ISOLATION OF ANTIBIOTIC RESISTANT BACTERIA FROM HEALTHY INDIVIDUALS IN NILAI, NEGERI SEMBILAN

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Abstract- The emergence of antibiotic resistant bacteria is a major problem worldwide. Some of the common cause for the spread of antibiotic resistant bacteria include the improper use of antibiotics and poor hygiene practices. Healthy individuals can be carriers of antibiotic resistant bacteria. One of such antibiotic resistant bacteria is the methicillin resistant S. aureus (MRSA), a common skin microflora. These individuals can transmit antibiotic resistant bacteria to others causing outbreaks of diseases. Thus, the aim of this study was to isolate antibiotic resistant bacteria from the nasal cavity and axilla of 28 healthy individuals in Nilai, Negeri Sembilan. The pure culture of the respective isolates were grown on Mannitol Salt Agar (MSA) and subjected to catalase test for identification. Antibiotic susceptibility testing was then conducted to determine the susceptibility of bacteria towards the tested antibiotics using the disc diffusion method. Out of the 40 isolates, 36 isolates showed resistance based on the BSAC guidelines, version 6.0, 2016.The bacteria that were Gram-positive cocci with cluster-like cell arrangement, catalase positive, and resistant to oxacillin were subjected to inoculation onto Brilliance MRSA 2 agar. Twenty-four MRSA were isolated while 12 MRSE might have been isolated. Although further studies are required, this preliminary finding can be used to create an awareness among the public to prevent the spread of antibiotic resistant bacteria.

Keywords- Antibiotic Resistant Bacteria, Carriers, Disc Diffusion, Oxacillin, Methicillin Resistant S. aureus(MRSA), Methicillin Resistant S. epidermidis(MRSE)

I. INTRODUCTION

Antibiotic resistant bacteria are bacteria that have acquired resistance to overcome the effects of antibiotics. Mutation and the misuse of antibiotics are some of the top reasons for the increase the antibiotic resistant bacteria (World Health Organization, 2015). Antibiotic-resistant traits can spread due to poor hygiene practices such as lack of hand washing (Collignon, Athukorala, Senanayake, & Khan, 2015).
The development and spread of resistant genes among the healthcare institutions and society has limited the efficiency of treating common bacterial infections, resulting in a higher treatment failure rate, in serious case, culminating in death (World Health Organization, 2015). For example, patients infected with MRSA are estimated to have 64% higher death rate than patients with non-resistant form of infection (World Health Organization, 2015). In 2015, CDC reported that at least two million people were infected with antibiotic resistant bacteria and at least 23 thousand people died from the infection in USA annually (Centers for Diseases Control and Preventions, 2015).
Methicillin-resistant Staphylococcus aureus (MRSA) is one of the deadly types of antibiotic-resistant bacteria found on the skin. This bacterium causes a range of skin diseases to sepsis and bloodstream infection. MRSA is resistant to methicillin and beta-lactam antibiotics. This makes it hard to treat MRSA infections.
Healthy individuals can be carriers of MRSA as well as Methicillin-resistantStaphylococcus epidermidis (MRSE). These carriers are usually unaware of the existence of these bacteria because MRSA and MRSE normally do not cause infections in healthy individuals (Minnesota Department of Health, 2014). These bacteria are commonly isolated from the skin such as axilla and the nasal cavity(Rath, Christmas, Picardo, & Westbay, 2015). Hence, the resistant bacteria can easily be transmitted from the carriers to another. The aim of this study was to isolate antibiotic resistant bacteria from the nasal cavity and axilla of healthy individuals in Nilai using the disc diffusion assay.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

A total of 28 healthy individuals participated in this study from which 8 of them provided both their nasal cavity and axilla samples giving a total of 20 samples from the nasal cavity and axilla respectively. Consent and survey forms were distributed before the start of the study.Sterile cotton swab was used to collect the samples and was placed into nutrient broth (Oxoid) for growth. The respective overnight culture mediawere then inoculated onto nutrient agar (Oxoid). Following that, the single colonies that were morphologically different were sub-cultured again and subjected to gram staining and catalase test. In addition, the pure cultures were grown onMannitol Salt agar (Oxoid). Then, the overnight pure cultures of isolates were diluted to match with the 0.5 McFarland standard turbidity. This gives an estimated concentration of 1.5×10^8 colony forming units / mL (CFU/mL) (Hardy Diagnostics, 2016), a sufficient concentration to lawn onto the Mueller-Hinton agar (Oxoid).Then, antibiotics discs of cephazolin, oxacillin, and clindamycin (Thermo Scientific) were placed onto the inoculated-Mueller-Hinton agar. Empty sterile antibiotic assay discs containing only

sterile water served as negative control. The diameter (mm) of the zone of inhibition was measured by using metric ruler after the overnight incubation and compared to those in British Society for Antimicrobial Chemotherapy (BSAC) guidelines, version 6.0, 2016 (Eucast, 2016).

III. RESULTS AND DISCUSSION

3.1. Gram-Staining and Catalase Reactions

All of the 40 isolates were Gram positive and catalase positive bacteria. Thirty-six isolates showed cluster-like cell arrangement as shown in Fig. 1a indicating that they were either S. aureus or S. epidermidis, the common microflora of the skin and the nasal cavity(Acharya, 2013). The other four were rod-shaped bacteria as shown in Fig.1b. This could be P. acnes.

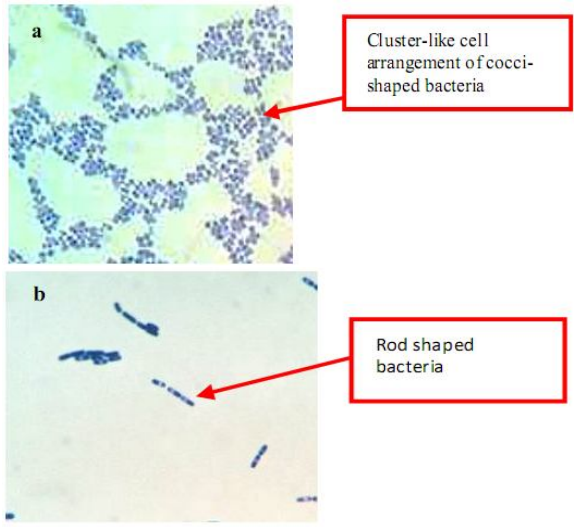

Fig.1. (a & b) Gram stained isolates viewed at 1000x (oil immersion). All cells were Gram positive (violet in colour).

3.2. Growth on Mannitol Salt Agar (MSA)

Fig.2 shows that out of the 40 isolates, 24 (60.00%) isolates were mannitol fermenters as they grew and produced acid turning the red medium to yellow, whereas another 15 isolates (37.50%) produced pink colonies and changed the media from red to pink. One isolate (2.50%) did not grow on the media indicating that they could be P. acnes, a Gram positive, catalase positive, skin bacteria that are not tolerant to the 7.5 % of NaCl found in MSA.

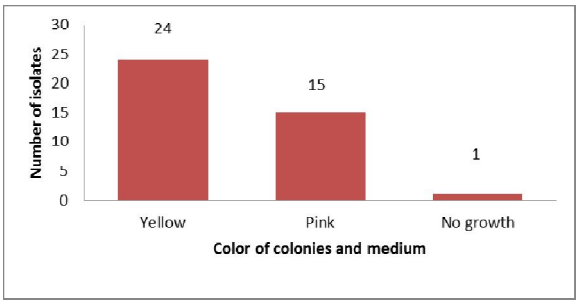

Fig.2 Number of isolates and the colour changes on MSA.

3.3. Antibiotic Susceptibility Testing

Four (11.11%) out of the 36 isolates with cluster-cell like arrangement were resistant to only oxacillin (Fig.3). Twenty-seven of the isolates (75.00%) were resistant to oxacillin and clindamycin as no zone of inhibition was detected surrounding these discs (Fig.3). Four isolates (11.11%) were resistant to all 3 antibiotic discs (Fig.3) as no zone of inhibition were observed (Fig.4).

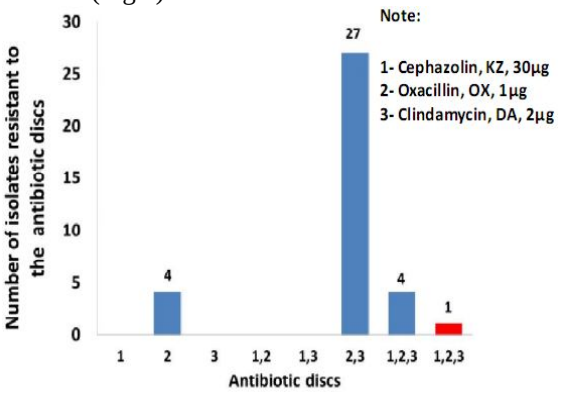

Fig.3. Number of bacteria resistant to the antibiotics tested

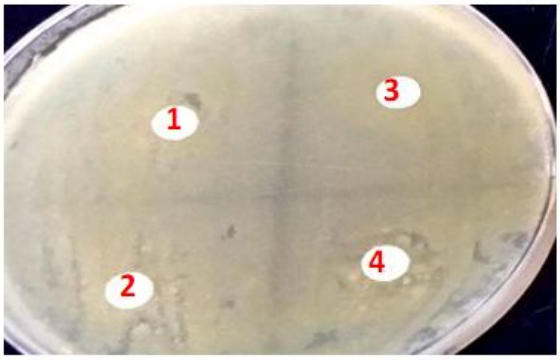

Fig.4. The Antibiotic susceptibility testing indicating the complete resistance of an isolate to all 3 antibiotics. Discs 1-3 denote antibiotics discs of cephazolin, oxacillin and clindamycin. Disc 4 is negative control.

3.4. Growth on Brilliance MRSA 2 agar

Isolates (n=36) that were resistant to oxacillin were streaked onto Brilliance MRSA 2 agar as resistance to oxacillin strongly indicates the likely presence of MRSA (Jorgensen& Ferraro, 2009). 24 isolates out of 36 isolates (Fig.5) produced blue colonies on the Brilliance MRSA 2 agar (Fig.6). Thus, these 24 isolates are confirmed to be MRSA. The 12 isolates from the 36 resistant isolates that did not grow on the Brilliance MRSA 2 agar (Fig.5) are likely to be MRSE.However, further tests are reuired to conclude this assumption.The antibiotic resistance of 4 isolates (rod-shaped gram positive bacteria) could not be determined using the zone of inhibition as indicated in the BSAC guidelines, version 6.02. Further analysis must be conducted to confirm their resistance. This study also revealed that 27 out of 28 healthy individuals (96%) who participated in this study were carriers of antibiotic resistant bacteria such as MRSA. The percentage of the carriers is likely to be higher than 50% in a larger sample size.

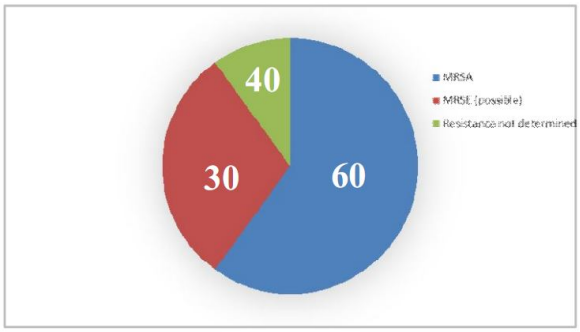

Fig.5.The types of antibiotic resistant bacteria in percentage. MRSA comprised the largest group of the total resistant isolates (60%).

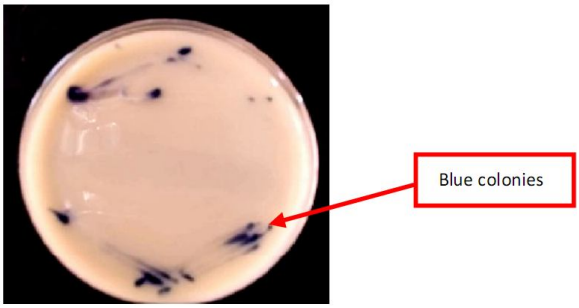

Fig.6.Growth of an isolate on Brilliance MRSA 2 agar. The blue colonies indicate positive for MRSA.

CONCLUSIONS

The antibiotic resistance of isolates from 28 healthy individuals in Nilai revealed:

1. 24 MRSA isolates whereas 12 likely to be MRSE (unconfirmed).
2. High number ofhealthy individuals are carriers of antibiotic resistant bacteria such as MRSA. Although only 28 participants provided their samples in this study, 96% of them are carriers of antibiotic resistant bacteria such as MRSA.
3. Although preliminary, the findings from this study can be used to create awareness to prevent the possible outbreaks of diseases caused by antibiotic resistant bacteria.

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