REIDENTIFICATION OF ANTIBIOTIC RESISTANT BACTERIA ISOLATED FROM THE HANDPHONES OF MALE AND FEMALE USERS IN NILAI

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ABSTRACT

Although handphones have many functions, the overuse of handphones can lead to transmission of bacteria including antibiotic resistant bacteria. Previous study had successfully isolated bacteria from handphones, some of the isolates were poorly identified. In addition, the susceptibility pattern of the isolate were not convincing. Thus, the objectives of this study were to re-isolate and re-identify the bacterial isolates obtained by previous study as well as to reconfirm the resistance patterns of the bacterial isolates and to compare the distribution of the antibiotic resistant bacteria between the handphones of male and female users in Nilai. The pure cultures of isolates were obtained using the dilution streak technique and subjected to gram staining and biochemical assays before proceeding to antibiotic susceptibility test using vancomycin, rifampicin, ciprofloxacin, gentamycin, ampicillin, cefoxitin, streptomycin, ceftriaxone, and ofloxacin. The isolates were confirmed using Bergey's Manual of Determinative Bacteriology. The antibiotic resistant pattern were determined using the Clinical and Laboratory Standard Institute, 2017. Few possible genus and species were isolated, which were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Propionibacterium sp*, *Neisseria sp*, *Enterobacter sp*, and *Stomatococcus sp*. Fifty-six isolates were obtained in which, 48 isolates were gram positive while 8 were gram negative. 30 (53%) isolates showed resistance towards at least one antibiotic. However, 11 (20%) isolates were susceptible to all the tested antibiotics. However, the susceptibility of 15 (27%) isolates could not be defined because the genera of the isolates could not be identified. The data obtained from the antibiotic susceptibility tests were analysed statistically using the chi-square and indicated that there were no significant differences on the number of antibiotic resistant bacteria isolated from handphones of male and female users.

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LIST OF ABBREVIATION

ARB	Antibiotic resistant bacteria
ATM	Automated teller machine
CLSI	Clinical and Laboratory Standards Institute
CONS	Coagulase negative *Staphylococcus*
°C	Degree Celsius
ESBL	Extended spectrum beta-lactamases
g	Gram
H_2O_2	Hydrogen peroxide
MSA	Mannitol Salt Agar
MRSA	Methicillin-resistant *Staphylococcus aureus*
µL	Microliter
mL	Mililiter
MH	Mueller Hinton
MDROs	Multidrug-resistant organisms
P. notatum	*Penicillium notatum*
TSI	Triple Sugar Iron
UTI	Urinary Tract Infection
VRE	Vancomycin-resistant *Enterococci*
VRSA	Vancomycin-resistant *Staphylococcus aureus*
VP	Voges-Proskauer

CHAPTER 1

INTRODUCTION

Mobile phones are important tools of communication and are widely used for many reasons (Ibrahim, Akenroye, Opawale zs& Osabiya, 2013; Rahangdale, Kokate & Surpam, 2014).

However, mobile phones can cause several disadvantages to the users. For instance, mobile phones can easily be contaminated with microorganisms leading to the spread of microorganisms in the environment (Sharma, Solanki, Parihar, Khatri, Chandora & Bora, 2014; Vivekanandan, 2017). Studies have shown that, many mobile phones users do not clean their phones (Gashaw, Abtew & Addis, 2014). Most people touch their mobile phones without washing their hands before or after their activities, such as after using the toilet and before having a meal (Gashaw, Abtew & Addis, 2014). This has led to high microbial count on the surface of mobile phones.

Al-Abdalall (2010) reported that the heat generated by mobile phones provide a suitable environment for the microbes especially those found on human skin to survive. A study done by Shahaby et al (2012) showed that more of gram positive than gram negative bacteria isolated from mobile phone of male than female users. In addition, more bacterial isolates were cultured from handphones of male users compared to female users. One of the most commonly isolated bacteria from mobile phones is coagulase negative *Staphylococcus* (CONS) (Shahaby, Awad, El-Tarras & Bahobial, 2012). Apart from that, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) are examples of resistant bacteria that have been isolated from mobile phones (Shahaby et al., 2012). With the spread of such resistant bacteria through mobile phones, mobile phone users should take precautionary steps to improve their personal hygiene to prevent the transmission of pathogens (Shahaby et al., 2012).

A previous study by Thiagu (2017) has shown that all of the gram-negative isolates from handphones were antibiotic resistant bacteria. Apart from that, 32 antibiotic resistant bacteria were isolated from 48 of gram-positive isolates. However, the antibiotic susceptibility pattern for 16 bacterial isolates could not be defined and confirmed.

Thus, the objectives of this study were to re-isolate and re-identify the isolates obtained by Thiagu (2017) using various biochemical tests. The antibiotic susceptibility pattern of the isolates was determined using disk diffusion technique with a wider range of antibiotics before determining the significant difference in the number of antibiotic resistant bacteria from mobile phones between genders using the chi square test.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTIC

Antibiotics are chemical compounds produced by microbes such as bacteria and fungi which kills and inhibits the growth of susceptible bacteria (Bayarski, n.d.).

In 1928, Alexander Fleming discovered the very first antibiotic, penicillin produced by a fungus, *Penicillium notatum* (Learn Genetics, 2014). Fleming was observing the staphylococcal cultures and he found the growth of blue-green mould on the plates inhibited the growth of the bacterial culture (Explorable, 2010). Fleming decided to isolate the mould and found out that it was *P. notatum* that produced the inhibitory agent (Explorable, 2010).

Years later, semi-synthetic penicillin was produced by hydrolyzing natural penicillin followed by amidation of different donor chains of carboxylic acyl (Volpato, Rodrigues & Fernandez, 2010). Examples of semi-synthetic penicillin include ampicillin, amoxicillin, and methicillin (Rolinson, 1998). These semi-synthetic penicillins were produced because bacteria had became resistant to the natural antibiotics. Figure 1 shows the time-line of the year of antibiotic were introduced as well as the emergence of antibiotic resistant bacteria.

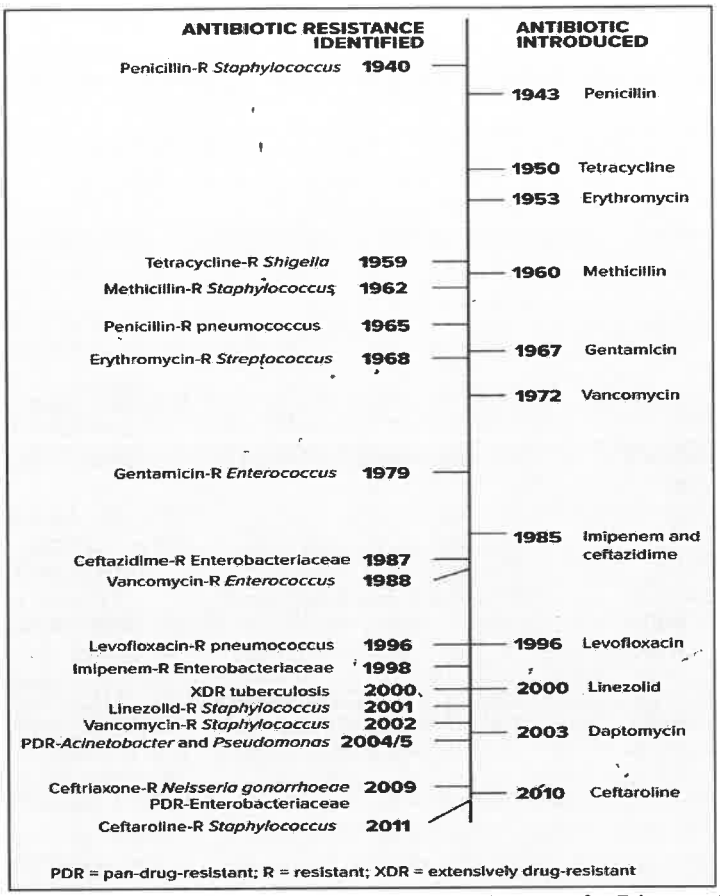

Figure 1. Timeline of antibiotic resistance events (Centers for Disease, 2017).

2.1.1 Classification of Antibiotics Based on their Mode of Action

Antibiotics can be categorized based on their mode of action and their targeted bacterial cells (Michigan State University, 2011a). Broad spectrum antibiotics kill or inhibit the growth of many bacteria such as gram-positive and gram-negative bacteria (MSU, 2011b). Narrow spectrum antibiotics however, are limited on the number or type of bacteria that they target. (MSU, 2011b). Vancomycin, and cefoxitin are some examples of narrow spectrum and broad spectrum antibiotics respectively.

Antibiotics work in two ways, which are bactericidal or bacteriostatic (Explorable, n.d.). A bactericidal antibiotic interferes with the formation of cell walls of bacteria which kills the bacteria (Pankey & Sabath, 2004). On the other hand, a bacteriostatic antibiotic inhibits the growth of bacteria by stopping their cell division which keeps the growth of the bacterial cells at the stationary phase (Pankey & Sabath, 2004).

Table 1 shows classifications of antibiotics based on their specific mode of action, whereas, Figure 2 shows the different target sites of each antibiotic.

Table 1. Mode of action of antibiotic with its specific example(s).

Mode of Action	Example(s) of Antibiotic	Reference
Inhibition of cell wall synthesis: – The active compound of the antibiotic inactivates transpeptidases (PBPs) which inhibit the cross-linking of peptidoglycan. – The active compound will inhibit transglycosylation.	Ampicillin, cefoxitin, vancomycin, and ceftriaxone	(Etebu & Arikekpar, 2016; Varun, 2012).
Inhibition of cell membrane function: – Active compound of the antibiotic inhibit the biosynthesis of ergosterol.	Colistin and daptomycin	(Michigan State University, 2011a).
Inhibition of protein synthesis: – The inhibition is done by either binding to 50S or 30S ribosomal subunit.	Gentamycin and streptomycin	(Varun, 2012; Biomikazi, 2008; Etebu & Arikekpar, 2016).
Inhibition of nucleic acid synthesis: – The active compound of this type of antibiotics works by inhibiting nucleic acid biosynthesis, mRNA synthesis, and DNA gyrase as well as topoisomerase.	Ciprofloxacin, norfloxacin, and rifampicin/rifampin	(Biomikazi, 2008; Etebu & Arikekpar, 2016).
Inhibition of key metabolic pathways: – The active compound of antibiotic inhibits the biosynthesis of mycolic acid. – Some antibiotics of this type work by interfering cell respiration and ubiquinone biosynthesis.	Sulphonamides and trimethoprim	(Varun, 2012; Biomikazi, 2008).