

ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF MRSA ISOLATED FROM
HEALTHY INDIVIDUALS IN NILAI, NEGERI SEMBILAN, MALAYSIA

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most antibiotic-resistant pathogens and is present worldwide, causing significant health problems and increase in mortality and morbidity. MRSA have acquired resistance to different classes of antibiotics, making MRSA infections difficult to treat. Hence, it is important to carry out in vitro antibiotic susceptibility testing to obtain the antibiotic sensitivity patterns of MRSA thus utilizing the results as a guidance for antibiotic therapy of MRSA infections and formulating antibiotic policy as well as appropriate control measures. This study was aimed at determining the antibiotic susceptibility patterns of MRSA isolated from samples that were collected from healthy individuals in Nilai, Negeri Sembilan. Culture samples were inoculated on the nutrient agar plates to obtain single colonies, followed by sub-culturing and series of confirmatory tests such as Gram staining, catalase test, Mannitol Salt Agar, oxacillin disk-diffusion test and Brilliance MRSA 2 agar to obtain pure MRSA isolates. A total of 20 MRSA strains from 23 pure culture isolates were obtained and subjected to antibiotic susceptibility test on Mueller Hinton agar using Kirby-Bauer disk diffusion technique. All 20 MRSA isolates (100%) showed resistance towards oxacillin, penicillin G and vancomycin; 85% of them were resistant to erythromycin; 80% showed resistance to clindamycin, teicoplanin and linezolid; 65% of them were resistant to nalidixic acid while only 5% of them showed resistance to azithromycin and cefazolin respectively. All isolates (100%) were susceptible to ciprofloxacin. Five different antibiogram patterns were obtained from these 20 MRSA isolates with resistance towards 8 out of 11 types of antibiotics used in this study. This finding which is a surveillance control method of antibiotic resistant crisis is important as the results could provide a basis for the antibiotic therapy of MRSA infections in Malaysia.

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LIST OF ABBREVIATIONS

AZM	Azithromycin
°C	Degree celcius (temperature)
CA-MRSA	community-acquired MRSA
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
DA	Clindamycin
E	Erythromycin
g	gram
HA-MRSA	hospital acquired MRSA
HGT	Horizontal gene transfer
KZ	Cefazolin
L	litre
LZD	Linezolid
mL	millilitre
mm	millimetre
mrMRSA	multi-resistant MRSA
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSA	Mannitol Salt Agar
MSSA	Methicillin-susceptible <i>Staphylococcus aureus</i>
NA	Nalidixic acid
ORSA	Oxacillin-resistant <i>Staphylococcus aureus</i>
OX	Oxacillin
P	Penicillin G
TEC	Teicoplanin
VA	Vancomycin

CHAPTER 1

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with health care and community-acquired infections in Malaysia and all around the world. These infections include post-operative toxic shock syndrome, wound sepsis, osteomyelitis and septicemia which result in significant morbidity and mortality (Shittu & Lin, 2006). According to a study, 10 out of 1000 admissions in a tertiary hospital in Malaysia were reported to be infected by MRSA (Lim, Hanifah, Mohd Yusof, Ito, & Thong, 2013).

Before the existence of antibiotics in medical field, *S. aureus* infections were always incurable. Methicillin was the first beta-lactamase resistant penicillin produced in 1960. However, the first MRSA case was confirmed in 1961 which was followed by MRSA outbreaks in Europe thereafter. Oxacillin then became the alternative to methicillin to treat MRSA infections resulted in term “Oxacillin-resistant *Staphylococcus aureus*” (ORSA) that was resistant to various antibiotics (Al-Zoubi, Al-Tayyar, Hussein, Jabali, & Khudairat, 2015).

The variability of MRSA with varied antibiotic resistance patterns among countries as well as various clonal types are the main reasons that led to the development of multi-drug resistant strains of MRSA (Shittu & Lin, 2006). The unique mechanism that these microorganisms harbour, confer protection from beta-lactam antibiotics, thereby making these MRSA infections difficult and costly to treat (Al-Zoubi et al., 2015).

Nowadays, the last option to treat MRSA infections is vancomycin which has been accepted throughout the world. Lately, there was a report about the emergence of clinical isolates of vancomycin-resistant *Staphylococcus aureus* (VRSA) and *S.aureus* isolates that are resistant to an extensive range of antibiotics which are structurally unrelated. This makes MRSA more dangerous than before, not only in

hospital environment but also in the community (Al-Zoubi et al., 2015). In order to treat MRSA infections effectively, it is utmost important to carry out antibiotic susceptibility test in vitro to predict in vivo success or failure of antibiotic treatment. The results can be served as a guide to select the most appropriate antibiotic to treat patients with MRSA-related infections.

Therefore, the objective of this study was initiated to determine the antibiotic susceptibility pattern of MRSA strains isolated from healthy individuals in Nilai, Negeri Sembilan, Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTIC RESISTANCE

Antibiotics have been used in the treatment of bacterial infections for more than 70 years (CDC, 2016). Antibiotics have played an important role in greatly reducing mortality since 1940's (Ventola, 2015). However, the extended and improper usage of antibiotics had resulted in adaptation and development of resistance in pathogenic bacteria which have become less susceptible to antibiotics. Scientists named this phenomena 'antibiotic resistance', which is the ability of bacteria to overcome the effects of antimicrobial agents. This renders the original drugs used to treat an infectious disease, ineffective or less effective, resulting in persistent infectious diseases. In the United States alone, an average of 2 millions people infected by antibiotic-resistant bacteria were reported (CDC, 2016). This resulted in at least 23,000 deaths each year (CDC, 2016). The difficulties in treating antibiotic-resistant bacteria caused increasing mortalities as there is no specific cure for the infections.

2.2 WHAT CAUSES INCREASED INCIDENCE IN ANTIBIOTIC RESISTANCE

Antibiotics are one of the most commonly and universally prescribed drugs to treat human diseases caused by bacterial pathogens. However, the indiscriminate use of antibiotics is one of the major factors that has led to the increased incidence of antibiotic resistance. According to several studies, 30% to 50% of antibiotic therapy cases are incorrect in terms of the choice of agent, duration, or treatment indication (Ventola, 2015). Furthermore, 30% to 60% of the antibiotics used in the intensive care units have also been found to be inappropriate thus unnecessary (Luyt, Bréchet, Trouillet, & Chastre, 2014).

In addition, increased mutagenesis and horizontal gene transfer (HGT) clearly drives and promotes the evolution of antibiotic resistance (Read & Woods, 2014). The

antibiotic resistance genes can emerge spontaneously through mutation and can be easily spread and transferred among different species of bacteria through HGT (Read & Woods, 2014).

One of the contributing factors could be that antibiotic usage is not strictly regulated and easily accessible over the counter without prescription in some countries. This causes the drug to be plentiful and cheaper than in countries where antibiotics are regulated hence promotes overuse (Michael, Dominey-Howes, & Labbate, 2014). Overuse of antibiotics has also contributed to the increase in infections due to antibiotic resistant bacteria. Antibiotics should solely be used to kill the bacteria which the human immunity is unable to fight off instead they have been used widely even against weak bacteria. Overuse of antibiotics happens in agricultural sector as well. In the U.S., 80% of antibiotics sold are used in animals to prevent infection and enhance growth of the animals (Bartlett, Gilbert, & Spellberg, 2013). As a result, the surviving bacteria develop resistance and are transmitted to humans through the food supply (Bartlett, Gilbert, & Spellberg, 2013). The resistant bacteria strains which can also be spread either from person to person or from a non-human source to human promoting the growth of antibiotic resistance.

2.3 METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

2.3.1 Why MRSA is such a problem?

Methicillin-resistant *Staphylococcus aureus*, commonly abbreviated as MRSA, is a strain of *S. aureus* bacteria which is resistant to large group of beta-lactam antibiotics because of the acquisition of an enzyme, transpeptidase (PBP2a), that is encoded by *mecA* gene. Transpeptidase is involved in the synthesis of the bacterial cell wall that has a low affinity to the beta-lactam class of antibiotics. MRSA is the most commonly identified antibiotic-resistant pathogen and is present worldwide including in America, Europe, Middle East, East Asia and North Africa, causing significant health problems (Ippolito, Leone, Lauria, Nicastrì, & Wenzel, 2010). MRSA has caused an increase in morbidity and mortality particularly in immuno-compromised people, causing elevated costs in health care. There was an estimation of 1650 blood stream infections caused by MRSA in Australia, with 500 deaths in Australia and 11, 000

deaths in the United States caused by MRSA annually. MRSA can cause severe soft tissue and skin infections, surgical wound infections, abscess in deep organs and also implicated in toxin-mediated diseases. In addition, the most commonly reported MRSA-related conditions include endocarditis (19%), septic shock (56%), bacteremia (10%), cellulitis (6%) as well as pneumonia (32%) which are all life-threatening. Figure 2.1 indicates the percentage of prevalence of MRSA in many countries which include the UK (44%), Japan (74%), the US (55%), Hong Kong (80%) and Singapore (63%) while fairly low prevalence in Netherlands (<1%) as well as in Canada (2.3%).

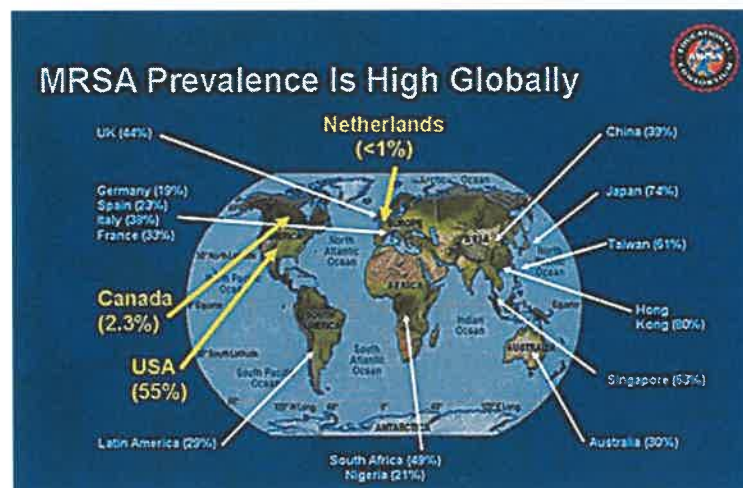


Figure 2.1 Prevalence of MRSA in several countries (adapted from Medscape, 2016).

2.3.2 MRSA in patients and carriers

Most MRSA infections occur in patients who have been hospitalized or are in other health care settings like dialysis centres and nursing homes. These can be categorized as health care-associated MRSA or hospital acquired MRSA (HA-MRSA). According to studies, HA-MRSA has a high incidence rate in North Africa, Australia, East Asia and the Middle East. The compromised immune system of in-patients is the main risk factor for HA-MRSA infections while the additional risk factors include the length of stay in health care settings and hospitals, the rate of exposure to antibiotics and people who are infected by MRSA. People who most at risk are often the elderly, burn victims, infants, chronically ill patients, those with AIDS, cancer and diabetic patients as well as organs transplant recipients.