# Survey of the nasal carriage of *Staphylococcus aureus* in Microbiology class students


## Abstract

*Staphylococcus aureus* is one of the most common cause of community or in hospital infections; in Australia, up to 30% of the population is a persistent carrier of the bacteria, while general colonization is harmless; but the increasing prevalence rate in the community is posting threat to the high risk population such as immunosuppressed patients, elderly or newborns. Moreover, *S. aureus* is also a major food pathogen, cross-contamination from the food handlers will lead to poor quality and safety of food products. Hence, monitoring of nasal carriage rate is essential. In this survey, the nasal carriage rate of *S. aureus* among 912 RMIT Microbiology students between 2013-2019 were tested for *S. aureus* , sampling were obtained through nasal swabs, followed by inoculation in selective medium, identification through colonial and gram morphology as well as biochemical tests. 228 positive isolates were reported for the total of 7 years study period, giving an average of 25% carriage rate. The results are comparable to the general community of 30% carriage rate, and is expected to be lower than that of the health care workers. However, the reliability of the results are relatively low due to the limitation of experimental designs and lack of information from the subjects group such as age, gender, race, health conditions and professions.


## Introduction

*Staphylococcus aureus* , the gram-positive and coagulase positive round shape bacteria which is widely known as "*Golden Staph*" can be commonly found on human skin, hair and nose (Todar, 2012). Up to 50% of the population carries *S. aureus* in the nasal passage in any given time of their life , and this colonization is generally harmless, however, once the bacteria successfully enter the body it can cause infection in skin, upper respiratory tract and wounds or other serious illness such as blood poisoning or pneumonia; posting major threat especially to the high risk immunosuppressed population. Transmission of *S. aureus* is often through direct or indirect contact such as skin to skin , discharging wounds, respiratory mucus of infected individuals or contaminations of surface and medical equipment (Department of Health & Human Services, 2018).

*S. aureus* has numerous surface proteins and antigens which allows it to attach to the affected host cell and protect itself from phagocytic engulfment, it also has the ability of secreting exoproteins such as toxins and enzymes that break down the host cells and transform the host tissue to nutrients supporting the bacteria growth. These virulence factors work together to avoid the host immunity defence , promote bacteria growth and multiplication (Todar, 2012). Most *S. aureus* infection can be treated by antibiotics, while others have developed antibiotic resistance. More than 95% of *S. aureus* isolates currently found is Penicillin resistance which was first discovered in 1940s, then followed by the first detection of Methicillin resistance *S. aureus* (MRSA) strain in Australia back in 1960s (Department of Health & Human Services, 2018). MRSA strain is found to be resistant not only to methicillin, but also amoxicillin,

penicillin , oxacillin and increasing resistance to vancomycin too (Newman, 2017). MRSA can be classified into two groups : 1) hospital-acquired (HA-MRSA) which is a multi-antibiotic resistant clones and 2) community-acquired (CA-MRSA), the non-multi antibiotic resistant clones that are sensitive to the traditional anti-staphylococcal antibiotics (Department of Health & Human Services, 2018). It is believed the excessive use of antibiotics over the years and production of biofilm by the *S. aureus* facilitates the antibiotic resistance by mutation (Savage, Chopra, & O'Neill, 2013). Although HA-MRSA has remained the main case of MRSA infection in Australia until early 2000s, a increasing trend of CA-MRSA was shown in a nation survey of *S. aureus* in 2012 (Agostino, Ferguson, Eastwood, & Kirk, 2017).

Due to the high prevalence of *S. aureus* and the MRSA strains , surveillance of carriage rate is essential especially in the hospital or heath care sectors, where *S. aureus* can be transmitted between staffs or to patients, causing severe infections and illness to the immune-weaken patients, elderly and newborns; research has also shown a strong relationship between nasal carriage and colonization of *S. aureus* , especially with the MRSA strains, with increasing number of in-hospital and post-hospital infections ( Tur, Kielar, Kłaczkowska, Teodorowicz, & Lewczuk, 2010). Moreover, *S. aureus* is also a common food pathogen, causing food poisoning by releasing toxins on the affected food, usually through contaminations by food handlers who are the carriage of *S. aureus*, as well as inappropriate handling practises and storage (U.S. Department of Health & Human Services, 2019). The continuous rise of CA-MRSA carriage is posting threat to public health and causing the rise of community outbreaks of Golden Staph infections, the incidence of community associated infection has increase significantly during the study period between 2011-2016 with 8% in Victoria and 6% in Western Australia each year according to a research reported in Medical Journal of Australia (Swannell, 2019).

The survey of *S. aureus* carriage rate in community, clinical staffs and food handlers are useful as an indicator of potential outbreaks, so appropriate disease control and preventative measures can be taken accordingly, such as education of general public about the importance of hand washing and personal hygiene, precautions on wound handling, avoidance of crowded area , cleaning and sanitizing of shared public area, cooking and correct storage of food products etc (NSW Ministry of Health, 2019).

Methods of studying the *S. aureus* carriage rate of particular population of interest usually involves a collection of nasal swabs of selected group , with laboratory culture in specific agar medium, followed by microbiological tests, biochemical indication of strains and antibiotic resistance (Shibabaw, Abebe, & Mihre, 2013). Other investigations have done through DNA extractions with PCR analysis to determine the antimicrobial susceptibility (Vatansever, Sezer, & Bilge, 2016). Due to time constraints, a rapid identification of MRSA colonies are often performed through direct agglutination reaction with specific antibody in clinical settings. (French, 2009)

The aim of this survey is to determine the nasal carriage of *S. aureus* among RMIT Microbiology student in 2019 and compare that with the previous years, the general community , health care workers and food handlers.

## Materials and Methods

The materials used in this survey of nasal carriage of *S. aureus* were prepared and supplied by the Microbiology laboratory of RMIT University, including:

- Mannitol salt agar (MSA) plate (Oxoid CM0085)
- Nutrient Agar (NA) plate (Oxoid CM0003)
- Sterile swab
- Loop
- Gram Stain required materials
- Slide and Tube Coagulase Test required materials
- Catalase Test required materials

A total number of 146 Microbiology students with both gender were tested. Sterile swab was moisten using sterile saline, samples from both the left and right nostrils were taken by the individual using the swab and cultured on a MSA plate with streak dilution. The MSA plate was then incubated at 35$^{o}$C under aerobic conditions for 44-48 hours. A presumptive identification of *S. aureus* was determined based on colonial morphology on MSA, followed by confirmation with gram morphology and slide coagulation Test. For any negative slide coagulase results, the isolate was then sub-cultured to NA plate for further confirmation with tube coagulase and catalase tests.

Formulation of medium agar plate used can be obtained from the Thermofischer (Oxoid) website. Details on test procedures and materials required for each test can be found on Microbiology 1 Laboratory Manual (Microbiology Teaching Team, 2019) and Glossary and Electronic Resources of Microbiology Methods (G.E.R.M.M) (Microbiology teaching team, 2017).

## Result

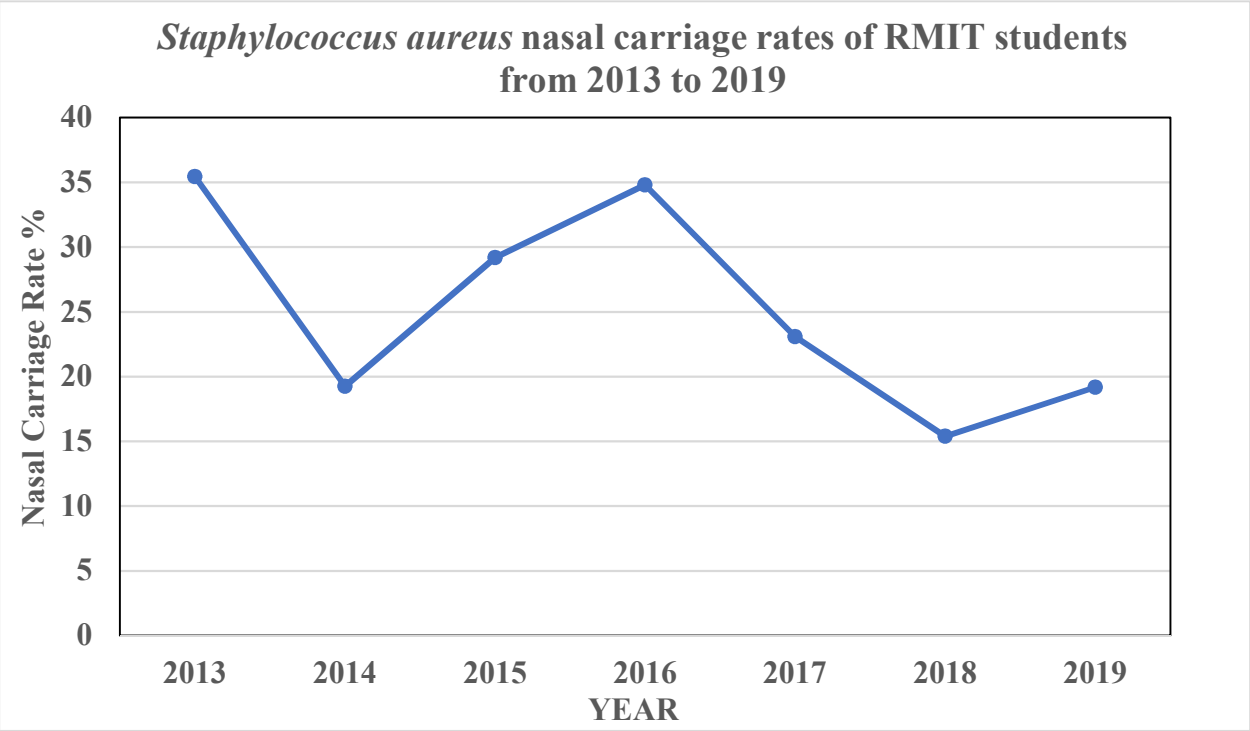
Presumptive isolates of *S. aureus* is determined by the colonial morphology with a round yellow colonies and yellow halo on MSA (Microbiology teaching team, 2017), followed by confirmation of a purple cocci gram positive microscopic morphology , a positive tube coagulase and positive catalase test (Microbiology Unit, 2019).

Results for positive carriage of *S. aureus* of Microbiology students between 2013-2019 are listed in “Table 1 Numbers of *Staphylococcus aureus* nasal carriage of RMIT students from 2013 to 2019” and the nasal carriage rate is presented in “Graph 1 *Staphylococcus aureus* nasal carriage rates of RMIT students from 2013 to 2019”.

Table 1 Numbers of *Staphylococcus aureus* nasal carriage of RMIT students from 2013 to 2019

| **Year** | **Numbers of positive *S. aureus* student carrier** | **Total numbers of student tested** | **Nasal Carriage rate %** |
|---|---|---|---|
| 2013 | 39 | 110 | 35% |
| 2014 | 26 | 135 | 19% |
| 2015 | 33 | 113 | 29% |
| 2016 | 47 | 135 | 35% |
| 2017 | 39 | 169 | 23% |
| 2018 | 16 | 104 | 15% |
| 2019 | 28 | 146 | 19% |
| Average | 33 | 130 | 25% |

Graph 1 *Staphylococcus aureus* nasal carriage rates of RMIT students from 2013 to 2019



## Discussion

The average nasal carriage rate of *S. aureus* among RMIT microbiology students for the past 7 years between 2013-2019 is 25% as stated in Table 1; which agrees to previous research showing around 20%-30% of healthy adults are persistent *S. aureus* nasal carrier (Kluytmans , Belkum, & Verbrugh, 1997). However, referring to Graph 1 of the nasal carriage rate % for the whole study period, it has shown firstly a declining trend between 2013-2014, followed by a rising trend up until 2016, then another massive drop towards 2018 with a slight increase in 2019, the nasal carriage rate % varies from the highest 35% in 2013 and 2016 with the lowest being 15% in 2018. The results are fairly inconsistent. This can be expected due to the differences in subject size and sample population background.

Important factors such as age, gender, race/ethnicity and health status were not taken into consideration when the survey was performed. Research investigating the age and gender association with nasal carriage of *S. aureus* in Norway has found that younger age male between 30-44 years old has the highest carriage rate among the general population (Sangvik, et al., 2011). Another study in United States, has also confirmed that there is a difference in carriage rates with respect to age, sex and ethnicity (Kuehnet, et al., 2006). Moreover, the carriage rate is highly dependent on the general health condition of the tested individuals, existence of even non-influenza respiratory virus can increase the host cell susceptibility of *S. aureus* colonization (Morgene, et al., 2018).

In addition, variation in sampling as well as inoculation techniques can impact on the identification of bacteria strain. Swabbing was performed by individual student without standardization of method, failure of proper streak dilution or aseptic technique during initial inoculation and subculturing could result in no isolated colonies available for further biochemical confirmation or possible contamination. Due to time constraint, results of tube coagulase test were to be determined within 3 hours instead of the 4 hours initial check and 24 hours confirmation as stated (Microbiology teaching team, 2017). All these experimental errors could have influenced the results.

Comparing to three other similar studies carried out around the world with samples taken by nasal swabbing and confirmation of isolates using the same biochemical tests ; it shows 17.5% of positive *S. aureus* carriage in Zakho University students in Iraq (Assafi, Mohammed, & Hussein, 2015), 23.1% rate among three public school students in Yamen (AL-Haj, Hauter, Al-Bulili, Al-Hotami, & Al-Horaibi, 2018), and 21.6% rates on a cross-sectional study among the general communities in nine European countries (Heijer, et al., 2013), the average carriage rate of 25% in our survey is within the expected range even though it is slightly higher. However, besides of all the possible experimental errors and unknown factors mentioned above in our survey, the results obtained are far from being reliable and representative, mainly due to the small subject numbers of only 130 subjects were tested on average, while all the other three studies were carried out on 432, 450, 32,306 subjects respectively.

Another limitation of our survey was the absent of the subject's past and current work experiences information; previous exposure and work environment can also influence the carriage rate. One study investigating the prevalence of health care workers in Tanzania shows 41.4% carriage rate and one-third of the positive cases were confirm to be MRSA clones (Joachim, et al., 2018), while another research in Argentina has found a rate of 30% with one -fifth of those being MRSA clones (Boncompaina, Suárez, & Morbidoni, 2017) , the carriage

rates among health care workers are comparably higher than the general public. This further emphasise the importance of disease control especially within the clinical / hospital settings to reduce the chances of transmission.

Our survey results are found to be consistent to those of food handlers, the prevalence of *S.aureus* in nose of a recent survey in Portugal shows 19.8% of positive carriage and none of them was the MRSA strain (Castro, Santos, Meireles, Silva, & Teixeira, 2016); which is also within the expected range of the general community as stated above. No antibiotic resistance was tested for in the positive isolates of our survey , further investigation on MRSA clones in our subjects will be beneficial. Although the prevalence of food handlers are comparingly low, nasal carriage investigation still serves as a good indicator during a *S.aureus* food poisoning outbreak; good manufacturing practises and proper training for food handling shall be maintained to ensure the safety and quality of food products.

## Conclusion

The nasal carriage rate of *Staphylococcus aureus* among RMIT microbiology students from 2013 to 2019 in this survey is found to be consistent with the expected range of general community. However, a few major physical and environmental factors, such as age, gender, race/ethnicity and health conditions were not taken into consideration. With limitations in sampling methods and small subject size, the results should only be used as a brief screening of prevalence.

## Reference

Agostino, J. W., Ferguson, J. K., Eastwood, K., & Kirk, M. D. (2017). The increasing importance of community-acquired methicillin-resistant *staphylococcus aures* infections. *Medical Journal of Australia*.

AL-Haj, N., Hauter, J. M., Al-Bulili, N. H., Al-Hotami, R., & Al-Horaibi, M. T. (2018). Nasal Carriage of *Staphylococcus aureus* Among Students of Public Schools in Sana'a, Yamen. *Research Journal of Microbiology*, 13(1): 65-69 .

Assafi, M., Mohammed, R., & Hussein, N. (2015). Nasal Carriage Rates of *Staphylococcus aureus* and CA-Methicillin Resistant *Staphylococcus aureus* among University Students. *Journal of Microbiology Research*, 5(4):123-127.

Boncompaina, C. A., Suárez, C. A., & Morbidoni, H. R. (2017). *Staphylococcus aureus* nasal carriage in health care workers: First report from a major public hospital in Argentina. *Revesta Argentina De Microbiologia*, 49(2):125-131.

Castro, A., Santos, C., Meireles, H., Silva, J., & Teixeira, P. (2016). Food handlers as potential sources of dissemination of virulent strains of *Staphylococcus aures* in the community. *Journal of Infection and Public Health*, 153-160.

Department of Health & Human Services, S. G. (2018). *Staphylococcal infections*. Retrieved from Health Victoria : www2.health.vic.gov.au

French, G. .. (2009). Methods for screening for methicillin-resistant *Staphylococcus aureus* carriage. *Clinical Microbiology and Infection*, Volume 15, Supplement 7, Pages 10-16.

Heijer, C. D., Bijnen, E. M., Paget, J. W., Pringle, M., Gossens, H., Bruggeman, C., . . . Stobberingh, E. E. (2013). Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin-resistant S aureus, in nine European countries: a cross-sectional study. *The Lancet Infectious Diseases* , Volume 13, Issue 5, Pages 380-381.

Joachim, A., Moyo, S., Nkinda, L., Majigo, M., Rugarabamu, S., Mkashabani, E., . . . Lyamuya, E. (2018). Nasal Carriage of Methicillin-Resistant *Staphylococcus* among Health Care Workers in Tertiary and Regional Hospitals in Dar es Salam, Tanzania. *International Journal of Microbiology*.

Kluytmans , J., Belkum, V., & Verbrugh, H. (1997). Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clinical Microbiology Reviews*, 10(3):505-20.

Kuehnet, M., Kruszon-Moran, D., Hill, H., McQuillan, G., McAllister, S., Fosheim, G., . . . Chaitram, J. (2006). Prevalence of *Staphylococcus aureus* Nasal Colonization in the United States, 2001–2002. *The Journal of Infectious Diseases*, Volume 193, Issue 2, Pages 172–179.

Microbiology Teaching Team, R. U. (2019). *BIOL2158/BIOL2173 Microbiology 1 Laboratory Manual Semester 1, 2019.*

Microbiology teaching team, S. o. (2017). *GERMM Glossary and Electronic Resources of Microbiology Methods* . Retrieved from https://sites.google.com/a/rmit.edu.au/germm/home

Microbiology Unit, R. (2019). *IDENTIFICATION TABLES FOR BACTERIA.* Biosciences and Food Technology, School of Science, RMIT.

Morgene, M., Botelho-Nevers, E., Grattard, F., Pillet, S., Berthelot, P., Pozzetto, B., & Verhoeven, P. (2018). *Staphylococcus aureus* colonization and non-influenza respiratory viruses: Interactions and synergism mechanisms. *Virulence*, 9(1): 1354–1363.

Newman, T. (2017, November 13). *All you need to know about MRSA*. Retrieved from Medical News Today: https://www.medicalnewstoday.com

NSW Ministry of Health, N. G. (2019). *MRSA in the community control guideline*. Retrieved from NSW Ministry of Health: https://www.health.nsw.gov.au/Infectious/controlguideline/Pages/methicillin_res_stap.aspx

Sangvik, M., Olsen, R. S., Olsen, K., Simonsen, G. S., Furberg, A.-S., U, J., & Sollid, E. (2011). Age- and Gender-Associated *Staphylococcus aureus* spa Types Found among Nasal Carriers in a General Population: the Tromsø Staph and Skin Study. *Journal of Clinical Microbiology*.

Savage, V. J., Chopra, I., & O'Neill, A. J. (2013). *Staphylococcus aureus* Biofilms Promote Horizontal Transfer of antibbiotic resistance. *American Society for Microbiology*.

Shibabaw, A., Abebe, T., & Mihre, A. (2013). Nasal carriage rate of methicillin resistant *Staphylococcus aureus* among Dessie Referral Hospital Health Care Workers; Dessie, Northeast Ethiopia. *Antimicrob Resist Infect Control*, 2: 25.

Swannell, C. (2019). Community-acquired golden staph infections on the rise. *The Medical Journal of Australia*.

Todar, K. (2012). Todar's Online Textbook of Bacteriology. Wisconsin: University of Wisconsin .

Tur, W., Kielar, M., Kłaczkowska, A., Teodorowicz, E., & Lewczuk, A. (2010). Nasal carriage of MRSA strains among hospital employees in a surgical department. *Polish Journal of Surgery* , Volume 82: Issue 3 , page 166-169.

U.S. Department of Health & Human Services, W. D. (2019, April 2). *Staphylococcus*. Retrieved from Foodsafety.gov: https://www.foodsafety.gov/poisoning/causes/bacteriaviruses/staphylococcus/index.html

Vatansever, L., Sezer, Ç., & Bilge, N. (2016). Carriage rate and methicillin resistance of *Staphylococcus aureus* in food handlers in Kars City, Turkey. *Springerplus*, 5: 608.