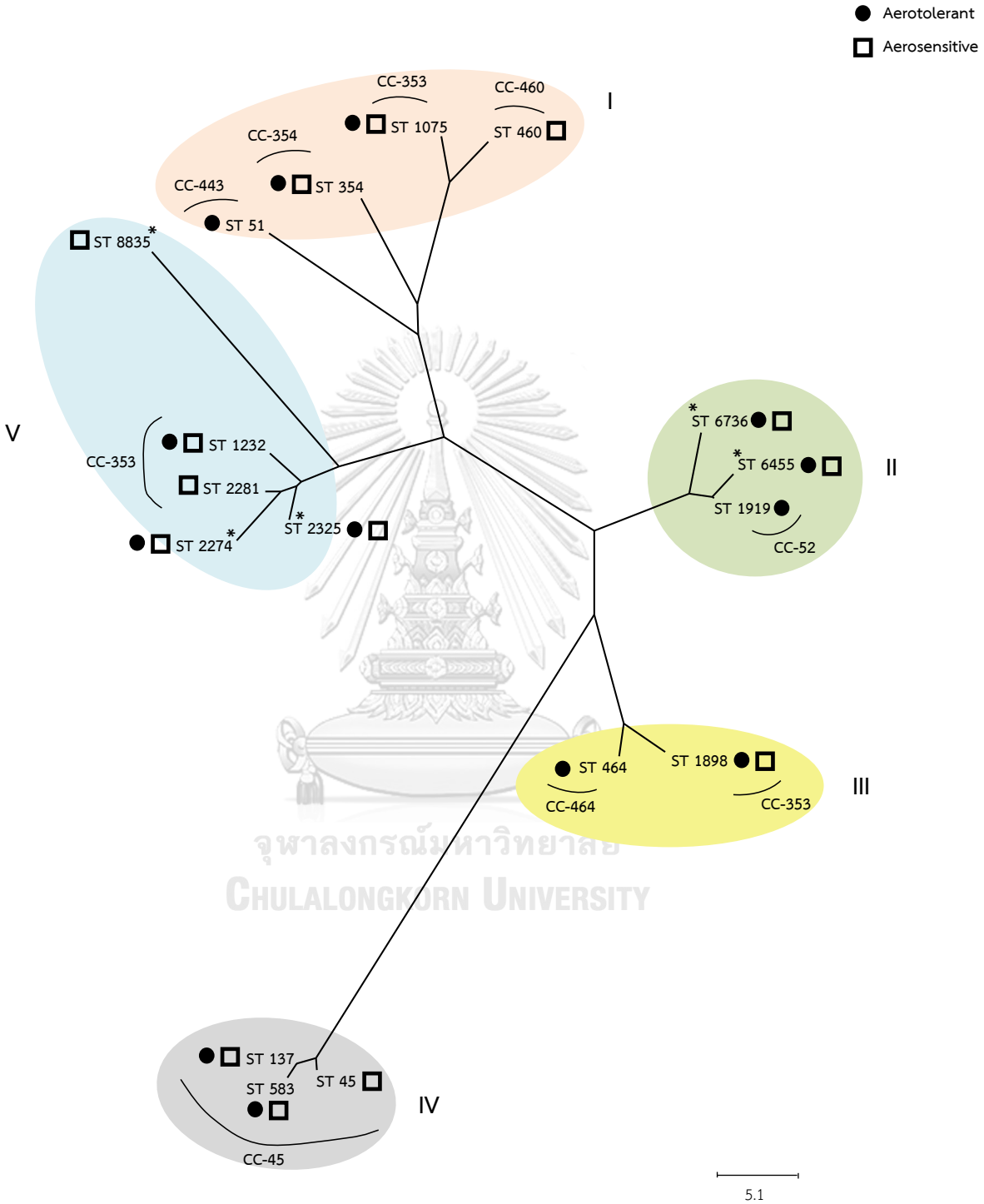

Figure 13 Phylogenetic relationship of aerosensitive and aerotolerant *Campylobacter jejuni* strains. Distribution of sequence types in aerosensitive and aerotolerant strains were represented by different geometric shapes. Asterisk (*) defined as unassigned clonal complexes.

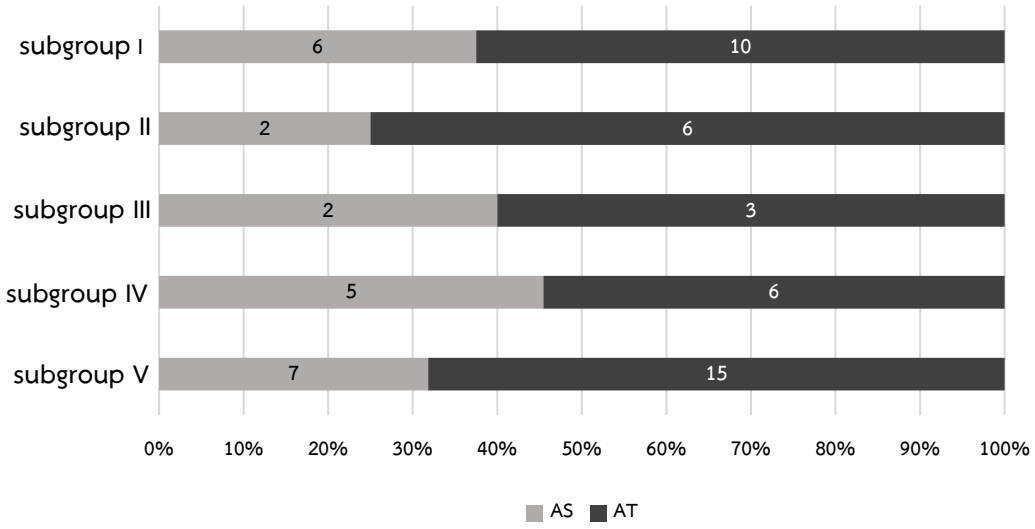

Figure 14 Proportion of aerosensitive and aerotolerant *C. jejuni* strains among isolates in each subgroup. AS, aerosensitive; AT, aerotolerant. The numbers inside the bar chart represent the number of isolates in each subgroup.

4.3.3 Correlation between *flaA* SVR and MLST

Generally, certain *flaA* SVR allele types were found to be associated with specific sequence types. For example, *flaA* SVR allele number 142 was only found in isolates from aerosensitive strain and they were all genotyped as ST-460. Likewise, *flaA* SVR allele number 21 was detected in aerotolerant strain and was assigned as ST-51. *flaA* SVR allele number 112 and 210 found in both aerosensitive and aerotolerant strains were classified as ST-2274 and ST-354, respectively. Conversely, single *flaA* SVR allele type could result in multiple sequence types as well. For instance, *flaA* SVR allele number 22 was genotyped as ST-1075 in aerotolerant strains, while this *flaA* SVR allele type belonged to ST-45 and ST-8835 in aerosensitive strains. Similarly, *flaA* SVR allele number 783 was genotyped as ST-1232 in aerotolerant strains, whereas this *flaA* SVR allele type was identified as ST-1075 and ST-2281 in aerosensitive strains. Likewise, *flaA* SVR allele number 288 was assigned as ST-583 but this *flaA* SVR genotype could also be classified as ST-137. In addition, *flaA* SVR allele number 54 was identified as

ST137 and 464 in aerotolerant strain. Moreover, *flaA* SVR number 67 found in both aerosensitive and aerotolerant *C. jejuni* strains was identified as ST-1919 in aerotolerant strain and as ST-6455 and 6736 in both aerosensitive and aerotolerant strains. *flaA* SVR allele number 97 was classified as ST-1898 in both aerosensitive and aerotolerant strains, while ST-137 was assigned from aerosensitive strain. In addition, ST-2325 in both aerosensitive and aerotolerant strains and ST-1232 in aerotolerant strain were all assigned *flaA* SVR allele number 287 (Table 6). On the contrary, individual ST was linked to multiple distinct *flaA* SVR allele types. For instance, ST- 1232 was genotyped from *flaA* SVR allele number 287, 596 and 783. Likewise, ST-1075 was identified from *flaA* SVR allele number 22 in aerotolerant strains and *flaA* SVR allele number 783 in aerosensitive strain. ST-137 was genotyped from *flaA* SVR allele number 54 in aerotolerant strain and *flaA* SVR allele number 97 in aerosensitive strain. In addition, this sequence type was identified from *flaA* SVR allele number 288 in both aerosensitive and aerotolerant strains (Table 7). In this study, some of the *flaA* SVR allele types appear to be associated with particular genotypes identified by MLST method. Notably, genotypic differences between aerosensitive and aerotolerant strains suggested that aerotolerant status of *C. jejuni* isolates could be related to particular genotypes.

Table 6 Comparison of *C. jejuni* genotypes identified by *flaA* SVR sequencing and MLST techniques

***flaA* SVR allele type** (no. of isolates)	**MLST sequence type** (no. of isolates)	
	Aerotolerant strain	**Aerosensitive strain**
21 (4)	51 (4)	N/A
22 (7)	1075 (5)	45 (1) 8835 (1)
54 (2)	137 (1) 464 (1)	N/A
67 (8)	1919 (1) 6455 (1) 6736 (4)	6455 (1) 6736 (1)
97 (5)	1898 (2)	137 (1) 1898 (2)
112 (6)	2274 (4)	2274 (2)
142 (4)	N/A	460 (4)
210 (2)	354 (1)	354 (1)
287 (3)	1232 (1) 2325 (1)	2325 (1)
288 (8)	137 (2) 583 (3)	137 (1) 583 (2)
596 (4)	1232 (2)	1232 (2)
783 (9)	1232 (7)	1075 (1) 2281 (1)

N/A, no aerosensitive or aerotolerant *C. jejuni* strain in each sequence type was identified from a particular *flaA* SVR allele type.

Table 7 Correlation between genotypes of *C. jejuni* isolates identified by *flaA* SVR sequencing and MLST techniques

Sequence type (no. of isolates)	***flaA* SVR allele type** (no. of isolates)	
	Aerotolerant strain	**Aerosensitive strain**
45 (1)	N/A	22 (1)
51 (4)	21 (4)	N/A
137 (5)	54 (1) 288 (2)	97 (1) 288 (1)
354 (2)	210 (1)	210 (1)
460 (4)	N/A	142 (4)
464 (1)	54 (1)	N/A
583 (5)	288 (3)	288 (2)
1075 (6)	22 (5)	783 (1)
1919 (1)	67 (1)	N/A
1232 (12)	287 (1) 596 (2) 783 (7)	596 (2)
1898 (4)	97 (2)	97 (2)
2274 (6)	112 (4)	112 (2)
2281 (1)	N/A	783 (1)
2325 (2)	287 (1)	287 (1)
6455 (2)	67 (1)	67 (1)
6736 (5)	67 (4)	67 (1)
8835 (1)	N/A	22 (1)

N/A, no aerosensitive or aerotolerant *C. jejuni* strain in each *flaA* SVR allele type was identified from a particular sequence type.

CHAPTER V

DISCUSSION

5.1 Occurrence of aerotolerant *C. jejuni* in poultry production processes

In this study, the occurrence of aerotolerant *C. jejuni* in farms, slaughterhouses and retail markets was compared. According to our hypothesis, exposure to higher level of oxygen for longer period might result in higher level of aerotolerance. There was a study reported that frequent oxygen exposure might induce the bacteria to become more aerotolerant (O'Kane and Connerton, 2017). Samples from retail markets were expected to have the highest level of aerotolerant *C. jejuni* since they may expose to higher level of oxygen for longer duration than samples from other stages. However, the results of our study showed that the occurrence of aerotolerant *C. jejuni* in farm, slaughterhouse and retail market stage was not significantly different. The level of aerotolerant *C. jejuni* among isolates from farms, slaughterhouses and retail markets was 41.3%, 56% and 51%, respectively, while the study of Oh et al. (2015) found that 71.4% of *C. jejuni* in retail chicken products were aerotolerant strain. In addition, the study conducted in Japan showed that 58.6% of *C. jejuni* isolates from various sources remained viable under aerobic condition at least 12 h (Kiatsomphob et al., 2019). Interestingly, hyper-aerotolerant strain was not found in this study as no bacterial strains survived 24 hours of aerobic shaking. Unlike our study, hyper-aerotolerant *C. jejuni* in the study of Oh et al. (2015) and Kiatsomphob et al. (2019) were 35.71% and 40%, respectively. Aerotolerant status of *C. jejuni* might be affected by sources and strains of bacterial isolates. *C. jejuni* isolates included in this study were collected only from chickens, while isolates in other studies were obtained from various sources (e.g., human, cattle and environment) (Oh et al., 2015; Kiatsomphob et al., 2019). Moreover, discrepancies within bacterial strains (e.g., gene expression) could affect survival

capacity under aerobic condition (Rodrigues et al., 2015). According to Ruiz et al. (2018), there was a correlation between sample type as well as genotype and oxidative stress sensitivity which resulted in different level of aerobic stress tolerance.

Among samples collected from the slaughterhouse stage, we speculated that *C. jejuni* isolates from carcass rinse would have higher proportion of aerotolerant strain than isolates from cecum as this type of sample might expose to higher level of oxygen. There was evidence showed that repeat oxygen exposure of *C. jejuni* increased its aerotolerance (O'Kane and Connerton, 2017). However, our results demonstrated that *C. jejuni* isolates from cecal contents had higher proportion of aerotolerant strain than isolates from carcass rinses although the difference was not statistically significant. This finding was different from the study of Ruiz et al. (2018), which found that *C. jejuni* isolates obtained from cecal contents showed less resistant to aerobic stress than isolates from chicken meats and neck skins. The discrepancies of the results between studies suggested that besides oxygen exposure (<1.4% in cecum and 21% in the atmosphere), other factors, such as bacterial sources and strains can contribute to the aerotolerance level of *C. jejuni* (Rodrigues et al., 2015; O'Kane and Connerton, 2017; Ugarte-Ruiz et al., 2018).

Our study and the study of Oh et al. (2015) conducted the aerotolerance test at 42°C, while the study of Kiatsomphob et al. (2019) performed the test at 37°C. Even though 42°C is the optimal growth temperature for *C. jejuni*, Garenaux et al. (2008) reported that *C. jejuni* was more sensitive to oxidative stress at this temperature than at lower temperature. At low temperature (e.g., 4°C), over-expression of oxidative stress proteins was found in *C. jejuni* in response to cold shock which allows the bacteria to be more resistant to aerobic stress. Unsurprisingly, the higher prevalence of hyper-aerotolerant *C. jejuni* was found when the aerotolerance test was conducted at 37°C.

Many factors seem to play a vital role in the aerotolerance of *C. jejuni.* Although there might be other unknown factors that affect this feature, aerotolerant trait was

proved to be stable. In addition, the study of O'Kane and Connerton (2017) also showed that aerotolerant *C. jejuni* were effective in chicken colonization. Moreover, aerotolerant *C. jejuni* strain retained its aerotolerance after it was recovered from challenged chickens.

5.2 Distribution and genetic relatedness of aerotolerant *C. jejuni* strains from poultry production chains

Genetic profiles of representative aerosensitive and aerotolerant *C. jejuni* strains were identified by *flaA* SVR sequencing technique and MLST. In this study, most genotypes found at the farm and slaughterhouse levels were similar. Our result was in agreement with the study of Prachantasena et al. (2016) reporting that genetic similarity between broiler flocks and final processed products from slaughterhouses was observed. According to Hiett et al. (2007), broilers served as the main sources of contamination in the final carcass products which results in detection of similar *flaA* SVR genotypes at both production sites. However, in some cases, *flaA* SVR allele types found in broiler flocks could differ from those identified in slaughterhouses (Hiett et al., 2007). The additional sources of different genotypes such as residues from the previous processed carcasses may lead to contamination of the final products. Thus, resulting in different *flaA* SVR allele types between broiler flocks and final processed products (Hiett et al., 2007). Although there was no report of relationship between specific *flaA* SVR allele types and aerotolerant status, the results of this study showed that aerotolerance and aerosensitive may link to specific *flaA* SVR allele type. For example, we found that *flaA* SVR allele number 142 and 854 detected at the farm and slaughterhouse levels were all identified as aerosensitive and aerotolerant strain, respectively. Likewise, *flaA* SVR allele number 21 and 524 found in all of the production stages were classified as aerotolerant and aerosensitive strain, respectively.

In terms of the MLST results, the main clonal complexes identified in this study were CC-353, followed by CC-45. These clonal complexes found in both aerotolerant and aerosensitive *C. jejuni* strains. CC-353 was one of the most common clonal complexes identified in *C. jejuni* isolates from human and poultry sources (Ragimbeau et al., 2008; Sheppard et al., 2009). Meanwhile, CC-45 was described as environmentally adapted complex and *C. jejuni* that belonged to this CC were able to survive under aerobic condition (Habib et al., 2010). The results of our study were concordant with the study of Prachantasena et al. (2016) which found that these two CCs were frequently detected among *C. jejuni* isolates in Thai poultry production system. Compared to the studies in Canada and Japan, CC-21 was the most frequently detected CCs in both aerotolerant and aerosensitive strains (Oh et al., 2015; Kiatsomphob et al., 2019). According to the study of Colles and Maiden (2012), CC-21 was found in a variety of sources as they suggested that this CC may evolve to survive in diverse environment and hosts. In addition, CC-21 and CC-45 were the most dominant genotypes identified from gastroenteritis patients (Nielsen et al., 2010). Mouftah et al. (2020) reported that isolates in CC-21 and CC-45 contained certain genetic of fitness which allows them to be more aerotolerant and survive in stressful environment encountered during poultry processing. Despite the frequent detection of CC-21 among *C. jejuni* population in many studies, this CC was not observed in our study. According to MLST database (http://pubmlst.org/campylobacter), CC-21 was uncommon in Thailand. The differences in clonal complexes observed among studies could possibly be explained by the study of Manning et al. (2003) which indicated that source of isolates or host specificity may play an important role in different predominant clonal complexes found in *C. jejuni* isolates in each country.

In this study, ST-1232 was the most frequently detected sequence type in aerotolerant *C. jejuni* strain. This sequence type was detected among *C. jejuni* population in Thailand including isolates obtained from chicken and human sources

(Prachantasena et al., 2016; Wongbundit et al., 2016). Likewise, ST-1075 was the second most predominant genotype identified only in aerotolerant *C. jejuni* strain. Majority of this sequence type was found in Thailand (http://pubmlst.org/campylobacter) and was mainly isolated from chickens and gastroenteritis patients. In contrast, CC-460 was the predominant CC found in aerosensitive *C. jejuni* strain in this study. Unlike our study, the study of Kiatsomphob et al. (2019) found that CC-460 was identified in aerotolerant and hyper-aerotolerant strains isolated from humans, broilers and cattle in Japan. In Thailand, this CC was previously detected in human and chicken isolates (Ngulukun et al., 2016). Another common sequence type observed in this study was ST-2274, which was found in both aerotolerant and aerosensitive *C. jejuni* strains. Although this sequence type was uncommon in other countries, it was quite common in China and Thailand. Previous studies reported that ST-2274 was frequently isolated from diarrheal patients in China and broilers in Thailand (Chokboonmongkol et al., 2013; Zhang et al., 2020). Similarity between genotypes previously identified in human clinical cases and broilers indicated that poultry was one of the most important sources of human Campylobacteriosis.

In this study, *C. jejuni* isolates in the retail market level were obtained from poultry products of two supermarket chains located in Bangkok and vicinity. The MLST results showed that the predominant genotype in retail market chain A was ST-1232, while ST-1075 was the most frequently detected genotype in chain B. Only 2 STs (ST-583 and ST-1075) were found to be overlapped between these two chains. According to Mullner et al. (2010), each retail company has its own genetic structure and diversity due to different level of biosecurity in the production line which resulted in different genotypes distribution among retail market chains. Interestingly, ST-1075 was all identified as aerotolerant strain. Likewise, majority of isolates genotyped as ST-1232 were also aerotolerant strain. This possibly indicated that these two STs might be more tolerant to oxygen than other strains leading to superior survival of these strains along

the poultry production line and detection of these STs in the final products (O'Kane and Connerton, 2017). As enhanced aerotolerance of *C. jejuni* possibly increases viability of bacteria in poultry products and consequently increases the chance of transmission to human (Oh et al., 2015), detection of these two sequence types in broiler chickens suggested that prevention of *Campylobacter* contamination in poultry products is required in order to decrease *C. jejuni* transmission to human.

The results provided by *flaA* SVR sequencing technique and MLST in this study were mostly concordant. In some cases, particular *flaA* SVR allele type linked to multiple sequence types. Conversely, certain sequence type was genotyped from several *flaA* SVR allele numbers. For example, ST-1075 was identified from *flaA* SVR allele number 22 in aerotolerant strain and *flaA* SVR allele number 783 in aerosensitive strain. On the other hand, *flaA* SVR allele number 54 was genotyped as ST-137 and ST-464. In addition, association between *flaA* SVR allele types and sequence types was also reported in other studies (Dingle et al., 2005; Prachantasena et al., 2016). According to the study of Dingle et al. (2005), ST-825 was classified from *flaA* SVR allele number 16, 253, 299 and 336, while *flaA* SVR allele number 16 was genotyped as ST-825, 829, 891 and 899. Moreover, ST-464 was identified from *flaA* SVR allele number 54 in this study, but this sequence type could be genotyped from *flaA* SVR allele number 783 as well (Prachantasena et al., 2016). The genotyping results of our study and the study of Dingle et al. (2005) and Prachantasena et al. (2016) suggested that a combination of *flaA* SVR sequencing technique and MLST provided a high discriminatory power in genetic identification of *Campylobacter* population. Therefore, these two molecular genotyping methods should be used in the study of *Campylobacter* epidemiology.

CHAPTER VI
CONCLUSION AND SUGGESTION

Superior survival of aerotolerant *Campylobacter* in the food chain consequently increases the chance of human transmission. In Thailand, a high prevalence of *C. jejuni* was found in the poultry production chain, but the information on aerotolerant *C. jejuni* is limited. In this study, the occurrence of aerotolerant *C. jejuni* at the farm, slaughterhouse and retail market levels was investigated. In addition, genotypes of *C. jejuni* strains distributed in the poultry production chain were also identified. The highest occurrence of aerotolerant *C. jejuni* was found at the slaughterhouse stage (56%), followed by retail market stage (51%) and farm stage (41.33%). Hyper-aerotolerant *C. jejuni* strain was not detected in this study as no bacterial isolates survived 24 h of aerobic culture. The longest viability of bacterial strains cultured under aerobic condition at each production stage was 18 h. Moreover, the occurrence of aerotolerant *C. jejuni* between different sample types at the farm and slaughterhouse level was examined. At the farm stage, manures (43.24%) had a higher level of aerotolerant *C. jejuni* strains than cloacal swabs (39.47%). Surprisingly, in slaughterhouses, cecal contents (58%) had a higher proportion of aerotolerant *C. jejuni* strain than carcass rinses (54%). However, the differences in the occurrence of aerotolerant *C. jejuni* between different sample types in these two stages were not statistically significant. Additionally, viable counts of aerotolerant *C. jejuni* strains at 12 h, 15 h, 18 h and 24 h of aerobic culture were evaluated. The mean bacterial counts among aerotolerant strain at the farm level at 12 h, 15 h and 18 h of aerobic shaking were 5.60 log CFU/ml (3.41-7.19 log CFU/ml; SE=0.16), 4.16 log CFU/ml (1.95-5.69 log CFU/ml; SE=0.22) and 2.59 log CFU/ml (2-3.14 log CFU/ml; SE=0.29), respectively. Likewise, the mean viable counts at the slaughterhouse stage at the same time period were 5.14 log CFU/ml (1.90-7.62 log CFU/ml; SE=0.17), 4.30 log CFU/ml (1.90-6.47 log

CFU/ml; SE=0.26) and 3.53 log CFU/ml (2.20-5.44 log CFU/ml; SE=0.55), respectively. At the retail market stage, the mean bacterial counts were 5.39 log CFU/ml (2.34-7.39 log CFU/ml; SE=0.16), 3.91 log CFU/ml (2.11-5.55 log CFU/ml; SE=0.27) and 3.06 log CFU/ml (2.46-3.67 log CFU/ml; SE=0.60) at 12 h, 15 h and 18 h of aerobic culture, respectively.

In terms of genetic identification at the farm level, *flaA* SVR allele number 142 and 783 was the predominant genotype in aerosensitive and aerotolerant *C. jejuni* strain, respectively. At the slaughterhouse level, *flaA* SVR allele number 142 was the most frequently detected genotypes in aerosensitive strain, whereas *flaA* SVR allele number 67 was the predominant genotype found in aerotolerant strain. The most common genotype identified in both aerosensitive and aerotolerant strains at the retail market stage was *flaA* SVR allele number 22. In this study, similar *flaA* SVR allele types were found between *C. jejuni* isolates from farms and slaughterhouses. However, in some cases the differences between genotypes in both production sites were observed suggesting that good hygienic practices must be implemented in poultry production processes to reduce contamination of the final products from external sources. Additionally, representatives of *flaA* SVR genotyped isolates were selected for further genetic identification by MLST. CC-45 and CC-353 were the predominant CCs identified in this study. Although CC-21 was frequently detected in aerotolerant *C. jejuni* strain in other countries, this genotype was not found in our study as various factors (e.g., sources of isolates and geographical isolation) could contribute to differences in *Campylobacter* genotypes among countries. In addition, most of the sequence types (e.g., ST-460, 1075 and 1232) found in aerosensitive and aerotolerant strains in all of the production stages were similar to the sequence types previously identified in chickens and gastroenteritis patients in Thailand which emphasized the importance of chickens as one of the main sources in human infection. Interestingly, we found that several genotypes might link to aerotolerance status of *C. jejuni* (e.g., ST-460, 1075 and

1232). This finding was supported by the data reported in many studies describing certain genotypes may carry genetic of fitness which allow superior survival than other strains. In order to obtain a high discriminatory power in genetic identification of *Campylobacter* population, a combination of *flaA* SVR sequencing technique and MLST is recommended in the epidemiological study of *Campylobacter*.

This study revealed that aerotolerant *C. jejuni* were distributed throughout the entire poultry production processes. Further study will be required to assess the risk that aerotolerant *C. jejuni* have on human health as aerotolerance feature enhances bacterial survival along the food chain. Consequently, increasing the chance of *Campylobacter* transmission to humans. The data obtained from this study emphasizes the reduction of *Campylobacter* contamination level in the poultry production system, especially in the plucking and evisceration steps which contribute to the highest contamination level in chicken carcasses. Besides decreasing contamination from the beginning of the production process, consumers awareness of cross-contamination while handling food could also help minimize the risk of *Campylobacte*r exposure and eventually decrease human infection.

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APPENDIX A

Campylobacter jejuni growth media

1. Modified Charcoal-Cefoperazone-Deoxycholate (mCCDA) (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom)

Typical formula	**(g/litre)**
- Nutrient Broth No. 2	25.0
- Bacteriological charcoal	4.0
- Casein hydrolysate	3.0
- Sodium deoxycholate	1.0
- Ferrous sulphate	0.25
- Sodium pyruvate	0.25
- Agar	12.0
- Cefaperazone	32 mg
- Amphotericin B	10 mg

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

Typical formula	**(g/litre)**
- Beef Extract Powder	2.0
- Acid Digest Hydrolysate of Casien	17.5
- Starch	1.5
- Agar	1.0

3. Muller Hinton Broth (MHA) (Difco™, MD, USA)

Typical formula for 1 litre	**(g/litre)**
- Beef Extract Powder	2.0
- Starch	1.5
- Casein hydrolysate	17.5

4. Preston Broth (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom)

Typical formula for 1 litre	**(g/litre)**
- Lab-Lemco powder	10.0
- Peptone	10.0
- Sodium chloride	12.0
- Polymyxin B	5,000 IU
- Rifampicin	10.0 mg
- Trimethoprim	10.0 mg
- Amphoteracin B	10.0 mg
- Sheep blood	50 ml

APPENDIX B

Chemical substances

1. 50X Tris acetate EDTA (TAE)

Typical formula	**(g/litre)**
- Tris	220
- Gracial acetic acid	57.1
- EDTA (0.5 Molar) pH 8	100 ml
- Adding deionized water to reach 1,000 ml	

2. 1X TAE buffer

Typical formula	**(ml/litre)**
- 50X TAE	20
- Deionized water	980

APPENDIX C

Aerotolerant test procedure and drop plate technique for bacterial enumeration

1. Aerotolerant test procedure

Aerotolerant test in this study was carried out by growing *C. jejuni* colonies onto mCCDA plates and incubating at 42°C under microaerobic condition for 48 h. Thereafter, the bacteria were subcultured onto MHA and incubated at 42°C under microaerobic condition for 24 h. Overnight culture of *C. jejuni* colonies were inoculated into 15 mm x 97 mm glass test tube (Pyrex®) containing a final volume of MHB equivalent to 8 ml with an adjusted OD_{600} of 0.2 to obtain bacterial concentration of 10^8 CFU/ml. Subsequently, test tubes were covered with loose plastic cap and shaken in the shaking incubator (New Brunswick Scientific™) at 150 rpm under aerobic condition at 42°C for 24 h. Samples were collected to check for the number of bacterial viability at 12 h, 15 h, 18 h and 24 h of aerobic culture.

2. Drop plate technique for bacterial enumeration

To evaluate viable counts of aerotolerant *C. jejuni* strains, ten-fold dilution was performed in the 96-well microplate with U-shape bottom. One hundred microliters of samples collected at 12 h, 15 h, 18 h and 24 h were added to the first well of microplates. To obtain initial dilution of 10^{-1}, 20 µl of samples from the first well were transferred to the next well containing 180 µl of normal saline to achieve a total volume of 200 µl. Subsequently, 20 µl of properly mixed samples in the first dilution were drawn and then added in the next well filled with 180 µl of normal saline to obtain the dilution of 10^{-2}. The samples were serially diluted until the final dilution of 10^{-5} was obtained. Thereafter, 10 µl/spot for each dilution were plated onto MHA agar plates. After incubation at 42°C for 48 h under microaerobic condition, dilution with

the sum of 5 spots containing 3-30 visible colonies were selected. A total count of 5 spots were multiplied by 20 and the inverse of the dilution factor to obtain the bacterial viability counts in CFU/ml (O'Kane and Connerton, 2017).

APPENDIX D

Table D-1 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from farms

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
1	B2-26C	*C. jejuni*	cloacal swab	aerosensitive	948	
2	CH7C	*C. jejuni*	cloacal swab	aerosensitive	1173	
3	J4C	*C. jejuni*	cloacal swab	aerosensitive	142	
4	J7C	*C. jejuni*	cloacal swab	aerosensitive	142	
5	J8C	*C. jejuni*	cloacal swab	aerosensitive	142	460
6	J9C	*C. jejuni*	cloacal swab	aerosensitive		
7	P2C	*C. jejuni*	cloacal swab	aerosensitive	67	6455
8	P5C	*C. jejuni*	cloacal swab	aerosensitive		
9	PP4-4C	*C. jejuni*	cloacal swab	aerosensitive	524	
10	PP4-5C	*C. jejuni*	cloacal swab	aerosensitive		
11	S4C	*C. jejuni*	cloacal swab	aerosensitive	34	
12	S9C	*C. jejuni*	cloacal swab	aerosensitive		
13	V3C	*C. jejuni*	cloacal swab	aerotolerant	783	1232
14	V10C	*C. jejuni*	cloacal swab	aerosensitive	97	1898
15	V13C	*C. jejuni*	cloacal swab	aerotolerant	783	1232
16	V11C	*C. jejuni*	cloacal swab	aerosensitive	97	1898
17	K12C	*C. jejuni*	cloacal swab	aerotolerant		
18	K13C	*C. jejuni*	cloacal swab	aerosensitive	112	2274
19	K14C	*C. jejuni*	cloacal swab	aerosensitive		
20	K19C	*C. jejuni*	cloacal swab	aerosensitive	1670	
21	K20C	*C. jejuni*	cloacal swab	aerotolerant	854	
22	cs3801k1	*C. jejuni*	cloacal swab	aerosensitive		
23	cs3114s5_4	*C. jejuni*	cloacal swab	aerosensitive		

Table D-1 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from farms (continued)

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
24	cs3508s5	*C. jejuni*	cloacal swab	aerosensitive		
25	cs3108k1	*C. jejuni*	cloacal swab	aerosensitive		
26	LScs53km1	*C. jejuni*	cloacal swab	aerosensitive		
27	LScs503k1	*C. jejuni*	cloacal swab	aerosensitive		
28	cs3503k1	*C. jejuni*	cloacal swab	aerotolerant		
29	cs3104k1	*C. jejuni*	cloacal swab	aerotolerant		
30	csb21d07c6_kpi	*C. jejuni*	cloacal swab	aerotolerant		
31	cs3111s5_3	*C. jejuni*	cloacal swab	aerotolerant		
32	cs3512s5_3	*C. jejuni*	cloacal swab	aerotolerant		
33	csb32d07c10kpi	*C. jejuni*	cloacal swab	aerotolerant		
34	cs3505k1	*C. jejuni*	cloacal swab	aerotolerant		
35	cs3103k1	*C. jejuni*	cloacal swab	aerotolerant		
36	csb32d09c10_kpi	*C. jejuni*	cloacal swab	aerotolerant		
37	cs2817s5_3	*C. jejuni*	cloacal swab	aerotolerant		
38	csb28d07c5su_1	*C. jejuni*	cloacal swab	aerotolerant		
39	B2-26F	*C. jejuni*	manure	aerotolerant	854	
40	CH2F	*C. jejuni*	manure	aerosensitive		
41	CH4F	*C. jejuni*	manure	aerotolerant	112	2274
42	CH5F	*C. jejuni*	manure	aerosensitive	288	137
43	CH7F	*C. jejuni*	manure	aerosensitive	607	
44	CH8F	*C. jejuni*	manure	aerosensitive		
45	J3F	*C. jejuni*	manure	aerosensitive		
46	J4F	*C. jejuni*	manure	aerosensitive	142	
47	J7F	*C. jejuni*	manure	aerosensitive	142	
48	J8F	*C. jejuni*	manure	aerosensitive	142	460
49	J9F	*C. jejuni*	manure	aerosensitive		

Table D-1 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from farms (continued)

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
50	K13F	*C. jejuni*	manure	aerosensitive	67	6736
51	K14F	*C. jejuni*	manure	aerosensitive		
52	K20F	*C. jejuni*	manure	aerotolerant	854	
53	P2F	*C. jejuni*	manure	aerotolerant	112	2274
54	P5F	*C. jejuni*	manure	aerosensitive		
55	P6F	*C. jejuni*	manure	aerotolerant		
56	P7F	*C. jejuni*	manure	aerosensitive	67	
57	PP4-5F	*C. jejuni*	manure	aerosensitive	524	
58	S4F	*C. jejuni*	manure	aerosensitive	34	
59	S7F	*C. jejuni*	manure	aerosensitive		
60	S9F	*C. jejuni*	manure	aerosensitive		
61	V3F	*C. jejuni*	manure	aerotolerant	783	1232
62	V10F	*C. jejuni*	manure	aerotolerant	97	1898
63	V11F	*C. jejuni*	manure	aerosensitive	97	
64	V12F	*C. jejuni*	manure	aerotolerant	783	
65	V13F	*C. jejuni*	manure	aerotolerant	783	1232
66	TC3F	*C. jejuni*	manure	aerotolerant		
67	TC4F	*C. jejuni*	manure	aerotolerant	21	51
68	TC5F	*C. jejuni*	manure	aerotolerant	52	
69	TC11F	*C. jejuni*	manure	aerotolerant	21	51
70	F-mkn4-1	*C. jejuni*	manure	aerosensitive		
71	F-ps5-1	*C. jejuni*	manure	aerosensitive		
72	F-mc5-3	*C. jejuni*	manure	aerotolerant		
73	F-mc1-2	*C. jejuni*	manure	aerotolerant		
74	F-mc6-1	*C. jejuni*	manure	aerotolerant		
75	F-mc4-2	*C. jejuni*	manure	aerosensitive		

Table D-2 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from slaughterhouses

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
76	541	*C. jejuni*	carcass rinse	aerosensitive		
77	543	*C. jejuni*	carcass rinse	aerosensitive		
78	544	*C. jejuni*	carcass rinse	aerosensitive		
79	545	*C. jejuni*	carcass rinse	aerosensitive		
80	546	*C. jejuni*	carcass rinse	aerosensitive		
81	A2CR	*C. jejuni*	carcass rinse	aerotolerant		
82	A3CR	*C. jejuni*	carcass rinse	aerotolerant		
83	A4CR	*C. jejuni*	carcass rinse	aerosensitive		
84	A5CR	*C. jejuni*	carcass rinse	aerosensitive		
85	A7CR	*C. jejuni*	carcass rinse	aerotolerant		
86	B1-13CR	*C. jejuni*	carcass rinse	aerotolerant	589	
87	B1-15CR	*C. jejuni*	carcass rinse	aerotolerant	67	1919
88	B1-16CR	*C. jejuni*	carcass rinse	aerosensitive		
89	B1-17CR	*C. jejuni*	carcass rinse	aerotolerant	1527	
90	B2-19CR	*C. jejuni*	carcass rinse	aerotolerant		
91	B2-25CR	*C. jejuni*	carcass rinse	aerotolerant	948	
92	B2-26CR	*C. jejuni*	carcass rinse	aerosensitive		
93	CH2CR	*C. jejuni*	carcass rinse	aerosensitive		
94	CH4CR	*C. jejuni*	carcass rinse	aerotolerant	112	2274
95	CH5CR	*C. jejuni*	carcass rinse	aerosensitive	112	2274
96	CH7CR	*C. jejuni*	carcass rinse	aerotolerant	112	
97	J3CR	*C. jejuni*	carcass rinse	aerosensitive		
98	J4CR	*C. jejuni*	carcass rinse	aerotolerant	22	
99	J7CR	*C. jejuni*	carcass rinse	aerosensitive	142	
100	J8CR	*C. jejuni*	carcass rinse	aerosensitive	142	460

Table D-2 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from slaughterhouses (continued)

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
101	J9CR	*C. jejuni*	carcass rinse	aerosensitive		
102	K13CR	*C. jejuni*	carcass rinse	aerotolerant	67	6736
103	K14CR	*C. jejuni*	carcass rinse	aerosensitive	902	
104	K19CR	*C. jejuni*	carcass rinse	aerotolerant	783	1232
105	K20CR	*C. jejuni*	carcass rinse	aerotolerant	253	
106	P2CR	*C. jejuni*	carcass rinse	aerotolerant	295	
107	P5CR	*C. jejuni*	carcass rinse	aerosensitive	67	
108	P6CR	*C. jejuni*	carcass rinse	aerotolerant	67	6736
109	P7CR	*C. jejuni*	carcass rinse	aerosensitive	902	
110	PJ2-4CR	*C. jejuni*	carcass rinse	aerosensitive		
111	PP4-1CR	*C. jejuni*	carcass rinse	aerosensitive		
112	PP4-2CR	*C. jejuni*	carcass rinse	aerotolerant	288	583
113	PP4-3CR	*C. jejuni*	carcass rinse	aerosensitive	440	
114	S2CR	*C. jejuni*	carcass rinse	aerosensitive		
115	S4CR	*C. jejuni*	carcass rinse	aerotolerant		
116	S7CR	*C. jejuni*	carcass rinse	aerotolerant		
117	S8CR	*C. jejuni*	carcass rinse	aerotolerant	34	
118	TC3CR	*C. jejuni*	carcass rinse	aerosensitive	524	
119	TC4CR	*C. jejuni*	carcass rinse	aerotolerant	21	51
120	TC5CR	*C. jejuni*	carcass rinse	aerotolerant	16	
121	TC11CR	*C. jejuni*	carcass rinse	aerotolerant	16	
122	TC16CR	*C. jejuni*	carcass rinse	aerotolerant		
123	V3CR	*C. jejuni*	carcass rinse	aerotolerant	288	137
124	V11CR	*C. jejuni*	carcass rinse	aerotolerant	287	
125	V12CR	*C. jejuni*	carcass rinse	aerotolerant	97	1898

Table D-2 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from slaughterhouses (continued)

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
126	A2CC	*C. jejuni*	cecal content	aerosensitive		
127	A3CC	*C. jejuni*	cecal content	aerosensitive		
128	A5CC	*C. jejuni*	cecal content	aerosensitive		
129	B1-13CC	*C. jejuni*	cecal content	aerosensitive	316	
130	B1-15CC	*C. jejuni*	cecal content	aerosensitive	589	
131	B1-16CC	*C. jejuni*	cecal content	aerosensitive		
132	B1-17CC	*C. jejuni*	cecal content	aerotolerant	854	
133	B2-26CC	*C. jejuni*	cecal content	aerosensitive	948	
134	B2-19CC	*C. jejuni*	cecal content	aerotolerant		
135	B2-25CC	*C. jejuni*	cecal content	aerotolerant	985	
136	B2-27CC	*C. jejuni*	cecal content	aerotolerant		
137	B2-28CC	*C. jejuni*	cecal content	aerotolerant		
138	CH2CC	*C. jejuni*	cecal content	aerosensitive		
139	CH5CC	*C. jejuni*	cecal content	aerotolerant	112	2274
140	CH7CC	*C. jejuni*	cecal content	aerosensitive	189	
141	J8CC	*C. jejuni*	cecal content	aerosensitive	142	460
142	K13CC	*C. jejuni*	cecal content	aerotolerant	67	6736
143	K19CC	*C. jejuni*	cecal content	aerotolerant	67	6736
144	K20CC	*C. jejuni*	cecal content	aerotolerant	1527	
145	P5CC	*C. jejuni*	cecal content	aerotolerant		
146	P6CC	*C. jejuni*	cecal content	aerotolerant	67	6455
147	PP4-1CC	*C. jejuni*	cecal content	aerotolerant		
148	PP4-2CC	*C. jejuni*	cecal content	aerotolerant	288	583
149	PP4-4CC	*C. jejuni*	cecal content	aerosensitive		
150	V3CC	*C. jejuni*	cecal content	aerosensitive	97	137

Table D-2 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from slaughterhouses (continued)

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
151	V10CC	*C. jejuni*	cecal content	aerotolerant	288	137
152	V11CC	*C. jejuni*	cecal content	aerotolerant	288	
153	V12CC	*C. jejuni*	cecal content	aerotolerant	783	1232
154	S8CC	*C. jejuni*	cecal content	aerotolerant	34	
155	S9CC	*C. jejuni*	cecal content	aerotolerant		
156	TC4CC	*C. jejuni*	cecal content	aerotolerant	16	
157	TC11CC	*C. jejuni*	cecal content	aerosensitive	16	
158	s9690_1B	*C. jejuni*	cecal content	aerosensitive		
159	s4940_4B	*C. jejuni*	cecal content	aerosensitive		
160	s8270_3B	*C. jejuni*	cecal content	aerosensitive		
161	s9290_3C	*C. jejuni*	cecal content	aerosensitive		
162	s8990_3C	*C. jejuni*	cecal content	aerosensitive		
163	s9490_5C	*C. jejuni*	cecal content	aerosensitive		
164	s9390_3B	*C. jejuni*	cecal content	aerosensitive		
165	s10090_6B	*C. jejuni*	cecal content	aerosensitive		
166	s9790_2C	*C. jejuni*	cecal content	aerotolerant		
167	s5340_1C	*C. jejuni*	cecal content	aerotolerant		
168	s110110_5B	*C. jejuni*	cecal content	aerotolerant		
169	s8680_1B	*C. jejuni*	cecal content	aerotolerant		
170	s7860_2b	*C. jejuni*	cecal content	aerotolerant		
171	s7950_3b	*C. jejuni*	cecal content	aerotolerant		
172	s109100_4B	*C. jejuni*	cecal content	aerotolerant		
173	s9090_2C	*C. jejuni*	cecal content	aerotolerant		
174	s49405B	*C. jejuni*	cecal content	aerotolerant		
175	s8170_5B	*C. jejuni*	cecal content	aerotolerant		

Table D-3 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from retail markets

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
176	1.2	*C. jejuni*	raw chicken products	aerosensitive	22	
177	4.2	*C. jejuni*	raw chicken products	aerotolerant		
178	13.2	*C. jejuni*	raw chicken products	aerosensitive		
179	15.2	*C. jejuni*	raw chicken products	aerotolerant	22	1075
180	16.1	*C. jejuni*	raw chicken products	aerosensitive		
181	17.1	*C. jejuni*	raw chicken products	aerosensitive		
182	18.1	*C. jejuni*	raw chicken products	aerotolerant	97	
183	21.1	*C. jejuni*	raw chicken products	aerosensitive		
184	22.2	*C. jejuni*	raw chicken products	aerotolerant	45	
185	24.2	*C. jejuni*	raw chicken products	aerosensitive		
186	25.1	*C. jejuni*	raw chicken products	aerosensitive	14	
187	25.2	*C. jejuni*	raw chicken products	aerosensitive	14	
188	27.1	*C. jejuni*	raw chicken products	aerosensitive	783	1075
189	27.2	*C. jejuni*	raw chicken products	aerotolerant	22	1075
190	27.3	*C. jejuni*	raw chicken products	aerosensitive		
191	27.4	*C. jejuni*	raw chicken products	aerosensitive	979	
192	27.5	*C. jejuni*	raw chicken products	aerotolerant	22	
193	28.1	*C. jejuni*	raw chicken products	aerotolerant	22	1075
194	28.2	*C. jejuni*	raw chicken products	aerotolerant	22	
195	29.2	*C. jejuni*	raw chicken products	aerosensitive		
196	30.2	*C. jejuni*	raw chicken products	aerosensitive		
197	34.2	*C. jejuni*	raw chicken products	aerosensitive		
198	37.1	*C. jejuni*	raw chicken products	aerosensitive		
199	38.2	*C. jejuni*	raw chicken products	aerotolerant	769	
200	39.1	*C. jejuni*	raw chicken products	aerosensitive		

Table D-3 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from retail markets (continued)

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
201	43.1	*C. jejuni*	raw chicken products	aerotolerant	21	51
202	44.2	*C. jejuni*	raw chicken products	aerotolerant		
203	45.1	*C. jejuni*	raw chicken products	aerotolerant	45	
204	46.2	*C. jejuni*	raw chicken products	aerotolerant		
205	52.1	*C. jejuni*	raw chicken products	aerosensitive		
206	56.2	*C. jejuni*	raw chicken products	aerotolerant		
207	57.2	*C. jejuni*	raw chicken products	aerosensitive		
208	59.1	*C. jejuni*	raw chicken products	aerotolerant	112	
209	60.1	*C. jejuni*	raw chicken products	aerosensitive		
210	61.1	*C. jejuni*	raw chicken products	aerosensitive		
211	62.2	*C. jejuni*	raw chicken products	aerosensitive		
212	63.1	*C. jejuni*	raw chicken products	aerosensitive		
213	65.2	*C. jejuni*	raw chicken products	aerosensitive		
214	66.2	*C. jejuni*	raw chicken products	aerotolerant		
215	67.2	*C. jejuni*	raw chicken products	aerosensitive	177	
216	73.1	*C. jejuni*	raw chicken products	aerotolerant	22	1075
217	73.2	*C. jejuni*	raw chicken products	aerotolerant	22	
218	74.1	*C. jejuni*	raw chicken products	aerotolerant	979	
219	74.2	*C. jejuni*	raw chicken products	aerotolerant	208	
220	74.3	*C. jejuni*	raw chicken products	aerotolerant		
221	74.4	*C. jejuni*	raw chicken products	aerosensitive	22	8835
222	75.1	*C. jejuni*	raw chicken products	aerotolerant	1530	
223	75.2	*C. jejuni*	raw chicken products	aerotolerant	1530	
224	79.1	*C. jejuni*	raw chicken products	aerotolerant	36	
225	79.2	*C. jejuni*	raw chicken products	aerotolerant	36	

Table D-3 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from retail markets (continued)

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
226	79.3	*C. jejuni*	raw chicken products	aerotolerant		
227	84.1	*C. jejuni*	raw chicken products	aerosensitive		
228	85.1	*C. jejuni*	raw chicken products	aerosensitive	210	
229	85.3	*C. jejuni*	raw chicken products	aerotolerant	210	
230	86.2	*C. jejuni*	raw chicken products	aerosensitive	210	354
231	86.4	*C. jejuni*	raw chicken products	aerotolerant	210	354
232	88.1	*C. jejuni*	raw chicken products	aerotolerant	54	464
233	88.2	*C. jejuni*	raw chicken products	aerosensitive	783	2281
234	95.1	*C. jejuni*	raw chicken products	aerosensitive	287	2325
235	95.2	*C. jejuni*	raw chicken products	aerotolerant	287	2325
236	96.3	*C. jejuni*	raw chicken products	aerosensitive	288	583
237	96.4	*C. jejuni*	raw chicken products	aerosensitive		
238	96.5	*C. jejuni*	raw chicken products	aerotolerant	288	583
239	107.1	*C. jejuni*	raw chicken products	aerosensitive	48	
240	107.2	*C. jejuni*	raw chicken products	aerotolerant		
241	107.3	*C. jejuni*	raw chicken products	aerotolerant	596	1232
242	107.4	*C. jejuni*	raw chicken products	aerosensitive	596	1232
243	108.1	*C. jejuni*	raw chicken products	aerotolerant	963	
244	108.2	*C. jejuni*	raw chicken products	aerosensitive	253	
245	117.2	*C. jejuni*	raw chicken products	aerotolerant	287	1232
246	117.3	*C. jejuni*	raw chicken products	aerosensitive	596	1232
247	117.4	*C. jejuni*	raw chicken products	aerosensitive		
248	118.2	*C. jejuni*	raw chicken products	aerotolerant	596	1232
249	120.1	*C. jejuni*	raw chicken products	aerosensitive		
250	121.1	*C. jejuni*	raw chicken products	aerosensitive	22	

Table D-3 Genotypes and aerotolerant status of *Campylobacter jejuni* isolated from retail markets (continued)

No.	Strain ID	Species	Type of sample	Aerotolerant status	*flaA* SVR allele no.	MLST (ST)
251	122.1	*C. jejuni*	raw chicken products	aerosensitive	22	
252	126.1	*C. jejuni*	raw chicken products	aerotolerant		
253	128.1	*C. jejuni*	raw chicken products	aerosensitive	22	45
254	128.4	*C. jejuni*	raw chicken products	aerotolerant	54	137
255	129.1	*C. jejuni*	raw chicken products	aerotolerant		
256	130.6	*C. jejuni*	raw chicken products	aerosensitive		
257	131.1	*C. jejuni*	raw chicken products	aerotolerant		
258	132.1	*C. jejuni*	raw chicken products	aerotolerant	783	1232
259	132.3	*C. jejuni*	raw chicken products	aerotolerant	783	
260	132.4	*C. jejuni*	raw chicken products	aerotolerant	783	
261	136.1	*C. jejuni*	raw chicken products	aerosensitive		
262	137.1	*C. jejuni*	raw chicken products	aerosensitive	524	
263	137.2	*C. jejuni*	raw chicken products	aerosensitive	315	
264	138.2	*C. jejuni*	raw chicken products	aerotolerant		
265	138.3	*C. jejuni*	raw chicken products	aerosensitive	288	583
266	139	*C. jejuni*	raw chicken products	aerosensitive		
267	143	*C. jejuni*	raw chicken products	aerotolerant		
268	146	*C. jejuni*	raw chicken products	aerosensitive		
269	148.1	*C. jejuni*	raw chicken products	aerotolerant		
270	149	*C. jejuni*	raw chicken products	aerotolerant		
271	150	*C. jejuni*	raw chicken products	aerotolerant		
272	152.1	*C. jejuni*	raw chicken products	aerotolerant	22	1075
273	156	*C. jejuni*	raw chicken products	aerotolerant		
274	157	*C. jejuni*	raw chicken products	aerotolerant		
275	160.2	*C. jejuni*	raw chicken products	aerosensitive		

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