Prevalence and antimicrobial profiles of *Staphylococcus aureus* in a university environment

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Abstract

Microorganisms such as *Staphylococcus aureus* are one of the greatest problematic pathogens to human, due to the rise in antibiotic resistance or methicillin resistance *S. aureus* (MRSA). This pathogen normally causes diseases such as pneumonia, skin infection, necrotizing and bacteraemia in human beings. Community associated antibiotic resistant *S. aureus* infections had been reported and environmental surfaces can be a reservoir of the bacteria. The aim of this study was to isolate and investigate the antimicrobial profile of *S. aureus* in Nilai University environment. Sampling using wet cotton swabs had been conducted by collecting samples from 10 randomly selected surfaces. These include the library computer keyboard, washroom water taps, canteen water taps, canteen bench and activity room piano keys. Swab samples were inoculated on mannitol salt agar for screening for staphylococcus. A total of 42 typical colonies from these areas had been isolated for further characterisation by biochemical tests which were gram staining, latex agglutination test and catalase test. Based on these, 11 isolates were confirmed to be *S. aureus*. The highest sensitivity of *S. aureus* isolated was reported to gentamycin and tetracycline (100%) which were found to be effective drugs for treating multi-drug resistant strains. Nine of the *S. aureus* isolates were sensitive towards all the antibiotics tested. There were two isolates that showed reduced sensitivity to vancomycin, and resistant to penicillin G and erythromycin, suggesting possible multi-drug resistant strains. Data from this study ascertain that *S. aureus* contamination is prevalent on human contact surfaces in a university environment and such objects or surfaces could be a reservoir for multi- drug resistant organisms.

Keywords: *Staphylococcus aureus; antimicrobial profile; antibiotic resistance*

1. INTRODUCTION

Staphylococci, also known as staph are Gram-positive spherical bacteria that divide in more than one plane to form microscopic clusters resembling grapes. There are more than 20 species and eight subspecies in the genus *Staphylococcus* and only *Staphylococcus epidermidis* and *Staphylococcus aureus* (*S. aureus*) are found to interact with human (Wang *et al*., 2020). *S. aureus* mainly colonises the nasal passage, skin, gastrointestinal tract and oral cavity (Todar, 2020). *S. aureus* have become the important pathogen due to the rise in antibiotic resistance (Roberts *et al*., 2011). In year 1942, penicillin was introduced and effective in treating *S. aureus*

until it became antibiotic resistant 2 years later (Peter, 2012). Subsequently, methicillin-resistant *S. aureus* (MRSA) was reported in 1961, 2 years after the application of methicillin in clinical practice. Multi-drug resistant *S. aureus* had been reported since the 1970s and have infected patients in hospitals worldwide. More recently, vancomycin resistant *S. aureus* (VRSA) has been isolated in Japan while those with partial resistant to vancomycin were noted in United States, Australia, France, Brazil, Belgium, Germany, United Kingdom and India (Peter, 2012).

The increase in incidence of MRSA, and its cross transmission in hospitals and community associated infections have posed an immense challenge for infection pathophysiological medicine (Thapaliya et al., 2017; Jaradat et al., 2020). *S. aureus* has been found to contaminate a number of environmental surfaces especially those with frequent human contact, in common shared facilities such as children's playgrounds (Thapaliya *et al*., 2019), nursing homes (Cheatham *et al*., 2019) and fitness facilities (Dalman *et al*., 2019). In all these cases, MRSA/ multi drug-resistant *S. aureus* were reported with the overall prevalence of 3.9%,-36.3%.

Similar studies were also done to investigate the prevalence of *S. aureus* and MRSA in educational institutions including elementary schools in China (Lin et al., 2018), secondary schools in China (Wang et al., 2020) and universities in US (Roberts et al., 2011; Thapaliya et al., 2017). Thapaliya et al. (2017) reported the overall contamination of *S. aureus* and MRSA of 22.4% and 5.9% respectively, from surfaces such as door knob, light switch, classroom tables, elevator button, toilet handle and so on in a large university in US. In a study carried out in Malaysia, MRSA was isolated from 8% of the samples collected from cell phones of college students (Amini et al., 2012). However, to the best of our knowledge, there was no report on the prevalence of *S. aureus* on environmental surfaces in a university environment in Malaysia. Therefore, this study aimed to give an insight into the prevalence of *S. aureus* on Nilai University campus by isolating and investigating their antimicrobial profiles. The specific objectives of this study were:

1. To investigate the extent of *S. aureus* contamination in university environmental surfaces; and
2. To determine the antibiotic resistance profile of the isolated *S. aureus* strains by disc diffusion method.

2. METHODOLOGY

2.1 Sample collection and processing

Environmental swabs were obtained from 10 randomly selected surfaces at Nilai University. These include door handle, computer lab keyboards, taps in common washrooms, toilet flush handles, staircase handrails, elevator buttons, canteen bench and activity room piano keys. These surfaces were selected due to frequent human contact and may be a reservoir of microorganisms. Samples were collected during academic term over a period of 2 weeks. Swabbing was done by using sterile cotton-tipped swabs moistened with sterile saline solution, for about 10 seconds in each sampling area. All specimens were transferred to laboratory within 2 hours for further processing.

2.2 Screening and isolation of *S. aureus*

Each swab sample was streaked directly onto mannitol salt agar (MSA) by 60° streaking pattern. All the plates were incubated at 37°C for 24 hours before examination. After 24 hour incubation, the number of colonies in each MSA was recorded. Streaking was by using sterilized wire loop to obtain discrete colonies. Presumptive *S. aureus* colonies on MSA are small cluster in 1-2 mm diameter surrounded by yellow zones.

All the yellow colonies on MSA were sub-cultured onto nutrient agar and incubated for 24 hours for further characterization by gram staining, catalase test, and latex agglutination test. For gram staining, gram positive cocci were identified and further analysed by catalase test and latex agglutination test. The latex agglutination test was performed by PROLEX™ Staph Latex kit.

2.3 Antimicrobial susceptibility testing

The antimicrobial profile of the of *S. aureus* was evaluated by Kirby- Baur disc diffusion method. At least five well-isolated colonies of the *S. aureus* were selected. The top of each colony is touched with a loop and transferred into a tube containing 2 ml of saline water. Cell density was adjusted to optical density of 0.08 to 0.14 at 625 nm for 0.5 McFarland. Within 15 minutes after adjusting the turbidity suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level before streaking over the dried surface of Mueller Hinton agar plate. All the cultured plates were allowed to dry for 15 minutes at room temperature before placing the antibiotic discs by using forceps. The antibiotics tested were Gentamycin (10 µg), Chloramphenicol (30 µg), Tetracycline (30 µg), Vancomycin (30 µg), Penicillin G (10 µg), Erythromycin (15 µg), and Cefoxitin (30 µg) for indication of MRSA. The antibiotic discs were pressed down to ensure complete contact with the agar surface. The plates were incubated at 37 °C for 24 hours. The diameter of the zone of inhibition was measured by a ruler and results interpreted according to the Clinical and Laboratory Standards Institute (CLSI).

3. RESULTS AND DISCUSSION

A total of 62 samples were collected from the 10 selected sampling environmental surfaces. From these, 42 samples yielded typical colonies on MSA. It is interesting to note that the library computer keyboards had apparently more numbers of isolates compared with the other sampling locations. This is probably because the computers in the library are shared facilities and are frequently used.

From the MSA, 11 isolates were Gram positive cocci, catalase and latex agglutination positive, and were identified as *S. aureus*. The distribution of the isolates among the samples is shown in Table 1. Thus, the prevalence of *S. aureus* among the samples is 18% (11/62). This was slightly lower compared to studies conducted in a large university in US (22.4%, n=152) (Thapaliya *et al.*, 2017), nursing homes (28.6%, n=259) (Cheatham *et al.*, 2019) and fitness facilities (38.2%, n=288) (Dalman et al., 2019) but higher than a study conducted in elementary schools in China (4.6%, n=1240) (Lin et al., 2018).

Table 1. Distribution of presumptive *S. aureus* isolates among the sampled sites.

Sampled sites	Gram positive cocci	Catalase positive	Latex agglutination positive
Canteen bench	3	3	3
Canteen water tap	2	2	1
Canteen washroom water tap	2	2	2
Library computer keyboard	7	4	4
Piano key	3	1	1
Total number of isolates	**17**	**12**	**11**

All the 11 strains of *S. aureus* isolated were subjected to antibiotic susceptibility tests by Kirby-Baur disc diffusion. Diameters of the zone of inhibition on Mueller Hinton agar (Figure 1) were measured and interpretation was made based on CLSI guidelines. A summary of the antibiotic resistance profiles of *S. aureus* is showed in Table 2. In this study, 7 of the *S. aureus* isolates were sensitive to all antibiotics. The trends of zone of inhibitions for resistant isolates (%) is as follow: gentamycin and tetracycline (100 %) > chloramphenicol (90.9 %) > vancomycin (81.8 %) > erythromycin (72.7 %) > penicillin G (63.6 %). All isolates were sensitive to cefoxitin, indicating that none of them were MRSA. There were two isolates that showed reduced sensitivity to vancomycin, and resistant to penicillin G and erythromycin, suggesting possible multi-drug resistant *S. aureus* strains.

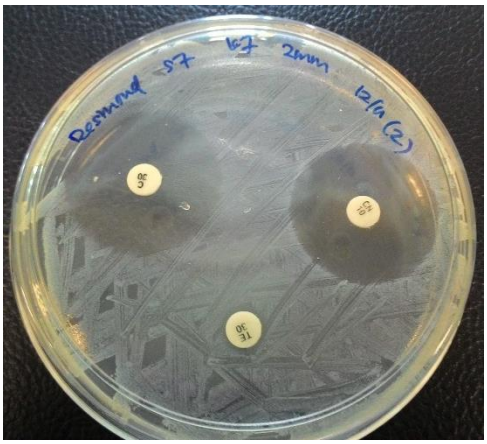

Fig. 1. Zone of inhibition of an *S. aureus* isolate on Mueller Hinton agar.

Table 2. Summary of the antibiotic resistance profiles of *S. aureus*.

Samples	Zone of Inhibition for resistant isolates, No (%) isolates (n=11)		
	Sensitive	**Intermediate**	**Resistance**
Gentamycin (10ug)	11 (100)	0	0
Chloramphericol (30 µg)	10 (90.9)	1 (9.1)	0
Cefoxitin (30 µg)	11 (100)	0	0
Tetracyclin (30 µg)	11(100)	0	0
Vancomycin (30 µg)	9 (81.8)	- *	- *
Penicillin G (10 µg)	7 (63.6)	4 (36.4)	0
Erythromycin (15 µg)	8 (72.7)	2 (18.2)	1 (9.1)

* (-) No interpretive criteria for vancomycin zone of inhibition of < 15mm as there has not been a sufficient number of non-susceptible isolates to develop resistant and intermediate breakpoints (CLSI).

4. CONCLUSION

Data from this study ascertain that *S. aureus* contamination is prevalent on human contact surfaces in a university environment. The antimicrobial resistance profile of the isolates suggests the presence of possible multi- drug resistant organisms thus may potentially be spread from one person to another through such objects or surfaces. Future studies may involve increasing sampling size and area, and carry out molecular characterisation of the isolates.

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