

Antibiogram Patterns of Staphylococci isolated from the Nasal and Axilla of Healthy Individuals in Nilai

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Abstract

An antibiogram study suggests the prescription patterns of antibiotics in a location through which, the prescription of certain antibiotics can be controlled to minimize the rise of antibiotic resistant bacteria. *S. aureus*, *S. epidermidis* and *P. acnes* are common bacteria of the skin, nasal and axillae. However, many of these bacteria are gaining resistance towards antibiotics. Thus, the objective of this study was to discover the antibiogram of the common microflora isolated from the axillae and nasal cavity of 25 healthy individuals aged 18-50 in Nilai. The bacterial samples were aseptically obtained, cultured in nutrient broth and streaked onto nutrient agar to obtain pure cultures. The pure cultures were subjected to Gram-staining, catalase test, and grown on mannitol salt agar. The antibiotic susceptibility testing were performed to characterize the susceptibility of the bacterial isolates towards amoxicillin, cefazolin, erythromycin, cefoxitin, trimetoprima/ sulfametoxazol, tetracycline and gentamycin on Mueller Hinton agar. Pure cultures of 78 axillae and nasal samples were screened in this study. 65.38% of the bacterial isolates were gram positive bacteria comprising of *S. aureus*, *S. epidermidis* and possibly *Propionibacterium* and *Corynebacterium*. Out of the 24 isolates of *S. aureus* and *S. epidermidis*, 18.42% were found to be sensitive to the tested antibiotics whereas, 7.89 % showed resistance to more than one antibiotic. 28% of the staphylococci showed resistance towards amoxicillin. Although amoxicillin-resistance among gram positive staphylococci has been reported previously, it is also crucial to note that the transfer of resistant bacteria is likely to increase with imprudent prescriptions of antibiotics and improper hygiene practices of individuals. Although the sample size used in this were rather small, the findings in this study could be used for a definitive antibiotic therapy among gram positive infections in Malaysia.

Keywords

Antibiogram, antibiotic, sensitive, resistant

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Introduction

Staphylococcus aureus and *Staphylococcus epidermidis* are common skin microflorae (Davis, 1996). However, they can be a threat when there is an open wound. The common antibiotics used to treat diseases caused by these bacteria include amoxicillin, cefazolin, erythromycin, ceftiofur, trimethoprim/sulfamethoxazole, tetracycline and gentamycin. However, studies have shown that these microflorae are gaining resistance towards antibiotics (Himashi et al. 2018).

Antibiotic resistance is a worldwide crisis. This has caused a shortage of effective antibiotics in treating diseases caused by bacteria (Ventola, 2015). Some of the reasons for the increase of resistance include overuse, and inappropriate prescriptions of antibiotics as well as lack of drug development by pharmaceutical companies. Some countries also allow over-the-counter purchase of antibiotics without a prescription (Nature, 2013). These collective conditions have led to natural selection causing the increase and spread of resistant bacteria.

One of the global efforts to combat antibiotic resistance is through monitoring antibiogram patterns (National Committee for Clinical Laboratory Standards, & Fridkin, 2000). An antibiogram is an accumulation of localized data regarding the susceptibility of specific bacterial species to specific antibiotics (Joshi, 2010). Antibiotic resistance change over time and hence, these antibiograms have to be constantly updated (Joshi, 2010). Therefore, the objective of this research is to characterize the antibiogram patterns of staphylococci isolated from the nasal cavity and axillae of healthy individuals in Nilai.

Methodology

Sample Collection And Characterization

A consent form and a survey form were given to 25 individuals who participated in this study. Sterile moist cotton swabs were rubbed vigorously for 5 to 15 seconds at the nasal and axilla regions respectively. The swabs were inoculated into 2mL of nutrient broth (OXOID) at 200 rpm, 37°C for 24 hours. A loopful of the mixed cultures were streaked onto nutrient agar and incubated at 37°C overnight. Homogenous looking colonies were subcultured and gram stained. Gram positive cocci were subjected to the catalase test. The catalase positive staphylococci were streaked onto Mannitol Salt Agar (OXOID).

Antibiotic Susceptibility Testing

The Kirby Bauer method was used to characterize the antibiotic susceptibility of the gram positive isolates (Bauer et al. 1966). 50µL of the overnight bacterial cultures comparable to the 0.5 McFarland standard was spread evenly on Mueller-Hinton agar (OXOID). The agar was left to dry for several minutes before placing the antibiotic disks of amoxicillin (OXOID, 25 µg), cefazolin (OXOID 30 µg), erythromycin (OXOID 15 µg), ceftiofur (OXOID 30 µg), trimethoprim/sulfamethoxazole (OXOID 5 µg), tetracycline (OXOID 30 µg) and gentamycin (OXOID 10 µg) at

designated locations and incubated at 37°C overnight. An empty paper disk served as a negative control. This assay was conducted in triplicates. The zones of inhibition were measured and the susceptibility of the isolates were characterized according to the Clinical and Laboratory Standards Institute CLSI, 2016.

Results and Discussion

Collection of Samples

A total of 50 samples were collected from the nasal and axilla of 25 healthy individuals. Healthy participants were targeted as this research as many studies show that healthy individuals have resistant bacteria and also have a high possibility to transfer the resistant bacteria to others or to inanimate objects (Tsiouris & Tsiouri, 2017). Out of the 25 individuals, 28% were male and 72% were female. All of them were residing in Nilai, Negeri Sembilan at the time of sample collection. 84% of the participants were also known to be using facial and healthcare products with antibacterial agents for various reasons. 12% of the individuals also had visited healthcare facilities recently

Isolate Characterization

78 pure isolates were obtained in this study out of which, 65.38% were gram positive (Figure 1). This is in consensus to the findings of many studies that show gram positive bacteria to be predominant bacterial species of the skin and nasal regions (Davis, 1996 and Kassam et al., 2017). This is mainly due to the presence of the thick peptidoglycan layer in their cell wall enabling them to survive the dry conditions of the skin.

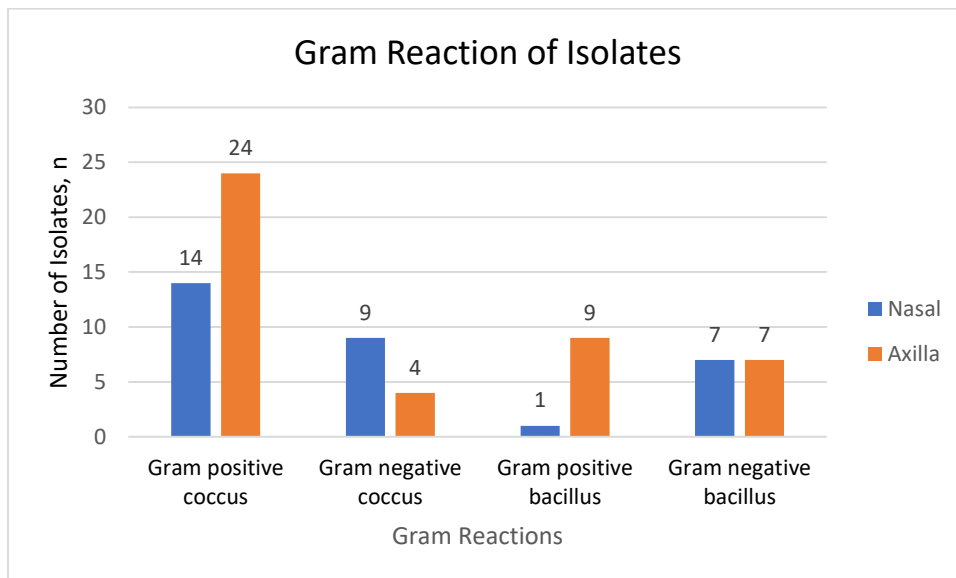


Figure 1. Gram Reaction of the nasal and axillae isolates.

In addition, 74.5% of the gram positive isolates were identified as gram positive cocci with staphylococcal arrangement. Out of this, 31.37% and 35.29% were *S. aureus* and *S. epidermidis*

as all produced catalase and grew producing yellow and pink colonies on mannitol salt agar respectively (Figure 2). Staphylococcal species are able to grow on mannitol salt agar because of their halotolerant nature that enables them to survive the salty secretion of the sweat gland (Dickgiesser, 1978). 19.61% of the gram positive bacilli were probably mixtures of species of *Propionibacterium* and *Corynebacterium* (Figure 1). These bacteria are also common inhabitants of the skin (Grice & Segre, 2011). However, the species could not be determined during the course of study but will be identified in another study using 16sRNA gene sequencing before characterizing their antibiotic susceptibility patterns.

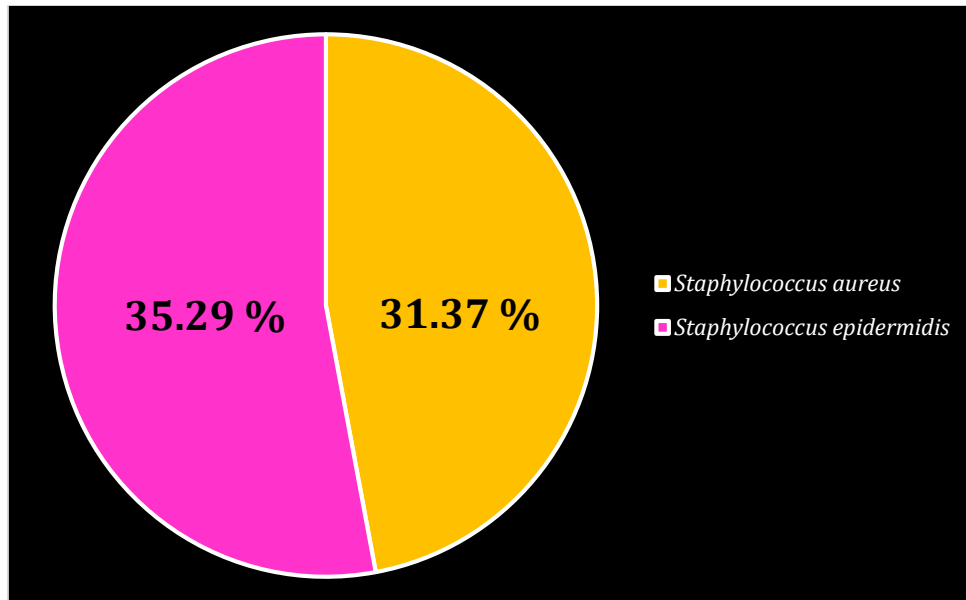


Figure 2. Characterization of staphylococci based on their growth on Mannitol Salt Agar.

Antibiotic Susceptibility Patterns

18.42% of the staphylococci were sensitive to all antibiotics. 7.89% were resistant to more than one antibiotics while, the remaining isolates were found to be resistant only to amoxicillin (Table 1). Another isolate was identified to be resistant to erythromycin but sensitive to the rest of antibiotics including amoxicillin. Amoxicillin is a beta-lactam antibiotic that targets penicillin-binding proteins (PBPs) in the inner membranes of the growing cells, disrupting the cross-links of peptidoglycans (O'Rourke et al., 2020). It is prescribed to treat various bacterial-related diseases including skin infections which is often caused by staphylococci (<https://www.drugs.com/amoxicillin.html>). This antibiotic is also reported to be one of the most frequently prescribed antibiotics in primary health care facilities (Anong & Akoachere, 2018). Studies by Deyno, Fekadu, & Astatkie (2017) and Eladli et al. (2018) showed that amoxicillin resistance is common in *S. aureus* and *S. epidermidis*. These studies indicate that the resistant bacteria were acquired from the community and through visits to the healthcare facilities. Since 57.89% of the staphylococci showed resistance towards amoxicillin, there is a high possibility of amoxicillin being frequently prescribed in the healthcare facilities in Nilai. This is because overprescription of antibiotics sets a natural selection condition that causes bacteria to become

resistant (Al-Momany et al., 2009). It is also possible that the participants of this study did not complete the prescription of antibiotics (Rather et al., 2017). In addition, they could have also indirectly acquired the resistant bacteria from the environment through surface contact. Their improper practice of handwashing and improper use of hand sanitizers and handwashing detergents or soap could also contributed to this (Bird et al., 2010).

In this study, 4 antibiogram patterns were observed in isolates of *S. aureus* whereas, 3 patterns were observed in isolates of *S. epidermidis* (Table 1). It is very likely for these isolates to be of a different strains as resistance is attributed by the presence of the resistant gene in the genome or plasmid (Himashi et al., 2018). Although a small number of isolates of staphylococci were obtained in this study, the fact that more than 50% of the staphylococci is resistant to amoxicillin strongly suggests the possible transmission of resistant bacteria in Nilai. However, this can be prevented if the healthcare practitioners are more prudent when prescribing antibiotics targeting gram positive bacteria. It is also noted that the resistant patterns among staphylococci to change over time as indicated in many studies. This is because the emergence of resistant bacteria changes mainly due to the prescription pattern of antibiotics (Tascini et al., 2016). An alternate use of several other effective antibiotics will reduce the incidence of resistant bacteria in a community but this must be complemented with the proper use of antibiotics by patients as well as personal hygiene.

Table 1. Antibiogram summary of staphylococci

| Bacterial species | Region | Number of Isolates | Resistant trend |
|-----------------------|--------|--------------------|--|
| <i>S. aureus</i> | Nasal | 1 | ERY ^S AMX ^S CFZ ^S GEN ^S SXT ^S TET ^S |
| | | 6 | ERY ^S AMX ^R CFZ ^S GEN ^S SXT ^S TET ^S |
| | | 1 | ERY ^S AMX ^R CFZ ^S GEN ^S SXT ^S TET ^R |
| | Axilla | 1 | ERY ^S AMX ^S CFZ ^S GEN ^S SXT ^S TET ^S |
| | | 1 | ERY ^S AMX ^R CFZ ^S GEN ^S SXT ^S TET ^S |
| | | 1 | ERY ^S AMX ^R CFZ ^{IR} GEN ^S SXT ^S TET ^S |
| <i>S. epidermidis</i> | Nasal | 5 | ERY ^S AMX ^S CFZ ^S GEN ^S SXT ^S TET ^S |
| | | 1 | ERY ^R AMX ^R CFZ ^S GEN ^S SXT ^S TET ^S |
| | Axilla | 1 | ERY ^R AMX ^S CFZ ^S GEN ^S SXT ^S TET ^S |
| | | 1 | ERY ^S AMX ^R CFZ ^S GEN ^S SXT ^S TET ^S |

Note: S-sensitive, IR-intermediate resistant, R-resistant

Conclusions

In this study, 65.38% of the isolates were identified as gram positive cocci and bacillus with the former being more predominant. 74.5% of the gram positive isolates were identified as *S. aureus* and *S. epidermidis*. From the antibiogram patterns, 81.58% of the staphylococci were resistant to at least one antibiotic in which, most of these isolates were found to be resistant to amoxicillin compared to other antibiotics. This strongly suggests that amoxicillin could be highly prescribed

in the healthcare facilities in Nilai. The different antibiogram patterns also indicates the presence of different strains of *S. aureus* and *S. epidermidis* on the individuals.

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