TRANSFERABLE R PLASMIDS IN *ESCHERICHIA COLI* ISOLATED FROM MEAT DUCKS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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อาร์พลาสมิดที่ถ่ายทอดได้ใน *Escherichia coli* ที่แยกได้จากเป็ดเนื้อ

นายซูโลกาเมน บาร์คา

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ซูโลกาเมน บาร์คา : อาร์พลาสมิดที่ถ่ายทอดได้ใน *Escherichia coli* ที่แยกได้จากเป็ดเนื้อ . (TRANSFERABLE R PLASMIDS IN *ESCHERICHIA COLI* ISOLATED FROM MEAT DUCKS) อ.ที่ปรึกษาหลัก : PROF. DR.รุ่งทิพย์ ชวนชื่นD.V.M., M.Sc., Ph.D.

การศึกษานี้มีวัตถุประสงค์เพื่ออธิบายลักษณะการดื้อยาและอาร์พลาสมิด (R plasmids) ที่ถ่ายทอดได้ใน *Escherichia coli* ที่แยกได้จากเป็ดเนื้อเลี้ยงในระบบเปิด โดยได้รับ commensal *E. coli* จำนวน 177 isolates ที่แยกจาก cloacal swab ในการศึกษาก่อนหน้านี้ ทำการยืนยันเชื้อด้วยวิธีทางชีวเคมีและทดสอบความไวต่อยาปฏิชีวนะ 15 ชนิดด้วยเทคนิค broth microdilution ทดสอบการถ่ายทอดของอาร์พลาสมิดด้วยวิธี biparental mating จากนั้นจึงตรวจหาชนิดของพลาสมิดด้วยวิธี plasmid replicon typing (PBRT) และ plasmid multi-locus sequence typing (pMLST) จากผลการทดความไวต่อยาปฏิชีวนะ พบว่ายาปฏิชีวนะที่มีอัตราการดื้อยาสูงที่สุดคือ ampicillin (83.0%) และ tetracycline (81.9%) เชื้อส่วนใหญ่ดื้อยาหลายชนิดพร้อมกัน (86.4%) และจำนวน 9 isolates สามารถผลิตเอนไซม์ Extended-spectrum beta-lactamases (ESBLs) ได้ อาร์พลาสมิดสามารถถ่ายทอดได้ด้วยวิธีการ conjugation ในยาปฏิชีวนะที่เป็น selective pressure ต่างๆ คือ tetracycline ชนิดเดียว (n=4) ampicillin ชนิดเดียว (n=3) chloramphenicol ชนิดเดียว (n=3) และทั้ง ampicillin/tetracycline (n=3) พบ replicon type จำนวน 5 ชนิด ซึ่ง IncFrepB เป็นชนิดที่พบมากที่สุด (38.4%) ในตัวให้ (n=13) และใน transconjugants (31.2%) (n=16) จากการ subtyping พลาสมิดในกลุ่ม F โดยใช้ replicon sequence typing (RST) scheme (n=6) พบรูปแบบ replicons 5 แบบ คือ F47:A-:B- (n=2), F29:A-:B23 (n=1), F29:A-:B- (n=1), F18:A-:B- (n=1) และ F4:A-:B- (n=1) คุณลักษณะการดื้อยา พบว่ามีความสัมพันธ์กันเชิงบวกทางสถิติอย่างมีนัยยะสำคัญ ($p<0.05$) โดยเฉพาะอย่างยิ่ง การดื้อยา chloramphenicol มีความสัมพันธ์สูงกับการดื้อยาชนิดอื่นๆ โดยสรุป การพบอัตราการดื้อยาปฏิชีวนะที่มีความสำคัญทางคลินิกในระดับสูงในการศึกษานี้บ่งชี้ถึงความสำคัญของเป็ดเนื้อเลี้ยงในระบบเปิดต่อการการแพร่กระจายของเชื้อดื้อยาซึ่งสามารถก่ออันตรายต่อมนุษย์และสิ่งแวดล้อมได้ รวมทั้งยังยันยืนว่าปัญหาเชื้อดื้อยาเป็นปัญหาสุขภาพหนึ่งเดียว ควรมีการตรวจติดตามและเฝ้าระวังการดื้อยาในแบคทีเรียที่แยกได้จากเป็ดเนื้ออย่างเป็นประจำ

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Zulqarnain Baqar : TRANSFERABLE R PLASMIDS IN *ESCHERICHIA COLI* ISOLATED FROM MEAT DUCKS. Advisor: PROF. DR. RUNGTIP CHUANCHUEN, D.V.M., M.Sc., Ph.D.

This study aimed to describe the AMR characteristics and transferable R plasmids in *Escherichia coli* isolated from meat ducks reared in an open house system. One hundred seventy-seven (n=177) commensal *E. coli* were previously isolated from duck cloacal swabs. In this study, all were biochemically confirmed and examined for their susceptibilities to 15 antimicrobial agents by broth microdilution method. Transfer of R plasmids was tested by biparental mating method followed by plasmid replicon typing (PBRT) and plasmid multi-locus sequence typing (pMLST). The highest resistance rates were observed for ampicillin (83.0%) and tetracycline (81.9%) while multidrug resistance was common (86.4%). Extended-spectrum beta-lactamases (ESBLs) production were confirmed in nine isolates. R plasmids were conjugally transferred using only tetracycline (n=4), only ampicillin (n=3), only chloramphenicol (n=3) and ampicillin/tetracycline (n=3) as selective pressure. Five replicon types were identified, of which IncFrepB was most common (38.4%) in donors (n=13) and (31.2%) in transconjugants (n=16). Subtyping F type plasmids using replicon sequence typing (RST) scheme (n=6) revealed five distinct replicons combinations, including F47:A-:B- (n=2), F29:A-:B23 (n=1), F29:A-:B- (n=1), F18:A-:B- (n=1) and F4:A-:B- (n=1). AMR phenotypes were found to have a significant statistically positive correlation ($p<0.05$). In particular, chloramphenicol resistance was highly correlated with other AMR phenotypes. In conclusion, the high resistance rates to clinically important antimicrobial agents in this study highlight the important role of meat ducks raised in open house farming system in the dissemination of AMR bacteria that are potentially hazardous to human and environment. This confirms AMR as one health issue and routine monitoring and surveillance of AMR among bacteria from meat ducks is suggested.

Field of Study:	Veterinary Science and technology	Student's Signature ....
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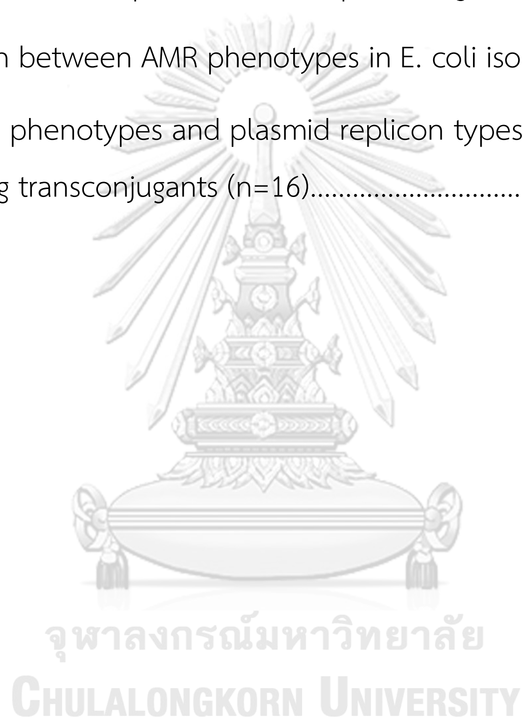
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LIST OF ABBREVIATIONS

AMR	antimicrobial resistance
bp	base pair
DNA	deoxyribonucleic acid
DW	distilled water
et al.	at alii, and others
g	gram
M	molar
MDR	multidrug resistance
mg	milligram
MIC	minimum inhibitory concentration
ml	milliliter
n	sample size/number
PCR	polymerase chain reaction
PBRT	PCR based replicon typing
pMLST	plasmid multilocus sequence typing
TAE	tris-acrtate-EDTA
µg	microgram
µl	microliter

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CHAPTER 1 INTRODUCTION

Importance and Rationale

Antimicrobial resistance (AMR) is a global threat according to the One Health perspective (Osman et al., 2019). This problematic issue causes 700,000 human fatalities annually on a global scale (O'Neill, 2016). If no quick action is made to control the AMR problem, it is anticipated that 10 million human deaths will result from AMR bacterial infections, and the cost of AMR will exceed 100 trillion USD by 2050 (O'Neill, 2016). The burden of the AMR problem is far greater in low- and middle-income countries than in developed nations (Pokharel et al., 2019). Antimicrobial use (AMU) in livestock, particularly in poultry production to treat bacterial infections, promote growth, and prevent disease, is considered as a major contributor to emergence and spread of AMR. For its control and prevention, it is necessary to raise the awareness of AMU among livestock sector stakeholders (Poole and Sheffield, 2013).

The duck production is now an integral part of poultry industry in Thailand. Duck meat is delicious, rich in amino acids and polyunsaturated fatty acids but low in fat, and is regarded as an excellent source of protein for human consumption (Adzitey et al., 2012b; Assawatheptawee et al., 2022). As a result, chicken meat is replacing by duck meat day by day across several countries, including Thailand, where the rate of production of ducks for meat has increased dramatically (Sinwat et al., 2021). Thailand has been among the top 10 countries for duck meat for the past decade. A 1% rise from the previous year put the expected export value of duck meat products at USD 18 million in 2019 (MOC, 2019). Thailand produces 7,000,000 meat-type ducks annually, the largest population of ducks reared for meat in the world (OIE, 2016). Due to this enormous duck production, it is anticipated that antimicrobials have been increasingly used to cure and prevent the transmission of infectious diseases among ducks (Sinwat et al., 2021). According to a survey of multiple farms, amoxicillin, colistin, doxycycline, oxytetracycline, and tilmicosin are commonly administered as prophylactic treatment (Wongsuvan et al., 2018). Rearing ducks in an open house farming system is common in Thailand and many developing countries (Charoensook

et al., 2021). Such rearing system has become an issue for public health concern, because of insufficient biosecurity measures in animal care and farm management. Importantly, ducks that may look healthy, yet they can spread bacteria including AMR pathogens to humans via either direct or indirect contact (Assawatheptawee et al., 2022).

Monitoring and surveillance of AMR in bacteria from food animals included commensal *Escherichia coli* as a Gram negative representative indicator (EFSA, 2012). Commensal *E. coli* are commonly found in the large intestines of both humans and animals, where they serve as reservoirs for AMR determinants that can be transmitted to other bacterial pathogens (Madec and Haenni, 2018). These bacterial species are usually resistant to many antibiotics at the same time. Commensal *E. coli* possess a variety of conjugative R plasmids carrying genes encoding resistance to one or more antibiotics. These R plasmids are vehicles for the dissemination of AMR determinants (Carattoli, 2013; Madec and Haenni, 2018).Transferable R plasmids play a major role in the evolution and spread of AMR.

Incompatibility (Inc) grouping is a technique for plasmid identification and classification (Rozwandowicz et al., 2018). Plasmids from the same Inc group can neither coexist in the same bacterial cells nor be transmitted between them because they share the same replication control or partitioning mechanisms (Novick, 1987). The existence of bacterial strains containing plasmids from the same Inc group in those from diverse sources indicates the horizontal transmission of such plasmids with close phylogenetic relationships (Carattoli, 2009). Prior research demonstrated that particular Inc groups was related to particular bacterial species or genera. For example, the Enterobacteriaceae family, which includes *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enterica*, frequently contains IncF plasmids (Carattoli, 2013). IncX plasmids have been identified in *Salmonella* (Sinwat et al., 2016; Trongjit et al., 2017) and *E. coli* (Lay et al., 2012; Trongjit et al., 2016), but they are also present in *Pseudomonas* spp., *Acinetobacter* spp. and *Aeromonas* spp. (Lukkana et al., 2011; Poonsuk et al., 2012). The relationship between particular Inc groups and bacterial species may be due to certain plasmids' capacity for stable replication in particular bacterial hosts (Carattoli, 2013; Rozwandowicz et al., 2018; Puangseree et al., 2022).

The horizontal transfer of plasmids is a crucial factor in the spread of AMR. These place the investigation of plasmids as an epidemiological marker for AMR surveillance (Puangseree et al., 2022).

To date, AMR studies have been extensively conducted in livestock, especially pigs and broilers. Previous studies reported AMR extent, distribution, genetic characteristics including plasmid replicons in livestock, meat and humans (Trongjit et al., 2016; Pungpian et al., 2021). The predominant type of replicon was IncF in *E. coli* isolated from pigs, pork and humans in Thailand (Puangseree et al., 2022). However, the data is still limited in bacteria of duck origin. This study aimed to describe the AMR characteristics and transferable plasmids in *E. coli* from meat ducks in Thailand.

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Objectives of the Study

1. To phenotypically examine AMR in commensal *E. coli* in meat ducks.
2. To characterize R plasmids in commensal *E. coli* isolated from meat ducks in Thailand.

Research Questions of the Study

1. What is phenotypic AMR of commensal *E. coli* isolated from meat ducks?
2. What are the characteristics and role of R plasmids in spreading of AMR in commensal *E. coli* in meat ducks in Thailand?

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CHAPTER 2 LITERATURE REVIEW

1. General characteristics of *Escherichia coli*

Escherichia coli is a rod-shaped, gram-negative and facultatively anaerobic bacteria. This Enterobacteriaceae family member is typically found in the lower intestine of warm-blooded animals and humans. The optimal conditions for development are a pH of 6-7 and a temperature of 37 °C. Eosin methylene blue (EMB) and MacConkey agar are recognized as selective and distinguishing media for identifying *E. coli* from gram-negative organisms that do not digest lactose (Na et al., 2019).

2. Pathogenesis of *Escherichia coli* infection

Although the majority of *E. coli* strains are not harmful, some of them can be harmful if ingested in food. The pathogenesis of *E. coli* can begin locally in the digestive system and spread to other body regions. Urinary tract infections, gastroenteritis, and neonatal meningitis are common infections by *E. coli* in humans. Enterotoxigenic *E. coli* (ETEC), entero-pathogenic *E. coli* (EPEC), entero-invasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) are six distinct pathotypes that can cause enteric *E. coli* infections (Stenutz et al., 2006). Colibacillosis in newborn animals is mostly caused by the bacterium ETEC, which also causes substantial economic damage worldwide (Barros et al., 2023). Additionally, ETEC frequently results in traveler's diarrhea in underdeveloped nations (Nagy and Fekete, 2005). The most prevalent outbreak strain of EHEC O157:H7 in people is the one that causes hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Robins and Hartland, 2002). Commensal *E. coli*, on the other hand, functions as normal gut flora to prevent dangerous bacteria from inhabiting the intestines. Commensal *E. coli* are now more of a problem than ever since they may serve as a source of resistance genes and determinants that could spread to pathogenic bacteria.

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3. AMR in commensal *E. coli* in poultry

As a member of the Enterobacteriaceae family, *E. coli* is a Gram-negative bacterium. Large intestines of animals and birds commonly contain the *E. coli* bacteria. The majority of *E. coli* are harmless and necessary for a healthy digestive system. *E. coli* has therefore been utilized as a marker for fecal contamination of food and water. In particular, *E. coli* has been utilized as a sentinel for AMR monitoring (EFSA, 2015). The primary source of resistance genes that can spread to other bacterial species is *E. coli*. *E. coli* evolved to become resistant to the antibiotics through genetic changes or the addition of mobile genetic components. Resistance to last-line antibiotics, such as third-generation cephalosporins, the antibiotic of choice for severe infections, carbapenem, has been reported to be on the rise, along with multidrug-resistant (MDR) *E. coli* (Paitan., 2018; Liu et al., 2023). Antimicrobials are used in animals raised for food for a variety of purposes, including treatment, disease management and prevention, and growth enhancement. Increased antimicrobials can accelerate and propagate AMR among bacteria, which might be transmitted to humans through the food chain (Sinwat et al., 2016). According to previous study prevalence of *E. coli* is reported 39% in raw poultry meat in Bangkok, Thailand (Trongjit et al., 2016). Commensal *E. coli* are currently causing more concern than ever since they may serve as a reservoir for resistance genes and determinants that might be passed on to pathogenic bacteria.

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4. Duck production in Thailand

Meat-type duck production plays a significant role in the livestock industry of Thailand, meeting the growing demand for high-quality duck meat both domestically and internationally. Thailand has developed specialized production systems to provide meat ducks to market. According to a study large-scale commercial farms in Thailand employ intensive production systems that focus on rapid growth and efficient feed conversion (Biswas et al., 2019). These farms often utilize selected meat-type breeds, such as the Pekin breed, known for its high growth rate and meat yield. On average 7,000,000 ducks are reared annually for meat purposes (OIE, 2016). Moreover, previous studies have explored the nutritional requirements and feeding strategies for meat-

type ducks in Thailand, aiming to optimize growth performance and carcass quality (Baéza, 2016). Furthermore, it has been investigated that meat quality characteristics and sensory attributes of duck meat produced in Thailand, providing valuable insights for product development and consumer acceptance (Prahkarnkaeo et al., 2017). The advancements in meat-type duck production in Thailand highlight the industry's commitment to meet the increasing market demands for high-quality, nutritious duck meat.

5. *E. coli* in ducks

Studies on the features of commensal *Escherichia coli* that are resistant to several kinds of antibiotics, including tetracyclines, sulfonamides, and fluoroquinolones, have been conducted in Thailand in chickens and ducks (Nhung et al., 2016). These findings imply that the selection and dissemination of antibiotic-resistant bacteria have been caused by the usage of antibiotics in Thailand's poultry and duck industries. Several investigations have documented the existence of transferable resistance plasmids in commensal *E. coli* in poultry and other food animals in Thailand in addition to high levels of resistance (Chotinantakul et al., 2022; Trongjit et al., 2022). These plasmids contribute to the spread of antibiotic resistance by dispersing resistance genes to other bacteria, including human pathogenic bacteria. Overall, research on the AMR traits of commensal *E. coli* in Thai poultry and ducks has revealed the necessity to regulate antibiotic usage in the poultry and duck business and stop the development of resistant bacteria. This includes limiting the use of antibiotics in animals used for food production, putting in place infection control procedures to stop the spread of resistant bacteria, and encouraging the creation and application of substitute techniques for treating bacterial infections in chickens and ducks.

According to a recent study, duck-derived commensal *E. coli* may indirectly affect human and animal health by acting as possible reservoirs for resistant genes and antibiotic resistance (Assawatheptawee et al., 2022). Duck isolates were shown to be 39.7% MDR by the French surveillance network for AMR in diseased animals, which examined isolates from 2012 to 2016 in seven animal species (Bourély et al., 2019).

According to a recent study in South Korea, AMR in *E. coli* isolates from healthy ducks' feces was relatively high (54.0%) (Na et al., 2019). According to a study conducted in China it is reported that the highest prevalence of MDR *E. coli* in isolates from ducks (100%) (Yassin et al., 2017). The prevalence of *E. coli* reported highest in duck feces (87.93%) (Adzitey et al., 2012a). Data on *E. coli* in ducks, however, are limited. Additionally, trends of AMR in commensal bacteria might indicate the antimicrobials that were used during animal production (EFSA, 2012). According to particular research, the prolonged use of antimicrobials in animal production at sub-therapeutic doses may increase the predominance of bacteria that are resistant to treatment (Gullberg et al., 2011). The use of antimicrobials blended in animal feed as a growth booster is now forbidden in several EU and Thai counties, among other places (Elliott, 2015; Rychen et al., 2017).

6. Transfer of AMR determinants and role of plasmids in AMR distribution

The development of AMR among pathogenic bacteria is considered as a hazard for public health. Plasmids play a significant role in horizontal gene transfer, which has contributed to the continuous growth of AMR bacteria (Rozwandowicz et al., 2018) i.e., 1. vertical transfer of resistant bacteria occurs in a clonal proliferation of one resistance strain and 2. horizontal transmissions of AMR genes make up the major mechanisms of transfer of resistance determinants in pathogenic bacteria (Von Wintersdorff et al., 2016). Bacteriophages may transfer bacterial DNA from previously infected donor cells to other cells through transduction, which requires cell-to-cell contact (Devanga Ragupathi et al., 2019). Plasmids are extra-chromosomal circular DNA structures that can replicate on their own in a host cell. Other mechanisms of horizontal gene transfer include conjugation, which requires cell-to-cell contact to transfer DNA from donor cell to recipient cell (Figure 1); transformation, which is the uptake of naked DNA mobile genetic elements (MGEs) were substantially related with the distribution of AMR determinants in Enterobacteriaceae and had the capacity to transmit the resistance determinants by horizontal gene transfer via conjugative plasmids (Szmolka and Nagy, 2013).

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Furthermore, plasmids, particularly the R plasmid, which contains resistant genes for AMR, are giving their bacterial host cell new functions like AMR. It gives their host cells an advantage when confronted with antimicrobial pressure. The fast-global expansion of bacterial families like Enterobacteriaceae and the prevalence of MDR bacteria have been attributed in large part to this free movement of R-plasmids (Rozwandowicz et al., 2018). Unfortunately, it has been shown that many significant antimicrobial classes can coexist on the same conjugative plasmid. For instance, the *E. coli mcr-1* and $bla_{CTX\text{-}M\text{-}1}$ genes can coexist with genes that confer resistance to sulfonamides and tetracyclines on a large conjugative plasmid, plasmid co-hosting *mcr-1* and $bla_{CTX\text{-}M\text{-}55}$ in *Salmonella* in poultry in China and *E. coli* from cattle in France (Haenni et al., 2016).

As a result, numerous AMR genes can be concurrently acquired by acquiring a single R-plasmid. Plasmids are significant because they may contain multiple resistance genes, which may allow bacteria to acquire new resistance genes and propagate AMR (Thomas and Nielsen, 2005). Recent studies of the genetic stability of plasmids and the frequent discovery of resistance plasmids in isolates of several food-borne infections indicate that plasmids as a significant source of AMR genes that might pose a significant public health hazard.

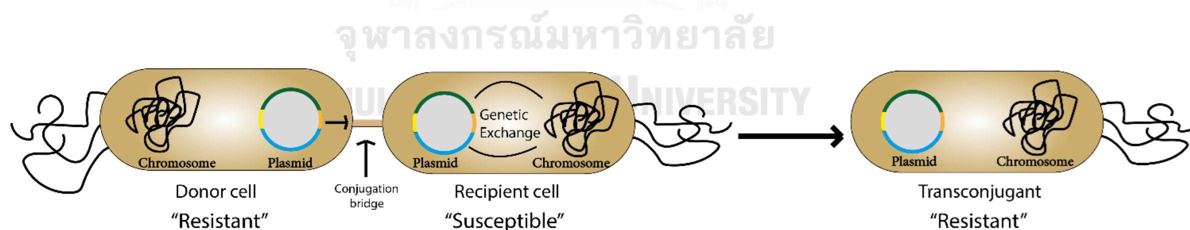

Figure 1 Schematic picture of horizontal gene transfer by conjugation. In conjugation, resistant donor cells carry resistance genes on plasmid and horizontally transfer R-plasmids to susceptible recipient cells. Transconjugants are recipient cells acquiring R-plasmids and exhibiting corresponded resistance phenotype.

7. PCR- based plasmid replicon typing (PBRT) for R plasmids identification

A plasmid is a circular fragment of extrachromosomal DNA that can replicate on its own inside a bacterial cell. By containing antimicrobial resistance genes, plasmids can impart resistance to the major antibiotic classes (Carattoli et al., 2005). Plasmids play a significant role in the propagation of AMR through horizontal bacterial population interchange (Zhang et al., 2019). Incompatibility (Inc) groups are a systematic approach for classifying plasmids. To identify the replicons of the major plasmid groups, PCR-based replicon typing has been established (Carattoli et al., 2005). This technique was used to find 18 different replicon types and is based on 5 multiplex and 3 simplex PCR (Rozwandowicz et al., 2018).

8. Plasmid multilocus sequence typing (pMLST) for R plasmids classification

A technique used to research the genetic diversity and development of AMR in bacteria is called plasmid multilocus sequence typing (pMLST). Understanding the processes by which resistance develops and spreads is crucial for addressing the huge global health concern posed by AMR. Because plasmids are mobile genetic components that may be rapidly transmitted across bacteria, allowing for the quick dissemination of resistance genes, pMLST is a potent method for studying plasmid-mediated AMR in bacteria. One can recognize and monitor the spread of certain plasmids that carry AMR genes by sequencing the plasmid at various loci and comparing the sequences to a database of previously identified plasmids. pMLST can be used to follow the spread of AMR, as well as to figure out the mechanism of resistance and the ancestry and evolution of resistance genes. This data may be utilized to design novel antimicrobial medicines for bacterial infections as well as curb the spread of AMR. In conclusion, pMLST is employed in AMR research because it offers insightful data on the genetic diversity and evolution of AMR genes, facilitating a better comprehension of how resistance spreads and develops as well as informing the creation of strategies to stop the spread of AMR.

pMLST is an additional tool for grouping plasmids that are in the same Inc group and for classifying plasmids in each Inc group into sequence types based on different DNA sequences at the specific loci of each plasmid. This scheme is used to analyze

the various sequence types by choosing a number of target genes (Carattoli et al., 2014). pMLST was designed to recognize and subtype plasmid IncF, I1, N, HI1, HI2, and A/C at this time (Villa et al., 2010; Hancock et al., 2017). The epidemiological research of plasmid Inc groups has been used to follow the horizontal transmission of AMR genes among the Enterobacteriaceae or to monitor the circulation of plasmids among bacterial strains from various sources (Carattoli et al., 2005)

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CHAPTER 3 MATERIALS AND METHODS

This study consisted of 4 phases (Figure 2). The first phase included confirmation of the bacterial isolates. In the second phase, detection of AMR phenotypes was done. The third phase was the genetic characterization of R plasmids. The fourth phase was the statistical analysis.

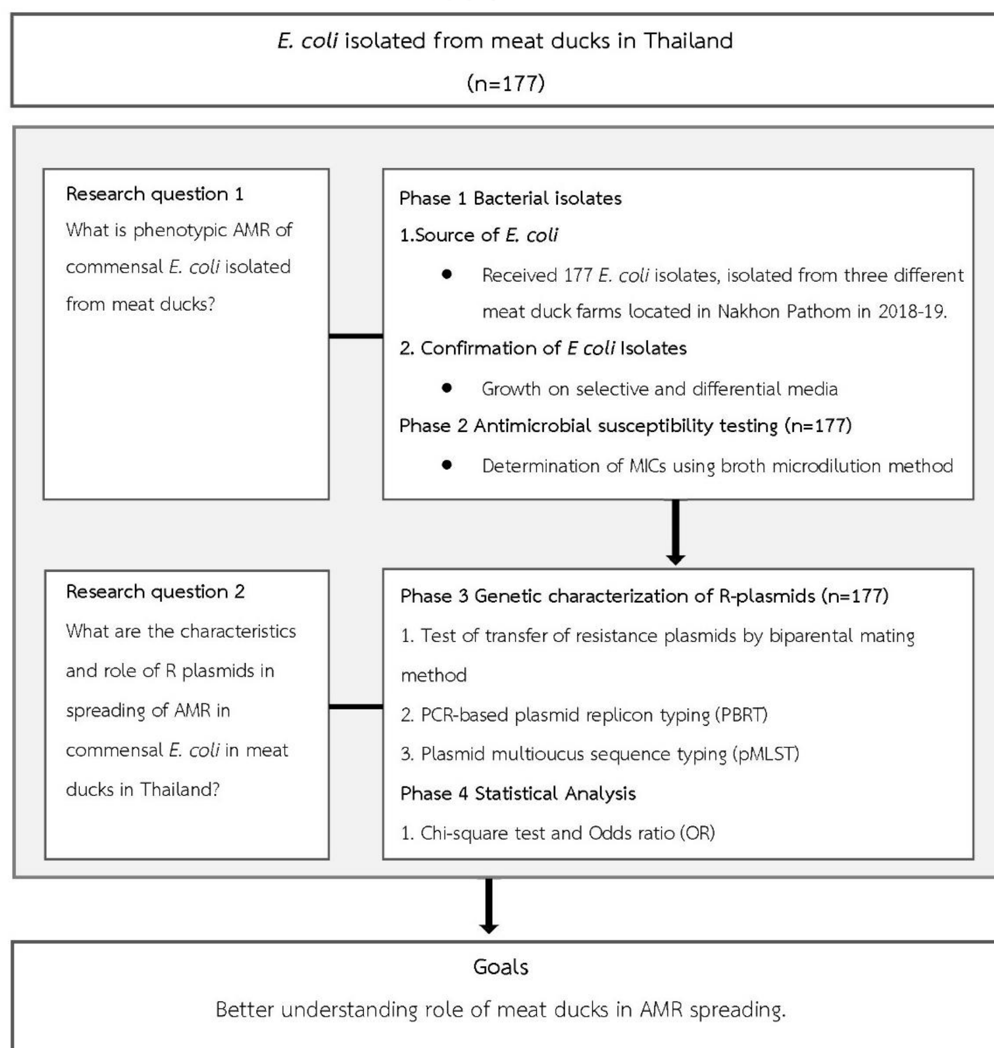

Figure 2 Experimental design of the study

Phase 1 Bacterial isolates

1.1 Source of *E. coli*

E. coli isolates (n=177) were obtained from the Department of Farm Resources and Production Medicine, Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom, Thailand. All the *E. coli* strains were isolated from fecal samples obtained by cloacal swab taken randomly from the birds. They were collected from three different meat-duck farms, located in Nakhon Pathom. On average, two farms have 4000 ducks and one farm has 2000 ducks. The open house farming system was used to rear all of the ducks, and each farm only had one flock. All this sample collection was done from 2018 to 2019. The samples were collected from meat ducks at 62-70 days of age with a normal health status by cloacal swab to ensure that they are commensal, when they sent to slaughterhouse for further processing. Amoxicillin is the only antibiotic that is used in the sampling farms, of which the purpose for disease treatment. According to the technical specifications of AMR monitoring, *E. coli* from the same sample in the similar epidemiological unit have the same AMR characteristics and show the similar resistance pattern. Therefore, only 1 isolate obtained from each positive sample to maintain representativeness.

The province of Nakhon Pathom, which is well known for raising livestock, is situated in the core economic zone of Thailand's central region. In Thailand, where 7,000,000 meat-type ducks are bred annually, this province has the most significant population of ducks raised for meat. In Nakhon Pathom, ducks are commonly raised in an open farming system.

1.2 Confirmation of *E. coli* isolates

All the *E. coli* isolates (n=177) were confirmed by growing on the Eosin methylene blue (EMB) agar plates and MacConkey agar plates (Na et al., 2019). The isolates with typical characteristics on selective agar were purified and stored as 20% glycerol stock at -80 °C for further processing (Figure 3).

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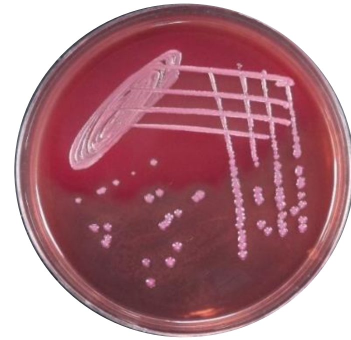

(a) (b)

Figure 3 Colony appearance of *E. coli* on selective media (a) *E. coli* showing metallic green sheen colonies on Eosin methylene blue (EMB) agar and (b) pinkish colonies on MacConkey agar

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Phase 2

2.1 Antimicrobial susceptibility testing (n=177)

All *E. coli* isolates (n=177) were examined for their susceptibilities to 15 antimicrobial agents by broth microdilution method using a Sensititre™, automatic machine (Thermo Scientific, Kansas, USA). The antimicrobial agents included (clinical breakpoints in parentheses): ampicillin (AMP, 32 µg/ml), azithromycin (AZI, 32 µg/ml), cefotaxime (FOT, 4 µg/ml), ceftazidime (TAZ, 16 µg/ml), chloramphenicol (CHL, 32 µg/ml), ciprofloxacin (CIP, 4 µg/ml), colistin (COL, 4 µg/ml), gentamicin (GEN, 16 µg/ml), meropenem (MERO, 4 µg/ml), nalidixic acid (NAL, 32 µg/ml), streptomycin (STR, 16 µg/ml), tetracycline (TET, 16 µg/ml), tigecycline (TGC, 1 µg/ml), trimethoprim (TMP, 16 µg/ml) and sulfamethoxazole (SMX, 512 µg/ml). The reference strains *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 were utilized as a quality control following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2018).

2.2 ESBL screening and confirmation (n=10)

Following the initial AST, *E. coli* isolates (n=10) that were resistant to the ESBL indicator antibiotics (cefotaxime and/or ceftazidime) were further analyzed for the confirmation of ESBL production. The Sensititre™ EUVSEC2 plate (TREK Diagnostic Systems, West Sussex, UK) was used for this confirmation step. This plate is specifically designed for the susceptibility testing of Enterobacteriaceae and includes the combination of cefotaxime/clavulanate and ceftazidime/clavulanate for ESBL confirmation. ESBL production was confirmed based on the MIC results for the ESBL indicator antibiotics (cefotaxime, ceftazidime, cefotaxime/clavulanate, and ceftazidime/clavulanate) according to the interpretive criteria outlined by the CLSI guidelines and reference strain *E. coli* ATCC 25922 was used (CLSI, 2018). i.e. any isolate showing a ≥ 3 twofold concentration decrease in the MIC for either cefotaxime or ceftazidime tested in combination with clavulanic acid versus its MIC when tested alone was confirmed as an ESBL-producer.

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Phase 3 Genetic characterization of R-Plasmids (n=148)

3.1 Test of transfer of resistance plasmids by conjugation

Horizontal transfer of resistance plasmids was tested by utilizing the biparental mating technique (Khemtong and Chuanchuen, 2008; Pungpian et al., 2021). MDR *E. coli* isolates (n=148) served as donors using ampicillin or tetracycline (n=77), ampicillin or tetracycline or colistin (n=15), ampicillin or tetracycline or chloramphenicol (n=10), ampicillin or colistin or chloramphenicol (n=1), ampicillin or tetracycline or chloramphenicol or colistin (n=32), ampicillin (n=6), tetracycline (n=6) and colistin (n=1) as selective pressure. Only one antibiotic was used as selective pressure in each plate. Rifampicin resistant *Salmonella* Enteritidis (SE12) strains (SE12 rif^r, MIC=256 µg/ml) was used as recipient and it is susceptible to all antibiotics tested, MIC values in parentheses. (CHL, 4 µg/ml; AMP, 1 µg/ml; TET, 1 µg/ml; COL, 0.0125 µg/ml) (Khemtong and Chuanchuen, 2008; Pungpian et al., 2021).

Briefly, the donor and recipient strains overnight cultures were diluted by mixing 80 ml of the culture with 4 ml of fresh Luria Bertani broth (Difco®, New Jersey, USA). In a microcentrifuge, the mating of donor and recipient cultures was made in a ratio of 1:1. Centrifugation at 8,000 rpm for 1 minute was used to collect the bacterial cells, which were then disseminated out on LB agar plates with 0.45 mm-sized filters (Millipore™, Merck, Darmstadt, Germany) and cultured at 37°C overnight. After that, the conjugation mixture was scraped and washed off the filter into a new microcentrifuge with 0.9% NaCl solution. On LB agar plates with rifampicin (32 µg/ml) and the appropriate antibiotic i.e. ampicillin (150 µg/ml), tetracycline (15 µg/ml), chloramphenicol (25 µg/ml) and colistin (2 µg/ml), the conjugation cells were collected, resuspended in 200 mL of 0.9% NaCl solution, and distributed on antibiotic plates. Transconjugants were further confirmed on Xylose Lysine Deoxycholate (XLD) agar (Difco®, New Jersey, USA) containing one of the following 4 antibiotics: ampicillin (150 µg/ml), tetracycline (15 µg/ml), chloramphenicol (25 µg/ml) and colistin (2 µg/ml). Resistance phenotype of all transconjugants were examined. The transconjugants were stored as 20% glycerol stock at -80 °C for further process. Overview of the conjugation experiment shown in Figure 4.

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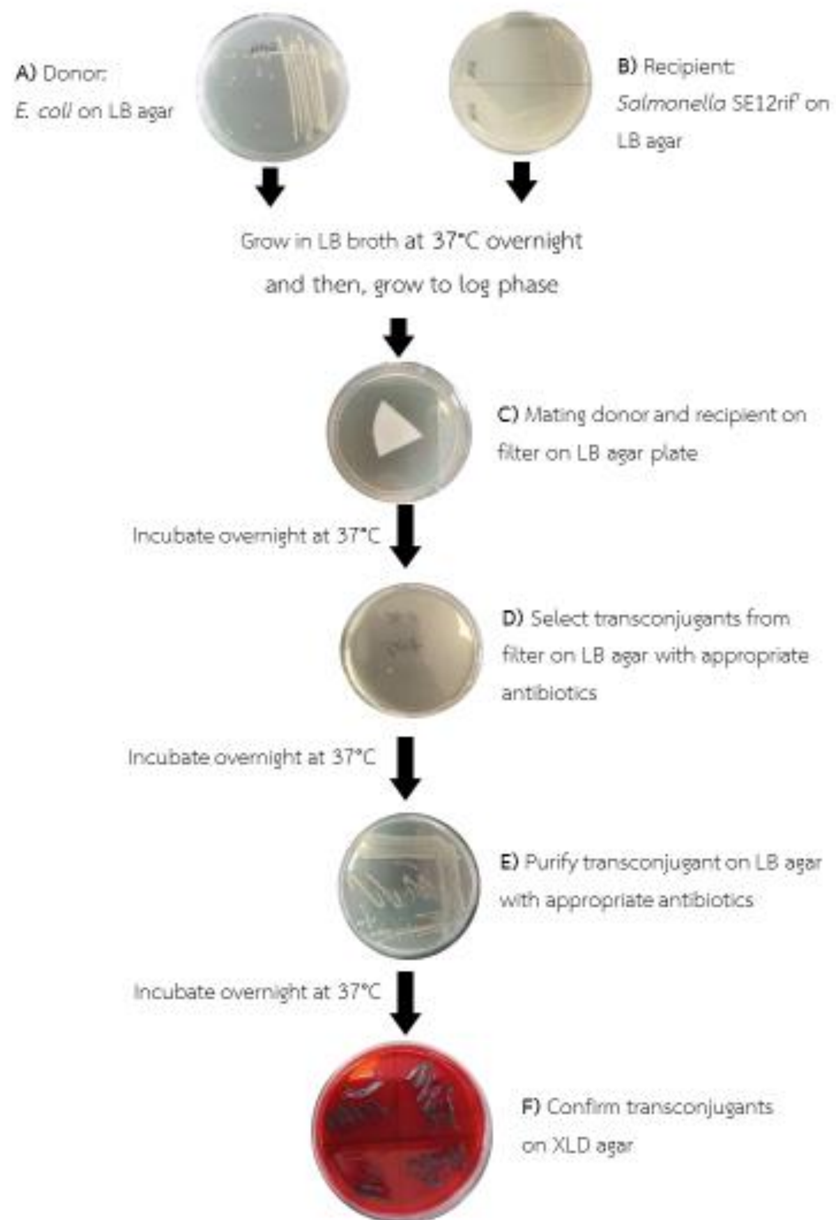

Figure 4 Flow of biparental mating conjugation experiment

3.2 Polymerase chain reaction (PCR)

All the polymerase chain reaction (PCR) primers are enlisted in Table 2 and PCR conditions are shown in Table 1.

Table 1 PCR conditions used for genetic characterization of R-plasmids in this study

PCR condition						
	Initial denaturatio	Denaturation	Annealing	Extension	Final extension	No. of cycles
PBRT[a]	94°C 5 min	94°C 1 min	60°C 30 sec	72°C 1 min	72°C 5 min	30
IncF pMLST[b]	94°C 5 min	94°C 1 min	60°C 30 sec	72°C 1 min	72°C 5 min	30

[a] Simplex-F was performed under PBRT PCR condition except the annealing temperature was 52 °C for 30 sec.

[b] FII was performed under pMLST PCR condition except the annealing temperature was 54 °C for 30 sec.

3.2.1 PCR-based replicon typing (PBRT)

The *E. coli* isolates were selected based on the results of conjugation experiment. The *E. coli* donors (n=13) that conjugally transferred plasmids when using ampicillin (n=3), tetracycline (n=4), chloramphenicol (n=3) and ampicillin/tetracycline (n=3) as selective pressure and one of their corresponding transconjugants (n=16) were selected. Screening of 18 Inc groups of plasmids was conducted using five multiplex PCRs (i.e., HI1/ HI2/I1-I g, X/L-M/N, FIA/FIB/W, Y/P/FIC, and A-C/T/ FIIs) and three simplex PCRs (i.e., F, K, and B/O) (Carattoli et al., 2005) (Table 1).

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3.2.2 Plasmid multilocus sequence typing (pMLST)

Based on PBRT results, six donor *E. coli* isolates (i.e. A144, A183, C248, C249, C250 and C253) that possess IncF replicons were selected for further characterization of IncF plasmids and subtyped by the pMLST scheme (Carattoli et al., 2014). The FIA, FIB, FIC, and FIIs were PCR amplified, purified and submitted for nucleotide sequencing (Villa et al., 2010). The Fasta files of individual allele specific sequences were uploaded for identification of allele number and sequence type (ST) assignment by using the pMLST database (www.pubmlst.org/plasmid/). The process is explained in a schematic diagram (Figure 5).

Because of the distinct multi-replicon characteristics of IncF plasmids, the IncF replicon sequence typing (RST) was carried out independently. FII, FIIs, FIA, FIC and FIB-carrying isolates were included. Each of the four replicon types FII, FIIs, FIA, FIC and FIB identified sequence variations was given an allele number. The mix of allele types found in each replicon were served as the basis for each plasmid's FAB formula (FII, FIIs, FIA and FIB). For instance, the FII allele 1, FIA allele 1, and FIB allele 1 were used to create the formula F1:A1:B:1. From FII allele 1, the formula F1:A-:B- was allocated. The symbols A- and B- indicate the lack of FIA and FIB replicons, respectively.

Phase 4 Statistical Analysis

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Descriptive statistics was used to examine the percentage of AMR using Microsoft excel program, in this investigation. Association of ampicillin resistance and tetracycline resistance with other antibiotics were determined by using Chi-square test and calculating odds ratio (OR) by SPSS program version 22.0. A p-value <0.05 was considered statistically significant. Odds ratio value <1 and >1 shows negative and positive associations, respectively.

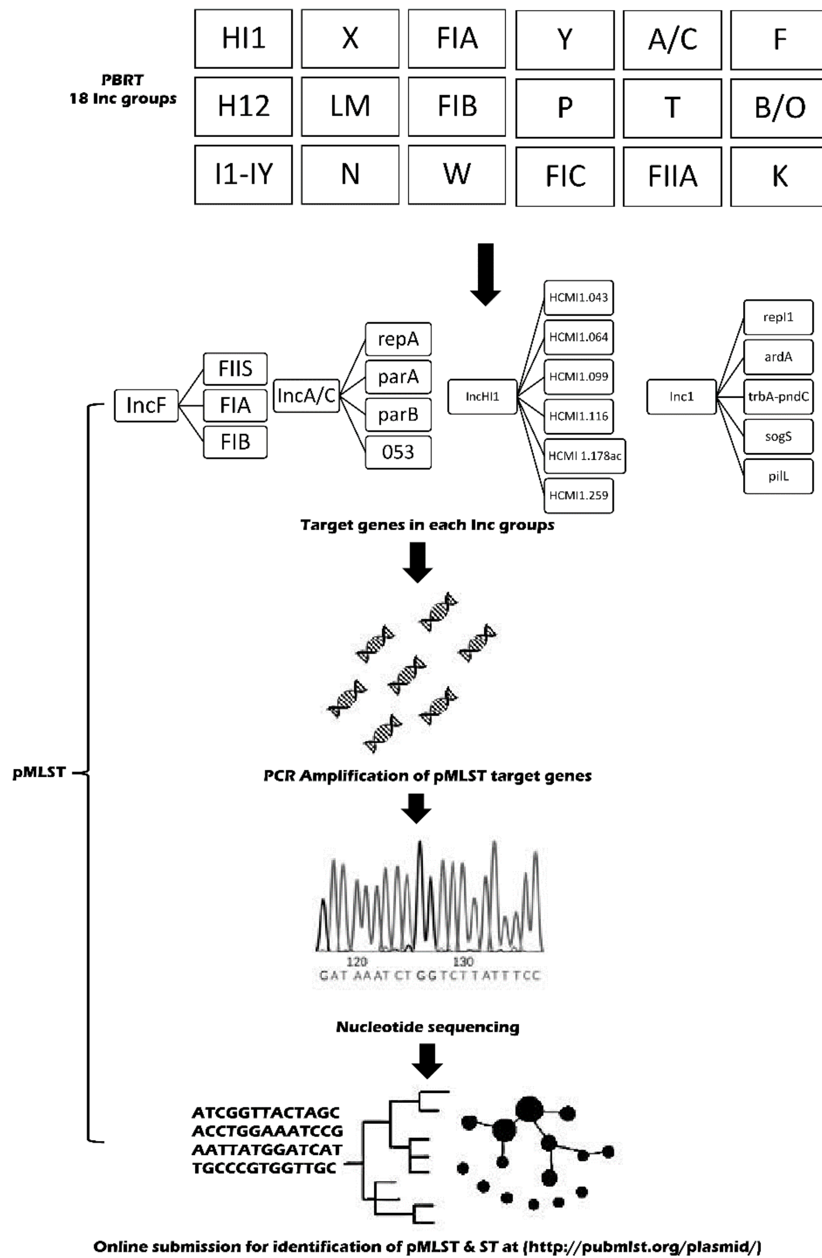

Figure 5 PBRT and pMLST scheme. Inc groups identification by PBRT was first conducted, followed by PCR amplification and nucleotide sequencing of pMLST target genes for each Inc groups. Nucleotide sequences were submitted to pMLST online database for ST identification.

Table 2 Primers used in this Study

Target		Primer	Sequence (5'–3')	Amplicon size	Reference
PBRT	IncHI1	HI1-F	GGAGCGATGGATTACTTCAGTAC	471	(Carattoli et al., 2005)
		HI1-R	TGCCGTTTCACCTCGTGAGTA		
	IncHI2	HI2-F	TTTCTCCTGAGTCACCTGTTAACAC	644	(Carattoli et al., 2005)
		HI2-R	GGCTCACTACCGTTGTCATCCT		
	IncI1	I1-F	CGAAAGCCGGACGGCAGAA	139	(Carattoli et al., 2005)
		I1-R	TCGTCGTTCCGCCAAGTTCGT		
	IncX	X-F	AACCTTAGAGGCTATTTAAG TTGCTGAT	376	(Carattoli et al., 2005)
		X-R	TGAGAGTCAATTTTTATCTCATGTTTT AGC		
	IncL/M	L/M-F	GGATGAAAACTATCAGCATCTGAAG	785	(Carattoli et al., 2005)
		L/M-R	CTGCAGGGGCGATTCTTTAGG		
	IncN	N-F	GTCTAACGAGCTTACCGAAG	559	(Carattoli et al., 2005)
		N-R	GTTTCAACTCTGCCAAGTTC		
	IncFIA	FIA-F	CCATGCTGGTTCTAGAGAAGGTG	462	(Carattoli et al., 2005)
		FIA-R	GTATATCCTTACTGGCTTCCGCAG		
	IncFIB	FIB-F	GGAGTTCTGACACACGATTTTCTG	702	(Carattoli et al., 2005)
		FIB-R	CTCCCGTCGCTTCAGGGCATT		
	IncW	W-F	CCTAAGAACAACAAAGCCCCCG	242	(Carattoli et al., 2005)
		W-R	GGTGCGCGGCATAGAACCGT		
	IncY	Y-F	AATTCAAACAACACTGTGCAGCCTG	765	(Carattoli et al., 2005)
		Y-R	GCGAGAATGGACGATTACAAAACTTT		
	IncP	P-F	CTATGGCCCTGCAAACGCGCCAGAAA	634	(Carattoli et al., 2005)
		P-R	TCACGCGCCAGGGCGCAGCC		
	IncFIC	FIC-F	GTGAACTGGCAGATGAGGAAGG	262	(Carattoli et al., 2005)
		FIC-R	TTCTCCTCGTCGCCAAACTAGAT		
	IncA/C	A/C-F	GAGAACCAAAGACAAAGACCTGGA	465	(Carattoli et al., 2005)
		A/C-R	ACGACAAACCTGAATTGCCTCCTT		
	IncT	T-F	TTGGCCTGTTTGTGCCTAAACCAT	750	(Carattoli et al., 2005)
		T-R	CGTTGATTACACTTAGCTTTGGAC		
	IncFIIA	FIIs-F	CTGTCGTAAGCTGATGGC	270	(Carattoli et al., 2005)
		FIIs-R	CTCTGCCACAAACTTCAGC		
	IncF	F-F	TGATCGTTTAAGGAATTTTG	270	(Carattoli et al., 2005)
		F-R	GAAGATCAGTCACACCATCC		
	IncK	K-F	GCGGTCCGGAAAGCCAGAAAAC	160	(Carattoli et al., 2005)
		K-R	TCTTTCACGAGCCCGCCAAA		
	IncB/O	B/O-F	GCGGTCCGGAAAGCCAGAAAAC	159	(Carattoli et al., 2005)

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Target		Primer	Sequence (5'–3')	Amplicon size (bp)	Reference
		B/O-R	TCTGCGTTCCGCCAAGTTCGA		
IncF-RST	FII	FII-F	CTGATCGTTTAAGGAATTTT	258–262	(Villa et al., 2010)
		FII-R	CACACCATCCTGCACTTA		
	FIIs	FIIS-F	CTAAAGAATTTTGATGGCTGGC	259–260	(Villa et al., 2010)
		FIIS-R	CAGTCACTTCTGCCTGCAC		
	FIA	FIA-F	CCATGCTGGTTCTAGAGAAGGTG	462	(Villa et al., 2010)
		FIA-R	GTATATCCTTACTGGCTTCCGCAG		
	FIB	FIBs-F	TGCTTTTTATTCTTAAACTATCCAC	683	(Villa et al., 2010)
		FIB-R	CTCCCGTCGCTTCAGGGCATT		

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Chapter 4 Results

1. Antimicrobial susceptibilities of *E. coli* isolates

All *E. coli* isolates in this study were resistant to at least one antimicrobial agent. The highest percentage resistance rate was observed in *E. coli* isolates to ampicillin (83%) and tetracycline (81.9%), followed by streptomycin (75.7%), tigecycline (72.8%) and sulfamethoxazole (60.4%). Only 2.2% of *E. coli* isolates were resistant to ciprofloxacin. Resistance rates to colistin and chloramphenicol were 27.6% and 24.2%, respectively. None of the isolates were resistant to meropenem. Multidrug resistance (MDR, being resistant to at least three antimicrobials in different classes) was observed in 86.4% of *E. coli* isolates (Figure 6). The most prevalent resistance patterns were AMP-STR-TET-TGC (6.2%), AMP-STR-TET (5.6%) and AMP-TET-TGC (5.6%) (Table 3).

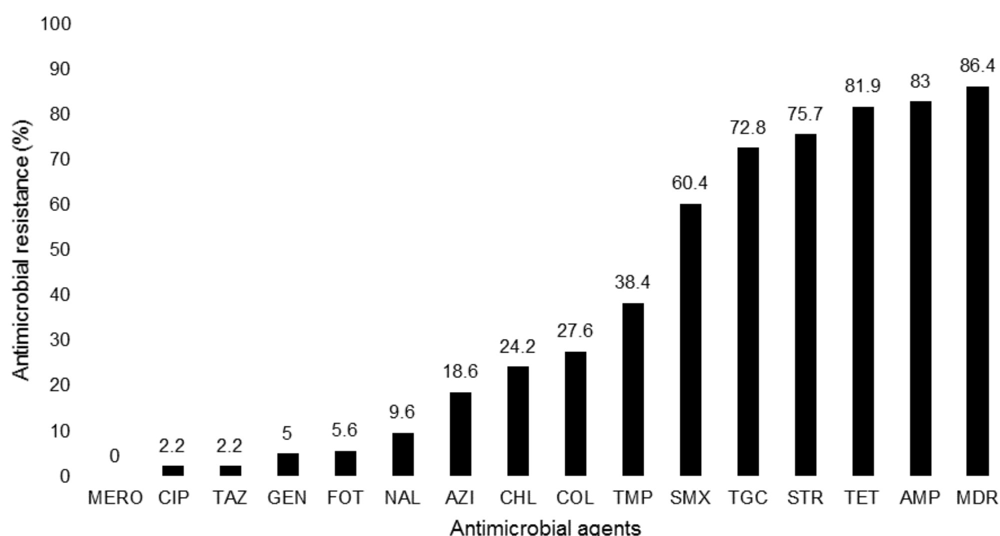

Figure 6 Distribution of AMR among *E. coli* isolates from meat ducks (n=177)
Abbreviations: AMP, ampicillin; AZI, azithromycin; FOT, cefotaxime; TAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; GEN, gentamicin; MERO, meropenem; NAL, nalidixic acid; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TGC, tigecycline; TMP, trimethoprim; MDR, multidrug resistance

Table 3 Resistance pattern of the *E. coli* isolates isolated from meat ducks (n=177)

AMR pattern	N0. of isolates (n)
STR	1 (0.5)
SMX	3 (1.6)
TET	1 (0.5)
TGC	1 (0.5)
AMP-SMX	1 (0.5)
AMP-TET	4 (2.2)
STR-TGC	3 (1.6)
STR-TET	3 (1.6)
STR-SMX	4 (2.2)
SMX-TGC	3 (1.6)
AMP-STR-SMX	1 (0.5)
AMP-SMX-TGC	1 (0.5)
AMP-STR-TET	10 (5.6)
AMP-TET-TGC	10 (5.6)
COL-STR-TGC	1 (0.5)
STR-SMX-TGC	5 (2.8)
STR-SMX-TMP	1 (0.5)
STR-TET-TGC	1 (0.5)
SMX-TET-TGC	2 (1.1)
SMX-TGC-TMP	1 (0.5)
AMP-AZI-TET-TMP	1 (0.5)
AMP-FOT-TET-TGC	2 (1.1)
AMP-COL-TET-TGC	2 (1.1)
AMP-COL-STR-TET	1 (0.5)
AMP-STR-SMX-TET	3 (1.6)
AMP-STR-TET-TGC	11 (6.1)
AMP-STR-TET-TMP	1 (0.5)
AMP-TET-TGC-TMP	1 (0.5)
STR-SMX-TET-TGC	1 (0.5)
AMP-STR-SMX-TET-TGC	2 (1.1)
AMP-STR-SMX-TGC-TMP	3 (1.6)
AMP-AZI-STR-SMX-TET	2 (1.1)
AMP-AZI-SMX-TET-TGC	2 (1.1)
AMP-CHL-SMX-TET-TMP	1 (0.5)
AMP-STR-SMX-TET-TMP	3 (1.6)
AMP-AZI-STR-TET-TGC	6 (3.3)
AMP-COL-STR-TET-TGC	2 (1.1)
AMP-COL-STR-SMX-TET	1 (0.5)
AMP-COL-STR-TET-TMP	1 (0.5)
AMP-FOT-CHL-COL-TGC	1 (0.5)
AMP-FOT-STR-TET-TGC	1 (0.5)
AZI-STR-SMX-TET-TGC	1 (0.5)

GEN-STR-SMX-TET-TGC	1 (0.5)
AMP-STR-TET-TGC-TMP	5 (2.8)
AMP-SMX-TET-TGC-TMP	1 (0.5)
AMP-AZI-TET-TGC-TMP	1 (0.5)
AMP-NAL-STR-TGC-TMP	1 (0.5)
AMP-STR-SMX-TET-TGC-TMP	8 (4.5)
AMP-AZI-STR-SMX-TET-TGC	2 (0.5)
AMP-CHL-STR-SMX-TET-TMP	2 (1.1)
AMP-COL-STR-SMX-TET-TGC	1 (0.5)
AMP-NAL-STR-TET-TGC-TMP	1 (0.5)
AMP-NAL-STR-SMX-TET-TGC	2 (1.1)
AMP-AZI-COL-STR-SMX-TET-TGC	1 (0.5)
AMP-AZI-CHL-COL-STR-SMX-TET	1 (0.5)
AMP-AZI-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-CHL-COL-SMX-TET-TGC-TMP	1 (0.5)
AMP-COL-STR-SMX-TET-TGC-TMP	4 (2.2)
AMP-COL-NAL-STR-SMX-TET-TGC	1 (0.5)
AMP-CHL-COL-STR-SMX-TET-TMP	1 (0.5)
AMP-CHL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-AZI-COL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-AZI-CHL-STR-SMX-TET-TGC-TMP	2 (1.1)
AMP-AZI-CHL-COL-STR-SMX-TET-TGC	3 (1.6)
AMP-AZI-CHL-COL-SMX-TET-TGC-TMP	1 (0.5)
AMP-FOT-NAL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-CHL-COL-STR-SMX-TET-TGC-TMP	7 (3.3)
AMP-CHL-COL-NAL-STR-SMX-TET-TGC	1 (0.5)
AMP-AZI-CHL-COL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET	1 (0.5)
AMP-CHL-COL-NAL-STR-SMX-TET-TGC-TMP	5 (2.8)
AMP-CHL-CIP-NAL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-CHL-CIP-COL-NAL-STR-SMX-TET-TMP	1 (0.5)
AMP-AZI-CHL-COL-GEN-STR-SMX-TET-TGC-TMP	3 (1.6)
AMP-AZI-CHL-GEN-NAL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-AZI-FOT-CHL-COL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC	3 (2.2)
AMP-CHL-CIP-COL-NAL-STR-SMX-TET-TGC-TMP	1(0.5)
AMP-AZI-CHL-CIP-COL-NAL-STR-SMX-TET-TGC-TMP	1 (0.5)
Total	177 (100)

Abbreviations: AMP, ampicillin; AZI, azithromycin; FOT, cefotaxime; TAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; GEN, gentamicin; MERO, meropenem; NAL, nalidixic acid; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TGC, tigecycline; TMP, trimethoprim

2. ESBL producing *E. coli* isolates

Only ten isolates out of 177 *E. coli* isolates were resistant to cefotaxime and ceftazidime in this study. Nine isolates except C220 were confirmed to be the ESBL-producer (Table 4).

Table 4 Antibiotic resistance pattern of ESBL producing *E. coli* Isolates (n=9)

Strain ID	Antibiotic resistance pattern
A197	AMP-FOT-TET-TGC
A198	AMP-FOT-TET-TGC
B129	AMP-FOT-CHL-COL-TGC
B131	AMP-AZM-FOT-CHL-COL-STR-SMX-TET-TGC-TMP
C172	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC
C177	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET
C201	AMP-FOT-STR-TET-TGC
C249	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC
C250	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC

Abbreviations: AMP, ampicillin; AZI, azithromycin; FOT, cefotaxime; TAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; GEN, gentamicin; MERO, meropenem; NAL, nalidixic acid; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TGC, tigecycline; TMP, trimethoprim

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3. Transfer of R plasmids and conjugation efficiency

Thirteen *E. coli* donors yielded transconjugants in the presence of a single antibiotic selective pressure including tetracycline (n=4/13), ampicillin (n=3/13) and chloramphenicol (n=3/13). Three isolates yielded transconjugants in either ampicillin or tetracycline selective pressure. None of the transconjugants were obtained in the presence of colistin. All transconjugants were resistant to additional antibiotics besides the antibiotic selective pressures and most of them were multidrug resistant. Conjugation rates vary from 4.76×10^{-8} to 9.5×10^{-7} (Table 6).

4. Plasmid replicon typing

4.1. Plasmid replicons among the *E. coli* isolates from meat ducks

Overall, five replicons types were found among the *E. coli* donors (n=13) and their respective transconjugants (n=16). The most common replicon identified in *E. coli* donors was IncFrepB (n=5/13), followed by IncFIC (n=2/13). The other replicons identified were $IncI_1$ (n=1/13), IncY (n=1/13) and IncFIB (n=1/13). In transconjugants, the most common replicon identified was IncFrepB (n=5/16). Interestingly, IncFrepB found in both donors and transconjugants. Some donors and transconjugants did not carry Inc plasmids tested in this study (Table 6).

4.2. Replicon sequence type (RSTs) of IncF plasmids in *E. coli* isolates (n=6)

Due to the numerous replicon status of F-type plasmids, the RST scheme was initially created for subtyping and the FAB formula of each plasmid was identified. Six selected *E. coli* isolates that belong to the F replicon were found to possess different IncF replicon sequence types by pMLST analysis. Five FAB formula were identified including C249, C250, F47:A-:B-; A144, F29:A-:B23; A183, F29:A-:B-; C248, F18:A-:B- and C253, F4:A-:B- (Table 6).

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5. Association among AMR phenotypes in *E. coli* isolates (n=177)

There were different types of associations between AMR phenotypes in *E. coli* isolates (n=177) revealed in Table 5. Overall, more positive associations were observed between resistance phenotypes than negative associations. The strongest positive association were observed between ampicillin and tetracycline resistance (OR=50.3, Cl: 17-148), followed by chloramphenicol and sulfamethoxazole resistance (OR=44.5, Cl: 5.9-333).

Chloramphenicol resistance was positively associated to all antibiotics tested except tigecycline. The strong positive association (OR>10) was observed between chloramphenicol resistance and resistance to colistin, gentamicin, sulfamethoxazole and tetracycline. There was no positive association between MDR and AMR phenotypes

but some were associated significantly ($p<0.05$). There associations were between AMR phenotypes.

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Table 5 Association between AMR phenotypes in *E. coli* isolates (n=177)

		Associations between AMR phenotypes											
	N	AMP	AZI	FOT	CHL	COL	GEN	NAL	STR	SMX	TET	TGC	TMP
AMP	147	ns	8.0 (1.0-61.5)	-	1.2 (1.1-1.4)	14.6 (1.8-106)	-	-	-	-	50.3 (17-148)	-	11.4 (2.6-49.6)
AZI	33	8.0 (1.0-61.5)	ns	-	3.4 (1.5-7.6)	-	3.8 (0.9-15.1)	-	-	2.3 (0.9-5.5)	1.2 (1.1-1.4)	3.1 (1-9.6)	-
FOT	10	-	-	ns	5.2 (1.4-19.6)	4.3 (1.1-1)	21.6 (4.5-101)	-	-	-	-	-	-
TAZ	4	-	-	28.8 (13.1-63.2)	4.4 (3.3-5.8)	3.8 (2.9-2.9)	34.6 (14.5-82)	-	-	-	-	-	-
CHL	43	1.2 (1.1-1.4)	3.4 (1.5-7.6)	5.2 (1.4-19.6)	ns	24.3 (10-58)	30.4 (3.6-251)	7.3 (2-21)	3 (1.1-8.2)	44.5 (5.9-333)	12.6 (1.6-95)	-	7.9 (3.6-17.3)
CIP	4	-	-	-	4.4 (3.3-5.8)	8.2 (0.8-82.6)	-	-	-	-	-	-	2.7 (2.2-3.28)
COL	49	14.6 (1.8-106)	-	4.3 (1.1-16.0)	24.3 (10-58)	ns	10.5 (2-52.0)	4.3 (1.5-12)	3.7 (1-10)	4.8 (2-11)	7.1 (1.6-31.3)	2.8 (1.1-6.8)	3.3 (1.6-6.5)
GEN	9	-	3.8 (0.9-15.1)	21.6 (4.5-101)	30.4 (3.6-251)	10.5 (2.5-51)	ns	-	-	1.7 (1.5-1.9)	-	-	-
NAL	17	-	-	-	7.3 (2.5-21.3)	4..3 (1.5-12)	-	ns	1.3 (1.2-1.5)	5.5 (1.2-25)	-	6.6 (0.8-51.6)	6.2 (1.9-19)
STR	134	-	-	-	3 (1.1-8.2)	3.7 (1.36-10)	-	1.3 (1.2-1.5)	ns	2.4 (1.2-4.8)	-	-	3.5 (1.5-8.2)
SMX	107	-	2.3 (0.9-5.5)	-	44.5 (5.9-333)	4.8 (2-11)	1.7 (1.5-1.9)	5.5 (1.2-25)	2.4 (1.2-4.8)	ns	-	-	5.3 (2.5-11)
TET	145	50.3 (17-148)	1.2 (1.1-1.4)	-	12.6 (1.6-95)	7.1 (1.6-31.3)	-	-	-	-	ns	-	3.2 (1.2-8.3)
TGC	129	-	3.1 (1-9.6)	-	-	2.8 (1.1-6.8)	-	6.6 (0.8-51)	-	-	-	ns	-
TMP	68	11.4 (2.6-49.6)	-	-	7.9 (3.6-17.3)	3.3 (1.6-6.5)	-	6.2 (1.9-19)	3.5 (1.5-8.2)	5.3 (2.5-11)	3.2 (1.2-8.3)	-	ns

N, Number of isolates resistant to corresponding antimicrobial agents [b] Odds ratio (OR) for significant associations between AMR phenotypes (95% confidence interval in parenthesis); OR>1 and <1 shows positive and negative associations respectively; -, no statistically significant ($p \geq 0.05$); ns, no statistics determined

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Table 6 Resistance phenotypes and plasmid replicon types of donors (n=13) and their corresponding transconjugants (n=16)

Donors				Transconjugants				Conjugation
ID	Resistance pattern	Inc group	FAB formula	Selective pressure	ID	Resistance pattern	Inc group	rate
A144	AMP-STR-TET-TGC	FrepB, FIC	F29:A-:B23	Ampicillin	A144Z1	AMP-TET-TGC	FrepB	4.76×10^{-8}
A183	AMP-STR-TET-TGC	FrepB	F29:A-:B-	Ampicillin	A183Z1	AMP	FrepB	9.5×10^{-7}
B206	AMP-STR-SMX-TET-TMP	-	-	Tetracycline	B206Z1	AMP-STR-SMX-TET-TGC-TMP	-	9.5×10^{-7}
B136	AMP-COL-STR-SMX-TET-TGC-TMP	-	-	Tetracycline	B136Z1	AMP-STR-SMX-TET-TMP	-	9.5×10^{-7}
B170	AMP-CHL-COL-STR-SMX-TET-TMP	-	-	Chloramphenicol	B170Z1	CHL	-	1.11×10^{-8}
B173	AMP-CHL-COL-STR-SMX-TET-TGC-TMP	-	-	Chloramphenicol	B173Z1	CHL-COL	-	2.7×10^{-7}
C248	AMP-CHL-COL-NAL-STR-SMX-TET-TGC-TMP	FIB, FIC, FrepB	F18:A-:B-	Chloramphenicol	C248Z1	CHL	FrepB	6.3×10^{-7}
C250	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC	FrepB	F47:A-:B-	Tetracycline	C250Z1	AMP-AZI-COL-TET	-	2.1×10^{-7}
C200	AMP-STR-SMX-TET-TGC-TMP	-	-	Ampicillin	C200Z1	AMP-STR-SMX-TET-TGC-TMP	-	2.1×10^{-7}
A175	AMP-STR-TET	I_1	-	Tetracycline	A175Z1	TET	-	9.5×10^{-7}
A198	AMP-FOT-TET-TGC	-	-	Ampicillin	A198Z1	AMP-FOT-TET	-	6×10^{-7}
				Tetracycline	A198Z2	AMP-FOT-TET	-	6×10^{-7}
C249	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC	FrepB	F47:A-:B-	Ampicillin	C249Z1	AMP-FOT-TAZ-CHL-GEN-STR-TET-TGC	FrepB	2.1×10^{-7}
				Tetracycline	C249Z2	AMP-FOT-TAZ-CHL-GEN-STR-TET	FrepB	4.23×10^{-7}
C253	AMP-STR-TET-TGC	-	F4:A-:B-	Ampicillin	C253Z1	AMP-STR-TET-TGC	-	9.5×10^{-7}
				Tetracycline	C253Z2	AMP-STR-SMX-TET-TGC	-	1.9×10^{-8}

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Chapter 5 Discussion

The spread of antibiotic resistance is a growing global health concern, and the identification of sources of resistance is important for developing strategies to reduce the spread of resistant bacteria. Although it is known that commensal bacteria in animals may serve as a reservoir for AMR, there is currently limited proof connecting the presence of these organisms in livestock and poultry to their presence in meat products (Thorsteinsdottir et al., 2010). Duck production is increasing day by day due to enormous duck meat demand. Rearing ducks in an open house farming system is common in many developing countries (Charoensook et al., 2021). Such rearing system has become an issue for public health concern, because of insufficient biosecurity measures in animal care and farm management. Importantly, ducks that may look healthy, yet they can spread bacteria including AMR pathogens to humans via either direct or indirect contact (Assawatheptawee et al., 2022).

One of the major findings of this study is the observation of MDR *E. coli* in fecal samples from the meat ducks raised in an open house farming system. Currently, there is still limited information on AMR in bacteria that originate from ducks and most AMR research has been conducted on poultry and livestock. Therefore, published data of livestock was additionally employed for the comparison and discussion of the findings in the study.

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The *E. coli* isolates in this collection were mostly MDR (86.4%) compared with *E. coli* isolates from duck feces raised in open farming system in other countries, the percentage of MDR isolates in our study was higher than that of *E. coli* from ducks in Tanzania (Kissinga et al., 2018) and Korea (Na et al., 2019). The MDR *E. coli* prevalence was in agreement with its prevalence in pigs, pig carcasses and in human in Thailand (Pungpian et al., 2021). The *E. coli* isolates exhibited the highest resistance rates to ampicillin (83.0%) and tetracycline (81.9%). This is likely a result of the extensive use of the two antibiotics in the livestock and poultry production including ducks for a long period of time. The resistance rate to tetracycline was higher than Tanzania (Kissinga

et al., 2018), and Korea (Na et al., 2019) and lower than those of the pathogenic *E. coli* from ducks in China (Yassin et al., 2017). Resistance rates of ampicillin in *E. coli* from ducks were higher than in Korea (Na et al., 2019). These differences in AMR rates between countries may be brought about by differences in geographical location, antimicrobial usage forms, prescription patterns, availability of antibiotics and antibiotics administration. Future investigation of AMU situation analysis is suggested to better understanding AMR dynamics.

All of the *E. coli* isolates were resistant to at least one antibiotic, which was consistent with a previous study conducted on commensal *E. coli* isolated from Thai chicken (Boonyasiri et al., 2014). High resistance rates to commonly used antibiotics such as ampicillin and tetracycline were consistent with resistance rates of ampicillin and tetracycline in *E. coli* isolated from many other animals in other countries, e.g., pigs in Vietnam (Van et al., 2012), hens in Thailand (Boonyasiri et al., 2014), healthy swine in Thailand (Lay et al., 2012) and chickens in China (Tong et al., 2015). A previous study demonstrated that the broad use of ampicillin and tetracycline in the livestock and poultry industry including ducks has created the selective pressure for the resistant strains to emerge and thrive, which has led to the widespread resistance to these drugs (Van Boeckel et al., 2015).

Ciprofloxacin, which is a broad-spectrum fluoroquinolone and termed a last-line antibiotic, have a low resistance rate (2.2%) which was in disagreement with higher resistance rate in *E. coli* isolates from pigs in Vietnam (Van et al., 2012) and Thailand (Trongjit et al., 2016). The possible explanation is the differences in antibiotic usage patterns and regulations regarding the limited use of fluoroquinolones in livestock and poultry industries between countries may be responsible for the difference in resistance rates.

Concern has been raised in particular about bacteria of food animal origin developing resistance to last-line antibiotics (e.g., third-generation cephalosporins, colistin, and meropenem) that can be transmitted to humans through food products and/or the environment. The latter could be exacerbated by food animals raised in an

open house farming. Colistin is regarded as one of the last-resort antibiotics for the treatment of MDR infections in people, thus even if its rate was lower than those shown for other antimicrobials, it still raised alarm (Magiorakos et al., 2012). Meropenem is a carbapenem antibiotic that is the last choice for the treatment of severe MDR bacterial infections, hence it was encouraging that no meropenem resistance was found in this study (Nordmann et al., 2012). Previous studies also reported that there was no resistance observed for colistin and meropenem in *E. coli* isolates from ducks (Na et al., 2019). The possible explanation for no resistance against these antibiotics, may be limited use of colistin and carbapenems in ducks.

In this study, resistance rates to cefotaxime (5.6%) and ceftazidime (2.2%) were still low. The observation of low resistance rates to these two cephalosporins and the other clinically important antibiotics are likely attributed to limited use in meat ducks that was in line with the farm owners' disclosure of their history of antibiotic use. Cefotaxime and ceftazidime are the indicators for screening of ESBL production. It was observed that the prevalence of the ESBL producing *E. coli* from ducks raised in an open house farming system (5.0 %, n=9/177) were noted that was lower than that from the backyard ducks (36.6%) and chicken (24.9%) from Thailand (Tansawai et al., 2019). However, as waterfowl, ducks generally discharge their feces directly into water reservoir, hence boosting the rapid spread of ESBL-producing *E. coli* among the duck populations. In contrast, another study conducted in China also revealed that there was the highest prevalence of ESBL-producing *E. coli* (>50%) in backyard ducks which was higher than our findings (Ma et al., 2012). The possible reason is that, in the country ceftiofur injection is used to treat day-old ducks for colibacillosis subcutaneously, which could provide the selection pressure for the colonization of resistant bacteria in the gastrointestinal system. Although only a small percentage of ESBL-producing bacteria were found in this investigation, but all the positive ESBL isolates were MDR. Therefore, the presence of ESBL-producing bacteria that carried MDR denotes a hazard to the general public health (Jeamsripong et al., 2023).

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Biparental mating experiment was conducted in all *E. coli* isolates to explore the contribution of horizontally-transferable plasmids in conventionally raised meat

ducks in AMR distribution. However, only 13 of the 177 *E. coli* isolates transferred their resistance to the recipients. It should be noted that *in vitro* conjugation of plasmids may not accurately mirror horizontal transfer plasmid *in vivo*. There may be some lacking factors that lower the transfer efficacy under *in vitro* conditions. *Salmonella* was used as the conjugation recipient to test interspecies transfer. The recipient *Salmonella* SE12rif^{r} was originally a field isolate that is susceptible to all antimicrobials tested and does not harbor plasmids of any size and has been used as recipient for *E. coli* donors in previous studies (Lay et al., 2012). Therefore, this should not have significantly contributed to the rejection of other plasmids.

In this study, the conjugative transfer of resistance plasmids under the selection pressure of tetracycline and ampicillin was observed and in agreement with previous studies (Sirichote et al., 2010; Adams et al., 2018; Lerminiaux and Cameron, 2019). Conjugative transfer of resistance plasmids using chloramphenicol as a selective pressure was shown which was in agreement with previous studies (Dang-Van et al., 1978; Zhao et al., 2020). In this study, antimicrobials that are often used in Asian food animals were examined (Chuanchuen et al., 2014). It is intriguing to see chloramphenicol resistance in *E. coli* isolates despite the fact that the antibiotic is no longer permitted to be used on animals raised for food and has been banned since 1994. This response may be the consequence of co-selection and/or cross-resistance produced by other antibiotics, according to a previous explanation (Bischoff et al., 2005; Chuanchuen et al., 2008) and described the effective transmission of chloramphenicol resistance determinants horizontally (Karczmarczyk et al., 2011). Nevertheless, analysis of the isolates that are resistant to chloramphenicol indicates that even removing some antimicrobial selection pressures could not totally eradicate AMR.

Under colistin selective pressure, no transconjugants were observed which is consistent with a Chinese investigation that found no transferrable colistin resistance among *E. coli* isolates from food animals (Liu et al., 2016). This could be due to a variety of factors, including the lack of mobile genetic elements carrying colistin

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resistance genes, plasmid incompatibility, bacterial strain factors, low transfer frequency, or experimental conditions (Xavier et al., 2016).

The most prevalent resistance phenotypes and frequent conjugal transfer of resistance of ampicillin and tetracycline were in accordance with earlier Southeast Asian research conducted on *Salmonella* from chicken and pork and *E. coli* from livestock farms (Sirichote et al., 2010; Nhung et al., 2015). In the present study, tetracycline co-selected resistance to ampicillin, streptomycin, and tigecycline, whereas ampicillin selective pressure co-selected resistance to tetracycline, streptomycin, and tigecycline. It is in agreement with previous studies in different food producing animals in different countries, e.g., poultry in republic of Serbia (Ljubojević et al., 2017) and beef, pork and poultry in Austria (Mayrhofer et al., 2004). The co-selection phenomenon is important because it implies that using a single antimicrobial drug would promote the spread of resistance to several antibiotics, including those from other classes (Andersson and Hughes, 2010). This confirms that AMR containment should base on reducing the overall use of antibiotics and responsible use of antibiotics.

In this study, the degree of correlation between the AMR phenotypes was statistically measured and the correlations varied. The strongest association was between ampicillin and tetracycline resistance. This well corresponded to the observation of high resistance rates to these two antibiotics and their resistance genes are commonly plasmid borne (Bischoff et al., 2005). Chloramphenicol resistance was positively associated with almost all antibiotics tested, while the strong correlation (OR>10) was observed between chloramphenicol resistance and resistance to antibiotics commonly used in livestock and poultry. This suggests co-localization of genes encoding resistance to chloramphenicol and the others on the same plasmid. The latter leads to co-selection of chloramphenicol resistance genes by other antibiotics and explains the persistence of chloramphenicol resistant-bacterial strains despite the ban of chloramphenicol. These results highlight that selective pressure of resistance to various antimicrobials are linked and that the emergence and spread of AMR is a dynamic issue. Therefore, regulation of antimicrobial use should be conducted

using a whole-system approach, not at individual drug level. Therefore, regulating the use of antibiotics should be done holistically rather than at the level of individual drugs. In addition, no significant associations were found with streptomycin resistance, cefotaxime resistance, ceftazidime resistance or sulfamethoxazole resistance. The presence of genes encoding resistance to these antibiotics on plasmids in the same incompatibility group may be the justification for such negative correlations (Boerlin et al., 2005). Another possible explanation may be the co-resistance phenomenon in which two or more resistance genes present in a same bacterium (Stokes and Gillings, 2011).

Previous studies have revealed that many environmental and clinical isolates of *E. coli* and other Gram-negative bacteria contain a high incidence of resistance genes on plasmids, notably those from the IncF and IncI families (Carattoli, 2011). Five types of replicons were found in this study and this variety is consistent with that was found in bacterial isolates from the environment, containing a wide range of plasmid incompatibility groups (Zhang et al., 2012; Nakayama et al., 2015). IncF was identified as the most prevalent Inc group in Enterobacteriaceae, which is also consistent with our findings (Villa et al., 2010). The previous study demonstrated that the IncF plasmid contains a number of virulence plasmids (Silva et al., 2017), therefore the use of antibiotics may co-select genes for virulence and resistance (Carattoli, 2007). However, the detection of virulence genes was not pursued in this study.

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In this study IncFrepB plasmid was the most common in the MDR *E. coli* isolates from meat ducks raised in an open house farming system that is consistent with research in MDR *E. coli* from animals in China (Yang et al., 2015). The high prevalence of IncFrepB (48.9%) in *E. coli* and IncFIIs plasmids (9.9%) found in *Salmonella* isolates from pigs, pork and human in Thailand suggested that these plasmids may play a role in the spread of antibiotic resistance (Puangseree et al., 2022). Another study conducted in Thailand discovered a high prevalence of IncFrepB plasmids in isolates of MDR *E. coli* from swine and chickens (Nakayama et al., 2015), corroborating the idea that IncFrepB plasmids may be crucial in the spread of antimicrobial resistance genes in food animals in Southeast Asia.

In this study, the selection of donors for conjugation experiment were based on their resistance phenotype. It was interesting to observe that donors and their corresponded transconjugants did carry Inc plasmids detected despite the transfer of resistance phenotypes. Since the methodology used in this study detected 18 Inc plasmids. Therefore, the detection scheme of Inc plasmids should be revised to cover many other different Inc groups. According to earlier research, there is a high degree of genetic diversity among IncF plasmids in Enterobacteriaceae, including *E. coli* and *Salmonella* spp., isolated from food animals in Southeast Asia (Villa et al., 2010; Cheng et al., 2013). This was consistent with our findings where IncF was the most common Inc group found in the AMR bacteria from the meat ducks despite the low number of total isolates. Another study in Thailand found that *Salmonella* isolates from commercial pigs had a wide variety of IncF replicon sequence types, underscoring the significance of these plasmids in the spread of antimicrobial resistance genes in this area (Pornsukarom and Thakur, 2017). However, the FAB formula for *E. coli* from food animals in Thailand has not been previously published.

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CHAPTER 6 CONCLUSION

In conclusion our objectives were achieved. Our results emphasized that meat ducks play an important role as reservoirs for MDR *E. coli* carrying a range of plasmids. These findings yielded epidemiological information on *E. coli* and replicon types in Thailand. These results emphasize that veterinarians and farm owners must use antimicrobial agents prudently and practice proper antimicrobial use guidelines. While data on AMR in duck origin is still limited, the majority of AMR monitoring and surveillance systems concentrate mostly on other food-producing animals. We fervently advocate for the inclusion of duck-associated bacteria in AMR monitoring and surveillance programs as a beneficial element of the One Health concept. It should also be urged to monitor antimicrobial usage in ducks in great detail.

Applications

The results obtained from this study can be applied as follows:

1. The information on the occurrence and distribution of AMR could be used as part of national AMR surveillance.
2. The results could be used to support the development guidelines on the antimicrobials use in food animals, in particular meat ducks.
3. Data can be used in combination with data of food animals, foods, and humans to explain the linkage of AMR using One Health concept.

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Suggestions

1. To address the growing threat of AMR, the effectiveness of AMR surveillance and continuous monitoring programs at the local, national, and global levels is required. One Health approach to national AMR surveillance in human and animal populations is required to strengthen the understanding and support control and prevention strategic actions. Studies on ducks raised in an open house farming system and other animals kept in the similar symptoms should be implemented to better understanding environmental aspects of AMR.

2. The prevalence and genetic characteristics of AMR in *E. coli* from ducks and other food-producing animals should be studied in a larger population across the region.
3. A genetic and clonal relationship between *E. coli* and other bacteria from humans and food-producing animals should be investigated to characterize the plasmid-mediated AMR in *E. coli* and other bacteria from ducks and food animals will offer valuable information about the evolution, circulation, and spread of plasmid-mediated resistance genes in the region.
4. National monitoring and surveillance of antimicrobial use in ducks should be performed. Together national AMR data, this will support the development and implementation of control and prevention strategic action plan to contain AMR.

Further investigations

To date, data and activity on AMR related to ducks is still limited. Further investigations are warranted as follows:

1. Additional studies with larger sample size are suggested.
2. Association between resistance and virulence genes in *E. coli* isolate from ducks should be determined.
3. Study on other mobile genetic elements and transfer of AMR determinants in commensal *E. coli* should be performed.
4. R plasmids obtained from this study can be used for further studies to identify their genetic elements.
5. Whole genome sequencing analysis of the bacterial isolates obtained is suggested.
6. Situation analysis of antimicrobial use and consumption is suggested.

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APPENDIX A

Bacterial Growth Media

1. Eosin Methylene Blue agar, Modified (Difco®, New Jersey, USA).

Pancreatic digest of gelatin	10.0 g
Lactose	5.0 g
Sucrose	5.0 g
Dipotassium phosphate	2.0 g
Eosin Y	0.4 g
Methylene blue	65.0 g
Agar	13.5 g

2. MacConkey agar (Difco®, New Jersey, USA).

Peptone	20.0 g
Lactose	10.0 g
Bile salts	5.0 g
Agar	12.0 g
Natural red	0.075 g

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3. Luria Bertani agar (Difco®, New Jersey, USA).

Typhone	10.0 g
Yeast extract	5.0 g
Sodium chloride	10.0 g
Agar	15.0 g

4. Xylose Lysine Deoxycholate Agar (Difco®, New Jersey, USA).

Xylose	3.5 g
L-lysine	5.0 g
Lactose	7.5 g
Saccharose	7.5 g
Sodium chloride	15.0 g
Yeast extract	3.0 g
Phenol red	0.08 g

5. Luria Bertani broth (Difco®, New Jersey, USA).

Typhone	10.0 g
Yeast extract	5.0 g
Sodium chloride	10.0 g

Chemicals

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1. 50X TAE (Tris-Acetate Buffer)

Tris base	242.0 g
Acetic acid	57.1 g
0.5M EDTA pH 8.0	100.0 ml
Distilled water	1000.0 ml

2. 0.5M EDTA, pH 8.0

Disodium ethylene diamine tetraacetate. H_2O	121.1 g
Distilled ionized water	800.0 ml
0.5M EDTA pH 8.0	100.0 ml

3. Agarose gel (Sigma-Aldish®, Missouri, United States)

Agarose (ultra-pure)	1.5 g
1x TAE Buffer	100 ml

4. Other chemicals

TAE buffer (Tris 10mM and EDTA 1Mm)

NaOH (0.2M)

DNA marker (DNA ladder, Thermo Scientific™, Waltham, U.S.A.)

Loading Dye (Tristrack DNA loading Dye, Thermo Scientific™, Waltham, U.S.A.)

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APPENDIX B

Antimicrobial agents used for antibiotic sensitivity testing

Antibiotics	Concentration range (µg/ml)	Clinical breakpoints (µg/ml)
Ampicillin (AMP)	0.5-128	32
Azithromycin (AZI)	0.5-64	32
Cefotaxime (FOT)	0.06-16	4
Ceftazidime (TAZ)	0.12-64	16
Chloramphenicol (CHL)	1-256	32
Ciprofloxacin (CIP)	0.015-16	4
Colistin (COL)	0.12-16	4
Gentamicin (GEN)	0.25-128	16
Meropenem (MERO)	0.008-8	4
Nalidixic acid (NAL)	1-128	32
Streptomycin (STR)	1-256	16
Sulfamethoxazole (SMX)	1-1024	512
Tetracycline (TET)	1-256	16
Tigecycline (TGC)	0.25-16	1
Trimethoprim (TMP)	0.25-256	16

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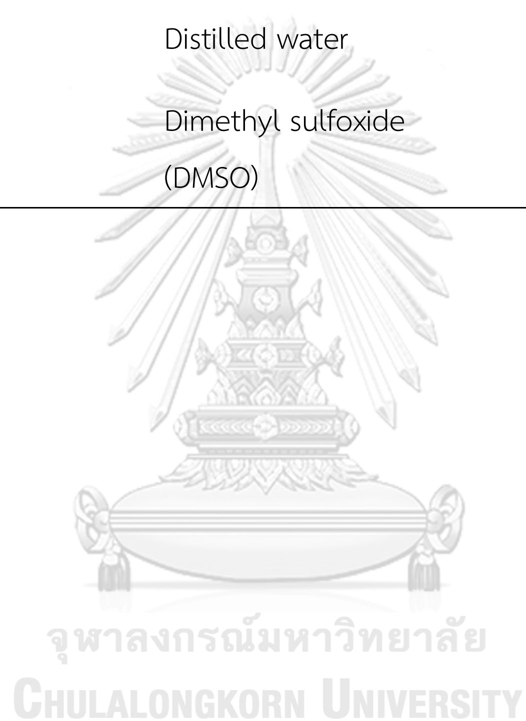
Antimicrobial agents used in conjugation experiment as selective pressures

Antibiotics	Solvents	Concentration (µg/ml)
Ampicillin (AMP)	Distilled water	150
Tetracycline (TET)	70% ethanol	15
Chloramphenicol (CHL)	95% ethanol	25
Colistin (COL)	Distilled water	2
Rifampicin (RIF)	Dimethyl sulfoxide (DMSO)	32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Appendix C

Table Primers used in this study

Target		Primer	Sequence (5'–3')	Amplicon size (bp)	Reference
PBRT	IncHI1	HI1-F	GGAGCGATGGATTACTTCAGTAC	471	(Carattoli et al., 2005)
		HI1-R	TGCCGTTTCACCTCGTGAGTA		
	IncHI2	HI2-F	TTTCTCCTGAGTCACCTGTTAACAC	644	(Carattoli et al., 2005)
		HI2-R	GGCTCACTACCGTTGTCATCCT		
	IncI1	I1-F	CGAAAGCCGGACGGCAGAA	139	(Carattoli et al., 2005)
		I1-R	TCGTCGTTCCGCCAAGTTCGT		
	IncX	X-F	AACCTTAGAGGCTATTTAAG TTGCTGAT	376	(Carattoli et al., 2005)
		X-R	TGAGAGTCAATTTTTATCTCATGTTT TAGC		
	IncL/M	L/M-F	GGATGAAAACTATCAGCATCTGAAG	785	(Carattoli et al., 2005)
		L/M-R	CTGCAGGGGCGATTCTTTAGG		
	IncN	N-F	GTCTAACGAGCTTACCGAAG	559	(Carattoli et al., 2005)
		N-R	GTTTCAACTCTGCCAAGTTC		
	IncFIA	FIA-F	CCATGCTGGTTCTAGAGAAGGTG	462	(Carattoli et al., 2005)
		FIA-R	GTATATCCTTACTGGCTTCCGCAG		
	IncFIB	FIB-F	GGAGTTCTGACACACGATTTTCTG	702	(Carattoli et al., 2005)
		FIB-R	CTCCCGTCGCTTCAGGGCATT		
	IncW	W-F	CCTAAGAACAACAAAGCCCCCG	242	(Carattoli et al., 2005)
		W-R	GGTGCGCGGCATAGAACCGT		
	IncY	Y-F	AATTCAAACAACACTGTGCAGCCTG	765	(Carattoli et al., 2005)
		Y-R	GCGAGAATGGACGATTACAAAACTTT		
	IncP	P-F	CTATGGCCCTGCAAACGCGCCAGAAA	634	(Carattoli et al., 2005)
		P-R	TCACGCGCCAGGGCGCAGCC		
	IncFIC	FIC-F	GTGAACTGGCAGATGAGGAAGG	262	(Carattoli et al., 2005)
		FIC-R	TTCTCCTCGTCGCCAAACTAGAT		
	IncA/C	A/C-F	GAGAACCAAAGACAAAGACCTGGA	465	(Carattoli et al., 2005)
		A/C-R	ACGACAAACCTGAATTGCCTCCTT		
	IncT	T-F	TTGGCCTGTTTGTGCCTAAACCAT	750	(Carattoli et al., 2005)
		T-R	CGTTGATTACACTTAGCTTTGGAC		
	IncFIIA	FIIs-F	CTGTCGTAAGCTGATGGC	270	(Carattoli et al., 2005)
		FIIs-R	CTCTGCCACAAACTTCAGC		
	IncF	F-F	TGATCGTTTAAGGAATTTTG	270	(Carattoli et al., 2005)
		F-R	GAAGATCAGTCACACCATCC		
	IncK	K-F	GCGGTCCGGAAAGCCAGAAAAC	160	(Carattoli et al., 2005)
		K-R	TCTTTCACGAGCCCGCCAAA		
	IncB/O	B/O-F	GCGGTCCGGAAAGCCAGAAAAC	159	(Carattoli et al., 2005)

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Target		Primer	Sequence (5’–3’)	Amplicon size (bp)	Reference
		B/O-R	TCTGCGTTCCGCCAAGTTCGA		
IncF-RST	FII	FII-F	CTGATCGTTTAAGGAATTTT	258–262	(Villa et al., 2010)
		FII-R	CACACCATCCTGCACTTA		
	FIIs	FIIS-F	CTAAAGAATTTTGATGGCTGGC	259–260	(Villa et al., 2010)
		FIIS-R	CAGTCACTTCTGCCTGCAC		
	FIA	FIA-F	CCATGCTGGTTCTAGAGAAGGTG	462	(Villa et al., 2010)
		FIA-R	GTATATCCTTACTGGCTTCCGCAG		
	FIB	FIBs-F	TGCTTTTTATTCTTAAACTATCCAC	683	(Villa et al., 2010)
		FIB-R	CTCCCGTCGCTTCAGGGCATT		

Table Resistance pattern of the *E. coli* isolates isolated from meat ducks

AMR pattern	N0. of isolates (n)
STR	1 (0.5)
SMX	3 (1.6)
TET	1 (0.5)
TGC	1 (0.5)
AMP-SMX	1 (0.5)
AMP-TET	4 (2.2)
STR-TGC	3 (1.6)
STR-TET	3 (1.6)
STR-SMX	4 (2.2)
SMX-TGC	3 (1.6)
AMP-STR-SMX	1 (0.5)
AMP-SMX-TGC	1 (0.5)
AMP-STR-TET	10 (5.6)
AMP-TET-TGC	10 (5.6)
COL-STR-TGC	1 (0.5)
STR-SMX-TGC	5 (2.8)
STR-SMX-TMP	1 (0.5)
STR-TET-TGC	1 (0.5)
SMX-TET-TGC	2 (1.1)
SMX-TGC-TMP	1 (0.5)
AMP-AZI-TET-TMP	1 (0.5)
AMP-FOT-TET-TGC	2 (1.1)
AMP-COL-TET-TGC	2 (1.1)
AMP-COL-STR-TET	1 (0.5)
AMP-STR-SMX-TET	3 (1.6)
AMP-STR-TET-TGC	11 (6.1)
AMP-STR-TET-TMP	1 (0.5)
AMP-TET-TGC-TMP	1 (0.5)
STR-SMX-TET-TGC	1 (0.5)
AMP-STR-SMX-TET-TGC	2 (1.1)
AMP-STR-SMX-TGC-TMP	3 (1.6)
AMP-AZI-STR-SMX-TET	2 (1.1)
AMP-AZI-SMX-TET-TGC	2 (1.1)
AMP-CHL-SMX-TET-TMP	1 (0.5)
AMP-STR-SMX-TET-TMP	3 (1.6)
AMP-AZI-STR-TET-TGC	6 (3.3)
AMP-COL-STR-TET-TGC	2 (1.1)
AMP-COL-STR-SMX-TET	1 (0.5)
AMP-COL-STR-TET-TMP	1 (0.5)
AMP-FOT-CHL-COL-TGC	1 (0.5)
AMP-FOT-STR-TET-TGC	1 (0.5)
AZI-STR-SMX-TET-TGC	1 (0.5)

GEN-STR-SMX-TET-TGC	1 (0.5)
AMP-STR-TET-TGC-TMP	5 (2.8)
AMP-SMX-TET-TGC-TMP	1 (0.5)
AMP-AZI-TET-TGC-TMP	1 (0.5)
AMP-NAL-STR-TGC-TMP	1 (0.5)
AMP-STR-SMX-TET-TGC-TMP	8 (4.5)
AMP-AZI-STR-SMX-TET-TGC	2 (0.5)
AMP-CHL-STR-SMX-TET-TMP	2 (1.1)
AMP-COL-STR-SMX-TET-TGC	1 (0.5)
AMP-NAL-STR-TET-TGC-TMP	1 (0.5)
AMP-NAL-STR-SMX-TET-TGC	2 (1.1)
AMP-AZI-COL-STR-SMX-TET-TGC	1 (0.5)
AMP-AZI-CHL-COL-STR-SMX-TET	1 (0.5)
AMP-AZI-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-CHL-COL-SMX-TET-TGC-TMP	1 (0.5)
AMP-COL-STR-SMX-TET-TGC-TMP	4 (2.2)
AMP-COL-NAL-STR-SMX-TET-TGC	1 (0.5)
AMP-CHL-COL-STR-SMX-TET-TMP	1 (0.5)
AMP-CHL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-AZI-COL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-AZI-CHL-STR-SMX-TET-TGC-TMP	2 (1.1)
AMP-AZI-CHL-COL-STR-SMX-TET-TGC	3 (1.6)
AMP-AZI-CHL-COL-SMX-TET-TGC-TMP	1 (0.5)
AMP-FOT-NAL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-CHL-COL-STR-SMX-TET-TGC-TMP	7 (3.3)
AMP-CHL-COL-NAL-STR-SMX-TET-TGC	1 (0.5)
AMP-AZI-CHL-COL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET	1 (0.5)
AMP-CHL-COL-NAL-STR-SMX-TET-TGC-TMP	5 (2.8)
AMP-CHL-CIP-NAL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-CHL-CIP-COL-NAL-STR-SMX-TET-TMP	1 (0.5)
AMP-AZI-CHL-COL-GEN-STR-SMX-TET-TGC-TMP	3 (1.6)
AMP-AZI-CHL-GEN-NAL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-AZI-FOT-CHL-COL-STR-SMX-TET-TGC-TMP	1 (0.5)
AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC	3 (2.2)
AMP-CHL-CIP-COL-NAL-STR-SMX-TET-TGC-TMP	1(0.5)
AMP-AZI-CHL-CIP-COL-NAL-STR-SMX-TET-TGC-TMP	1 (0.5)
Total	177 (100)

Table Antibiotic resistance pattern of ESBL producing *E. coli* Isolates (n=19)

Strain ID	Antibiotic resistance pattern
A197	AMP-FOT-TET-TGC
A198	AMP-FOT-TET-TGC
B129	AMP-FOT-CHL-COL-TGC
B131	AMP-AZM-FOT-CHL-COL-STR-SMX-TET-TGC-TMP
C172	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC
C177	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET
C201	AMP-FOT-STR-TET-TGC
C249	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC
C250	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC

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Table PCR conditions used for genetic characterization of R-plasmids in this study

	PCR condition					
	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
PBRT[a]	94°C 5 min	94°C 1 min	60°C 30 sec	72°C 1 min	72°C 5 min	30
IncF pMLST[b]	94°C 5 min	94°C 1 min	60°C 30 sec	72°C 1 min	72°C 5 min	30

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Table. Resistance phenotypes and plasmid replicon types of donors (n=13) and their corresponding transconjugants (n=16)

Donors				Transconjugants				Conjugation
ID	Resistance pattern	Inc group	FAB formula	Selective pressure	ID	Resistance pattern	Inc group	rate
A144	AMP-STR-TET-TGC	FrepB, FIC	F29:A-:B23	Ampicillin	A144Z1	AMP-TET-TGC	FrepB	4.76×10^{-8}
A183	AMP-STR-TET-TGC	FrepB	F29:A-:B-	Ampicillin	A183Z1	AMP	FrepB	9.5×10^{-7}
B206	AMP-STR-SMX-TET-TMP	-	-	Tetracycline	B206Z1	AMP-STR-SMX-TET-TGC-TMP	-	9.5×10^{-7}
B136	AMP-COL-STR-SMX-TET-TGC-TMP	-	-	Tetracycline	B136Z1	AMP-STR-SMX-TET-TMP	-	9.5×10^{-7}
B170	AMP-CHL-COL-STR-SMX-TET-TMP	-	-	Chloramphenicol	B170Z1	CHL	-	1.11×10^{-8}
B173	AMP-CHL-COL-STR-SMX-TET-TGC-TMP	-	-	Chloramphenicol	B173Z1	CHL-COL	-	2.7×10^{-7}
C248	AMP-CHL-COL-NAL-STR-SMX-TET-TGC-TMP	FIB, FIC, FrepB	F18:A:-B-	Chloramphenicol	C248Z1	CHL	FrepB	6.3×10^{-7}
C250	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC	FrepB	F47:A:-B-	Tetracycline	C250Z1	AMP-AZI-COL-TET	-	2.1×10^{-7}
C200	AMP-STR-SMX-TET-TGC-TMP	-	-	Ampicillin	C200Z1	AMP-STR-SMX-TET-TGC-TMP	-	2.1×10^{-7}
A175	AMP-STR-TET	I_1	-	Tetracycline	A175Z1	TET	-	9.5×10^{-7}
A198	AMP-FOT-TET-TGC	-	-	Ampicillin	A198Z1	AMP-FOT-TET	-	6×10^{-7}
				Tetracycline	A198Z2	AMP-FOT-TET	-	6×10^{-7}
C249	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC	FrepB	F47:A-:B-	Ampicillin	C249Z1	AMP-FOT-TAZ-CHL-GEN-STR-TET-TGC	FrepB	2.1×10^{-7}
				Tetracycline	C249Z2	AMP-FOT-TAZ-CHL-GEN-STR-TET	FrepB	4.23×10^{-7}
C253	AMP-STR-TET-TGC	-	F4:A-:B-	Ampicillin	C253Z1	AMP-STR-TET-TGC	-	9.5×10^{-7}
				Tetracycline	C253Z2	AMP-STR-SMX-TET-TGC	-	1.9×10^{-8}

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Table Association between AMR phenotypes in *E. coli* isolates (n=177)

		Associations between AMR phenotypes											
	N	AMP	AZI	FOT	CHL	COL	GEN	NAL	STR	SMX	TET	TGC	TMP
AMP	147	ns	8.0 (1.0-61.5)	-	1.2 (1.1-1.4)	14.6 (1.8-106)	-	-	-	-	50.3 (17-148)	-	11.4 (2.6-49.6)
AZI	33	8.0 (1.0-61.5)	ns	-	3.4 (1.5-7.6)	-	3.8 (0.9-15.1)	-	-	2.3 (0.9-5.5)	1.2 (1.1-1.4)	3.1 (1-9.6)	-
FOT	10	-	-	ns	5.2 (1.4-19.6)	4.3 (1.1-1)	21.6 (4.5-101)	-	-	-	-	-	-
TAZ	4	-	-	28.8 (13.1-63.2)	4.4 (3.3-5.8)	3.8 (2.9-2.9)	34.6 (14.5-82)	-	-	-	-	-	-
CHL	43	1.2 (1.1-1.4)	3.4 (1.5-7.6)	5.2 (1.4-19.6)	ns	24.3 (10-58)	30.4 (3.6-251)	7.3 (2-21)	3 (1.1-8.2)	44.5 (5.9-333)	12.6 (1.6-95)	-	7.9 (3.6-17.3)
CIP	4	-	-	-	4.4 (3.3-5.8)	8.2 (0.8-82.6)	-	-	-	-	-	-	2.7 (2.2-3.28)
COL	49	14.6 (1.8-106)	-	4.3 (1.1-16.0)	24.3 (10-58)	ns	10.5 (2-52.0)	4.3 (1.5-12)	3.7 (1-10)	4.8 (2-11)	7.1 (1.6-31.3)	2.8 (1.1-6.8)	3.3 (1.6-6.5)
GEN	9	-	3.8 (0.9-15.1)	21.6 (4.5-101)	30.4 (3.6-251)	10.5 (2.5-51)	ns	-	-	1.7 (1.5-1.9)	-	-	-
NAL	17	-	-	-	7.3 (2.5-21.3)	4..3 (1.5-12)	-	ns	1.3 (1.2-1.5)	5.5 (1.2-25)	-	6.6 (0.8-51.6)	6.2 (1.9-19)
STR	134	-	-	-	3 (1.1-8.2)	3.7 (1.36-10)	-	1.3 (1.2-1.5)	ns	2.4 (1.2-4.8)	-	-	3.5 (1.5-8.2)
SMX	107	-	2.3 (0.9-5.5)	-	44.5 (5.9-333)	4.8 (2-11)	1.7 (1.5-1.9)	5.5 (1.2-25)	2.4 (1.2-4.8)	ns	-	-	5.3 (2.5-11)
TET	145	50.3 (17-148)	1.2 (1.1-1.4)	-	12.6 (1.6-95)	7.1 (1.6-31.3)	-	-	-	-	ns	-	3.2 (1.2-8.3)
TGC	129	-	3.1 (1-9.6)	-	-	2.8 (1.1-6.8)	-	6.6 (0.8-51)	-	-	-	ns	-
TMP	68	11.4 (2.6-49.6)	-	-	7.9 (3.6-17.3)	3.3 (1.6-6.5)	-	6.2 (1.9-19)	3.5 (1.5-8.2)	5.3 (2.5-11)	3.2 (1.2-8.3)	-	ns

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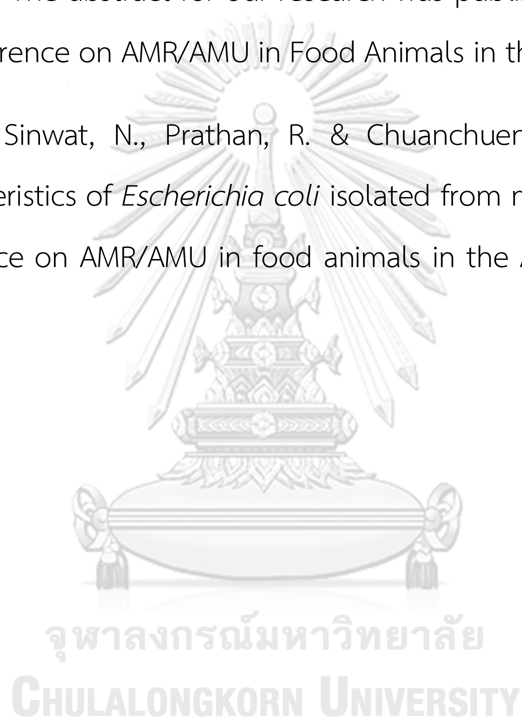
Output

The results from this study were presented as poster presentation at the 1st Research Conference on AMR/AMU in Food Animals in the Asia-Pacific Region from 6-8 February 2023 held virtually and organized by Food and Agriculture organization of the United Nations and Faculty of Veterinary Sciences, Chulalongkorn University, Bangkok, Thailand. The abstract for our research was published in the proceedings of 1st Research Conference on AMR/AMU in Food Animals in the Asia-Pacific Region 2023.

Baqar, Z., Sinwat, N., Prathan, R. & Chuanchuen, R. (2023). Antimicrobial resistance characteristics of *Escherichia coli* isolated from meat ducks in Thailand, 1st research conference on AMR/AMU in food animals in the Asia-Pacific region 2023 pp 54-55.



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