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ANTIBIOGRAM PATTERNS OF STAPHYLOCOCCAL SPECIES ISOLATED FROM HEALTHY INDIVIDUALS IN NILAI, NEGERI SEMBILAN, MALAYSIA

HIMASHI IMANDA GURUDENIYA, GEETHA SUBRAMANIAM*, ERIC CHUAH HAN LIM, OOI HOOI YI AND LALITA AMBIGAI SIVASAMUGHAM

Faculty of Health and Life Sciences, INTI International University,
Persiaran Perdana BBN, 71800 Nilai, Negeri Sembilan, Malaysia

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Abstract– Antibiotic resistance is an alarming phenomenon worldwide, challenging the effectiveness of antibiotics which are used to treat infectious diseases. In this study, pure cultures of forty skin and nasal samples from healthy individuals in Nilai, Negeri Sembilan, were screened. 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, as well as cefoxitin disk-diffusion test to obtain pure MRSA and MRSE isolates. A total of 13 MRSA strains and one MRSE strain were obtained and subjected to antibiotic susceptibility testing on Mueller Hinton agar using Kirby-Bauer disk diffusion technique. All cefoxitin-resistant isolates were resistant to penicillin G, erythromycin, linezolid, and vancomycin; 78% of them were resistant to teicoplanin, clindamycin, quinupristin/dalfopristin; 56% showed resistance to nalidixic acid and cefazolin while 33% of them showed resistance to cefuroxime. All isolates (100%) were susceptible to doxycycline, chloramphenicol, and ciprofloxacin. Eight different antibiogram patterns were obtained from these 14 isolates with resistance towards 11 out of 16 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 an indication of the resistance patterns circulating among healthy individuals, and could contribute towards a more definitive antibiotic therapy of staphylococcal species infections in Malaysia.

INTRODUCTION

The discovery of antibiotics in the 1950s (Bérdy, 2012) is unquestionably one of the most significant discoveries that have benefited mankind. These infectious microorganisms when faced with the challenge of survival against antibiotics, have developed the ability to overcome the effects of antibiotics through the process called natural selection leading to antibiotic resistance (Kleinman, 2016). Furthermore, the effect of antibiotics decreases against these surviving bacteria which is the basis of antibiotic resistance. This is due to the changes in a microorganism’s structure which could be the result of overuse and misuse of antibiotics. The infections caused by these “superbugs” such as methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant Staphylococcus epidermidis, vancomycin-resistant Staphylococcus aureus (VRSA) and vancomycin-resistant Enterococcus (VRE) are generally difficult to treat.

The authorities responsible for detecting and responding to antibiotic-resistance threats are limited even though the emergence of antibiotic-resistance is an alarming healthcare problem throughout the world. Staphylococcus aureus is a commonly isolated pathogen in both nosocomial and community-acquired infections (Appelbaum, 2007). These bacteria normally exist as commensals and are found in the anterior nares, axilla and skin of health individuals. S. aureus has the capability to develop resistance rapidly to a wide range of antibiotics. The emergence of Methicillin-resistant Staphylococcus aureus (MRSA) has contributed to the growing threat of antibiotic resistance globally, and limits the therapeutic options to treating infections caused by S. aureus. Knowing this is important as healthy individuals can be the carriers of MRSA

*Corresponding author’s email: geetha.subramaniam@newinti.edu.my
which could lead to the spread of these pathogens. Some of the research on the isolation of MRSA from the nasal cavity of healthy individuals has been done in the United States (Rath 

et al., 2015) and Nigeria (Ugwu, et al., 2016). However, there are very few studies on the isolation of antibiotic-resistant bacteria from axilla of healthy individuals. The objective of this study is to assess the prevalence of MRSA and MRSE colonization among healthy individuals in Nilai, Negeri Sembilan, Malaysia, and to determine the antibiogram patterns of these pathogens.

MATERIALS AND METHODS

Screening for nasal and axilla carriage

Forty samples were obtained from nasal and axilla sites of twenty-eight healthy volunteers in Nilai, Negeri Sembilan, Malaysia. Specimens were obtained using sterile cotton swabs moistened with sterile water. The swabs were inoculated into Nutrient broth and incubated overnight at 37°C with aeration. The resultant cultures were sub-cultured onto Nutrient agar and incubated at 37°C for 24 hours.

Biochemical identification

Pure cultures were subjected to standard biochemical tests including Gram staining, catalase test, and sub-culturing onto Mannitol Salt agar (MSA). Only cultures that were gram positive cocci with the Gram staining reaction were subjected to the other two tests. All cefoxitin resistant isolates that grew as yellow colonies on MSA were further tested using MRSA Select agar (BioRad).

Antimicrobial susceptibility testing

Staphylococcus aureus and Staphylococcus epidermidis isolates were subjected to antibiotic susceptibility testing using the Kirby Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI). The antibiotics used in this study included oxacillin (1 μg), teicoplanin (30 μg), cefuroxime (30 μg), clindamycin (2 μg), azithromycin (15 μg), doxycycline (30 μg), chloramphenicol (10 μg), nalidixic acid (30 μg), quinupristin/dalfopristin (15 μg), vancomycin (30 μg), ciprofloxacin (10 μg), ceftriaxone (30 μg), tetracycline (30 μg), erythromycin (15 μg), cefazolin (30 μg), penicillin G (10 iU) and linezolid (30 μg) (CLSI, 2017).

RESULTS

Out of the 28 healthy individuals analysed, forty samples were obtained, out of which 36 (90%) were Gram positive cocci and the other 10% were gram positive rods. On further investigation, these gram positive cocci were determined to be S. aureus (85%) and S. epidermidis (5%). An equal number of S. aureus (17 isolates each) was obtained from both nasal and axilla samples.

The differentiation of resistant strains and susceptible strains was carried out using the cefoxitin disc diffusion assay. MRSA isolates were confirmed using the Brilliance MRSA agar. A total of nine MRSA (6 strains from nasal cavity; 3 strains from axilla) and one MRSE (from axilla) were detected. The maximum MRSA isolates were found in the nasal cavity compared to the axilla. The only MRSE strain was isolated from the axilla of an individual who harboured an MRSA isolate in his nasal cavity.

The interpretive categories and zone diameter breakpoints of five antibiotics cefuroxime, nalidixic acid, vancomycin, ceftriaxone and cefazolin could not be determined from the CLSI document. In this study, it could be described as susceptible if these MRSA and MRSE isolates showed zones of inhibition of more than 24 mm around these five antibiotic discs. Table 2 shows that all the ten MRSA and MRSE isolates that were isolated from healthy individuals in Nilai, Negeri Sembilan, Malaysia were resistant to cefoxitin, penicillin G, erythromycin, linezolid, and vancomycin but were susceptible to doxycycline, chloramphenicol, ciprofloxacin (Table 2). The nine MRSA isolates from healthy individuals also showed resistance to other antibiotics including teicoplanin (78%), clindamycin (78%), quinupristin/dalfopristin (78%), cefazolin (56%), nalidixic acid (56%), cefuroxime (33%) and ceftriaxone (22%).

Table 1. Prevalence of MRSA and MRSE on isolated samples

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Percentage</th>
<th>MRSA</th>
<th>MSSA</th>
<th>MRSE</th>
<th>MSSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal cavity</td>
<td>20 (44.4%)</td>
<td>3 (15%)</td>
<td>14 (70%)</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Axilla</td>
<td>25 (55.6%)</td>
<td>4 (16%)</td>
<td>19 (76%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>
Table 3 shows nine different antibiogram patterns out of which 8 of them (A, B, C, D, E, F, G, and H) were antibiograms of the nine MRSA isolates whereas I was the antibiogram pattern of the single MRSE isolated in this study. All the MRSA isolates were resistant to at least six of the antibiotics tested.

**DISCUSSION**

The increasing prevalence of MRSA in the community (CA-MRSA) has resulted in the need to screen for this pathogen among healthy individuals. In previous studies, the MRSA screening was performed on the nasal cavities of individuals (Ouidri, 2017, Onanuga et al., 2011; Bishoff et al., 2004). However, the importance of screening other sites including the axilla have become increasingly important for the detection of MRSA in healthy carriers (Lee et al., 2015; French, 2009).

Out of the 28 individuals screened, we obtained forty isolates from the axilla and/or nasal cavity of the volunteers. The frequency of *S. aureus* carriage was 85% compared to 5% of *S. epidermidis* which showed a prevalence of *S. aureus* and is in accordance with previous studies studies (Otto, 2010). In addition, an equal number of *S. aureus* were isolated from the nasal cavity as well as the axilla, which would have been missed had the study been confined to obtaining samples solely from the nares.

All the MRSA strains used in this study were resistant to antibiotic cefoxitin, penicillin G, erythromycin, linezolid and vancomycin. Vancomycin is bactericidal, glycopeptide antibiotic which inhibits the bacteria growth by disrupting the growth of bacterial cell wall. This glycopeptide antibiotic acts with peptidoglycan D-Ala-D-Ala, the

### Table 3. Antibiogram patterns of MRSA isolated from healthy individuals.

<table>
<thead>
<tr>
<th>List</th>
<th>Antibiogram patterns</th>
<th>Number of isolates</th>
<th>Percentage (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Axilla</td>
<td>Nasal</td>
</tr>
<tr>
<td>A</td>
<td>FOX(^8), VA(^6), E(^5), P(^5), LZD(^5), TEC(^5), DA(^8), QD(^8)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>FOX(^5), CXA(^6), NA(^6), VA(^6), CRO(^5), E(^5), KZ(^5), P(^6), LZD(^5)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>FOX(^3), VA(^6), E(^5), KZ(^5), P(^6), LZD(^5), TEC(^5), DA(^5), OD(^8)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>FOX(^3), CXA(^6), NA(^6), VA(^6), E(^5), KZ(^6), P(^6), LZD(^6), TEC(^5), DA(^5), QD(^8)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>FOX(^5), NA(^5), VA(^5), E(^5), KZ(^5), P(^6), LZD(^5), TEC(^5), DA(^5), QD(^8)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>FOX(^3), NA(^5), VA(^5), E(^5), KZ(^5), P(^6), LZD(^5)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>FOX(^5), CXA(^6), NA(^6), VA(^6), CRO(^5), E(^5), KZ(^5), P(^6), LZD(^5), TEC(^5), DA(^5), QD(^8)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>FOX(^3), VA(^6), E(^5), KZ(^5), P(^6), LZD(^5), TEC(^5), DA(^5), QD(^8)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>FOX(^5), NA(^5), VA(^5), CRO(^5), E(^5), P(^6), LZD(^5), TEC(^5), DA(^5), OD(^8)</td>
<td>MRSE</td>
<td>-</td>
</tr>
</tbody>
</table>

MRSE
peptide stem terminus of the side chain of peptidoglycan precursors, thereby interfering cell wall formation in Gram-positive bacteria (Sujatha, & Praharaj, 2012). Vancomycin was pre-defined to have no effect on all MRSA and MRSE isolates used in this study as they had zones of inhibition of less than 24 mm. It also showed substantial reduction of susceptibility against MRSA strains compared to the research done in Hospital UniversitiSains Malaysia (HUSM) during 2002-2007 when vancomycin had 100% susceptibility towards MRSA isolates (Al-Talib et al., 2010). However, CLSI recommended that in order to confirm the susceptibility of these S. aureus strains to vancomycin, minimum inhibitory concentration (MIC) tests need to be done as vancomycin disk diffusion assay may produce erratic results and cannot differentiate vancomycin-susceptible S. aureus from vancomycin-resistant S. aureus (Holmes et al., 2012). 85% of MRSA isolates showed resistance towards teicoplanin proved that this glycopeptide antibiotic agent might not be an effective agent to cure MRSA infections in Malaysia as it did show relatively good susceptibility against MSSA strain in this study.

All the MRSA and MRSE isolates in this study showed resistance towards erythromycin and penicillin G. It is not surprising that these antibiotics was practically ineffective towards MRSA and MRSE in this finding as several studies in Malaysia had shown that the resistance rate of MRSA isolates towards erythromycin and â-lactam antibiotics reached up to 98% during 2002-2007 and 100% in the recent years (Al-Talib et al., 2015). In addition, â-lactam antibiotics are generally not prescribed to treat MRSA infections as the mecA gene encodes for penicillin binding proteins (PBPs) which result in an overall lowered binding affinity for these antibiotics, resulting in resistance to all â-lactams (Fuda et al., 2004).

Linezolid was another antibiotic which the MRSA isolates exhibited 100% resistance to, which is not in accordance to the findings of Watkins et al. (2012). Based on their study, the Infectious Diseases Society of America (IDSA) recommended linezolid as an initial of alternative therapy for pneumonia, osteomyelitis, septic arthritis and meningitis caused by MRSA. These conflicting results could be due to the changing patterns in MRSA resistance and more importantly the type of antibiotics prescribed to treat MRSA infections in Malaysia which would affect the development of antibiotic resistance towards Linezolid.

Clindamycin and Quinupristin/Dalfopristin which showed good activity against MRSA isolates in previous findings had resistance rate as high as 85% in MRSA isolates in this study (Adhikari et al., 2017; Drew et al., 2000). This is a worrying phenomenon as Quinupristin/Dalfopristin is a combination of two antibiotics that is used worldwide to treat MRSA infections (Gurk-Turner, 2000). The possible root causes of the increase in the resistance rate to this antibiotic could be due to the overuse and misuse of these particular antibiotics in the recent years, wide spreading of antibiotic resistance genes among bacteria. Nonetheless, penicillin G, erythromycin, linezolid, and vancomycin still showed a relatively good effect against MSSA and MSSE isolate in this study.

The interpretive criteria and zone of inhibition breakpoints of nalidixic acid could not be determined from CLSI document but it was pre-defined to exhibit less effect against MRSA strains by having 42% resistance rate in MRSA strains as nalidixic acid was known to show effectiveness primarily against most type of Gram-negative bacteria (Campos et al., 2006) but with minor effect against gram-positive bacteria. This could explain why Nalidixic acid was less effective against MRSA in this study.

All the resistant staphylococcal isolates exhibited 100% susceptibility towards doxycycline, chloramphenicol and ciprofloxacin in this study. A study by Bhamri and Kim (2009) have demonstrated the effectiveness of doxycycline in the treatment of cutaneous community-acquired MRSA (CA-MRSA). Doxycycline is a bacteriostatic antibiotic that belongs to tetracycline class of antibiotics that inhibit the growth of bacteria by inhibiting protein synthesis (Arikekar & Etebu, 2016). Hence, this antibiotic can be considered as a treatment option for MRSA infections in Malaysia.

Antibiogram patterns of MRSA isolates showed that all of the isolates in this study exhibited resistance to at least six of the antibiotics tested while some showed resistance toward eleven out of sixteen antibiotics. This could be due to the inclusion of antibiotics such as penicillin G, the beta-lactams (cefoxitin, cephazolin, cefuroxime and ceftriaxone) are commonly prescribed antibiotics thereby are ineffective towards the MRSA isolates in this study. It was interesting to note that although beta-lactams are no longer used to treat MRSA, however, the isolates in this study were not completely resistant to the three beta-lactam...
antibiotics tested.

The varying antibiograms among the MRSA isolates have been demonstrated in other studies and could be attributed to indiscriminate use of antibiotics and self-medication (Ike et al., 2016; Onanuga and Temedie, 2011).

This study shows that MRSA isolates are becoming more resistant against most antibiotics tested, indicating the challenge in treating diseases caused by these pathogens. Vancomycin, clindamycin, and linezolid which were known to show relatively good activity against MRSA strains in previous studies, showed reduced effects against these MRSA isolates. Persistent increase of multi-drug resistant bacteria constitutes a growing problem in the medical field as this could narrow down the choices of antibiotic therapy and render MRSA infections untreatable in future. Hence, it is extremely important to know the sensitivity patterns of MRSA isolates to ensure the effectiveness of antibiotic treatments in vivo and utilize the results obtained to develop more restrictive antibiotic policy and implement appropriate control measures to promote rational use of antibiotics in Malaysia.

CONCLUSION

Detection of antibiotic susceptibility patterns in MRSA isolates are important to help determine the most effective antibiotics for the treatment of these pathogens. More studies should be carried out on a larger number of healthy volunteers in order to obtain a more accurate representation of the antibiograms of MRSA isolates that are circulating in Malaysia. As MRSA has constituted a major problem in the medical field, policies on prudent use of antibiotics should be implemented to prevent increasing of antibiotic-resistant cases, as well as proper hygiene, should always be practiced based on a daily basis in order to reduce transmission of MRSA strains among individuals.

ACKNOWLEDGEMENT

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