

TABLE OF CONTENTS

TITLE PAGE	i
APPROVAL SHEET	ii
DECLARATION OF AUTHENTICITY	iii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF APPENDICES	xi
ABSTRACT	xii
INTISARI	xiii
CHAPTER I.....	1
1.1 Background	1
1.2 Research objectives.....	4
1.3 Research Benefits.....	4
CHAPTER II.....	5
2.1 Antibiotics.....	5
2.2 History of Quinolones and fluoroquinolones.....	6
2.3 Usage of quinolones and fluoroquinolones in Japan.....	9
2.4 Quinolones and fluoroquinolones mode of action.....	10
2.4.1 Quinolone target: DNA gyrase and topoisomerase IV.....	11
2.4.2 Quinolone action.....	14
2.5 Quinolone resistance mechanisms	15
2.5.1 Chromosomal mutations	16
2.5.2 Plasmid-mediated quinolone resistance	17
2.6 Emergence and impact of antibiotic resistance	19
2.7 The role of aquatic ecosystems as reservoirs of antibiotic resistance genes	20
2.8 <i>Escherichia coli</i>.....	21
CHAPTER III	22
3.1 Research time and location	22
3.2 Materials	22
3.3 Methods.....	22

3.3.1 Study area.....	22
3.3.2 Bacterial isolates	24
3.3.3 Detection of Plasmid-Mediated Quinolone Resistance (PMQR) genes.....	25
3.3.4 Detection of Quinolone-Resistance Determining Region (QRDR) genes	27
3.3.5 Antibiotic susceptibility test.....	28
CHAPTER IV	30
4.1 PMQR genes possessed by <i>E. coli</i> isolates from Akagawa Water System	30
4.2 QRDR genes possessed by <i>E. coli</i> isolates from Akagawa Water System.....	34
4.3 Antibiotic resistance profile of <i>E. coli</i> isolates from Akagawa Water System.	37
CHAPTER V	43
5.1 Conclusions	43
5.2 Recommendations	43
REFERENCES	44
APPENDICES	51

LIST OF TABLES

Table 2.1 Major classes of antibiotics and how they work	5
Table 2.2 Available quinolones in Japan (Kusama et al., 2020)	9
Table 3.1 Primer set used for detection of PMQR genes	25
Table 3.2 Amplification program for detection of PMQR genes	26
Table 3.3 Primer set used for detection of QRDR genes	27
Table 3.4 Amplification program for detection of QRDR genes	28
Table 4.1 Detection rates of PMQR genes	30
Table 4.2 Detection rates of QRDR genes	34
Table 4.3 The number of drug-resistant <i>E. coli</i> at different sampling sites	37
Table 4.4 Drug susceptibility of <i>E. coli</i> isolated from Akagawa Water System	39
Table 4.5 Ciprofloxacin- and levofloxacin-resistant <i>E. coli</i> isolates.....	40

LIST OF FIGURES

Figure 2.1 Structure of nalidixic acid and quinolone core.	7
Figure 2.2 Structure of norfloxacin and ciprofloxacin.	8
Figure 2.3 Structure of levofloxacin and moxifloxacin.	9
Figure 2.4 Structure of DNA gyrase and Topoisomerase IV (Sutormin et al., 2021).....	11
Figure 2.5 Supercoiling, relaxation, catenation, and decatenation of DNA molecules (Sutormin et al., 2021).	12
Figure 2.6 Domain structure of bacterial DNA gyrase and topoisomerase IV and human topoisomerase II α (Pham et al., 2019).	13
Figure 2.7 Intracellular action of quinolones. (a) chromosomal break and (b) bacterial SOS response (Sutormin et al., 2021)	14
Figure 2.8 Mechanisms of quinolone resistance	16
Figure 3.1 Geographic locations of the seven sampling sites along the Akagawa Water System.....	24
Figure 4.1 Distribution of PMQR genes in the samples from different sampling sites in Akagawa Water System.....	32
Figure 4.2 Distribution of the PMQR-positive <i>E. coli</i> isolates among phylogenetic groups	33
Figure 4.3 Distribution of QRDR genes in the samples from different sampling sites in Akagawa Water System.....	36
Figure 4.4 Distribution of the QRDR-positive <i>E. coli</i> isolates among phylogenetic groups	37

LIST OF APPENDICES

Appendix 1 96-well PCR plate template example	51
Appendix 2 Sheet for documenting the PCR results.....	52
Appendix 3 PCR result visualized under an ultraviolet illumination.....	53