This report should be submitted within 2 weeks after you return to Japan. Please do not change the formatting

（Abroad・Domestic）Internship report form（Student） 2023/06/13 (Year/Month/Day)

Name	Jirachaya Toyting
Laboratory	Division of Bioresources
Year (Grade)	D3
Internship institution	Genomic Epidemiology Research Group, National Food Institute, Technical University of Denmark
Internship period	Internship period: 05/08/2023 - 06/02/2023 (Departure Date from Sapporo: 05/06/2023, Arrival Date in Sapporo: 06/04/2023)
Purpose	To gain hands-on practice in the field of genomic epidemiology research by conducting experiments with the experienced research team and experiencing the teaching, mentoring, and research system in the top research university.

- The reason why you chose this institute

Technical University of Denmark (DTU) is one of Denmark's foremost research universities. In 2020, DTU was ranked as the second-best university in the world and number one in Europe by World University Research Rankings. The National Food Institute focuses on public health in relation to human nutrition, food safety, environment, and health. Specifically, the Genomic Epidemiology Research Group is a research group that has a primary task to conduct targeted research with the aim of predicting and preventing infectious diseases in humans and support global detection and control. This research group also provides advisory services to national and international authorities regarding health risks related to the presence of pathogenic microorganisms. Additionally, this research group is a pioneer in conducting antimicrobial resistance research in environmental samples. This research group initiated the Global Sewage Surveillance Project to explore the potential of using sewage for continuous monitoring of AMR. The project now continues as Global Surveillance of Antimicrobial Resistance, which is expected to initiate the first global real-time, large-scale AMR surveillance of human populations. I consider this a terrific opportunity to gain working experience in a leading research university among top researchers, who conduct a project that facilitates better and faster detection of AMR, leading to a paradigm shift in the way AMR surveillance is conducted and the real-time results are shared and analyzed.

- Result of the activity （about 800 words provide photos, tables and figures that clearly show the activities during the period）

During my internship, I worked on the project of genome characterization of *Salmonella* spp. isolated from Bangkok canal water. In this project, I had the opportunity to delve into the analysis of whole genome data obtained from 30 *Salmonella* spp. strains isolated from Bangkok canal water. By leveraging a range of bioinformatic tools, I gained proficiency in genome analysis and drawing insightful conclusions. The analysis programs were summarized in the following table.

Analysis	Program	Platform format
Sequence quality and genome assembly	FoodQC pipeline	Unix (Command line)
Species confirmation	KmerFinder 3.2	Web tool
Serotype prediction	SeqSero 1.2	Web tool
ST type identification	MLST 2.0	Web tool
Plasmid replicon identification	PlasmidFinder 2.1	Web tool
Antimicrobial resistance gene detection	ResFinder 4.1	Web tool
Phylogenetic analysis	CSI Phylogeny 1.4	Web tool
Phylogenetic visualization	iTOL v6	Web tool
Flanking region	Flankophile	Unix (Command line)
Gene cluster comparison	Clinker	Unix (Command line)

To begin, I employed the FoodQC pipeline to assess sequence quality and perform genome assembly. This initial step ensured that the subsequent analyses were built on a solid foundation of reliable and accurate genomic data.

Subsequently, I utilized KmerFinder 3.2 to confirm the species of the isolates as *Salmonella enterica*.

Further characterization of the isolates involved serotype prediction using SeqSero 1.2. This analysis revealed the major serotype distribution within the dataset. Notably, the most prevalent serotype was *S*. Agona, accounting for 31% of the isolates followed by *S*. Stanley (10%) and *S*. Mbandaka (10%). These findings provided insights into the prevalence and diversity of *Salmonella* serotypes present in the Bangkok canal water samples.

In addition to serotyping, I utilized MLST 2.0 to identify the Sequence Types (STs) of the isolates. This information contributes to the understanding of the genetic relatedness and population structure of the *Salmonella* strains. The analysis revealed that ST13 was the most predominant type, accounting for 31% of the isolates. ST29 (10%) and ST413 (10%) were also observed as significant STs within the dataset. The distribution of ST types aligned with the major serotypes identified, further validating the genetic associations between serotype and sequence type.

To explore the plasmid replicons present in the isolates, I employed PlasmidFinder 2.1. The analysis identified 15 different plasmid replicons among the strains. Notably, the most prevalent plasmid replicon was Col(pHAD28), suggesting its potential role in facilitating horizontal gene transfer and the dissemination of genetic elements among the *Salmonella* strains. This observation underscores the importance of plasmids in conferring antimicrobial resistance and other adaptive traits in bacteria.

Of particular interest in this study was the detection of antimicrobial resistance genes. By employing ResFinder 4.1, I identified a total of 37 acquired antimicrobial resistance genes in the *Salmonella* isolates. These genes conferred resistance to various classes of antibiotics, including beta-lactams, aminoglycosides, quinolones, tetracyclines, sulphonamides, trimethoprim, phenicols, fosfomycin, and disinfectants. This extensive repertoire of antimicrobial resistance genes highlights the potential for these strains to evade treatment and underscores the significance of continued surveillance in environmental samples and prudent use of antibiotics.

Within the context of antimicrobial resistance, I focused specifically on plasmid-mediated fluoroquinolone resistance (PMQR) genes. The analysis revealed the presence of *qnrS1*, *qnrB19*, *qnrD1*, *oqxAB*, and *aac(6')-Ib-cr* genes.

These genes contribute to the reduced susceptibility of *Salmonella* to fluoroquinolone antibiotics. In terms of point mutations associated with fluoroquinolone resistance, the majority of the isolates (93%) exhibited the *parC* T57S mutation. However, this mutation is associated with enhanced bacterial fitness rather than contribute to antimicrobial resistance. Additionally, one isolate displayed the *parC* S80R mutation, while nine isolates carried mutations in *gyrA* at positions S83F (n = 5), S83Y (n = 3), and D87G (n = 1). These point mutations are known to confer varying degrees of resistance to fluoroquinolones, further emphasizing the potential clinical implications of these findings.

To gain insights into the genetic relationships and population structure of the *Salmonella* strains, I conducted phylogenetic analyses with *S*. Agona, *S*. Stanley, and *S*. Mbandaka using CSI Phylogeny 1.4. The resulting phylogenetic trees provided visual representations of the genetic relatedness among the isolates. Notably, strains isolated from the same canal and during the same year tended to cluster together, indicating close genetic relationships and potential local transmission patterns. Moreover, these strains exhibited similar patterns of plasmid replicons, acquired antimicrobial resistance genes, and point mutations, suggesting the dissemination of specific Salmonella lineages in the canal water.

Focusing on the *qnrS* gene, I performed flanking region analysis to understand the genetic context and potential mobility of this resistance determinant. Initially, I employed Flankophile; however, due to its limitation of excluding isolates with contigs shorter than 1,500 bp, I turned to Clinker for further analysis. The flanking region analysis revealed dynamic changes in the gene organization pattern over time, suggesting ongoing genetic rearrangements and potential horizontal gene transfer events. This finding sheds light on the evolutionary dynamics of antimicrobial resistance genes and highlights the importance of understanding their mobility and impact on public health.

By employing Clinker for gene cluster comparison, I aimed to identify conserved genetic elements and ascertain potential associations between genetic features and epidemiological factors. The results revealed that strains isolated from the same canal exhibited consistent gene organization patterns, irrespective of serotype or year of isolation. This observation suggests the existence of specific genetic lineages associated with particular canals, reinforcing the notion of localized transmission and potentially shared sources of contamination. However, intriguingly, a particular gene cluster pattern was distributed across various

canals, serotypes, and years of isolation, indicating potential inter-canal dissemination or shared genetic ancestry.

In summary, the comprehensive analysis of the whole genome data from *Salmonella* isolates obtained from Bangkok canal water provided valuable insights into the serotypes, acquired antimicrobial resistance genes, and point mutation patterns of these strains. The results shed light on the genomic epidemiology of *Salmonella* spp. in this environmental context and contribute to our understanding of the genetic factors influencing antimicrobial resistance. The findings of this research hold potential for publication as a research article in an academic journal and could serve as a basis for future collaborations with researchers at DTU, fostering further advancements in this field of study.

- What do you think the positive impact of the activity will have on your further career path?

The internship afforded me a unique and invaluable opportunity to engage in collaborative research alongside seasoned researchers within a world-renowned research environment. Through this experience, I acquired firsthand knowledge and skillset in the field of genomic epidemiology research, with a specific focus on the comprehensive analysis of bacterial genomes. This immersive involvement enabled me to cultivate essential technical proficiencies and analytical skills requisite for a successful career as a researcher.

During the internship, participation in research group meetings provided me with an understanding of ongoing research within the group, as well as prevailing research trends and the latest technological advancements implemented to this field. Armed with this knowledge, I will be able to make meaningful contributions to ongoing and future research endeavors. The internship also encompassed mentorship and professional development opportunities, affording me the privilege of working closely with esteemed experts who provided invaluable guidance and constructive feedback on my work. Additionally, I had the opportunity to observe the teaching and mentoring system, thereby enhancing my awareness of effective strategies for future endeavors as a university lecturer.

The noteworthy research achievement during the internship involved

the identification of antimicrobial resistance genes, as well as the exploration of genetic relationships and gene synteny among *Salmonella* strains isolated from Bangkok canal water. The outcomes of this research hold potential for dissemination through scientific journal publications and conference presentations, thereby contributing to the advancement of knowledge and comprehension within the realm of genomic epidemiology. Moreover, the diverse range of research studies undertaken in this research group also served as a source of inspiration for my future research pertaining to antimicrobial resistance in environmental samples, hence facilitating sustainable interventions in addressing antimicrobial resistance within the One Health framework.

In addition to the academic facets, the internship facilitated the expansion of my professional network and fostered vital relationships with researchers affiliated with DTU. These connections not only provided insights into prospective job opportunities but also presented me with recommendations to consider pursuing a career in Denmark. Furthermore, I had the privilege of observing the advisory services provided by the head of the research group to governmental bodies, specifically relating to antimicrobial registration. This exposure is anticipated to be advantageous in my envisioned role as a zoonosis control expert. Lastly, the cross-cultural exchange during the internship allowed me to gain firsthand experience of Danish working culture and engage in fruitful discussions concerning working environments in Japan, Thailand, and Denmark. This exposure profoundly influenced my perceptions regarding desirable work settings and has undoubtedly shaped my trajectory for future career aspirations.

- Advice for your junior fellows

First and foremost, I recommend exploring multiple internship options and gathering as much information as possible about potential candidates. Consider aligning your choices with the field of your research interests, the organizations you aspire to work with in the future, or even the countries where you wish to immerse yourself in the local living and working culture. This preliminary research will enable you to make an informed decision and select an internship opportunity that aligns with your goals and aspirations.

It is important to be prepared for the intricate process of obtaining the

necessary work permits and visas for your chosen destination. This often involves dealing with a multitude of documents and navigating bureaucratic procedures. Additionally, keep in mind that there may be expenses associated with the application process. Being aware of these requirements and planning accordingly will ensure a smoother transition into your internship.

Resilience is a key attribute to cultivate during your internship journey. Challenges and obstacles may arise, but maintaining a positive mindset and perseverance will help you overcome them. Remember that setbacks are opportunities for growth and learning. Embrace them as part of your personal and professional development.

One of the most enriching aspects of an internship is the opportunity for cultural exchange. Immerse yourself in the local culture, traditions, and customs of the country where you are interning. Engage with your colleagues, make new friends, and learn from their experiences. Embracing cultural diversity will broaden your horizons and enhance your understanding of different perspectives.

While pursuing your internship, it is crucial to prioritize work-life balance and prioritize your well-being. Take care of yourself mentally, physically, and spiritually. Engage in activities outside of work that bring you joy and relaxation. Whether it is exploring the local sights, indulging in hobbies, or simply taking time for self-reflection, remember to find a healthy balance between your professional and personal life.

Seize every moment during your internship. Recognize that some experiences may be once-in-a-lifetime opportunities. Embrace new challenges, step out of your comfort zone, and make the most of the knowledge and skills you acquire. Take initiative, be proactive, and contribute actively to the projects and activities you are involved in. By fully immersing yourself in the internship experience, you will maximize the value of your time and leave with a sense of accomplishment.

Finally, always remember that your life and career decisions are ultimately yours to make. Embrace the freedom to choose the path that aligns with your passions, interests, and aspirations. Seek advice from mentors and trusted individuals but trust your instincts and make decisions that resonate with your goals and values.

Approval of supervisor	Institution・Official title・Name International Institute for Zoonosis Control Prof. Yasuhiko Suzuki

※1 Send the electronic file to the WISE Program Office
※2 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
※3 The Steering Committee for the WISE Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

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