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Prevalence and antibiotic sensitivity profiles of *Staphylococcus aureus* nasal carriage among preclinical and clinical medical students in a Malaysian university

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ABSTRACT

Aims: Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains in healthcare (HA-MRSA) and community (CA-MRSA) incurred costly morbidity and mortality. This study assessed the prevalence and antibiotic sensitivity profile of *S. aureus* and MRSA isolates from medical students.
Methodology and results: A cross-sectional study of nasal swabs from 60 medical students yielded 93% positive *S. aureus*. In this study, erythromycin, fusidic acid, gentamicin, penicillin, vancomycin and methicillin were used. The most significant antibiotic sensitivity against *S. aureus* was fusidic acid (*p*-value = 0.0042). The *S. aureus* and MRSA isolates from clinical students were more resistant than those of preclinical students against erythromycin (44%; 15%), fusidic acid (33.3%; 10%), penicillin (85%; 86.9%), vancomycin (11.1%; -) and methicillin (19.4%; 15%) respectively while the isolates from preclinical students were more resistant than those of clinical students against gentamicin (5%; -).
Conclusion, significance and impact of study: In this study, gender, age and duration of clinical exposure had no significant bearing on the prevalence of nasal *S. aureus* and MRSA respectively. No MRSA infections were detected in preclinical (15%) and clinical (19%) students positive for MRSA, suggesting that these students may be carriers of CA-MRSA. A larger study will be implemented to provide baseline data for monitoring CA-MRSA infections, genotyping and constructing of phylogenetic tree.

Keywords: Methicillin-resistant, *Staphylococcus aureus*, medical students, nasal

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacteria, cluster-forming cocci and normal flora found on nasal passages, skin and mucous membranes of human. It is present in approximately 30% of healthy people population (von Eiff *et al*., 2001). Staphylococci, ubiquitous colonisers of human epithelia are implicated in soft tissue infections and invasive diseases namely osteomyelitis, necrotizing fasciitis, severe sepsis and endocarditis (Miller *et al*., 2005; Klevens *et al*., 2007). Methicillin-Resistant *Staphylococcus aureus* (MRSA) was first documented in 1960 (Barber, 1961) and classified into Hospital-acquired Methicillin-Resistant *Staphylococcus aureus* (HA-MRSA) and Community-acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) (Naimi *et al*., 2003; Kluytmans-Vandenbergh and Kluytmans, 2006; David and Daum, 2010).

Previous studies had shown that developing countries for instance, Nigeria (14%), India (16%) and Malaysia (26%) had lesser *S. aureus* carriers than developed countries like United State (32%) and Netherlands (35%) (Klevens *et al*., 2007; Alvarez-Uria and Reddy 2012, Al-Talib *et al*., 2013; Dulon *et al*., 2014). However, Asia is currently among the regions with the highest prevalence rates of HA-MRSA and CA-MRSA in the world (Song *et al*., 2011; Chen and Huang, 2014; You *et al*., 2017). Prevalence of HA-MRSA infections had been reported to be 70-80% in Asia region (Boucher and Corey, 2008; Song *et al*., 2011). While a study reported 7% of adults were carrying MRSA in their nasal passageway (Hidron *et al*., 2005), nasal carriage MRSA colonization rate was found higher in healthcare setting than in community (Berthelot *et al*., 2004; Trepanier *et al*., 2013; Dulon *et al*., 2014). Studies also suggested that healthy individual may act as medium that transferred MRSA among the communities causing an increase in MRSA infections (Kluytmans-Vandenbergh and Kluytmans, 2006; David and Daum, 2010; Conceicao *et al*., 2013; Conceicao *et al*., 2014). CA-MRSA showed high resistance against

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beta-lactam antibiotics, hence they were more difficult to treat (Kluytmans-Vandenbergh and Kluytmans, 2006). Conversely, HA-MRSA had better resistance against clindamycin and doxycycline than CA-MRSA (Alvarez-Uria and Reddy, 2012; Lim *et al*., 2013). Although CA-MRSA infections are usually mild, but once prolonged may be severe and requires hospitalization or even causes death. CA-MRSA had a larger spread to the community than HA-MRSA which was limited to the healthcare setting and easier to control since the strain can only be acquired and developed inside the hospital or due to healthcare facilities (Chen and Huang, 2014). Therefore, it is pertinent to assess the prevalence of MRSA, its strain, exposure to the community especially in Malaysia given the ease of traveling within the ASEAN regions and its antibiotic sensitivity profile to aid in reducing the morbidity and mortality MRSA may trigger.

MATERIALS AND METHODS

Study population

This was a cross-sectional study involving 24 preclinical and 36 clinical students from the Faculty of Medicine and Health Sciences (FMHS), Universiti Malaysia Sarawak (UNIMAS). These preclinical students had little or no patient encounters due to their lecture-based curriculum while clinical students were more involved in bedside learning. The study was approved by the Ethics Committee of the National Institute of Health (Ministry of Health) and Medical Ethical Committee, FMHS, UNIMAS.

Sampling

Nasal swab samples were collected and were kept at room temperature for 3 h before culturing and microbiology examinations were conducted. Primary culture was streaked on blood agar (OXOID, UK) (Figure 1) and mannitol salt agar (MSA) (OXOID, UK) (Figure 2) to culture and isolate bacteria. Microbiological tests for *S. aureus* including Gram-staining (HIMEDIA, India), catalase test (Sigma) and coagulase test (Sigma) were performed. Colony suspension in Mueller-Hinton (MH) broth (OXOID, UK) was obtained from single colony positive on MSA agar to create bacterial lawn on MH agar. Standard Antibiotic discs (OXOID, UK) namely Erythromycin (15 µg), Fusidic acid (10µg), Gentamicin (10 µg), Methicillin (5 µg), Penicillin (10µg) and Vancomycin (30 µg) were placed on the bacterial lawn. The agars were incubated for 24 h at 37 °C. Inhibition zones were measured and recorded (Figure 3) while in the absent of inhibition zone, the culture samples were identified as resistant against antibiotic of interest (Figure 4) The antibiotic sensitivity was interpreted using the antibiotic sensitivity chart provided by OXOID (Table 1). The final antibiotic resistance validation was done on Brilliance MRSA agar (ThermoFisher Scientific) (Figure 5).

Figure 1: Blood agar with presence of *S. aureus*. In the Blood Agar plate, most of the organisms appeared as colourless to yellowish-white colonies of various sizes (small, medium, big).

Figure 2: Mannitol salt agar (MSA) with growth of bacteria. Since *S. aureus* fermented mannitol, the presence of it was indicated by the changes of the MSA reddish colour into yellow.

Figure 3: Inhibition zone was measured and recorded. The smaller the inhibition zone, the more resistant the bacteria were towards the antibiotic of interest.

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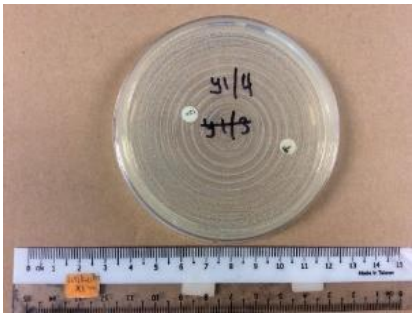

Figure 4: Inhibition zone was absent. The absent of inhibition zone was a clear indication of the bacteria resistant towards the antibiotic of interest.

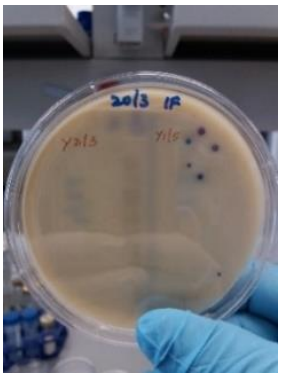

Figure 5: Identification of MRSA using Brilliance MRSA agar (ThermoFisher Scientific, USA). MRSA obtained from nasal swab and tested resistant against Methicillin was further identified through chromoagar culturing on Brilliance MRSA. Methicillin-sensitive *S. aureus* (MSSA) appeared pink (left) while MRSA appeared blue (right).

Table 1: Antibiotics sensitivity range as provided by OXOID, UK. This table summarized the standard antibiotic discs (µg) inhibition zone measurement (mm) of resistance (R), intermediate (I) and sensitive (S) based on their standard *S. aureus* from ATCC.

	Resistance (R)	Intermediate (I)	Sensitive (S)
Erythromycin (E 15)	≤ 13	14 - 22	≥ 23
Fusidic Acid (FD 10)	≤ 18	17 - 20	≥ 21
Gentamicin (CN 10)	12	13 - 14	15
Penicillin (P 10)	≤ 28	-	≥ 29
Vancomycin (VA 30)	≤ 9	10 - 11	≥ 12
Methicillin (MET 5)	≤ 9	10 - 13	≥ 14

RESULTS AND DISCUSSION

A total of 60 nasal swab samples were collected from 20 preclinical year students and 36 clinical year students respectively. From these samples, 56 students were positive for *S. aureus* while 4 samples collected from preclinical year students were negative for *S. aureus* (Table 2). Some of the nasal swab samples yielded both *S. aureus* and MRSA from individuals. The antimicrobial sensitivity profiles of *S. aureus* samples (Table 3) from clinical students were found to be more resistant than preclinical students against erythromycin (29%; 5%), fusidic acid (21%; 4%), penicillin (57%; 30%), vancomycin (7%; -%) and methicillin (13%; 5%) respectively. On the contrary, the *S. aureus* samples from preclinical students were found to be more resistant than clinical students against gentamicin (0%; 2%). Overall, there was a significant difference between preclinical and clinical year students against fusidic acid (p= 0.042) while less significantly associated with erythromycin (p= 0.082), penicillin (p= 0.691), vancomycin (p= 0.218) and methicillin (p= 0.780). In this study, 93% of the samples collected were positive for *S. aureus* in contrast with previous studies conducted in West Malaysia at 26% and 28.7% and China at 46% respectively (Neela *et al*., 2010; Ma *et al*., 2011; Al-Talib *et al*., 2013). Of the positive *S. aureus* samples isolated, 17.8% of these were found to be MRSA based on antibiotic susceptibility test and growth on MRSA Brilliance agar. This result was lower than those reported in HUSM (21.5%) (Al-Talib *et al*., 2010; Al-Talib *et al*., 2013). This study also demonstrated no significant association between the duration of clinical exposure and the nasal carriage rate of *S. aureus* (p= <0.001), concurring with the previous findings (Chen *et al*., 2012). However, culture samples from the clinical students showed smaller inhibition zone than those of preclinical students. There was a statistically significance difference associated with fusidic acid (p= 0.0042) between preclinical and clinical medical students with 86% of fusidic acid resistant *S. aureus* contributed by clinical year students.

This study showed gender, age and duration of clinical exposure had no significant bearings on the prevalence of *S. aureus* and MRSA respectively. The results also demonstrated both clinical (19%) and preclinical students (15%) carried MRSA in their nares with the clinical students accorded a slightly higher prevalence. The results further suggested that these medical students may have CA-MRSA rather than HA-MRSA as the prevalence of MRSA in both cohorts of students were similar.

Table 2: *Staphylococcus aureus* positive and *S. aureus* negative from nasal swab samples. A tabulated data of 2 cohorts of students, namely preclinical and clinical students and the prevalence of *S. aureus* in number and percentage with Chi-square and p-value.

Cohort	*S. aureus* Positive n (%)	*S. aureus* negative n (%)	Chi-square value	*p* value
Preclinical	20 (83.3)	4 (16.7)	38.57	<0.001[a]
Clinical	36 (100.0)	0 (0.0)		

[a]Fisher exact test

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Table 3: Effects of antibiotic sensitivity on *S. aureus* cultured from preclinical and clinical students. A tabulated data depicting the different antibiotics (erythromycin, fusidic acid, gentamicin, penicillin, vancomycin and methicillin) of interest tested on nasal swab samples obtained from 24 preclinical and 36 clinical students respectively. The antibiotic profiles (sensitive, intermediate, resistant) were recorded and percentages were calculated with Chi-square values and p-values. Note: Some nasal samples from students yielded both *S. aureus* and MRSA.

Antibiotics	Year	Sensitive, n (%)	Intermediate, n (%)	Resistant, n (%)	Chi square value	*P*-value
Erythromycin	Preclinical	14 (70.0)	3 (15.0)	3 (15.0)	5.01	0.082
	Clinical	16 (44.4)	4 (11.1)	16 (44.4)		
Fusidic acid	Preclinical	18 (90.0)	0 (0.0)	2 (10.0)	6.32	0.042
	Clinical	21 (58.3)	3 (8.3)	12 (33.3)		
Gentamicin	Preclinical	19 (95.0)	0 (0.0)	1 (5.0)	1.83	0.357[a]
	Clinical	36 (100.0)	0 (0.0)	0 (0.0)		
Penicillin	Preclinical	3 (15.0)	0 (0.0)	17 (85.0)	0.67	0.691[a]
	Clinical	4 (11.1)	0 (0.0)	32 (86.9)		
Vancomycin	Preclinical	20 (100.0)	0 (0.0)	0 (0.0)	3.05	0.218
	Clinical	31 (86.1)	1 (2.8)	4 (11.1)		
Methicillin	Preclinical	15 (75.0)	2 (10.0)	3 (15.0)	0.498	0.780
	Clinical	27 (75.0)	2 (5.6)	7 (19.4)		

[a] Fisher's exact test

Albeit no significant association between the duration of clinical exposure and antibiotic susceptibility (p= 0.673), the highest number of antibiotic resistant *S. aureus* was those of penicillin (preclinical: 85%; clinical: 86.9%) as also observed in other studies (Du *et al*., 2011; Syafinaz *et al*., 2012). In this study, the most sensitive antibiotic was vancomycin (preclinical: 100%; clinical: 86.1%) as observed by other researchers (Onanuga and Temedie, 2011; Syafinaz *et al*., 2012) and 31.6% of the SA isolated were resistant against macrolide erythromycin. Due to the relatively small sample size, the risk factors for the acquisition of MRSA among the medical students in current study could not be identified. It was noted that there was a slight indication of a change in pattern of *S. aureus* nasal carriage, but a bigger sample size would be needed to provide a sound analysis.

CONCLUSION

This study demonstrated the high prevalence of *S. aureus* nasal carriage among these 60 medical students, indicative of a probable change in S*. aureus* nasal carriage pattern. While MRSA nasal colonization among these students was low, penicillin-resistant *S. aureus* was prevalent. We suggest a larger sample size study in different medical schools and hospitals to provide essential epidemiological information on MRSA in healthcare settings.

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REFERENCES

Al-Talib, H., Yean, C. Y., Hasan, H., Nik Zuraina, N. M. and Ravichandran, M. (2013). Methicillin-resistant *Staphylococcus aureus* nasal carriage among patients and healthcare workers in a hospital in Kelantan, Malaysia. *Polish Journal of Microbiology* **62(1), 109-112.**

Al-Talib, H. I., Yean, C. Y., Al-Jashamy, K. and Hasan, H. (2010). Methicillin-resistant *Staphylococcus aureus* nosocomial infection trends in Hospital Universiti Sains Malaysia during 2002-2007. *Annals of Saudi Medicine* **30(5), 358-363.**

Alvarez-Uria, G. and Reddy, R. (2012). Prevalence and antibiotic susceptibility of community-associated Methicillin-Resistant *Staphylococcus aureus* in a rural area of India: Is MRSA replacing Methicillin-Susceptible Staphylococcus aureus in the Community? *ISRN Dermatology* **2012, 248951.**

Barber, M. (1961). Methicillin-resistant staphylococci. *Journal of Clinical Pathology* **14, 385-393.**

Berthelot, P., Grattard, F., Fascia, P., Martin, I., Mallaval, F. O., Ros, A., Pozzetto, B. and Lucht, F. (2004). Is nasal carriage of methicillin-resistant *Staphylococcus aureus* more prevalent among student healthcare workers? *Infection Control and Hospital Epidemiology* **25(5), 364-365.**

Boucher, H. W. and Corey, G. R. (2008). Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clinical Infectious Diseases* **46(Supplement_5), S344-S349.**

Chen, C. J. and Huang, Y. C. (2014). New epidemiology of *Staphylococcus aureus* infection in Asia. *Clinical Microbiology and Infection* **20(7), 605-623.**

Chen, C. S., Chen, C. Y. and Huang, Y. C. (2012). Nasal carriage rate and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among medical students at a Taiwanese university.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

International Journal of Infectious Diseases **16(11), e799-803.**

Conceicao, T., Diamantino, F., Coelho, C., de Lencastre, H. and Aires-de-Sousa, M. (2013). Contamination of public buses with MRSA in Lisbon, Portugal: A possible transmission route of major MRSA clones within the community. *PLoS One* **8(11), e77812.**

Conceicao, T., Santos Silva, I., de Lencastre, H., and Aires-de-Sousa, M. (2014). *Staphylococcus aureus* nasal carriage among patients and health care workers in Sao Tome and Principe. *Microbial Drug Resistance* **20(1), 57-66.**

David, M. Z. and Daum, R. S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: Epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews* **23(3), 616-687.**

Du, J., Chen, C., Ding, B., Tu, J., Qin, Z., Parsons, C., Salgado, C., Cai, Q., Song, Y., Bao, Q., Zhang, L., Pan, J., Wang, L. and Yu, F. (2011). Molecular characterization and antimicrobial susceptibility of nasal *Staphylococcus aureus* isolates from a Chinese medical college campus. *PLoS One* **6(11), e27328.**

Dulon, M., Peters, C., Schablon, A. and Nienhaus, A. (2014). MRSA carriage among healthcare workers in non-outbreak settings in Europe and the United States: A systematic review. *BMC Infectious Diseases* **14(1), 363.**

Hidron, A. I., Kourbatova, E. V., Halvosa, J. S., Terrell, B. J., McDougal, L. K., Tenover, F. C., Blumberg, H. M. and King, M. D. (2005). Risk factors for colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) in patients admitted to an urban hospital: Emergence of community-associated MRSA nasal carriage. *Clinical Infectious Diseases* **41(2), 159-166.**

Klevens, R. M., Morrison, M. A., Nadle, J., Petit, S., Gershman, K., Ray, S., Harrison, L. H., Lynfield, R., Dumyati, G., Townes, J. M., Craig, A. S., Zell, E. R., Fosheim, G. E., McDougal, L. K., Carey, R. B., Fridkin, S. K. and Active Bacterial Core surveillance, M. I. (2007). Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* **298(15), 1763-1771.**

Kluytmans-Vandenbergh, M. F. and Kluytmans, J. A. (2006). Community-acquired methicillin-resistant *Staphylococcus aureus*: Current perspectives. *Clinical Microbiology and Infection* **12(Supplement_1), 9-15.**

Lim, K. T., Hanifah, Y. A., Mohd Yusof, M. Y., Ito, T. and Thong, K. L. (2013). Comparison of methicillin-resistant *Staphylococcus aureus* strains isolated in 2003 and 2008 with an emergence of multidrug resistant ST22: SCCmec IV clone in a tertiary hospital, Malaysia. *Journal of Microbiology, Immunology and Infection* **46(3), 224-233.**

Ma, X. X., Sun, D. D., Wang, S., Wang, M. L., Li, M., Shang, H., Wang, E. H. and Luo, E. J. (2011). Nasal carriage of methicillin-resistant *Staphylococcus aureus* among preclinical medical students: Epidemiologic and molecular characteristics of methicillin-resistant S. aureus clones. *Diagnostic Microbiology and Infectious Disease* **70(1), 22-30.**

Miller, L. G., Perdreau-Remington, F., Rieg, G., Mehdi, S., Perlroth, J., Bayer, A. S., Tang, A. W., Phung, T. O. and Spellberg, B. (2005). Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. *The New England Journal of Medicine* **352(14), 1445-1453.**

Naimi, T. S., LeDell, K. H., Como-Sabetti, K., Borchardt, S. M., Boxrud, D. J., Etienne, J., Johnson, S. K., Vandenesch, F., Fridkin, S., O'Boyle, C., Danila, R. N. and Lynfield, R. (2003). Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* **290(22), 2976-2984.**

Neela, V., Ghasemzadeh Moghaddam, H., van Belkum, A., Horst-Kreft, D., Mariana, N. S. and Ghaznavi Rad, E. (2010). First report on methicillin-resistant *Staphylococcus aureus* of Spa type T037, Sequence Type 239, SCCmec type III/IIIA in Malaysia. *European Journal of Clinical Microbiology and Infectious Diseases* **29(1), 115-117.**

Onanuga, A. and Temedie, T. C. (2011). Nasal carriage of multi-drug resistant *Staphylococcus aureus* in healthy inhabitants of Amassoma in Niger delta region of Nigeria. *African Health Sciences* **11(2), 176-181.**

Song, J. H., Hsueh, P. R., Chung, D. R., Ko, K. S., Kang, C. I., Peck, K. R., Yeom, J. S., Kim, S. W., Chang, H. H., Kim, Y. S., Jung, S. I., Son, J. S., So, T. M., Lalitha, M. K., Yang, Y., Huang, S. G., Wang, H., Lu, Q., Carlos, C. C., Perera, J. A., Chiu, C. H., Liu, J. W., Chongthaleong, A., Thamlikitkul, V., Van, P. H. and Group, A. S. (2011). Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: An ANSORP study. *Journal of Antimicrobial Chemotherapy* **66(5), 1061-1069.**

Syafinaz, A. M., Nur Ain, N. Z., Nadzirahi, S. N., Fatimah, J. S., Shahram, A. and Nasir, M. D. (2012). *Staphylococcus aureus* nasal carriers among medical students in a medical school. *Medical Journal of Malaysia* **67(6), 636-638.**

Trepanier, P., Tremblay, C. and Ruest, A. (2013). Methicillin-resistant *Staphylococcus aureus* colonization among medical residents. *Canadian Journal of Infectious Diseases and Medical Microbiology* **24(2), e39-41.**

von Eiff, C., Becker, K., Machka, K., Stammer, H. and Peters, G. (2001). Nasal carriage as a source of *Staphylococcus aureus* bacteremia. *The New England Journal of Medicine* **344(1), 11-16.**

You, J. H. S., Choi, K. W., Wong, T. Y., Ip, M., Ming, W. K., Wong, R. Y., Chan, S. N., Tse, H. T., Chau, C. T. S. and Lee, N. L. S. (2017). Disease burden, characteristics, and outcomes of Methicillin-resistant *Staphylococcus aureus* bloodstream infection in Hong Kong. *Asia Pacific Journal of Public Health* **29(5), 451-461.**

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