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## The Prevalence of *Staphylococcus aureus* in the Oral Cavity of Healthy Adults in Malaysia

(Kelaziman *Staphylococcus aureus* pada Kaviti Oral Dewasa Sihat di Malaysia)

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### ABSTRACT

The aim of this study was to determine the prevalence of *Staphylococcus aureus* in the oral cavity of healthy adults and the factors that may influence the presence of the bacteria. A cross-sectional study was conducted on a number of selected healthy adults in a district in Malaysia, during which, information about their socio-demographic background and oral hygiene practices was obtained. Oral rinse samples of the respondents were also collected using phosphate buffered saline and the data obtained was subsequently analyzed using SPSS. A total of 140 oral rinse samples were collected and the results of the analysis conducted showed a prevalence of approximately 40% of the *Staphylococcus aureus* in the oral cavity of the participants. There was no significant association observed between both the socio-demographic factor and oral hygiene practices with the presence of *Staphylococcus aureus*. The use of prostheses was found to be a significant factor for a higher prevalence of *Staphylococcus aureus* in the oral cavity (OR 5.13;  $P < 0.05$ , 95%CI 1.410 - 18.76). The prevalence of *Staphylococcus aureus* in the oral cavity of healthy adults was high and the use of prostheses was a factor associated with the presence of the bacteria. This accentuates the importance of a good oral hygiene, as oral cavity can be the primary route for *Staphylococcus aureus* to cause potential systemic infections.

Keywords: Adults; infection; oral cavity; oral health; *Staphylococcus aureus*

### ABSTRAK

Tujuan kajian ini adalah untuk mengenal pasti prevalen bakteria *Staphylococcus aureus* di dalam rongga mulut orang dewasa yang sihat dan faktor yang mungkin mempengaruhi kewujudan bakteria tersebut. Suatu kajian keratan lintang telah dijalankan ke atas sejumlah orang dewasa yang sihat di satu daerah di Malaysia dan semasa kajian tersebut, maklumat mengenai latar belakang sosio-demografi dan amalan-amalan penjagaan kebersihan mulut peserta telah diperolehi. Larutan bergaram tertimbang fosfat iaitu sampel air kumuran daripada peserta dikumpulkan dan data yang diperolehi seterusnya dianalisis menggunakan perisian SPSS. Sebanyak 140 sampel dikumpul dan hasil kajian menunjukkan peratusan prevalen *Staphylococcus aureus* di dalam rongga mulut peserta setinggi 40%. Selain itu, didapati bahawa tidak wujud perkaitan signifikan antara kedua-dua faktor sosio-demografi dan amalan kebersihan mulut dengan kehadiran *Staphylococcus aureus*. Sementara itu, kajian juga menunjukkan bahawa protesis gigi ialah faktor penyumbang utama kepada peratusan tinggi prevalen *Staphylococcus aureus* di dalam rongga mulut (OR 5.13;  $P < 0.05$ , 95% CI 1.410 – 18.76). Prevalen *Staphylococcus aureus* di dalam rongga mulut orang dewasa yang sihat adalah tinggi dan protesis gigi ialah faktor yang dikaitkan dengan kewujudan bakteria tersebut di dalam rongga mulut. Ini menegaskan lagi betapa pentingnya kebersihan mulut yang baik, kerana rongga mulut boleh menjadi laluan utama bagi *Staphylococcus aureus* yang akan menyebabkan kemungkinan berlakunya jangkitan sistemik.

Kata kunci: Dewasa; jangkitan; kesihatan mulut; rongga mulut; *Staphylococcus aureus*

### INTRODUCTION

Among staphylococci, *Staphylococcus aureus* is one of the most common micro-organisms that can cause opportunistic infections, such as hospital-acquired infections and community-acquired infections (Davis et al. 2007; Harbarth et al. 2008). *Staphylococcus aureus* is part of human microflora capable of inflicting potential life-threatening infections such as pneumonia, urinary tract infection, bloodstream infection and infective endocarditis (Irek et al. 2018). Furthermore, studies have proven *S. aureus* and gram-negative bacilli to be the dominant oral pathogen in patients under intensive care treatment and

elderly patients under chronic care (Russell et al. 1999; Scannapieco & Cantos 2016). The risk of infection caused by *S. aureus* increases in patients suffering from dermatologic conditions (Fridkin et al. 2005), intravascular catheters (Hetem et al. 2011) and diabetics (Mantey et al. 2000). The same effect is observed in immunocompromised patients (Klevens et al. 2006), surgical patients (Kim et al. 2010) and health-care workers (Albrich & Harbarth 2008). The increasing prevalence of the resistant *S. aureus* at the hospital level has raised concerns about its occurrence at the community levels. Treatments against *S. aureus* infection can be complicated due to the increase in the

number of multi-drug resistant strain of *S. aureus*, both at the hospital and the community levels (Wertheim et al. 2005).

Nasal cavity has long been considered as the main reservoir for *S. aureus* (Sakr et al. 2018; Von Eiff et al. 2001). A study on healthy individuals reported that, a total of 36.4% of *S. aureus* was isolated from nasal area, while 12.4 % of the bacteria was isolated from the throat area (Mertz et al. 2009). The same trends were also found in a hospital-based study, where approximately half of the patients on admission and health care workers had a higher prevalence of *S. aureus* in the nasal area, compared to the throat area (Nilsson & Ripa 2006). The prevalence of *S. aureus* in a hospital setting varied in the range of 45 % to 80 % (Norazah et al. 2003). Besides nasal, skin and throat areas, oral cavity has also been reported as one of the potential reservoirs for *S. aureus*, despite its being considered as being transient and not permanent in nature (Koukos 2015; Marsh 2000). However, there is a concern that it may potentially become the primary reservoir for *S. aureus* (Smith et al. 2001). Studies on the oral cavity among healthy children and adults in other regions reported that, the prevalence of *S. aureus* was rather high, in the range of 33% to 64%, and 4% to 36%, respectively (Jackson 2000; Smith et al. 2001).

With regards to oral infections, a review on oro-facial bacterial infections diseases reported that, *S. aureus* was associated with facial cellulitis and a bullous type of facial infections around the nose and the mouth (Irani 2017). *S. aureus* has also been reported to be associated with other oro-facial infections, such as, angular cheilitis (Oza & Doshi 2017; Smith et al. 2003), denture wearers (O'Donnell et al. 2016a), dry mouth (Murakami et al. 2015) and acute dentoalveolar abscess (Bahl et al. 2014). Similar studies have shown that, *S. aureus* in the oral cavity may lead to systemic infections and life-threatening events, such as, infective endocarditis (Lockhart et al. 2009), rheumatoid arthritis (Friedlander 2010; Jackson et al. 1999) and aspiration pneumonia (Terpenning et al. 2001). The risk of infections increases with the increase in the number of colonization, thus altering oral and general health conditions.

Therefore, this study aimed to determine the prevalence of *S. aureus* in the oral cavity of healthy adults and to ascertain the association between the socio-demographic factor and oral hygiene practices with the presence of *S. aureus* in the oral cavity.

## MATERIALS AND METHODS

### DESIGN AND SAMPLING

This study was a cross-sectional study conducted on a group of healthy adults in Ampang Jaya district, Kuala Lumpur, Malaysia. Convenience sampling was used in this study because the recruitment was done at a Municipal Centre which is easily assessable and has a reasonable

logistic. The centre is open to the residents living in the district, five to six days a week. Furthermore, the study had a limited time frame. Therefore, those who had fulfilled the set criteria were invited to participate in the study. As their involvement for the study was on a voluntary basis, only those who agreed to participate in the study were enrolled. There were two main inclusion criteria for the selection of the participants, namely, healthy adults (aged 18 years old and above) and no records of being hospitalized or having taken any antibiotics for the past three months. Hospitalizations may expose a person to nosocomial bacteria, while taking antibiotics, such as, Penicillin or Erythromycin may influence the number of existing bacteria in the oral cavity (Costelloe et al. 2010; Khan et al. 2017).

### SETTING

Prior to the commencement of the study, the ethical approval was obtained from the Ethics Committee of Faculty of Dentistry (USIM/FPg-MEC/2016/no. (43)), Universiti Sains Islam Malaysia, Kuala Lumpur, research registration number PPPI/TB/FPG/18618. The respondents were from Ampang Jaya district, Kuala Lumpur and the recruitment was made at Menara MPAJ, Ampang Jaya Municipal Council, Kuala Lumpur, prior to which, the required permission was obtained from the Municipal Council office. A booth was set-up at the main lobby area which provided basic facilities during the duration of the study (July 2018 - October 2018). Each of the participants was provided with an information sheet explaining the study process, together with an informed consent form. Only those who signed the form in the affirmative proceeded to participate in the study. The research was conducted in full compliance with the World Medical Association Declaration of Helsinki. The participants were assured that their data would be kept confidential.

### SAMPLE SIZE

The minimum sample size required for this study was determined based on a previous study conducted on nasal carriage of *S. aureus* in Malaysia (Choi et al. 2006). As the oral cavity is not the main reservoir for the bacteria, the percentage of *S. aureus* in the oral cavity was assumed to be less than that in the nasal area. Therefore, a percentage of approximately 10 % was set up for the sample size calculation. The minimum sample size required for this study was 140, with a 95 % confidence interval.

### DATA COLLECTION

Information about the socio-demographic background and oral hygiene practices of the participants was obtained via a self-administered question form. Questions on socio-demographic background were about gender, age, ethnic, and occupation, while those on oral hygiene practices were about the date of the most recent dental visit, wearing a

dental prosthesis (such as a removable or fixed dental prosthesis), frequency of tooth brushing, type of toothpastes, and additional oral aids used, such as mouthwash and floss. It was also determined in the study if the parents of the respondents had systemic diseases, to look for possible associations between parent's background and the presence of *S. aureus*. Besides, a question about the relationships between oral health and general health was asked to determine their awareness level on the associations between them.

#### MICROBIOLOGICAL PROCEDURES

Oral rinse technique was used for sample collection, where, a freshly-prepared volume of 10 mL of sterile phosphate buffered saline solution (0.1M, pH 7.2) was provided to each of the participants. They were subsequently required to hold the solution in their mouths for 60 s before expelling it into a sterilized conical tube (Falcon, UK) (Loberto et al. 2004; Samaranayake et al. 1986). Each of the tubes was immediately labelled and placed in an icebox to be transported to the laboratory. This was followed by a microbiology culturing process, conducted within a period of three hours, in the microbiology laboratory of the medical faculty (Loberto et al. 2004). Serial dilutions were performed tenfold for each sample with sterilized phosphate buffered saline (0.1M, pH 7.2) and 100  $\mu$ L of the diluted samples was aseptically plated onto mannitol salt agar (MSA) (Oxoid, UK), before it was subsequently incubated for 48 h at 37°C. The test was performed in duplicates to ensure an optimal fidelity. The number of yellow colonies with yellow zones formed was counted for each media plate with the aid of a colony counter. Following this, the colony-forming units per milliliters of samples (cfu/mL) were calculated. The prevalence and the total counts of viable *S. aureus* were reported based on the limit of quantification (or the lowest amount of growth that can be quantitatively determined under experimental conditions) and the limit of detection (or the lowest amount of growth that can be detected in the sample) (Tortora et al. 2016).

#### DATA ANALYSES

Data was collected and subsequently analyzed using the *Statistical Package for the Social Sciences* (SPSS), version 23.0 (SPSS Inc. Chicago, IL, USA). Descriptive statistics for categorical data were produced for socio-demographic and oral hygiene practices. Bivariate analyses using *Chi-square* tests ( $\chi^2$ ) were performed to assess factors associated with the presence of *S. aureus*. A two-tailed *P-value* < 0.05 was considered statistically significant. Meanwhile, multivariate logistic regression analyses were carried out to determine the factors that could possibly be associated with the presence of *S. aureus* in the oral cavity. Finally, the relationships between the independent variables and dependent variables were determined by odds ratios (OR).

## RESULTS AND DISCUSSION

### RESULTS

A total of 300 healthy adults were approached during the recruitment at the Municipal centre, 140 of whom agreed to participate in the study. The profiles of the subjects are presented in Table 1. The majority of the participants were Malay (91.4%, 95% CI=85.5, 95.5) and more than half of them were female (59.3%, 95% CI=50.7, 67.5). Slightly over a quarter of them were aged 36 years old and above, with the mean age of 30.8 (27.9%, 95% CI=20.6, 36.1) and only about 10% of them were dental prostheses wearers (95% CI=5.6, 16.2). A high percentage of them claimed that, they brushed their teeth at least twice a day (87.9%, 95% CI=81.3, 92.8) and used fluoridated toothpastes (77.9%, 95% CI=70.1, 84.4). Less than half of them claimed that, their parents had systemic diseases (39.3%, 95% CI=31.1, 47.9). Approximately a quarter of them were found to have either cardiovascular or respiratory diseases (29.3%, 95% CI=21.9, 37.6). Less than three quarters of the participants (67.9%, 95% CI=59.4, 75.5) were aware of the associations between oral health and general health.

Chi-square ( $\chi^2$ ) analyses were performed to identify the associations between the presence of *S. aureus* with socio-demographic background and oral hygiene practices. There was no significant relationship found between the socio-demographic background and oral hygiene practices ( $P>0.05$ ). Similarly, there was also no association found between the presence of *S. aureus* and the background of the parents ( $P>0.05$ ). Nonetheless, there was a significant difference between the presence of *S. aureus* and the use of dental prostheses ( $P=0.022$ ).

Almost half of the population studied (41.4%, 95% CI=33.2, 50.1) were found to have at least  $3.0 \times 10^2$  cfu/mL for the limit of quantification, while a high percentage of them (76.4%, 95% CI=68.5, 83.2), were found to have more than  $1.0 \times 10^2$  cfu/mL for the limit of detection (Table 3).

In the adjusted regression analyses, having a dental prosthesis was found to be the key factor significantly associated ( $P=0.013$ ) with the presence of *S. aureus*, based on the limit of quantification (Table 4). Dental prostheses wearers were found to have the likelihood to be associated with the presence of *S. aureus* five times higher, compared to those who were not (OR 5.13, 95% CI 1.41, 18.76,  $P<0.05$ ). In terms of the level of detection, none of the factors was significantly associated with the presence of *S. aureus* in the oral cavity.

### DISCUSSION

The carriage of *S. aureus* may be harmful as it has the potential to cause life threatening consequences. Most studies have focused on the nasal carriage as the main reservoirs for *S. aureus*, oblivious of the potential harms caused by the oral carriage as one. This study showed that, the prevalence of *S. aureus* in the oral cavity of healthy

TABLE 1. Participants profile

N=140	Number (n)	Percentage (%)
Gender		
Male	57	40.7
Female	83	59.3
Ethnic group		
Malay	128	91.4
Others	12	8.6
Age		
35 years old and less	101	72.1
36 years old and more	39	27.9
Employment		
Gov/Private sector	96	68.6
Pensioner/Unemployed	44	31.4
Last dental visit		
One year and less	73	52.1
More than one year	67	47.9
Prostheses		
None	126	90.0
Presence	14	10.0
Brushing frequency		
Less than twice a day	17	12.1
Twice or more a day	123	87.9
Type of toothpaste		
Fluoridated	109	77.9
Non-fluoridated	31	22.1
Additional oral aids		
None	73	52.1
Present	67	47.9
Parents had/having systemic diseases		
None	85	60.7
Present	55	39.3
CVD or respiratory disease		
No	99	70.7
Yes	41	29.3
Association between oral and general health		
No	45	32.1
Yes	95	67.9

adults is high, with approximately 40 % of them at the level of quantification and more than three quarters (76.4%) at the level of detection, indicating oral cavity to be another potential reservoir of *S. aureus* in human. These results are consistent with those found in a previous study on oral cavities of healthy adults in Japan, with a slightly higher percentage of 46 % (Ohara-Nemoto et al. 2008). It was also observed that, the prevalence of *S. aureus* in the oral cavity was in the range of approximately 33 % in the dental plaque, to 47% in the saliva. The same study reported that, the prevalence of *S. aureus* from nasal swab was high (45 %). However, the samples were collected from those who had positive oral *S. aureus*. In contrast, a study in Malaysia found a lower prevalence of *S. aureus*, at approximately 23 % from the nasal swab of healthy adults (Choi et al. 2006). Thus, this indicates that, the prevalence of *S. aureus* in the oral cavity is higher compared to that in the nasal

area among healthy adults, and the bacteria can colonize and cause serious potential problems if the conditions permit.

A number of studies have reported that, *S. aureus* has been frequently isolated from the oral cavity of particular groups, namely, frail people, such as sick children (Ullman et al. 2011), the elderly (Abe et al. 2001), stroke patients (Ab Malik et al. 2017), rheumatoid arthritis patients (Smith et al. 2001) and terminally-ill individuals (Ullman et al. 2011). In general, the prevalence of *S. aureus* was higher among them, as compared to their healthy peers, regardless of the colonization site (Miyake et al. 1991; Norazah et al. 2003). The harbour of *S. aureus* in the hospital setting has also been reported to be higher, as compared to the community setting (Lewis et al. 2015a).

The high detection rate of *S. aureus* in the oral cavity has been found in an approximately three quarters of the

TABLE 2. Association between presence of *S. aureus* and participant's profiles

Factor	<i>Staphylococcus aureus</i>		$\chi^2$ statistics (df)	p-value
	Positive No. (%)	Negative No. (%)		
Gender				
Male	25 (43.9%)	32 (56.1%)	0.234	0.727
Female	33 (39.8%)	50 (60.2%)	(1)	
Ethnic group			0.000	
Malay	53 (41.4%)	75 (58.6%)	(1)	0.608
Others	5 (41.7%)	7 (58.3%)		
Age			0.104	
Less than 35 yr. old	41 (40.6%)	60 (59.4%)	(1)	0.849
35 yr. old and more	17 (43.6%)	22 (56.4%)		
Employment				
Gov/Private sector	40 (41.7%)	56 (58.3%)	0.007	1.000
Pensioner/Unemployed	18 (40.9%)	26 (59.1%)	(1)	
Last dental visit				
One year and less	30 (41.1%)	43 (58.9%)	0.007	1.000
More than one year	28 (41.8%)	39 (58.2%)	(1)	
Prostheses				
None	48 (38.1%)	78 (61.9%)	5.770	0.022*
Presence	10 (71.4%)	4 (28.6%)	(1)	
Brushing frequency				
Less than twice a day	10 (58.8%)	7 (41.2%)	2.413	0.188
Twice or more a day	48 (39.0%)	75 (61.0%)	(1)	
Type of toothpaste				
Fluoridated	46 (42.2%)	63 (57.8%)	0.121	0.837
Non-fluoridated	12 (38.7%)	19 (61.3%)	(1)	
Additional oral aids				
None	34 (46.6%)	39 (53.4%)	1.665	0.231
Present	24 (35.8%)	43 (64.2%)	(1)	
Parents had/having systemic diseases				
None	35 (41.2%)	50 (58.8%)	0.006	1.000
Present	23 (41.8%)	32 (58.2%)	(1)	

TABLE 3. Percentage of *S. aureus* based on quantification and detection level

N=140	Number (n)	Percentage (%)
Limit of quantification		
< $3.0 \times 10^2$ cfu/mL	82	58.6
$\geq 3.0 \times 10^2$ cfu/mL	58	41.4
Limit of detection		
$\leq 1.0 \times 10^2$ cfu/mL	33	23.6
$> 1.0 \times 10^2$ cfu/mL	107	76.4

subjects, a matter of serious concerns. A study conducted on healthy children reported that, the persistent presence of *S. aureus* in the oral cavity may further serve as a reservoir for Methicillin-Resistant *Staphylococcus aureus* (MRSA), which has the potential to cause nosocomial infections (Suzuki et al. 1997). Therefore, oral colonizations by potential respiratory pathogens, such as *S. aureus*, may influence the beginning and progression of systemic infections, mainly in high-risk individuals (Paju & Scannapieco 2007).

Both factors of socio-demographic background and oral hygiene practices were not significantly associated

with the presence of *S. aureus* in the oral cavity. This finding was in agreement with that in a similar study on nasal carriage in Malaysia, also involving predominantly Malay subjects, where there was no significant associations found between the presence of *S. aureus* in the nasal area and age, gender as well as ethnicity (Choi et al. 2006). In this study, having a dental prosthesis was found to be associated with the presence of *S. aureus* in the oral cavity. This finding is well in agreement with that in a previous report, where the use of dental prostheses caused an increase in the number of pathogens in the oral cavity and among the pathogens, *S. aureus* was persistently present



TABLE 4. Factors associated with presence of *S. aureus* in the oral cavity

N=140	B	SE	OR	95%CI	P-value
<i>Socio-demographic</i>					
Gender					
Female	0.02	0.41	1.02	0.46, 2.25	0.963
Male					
Ethnic					
Malay	0.33	0.68	1.40	0.37, 5.33	0.625
Others					
Age					
Less than 35	0.05	0.47	1.05	0.42, 2.68	0.912
36 and above					
Occupation					
Unemployed	0.06	0.45	1.06	0.44, 2.57	0.888
Employed					
<i>Oral hygiene</i>					
Last dental visit					
More than 1 year	0.00	0.39	1.00	0.47, 2.13	1.000
Less than 1 year					
Dental Prostheses					
Yes	1.64	0.66	5.13	1.41, 18.76	0.013*
No					
Brushing frequency					
Twice and more	-0.96	0.58	2.67	1.12, 1.21	0.102
Less than twice a day					
Toothpaste					
Non-fluoridated	-0.24	0.44	0.78	0.33, 1.88	0.586
Fluoridated					
Oral aids					
Yes	0.42	0.38	1.52	0.73, 3.19	0.267
No					
<i>Parents illnesses</i>					
Having systemic diseases					
Yes	-0.01	0.38	0.99	0.48, 2.09	0.993
No					

at the different stages of assessments (i.e. one month, six months and more than 12 months) (Nair et al. 2016). Prostheses have also been considered as potential reservoirs for respiratory pathogens (Lewis et al. 2015b; O'Donnell et al. 2016b). Therefore, good oral hygiene and proper prosthesis care are significantly important among healthy adults. Findings from regression analyses showed that having a dental prosthesis is the factor associated with the presence of *S. aureus* in the oral cavity. This implies that, the oral hygiene related to dental prostheses is crucial.

A 10-year retrospective laboratory study showed that, out of eleven thousand specimens, two thousands of them (18%) were found to contain *S. aureus*. Most of the *S. aureus* was isolated through oral rinse technique and followed by a tongue swab (McCormack et al. 2015). In this study, mannitol salt agar (MSA) was utilized in the microbiological procedures as a selective and differential medium for the growth of pathogenic *S. aureus*, as recommended by American Public Health Association (1993). It contains 7.5 % sodium chloride as a selective

agent for the isolation of staphylococci, which serves to inhibit most organisms, except staphylococci. In addition, the incorporation of phenol red indicator enabled this medium to display the capability of *S. aureus* to ferment mannitol (Chapman 1945). Fermentation of mannitol will result in acid production and the formation of yellow zone colonies. The non-fermenters staphylococci colonies will exhibit a purple or red zone.

There were some limitations to this study, despite its being population-based in nature. Firstly, the oral hygiene practices could not be directly related to the prevalence of *S. aureus*, as no clinical examination was performed. Therefore, further studies are required to determine the actual relationships between the oral hygiene status and the *S. aureus* oral carriage among healthy adults. Secondly, the self-administered questionnaire was used to collect information on socio-demographic and oral hygiene practices only. Even though the presence of oral carriage of *S. aureus* may vary in different population, geographical areas and under different measurement approaches (Choi

et al. 2006), the general findings showed that, the prevalence of *S. aureus* in the oral cavity was higher than that in the nasal site.

#### CONCLUSION

The high prevalence of *S. aureus* in the oral cavity among healthy adults is a major concern, as it may pose significant community-associated infections and increase the strains that are resistant to antibiotics at the community levels. Furthermore, it may also increase the risk for MRSA infection at the community level. Identifying the oral condition with *S. aureus* colonization could target the high-risk population with interventions that may help to reduce the risk for subsequent *S. aureus* infections, in the form of oral or systematic infections.

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