Nasal carriers are more likely to acquire exogenous *Staphylococcus aureus* strains than non-carriers

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Abstract

We performed a prospective observational study in a clinical setting to test the hypothesis that prior colonization by a *Staphylococcus aureus* strain would protect, by colonization interference or other processes, against *de novo* colonization and, hence, possible endo-infections by newly acquired *S. aureus* strains. Three hundred and six patients hospitalized for >7 days were enrolled. For every patient, four nasal swabs (days 1, 3, 5, and 7) were taken, and patients were identified as carriers when a positive nasal culture for *S. aureus* was obtained on day 1 of hospitalization. For all patients who acquired methicillin-resistant *S. aureus* (MRSA) or methicillin-susceptible *S. aureus* via colonization and/or infection during hospitalization, strains were collected. We note that our study may suffer from false-negative cultures, local problems with infection control and hospital hygiene, or staphylococcal carriage at alternative anatomical sites. Among all patients, 22% were prior carriers of *S. aureus*, including 1.9% whom carried MRSA upon admission. The overall nasal staphylococcal carriage rate among dermatology patients was significantly higher than that among neurosurgery patients (n = 25 (55.5%) vs. n = 42 (16.1%), p 0.005). This conclusion held when the carriage definition included individuals who were nasal culture positive on day 1 and day 3 of hospitalization (p 0.0001). All MRSA carriers were dermatology patients. There was significantly less *S. aureus* acquisition among non-carriers than among carriers during hospitalization (p 0.005). The mean number of days spent in the hospital before experiencing MRSA acquisition in nasal carriers was 5.1, which was significantly lower than the score among non-carriers (22 days, p 0.012). In conclusion, we found that nasal carriage of *S. aureus* predisposes to rather than protects against staphylococcal acquisition in the nose, thereby refuting our null hypothesis.

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Introduction

The recent introduction of metagenomic technologies has highlighted the great diversity of bacterial species that live in or on the human body. These bacteria play an important role in balancing our health status. However, this natural balance may be disturbed, and some species can transform from colonizers into invasive pathogens. The causes of such changes are ill-

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defined, and both human and microbial physiology will be influential, as are bacterial interspecies interactions. Certain species may preclude the presence of others, and the dynamics of such interactions may be important in homeostasis. *Staphylococcus aureus* is one of the best-known human (nasal) colonizers that is also very capable of pathogenic behaviour.

Being the flexible opportunist that it is, *S. aureus* is a well-recognized nosocomial pathogen. Of all nosocomial infections, *S. aureus* accounts for approximately 15% [1–4]. This includes bacteraemia and wound infections, which lead to increased hospital stay, additional antibiotic use, and elevated costs, morbidity, and mortality [4–9]. Patients who are nasal carriers (20–50%) are at increased risk of developing autoinfection [8,10–14]. Thus, eradication of *S. aureus* nasal carriage may prevent nosocomial infections, and such interventions have been proven to be clinically effective [8,14–17]. For instance, mupirocin treatment combined with chlorhexidine washings was effective in eliminating *S. aureus* from both the nares and skin [18,19], which reduced the number of nosocomial *S. aureus* endo-infections.

Whether prior nasal colonization with a methicillin-susceptible *S. aureus* (MSSA) strain protects against acquisition of exogeneous MSSA or methicillin-resistant *S. aureus* (MRSA) is still largely unknown [20,21]. Consequently, the effect of prior colonization on *S. aureus* endo-infection vs. exo-infection is unclear. It was shown that patients re-admitted to hospital benefited from a protective effect of being colonized with MSSA [22,23]. The protective efficacy of such colonization in the prevention of acquisition of MRSA was calculated to be 78%. The studies lacked information on real hospital-based events, including the occurrence of infections, and were considered to be inconclusive.

In this study, we investigated the frequency of *de novo* colonization by *S. aureus* in patients in a single hospital where a highly endemic MRSA clone was circulating. We studied whether patients who carried *S. aureus* upon admission were more susceptible to or protected from acquisition of another *S. aureus* strain.

Materials and methods

Study design

We performed a single-centre prospective observational follow-up study. The study involved patients admitted to Hospital Kuala Lumpur (HKL), which is a 2200-bed acute-care and teaching hospital in Malaysia. The current study covered two departments: neurosurgery, which has three wards (intensive-care unit; male ward A; and female ward B); and dermatology. The majority of the neurosurgery patients were trauma cases without underlying diseases. Patients in all departments previously showed a higher rate of *S. aureus* (MSSA and MRSA) colonization and infections and more extended hospitalization periods than patients in other but clinically similar wards (unpublished data). Patients were followed until the moment at which they left the hospital.

Inclusion criteria

Adult patients (aged $\geq$18 years) were included in the study, after informed consent had been obtained, when the patient was not pregnant, did not have obvious staphylococcal infection upon admission, was not hospitalized in the 6 months before, had a hospital stay of $\geq$7 days at the end of the study period, and provided the possibility of performing frequent microbiological culture. Patients diagnosed with staphylococcal infection within <24 h after hospitalization were excluded from the study.

Ethics and informed consent

The study was performed according to the rules of Good Clinical Practice and according to the Malaysian statutory definition of clinical trials. Patients received verbal and written information on screening for nasal carriage with *S. aureus* by nursing personnel. Patients or relatives provided oral consent, which was noted in the nursing record. Patients had to be mentally and physically capable of taking the decision to take part in the study. For patients unable to make such a decision, guardians were informed, and their initial agreement was obtained. Before official participation, either the patients or their guardians had to provide official written consent. Patients could stop participating at any time without having to provide a particular reason. Furthermore, the study investigator ended the participation whenever the patient's wellbeing was considered to be at risk.

Definition of staphylococcal carriage state

In the current study, we chose the simplest of carriage definitions: carriers were those subjects who had a positive nasal culture taken on their admission day (day 1 of hospitalization). All other patients were defined as non-carriers, irrespective of whether they had one or more positive cultures for the nasal swabs taken on days 3, 5 and 7 after admission (Fig. 1). The likelihood of acquiring one or more new *S. aureus* strains during hospitalization in carriers and non-carriers was examined.

Extranasal and nasal *S. aureus* acquisition

S. aureus isolation from the nose at >48 h after hospitalization and acquisition of a strain with a different *spa* type among non-carriers was considered to be proof of *de novo* nasal acquisition. To determine acquisition of *S. aureus* during hospital stay, data

First definition for carriage status	*Second definition for* carriage status	No. of cases	Cases with similar strains isolated on different days	Nasal culture results during first week of admission			
				Day 1	Day 3	Day 5	Day 7
Carrier (*n* = 67)	Carrier (*n* = 89)	27	19	P	P	P	P
		5	3	P	P	P	
		6	3	P	P	P	NP
		3	2	P		P	P
		7	2	P	P		
		2	2	P	P		NP
		1	1	P			P
		1	-	P		P	NP
		15	-	P			
Non-carrier (*n* = 238)		11	8		P	P	P
		3	-		P		
		3	2		P	P	NP
		2	1	NP	P	P	
		3	2		P		P
	non-carrier (*n* = 216)	199	-				
		6	3			P	P
		9	-				P
		4	-			P	

FIG. 1. Nasal cultivation results. 'No. of cases' shows the number of patients who showed the same sequence of culture positivity as highlighted in yellow on the right. 'Cases with similar strains isolated on different days' shows only the number of patients in whom all of the strains were genetically indistinguishable. NP, not possible to take swabs; P, positive nasal culture; –, negative nasal culture.

on *S. aureus* infections during the hospital stay were collected by evaluation of patients' microbiological culture data and by consulting their physicians and their medical records. Swabs for MSSA and MRSA cultures were taken twice weekly in cases of suspicion of infection. Usually, a tracheal swab and/or a urine specimen were taken for such monitoring. Infections were retrospectively defined according to the CDC/NHSN Surveillance Definitions for Specific Types of Infections (Version January 2015, as modified in April 2015). In the case of acquisition of more than one *S. aureus* strain (both MSSA and MRSA), the first acquired strain was included in the statistical and epidemiological analyses. A strain with the same *spa* type found at additional body sites was considered to be of endogenous origin, i.e. the same as the nasal isolate cultured on the admission day (day 1). Isolation of a different *spa* type from another body site was considered to be an exogenous acquisition event.

Nasal swabs

Nasal swabs were taken on days 1, 3, 5 and 7 of hospitalization by trained healthcare personnel. A sterile Transswab (Medical Wire and Equipment, England, UK) was rotated four times in both anterior nasal vestibules. The swab was immersed in 0.5 mL of transport medium, and was transported to the research laboratory of the Department of Medical Microbiology, University Putra Malaysia, together with the patient's informed consent form. The swabs were plated on mannitol salt agar (Merck, Selangor, Malaysia) and Chrome Agar for *S. aureus* isolation (Becton Dickinson, Sparks, MD, USA) within 6 h of collection. All cultures that were negative after 48 h at 37°C were incubated for an additional 5 days at room temperature until a final review. A single colony of the culture-positive Petri dishes was subjected to Gram staining, catalase testing and coagulase tube testing to confirm the presence of *S. aureus*. Strains were stored at −70°C in LB broth (Merck) with 20% glycerol. Isolates from potentially MRSA-infected or MSSA-infected patients were also collected and stored at −70°C. All isolates were subjected to *Sa442* PCR for definitive species confirmation [24]. MRSA screening was performed with a cefoxitin disk diffusion test on a Mueller–Hinton agar plate. Whenever resistance to cefoxitin (inhibition zone of $\leq$21 mm) was observed, MRSA status was confirmed by PCR of the *mecA* gene [25].

Staphylococcal genotyping

All strains collected during the present study were subjected to genotyping. Chromosomal DNA was extracted with the DNeasy Kit (QIAGEN Biotechnology Malaysia Sdn Bhd, Kuala Lumpur, Malaysia), according to the manufacturer's instructions, and *spa* typing was performed [26]. The amplified *spa* gene fragment was purified and sequenced, and *spa* types were assigned by use of the Spa-server (Ridom, Würzburg, Germany) [27]. SCC*mec* typing of MRSA strains was performed as described previously [25]. Strains that were non-typeable for

TABLE 1. Demographic data and carriage status of participating patients

Patient		Gender, no. (%)			Ethnic background, no. (%)				Department, no. (%)	
Group	No. (%)	Male	Female	Age (years), median (mean)	Malay	Chinese	Indian	Others	NS	Dermatology
Carrier	67 (22)	39 (58.2)	28 (41.8)	45 (45.6)	36 (53)	16 (23.8)	9 (13.4)	6 (9)	42 (62.7)	25 (37.3)
Non-carrier	238 (78)	161 (67.6)	77 (32.4)	49 (48.6)	125 (47)	59 (76.2)	31 (86.6)	23 (91)	218 (37.3)	20 (62.7)
Total	305 (100)	200 (65.6)	105 (34.4)	49 (47.8)	161 (52.8)	75 (24.6)	40 (13.1)	29 (9.5)	260 (85.2)	45 (14.8)

Note that the median age for the entire group would be 49.0 years.
NS, neurosurgery.

spa were subjected to multilocus sequence typing for definition of sequence types (STs) of MSSA and MRSA. STs and clonal clusters were assigned by use of the *S. aureus* multilocus sequence typing database (www.mlst.net) hosted by Imperial College in London, UK.

Statistics

Relative risks with CIs not containing unity were considered to be significant. Differences per group in duration of hospitalization and time to infection were tested for significance with the independent *t*-test and the Mann–Whitney *U*-test, based on the result of a normality test. p-Values of <0.05 were considered to be significant.

Results

Patients and carriage characteristics

The inclusion period was from 16 May 2011 to 16 February 2012, and 342 patients were included. Among them, 37 were excluded because they were hospitalized for <7 days. In the end, we included 305 patients in the final cohort (260 neurosurgery patients and 45 dermatology patients). According to the three major ethnic groups in Malaysia, participants were Malay (n = 161, 52.8%), Chinese (n = 75, 24.6%), Indian (n = 40, 13.1%), or 'other' (n = 29, 9.5%). The male/female ratio was 65.6%/34.4%. The median age of the patients was 49.0 years (range, 18–89 years). Rates of carriage were not significantly different between ethnic groups (Table 1; chi-square p > 0.05 for all combinations). Carriage among dermatology patients was significantly higher than among neurosurgery patients (62.6% vs. 37.3%, p < 0.05), and all MRSA carriers were detected among dermatology patients (Table 2). Among the 305 participants, 67 (22%) were identified as carriers, and 238 (78%) were classified as non-carriers. The acquisition rate for new strains was not different between the neurosurgery and the dermatology groups (65/260 vs. 14/45 (p 0.245, Fisher exact test), respectively). For this reason, we decided to analyse the group as a whole without subanalyses for the two different departments. Among the carriers, 63 (20.1%) carried MSSA and four (1.9%) carried MRSA (Tables 1 and 2). The average length of stay in the hospital was 16.2 days.

Strain typing

Three hundred and seventy-six strains were isolated from 135 patients. Among these strains, 263 were of nasal origin, and 113 were from extranasal sites. Overall, we identified 73 different *spa* types. Among these were ten new *spa* types and two new STs, which have been deposited in the relevant databases (Ridom and www.mlst.net). In 34 of the 67 nasal carriers, all nasal strains isolated on different days shared the same *spa* type (Fig. 1). Two different *spa* types were obtained from 16 patients, and three different *spa* types were obtained from four patients. During the week of nasal screening, 57 acquisitions of strains with new *spa* types were detected. The highest rate of nasal acquisition was documented on day 3 after admission

TABLE 2. Distribution of carriers (methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA)) and source of acquired *S. aureus* strains among patients from different wards

	Presence of *S. aureus* on body sites than the nose, detected during the follow-up period							
	MSSA and MRSA		MSSA			MRSA		
	Presence (n = 79)			Acquisition (n = 53)			Acquisition (n = 26)	
Ward	Same type as in nose on day 1	Acquired	Carrier	Same type as in nose on day 1	Exogenous	Carrier	Same type as in nose on day 1	Exogenous
Neurosurgery, no.	6	59	42	6	40	0	0	19
Dermatology, no.	5	9	21	4	3	4	1	6
Total, no. (%)	11 (3.6)	68 (20)	63	10 (18.9)	43 (81.1)	4	1 (3.9)	25 (96.1)

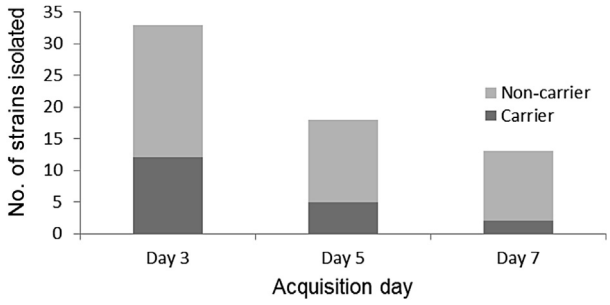

FIG. 2. Nasal acquisition day among carriers and non-carriers.

(*n* = 33). We found 18 acquisitions on day 5, and 13 acquisitions on day 7 (Fig. 2).

Among the six MRSA strains isolated from the anterior nares of dermatology patients, four were typed as t37/ST239/SCC*mec*III, and other two strains were typed as t421/ST239/SCC*mec*III and t10562/ST573/SCC*mec*V. The three MRSA strains isolated from neurosurgery patients shared the same genotype, and were typed as t37/ST239/SCC*mec*III. *Spa* and SCC*mec* typing of acquired MRSA strains (*n* = 26) revealed t37/ST239/SCC*mec*III (*n* = 21, 80.7%), t4184/ST22/SCC*mec*IVh (*n* = 4, 15.5%), and t10562/ST573/SCC*mec*V (*n* = 1, 4%). *Spa* type t10562 was acquired by a dermatology patient, whereas t4184 strains were detected among inpatients in the neurosurgery wards only.

Frequency and sites of acquisition

During the study period, 101 study cases acquired *S. aureus*, and the risk of acquisition among carriers was significantly higher than that among non-carriers (p 0.005). According to culture and typing results, the 101 patients (32 carriers and 69 non-carriers) acquired one or more strains in their nose or other body sites during hospital stay (57 and 72 isolates, respectively). Among them were 82 MSSA and 34 MRSA acquisitions (Table 3). The majority of the strains cultured from clinical samples (*n* = 60, 51.7%) were isolated from tracheal aspirates (49 MSSA and 11 MRSA). Fifty-two strains (44.8%) were isolated from the nose (44 MSSA and eight MRSA), 18 strains (15.5%) were isolated from pus (eight MSSA and ten MRSA), and three strains were isolated from blood cultures (one MSSA and two MRSA). Another two strains were isolated from urine (one MSSA and one MRSA), and the final MRSA strain was isolated from a wound (Fig. 3).

Endogenous vs. exogenous events

When nasal acquisition was excluded, 53 MSSA acquisitions at alternative body sites were detected in the study group: 46 in neurosurgery patients, and seven in dermatology patients (Table 2). Twenty-six MRSA acquisitions were noted: 19 in neurosurgery patients, and seven in dermatology patients. Comparison of the *spa* types of the MSSA and MRSA strains isolated from the alternative body sites with the nasal isolates (on day of admission) revealed an endogenous origin of 11 strains (ten MSSA and one MRSA), and an exogenous origin of 68 strains (43 MSSA and 25 MRSA) (Table 2). Among 42 MSSA carriers in the neurosurgery ward, six were colonized with an MSSA strain sharing the same *spa* type; and among 21 MSSA carriers in the dermatology ward, four had endogenous MSSA cultured (Table 2). One of four MRSA carriers among dermatology patients harboured the same strain as the one isolated from the anterior nares in an extranasal site. Ten of 63 MSSA carriers acquired a strain with a similar *spa* type as the one from their anterior nares in an extranasal site. The majority of MSSA (81.1%) and MRSA (96.1%) acquisitions were of exogenous origin (Table 2).

S. aureus acquisition among carriers vs. non-carriers

Among the patients, 82 MSSA acquisitions were documented: 26 among carriers, and 59 among non-carriers (Table 3). Thirty-four MRSA acquisitions were documented: eight among carriers, and 26 among non-carriers. There was a significant difference between carriers and non-carriers MSSA acquisition

TABLE 3. Comparison of risk of acquiring methicillin-susceptible *Staphylococcus aureus* (MSSA) or methicillin-resistant *S. aureus* (MRSA) in nasal carriers vs. non-carriers

		MSSA and MRSA acquisition (no.)		MSSA acquisition (no.)		MRSA acquisition (no.)		Start day of acquisition (mean days)	
Carrier definition	**Patient category**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**MSSA**	**MRSA**
Based on first-day nasal swab results	Carrier (*n* = 67)	32	35	26	41	8	59	2.8	5.1
	Non-carrier (*n* = 238)	69	169	59	182	26	212	7	22
	Total (*n* = 305)	101	204	82	223	34	271	5.7	17.4
		p 0.005[a]		**p 0.019**[a]		p 0.827[a]		p 0.142[b]	**p 0.012**[c]

Bold type indicates statistical significance.
[a]Fisher exact test.
[b]Mann–Whitney *U*-test.
[c]Independent *t*-test.

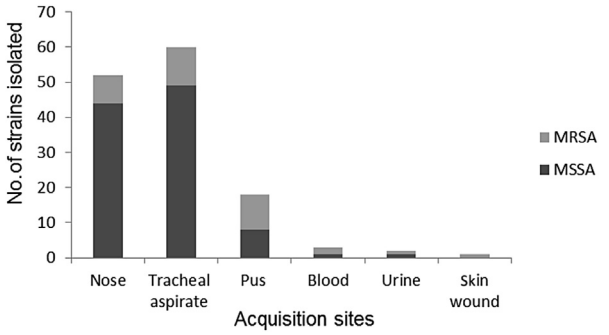

FIG. 3. Sites of acquisition of different *Staphylococcus aureus* strains. MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

(p 0.019) during hospitalization, whereas no difference was observed for MRSA acquisition (p = 0.827). However, carriers acquired MRSA faster than non-carriers (mean of 5.1 days vs. mean of 22 days, respectively) (t_{26} = 2.893, p 0.012). No significant difference was observed for time between admission and day of MSSA acquisition among carriers (mean of 2.8 days) vs. non-carriers (mean of 7 days) (Table 3).

Discussion

Colonization by *S. aureus* is a multifactorial process, with both host and bacterial factors being involved. It is not well known whether or not the prior presence of an *S. aureus* strain predisposes to or protects against colonization by a new strain. Precisely tuned natural human (re)colonization schemes are not available [28,29], although it has been demonstrated previously that persistent carriers can change their nasal strain over time while staying phenotypically persistent carriers [30]. In this study, we have demonstrated that existing nasal carriage of *S. aureus* is a significant determinant for acquiring new *S. aureus* strains, which is in contrast to previous data [23].

A 33.7% MRSA carriage rate was reported previously for HKL patients [31,32]. Previous investigations on the predominant MRSA lineages in HKL revealed that ST239/SCC*mec*III or ST239/SCC*mec*IIIa (92.5%) and ST22/SCC*mec*IVh (1.5%) are the most successful lineages isolated from infection sites [33]. The majority of the MRSA infections (92.5%) among patients in the current study were also caused by ST239 MRSA strains. This finding shows that being a nasal carrier of *S. aureus* is not protective against the acquisition of MRSA strains circulating in the environment, although antibiotic usage may be influential. Unfortunately, we lack data on antibiotic usage, but, given the lack of precise antibiotic policies in the hospital concerned, we assume that, during the present study, this was sufficiently randomized. The variable genotypes of the MSSA strains isolated from the nose and/or body sites was in concordance with the results of previous studies [34,35].

It has been shown recently that nosocomial acquisition of MRSA results in morbidity and mortality upon re-admission of elderly persons to the hospital [36]. This suggests that screening for MRSA acquisition and management of its risks is clinically important. We conclude that nasal carriage of *S. aureus* predisposes to nosocomial acquisition of new strains, both in the nose and in extranasal body sites. It has been shown that resident populations of *S. aureus* may be heterogeneous, with multiple genotypes being present simultaneously. Our current data are unlikely to be confounded by such phenomena, as we only used culture positivity as a single marker for carriage. Strain heterogeneity or mixed colonization, for instance, would not affect this type of purely numerical and statistical analysis. However, there are some inherent weaknesses in the design of our study. Major weaknesses that need to be taken into account are the fact that the first nasal culture may be falsely negative or that there may be a strong correlation between cross-colonization and the local level of hygiene practices. We were also unable to include throat and perianal cultures in order to screen for secondary carriage of *S. aureus* at these alternative anatomical sites. Second, because prospective studies are very difficult in our field of study, we had to use a simplified test for definition of carriage, and not repetitive serial culture [29]. In any case, our findings again underscore the relevance of regular sampling of the nasal cavity of patients, especially those who were previously identified as persistent carriers, in order to continuously minimize risks. In addition, it demonstrates that being a non-carrier in a hospital setting has a protective effect that is probably purely host defined. Investigation into this protective effect may reveal novel methods for protecting patients from staphylococcal disease.

Transparency declaration

The authors declare no conflicts of interest in relation to the studies presented here. A. van Belkum is an employee of bioMérieux, a company that develops and sells infectious disease diagnostics.

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