DUPLEX PCR DETECTION OF *bla*$_{SHV}$ AND *bla*$_{TEM}$ GENES IN UROPATHOGENIC *Escherichia coli* (UPEC) ISOLATES FROM PATIENTS

By

LEE YIN YING

A project report submitted to the Department of Allied Health Science

Faculty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Biomedical Science

April 2022

ABSTRACT

DUPLEX PCR DETECTION OF *bla*SHV AND *bla*TEM GENES IN UROPATHOGENIC *Escherichia coli* (UPEC) ISOLATES FROM PATIENTS

Lee Yin Ying

The emergence of multidrug-resistant and extended spectrum beta-lactamases-producing uropathogenic *Escherichia coli* (ESBLs-producing UPEC), which is the leading aetiological agent of urinary tract infections (UTIs), causing a significant reduction in the efficacy of antimicrobial treatment and increase in mortality. This study aimed to investigate the prevalence of *bla*SHV and *bla*TEM genes, and to determine the antibiotic resistance profile among the UPEC isolates as well as the association between the phenotypic and genotypic data. UPEC strains (n=60) were isolated from patients' urine samples. The isolates had undergone bacterial strain identification using MALDI-TOF by our collaborator in Hospital Raja Permaisuri Bainun in Ipoh. Kirby-Bauer disk diffusion was performed with nine antimicrobial agents that come from five different classes to determine the antibiotic resistance profile. The fast boil method was carried out for DNA extraction from the UPEC isolates. To screen the *bla*SHV and *bla*TEM genes, duplex PCR was conducted. The antibiotic susceptibility result showed that 71.67% of the isolates were resistant to ampicillin, 51.67% to tetracycline, 50.00% to nalidixic acid, 33.33% to SXT, 31.67% to ciprofloxacin, 26.67% to levofloxacin, 16.67% to chloramphenicol,

1.67% to minocycline, while all the isolates were susceptible to imipenem. The molecular result revealed that *bla*TEM gene was detected in most of the UPEC isolates (60.00%, n=36) while none of the isolates was detected with the presence of *bla*SHV gene, suggesting that there are variants of *bla*SHV gene that may not be able to detect in this study. The positive associations between *bla*TEM gene and four of the tested antibiotics: ampicillin (p=0.014), ciprofloxacin (p=0.041), levofloxacin (p=0.043) and tetracycline (p=0.001), were found to be significant. Distribution of *bla*TEM was revealed to be predominant in female patients and in patients who aged 60 years and above. However, negative association was observed between the *bla*TEM genes and gender as well as age.

ACKNOWLEDGEMENTS

Without the help of a number of people, this project would not have been feasible. My first, sincere and big appreciation goes to my supervisor, Dr. Chew Choy Hoong, for her invaluable assistance, unwavering patience and great insights leading to this project.

Further, I would like to thanks to all the UTAR laboratory officers, Ms. Fatin, Ms. Hemaroopini, and Mr. Saravanan especially. Also, special thanks to all the lab assistants, Mrs. Siti Nor Ain, Mrs. Natrah, Mr. Mohd Zaini, Mrs. Nur Izzati and Mrs. Nurfarhana. Their detailed explanation and feedback for the doubts I faced during the lab works were very important to me. Another special appreciation to the postgraduate student, Chin Jia Jin, for his generous engagement in guiding and constructive advices that contributes to the completion of this project.

Moreover, I would also like to express my deepest gratitude to my lab mates, Lee Hui Mei, Lee Shuet Yi, Joey Sim Chin Yee and Theo Chun Hao for their valuable suggestion and inspirations. Last but not least, my heartfelt thanks go to my family members who were the constant love, support and strength providers to me, keeping me motivated throughout the project.

DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

LEE YIN YING

APPROVAL SHEET

This project report entitled **"DUPLEX PCR DETECTION OF *bla*$_{SHV}$ AND *bla*$_{TEM}$ GENES IN UROPATHOGENIC *Escherichia coli* (UPEC) ISOLATES FROM PATIENTS"** was prepared by **LEE YIN YING** and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:

Dr. Chew Choy Hoong Date: 30/5/2022

Associate Prof. /Supervisor

Department of Allied Health Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 21/04/2022

PERMISSION SHEET

It is hereby certified that **LEE YIN YING** (ID No: **18ADB02333**) has completed this final year project thesis entitled **"DUPLEX PCR DETECTION OF *bla*$_{SHV}$ AND *bla*$_{TEM}$ GENES IN UROPATHOGENIC *Escherichia coli* (UPEC) ISOLATES FROM PATIENTS"** under the supervision of Dr. Chew Choy Hoong from the Department of Allied Health Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(LEE YIN YING)

TABLE OF CONTENTS

		Page
ABSTRACT		**ii**
ACKNOWLEDGEMENTS		**iv**
DECLARATION		**v**
APPROVAL SHEET		**vi**
PERMISSION SHEET		**vii**
TABLE OF CONTENTS		**viii**
LIST OF TABLES		**x**
LIST OF FIGURES		**xi**
LIST OF APPENDICES		**xii**

Chapter

1	INTRODUCTION	1
2	LITERATURE REVIEW	4
	2.1 Urinary Tract Infection	4
	2.1.1 Overview	4
	2.1.2 Uropathogenic *Escherichia coli*	4
	2.2 Antimicrobial Resistance in UPEC	5
	2.2.1 Overview	5
	2.2.2 Beta-lactams	6
	2.2.3 Quinolones	8
	2.2.4 Tetracyclines	9
	2.3 Antibiotic Resistance Gene	11
	2.3.1 Overview	11
	2.3.2 bla_{SHV} and bla_{TEM} genes	11
	2.3.3 bla_{CTX-M} gene	16
	2.3.4 bla_{OXA} gene	16
3	MATERIALS AND METHODS	17
	3.1 Materials	17
	3.2 Methodology	18
	3.2.1 Sample Collection and Identification	18
	3.2.2 Antimicrobial Susceptibility Testing	19
	3.2.3 Extraction of Genomic DNA	21
	3.2.4 Duplex PCR Optimisation and Screening of Samples	21
	3.2.5 Agarose Gel Electrophoresis	24
	3.2.6 Statistical Analysis	24
4	RESULTS	25
	4.1 Demographic Profile of the UPEC Isolated	25
	4.2 Antimicrobial Susceptibility Test	27
	4.3 Concentration and Purity of Extracted DNA	30
	4.4 Duplex PCR Screening of bla_{TEM} and bla_{SHV}	30
	4.5 Gene Prevalence based on Gender and Age	33

4.6 Association between Phenotypic and Genotypic Features of UPEC Isolates 35

5 DISCUSSION 38
5.1 Overview 38
5.2 Antimicrobial Susceptibility Profile among UPEC Isolates 38
5.3 Prevalence of *bla*TEM and *bla*SHV among the UPEC Isolates 40
5.4 Association of Phenotypic and Genotypic Data 41
5.5 Prevalence of UPEC Strain based on Gender and Age 43
5.6 Limitations and Future Studies 44

6 CONCLUSION 45

REFERENCES 47

APPENDICES 54

LIST OF TABLES

Table	Title	Page
2.1	Compilation of the SHV-type extended spectrum beta-lactamases-producing bacteria, the pI values of the enzymes, the geographical distribution, and their year of isolation	13
3.1	Chemicals and reagents utilised along with their corresponding manufacturer	17
3.2	The interpretation standards for inhibition zone (in millimetres) proposed by Clinical Laboratory Standards Institute (CLSI) for the nine chosen antibiotics	20
3.3	Cycling conditions of gradient PCR	22
3.4	Components of a standard duplex PCR reaction mixture in a final volume of 25 μL for the detection of bla_{SHV} and bla_{TEM} genes	23
3.5	Primer sequences and expected product sizes for bla_{SHV} and bla_{TEM} genes	23
3.6	Cycling conditions of duplex PCR	24
4.2	Distribution of bla_{TEM} genes in accordance with gender and age of the patients	35
4.3	Association of bla_{TEM} gene with antibiotic susceptibility of UPEC isolates	37

LIST OF FIGURES

Figure	Title	Page
2.1	Classification of beta-lactams according to the core ring's structure	7
2.2	The mechanism of action of quinolones	9
2.3	The mechanism of action of tetracyclines	10
4.1	The attributes of sample by gender and age	26
4.2	Representative figures of antimicrobial susceptibility test on UPEC isolates	28
4.3	Distribution (%) of resistance to antibiotics among UPEC isolates	29
4.4	Optimisation of PCR condition based on annealing temperature gradient	32
4.5	Representative gel image of duplex PCR screening for *bla*$_{TEM}$ and *bla*$_{SHV}$ on 1.5% agarose gel	32
4.6	Distribution of *bla*$_{TEM}$ gene according to the (A) gender and (B) age of the patients	34

LIST OF APPENDICES

Appendix	Title	Page
A	Demographic data of each UPEC isolate tested in this study	54
B	Antimicrobial susceptibility profile of UPEC isolates	56
C	Concentration and purity (A260/280 ratio) of the extracted DNA samples from each UPEC isolates	59
D	Results of duplex PCR for detection of *bla*TEM and *bla*SHV in 60 UPEC isolates	61
E	Representative statistical analysis of negative and positive association	62

CHAPTER 1

INTRODUCTION

According to Centres for Disease Control and Prevention (2022), urinary tract infection (UTI) is the fifth-ranking most common type of nosocomial infection. The most common causative agent is uropathogenic *Escherichia coli* (UPEC). This bacterial strain is originated from extraintestinal pathogenic *Escherichia coli* (ExPEC), it evolves by acquiring a combination types of survival ability, therefore, it can live, colonise, and infect the normally sterile urinary system (Vila et al., 2016).

Previous research had shown that curative treatment for UTIs using penicillin and tetracycline is ineffectual due to the high resistance prevalence of UPEC strain to the mentioned antibiotics (Momtaz et al., 2013). Subsequently, trimethoprim-sulfamethoxazole became the commonly prescribed drug for UTI therapy, and recently, trimethoprim-sulfamethoxazole resistance UPEC strain has still emerged (Moura et al., 2009). The decreasing effectiveness of the aforementioned antimicrobial agents used for UTIs treatment has driven the prescription of quinolones or fluoroquinolones as first-line UTI therapy. This type of drug effectively cures UTIs for some time, yet the emergence of the resistance strains has arisen due to the indiscriminate prescription (Moura et al., 2009; Momtaz et al., 2013).

The acquisition of antibiotic resistance genes is one of the key mechanisms that leads to the emergence of antibiotics- or multidrug-resistant UPEC strains. These genes are predominantly spread through the transmission of mobile genetic elements, particularly plasmid. Beta-lactamase genes are the widely known antibiotic resistance genes found in most Gram-negative bacteria, especially *E. coli*. The presence of beta-lactamase genes in the bacteria not only confers resistance towards beta-lactam antibiotics but also associates with the gain of consolidated resistance trait against other classes of antibiotics (Pishtiwan and Khadija, 2019).

Production of beta-lactamases is the main resistance mechanism of *E. coli* (Xiao et al., 2019). Two popular examples of the plasmid-mediated beta-lactamases genes are bla_{SHV} and bla_{TEM} genes, some of their variants are encoded for extended spectrum beta-lactamases (ESBLs). These two types of genes are determined to be the common type of beta-lactams resistance gene that is responsible for the production of ESBLs among the pathogenic bacteria strains and thus contribute to the alarming emergence of antibiotic resistant disease-causing bacteria, including the UPEC strains (Ibrahim et al., 2021). These synthesised enzymes inactivate the beta-lactams by hydrolysis of the core four-membered ring structure. Both genes have over hundred mutant derivatives that were being reported. In the comparison between both genes, bla_{TEM} gene is rather explosively disseminated and thus has a higher worldwide prevalence (Pishtiwan and Khadija, 2019).

The prevalence rate of multidrug-resistant UPEC strain is geographically different, and it will vary with time (Raeispour and Ranjbar, 2018). In addition to that, Malaysia lacks the research on antimicrobial resistance (AMR) patterns in UPEC strains, and to the best of our knowledge, there is no published study that aims to assess the multidrug resistance (MDR) profile in the UPEC strain in the Malaysian context. Hence, the purpose of this presented study is to detect the prevalence of two of the beta-lactamase genes in the UPEC strain isolated from patients' urine samples in Malaysia, which can provide an insight into the AMR or MDR mechanism of UPEC strain and raise awareness of fast-acting to combat the spreading of AMR.

Therefore, the following objectives were set out in this research:

1. To detect the presence of *bla*SHV and *bla*TEM genes in UPEC isolates from patients via duplex PCR.
2. To analyse the antimicrobial resistance phenotypes and genotypes of the UPEC isolates.
3. To investigate the association of prevalence of *bla*SHV and *bla*TEM genes to the demographic profile.

CHAPTER 2

LITERATURE REVIEW

2.1 Urinary Tract Infection

2.1.1 Overview

Urinary tract infection (UTI) is a microbial infection that happens in the urinary system, in which bacteria are the most frequent causative agent, followed by fungi and viruses (Healthline, 2022). The lower urinary tract (urethra and bladder) is more commonly to be the infected part in comparison to the upper urinary tract (ureters and kidneys), which is the part that will result in a more severe clinical manifestation if it is involved in the infection (National Institute of Diabetes and Digestive and Kidney Diseases, 2019). It is known as the second-highest global prevalence of bacterial infectious disease in humans (Sadeghi et al., 2020). The prevalence of UTI has a direct relationship with age, and older adults are the highest-risk group to suffer from UTI. In addition, females are more prone to this disease as compared to males (Medina and Castillo-Pino, 2019).

2.1.2 Uropathogenic *Escherichia coli*

Escherichia coli (*E. coli*) is a Gram-negative bacterium, that not only is a commensal gastrointestinal tract bacterium, but as well a human opportunistic pathogen. One of its pathotypes named UPEC (uropathogenic *E. coli*) strain is broadly recognised as the major aetiological agent of UTI (Vila et al., 2016). The UPEC strain is originated from the commensal intestinal *E. coli* microbiome, it acquires some virulence genes and thus gains the capabilities to

survive, disseminate and colonise in the urinary organ system. Hence, it is also categorised as extraintestinal pathogenic *E. coli* (ExPEC) (Mann et al., 2017). Other than virulence factors, antimicrobial resistance is also one of the significant factors that lead to the epidemiological success of UPEC. Multidrug resistance conveys a higher fitness to the UPEC in specific ecological niches (Vila et al., 2016). The acquisition of antimicrobial resistance genes among UPEC strains may be through horizontal transfer mechanisms or alteration in DNA sequences owing to their flexible genomic pool (Jahandeh et al., 2015). To add on, the antimicrobial resistance genes not only can be located in the bacterial chromosomal DNA, but as well can be resided on plasmid, transposons and integrons (Jahandeh et al., 2015).

2.2 Antimicrobial Resistance in UPEC

2.2.1 Overview

In recent times, the emergence and increase of antimicrobial resistance (AMR) have become a global public health concern. The excessive, imprudent, and inappropriate use of antimicrobial drugs has led to this alarming worldwide community health peril. AMR complicates microbial infectious diseases, causing prolonged illness. In consequence, it gives rise to the mortality rate apart from the fact that it financially burdens the patients. The medical treatment for UTI has a significant drop in efficacy as a result of AMR, especially for the commonly prescribed drugs or the broad-spectrum drugs like ampicillin. Therefore, AMR has obtained global urgent attention to come out with proper management of antimicrobial agents and development of safe, effective as well as affordable antimicrobial drugs (Lien et al., 2017).

2.2.2 Beta-lactams

Beta-lactams, a huge group of antibiotic agents named for the same core four-membered beta-lactam ring system. It is subdivided into four major groups: penicillins, cephalosporins, monobactams and carbapenems, according to the core ring's structure as shown in Figure 1. This group of antibiotics exerts a bactericidal effect, in which the core beta-lactam ring forms covalent bindings with the active site of the enzymes namely penicillin-binding proteins (PBPs). These beta-lactams targeted enzymes (transpeptidases, endopeptidases and carboxypeptidases) are important in the terminal stage of bacterial cell wall synthesis. This class of antibiotics can function to interrupt the cell wall synthesis process due to its mimicry of the natural substrate of the PBPs, which is the D-Ala-D-Ala peptide terminus. Thus, the binding between the PBPs with the 'fake' substrate results in the failure of cell wall formation (Denis et al., 2010; Ring Biotechnology Co Ltd, 2018).

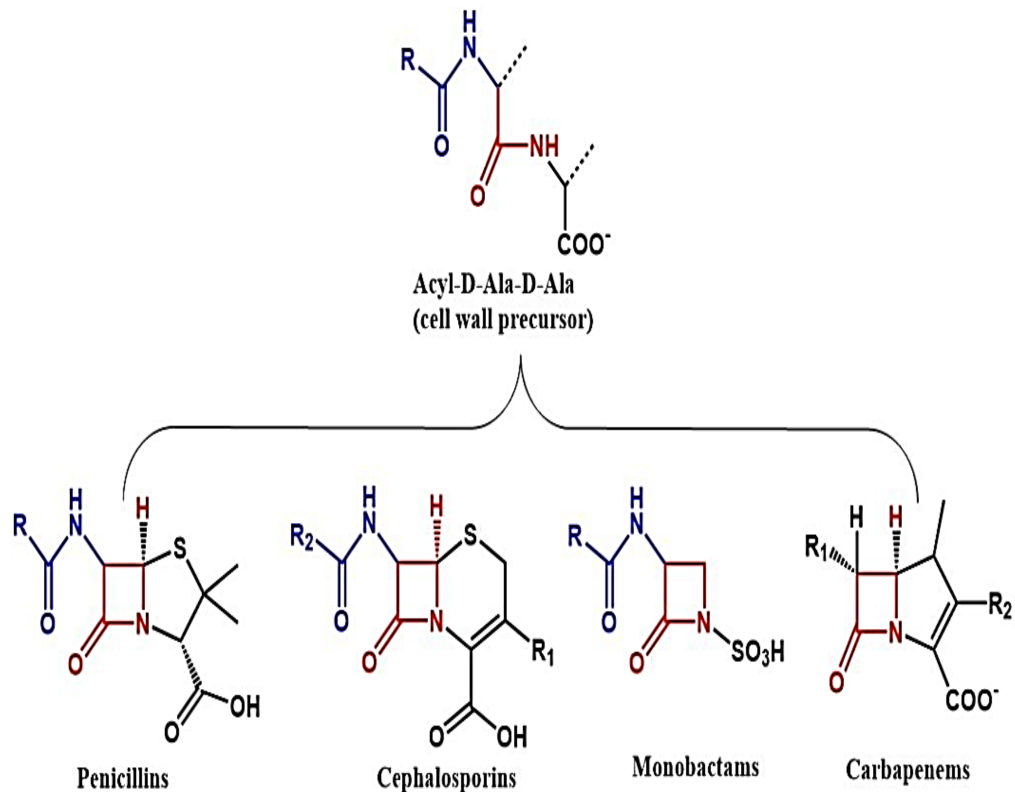


Figure 2.1: Classification of beta-lactams according to the core ring's structure (Adapted from Ring Biotechnology Co Ltd, 2018).

On the whole, there are four key resistance mechanisms against beta-lactams: beta-lactamases resistance, mutations in PBPs, decreased permeability of cell wall and the presence of efflux pumps. The acquisition of the ability to produce beta-lactamases is the most typical and important resistance mechanism that contributes to the distressing emergence of beta-lactams resistant Gram-negative bacterial strains among the listed mechanisms (Ring Biotechnology Co Ltd, 2018; Pandey and Cascella, 2019). These enzymes inactivate the beta-lactams by hydrolysis of the core four-membered ring structure. Additionally, the modifications in the bacterial permeability barrier like altered porin (the entrance channel of beta-lactams into Gram-negative bacteria) structures or downregulation of porin production and increased

synthesis of efflux pumps in Gram-negative bacteria aids in their resistance towards the beta-lactam antibiotics (Bush, 2010).

2.2.3 Quinolones

Quinolones are a group of bactericidal antibiotics that has evolved into four generations. It acts by inhibiting the activity of two crucial microbial enzymes, in the sense of bacterial viability, which are DNA gyrase (topoisomerase II) and topoisomerase IV. The inactivation of the two enzymes leads to the failure of DNA replication in the bacterial cells, thus resulting in bacterial death (Fàbrega et al., 2009). The schematic mechanism of action for quinolones is shown in Figure 2.2. Generally, the emergence of quinolone resistance strain is due to chromosomal mutations, specifically, the spontaneous mutations that happen in the quinolone resistance determining regions (QRDR) of *gyrA* and *parC* genes (Kotb et al., 2019). Additionally, it is also associated with upregulation of the expression of drug efflux pumps and alteration of porins in the bacteria (Reis et al., 2016). Besides that, plasmid-mediated quinolone resistance (PMQR) genes were also found to be implicated in the emergence of resistance strains. PMQR genes encode for pentapeptide repeat proteins (PRP) that function to protect the DNA gyrase and topoisomerase IV from the suppression of quinolones by binding to the enzymes (Kareem et al., 2021). The major PMQR genes that have been discovered include *qnrA*, *qnrB* and *qnrS* genes. These genes play a role in the increased levels of quinolone resistance, even though their occurrence is claimed to confer only a low-level resistance, it has the reasonable possibility to promote the QRDR mutations (Kotb et al., 2019).

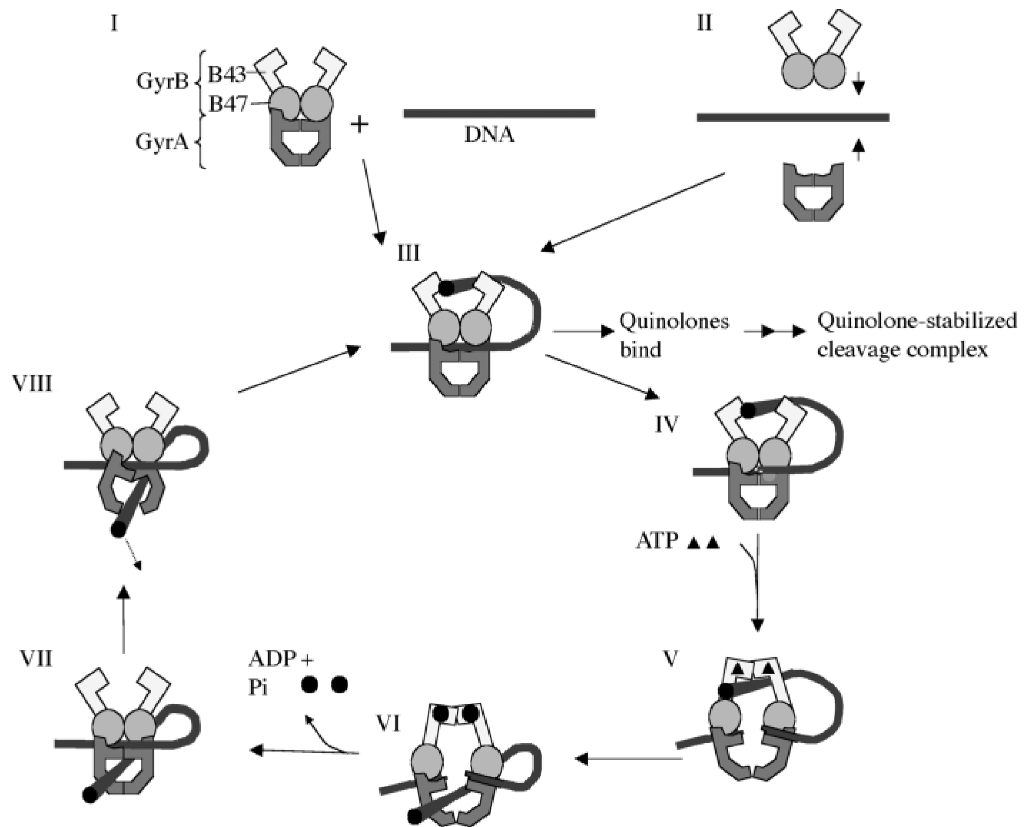


Figure 2.2: The mechanism of action of quinolones (Adapted from Hawkey, 2003).

2.2.4 Tetracyclines

Tetracyclines are a well-known antibiotic family with a broad antimicrobial spectrum activity. This group of antimicrobial agents exert its bacteriostatic effect, which is halting the growth and spread of bacteria mainly by inhibiting the protein synthesis. In another word, interrupting the translation process. It achieves its goal by interacting with the bacterial 30S ribosomal subunit and also binding to the bacterial 50S ribosomal subunit marginally. This interaction arrests the translation process as the docking of the incoming aminoacyl-transfer RNA is blocked by the antimicrobial agent (Grossman, 2016). The schematic mechanism of action for tetracyclines is shown in Figure 2.3. In brief, the most common resistance mechanism against tetracyclines in

Gram-negative bacteria is the acquisition of tetracycline-specific resistance genes (*tet* genes). Some of these genes encode for tetracycline-specific efflux pumps while some of them encode for ribosomal protection proteins (RPPs). The efflux pumps will confer the antibiotic resistance to the bacteria through the extrusion of tetracyclines out of the bacterial cell (Chopra and Roberts, 2001). For the RPPs, they act by dissociating the tetracyclines from the ribosome binding site (Chopra and Roberts, 2001). As a consequence, the tetracyclines cannot exhibit their antibacterial function.

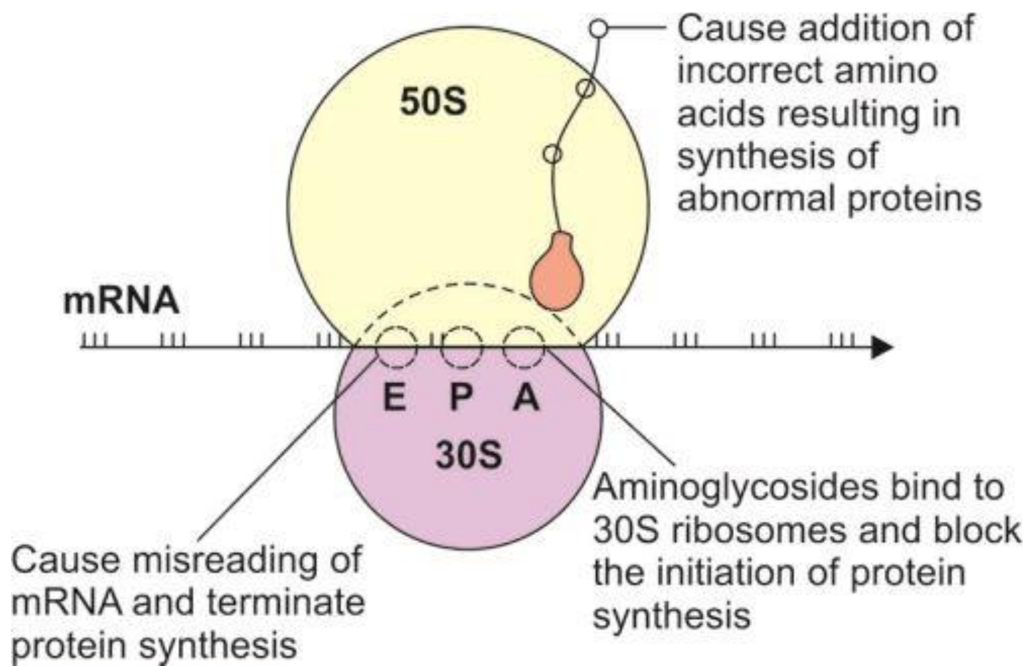


Figure 2.3: The mechanism of action of tetracyclines (Adapted from Padmaja, 2017).

2.3 Antibiotic Resistance Gene

2.3.1 Overview

Antibiotics are the cure for many bacterial infections, nonetheless, the imprudent use of antibiotics can lead to antibiotic resistance among bacteria. Most antibiotic resistance genes are found to be carried on mobile genetic elements such as plasmids and transposons, which are the common culprit of the emergence of multidrug-resistant bacterial strains. There are three natural mechanisms of the transmission of those genes in the bacterial community which are transduction, conjugation and transformation (Coleman and Smith, 2014). The most well-known plasmid-mediated resistance gene is the extended-spectrum beta-lactamase (ESBL) gene. ESBL genes encoded for beta-lactamases, in that a group of enzymes with a high capability in inhibiting a wide range of beta-lactam antibiotics inclusive of third-generation cephalosporins and monobactams (Pishtiwan and Khadija, 2019).

2.3.2 *bla*SHV and *bla*TEM genes

Two popular examples of the plasmid-mediated beta-lactamases genes are *bla*SHV and *bla*TEM genes, some of their variants are encoded for ESBLs. SHV refers to the sulfhydryl variable while TEM is named due to the fact that it is first isolated from a patient named Temoneira. These two types of genes are determined to be the common type of beta-lactams resistance gene that is responsible for the production of ESBLs among the *E. coli* strains (Pishtiwan and Khadija, 2019). The synthesised beta-lactamases will mostly be periplasmic localised in Gram-negative bacteria to inhibit the incoming beta-

lactams by breaking the amide bond in the core beta-lactam ring (Livermore, 1995).

TEM-1, TEM-2, and SHV-1 are the parent types for the majority of ESBL genes that encode for the ESBLs with the variation in the active site amino acid configuration, yet they are not encoding for ESBLs. In the aspect of SHV-type genes that were the cipher for SHV-type beta-lactamases, the evolution into ESBLs is through the glycine to serine substitution at the 238 position. Table 2.1 shows the compilation of the SHV-type extended spectrum beta-lactamases producing bacteria and their geographical locations as well as the respective year of isolation for each bacterium. For TEM-type beta lactamases, the adaptation mechanism used under the antibiotic selection pressure to develop into ESBLs is also the modification of active site residues either by amino acid substitutions or deletion (Paterson and Bonomo, 2005).

According to Alipour and Jafari (2019), the prevalence of AMR and AMR genes among UPEC strains varies in different countries. This study utilised multiplex PCR to detect the presence of *bla* genes in the UPEC isolates sampled from the UTI patients in Iran. Out of 192 isolates, 45 of the isolates (23.44%) identified as ESBL producers via phenotypic testing. The PCR of the 45 ESBL-producing *E. coli* isolates demonstrated that the *bla*$_{TEM}$ was the most abundant gene (89%, n = 40), followed by *bla*$_{SHV}$ (20%, n = 9). Another similar study conducted in Kenya, a total of 23 (24.2%) of the 95 UPEC isolates were confirmed as ESBL producers with *bla*$_{TEM}$ (95.6%, n = 22) and *bla*$_{SHV}$ (21.7%, n = 5) genes detected (Muriuki et al., 2022).

Table 2.1: Compilation of the SHV-type extended spectrum beta-lactamases-producing bacteria, the pI values of the enzymes, the geographical distribution, and their year of isolation (Adapted from Liakopoulos et al., 2016).

Gene	Bacterial Species	pI	Country	Year	Accession Number
bla_{SHV-1}	*E. coli*	7.6	NA	1972	AF148850
bla_{SHV-2}	*K. ozaenae*	7.6	Germany	1983	AF148851
bla_{SHV-2a}	*K. pneumoniae*	7.6	Germany	1987-1988	X98102
bla_{SHV-3}	*K. pneumoniae*	7.0	France	1986	KX092356
bla_{SHV-4}	*K. pneumoniae*	7.8	France	1987	NA
bla_{SHV-5}	*K. pneumoniae*	8.2	Chile	1987	X55640
bla_{SHV-6}	*K. pneumoniae*	7.6	France	1991	Y11069.1
bla_{SHV-7}	*E. coli*	7.6	USA	1993	U20270
bla_{SHV-8}	*E. coli*	7.6	USA	1990	U92041
bla_{SHV-9}	*E. coli; K. pneumoniae; S. marcescens*	8.2	Greece	1995	S82452.1
bla_{SHV-11}	*K. pneumoniae*	8.2	Switzerland	1993-1995	X98101
bla_{SHV-12}	*E. coli; K. pneumoniae*	8.2	Switzerland	1993-1995	JX268741
bla_{SHV-13}	*K. pneumoniae*	7.6	Netherlands	1994	AF164577
bla_{SHV-15}	*E. coli*	ND	India	1998	AJ011428.2
bla_{SHV-16}	*K. pneumoniae*	7.6	France	1996	AF072684.2
bla_{SHV-18}	*K. pneumoniae*	7.8	USA	1994	AF132290
bla_{SHV-23}	*K. pneumoniae*	ND	South Africa	1990	AF117747
bla_{SHV-24}	*E. coli*	7.5	Japan	1996	AB023477
bla_{SHV-27}	*K. pneumoniae*	8.2	Brazil	1999	AF293345.1
bla_{SHV-30}	*E. cloacae*	6.7	USA	2003	AY661885

Table 2.1 (continued)

bla$_{\text{SHV-31}}$	*K. pneumoniae*	7.8	Netherlands	2001	AY277255
bla$_{\text{SHV-34}}$	*C. koseri; E. coli; K. pneumoniae*	ND	USA	1998-2000	AY036620
bla$_{\text{SHV-38}}$	*K. pneumoniae*	7.6	France	2001	AY079099
bla$_{\text{SHV-40}}$	*K. pneumoniae*	7.6	Canada	1999-2000	AF535128
bla$_{\text{SHV-41}}$	*K. pneumoniae*	7.6	Canada	1999-2000	AF535129
bla$_{\text{SHV-42}}$	*K. pneumoniae*	7.6	Canada	1999-2000	AF535130
bla$_{\text{SHV-45}}$	*K. pneumoniae*	8.2	Brazil	NA	AF547625
bla$_{\text{SHV-46}}$	*K. oxytoca*	8.2	New York	1998	AY210887
bla$_{\text{SHV-55}}$	*K. pneumoniae*	ND	Portugal	NA	DQ054528
bla$_{\text{SHV-57}}$	*E. coli*	8.3	Taiwan	1998	AY223863
bla$_{\text{SHV-64}}$	*K. pneumoniae*	ND	China	2000-2002	DQ174304
bla$_{\text{SHV-66}}$	*K. pneumoniae*	ND	China	2000-2002	DQ174306
bla$_{\text{SHV-70}}$	*E. cloacae*	7.6	China	2003-2004	DQ013287
bla$_{\text{SHV-86}}$	*K. pneumoniae*	8.2	Columbia	2003	DQ328802
bla$_{\text{SHV-90}}$	*K. pneumoniae*	8.2	Portugal	2003	NA
bla$_{\text{SHV-91}}$	*K. pneumoniae*	7.6	Portugal	2003	NA
bla$_{\text{SHV-98}}$	*K. pneumoniae*	7.6	Algeria	2005	AM941844
bla$_{\text{SHV-99}}$	*K. pneumoniae*	7.8	Algeria	2005	AM941845
bla$_{\text{SHV-100}}$	*K. pneumoniae*	7.2	Algeria	2005	AM941846
bla$_{\text{SHV-102}}$	*E. coli*	ND	Spain	2003-2004	EU024485
bla$_{\text{SHV-104}}$	*K. pneumoniae*	7.3/8.6	Tunisia	2004	EU274581
bla$_{\text{SHV-105}}$	*K. pneumoniae*	ND	USA	NA	FJ194944
bla$_{\text{SHV-106}}$	*K. pneumoniae*	7.6	Portugal	1999	AM941847
bla$_{\text{SHV-128}}$	*E. cloacae*	8.6	Tunisia	2009	GU932590

Table 2.1 (continued)

*bla*SHV-129	*E. coli*	ND	Italy	2008	GU827715
*bla*SHV-134	*K. pneumoniae*	ND	Spain	2009	HM559945
*bla*SHV-183	*E. cloacae*	ND	NA	NA	HG934764

'ND' stands for not determined, and 'NA' stands for not available.

2.3.3 bla_{CTX-M} gene

Another subtype of ESBL gene, the bla_{CTX-M} gene is become increasingly prevalent worldwide and had reported to be more predominant than bla_{SHV} and bla_{TEM} genes in many studies since 21st century due to its higher proliferation rate (Bevan et al., 2017). CTX infers the synthesised CTX-M type of ESBLs are preferentially hydrolyse cefotaxime and against cefepime as well with the ability of resisting the bactericidal effect exerted by cephalosporin into the bargain. To add on, it is also having a rapid rate of expanding its variant family (Pishtiwan and Khadija, 2019).

2.3.4 bla_{OXA} gene

In view of the ability of prompt hydrolysis of oxacillin, the beta-lactamases type was named as OXA, the genes that encoded this type of enzymes was also named as bla_{OXA} genes. This type of gene has been detected in many Gram-negative bacteria. For *E. coli*, OXA-1 is the most common harboured OXA-type beta-lactamase variant among the isolates (Paterson and Bonomo, 2005). It is well known for its great heterogeneity as some of the members only acquire a rather narrow hydrolysis spectrum while others have a relatively broad spectrum of hydrolysis, in the sense that some of the variants in this OXA family are able to hydrolyse carbapenems, for example, the OXA-48. The ESBLs that derived from this subgroup of beta-lactamases are produced from the mutated form of the narrow spectrum OXA-type of *bla* genes (Poirel et al., 2009).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The chemicals and reagents utilised in this study are compiled in Table 3.1 along with their corresponding manufacturer.

Table 3.1: Chemicals and reagents utilised along with their corresponding manufacturer.

Chemical and reagent	Manufacturer, Country
Agarose powder	1st BASE Laboratories, Singapore
Mueller Hinton agar	HiMedia Laboratories Pvt. Ltd., India
Nutrient broth	HiMedia Laboratories Pvt. Ltd., India
Tryptic soy agar	Merck KGaA, Germany
Ciprofloxacin, Levofloxacin antibiotic disks	HiMedia Laboratories Pvt. Ltd., India
Minocycline, Tetracycline, Trimethoprim-sulfamethoxazole (SXT), Ampicillin, Chloramphenicol antibiotic disks	Oxoid Ltd., United Kingdom
Nalidixic acid, Imipenem antibiotic disks	Liofilchem s.r.l., Italy
5X *Taq* reaction buffer	Promega Corporation, United States

Table 3.1 (continued)

Taq DNA polymerase	Promega Corporation, United States
Deoxynucleotide triphosphates (dNTP) mix	Promega Corporation, United States
Magnesium chloride ($MgCl_2$)	Promega Corporation, United States
Forward primers, reverse primers	1st BASE Laboratories, Malaysia
100 bp DNA ladder	SMOBIO Technology, Inc., Taiwan
Tris base	Thermo Fisher Scientific, United States
Boric acid	Merck KGaA, Germany
EDTA disodium salt	Grupo RNM, Portugal
GelRed Nucleic Acid Stain	Yeastern Biotech Co., Ltd., Taiwan

3.2 Methodology

3.2.1 Sample Collection and Identification

Collection of UPEC isolates from patients' urine samples was done by senior and kept as glycerol stock cultures. The ethical approval was obtained from Medical Research and Ethics Committee prior to the study. The isolates had undergone bacterial strain identification by means of MALDI-TOF by our collaborator in Hospital Raja Permaisuri Bainun in Ipoh, Malaysia. Random 60 samples tested in this study were verified as UPEC strains. The patients' data such as age and gender were recorded for analysis (Appendix A). UPEC isolates were grown on tryptic soy agar (TSA) at 37°C and stored at 4°C routinely to ensure that the samples remain fresh for subsequent testing.

3.2.2 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test was conducted via the Kirby-Bauer disk diffusion method (Hudzicki, 2009). Colony suspension was prepared by inoculating the isolated bacterial colonies into a 5 mL sterile saline solution. A 0.5 McFarland Standard was used as a turbidity reference for the standardisation of the test. The inoculum from each sample was spread evenly on Mueller Hinton agar using a sterile swab. This was followed by the careful placing of the nalidixic acid (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), tetracycline (30 µg), minocycline (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (25 µg), ampicillin (10 µg) and imipenem (10 µg) antibiotic disks onto the surface of agar with sterile forceps. Then, the diameters of growth inhibition zones were measured in millimetres using a ruler after the 16-18 hours incubation of the inoculated plates in an aerobic atmosphere at 37°C. The phenotypic profiles of the isolates were determined as resistant (R), intermediate (I) or susceptible (S) according to the interpretation standards proposed by Clinical Laboratory Standards Institute (CLSI) as shown in Table 3.2 (Clinical and Laboratory Standards Institute, 2021). *E. coli* ATCC 25922 was used as a control strain for the test.

Table 3.2: The interpretation standards for inhibition zone (in millimetres) proposed by Clinical Laboratory Standards Institute (CLSI) for the nine chosen antibiotics.

CLSI guidelines	**Tetracycline (30 µg)**	**Minocycline (30 µg)**	**SXT (25 µg)**	**Ciprofloxacin (5 µg)**	**Nalidixic acid (30 µg)**	**Levofloxacin (5 µg)**	**Imipenem (10 µg)**	**Chloramphenicol (30 µg)**	**Ampicillin (10 µg)**
Susceptible	≥15	≥16	≥16	≥26	≥19	≥21	≥23	≥18	≥ 17
Intermediate	12-14	13-15	11-15	22-25	14-18	17-20	20-22	13-17	14-16
Resistant	≤11	≤12	≤10	≤21	≤13	≤16	≤19	≤12	≤ 13

(Adapted from Clinical and Laboratory Standards Institute, 2021)

3.2.3 Extraction of Genomic DNA

The genomic DNA of the UPEC isolates was extracted through the fast boil method (Kor et al., 2013). Preparation of bacterial suspension was done by inoculating each sample into 7 mL of nutrient broth followed by 18-24 hours of incubation in a shaking incubator at 37°C, 220 rpm. A volume of 1.5 mL of the overnight UPEC isolates suspension had been aspirated into 1.5 mL microcentrifuge tube and centrifuged at 12,000 rpm for 5 min. The supernatant was discarded, and the pellet was resuspended in 300 µL of sterile deionised water. Cell lysis was then performed by using a heat block which was set at 100°C for 5 min. The cell debris was pelleted for 2 min at 12,000 rpm to obtain the supernatant after 2-min of incubation on ice. The supernatant was then transferred into a sterile microcentrifuge tube while the pellet was discarded. The quality and yield of DNA samples were examined via the absorbance reads using Thermo Scientific Nanodrop™ 1000 Spectrophotometer. Samples with DNA purity of about 1.8 were qualified for the subsequent analysis, while the samples with undesired DNA purity had been marked as contaminated and the DNA from those samples were reextracted. The extracted DNA samples were stored at -20°C immediately until the subsequent PCR analysis step.

3.2.4 Duplex PCR Optimisation and Screening of Samples

Genotypic confirmation of the UPEC isolates for the targeted *bla* genes was done through the duplex PCR technique. Prior to the conduction of duplex PCR to screen for the targeted genes, a gradient PCR program was run as shown in Table 3.3 to optimise the assay condition. The optimal annealing

temperature was determined via the gradient PCR and was being used in the succeeding duplex PCR screening of UPEC isolates. One microlitre of the extracted DNA was subjected to a 24 µL reaction mixture that was prepared for the duplex PCR amplification as stated in Table 3.4. The primer sequences and PCR parameters are listed in Tables 3.5 and 3.6, respectively. Bio-Rad T100 Thermal Cycler was utilised for the PCR in this study.

Table 3.3: Cycling conditions of gradient PCR.

Step	Temperature (°C)	Duration (sec)	Number of cycle (s)
Initial denaturation	94	300	1
Denaturation	94	30	
Annealing	52-60	30	32
Extension	72	60	
Final extension	72	600	1

Table 3.4: Components of a standard duplex PCR reaction mixture in a final volume of 25 µL for the detection of bla_{SHV} and bla_{TEM} genes.

Component	Initial Concentration	Final Concentration	Final volume (µL)
Deionised water	-	-	9.3
Taq buffer	5X	1X	5.0
dNTP	10 mM	0.2 mM	0.5
$MgCl_2$	25 mM	1.5 mM	1.5
bla_{SHV} primers (F)	10 µM	0.5 µM	1.25
(R)	10 µM	0.5 µM	1.25
bla_{TEM} primers (C)	10 µM	1 µM	2.5
(H)			2.5
Taq DNA polymerase	5 U	1 U	0.2
Extracted DNA	-	~180 ng/µL	1.0
		Total volume	**25.0**

Table 3.5: Primer sequences and expected product sizes for bla_{SHV} and bla_{TEM} genes.

Primer	Sequence (5'-3')	Expected PCR product size (bp)	Reference
bla_{SHV}_F	AGG ATT GAC TGC CTT TTT G	392	(Colom et al., 2003)
bla_{SHV}_R	ATT TGC TGA TTT CGC TCG		
bla_{TEM}_C	ATC AGC AAT AAA CCA GC	516	(Mabilat and Courvalin, 1990)
bla_{TEM}_H	CCC CGA AGA ACG TTT TC		

Table 3.6: Cycling conditions of duplex PCR.

Step	Temperature (°C)	Duration (sec)	Number of cycle (s)
Initial denaturation	94	300	1
Denaturation	94	30	32
Annealing	56	30	
Extension	72	60	
Final extension	72	600	1

3.2.5 Agarose Gel Electrophoresis

The PCR products were then be resolved on a 1.5% (w/v) agarose gel containing GelRed in 1× TBE (1 M Tris base, 1 M boric acid and 0.02 M EDTA disodium salt) at 100 V for 45 min. Five microlitres of each PCR product and 3 μL of DNA ladder were loaded into the wells of the solidified gel prior to the electrophoresis. Visualisation of DNA and capture of gel image by using Gel Imaging System (UV transilluminator) were carried out. The presence of amplicons was recorded.

3.2.6 Statistical Analysis

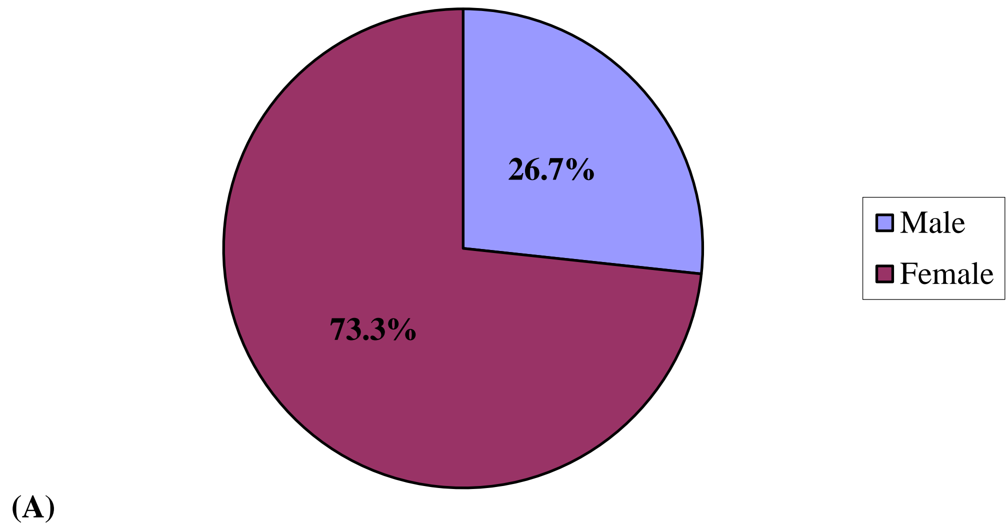
Statistical analysis of the subjects' demographical data (age and gender), the prevalence of the targeted genes and the association between the prevalence of the targeted genes and the mentioned demographics was conducted using IBM® SPSS® Statistics 25.0. A p-value of < 0.05 was considered statistically significant.

CHAPTER 4

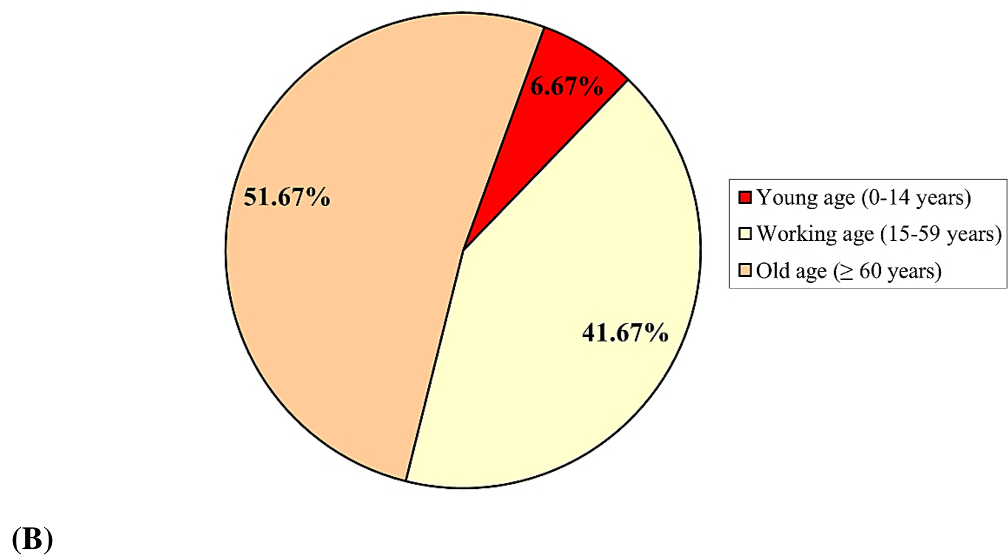
RESULTS

4.1 Demographic Profiles of the UPEC Isolated

Gender and age were the demographic parameters selected to determine their possible relationships with the target genes' prevalence. The collected demographic data from the random 60 samples were recorded in Appendix A whereas the gender and age distribution of sample was shown in Figure 4.1. Most of the isolates were obtained from females as indicated by the markedly higher percentage (73.33%) as compared to males (26.67%). The categorisation of age group was modified from the Department of Statistics Malaysia, in which the maximum age for the working age group was adjusted to be 59 years old instead of 64 years old as 60 years old is the maximum retirement age in Malaysia (Department of Statistics Malaysia, 2021; Singh, 2021). Overall, a higher proportion of UPEC isolates were obtained from the elderly (age ≥ 60 years old) than of other age groups was observed in the sample.



(A)



(B)

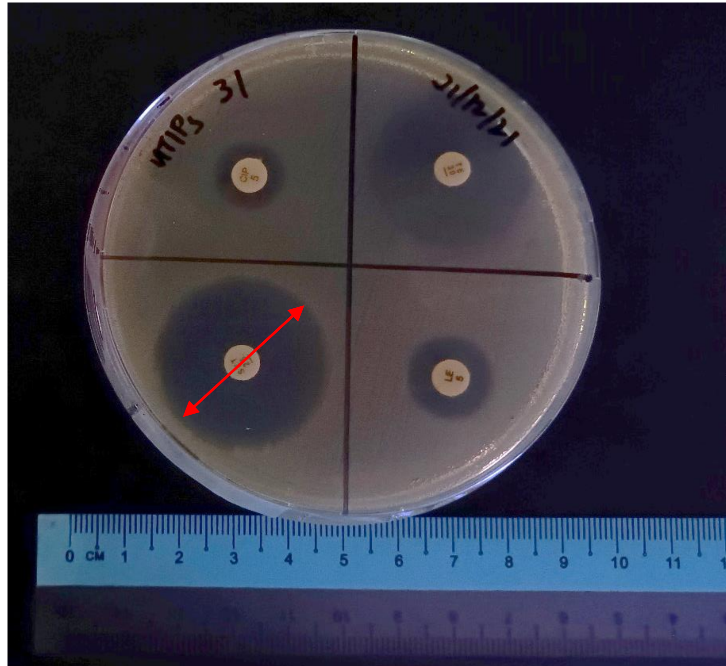
Figure 4.1: The attributes of sample by gender and age.

(A) Gender distribution of the tested UPEC isolates. (B) Age distribution of the tested UPEC isolates.

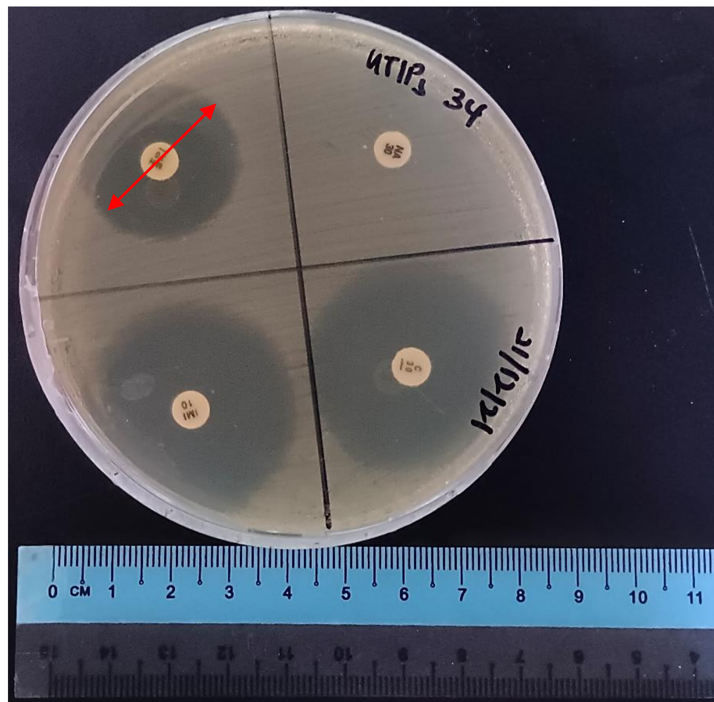
4.2 Antimicrobial Susceptibility Test

Nine antibiotics were used in the antibiotic susceptibility testing of 60 UPEC isolates. The chosen antibiotics were from beta-lactams (ampicillin, imipenem), quinolones (nalidixic acid, ciprofloxacin, levofloxacin), tetracyclines (tetracycline, minocycline), folate pathway antagonists (trimethoprim-sulfamethoxazole) and phenicol (chloramphenicol). Isolates that had intermediate susceptibility were considered as resistant to those particular antibiotics for easier analysis. The results obtained for the test are shown in Figures 4.2 and 4.3. The detailed results for each of the isolates were summarised in Appendix B.

The isolates showed a distinctive resistance status to beta-lactams (71.67% to ampicillin and 0.00% to imipenem) and tetracyclines (51.67% to tetracycline and 1.67% to minocycline). For quinolones, the resistance profile was considered high in this study (50.00% to nalidixic acid, 31.67% to ciprofloxacin and 26.67% to levofloxacin). Resistances of isolates to SXT and chloramphenicol were 33.33% and 16.67% respectively. In general, the isolates showed the greatest susceptibility to imipenem and the greatest resistance against ampicillin.



(A)



(B)

Figure 4.2: Representative figures of antimicrobial susceptibility test on UPEC isolates.

Positive results were indicated by the absence or small inhibition zone by referring to CLSI guidelines. Isolates (A) UTIPS 31 was shown to be resistant to ciprofloxacin and levofloxacin but susceptible to tetracycline and trimethoprim-sulfamethoxazole while (B) UTIPS 34 was shown to be resistant to nalidixic acid but susceptible to minocycline, imipenem and chloramphenicol.

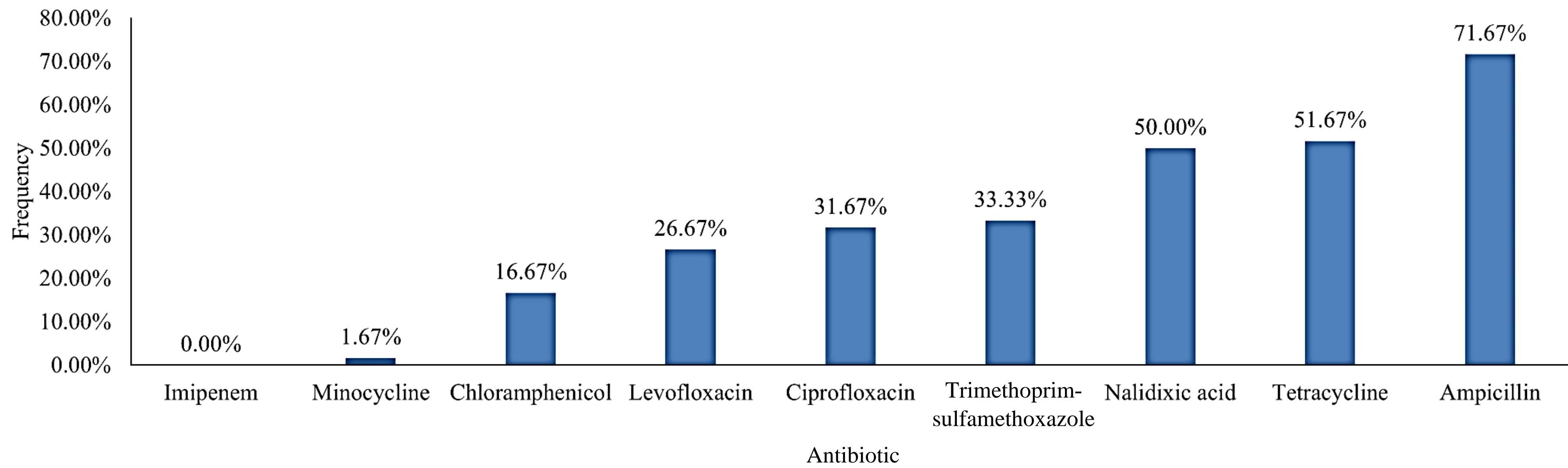


Figure 4.3: Distribution (%) of resistance to antibiotics among UPEC isolates.

4.3 Concentration and Purity of Extracted DNA

The bacterial DNA templates were extracted from all the 60 UPEC isolates by performing the fast boil method as described in Section 3.2.3. Before the screening for targeted genes, the concentration and purity of DNA samples were examined by using Thermo Scientific Nanodrop™ 1000 Spectrophotometer. The DNA purity was assessed by using the A260/280 ratio. Samples with DNA purity of about 1.8 and in the range of 1.8 to 2.0 were quantified for the subsequent analysis. The DNA samples that had a purity lower than 1.6 were reextracted as the DNA samples might be contaminated by phenols or proteins or other reagents. The obtained absorbance reading for the DNA concentration and A260/280 ratio of all the UPEC isolates are listed in Appendix C.

4.4 Duplex PCR Screening of bla_{TEM} and bla_{SHV}

All the 60 samples were screened for bla_{TEM} and bla_{SHV} simultaneously. Prior to that, optimisation was done via gradient PCR and the result obtained from the run gradient PCR program was shown in Figure 4.4. Among the four different temperatures, 52°C and 54°C were opted out due to the primer-dimer formation, meanwhile the band intensity of bla_{SHV} shown in lane 5 is far lower in comparison to lane 4. Therefore, the annealing temperature of 56°C was chosen as the optimum temperature for the duplex PCR. A total of 36 out of 60 (60.00%, n = 36) UPEC isolates were screened positive for bla_{TEM} whereas none of the isolates was screened positive for bla_{SHV}. The representative gel image of the duplex PCR screening for bla_{TEM} and bla_{SHV} genes was shown in

Figure 4.5. The duplex PCR screening results for each of the isolates were summarised in Appendix D.

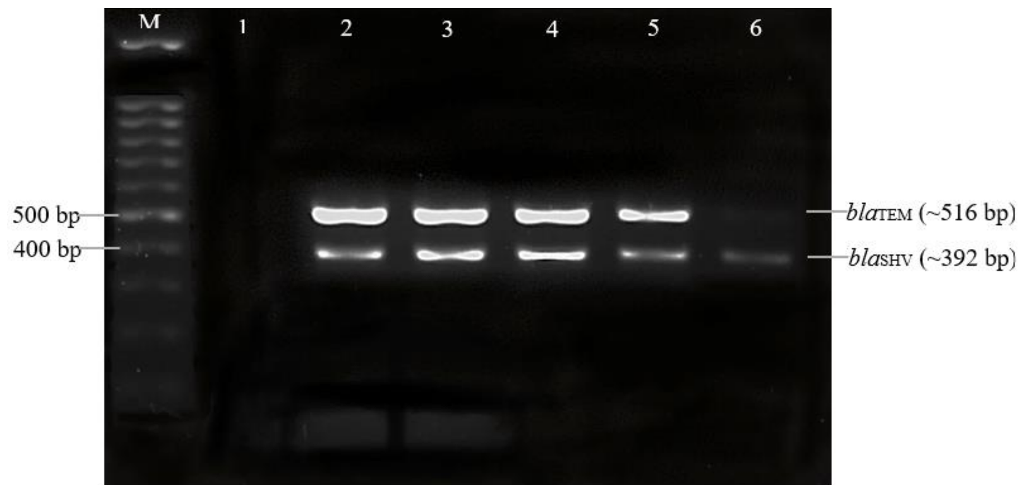


Figure 4.4: Optimisation of PCR condition based on annealing temperature gradient.

Lane M represents 100 bp ladder. Lanes 2-6 were loaded with H65 and subjected to the gradient PCR with the annealing temperatures of 51.9°C, 53.8°C, 56.1°C, 58.0°C and 60.0°C accordingly. Lane 1 was loaded with negative control. The expected amplicon sizes for *bla*SHV and *bla*TEM were 392 bp and 516 bp respectively.

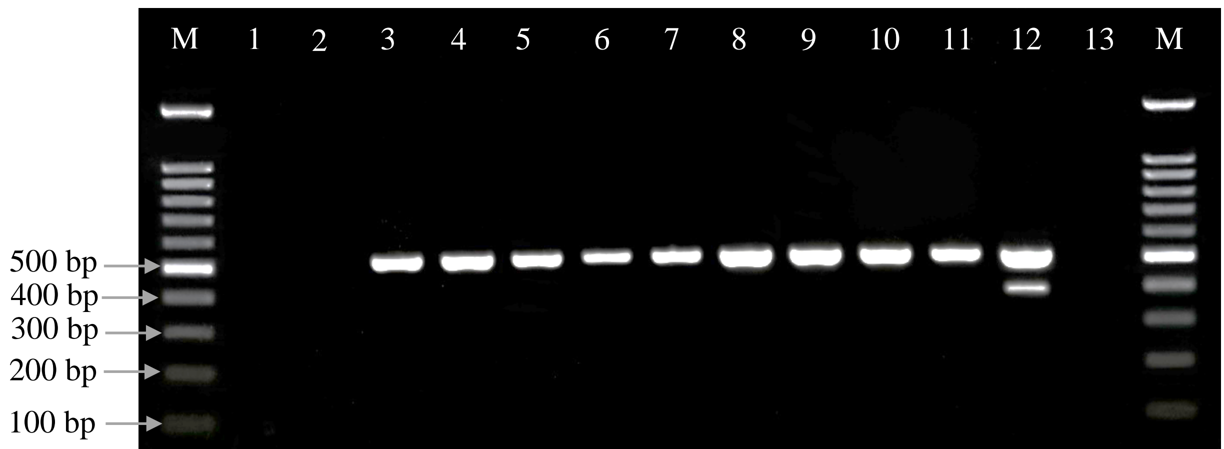


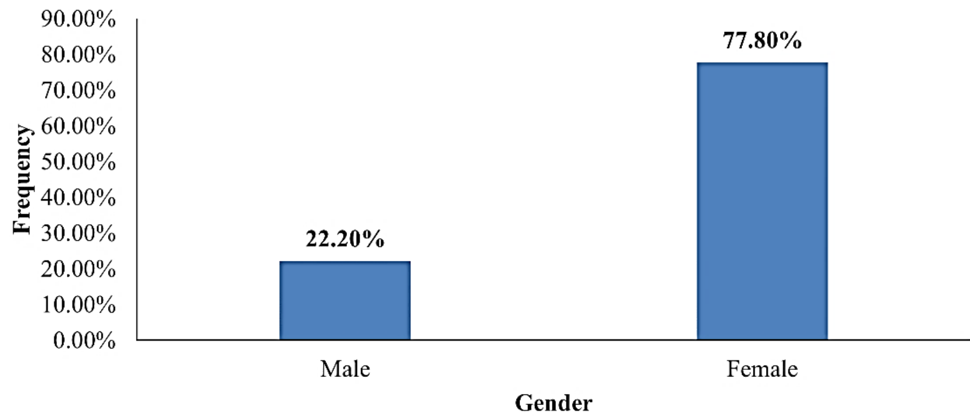
Figure 4.5: Representative gel image of duplex PCR screening for *bla*TEM and *bla*SHV on 1.5% agarose gel.

Lanes M represent 100 bp ladder. Lanes 1-11 were loaded with UTIPS 36, UTIPS 53, UTIPS 2, UTIPS 4, UTIPS 7, UTIPS 9, UTIPS 15, UTIPS 21, UTIPS 24, UTIPS 34 and UTIPS 47 accordingly. Lane 12 was loaded with H65, which was the positive control while Lane 13 was loaded with negative control (template with deionised distilled water). The expected amplicon sizes for *bla*SHV and *bla*TEM were 392 bp and 516 bp respectively.

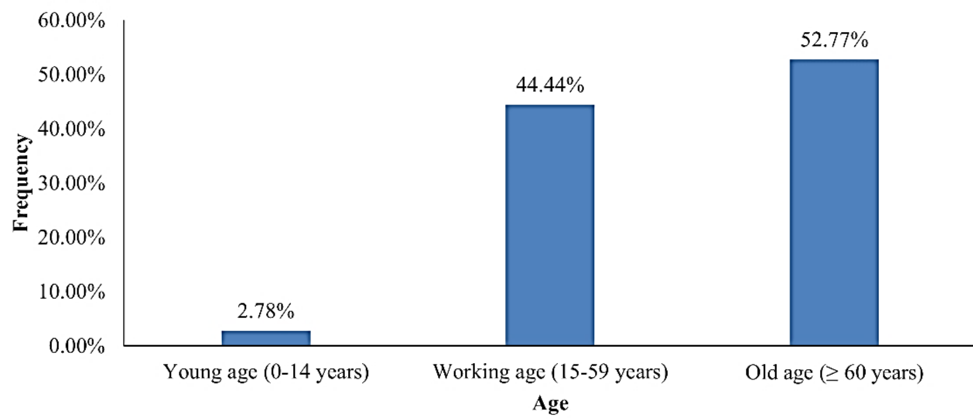
4.5 Gene Prevalence based on Gender and Age

The distribution of the targeted genes based on gender and age as well as the statistical analysis of associations between gene prevalence and the two variables (gender and age) were only performed for bla_{TEM} gene due to the absence of bla_{SHV} gene in all the isolates. Figure 4.6 displays the distribution of bla_{TEM} gene according to gender and age respectively.

In the aspect of gender, females occupied a far larger percentage (28 out of 36 isolates, 77.78%) as compared to males (8 out of 36 isolates, 22.22%), which means that the bla_{TEM} gene-containing isolates were predominantly originated from female patients. However, the association between the bla_{TEM} gene and gender was found to be not significant (negative association) via statistical analysis shown in Table 4.2 ($p = 0.340$). In terms of age, the isolates that carried bla_{TEM} gene were mostly found in elderly patients (aged 60 years old and above). This age group had a notably high frequency, which was 19 out of 36 isolates (52.77%, n = 19). It was followed by the 15 to 59 age group (44.44%). The graphical illustration also pointed out that the young age group (aged 0 to 14) had the lowest percentage (2.78%) in terms of the presence of bla_{TEM} gene. The association between the bla_{TEM} gene and gender was also found to be not significant (negative association) as shown in Table 4.2 ($p = 0.328$).



(A)



(B)

Figure 4.6: Distribution of *bla*TEM gene according to the (A) gender and (B) age of the patients.

Table 4.2: Distribution of *bla*TEM genes in accordance with gender and age of the patients.

Demographic Profile		Presence of genes		
		***bla*TEM +**	***bla*TEM -**	***p*-value***
Gender	Male (n=16)	8 (50.00%)	8 (50.00%)	0.340
	Female (n=44)	28 (63.64%)	16 (36.36%)	
Age group	Young age (n=4)	1 (25.00%)	3 (75.00%)	0.328
	Working age (n=25)	16 (64.00%)	9 (36.00%)	
	Old age (n=31)	19 (61.29%)	12 (38.71%)	

A *p*-value that is <0.05 is considered as statistically significant and is indicated by *.

4.6 Association between Phenotypic and Genotypic Features of UPEC Isolates

The prevalence of *bla*TEM gene among the resistant and susceptible UPEC isolates for each antibiotic is listed in Table 4.3. Most of the ampicillin-resistant isolates (69.77%, 30/43) were corresponded well with the detection of *bla*TEM gene, yet six ampicillin-susceptible (35.29%, 6/17) and 36 imipenem-susceptible (60.00%, 36/60) isolates were observed to harbour *bla*TEM gene. The positive associations between the *bla*TEM gene prevalence and four of the tested antibiotics which are ampicillin, ciprofloxacin, levofloxacin and tetracycline, were found to be significant (p = 0.014; p = 0.041; p = 0.043; p = 0.001) as shown in Table 4.3. In contrast, the resistance profiles of isolates for nalidixic acid, minocycline, SXT and chloramphenicol were found to be not associated with *bla*TEM gene (p = 0.114; p = 0.410; p = 0.094; p = 0.157). The association between the resistance profile of isolates for

imipenem and *bla*$_{TEM}$ gene prevalence cannot be determined due to the absence of imipenem-resistant isolates in the current study.

Table 4.3: Association of *bla*TEM gene with antibiotic susceptibility of UPEC isolates.

Antibiotic susceptibility		Presence of genes		
		***bla*TEM +**	***bla*TEM -**	***p*-value***
Ampicillin	R (n=43)	30 (69.77%)	13 (30.23%)	0.014*
	S (n=17)	6 (35.29%)	11 (64.71%)	
Imipenem	R (n=0)	-	-	- [a]
	S (n=60)	36 (60.00%)	24 (40.00%)	
Nalidixic acid	R (n=30)	21 (70.00%)	9 (30.00%)	0.114
	S (n=30)	15 (50.00%)	15 (50.00%)	
Ciprofloxacin	R (n=19)	15 (78.95%)	4 (21.05%)	0.041*
	S (n=41)	21 (51.22%)	20 (48.78%)	
Levofloxacin	R (n=16)	13 (81.25%)	3 (18.75%)	0.043*
	S (n=44)	23 (52.27%)	21 (47.73%)	
Tetracycline	R (n=31)	25 (80.65%)	6 (19.35%)	0.001*
	S (n=29)	11 (37.93%)	18 (62.07%)	
Minocycline	R (n=1)	1 (100.00%)	0 (0.00%)	0.410
	S (n=59)	35 (59.32%)	24 (40.68%)	
Trimethoprim-sulfamethoxazole	R (n=20)	15 (75.00%)	5 (25.00%)	0.094
	S (n=40)	21 (52.50%)	19 (47.50%)	
Chloramphenicol	R (n=10)	4 (40.00%)	6 (60.00%)	0.157
	S (n=50)	32 (64.00%)	18 (36.00%)	

'R' stands for resistant, and 'S' stands for susceptible.
A *p*-value that is <0.05 is considered as statistically significant and is indicated by *.
[a] Antibiotic susceptibility profile for imipenem is a constant thus no *p*-value is generated.

CHAPTER 5

DISCUSSION

5.1 Overview

This study attempted to accomplish the set three main objectives which are to detect the presence of *bla*TEM and *bla*SHV genes in UPEC isolates from UTI patients via duplex PCR, to analyse the quinolone-resistance phenotypes and genotypes of the UPEC isolates by using three different quinolone antibiotics and to investigate the association of prevalence of *bla*TEM and *bla*SHV genes to the demographic.

5.2 Antimicrobial Susceptibility Profile among UPEC Isolates

From the antimicrobial susceptibility test results (Figure 4.3), the isolates showed a distinctive resistance status to beta-lactams and also tetracyclines. The isolates were in the main showed the greatest susceptibility to imipenem and the greatest resistance against ampicillin, which correlates to the previous research findings, in which, the clinical efficacy and the in vitro bactericidal effect of imipenem are still sustained (Rodloff et al., 2006). According to Joly-Guillou et al. (2010), imipenem is very active against Gram-negative bacteria, including ESBLs-producing *E. coli*. Imipenem is commonly being prescribed to patients in the combination with cilastatin (dehydropeptidase inhibitor) or relebactam (beta-lactamase inhibitor) as a treatment for UTI patients (Kuiper et al., 2020). This combination antibacterial therapy provides an enhanced antibacterial activity to the drug as the combination product is more difficult

for the disease-causing bacteria to develop resistance against it (Brennan-Krohn, 2018).

For quinolones, the resistance profile was considered high in this study. The trend of resistance rates for quinolones is consistent with the result proposed by a previous study (Malekzadegan et al., 2019). The older generation of quinolones has a relatively lower potency as compared to the newer generation, thus the antimicrobial effect exerted by nalidixic acid (first-generation) is the weakest while levofloxacin (third-generation) has the strongest effect among the chosen quinolones in the current study in killing the bacterial isolates.

The same explanation can be applied for the distinctive resistance of UPEC isolates to tetracyclines, in which minocycline (second-generation) can exhibit a better antibacterial efficacy as compared to the first-generation tetracycline. The newer generation of antibiotics is certainly the more recent developed or discovered drug with the aim to overcome the resistance issue faced by the older generation of antibiotic, therefore the newer generation of antibiotics will undoubtedly be more effective against the causative agents (Wellcome, 2020). In addition to that, the lack of time for the causative agents to develop resistance against the newer generation is one of the contributing factors to the higher effectiveness of the newer generation antibiotics in killing or preventing the growth of the causative agents (Peterson and Kaur, 2018).

5.3 Prevalence of *bla*$_{TEM}$ and *bla*$_{SHV}$ among the UPEC Isolates

The worldwide distribution of extended-spectrum beta-lactamase (ESBL) genes lead to the epidemic spread of antimicrobial resistance, not only against beta-lactams but also other antibiotic groups. Two of the famous ESBL genes are the targeted genes in this study, which are *bla*$_{TEM}$ and *bla*$_{SHV}$ genes. Both genes are widespread among Gram-negative bacteria, with the commonest representative, *E. coli* (Rybak et al., 2022). In this study, the prevalence of *bla*$_{TEM}$ was considered moderately high (60.00%, n = 36), of which 36 out of 60 isolates were screened positive for *bla*$_{TEM}$ gene whereas none of the isolates was screened positive for *bla*$_{SHV}$ which is in agreement with previous studies (Alqasim et al., 2018; Valadbeigi et al., 2020). It indicates that *bla*$_{TEM}$ has a higher prevalence than *bla*$_{SHV}$ in the tested sample population, the results are in accordance a previous study by Hashemizadeh et al. (2018) (*bla*$_{TEM}$ = 74.8%; *bla*$_{SHV}$ = 1.2%). Moreover, another study revealed a similar gene prevalence pattern where *bla*$_{TEM}$ (45%) gene predominant over *bla*$_{SHV}$, however, the study somehow contradict to the finding as the *bla*$_{SHV}$ carriage was detected at 5.4% (Halaji et al., 2020). This discrepancy can be due to the difference in geographical regions. Apart from that, there is still a possibility that some of the isolates did contain *bla*$_{SHV}$, but the other harboured SHV variants might could not be exemplified by the primers used for *bla*$_{SHV}$ (Pitout and Laupland, 2008).

5.4 Association of Phenotypic and Genotypic Data

The positive associations between the *bla*TEM gene and four of the tested antibiotics: ampicillin, ciprofloxacin, levofloxacin and tetracycline, were found to be statistically significant. *bla*TEM is one of the predominant ESBL genes that encode for the enzymes, ESBLs, in which has a direct relationship with beta lactams as beta-lactamases function to hydrolyse beta-lactams. Ampicillin is one of the commonly prescribed beta-lactam antibiotics and thus many studies had reported the resistance against it primarily due to the production of beta-lactamases among Gram-negative bacteria, especially the beta-lactamases encoded by *bla*TEM (Pandey and Cascella, 2019). Most of the ESBLs are still susceptible to carbapenems nonetheless, and this could explain the phenomenon of all the isolates were susceptible to imipenem in the current study as imipenem is a carbapenem antibiotic (Paterson and Bonomo, 2005).

With regard to those isolates that were screened positive for *bla*TEM yet susceptible to ampicillin (35.29%, n = 6), it could be due to the beta-lactamases encoded by the harboured *bla*TEM gene variants are not strong enough to protect the isolates from the bactericidal effect of ampicillin or the resistance genes harboured were silenced or unexpressed (Enne et al., 2006). Aside from that, those isolates that were screened negative for *bla*TEM yet resistant to ampicillin (30.23%, n = 13), it may result from the acquisition and expression of other subtypes of ESBL genes by those isolates. Plus, other resistance mechanisms that were not looked into in the current study, for instance, the mutation in PBPs and upregulation of efflux pumps, would also be responsible for the susceptibility profile for ampicillin among the *bla*TEM-

negative isolates (Eguale et al., 2017). Besides, *bla*$_{TEM}$ does not always confer abilities that can interfere or inhibit the mechanisms of non-beta-lactam antibiotics (Tamma et al., 2021). Therefore, it is reasonable for those *bla*$_{TEM}$-containing isolates to be susceptible to other group of antibiotics.

Multiple studies disclosed the close relationship between quinolones resistance and *bla* genes. These genes are often being acquired by the bacteria through uptaking of transferable plasmid or other mobile genetic elements (MGEs) that consists of them. The transferable plasmids could carry PMQR genes which function to transfer a low-level of quinolone resistance to the pathogen, causing a phenomenon of co-resistance against beta-lactams and quinolones or/and co-existence of ESBL and PMQR genes in a bacterial isolate that harboured with the multi-resistance plasmid (Salah et al., 2019). Additionally, several studies also revealed the significant positive association between ESBL and *tet* genes, in which, the co-occurrence of both types of genes is commonly observed in *E. coli* (Salvador-Membreve and Rivera, 2021). In brief, the *bla* genes usually being spread through the MGEs that co-carriage with the resistance genes for other group of antibiotics that can reside in the MGEs, hence, the *bla* genes are oftentimes stated to be positively associated with multidrug resistance.

5.5 Prevalence of UPEC Strain based on Gender and Age

The majority of the UPEC isolates were sampled from females, where the distribution across gender showed 73.33% (n = 44) for females. This result is following the global trend, in which females are more prone to UTI as compared to males (Ramírez-Castillo et al., 2018). The anatomical differences in both genders contribute to the situation, females have a significantly shorter urethra, providing an easier and short pathway to the infectious agents (Huston, 2018). With respect to age, the 60-year-old and above age group recorded the highest proportion of UPEC strain (51.67%, n = 31). Ageing is one of the predisposing factors to UTI, the elderly is more prone to the infection. Several factors can be proposed such as a weak immune system, weak bladder sphincter and loss of ambulation (Amadu et al., 2019).

According to Abrar et al. (2019), ESBL-positive *E. coli* was more prevalent in females (53%, n = 138) which is in accordance with the findings in the current study (77.8%, n = 28). Aside from that, the mentioned study also revealed that the ESBL-infectivity rate in *E. coli* was higher in old age group (36%, n = 94) which was as well in agreement with the findings in the current study (52.77%, n = 19). The difference between the mentioned studies and the current study is the method of determination of ESBL-producer, in which the phenotypic screening for beta-lactamase production was utilised in the mentioned studies instead of the molecular screening for *bla* genes that was used in the current study. Regarding to the negative association shown via the statistical analysis performed for the prevalence of bla_{TEM} gene and the demographic profiles, there is an absence of proof that can be used as a possible explanation due to

the lack of studies in investigating the association between the prevalence of gene with the two categorical variables (age and gender).

5.6 Limitations and Future Studies

Several limitations are found in this study: small sample size, the sampled patients are specified only for hospitalised patients and the UPEC isolates were collected only from a city. The drawbacks of the mentioned limitations are the failure in providing accurate and precise antimicrobial resistance trends in other regions in Malaysia as well as the failure in assessing the epidemiological changes in Malaysia population. Thus, a wider and larger sample population ought to be included in future studies. Besides, there is a lack of phenotypic testing for ESBL-production (ESBLs screening test) as a supportive test in the current study as this study just aim for molecular characterisation of beta-lactamases.

Furthermore, the non-fulfilment for the association between the prevalence of genes and the demographic profiles needs to be followed up for the possible explanation that may be obtained from the future research. In addition to the above, PCR screening for other *bla* genes subtypes such as *bla*CTX-M and variants for *bla*TEM and *bla*SHV can be performed. The sequence obtained should be sent for direct sequencing for analysis by comparing to the databases via Nucleotide BLAST programme in future studies so as to verify the identity of amplicons obtained from the run PCR assay.

CHAPTER 6

CONCLUSION

In this study, a rather comprehensive antibiotics resistance pattern among UPEC strains is provided with the intention to raise the attention to the serious issue of antibiotic resistance with globally unprecedented momentum. Most isolates were resistant to ampicillin, following by tetracycline, nalidixic acid, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, chloramphenicol, and minocycline. Prescription of ampicillin as a treatment for UTI seems to be vain according to the result obtained. In contrast, all the isolates were susceptible to imipenem, indicating its effectiveness in the treatment of UTI was still sustained. In terms of the gene prevalence, *bla*TEM gene was detected in most of the UPEC isolates (60%, n = 60). However, none of the isolates was detected with the presence of *bla*SHV gene, suggesting that other *bla*SHV variants may be present in the isolates. In addition to that, the presence of *bla*TEM gene is proposed to be positively associated with the ampicillin, ciprofloxacin, levofloxacin and tetracycline resistance of UPEC strain, implying that *bla* genes are associated with multidrug resistance. The co-carriage of resistance genes, which are *bla*, PMQR and *tet* genes in the transferable genetic elements is proposed to be the factor for the associations. However, no significant correlation is observed between the *bla* genes and gender as well as age of patients.

The high proportion of *bla*$_{TEM}$-harboured and multidrug-resistant UPEC isolates that revealed in the current study showing a serious clinical challenge. Hence, a more extensive study on the antibiotic genes and their mechanism as well as the association with the antimicrobial resistance profile needs to be conducted to have better insight in the severity of antibiotic resistance among UPEC strains in this locality. This type of study should be periodically conducted as a follow-up of the resistance pattern of the UPEC strain as it not merely can aid in the judicious management and formulation of antibiotics usage in clinical settings, but as well very useful for the ongoing development of more effective antibiotics against UTI.

REFERENCES

Abrar, S. et al., 2019. Distribution of *bla*CTX − M, *bla*TEM, *bla*SHV and *bla*OXA genes in extended-spectrum-β-lactamase-producing clinical isolates: A three-year multi-center study from Lahore, Pakistan. *Antimicrobial Resistance & Infection Control*, 8(1), pp.1-10.

Alipour, M. and Jafari, A., 2019. Evaluation of the prevalence of *bla*SHV, *bla*TEM, and *bla*CTX genes in *Escherichia coli* isolated from urinary tract infections. *Avicenna Journal of Clinical Microbiology and Infection*, 6(3), pp.83-87.

Alqasim, A., Abu Jaffal, A. and Alyousef, A.A., 2018. Prevalence of multidrug resistance and extended-spectrum β-lactamase carriage of clinical uropathogenic *Escherichia coli* isolates in Riyadh, Saudi Arabia. *International Journal of Microbiology*, 2018, pp.1-9.

Amadu, D. et al., 2019. Prevalence and associated factors associated with uropathogenic *Escherichia coli* isolates from catheterized persons at Ilorin Tertiary Hospital, Nigeria. *Afro-Egyptian Journal of Infectious and Endemic Diseases*, 9(2), pp.119–128.

Bevan, E.R., Jones, A.M. and Hawkey, P.M., 2017. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *Journal of Antimicrobial Chemotherapy*, 72(8), pp.2145-2155.

Brennan-Krohn, T., 2018. *Combination antibiotic testing: when 2 drugs are better than 1 (or 2).* [online] Available at: <https://asm.org/Articles/2018/September/Combination-Antibiotic-Testing-When-2-Drugs-are-Be> [Accessed 1 April 2022].

Bush, K., 2010. *Antibiotic and Chemotherapy*. 9th ed. New York: Saunders.

Centres for Disease Control and Prevention, 2022. *7 Catheter-associated Urinary Tract Infection (CAUTI).* [online]. Available at: <https://www.cdc.gov/nhsn/pdfs/pscmanual/7psccauticurrent.pdf.> [Accessed 20 March 2022].

Chopra, I. and Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*, 65(2), pp.232–260.

Clinical and Laboratory Standards Institute, 2021. *CLSI M100 ED31:2021 — Performance standards for antimicrobial susceptibility testing*. 31st ed. Malvern: Clinical and Laboratory Standards Institute.

Coleman, J.P. and Smith, C.J., 2014. *Reference module in biomedical sciences*. Amsterdam: Elsevier.

Colom, K. et al., 2003. Simple and reliable multiplex PCR assay for detection of *bla*TEM, *bla*SHV and *bla*OXA-1 genes in *Enterobacteriaceae*. *FEMS Microbiology Letters*, 223(2), pp.147-151.

Denis, O., Rodriguez-Villalobos, H. and Struelens, M.J., 2010. *Antibiotic and chemotherapy*. 9th ed. New York: Saunders.

Department of Statistics Malaysia, 2021. *Current population estimates, Malaysia, 2021*. [online] Available at: <https://www.dosm.gov.my/v1/index.php?r=column/cthemeByCat&cat=155&bul_id=ZjJOSnpJR21sQWVUcUp6ODRudm5JZz09&menu_id=L0pheU43NWJwRWVSZklWdzQ4TlhUUT09> [Accessed 29 March 2022].

Eguale, T. et al., 2017. Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal *Salmonella* isolates from humans and animals in central Ethiopia. *Antimicrobial Resistance & Infection Control*, 6(1), pp.1-10.

Enne, V.I., Delsol, A.A., Roe, J.M. and Bennett, P.M., 2006. Evidence of antibiotic resistance gene silencing in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, 50(9), pp.3003–3010.

Fàbrega, A., Madurga, S., Giralt, E. and Vila, J., 2009. Mechanism of action of and resistance to quinolones. *Microbial Biotechnology*, 2(1), pp.40–61.

Grossman, T.H., 2016. Tetracycline antibiotics and resistance. *Cold Spring Harbor Perspectives in Medicine*, 6(4), p.a025387.

Halaji, M. et al., 2020. Characterization of extended-spectrum β-lactamase-producing uropathogenic *Escherichia coli* among Iranian kidney transplant patients. *Infection and Drug Resistance*, 13, pp.1429-1437.

Hashemizadeh, Z., Kalantar-Neyestanaki, D. and Mansouri, S., 2018. Clonal relationships, antimicrobial susceptibilities, and molecular characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates from urinary tract infections and fecal samples in Southeast Iran. *Revista da Sociedade Brasileira de Medicina Tropical*, 51(1), pp.44-51.

Hawkey, P.M., 2003. Mechanisms of quinolone action and microbial response. *Journal of Antimicrobial Chemotherapy*, 51(1), pp.29-35.

Healthline, 2022. *Urinary tract infection: symptoms, diagnosis, and treatment*. [online] Available at: <https://www.healthline.com/health/urinary-tract-infection-adults#risks-for-women.> [Accessed 19 March 2022].

Hudzicki, J., 2009. *Kirby-bauer disk diffusion susceptibility test protocol*. Washington: American Society for Microbiology.

Huston, K., *2018. Blame your anatomy: women are more prone to UTI than men | Norton Healthcare Louisville, Ky*. [online] Available at: <https://nortonhealthcare.com/news/uti-ecare//> [Accessed 8 September 2021].

Ibrahim, M., Algak, T., Abbas, M. and Elamin, B., 2021. Emergence of *bla*TEM, *bla*CTX-M, *bla*SHV and *bla*OXA genes in multidrug-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* in Saudi Arabia. *Experimental and Therapeutic Medicine*, 22(6), pp.1-11.

Jahandeh, N., Ranjbar, R., Behzadi, P. and Behzadi, E., 2015. Uropathogenic *Escherichia coli* virulence genes: invaluable approaches for designing DNA microarray probes. *Central European Journal of Urology*, 68(4), pp.452-458.

Joly-Guillou, M.L. et al., 2010. Comparative in vitro activity of meropenem, imipenem and piperacillin/tazobactam against 1071 clinical isolates using 2 different methods: a French multicentre study. *BMC Infectious Diseases*, 10(1), pp.1-9.

Kareem, S. M. et al., 2021. Detection of *gyrA* and *parC* mutations and prevalence of plasmid-mediated quinolone resistance genes in *Klebsiella pneumoniae*. *Infection and drug resistance*, 14, pp.555-563.

Kor, S.B., Choo, Q.C. and Chew, C.H., 2013. New integron gene arrays from multiresistant clinical isolates of members of the *Enterobacteriaceae* and *Pseudomonas aeruginosa* from hospitals in Malaysia. *Journal of Medical Microbiology*, 62(3), pp.412-420.

Kotb, D.N., Mahdy, W.K., Mahmoud, M. S. and Khairy, R. M.M., 2019. Impact of co-existence of PMQR genes and QRDR mutations on fluoroquinolones resistance in *Enterobacteriaceae* strains isolated from community and hospital acquired UTIs. *BMC Infectious Diseases*, 19(1), pp.1-8.

Kuiper, S.G., Leegwater, E., Wilms, E.B. and van Nieuwkoop, C., 2020. Evaluating imipenem + cilastatin + relebactam for the treatment of complicated urinary tract infections. *Expert Opinion on Pharmacotherapy*, 21(15), pp.1805-1811.

Liakopoulos, A., Mevius, D. and Ceccarelli, D., 2016. A review of SHV extended-spectrum β-lactamases: neglected yet ubiquitous. *Frontiers in Microbiology*, 7, pp.1-27.

Lien, L.T.Q. et al., 2017. Antibiotic resistance and antibiotic resistance genes in *Escherichia coli* isolates from hospital wastewater in Vietnam. *International Journal of Environmental Research and Public Health*, 14(7), pp.81-91.

Livermore, D.M., 1995. Beta-Lactamases in laboratory and clinical resistance. *Clinical Microbiology Reviews*, 8(4), pp.557–584.

Mabilat, C. and Courvalin, P., 1990. Development of "oligotyping" for characterization and molecular epidemiology of TEM beta-lactamases in members of the family *Enterobacteriaceae*. *Antimicrobial Agents and Chemotherapy*, 34(11), pp.2210-2216.

Malekzadegan, Y. et al., 2019. Prevalence of quinolone-resistant uropathogenic *Escherichia coli* in a tertiary care hospital in south Iran. *Infection and Drug Resistance*, 12, pp.1683-1689.

Mann, R., Mediati, D. G., Duggin, I. G., Harry, E. J. and Bottomley, A. L., 2017. Metabolic adaptations of uropathogenic *E. coli* in the urinary tract. *Frontiers in Cellular and Infection Microbiology*, 7, pp.1-15.

Medina, M. and Castillo-Pino, E., 2019. An introduction to the epidemiology and burden of urinary tract infections. *Therapeutic advances in urology*, 11, pp.3-7.

Momtaz, H. et al., 2013. Uropathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. *Annals of clinical microbiology and antimicrobials*,12(8), pp.1-12.

Moura, A., Nicolau, A., Hooton, T. and Azeredo, J., 2009. Antibiotherapy and pathogenesis of uncomplicated UTI: difficult relationships. *Journal of Applied Microbiology*, 106(6), pp.1779-1791.

Muriuki, C.W. et al., 2022. Phenotypic and genotypic characteristics of uropathogenic *Escherichia coli* isolates from Kenya. *Microbial Drug Resistance*, 28(1), pp.31-38.

National Institute of Diabetes and Digestive and Kidney Diseases, 2019. *Bladder infection (Urinary Tract Infection—UTI) in adults | NIDDK*. [online]. Available at: <https://www.niddk.nih.gov/health-information/urologic-diseases/bladder-infection-uti-in-adults.> [Accessed 19 March 2022].

Padmaja, U., 2017. *Pharmacology for dental and allied health sciences*. 4th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd.

Pandey, N. and Cascella, M., 2019. *Beta lactam antibiotics*. [online] Available at: <https://www.ncbi.nlm.nih.gov/books/NBK545311/> [Accessed 21 March 2022].

Paterson, D.L. and Bonomo, R.A., 2005. Extended-spectrum β -lactamases: a clinical update. *Clinical Microbiology Reviews*, 18(4), pp.657-686.

Peterson, E. and Kaur, P., 2018. Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. *Frontiers in Microbiology*, 9, pp.1-21.

Pishtiwan, A.H. and Khadija, K.M., 2019. Prevalence of *bla*TEM, *bla*SHV, and *bla*CTX-M genes among ESBL-producing *Klebsiella Pneumoniae* and *Escherichia coli* isolated from thalassemia in Erbil, Iraq. *Mediterranean Journal of Hematology and Infectious Diseases*, 11(1), p.e2019041.

Pitout, J.D. and Laupland, K.B., 2008. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. *The Lancet Infectious Diseases*, 8(3), pp.159-166.

Poirel, L., Naas, T. and Nordmann, P., 2009. Diversity, epidemiology, and genetics of Class D β-Lactamases. *Antimicrobial Agents and Chemotherapy*, 54(1), pp.24-38.

Raeispour, M. and Ranjbar, R., 2018. Antibiotic resistance, virulence factors and genotyping of uropathogenic *Escherichia coli* strains. *Antimicrobial Resistance & Infection Control*, 7(1), pp.1-9.

Ramírez-Castillo, F.Y. et al., 2018. An evaluation of multidrug-resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. *Annals of Clinical Microbiology and Antimicrobials*, 17(1), pp.1-13.

Reis, A.C. et al., 2016. Ciprofloxacin resistance pattern among bacteria isolated from patients with community-acquired urinary tract infection. *Revista do Instituto de Medicina Tropical de Sao Paulo*, 58, pp.1-6.

Ring Biotechnology Co Ltd, 2018. *Beta lactams antibiotics: the greatest discovery, the biggest danger.* [online] Available at: <http://www.ringbio.com/press-release/introduction-of-beta-lactams-antibiotics.> [Accessed 21 March 2022].

Rodloff, A.C., Goldstein, E.J.C. and Torres, A., 2006. Two decades of imipenem therapy. *Journal of Antimicrobial Chemotherapy*, 58(5), pp.916-929.

Rybak, B. et al., 2022. Antibiotic resistance, virulence, and phylogenetic analysis of *Escherichia coli* strains isolated from free-living birds in human habitats. *PLOS ONE*, 17(1), p.e0262236.

Sadeghi, A., Halaji, M., Fayyazi, A. and Havaei, S. A., 2020. Characterization of plasmid-mediated quinolone resistance and serogroup distributions of uropathogenic *Escherichia coli* among Iranian kidney transplant patients. *BioMed Research International*, 2020, pp.1-7.

Salah, F.D. et al., 2019. Distribution of quinolone resistance gene (*qnr*) in ESBL-producing *Escherichia coli* and *Klebsiella spp*. in Lomé, Togo. *Antimicrobial Resistance & Infection Control*, 8(1), pp.1-8.

Salvador-Membreve, D.M. and Rivera, W.L., 2021. Predominance of *bla*TEM and *tetA* genes in antibiotic-resistant *Escherichia coli* isolates from Laguna Lake, Philippines. *Journal of water, sanitation and hygiene for development*, 11(5), pp.814-823.

Singh, S., 2021. Extending retirement age possible. *The Star Online*, [online] 26 Nov. Available at: <https://www.thestar.com.my/news/nation/2021/11/26/extending-retirement-age-possible> [Accessed 29 March 2022].

Tamma, P.D. et al., 2021. Infectious Diseases Society of America Guidance on the treatment of extended-spectrum β-lactamase producing *Enterobacterales* (ESBL-E), Carbapenem-Resistant *Enterobacterales* (CRE), and *Pseudomonas aeruginosa* with Difficult-to-Treat Resistance (DTR-*P. aeruginosa*). *Clinical Infectious Diseases*, 72(7), pp.1109-1116.

Valadbeigi, H., HatamiLak, M., Maleki, A., Kouhsari, E. and Sadeghifard, N., 2020. Molecular characteristics, antimicrobial resistance profiles, and antibiotic resistance determinants in uropathogenic fluoroquinolone resistant-*Escherichia coli* isolates. *Gene Reports*, 18, p.100584.

Vila, J. et al., 2016. *Escherichia coli*: an old friend with new tidings. *FEMS Microbiology Reviews*, 40(4), pp.437-463.

Wellcome, 2020. *Why is it so hard to develop new antibiotics?* [online] Available at: <https://wellcome.org/news/why-is-it-so-hard-develop-new-antibiotics> [Accessed 30 March 2022].

Xiao, L. et al., 2019. Characterization of beta-lactamases in bloodstream-infection *Escherichia coli*: dissemination of *bla*ADC–162 and *bla*CMY–2 among bacteria via an IncF Plasmid. *Frontiers in Microbiology*, 10, pp. 1-10.

APPENDICES

Appendix A

Table 1: Demographic data of each UPEC isolate tested in this study.

Sample	Gender	Age
UTIPS 1	M	2
UTIPS 2	F	78
UTIPS 3	F	48
UTIPS 4	F	26
UTIPS 5	F	67
UTIPS 6	F	36
UTIPS 7	F	77
UTIPS 8	F	76
UTIPS 9	M	69
UTIPS 10	M	52
UTIPS 12	F	66
UTIPS 13	F	24
UTIPS 14	F	74
UTIPS 15	M	40
UTIPS 16	F	41
UTIPS 17	F	30
UTIPS 18	F	51
UTIPS 19	M	63
UTIPS 20	F	88
UTIPS 21	F	53
UTIPS 22	F	63
UTIPS 23	F	63
UTIPS 24	M	77
UTIPS 25	F	79
UTIPS 26	F	61
UTIPS 27	F	76
UTIPS 28	F	66
UTIPS 29	F	38
UTIPS 30	F	73
UTIPS 31	M	63
UTIPS 32	M	68
UTIPS 33	F	68
UTIPS 34	F	64
UTIPS 35	F	56

Table 1 (continued)

UTIPS 36	F	64
UTIPS 37	F	59
UTIPS 38	F	79
UTIPS 39	F	86
UTIPS 40	F	35
UTIPS 41	F	70
UTIPS 42	F	17
UTIPS 43	F	17
UTIPS 44	M	57
UTIPS 45	F	30
UTIPS 46	F	48
UTIPS 47	F	26
UTIPS 48	M	82
UTIPS 49	M	87
UTIPS 50	F	53
UTIPS 51	F	75
UTIPS 52	M	13
UTIPS 53	F	5
UTIPS 54	F	39
UTIPS 55	M	53
UTIPS 56	M	49
UTIPS 57	F	67
UTIPS 58	F	44
UTIPS 59	M	82
UTIPS 60	F	3
UTIPS 61	M	68

‘M’ stands for male and ‘F’ stands for female.

Appendix B

Table 2: Antimicrobial susceptibility profile of UPEC isolates.

Sample	NAL	CIP	LEX	TET	MIN	SXT	AMP	CHL	IPM
UTIPS 1	S	S	S	S	S	S	S	R	S
UTIPS 2	R	R	S	R	S	S	R	S	S
UTIPS 3	S	S	S	S	S	S	S	S	S
UTIPS 4	R	R	R	R	S	R	R	S	S
UTIPS 5	R	R	R	R	S	S	R	S	S
UTIPS 6	S	S	S	S	S	S	R	S	S
UTIPS 7	R	R	R	R	S	R	R	R	S
UTIPS 8	S	S	S	R	S	S	R	S	S
UTIPS 9	R	R	R	R	S	R	R	S	S
UTIPS 10	R	S	S	R	S	R	R	R	S
UTIPS 12	S	S	S	S	S	S	R	R	S
UTIPS 13	S	S	S	R	S	S	R	S	S
UTIPS 14	R	R	R	R	S	R	R	S	S
UTIPS 15	R	R	R	R	S	R	R	S	S
UTIPS 16	R	R	R	R	S	R	R	S	S
UTIPS 17	S	S	S	R	S	R	R	S	S
UTIPS 18	R	R	R	R	S	R	R	S	S
UTIPS 19	R	S	S	R	S	R	R	R	S

Table 2 (continued)

UTIPS 20	S	S	S	R	S	S	R	R	S
UTIPS 21	R	R	R	S	S	S	R	S	S
UTIPS 22	S	S	S	R	S	R	S	S	S
UTIPS 23	S	S	S	S	S	S	S	S	S
UTIPS 24	R	R	R	S	S	S	R	S	S
UTIPS 25	S	S	S	S	S	S	R	S	S
UTIPS 26	S	S	S	S	S	S	R	S	S
UTIPS 27	R	S	S	S	S	S	R	S	S
UTIPS 28	S	S	S	S	S	S	S	S	S
UTIPS 29	S	S	S	R	S	S	R	R	S
UTIPS 30	R	S	S	S	S	S	R	S	S
UTIPS 31	R	R	R	S	S	S	R	S	S
UTIPS 32	R	R	R	R	S	S	R	R	S
UTIPS 33	R	S	S	S	S	S	R	S	S
UTIPS 34	R	R	R	R	S	R	R	S	S
UTIPS 35	R	S	S	S	S	S	S	S	S
UTIPS 36	S	S	S	S	S	S	S	S	S
UTIPS 37	S	S	S	S	S	S	S	R	S
UTIPS 38	S	S	S	R	S	S	R	S	S
UTIPS 39	R	S	S	R	S	S	R	S	S
UTIPS 40	S	S	S	S	S	R	R	S	S
UTIPS 41	R	R	S	R	S	R	R	S	S
UTIPS 42	R	S	S	R	S	R	R	S	S
UTIPS 43	R	S	S	R	S	R	R	S	S

Table 2 (continued)

UTIPS 44	S	S	S	S	S	S	R	S	S
UTIPS 45	R	S	S	S	S	R	S	S	S
UTIPS 46	S	S	S	S	S	S	S	S	S
UTIPS 47	R	R	S	R	S	S	R	S	S
UTIPS 48	S	S	S	S	S	S	S	S	S
UTIPS 49	R	R	R	S	S	S	S	S	S
UTIPS 50	S	S	S	R	S	R	R	S	S
UTIPS 51	S	S	S	S	S	S	S	S	S
UTIPS 52	S	S	S	R	S	S	R	S	S
UTIPS 53	S	S	S	S	S	S	R	R	S
UTIPS 54	R	S	S	R	S	R	R	S	S
UTIPS 55	R	R	R	R	S	R	R	S	S
UTIPS 56	S	S	S	R	R	S	S	S	S
UTIPS 57	S	S	S	S	S	S	S	S	S
UTIPS 58	S	S	S	S	S	S	S	S	S
UTIPS 59	S	S	S	S	S	S	S	S	S
UTIPS 60	S	S	S	R	S	S	R	S	S
UTIPS 61	R	R	R	S	S	S	R	S	S

‘R’ stands for resistant, and ‘S’ stands for susceptible.
‘NA’ denotes nalidixic acid, ‘CIP’ denotes ciprofloxacin, ‘LEX’ denotes levofloxacin, ‘TET’ denotes tetracycline, ‘MIN’ denotes minocycline, ‘SXT’ denotes trimethoprim-sulfamethoxazole, ‘AMP’ denotes ampicillin, ‘CHL’ denotes chloramphenicol, ‘IMP’ denotes imipenem.

Appendix C

Table 3: Concentration and purity (A260/280 ratio) of the extracted DNA samples from each UPEC isolates.

Sample	DNA concentration (ng/µl)	A260/280 ratio
UTIPS 1	182.52	1.84
UTIPS 2	195.10	1.90
UTIPS 3	211.00	1.88
UTIPS 4	223.60	1.76
UTIPS 5	186.97	1.84
UTIPS 6	196.85	1.90
UTIPS 7	244.06	1.82
UTIPS 8	189.48	1.86
UTIPS 9	222.32	1.93
UTIPS 10	149.31	1.84
UTIPS 12	222.47	1.77
UTIPS 13	247.61	1.88
UTIPS 14	265.59	1.75
UTIPS 15	228.27	1.81
UTIPS 16	209.98	1.89
UTIPS 17	268.06	1.84
UTIPS 18	228.72	1.85
UTIPS 19	151.45	1.93
UTIPS 20	169.07	1.83
UTIPS 21	199.92	1.97
UTIPS 22	175.11	1.75
UTIPS 23	167.75	1.92
UTIPS 24	237.89	1.90
UTIPS 25	187.85	1.78
UTIPS 26	134.36	1.92
UTIPS 27	195.65	1.81
UTIPS 28	147.94	1.93
UTIPS 29	173.10	1.75
UTIPS 30	150.39	1.76
UTIPS 31	186.12	1.92
UTIPS 32	210.41	1.97
UTIPS 33	186.08	1.93
UTIPS 34	179.50	1.81
UTIPS 35	167.08	1.81
UTIPS 36	188.58	1.98

Table 3 (continued)

UTIPS 37	206.35	1.78
UTIPS 38	131.62	1.91
UTIPS 39	192.39	1.91
UTIPS 40	224.02	1.97
UTIPS 41	158.59	1.88
UTIPS 42	160.28	1.82
UTIPS 43	156.76	1.82
UTIPS 44	159.63	1.83
UTIPS 45	184.43	1.96
UTIPS 46	122.98	1.99
UTIPS 47	198.54	1.91
UTIPS 48	223.63	1.85
UTIPS 49	164.18	1.92
UTIPS 50	156.13	1.94
UTIPS 51	207.73	1.92
UTIPS 52	150.46	1.83
UTIPS 53	134.55	1.80
UTIPS 54	197.75	1.87
UTIPS 55	170.40	2.00
UTIPS 56	211.77	1.95
UTIPS 57	153.31	1.81
UTIPS 58	208.89	1.90
UTIPS 59	190.67	1.95
UTIPS 60	182.55	1.92
UTIPS 61	225.89	1.88

Appendix D

Table 4: Results of duplex PCR for detection of *bla*TEM and *bla*SHV in 60 UPEC isolates.

Sample	*bla*TEM	*bla*SHV	Sample	*bla*TEM	*bla*SHV
UTIPS 1	-	-	UTIPS 32	+	-
UTIPS 2	+	-	UTIPS 33	+	-
UTIPS 3	-	-	UTIPS 34	+	-
UTIPS 4	+	-	UTIPS 35	-	-
UTIPS 5	+	-	UTIPS 36	-	-
UTIPS 6	-	-	UTIPS 37	-	-
UTIPS 7	+	-	UTIPS 38	+	-
UTIPS 8	+	-	UTIPS 39	+	-
UTIPS 9	+	-	UTIPS 40	+	-
UTIPS 10	-	-	UTIPS 41	-	-
UTIPS 12	+	-	UTIPS 42	+	-
UTIPS 13	+	-	UTIPS 43	+	-
UTIPS 14	+	-	UTIPS 44	+	-
UTIPS 15	+	-	UTIPS 45	-	-
UTIPS 16	+	-	UTIPS 46	-	-
UTIPS 17	+	-	UTIPS 47	+	-
UTIPS 18	+	-	UTIPS 48	-	-
UTIPS 19	-	-	UTIPS 49	-	-
UTIPS 20	+	-	UTIPS 50	-	-
UTIPS 21	+	-	UTIPS 51	+	-
UTIPS 22	+	-	UTIPS 52	-	-
UTIPS 23	-	-	UTIPS 53	-	-
UTIPS 24	+	-	UTIPS 54	+	-
UTIPS 25	-	-	UTIPS 55	+	-
UTIPS 26	-	-	UTIPS 56	+	-
UTIPS 27	+	-	UTIPS 57	+	-
UTIPS 28	-	-	UTIPS 58	+	-
UTIPS 29	-	-	UTIPS 59	+	-
UTIPS 30	-	-	UTIPS 60	+	-
UTIPS 31	-	-	UTIPS 61	-	-

‘ + ’ indicates the presence of *bla* genes, ‘-’ indicates the absence of *bla* genes.

Appendix E

Table 5: Representative statistical analysis of negative association (association between gender and *bla*TEM gene prevalence).

Crosstab

			Gender		
			female	Male	Total
Presence of bla_TEM	Absence	Count	16	8	24
		% within Presence of bla_TEM	66.7%	33.3%	100.0%
		% within Gender	36.4%	50.0%	40.0%
		% of Total	26.7%	13.3%	40.0%
	Present	Count	28	8	36
		% within Presence of bla_TEM	77.8%	22.2%	100.0%
		% within Gender	63.6%	50.0%	60.0%
		% of Total	46.7%	13.3%	60.0%
Total		Count	44	16	60
		% within Presence of bla_TEM	73.3%	26.7%	100.0%
		% within Gender	100.0%	100.0%	100.0%
		% of Total	73.3%	26.7%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.909[a]	1	.340		
Continuity Correction[b]	.430	1	.512		
Likelihood Ratio	.898	1	.343		
Fisher's Exact Test				.383	.255
N of Valid Cases	60				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.40.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	-.123	.340
	Cramer's V	.123	.340
N of Valid Cases		60	

Table 6: Representative statistical analysis of positive association (association between ampicillin susceptibility profile and *bla*TEM gene prevalence).

Crosstab

			AMP		
			Resistant	Susceptible	Total
Presence of bla_TEM	Absent	Count	13	11	24
		% within Presence of bla_TEM	54.2%	45.8%	100.0%
		% within AMP	30.2%	64.7%	40.0%
		% of Total	21.7%	18.3%	40.0%
	Present	Count	30	6	36
		% within Presence of bla_TEM	83.3%	16.7%	100.0%
		% within AMP	69.8%	35.3%	60.0%
		% of Total	50.0%	10.0%	60.0%
Total		Count	43	17	60
		% within Presence of bla_TEM	71.7%	28.3%	100.0%
		% within AMP	100.0%	100.0%	100.0%
		% of Total	71.7%	28.3%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.033[a]	1	.014		
Continuity Correction[b]	4.682	1	.030		
Likelihood Ratio	5.984	1	.014		
Fisher's Exact Test				.020	.016
N of Valid Cases	60				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.80.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	-.317	.014
	Cramer's V	.317	.014
N of Valid Cases		60	

Document Viewer

Turnitin Originality Report

Processed on: 19-Apr-2022 21:51 +08
ID: 1814495420
Word Count: 6516
Submitted: 1

Thesis By Yin Ying Lee

Similarity Index	Similarity by Source	
6%	Internet Sources:	2%
	Publications:	5%
	Student Papers:	1%

include quoted   include bibliography   excluding matches < 8 words   mode: quickview (classic) report   Change mode   print   download

1% match (publications)
"Poster Sessions", Clinical Microbiology and Infection, 04/2012

1% match (publications)
"Posters", Clinical Microbiology and Infection, 5/2008

1% match (publications)
"13th European Congress of Clinical Microbiology and Infectious Diseases", Clinical Microbiology and Infection, 2003

<1% match ()
Archer, Gareth T., Elhawaz, Alaa et al. "Validation of four-dimensional flow cardiovascular magnetic resonance for aortic stenosis assessment", 'Springer Science and Business Media LLC', 2020

<1% match (Internet from 12-Mar-2022)
http://openaccess.ogu.edu.tr:8080

<1% match ()
Masoud Zarei, Moghaddameh Mirzaee, Hosniyeh Alizadeh, Yunes Jahani. "Investigation of the affective factors on the survival rate of patients with laryngeal cancer using Cox proportional hazards and Lin -Ying's additive hazards models", Medical Journal of the Islamic Republic of Iran

Universiti Tunku Abdul Rahman			
Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)			
Form Number: FM-IAD-005	Rev No.: 1	Effective Date: 3/10/2019	Page No.: 1of 1

FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	LEE YIN YING
ID Number(s)	18ADB02333
Programme / Course	BACHELOR OF SCIENCE (HONS) BIOMEDICAL SCIENCE
Title of Final Year Project	DUPLEX PCR DETECTION OF *bla*SHV AND *bla*TEM GENES IN UROPATHOGENIC *Escherichia coli* (UPEC) ISOLATES FROM PATIENTS

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
Overall similarity index: 6 **%** **Similarity by source** Internet Sources: 2 % Publications: 5 % Student Papers: 1 %	All in range
Number of individual sources listed of more than 3% similarity: 0	

Parameters of originality required and limits approved by UTAR are as follows:

(i) **Overall similarity index is 20% and below, and**
(ii) **Matching of individual sources listed must be less than 3% each, and**
(iii) **Matching texts in continuous block must not exceed 8 words**

Note: Parameters (i) – (ii) shall exclude quotes, bibliography and text matches which are less than 8 words.

Note Supervisor/Candidate(s) is/are required to provide softcopy of full set of the originality report to Faculty/Institute

Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.

Signature of Supervisor
Name: Dr. Chew Choy Hoong
Date: 21/04/2022