ISOLATION OF ANTIBIOTIC RESISTANT BACTERIA FROM THE TOILET DOOR HANDLES IN INTI INTERNATIONAL UNIVERSITY

MELISSA KONG SUK YI

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMEBTS FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI, MALAYSIA

2016

NON-PLAGIARISM DECLARATION

By this letter I declare that I have written this thesis completely by myself, and that I have used no other sources or resources than the ones mentioned.

I have indicated all quotes and citations that were literally taken from publications, or that were in close accordance with the meaning of those publications, as such. All sources and other resources used are stated in the references.

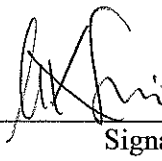
Moreover I have not handed in a thesis similar in contents elsewhere.

In case of proof that the thesis has not been constructed in accordance with this declaration, the Faculty of Health and Life Sciences has the right to consider the research thesis as a deliberate act that has been aimed at making correct judgment of the candidate's expertise, insights and skills impossible.

I acknowledge that the assessor of this item may, for the purpose of assessing this item,

- reproduce this assessment item and provide a copy to another member of the University; and/or,
- communicate a copy of this assessment item to a plagiarism checking service (which may then retain a copy of the assessment item on its database for the purpose of future plagiarism checking).

In case of plagiarism the examiner has the right to fail me and take action as prescribed by the rules regarding Academic Misconduct practiced by INTI International University.

Melissa Kong Suk Yi	
Name	Signature
I14006026	16th December 2016
I.D.Number	Date

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged, and completed under the supervision of Ms. Lalita Ambigai Sivasamugham.

Melissa Kong Suk Yi

I14006026

14th November 2016

Ms. Lalita Ambigai Sivasamugham

(SUPERVISOR)

Dr. Geetha Subramaniam

(CO-SUPERVISOR)

ACKNOWLEDGEMENTS

First of all, I would like to take this chance to acknowledge and appreciate the help that have been given from everyone for my project. A sincere thank to my supervisor, Ms Lalita Ambigai and my co-supervisor, Dr. Geetha Subramaniam for the continuous support throughout my final year project. I would like to appreciate and thank Ms Lalita for her guidance, patience, expertise, support and assistance in my thesis writing and also lab experiments. I would like to thank all the laboratory technicians who had given me assistant in lab work and appreciate their tolerance throughout the lab work. Lastly, I would like to thank my family, my friends; Shee Hui Ping, Lee Chee Yan, Abegal Then and Liew Zeh San and my seniors for their encouragement, love and moral support. Without all of these people, I would not be able to complete my dissertation.

ABSTRACT

The inanimate surfaces are reservoirs of various bacteria. Many bacteria can survive for weeks and months on the surfaces. One of the common surfaces that contain bacteria and can cause the transmission is the toilet door handle. Individuals that do not wash their hands after using the toilet touch the door handles and leads to the possible transmission of common bacteria, pathogens as well as antibiotic resistant bacteria from one individual to another. This study is aimed at isolating antibiotic resistant bacteria from the toilet cubicles' door handles of the female toilet at the academic block in INTI International University. All samples were inoculated on nutrient agar plate and incubated at 37 °C for 24 hours in a shaking incubator. All isolates were sub cultured and identified by a series tests such as Gram staining, catalase test, oxidase test and IMVIC test. These isolates were also grown on MSA and MacConkey Agar. A total of 56 pure isolates were isolated from the toilet door handles samples. Eight pure isolates showed Gram-negative reaction while 48 pure isolates showed Gram-positive reaction. Based on the Bergey's Manual 9th edition, the possible bacterial species of *Pseudomonas spp.* (8.93%), *Staphylococcus epidermidis* (21.43%) and *Staphylococcus aureus* (26.79%) were isolated from the toilet door handles. There were 24 isolates (42.86%) that could not be identified due to lack of confirmatory tests. The antibiotic susceptibility test for the isolates were carried out using the Disk Diffusion Method on Mueller-Hinton agar and the zone of inhibition were compared to BSAC guidelines, version 12 May 2013. From the 36 Gram-positive isolates, six isolates (16.67%) were resistant only to oxacillin and five of the isolates (13.89%) were only resistant to clindamycin. The antibiotic resistance of Gram-negative isolates could not be determined due to lack of information in the BSAC guidelines. A total of 11 out of 56 isolates (19.64%) were resistant to antibiotics. Hence, the chance of transmission of antibiotic resistant bacteria from the toilet door handles is relatively low and unlikely to cause a bacterial disease outbreak among the individual at INTI International University.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF ABBREVIATIONS

AK	Amikacin
ARB	Antibiotic Resistant Bacteria
BSAC	British Society for Antimicrobial Chemotherapy
C	Chloramphenicol
CE	Common era
CFU/mL	Colony forming units/milliliter
CLSI	Clinical & Laboratory Standards Institute
DA	Clindamycin
DNA	Deoxyribonucleic acid
H_2O_2	Hydrogen peroxide
KZ	Cephazolin
MDR-TB	Multi-drug-resistant *Mycobacterium Tuberculosis*
mL	Millilitre
mm	Millimetre
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
MR-VP	Methyl Red-Voges-Proskauer
MSA	Mannitol Salt Agar
OX	Oxacillin
RNA	Ribonucleic acid
rpm	Revolutions per minute
TE	Tetracycline
TSI	Triple Sugar Iron

VRE	Vancomycin-resistant *Enterococci*
WHO	World Health Organization
°C	Degree celcius
µg	Microgram
µL	Microlitre

CHAPTER 1

INTRODUCTION

Antibiotics are commonly used to prevent and treat various bacteria-related diseases (Davies & Davies, 2010). In 1928, Sir Alexander Fleming discovered penicillin (Ventola, 2015). Until now, antibiotics have saved thousands and millions of lives. However, almost all of the developed antibiotics are resistant to bacteria (Ventola, 2015). Bacteria can acquire resistance gene through conjugation of plasmid (Alanis, 2005). The bacterial strains harbouring multidrug-resistance (MDR), is a result of the new mechanisms that drive the development of different classes of antibiotic resistance, causing the emergence of "superbugs" that claim many lives of humans, and even animals (Alanis, 2005).

The biggest factor leading to the formation of MDR is the improper and indiscriminate use of antibiotics (Alanis, 2005). Also, some people with little knowledge of medicines tend to self-medicate for common flu that is usually caused by viruses. This too contributes to the evolution of antibiotic resistance. The evolution of antibiotic resistant bacteria can be deadly, as it can cause severe complications and multiple organ failure (Alanis, 2005). Hence, scientists are in search for new antibiotics for diseases cause by MDR, but it will take a long time and might cost a lot of lives, as inventing for new antibiotics is not an easy task that might takes a long time (Alanis, 2005). During the wait for new antibiotics for MDR-caused disease, some patients may not survive due to severe complications.

A university is a community with people coming from different places. Like anywhere else, the students, and the staff are constantly in contact with inanimate surfaces such as toilet door handles. The toilet door handles can act as a bacteria reservoir, as well as serving as a source of transmission of pathogenic bacteria (Hammuel et al., 2014). The microbes on the inanimate surfaces can remain infectious even after weeks of the event of contamination (Carvahlo et al., 2007). Thus, these microbes can easily be transmitted to humans who encounter such surfaces. Transmission of pathogens is likely to cause diseases in individuals (Sabra, 2013).

According to a study, the types of bacteria that will cause common diseases like food contamination, diarrhea, sore throat and urinary tract infections are *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Streptococcus pyogenes*, which are also commonly found on the inanimate surfaces of toilet (Chengula et al., 2014).

Thus, the aim of this study was to isolate and identify antibiotic resistant bacteria from the toilet door handles of block A, B and C female toilets in the academic blocks of INTI International University. Knowing this will indicate if the door handles can transmit antibiotic resistant bacteria to the users eventually causing an outbreak if not contained. Perhaps, through this study, the users can be emphasized to be hygienic to prevent the outbreak or spread of antibiotic resistant bacteria.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTICS

Antibiotics are substances that can be used to treat infections and diseases caused by bacteria (Davies & Davies, 2010). Antibiotic can cause cell death by inhibiting the cellular function through the interaction of the primary drug target (Kohanski et al., 2010). Antibiotic can be divided into two types, which are broad-spectrum antibiotics and narrow spectrum antibiotics. Broad-spectrum antibiotics work against a wide range of bacteria, whereas narrow spectrum antibiotics work with only one or a few type of bacteria (Nordqvist, 2016). There are three modes of actions of antibiotics, which are bactericidal, bacteriostatic and bacteriolytic. Bactericidal antibiotics kill the bacteria, while bacteriostatic antibiotics will prevent growth by inhibiting the synthesis of DNA, RNA, cell wall or protein (Kohanski et al., 2010). Bacteriolytic antibiotics will kill bacteria through lysing the cells (Kohanski et al., 2010).

2.1.1 Background of Antibiotics

In 350 to 550 CE, a long time before the discovery of penicillin in 1928, traces of a type antibiotic, which is tetracycline, were discovered in the remains of human skeleton from Sudanese Nubia population (Aminov, 2010). The studies have found out that the bones of these ancient people have tetracycline distribution that can only be possible through ingesting tetracycline (Aminov, 2010). Scientist predicted that the ancient people ingested tetracycline to protect themselves against infections. Also, report shows that the infectious diseases recorded in the population of Sudanese Nubian were very low (Aminov, 2010). This proves that humans already had the knowledge of using antibiotics even during the ancient time.

In 1928, Alexander Fleming believed that *Penicillium* has the antimicrobial activity and after 12 years of persistent belief, he approached chemists to solve the problems of active substance's purification and stability (Markel, 2013). In 1940, when

Fleming finally gave up this idea, an Oxford team under the lead of Howard Florey and Ernest Chain incorporated to publish a journal regarding the description of penicillin purification that were enough to contribute to clinical testing (Markel, 2013). This had successfully contributed to the mass production and distribution of penicillin (Aminov, 2010). Alexander Fleming's screening method for antibiotic resistance on the agar plates lawned with pathogens by measuring the zone of inhibition had minimized the resources needed to test on animal models (Aminov, 2010). This method became popular and was widely used to screen microbes that produce antibiotic and also used in research and industry as well (Aminov, 2010). Fleming was one of the first people to warn about the potential penicillin resistance if the medication is overused or used too little in a disease treatment (Markel, 2013).

2.1.2 Commonly Used Antibiotics

Different types of bacteria cause different types of infections. Hence, there are lots of antibiotics used to treat various bacterial infections.

2.1.2.1 Penicillin

Penicillin has an original form, which is known as penicillin G (Ross-Flanigan & Uretsky, 2006). Penicillin can be used against anaerobic bacteria. However, the strong acid in the stomach can destroy it, which makes it a type of narrow spectrum antibiotic (Ross-Flanigan & Uretsky, 2006). Due to the vulnerability of penicillin towards stomach acid, penicillin V, a new type of penicillin was introduced (Ross-Flanigan & Uretsky, 2006). Penicillin often combined with beta-lactamase inhibitors, prevent the enzyme of bacteria from destroying it before the penicillin can act on it (Ross-Flanigan & Uretsky, 2006).

2.1.2.2 Cephalosporin

Cephalosporin is usually used during surgical procedures, as it will prevent the infection of bacteria during or after surgery (Ross-Flanigan & Uretsky, 2004). Other than this, cephalosporin is also used to treat several infections of throat, skin, lungs, ears, nose and sinuses (Ross-Flanigan & Uretsky, 2004). Cephalosporin now has five