

Distribution of Hyaluronidase-producing *Staphylococcus aureus* and *Staphylococcus epidermidis* Isolated from Palm Skin and Anterior Nares of Healthy Malaysian Adults

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Received

17th December 2019

Received in revised form

2nd March 2020

Accepted

9th March 2020

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ABSTRACT

Introduction: The distribution of *Staphylococcus aureus* and *Staphylococcus epidermidis* among Malaysian healthy adults and their capability to produce enzyme hyaluronidase are less reported. Hyaluronidase degrade hyaluronic acid in animal connective tissue and facilitate bacterial spreading in host body. This study aims to identify the distribution of both *Staphylococci* species in healthy subject, the hyaluronidase producer among the isolates and the association of the latter with site of isolation (palm skin and anterior nares) and gender of the host. **Methods:** A total of 108 swab samples were collected from anterior nares and palm of 54 healthy subjects. The bacteria were identified through microscopic and biochemical tests, before screened for hyaluronidase production using hyaluronic acid diffusion rapid plate method. **Results:** Total of 139 bacterial isolates were identified; 68 isolates are *S. aureus*, 63 *S. epidermidis* and 8 other bacterial species. *Staphylococcus aureus* was highly isolated from palm (57%) than anterior nares (47%). On the contrary for *S. epidermidis* was highly isolated from anterior nares (53%) than from palm skin (43%). Equal proportion was found for both species in male and female subject. A total of 77 (59%) isolates produced hyaluronidase; 55% are *S. aureus* and 45% are *S. epidermidis*. Hyaluronidase-producer isolates are equally found between anterior nares (56%) and palm skin (61%) or male (57%) and female subject (60%) regardless of *Staphylococcal* species. No significant value was recorded for any analysis. **Conclusion:** Capability of commensal *S. aureus* and *S. epidermidis* isolated from healthy subject to produce hyaluronidase may indicate their potential as opportunistic pathogen whenever the opportunity arises in any way.

KEYWORDS: *Staphylococcus aureus*, *Staphylococcus epidermidis*, hyaluronidase, skin, anterior nares, gender

INTRODUCTION

Staphylococcus aureus and *Staphylococcus epidermidis* are frequently found on human skin, anterior nares, and mucous membrane as commensal or normal flora [1, 2]. On human body, *S. aureus* was reported to colonize almost 30% of healthy adults [3]. This bacterium which could be present as commensal or pathogen can cause variety of infections including skin and soft tissue infections, bacteraemia, endocarditis and device-related diseases [4, 5]. Based on Malaysia National Surveillance of Antimicrobial Resistance, total *S. aureus* isolates recorded in few hospitals in Malaysia range from 37 000 to 40 000 isolates in 2016 to 2017 [6]. In contrast to *S. aureus*, the coagulase positive *Staphylococci* which frequently studied because it

causes severe infections, coagulase negative *Staphylococci* are less reported to cause severe diseases [1]. However, increasing number of infections caused by coagulase negative *Staphylococci* recently trigger the interest of researchers to understand the pathogenicity of this bacterial group [7, 8]. Among more than 40 species of coagulase negative *Staphylococci*, *S. epidermidis* is the most frequent species related to clinically significant infections especially implant- or device-related infections [9]. In 2009, approximately 34% of total 2354 *staphylococci* isolates recorded in one of University teaching hospital in Malaysia are caused by *S. epidermidis* [10]. Prevalence data of *S. aureus* and *S. epidermidis* are more established among patients or clinically-related

subject [11, 12]. Although the clinical isolates always being targeted in pathogenesis study due to the disease-agent relationship, contribution of normal flora or commensal strain to the invasive diseases are evidenced, with limited source of reference [13].

Hyaluronidase is a class of enzyme that has ability to breakdown hyaluronic acid [14]. There are three groups of hyaluronidases which are categorized based on enzymatic reaction products; hyaluronate-4-glycanohydrolases, hyaluronate-3-glycanohydrolases, and hyaluronate lyases. Hyaluronate-4-glycanohydrolases are found in mammalian spermatozoa, lysosomes, venoms of various insects and snake while hyaluronate-3-glycanohydrolases are found in leeches and some hookworms [15]. Hyaluronate lyase which is also known as microbial hyaluronidase was found in various bacterial species and fungi [16]. The substrate for hyaluronidase, hyaluronic acid (HA) is an essential component in animal connective tissue particularly in extracellular matrix (ECM). This substance is abundantly present in human skin and other tissues including umbilical cords, synovial fluid, vitreous of eyes, heart valves and lung epithelial cells [17, 19, 20]. It plays a role in various biological functions including initiate cellular signaling, provide viscoelasticity to synovial fluid and vitreous humor of eye and control tissues hydration and transportation of water molecules [21].

Degradation of HA by hyaluronidase increases the tissue permeability thus aid for greater diffusion and spreading of the pathogen into deeper host tissues [22]. Therefore, it has been used in various field including therapeutic uses in drug delivery for cancer treatment, cosmetic dermatology, aesthetics practice and ophthalmic surgery [23]. In bacteria, hyaluronidase act as virulence factor since it facilitates the invasion of the organisms or their secreted products into human tissue. In most of the studies, hyaluronidase was investigated from pathogenic bacterial strain, where the function of this enzyme in bacterial colonization and invasion were evaluated [23]. As compared to pathogenic isolates, less attention was paid on commensal Staphylococci isolates. It is unclear if the enzyme also have the same effect in commensal strain, due to less study performed on these isolates. Therefore, studying this enzyme among commensal will extend the understanding of

hyaluronidase potentiality to increase risk of commensal Staphylococci to become pathogenic strain causing invasive diseases.

Previous studies suggest that bacterial hyaluronidase production is associated with the presence of the substrate in the surroundings [16]. Since the HA level are differently produced in different gender and different parts of human body therefore, it is interesting to study the production or activity of hyaluronidase in bacteria residing the skin and respiratory site of the host with different gender [24, 25].

MATERIALS AND METHODS

Ethical Approval

The study was conducted in International Islamic University Malaysia (IIUM) Kuantan campus involving subject of undergraduate students fulfilling the inclusion and exclusion criteria. The ethical approval was obtained from IIUM Research Ethics Committee (IREC) (IREC 2017-042). Consent was taken from all subjects prior to sampling.

Sample Collection and Bacterial Identification

Swab samples were collected from 54 healthy male (n=21) and female (n=33) adults, in the age range of 20 to 40 years old. The sampling was done within six month period, where the sample done was taken once from each subject. This voluntary basis study excluded individuals who have skin and throat infections and /or under antibiotic treatments within one month prior to sample collection. The sample size was calculated (www.surveysystem.com) by considering 95% confidence level and 10% confidence interval with estimation of 3570 population size (the Kuantan campus population). The targeted sample size is 94 subjects (excluding 10% withdrawal rates). However, only 54 subjects agreed to join the study and proceeded to the analysis. Palm skin and anterior nares of all subjects were swabbed by using sterile cotton bud and streaked on Mannitol Salt agar plate (MSA) (Merck, USA) before incubated at 37°C for 24 hours. All the grown colonies on agar plate were identified by Gram staining and standard biochemical tests including catalase, coagulase and novobiocin sensitivity test. Confirmed *S. aureus* and *S. epidermidis* isolates were

stored in 10% glycerol stock with Brain Heart Infusion (BHI) as culture medium.

Hyaluronic Acid Agar Preparation

Hyaluronic acid (HA) agar plate was prepared by using BHI as culture medium (BHI-HA agar) following method of Smith and Willet [26]. The BHI agar powder (14.1g) (Oxoid, UK) were dissolved in 210ml distilled water and autoclaved at 121°C. The agar medium then cooled to 46°C in water bath and added with 30ml of sterilized Bovine Serum Albumin (BSA) (10%) to give a final concentration of 1%. Then, 60ml of 2mg/ml sterilized sodium hyaluronate (ACROS Organic, USA) were added to the mixture to a final concentration of 400mg/ml. The molten media were swirled for homogeneous mixture and poured into petri dishes and left to solidify. The agar plates were incubated at 37°C for 24 hours prior to store at 8°C.

Screening of *S. aureus* and *S. epidermidis* Producing Hyaluronidase

One loopful of 24 hours incubated bacterial colony was streaked on BHI-HA agar in a circular motion around 1cm in diameter and incubated at 37°C for 15 to 18 hours. Acetic acid (2N) were flooded onto the plate for 10 minutes before observed. The halo zone surrounding the colony indicate the presence of hyaluronidase. The positive and negative control experiment was performed using commercial hyaluronidase enzymes (from bovine testes) (Sigma-Aldrich, USA) and culture broth without bacterial inoculum respectively. The number of hyaluronidase producer among *S. aureus* and *S. epidermidis* was compared to each other by taking the factor of different site of isolation and gender of the host. The data were statistically analyzed through chi-square test by using SPSS software. *p*-value of less than 0.05 was considered as significant value.

RESULTS

The Distribution of *S. aureus* and *S. epidermidis*

In this study, 139 bacterial isolates were isolated from two sites (palm skin and anterior nares) of 54 healthy adults. Sixty-eight (account for 49%) of them are *S. aureus*, 63 (45%) are *S. epidermidis* and remaining 8 isolates are other species. *Staphylococcus aureus* were

highly found on palm skin (57%), while *S. epidermidis* highly found in anterior nares (53%) (Table 1). Similarly, a slightly similar distribution was identified for both bacterial species in male and female host. No significant differences were identified for the distribution of *S. aureus* and *S. epidermidis* in different site of isolation or in different gender of the host (Table 1).

Table 1 Distribution of *Staphylococcus aureus* and *Staphylococcus epidermidis* according to site of isolation and gender of the host

Variables		<i>S. aureus</i> , n (%)	<i>S. epidermidis</i> , n (%)	<i>p</i> -value
Site of isolation	Anterior nares	30 (47%)	34 (53%)	0.260
	Palm skin	38 (57%)	29 (43%)	
Gender	Male	22 (45%)	27 (55%)	0.214
	Female	46 (56%)	36 (44%)	

Hyaluronidase Production by *S. aureus* and *S. epidermidis*

In total, 77 out of 131 (58.8%) isolates are positive for hyaluronidase production, regardless of Staphylococci species. In specific, higher number of hyaluronidase-producing isolates was observed; accounting 62% in *S. aureus* and 56% in *S. epidermidis*, than non-producing isolates. In term of sites of isolation, 56% and 61% positive hyaluronidase isolates were identified from anterior nares and palm skin, respectively. Similarly, almost similar percentage of positive hyaluronidase producer were identified from both males (57%) and females (60%) subjects. Significant association neither identified between hyaluronidase production and bacterial species, site of isolation nor gender of the host (Table 2).

Table 2 Hyaluronidase production by *Staphylococcus aureus* and *Staphylococcus epidermidis* based on site of isolation and gender of the host

Variables		Hyaluronidase -producer	Hyaluronidase non-producer	<i>p</i> - value
Bacteria	<i>S. aureus</i>	42 (62%)	26 (38%)	0.362
	<i>S. epidermidis</i>	35 (56%)	28 (44%)	
Site of isolation	Anterior nares	36 (56%) ^a	28 (44%)	0.353
	Palm skin	41 (61%) ^b	26 (39%)	
Gender	Male	28 (57%) ^c	21 (43%)	0.509
	Female	49 (60%) ^d	33 (40%)	

^a*S. aureus* - 18 isolates; *S. epidermidis* - 18 isolates
^b*S. aureus* - 24 isolates; *S. epidermidis* - 17 isolates
^c*S. aureus* - 13 isolates; *S. epidermidis* - 15 isolates
^d*S. aureus* - 29 isolates; *S. epidermidis* - 20 isolates

DISCUSSION

The Distribution of *S. aureus* and *S. epidermidis*

In this study, the proportion of *S. aureus* isolated from palm skin (57%) are higher than anterior nares (47%) (Table 1). *Staphylococcus aureus* are more likely to be isolated from anterior nares than other non-sterile sites including hand or forehead [27, 28], while *S. epidermidis* which typically found on human skin were found higher in anterior nares (53%) compared to the palm skin (43%). It is hard to claim strong contradiction between current and previous study due to small sample size and the low frequency of sampling in each subject. However, the bacterial distribution of specific species in human body depends on their preferences to reside different anatomical niches. It depends on their capability to survive and suit to specific condition, for example, oxygen availability and temperature. Bacteria with flexible metabolic capacity are able to withstand several situations, thus favor them to inhabit particular body surfaces [24]. Forbes et al. [29] stated that *S. aureus* is the commonest bacteria isolated from human skin, anterior nares and nasopharynx while *S. epidermidis* is likely to inhabit at skin and mucosa membrane. Factor that enhance the colonization of Staphylococci species at sweat gland on the skin basically is due to their capability to survive in high salt concentration [1, 30].

In terms of gender, percentage of both Staphylococci was found to be equally distributed among male or female host, where higher number of isolates were observed among female subjects (Table 1). In addition to the uneven sampling size between male and female subjects (more samples), the bacterial distribution could be due to the usage of cosmetic which typically applied by females. Cosmetic ingredients or compounds may affect the difference in skin properties thus indirectly suggest its contribution to different bacterial colonization [31]. Yurkovetskiy et al. [32] stated that the elements such as hormone metabolism, hair growth, surface pH or fat accumulation might contribute to the differences of resident microbiota in different genders. Sebum secretion which is greatly produced by male are linked to the higher number of *Propionibacterium* bacteria [33]. Such factor is yet to

be investigated towards both *S. aureus* and *S. epidermidis*. However, this study found no significant association ($p = 0.214$) between the distribution of both *S. aureus* and *S. epidermidis* and gender of the host (Table 1). This result may suggested that both Staphylococci species are equally distributed and suit to both sexes. However, the mechanisms concerning the difference in the prevalence of commensal bacteria between male and female remain unexplained.

Hyaluronidase Production by *S. aureus* and *S. epidermidis*

Hyaluronidase is also known as spreading factor due to its capability to degrade hyaluronic acid (HA) [34]. Degradation of HA by hyaluronidase in the snake venom decrease tissue viscosity while increase tissue permeability, thus allow the spreading of the toxin [35]. In similar mechanism, bacterial hyaluronidase facilitates spreading of bacteria and other molecules to many parts in human and animal body [36]. Previous studies examined the presence of hyaluronidase in various Staphylococcal species, where many of the hyaluronidase producer were detected among Staphylococcal strains with positive coagulase and DNase activity [37, 38]. However, the association of both characteristics with hyaluronidase production remain unexplained. This recent study supported findings by Hart et al. [39] who was found 98% of *S. aureus* strain produced hyaluronidase, albeit lower percentage for the former (68%). Interestingly, the similar study found no hyaluronidase producing among tested *S. epidermidis* which is contrast to ours. In early years of hyaluronidase discovery, a big difference between coagulase positive and negative staphylococci producing hyaluronidase; 217/218 (99.5%) and 1/150 (0.7%) respectively, was observed by Essers and Radebold [38] suggest that coagulase could be a possible factor that may be associated with the hyaluronidase production. However, in recent years, hyaluronidase has been reported in various coagulase staphylococci isolates. The exact contribution of hyaluronidase in *S. epidermidis* pathogenicity are undervalued due to less study was performed on this bacterium [1]. High production of hyaluronidase in *S. epidermidis* as identified in the present study suggest that this bacterium have potential to cause severe cases

as demonstrated by *S. aureus*. In pathogenic model, hyaluronidase had demonstrated its capability in bacterial colonization, invasion and in biofilm dispersal. The ability of commensal isolates to exhibit this virulence factor may contribute in bacterial colonization, but potentially may lead to invasive diseases under certain condition such as in immune-compromised individual. Therefore, investigation on *S. epidermidis* should not be neglected.

The activity of hyaluronidase was shown to be affected by the presence of HA [16]. The amount of HA differed in different parts of the body where the highest amount are measured in the eyes, skin and joints [20]. High HA in the skin is due to their role to balance the skin moisture, while HA in the anterior nares possess valuable role in the function of mucociliary clearance by surface of epithelial [40]. It is also found to be high in certain parts of the male body as compared to females [41, 42]. Therefore, the present study was conducted to find if the distribution of hyaluronidase producer is related to the sites of isolation and gender factor. In the present study, it was found that the distribution of hyaluronidase-producing among both Staphylococcal species are equal between anterior nares and palm skin (Table 2). It may suggest that the production of the enzyme is not affected by the site of isolation (at least to the anterior nares and palm skin). Similarly, no significant association was found between hyaluronidase-producing in isolates from male (57%) and female (60%), regardless of the species. This could be due to the adherence of both Staphylococci species to the keratinocytes, the outermost layer of epidermis, but do not penetrate deeper into epidermis and dermis [43]. Since HA are embedded deep in the dermis layer, it is not reachable to affect hyaluronidase production by the resident microbiota [44].

CONCLUSION

In conclusion, high isolation of commensal *S. aureus* and *S. epidermidis* with capability to produce hyaluronidase show their potential to become opportunistic pathogen regardless of species, site of isolation or gender of the host. Therefore, future strategies on combating and preventing Staphylococci

infections need to consider the commensal Staphylococci in investigation.

Conflict of Interest

Authors declare none

Acknowledgement

We would like to thank staff of Department of Biomedical Science, Kulliyah of Allied Health Sciences, International Islamic University Malaysia who directly or indirectly facilitated the study. This study was supported by Research Initiative Grant Scheme (RIGS) (RIGS 15-018-0018).

Author's Contribution

Nabila Huda Abdul Halim involved in acquisition, analysis and interpretation of data as well as drafting the manuscript. Nur Syarafina Mohd Zahir contributed in term of sample and data collections. Nor Munirah Mohd Amin involved in data collection and manuscript writing preparation. Hanani Ahmad Yusof contributed in the study design, analysis and interpretation of data, as well as in manuscript writing.

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