



**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**WHOLE GENOME SEQUENCING
ANALYSIS OF ANTIMICROBIAL
RESISTANT *Escherichia coli*:
FROM FOOD TO HUMAN**

**GUO SIYAO
SCHOOL OF CHEMICAL AND BIOMEDICAL
ENGINEERING
2020**

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ANALYSIS OF ANTIMICROBIAL
RESISTANT *Escherichia coli*:
FROM FOOD TO HUMAN**

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A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for
the degree of Doctor of Philosophy

2020

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.



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Guo Siyao

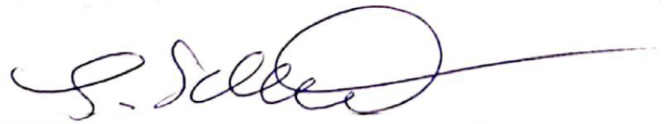
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3 April 2020

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Joergen Schlundt

Authorship Attribution Statement

This thesis contains material from 2 papers published in the following peer-reviewed journals in which I am listed as an author.

Chapter 3 is published as

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Guo, S., Tay, M.Y., Thu, A.K., Seow, K.L.G., Zhong, Y., Ng, L.C. and Schlundt, J., 2019. Conjugative IncX1 Plasmid Harboring Colistin Resistance Gene mcr-5.1 in Escherichia coli Isolated from Chicken Rice Retailed in Singapore. Antimicrobial agents and chemotherapy, 63(11).

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The contributions of the co-authors are as follows:

- Prof Joergen Schlundt provided the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts. The manuscript was revised by Dr Moon and Mr. Aung K.T.
- I co-designed the study with Prof Joergen Schlundt, Dr Moon and Mr. Aung K.T and performed most of the laboratory work at the School of Chemical and Biomedical Engineering and Environmental Health Institute. I also analyzed the data.
- All DNA extraction, including sample preparation and quality control, was conducted by Kelyn and Zhong Yang.
- Ng, L.C., Purbojati, R.W., Drautz-Moses, D.I., Schuster, S.C. provide suggestions to experiments design and data analysis, and they also revised the manuscript.

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Summary

The main work of this study is to analyze antimicrobial resistant *E. coli* based on whole genome sequencing data. Three stages were included for isolates from different sources (ready-to-eat food, retail raw meats and human patients).

At the first stage, a retrospective study for antimicrobial resistant *E. coli* in ready-to-eat (RTE) food sold in retail food premises in Singapore was performed in collaboration with Environmental Health Institute under NEA. A total of 99 *E. coli* isolates from poultry-based dishes (n=77) and fish-based dishes (n=22) were obtained between 2009 and 2014 during the surveillance project. All the isolates were included for disk diffusion testing for antimicrobial susceptibility testing. Of the 99 isolates, 24 (24.2%) were resistant to at least one antimicrobial agent. These isolates were then subjected to broth microdilution testing against 33 antimicrobial agents, including β -lactams, aminoglycosides, tetracycline, fluoroquinolones and polymyxin E (colistin), to determine the minimum inhibitory concentration (MIC) of isolates. Whole genome sequence (WGS) was carried out on the strains in order to correlate resistant phenotypes to putative antimicrobial resistance-related genes. Of the 24 isolates, 15 (62.5%) were found to be resistant to three or more classes of antimicrobials and thus were defined as multi-drug resistant strains. Two isolates (8.3%) were confirmed as Extended-Spectrum β -lactamase (ESBL)- producing *E. coli* by double disk synergy test. Based on WGS data, online analysis tool ResFinder detected 7 classes of antimicrobial resistance genes and resistance-related chromosomal point mutations in 19 of the 24 *E. coli* isolates. By analyzing the WGS contigs using BLASTn and KmerFinder, ESBL genes and transferable colistin resistance gene

mcr-1 (2/24) and *mcr-5* (1/24) were determined to be located on plasmids, which could pose a greater risk of AMR transfer among bacteria. Mutations were detected in four isolates within genes previously shown to confer resistance to quinolones (*gyrA* and *parE*) and tetracycline (*rrsB*). Prediction of AMR using WGS data was evaluated for six antimicrobials including ampicillin, chloramphenicol, colistin, fluoroquinolones, tetracycline and trimethoprim. The evaluation indicates WGS-based genotype and phenotype showed high consistency, however, for some antimicrobial resistance whose mechanism is not totally clear yet (such as colistin), there is challenge for resistance gene detection.

To have a better understanding of genetic environment and mobility of colistin resistance gene *mcr-5.1*, the isolates carrying *mcr-5.1* were sequenced using long-read sequencing technology. The plasmid sequences were assembled into a closed circle using both long-read sequencing data and short-read sequencing data. The blasting result showed the closest plasmid sequence to pSGMCR103 in NCBI is plasmid pYD786-3 (accession number KU254580.1) with 77% query coverage and 99% identity, which was carried by one *E. coli* isolate from human urine in USA. They share antimicrobial resistance gene *aph(3')-Ia*, *aadA1* (aminoglycoside resistance), *bla*_{TEM-176} (beta-lactam resistance) and *sul3* (sulphonamide resistance). Gene *mcr-5.1* was harbored on a Tn3 transposon-like element, which is similar with pSE13-SA01718 (accession number KY807921.1) carried by a *Salmonella* isolate reported before. Also, other insertion elements such as IS5, IS6, IS91, IS256 family were found on the plasmid, which may indicate the recombination activity of the plasmid. Moreover, the mobility of this

plasmid was confirmed by the conjugation experiment. The frequency of conjugation after 24 hours is 10^{-6} .

After knowing the AMR profile in ready-to-eat food in Singapore, at the second stage, an important resistance type was studied: Extended-Spectrum Beta-Lactamase (ESBL)-caused resistance to most of beta-lactams. We collected 634 meat samples including chicken, pork and beef from 97 supermarkets and 65 wet markets in Singapore during June 2017-October 2018. The samples were enriched before bacteria isolation. Presumptive ESBLs were screened by Brilliance™ ESBL Agar and confirmed by Double Disk Synergy Test (DDST). *E. coli* isolates were identified by EMB agar and indole test. The genomic DNA of ESBL-producing *E. coli* were extracted and sent for WGS. Besides the analysis for AMR genes, MLST, annotation and genetic environment, these sequence collection was also compared with sequence data of ESBL *E. coli* isolated from community in Singapore for phylogenetic study based on SNPs. A total of 225 ESBL-producing *E. coli* were isolated from 184 samples. The prevalence of ESBL in chicken, pork and beef was 51.2% (109/213), 26.9% (58/216), 7.3% (15/205), respectively. The most common AMR genes in all 225 ESBL isolates were beta-lactam-resistance genes (100%), aminoglycoside resistance genes (92.4%), sulphonamide resistance genes (86.2%). In terms of beta-lactam resistance genes, 172 of isolates (76.4%) carry *bla*_{CTX-M} genes, 102 (45.3%) of isolates carry *bla*_{TEM} genes and 52 of isolates (23.1%) carry *bla*_{SHV} genes. Besides these most common three beta-lactamase genes, *bla*_{CMY-2}, *bla*_{OXA} and *bla*_{DHA} were also found. Gene *bla*_{CTX-M-55} (57/225, 25.3%) and *bla*_{CTX-M-65} (40/225, 17.8%) were the most frequent ESBL genes. Among all these classes of

antimicrobials, beta-lactam-resistance genes and aminoglycoside resistance genes exhibit great variety. The last-resort antimicrobial colistin resistance *mcr* genes exist in 15.6% of all isolates (33 isolates carry *mcr-1*, one carries *mcr-3.1* and one carries *mcr-5*). Phylogeny tree based on SNPs of our isolates and previous ESBL isolates from human community in Singapore shows obvious separate human clusters and food clusters, however, two *E. coli* isolates from human fell into food clusters and showed high similarity with our isolates from meats, which indicates the possible transmission of resistant *E. coli* from meats to human may exist. Occurrence of AMR genes especially for last resort drug resistance genes was observed, raising concerns on food safety and public health.

At the last stage, we applied WGS to the analysis of clinical isolates. We collaborated with the university in Thailand to get 28 ESBL-producing *E. coli* isolated from diarrhea patients hospitalized at the Phayao Ram Hospital in Thailand. Result shows all *E. coli* carried CTX-Ms beta-lactamase (including CTX-M-14, CTX-M-15, CTX-M-27, CTX-M-55), and half of these isolates (14/28) belong to the important pathogenic cluster ST131. CTX-M-55s were detected only in non-ST131s. Two serotypes O16:H5 (6/14) and O25:H4 (8/14) were observed in ST131 isolates. Generally, ST131 isolates showed different virulence factor patterns with non-ST131 isolates. BLAST results indicate that for half of ST131 isolates, *bla*_{CTX-M} genes are located on chromosome adjacent to insert sequences (IS). For the other half ST131s, *bla*_{CTX-M} genes are located on plasmids. Besides CTX-Ms, other beta-lactamases such as TEM-1B, OXA-1 and CMY-2 were also observed in our study. Phylogenetic analysis for a global collection and our clinical isolates in Thailand based on SNPs showed the closest

isolates with our isolates are from Thailand, Singapore, Australia, Laos and New Zealand. A special strain cluster O16:H5-ST131 was found from 2015-2017 as well as other previous Thailand studies. These isolates showed high similarity in term of serotype, MLST, virulence factor / AMR patterns, and phylogeny, which indicates the persistence and spread of this cluster. This study provides an insight on characteristics of clinical ESBL-producing *E. coli* with special focus on ST131 in Thailand.

It is noteworthy that four isolates in our study showed same serotype, ST (O16:H5-ST131), same virulence factor pattern (*cnf1*, *iha*, *sat*, *senB*) and even resistance gene pattern. In terms of SNP analysis, these isolates were located the same cluster 3 and the SNP difference range 17-20, which indicate the high similarity of these strains. But actually they are isolated in different years from 2015-2017, respectively. In cluster 3, other four isolates from Thailand (ERR1218557, ERR1218609, ERR1218624, ERR1218628) in a previous study also showed less than 30 SNPs difference with our four isolates. This also strengthens the hypothesis that these isolates diverged from one ancestor and this cluster of isolates are persistent in Thailand in recent years. This strain should raise our attention of further spread. The phylogenetic study indicates that the closest isolates with Thailand ST131 isolates are from Oceania and Southeast Asia. Our data based on whole genome add more evidence on ubiquity of ESBL-ST131 *E. coli* in Thailand. The co-existence of multi-resistance and multi-virulence factors adds challenges to clinical treatment. Although resistances to carbapenem and/or colistin are rare in this study, more epidemiology studies are needed to verify their actual prevalence.

Overall speaking, WGS is a useful tool for prevalence and epidemiological analysis and source tracking. With the development of sequencing technology and the decrease of cost, whole-genome-based analysis is becoming more and more necessary for AMR study.

Publications

- **Guo, Siyao**, Moon YF Tay, Kyaw Thu Aung, Kelyn LG Seow, Lee Ching Ng, Rikky W. Purbojati, Daniela I. Drautz-Moses, Stephan C. Schuster, and Joergen Schlundt. “Phenotypic and genotypic characterization of antimicrobial resistant *Escherichia coli* isolated from ready-to-eat food in Singapore using disk diffusion, broth microdilution and whole genome sequencing methods.” *Food control* 99 (2019): 89-97.
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Abbreviations

AMR	Antimicrobial resistance
AVA	Agri-food & Veterinary Authority
CAESAR	Central Asian and Eastern European Surveillance of Antimicrobial Resistance
CDC	Center for Disease Control
DAEC	Diffusely adherent <i>E. coli</i>
DDD	Defined daily dose
EAEC	Enteropathogenic <i>E. coli</i>
EARS-Net	European Antimicrobial Resistance Surveillance Network
<i>E. coli</i>	<i>Escherichia coli</i>
EIEC	Enteroinvasive <i>E. coli</i>
ENA	European Nucleotide Archive
EHEC	Enterohemorrhagic <i>E. coli</i>
EHl	Environmental Health Institute
EPEC	Enteropathogenic <i>E. coli</i>
ESBL	Extended-Spectrum Beta-Lactam
ETEC	Enterotoxigenic <i>E. coli</i>
ExPEC	Extra-intestinal pathogenic <i>E. coli</i>
FDA	Food and drug administration
GLASS	Global Antimicrobial Resistance Surveillance System
HGT	Horizontal gene transfer
InPEC	Intestinal pathogenic <i>E. coli</i>
LPS	Lipopolysaccharide
MDR	Multi-drug resistance
MIC	Minimum inhibitory concentration
MGE	Mobile genetic elements
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MOH	Ministry of Health
NCBI	National Center of Biotechnology Information
NEA	National Environmental Agency
NGS	Next generation sequencing

NPHL	National Public Health Lab
OECD	The Organization for Economic Co-operation and Development
PBPs	Penicillin binding proteins
PCR	Polymerase chain reaction
PCU	Population correction unit
PUB	Public Utilities Board
ReLAVRA	Latin American Network for Antimicrobial Resistance Surveillance
RTE	Ready-to-eat
SFA	Singapore Food Agency
SNP	Single nucleotide polymorphism
ST	Sequence type
UTI	Urinary tract infection
VF	Virulence factor
WGS	Whole genome sequencing
WHO	World Health Organization

Chapter 1 Introduction

Research background

Antimicrobial resistance (AMR) greatly narrows down the drug choices during infection. If no effective measures are taken, the world is heading towards the post-antibiotic era in which common infections can kill. In the face of possible crisis, World Health Organization (WHO) launched the global action on AMR in 2015. Five objectives are raised based on the goal of the global action (Figure 1) [1]. In the following year, a high-level meeting of the United Nation General Assembly on antimicrobial resistance was held. All member states affirmed the blueprint for tackling AMR and adopted 'One Health' approach. Singapore joined the global call and published the Singapore Strategic Action Plan (NSAP) in 2017. Five core strategies were set up including education, surveillance and risk assessment, research, prevention and control of infection, optimization of antimicrobial use [2]. The Ministry of Health (MOH), National Environment Agency (NEA), Agri-food & Veterinary Authority (AVA) and Public Utilities Board (PUB) were involved and collaborated to combat AMR.



Figure 1. Five objectives outlined in global action plans on AMR by WHO in 2015

In terms of AMR surveillance and risk assessment in Singapore, the use of antimicrobials for human is regulated by the Health Science Authority. Most of antimicrobials are prescription-only. MOH also works with healthcare institute to monitor AMR. All public hospital labs and National Public Health Labs (NPHLs) have capability to detect AMR. However, further works are needed including lab detection methods harmonizing and data reporting, national AMR reference laboratory establishment and extending surveillance to private hospitals and community. For animal and food part, farms with food-producing animals are regulated by AVA (split into SFA and NParks on April 1, 2019) and

all imported and locally produced food products are monitored by Singapore Food Agency (SFA). The related analysis is mainly focusing on antimicrobial residues and important foodborne pathogens especially resistant types [3]. However, these are far from enough to set up a harmonized AMR surveillance system. Although the antimicrobials can be passed out of the animals' physiological systems after withdrawal period, the antimicrobial resistant bacteria may still remain and transfer. That is the reason why antimicrobial residues detection is not enough and AMR detection and surveillance for animals and food should be strengthened.

Routine AMR detection methods are phenotype-based antimicrobial susceptibility testing including disk diffusion method and minimum inhibitory concentration (MIC) testing. These methods are relatively easy to perform and cost-effective especially for low or middle-income countries. These methods provide information for surveillance and clinical management but no genetic information about resistance mechanism such as resistance genes or related mutations. Therefore, genotypic detection is worthy as a supplementary method for deeper understanding of AMR molecular epidemiology. Common genotypic detection such as polymerase chain reaction (PCR) or microarray is target region-based, which needs to design a primer or probe. However, the new rising technology – Whole Genome Sequencing (WGS) is able to provide comprehensive genomic information. In addition, it also provides other important information such as virulence factor, plasmids type, serotype and others, which are valuable data for epidemiology study and AMR mechanism exploration [4].

‘One Health’ is a collaborative and multi-sectoral approach to achieve optimal health outcome realizing the non-neglectable interconnection between human, animal and environment. The area of work in One Health is particularly related to food safety, zoonosis control and AMR [5]. One Health concept has been widely considered in control and prevention of diseases and optimization of public health by WHO and CDC.

NTU Food Technology Centre (NAFTEC) was established in 2016 to work with Singapore local national agencies to enhance food safety and security following One Health approach. Under the AMR subgroup, my research during PhD period mainly focused on surveillance and characterization of AMR in food based on WGS and bioinformatic analysis, further analysis of isolates from human (in community and in hospital) was also performed for comparison. This study provided reference information for national surveillance in food sector and cornerstone of further risk assessment.

Chapter 2 Literature review

2.1 Antimicrobial resistance

2.1.1 Antimicrobial agents

Infectious diseases were leading causes of high mortality and morbidity in the history. This situation was not improved until the antimicrobial agents were discovered [6]. The first antimicrobial agent arsphenamine was synthesized in 1907, and then marketed with trade name Salvarsan for treatment of syphilis [7]. In 1935, the discovery of sulfonamides was published. This was the first medicine to treat bacterial infections inside body [8]. However, the use of these drugs were limited by the efficacy and safety of synthetic compounds. Until 1940s, penicillin was applied to clinics. Actually penicillin, an antimicrobial agent produced by a fungus, was discovered by Fleming accidentally in 1928. Fleming is not the first one to observe antibiosis phenomenon, however, he is the first one to study the substance-penicillin [9]. From that, it was found that some microorganisms could inhibit other organisms by producing certain substance. This was an important milestone in the history of the discovery and development of antimicrobial agents [10] (Strictly speaking, antimicrobials including antibacterials, antifungals, antivirals and antiparasitics, however, in this thesis, only antibacterials are focused). The emergence of penicillin saved a lot of lives of soldiers during World War II, and penicillin and its derivatives are still widely used in the world [6] (Figure 2).

Antimicrobial discovery was booming during 1940s-1960s. In fact, the most of antimicrobials we use today are the compounds or their derivatives discovered

during that period called ‘golden era of antimicrobial discovery’ [11]. The early methods are mainly screenings of actinomycetes in the soil, such platform was called ‘Waksman platform’ [12], and it earned the inventor Waksman a Nobel prize. However, the source soon became exhausted and the speed of drug discovery slowed down. The modification of existed antimicrobial agents brought limited benefits [13]. After 1960s, few new class of antimicrobials were discovered, one is daptomycin which targets the cell membrane. Another one is bedaquiline, a narrow spectrum against tuberculosis (Table 1). A high-throughput screening platform targeting specific site was developed for synthetic antimicrobials during 1990s, nevertheless, it proved challenging to get effective compounds *in vivo* because of the difficulty to penetrate bacterial cell wall. Because of the low rate of return, many large drug companies left this area. Only a few companies such as GlaxoSmithKline, Merck, Novartis and Roche are still active in antimicrobial research and development [14].

Besides the low productivity of anti-bacterial drug discovery, the antibacterial market is losing its attractiveness for pharmacies. Most of antibacterial drugs are for short courses of therapy. Compared with drugs for long-term diseases (such as cancers and chronic diseases, which need months even years of treatment), antibacterial drugs discovery is less cost-effective for pharmaceuticals. In addition, anti-infective drugs are considered ‘life-saving’ medicines and under strict price control in many countries. Under such a policy circumstance, big pharma are getting out of antibacterial drug discovery because of increased risk and decreased profits [15].

There is another dilemma that impedes the antimicrobial discovery by drug industries. To guarantee the clinical efficacy of antimicrobials, physicians and hospitals need to control the use of antimicrobials to reduce the emergence of resistance, which inevitably affects the sale of drugs and interest of drug manufacturer. This increases the risk of companies to develop antimicrobial drugs compared to other drugs. That is the reason why antimicrobial research and development require more support from government side [13].

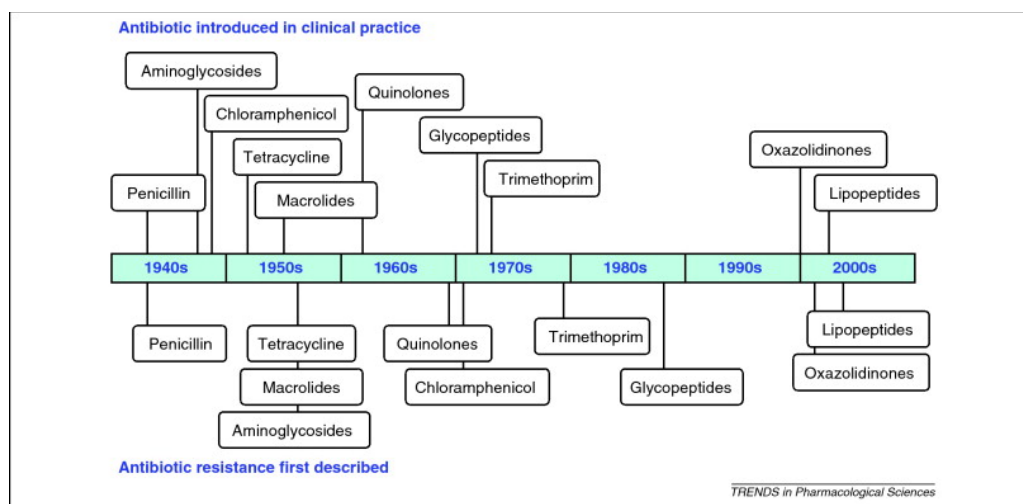


Figure 2. The history and development of antimicrobial agents and antimicrobial-resistant bacteria

Source: reference [16]

Table 1. Timeline of the discovery and introduction of antimicrobials

Antibiotic class; example	Year of discovery	Year of introduction	Year resistance observed	Mechanism of action	Activity or target species
Sulfadiazine; prontosil	1932	1936	1942	Inhibition of dihydropteroate synthetase	Gram-positive bacteria
β -lactams; penicillin	1928	1938	1945	Inhibition of cell wall biosynthesis	Broad-spectrum activity
Aminoglycosides; streptomycin	1943	1946	1946	Binding of 30S ribosomal subunit	Broad-spectrum activity
Chloramphenicols; chloramphenicol	1946	1948	1950	Binding of 50S ribosomal subunit	Broad-spectrum activity
Macrolides; erythromycin	1948	1951	1955	Binding of 50S ribosomal subunit	Broad-spectrum activity
Tetracyclines; chlortetracycline	1944	1952	1950	Binding of 30S ribosomal subunit	Broad-spectrum activity
Rifamycins; rifampicin	1957	1958	1962	Binding of RNA polymerase β -subunit	Gram-positive bacteria
Glycopeptides; vancomycin	1953	1958	1960	Inhibition of cell wall biosynthesis	Gram-positive bacteria
Quinolones; ciprofloxacin	1961	1968	1968	Inhibition of DNA synthesis	Broad-spectrum activity
Streptogramins; streptogramin B	1963	1998	1964	Binding of 50S ribosomal subunit	Gram-positive bacteria
Oxazolidinones; linezolid	1955	2000	2001	Binding of 50S ribosomal subunit	Gram-positive bacteria
Lipopeptides; daptomycin	1986	2003	1987	Depolarization of cell membrane	Gram-positive bacteria
Fidaxomicin (targeting <i>Clostridium difficile</i>)	1948	2011	1977	Inhibition of RNA polymerase	Gram-positive bacteria
Diarylquinolines; bedaquiline	1997	2012	2006	Inhibition of F_1F_0 -ATPase	Narrow-spectrum activity (<i>Mycobacterium tuberculosis</i>)

Source: reference [11]

In spite of the decrease of number of novel antimicrobials, the use of antimicrobial drugs is increasing with time. The latest study to determine the trend of antimicrobial use in 76 countries during 2010-2015 found that the total antimicrobial consumption (in defined daily dose, DDD) increased 65% (from 21.1 to 34.8 billion DDDs). The low & middle-income countries have much higher increasing rate compared to high income countries. And it is noteworthy that the consumption of newer and last-resort antimicrobial drugs (such as carbapenems and colistin) increased across all country income groups during these 15 years. The increase of antimicrobial consumption worldwide undoubtedly worsens the situation of antimicrobial resistance [17].

In fact, the use of antimicrobials in food animals contributed to a large fraction of total use. For example, around 80% of the nation's annual antimicrobial consumption in US is for food animal use in 2010, among which, a great fraction of antimicrobials are also used for human medicine [18]. As a result, the resistant bacteria may also cause challenges for human infection treatment. The use of antimicrobial agents in livestock as growth promoters, prophylactics and therapeutics is placing selection pressure to the bacteria in food animals. A study estimated that the global consumption of antimicrobials in food animals would rise by 67% from 2010 ($63,151 \pm 1,560$ tons) to 2030 ($105,596 \pm 3,605$ tons) if no regulatory action is taken. In terms of types of livestock, cattle have a lower mean antimicrobial use (in per population correction unit, PCU) than chicken and pigs (Figure 3). The higher dispersion of the distribution of chicken indicates a wider range of intensity of antimicrobials use worldwide. In addition, aquaculture may also play an important role in antimicrobial consumption [19]. All these data calls for the urgent action in all countries to limit the overuse of antimicrobial drugs in food animals.

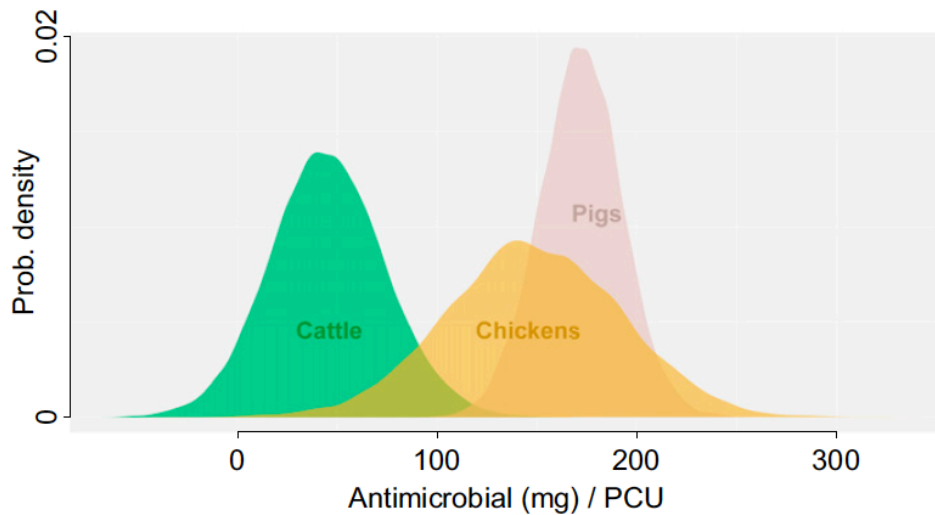


Figure 3. Posterior distributions for estimates of antimicrobial consumption in cattle, chickens, and pigs in OECD countries.

PCU: Population Correction Unit; OECD: Organization for Economic Co-operation and Development

Source: reference [19]

Misuse of antimicrobials is another issue. A systematic review found that non-prescription use accounted for 19-100% of antimicrobial use outside North America and northern Europe during 1970-2009 [20]. There are evidences to show non-prescription antimicrobial use is commonly associated with inappropriate drug types, dose and courses. Non-prescription antimicrobial use is common for non-bacterial caused diseases (for example, many people use anti-bacterial drugs to treat viral influenza by mistake without prescription). This could directly leads to adverse drug reaction and drug resistance [21]. As a result, the monitoring of non-prescription antimicrobial use is necessary and related stewardship must be paid attention as well.

2.1.2 The mechanism of antimicrobial resistance

Generally speaking, antimicrobials mainly have four mechanisms to inhibit the growth and even cause bacterial cell death: (1) inhibition of double DNA formation by inhibiting the DNA gyrase (eg. Fluoroquinolones); (2) inhibition of RNA synthesis (eg. Rifamycins); (3) inhibition of protein synthesis by inhibiting the 50S or 30S ribosome (eg. Macrolides, aminoglycosides, tetracyclines); (4) inhibition of cell wall synthesis (eg. Beta-lactams, glycopeptides, lipopeptides). Other mechanisms such as disadvantageous bacterial cell response to drug-induced stress were also reported [22].

Antimicrobial resistance is the evolution mechanism to adapt for antimicrobials existing in the natural environment, which is in accord with Darwinian theory of evolution. So the history of antimicrobial resistance is far longer than that of clinical antimicrobial use. For instance, beta-lactamases, the main resistance mechanism to beta-lactam class of antimicrobials, have exist for millions of years [23]. Wide-spread antimicrobial resistance determinants exist in the environment such as soil, and only a fraction of them has been reported in human pathogens [24]. Nevertheless, the human use of antimicrobials (including for animals and agriculture) is still regarded the main driver of antimicrobial resistance [25].

Bacterial antimicrobial resistance can be caused by intrinsic or acquired mechanisms. Intrinsic resistance is normally determined by the genes naturally located on bacterial chromosome. The specific causes include: 1) lack of drug affinity for the bacterial target 2) extrusion of antimicrobials by bacterial

exporters 3) inaccessibility of the antimicrobials into bacterial cell 4) innate enzymes produced to inactivate antimicrobials [26]. This trait is universally found and independent with antimicrobial selective pressure. The representative examples are Gram-negative outer membrane, which is impermeable to many antimicrobial agents, and multi-drug resistance (MDR) efflux pumps, which reduce the intracellular antimicrobial concentration [27]. In addition to intrinsic resistance, the resistance can be acquired either by spontaneous site mutation of genes located on bacterial chromosome or horizontal gene transfer. The alteration of drug target caused by chromosomal mutations decreases the antimicrobial susceptibility of bacteria. For example, mutations within DNA gyrase and topoisomerase are one of important causes of fluoroquinolone resistance. The decreased expression level of porin protein caused by mutation also decreases the permeability of some antimicrobials such as imipenem. Horizontally acquired resistance genes are usually located on mobile genetic elements (MGE), which encode enzymes that degrade (such as beta lactamase) or modify (such as aminoglycoside-modifying proteins) antimicrobials [28].

Actually, bacteria usually combine various strategies to defense themselves and develop multi-drug resistance, and the understanding of resistance mechanism helps us to discover new drugs and control infectious diseases.

2.1.3 Global Antimicrobial Resistance Surveillance System

Global Antimicrobial Resistance Surveillance System (GLASS) was launched by WHO in 2015 to share global data on AMR. This is part of the implementation

of Global Action Plan. GLASS initially focus on the global data collection on bacterial pathogen in humans and then will progressively expand the range to food chain, environment and antimicrobial use [29]. By December 2018, 71 countries have been enrolled in GLASS. WHO also has close collaboration with regional surveillance system such as ReLAVRA (Latin American Network for Antimicrobial Resistance Surveillance), EARS-Net (European Antimicrobial Resistance Surveillance Network), and CAESAR (Central Asian and Eastern European Surveillance of Antimicrobial Resistance). The early implementation phase (2015-2019) of GLASS aims to collection data for selected priority bacteria that can cause human diseases including *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, and *Streptococcus pneumoniae*. The samples collected included blood, urine, stool, cervical and urethral specimens, which are for routine clinical diagnosis.

According to the report published by WHO in 2018 [30], in total, 3097 hospitals and 2358 outpatient's clinics reported data to GLASS. The most frequently reported bacteria were in order *E. coli*, *K. pneumoniae*, *Salmonella* spp., *Acinetobacter* spp., *S. aureus*, *S. pneumoniae*, *N. gonorrhoea*, and *Shigella* spp.

Figure 4 shows the countries involved in GLASS and status of AMR reporting and national surveillance system. Figure 5 indicates the existence of functioning national AMR surveillance plan by region, which shows that there are existing national surveillance plans on AMR in most of countries. Compared with the first data in 2017, more countries are approving budget for the surveillance plans.

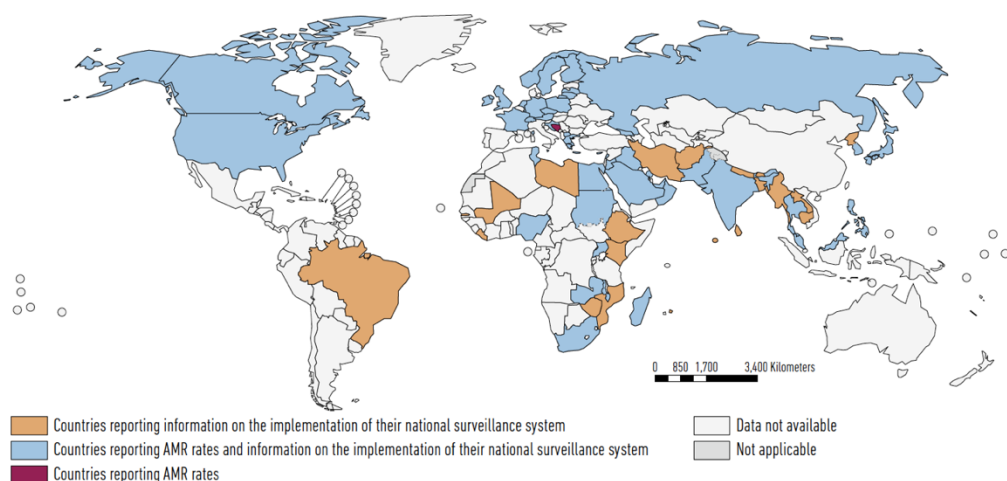


Figure 4. Map with countries providing implementation data only / Implementation data + AMR data

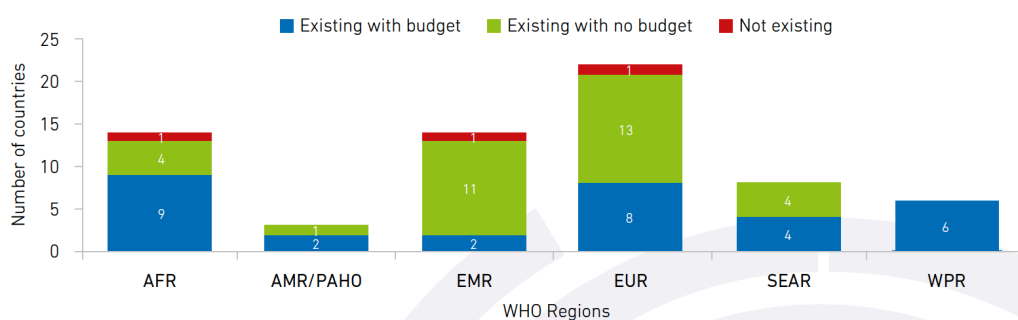


Figure 5. Existence of functioning national AMR surveillance plan per country by region

AFR: African Region; AMR/PAHO: Region of the Americas; EMR: Eastern Mediterranean Region;
 EUR: European Region; SEAR: South-East Asia Region; WPR: Western Pacific Region

ResistanceMap (<https://resistancemap.cddep.org/index.php>) is a web-based tool for global data visualization, and it was firstly developed by the Center for Disease Dynamics, Economics & Policy (CDDEP). It presents the antimicrobial resistance data from 46 countries and antimicrobial consumption data from 76 countries. Figure 6 is an example of ResistanceMap page which shows the global

status of resistance of *E. coli* to fluoroquinolones. Twelve common clinical organisms are included.

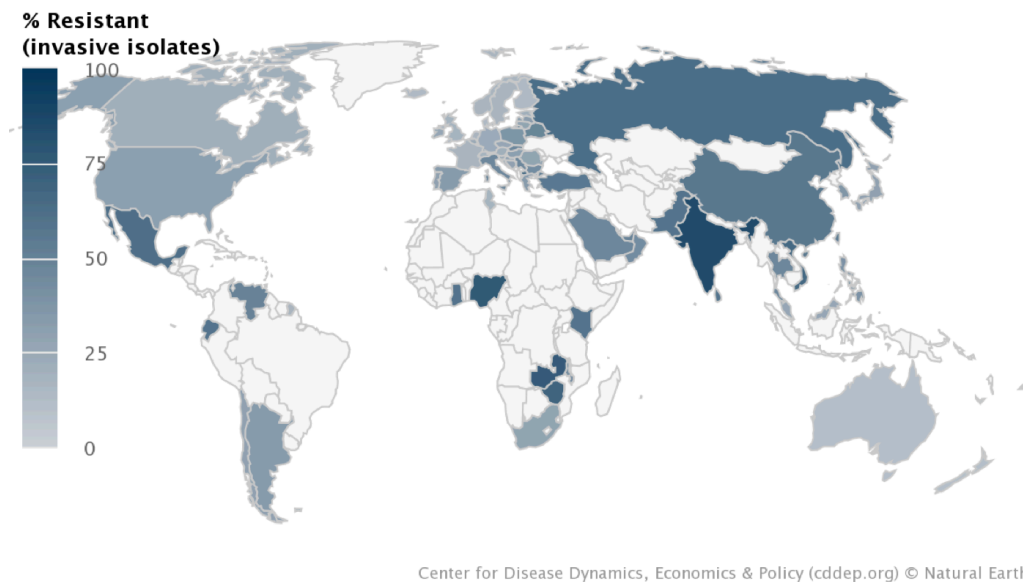


Figure 6. Worldwide resistance of *E. coli* to fluoroquinolones

Because of the limitation of GLASS data collection regards to representativeness and quality, the AMR status between countries and regions was not compared directly. Nevertheless, the data showed high levels of antimicrobial resistance found worldwide, which confirmed the serious situation of global antimicrobial resistance. The AMR prevalence of different countries ranged tremendously. For instance, the proportion of bacteria that resistant to at least one common antimicrobials among patients with suspected bloodstream infection ranged from zero to 82% in GLASS 2018 report [31].

Although the establishment of global surveillance is ongoing, the actual number of infections caused by resistant organism is yet unknown. Some effort was made to estimate the number by modeling study. Elizabeth Temkin et.al estimated the

number of infections caused by third-generation cephalosporin-resistant and carbapenem-resistant *E. coli* and *K. pneumoniae* in 2014 [32]. It was estimated that third-generation cephalosporin-resistant *E. coli* and *K. pneumoniae* caused 6.4 million (additive model) or 4.6 million (50% replacement model) bloodstream infections in 2014. Carbapenem-resistant strains were estimated to have caused 0.5 million (additive model) or 0.4 million (50% replacement model) bloodstream infections. The study carried by the Global Burden of Disease (GBD) in 2016 estimated that 126,000 deaths were caused by multidrug-resistant and extensively drug-resistant tuberculosis [33]. While O'Neill estimated around 700 000 deaths were caused by AMR bacterial infections each year globally in a review published in 2014 [34].

Singapore perspective

Alvin et.al reviewed the changes in Singapore in terms of antimicrobial uses, regulation and AMR epidemiology in the past ten years, mainly focusing on hospital sector [35]. The antibiotic (only antibacterial drugs are included) usage during 2000-2015 in Singapore is shown in Figure 7. Antimicrobial stewardship program (ASP) has been implemented in local public sector hospitals, however, private hospitals and primary healthcare sectors have not been fully involved. Antimicrobial prescription data from private hospitals and primary healthcare sectors are not available currently. AMR data from private hospitals and community hospitals is available but not shared at national level. As a result, in the healthcare sector, the surveillance system was improved in the past decade but more work is needed to make a more comprehensive, effective and transparent system.

As Singapore is one of the global economy and trade centers, the strategic geographical location of Singapore may contribute to the diversity of multidrug-resistant organisms. ESBL and AmpC beta-lactamase-producing Enterobacteriaceae, the main causes of third-generation cephalosporin resistance, was firstly reported in Singapore in 1986 and 2003, respectively [36]. In 2006, 30.3% *Klebsiella* spp. from hospital in Singapore are ESBL-producer, and 5.6% of these bacteria also produce AmpC, For *E. coli*, the percentages are 19.6% and 8.5%, respectively. Another study in 2014 showed 12.4% (124/1006) and 1.8% (18/1006) of patients in a public tertiary hospital carried ESBL-producing Enterobacteriaceae, methicillin-resistant *Staphylococcus aureus* (MRSA), respectively [37]. In community setting, 26.2% (80/305) of healthy people in community in Singapore carried at least one ESBL-producing Enterobacteriaceae isolates in 2018 [38]. It is noteworthy that resistance to last-resort drug such as colistin also appeared in Singapore. The presence of mobile colistin resistance gene carried by clinical isolates was firstly reported in 2016 in a retrospective study, which means it has been existed before the detection [39]. A recent study firstly reported the prevalence of human fecal carriage of *mcr-I*, the most common mobile colistin resistance gene. 8.0% stool samples in hospital were *mcr-I* positive by direct stool PCR [40].

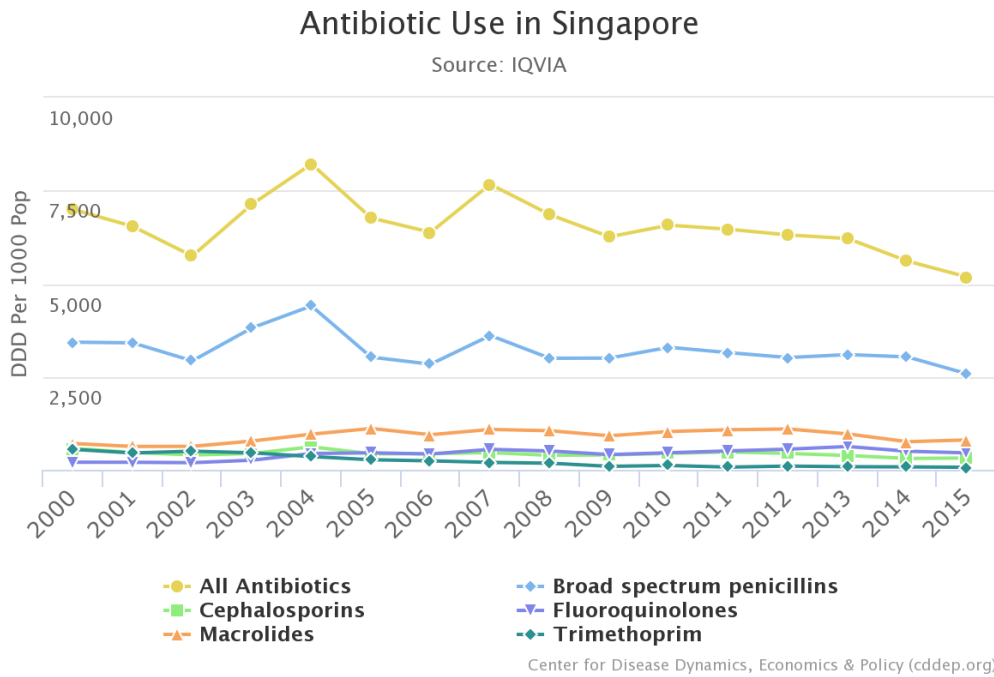


Figure 7. Antibiotic use (only antibacterial drugs are included) in Singapore (2000-2015)

2.2 ESBL-producing bacteria

2.2.1 *Beta-lactam antimicrobials*

Beta-lactam antimicrobials are a broad group of antimicrobials which share a same structure feature - beta-lactam ring. They are the first batch of antimicrobials to be described in the history starting from the discovery of penicillin [41]. This class of antimicrobials includes penicillins, cephalosporins, cephamycins, carbapenems, monobactams and beta-lactamase inhibitors. This class of drugs inhibits the growth of bacteria by inactivating enzymes involved in the third stage of bacterial cell wall synthesis, which are located on the cell membrane. These related proteins are called penicillin binding proteins (PBPs). As they play unique roles related to the synthesis of bacterial cell wall at different stages, the binding of beta lactam to certain PBPs may result in characteristic

effects on bacterial growth inhibition or death [42]. Other bactericidal mechanisms may also exist such as beta-lactam-caused autolytic system [43].

Currently beta-lactam is the most used class of antimicrobials for infectious diseases. It accounts for 65% of injectable antimicrobial prescriptions in US during 2004-2014 (Figure 8), and among which, half of beta-lactams prescribed are cephalosporins (Table 2). This class of antimicrobials are efficacious especially for the treatment of serious infection and widely prescribed clinically [44].

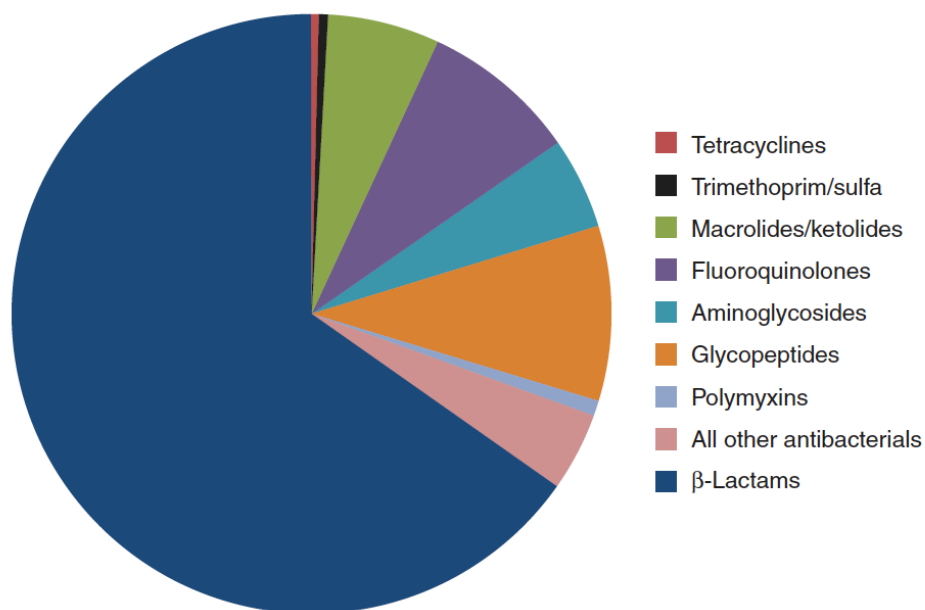


Figure 8. Proportion of prescriptions in the United States for injectable antimicrobials by class for years 2004–2014.

Table 2. Usage of parenteral beta-lactams by class from 2004 to 2014 in US

Class of β -lactam	Percentage of prescriptions ^a
Narrow spectrum penicillins	3.12
Broad spectrum penicillins ^b	36.54
Cephalosporins	47.49
Monobactams	1.66
Carbapenems	11.20

^aThe percentage for each injectable antimicrobial class prescribed in the United States from 2004 to 2014 (Data from the IMS MDART Quarterly Database on file at AstraZeneca).

^bBroad-spectrum penicillins include the beta-lactam/ beta-lactam-inhibitor combinations piperacillin-tazobactam, ticarcillin-clavulanate, and ampicillin-sulbactam.

2.2.2 Emergence of ESBL

With the wide use of beta-lactams, the corresponding resistance soon developed. Extended-spectrum beta-lactamases (ESBL) are enzymes that can hydrolyze most of beta-lactams including penicillins, cephalosporins and monobactams. And thus, ESBL-producing bacteria confer resistance to these antimicrobials and this has resulted in poor clinical outcomes [45].

ESBLs are mainly produced by the Enterobacteriaceae family of Gram-negative bacteria, and typical ESBL-producing bacteria are *E. coli* and *Klebsiella pneumonia* [46]. In addition, some nonfermentative Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* can also produce ESBLs [47]. The most common types of ESBLs are TEM, SHV and CTX-M. Besides these, there are still OXA, PER and other uncommon ESBL types which have been found in a wide range of geographic locations [48].

TEM type

TEM type ESBL is derived from TEM-1 and TEM-2. TEM refers to ‘Temoneira’, the name of patient from whom the first TEM-1 was isolated in 1965. TEM-1 and TEM-2 are able to hydrolyze ampicillin and has weak activity against extended-spectrum cephalosporins. However, an enzyme found in 1984 in France, now termed as TEM-3, has enhanced activity against cefotaxime. This enzyme differs from TEM-2 by only two amino acid. But actually, TEM-12, isolated from *Klebsiella* in England, in 1982, is found earlier than TEM-3. Currently, there are more than 140 TEM type ESBLs, of which, TEM-10, TEM-12 and TEM-26 are the most common types.

SHV type

SHV refers to sulfhydryl variable. The native SHV-1 was found in *K. pneumoniae*, which is resistant to penicillins and 1st generation cephalosporins. Similar with TEM-1, specific mutations of SHV-1 expanded its hydrolysis capabilities to extended-spectrum cephalosporins and monobactams. More than 80 types of SHV ESBLs were discovered and most common types are SHV-5 and SHV-12.

CTX-M type

The name CTX-M reflects the hydrolysis activity against cefotaxime. CTX-M type hydrolyzes cefepime with higher efficiency than other types of ESBL. CTX-M beta-lactamases show 40% or less identity with SHV and TEM types of ESBLs. From the beginning of 21st century, the predominant types changed from SHV and TEM because of the emergence of CTX-M. This enzyme was originally encoded by *Kluyvera* spp., however, this gene was horizontally transferred to

other species and now it has become a non-neglectable member of ESBLs especially in *E. coli* and *K. pneumonia* [46]. The current common types of CTX-M are CTX-M-2, CTX-M-3, CTX-M-14 and CTX-M-15.

OXA type

OXA refers to oxacillinases. OXA type beta-lactamase is characterized by its hydrolysis rates for cloxacillin and oxacillin greater than 50% that for benzylpenicillin. The OXA-type ESBLs were found originally in *Pseudomonas aeruginosa* isolated from a hospital in Turkey. This type of ESBLs are usually produced by *Pseudomonas aeruginosa* but they have been also found in observed in other Gram-negative bacteria.

PER type and other types

PER type ESBLs have only 25-27% homology with TEM and SHV types of ESBLs. PER-1 was firstly found in *Pseudomonas aeruginosa*. PER-2 shares 86% homology to PER-1. They are able to efficiently hydrolyze penicillins and cephalosporins and are sensitive to clavulanic acid. It is worrying that a *Pseudomonas aeruginosa* isolate carrying PER-1 and the carbapenemase VIM-2 was discovered. These two enzymes make the isolate resistant to all beta-lactams.

There are still other ESBL types which are not derivatives of any known beta-lactamases by point mutations. For instance, VEB-1 has 38% homology with PER-1 and PER-2, and it is highly resistant to ceftazidime, cefotaxime and aztreonam. GES, BES, TLA, SFO, IBC are also ESBL types found worldwide.

2.2.3 Prevalence of ESBL in food and food-producing animals (worldwide and in Singapore)

The presence of ESBL-producers in food and food-producing animals have been reported in many countries, however, the surveillance network for ESBL has not been established widely. As a result, the prevalence data in this field is still limited. On the other hand, the isolation and detection methodology used by researchers worldwide are not unified, which makes the results not comparable. For instance, the prevalence data of ESBL-producer from raw meats in different countries are shown in Table 3. The prevalence of ESBL in raw meats has a wide range from less than 10% to more than 90%. The discrepancy may be influenced by many factors such as geography, local antimicrobial administration, farming system, animal species and so on. The complexity of ESBL epidemiology makes the need for a consistent and scientific global detection system.

The data of ESBL in Singapore is also limited. On the clinical side, in 2008, Tse Hsien Koh reviewed the clinical ESBL situation in Singapore, which pointed out that the clinical ESBL *E. coli* percentage increased from less than 3% to more than 20% during 1981-2007, for *Klebsiella*, this number went to 35%-40%, which made the ESBL Enterobacteriaceae the biggest Gram-negative resistance problem in clinical situation [36]. Although ESBL percentage in Singapore is relatively low compared with other Southeast countries like Vietnam, Philippines and Thailand [49], actions need to be taken to inhibit its further spread. On the food side, governmental agencies like Singapore Food Agency (SFA) has started to put ESBL into routine test, however, the data is not accessible to public now. High prevalence (96.4%) of ESBL Enterobacteriaceae was reported in raw chicken with limited sample size [50]. Our study is the first systematic study to

report the ESBL *E. coli* in raw meats in Singapore. The detailed analysis will be discussed in Chapter 3.

Table 3. Prevalence of ESBL-producer in raw meats in different countries

Country	Year	Isolate type	Chicken	Pork	Beef	Reference
Singapore	2016	ESBL Enterobacteriaceae	78.9% (15/19)	--	--	[50]
Singapore	2017-2018	ESBL <i>E. coli</i>	51.2% (109/213)	26.9% (58/216)	8.3% (17/205)	Our study
UK	2013-2014	ESBL <i>E. coli</i>	65.4% (104/159)	2.5% (2/79)	1.9% (1/159)	[51, 52]
Netherland	2011	ESBL <i>E. coli</i>	94% (92/98) 67.0% (fresh meat); 84.4%	--	--	[53]
Netherland	2014	ESBL isolates	(import)	2.7%	2.2%	[54]
Netherland	2018	ESBL/AmpC <i>E. coli</i>	13.7 (fresh);1.3% (import)	0	1.3%	[55]
Denmark	2016	ESBL <i>E. coli</i>		--	--	[56]
Denmark	2018	ESBL <i>E. coli</i>	4%	--	--	DANMAP
Thailand	2014	ESBL <i>E. coli</i>	--	20% (3/15)	--	[57]
Turkey	2016	ESBL <i>E. coli</i>	86.6%	--	--	[58]
China	2011-2014	ESBL Enterobacteriaceae	23.80%	13.7%	13.3%	[59]
China	2013	ESBL Enterobacteriaceae	--	7.5%	--	[60]
Switzerland	2009-2011	ESBL Enterobacteriaceae	--	0%	0%	[61]
Austria	2012 (publish year)	ESBL <i>E. coli</i>	35.90%	--	--	[11]
Spain	2012 (publish year)	ESBL Enterobacteriaceae	84%	55%	59%	[62]

2.3 Colistin and colistin resistance

Colistin is also called polymyxin E, belonging to the class of polypeptide antimicrobials called polymyxins with a broad antibacterial spectrum of Gram-negative bacteria. It is mainly comprised of colistin A and B, which was used in hospitals since 1960s. Due to its toxicity, it was gradually replaced by other antimicrobials, however, with the emergence of multidrug-resistant bacteria, colistin once again gained to the people's attention and is usually regarded as the last resort of Gram-negative bacterial infection [63].

Different from most of antimicrobials, the bactericidal effect of colistin is not depended on the inhibition of bacterial metabolic activity, it kills bacteria mainly by destroying the outer membrane of Gram-negative bacteria. Similar with detergent effect, it can replace the magnesium(Mg^{2+}) and calcium (Ca^{2+}) ions from the phosphate groups of lipopolysaccharide (LPS) on the membrane, which leads to the leakage of cell contents [64]. Besides this, colistin also has anti-endotoxin activity. Endotoxin releases after the destruction of cell membrane. Endotoxin refers LPS in outer membrane in most of Gram-negative bacteria, and the hydrophobic component lipid A on LPS is mainly responsible for the bioactivity of endotoxin. It can be recognized by immune system as pathogen-associated molecule through Toll-like receptor 4 [65]. Colistin can bind and neutralize LPS to prevent the ability of endotoxin [64].

Colistin resistance is mainly caused by less affinity of colistin to bacterial outer membrane due to the modification of LPS. Two-component regulatory systems

PhoPQ and PmrAB as signal transduction system as well as response regulator (including an ethanolamine transferase) can reduce the interaction by adding ethanolamine moieties or 4-amino-4-arabinose to the lipid A and reducing the negative charge of outer membrane [64]. Other mechanisms including efflux pump systems, overproduction of capsule polysaccharide, colistinase and total loss of LPS (rare) can also result in colistin resistance [64, 66]. Besides these, the transferable gene *mcr* related to colistin resistance was discovered in 2016, which means the possible horizontal colistin resistance gene transfer (plasmid-mediated). MCR-1 is one of the phosphoethanolamine transferase enzymes which leads to colistin resistance in *E. coli* by adding phosphoethanolamine to lipid A on LPS [67]. Since then, *mcr* and their variants were discovered in succession (Table 4). Since the *mcr* genes' discovery, many retrospective studies were done to explore the prevalence of *mcr* genes. MCR has been found in five continents including Europe, Southern America, Northern America, Asia, Africa and Oceania. There have not been any related reports in Antarctica yet [68]. It was firstly found in *E. coli*, and it was also found in other Enterobacteriaceae like *Salmonella*, *Klebsiella*, *Shigella* and others [69, 70]. The prevalence of *mcr*-mediated colistin resistance is not high currently [71, 72], however, the use of colistin and transferability of resistance genes will speed up the selection and spread of resistant bacteria. Regular and large screening and surveillance is still needed, after all, colistin is regarded as the last resort of multidrug-resistance infection nowadays.

Table 4. Summary of *mcr* genes and their variants

Gene	Date	Bacteria	Country	Plasmid	Accession	Reference
<i>mcr-1</i>	02/01/16	<i>E. coli</i>	China	IncI2	KP347127	[67]
<i>mcr-1.2</i>	09/01/16	<i>K. pneumoniae</i>	Italy	IncX4	KX236309	[73]
<i>mcr-1.3</i>	02/01/17	<i>E. coli</i>	China	IncI2	KU934208	
<i>mcr-1.4</i>	10/01/17	<i>E. coli</i>	China	IncX4	KY041856.1	[74]
<i>mcr-1.5</i>	12/01/16	<i>E. coli</i>	Argentina		KY283125	
<i>mcr-1.6</i>	03/01/17	<i>S. Typhimurium</i>	China	IncP	KY352406	[75]
<i>mcr-1.7</i>	10/01/17	<i>E. coli</i>	China	IncX4	KY488488	[74]
<i>mcr-1.8</i>	03/01/17	<i>E. coli</i>	UK		KY683842	
<i>mcr-1.9</i>	04/01/18	<i>E. coli</i>	China	IncI2	MG946761	[76]
<i>mcr-1.10</i>	08/01/17	<i>Moraxella</i> spp	UK		MF176238	[77]
<i>mcr-1.11</i>	06/01/18	<i>E. coli</i>	USA		NG_055784.2	
<i>mcr-1.12</i>	12/01/17	<i>E. coli</i>	Japan		LC337668.1	
<i>mcr-1.13</i>	03/01/18	<i>E. coli</i>	Germany		MG384739.1	
<i>mcr-1.14</i>	05/01/19	<i>E. coli</i>	China		NG_057460.1	
<i>mcr-1.15</i>	08/23/18	<i>K. pneumoniae</i>	China		NG_061610.1	
<i>mcr-1.16</i>	05/01/19	<i>E. coli</i>	China		NG_064787.1	
<i>mcr-1.17</i>	05/01/19	<i>E. coli</i>	China		NG_064788.1	
<i>mcr-1.18</i>	05/01/19	<i>E. coli</i>	China		NG_064789.1	
<i>mcr-1.19</i>	06/27/19	<i>S. Typhimurium</i>	China		NG_065449.1	
<i>mcr-1.20</i>	06/27/19	<i>E. coli</i>	UK		NG_065450.1	
<i>mcr-1.21</i>	06/27/19	<i>E. coli</i>	China		NG_065451.1	
<i>mcr-1.22</i>	08/26/19	<i>E. coli</i>	Nigeria		NG_065944.1	
<i>mcr-1.23</i>	01/21/20	<i>Salmonella</i> .sp.	Bangladesh		NG_067235.1	
<i>mcr-1.24</i>	01/21/20	<i>E. coli</i>	Bangladesh		NG_067236.1	
<i>mcr-1.25</i>	01/21/20	<i>E. coli</i>	Bangladesh		NG_067237.1	
<i>mcr-2</i>	06/01/16	<i>E. coli</i>	Belgium	IncX4	LT598652	[78]
<i>mcr-2.2</i>	01/25/18	<i>Moraxella pluranimalium</i>	Spain		NG_055496.1	
<i>mcr-2.3</i>	06/27/19	<i>E. coli</i>	Thailand		NG_065452.1	
<i>mcr-3</i>	06/01/17	<i>E. coli</i>	China	IncHI2	KY924928.	[79]
<i>mcr-3.2</i>	09/16/18	<i>S. enterica</i>	Canada	IncHI-2	MH114596	[80]
<i>mcr-3.3</i>	10/24/17	<i>Aeromonas veronii</i>	China	chromosome	MF495680	[81]
<i>mcr-3.4</i>					FLXA01000011.1	

Gene	Date	Bacteria	Country	Plasmid	Accession	Reference
<i>mcr-3.5</i>	11/22/17	<i>E. coli</i>	China	IncP	MF489760.1	[82]
<i>mcr-3.6</i>	01/31/18	<i>A.allosaccharophila</i>	Germany		MF598076.1	[83]
<i>mcr-3.7</i>	01/31/18	<i>A. media</i>	Germany		MF598077.1	[83]
<i>mcr-3.8</i>	01/31/18	<i>A. jandaei</i>	Germany		MF598078.1	[83]
<i>mcr-3.9</i>	01/31/18	<i>A. hydrophila</i>	Germany		MF598080.1	[83]
<i>mcr-3.10</i>	01/25/18	<i>A. caviae</i>	China	IncI2	MG214531.1	[84]
<i>mcr-3.11</i>		<i>E. coli</i>	China		MG489958.1	
<i>mcr-3.12</i>	04/30/18	<i>E. coli</i>	Brazil	IncA/C ₂	MG564491.1	[85]
<i>mcr-3.13</i>	08/27/18	<i>A. caviae</i>	China		MH332763	[86]
<i>mcr-3.14</i>	08/27/18	<i>A. bivalvium</i>	China		MH332764	[86]
<i>mcr-3.15</i>	08/27/18	<i>A. media</i>	China		MH332765	[86]
<i>mcr-3.16</i>	08/27/18	<i>A. salmonicida</i>	China		MH332766	[86]
<i>mcr-3.17</i>	08/27/18	<i>A. allosaccharophila</i>	China		MH332767	[86]
<i>mcr-3.18</i>	08/27/18	<i>A. caviae</i>	China		MH332768	[86]
<i>mcr-3.19</i>	03/14/19	<i>A. veronii</i>	India	chromosome	CP032839	[87]
<i>mcr-3.21</i>	11/26/19	<i>K. pneumoniae</i>	France	IncFII	CP035205	[88]
<i>mcr-3.22</i>	06/27/19	<i>K. pneumoniae</i>	Thailand		NG_060581.2	
<i>mcr-3.23</i>	11/09/18	<i>K. pneumoniae</i>	Thailand		NG_060583.1	
<i>mcr-3.24</i>	11/09/18	<i>Shigella sonnei</i>	Thailand		NG_060580.1	
<i>mcr-3.25</i>	11/09/18	<i>Aeromonas veronii</i>	China		NG_060585.1	
<i>mcr-3.26</i>	11/26/19	<i>K. pneumoniae</i>	France	IncFII	CP035200	[88]
<i>mcr-3.27</i>	04/04/19	<i>A. hydrophila</i>	Malaysia		MH131694.1	
<i>mcr-3.28</i>	11/26/19	<i>K. pneumoniae</i>	France	IncP1	CP035195	[88]
<i>mcr-3.29</i>	02/20/19	<i>E. coli</i>	Vietnam		MK521074.1	
<i>mcr-3.30</i>	06/27/19	<i>A. veronii</i>	India		NG_065456.1	
<i>mcr-4</i>	08/01/17	<i>Salmonella and E. coli</i>	Italy, Spain, Belgium	ColE	MF543359	[89]
<i>mcr-4.2</i>	01/11/18	<i>S. Typhimurium</i>	Italy		MG581979	[90]
<i>mcr-4.3</i>	02/22/18	<i>S. enterica</i>	Denmark		ERS1801979	[91]
<i>mcr-4.4</i>	04/07/18	<i>E. coli</i>	Spain		MG822665	[92]
<i>mcr-4.5</i>	04/07/18	<i>E. coli</i>	Spain		MG822664	[92]
<i>mcr-4.6</i>	12/13/17	<i>E. cloacae</i>	Singapore	ColE10	MG026621	[91]
<i>mcr-5</i>	09/01/17	<i>S. enterica</i>	Germany	ColE	KY807921	[93]
<i>mcr-5.2</i>	02/12/18	<i>E. coli</i>	Germany		MG384740	[94]
<i>mcr-5.3</i>	09/07/18	<i>E. coli</i>	Brazil		MG886287	[95]

Gene	Date	Bacteria	Country	Plasmid	Accession	Reference
<i>mcr-5.4</i>	08/23/19	Metagenome	Netherlands		MK965519	[96]
<i>mcr-6.1</i> (<i>mcr-2.2</i>)	08/01/17	<i>Moraxella spp</i>	UK			[77]
<i>mcr-7</i>	03/01/18	<i>K. pneumoniae</i>	China	IncI2	MG267386	[97]
<i>mcr-8</i>	07/01/18	<i>K. pneumoniae</i>	China	IncFII	MG736312	[98]
<i>mcr-8.2</i>	12/11/19	<i>K. pneumoniae</i>	China	IncQ, IncR, IncFII	MH703569	[99]
<i>mcr-8.3</i>		<i>K. pneumoniae</i>	Laos	IncFII	AXI82467	[88]
<i>mcr-8.4</i>	02/15/19	<i>Raoultella ornithinolytica</i>	China	IncFII	MH791448	[100]
<i>mcr-9</i>	05/07/19	<i>S. Typhimurium</i>	USA	IncHI2	NZ_NAAN010 00063.1	[101]

2.4 AMR in food chain and its transmission to human

There is growing concern about the transmission of AMR via the food chain, although the relative contribution of the food chain to the global disease burden caused by antimicrobial resistant bacteria is under investigation [102].

There are many ways for food to be contaminated with antimicrobial-resistant bacteria which broadly exist in soil, water and human / animal feces. Animal meat production may be contaminated by feces during slaughter. Irrigation with contaminated water is the cause of AMR transfer in agriculture production. AMR may also transfer during food processing originating from human or other food, which is called cross-contamination [103]. Certain microorganisms may also be added by humans during food processing for starter culture (yogurt production), probiotics and biopreserving. These microorganisms have been proved to be potential reservoir of AMR long time ago [104, 105]. The AMR gene transfer by

conjugation or transformation in food (in fermented milk [106-108], sausages and cheese [109]) was also reported.

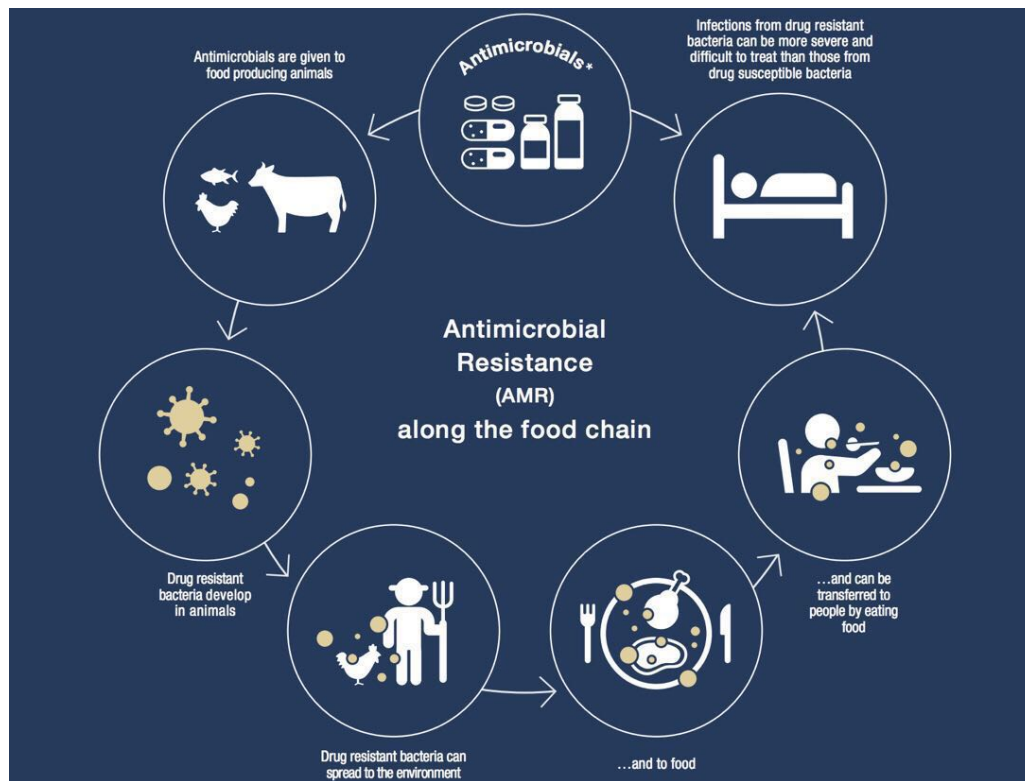


Figure 9. AMR transmission along the food chain

Source: WHO

Foods of animal origins are regarded as an important source of antimicrobial resistant bacteria on food chain. Resistant bacteria in food-producing animals' guts can be selected by antimicrobials used in veterinary husbandry. Resistant bacteria can be spread by cross-contamination between animals and humans in the farm, and resistant bacteria can also spread to surface of animal meats during meat processing. Finally human acquire the bacteria by food consumption. It is estimated that 50% antimicrobials are used in food-producing animals in Europe and North America. Antimicrobial resistant bacteria can persist in animals for a

long time even the antimicrobial residues have been degraded and undetectable [110].

Human and animals use 118 mg/population correction unit (PCU) and 133 mg/kg of antimicrobials, respectively. However, unlike in humans, antimicrobials used in food animals are usually for growth promoter and mass prophylaxis. According to US FDA, 80% of antimicrobials (17,000 tons) sold in the US were for livestock use by 2014. Realizing the potential effect on AMR development, EU and US banned the use of antimicrobial growth promoters in 2006 and 2017 [111]. These uses through feed and low-dose pattern promote the evolution of AMR. This suggests that food animals can be a big reservoir for AMR [112]. However, the role of farm animals in the dissemination of AMR to human has been controversial. It is hypothesized that there are two ways of AMR transmission: antimicrobial resistant bacteria from food animal and their AMR determinants transfer. Dishon et.al [113] systematically reviewed the studies about current evidence on AMR transmission from food animals to human. It was found that eight studies (18%) suggested transmission from food animals to human and 25 studies suggested transmission between animals and humans with no specific directions. The resolution of typing method in these studies are variable so that robust conclusions on the direction of transmission cannot be drawn in some studies. This also highlights the need of application of high-resolution typing method like whole genome sequencing in this area.

The use of antimicrobials in animal husbandry industry increases the antimicrobial resistance in human through the consumption of food. A

metagenome analysis for 252 fecal samples from human suggests that AMR genes were more abundant for those antimicrobials which have been used in clinical setting longer and for those which are approved for use in livestock than the antimicrobials introduced late and not used in livestock [114]. The AMR genes for antimicrobials used in livestock are the most abundant in human gut microbiome. This study supports the “farm-to-fork” hypothesis.

In fact, the types of antimicrobials used in animal feeds for growth promotion, prophylactic or therapeutic purposes are usually the same, or relevant to those used in human. In 2017, WHO updated the 5th version *Critically important antimicrobials for human medicine* to raise researchers’ and stakeholders’ attention [115]. This list helps to develop risk management strategies of antimicrobial use in food-producing animals.

AMR genes transfer in human gut

The gastrointestinal tract colonized by a complex microbiota provides a suitable environment for AMR gene-carrying plasmid transfer between commensals and pathogens [116]. The lower gastrointestinal tract is the largest microbial community of human body, which is consist of up to 10^{14} bacterial cells of over 500 species from nine bacterial divisions [117]. High cell density, antimicrobial agent exposure and subsequent selection followed by the innate ability for gene transfer through a variety of different mechanisms contribute to the favorable conditions for gene transfer [118]. Metagenomic analysis of human gut microbiome suggests high level AMR and related mobile genetic elements. As a

reservoir, the human gut has more AMR genes than any other studied natural environment including ocean, lake and soil [119-121].

Different *in vitro* methods including filter, plate and liquid mating have been used to study the transfer of AMR genes [122, 123]. And Chemostat system with a constant culture environment is suitable for mimicking the gut system. It has been applied to the establishment of *in vitro* gut model for gene transfer. Recently, an *in vitro* chicken gut model was established to explore the multidrug resistance plasmid transfer from *Salmonella* to commensal *E. coli*. This system demonstrates that new cefotaxime-resistant *E. coli* appeared after adding cefotaxime-resistant *Salmonella*. Plasmid harboring AMR genes was detected and the plasmid transfer rate ranged from 2.2×10^{-9} to 6.4×10^{-10} [124]. Similarly, A. Smet et al proved *bla*_{TEM-52}-carrying plasmid could be transferred from *E. coli* of broiler origin to that of human origin in a human intestinal tract simulating system even without antimicrobial treatment, which highlight the risk for public health related to ESBL-producing Enterobacteriaceae in food chain [125]. *In vivo* model based on rats or mice is also applied to this field. No matter the “worst case” like gnotobiotic rats/mice [126-129] or the animals with normal bacterial flora which better mimic the human gastrointestinal tract [130-132], they all suggest the AMR gene can be spread in species [131] [132] or between different species [130]. Such cases also happened in clinical environments. A one-year-old Tunisian boy acquired *Klebsiella pneumonia* carrying a plasmid with AAC-1 AmpC, and then ACC-1 AmpC was transferred to an ampicillin-resistant *E. coli* in vivo which caused a urinary tract infection after cefotaxime therapy [133]. A study in Sweden also found one *E. coli* strain in an infant gut acquired ampicillin resistance through the transfer of *bla*_{TEM-1B}-encoding plasmid, pNK29 from

another *E. coli* strain after antimicrobial treatment [134]. These studies highlight the importance to monitor the enteric flora on hospital admission and antimicrobial chemotherapy because of the AMR gene transfer in human gut .

It is easy to understand that antimicrobial use poses a selective function to antimicrobial-resistant bacteria because of their survival advantages, and mobile resistance genetic elements may be lost when the antimicrobial no longer exist. However, co-selection makes the situation more complex. Co-selection refers to the phenomenon that different resistance genes on the same genetic element like plasmid can be selected by any of the antimicrobials which they are resistant to. This is one of the reason why the phenotype of resistance is hard to reverse. For example, plasmids carrying ESBL genes frequently carry other resistance genes at the same time. The wide range of resistance genes make the bacteria easy to be selected with any one of these antimicrobials even without beta-lactam [135]. Anthropogenic activities also pose an impact on resistance selection [136]. It has been reported that the exposure of antimicrobial residues and/or detergents increased the prevalence of mobile genetic elements (class 1 integrons) carrying resistance genes specifically in sewage sludge and pig slurry. Detergents and biocides in domestic wastewater and farm animal waste co-select for antimicrobial resistance even in the absence of antimicrobials, because the quaternary ammonium compound (QAC) resistance genes and antimicrobial resistance genes co-exist on the same integron [136]. Transmission to humans may occur when meat containing antimicrobial-resistant bacteria and resistance genes, most often originating from the gastro-intestinal tract, are consumed or contaminated by other food products [137]. Investigations into the relative contributions of the food animal industry to the emergence of resistant human

pathogens have focused primarily on the most commonly consumed meat products, poultry, beef, and pork, since they represent a greater degree of exposure for the consumer [138].

2.5 AMR Genes and Horizontal Gene Transfer

Spontaneous mutation is one of the causes for AMR, however, it would take a long time to develop multi-drug resistance if only mutation-based evolution is relied on. Compared with mutation, horizontal gene transfer (HGT) is a faster way to exchange genetic elements within species and among diverse species, which greatly speed the transmission of AMR and MDR [139].

HGT was first described in bacteria in 1947 [140]. HGT is a process of sharing genetic materials between different organisms that are not in vertical relationship (parent-offspring). The most recognized mechanisms of HGT in bacteria are: 1) conjugation 2) transformation 3) transduction [141] (Figure 10).

Conjugation requires the direct contact of donor bacteria and recipient bacteria through pilus. The donor cell produces pilus and carries F-plasmid, which is called F-positive (denoted by F^+). Whereas the recipient cell without F-plasmid is called F-minus (F^-). Once the conjugation process is done, the recipient cell becomes a new donor cell carrying F-plasmid. This process is also called mating, however, this is not a sexual reproduction since no new generation is created. The conjugative genetic elements (most likely plasmids or transposons) are transferred via a bridge-like structure between two cells, which can result in the acquisition of AMR genes or other fitness factors [142]. Transformation is a

process that bacteria can uptake foreign genetic material (naked DNA released by lysed bacteria) from environment. This process requires the ability of bacteria to take up free genetic materials. The bacteria in that status are called competent cells. Artificial competent cells can be made by chemical method or electrical pulse method. As a result, transformation is an useful tool in genetic engineering [143]. Transduction is mediated by bacteriophage, during which genetic material of host bacteria can be integrated into the genome of bacteriophage and then transfer to other bacteria via the re-infection of phage. More recently, other mechanisms of HGT were discovered. For example, cell fusion is a conjugation-like process but the genetic material exchange is bi-directional. Another transduction-like activity is mediated by gene transfer agents (GTAs). GTAs are phages that only randomly carry host DNA and no long recognize their own DNA, and they can integrate into the genome of host bacteria [141].

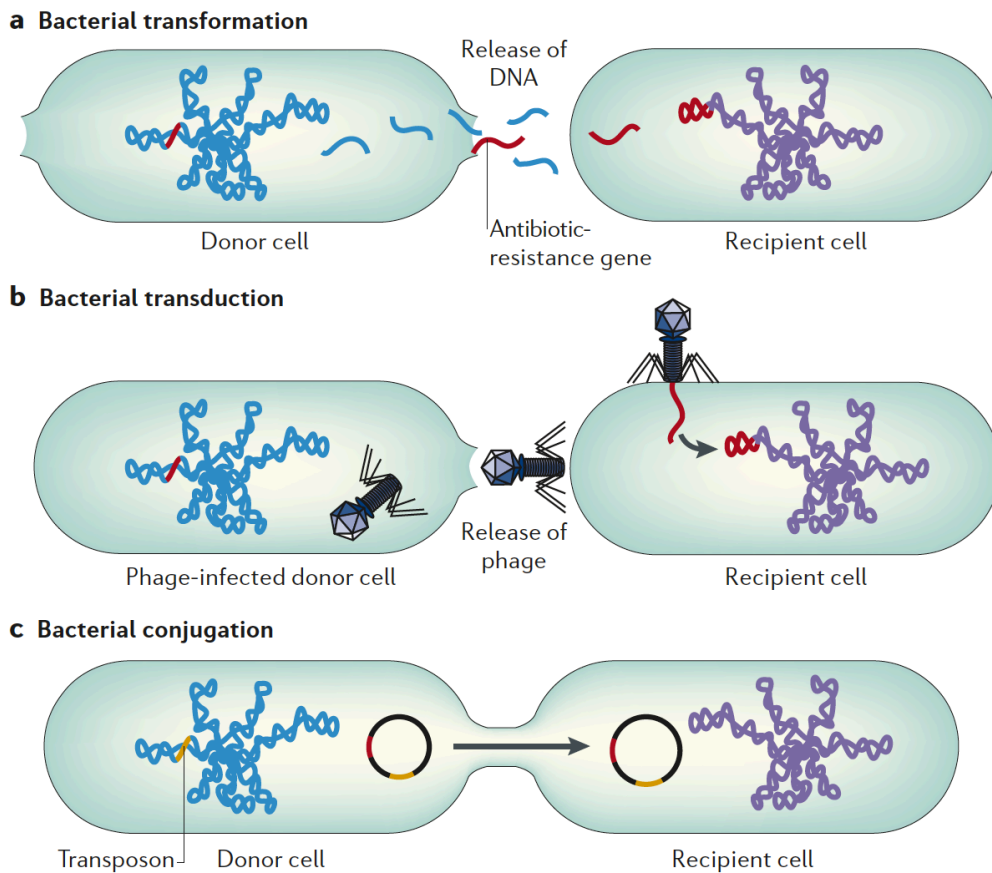


Figure 10. Horizontal gene transfer between bacteria

Source: reference [142]

Mobile genetic elements associated with AMR

The acquisition and spread of AMR genes is largely achieved by activities of mobile genetic elements (MGEs). MGEs refer to the genetic elements with either intracellular mobility (e.g. between chromosome and plasmid or between plasmids) or/and intercellular mobility, which include insertion sequence (IS), transposons (Tn), gene cassettes / integrons, plasmids and integrative conjugative elements (ICEs). Their possible modular composition is shown in Figure 11. But actually IS and Tn are able to move themselves (and resistance genes) almost randomly to new locations within a single cell, whereas integrons move resistance genes using site-specific recombination, as a result, the movement

happen between specific sites [144]. Plasmid is an important vehicle for the transmission of other MGEs and AMR genes. One resistance plasmid is typically composed of one or more AMR genes and associated MGEs mentioned above such as IS and Tn. All these elements play a crucial role in facilitating horizontal gene transfer and the spread of AMR genes in both Gram-negative and Gram-positive bacteria [144].

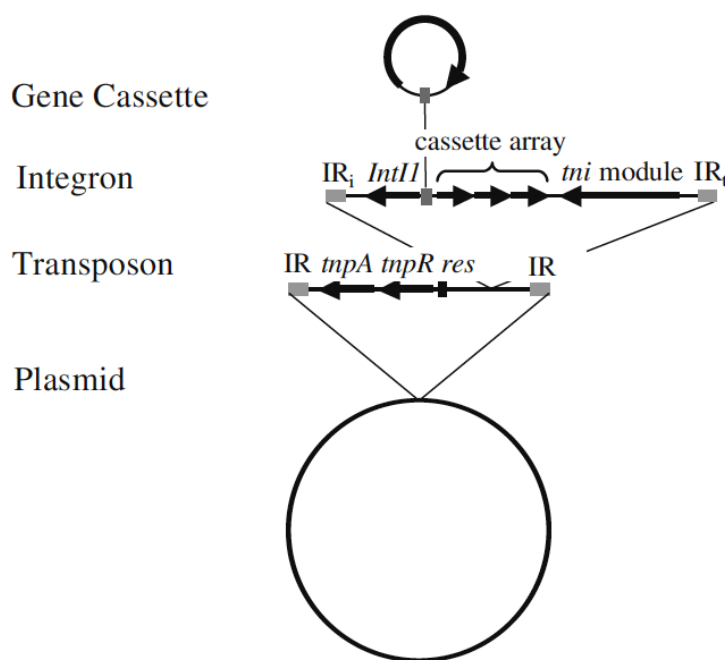


Figure 11. The modular and hierarchal composition of mobile genetic elements

2.6 AMR control from One Health perspective

In the early years of 21st century, emerging severe zoonotic infection caused by viruses such as severe acute respiratory syndrome (SARS) and avian influenza (H5N1) made scientists and policy-makers realize that interdisciplinary collaboration should be built to solve the problem, and this collaboration should involve not only physicians and veterinarians, but also environmentalists,

wildlife experts and so on. This need for collaboration is not locally or nationally, but on a worldwide scale. In 2005, The Veterinary Record and BMJ co-published various articles under the theme of “one medicine”, which highlighted important link between human health and animal health. At the same period, Wildlife Conservation Society (WCS) in New York held a conference and raised the term “One World-One Health”, which emphasized the medicine and ecosystem health. Twelve recommendations (called “Manhattan Principle”) for a more holistic approach to preventing epidemic diseases were raised. In 2006, the One Health Initiative Task Force (OHITF) was established by the American Veterinary Medical Association (AVMA). The concept of “One Health” became well-recognized worldwide since then [145]. By the definition of OHITF, One Health is "the collaborative efforts of multiple disciplines working locally, nationally, and globally, to attain optimal health for people, animals and our environment" [146].

One Health approach has been applied in different areas to solve problems including AMR. AMR is a complex and multifaceted issue not only existing in human clinical situation, its emergence and transmission involve multiple sectors (Figure 12). As a result, to control and decrease AMR requires interdisciplinary efforts globally. At the preliminary stage, One Health approach on AMR mainly focus on the reduction of antimicrobial use in food animals. The use of antimicrobials in food animals has been banned in 2006, and many other countries followed after that, especially for the last-resort drugs. However, based on the report from the World Organization for Animal Health (known as OIE), 45 countries are still using antimicrobials as growth promoters, among which 18

are in the Americas, 14 are in Asia and Oceania, 10 are in Africa, two are in Europe and one is in the Middle East [147]. WHO also published the new guideline to limit the antimicrobial use in healthy food animals [148]. Besides food animals, more comprehensive measures including wildlife, aquaculture, and environment should be taken step by step. Wildlife ecosystem is a potential reservoir for antimicrobial resistant bacteria and AMR genes. Resistant bacteria and AMR determinants have been reported in various wild animals [149, 150] as well as aquaculture [151]. More and more countries are making guidelines or policy based on One Health concept and principle.

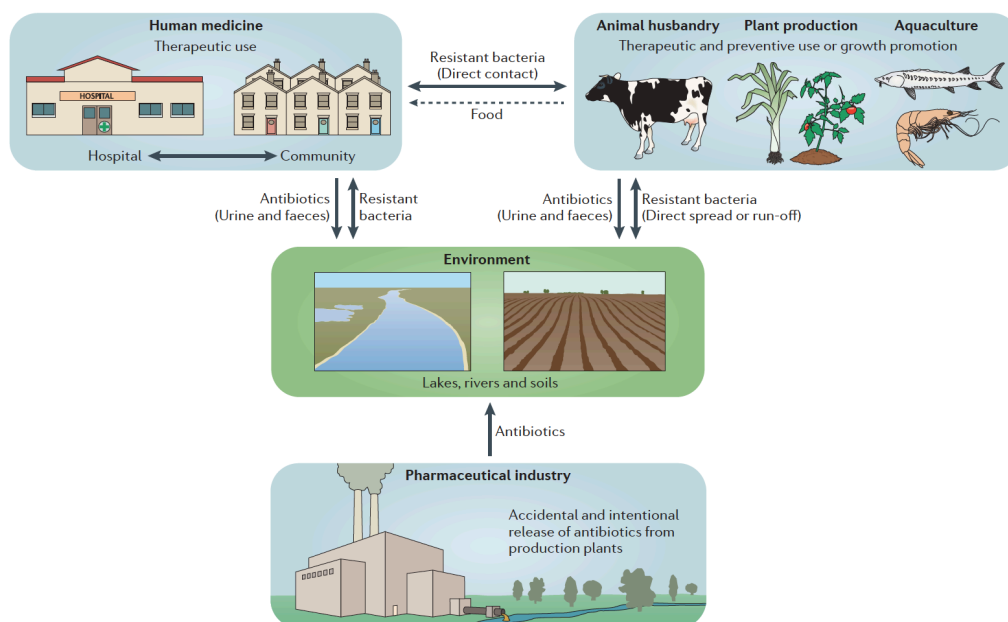


Figure 12. Ecosystem of spread of AMR from one health perspective

Source: reference [152]

2.7 Enterobacteriaceae and *E. coli*

Enterobacteriaceae are Gram-negative, rod shaped bacteria, which are parts of normal commensal microbial flora in human and animals. Many bacteria in this large family are familiar pathogens such as *Salmonella*, *Yersinia pestis*, and *Shigella*. Others are opportunistic pathogens which can only cause diseases under certain conditions or in certain hosts, like *E. coli*, *Klebsiella*, *Proteus*, *Serratia*, *Morganella*, *Enterobacter*, *Providencia*. These pathogens may cause infection of urinary tract, respiratory tract, wound and bloodstream [153, 154]. Enterobacteriaceae has developed several mechanisms to be resistant to different antimicrobial agents, which made it a great concern worldwide. In the drug-resistant bacteria ranking list released in 2017 by WHO aiming to drive the development of much-needed antimicrobials, antimicrobial-resistant Enterobacteriaceae has been ranked top three [155].

E. coli is the most-studied bacterium by researchers worldwide. It colonizes the gastrointestinal track of human infants typically within several hours after their birth. It is one type of common commensal bacteria in gastrointestinal tract of human and animals as well as important pathogen. The commensal *E. coli* usually co-exist with healthy human without causing diseases. Extraintestinal diseases can be caused by these *E. coli* only in the presence of a large inoculum or in the immunocompromised hosts [156, 157]. Because of its commensal trait, *E. coli* is one of indicator bacteria for fecal contamination in water [158].

However, during the evolution process, some *E. coli* clones acquired specific virulence determinants and developed to pathogenic *E. coli*, which can colonize

to new niches and cause diseases in humans. These virulence attributes might once have been mobile, but now inserted into chromosomes of *E. coli* already. Pathogenic *E. coli* are mainly categorized to types based their locations in human body: intestinal pathogenic *E. coli* (InPEC) and extraintestinal pathogenic *E. coli* (ExPEC). InPEC cause enteric/diarrhoeal diseases and are consist of six well-characterized groups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC). While ExPEC are associated with urinary tract infections (UTIs) and sepsis/meningitis [159]. The three pathotypes (commensal, InEPC, ExPEC) are characterized by the different accessory traits, that is, virulence factors (VF). VF of pathogenic *E. coli* are usually located on mobile genetic elements such as plasmids, transposons and bacteriophages, which makes it possible to transfer into different strains and create novel VF patterns [156].

In terms of ExPEC, many virulence factors have been associated to the pathogenicity of ExPEC. ExPEC exhibit great genome diversity and carry a broad range of virulence factors including adhesins, toxins, capsules and so on. The detailed list is shown in Table 5. Whether a commensal *E. coli* is able to develop to ExPEC not only depends on the genes directly contributing to pathogenesis, but also certain factors for successful colonization that enhance the fitness and adaptation to the host environment [160].

Table 5. Virulence-associated factors present in ExPEC

ADHESINS	
<i>F10 papA</i>	P fimbriae subunit variant
<i>papC</i>	<i>papACEFG</i> , genes of P fimbriae operon
<i>papEFG</i>	<i>papACEFG</i> , genes of P fimbriae operon
<i>sfa/foc</i>	S or F1C fimbriae
<i>focG</i>	F1C fimbriae adhesin
<i>iha</i>	Adhesion siderophore
<i>fimH</i>	Type 1 fimbriae
<i>tsh</i>	temperature sensitive hemagglutinin
<i>hra</i>	Heat-resistant agglutinin
<i>afa/draBC</i>	Dr-binding adhesins
TOXIN	
<i>hlyD</i>	α -Hemolysin
<i>sat</i>	Secreted autotransporter toxin
<i>pic</i>	Serine protease
<i>vat</i>	Vacuolating toxin
<i>astA</i>	Enteroaggregative <i>E. coli</i> toxin
<i>cnf1</i>	Cytotoxic necrotizing factor
SIDEROPHORES	
<i>iroN</i>	Salmochelins (siderophore) receptor
<i>fyuA</i>	Yersiniabactin (siderophore) receptor
<i>ireA</i>	Siderophore receptor
<i>iutA</i>	Aerobactin (siderophore) receptor
CAPSULE	
<i>kpsM II</i>	<i>kpsM II</i> group 2 capsule
K1	K1 group 2 capsule variants
K2	K2 group 2 capsule variants
K5	K5 group 2 capsule variants
<i>kpsMT III</i>	Group 3 capsule
MISCELLANEOUS	
<i>usp</i>	Uropathogenic-specific protein
<i>traT</i>	Serum resistance-associated
<i>ompT</i>	Outer membrane protease T
<i>iss</i>	Increased serum survival
H7 <i>fliC</i>	Flagellin variant
<i>malX</i>	Pathogenicity island marker

Source: reference [160]

2.8 Next generation sequencing

History of DNA sequencing

Actually, there are different opinions regarding the definition of sequencing generation, however, this does not influence our understanding of technology

innovation. Here we introduce one of classifications of different sequencing platforms [161-164].

The first generation sequencing

The first generation of DNA sequencing emerged in the 1970s. Two methods Maxam Gilbert sequencing (fragmentation technology) and Sanger sequencing (chain termination technology) were developed. However, because Sanger sequencing requires less toxic chemicals and radioisotopes, it became the main sequencing technology for the next 30 years [165]. The principle of Maxam Gilbert sequencing is to fragment the DNA by chemical modification, and the site of cleavage can be controlled by adjustment of modifying agent concentration. The 5'-end of the DNA molecular is radioactively labeled. The different sized DNA fragments can be visualized under X-ray. Whereas Sanger sequencing uses single-stranded DNA as the template. DNA extension is performed by adding DNA primer, DNA polymerase, dNTPs, ddNTPs. Four PCR reactions are processed by adding different ddNTPs (shown as below). The extension reactions are terminated at specific nucleotide site because of the presence of ddNTPs (ddNTP lacks hydroxyl group at C3 of ribose sugar, which cannot make phosphodiester bond with the next nucleotide). The different-size DNA fragments in reaction mixture can be separated by electrophoresis, and the position of the band indicate the nucleotide types .

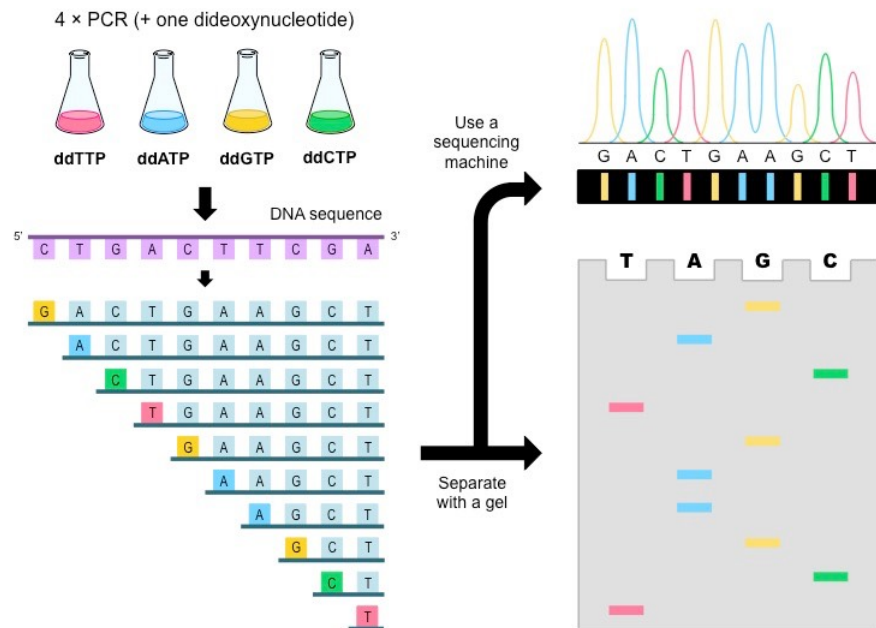


Figure 13. Illustration of Sanger sequencing.

Source:

<https://www.onlinebiologynotes.com/sangers-method-gene-sequencing/ii>

Sanger sequencing technology opened a new era of molecular biology, which ultimately enable the completion of the first human genome sequence in 2004. However, this technology has some limitations: 1) the first 15-40 bases of sequencing result is usually with poor quality; 2) the efficiency decreases greatly after 700-900 bases; 3) not cost-effective for high number of targets 4) low scalability due to increasing sample input requirements. A growing demand for increased throughput requires the technology revolution.

The second generation sequencing

454 Pyrosequencing—this method is based on the detection of the pyrophosphate released during the PCR reaction. The pyrosequencing is has the

major advantage of fast speed. However, it requires more chemical steps than sanger sequencing. Because of the high cost and low demand, Roche discontinued 454 Pyrosequencing in 2013.

Illumina technology—Illumina is the major player in the second generation sequencing. The principle and process of sequencing is shown below. The genomes are randomly fragmented for the library preparation, and the DNA fragments are hybridized to a solid support by adapters. The DNA fragments are amplified to create clonal “clusters”, and this step is for signal amplification. Next, parallel cluster synthesis reactions are repeated for 300 or more rounds. During synthesis, the fluorescent labeled nucleotides are incorporated and detected in real time.

The critical difference between Sanger sequencing and Illumina sequencing technology is sequencing volume. Illumina sequence millions of fragments in parallel per run, while Sanger methods only sequences a single fragment at a time. This greatly increases the cost-effectiveness of Illumina sequencing for massive sequencing targets. However, it is time-consuming and less cost-effective compared with Sanger sequencing if there are low number of sequencing target (<20) [166].

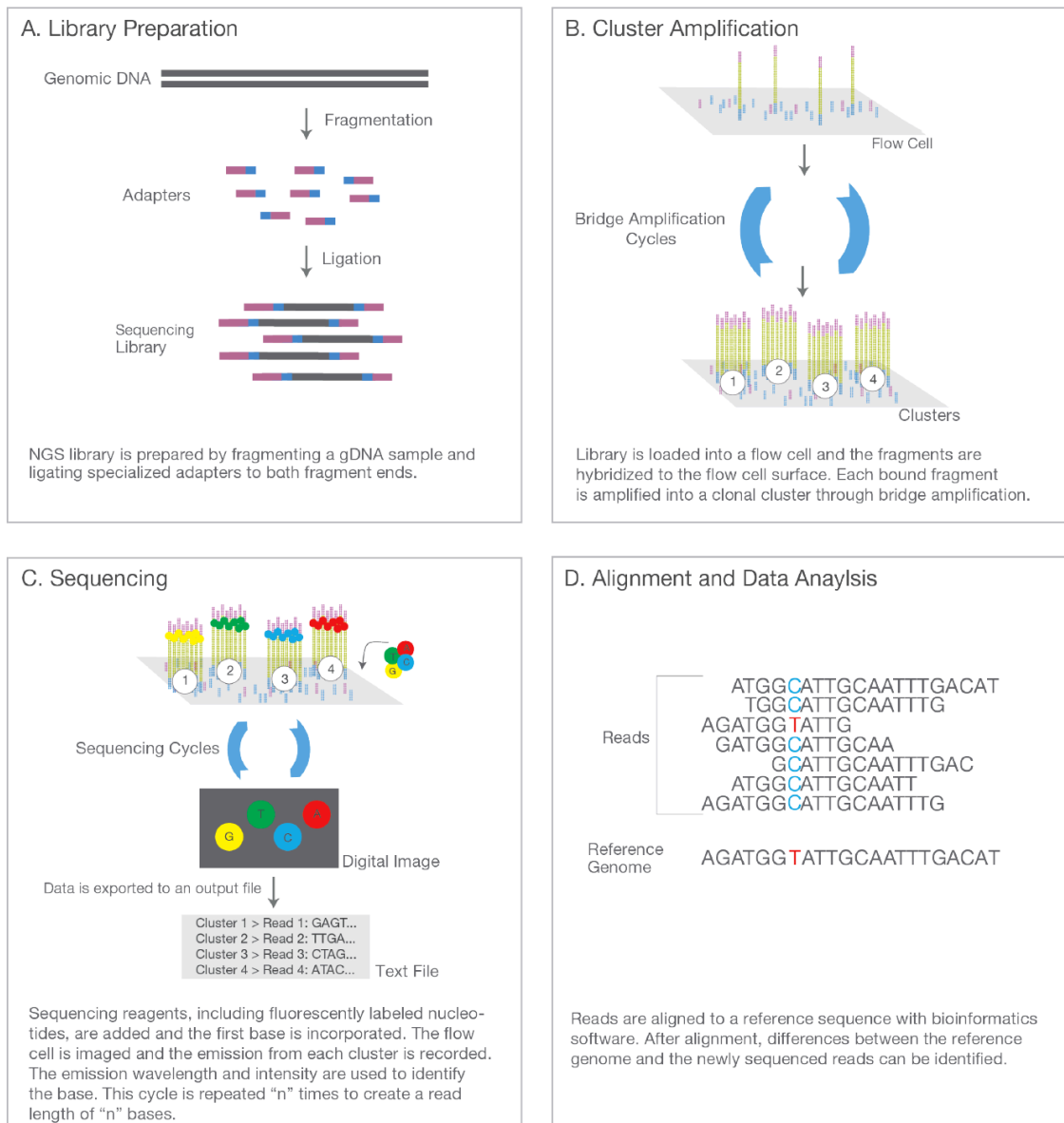


Figure 14. The principle of Illumina sequencing technology

Source: Illumina website

Ion Torrent—Ion Torrent semiconductor sequencing also uses a “sequencing by synthesis” approach, which is same with Illumina sequencing. During a DNA synthesis reaction, when a nucleotide is incorporated in a extending DNA chain, a hydrogen ion is released, which can change the pH of the solution. This change is recorded as voltage changed recorded by an ion sensor. This changes chemical information into sequencing information by a semiconductor chip.

Ion torrent sequencing does not need any light source and scanner, as a result, it is cheaper and faster than many of the other methods, however, it is difficult to differentiate the repetitive bases, which limits its application.

The third generation sequencing

PacBio sequencing—also called SMRT (Single Molecular Real Time) sequencing, can sequence long fragments up to 30-50k, which is the main difference with previous technology. Its principle involves a small chamber called ZMW (zero-mode waveguide). Imaging occurs only at the bottom of the ZMW where DNA is bound to the polymerase. When the nucleotide labeled with different fluorophores is incorporated into the growing DNA chain, the fluorescent signal is recorded.

Besides long reads, a unique advantage of SMRT is that the rate of nucleotide addition can be measured so that the nucleotides with modifications (such as methylations) can be identified as well. This can be applied in epigenetic studies. However, SMRT has relatively high error rate compared with other technologies. As a result, the combination with other methods such as Illumina sequencing is often used to increase sequencing accuracy.

Nanopore sequencing—this sequencing technology was firstly introduced in the late 1990s and commercialized recently. Its principle is monitor the electrical current as nucleic acids are passed through a protein nanopore in an electrically resistant polymer membrane. When a nucleotide passes through the pore, the current that has been applied to the nanopore is disrupted and recorded as a signal.

Nanopore sequencing is able to detect DNA modification like SMRT. PCR amplification and any chemical labeling are not required for sequencing. Nanopore sequencing makes long-reads sequencing much faster and cheaper than before. More than 2Mb read length has been achieved, which makes it possible to sequence entire repetitive regions and reconstruct complex genome. In addition, nanopore sequencers can be very small (handheld size), its portability is a great advantage compared with other technologies, which makes it applied in environmental and metagenomic sample sequencing. However, like Pacbio sequencing, nanopore sequencing also has a high error rate, this can be circumvented by the large number of sequenced molecules [167, 168].

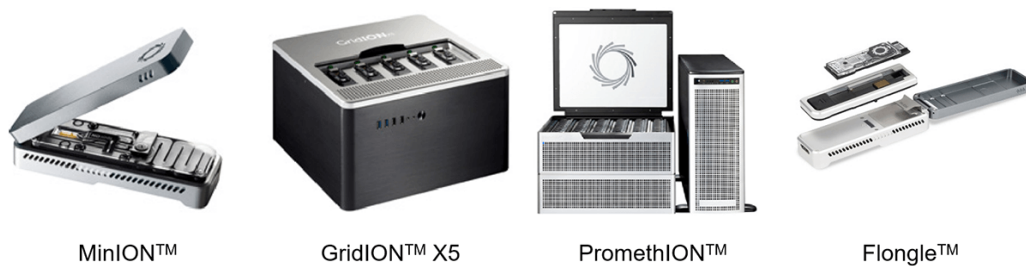


Figure 15. Nanopore sequencers

Table 6. Comparison of different sequencing platforms [169, 170]

Platform	Maximum read length (bp)	Sequencing principle	Accuracy (%)	Maximum output per run	Advantages	Disadvantages
Sanger sequencing	400-900	Dideoxy chain termination	99.999	1.9~84 Kb	High quality; long read length	High cost; low throughput
454	700	Sequencing by synthesis	99.9	700 Mb	Long read length	Homopolymer errors; high cost
MiSeq	2x300	Sequencing by reversible termination	99.9	15 Gb	High accuracy; low cost	GC bias
HiSeq	2x125	Sequencing by reversible termination	99.9	1000 Gb	High accuracy; high throughput	GC bias; short read length
Ion Torrent S55	600	Sequencing by synthesis	99	10-15 Gb	Long read length	Homopolymer errors
PacBio RS II	10,000	Single molecule real-time sequencing	95	400 Mb	Long read length	High error rate
MinION	6000	Nanopore sequencing	98	500 Mb	Long read length	High error rate

With the fast development of WGS, related bioinformatic tools are also emerging. The analysis of raw reads of WGS consists multiple steps: 1) read quality control 2) genome assembly 3) genome annotation 4) bacterial typing 5) genome characteristic identification (such as AMR genes, virulence genes) 6) single-nucleotide polymorphism (SNP) calling and phylogenetic analysis, etc. The tools were also reviewed and summarized in several studies [171]. For users without coding and computer study background, web-based tools such as Galaxy (<https://usegalaxy.org/>) and the tools developed by Technical University of Denmark (<https://cge.cbs.dtu.dk/services/>) seem more user-friendly. The input of sequencing data is required by online uploading. The analysis will be processed after parameter setting. The first step of raw reads analysis is the quality control of raw reads. It removes poor-quality sequencing data and trims the reads to remove adapters if necessary. For the second step---genome assembly, *de novo* assembly is used in this thesis. The assembly is based on the overlapping of reads, and contigs or scaffolds (assembled sequence fragments) are obtained. There is another assembly method, that is mapping reads to a reference genome. For genome annotation, species typing and other characteristic identification (such as AMR gene identification), the basic principle is sequence alignment. Unknown sequence regions are compared with sequences (known function) in the database. While SNPs calling is to find the genome difference (on single nucleotide level) with the reference genome, and the phylogenetic distance can be calculated based on SNPs. It is obvious that available tool especially web-based tools bring great convenience to researchers, however, there are some limitations while using these tools: uploading or downloading large sequencing files can be very slow and programs may fail because of overloading. Web-based tools are also “black

boxes”, which make users hard to understand how the operation is performed and how to adjust parameters. In addition, it still requires professional knowledge to interpret output result, and sometimes it is challenging for users without bioinformatics background. With WGS becoming more and more useful for genome study and realistic applications, the needs for bioinformatic analysis tools is also increasing greatly. It is believed that the limitations above are going to be overcome and more useful bioinformatic tools is going to be developed in the future [171].

The application of next generation sequencing

Starting from the first generation sequencing using the Sanger chain termination method in 1977 [172], the sequencing technology and genomic science have made significant progress. The data output of next-generation sequencing has outpaced Moore’s law, more than doubling each year, however, the cost of sequencing is decreasing dramatically (Figure 16).

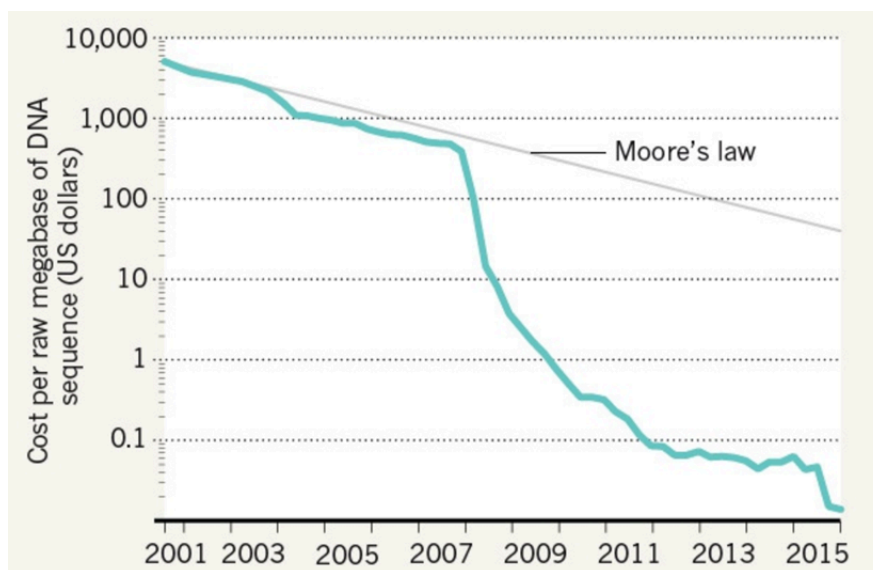


Figure 16. Plunging cost of sequencing.

Since 2008, new sequencing technologies have driven the costs of DNA sequencing down faster than the rapid improvement in microprocessor power represented by Moore's Law. Source: Nature news

Traditional antimicrobial susceptibility testing is performed by MIC determination or disk diffusion with the problem of time delay and limited antimicrobials used for detection. Further genetic characterization is often followed by the routine surveillance, which adds the cost of time and money [173]. Compared with traditional phenotypic methods, whole genome sequencing has the potential to provide data about all the resistance genes or related mutations, which could be used to analyze genotypically inferred antimicrobial resistance, even to predict susceptibility. In addition, it is expected to be easier to standardize a procedure for AMR detection in WGS data, which is good to compare results between different laboratories. Previous genotypic testing like PCR and Sanger sequencing has the limitation of detection to known resistant targets [174]. Routine genotypic prediction of AMR is used only in limited contexts, typically with single gene targets known to be highly associated with resistance, for example, *mecA* is used to determine methicillin-resistant *Staphylococcus aureus* (MRSA), however, this assay is hard to handle the current complex patterns of antimicrobial resistance [175]. WGS can provide the whole snapshot of the genetic data instead of a few target genes. The main principle of next generation sequencing is to produce short reads and assembly according to the reference genome, which could realize the goal of high-throughput sequencing. Resistance genes are detected by BLAST-based tools such as ResFinder and ARG-ANNOT [176].

Currently the evidence that WGS as a tool can take place of traditional phenotype testing for *Enterobacteriaceae* is limited. However, some researchers have proved WGS is a useful tool for AMR prediction and shows high level of concordance (95% [175] and 99.75% [173]) with phenotypic testing. WGS has great potential application in epidemiological typing, resistance profiling, novel AMR genes discovery and determination of genetic context [174]. With the deepening of knowledge of relationship between phenotype and genotype as well as the AMR mechanism, WGS would be more powerful for clinical use.

Chapter 3 Phenotypic and Genotypic Characterization of Antimicrobial Resistant *E. coli* in Ready-to-eat Food in Singapore

3.1 Introduction

The spread of AMR *E. coli* and AMR genes are a major challenge for the treatment of human illnesses. In particular, the Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli* are emerging all over the world [177]. The treatment of infections by ESBL-producing bacteria is quite challenging due to their resistance to most beta-lactam antimicrobials, including penicillin, cephalosporines (especially 3rd generation) and monobactams [46]. Most of the ESBL genes are located on plasmids co-existing with other resistance genes, which accelerate the spread of AMR [177].

With the significant advances in sequencing technology and genomic science, as well as the decrease of sequencing cost, whole genome sequencing (WGS) is becoming a useful tool to detect and study AMR [178]. WGS has the potential to provide data about all known resistance genes or related mutations in the database, which could be used to analyze genotypically inferred AMR and to predict susceptibility [174]. Indeed, some researchers have validated the usefulness of WGS as a useful tool for AMR prediction [173, 175]. WGS has great potential for application in epidemiological typing, resistance profiling and determination

of genetic context [174]. The potential for WGS to deepen our knowledge of the relationship between phenotype and genotype makes this technology a powerful tool for clinical use and scientific discovery.

Internationally, some research papers have explored the occurrence of AMR among *E. coli* in food, including raw meat [51, 179], vegetables [51, 180], milk [181], and sandwiches [180, 182]. However, few papers have explored AMR bacteria in other types of food, including cooked food in Singapore. In the present study, the frequency of AMR in a collection of *E. coli* isolated from retail ready-to-eat (RTE) food (foods that have been prepared so they can be consumed as is without any additional cooking) sold in Singapore was assessed. Although the majority of the foods were cooked, some raw RTE commodities were also included in the study. Antimicrobial susceptibility of the *E. coli* isolates was tested using two traditional methods (i.e. disk diffusion assay and minimum inhibitory concentration (MIC) determinations), followed by WGS on selected isolates displaying resistance to at least one antimicrobial. The method of AMR prediction using WGS data was compared with traditional antimicrobial susceptibility testing. This may help to deepen our understanding of the relationship between AMR phenotype and genotype, and the resistance profile of AMR *E. coli* in RTE food in Singapore and provide a comparative evaluation of this alternative method to traditional AMR detection.

3.2 Material and Methods

3.2.1. *Bacterial isolates*

A total of 99 *E. coli* isolates from RTE retail food, obtained through the retail food surveillance program by the National Environmental Agency (NEA), during 2009-2014 period were included in this study. The isolates were obtained from cooked poultry-based dishes (n=77), raw fish (n=5) and cooked fish-based dishes (n=17) (Supplementary Table 1). The isolates were stored at - 80°C in Brain Heart Infusion (BHI) medium (Acumedia, USA) with 15% (v/v) glycerol until further analyzed.

3.2.2. *Phenotypic antimicrobial resistance characterization*

Disk diffusion method

Antimicrobial susceptibility of all *E. coli* isolates was determined by disk diffusion method using Mueller-Hinton (MH) agar (Thermo Fisher Scientific, USA) according to the standard procedure of the Clinical and Laboratory Standards Institute (CLSI) [183]. The 12 antimicrobials (Oxoid, Australia); amikacin (AK; 30 µg), ampicillin (AMP; 10 µg), amoxicillin/clavulanic acid (AMC; 20/10 µg), chloramphenicol (C; 30 µg), ceftriaxone (CRO; 30 µg), ciprofloxacin (CIP; 5 µg), gentamicin (GEN; 10 µg), nalidixic acid (NA; 30 µg), norfloxacin (NOR; 10 µg), sulphamethoxazole/trimethoprim (STX; 1.25/23.75 µg), tetracycline (TE; 30 µg), and meropenem (MEM; 10 µg) were selected based on their clinical or epidemiological significance to human and animal health. On the day of testing, the inoculum was prepared according to direct colony suspension method stated in CLSI. Briefly, three to five well-isolated colonies of

the same morphological type were picked from 18- to 24-hour tryptone soya agar (TSA) plates and were suspended in 5 ml of sterile saline. The suspension was mixed thoroughly and was adjusted to match the turbidity of 0.5 McFarland standard. After testing, inhibition zone diameters for respective antimicrobials were measured and interpreted in accordance with the CLSI guideline [183]. Quality control strains *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control for antimicrobial susceptibility testing.

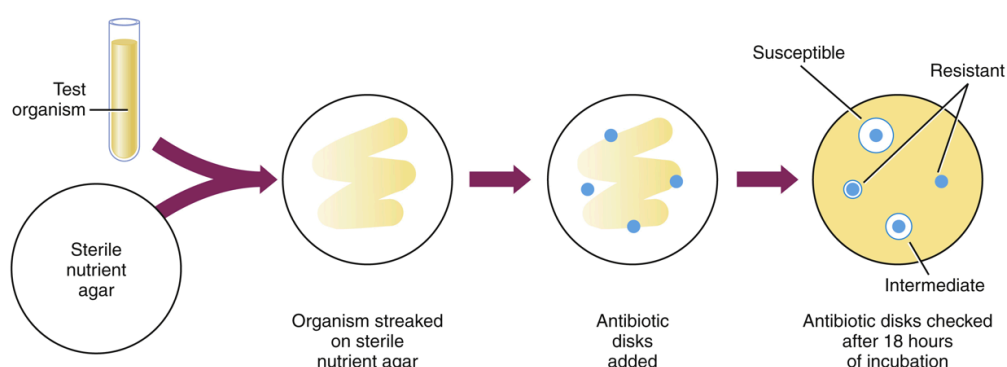


Figure 17. The schematic of disk diffusion

Broth microdilution method

The isolates resistant to at least one antimicrobial agent by disk diffusion method were subjected to broth microdilution, to determine the minimum inhibitory concentration (MIC) of each antimicrobial by using MicroScan Neg MIC Panel Type 40 (Beckman Coulter, Inc., Brea, CA, USA), in accordance with the manufacturer's instruction. The concentration ranges of 33 antimicrobials and their interpretation breakpoints have been shown in the Supplementary Table 4. The quality control strain *E. coli* ATCC 25922 was included in the testing. Based on the MIC results, isolates were then classified as sensitive, intermediate and resistant according to the EUCAST guideline [184]. For tetracycline,

trimethoprim and cefoxitin, no EUCAST interpretation was available for these antimicrobials and therefore, the CLSI standard was applied [183].

3.2.3. Extended-Spectrum Beta-Lactemase confirmatory testing

The isolates resistant to ceftriaxone by disk diffusion were confirmed by double-disk synergy test (DDST). The steps of DDST are almost the same with disk diffusion mentioned above, and the only difference is the disk used. A disk containing clavulanic acid was positioned adjacent to a cephalosporin disk. An elliptical clearing (also called “key-hole” shape) between the two discs indicates the inhibition of the β -lactamase by clavulanic acid. Whereas clavulanic acid has no effect on AmpC enzymes so it is able to distinguish ESBL from AmpC. The test was performed on agar with three disks of cephalosporins including ceftriaxone (CRO; 30 μ g), ceftazidime (CAZ; 30 μ g), and cefotaxime (CTX; 30 μ g) (Oxoid, UK), disks of amoxicillin/clavulanic acid (AMC; 20/10 μ g) were positioned next to these three disks of cephalosporin at a distance that is preset by the 8-disk dispenser (Thermo Fisher Scientific, USA). The test was regarded as positive when the inhibition zones around any of the cephalosporin disks are augmented in the direction of the AMC disks [185].

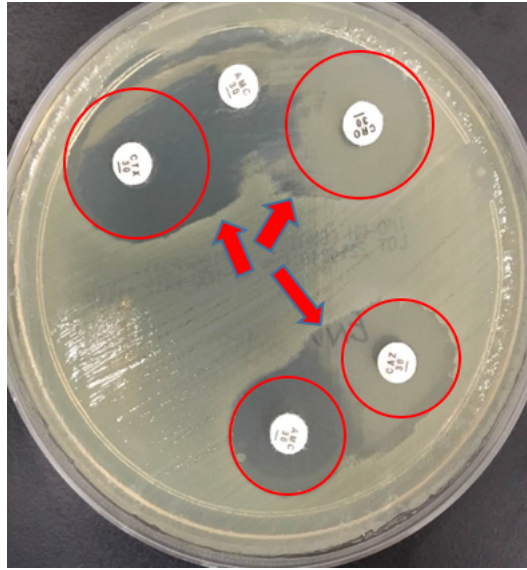


Figure 18. An example of ESBL-positive result of DDST.

The arrows indicate the “key-hole” shapes, which show the inhibition of clavulanic acid

3.2.4. DNA extraction and whole genome sequencing

A single colony from each isolate was picked from fresh nutrient agar culture and transferred to Luria-Bertani (LB) broth which was then incubated overnight at 37°C. On the following day, 1 ml of overnight culture was used for DNA extraction using QIAamp® DNA Mini Kit, according to manufacturer’s instructions (Qiagen, Germany). Prior to library preparation, DNA quantitation was carried out using PicoGreen ® Assay Kit (Invitrogen, USA). Library preparation was performed according to Illumina’s TruSeq Nano DNA Sample Preparation Protocol. The samples were sheared on a Covaris S220 to ~450 bp, following the manufacturer’s recommendations, and uniquely tagged with Illumina’s TruSeq HT DNA dual barcodes to enable library pooling for sequencing. Finished libraries were quantitated using Invitrogen’s PicoGreen assay and the average library size was determined on a Bioanalyzer 2100 (Agilent, USA) or a Tapestation 4200 (Agilent, USA). Library concentrations were then

normalized to 4 nM and validated by qPCR on a ViiA-7 real-time thermocycler (Applied Biosystems, USA), using qPCR primers recommended in Illumina's qPCR protocol, and Illumina's PhiX control library as standard. The libraries were then pooled at equimolar concentrations in batches of 96 samples and each pool was sequenced in 1 lane on an Illumina HiSeq2500 sequencer in rapid mode at a read-length of 250 bp paired-end. Raw sequence data were submitted to the European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena>) under study accession number: PRJEB26639. The complete list of genomic sequence accession number has been provided in the Supplementary Table 1. The isolate carrying *mcr-5.1* was also sequenced using Pacbio RS II system for long-reads sequencing.

3.2.5. Analysis of whole genome sequencing data

Genome assembly

The raw reads of Illumina sequencing were assembled using SPAdes version 3.10.1¹, with “-careful, -k auto and -cov-cutoff as off” parameters [186].

The closed plasmid carrying *mcr-5.1* was assembled by Unicycler on Galaxy platform (<https://usegalaxy.org/>) using both long and short reads data with default parameters [187].

Genome annotation and determination of AMR genes, virulence factor

¹ Compared with reference mapping, *De Novo* assembly identifies structural variants and complex rearrangements such as deletions, inversions, or translocations. It also clarifies similar or repetitive regions of genome. Most importantly, the key point of research is antimicrobial resistance gene, which is usually located on transferrable genetic elements such as transposons or plasmids. These elements may not map well on a reference genome. These are the reasons why *De Novo* assembly was used in the study

Genome annotation was done by Rapid Annotation using Subsystem Technology (RAST) [188-190] and corrected by the use of BLASTn [191]. The ResFinder (version 3.0) web server (<https://cge.cbs.dtu.dk/services/ResFinder/>) was used to identify chromosomal or acquired AMR genes and point mutations based on the following parameters - minimum length coverage of 60% and minimum identity of 90% [192]. The location of ESBL-encoding genes, quinolone and colistin resistance genes were determined by analyzing the contigs harboring related resistance genes using KmerFinder (version 2.5) [193, 194] and BLASTn. The genome with the highest score in KmerFinder was selected correspondingly for each isolate as the reference genome.

3.2.6 Evaluation of WGS for AMR prediction

The sensitivity, specificity and accuracy for genotypic resistance prediction were calculated for each class of antimicrobial against the gold standard for susceptibility testing, which is broth microdilution. The calculation formulas are – sensitivity = (the number of isolates that carried AMR genes and were resistant according to gold standard)/the number of isolates that were resistant according to gold standard; specificity = (the number of isolates that did not carry AMR genes and were sensitive or intermediate according to gold standard)/ the number of isolates that were sensitive or intermediate according to gold standard; accuracy = (the number of isolates that carried AMR genes and were resistant according to gold standard + the number of isolates that did not carry AMR genes and were sensitive or intermediate according to gold standard)/the number of all the isolates.

3.2.7 Conjugation experiment and stability testing of plasmid

Conjugation experiments were performed using the filter mating method [74]. Sodium azide resistant *E. coli* strain J53 was the recipient strain and transconjugants were selected using 4 µg/ml colistin plus 200 µg/ml sodium azide. The presence of *mcr-5.1* in transconjugants was confirmed by PCR after 24h co-culture [195]. The stability test for the plasmid carrying *mcr-5.1* was performed as previously described [196]. Briefly speaking, stability experiments were performed in triplicate. Three single colonies were picked and incubated in 5 ml LB broth with 4 µg/ml colistin for 24 hours at 30 °C with shaking at 200 r.p.m. The cultures were washed to remove colistin and resuspended in 1 ml saline. 4.88 µl of suspensions was transferred to 5ml LB broth and subcultured for 24 hours. The subculture process was repeated for 20 days. At the finishing point of each culture, bacteria solution was diluted and plated onto LB plates and incubated overnight. Fifty colonies were randomly picked for PCR for *mcr-5* detection.

3.3 Results

3.3.1. Phenotypic characterization analysis of antimicrobial resistance

Result of disk diffusion

Of the 99 *E. coli* isolates studied (Supplementary Table 1), 24.2% were resistant to at least one antimicrobial tested (Table 8). Resistance to tetracycline (17.2%), ampicillin (15.2%) and chloramphenicol (10.1%) were the most common. Interestingly, all 99 *E. coli* isolates were sensitive to amikacin,

amoxicillin/clavulanic acid and meropenem, which all belong to beta-lactam antibiotic class. Detailed results can be found in Supplementary Table 3.

Among these 24 isolates resistant to at least one antimicrobial, 14 (58.3%) were isolated from cooked chicken-based dishes, 9 (37.5%) from cooked duck-based dishes, 1 (4.2%) from a cooked fish-based dish, while no isolates from a raw fish-based dish showed resistance (Table 8). Overall, a higher resistance level to the tested antimicrobials was observed in isolates obtained from duck-based dishes (Figure 19, Table 7). However, the small sample size (n=99) limited the interpretation of the data. Further testing by DDST confirmed that two *E. coli* isolates, one from a cooked chicken-based dish and the other from a cooked duck-based dish, were ESBL-producing *E. coli*.

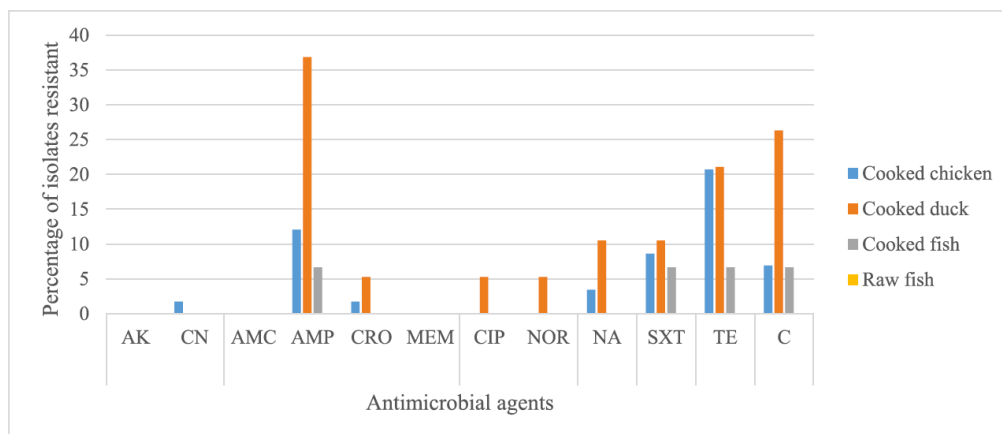


Figure 19. Resistance prevalence among *E. coli* isolates from ready-to-eat food.

The percentage of resistant isolates for each antimicrobial tested is plotted on y-axis. 12 antimicrobials, belonging to seven different antimicrobial classes were tested against 99 *E. coli* isolates in disk diffusion assay. The seven antibiotic classes are as follow: aminoglycoside, beta-lactam, fluoroquinolone, quinolone, sulphonamide, tetracycline and phenicol and are separated by lines in the figure. The 12 tested antimicrobials were amikacin (AK), ampicillin (AMP), amoxicillin/clavulanic acid (AMC), chloramphenicol (C), ceftriaxone (CRO), ciprofloxacin (CIP), gentamicin (CN), nalidixic acid (NA), norfloxacin (NOR), sulphamethoxazole/trimethoprim (STX), tetracycline (TE), meropenem (MEM)

Table 7. Antimicrobial resistance of *E. coli* isolated from cooked food based on disk diffusion

Poultry-related, n=77												
	AK	AMP	AMC	C	CRO	CIP	CN	NA	NOR	SXT	TE	MEM
S	76	30	68	68	75	75	76	68	76	69	61	76
I	1	33	9	0	0	1	0	5	0	1	0	1
R	0	14	0	9	2	1	1	4	1	7	16	0

Fish-related, n=23												
	AK	AMP	AMC	C	CRO	CIP	CN	NA	NOR	SXT	TE	MEM
S	23	12	23	22	23	23	23	23	23	22	22	23
I	0	10	0	0	0	0	0	0	0	0	0	0
R	0	1	0	1	0	0	0	0	0	1	1	0

S-sensitive; I-intermediate; R-resistant

Table 8. Number of antimicrobial resistant *Escherichia coli* isolated from ready-to-eat foods in Singapore, as determined by the disk diffusion method

	Resistance to number of antimicrobial							
	0	1	2	3	4	5	7	Total
Cooked chicken-based dish	44	5	5	2	1	0	1	58
Cooked duck-based dish	10	5	0	2	0	1	1	19
Cooked fish-based dish	14	0	0	0	1	0	0	15
Raw fish-based dish	7	0	0	0	0	0	0	7
Total	75	10	5	4	2	1	2	99

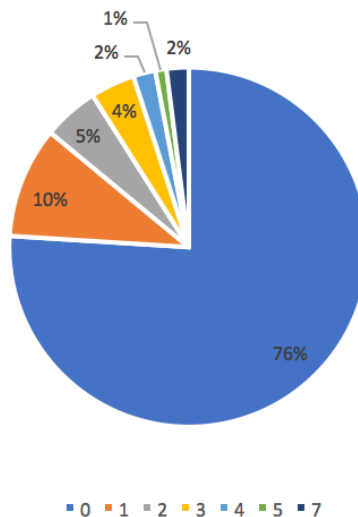


Figure 20. Resistance pattern of all *E. coli* isolates based on disk diffusion

*The numbers represent the quantity of resistance for one isolate

Result of MIC testing

In order to further characterize the resistance profiles of the 24 *E. coli* isolates that were resistant to at least one antimicrobial, the MICs of the 33 different antimicrobials belonging to nine classes (Supplementary Table 4) against these isolates were determined. Result showed that resistance to tetracycline (70.8%), chloramphenicol (50%), ampicillin (41.7%) and trimethoprim (41.7%) were most prevalent (Supplementary Table 5). All 24 isolates were sensitive to amikacin, nitrofurantoin and all carbapenems (imipenem, meropenem, doripenem, ertapenem). It is worth noting that about 2/3 of the isolates considered to be multidrug resistant (defined as resistant to three or more classes of antimicrobials) and among which, one was resistant to 19 antimicrobials (ENV210), the highest number that was observed in this study and it was also positive for ESBL production.

Result comparison of disk diffusion and MIC

The results in disk diffusion and microdilution for the commonly tested antimicrobials were compared in Figure 21. To interpret the results as resistance (shown in red in figure) or non-resistance (including intermediate and susceptible, shown in green in figure), high coherence (90.5%, 25/264) was observed between disk diffusion and microdilution. Results of amikacin, gentamicin, ceftriaxone, meropenem showed 100% coherence between MIC and disk diffusion. Except for ampicillin (AMP), microdilution showed higher sensitivity to antimicrobials.

	AK	CN	C	CRO	AMP	TE	NOR	CIP	MEM	SXT	AMC
ENV49											
ENV66											
ENV68											
ENV76											
ENV103											
ENV190											
ENV210											
ENV222											
ENV225											
ENV228											
ENV233											
ENV235											
ENV316											
ENV317											
ENV323											
ENV326											
ENV388											
ENV463											
ENV663											
ENV665											
ENV694											
ENV704											
ENV727											
ENV737											

Figure 21. Result comparison of disk diffusion and MIC testing

* AK: amikacin; AMP ampicillin; AMC: amoxicillin/clavulanic acid; C: chloramphenicol; CRO: ceftriaxone; CIP: ciprofloxacin; CN: gentamicin; NOR: norfloxacin; SXT :Sulphamethoxazole/Trimethoprim; TET: tetracycline; MEM: meropenem

* Red cell indicates the isolate was resistant in both two methods; green cell indicates the isolate was not resistant (intermediate, sensitive or not reported); orange cell indicates the isolate was resistant in MIC testing but not resistant in disk diffusion; pink cell indicates the isolate was resistant in disk diffusion but not resistant in MIC testing

3.3.2. Genotypic characterization analysis of antimicrobial resistance

All 24 *E. coli* isolates displaying resistance to at least one antimicrobial by disk diffusion were subjected to WGS. Genomics analysis showed that 10 out of 24 *E. coli* isolates carried at least five AMR genes, whereas no known AMR genes were found in five isolates (ENV228, ENV233, ENV235, ENV388 and ENV737). The highest number of resistance genes observed was 15 and it was found in one isolate (Table 9).

Aminoglycoside resistance genes were detected in 62.5% of the isolates. The most common aminoglycoside resistance genes detected were *aadA1* (33.3%), *aph(3')-Ic* (33.3%) and *strA* (20.8%), *strB* (20.8%). Beta-lactam resistance genes were detected in 41.7% of the isolates, which included *bla*_{TEM-176} (5/24), *bla*_{TEM-1A} (1/24), *bla*_{TEM-1B} (4/24) and *bla*_{SHV-12} (1/24). For two ESBL-producing *E. coli* isolates, one carried *bla*_{SHV-12} and *bla*_{TEM-1B}, and the other carried *bla*_{TEM-1B}. Colistin resistance genes were found in 12.5% of the isolates and only two types of resistance genes (*mcr-1*, 2/24 and *mcr-5*, 1/24) were identified. Quinolone/fluoroquinolone resistance genes were seen in 45.8% of the isolates, with the most prevalent resistance genes detected being *QnrS1* (33.3%). Other mechanisms of quinolone/fluoroquinolone resistance (eg. mutation in *gyrA* and *ParE* genes; 3/24) were also seen. Phenicol resistance genes against chloramphenicol were detected in 50% of the isolates and only three types of resistance genes were found, namely *floR*-like (29.2%), *cmlA1*-like (8.3%) and *catA1*-like (4.2%). Sulphonamide resistance genes were detected in 45.8% of the

isolates and all three currently known transferable *sul* genes (*sul1*, 2/24; *sul2*, 6/24 and *sul3*, 7/24) related to sulfonamide resistance were detected. Trimethoprim resistance genes were detected in 45.8% of the isolates and they are mainly *dfrA* genes (namely, *dfrA1*, *dfrA5*, *dfrA15*, *dfrA17* and *dfrA27*) and no *dfrB* genes were seen in these isolates. Tetracycline resistance genes were detected in 70.8% of the isolates. The most prevalent tetracycline resistance genes were *tet(A)* (20.8%) and *tet(A)*-like (37.5%). The other tetracycline resistance genes were *tet(B)* (8.3%), *tet(C)* (4.1%) and *tet(M)* (4.1%). Mutation in *rrsB* was also detected in one isolate that already had a *tet(A)* gene.

Table 9. The AMR genes detected in *E. coli* isolates

Isolate ID	No. of Resistance genes	Resistance gene
ENV233	0	
ENV235	0	
ENV388	0	
ENV737	0	
ENV228	0	
ENV694	1	<i>tet(B)</i>
ENV463	1	<i>tet(B)</i>
ENV190	4	<i>bla</i> _{TEM-1B} , <i>dfrA14</i> -like, <i>QnrS1</i> , <i>tet(A)</i>
ENV316	4	<i>aadA1</i> , <i>aadA2</i> , <i>cmlA1</i> -like, <i>sul3</i>
ENV317	4	<i>aadA1</i> , <i>aadA2</i> , <i>cmlA1</i> -like, <i>sul3</i>
ENV323	4	<i>dfrA14</i> -like, <i>QnrS1</i> , <i>tet(A)</i> -like, <i>tet(C)</i>
ENV663	4	<i>aadA5</i> , <i>dfrA17</i> , <i>sul2</i> , <i>tet(A)</i>
ENV665	4	<i>aadA5</i> , <i>dfrA17</i> , <i>sul2</i> , <i>tet(A)</i>
ENV727	4	<i>aph(3')-Ic</i> -like, <i>dfrA14</i> -like, <i>QnrS1</i> , <i>tet(A)</i> -like
ENV49	5	<i>aph(3')-IIa</i> -like, <i>strA</i> , <i>strB</i> , <i>sul2</i> , <i>tet(A)</i> -like
ENV222	6	<i>aph(3')-Ic</i> -like, <i>bla</i> _{TEM-176} , <i>QnrS1</i> , <i>strA</i> , <i>strB</i> , <i>tet(B)</i>
ENV326	6	<i>aph(3')-Ic</i> -like, <i>bla</i> _{TEM-176} , <i>dfrA14</i> -like, <i>floR</i> -like, <i>QnrS1</i> , <i>tet(A)</i> -like
ENV76	6	<i>aph(3')-Ic</i> -like, <i>bla</i> _{TEM-176} , <i>floR</i> -like, <i>mcr-1</i> , <i>QnrS1</i> , <i>tet(A)</i> -like
ENV66	8	<i>aadA1</i> -like, <i>aph(3')-Ic</i> -like, <i>bla</i> _{TEM-176} , <i>floR</i> -like, <i>fosA</i> -like, <i>mcr-1</i> , <i>QnrS1</i> , <i>sul3</i> , <i>tet(A)</i> -like
ENV103	10	<i>aadA1</i> -like, <i>aph(3')-Ic</i> -like, <i>bla</i> _{TEM-176} , <i>dfrA5</i> , <i>floR</i> -like, <i>mcr-5</i> , <i>QnrS1</i> , <i>sul3</i> , <i>tet(A)</i> , <i>tet(M)</i> -like

ENV704	10	<i>aadA1</i> -like, <i>aph(3')</i> -Ic-like, <i>bla</i> _{TEM-1B} , <i>dfrA14</i> -like, <i>floR</i> -like, <i>strA</i> -like, <i>strB</i> , <i>sul2</i> , <i>sul3</i> , <i>tet(A)</i> -like
ENV210	10	<i>aadA1</i> , <i>bla</i> _{TEM-1B} , <i>catA1</i> -like, <i>dfrA1</i> , <i>mph(B)</i> , <i>strA</i> , <i>strB</i> , <i>sul1</i> , <i>sul2</i> , <i>tet(A)</i>
ENV225	11	<i>aadA1</i> , <i>aadA2</i> , <i>bla</i> _{SHV-12} , <i>bla</i> _{TEM-1B} , <i>dfrA15</i> , <i>floR</i> -like, <i>QnrD</i> -like, <i>QnrS1</i> , <i>sul3</i> , <i>tet(A)</i> -like, <i>tet(B)</i>
ENV68	15	<i>aac(6')Ib-cr</i> , <i>aadA1</i> -like, <i>aadA16</i> -like, <i>aph(3')</i> -Ic-like, <i>ARR-3</i> , <i>bla</i> _{TEM-1A} , <i>dfrA27</i> , <i>floR</i> -like, <i>QnrB6</i> , <i>strA</i> , <i>strB</i> , <i>sul1</i> , <i>sul2</i> , <i>sul3</i> , <i>tet(A)</i> -like

3.3.3. Comparison of phenotypic and genotypic AMR data

To assess the correlation between resistance genotype and phenotype, we computed the sensitivity, specificity and accuracy of using WGS data to predict resistance phenotype by comparing resistance genotype to resistance phenotype that was determined by the gold standard for susceptibility testing, which is the broth microdilution method (Table 11). As shown in Table 11, the trimethoprim and chloramphenicol resistance genotype correlated with 100% sensitivity, whereas the colistin resistance genotype correlated with low sensitivity (37.5%) and 100% specificity. The accuracy ranged from 79.2% to 95.8%. The limitations of phenotype testing in this study were: i) there was no individual testing for sulphonamide and only testing for its combination with trimethoprim was carried out and ii) many aminoglycoside classes of antimicrobials were not included. As a result, these classes of antimicrobials were not included in the evaluation of WGS prediction. In general, except for colistin resistance, most resistance phenotypes correlated with resistance genotypes.

Table 10. Antimicrobial resistance phenotype and genotype of the 24 whole genome sequenced isolates

Isolate ID	Aminoglycoside		Beta-lactam		Colistin		Quinolone	
	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype
ENV49 [#]	–	<i>aph(3')-IIa-like, strA, strB</i>	–	–	–	–	MXF, OFL	<i>gyrA</i> mutation
ENV66 ⁺	–	<i>aadA1-like, aph(3')-Ic-like</i>	AMP, AMC*, A/S*, CRM, PI	<i>bla_{TEM-176}</i>	CL	<i>mcr-1</i>	MXF, NOR, OFL	<i>QnrS1, parE</i> mutation
ENV68 [^]	TO	<i>aac(6')Ib-cr, aadA1-like, aadA16-like, aph(3')-Ic-like, strA, strB</i>	AMP, A/S*, PI	<i>bla_{TEM-1A}</i>	–	–	–	<i>aac(6')Ib-cr, QnrB6</i>
ENV76 [#]	–	<i>aph(3')-Ic-like</i>	AMP, AMC*, A/S*, PI	<i>bla_{TEM-176}</i>	CL	<i>mcr-1</i>	MXF, NOR, OFL	<i>QnrS1</i>
ENV103 [#]	–	<i>aadA1-like, aph(3')-Ic-like</i>	AMP, A/S*, CRM, PI	<i>bla_{TEM-176}</i>	CL	<i>mcr-5</i>	MXF, NOR, OFL	<i>QnrS1</i>
ENV190 [#]	–	–	–	<i>bla_{TEM-1B}</i>	–	–	–	<i>QnrS1</i>
ENV210 [#]	CN, TO	<i>aadA1, strA, strB</i>	AMP, A/S*, AZT, CFT, CPD, CPE, CRM, CRO, PI	<i>bla_{TEM-1B}</i>	CL	–	NOR, OFL	–
ENV222 ⁺	–	<i>aph(3')-Ic-like, strA, strB</i>	AMP, A/S*, PI	<i>bla_{TEM-176}</i>	–	–	CIP, MXF, NOR, OFL	<i>QnrS1, gyrA</i> mutation
ENV225 ⁺	–	<i>aadA1, aadA2</i>	AMP, AMC*, A/S*, AZT, CAZ, CFT, CPD, CPE, CRO, PI	<i>bla_{SHV-12, bla_{TEM-1B}}</i>	–	–	MXF, NOR, OFL	<i>QnrD-like, QnrS1</i>
ENV228 [#]	–	–	CRM	–	–	–	–	–
ENV233 ⁺	–	–	–	–	–	–	–	–
ENV235 ⁺	–	–	–	–	CL	–	–	–
ENV316 ⁺	–	<i>aadA1, aadA2</i>	–	–	–	–	–	–
ENV317 ⁺	–	<i>aadA1, aadA2</i>	–	–	–	–	–	–
ENV323 [#]	–	–	–	–	–	–	–	<i>QnrS1</i>
ENV326 [#]	–	<i>aph(3')-Ic-like</i>	AMP, AMC*, A/S*, PI	<i>bla_{TEM-176}</i>	–	–	MXF	<i>QnrS1</i>
ENV388 ⁺	–	–	–	–	CL	–	–	–
ENV463 [#]	–	–	AMP, AMC*, CFX, CPD	–	–	–	–	–
ENV663 [#]	–	<i>aadA5</i>	–	–	–	–	–	–
ENV665 [#]	–	<i>aadA5</i>	–	–	–	–	–	–
ENV694 [#]	–	–	–	–	CL	–	–	–
ENV704 ⁺	TO	<i>aadA1-like, aph(3')-Ic-like, strA-like, strB</i>	A/S*, CRM, PI	<i>bla_{TEM-1B}</i>	CL	–	CIP, LVX, MXF, NOR, OFL	–
ENV727 [#]	–	<i>aph(3')-Ia-like</i>	–	–	–	–	CIP, MXF	<i>QnrS1</i>
ENV737 [#]	–	–	–	–	–	–	–	–

Continued Table

Isolate ID	Phenicol		Sulphonamide		Trimethoprim		Tetracycline	
	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype
ENV49 [#]	C	–	SXT*	<i>sul2</i>	SXT*	–	TE	<i>tet(A)</i> -like
ENV66 ⁺	C	<i>floR</i> -like	–	<i>sul3</i>	–	–	TE	<i>tet(A)</i> -like
ENV68 [^]	C	<i>floR</i> -like	SXT*	<i>sul1,sul2,sul3</i>	T, SXT*	<i>dfrA27</i>	TE	<i>tet(A)</i> -like
ENV76 [#]	C	<i>floR</i> -like	–	–	–	–	TE	<i>tet(A)</i> -like
ENV103 [#]	C	<i>floR</i> -like	SXT*	<i>sul3</i>	T, SXT*	<i>dfrA5</i>	TE	<i>tet(A),tet(M)</i> -like
ENV190 [#]	–	–	–	–	–	<i>dfrA14</i> -like	–	<i>tet(A)</i>
ENV210 [#]	C	<i>catA1</i> -like	SXT*	<i>sul1,sul2</i>	T, SXT*	<i>dfrA1</i>	TE	<i>tet(A)</i>
ENV222 ⁺	–	–	–	–	–	–	TE	<i>tet(B)</i>
ENV225 ⁺	C	<i>floR</i> -like	SXT*	<i>sul3</i>	T, SXT*	<i>dfrA15</i>	TE	<i>tet(A)</i> -like, <i>tet(B)</i>
ENV228 [#]	C	–	–	–	–	–	–	–
ENV233 ⁺	–	–	–	–	–	–	–	–
ENV235 ⁺	–	–	–	–	–	–	–	–
ENV316 ⁺	C	<i>cmlA1</i> -like	–	<i>sul3</i>	–	–	–	–
ENV317 ⁺	C	<i>cmlA1</i> -like	–	<i>sul3</i>	–	–	–	–
ENV323 [#]	–	–	SXT*	–	T, SXT*	<i>dfrA14</i> -like	TE	<i>tet(A)</i> -like, <i>tet(C)</i>
ENV326 [#]	C	<i>floR</i> -like	SXT*	–	T, SXT*	<i>dfrA14</i> -like	TE	<i>tet(A)</i> -like
ENV388 ⁺	–	–	–	–	–	–	–	–
ENV463 [#]	–	–	–	–	–	–	TE	<i>tet(B)</i>
ENV663 [#]	–	–	SXT*	<i>sul2</i>	T, SXT*	<i>dfrA17</i>	TE	<i>tet(A)</i> , 16S mutation (<i>rrsB</i>)
ENV665 [#]	–	–	SXT*	<i>sul2</i>	T, SXT*	<i>dfrA17</i>	TE	<i>tet(A)</i>
ENV694 [#]	–	–	–	–	–	–	TE	<i>tet(B)</i>
ENV704 ⁺	C	<i>floR</i> -like	SXT*	<i>sul2,sul3</i>	T, SXT*	<i>dfrA14</i> -like	TE	<i>tet(A)</i> -like
ENV727 [#]	–	–	SXT*	–	T, SXT*	<i>dfrA14</i> -like	TE	<i>tet(A)</i> -like
ENV737 [#]	–	–	–	–	–	–	TE	–

The antimicrobial resistance phenotype and genotype were determined by broth microdilution method and WGS, respectively. Sources of isolates are indicated by symbols #, + and ^ for cooked chicken-based dishes, cooked fish-based dishes and a raw fish-based dish, respectively.

* denotes that a combination of two different classes of antimicrobial agents was used for testing.

AMC: Amoxicillin/Clavulanic acid; AMP: Ampicillin; A/S: Ampicillin/Sulbactam; AZT: Aztreonam; C: Chloramphenicol; CAX: Ceftriaxone; CAZ: Ceftazidime; CFT: Cefotaxime; CFT: Cefotaxime; CFX: Cefoxitin; CIP: Ciprofloxacin; CL: Colistin; CN: Gentamicin; CPD: Cefpodoxime; CPE: Cefepime; CRM: Cefuroxime; CRO: Ceftriaxone; LVX: Levofloxacin; MXF: Moxifloxacin; NOR: Norfloxacin; OFL: Ofloxacin; PI: Piperacillin; SXT: Sulphamethoxazole/Trimethoprim; T: Trimethoprim; TE: Tetracycline; TO: Tobramycin

Table 11. Evaluation of genotypic analysis to predict antimicrobial resistance phenotype in *E. coli*

Antimicrobials	Phenotype: resistant		Phenotype: susceptible or intermediate		Sensitivity (%)	Specificity (%)	Accuracy (%)
	genotype: resistant (row %)	genotype: susceptible (row %)	genotype: resistant (row %)	genotype: susceptible (row %)			
Ampicillin	8 (33.3)	1 (4.2)	1 (4.2)	14 (58.3)	88.9	93.3	91.7
Colistin	3 (12.5)	5 (20.8)	0 (0.0)	16 (66.7)	37.5	100	79.2
Chloramphenicol	10 (41.7)	0 (0.0)	2 (8.3)	12 (50.0)	100	85.7	91.7
Fluoroquinolones	8 (33.3)	2 (8.3)	3 (12.5)	11 (45.8)	80.0	78.6	79.2
Trimethoprim	10 (41.7)	0 (0.0)	1 (4.2)	13 (54.2)	100	92.9	95.8
Tetracycline	16 (66.7)	1 (4.2)	1 (4.2)	6 (25.0)	94.1	85.7	91.7

Table 12. BLASTn result for the contigs containing resistance genes

Isolate ID	Reference genome	Related resistance	Resistance genes	Contig information			Location	BLASTn output for the best hit for the contigs containing resistance genes				
				ID	Length/bp	Coverage		Total score	Query cover	E value	Identity	Accession
ENV66	NC_011415.1	Colistin resistance	<i>mcr-1</i>	49	2943	53.3462	plasmid	6055	100%	0	100%	KX129782.1
		Quinolone resistance	<i>QnrS1</i>	45	3852	265.352	plasmid	13294	100%	0	99%	CP023167.1
ENV68	NZ_CP022959.1	Quinolone resistance	<i>aac(6')Ib-cr, QnrB6</i>	45	13427	89.8656	plasmid	70353	99%	0	99%	MG870194.1
ENV76	NC_011415.1	Colistin resistance	<i>mcr-1</i>	44	2943	160.463	plasmid	6055	100%	0	100%	KX129782.1
		Quinolone resistance	<i>QnrS1</i>	46	2283	212.208	plasmid	3596	85%	0	100%	CP027680.1
ENV103	NC_017641.1	Colistin resistance	<i>mcr-5</i>	110	3654	1425.51	plasmid	6812	100%	0	99%	KY807921.1
		Quinolone resistance	<i>QnrS1</i>	125	2283	314.48	plasmid	3596	85%	0	100%	CP027680.1
ENV190	NZ_CP024243.1	Quinolone resistance	<i>QnrS1</i>	48	8049	68.8271	plasmid	20729	100%	0	100%	CP020091.1
ENV210	NZ_CP007491.1	ESBL	<i>bla_{TEM-1B}</i>	48	2170	80.3059	plasmid	9883	100%	0	100%	CP022451.1
ENV222	NZ_CP032989.1	Quinolone resistance	<i>QnrS1</i>	66	2029	360.459	plasmid	3596	95%	0	100%	CP027680.1
ENV225	NC_017633.1	ESBL	<i>bla_{SHV-12}</i>	57	4715	176.179	plasmid	21615	100%	0	100%	KP975077.1
			<i>bla_{TEM-1B}</i>	47	9424	72.963	plasmid	19126	100%	0	99%	CP018662.1
			<i>QnrS1</i>	67	2673	177.963	plasmid	5147	100%	0	100%	MH884651.1
ENV323	NC_017633.1	Quinolone resistance	<i>QnrS1</i>	40	39096	418.817	plasmid	72284	99%	0	99%	MH121703.1
ENV326	NZ_CP012380.1	Quinolone resistance	<i>QnrS1</i>	67	2283	344.484	plasmid	3596	85%	0	100%	CP027680.1
ENV727	NZ_CP010235.1	Quinolone resistance	<i>QnrS1</i>	25	44804	250.02	plasmid	63789	75%	0	99%	KU254580.1

3.3.4. Location analysis of ESBL gene, and colistin and quinolone resistance gene

The whole genome sequence of ESBL-producing isolates and isolates carrying colistin and/or quinolone resistance were analyzed by KmerFinder². After comparing contigs and reference genome by BLASTn, all the related resistance genes could not be matched to reference genome. However, plasmids with high coverage and identity could be found by BLASTn to match to contigs that contained related resistance genes (Table 12). This suggested that the resistance genes were most likely to be located on plasmids.

3.3.5 Structure of the *mcr-5.1*-carrying plasmid

The sequence of pSGMCR103 revealed a circular plasmid of 58090 bp in length with 47.7% G+C content. PlasmidFinder showed it has 98.93% identity with the IncX1 replicon. IncX group of plasmids are commonly found in *Enterobacteriaceae* with narrow host range. They are known to encode fimbriae which enable conjugative transfer [197]. Among the five subtypes of the IncX group, only IncX4 was previously reported to be related to *mcr* genes in *E.coli*, *Salmonella* spp., *Klebsiella* spp. and etc [198, 199]. This is the first time that IncX1 group of plasmid carrying *mcr* gene is reported.

² K-mer is a short sequence with a number of k bases. For example, 10-mers means a short sequence with 10 basepairs. In principle, any sequences with high similarity must share k-mers. As a result, comparing k-mers of unknown genome with that of reference genomes in the database can quickly find the most similar genome. Details available at <https://www.coursera.org/lecture/wgs-bacteria/species-identification-kmerfinder-tool-description-and-applications-FTpuf>

The plasmid sequence closest to pSGMCR103 in NCBI is plasmid pYD786-3 (accession number KU254580.1) with 77% query coverage and 99% identity, which was carried by one *E. coli* isolate from human urine in USA. The comparison of these two plasmids is shown in Figure 23. They share antimicrobial resistance gene *aph(3')-la*, *aadA1* (aminoglycoside resistance), *bla*_{TEM-176} (beta-lactam resistance) and *sul3* (sulphonamide). In addition, pSGMCR103 also carries quinolone-resistance gene *QnrS1* and colistin-resistance gene *mcr-5.1*. Gene *mcr-5.1* was harbored on a Tn3 transposon-like element, which is similar with pSE13-SA01718 (accession number KY807921.1) carried by a *Salmonella* isolate reported before. Three components of Tn3 were all found on this plasmid: beta-lactamase (encoded by gene *bla*), Tn3 transposease (encoded by gene *tnpA*) and Tn3 resolvase (encoded by gene *tnpR*) (Figure 23). Its genetic environment is shown in Figure 23. Also, other insertion elements such as IS5, IS6, IS91, IS256 family were found on the plasmid, which may indicate the recombination activity of the plasmid.

3.3.6 Transferability and stability of *mcr-5* carrying plasmid

PCR confirmed the presence of *mcr-5* in transconjugants after 24h co-culture, and the amplicon was also sequenced and confirmed, suggesting *mcr-5.1* genes were able to be transferred to recipient *E. coli* strain J53. The transfer frequency was determined as 10^{-6} . The plasmid was stable after 20-day successive subculture in LB broth (~200 generations) without colistin selection, demonstrating the stability of the plasmid. The colistin MIC of transconjugants showed an 8-fold increase (from 1 to 8).

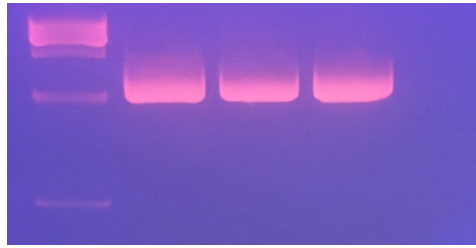


Figure 22. Electrophoresis result of PCR products.

Five lanes from left to right: marker, colony 1, colony 2, positive control, negative control.

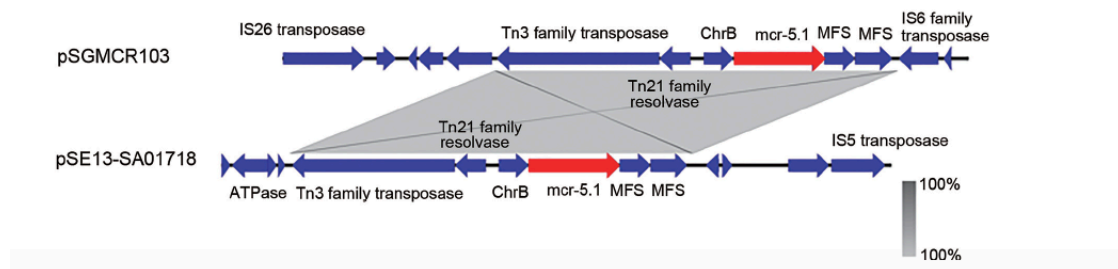
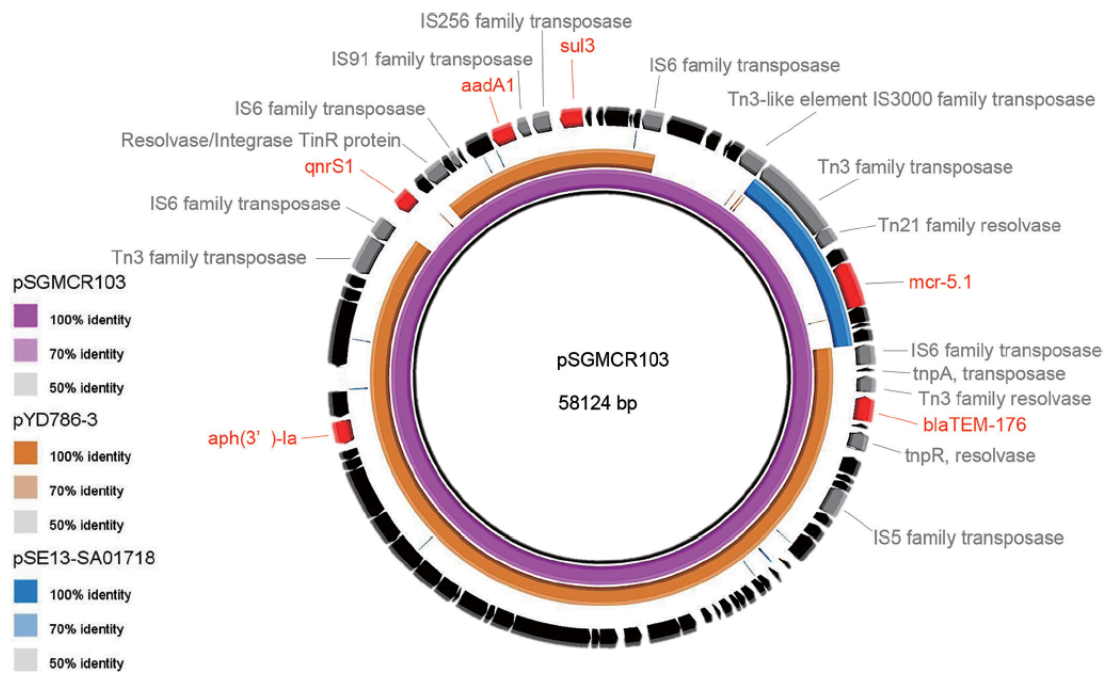


Figure 23. Genetic environment of *mcr-5* carrying plasmid

(Top panel) Sequence map of plasmids pSGMCR103, pYD786-3 (GenBank accession number KU254580.1), and pSE13-SA01718 (GenBank accession number KY807921.1). The outermost circle shows the predicted coding sequences of pSGMCR103. The red parts indicate antimicrobial resistance genes; the gray parts indicate the genes encoding mobile element protein. The figure was generated by the use of BRIG (<http://brig.sourceforge.net/>). (Bottom panel) Genetic environment of the *mcr-5.1* gene in comparison to pSE13-SA01718. ME, mobile element; MFS, gene encoding major facilitator superfamily (MFS)-type transporter; ChrB, gene encoding the protein involved in chromate resistance. The figure was drawn by the use of EasyFig 2.2.2 (<http://mjsull.github.io/Easyfig/>).

3.3.7 Detected virulence factor

In this study, 24 *E. coli* isolates were detected the presence of virulence genes. There were 11 virulence genes detected in total (Table 13). The most prevalent virulence genes were *gad* (23/24), *lpfA* (17/24), *iss* (9/24). For these 24 isolates, every isolate carried at least one virulence gene. Some of them are related to pathogenic *E. coli* such as *iss*, *iroN*, *astA*, *cnfI*. Nine out of 24 isolates contained three or more virulence genes. Although they are not able to cause pathogenicity due to the lack of specific virulence combination [200], they are still important reservoir for virulence genes which are feasible and easy to transfer among stains over time [201]. In particular, those who carrying multi virulence genes are mostly multi-resistant *E. coli* (7 out of 9). The combination of antimicrobial resistance and virulence factors would be great concern in infection treatment.

Table 13. Virulence factors found in 24 *E. coli* isolates

Virulence genes	Frequency	Protein function	Remark
<i>gad</i>	23/24	Glutamate decarboxylase	A prescreening marker for identifying pathogenic <i>E. coli</i> in foods[202].
<i>lpfA</i>	17/24	Long polar fimbriae	Lpf is one of the few adhesive factors of EHEC O157:H7 associated with colonization of the intestine[203]
<i>iss</i>	9/24	Increased serum survival	ExPEC-associated virulence marker[201]
<i>iroN</i>	3/24	Enterobactin siderophore receptor protein	ExPEC-associated virulence marker[201]
<i>cma</i>	2/24	Colicin M	
<i>mcmA</i>	1/24	Microcin M part of colicin H	
<i>capU</i>	1/24	Hexosyltransferase homolog	
<i>mchF</i>	1/24	ABC transporter protein MchF	
<i>astA</i>	1/24	EAST-1 heat-stable toxin	Occasionally detected in ETEC, EPEC, EAEC; normally detected in STEC/EHEC[204]
<i>cnfI</i>	1/24	Cytotoxic necrotizing factor	Occasionally detected in UPEC[204]
<i>senB</i>	1/24	Plasmid-encoded enterotoxin	

*ETEC: enterotoxigenic *E.coli*; EPEC: enteropathogenic *E.coli*; STEC:Shiga toxin-producing *E.coli*; EHEC: enterohemorrhagic *E. coli*; EIEC: enteroinvasive *E.coli*; EAEC: enteroaggregative *E.coli*; UPEC: uropathogenic *E.coli*; NMEC: neonatal meningitis *E.coli*; ExPEC: extraintestinal pathogenic *E. coli*

3.4 Discussion

Antimicrobials are widely used in poultry production and aquaculture [19]. Enteric bacteria isolated from food-producing animals are commonly resistant to a range of antimicrobials including ampicillin and tetracycline. Bacteria found in poultry can have an even broader resistance spectrum with quinolones and third-generation cephalosporins resistance [205]. The isolates used in this study were derived from samples that also contained other ingredients such as rice, seasoning and garnishings and as a result, we were not able to link the *E. coli* isolates specifically to poultry or fish, which was the main ingredient in the dish. However, AMR including multidrug resistance is common in poultry and other food animals. A seven-year surveillance project conducted in seven provinces in China found that 89.2% *E. coli* isolated from broiler chicken carried multidrug resistance. The most prevalent AMR phenotype displayed resistance to tetracycline, sulfisoxazole, and ampicillin [179]. Despite the different sources, the isolates in our study showed a similar resistance pattern. Our finding of the two resistance determinants, sulfamethoxazole and trimethoprim were also observed in another study in Germany, which showed high prevalence of resistances to trimethoprim (22%) and trimethoprim/sulfamethoxazole (21%) in the isolates from poultry (including livestock and food) during 1999-2001 [206]. Another review summarized AMR in *E. coli* isolated from broiler chickens in Europe and North America [207]. Although resistance patterns differed from country to country, resistance to tetracycline, ampicillin, streptomycin and trimethoprim/sulfamethoxazole were the most prevalent compared to other antimicrobials [207]. The emerging resistance to these antimicrobial agents is explicable due to their long history of use in animals [179]. In our study,

resistance to chloramphenicol was also prevalent (11/23, 47.8%), which was not commonly reported in other poultry-related studies [206, 207].

The detection and comparative analysis of AMR genes through a WGS approach in this study furthers our understanding of the mechanisms involved. The reliability of AMR prediction based on WGS has been discussed in several papers in recent years [208-211]. Our observation of *tet(A)* gene being the most common resistance gene (76.5%, 13/17) is in agreement with previous studies [212, 213]. Together with *tet (B)* and *tet (C)* genes, *tet(A)* gene codes for energy-dependent efflux proteins which help bacteria pump out tetracycline out of the cell [214]. Of the 17 isolates phenotypically displaying tetracycline resistance, one carried *tet (M)*-like gene, which codes for ribosomal protection proteins to disrupt the primary binding site of tetracycline with ribosome [214]. *Tet (M)* has not been commonly reported in *E. coli* from food, and thus has not been commonly included in polymerase chain reaction (PCR) based detection of tetracycline resistance genes. Thus, WGS provides an unbiased approach for the detection of known genes or mutations that are attributable to resistance, which could go undetected in targeted screening tests such as PCR-based assay. In addition, three of 17 isolates carried double *tet* genes, which may enhance the resistance to tetracycline. With the long and wide use of tetracycline in human and animals, the tetracycline resistance genes which can be horizontally transferred have been intensively studied. However, for some antimicrobial agents like colistin, its transferable resistance mechanism was not discovered until recently. The association of colistin resistance genes with transferable plasmids was first reported in 2015 [67]. The transferable colistin resistance genes *mcr-2* to -8 were

discovered shortly after [79, 89, 215-217]. In this study, *mcr-1* and *mcr-5* were detected in three isolates from eight colistin-resistant isolates. Gene *mcr-5*, a transposon-associated phosphoethanolamine transferase gene mediating colistin resistance, was first found in *Salmonella* in 2017 [93], followed by findings in *E. coli* isolated from pigs and poultry in farms from Japan, Germany and China [218-220]. To our knowledge, *mcr-5* has not been reported in *E. coli* or other enteric bacteria from RTE food. In Singapore, *mcr* genes had been reported in clinical isolates [39, 91, 221], however, this is the first time that *mcr* genes have been reported in bacteria from food. It is also the first time *mcr-5* gene was detected in Singapore. Colistin resistance in clinical isolates is relatively rare currently [64]; however, the discovery of such plasmid-mediated resistance genes in isolates from RTE retail food is a public health concern as horizontal gene transfer via contaminated RTE food can accelerate the further spread of resistance genes if no measures are taken in the future.

Interestingly, no *mcr* gene was detected in the 5 *E. coli* isolates which were phenotypically resistant to colistin. However, within the genomes of these 5 isolates, similar chromosome mutations were found on gene *pmrA/B*, which was reported to cause colistin resistance by modifying LPS [222]. These mutation sites have not been reported in the literature and we have planned further research to explore the relationship between these mutations and colistin resistance.

Resistance to beta-lactam antimicrobials is increasingly observed, among which the resistance caused by ESBL-producing bacteria has emerged as a serious

problem. A study in Belgium suggested that 45% (133/295) of *E. coli* isolated from broiler chickens produced ESBL even before the use of licensed cephalosporin in poultry [223]. This may be caused by co-selection of other antimicrobials. ESBL genes are generally derived from two sources, some are mutant derivatives from traditional penicillinase like *bla*_{TEM/SHV}, and others such as *bla*_{CTX-M} are acquired from environmental bacteria [224].

In Singapore, classical ESBLs (TEM- or SHV-type) have been the main contributors to AMR in Gram-negative bacteria over the past 30 years [36]. Unlike the screening result in South Korea, Europe and USA where CTX-M is the most prevalent ESBL type [224-227], no *bla*_{CTX-M} gene in *E. coli* isolates was detected in our study. All beta-lactam resistance related genes detected in this study were penicillinase gene derivatives. Gene *bla*_{TEM-1} and gene *bla*_{SHV-12} are widely exist in community and clinical isolates, as well as food-producing animals [228-231]. Gene *bla*_{TEM-1A} and *bla*_{TEM-1B} both code the same TEM-1 β -lactamase, which is able to hydrolyze penicillins and 1st generation cephalosporins [230, 232]. Gene *bla*_{TEM-176} is less common but also found in healthy pigs [233], community [229] and hospital or hospital environments [234]. No specific source of these ESBL genes was found in past studies. In our study, isolates carrying the same types of beta-lactam resistance related genes could show different resistance phenotypes and ESBL-producing isolate that did not carry any published ESBL genes could be observed. This may indicate the existence of a novel resistance mechanisms or it is also plausible that sequence information was not available due to the limitations of shotgun sequencing method.

ResFinder is capable of detecting not only AMR genes and known resistance-associated point mutations on the chromosome, but it also has utility for the discovery of previously unknown site mutations affecting resistance. The most common mechanism of fluoroquinolone resistance is the mutation of the target genes. This was also observed in this study. Gene *gyrA* mutations were found in two resistant isolates (ENV49, ENV222) while *parE* mutation was found in another isolate (ENV66). Both of these two genes code for type II topoisomerases. Such mutations are known to reduce the binding efficiency of fluoroquinolone drugs, thereby leading to resistance to these drugs. Other studies have also reported *gyrA* mutations being common among quinolone resistant *E. coli*. In particular studies examining avian pathogenic *E. coli* isolated in both USA and China [235, 236] noted the presence of *gyrA* mutations in quinolone resistant isolates. In contrast to our findings, these authors also reported the presence of mutations in *parC* which were not found here. Furthermore, regarding site mutations facilitating AMR, we also observed a mutation in *rrsB* in one mutant (ENV663) which is known to confer tetracycline resistance in other bacteria [237]. The identification of these point mutations along with known AMR genes helps to complete the picture of AMR resistance in these isolates.

Horizontal gene transfer drives the transmission of AMR genes between different species. Plasmids are important vectors for the transfer of AMR genes by conjugation [238]. Thus, it is important to determine whether AMR genes are located on the chromosome or plasmids in order to characterize the AMR bacteria. There is no direct way to determine which contigs belong to chromosome or

plasmids. However, if the majority of contigs are mapped to a reference genome, it is very likely that those remaining contigs with no homology to the reference genome are of plasmid origin [239]. In our study, further exploration was conducted to determine the location of ESBL genes, and colistin and quinolone resistance genes by comparing our sequences with those in the databases of KmerFinder and BLASTn. Results indicated that the contigs harboring ESBL, colistin and quinolone resistance genes were more likely from plasmids than chromosomes. It was noteworthy that *QnrSI*, the most prevalent quinolone resistance gene in this study, is commonly reported as transferable plasmid-mediated quinolone resistance (PMQR) determinant [240]. Although some isolates carrying this gene did not display resistance to quinolone, it may still keep as part of AMR gene reservoir and transfer to other bacteria. Moreover, the contigs containing AMR genes of isolate ENV210 was determined to be highly similar (100% identity) to plasmid pO83_CORR from adherent-invasive *E. coli* O83:H1 str. NRG 857C (accession number NC_017659.1). After mapping all contigs of ENV210 to this plasmid, all the AMR genes were accounted for with certain homology, thereby suggesting the plasmid as the source of these genes. This method also provided a way to explore the potential transfer route of AMR genes. Based on predicted serotypes (data not shown) defined by whole genome sequences, *E. coli* isolates in this study did not belong to enteropathogenic serotypes. However, the reference genomes selected by KmerFinder for two ESBL producing isolates were avian pathogenic *E. coli* strain ACN002 (accession number NZ_CP007491.1) and prototypical enterotoxigenic *E. coli* strain H10407 (accession number NC_017633.1) suggesting these two pathogenic strains were mostly similar to these two ESBL-producing isolates in

our study. This may imply the presence of potential virulence factors, in addition to the ability of being resistant to clinically important antimicrobials, in these two isolates.

Although there was strong correlation between resistant phenotypes and AMR gene profiles for most isolates, data inconsistencies will always give cause for debate. Many factors may contribute to the discrepancy such as gene silencing, undiscovered AMR genes and resistance mechanisms. In addition, incomplete sequences for AMR genes caused by constraints in current sequencing technologies may limit the detection tool. In this study, the low sensitivity (37.5%) displayed for colistin resistance is most likely caused by undiscovered site mutations on resistance-related genes. All of these factors may cause discrepancies between the AMR phenotype and genotype.

Although the discrepancies exist, it was demonstrated that WGS showed excellent potential for the prediction of AMR compared to traditional antimicrobial susceptibility testing methods and other molecular biology methods like PCR. Once WGS has been carried out on a particular isolate the sequence data is always available for present and future analysis. This offers superior utility over that generated by PCR based methods targeting only specific regions as with multi-locus sequencing or information acquired from microarrays. Therefore, one can easily go back to a data set to detect newly discovered genes [175, 192]. AMR genes, resistance-related point mutations, as well as other further analysis like plasmid detection, virulence detection, typing and others are

able to be done at the same time [173]. There is no need for a result expectation to design primers or probes which in fact narrows the scope of study. In addition, the sequence data could be preserved for a long time without worrying about the change of the quality over time compared with physical strain glycerol store. However, drawbacks also exist while using WGS. All of the detections are based on current knowledge, which limits its use in new AMR and mechanism discovery. The genotype may not be able to reflect the whole picture of AMR, and resistance phenotypes may result from complex gene networks which cannot be determined by occurrence of single genes [241]. High-throughput sequencing systems increase the efficiency of sequencing but break the whole genome into contigs which inevitably cause the deletion of gene information. Current analysis tools cannot differentiate plasmids and chromosomes based on WGS (short-read sequencing) data. This short-read sequencing technology is dominant in whole genome sequencing field, but it poses limitations as well, for example, it is hard to identify repetitive or highly homologous regions of genome, and fragments of genome instead of circular chromosome or plasmid are got after assembly, which may cause genetic information missing [242]. While the emerging long-read sequencing technology is able to make up for the deficiency. But currently the error rate of long-read sequencing is much high than short-read sequencing, as a result, the combination of short-reads data and long-reads are necessary to improve both accuracy and completeness of sequence especially for variable plasmids. In our study, the genetic environment of *mcr-5* gene (mobile colistin resistance gene) is hard to analysis only based on Illumina short-read sequencing, the application of long-read sequencing makes it achievable to get complete circular plasmid map. The possible recombination ways of this plasmid was

quickly speculated. It is still limited by the cost of two sequencing methods (especially the long-read sequencing method), however, it is expected that wider applications are being made in the near future with the decreasing cost.

RTE food may serve as potential vectors for the transfer of AMR bacteria to humans and become established in the gut microbiota. Food can be contaminated with AMR bacteria due to undercooking, cross contamination during food processing or post-cooking manipulation. The presence of multidrug resistant *E. coli* in local RTE food is a public health concern. Continuous food hygiene and safety reminders (such as proper sourcing of food from credible sources, thorough cooking, proper handling and storage of food) should be served to food handlers to minimize the risk of foodborne pathogens contamination in RTE food. This will also help in reducing the risk of contamination with AMR bacteria, which may play a role of AMR gene reservoirs and become parts of the resistome [243], in food and the environment.

3.5 Conclusion

In this study, we found the presence of AMR *E. coli* in RTE food in Singapore and related AMR genes, which raised our concern of AMR transmission from food to humans. WGS together with traditional AMR detection methods in our study provided more complete picture of AMR prevalence and characteristics. The relationship and discrepancy between AMR phenotype and genotype underlined the importance of investigating AMR mechanism and provided directions to explore unknown mechanisms. With the reductions in WGS price

and updating of sequencing technology, it will become more and more useful in the routine microbiology. However, challenges of sequence mapping and analysis remain significant. More user-friendly analytic tools for WGS data analysis are needed in the future.

Chapter 4 Prevalence and genomic analysis of Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli* from raw meats in Singapore

4.1. Introduction

Foodborne diseases threaten national economies, health care system as well as tourism and trade. Around 1 in 10 people in the world get illness after eating contaminated food and 420,000 people die from that. Infection caused by foodborne pathogen is the leading cause of foodborne disease, however, the resistance of antimicrobials which are essential to treat bacterial infections has worsened the situation. Contaminated raw meat is one of the main causes of foodborne illnesses and greatly contributes to the transmission of zoonotic infections [244]. There has been lots of evidence showing that antimicrobial resistant bacteria can be transferred from animal to human through food [245].

Currently beta-lactam is the most used class of antimicrobials for infectious diseases. It accounts for 65% of injectable antimicrobial prescriptions in US during 2004-2014 [44]. Extended-spectrum beta-lactamases (ESBL) are enzymes that can hydrolyze most of beta-lactams including penicillins, cephalosporins and monobactams. The emergence and spread of ESBL in Enterobacteriaceae is posing a great threat to public health globally. In 2017, WHO published a list of antimicrobial resistant “priority pathogens” and the list was divided into three categories based on the urgency of need for new

antimicrobials: critical, high and medium priority. ESBL-producing Enterobacteriaceae was listed in the critical group [246]. As the most important and common species, the prevalence of ESBL-producing *E.coli* has increased significantly [247]. The co-existence of other drug resistance especially for the resistance to last-resort drug such as colistin greatly narrows down the available drug choice for treatment [69].

Whole genome sequencing (WGS) is emerging as a useful tool for genomic epidemiology. Numerous applications of WGS have been documented in the area of AMR including molecular surveillance, transmission and evolution study of AMR, diagnostic test and so on [178]. The prevalence of ESBL in retail raw meats has been investigated in many countries [51, 52, 55, 58, 62, 248]. The WGS data of ESBL-producers has also been reported by many researchers [249-251]. However, the related prevalence data and genome information in Singapore are limited. In this study, the largest raw meat sample collection was performed in Singapore including supermarkets and wet markets. ESBL-producing *E .coli* were isolated and sent for whole genome sequencing. Bioinformatic analysis such as AMR gene detection, phylogeny studies were performed.

4.2. Methods

4.2.1. *Sample collection and processing*

A total of 634 chilled and frozen meat samples (chicken n=213, pork n=216, beef n=205) were purchased from wet markets and five main chain supermarkets in Singapore (details shown in Table 14) during June 2017-October 2018. 97 supermarkets and 65 wet markets all over the island were involved in this project

(Figure 24). All samples from wet markets were chilled. One sample was collected per stall. All the frozen samples were collected from supermarkets. Market surveys were done before sample collection to decrease bias of country source during collection. All samples were randomly selected in accordance with market share. After purchasing, all samples were put on ice and transferred to the laboratory. Ten grams of meat samples were put into a sterile plastic bag containing 90 grams of Universal Pre-Enrichment Broth (UPB). Then stomacher was used to homogenize the sample at 230rpm for 1 minute. Homogenate was put into incubator for overnight culture at 37°C.

Table 14. Information for collected samples

Total(n=634)	Chicken (n=213)		Pork (n=216)		Beef (n=205)	
	Chilled	Frozen	Chilled	Frozen	Chilled	Frozen
Supermarkets	90	68	100	60	89	66
Wet markets	55	0	56	0	50	0



Figure 24. Raw meats sampling map.

Red dots and blue dots indicate the wet market sites and supermarket sites involved in this study, respectively. The map was labeled and generated by Google Map.

4.2.2. Bacterial isolation and *E. coli* selection

The enrichment was streaked on Brilliance TM ESBL Agar³ (Oxoid, Hampshire, UK) and incubated at 37°C for 24 hours. Colonies were selected based on morphology. Each single presumptive colony for one color was streaked on Tryptic Soy Agar (TSA) and incubated at 37°C for 24 hours. Eosin methylene blue (EMB) agar and indole testing were used to determine *E. coli* species⁴. The isolates grown on EMB agar with a distinctive metallic green sheen were picked and incubated in peptone broth overnight at 37°C. Three drops of Kovac's reagent were added to the bacterial culture. A positive result is shown by the presence of a red or red-violet color in the surface of the broth. Glycerol store was made for strains with positive results and kept at -80°C for further experiments.

4.2.3. ESBL confirmation

The production of ESBL was confirmed by double-disk synergy test as previously described in Chapter 3 [252].

³ Brilliance TM ESBL Agar is a chromogenic screening plate for the detection of ESBL. Blue or pink colonies are presumptive positive for *E. coli* ESBLs.

⁴ EMB agar is a differential microbiological medium, providing a color indicator distinguishing between organisms that ferment lactose (*E. coli*) and those that do not. *E. coli* ferment the lactose produce acid which lowers the pH. This encourages dye absorption by the colonies and turns the colonies dark purple as the acid acts upon the dyes. In addition, certain lactose-fermenting bacteria produce flat, dark colonies with a green metallic sheen.

Indole test is used to determine the ability of *E. coli* to split amino acid tryptophan to form the compound indole. Tryptophan is hydrolyzed by tryptophanase to produce three possible end products – one of which is indole. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylamino benzaldehyde, this reacts with indole to produce a red-colored compound.

4.2.4. Minimum inhibitory concentration (MIC) determination

The antimicrobial susceptibility testing for *mcr*-carrying ESBL isolates was performed using MIC testing. The MicroScan Neg MIC Panel Type 40 (Beckman Coulter, Inc., Brea, CA, USA) was used in accordance with the manufacturer's instruction. A total of 31 antimicrobials were included. *E. coli* ATCC 25922 was used as quality control strain. The result interpretation was based on EUCAST v.3.1 [253]. For the antimicrobials not available in EUCAST, the CLSI standard [254] was applied.

4.2.5. DNA extraction and sequencing

The detailed methods of DNA extraction and sequencing were described previously in Chapter 3 [252]. Briefly speaking, genomic DNA of ESBL positive *E. coli* was extracted using QIAamp® DNA Mini Kit, following the instructions provided by manufacturer (Qiagen, Germany). The whole genome sequencing was performed at SCELSE using Illumina HiSeq2500 sequencer with 250 bp read length in paired-end mode. Raw sequence was deposit at the European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena>) under study accession number: PRJEB34067.

4.2.6. Sequence assembly and in silico analysis for AMR gene, Multi Locus Sequence Type (MLST), plasmid typing, virulence factor

The raw reads were assembled using FoodQCpipelien of DTU food which started with FastQC assessment and then trimmed by bbdduk2. De novo assembly was performed by SPAdes [186]. The AMR genes, sequence types, plasmid types,

virulence genes were determined using ResFinder, MLST, PlasmidFinder, VirulenceFinder, respectively. These tools are available on the website of Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>) [192, 255, 256]. Default setting were used.

4.2.7. Genetic environment analysis of gene mcr

The annotation of bacterial genome was done by RAST version 2.0 (<http://rast.theseed.org/>) [188, 257]. Contigs containing *mcr* genes and plasmid replicon were selected for genetic environment analysis. The contigs were compared and visualized by EasyFig 2.2.2 [258].

4.2.8. Phylogenetic analysis and tree building

The genome sequences of ESBL *E. coli* which were isolated from community in Singapore during June 2016 to April 2017 (n=67) used for comparison were downloaded from ENA for comparison [38]. The detailed information for these isolates was shown in Supplementary Table 8. The SNP tree was built using CSI phylogeny pipeline which is available on the website of Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>) [259]. The pair-end reads of all isolates were mapped to the reference genome *E. coli* str. K-12 substr. MG1655 (NC_000913.3) using Burrows–Wheeler Aligner (BWA) [260]. SNPs were called using SAMTools and filtered based on the following parameters: a minimum of 10x depth and 10% relative depth; a minimum of mapping quality of 25; a minimum of SNP quality of 30; a minimum of 10bp of prune zone [261].

The SNP tree was annotated and visualized using iTOL v4 (<https://itol.embl.de/>) [262].

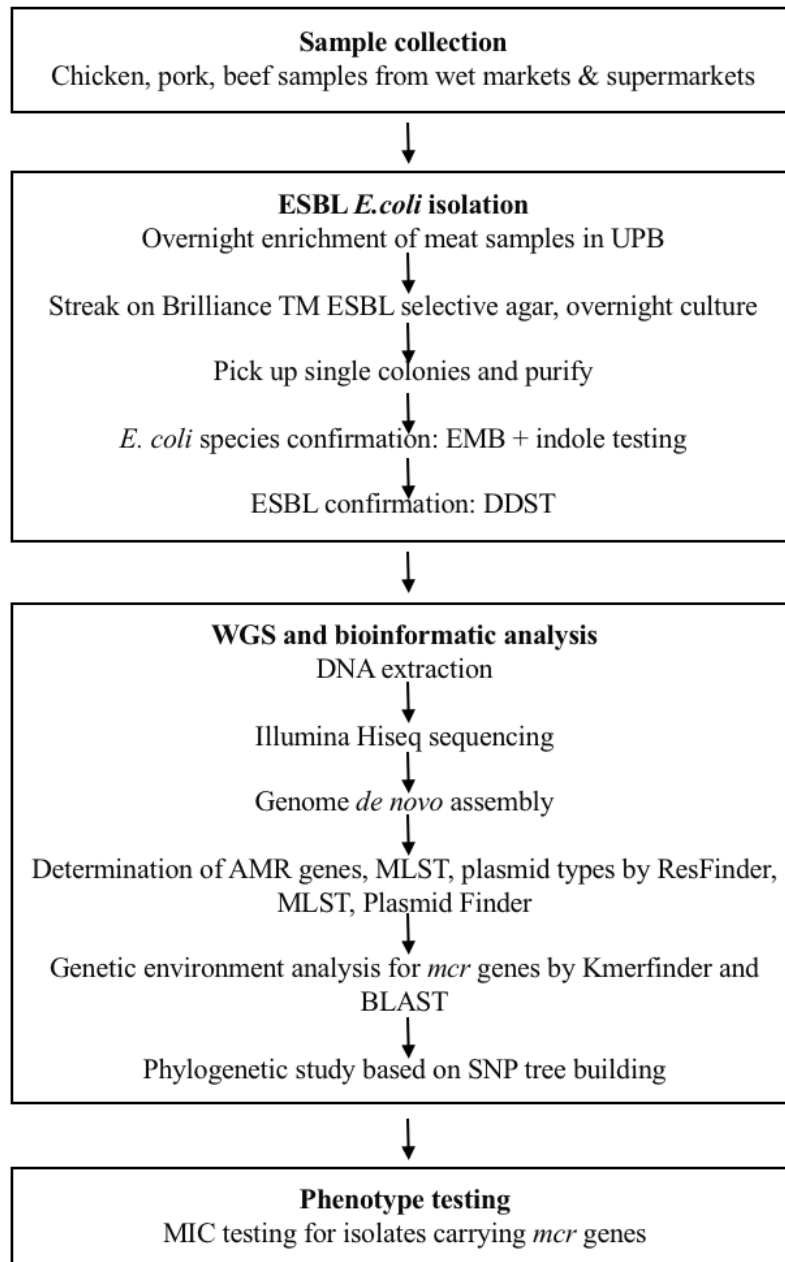


Figure 25. Flowchart of experiments and analysis

4.3. Results

*4.3.1. Prevalence of ESBL-producing *E. coli* in raw meat in*

Singapore

Among total 634 meat samples, 182 (28.7%) of them carried at least one type of ESBL-producing *E. coli*, and 35 of these samples (35/182, 19.2%) showed the presence of more than one types of ESBL-producing *E. coli*. A total of 225 ESBL-producing *E. coli* isolates were obtained from these 182 samples. If divided them into categories by meat types, the prevalence is 51.2% (109/213), 26.9% (58/216), 7.3% (15/205) in chicken, pork and beef, respectively. The detailed information for the samples is shown in Supplementary Table 6. The details of prevalence are shown in Figure 26. The prevalence rates of ESBL in frozen pork and beef decreased greatly as compared to chilled meats, namely, 5.0% and 0%. Only 3 frozen pork samples and none frozen beef carried ESBL-producing *E. coli*. However, over half of frozen chicken samples (51.5%) showed the presence of ESBL-producing *E. coli*. Generally speaking, the rank of prevalence of ESBL in three types of meats is chicken > pork > beef.

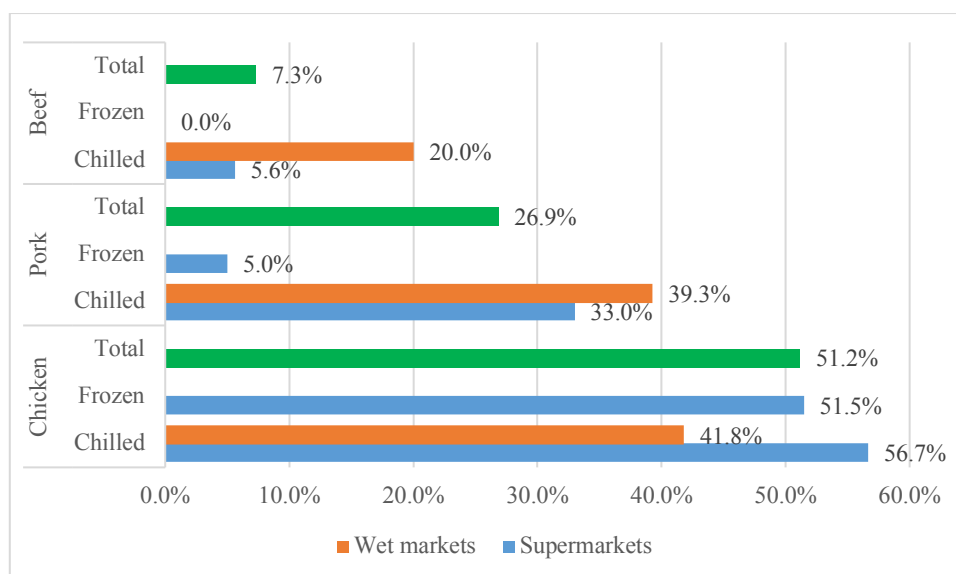


Figure 26. Prevalence of meat samples containing ESBL-producing *E. coli*. All frozen samples were collected from supermarkets, and chilled sample were from supermarkets or wet markets

Table 15. Prevalence of meat samples containing ESBL-producing *E. coli*

	Chicken			Pork			Beef		
	Chilled	Frozen	Total	Chilled	Frozen	Total	Chilled	Frozen	Total
Supermarkets	51/90 (56.7%)	35/68 (51.5%)	109/213 (51.2%)	33/100 (33%)	3/6 (5%)	58/216 (26.9%)	5/89 (5.6%)	0/66 (0%)	15/205 (7.3%)
Wet markets	23/55 (41.8%)			22/56 (39.3%)			10/50 (20.0%)		

For the samples from wet markets (n=161), the source countries were hard to trace because of the lack of labeling. For the samples from supermarkets (n=473), the country source and their sample size is shown in Figure 27. Among them, 442 samples were from 12 countries including Australia, Brazil, Malaysia, Indonesia, New Zealand, Holland, Spain, Thailand, USA, Japan, Singapore, Denmark, and 31 samples were from unknown countries. The bar chart indicates that the beef samples in Singapore supermarkets were mainly from Australia and chicken samples from Malaysia and Brazil, whereas pork samples mainly from Australia and Brazil. The percentage of ESBL-positive samples in each subcategory is shown as a form of heatmap (Figure 28). The red color indicates

the high prevalence, and NA means the category of samples were not collected. Generally speaking, the percentage of ESBL-positive samples from Indonesia (58.3%, 14/24) and Malaysia (60.3%, 41/68) are relatively high compared with other countries. All samples from Indonesia were chilled pork, and they are also the main contributor of ESBL-positive samples in all pork samples. For chicken samples, the ESBL-positive sample are mainly from Brazil and Malaysia. Although the inconsistent sample size for each country may cause bias for analysis, it still has the reference value.

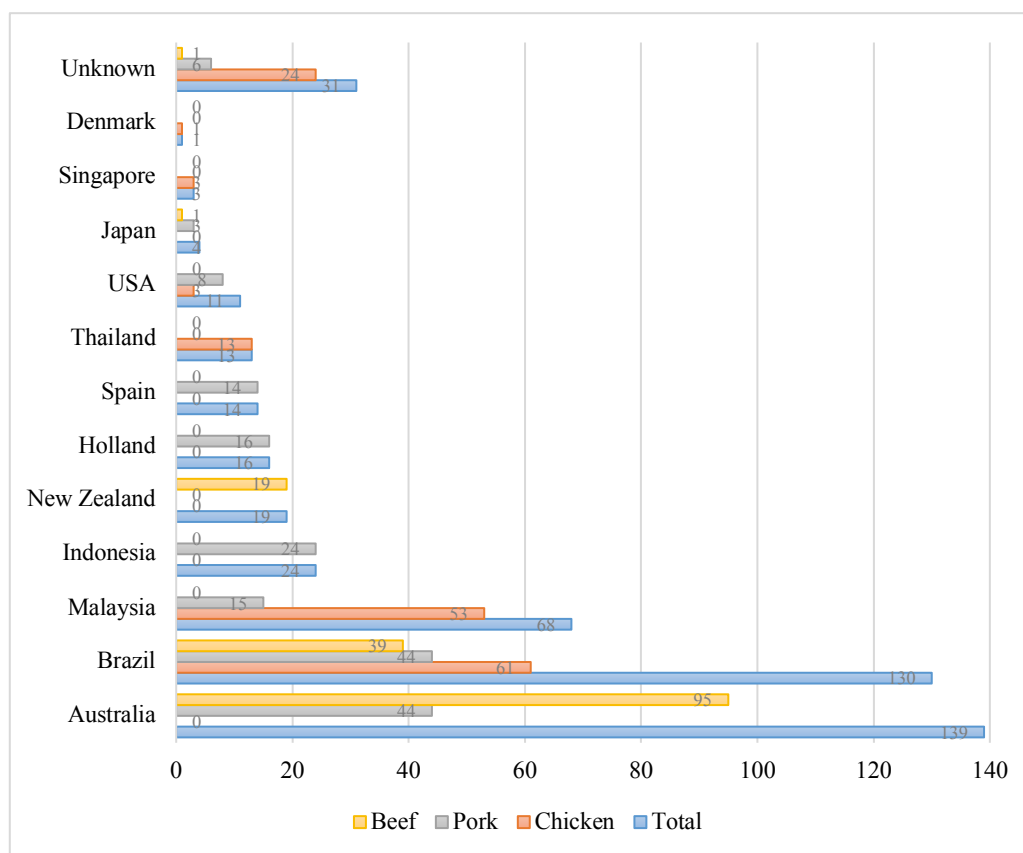


Figure 27. Distribution of sample source countries (samples from supermarkets in Singapore)

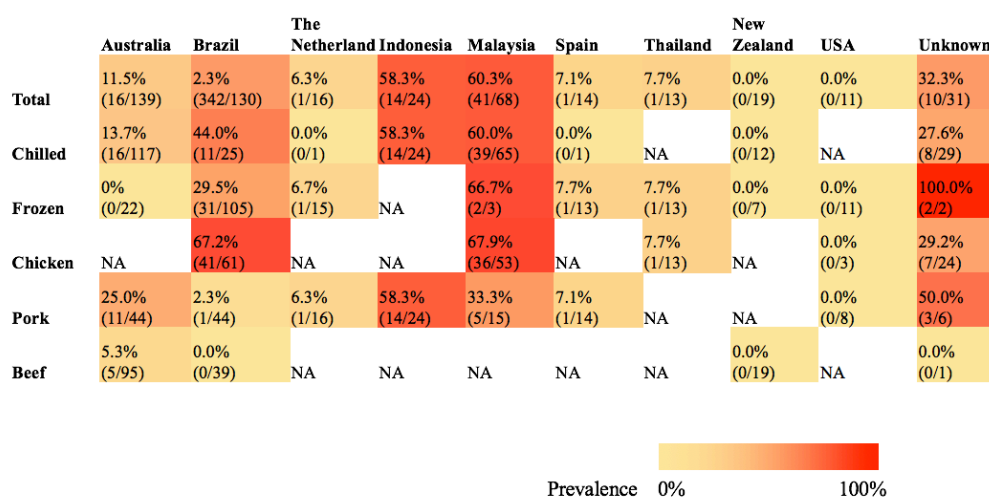


Figure 28. Heatmap for prevalence of ESBL in samples from supermarket based on countries and meat categories

NA means this category of samples were not collected. Samples from Denmark (n=1) Singapore (n=3) and Japan (n=4) were also collected, but not included in the heatmap because of the small sample size.

4.3.2. Types of ESBL genes and co-existence of other AMR genes

A total of 225 ESBL-producing *E. coli* isolates were obtained from 182 samples. Figure 29 shows the prevalence of AMR genes by class of antimicrobials. The top AMR genes in all 225 ESBL isolates are beta-lactam- resistance genes (100%), aminoglycoside resistance genes (92.4%), sulphonamide resistance genes (86.2%). For the last-resort antimicrobial colistin, its resistance genes exist in 15.6% of all isolates. Among all these classes of antimicrobials, beta-lactam-resistance genes and aminoglycoside resistance genes exhibit great variety. There are 16 and 20 types of resistance genes for these two classes. Among the 225 ESBL-producing *E. coli* isolates, 172 (76.4%) carry *bla*_{CTX-M} genes, 102 (45.3%)

of them carry *bla*_{TEM} genes and 52 (23.1%) carry *bla*_{SHV} genes. Besides these most common three beta-lactamase genes, *bla*_{CMY-2}, *bla*_{OXA} and *bla*_{DHA} were also found. In terms of ESBL genes, *bla*_{CTX-M-55} (57/225, 25.3%) and *bla*_{CTX-M-65} (40/225, 17.8%). Among all the resistance genes, phenicol resistance gene *floR* (149/225, 66.2%) and tetracycline resistance gene *tet(A)* (146/225, 64.9%) are the most frequent AMR genes.

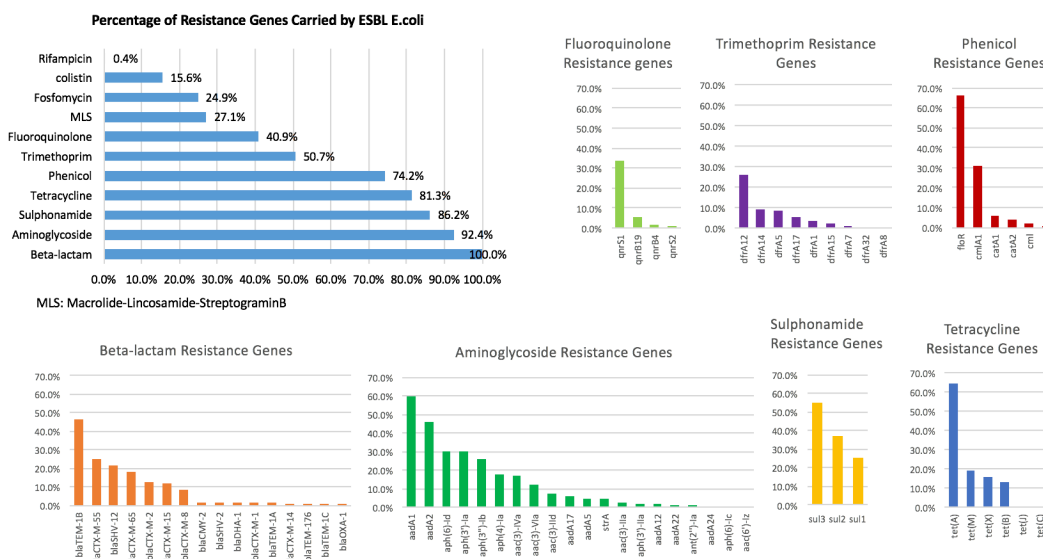


Figure 29. Percentage of AMR genes carried by ESBL-producing *E. coli* isolates

4.3.3. Genetic and phenotypic characterization of ESBL-producing isolates carrying *mcr* genes

Colistin resistance (*mcr*) gene was detected in 35 (15.6%) of ESBL-producing *E. coli* isolates *mcr-1*, n=33; *mcr-3.1*, n=1 and *mcr-5.1*, n=1). Interestingly, nearly all *mcr* genes (32/34, 94.1%) co-existed with ESBL gene *bla*_{CTX-55} (9/34, 26.5%) or *bla*_{CTX-M-65} (23/34, 67.6%) (Table 16). One exception is that *mcr-5.1* co-exist with *bla*_{SHV-12} and *bla*_{TEM-1B}, and another exception is one *mcr-1* gene co-exist with *bla*_{CTX-M-14} and *bla*_{TEM-1B}. The MIC against 31 antimicrobial agents of these

35 isolates were determined (detailed result shown in Supplementary Table 7). All these isolates showed characteristic resistance pattern of ESBL: resistant to penicillins most of cephalosprins and monobactams but sensitive to carbapenems. All the 35 isolates were confirmed as colistin resistance phenotypically (MIC $\geq$ 4 μ g/ml) except E8ESBLB1 carrying *mcr-5*. The MIC of E8ESBLB1 is equal or less 2 μ g/ml. Besides beta-lactam resistance and colistin resistance, resistance to chloramphenicol (35/35, 100%), tetracyclines (35/35, 100%), aminoglycosides (30/35, 85.7%), trimethoprim/ sulfamethoxazole (29/35, 82.9%), fosfomycin (26/35, 74.3%), fluoroquinolone (21/35, 60.0%) were also prevalent.

Replicons types including IncI2 (19/33), IncX4 (4/33), IncHI2 (2/33) and IncB/O/K/Z (1/33) were detected on 28 contigs carrying *mcr-1* (Table 16). No other AMR genes were found on the same contigs carrying *mcr* genes. For the other seven contigs carrying *mcr-1* but no replicons, three of them (E227ESBL_contig51, E534ESBLPP_contig37, E564ESBLPP_contig35) can be matched to IncI2-carrying contigs (Figure 30). One of the contigs E225ESBLB_contig37 is highly similar with part of contigs carrying IncHI2 (figure not shown). For the rest three contigs, there is no matchable contigs in our study, after blasting in NCBI, two of them can be aligned to IncHI1 plasmids and there are also IncHI1 replicon detected in their genomes. The other contig in the length of 70840 bp can be aligned to chromosome of *E. coli* strain CRE1540 (Genbank number CP019051.1) with 98.56% identity and 94% query coverage. This indicates that *mcr-1* gene in this isolate is located on the chromosome instead of plasmids. Detailed information of these contigs are shown in Supplementary Table 9.

Table 16. Co-existence of *mcr* genes and beta-lactamase genes and types of plasmid carrying *mcr*

		<i>mcr</i>							
		<i>mcr-5</i>	<i>mcr-3.1</i>	<i>mcr-1</i>					
					IncI2	IncX4	IncHI2	IncB/O/K/Z	unknown
Total	35	1	1	33	19	4	2	1	7
CTX-M-55	10	0	1	9	5	0	1	1	2
CTX-M-65	23	0	0	23	14	4	1	0	4
TEM-1B	25	1	0	24	12	4	2	0	6

4.3.4. Phylogenic analysis based on SNPs

Phylogeny trees based on SNP were built for 225 isolates in our study and 67 isolates from human community in Singapore. Supplementary Figure 1 shows the initial trees for all isolates, however, one of isolates E178ESBLW1 (shown in red on Figure 31) showed great difference with the rest of isolates. The closest genome of our isolates still has 37640 SNPs difference. As a result, the genome of E178ESBLW1 was removed on final figure (Figure 31) to have a better resolution for other genomes. For Figure 31 (a), the outer circle shows meat sources of isolates. The isolates from three meat types does not show obvious phylogroups but it could be observed that most of isolates from chicken and pork assembled on two sides of the circle. Isolates from beef are too few to show any obvious cluster.

MLST result is shown on the inner circle. Unknown ST or ST that appears less than twice are shown without color. The most prevalent ST is ST117 and all of these ST117 isolates are located on the same cluster (shadowed in yellow). Four isolates (three belong to ST3258 and one belongs to ST8261) are also located on

the same cluster. Actually, for the seven host gene for ST typing, there is only one allele difference between ST3258, ST8261 and ST117. This cluster consists of 41 isolates with two from pig, five from beef, all the rest from chicken.

The phylogenic comparison between isolates from meats and human (Figure 31 (b)) indicates most of isolates are located on source-dependent clusters. However, two isolates from human (shown in red squares) are quite close to isolates from meats: isolate with accession number SRR7371310 is quite close to E90ESBLB with 25 SNPs difference and isolate with accession number SRR7371348 is close to E437ESBLPP with 35 SNPs difference.

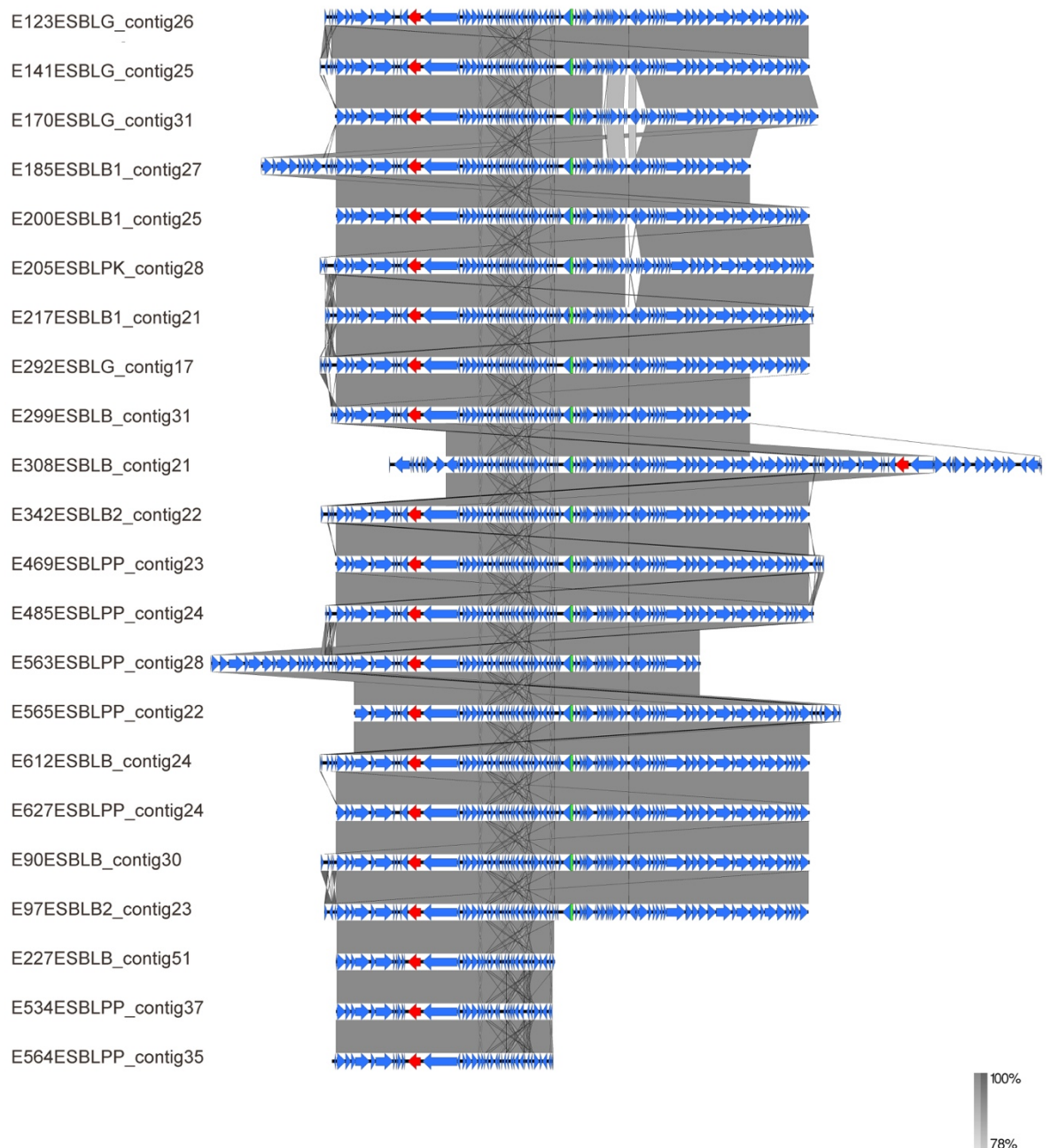


Figure 30. Genetic environment comparison of contigs carrying *mcr-1* and replicon IncI2.

Red arrows indicate the location of *mcr-1* and green rectangles indicate the location of IncI2 replicons. The last three contigs do not carry any replicons but their genetic environments are similar to the contigs on figure. The arrows indicate the direction of gene transcription.

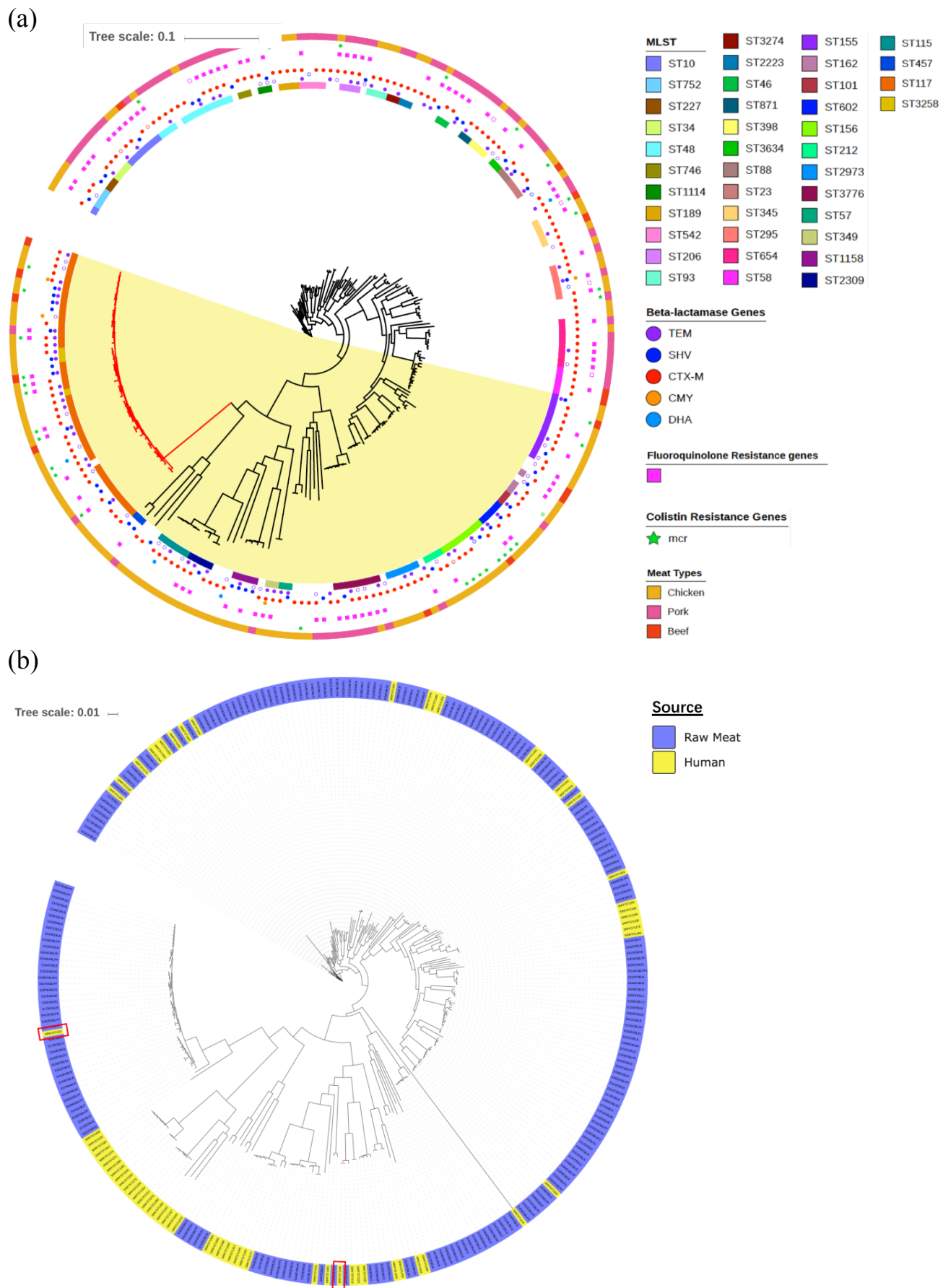


Figure 31. Phylogenetic analysis of ESBL-producing *E. coli* isolates based on SNPs.

(a) SNP tree of 224 genomes of ESBL-producing *E. coli* isolates in this study. (b) SNP tree of 224 genomes in this study and 67 ESBL-producing *E. coli* isolated from human community. Figures were visualized by iTOL.

4.4. Discussion

ESBL-producing bacteria especially *E. coli*, which are resistant to most of beta-lactams, have resulted in poor clinical outcomes and should be worth deeper investigation [45]. The existence of ESBL *E. coli* in food and their possible transmission to human is an important issue of public health. In view of this, we performed the largest meat sample collection in Singapore and did analysis for the prevalence and genetic contents of ESBL *E. coli* present in samples. In addition, the comparison between ESBL *E. coli* in this study and from human was performed based on SNP analysis.

Previously, Lim et al showed very high percentage (78.9%) of ESBL *E. coli* in retail raw chicken in Singapore with limited sample size (n=26), and CTX-M is ubiquitous ESBL genes in ESBL *E. coli* [50]. Our study supports the main conclusion with relatively lower prevalence, however, there are still more than half of samples (51.2%) carried ESBL *E. coli*. The prevalence of ESBL *E. coli* in frozen chicken (51.5%) does not show significant difference with that of chilled chicken (51.0%). Such systematic prevalence studies for retail meats were also performed in other countries such as UK [51] and China [52]. Although the results are not completely comparable because of different sampling and isolation methods, the prevalence trend among three types of meats are the same (chicken > pork > beef).

There are four classes of beta-lactamases, among these, class A and class C beta-lactamases are more commonly reported compared to class B and D. TEM, SHV and CTX-M all belong to class A and are usually found in Gram-negative bacteria, however, CTX-M did not emerge as predominant type of ESBL until the beginning of 21st century [263, 264]. Before that, most ESBLs were derivatives of TEM or SHV. In our study, the most prevalent beta-lactamase gene type is *bla*_{CTX-M} (76.4%), which is different from China (from 2011-2014) where *bla*_{TEM} is the predominant gene type [52]. The genes *bla*_{CTX-M-15} and *bla*_{CTX-M-14} are predominant in most of regions in the past publications [265], which is different with the situation in our study where the predominant *bla*_{CTX-M} types are *bla*_{CTX-M-55}, *bla*_{CTX-M-65}, *bla*_{CTX-M-2}, *bla*_{CTX-M-15}, *bla*_{CTX-M-8}, successively. *bla*_{CTX-M-14} only appeared in one isolate. But actually CTX-M-55 also belongs to the CTX-M-1 group and is a derivate of CTX-M-15 with one amino acid substitution and enhanced catalytic activity [266], and CTX-M-65 differs from CTX-M-14 by two amino acid substitutions [267]. The predominance of *bla*_{CTX-M-65} and *bla*_{CTX-M-55} was also observed in ESBL *E. coli* in chicken in South Korea [268]. This may indicate the predominant CTX-M types have developed a higher level of resistance. Whether *bla*_{CTX-M-55}/*bla*_{CTX-M-65} has taken the place *bla*_{CTX-M-15}/*bla*_{CTX-M-14} as the dominant type in other Asian countries needs further study in other countries.

ST117 is one of the most frequent ST of avian pathogenic *E. coli* (APEC), the subset of extraintestinal pathogenic *E. coli* (ExPEC). APEC are found in the normal intestinal flora of healthy birds and can cause severe disease. They are

the leading cause of mortality and morbidity in poultry [269]. Many human ExPEC share the ST, serotype and virulence genes with APEC. As a result, it is possible that poultry-related APEC become zoonotic pathogen and pose threat to human health [270]. ST117, one of most common ST of APEC, is regarded as an emerging human pathogen [271]. Most importantly, its carriage of ESBL has been reported many times [272-274]. This is also observed in our study. 24.0% (31/129) of ESBL isolates from chicken belong to ST117 group, which is the most prevalent ST in the isolate collection from chicken. In addition, 9 of them (29.0%) carry *mcr-1* genes. In spite of multi-resistance of ST117 isolates, we did not find at least 5 related virulence genes in these genomes of isolates, as a result, they cannot be defined as APEC by pathotype [275]. However, virulence genes such as *iss*, *astA* and *iroN* are detected in most of ST117 isolates. It would be quite troublesome if these multi-drug resistant isolates acquire specific virulence factors.

Colistin is regarded as the last resort for multidrug-resistant infections. Its main bactericidal mechanism is to replace the magnesium(Mg^{2+}) and calcium (Ca^{2+}) ions from the phosphate groups of lipopolysaccharide (LPS) on the membrane, which leads to the leakage of cell contents [64]. The emergence of transferable colistin resistance gene *mcr* recently has attracted great attention globally. In our study, the existence of *mcr* genes is observed in 15.6% (35/225) of all ESBL isolates. Among three types of meats, although ESBL isolates from beef are quite few (17/225 are from beef), four of them carry *mcr-1* and one of them carry *mcr-3.1*, which makes the prevalence of *mcr* genes not low (29.4%), even higher than that in chicken isolates (27/129, 20.9%). This may indicate the dissemination of

mcr genes in ESBL-producing isolates in beef. Whether this dissemination of *mcr* genes appear in all isolates from beef or they prefer to co-exist with ESBL isolates from beef needs further investigation.

All the assembled contigs carrying *mcr-1* genes are large (>20,000bp) compared with most of other AMR genes, which provided the possibility to explore the genetic environment of *mcr-1* and determine the plasmid type. The most prevalent types of plasmids carrying *mcr-1* in this study are IncI2 (19/33) and IncX4 (4/33). IncI2, IncX4 and IncHI2 accounted for more than 90% of reported *mcr-1* carrying plasmids [276]. Both IncI2 and IncX4 belong to narrow-host range plasmids in the family of Enterobacteriaceae and they are also most frequently reported plasmid types carrying *mcr-1* [277, 278]. Three isolates carry homologous regions with these contigs carrying *mcr-1* and IncI2, but IncI2 replicon was not detected in their genomes, this may indicate the flanking of this region into other types of plasmids. The ESBL isolates carrying the same *mcr* gene on the same types of plasmid with conserved genetic environment could be from different meat types and country sources. For example, the four isolates which carry *mcr-1* and IncX4 replicon on the same contig are from Holland, Brazil, Malaysia, and Australia, respectively. The meat types are pork, chicken, chicken, and beef accordingly. The variety of sources indicates the wide dissemination of this plasmid carrying *mcr-1* genes.

Transmission of antimicrobial resistant bacteria from food-producing animals to human has always been controversial, but there is evidence that whole bacterium transfer and mobile genetic elements-mediated transfer both play a role in AMR

transmission [279]. Our comparison of isolates from raw meats and human community in Singapore shows most of the isolates from different sources are located on different clusters based on SNPs. Two of isolates from human fecal samples showed close relationship with isolates from meats (25 SNPs difference and 35 SNPs difference). Although collection dates of human samples are ahead of our study, this could indicate bacterial transmission from the same source of our isolates to human previously by food consumption. However, the probability and quantitative analysis of this problem require more data and further investigation.

4.5. Conclusion

The high prevalence of ESBL in retail raw meats especially chicken showed in this study should draw our attention. Its co-existence with colistin resistance genes on plasmid even worsen the situation. Phylogenetic analysis indicates the divergence of ESBL-producing *E. coli* from raw meats and human community, however, possible bacteria transmission between food and human was found. Surveillance of ESBL-producing organism in imported meats in Singapore is necessary. More WGS data of ESBL in Singapore are needed. Further comparison of ESBL from food and clinical situation should be done in the future.

Chapter 5 ESBL-producing clinical *E. coli* from Thailand Hospital

5.1. Introduction

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) is a major human pathogen and the most common cause of urinary tract infections (UTI) and bacteremia, and the second most common cause of neonatal meningitis [280, 281]. The *E. coli* clonal group, sequence type 131 (ST131) is the most common and important lineage of ExPEC in this century. It was firstly reported in 2008, however, evidence shows that it may have risen to prominence as early as 2003 [282]. *E. coli* ST131 especially for clade C greatly changed the population structure of ExPEC. Its success could partially result from the acquisition of virulence factors and multidrug resistance, but it still remains unclear which unique feature made it such a prevalence compared with other lineages [280]. The characteristic antimicrobial resistances of *E. coli* ST131 are fluoroquinolone resistance (FQ-R) and broad cephalosporin resistance caused by extended-spectrum beta-lactamase (ESBL) production. ST131 is responsible for 60-90% of FQ-R ExPEC and 40-80% of ESBL ExPEC [282]. As a result, *E. coli* ST131 is becoming tough clinical challenge globally. In Thailand, a number of studies have reported the occurrence of ESBL-producing *E. coli* ST131 in hospitals [249], farms (farmers and chicken) [283], water environment [249, 284] and poultry meats [285], which indicates the wide spread of this type of strain.

Whole genome sequencing (WGS) is becoming a useful tool for the analysis of ST131 expansion. WGS-based studies revealed that ST131 has different clades (a group of organisms considered as having evolved from a common ancestor). Each of the clade is characterized by marker allele of *fimH* (a bacterial adhesion that helps pathogenic *E.coli* to adhere to human epithelial cells [286]) : *fimH41* in clade A, *fimH22* in clade B and *fimH30* in clade C (most prevalent clade, also known as H30-R). Subset of clade C containing the ESBL gene *bla*_{CTX-M-15} referred to as clade C2 or H30-Rx [287, 288]. Their distributions reflect the transmission and evolution history [289]. However, the epidemiology studies of ESBL-producing *E. coli* based on WGS are quite limited. Up-to-date genome-wide information for ESBL-producing *E. coli* (ESBL-EC) from patients is needed especially for ST131. Therefore, the purpose of this study was to characterize the features (such as AMR, virulence factors, ST and serotype) of ESBL-EC from diarrhea patients in Thailand, focusing especially on ST131 based on both phenotype testing and genome sequencing, their phylogenetic relationship with previous Thailand isolates and worldwide isolates was also explored.

5.2. Materials and Methods

5.2.1 Isolates collection

A total of 28 *Escherichia coli* strains were isolated from various samples including stool, blood urine and pus of diarrheal patients hospitalized at the Phayao Ram Hospital, Phayao Province, Thailand from 2015 to 2017. The study was conducted under ethical approval (No. 57 02 04 0020) granted by the Ethics

Committee of the University of Phayao. The detailed information of patients and isolates are shown in Table 17.

The samples including stool, blood urine and pus of diarrhea patients were streaked onto sterile MacConkey agar and incubated at 37 °C for 12 - 18 Hrs. Red colony referred as suspected *E. coli* colonies were inoculated to Triple Sugar Iron (TSI) slant⁵ (Biomedica, Nonthaburi, Thailand) and the species were also double confirmed by further genome sequencing results.

5.2.2 Determination of antimicrobial resistance profile and ESBL production

Susceptibility to antibiotics was performed using a disk diffusion method of the Clinical and Laboratory Standards Institute (CLSI) [290] with Ampicillin (AMP) 10 µg, Amoxicillin-clavulanate (AMC) 20/10 µg, Ceftazidime (CAZ) 30 µg, Cefotaxime (CTX) 30 µg, Cefepime (FEP) 30 µg, Cefoxitin (FOX) 30 µg, Ceftriaxone (CRO) 30 µg, Imipenem (IPM) 10 µg, Nalidixic acid (NA), Norfloxacin (NOR) 10 µg, Ciprofloxacin (CIP) 5 µg, Levofloxacin (LEV) 10 µg, Gentamicin (GEN) 10 µg, and Sulphamethox/trimethoprim (SXT) 1.25 µg/ 23.75 µg, (Oxoid, Hampshire, UK). *Escherichia coli* ATCC 25922 was used as a negative control strain. ESBL test was performed using the combination disk method according to CLSI criteria with both ceftazidime (30 µg), cefotaxime (30

⁵ TSI is to test a microorganism's ability to ferment sugars (lactose, sucrose, and glucose) and to produce hydrogen sulfide. When lactose (or sucrose) is fermented by *E. coli*, the acid produced turns the phenol red indicator yellow both in the butt and in the slant. The generated gases produces bubbles/cracks on the medium.

µg) alone and combined with clavulanic acid (10 µg) (Oxoid, Hampshire, UK). In-house known ESBL-producing *Escherichia coli* and ESBL-negative *Escherichia coli* strains ATCC 25922 were used as controls.

5.2.3 DNA extraction and whole genome sequencing

The detailed methods for DNA extraction and whole genome sequencing were described previously in Chapter 3 [252]. Briefly speaking, genomic DNA of isolates was extracted using QIAamp® DNA Mini Kit, and all the procedures followed the manufacturer's instructions (Qiagen, Germany). Sequencing was performed by Illumina HiSeq2500 sequencer in rapid model with 250bp paired-end reads. Raw sequence data have been submitted to the European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena>) under study accession number: PRJEB34463.

5.2.4 Genome Assembly and bioinformatic analysis

The assembly of raw reads were performed using DTU FoodQCPipeline (<https://bitbucket.org/RolfKaas/foodqcpipeline/src/master/>). In short, it started with FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) assessment and reads trimming by bbduk2 (<https://jgi.doe.gov/data-and-tools/bbtools/>). Then reads were assembled using SPAdes v3.11.05 [186]. The AMR related genes and site mutations were detected using ResFinder 3.2 [192]. Virulence factor detection was done by VirulenceFinder 2.0 [291]. Serotype was determined by SerotypeFinder 2.0 [292]. The default setting was used, that is,

threshold for 90% identity and 60% minimum length. Sequence type was determined by MLST (Multi-Locus Sequence Type) 2.0 [256].

5.2.5 Phylogenetic analysis

Raw reads of whole genome sequencing records for 442 ST131 isolates were downloaded from ENA. All of these and sequence data of ST131 in our study were mapped to the reference genome of ST131 representative strain SE15 (AP009378) [293] using Burrows–Wheeler Aligner (BWA) [260]. SNPs were called by SAMTools and filtered based on the following parameters: a minimum of mapping quality of 25; a minimum of 10x depth and 10% relative depth; a minimum of SNP quality of 30; a minimum of 10bp of prune zone [261]. The Trees were built by CSI phylogeny pipeline which is available on the website of Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>) [259]. Then the trees were annotated and visualized by iTOL v4 (<https://itol.embl.de/>) [262]. The accession numbers of all the sequence data used in this study and their origin countries were shown in Supplementary Table 12.

5.2.6 Genetic environment analysis of ESBL genes

Genome annotation was performed by RAST (<http://rast.theseed.org/FIG/rast.cgi>) [188] and manually corrected by NCBI BLAST [294]. The contigs carrying AMR genes and plasmid replicon were selected from assemblies. The location of *bla*_{CTX-M} (chromosome or plasmid) was determined by BLAST result of the contig against database. The insertion sequence (IS) was detected by ISfinder (<https://isfinder.biotoul.fr/>) [295].

5.3. Results

5.3.1 General information of isolates

For these 28 ESBL-positive *E.coli* isolates, 14 are isolated from male patients and 13 of them are from female patients. The gender information of one patient was unknown. The specimen types are urine (n=17), stool (n=7), pus (n=2) and blood (n=2). ST131 is the most common sequence type in these isolates (14/28, 50.0%). Other typical ExPEC ST such as ST405, ST410 were also observed. All the isolates were assigned to 13 STs (Table 17). Among ST131 isolates, 6 of them belong to serotype O16:H5 and other 8 belong to O25:H4.

Table 17. Meta-data of samples and isolates

Isolate	Sequence type	ST131 clade	Serotype	Virulence factor	Collect ion year	Specimen type	Age	Gender	Accession
BK_EC176	ST-131	A	O16:H5	<i>cnfI, iha, sat, senB</i>	2015	stool	7	2	ERR3528488
BK_EC441	ST-131	A	O16:H5	<i>cnfI, iha, sat, senB</i>	2016	pus	60	1	ERR3528497
BK_EC502	ST-131	A	O16:H5	<i>cnfI, iha, sat, senB</i>	2017	urine	28	1	ERR3528502
BK_EC503	ST-131	A	O16:H5	<i>cnfI, iha, sat, senB</i>	2017	urine	66	2	ERR3528503
BK_EC181	ST-131	A	O16:H5	<i>iha, sat, senB</i>	2015	stool	9	2	ERR3528489
BK_EC182	ST-131	A	O16:H5	<i>iha, sat, senB</i>	2015	stool	1	1	ERR3528490
BK_EC430	ST-131	C1-M27	O25:H4	<i>aafC, cnfI, gad, iha, ireA, iss, nfaE, sat</i>	2016	urine	75	2	ERR3528495
BK_EC373	ST-131	C1-M27	O25:H4	<i>ccI, cnfI, gad, iha, iss, sat, senB</i>	2016	pus	66	2	ERR3528494
BK_EC438	ST-131	C1-nM27	O25:H4	<i>ccI, cnfI, gad, iha, iss, sat, senB</i>	2016	urine	77	1	ERR3528496
BK_EC456	ST-131	C1-nM27	O25:H4	<i>cnfI, gad, iha, iss, sat, senB</i>	2016	blood	50	1	ERR3528499
BK_EC459	ST-131	SEA-C2 (H30-Rx)	O25:H4	<i>cnfI, gad, iha, iss, sat, senB</i>	2017	urine	0.5	1	ERR3528500
BK_EC58	ST-131	SEA-C2 (H30-Rx)	O25:H4	<i>cnfI, gad, iss, mchB, mchC, mchF, sat</i>	2017	urine	2	1	ERR3528506
BK_EC455	ST-131	SEA-C2 (H30-Rx)	O25:H4	<i>gad, iha, iss, sat, senB</i>	2016	urine	89	2	ERR3528498
BK_EC77	ST-131	SEA-C2 (H30-Rx)	O25:H4	<i>gad, iha, iss, sat, senB</i>	2017	urine	0.6	1	ERR3528508
BK_EC15	ST-10	-	O21:H27	<i>gad</i>	2015	urine	43	2	ERR3528487
BK_EC13	ST-206	-	O49:H10	<i>celb, cif, eae, espA, espF, espJ, gad, nleA, nleB, tir</i>	2015	stool	1	2	ERR3528485
BK_EC14	ST-206	-	O49:H10	<i>celb, cif, eae, espA, espF, espJ, gad, nleA, nleB, tir</i>	2015	stool	0.8	1	ERR3528486
BK_EC59	ST-354	-	O1:H34	<i>air, astA, eilA, gad, lpfA</i>	2017	urine	unknown	unknown	ERR3528507
BK_EC460	ST-38	-	O86:H18	<i>aap, air, astA, capU, eatA, eilA, gad, iss, nfaE</i>	2017	urine	50	1	ERR3528501
BK_EC57	ST-3858	-	O8:H10	<i>gad, lpfA</i>	2017	urine	47	2	ERR3528505
BK_EC364	ST-405	-	O102:H6	<i>air, eilA, gad</i>	2015	urine	81	2	ERR3528493
BK_EC101	ST-410	-	O-non typable: H34	<i>gad, iroN, iss, lpfA, mchF</i>	2015	urine	71	1	ERR3528483
BK_EC33	ST-4450	-	O53:H18	<i>gad, lpfA</i>	2015	blood	57	1	ERR3528492
BK_EC109	ST-648	-	O-non typable:H6	<i>air, eilA, gad, iha, lpfA, sat</i>	2015	stool	1	1	ERR3528484
BK_EC285	ST-710	-	O8:H30	<i>astA, gad, sepA</i>	2015	urine	0.6	2	ERR3528491
BK_EC100	ST-73	-	O2/O50:H1	<i>cnfI, gad, iha, ireA, iss, pic, sat, vat</i>	2015	stool	1	1	ERR3528482
BK_EC97	ST-73	-	O2/O50:H1	<i>cnfI, gad, iha, ireA, iss, pic, sat, vat</i>	2017	urine	64	2	ERR3528509
BK_EC517	ST-88	-	O-non typable:H12	<i>astA, cba, cma, gad, iroN, iss, lpfA</i>	2017	urine	64	2	ERR3528504

Table 18. AMR related genes and site mutations on genomes of isolates in this study

Isolate ID	AMR related genes or site mutations								
	Aminoglycoside	Sulphonamide	Quinolone	Trimethoprim	Tetracycline	Beta-lactam	Macrolide	Phenicol	Colistin
BK_EC13	<i>aac(3)-Ild</i> -like, <i>aadA1</i> -like, <i>aadA2</i> , <i>strA</i> , <i>strB</i>	<i>sul2</i> , <i>sul3</i>	<i>QnrS1</i> , <i>parC</i> p.A56T	<i>dfrA12</i>	<i>tet(A)</i> -like, <i>tet(X)</i> -like	<i>bla</i> _{CMY-2} , <i>bla</i> _{CTX-M-55} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	<i>catA2</i> -like, <i>cmlA1</i> -like, <i>floR</i> -like	--
BK_EC14	<i>aac(3)-Ild</i> -like, <i>aadA1</i> -like, <i>aadA2</i> , <i>strA</i> , <i>strB</i>	<i>sul2</i> , <i>sul3</i>	<i>QnrS1</i> , <i>parC</i> p.A56T	<i>dfrA12</i>	<i>tet(A)</i> -like, <i>tet(X)</i> -like	<i>bla</i> _{CMY-2} , <i>bla</i> _{CTX-M-55} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	<i>catA2</i> -like, <i>cmlA1</i> -like	--
BK_EC15	<i>aac(3)-Ild</i> -like	--	<i>QnrS1</i>	--	<i>tet(A)</i> -like	<i>bla</i> _{CTX-M-55}	<i>mph(A)</i>	<i>catA2</i> -like, <i>floR</i> -like	--
BK_EC33	<i>aac(3)-Ild</i> -like	<i>sul2</i>	<i>QnrS1</i>	--	--	<i>bla</i> _{CTX-M-55}	<i>mph(A)</i>	<i>catA2</i> -like, <i>floR</i> -like	<i>mcr-1</i>
BK_EC57	<i>aac(3)-Ild</i> -like, <i>strA</i> -like, <i>strB</i> -like	<i>sul2</i>	<i>QnrS1</i>	<i>dfrA14</i> -like	<i>tet(A)</i> -like	<i>bla</i> _{CTX-M-55} , <i>bla</i> _{TEM-1B}	<i>erm(B)</i> -like, <i>mph(A)</i>	<i>floR</i> -like	--
BK_EC58	<i>aac(3)-IIa</i> -like, <i>aadA5</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i> -like	<i>aac(6')Ib-cr</i> , <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>parE</i> p.I529L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-1}	<i>mph(A)</i> -like	<i>catB4</i>	--
BK_EC59	<i>aac(3)-IIa</i> -like, <i>aadA5</i>	<i>sul1</i> -like	<i>parC</i> p.S80I, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parE</i> p.I355T, <i>parE</i> p.S458A	<i>dfrA17</i>	<i>tet(B)</i>	<i>bla</i> _{CTX-M-55} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i> -like	--	--
BK_EC77	<i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>parE</i> p.I529L, <i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27}	<i>mph(A)</i>	--	--
BK_EC97	<i>aadA1</i> -like, <i>aadA5</i> -like, <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	--	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	<i>catA1</i> -like	--
BK_EC100	<i>aadA1</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	--	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	<i>catA1</i> -like	--
BK_EC101	<i>aac(3)-Ild</i> -like, <i>aadA1</i> , <i>aadA2</i>	<i>sul3</i>	<i>QnrS1</i> , <i>parE</i> p.S458A, <i>parC</i> p.S80I, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N	<i>dfrA12</i>	<i>tet(B)</i>	<i>bla</i> _{CTX-M-55} , <i>bla</i> _{TEM-1B}	<i>erm(B)</i> -like, <i>mph(A)</i>	<i>catA1</i> -like, <i>cmlA1</i> -like	--
BK_EC109	<i>aac(3)-IIa</i> -like, <i>aadA5</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i> -like	<i>aac(6')Ib-cr</i> , <i>parE</i> p.S458A, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parC</i> p.S80I	<i>dfrA17</i>	<i>tet(B)</i>	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-1} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	<i>catA1</i> -like, <i>catB4</i>	--
BK_EC176	<i>aac(3)-Ild</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>gyrA</i> p.S83L, <i>parE</i> p.I529L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	--	--
BK_EC181	<i>aac(3)-Ild</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>parE</i> p.I529L, <i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	--	--
BK_EC182	<i>aac(3)-Ild</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parE</i> p.I529L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	--	--
BK_EC285	<i>aac(3)-Ild</i> -like, <i>aadA1</i> , <i>aadA2</i> , <i>strA</i> , <i>strB</i>	<i>sul2</i> , <i>sul3</i>	<i>QnrS1</i>	<i>dfrA12</i>	<i>tet(A)</i> -like	<i>bla</i> _{CTX-M-55} , <i>bla</i> _{TEM-1B}	--	<i>cmlA1</i> -like, <i>floR</i> -like	--
BK_EC364	<i>aadA5</i> -like, <i>aac(6')Ib-cr</i>	<i>sul1</i> -like	<i>aac(6')Ib-cr</i> , <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parE</i> p.S458A, <i>parC</i> p.S80I	<i>dfrA17</i>	<i>tet(B)</i>	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-1}	<i>mph(A)</i>	<i>catB4</i>	--
BK_EC373	<i>aac(3)-Ild</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parE</i> p.I529L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	--	--
BK_EC430	<i>aac(3)-IIa</i> , <i>aac(6')Ib-cr</i>	--	<i>aac(6')Ib-cr</i> , <i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parE</i> p.I529L	--	--	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-1}	--	<i>catB4</i>	--
BK_EC438	<i>aac(3)-Ild</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>parE</i> p.I529L, <i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	--	--
BK_EC441	<i>aac(3)-Ild</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>gyrA</i> p.S83L, <i>parE</i> p.I529L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	--	--
BK_EC455	<i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parE</i> p.I529L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27}	<i>mph(A)</i>	--	--
BK_EC456	<i>aac(3)-IIa</i> -like, <i>aadA5</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i> -like	<i>aac(6')Ib-cr</i> , <i>parE</i> p.I529L, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parC</i> p.S80I, <i>parC</i> p.E84V	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-1}	<i>mph(A)</i> -like	<i>catB4</i>	--
BK_EC459	<i>aac(3)-IIa</i> -like, <i>aadA5</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i> -like	<i>aac(6')Ib-cr</i> , <i>parC</i> p.S80I, <i>parC</i> p.E84V, <i>gyrA</i> p.S83L, <i>gyrA</i> p.D87N, <i>parE</i> p.I529L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-1}	<i>mph(A)</i>	<i>catB4</i>	--
BK_EC460	<i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> , <i>sul2</i>	--	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27}	<i>mph(A)</i>	--	--
BK_EC502	<i>aac(3)-Ild</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>parE</i> p.I529L, <i>gyrA</i> p.S83L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	--	--
BK_EC503	<i>aac(3)-Ild</i> -like, <i>aadA5</i> , <i>strA</i> , <i>strB</i> -like	<i>sul1</i> -like, <i>sul2</i>	<i>parE</i> p.I529L, <i>gyrA</i> p.S83L	<i>dfrA17</i>	<i>tet(A)</i>	<i>bla</i> _{CTX-M-27} , <i>bla</i> _{TEM-1B}	<i>mph(A)</i>	--	--
BK_EC517	<i>aac(3)-Ild</i> -like, <i>strA</i> -like, <i>strB</i> -like	<i>sul2</i>	<i>QnrS1</i> , <i>gyrA</i> p.S83L	<i>dfrA14</i> -like	<i>tet(A)</i>	<i>bla</i> _{CTX-M-14} , <i>bla</i> _{CTX-M-55} , <i>bla</i> _{TEM-1B}	--	--	--

5.3.2 AMR phenotype and genotype

All isolates are multi-drug resistant (resistant to three or more classes of antimicrobials) related genes (Table 18). Aminoglycoside resistance genes and beta-lactam resistance genes exist in all isolates (28/28) in this study. The next prevalent AMR genes are sulphonamide resistance genes and tetracycline resistance genes (26/28, 92.9%). In terms of ESBL genes, *bla*_{CTX-M} type genes were detected in all isolates, which include *bla*_{CTX-M-14}, *bla*_{CTX-M-15}, *bla*_{CTX-M-27}, *bla*_{CTX-M-55}. Gene *bla*_{CTX-M-55} was only detected in non-ST131 isolates. Besides these, gene *bla*_{TEM-1B}, *bla*_{OXA-1} and *bla*_{CMY-2} were also observed. The patterns of beta-lactamase genes are shown in Table 19. Colistin resistance gene *mcr-1* exists in one isolate.

Table 19. Patterns of Beta-lactamase genes

ST131 (n=14)	Number
<i>bla</i> _{CTX-M-14} + <i>bla</i> _{TEM-1B}	2
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{OXA-1}	4
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{TEM-1B}	2
<i>bla</i> _{CTX-M-27}	2
<i>bla</i> _{CTX-M-27} + <i>bla</i> _{TEM-1B}	4
Non-ST131 (n=14)	Number
<i>bla</i> _{CTX-M-14} + <i>bla</i> _{CTX-M-55} + <i>bla</i> _{TEM-1B}	1
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{OXA-1}	1
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{OXA-1} + <i>bla</i> _{TEM-1B}	1
<i>bla</i> _{CTX-M-27}	1
<i>bla</i> _{CTX-M-27} + <i>bla</i> _{TEM-1B}	2
<i>bla</i> _{CTX-M-55}	2
<i>bla</i> _{CTX-M-55} + <i>bla</i> _{TEM-1B}	4
<i>bla</i> _{CTX-M-55} + <i>bla</i> _{TEM-1B} + <i>bla</i> _{CMY-2}	2

Detailed MIC result was shown in Supplementary Table 11. All isolates were sensitive to carbapenems and no related resistance genes were detected, either. Resistance to beta-lactams, tetracycline, colistin showed consistency in terms of

genotype and phenotype. For chloramphenicol, isolates carrying *catB4* did not show resistance but for other chloramphenicol resistance genes such as *cmlA1*, *floR*, *catA1*, the isolates showed corresponding resistance phenotype. For quinolones, the resistance-related site mutations were also detected. For those isolates with no quinolone resistance genes or site mutations (3/28), sensitivity phenotype to all tested quinolones were observed. Two isolates carrying only *QnrS1* (BK-EC33, BK-EC285) showed intermediate susceptibility to quinolones. For the rest of isolates, resistance to at least one quinolone antimicrobials were detected, which is in consistence of their genotypes. Other classes of antimicrobials were not included in the MIC testing so that comparison between genotype and phenotype was not performed.

5.3.3 Virulence factors and serotypes

A total of 35 types of virulence factors were found. Each isolate carried 1 to 10 virulence factor genes. Virulence factors present in ExPEC including adhesins (*iha*), toxins (*sat*, *pic*, *vat*, *astA*, *cnf1*), siderophores (*iroN*) and miscellaneous (*iss*) were observed in these isolates [160]. The most common virulence factors are *iha* (26/28), *gad* (22/28) and *sat* (17/28). For ST131 isolates, *sat* (14/14), *iha* (13/14) and *senB* (12/14) were most frequent. Whereas for non-ST131 isolates, *gad* (14/14), *lpfA* (6/14) and *iss* (5/14) most frequently appeared. Compared with ST131 isolates, the virulence factors in non-ST131 isolates showed higher diversity. The common virulence gene *senB* did not exist in any non-ST131 isolates.

For the serotypes of isolates (shown in Table 17), ST131s have two serotypes O16:H5 (6/14) and O25:H4 (8/14). For non-ST131s, the serotypes are quite various, and none of them have the same serotype with ST131. Three of them are O-non typable based on sequencing data.

5.3.4 Types and genetic environment of ESBL genes

There are 5 types of beta-lactamase patterns observed in ST131 isolates (Table 19). The ESBL CTX-M types of ESBL were detected in all isolates in this study. For ST131 isolates, the BLAST result of each contig carrying *bla*_{CTX-M} against NCBI database showed the presence of *bla*_{CTX-M} genes on chromosomes (8/14) and plasmids (6/14). Among the three types of CTX-Ms in ST131 isolates (CTX-M-14, CTX-M-15, CTX-M-27), CTX-M-15 and CTX-M-27 appeared on both plasmids and chromosomes. CTX-M-14 was observed only on chromosome. The ISfindre result showed that the adjacent mobile elements of *bla*_{CTX-M-14} and *bla*_{CTX-M-27} is IS903B, which belongs to IS5 family. The *bla*_{CTX-M-15} harboring on plasmids is adjacent to Tn3 family of transposon, and for those *bla*_{CTX-M-15}s on chromosome, their nearly mobile elements are ISEcp1 belong to IS1380 family.

Table 20. Location and genetic environment of *bla*_{CTX-M} carried by ST131 isolates

Pattern	Isolate ID	Location of <i>bla</i> _{CTX-M}	Adjacent mobile genetic elements
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{TEM-1B}	BK_EC181	<i>bla</i> _{CTX-M-15} : chromosome	ISEcp1 Family: IS1380
	BK_EC182	<i>bla</i> _{CTX-M-15} : chromosome	ISEcp1 Family: IS1380
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{OXA-1}	BK_EC430	<i>bla</i> _{CTX-M-15} : plasmid	Tn2 Family: Tn3
	BK_EC456	<i>bla</i> _{CTX-M-15} : plasmid	Tn2 Family: Tn3
	BK_EC459	<i>bla</i> _{CTX-M-15} : plasmid	Tn2 Family: Tn3
	BK_EC58	<i>bla</i> _{CTX-M-15} : plasmid	Tn2 Family: Tn3
<i>bla</i> _{CTX-M-27} + <i>bla</i> _{TEM-1B}	BK_EC176	<i>bla</i> _{CTX-M-27} : chromosome	IS903B Family: IS5

	BK_EC441	<i>bla</i> CTX-M-27: chromosome	IS903B Family: IS5
	BK_EC502	<i>bla</i> CTX-M-27: chromosome	IS903B Family: IS5
	BK_EC503	<i>bla</i> CTX-M-27: chromosome	IS903B Family: IS5
<i>bla</i> CTX-M-27	BK_EC455	<i>bla</i> CTX-M-27: plasmid	IS903B Family: IS5
	BK_EC77	<i>bla</i> CTX-M-27: plasmid	IS903B Family: IS5
<i>bla</i> CTX-M-14 + <i>bla</i> TEM-1B	BK_EC373	<i>bla</i> CTX-M-14: chromosome	IS903B Family: IS5
	BK_EC438	<i>bla</i> CTX-M-14: chromosome	IS903B Family: IS5

5.3.5 Comparison of ST131 isolates with global collection

The SNP tree (which ignore the branch length) for all ST131 isolates (genomes downloaded from database and those isolates in this study) collection is shown in Figure 32. The total number of genomes is 442. The maximum SNP difference is 7995. Metadata of all sequences was shown in Supplementary Table 12. Among this global collection, 24 isolates have less than 50 SNPs difference with at least one of our isolates. These isolates are from Thailand (8/24), Singapore (7/24), Australia (5/24), Laos (3/24) and New Zealand (1/24), mainly gathering in Southeast Asia and Oceania.

If the branch length is not ignored, the SNP tree is shown in Figure 33. To have a better view for the details, four sub-trees were made, and the numbers of genomes in these four clusters are 220, 84, 44, 74, respectively. None of our isolates was shown in cluster 4, as a result, cluster 4 was not shown in Figure 33. The 28 ST131 isolates in our study were distributed in the rest three clusters, which indicate the high diversity. For clade 1 based on 1113 SNPs, four of our isolates were included. The genomes which have less than 50 SNPs difference were marked with red dots. These are from Thailand (5/13), Singapore (3/13), Laos (3/13) and Australia (2/13). The SNPs difference ranges from 31 to 48. For clade 2 based on 1370 SNPs, five genomes showed less than 50 SNPs with our isolates (marked with blue dots). Three of them are from Singapore and two are

from Australia. The SNPs difference ranges from 40 to 48. For clade 3 based on 3087 SNPs in total, six genomes (marked with green dots) showed less than 50 SNPs with our isolates. Actually, the range is from 15 to 30 SNPs difference. Except that one from New Zealand, one from Australia, the rest four isolates are from previous Thailand study.

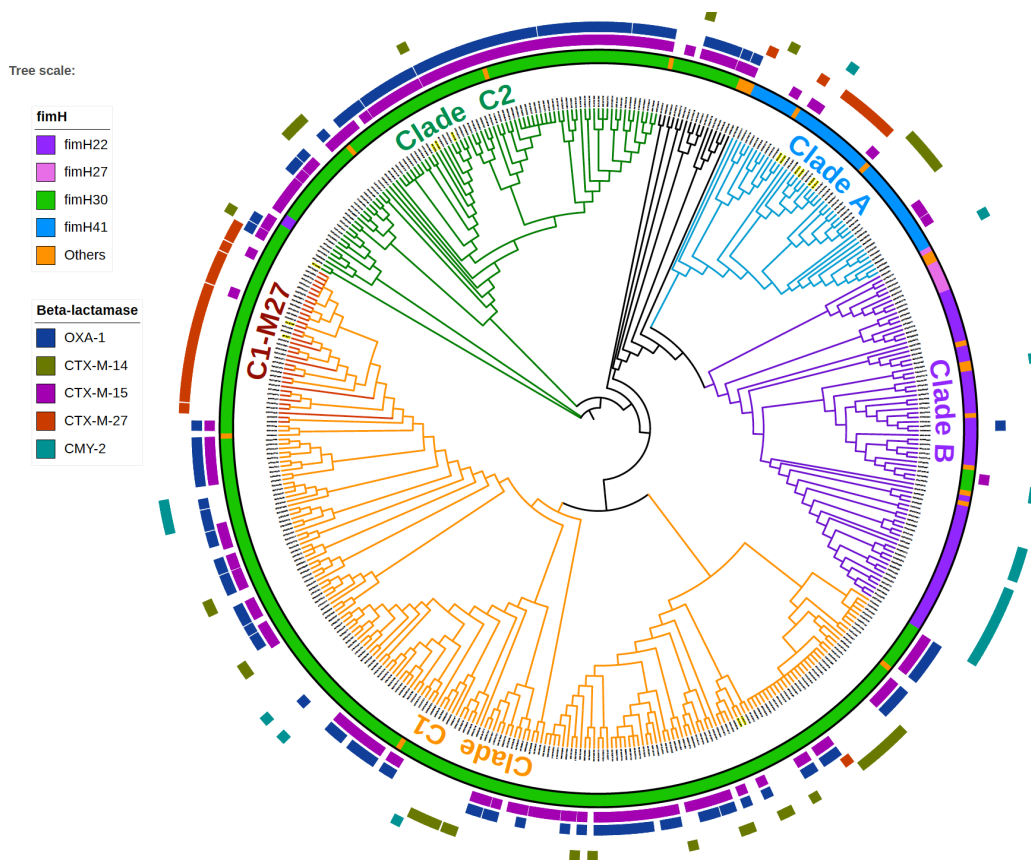


Figure 32. Phylogenetic tree based on SNPs for global ST131 collection

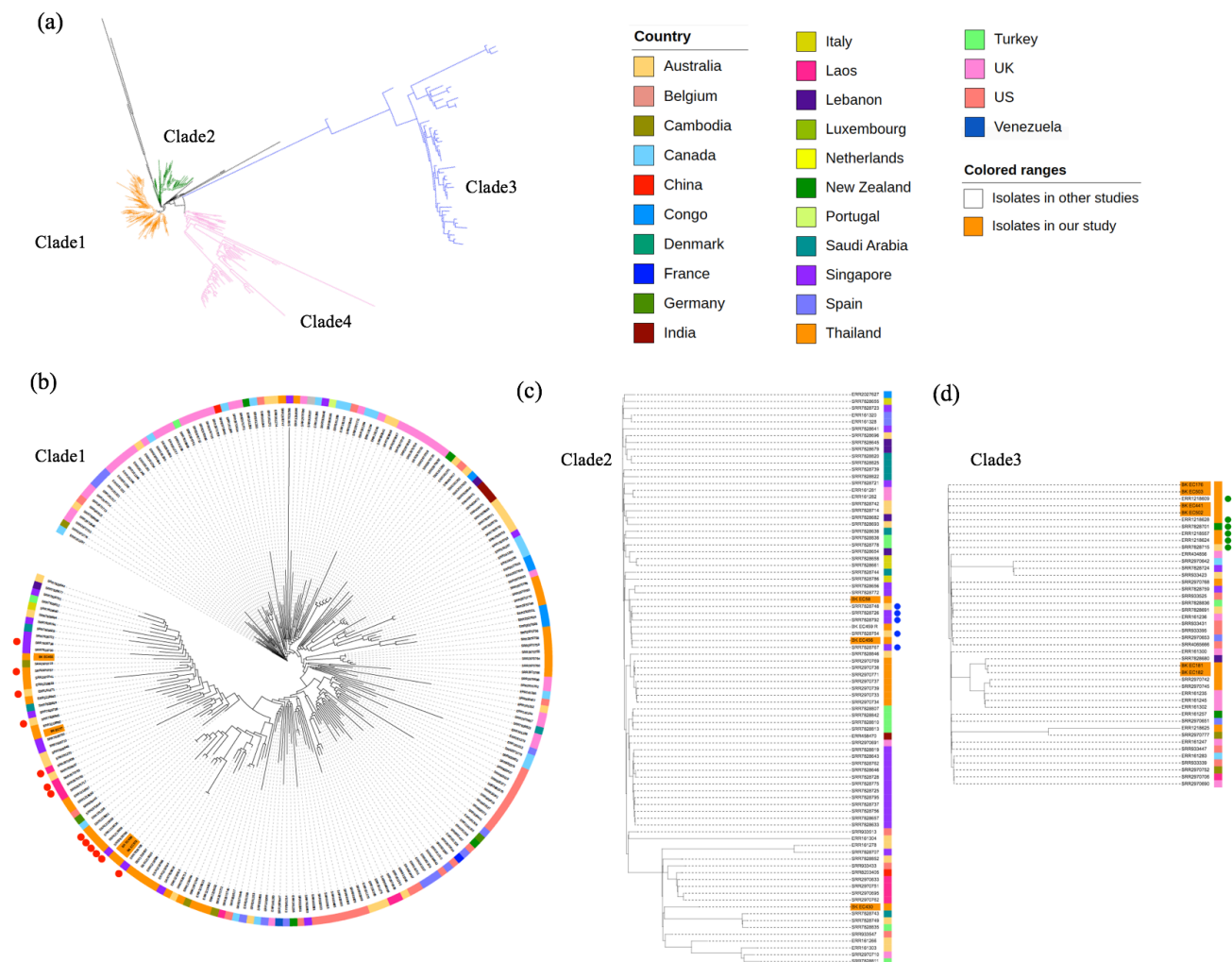


Figure 33. The phylogenetic trees for global ESBL-producing ST131 based on SNPs.

(a) is the SNP tree for all isolates which did not ignore the branch length; (b)(c)(d) are the trees for subgroups of isolates. The isolates ID shadowed in orange are our isolates in this study. The isolates marked with dots represent that they have less than 50 SNP difference with our isolates in the same cluster.

5.4 Discussion

Previously SHV and TEM were traditional ESBL types, however, ESBLs were dominated by CTX-M type since 2000. Epidemiology studies suggested that the fast spread of CTX-M was due to a single clone ST131 [296]. Our study supported the dominance of ST131 in ESBL-producing *E. coli* in Thailand hospital. All of these ESBLs carried *bla*_{CTX-M} genes. The genetic analysis suggested that these CTX-Ms were not only located on plasmids, which is the most common situation and easier way to transfer, but also located on chromosome (8/14). The IS elements around CTX-Ms suggested the possible transfer methods. It is noteworthy that all the *bla*_{CTX-M-27} and *bla*_{CTX-M-14} are flanked by IS903 which belongs to IS5 family, no matter they are located on chromosomes or plasmids. Gene *bla*_{CTX-M-15} on chromosome in this study is adjacent to *ISEcp1*. *ISEcp1* was reported as a mobile genetic element which plays an important role in beta-lactamase expression and transfer [297]. Its association with integration of CTX-M-15 into chromosome of *E. coli* was reported before [298]. It was also proved that *ISEcp1* was responsible for the integration of the gene *bla*_{CTX-M-2} into the chromosome of *Proteus mirabilis* [299], which may indicate the important role of *ISEcp1* in mobilization of CTX-Ms in Enterobacteriaceae.

CTX-M-15 is the most commonly reported in ESBL-producing ST131 worldwide, however, in Japan, ESBL-producing ST131 commonly carry *bla*_{CTX-M-14} and *bla*_{CTX-M-27} [300]. Despite of the limited sample size of our study, the prevalent CTX-M types are in concordance with the Japanese study. Recent study

in France also showed the dominance of *bla*_{CTX-M-27}-carrying ST131 [301]. Whether *bla*_{CTX-M-14} and *bla*_{CTX-M-27} are taking place of *bla*_{CTX-M-15} as new dominant ST131 strains worldwide needs further explorations from other countries.

Besides the CTX-Ms, other beta-lactamase genes (such as TEM-1B, OXA-1 and CMY-2) were also observed in both ST131s and non-ST131s. CMY-2 is the most common plasmid-encoded AmpC beta-lactamase in *E. coli* and other Enterobacteriaceae. Like ESBL, it results in resistance to cephalosporin, even worse, it causes resistance to carbapenem in combination with loss of outer membrane porins [302]. OXA belongs to class D beta-lactamases, which were among the earliest beta-lactamases discovered. In our study, OXA-1 appeared in one ST131 isolate and one non-ST131 isolates, both together CTX-M-15. TEM-1 and OXA-1 are both broad-spectrum beta-lactamase, which lead to the resistance to ampicillin, ticarcillin, piperacillin and cephalosporins [303]. Their co-existence with CTX-Ms add the resistance to beta-lactams. The co-existence of other AMR genes or related mutations is also common.

Phylogenetic analysis indicates the ST131 clones with close relationship appeared within limited regions (mainly in Asia and Oceania). Typical Southeast Asia ST131 clones (SEA-C2 clone [304]) were also observed in our study. This supports that high ESBL prevalence in this region is driven by expansion of one (or more) single local subclone. However, the mechanism behind this remains to be explored.

Chapter 6 Conclusion and Future work

Our study characterized the AMR profile of *E. coli* from ready-to-eat food and ESBL-producing *E. coli* from raw meats in Singapore based on whole genome sequencing technology, which to some extent fill the data gaps in these area. This also provide the basis for further risk assessment related to AMR in food chain. As a small country mainly depending on imported food from other countries and areas, Singapore observes stringent food safety standards to lower the incidence of food-borne diseases. The emerging AMR problem should raise more attention of Singapore authorities. Our data shows the prevalence of ESBL in raw meats in Singapore actually is not at a low level compared with other countries, which may reflect that potential risk exist as AMR has not been put into routine testing of imported food. Measures should be taken as soon as possible to avoid AMR transmission in the future.

Sequencing is a milestone technology in molecular biology. Whole genome sequencing is another great step which change our viewing angle from single or several target genes to genome global overview. This has spawned the emergence of comparative genomics and also improved the resolution of evolution studies. WGS can be widely used in epidemiology research. In our study, we applied WGS to characterize the prevalent AMR genes and other genetic features such as virulence factors and plasmids. This is valuable for global AMR transmission and source tracking research especially for important multi-drug resistance such ESBL and last-resort drug resistance such as colistin resistance. On the other hand, WGS plays a more important role in clinical diagnosis and retrospective

study. We analyzed past clinical ESBL *E. coli* from Thailand hospital focusing on important pathogen cluster ST131. Persistent strain type was observed, and it also exhibited the sign of regional transmission (Southeast Asia and Oceania). Genome characterization of pathogens like ST131 provide the foundation of mechanism study to explore the reason why specific sub-cluster exhibits high transmissibility.

However, there are shortcomings in this whole project because of the limitation of time and manpower. We did not manage to get Singapore clinical isolates, and isolates from community and from raw meats were not in the same isolation period, which makes it hard to directly prove the AMR transmission between food and human. To achieve “One Health” approach require comprehensive data from various sources including clinical and environmental isolates should be included. The possible complete transmission way needs to be confirmed in the future.

The advantages and strengths of WGS have been discussed in Chapter 4. Generally speaking, the major strength of WGS is its ability to detect all genes and genetic abnormalities (including substitution, insertion, duplication, deletions) across the entire genome. Compared with techniques with specific target such as PCR and microarray, WGS provides a unique overview of whole genome to greatly avoid missing information. This helps to discover hidden genetic secrets beyond our sights. Regards to sequencing capacity, WGS is able to sequence massive DNA in parallel, and its high-throughput characteristic makes sequencing technology one big step forward. Compared with traditional

Sanger sequencing, the cost of sequencing per base pair decreased dramatically. In 2006, the cost to sequence human genome by Sanger sequencing is around 20 million dollars to 25 million dollars, however, in 2016, this cost became less than 1000 dollars.

However, it should not be ignored that WGS also has disadvantages and limitations during application. First of all, the whole genome sequencing is actually not real “complete genome”. For example, Assemblies of Illumina sequencing are still in forms of contigs or scaffolds instead of one entire genome. The fragmentation of genome information actually limits its application especially for study of plasmids or recombination regions. It still needs other tools (such as BLAST) to determine whether the contigs belong to chromosome or plasmids, however, it not always works because some contigs can be very short, which is able to be matched to both a chromosome and a plasmid. Although long-read sequencing provides much more complete genome information, its high error rate become another disadvantage. If combining long-read sequencing and short-read sequencing (like the methods described in Chapter 4), the increased cost and complex operation become new problems.

Regards to the cost, although WGS decreased the price per base pair, it is still not realistic to use it as a routine test in clinical situation currently, not to mention the AMR detection in foods. WGS also requires bioinformatics systems, large data storage capabilities and professional data analysts, which can be much costly. In terms of a small number of targets, WGS is not cost-effective compared with PCR and Sanger sequencing.

There is also a large knowledge gap regards to genetic mechanisms behind the phenotype. Our study also showed the discrepancies between AMR genotype and phenotype, and this is inevitable before we have a deep understanding of AMR genes or other mechanisms. For many identified genetic abnormalities, the clinical significance is still unknown, and this makes the additional information provided by WGS become “useless” at the current stage. The main application of WGS is still focused on academic or research situations.

The emerging new technologies are always not perfect. Only appropriate application and development can maximize the value. For better utilizing whole genome sequencing data, worldwide efforts are made to form an international network. Global Microbial Identifier (GMI) is a platform for storing WGS data of microorganisms, for the identification of relevant genes and for the comparison of genomes to detect outbreak and emerging pathogens. GMI is a network of around 160 representatives from 32 countries with the goal of aggregating, sharing, mining and using microbiological genomic data for improving plant, animal, environmental and human health. By standardizing WGS data and related metadata, genomic information from every new outbreak sample will be able to be compared. To achieve this goal, member countries need to agree on rules for data sharing and appropriate using. This issue has not been totally solved and GMI is currently working on it. GMI is also providing a network of integrated surveillance because the various data source including human, environment and animals, which is the reflection of one health concept.

The more data we have, the more complete the surveillance picture is. Although there is still a long way to go, integrated surveillance network must be the future trend and WGS data will be important reference for decision makers.

In terms of AMR detection, phenotype testing is still the “gold standard” currently, sequencing is more like a tool to deepen our understanding of genes or mechanism behind. However, the direction from phenotype to genotype is an inevitable process and the resolution of our understanding towards genome must be higher and higher (from single gene level to single nucleotide level). The necessity of WGS will become more obvious in the near future.

The AMR transmission from food-producing animals to human has been paid attention for a long time. Although a lot of evidence showed the overlapping of antimicrobial resistant bacteria or their AMR determinants from food animals and humans, there is still lack of direct and quantitative conclusion for the AMR transmission. It is hypothesized that AMR transmission from animals to humans by two ways: 1) clonal transfer of antimicrobial resistant bacteria 2) horizontal transfer of AMR determinants. There is also difference in methods to study these two ways. Most commonly used method in previous studies is to confirm whether there is identical bacteria or AMR determinants both in animals and humans, which relies heavily on bacterial typing tools. Previous studies using traditional tools such as pulsed-field gel electrophoresis (PFGE) and MLST may not have sufficient resolution to distinguish bacteria. In such situation, WGS-based SNP analysis greatly increased the resolution to single nucleotide. Currently it is widely accepted that strains with less than SNP difference belong to the same

clone. Phylogenetic studies also show the phylogenetic distance between genomes. The study of whole bacterial transfer focus on the same species, whereas for study of horizontal transfer of AMR determinants, the situation may be more complicated because transfer can happen among different species. It also requires high resolution of molecular tools as well. Long-read sequencing is absolutely a better tool to generate plasmid structure compared with traditional molecular techniques. As a result, WGS will be the necessary tool for future AMR transmission study.

For the future research of investigating AMR transmission between food-producing animals and humans, the following points should be considered to have a solid conclusion. Firstly, antimicrobial use for both animals and humans should be clearly recorded and clarified before and during the research periods. Many previous studies did not include this information, which is an impactful factor for AMR; secondly, the population of study and their activity range should be clearly specified, and this also influences the exposure of bacteria; thirdly, high resolution of molecular tools should be used according to the aim and targets of research. If the aim is to investigate the transferable genetic elements, long-read sequencing is a better choice for a complete plasmid structure. Looking ahead, studies in this field will step into a new phase with the help of WGS.

Supplementary materials

Supplementary Table 1. Information of *E. coli* isolates in Chapter 3

No.	Isolate ID	Year of isolation	Types of dishes	Food Source	Accession number
1	SGEHI2009ENV42	2009	Poultry-based	Chicken rice	-
2	SGEHI2009ENV49	2009	Poultry-based	Chicken rice	ERX2597140
3	SGEHI2010ENV65	2010	Poultry-based	Duck rice	-
4	SGEHI2010ENV66	2010	Poultry-based	Duck rice	ERX2597141
5	SGEHI2010ENV76	2010	Poultry-based	Chicken rice	ERX2597143
6	SGEHI2010ENV77	2010	Poultry-based	Duck rice	-
7	SGEHI2010ENV90	2010	Poultry-based	Chicken rice	-
8	SGEHI2010ENV103	2010	Poultry-based	Chicken rice	ERX2597144
9	SGEHI2010ENV114	2010	Poultry-based	Chicken nasi briyani (rice-based dish prepared with spices, chicken, vegetables and egg)	-
10	SGEHI2010ENV116	2010	Poultry-based	Chicken rice	-
11	SGEHI2010ENV127	2010	Poultry-based	Duck rice	-
12	SGEHI2010ENV138	2010	Poultry-based	Duck rice	-
13	SGEHI2010ENV157	2010	Poultry-based	Chicken briyani	-
14	SGEHI2010ENV190	2010	Poultry-based	Chicken nasi briyani	ERX2597145
15	SGEHI2010ENV196	2010	Poultry-based	Duck rice	-
16	SGEHI2011ENV210	2011	Poultry-based	Doner chicken sandwich	ERX2597146
17	SGEHI2011ENV222	2011	Poultry-based	Duck rice	ERX2597147
18	SGEHI2011ENV224	2011	Poultry-based	Chicken rice	-
19	SGEHI2011ENV225	2011	Poultry-based	Duck rice	ERX2597148
20	SGEHI2011ENV228	2011	Poultry-based	Chicken rice	ERX2597149
21	SGEHI2011ENV230	2011	Poultry-based	Chicken rice	-
22	SGEHI2011ENV233	2011	Poultry-based	Duck rice	ERX2597150
23	SGEHI2011ENV235	2011	Poultry-based	Duck rice	ERX2597151
24	SGEHI2011ENV249	2011	Poultry-based	Braised duck	-
25	SGEHI2011ENV316	2011	Poultry-based	Duck rice	ERX2597152
26	SGEHI2011ENV317	2011	Poultry-based	Duck rice	ERX2597153
27	SGEHI2011ENV318	2011	Poultry-based	Steamed chicken-parson's nose (the triangular stub where tailfeathers grow on chicken)	-
28	SGEHI2011ENV323	2011	Poultry-based	Soy-sauced chicken drumsticks	ERX2597154
29	SGEHI2011ENV326	2011	Poultry-based	Chicken nasi briyani	ERX2597155
30	SGEHI2011ENV327	2011	Poultry-based	Chicken nasi briyani	-
31	SGEHI2011ENV332	2011	Poultry-based	Duck rice	-
32	SGEHI2011ENV342	2011	Poultry-based	Ayam kleo (Nyonyan chicken curry)	-
33	SGEHI2011ENV352	2011	Poultry-based	Chicken rice	-
34	SGEHI2010ENV357	2010	Poultry-based	Roasted chicken	-
35	SGEHI2010ENV363	2010	Poultry-based	Roasted chicken	-
36	SGEHI2010ENV369	2010	Poultry-based	Roasted chicken	-
37	SGEHI2010ENV372	2010	Poultry-based	Duck rice	-
38	SGEHI2010ENV377	2010	Poultry-based	Roasted chicken	-
39	SGEHI2010ENV380	2010	Poultry-based	Duck rice	-
40	SGEHI2010ENV384	2010	Poultry-based	Roasted chicken	-
41	SGEHI2010ENV388	2010	Poultry-based	Duck rice	ERX2597156

No.	Isolate ID	Year of isolation	Types of dishes	Food Source	Accession number
42	SGEHI2010ENV391	2010	Poultry-based	Roasted chicken	-
43	SGEHI2010ENV394	2010	Poultry-based	Duck rice	-
44	SGEHI2010ENV397	2010	Poultry-based	Roasted chicken	-
45	SGEHI2010ENV403	2010	Poultry-based	Roasted chicken	-
46	SGEHI2010ENV405	2010	Poultry-based	Chicken nasi briyani	-
47	SGEHI2010ENV408	2010	Poultry-based	Roasted chicken	-
48	SGEHI2010ENV438	2010	Poultry-based	Curry chicken	-
49	SGEHI2010ENV446	2010	Poultry-based	Curry chicken	-
50	SGEHI2010ENV452	2010	Poultry-based	Curry chicken	-
51	SGEHI2010ENV456	2010	Poultry-based	Curry chicken	-
52	SGEHI2011ENV463	2011	Poultry-based	Chicken nasi briyani	ERX2597157
53	SGEHI2011ENV473	2011	Poultry-based	Chicken rice	-
54	SGEHI2011ENV475	2011	Poultry-based	Chicken nasi briyani	-
55	SGEHI2013ENV537	2013	Poultry-based	Stuffed chicken wing	-
56	SGEHI2013ENV561	2013	Poultry-based	Chicken rice	-
57	SGEHI2013ENV565	2013	Poultry-based	Chicken rice ball	-
58	SGEHI2013ENV595	2013	Poultry-based	Chicken rice	-
59	SGEHI2013ENV621	2013	Poultry-based	Chicken rice	-
60	SGEHI2013ENV658	2013	Poultry-based	Chicken rice	-
61	SGEHI2013ENV661	2013	Poultry-based	Mandi chicken	-
62	SGEHI2013ENV663	2013	Poultry-based	Chicken wing	ERX2597158
63	SGEHI2013ENV665	2013	Poultry-based	Chicken kebab	ERX2597159
64	SGEHI2013ENV685	2013	Poultry-based	Chicken kebab	-
65	SGEHI2013ENV691	2013	Poultry-based	Chicken takoyaki	-
66	SGEHI2013ENV693	2013	Poultry-based	Mandi chicken	-
67	SGEHI2013ENV694	2013	Poultry-based	Chicken rice	ERX2597160
68	SGEHI2013ENV704	2013	Poultry-based	Duck rice	ERX2597161
69	SGEHI2013ENV710	2013	Poultry-based	Chicken rice	-
70	SGEHI2013ENV726	2013	Poultry-based	Nasi Ayam (roasted chicken rice)	-
71	SGEHI2013ENV727	2013	Poultry-based	Chicken rice	ERX2597162
72	SGEHI2013ENV737	2013	Poultry-based	Chicken rice	ERX2597163
73	SGEHI2013ENV764	2013	Poultry-based	Chicken rice	-
74	SGEHI2013ENV855	2014	Poultry-based	Chicken chop rice	-
75	SGEHI2013ENV863	2014	Poultry-based	Chicken rice	-
76	SGEHI2013ENV881	2014	Poultry-based	Chicken rice	-
77	SGEHI2013ENV885	2014	Poultry-based	Chicken chop rice	-
78	SGEHI2009ENV58	2009	Fish-based	Fish curry	-
79	SGEHI2010ENV68	2010	Fish-based	Grilled fish	ERX2597142
80	SGEHI2011ENV248	2011	Fish-based	Spicy fish	-
81	SGEHI2011ENV324	2011	Fish-based	Fish curry	-
82	SGEHI2011ENV330	2011	Fish-based	Spicy fish	-
83	SGEHI2010ENV419	2010	Fish-based	Fish bee hoon soup	-
84	SGEHI2010ENV426	2010	Fish-based	Fish bee hoon soup	-
85	SGEHI2010ENV430	2010	Fish-based	Fish bee hoon soup	-
86	SGEHI2010ENV433	2010	Fish-based	Sashimi*	-
87	SGEHI2010ENV436	2010	Fish-based	Fish bee hoon soup	-
88	SGEHI2010ENV440	2010	Fish-based	Sashimi*	-
89	SGEHI2010ENV443	2010	Fish-based	Fish bee hoon soup	-

No. Isolate ID	Year of isolation	Types of dishes	Food Source	Accession number
90 SGEHI2010ENV449	2010	Fish-based	Fish bee hoon soup	-
91 SGEHI2010ENV453	2010	Fish-based	Sashimi*	-
92 SGEHI2010ENV460	2010	Fish-based	Sashimi*	-
93 SGEHI2011ENV471	2011	Fish-based	Stingray fish	-
94 SGEHI2011ENV477	2011	Fish-based	Salmon sushi*	-
95 SGEHI2013ENV550	2013	Fish-based	Yu sheng (raw salmon)*	-
96 SGEHI2013ENV554	2013	Fish-based	Yu sheng (raw salmon)*	-
97 SGEHI2013ENV690	2013	Fish-based	Fishball	-
98 SGEHI2013ENV741	2013	Fish-based	Otah (grilled fish cake)	-
99 SGEHI2013ENV771	2013	Fish-based	Otah	-

The mark * means the food is raw. The ID were simplified in the main tables because of the limited space.

Supplementary Table 2. Quality information of assembled genomes in Chapter 3

Sample	No. of Contigs	Average Length	Total Assembly Size	N50 Contig Length	No. of Reads	No. of Read Bases	Depth Coverage
ENV103	315	15,170	4,778,768	77,344	3,360,304	843,436,304	176.50
ENV190	208	23,200	4,825,724	194,835	3,358,174	842,901,674	174.67
ENV210	182	27,158	4,942,869	208,942	3,850,958	966,590,458	195.55
ENV222	195	25,906	5,051,858	161,088	3,468,184	870,514,184	172.32
ENV225	268	18,342	4,915,716	150,843	3,634,890	912,357,390	185.60
ENV228	189	25,090	4,742,065	297,072	3,347,346	840,183,846	177.18
ENV233	254	19,638	4,988,063	172,349	3,487,878	875,457,378	175.51
ENV235	227	22,003	4,994,805	177,514	3,102,250	778,664,750	155.89
ENV316	200	24,512	4,902,574	201,989	2,856,680	717,026,680	146.26
ENV317	240	20,506	4,921,509	202,214	3,372,578	846,517,078	172.00
ENV323	271	18,517	5,018,273	109,800	3,049,024	765,305,024	152.50
ENV326	247	20,307	5,015,977	173,667	3,477,066	872,743,566	173.99
ENV388	229	22,098	5,060,644	224,979	3,346,576	839,990,576	165.98
ENV463	253	19,038	4,816,770	107,205	3,145,190	789,442,690	163.89
ENV49	251	18,974	4,762,694	104,219	3,293,740	826,728,740	173.58
ENV66	200	25,011	5,002,217	258,647	3,369,716	845,798,716	169.08
ENV663	200	24,354	4,870,917	211,477	3,275,464	822,141,464	168.79
ENV665	229	21,337	4,886,332	236,013	3,333,860	836,798,860	171.25
ENV68	228	21,477	4,896,869	142,355	3,519,602	883,420,102	180.41
ENV694	240	21,699	5,207,776	202,255	3,449,840	865,909,840	166.27
ENV704	238	20,461	4,869,744	131,936	3,363,976	844,357,976	173.39
ENV727	187	25,903	4,843,960	189,170	3,487,330	875,319,830	180.70
ENV737	214	22,164	4,743,283	236,055	3,413,086	856,684,586	180.61
ENV76	207	23,334	4,830,148	211,289	3,008,076	755,027,076	156.32

Supplementary Table 3. Disk diffusion result for whole genome sequenced *E. coli* isolates in chapter 3

Isolate ID	AK	AMP	AMC	C	CRO	CIP	CN	NA	NOR	SXT	TE	MEM
SGEHI2009ENV49	S	I	S	S	S	S	S	R	S	S	R	S
SGEHI2010ENV66	S	R	I	R	S	S	S	I	S	S	R	S
SGEHI2010ENV68	S	R	S	R	S	S	S	S	S	R	R	S
SGEHI2010ENV76	S	R	I	R	S	S	S	I	S	S	R	S
SGEHI2010ENV103	S	R	I	R	S	S	S	I	S	R	R	S
SGEHI2010ENV190	S	R	S	S	S	S	S	S	S	I	R	S
SGEHI2011ENV210	S	R	I	R	R	S	R	R	S	R	R	S
SGEHI2011ENV222	S	R	I	S	S	I	S	R	S	S	R	S
SGEHI2011ENV225	S	R	I	R	R	S	S	S	S	R	R	S
SGEHI2011ENV228	S	R	S	S	S	S	S	S	S	S	S	S
SGEHI2011ENV233	S	R	I	S	S	S	S	S	S	S	S	S
SGEHI2011ENV235	S	R	S	S	S	S	S	S	S	S	S	S
SGEHI2011ENV316	S	S	S	R	S	S	S	S	S	S	S	S
SGEHI2011ENV317	S	S	S	R	S	S	S	S	S	S	S	S
SGEHI2011ENV323	S	S	S	S	S	S	S	I	S	S	R	S
SGEHI2011ENV326	S	R	I	R	S	S	S	S	S	S	R	S
SGEHI2010ENV388	S	R	I	S	S	S	S	S	S	S	S	S
SGEHI2011ENV463	S	I	S	S	S	S	S	S	S	S	R	S
SGEHI2013ENV663	S	I	S	S	S	S	S	S	S	R	R	S
SGEHI2013ENV665	S	I	S	S	S	S	S	S	S	R	R	S
SGEHI2013ENV694	S	S	S	S	S	S	S	S	S	S	R	S
SGEHI2013ENV704	S	R	S	R	S	R	S	R	R	R	R	S
SGEHI2013ENV727	S	I	S	S	S	S	S	I	S	R	R	S
SGEHI2013ENV737	S	R	S	S	S	S	S	S	S	S	S	S

AK:amikacin; AMP ampicillin; AMC: amoxicillin/clavulanic acid; C: chloramphenicol; CRO:ceftriaxone; CIP: ciprofloxacin; CN:gentamicin; NA: nalidixic acid; NOR: norfloxacin; SXT :Sulphamethoxazole/Trimethoprim; TE: tetracycline; MEM: Meropenem

Supplementary Table 4. Antimicrobial agents used in MIC Testing in Chapter 3

Classes	Antimicrobial agents	Abbreviations	Concentrations ($\mu\text{g/ml}$)	Breakpoints ($\mu\text{g/ml}$)	
				S $\leq$	R $>$
Tetracycline	Tetracycline*	TE	4-8	4#	8#
	Tigecycline	TGC	0.25-2	1	2
Polymycin	Colistin	CL	2	2	2
Fluoroquinolone	Norfloxacin*	NOR	0.5-1	0.5	1
	Ofloxacin	OFL	0.5-1	0.5	1
	Ciprofloxacin*	CIP	0.5-1	0.5	1
	Levofloxacin	LVX	1-2	1	2
	Moxifloxacin	MXF	0.5-1	0.5	1
Aminoglycoside	Amikacin*	AK	8-16	8	16
	Gentamicin*	CN	2-4	2	4
	Tobramycin	TO	2-4	2	4

DHFR inhibitor	Trimethoprim	T	8	8#	16#
Amphenicol	Chloramphenicol*	C	8	8	8
Beta-lactams	Cefuroxime	CRM	1-8	8	8
	Cefoxitin	CFX	8-16	8#	32#
	Cefpodoxime	CPD	0.5-1	1	1
	Ceftazidime	CAZ	0.5-8	1	4
	Ceftriaxone*	CRO	0.5-2	1	2
	Cefotaxime	CFT	0.5-2, 16	1	2
	Cefepime	CPE	0.5-8	1	4
	Ampicillin*	AMP	0.5-8	8	8
	Piperacillin	PI	4-16	8	16
	Aztreonam	AZT	1, 8-16	1	4
	Doripenem	DOR	1-4	1	4
	Ertapenem	ETP	0.5-1	0.5	1
	Imipenem	IMP	1-8	2	8
	Meropenem*	MEM	0.5-8	2	8
Miscellaneous agents	Fosfomycin	FOS	32	32	32
Nitrofurantoin derivative	Nitrofurantoin	FD	64	64	64
Combination	Sulphamethoxazole/Trimethoprim*	SXT	2/38-4/76	2/38	4/76
	Ampicillin/Sulbactam	A/S	1/0.5-8/4	8/4	8/4
	Amoxicillin/Clavulanic acid*	AMC	2/1-8/4	8/4	8/4
	Piperacillin/Tazobactam	P/T	4-16	8	16

Asterisks* denotes antimicrobial agents or combinations that were used in the disk diffusion assay. All interpretations are based on EUCAST standard unless specified otherwise. # indicates no EUCAST interpretation was available for the antimicrobial; therefore, the CLSI standard was applied.

Supplementary Table 5. The result of MIC Testing in Chapter 3

Isolate ID	Antimicrobials MIC(μ g/ml)																														
	Tetracycline (TE)	Tigecycline (TGC)	Colistin (CL)	Norfloxacin (NOR)	Ofloxacin(OFL)	Ciprofloxacin (CIP)	Levofloxacin (LVX)	Moxifloxacin (MXF)	Amikacin (AK)	Gentamicin (CN)	Tobramycin (TO)	Trimethoprim (T)	Chloramphenicol (C)	Cefuroxime (CRM)	Cefoxitin (CFX)	Ceftazidime (CAZ)	Ceftriaxone (CRO)	Cefotaxime (CFT)	Cefepime (CPE)	Ampicillin (AMP)	Piperacillin (PI)	Aztreonam (AZT)	Ertapenem(ETP)	Imipenem (IMP)	Meropenem (MEM)	Fosfomycin (FOS)	Nitrofurantoin (FD)	Sulfamethoxazole/ Trimethoprim (SXT)	Ampicillin/Sulbactam (A/S)	Amoxicillin/Clavulanic acid (AMC)	Piperacillin/Tazobactam (P/T)
ENV49	>8	≤0.25	≤2	1	>1	1	2	>1	≤8	≤2	≤2	≤8	>8	4	≤8	≤0.5	≤0.5	≤0.5	≤0.5	4	16	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	2/1	4/2	≤4
ENV66	>8	≤0.25	>2	>1	>1	≤0.5	≤1	>1	≤8	≤2	4	≤8	>8	>8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	>8	>16	≤4	≤0.5	≤4	≤0.5	>32	≤64	≤2/38	>8/4	>8/4	≤4
ENV68	>8	≤0.25	≤2	1	≤0.5	≤0.5	≤1	1	≤8	≤2	>4	>8	>8	2	≤8	≤0.5	≤0.5	≤0.5	≤0.5	>8	>16	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	>8/4	8/4	≤4
ENV76	>8	≤0.25	>2	>1	>1	1	≤1	>1	≤8	≤2	4	≤8	>8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	>8	>16	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	>8/4	>8/4	≤4
ENV103	>8	1	>2	>1	>1	1	≤1	>1	≤8	≤2	≤2	>8	>8	>8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	>8	>16	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	>8/4	8/4	≤4
ENV190	≤4	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	>8	>8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	8	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	4/2	8/4	≤4
ENV210	>8	≤0.25	>2	>1	>1	1	≤1	1	≤8	>4	>4	>8	>8	8	≤8	2	>2	>16	>8	>8	>16	16	≤0.5	≤4	≤0.5	>32	≤64	>4/76	>8/4	8/4	≤4
ENV222	>8	≤0.25	≤2	>1	>1	>1	2	>1	≤8	≤2	≤2	≤8	≤8	4	≤8	≤0.5	≤0.5	≤0.5	≤0.5	>8	>16	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	>8/4	8/4	≤4
ENV225	>8	≤0.25	≤2	>1	>1	≤0.5	≤1	>1	≤8	≤2	4	>8	>8	≤1	≤8	>8	>2	>16	>8	>8	>16	>16	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	>8/4	>8/4	≤4
ENV228	≤4	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	>8	>8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	8	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	4/2	4/2	≤4
ENV233	≤4	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	≤8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	8	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	4/2	8/4	≤4
ENV235	≤4	≤0.25	>2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	≤8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	8	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	4/2	8/4	≤4
ENV316	≤4	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	>8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	4	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	2/1	4/2	≤4
ENV317	≤4	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	>8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	4	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	2/1	4/2	≤4
ENV323	>8	≤0.25	≤2	1	1	≤0.5	≤1	≤0.5	≤8	≤2	≤2	>8	≤8	2	≤8	≤0.5	≤0.5	≤0.5	≤0.5	4	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	8/4	≤2/1	≤4
ENV326	>8	≤0.25	≤2	≤0.5	1	≤0.5	≤1	>1	≤8	≤2	4	>8	>8	4	≤8	≤0.5	≤0.5	≤0.5	≤0.5	>8	>16	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	>8/4	>8/4	≤4
ENV388	≤4	≤0.25	>2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	≤8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	8	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	4/2	8/4	≤4
ENV463	>8	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	≤8	4	>16	≤0.5	≤0.5	≤0.5	≤0.5	>8	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	8/4	>8/4	≤4
ENV663	>8	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	>8	≤8	4	≤8	≤0.5	≤0.5	≤0.5	≤0.5	4	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	2/1	4/2	≤4
ENV665	>8	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	4	>8	≤8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	4	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	2/1	4/2	≤4
ENV694	>8	≤0.25	>2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	≤8	2	≤8	≤0.5	≤0.5	≤0.5	≤0.5	2	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	8/4	≤2/1	≤4
ENV704	>8	≤0.25	>2	>1	>1	>1	>2	>1	≤8	≤2	>4	>8	>8	>8	>8	16	≤0.5	≤0.5	≤0.5	>8	>16	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	>8/4	8/4	≤4
ENV727	>8	≤0.25	≤2	1	1	>1	≤1	>1	≤8	≤2	4	>8	≤8	8	≤8	≤0.5	≤0.5	≤0.5	≤0.5	4	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	>4/76	2/1	4/2	≤4
ENV737	>8	≤0.25	≤2	≤0.5	≤0.5	≤0.5	≤1	≤0.5	≤8	≤2	≤2	≤8	≤8	4	≤8	≤0.5	≤0.5	≤0.5	≤0.5	4	≤4	≤4	≤0.5	≤4	≤0.5	≤32	≤64	≤2/38	4/2	8/4	≤4

≤4	Susceptible
>4	Intermediate
>8	Resistant

Supplementary Table 6. Metadata of ESBL *E. coli* from raw meats in Chapter 4

	Run	BioSample	Collection date	Source	Strain	ST-type
1	ERR3503842	ERS3718632	2/10/17	Chicken	E109ESBLB	453
2	ERR3503811	ERS3718601	10/7/17	Pork	E10ESBLB11	226
3	ERR3503843	ERS3718633	2/10/17	Beef	E112ESBLB	2973
4	ERR3503844	ERS3718634	2/10/17	Chicken	E113ESBLB1	117
5	ERR3503845	ERS3718635	2/10/17	Pork	E115ESBLB	398
6	ERR3503846	ERS3718636	23/10/17	Pork	E121ESBLB	2325
7	ERR3503847	ERS3718637	23/10/17	Chicken	E123ESBLG	1072
8	ERR3503848	ERS3718638	23/10/17	Chicken	E124ESBLB	117
9	ERR3503812	ERS3718602	10/7/17	Chicken	E12ESBLBK11	295
10	ERR3503849	ERS3718639	23/10/17	Chicken	E130ESBLB	117
11	ERR3503850	ERS3718640	23/10/17	Pork	E131ESBLB1	1244
12	ERR3503851	ERS3718641	30/10/17	Chicken	E141ESBLG	156
13	ERR3503852	ERS3718642	30/10/17	Pork	E143ESBLB	1607
14	ERR3505110	ERS3718643	30/10/17	Chicken	E147ESBLB1	115
15	ERR3505111	ERS3718644	30/10/17	Chicken	E148ESBLG	1158
16	ERR3505112	ERS3718645	30/10/17	Chicken	E148ESBLPK	2309
17	ERR3503813	ERS3718603	11/7/17	Chicken	E14ESBLB11	115
18	ERR3503814	ERS3718604	11/7/17	Chicken	E14ESBLG2	2732
19	ERR3505113	ERS3718646	6/11/17	Chicken	E151ESBLB1	212
20	ERR3505114	ERS3718647	6/11/17	Chicken	E151ESBLB2	3258
21	ERR3505115	ERS3718648	6/11/17	Chicken	E151ESBLG	93
22	ERR3505116	ERS3718649	6/11/17	Pork	E152ESBLB	641*
23	ERR3505117	ERS3718650	6/11/17	Pork	E158ESBLB	48
24	ERR3505118	ERS3718651	6/11/17	Beef	E165ESBLB	117
25	ERR3505119	ERS3718652	6/11/17	Beef	E165ESBLG	189
26	ERR3505120	ERS3718653	6/11/17	Pork	E166ESBLB	117
27	ERR3505121	ERS3718654	7/11/17	Chicken	E170ESBLG	117
28	ERR3505122	ERS3718655	7/11/17	Pork	E171ESBLB1	48
29	ERR3505123	ERS3718656	7/11/17	Chicken	E174ESBLB	155
30	ERR3505124	ERS3718657	4/12/17	Chicken	E178ESBLB	117
31	ERR3505125	ERS3718658	4/12/17	Chicken	E178ESBLG1	752
32	ERR3505126	ERS3718659	4/12/17	Chicken	E178ESBLW1	770*
33	ERR3503815	ERS3718605	11/7/17	Pork	E17ESBL1	6256
34	ERR3503816	ERS3718606	11/7/17	Pork	E17ESBLB22	898
35	ERR3505127	ERS3718660	4/12/17	Chicken	E181ESBLPP	23
36	ERR3505128	ERS3718661	4/12/17	Chicken	E182ESBLB1	212
37	ERR3505129	ERS3718662	4/12/17	Chicken	E182ESBLG1	1158
38	ERR3505130	ERS3718663	4/12/17	Chicken	E185ESBLB1	117
39	ERR3505131	ERS3718664	4/12/17	Chicken	E185ESBLG	1485*

	Run	BioSample	Collection date	Source	Strain	ST-type
40	ERR3505132	ERS3718665	4/12/17	Chicken	E189ESBLB21	117
41	ERR3503817	ERS3718607	18/7/17	Pork	E18ESBLPK1	542
42	ERR3505133	ERS3718666	11/12/17	Chicken	E200ESBLB1	117
43	ERR3505134	ERS3718667	11/12/17	Chicken	E202ESBLB	162
44	ERR3505135	ERS3718668	11/12/17	Chicken	E205ESBLB1	2973
45	ERR3505136	ERS3718669	11/12/17	Chicken	E205ESBLB2	115
46	ERR3505137	ERS3718670	11/12/17	Chicken	E205ESBLPK	156
47	ERR3505138	ERS3718671	11/12/17	Pork	E207ESBLB	654
48	ERR3505139	ERS3718672	11/12/17	Pork	E207ESBLG	Unknown
49	ERR3505140	ERS3718673	11/12/17	Pork	E209ESBLB	4937
50	ERR3505141	ERS3718674	11/12/17	Pork	E210ESBLB1	354
51	ERR3505142	ERS3718675	11/12/17	Pork	E210ESBLG	3776
52	ERR3505143	ERS3718676	11/12/17	Chicken	E211ESBLB	155
53	ERR3505144	ERS3718677	18/12/18	Chicken	E217ESBLB1	162
54	ERR3505145	ERS3718678	18/12/18	Chicken	E221ESBLB1	349
55	ERR3505146	ERS3718679	18/12/18	Chicken	E221ESBLG1	295
56	ERR3505147	ERS3718680	18/12/18	Chicken	E222ESBLB2	349
57	ERR3505148	ERS3718681	18/12/18	Chicken	E225ESBLB	345
58	ERR3505149	ERS3718682	18/12/18	Chicken	E227ESBLG	Unknown
59	ERR3505150	ERS3718683	18/12/18	Pork	E228ESBLB1	1114
60	ERR3505151	ERS3718684	18/12/18	Pork	E228ESBLG	101
61	ERR3505152	ERS3718685	18/12/18	Pork	E229ESBLB1	3776
62	ERR3505153	ERS3718686	18/12/18	Pork	E229ESBLB2	34
63	ERR3505154	ERS3718687	18/12/18	Pork	E229ESBLG	48
64	ERR3505155	ERS3718688	18/12/18	Chicken	E230ESBLB	6697
65	ERR3505156	ERS3718689	26/12/17	Chicken	E241ESBLB	48
66	ERR3505157	ERS3718690	26/12/17	Chicken	E241ESBLPP	602
67	ERR3505158	ERS3718691	26/12/17	Chicken	E242ESBLB	752
68	ERR3506432	ERS3718692	26/12/17	Chicken	E244ESBLP	93
69	ERR3506433	ERS3718693	8/1/18	Chicken	E256ESBLG	398
70	ERR3506434	ERS3718694	8/1/18	Chicken	E262ESBLB	117
71	ERR3506435	ERS3718695	9/1/18	Pork	E265ESBLB	654
72	ERR3506436	ERS3718696	9/1/18	Pork	E265ESBLG	48
73	ERR3506437	ERS3718697	9/1/18	Pork	E267ESBLG	871
74	ERR3506438	ERS3718698	9/1/18	Pork	E267ESBLP	3776
75	ERR3506439	ERS3718699	9/1/18	Chicken	E270ESBLBR	1158
76	ERR3506440	ERS3718700	9/1/18	Pork	E274ESBLY2	227
77	ERR3506441	ERS3718701	9/1/18	Chicken	E278ESBLB	57
78	ERR3506442	ERS3718702	16/1/18	Chicken	E289ESBLB	2223
79	ERR3506443	ERS3718703	5/2/18	Chicken	E292ESBLB	3014
80	ERR3506444	ERS3718704	5/2/18	Chicken	E292ESBLG	23
81	ERR3506445	ERS3718705	5/2/18	Chicken	E296ESBLG	1158

	Run	BioSample	Collection date	Source	Strain	ST-type
82	ERR3506446	ERS3718706	5/2/18	Chicken	E296ESBLY1	345
83	ERR3506447	ERS3718707	5/2/18	Chicken	E298ESBLG	156
84	ERR3506448	ERS3718708	5/2/18	Chicken	E299ESBLB	117
85	ERR3503818	ERS3718608	31/7/17	Chicken	E29ESBLB21	2973
86	ERR3503804	ERS3718594	17/6/17	Chicken	E2ESBLW122	2309
87	ERR3506449	ERS3718709	5/2/18	Chicken	E306ESBLB	2309
88	ERR3506450	ERS3718710	19/2/18	Chicken	E308ESBLB	156
89	ERR3506451	ERS3718711	19/2/18	Chicken	E312ESBLB	155
90	ERR3506452	ERS3718712	19/2/18	Pork	E316ESBLB2	58
91	ERR3506453	ERS3718713	19/2/18	Chicken	E317ESBLB	57
92	ERR3506454	ERS3718714	19/2/18	Chicken	E319ESBLB1	3258
93	ERR3506455	ERS3718715	5/5/18	Pork	E336ESBLPP	3776
94	ERR3506456	ERS3718716	5/5/18	Chicken	E341ESBLB2	457
95	ERR3506457	ERS3718717	5/5/18	Chicken	E341ESBLG2	155
96	ERR3506458	ERS3718718	5/5/18	Chicken	E342ESBLB2	117
97	ERR3506459	ERS3718719	5/5/18	Chicken	E343ESBLPP2	115
98	ERR3506460	ERS3718720	5/5/18	Pork	E345ESBLB	3776
99	ERR3506461	ERS3718721	5/5/18	Chicken	E350ESBLB	38
100	ERR3506462	ERS3718722	5/5/18	Chicken	E354ESBLB	1011
101	ERR3506463	ERS3718723	5/5/18	Pork	E357ESBLG	3634
102	ERR3506464	ERS3718724	9/4/18	Chicken	E360ESBLB	2973
103	ERR3506465	ERS3718725	9/4/18	Chicken	E364ESBLB	3258
104	ERR3506466	ERS3718726	9/4/18	Chicken	E367ESBLB	362
105	ERR3506467	ERS3718727	9/4/18	Chicken	E367ESBLG	2223
106	ERR3506468	ERS3718728	24/4/18	Chicken	E368ESBLB	602
107	ERR3503819	ERS3718609	1/8/17	Pork	E36ESBLB1	3634
108	ERR3506469	ERS3718729	24/4/18	Pork	E370ESBLB	34
109	ERR3506470	ERS3718730	24/4/18	Chicken	E373ESBLPP	752
110	ERR3506471	ERS3718731	24/4/18	Chicken	E374ESBLB	117
111	ERR3506472	ERS3718732	8/5/18	Chicken	E379ESBLB	117
112	ERR3506473	ERS3718733	8/5/18	Chicken	E385ESBLG2	155
113	ERR3506474	ERS3718734	8/5/18	Chicken	E387ESBLB	351
114	ERR3506475	ERS3718735	8/5/18	Chicken	E389ESBLB	10*
115	ERR3506476	ERS3718736	8/5/18	Pork	E390ESBLG	206
116	ERR3506477	ERS3718737	8/5/18	Chicken	E393ESBLG	3776
117	ERR3506478	ERS3718738	15/5/18	Pork	E395ESBLPP	88
118	ERR3506479	ERS3718739	15/5/18	Pork	E396ESBLPP	Unknown
119	ERR3506480	ERS3718740	15/5/18	Chicken	E398ESBLPP	117
120	ERR3506481	ERS3718741	15/5/18	Pork	E405ESBLB	10
121	ERR3506785	ERS3718742	15/5/18	Chicken	E411ESBLB	3576
122	ERR3506786	ERS3718743	15/5/18	Chicken	E413ESBLB	117
123	ERR3506787	ERS3718744	15/5/18	Chicken	E414ESBLB	117

	Run	BioSample	Collection date	Source	Strain	ST-type
124	ERR3506788	ERS3718745	15/5/18	Pork	E416ESBLG	206
125	ERR3506789	ERS3718746	15/5/18	Pork	E419ESBLB	3776
126	ERR3506790	ERS3718747	15/5/18	Pork	E419ESBLG2	871
127	ERR3503820	ERS3718610	1/8/17	Chicken	E41ESBLB1	295
128	ERR3506791	ERS3718748	22/5/18	Beef	E421ESBLPP	10*
129	ERR3506792	ERS3718749	22/5/18	Pork	E422ESBLP	218
130	ERR3506793	ERS3718750	22/5/18	Pork	E422ESBLW	48
131	ERR3506794	ERS3718751	10/7/18	Chicken	E425ESBLPP	155*
132	ERR3506795	ERS3718752	10/7/18	Chicken	E426ESBLPP	1551
133	ERR3503821	ERS3718611	1/8/17	Chicken	E42ESBLB1	117
134	ERR3503822	ERS3718612	1/8/17	Chicken	E42ESBLG12	1266
135	ERR3506796	ERS3718753	10/7/18	Chicken	E430ESBLPP	117
136	ERR3506797	ERS3718754	10/7/18	Chicken	E435ESBLW1	Unknown
137	ERR3506798	ERS3718755	17/7/18	Pork	E436ESBLPP	88
138	ERR3506799	ERS3718756	17/7/18	Pork	E437ESBLPP	394
139	ERR3506800	ERS3718757	17/7/18	Chicken	E440ESBLP	7506
140	ERR3506801	ERS3718758	17/7/18	Chicken	E449ESBLPP	4450
141	ERR3506802	ERS3718759	17/7/18	Chicken	E451ESBLPP	602*
142	ERR3506803	ERS3718760	25/7/18	Chicken	E454ESBLPP	602*
143	ERR3506804	ERS3718761	25/7/18	Chicken	E458ESBLY	93
144	ERR3506805	ERS3718762	25/7/18	Chicken	E460ESBLPP	117*
145	ERR3506806	ERS3718763	25/7/18	Chicken	E461ESBLP	117*
146	ERR3506807	ERS3718764	25/7/18	Chicken	E461ESBLW	8261
147	ERR3506808	ERS3718765	25/7/18	Chicken	E464ESBLPP	654
148	ERR3506809	ERS3718766	25/7/18	Chicken	E465ESBLPP	117
149	ERR3506810	ERS3718767	25/7/18	Chicken	E467ESBLPP	155
150	ERR3506811	ERS3718768	25/7/18	Chicken	E469ESBLPP	156
151	ERR3503823	ERS3718613	29/8/17	Pork	E47ESBLB	540
152	ERR3506812	ERS3718769	31/7/18	Beef	E483ESBLPP	162
153	ERR3506813	ERS3718770	31/7/18	Chicken	E485ESBLPP	155
154	ERR3506814	ERS3718771	31/7/18	Pork	E486ESBLPP	398
155	ERR3503824	ERS3718614	29/8/17	Chicken	E48ESBLG	189
156	ERR3506815	ERS3718772	28/8/18	Chicken	E492ESBLPP	117
157	ERR3503805	ERS3718595	17/6/17	Pork	E4ESBLB11	48
158	ERR3503806	ERS3718596	17/6/17	Pork	E4ESBLB121	48
159	ERR3503807	ERS3718597	17/6/17	Pork	E4ESBLG12	48
160	ERR3503825	ERS3718615	29/8/17	Pork	E50ESBLB1	730
161	ERR3506816	ERS3718773	28/8/18	Pork	E510ESBLPP1	10
162	ERR3506817	ERS3718774	28/8/18	Chicken	E511ESBLPP	101
163	ERR3506818	ERS3718775	28/8/18	Beef	E512ESBLPP	117
164	ERR3506819	ERS3718776	28/8/18	Pork	E515ESBLPP	191
165	ERR3506820	ERS3718777	11/9/18	Pork	E524ESBLPP1	654

	Run	BioSample	Collection date	Source	Strain	ST-type
166	ERR3506821	ERS3718778	11/9/18	Pork	E526ESBLW1	746
167	ERR3506822	ERS3718779	11/9/18	Pork	E532ESBLPP	46
168	ERR3506823	ERS3718780	11/9/18	Chicken	E534ESBLPP	117
169	ERR3506824	ERS3718781	18/9/18	Chicken	E537ESBLPP	117
170	ERR3506825	ERS3718782	18/9/18	Chicken	E547ESBLPP	10
171	ERR3506826	ERS3718783	18/9/18	Beef	E548ESBLW1	58
172	ERR3506827	ERS3718784	18/9/18	Pork	E549ESBLPP	46*
173	ERR3506828	ERS3718785	18/9/18	Pork	E552ESBLPP	542
174	ERR3506829	ERS3718786	18/9/18	Chicken	E553ESBLPP	48
175	ERR3506830	ERS3718787	18/9/18	Chicken	E554ESBLPP	457
176	ERR3506831	ERS3718788	18/9/18	Beef	E555ESBLPP	117
177	ERR3506832	ERS3718789	25/9/18	Chicken	E563ESBLPP	156
178	ERR3506833	ERS3718790	25/9/18	Pork	E564ESBLPP	117
179	ERR3506834	ERS3718791	25/9/18	Chicken	E565ESBLPP	2614
180	ERR3506835	ERS3718792	25/9/18	Pork	E575ESBLPP	58
181	ERR3506836	ERS3718793	25/9/18	Chicken	E577ESBLPP	117
182	ERR3503826	ERS3718616	18/9/17	Chicken	E57ESBLB	115
183	ERR3506837	ERS3718794	25/9/18	Pork	E585ESBLY	206*
184	ERR3506838	ERS3718795	2/10/18	Pork	E587ESBLB	654
185	ERR3506839	ERS3718796	2/10/18	Chicken	E589ESBLB	10
186	ERR3503827	ERS3718617	18/9/17	Chicken	E58ESBLB	117
187	ERR3506840	ERS3718797	2/10/18	Pork	E590ESBLB	34
188	ERR3506841	ERS3718798	2/10/18	Pork	E590ESBLG	227
189	ERR3506842	ERS3718799	2/10/18	Beef	E591ESBLG	1730
190	ERR3506843	ERS3718800	2/10/18	Pork	E594ESBLB	654
191	ERR3506844	ERS3718801	2/10/18	Pork	E594ESBLG	48
192	ERR3506845	ERS3718802	2/10/18	Beef	E595ESBLB	3580
193	ERR3506846	ERS3718803	2/10/18	Chicken	E596ESBLB	155
194	ERR3506847	ERS3718804	2/10/18	Beef	E599ESBLB2	345
195	ERR3503828	ERS3718618	18/9/17	Pork	E59ESBLB1	1114
196	ERR3506848	ERS3718805	2/10/18	Chicken	E600ESBLB	2309
197	ERR3506849	ERS3718806	2/10/18	Pork	E601ESBLB	295
198	ERR3506850	ERS3718807	2/10/18	Chicken	E603ESBLB	295
199	ERR3506851	ERS3718808	2/10/18	Pork	E604ESBLB	654
200	ERR3506852	ERS3718809	2/10/18	Pork	E604ESBLG	746
201	ERR3506853	ERS3718810	2/10/18	Beef	E607ESBLB	117
202	ERR3506854	ERS3718811	2/10/18	Beef	E607ESBLG	23
203	ERR3506855	ERS3718812	9/10/18	Beef	E610ESBLB	155
204	ERR3506856	ERS3718813	9/10/18	Chicken	E611ESBLB	345
205	ERR3506857	ERS3718814	9/10/18	Beef	E612ESBLB	156
206	ERR3506858	ERS3718815	9/10/18	Chicken	E613ESBLB	2179
207	ERR3506859	ERS3718816	9/10/18	Chicken	E619ESBLB	117

Run	BioSample	Collection date	Source	Strain	ST-type	
208	ERR3503830	ERS3718620	18/9/17	Pork	E61ESBLB11	1421
209	ERR3503829	ERS3718619	18/9/17	Pork	E61ESBLB3	5766
210	ERR3503831	ERS3718621	18/9/17	Pork	E61ESBLG	3274
211	ERR3506860	ERS3718817	16/10/18	Beef	E627ESBLPP	117
212	ERR3506861	ERS3718818	23/10/18	Beef	E637ESBLB	58
213	ERR3503832	ERS3718622	19/9/17	Pork	E66ESBLB	542
214	ERR3503833	ERS3718623	19/9/17	Pork	E66ESBLG	846
215	ERR3503834	ERS3718624	19/9/17	Chicken	E69ESBLB1	117
216	ERR3503835	ERS3718625	25/9/17	Chicken	E77ESBLG	212
217	ERR3503836	ERS3718626	25/9/17	Pork	E80ESBLPK	8165
218	ERR3503837	ERS3718627	25/9/17	Pork	E80ESBLW	3274
219	ERR3503808	ERS3718598	28/6/17	Pork	E8ESBLB1	4589
220	ERR3503809	ERS3718599	28/6/17	Pork	E8ESBLG1	10
221	ERR3503810	ERS3718600	28/6/17	Pork	E8ESBLPK1	542
222	ERR3503838	ERS3718628	26/9/17	Chicken	E90ESBLB	117
223	ERR3503839	ERS3718629	2/10/17	Chicken	E97ESBLB1	2973*
224	ERR3503840	ERS3718630	2/10/17	Chicken	E97ESBLB2	117
225	ERR3503841	ERS3718631	2/10/17	Chicken	E97ESBLG1	189

Supplementary Table 7. MIC result in Chapter 4

Antimicrobials	E230B	E292B	E292G	E308B	E141G	E200B1	E227G	E534PP	E166B	E627PP	E90B	E612B	E610B	E619B	E454PP	E553PP	E485PP
Ampicillin (Am)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Piperacillin (Pi)	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Cephalothin(Cf)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Cefotaxime (Cft)	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
Cefuroxime (Crm)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Ceftazidime (Caz)	>16	>16	2	4	2	2	8	>16	<=1	2	>16	4	4	>16	2	4	<=1
Cefepime (Cpe)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Cefoxitin (Cfx)	>16	>16	<=8	>16	<=8	<=8	16	>16	<=8	<=8	<=8	<=8	<=8	<=8	<=8	>16	<=8
Colistin (Cl)	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	4	>4	>4
Doripenem (Dor)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Imipenem (Imp)	<=1	2	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Meropenem (Mer)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Ertapenem(Etp)	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5
Aztreonam (Azt)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Ciprofloxacin (Cp)	>2	>2	<=0.5	>2	<=0.5	<=0.5	2	>2	1	<=0.5	>2	<=0.5	<=0.5	>2	<=0.5	>2	<=0.5
Levofloxacin (Lvx)	>4	>4	<=1	4	<=1	<=1	2	>4	<=1	<=1	>4	<=1	<=1	>4	<=1	>4	<=1
Norfloxacin (Nxn)	>1	>1	<=0.5	>1	1	1	>1	>1	1	1	>1	>1	<=0.5	>1	1	>1	<=0.5
Nitrofurantoin (Fd)	<=32	<=32	<=32	<=32	<=32	<=32	>64	<=32	<=32	<=32	<=32	<=32	<=32	<=32	<=32	<=32	<=32
Chloramphenicol (C)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Tetracycline (Te)	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8
Tigecycline (Tgc)	<=1	>2	<=1	>2	2	2	>2	2	<=1	>2	<=1	>2	>2	<=1	<=1	2	2
Amikacin (Ak)	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8
Gentamicin (Gm)	>8	>8	>8	>8	>8	>8	>8	<=2	>8	>8	>8	>8	>8	>8	>8	>8	>8
Tobramycin (To)	8	>8	>8	>8	>8	>8	>8	<=2	>8	>8	>8	>8	>8	>8	>8	>8	>8
Nalidixic Acid(NA)	>16	>16	<=16	>16	>16	<=16	>16	>16	>16	<=16	>16	>16	<=16	>16	<=16	>16	<=16
Minocycline(Min)	8	8	8	>8	<=4	<=4	8	8	<=4	<=4	>8	8	<=4	<=4	<=4	8	<=4
Fosfomycin (Fos)	<=32	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	<=32	<=32	<=32	>64	>64	<=32
Amoxicillin/K Clavulanate (Aug)	16/8	>16/8	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	>16/8	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	16/8	<=8/4
Ampicillin/sulbactam (A/S)	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	<=8/4	>16/8	>16/8	16/8	>16/8	>16/8	>16/8
Piperacillin/Tazobactam (P/T)	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8
Trimethoprim/ Sulfamethoxazole (T/S)	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	<=2/38	<=2/38	<=2/38	>4/76	>4/76	>4/76	<=2/38

Continued Table

Antimicrobials	E454PP	E469PP	E565PP	E591G	E563PP	E603B	E299B	E342B2	E217B1	E225B	E205PK	E185B1	E170G	E165G	E97B2	E123G	E8B1
Ampicillin (Am)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Piperacillin (Pi)	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Cephalothin(Cf)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Cefotaxime (Cft)	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
Cefuroxime (Crm)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Ceftazidime (Caz)	>16	4	4	>16	4	4	>16	>16	2	<=1	4	>16	>16	4	4	4	>16
Cefepime (Cpe)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	4
Cefoxitin (Cfx)	<=8	16	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8
Colistin (Cl)	>4	>4	>4	4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4	<=2
Doripenem (Dor)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Imipenem (Imp)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Meropenem (Mer)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Ertapenem(Etp)	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5
Aztreonam (Azt)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Ciprofloxacin (Cp)	>2	>2	<=0.5	<=0.5	1	1	>2	2	>2	>2	2	>2	>2	<=0.5	<=0.5	<=0.5	<=0.5
Levofloxacin (Lvx)	>4	>4	<=1	<=1	<=1	<=1	>4	>4	>4	>4	2	>4	>4	<=1	<=1	<=1	<=1
Norfloxacin (Nxn)	>1	>1	<=0.5	1	>1	>1	>1	>1	>1	>1	>1	>1	>1	<=0.5	1	>1	<=0.5
Nitrofurantoin (Fd)	<=32	<=32	<=32	<=32	<=32	<=32	64	64	<=32	<=32	64	64	<=32	64	<=32	<=32	<=32
Chloramphenicol (C)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Tetracycline (Te)	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8
Tigecycline (Tgc)	>2	>2	2	<=1	>2	2	2	<=1	<=1	<=1	>2	<=1	<=1	>2	2	<=1	<=1
Amikacin (Ak)	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8
Gentamicin (Gm)	>8	>8	>8	>8	>8	>8	>8	<=2	>8	>8	>8	<=2	<=2	>8	>8	>8	<=2
Tobramycin (To)	>8	>8	>8	8	>8	>8	>8	<=2	>8	>8	>8	<=2	4	>8	>8	>8	<=2
Nalidixic Acid(NA)	>16	>16	<=16	<=16	>16	<=16	>16	>16	>16	>16	>16	>16	>16	<=16	<=16	>16	<=16
Minocycline(Min)	>8	>8	8	<=4	>8	>8	<=4	<=4	<=4	>8	>8	<=4	<=4	>8	<=4	<=4	>8
Fosfomycin (Fos)	>64	>64	>64	>64	>64	>64	>64	<=32	>64	>64	>64	>64	<=32	>64	>64	>64	<=32
Amoxicillin/K Clavulanate (Aug)	<=8/4	16/8	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	16/8	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4	<=8/4
Ampicillin/sulbactam (A/S)	>16/8	>16/8	>16/8	16/8	>16/8	>16/8	>16/8	<=8/4	>16/8	>16/8	>16/8	16/8	<=8/4	>16/8	16/8	>16/8	16/8
Piperacillin/Tazobactam (P/T)	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8
Trimethoprim/ Sulfamethoxazole (T/S)	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	<=2/38	>4/76	>4/76

Supplementary Table 8. Metadata of ESBL *E. coli* isolates from Singapore community in Chapter 4

			Collection				
Run	BioSample	Experiment	date	Source	Strain	ST-type	
1	SRR7371270	SAMN09460070	SRX4244290	10/2/17	rectal swab	1785-ESBL	1193
2	SRR7371271	SAMN09460114	SRX4244289	9/2/17	rectal swab	816-ESBL	131
3	SRR7371272	SAMN09460107	SRX4244288	8/2/17	rectal swab	667-ESBL	131
4	SRR7371273	SAMN09460061	SRX4244287	14/2/17	rectal swab	1519-ESBL	450
5	SRR7371274	SAMN09460062	SRX4244286	17/2/17	rectal swab	1584-ESBL	Unknown
6	SRR7371277	SAMN09460065	SRX4244283	17/2/17	stool	1657-ESBL	38
7	SRR7371278	SAMN09460066	SRX4244282	16/2/17	rectal swab	1678-ESBL	7394
8	SRR7371279	SAMN09460067	SRX4244281	17/2/17	rectal swab	1716-ESBL	131
9	SRR7371281	SAMN09460112	SRX4244279	13/2/17	rectal swab	71-ESBLB	2179
10	SRR7371282	SAMN09460115	SRX4244278	8/2/17	stool	897-ESBL	131
11	SRR7371283	SAMN09460111	SRX4244277	13/2/17	rectal swab	71-ESBLA	2179
12	SRR7371284	SAMN09460049	SRX4244276	17/2/17	rectal swab	1204-ESBL	443
13	SRR7371285	SAMN09460048	SRX4244275	10/2/17	stool	115-ESBL	38
14	SRR7371286	SAMN09460047	SRX4244274	13/2/17	rectal swab	1101-ESBL	4040
15	SRR7371287	SAMN09460046	SRX4244273	17/2/17	rectal swab	1097-ESBL	69
16	SRR7371288	SAMN09460045	SRX4244272	14/2/17	rectal swab	1056-ESBL	131
17	SRR7371289	SAMN09460044	SRX4244271	10/2/17	rectal swab	1053-ESBL	1193
18	SRR7371290	SAMN09460043	SRX4244270	13/2/17	rectal swab	1050-ESBL	10
19	SRR7371291	SAMN09460042	SRX4244269	18/2/17	rectal swab	1034-ESBL	23
20	SRR7371292	SAMN09460041	SRX4244268	13/2/17	rectal swab	1014-ESBL	196
21	SRR7371294	SAMN09460117	SRX4244266	9/2/17	rectal swab	955-ESBL	131
22	SRR7371295	SAMN09460116	SRX4244265	13/2/17	rectal swab	913-ESBL	38
23	SRR7371296	SAMN09460080	SRX4244264	18/2/17	stool	2060-ESBL	10
24	SRR7371297	SAMN09460105	SRX4244263	9/2/17	rectal swab	578-ESBL	998
25	SRR7371299	SAMN09460100	SRX4244261	8/2/17	rectal swab	484-ESBL	744
26	SRR7371300	SAMN09460099	SRX4244260	16/2/17	rectal swab	457-ESBL	38
27	SRR7371301	SAMN09460092	SRX4244259	15/2/17	rectal swab	282-ESBLB	23
28	SRR7371302	SAMN09460091	SRX4244258	15/2/17	rectal swab	282-ESBLA	3045
29	SRR7371303	SAMN09460094	SRX4244257	14/2/17	rectal swab	384-ESBL	101
30	SRR7371304	SAMN09460093	SRX4244256	10/2/17	stool	348-ESBL	617
31	SRR7371305	SAMN09460096	SRX4244255	9/2/17	rectal swab	395-ESBLB	93
32	SRR7371306	SAMN09460095	SRX4244254	9/2/17	rectal swab	395-ESBLA	93
33	SRR7371307	SAMN09460098	SRX4244253	17/2/17	rectal swab	403-ESBL	131
34	SRR7371308	SAMN09460097	SRX4244252	17/2/17	rectal swab	399-ESBL	657
35	SRR7371310	SAMN09460055	SRX4244250	9/2/17	stool	128-ESBL	117
36	SRR7371311	SAMN09460058	SRX4244249	18/2/17	stool	1409-ESBL	38
37	SRR7371312	SAMN09460057	SRX4244248	18/2/17	stool	1407-ESBL	1193

38	SRR7371313	SAMN09460052	SRX4244247	13/2/17	rectal swab	1270-ESBL	394
39	SRR7371315	SAMN09460054	SRX4244245	17/2/17	rectal swab	1286-ESBL	648
40	SRR7371316	SAMN09460053	SRX4244244	10/2/17	rectal swab	1280-ESBL	603
41	SRR7371317	SAMN09460078	SRX4244243	14/2/17	stool	1911-ESBL	28
42	SRR7371318	SAMN09460077	SRX4244242	9/2/17	stool	18-ESBLB	131
43	SRR7371319	SAMN09460076	SRX4244241	9/2/17	stool	18-ESBLA	48
44	SRR7371320	SAMN09460075	SRX4244240	8/2/17	rectal swab	188-ESBLB	48
45	SRR7371322	SAMN09460059	SRX4244238	14/2/17	rectal swab	1420-ESBL	69
46	SRR7371323	SAMN09460072	SRX4244237	13/2/17	stool	1802-ESBL	405
47	SRR7371324	SAMN09460071	SRX4244236	8/2/17	rectal swab	178-ESBLA	1193
48	SRR7371325	SAMN09460050	SRX4244235	14/2/17	rectal swab	1216-ESBL	131
49	SRR7371326	SAMN09460073	SRX4244234	10/2/17	rectal swab	1849-ESBL	10
50	SRR7371328	SAMN09460113	SRX4244232	17/2/17	rectal swab	725-ESBLA	95
51	SRR7371329	SAMN09460106	SRX4244231	9/2/17	rectal swab	579-ESBL	38
52	SRR7371330	SAMN09460103	SRX4244230	14/2/17	rectal swab	573-ESBLA	648
53	SRR7371331	SAMN09460102	SRX4244229	8/2/17	stool	494-ESBL	Unknown
54	SRR7371332	SAMN09460108	SRX4244228	10/2/17	stool	66-ESBLB	3580
55	SRR7371333	SAMN09460120	SRX4244227	13/2/17	rectal swab	XESBL	10
56	SRR7371334	SAMN09460101	SRX4244226	17/2/17	rectal swab	492-ESBL	773
57	SRR7371335	SAMN09460104	SRX4244225	14/2/17	rectal swab	573-ESBLB	2732
58	SRR7371336	SAMN09460119	SRX4244224	10/2/17	rectal swab	998-ESBL	442
59	SRR7371337	SAMN09460089	SRX4244223	9/2/17	stool	254-ESBL	1163
60	SRR7371338	SAMN09460090	SRX4244222	10/2/17	stool	280-ESBL	38
61	SRR7371339	SAMN09460109	SRX4244221	8/2/17	rectal swab	671-ESBLA	1193
62	SRR7371342	SAMN09460084	SRX4244218	9/2/17	rectal swab	210-ESBLA	Unknown
63	SRR7371343	SAMN09460081	SRX4244217	20/2/17	rectal swab	2088-ESBL	349
64	SRR7371344	SAMN09460082	SRX4244216	14/2/17	rectal swab	208-ESBLA	Unknown
65	SRR7371345	SAMN09460087	SRX4244215	18/2/17	rectal swab	2182-ESBL	38
66	SRR7371346	SAMN09460088	SRX4244214	16/2/17	stool	243-ESBL	349
67	SRR7371348	SAMN09460086	SRX4244212	18/2/17	rectal swab	2173-ESBL	394

Supplementary Table 9. The information of contigs carrying *mcr* genes

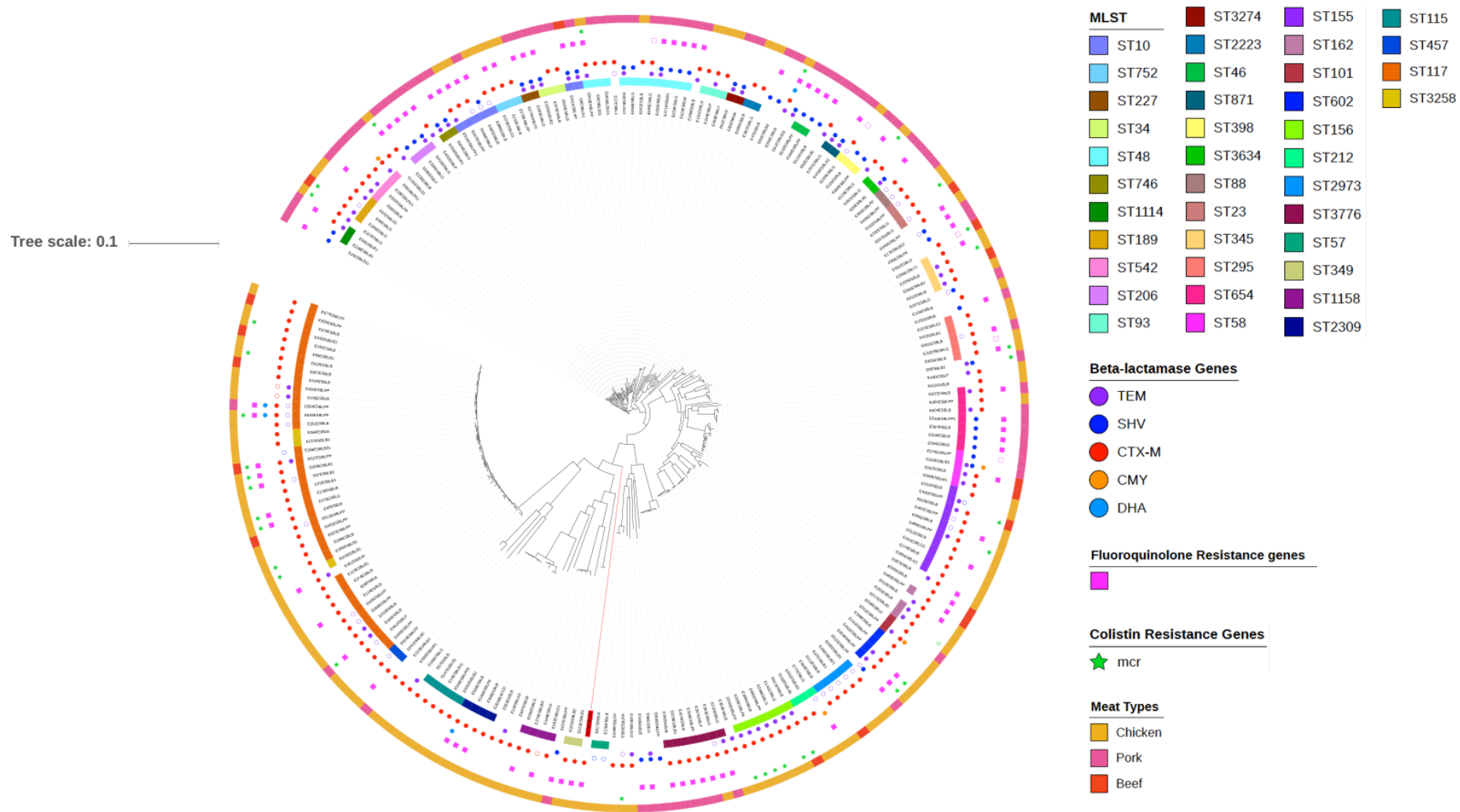
For *mcr* genes and replicon genes highlight in dark green: the genes in isolates has 100% identity with reference genes, and HSP/Query length equals to 1;

For *mcr* genes and replicon genes highlight in light green: the genes in isolates has less than 100% identity with reference genes, and HSP/Query length equals to 1;

For *mcr* genes and replicon genes highlight in gray: the genes in isolates has less than 100% identity with reference genes, and HSP/Query length is less than 1

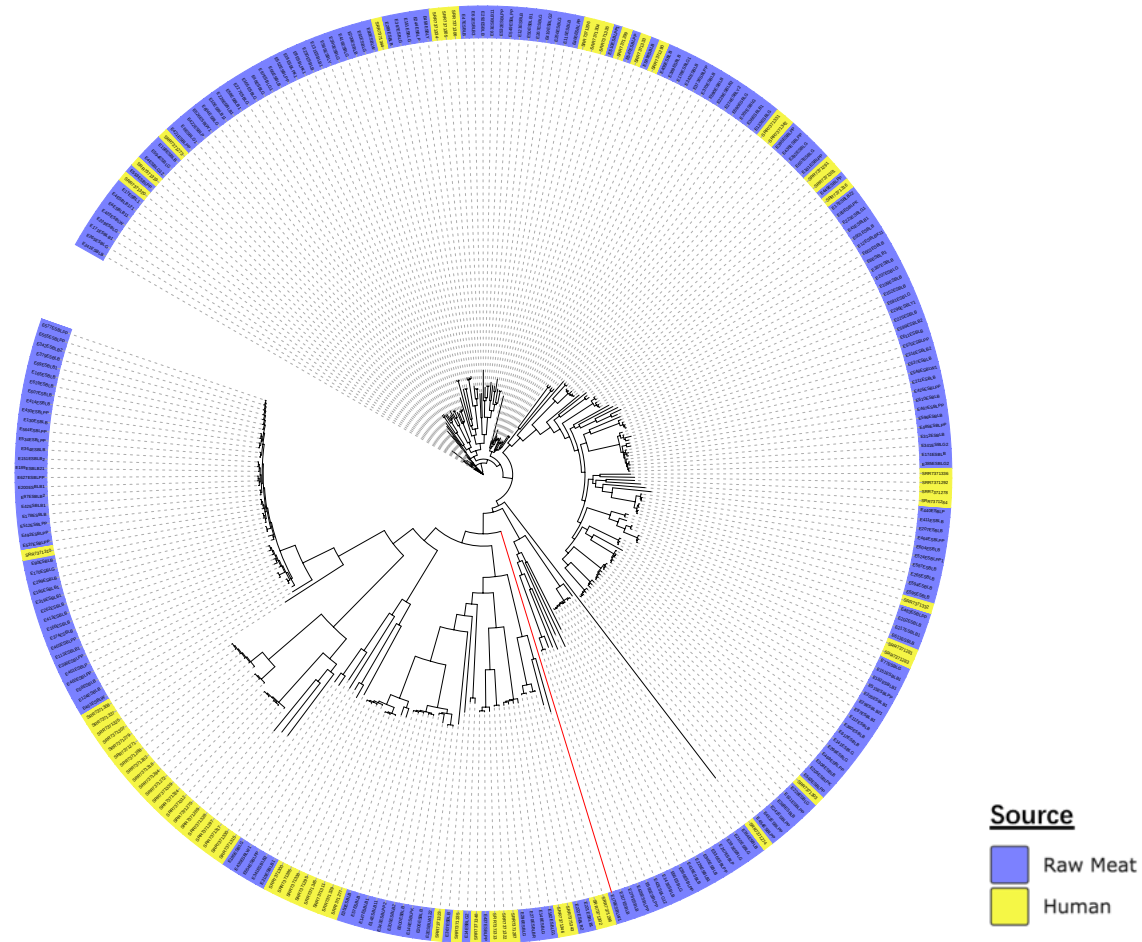
Isolate ID	ST	Source	<i>mcr</i> gene	Country	Market	Plasmid type	Contig information
E619ESBLB	ST117	Chicken	<i>mcr-1</i>	Malaysia	Wet market	IncB/O/K/Z	NODE 27 length 48059
E165ESBLG	ST189	Beef	<i>mcr-1</i>	Australia	Supermarket	IncHI2	NODE 9 length 188720
E230ESBLB	ST6697	Chicken	<i>mcr-1</i>	Unknown	Supermarket	IncHI2	NODE 5 length 180850
E123ESBLG	ST1072	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 26 length 60493
E141ESBLG	ST156	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 25 length 61200
E170ESBLG	ST117	Chicken	<i>mcr-1</i>	Unknown	Supermarket	IncI2	NODE 31 length 60393
E185ESBLB1	ST117	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 27 length 61217
E200ESBLB1	ST117	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 25 length 59180
E205ESBLPK	ST156	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 28 length 61733
E217ESBLB1	ST162	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 21 length 61088
E292ESBLG	ST23	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 17 length 61200
E299ESBLB	ST117	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 31 length 52490
E308ESBLB	ST156	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 21 length 81399
E342ESBLB2	ST117	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 22 length 61087
E469ESBLPP	ST156	Chicken	<i>mcr-1</i>	Unknown	Wet market	IncI2	NODE 23 length 61087
E485ESBLPP	ST155	Chicken	<i>mcr-1</i>	Unknown	Wet market	IncI2	NODE 24 length 61087
E563ESBLPP	ST156	Chicken	<i>mcr-1</i>	Unknown	Wet market	IncI2	NODE 28 length 61221
E565ESBLPP	ST2614	Chicken	<i>mcr-1</i>	Unknown	Wet market	IncI2	NODE 22 length 60852
E612ESBLB	ST156	Beef	<i>mcr-1</i>	Unknown	Wet market	IncI2	NODE 24 length 61200
E627ESBLPP	ST117	Beef	<i>mcr-1</i>	Brazil	Wet market	IncI2	NODE 24 length 59181
E90ESBLB	ST117	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 30 length 61087
E97ESBLB2	ST117	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncI2	NODE 23 length 60531
E166ESBLB	ST117	Pork	<i>mcr-1</i>	Holland	Supermarket	IncX4	NODE 41 length 32742
E451ESBLPP	ST602*	Chicken	<i>mcr-1</i>	Brazil	Supermarket	IncX4	NODE 31 length 32765
E454ESBLPP	ST602*	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	IncX4	NODE 31 length 32765
E610ESBLB	ST155	Beef	<i>mcr-1</i>	Australia	Supermarket	IncX4	NODE 49 length 23405
E225ESBLB	ST345	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	unknown	NODE 37 length 38476
E227ESBLG	Unknown	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	unknown	NODE 51 length 27372
E292ESBLB	ST3014	Chicken	<i>mcr-1</i>	Malaysia	Supermarket	unknown	NODE 54 length 22295
E534ESBLPP	ST117	Chicken	<i>mcr-1</i>	Malaysia	Wet market	unknown	NODE 37 length 27015
E553ESBLPP	ST48	Chicken	<i>mcr-1</i>	Malaysia	Wet market	unknown	NODE 54 length 24869
E564ESBLPP	ST117	Pork	<i>mcr-1</i>	Unknown	Wet market	unknown	NODE 35 length 27642
E603ESBLB	ST295	Chicken	<i>mcr-1</i>	Unknown	Wet market	unknown	NODE 25 length 70840
E591ESBLG	ST1730	Beef	<i>mcr-3.1</i>	Unknown	Wet market	unknown	NODE 80 length 5983

E8ESBLB1	ST4589	Pork	<i>mcr-5</i>	Unknown	Wet market	unknown	NODE_63_length_9001
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(a)

Tree scale: 0.01



(b)

Supplementary Figure 1. Complete phylogeny tree in Chapter 4.

a) phylogeny tree for all ESBL-producing *E. coli* isolates collected from raw meats in Singapore. The line in red is the genome of E178ESBLW1, which showed great difference with the rest of isolates.

b) phylogeny tree for all ESBL-producing *E. coli* isolates collected from raw meats in Singapore and ESBL-producing *E. coli* isolates from healthy human community in Singapore. The line in red is the genome of E178ESBLW1, which was collected from raw meats.

Supplementary Table 10. Information of assemblies in Chapter 5

ID	Genome size	No. of contigs	n50	MLST	MLST genes
1 BK_EC13	5153568	303	62880	ST-206	ADK_6,FUMC_7,GYRB_5,ICD_1,MDH_8,PURA_18,RECA_2
2 BK_EC14	5155318	310	58841	ST-206	ADK_6,FUMC_7,GYRB_5,ICD_1,MDH_8,PURA_18,RECA_2
3 BK_EC15	4727450	166	102688	ST-10	ADK_10,FUMC_11,GYRB_4,ICD_8,MDH_8,PURA_8,RECA_2
4 BK_EC33	5255740	270	126010	ST-4450	ADK_277,FUMC_8,GYRB_3,ICD_16,MDH_9,PURA_344,RECA_6
5 BK_EC57	4856475	89	223314	ST-3858	ADK_9,FUMC_6,GYRB_33,ICD_131,MDH_9,PURA_8,RECA_7
6 BK_EC58	5388703	183	227302	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
7 BK_EC59	5241516	105	192679	ST-354	ADK_85,FUMC_88,GYRB_78,ICD_29,MDH_59,PURA_58,RECA_62
8 BK_EC77	5103047	95	222568	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
9 BK_EC97	5264985	153	215669	ST-73	ADK_36,FUMC_24,GYRB_9,ICD_13,MDH_17,PURA_11,RECA_25
10 BK_EC100	5264263	148	215669	ST-73	ADK_36,FUMC_24,GYRB_9,ICD_13,MDH_17,PURA_11,RECA_25
11 BK_EC101	5109815	143	181152	ST-410	ADK_6,FUMC_4,GYRB_12,ICD_1,MDH_20,PURA_18,RECA_7
12 BK_EC109	5325893	158	204893	ST-648	ADK_92,FUMC_4,GYRB_87,ICD_96,MDH_70,PURA_58,RECA_2
13 BK_EC176	5164449	118	279970	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
14 BK_EC181	4926873	103	309404	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
15 BK_EC182	4925144	91	309404	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
16 BK_EC285	4982935	244	114366	ST-710	ADK_6,FUMC_153,GYRB_4,ICD_91,MDH_7,PURA_8,RECA_6
17 BK_EC364	5190223	175	112015	ST-405	ADK_35,FUMC_37,GYRB_29,ICD_25,MDH_4,PURA_5,RECA_73
18 BK_EC373	5273056	144	199043	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
19 BK_EC430	5386998	259	171364	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
20 BK_EC438	5270927	128	193020	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
21 BK_EC441	5355813	117	228159	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
22 BK_EC455	5089821	97	222569	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
23 BK_EC456	5304233	154	191062	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
24 BK_EC459	5300297	145	205503	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29

25	BK_EC460	5617288	227	151053	ST-38	ADK_4,FUMC_26,GYRB_2,ICD_25,MDH_5,PURA_5,RECA_19
26	BK_EC502	5165077	121	279970	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
27	BK_EC503	5166519	121	279970	ST-131	ADK_53,FUMC_40,GYRB_47,ICD_13,MDH_36,PURA_28,RECA_29
28	BK_EC517	5302377	195	194699	ST-88	ADK_6,FUMC_4,GYRB_12,ICD_1,MDH_20,PURA_12,RECA_7

Supplementary Table 11. MIC result in Chapter 5

	Non-ST131														ST131													
	EC13	EC14	EC15	EC57	EC59	EC97	EC100	EC101	EC109	EC285	EC33	EC364	EC460	EC517	EC58	EC77	EC176	EC181	EC182	EC373	EC430	EC438	EC441	EC455	EC456	EC459	EC502	EC503
Ampicillin (Am)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Piperacillin (Pi)	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Cephalothin(Cf)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Cefuroxime (Crm)	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Cefoxitin (Cfx)	>16	>16	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8
Cefotaxime (Cft)	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
Ceftazidime (Caz)	>16	>16	>16	>16	16	16	8	>16	>16	2	>16	>16	16	>16	16	8	8	8	16	2	>16	<=1	8	8	>16	>16	16	16
Cefepime (Cpe)	>16	>16	>16	>16	>16	>16	>16	>16	>16	4	>16	>16	>16	>16	>16	32	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Aztreonam (Azt)	>16	>16	>16	>16	>16	16	16	>16	>16	8	>16	>16	>16	>16	>16	>16	>16	>16	>16	8	>16	>16	16	16	>16	>16	>16	>16
Doripenem (Dor)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Imipenem (Imp)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Meropenem (Mer)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Ertapenem(Etp)	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5
Colistin (Cl)	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2	<=2
Tetracycline (Te)	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8	<=4	>8	>8	>8	>8	>8	>8	>8	>8	>8	<=4	>8	>8	>8	>8	>8	>8	>8
Tigecycline (Tgc)	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Minocycline(Min)	<=4	<=4	<=4	<=4	<=4	<=4	<=4	>8	>8	<=4	<=4	>8	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4
Amikacin (Ak)	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	16	<=8	<=8	16	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	16	32	<=8	<=8
Gentamicin (Gm)	>8	>8	>8	>8	>8	<=2	<=2	>8	>8	>8	>8	<=2	>8	>8	>8	<=2	>8	>8	>8	>8	>8	>8	>8	<=2	>8	>8	>8	>8
Tobramycin (To)	>8	>8	8	>8	8	<=2	<=2	>8	>8	>8	8	>8	>8	8	>8	<=2	8	8	8	>8	>8	>8	8	<=2	>8	>8	>8	8
Chloramphenicol (C)	>16	>16	>16	>16	<=8	>16	>16	>16	>16	>16	>16	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8	<=8
Ciprofloxacin (Cp)	<=0.5	1	1	1	>2	<=0.5	<=0.5	>2	>2	<=0.5	1	>2	<=0.5	>2	>2	>2	<=0.5	>2	>2	>2	>2	>2	<=0.5	>2	>2	>2	1	<=0.5
Norfloxacin (Nxn)	>1	>1	>1	>1	>1	<=0.5	<=0.5	>1	>1	1	1	>1	<=0.5	>1	>1	>1	1	>1	>1	>1	>1	>1	1	>1	>1	>1	>1	1
Levofloxacin (Lvxx)	<=1	<=1	<=1	<=1	>4	<=1	<=1	>4	>4	<=1	2	>4	<=1	2	>4	>4	<=1	>4	>4	>4	>4	>4	<=1	>4	>4	>4	<=1	<=1
Nalidixic Acid(NA)	>16	<=16	<=16	<=16	>16	<=16	<=16	>16	>16	<=16	<=16	>16	<=16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16

Supplementary Table 12. Meta data of global ST131 isolates in Chapter 5

	Accession	Isolate ID	Country	Source	Year
1	ERR3528488	BK_EC176	Thailand	human	2015
2	ERR3528489	BK_EC181	Thailand	human	2015
3	ERR3528490	BK_EC182	Thailand	human	2015
4	ERR3528494	BK_EC373	Thailand	human	2016
5	ERR3528495	BK_EC430	Thailand	human	2016
6	ERR3528496	BK_EC438	Thailand	human	2016
7	ERR3528497	BK_EC441	Thailand	human	2016
8	ERR3528498	BK_EC455	Thailand	human	2016
9	ERR3528499	BK_EC456	Thailand	human	2016
10	ERR3528500	BK_EC459	Thailand	human	2017
11	ERR3528502	BK_EC502	Thailand	human	2017
12	ERR3528503	BK_EC503	Thailand	human	2017
13	ERR3528506	BK_EC58	Thailand	human	2017
14	ERR3528508	BK_EC77	Thailand	human	2017
15	ERR1195703	Enterobase_79833_ESC_EA3756AA	Germany	human	2015
16	ERR1218535	PB164	Thailand	human	2014-2015
17	ERR1218536	PB191	Thailand	human	2014-2015
18	ERR1218542	PB289	Thailand	human	2014-2015
19	ERR1218544	PB363	Thailand	human	2014-2015
20	ERR1218545	PB370	Thailand	human	2014-2015
21	ERR1218557	PB390	Thailand	human	2014-2015
22	ERR1218582	PB13	Thailand	human	2014-2015
23	ERR1218589	PB84	Thailand	human	2014-2015
24	ERR1218592	PB100	Thailand	human	2014-2015
25	ERR1218598	PB169	Thailand	human	2014-2015
26	ERR1218601	PB195	Thailand	human	2014-2015
27	ERR1218606	PB236	Thailand	human	2014-2015
28	ERR1218607	PB239	Thailand	human	2014-2015
29	ERR1218609	PB251	Thailand	human	2014-2015
30	ERR1218616	PB335	Thailand	human	2014-2015
31	ERR1218622	PB368	Thailand	human	2014-2015
32	ERR1218623	PB375	Thailand	human	2014-2015
33	ERR1218624	PB382	Thailand	human	2014-2015
34	ERR1218625	PB385	Thailand	human	2014-2015
35	ERR1218628	PB409	Thailand	human	2014-2015
36	ERR1218632	PB419	Thailand	human	2014-2015
37	ERR1218633	PB420	Thailand	human	2014-2015
38	ERR1218637	PB474	Thailand	human	2014-2015
39	ERR1218682	W2_10_ERG1	Thailand	Environment	2015
40	ERR1218684	W2_11_ERB3	Thailand	Environment	2015
41	ERR1399397	Enterobase_67289_ESC_DA1317AA	Denmark	animal	2012
42	ERR161234	S1EC	UK	human	2007
43	ERR161235	S2EC	UK	human	2007
44	ERR161236	S5EC	UK	human	2007
45	ERR161237	S10EC	UK	human	2009
46	ERR161238	S11EC	UK	human	2009
47	ERR161239	S12EC	UK	human	2009
48	ERR161240	S15EC	UK	human	2009
49	ERR161241	S19EC	UK	human	2009
50	ERR161242	S21EC	UK	human	2009
51	ERR161243	S22EC	UK	human	2009
52	ERR161244	S24EC	UK	human	2009
53	ERR161245	S26EC	UK	human	2009
54	ERR161246	S30EC	UK	human	2007
55	ERR161247	S34EC	UK	human	2009
56	ERR161248	S39EC	UK	human	2004
57	ERR161249	S43EC	UK	human	2004

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58	ERR161250	S47EC	UK	human	2004
59	ERR161251	S53EC	UK	human	2004
60	ERR161252	MS2481	Australia	human	2007
61	ERR161253	MS2493	Australia	human	2007
62	ERR161254	B36EC	Australia	human	2007
63	ERR161255	S92EC	New Zealand	human	2009
64	ERR161256	S93EC	New Zealand	human	2009
65	ERR161257	S94EC	New Zealand	human	2009
66	ERR161258	S95EC	New Zealand	human	2009
67	ERR161259	S96EC	New Zealand	human	2010
68	ERR161260	S97EC	New Zealand	human	2010
69	ERR161261	S98EC	Australia	human	2008
70	ERR161262	S99EC	Australia	human	2009
71	ERR161263	S100EC	Australia	human	2009
72	ERR161264	S101EC	Australia	human	2009
73	ERR161265	S102EC	Australia	human	2010
74	ERR161266	S103EC	Australia	human	2010
75	ERR161267	S104EC	Australia	human	2008
76	ERR161268	S105EC	Australia	human	2008
77	ERR161270	S107EC	Australia	human	2010
78	ERR161271	S108EC	Australia	human	2009
79	ERR161272	S109EC	Australia	human	2009
80	ERR161273	S110EC	Australia	human	2009
81	ERR161274	S111EC	Australia	human	2009
82	ERR161275	S112EC	Australia	human	2009
83	ERR161276	S113EC	Australia	human	2009
84	ERR161277	S114EC	Australia	human	2011
85	ERR161278	S115EC	Australia	human	2011
86	ERR161279	S116EC	UK	human	2011
87	ERR161280	S117EC	UK	human	2011
88	ERR161281	S118EC	UK	human	2011
89	ERR161282	S119EC	UK	human	2011
90	ERR161283	S120EC	Canada	human	2009
91	ERR161284	S121EC	Canada	human	2000
92	ERR161285	S122EC	Canada	human	2003
93	ERR161286	S123EC	Canada	human	2001
94	ERR161287	S124EC	Canada	human	2003
95	ERR161288	S125EC	Canada	human	2002
96	ERR161289	S126EC	Canada	human	2002
97	ERR161290	S127EC	Canada	human	2002
98	ERR161291	S128EC	Canada	human	2004
99	ERR161292	S129EC	Canada	human	2004
100	ERR161293	S130EC	Canada	human	2004
101	ERR161294	S131EC	Canada	human	2002
102	ERR161295	S132EC	Canada	human	2005
103	ERR161296	S133EC	Canada	human	2005
104	ERR161297	S134EC	Canada	human	2005
105	ERR161298	S135EC	Canada	human	2005
106	ERR161299	S6EC	UK	human	2007
107	ERR161300	S31EC	UK	human	2007
108	ERR161301	S32EC	UK	human	2007
109	ERR161302	S37EC	UK	human	2009
110	ERR161303	S65EC	Australia	human	2009
111	ERR161304	S77EC	Australia	human	2010
112	ERR161305	S79EC	Australia	human	2009
113	ERR161306	HVM5	Spain	human	2010
114	ERR161307	P50EC	Spain	human	2011
115	ERR161308	HVM52	Spain	human	2010
116	ERR161309	P53EC	Spain	human	2011
117	ERR161310	P56EC	Spain	human	2011

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118	ERR161311	HVR83	Spain	human	2010
119	ERR161313	P146EC	Spain	human	2011
120	ERR161314	P189EC	Spain	human	2011
121	ERR161315	HVM277	Spain	human	2010
122	ERR161316	HVM826	Spain	human	2010
123	ERR161317	HVM834	Spain	human	2010
124	ERR161318	HVM1147	Spain	human	2010
125	ERR161320	HVM1299	Spain	human	2010
126	ERR161321	HVM1619	Spain	human	2010
127	ERR161322	HVM1997	Spain	human	2010
128	ERR161323	HVM2044	Spain	human	2010
129	ERR161325	HVM2289	Spain	human	2010
130	ERR161326	HVR2496	Spain	human	2010
131	ERR161328	HVM3017	Spain	human	2010
132	ERR161329	HVM3189	Spain	human	2010
133	ERR1656450	Enterobase_102535_ESC_FA4491AA	Spain	human	2016
134	ERR2027615	DRC_BKV_04	Congo	human	2014
135	ERR2027616	DRC_BKV_05	Congo	human	2014
136	ERR2027623	DRC_BKV_12	Congo	human	2014
137	ERR2027624	DRC_BKV_13	Congo	human	2014
138	ERR2027625	DRC_BKV_14	Congo	human	2014
139	ERR2027627	DRC_BKV_16	Congo	human	2014
140	ERR2091319	ERR2091319	Germany	food	2016
141	ERR2091373	ERR2091373	Germany	food	2016
142	ERR2091375	ERR2091375	Germany	food	2016
143	ERR2505731	DRC_BKV_03	Congo	human	2012/2014
144	ERR2694587	ERR2694587	Netherlands	food	2015
145	ERR2694588	ERR2694588	Germany	food	2015
146	ERR2694589	ERR2694589	France	food	2014
147	ERR2694918	VAR-33	Belgium	animal	2016
148	ERR2694919	ESBL20160056	Denmark	human	2016
149	ERR2694920	1603516	Luxembourg	animal	2016
150	ERR434856	Enterobase_22372_ESC_BA6920AA	UK	human	2009
151	ERR458470	IR18E	India	human	2009
152	ERR458471	IR49	India	human	2009
153	ERR458472	IR65	India	human	2009
154	ERR458473	IR68	India	human	2009
155	ERR712526	Enterobase_79551_ESC_EA3951AA	Germany	human	2013
156	SRR1201320	Enterobase_11188_ESC_BA8315AA	US	food	2013
157	SRR1220712	Enterobase_25288_ESC_BA8217AA	US	food	2013
158	SRR2970775	Enterobase_57787_ESC_CA4358AA	Cambodia	human	2009
159	SRR3345828	Enterobase_77096_ESC_DA3741AA	US	human	2014
160	SRR3618631	Enterobase_77374_ESC_DA3592AA	US	human	2014
161	SRR3982177	Enterobase_78134_ESC_EA2246AA	US	human	2012
162	SRR3982375	Enterobase_78137_ESC_EA2243AA	US	human	2013
163	SRR4065666	Enterobase_80603_ESC_EA4447AA	US	human	2014
164	SRR4294842	Enterobase_88019_ESC_EA8720AA	US	human	2016
165	SRR7828633	MER-199	Singapore	human	2015
166	SRR7828638	MER-366	Saudi Arabia	human	2017
167	SRR7828641	MER-206	Singapore	human	2015
168	SRR7828642	MER-205	Singapore	human	2015
169	SRR7828643	MER-257	Singapore	human	2015
170	SRR7828644	MER-298	Lebanon	human	2015
171	SRR7828645	MER-299	Lebanon	human	2015
172	SRR7828646	MER-214	Singapore	human	2015
173	SRR7828654	MER-297	Lebanon	human	2015
174	SRR7828655	MER-371	Italy	human	2016
175	SRR7828656	MER-215	Singapore	human	2015
176	SRR7828657	MER-216	Singapore	human	2015
177	SRR7828658	MER-374	Italy	human	2016

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178	SRR7828660	MER-372	Italy	human	2016
179	SRR7828661	MER-375	Italy	human	2016
180	SRR7828662	MER-209	Singapore	human	2015
181	SRR7828663	MER-210	Singapore	human	2015
182	SRR7828664	MER-207	Singapore	human	2015
183	SRR7828668	MER-184	Australia	human	2017
184	SRR7828671	MER-191	Australia	human	2017
185	SRR7828677	MER-307	Lebanon	human	2016
186	SRR7828679	MER-305	Lebanon	human	2016
187	SRR7828680	MER-304	Lebanon	human	2016
188	SRR7828682	MER-302	Lebanon	human	2015
189	SRR7828689	MER-178	Australia	human	2017
190	SRR7828691	MER-171	Australia	human	2017
191	SRR7828693	MER-173	Australia	human	2017
192	SRR7828694	MER-172	Australia	human	2017
193	SRR7828696	MER-174	Australia	human	2017
194	SRR7828697	MER-177	Australia	human	2017
195	SRR7828698	MER-176	Australia	human	2017
196	SRR7828701	MER-310	New Zealand	human	2016
197	SRR7828703	MER-317	Singapore	human	2016
198	SRR7828707	MER-319	Singapore	human	2017
199	SRR7828708	MER-320	Singapore	human	2017
200	SRR7828710	MER-161	Singapore	human	2017
201	SRR7828713	MER-167	Australia	human	2017
202	SRR7828714	MER-341	Australia	human	2017
203	SRR7828715	MER-342	Australia	human	2017
204	SRR7828721	MER-332	Singapore	human	2016
205	SRR7828722	MER-333	Singapore	human	2016
206	SRR7828723	MER-334	Singapore	human	2016
207	SRR7828724	MER-335	Singapore	human	2016
208	SRR7828725	MER-165	Singapore	human	2017
209	SRR7828726	MER-162	Singapore	human	2017
210	SRR7828728	MER-323	Singapore	human	2016
211	SRR7828730	MER-325	Singapore	human	2016
212	SRR7828736	MER-331	Singapore	human	2016
213	SRR7828737	MER-330	Singapore	human	2016
214	SRR7828738	MER-202	Singapore	human	2015
215	SRR7828739	MER-363	Saudi Arabia	human	2017
216	SRR7828742	MER-351	Australia	human	2017
217	SRR7828743	MER-367	Saudi Arabia	human	2017
218	SRR7828744	MER-368	Saudi Arabia	human	2017
219	SRR7828746	MER-370	Saudi Arabia	human	2016
220	SRR7828748	MER-345	Australia	human	2017
221	SRR7828749	MER-344	Australia	human	2017
222	SRR7828751	MER-350	Australia	human	2016
223	SRR7828752	MER-349	Australia	human	2016
224	SRR7828753	MER-348	Australia	human	2016
225	SRR7828754	MER-347	Australia	human	2016
226	SRR7828756	MER-154	Singapore	human	2017
227	SRR7828758	MER-152	Singapore	human	2017
228	SRR7828759	MER-151	Singapore	human	2017
229	SRR7828761	MER-149	Singapore	human	2017
230	SRR7828762	MER-148	Singapore	human	2017
231	SRR7828766	MER-241	Singapore	human	2015
232	SRR7828767	MER-242	Singapore	human	2015
233	SRR7828768	MER-243	Singapore	human	2015
234	SRR7828770	MER-237	New Zealand	human	2015
235	SRR7828772	MER-239	Singapore	human	2015
236	SRR7828775	MER-142	Singapore	human	2017
237	SRR7828778	MER-121	Turkey	human	2016

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238	SRR7828786	MER-376	Italy	human	2016
239	SRR7828792	MER-246	Singapore	human	2016
240	SRR7828793	MER-253	Singapore	human	2015
241	SRR7828794	MER-252	Singapore	human	2016
242	SRR7828795	MER-251	Singapore	human	2016
243	SRR7828798	MER-254	Singapore	human	2016
244	SRR7828807	MER-120	Turkey	human	2016
245	SRR7828808	MER-119	Turkey	human	2014
246	SRR7828810	MER-271	Turkey	human	2015
247	SRR7828811	MER-266	Turkey	human	2015
248	SRR7828812	MER-267	Turkey	human	2015
249	SRR7828813	MER-262	Turkey	human	2015
250	SRR7828816	MER-259	Singapore	human	2016
251	SRR7828819	MER-211	Singapore	human	2015
252	SRR7828820	MER-362	Saudi Arabia	human	2016
253	SRR7828821	MER-361	Saudi Arabia	human	2017
254	SRR7828822	MER-360	Saudi Arabia	human	2017
255	SRR7828823	MER-359	Saudi Arabia	human	2017
256	SRR7828824	MER-358	Saudi Arabia	human	2017
257	SRR7828825	MER-357	Saudi Arabia	human	2017
258	SRR7828828	MER-354	Saudi Arabia	human	2017
259	SRR7828835	MER-278	Turkey	human	2015
260	SRR7828836	MER-277	Turkey	human	2015
261	SRR7828838	MER-279	Turkey	human	2015
262	SRR7828842	MER-274	Turkey	human	2015
263	SRR7828844	MER-232	Australia	human	2015
264	SRR7828845	MER-227	Australia	human	2016
265	SRR7828846	MER-226	Australia	human	2016
266	SRR7828848	MER-230	Australia	human	2015
267	SRR7828852	MER-223	Australia	human	2016
268	SRR8203405	EC193	China	human	2014
269	SRR8203406	EC191	China	human	2015
270	SRR8203407	EC192	China	human	2015
271	SRR933339	JJ2055	US	human	2007
272	SRR933341	JJ2118	US	human	2008
273	SRR933343	JJ2134	US	human	2008
274	SRR933345	C001	US	human	2010
275	SRR933347	CD249	US	human	2005
276	SRR933349	JJ1999	India	human	2007
277	SRR933351	CD400	US	human	1992
278	SRR933353	CD466	US	avian	1990
279	SRR933355	CD467	US	avian	2009
280	SRR933357	CD471	US	unknown	1967
281	SRR933359	CD505	US	avian	1983
282	SRR933361	CU758	US	human	2009
283	SRR933363	G132	US	human	2010
284	SRR933365	G150	US	human	2010
285	SRR933367	G199	US	human	2010
286	SRR933369	G213	US	human	2010
287	SRR933371	G216	US	human	2010
288	SRR933373	H003	US	human	2010
289	SRR933375	H006	US	human	2010
290	SRR933377	H016	US	human	2010
291	SRR933379	H061	US	human	2011
292	SRR933381	H17	US	human	1985
293	SRR933385	JJ1901	US	human	2004
294	SRR933387	JJ2547	US	human	2009
295	SRR933389	JMI025	US	human	2000
296	SRR933391	JMI268	US	human	2006
297	SRR933393	MVAST0014	US	human	2010

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298	SRR933395	MVAST0020	US	human	2010
299	SRR933397	MVAST0036	US	human	2010
300	SRR933399	MVAST0038	US	human	2010
301	SRR933403	MVAST0077	US	human	2010
302	SRR933405	CD436	US	human	1997
303	SRR933407	JJ1908	US	human	2007
304	SRR933411	JJ2038	US	human	2007
305	SRR933413	JJ2050	US	human	2008
306	SRR933415	JJ2087	US	human	2003
307	SRR933417	JJ2183	US	human	2008
308	SRR933419	JJ2193	US	human	2008
309	SRR933421	JJ2643	US	human	2008
310	SRR933423	QU090	Australia	human	2008
311	SRR933425	MVAST0084	US	human	2010
312	SRR933427	MVAST0131	US	human	2010
313	SRR933429	MVAST0158	US	human	2010
314	SRR933431	MVAST0167	US	human	2010
315	SRR933433	MVAST0179	US	human	2010
316	SRR933435	SaT040	US	human	2007
317	SRR933437	SaT049	US	human	2007
318	SRR933439	SaT142	US	human	2003
319	SRR933441	SaT158	US	human	2003
320	SRR933443	U004	US	human	2010
321	SRR933445	U024	US	human	2010
322	SRR933447	U054	US	human	2010
323	SRR933449	ZH063	Canada	human	2002
324	SRR933451	ZH071	Canada	human	2002
325	SRR933453	ZH164	Canada	human	2004
326	SRR933455	ZH193	Canada	human	2004
327	SRR933457	CD301	US	turkey	2001
328	SRR933463	CD311	US	food	2002
329	SRR933467	CD340	US	monkey	2005
330	SRR933485	CU799	US	feline fecal	2008
331	SRR933489	JJ1887	US	human	2008
332	SRR933513	JJ2444	US	human	2008
333	SRR933515	JJ2508	US	human	2008
334	SRR933517	JJ2528	US	human	2007
335	SRR933519	JJ2550	US	human	2007
336	SRR933521	JJ2555	US	human	2007
337	SRR933523	JJ2578	US	human	2008
338	SRR933525	JJ2591	US	human	2006
339	SRR933527	JJ2608	US	human	2008
340	SRR933529	JJ2657	US	human	2009
341	SRR933531	JJ2668	US	human	2009
342	SRR933535	MH5800	Portugal	human	2005
343	SRR933537	KN1604	Korea	human	2003
344	SRR933539	QU300	Australia	human	2008
345	SRR933541	QUC02	Australia	canine	2008
346	SRR933543	QUC12	Australia	canine	2008
347	SRR933545	JJ2441	US	human	2008
348	SRR933547	JJ2489	US	human	2007
349	SRR2970633	la_5108	Laos	human	2007
350	SRR2970634	uk_P34091	UK	human	2005
351	SRR2970635	la_7619	Laos	human	2006
352	SRR2970636	la_8507	Laos	human	2007
353	SRR2970637	can_70883	Canada	human	2007
354	SRR2970639	can_70328	Canada	human	2007
355	SRR2970640	can_79159	Canada	human	2006
356	SRR2970641	can_1731_1	Canada	food	2006
357	SRR2970642	can_969	Canada	human	2007

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358	SRR2970643	can_1070	Canada	human	2007
359	SRR2970644	AZ657898	Germany	human	2010
360	SRR2970645	AZ600324	China	human	2009
361	SRR2970646	uk_P46212	UK	human	2005
362	SRR2970647	AZ647978	Venezuela	human	2010
363	SRR2970651	AZ779845	Spain	human	2011
364	SRR2970653	AZ735521	Spain	human	2011
365	SRR2970654	AZ684313	France	human	2010
366	SRR2970655	AZ844071	Germany	human	2012
367	SRR2970657	uk_P26250	UK	human	2005
368	SRR2970668	uk_UKUEC2	UK	human	2010
369	SRR2970680	uk_18B28B	UK	human	2010
370	SRR2970690	uk_8A9B	UK	human	2008
371	SRR2970691	uk_19B19L	UK	human	2010
372	SRR2970692	uk_19B22L	UK	human	2010
373	SRR2970693	uk_8A19D	UK	human	2008
374	SRR2970694	uk_7C26H	UK	human	2008
375	SRR2970695	la_12646	Laos	human	2008
376	SRR2970696	uk_18A18K	UK	human	2009
377	SRR2970697	uk_17B26A	UK	human	2009
378	SRR2970698	uk_18C29E	UK	human	2010
379	SRR2970699	uk_7C34K	UK	human	2008
380	SRR2970700	uk_17A7A	UK	human	2009
381	SRR2970701	uk_18A33A	UK	human	2009
382	SRR2970702	uk_18C16C	UK	human	2010
383	SRR2970703	uk_19A21D	UK	human	2010
384	SRR2970704	uk_18C23A	UK	human	2010
385	SRR2970705	uk_18B21F	UK	human	2010
386	SRR2970706	la_6169	Laos	human	2007
387	SRR2970707	uk_18B11D	UK	human	2010
388	SRR2970708	uk_19B17I	UK	human	2010
389	SRR2970709	uk_18C22I	UK	human	2010
390	SRR2970710	uk_18C14	UK	human	2010
391	SRR2970711	uk_8A16G	UK	human	2008
392	SRR2970712	uk_18A2G	UK	human	2009
393	SRR2970713	uk_18C4F	UK	human	2010
394	SRR2970714	uk_18B30B	UK	human	2010
395	SRR2970715	uk_17C26C	UK	human	2009
396	SRR2970716	uk_19A8G	UK	human	2010
397	SRR2970717	la_5220-3	Laos	human	2007
398	SRR2970718	uk_18B29L	UK	human	2010
399	SRR2970719	cam_1814	Cambodia	human	2010
400	SRR2970720	uk_18B18D	UK	human	2010
401	SRR2970728	la_11858	Laos	human	2008
402	SRR2970733	10B06164	Thailand	human	2010
403	SRR2970734	10B06797	Thailand	human	2010
404	SRR2970735	10B07395	Thailand	human	2010
405	SRR2970737	10B07488	Thailand	human	2010
406	SRR2970738	11B00062	Thailand	human	2011
407	SRR2970739	11B00094	Thailand	human	2011
408	SRR2970740	la_11242	Laos	human	2008
409	SRR2970741	11B00134	Thailand	human	2011
410	SRR2970742	11B00320	Thailand	human	2011
411	SRR2970743	11B00663	Thailand	human	2011
412	SRR2970744	11B00726	Thailand	human	2011
413	SRR2970745	11B00806	Thailand	human	2011
414	SRR2970746	11B01631	Thailand	human	2011
415	SRR2970747	11B01979	Thailand	human	2011
416	SRR2970748	10B07347	Thailand	human	2010
417	SRR2970749	cam_1439	Cambodia	human	2009

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418	SRR2970750	cam_2853	Cambodia	human	2009
419	SRR2970751	la_354-2	Laos	human	2008
420	SRR2970752	cam_1071	Cambodia	human	2008
421	SRR2970754	08B08158	Thailand	human	2008
422	SRR2970755	08B09891	Thailand	human	2008
423	SRR2970756	09B06064	Thailand	human	2009
424	SRR2970757	09B06312	Thailand	human	2009
425	SRR2970758	09B06460	Thailand	human	2009
426	SRR2970759	09B06574	Thailand	human	2009
427	SRR2970760	09B06576	Thailand	human	2009
428	SRR2970761	09B07697	Thailand	human	2009
429	SRR2970762	la_13105	Laos	human	2008
430	SRR2970763	09B09003	Thailand	human	2009
431	SRR2970764	09B09491	Thailand	human	2009
432	SRR2970765	09B11376	Thailand	human	2009
433	SRR2970766	09B13160	Thailand	human	2009
434	SRR2970767	09B13464	Thailand	human	2009
435	SRR2970768	10B05056	Thailand	human	2010
436	SRR2970769	10B05087	Thailand	human	2010
437	SRR2970770	10B05611	Thailand	human	2010
438	SRR2970771	10B05736	Thailand	human	2010
439	SRR2970772	cam_1531_1	Cambodia	human	2009
440	SRR2970773	uk_P16456	UK	human	2005
441	SRR2970774	cam_2254	Cambodia	human	2010
442	SRR2970776	cam_2917	Cambodia	human	2011
443	SRR2970777	cam_3162	Cambodia	human	2011
444	SRR2970778	can_80074	Canada	human	2008
445	SRR2970779	can_MS1007	Canada	human	2007
446	SRR2970780	la_12107_3	Laos	human	2008
447	SRR2970781	la_1424	Laos	human	2006
448	SRR2970782	la_10222	Laos	human	2008
449	SRR2970783	la_13792_2_pink	Laos	human	2009
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