

REISOLATION AND REIDENTIFICATION OF ANTIBIOTIC RESISTANT BACTERIA
FROM THE TOILET DOOR HANDLES IN INTI INTERNATIONAL UNIVERSITY

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

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

2018

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ACKNOWLEDGEMENTS

First of all, I would like to take this chance to thank God for His love and mercy upon my life. A sincere thank to my supervisor, Ms. Lalita Ambigai Sivasamugham and co-supervisor, Dr. Gheeta Subramaniam for the continuous guidance and support throughout my final year project. Last but not least, I want to thank my family ; Bapa dan Mama Waisimon, Kk Alfi, Kk Oli, Maito, Eki, Unu, Gio and Donna as well as all my beloved friends for their encouragement, love and moral support. Without all of these people, I would not be able to complete my dissertation.

ABSTRACT

Studies have shown that bacterial contamination are common on inanimate surfaces. One of the common surfaces that can transmit bacteria is toilet door handles. Improper hygiene practice of toilet users can be the cause of transmission of pathogens including antibiotic resistant bacteria. Antibiotic resistant bacteria were isolated from the toilet door handles in INTI International University from a previous study. However, these isolates were poorly identified, and the complete antibiogram pattern were not done as well as the pure cultures were not preserved. Thus, the aim of this study was to reisolate and reidentify the antibiotic resistant bacteria from the toilet door handles in INTI International University as well as to determine the resistant pattern of the isolates using the antibiotic disk diffusion method. Bacterial sample were obtained from each toilet cubicles in the female toilets in the academic block of INTI International University. The sample were inoculated in nutrient broth and incubated at 200rpm, 37°C for 16-18hr. The pure culture of 46 isolates were identified using several confirmatory tests such as gram staining, catalase test, oxidase test, TSI agar test and IMVIC test. Furthermore, the isolates were grown also grown onto MSA and MacConkey agar to further identify the genus and species. Out of 46 pure isolates, 42 isolates were able to be identified its genus and species using Bergey's Manual of Determinative Bacteriology 9th edition, whereas, 4 isolates cannot be identified. Several possible genus and species were identified such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus* spp and *Salmonella* spp. Different antibiotics such as amoxicillin, ampicillin, cefoxitin, penicillin G, ofloxacin, vancomycin as well as gentamycin were used to determine the antibiotic susceptibility pattern of gram positive and gram negative isolates based on the Clinical and Laboratory Standards Institute (CLSI) 27th Edition. From a total of 42 identified isolates, 10 isolates were resistant to antibiotics. Out of 11 isolates of *Staphylococcus aureus*, 1 isolate (2.38%) was resistance to cefoxitin explaining the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from the toilet door handles. Meanwhile, 5 isolates of *S. epidermidis* (11.90%) were resistant toward both penicillin G and cefoxitin showing the presence of multidrug resistant (MDR) bacteria. This study shows that the toilet door handles can be one of the significant inanimate surface to transmit antibiotic resistant bacteria from one individual to another in INTI International University. Personal hygiene is important to remove the spread of these pathogen in the environment.

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LIST OF ABBREVIATIONS

ARB	Antibiotic Resistant Bacteria
CLSI	Clinical and Laboratory Standards Institute
CFU/mL	Colony forming units/millilitre
°C	Degree Celcius
<i>E. Coli</i>	<i>Escherichia coli</i>
g	Gram
H ₂ O ₂	Hydrogen peroxide
MSA	Mannitol Salt Agar
µg	Microgram
µL	Microlitre
mm	Millimetre
mL	Milliliter
MIC	Minimal inhibitory concentration
min	Minute
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MR-VP	Methyl Red-Voges-Proskauer
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
rpm	Revolution per minute
TSI	Triple Sugar Iron
UTI	Urinary Tract Infection

CHAPTER 1

INTRODUCTION

Many bacteria or in most cases, pathogenic bacteria can live in unclean environments such as toilets which deposited from the toilet users (Bashir, Muhammad, Sani & Kawo, 2016). The microbes may have originated from the unclean hands of users and it can be transmitted from one user to another user through surface to surface contact (Bashir et al., 2016).

The transmission of pathogenic bacteria from public toilets such as those in a working environments as well as educational institutes can be vital because it concerns the public. An educational institute is an environment that contains its own community that often work closely, thus, a transmission of pathogen bacteria due to unhygienic practices can be deadly. Source of the outbreak of disease that have spread in such settings include the ones caused by *Staphylococcus aureus* or *Salmonella* spp. (Badger-Emeka, 2015). These bacteria can lead to several major health problems, for instance, food borne disease and urinary tract infection (UTI) (Chengula et. al., 2014). Hence, several prevention methods regarding personal hygiene has to be prioritized, in order to avoid the diseases caused by these pathogenic bacteria.

Antibiotics have been used to kill pathogenic bacteria. Antibiotic medication has been used from 1920s until now (Explorable, 2017). However, the improper use of antibiotics over decades has caused many bacteria to be resistant towards antibiotics. The resistance of bacteria towards some antibiotics is known as multidrug resistant (MDR), where the bacteria cannot be controlled or killed by antibiotics (Department of Public Health, 2016). For instance, in Sokoine University of Agriculture (SUA) in Tanzania, several pathogenic bacteria such as *Pseudomonas aeruginosa* and *S. aureus* were isolated from the toilets door handles, and were resistant to at least one of the tested antibiotic, such as Ampicillin, Amoxicillin and Gentamycin (Chengula, et. al., 2014). These pathogenic bacteria have also

caused diseases to the students in SUA, such as foodborne diseases, gastrointestinal infection and sore throat (Chengula, et. al., 2014).

A previous study by Melissa Kong (2016) showed the presence of bacteria on the toilet door handles in INTI International University. However, the isolated bacteria were poorly identified and a complete antibiogram of the resistant pattern was not done. Hence, the aim of this study was to reisolate and reidentify antibiotic resistant bacteria isolated from toilet door handles in INTI International University, by confirming the possible genus and species of the bacteria sample using Bergey's manual. In addition, the antibiotic resistance pattern of these bacteria were determined using the disk diffusion assay using several types of antibiotics.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTIC

Antibiotic is commonly used to kill or damage pathogenic bacterial cell. Antibiotic act by inhibiting bacterial cell wall synthesis, inhibiting of bacterial DNA and protein synthesis leading to cell death (Department of Health Antimicrobial Resistance Strategy Analytical Working Group, 2016). However, some bacteria have found ways to reduce the effectiveness of antibiotic by preventing the binding of antibiotic to the target molecule, hence leading to resistance of bacteria towards antibiotic (Department of Health Antimicrobial Resistance Strategy Analytical Working Group, 2016).

2.1.1 The History

In 350-550 CE, traces of tetracycline was found in the remains of humans in ancient Sudanese Nubia. Scientists believe that these remains were at least 1400 years old (Aminov, 2010). The intake of tetracycline benefitted the ancient Sudanese community by protecting the community from infectious diseases (Aminov, 2010). This finding shows that the use of antibiotic already existed even before the discovery of penicillin. Another trace of antibiotic-like compound was found in Jordan which was to treat skin infection (Aminov, 2010). The treatment using this compound has led to the production of several antibiotics such as actinomycin (Aminov, 2010). Also, the production of natural antibiotics have led to the development of some traditional medicine, specifically traditional-chinese medicine (TCM).

In 1921, an antimicrobial compound was discovered by Sir Alexander Fleming and 12 years after the discovery, he insisted a chemist to solve the purification and stability issues of the active compound derived from *Penicillium* (Aminov, 2010). Although the purification and stability issue was not solved, Fleming adhered to the method of purification published by Howard Florey and Ernest Chain leading to the mass production of pure penicillin in 1940s (Aminov, 2010).

2.1.2 Commonly Used Antibiotic

Commonly used antibiotics include naturally occurring antibiotic, semi-synthetic and synthetic antibiotic. Antibiotics can be classified based on the class as well as on the mode of action. In addition, there are broad spectrum and narrow spectrum antibiotics. Broad spectrum antibiotics act against wide range of bacteria unlike narrow spectrum antibiotic which only kill specific type of bacteria like gram positive or gram negative bacteria. Table 1 shows the mode of action of common antibiotics (Yocum, Rasmussen & Strominger, 1979).

Table 1. Summary of Commonly Used Antibiotics

Spectra	Antibiotic	Mode of Action	References
Broad Spectrum	Beta-lactams	Inhibit cell wall synthesis	(Adapted from Eickoff, 1992)
	Cephalosporin	Inhibit cell wall synthesis and activate autolysin	(Adapted from Courvalin, 1994)
	Fluoroquinolones	Inhibit DNA gyrase and prevent replication of bacterial DNA	(Adapted from Courvalin, 1994)
Narrow Spectrum	Aminoglycosides	Disrupt protein synthesis	(Adapted from Courvalin, 1994)
	Lipo-peptides	Disrupt cell membrane	(Adapted from Courvalin, 1994)
	Glyco-peptides	Prevent integration of NAG and NAM subunits into peptidoglycan matrix	(Adapted from Courvalin, 1994)

2.2 ANTIBIOTIC RESISTANT BACTERIA (ARB)

2.2.1 Background of ARB

Antibiotics used to treat bacterial infection have turned out to be a serious threat for public health due to the emergence of antibiotic resistant bacteria. Antibiotic resistant bacteria are bacteria that cannot be killed by antibiotics. World Health Organization (WHO) declared ARB as one of the three greatest threats to human health in 2011 (Nordberg, Monnet & Cars,

2004). Antibiotic resistant in bacteria has been attributed to various reasons such as, mutation in bacterial genome, overuse of the antibiotics and poor hygienic practices. In fact, during the discovery of penicillin, Fleming alerted the community by mentioning that the overuse or improper use of antibiotics could lead to the emergence of antibiotic resistant bacteria (Aminov, 2010). The emergence of resistant bacteria can be seen in Figure 1. It explains how the number of antibiotic resistance in bacteria increases from 1940 to 2011.

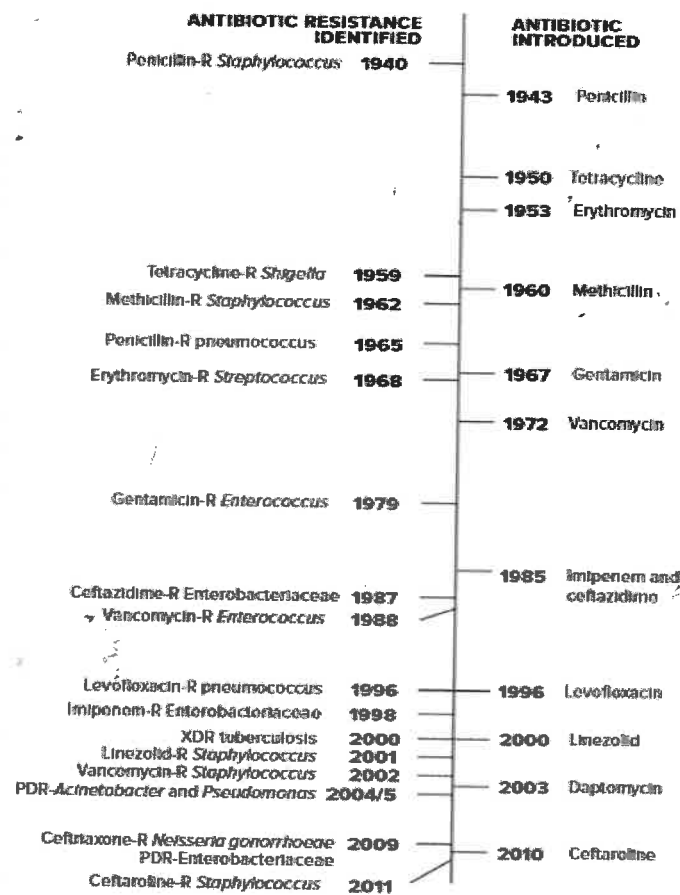


Figure 1. Timeline Development of Antibiotic Resistant Bacteria (Ventola, 2015). PDR = pan-drug-resistant; R = resistant; XDR = extensively drug-resistant.

2.2.2 Factor Causing ARB

Imprudent use of antibiotics has contributed to the evolution of antibiotic resistant bacteria (Aminov, 2010). Bacteria evolve in a short period of time because it does not take a longer time for them to replicate and adapt to the new environment (Alliance for the Prudent Use of Antibiotic, 2014). During the replication process, genetic mutation can occur where the bacterial cell find ways to either modify the antibiotic target enzyme or change its structure