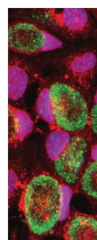

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RESEARCH ARTICLE

Staphylococcus aureus nasal isolates from healthy individuals cause highly variable host cell responses *in vitro*

The Tromsø Staph and Skin Study

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This study illustrates that a small series of nasal isolates of *Staphyloccoccus aureus*, belonging to different genotypes, do behave differently from a phenotypic standpoint (biofilm production, adhesion and invasion properties) but also in the host responses they induce (cytokine profiling). This underlines the caution we must exercise when trying to generalize observations in *S. aureus*.

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Keywords
growth rate; keratinocytes; adhesion; internalization; cytokine induction.

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Abstract

Studies on *Staphylococcus aureus* populations colonizing the nasal cavity reveal that some bacterial strains are more common, while others are rarely found. This study included five isolates with the most common *spa* types and five isolates with rare *spa* types from healthy population. Selected phenotypic traits and genomic content among nasal *S. aureus* isolates were compared. Besides the rather similar growth rates, our data revealed a high diversity among isolates; that is, in biofilm formation, the ability to attach to and be internalized in keratinocytes as well as ability to induce pro- and anti-inflammatory cytokines. The results showed that *S. aureus* isolates from healthy hosts are phenotypically diverse and cause highly variable host cell responses. Therefore, generalizing the results from one *S. aureus* isolate to all is highly questionable.

Introduction

Normal skin is exposed to various bacteria, including *Staphylococcus aureus. Staphylococcus aureus* persistently colonizes the anterior nares of 20–30% of healthy individuals (Costello *et al.*, 2009) and can cause several life-threatening infections such as endocarditis and sepsis (Kluytmans *et al.*, 1997; von Eiff *et al.*, 2001; Fowler *et al.*, 2003; Bardoel *et al.*, 2012). Nasal carriage is a risk factor for infection (von Eiff *et al.*, 2001; Ammerlaan *et al.*, 2009), and invasive disease is often caused by the *S. aureus* strain carried by the patient (Vaudaux & Schrenzel, 2004). Increased prevalence of antimicrobial-resistant strains combined with increasing numbers of immune compromised patients is a major clinical challenge for *S. aureus* treatment (Fowler *et al.*, 2005; Naber, 2009). Eradication of *S. aureus* carriage may reduce the numbers of nosocomial infections.

Nasal carriers of *S. aureus* are classified as persistent carriers and others (intermittent and noncarriers) (van Belkum *et al.*, 2009b). The persistent *S. aureus* nasal carriage rate varies between developed and developing countries, age, sex, smoking, and blood glucose levels (Sivaraman *et al.*, 2009). Colonization of the nasal cavity is a multifactorial process influenced by concerted activity of bacterial, host, and possibly environmental factors (Vaudaux & Schrenzel, 2004; van Belkum *et al.*, 2009a, DeLeo *et al.*, 2010). Bacteria contain several conserved molecules collectively referred to as pathogen-associated molecular patterns (PAMPs). These can be recognized by pattern recognition receptors such as Toll-like receptors (TLRs) present on the host cell, which can result in expression of

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antimicrobial peptides and proinflammatory cytokines (Nish & Medzhitov, 2011). Certain polymorphisms in TLR2, mannose-binding lectin, CRP, glucocorticoid receptor, and vitamin D receptor have been found to be associated with carriage (reviewed by Johannessen *et al.*, 2012). Moreover, the level of β-defensin-2 and α-defensins 1–3 was elevated in nasal secretions from carriers compared with noncarriers (Cole *et al.*, 2001).

Among the bacterial factors important for colonization are various molecules involved in adhesion and/or immune evasion such as clumping factor B (ClfB), iron-regulated surface determinant protein A (IsdA), and wall techoid acid (Edwards *et al.*, 2012; Weidenmaier *et al.*, 2012). Although *S. aureus* has been regarded as an extracellular bacterium, intracellular localization of *S. aureus* has been found in biopsies from the anterior part of the middle turbinate or tonsils from patients with recurrent rhinosinusitis or tonsillitis, respectively (Clement *et al.*, 2005; Zautner *et al.*, 2010). The bacteria can be internalized by various cell types in addition to the professional phagocytes (Garzoni & Kelley, 2009). Prolonged survival of internalized bacterial cells can result in the release of viable bacterial cells when the host cell dies. However, whether *S. aureus* is internalized in keratinocytes during colonization remains elusive.

The prevalence of virulence factors as well as surface proteins may vary between *S. aureus* isolates (Dreisbach *et al.*, 2010; Ziebandt *et al.*, 2010; Piechowicz *et al.*, 2011; McCarthy & Lindsay, 2012). Still, all *S. aureus* strains are able to induce infection, although there are discussions about whether some strains are, or can evolve to be, more virulent than others (Foster, 2004; Melles *et al.*, 2004; Lindsay, 2010). The population of clinical or nasal *S. aureus* strains has been grouped into different clusters based on multilocus sequence typing (MLST), amplified fragment length polymorphisms (AFLP), or *spa* typing (Melles *et al.*, 2004; Grundmann *et al.*, 2010; Lamers *et al.*, 2011; Sangvik *et al.*, 2011). *Spa* typing is based on sequencing of a single polymorphic variable number tandem repeat, which is the repeat region of the protein A gene. The *spa* typing of nasal isolates from healthy carriers has revealed that some types are commonly found in individuals from a general population, while other types are more seldom encountered (Sangvik *et al.*, 2011).

Host–microbe interaction studies are often carried out using one or a few isolates defined as invasive or colonizing. The aim of this study was to address whether colonizing isolates have common phenotypic traits such as growth rate, biofilm formation, and host cell attachment or internalization as well as the ability to induce host inflammatory response in keratinocytes. Furthermore, we aimed to investigate whether colonizing isolates of the more common *spa* types differ from those with rare *spa* types.

Materials and methods

Bacterial strains

In total, 1113 *S. aureus* nasal isolates from healthy participants were assigned to 368 *spa* types as described previously (Sangvik *et al.*, 2011). One isolate from each of the five most commonly found *spa* types (no. 1–5) and one of each of five rare *spa* types (no. 6–10; Table 1) were selected for further investigation. MLST types, *spa* clonal complex, and MLST clonal complex for all isolates are indicated in Table 1.

Staphylococcus aureus subsp. *aureus* Rosenbach MSSA476 and *S. aureus* subsp. *aureus* NCTC8325 were purchased from LGC standard AB (ATCC-BAA-1721, Sweden) and National Collection of Type Cultures. *S. aureus* subsp. *aureus* COL and *S. aureus* subsp. *aureus* N315 were kindly donated by A.R. Larsen (Statens Serum Institut, Denmark) and T. Ito (Juntendo University, Japan), respectively.

Mammalian cell line

HaCaT cells were purchased from PromoCell (Germany). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma Aldrich, Germany), supplemented with 10% (v/v) fetal bovine serum (FBS; Invitrogen Life Technologies), penicillin (100 units mL^{-1}), and 100 μg mL^{-1} streptomycin (Sigma Aldrich) in a CO_2 incubator (5% CO_2) at 37 °C.

Table 1 The overview of the 10 selected *Staphylococcus aureus* nasal isolates (*n* = 1113)

Isolate no.	Strain identity	*spa* type	Number of isolates	*spa* clonal complexes	MLST	MLST clonal complexes
1	61010029	t012	94	*spa*-CC012	ST30	CC30
2	61010044	t065	55	*spa*-CC065	ST45	CC45
3	61010165	t084	85	*spa*-CC084	ST15	CC15
4	61010527	t021	42	*spa*-CC012	ST30	CC30
5	61011308	t015	38	*spa*-CC065	ST45	CC45
6	61010305	t186	1	NA	ST78	CC88
7	61010555	t5229	1	NA	ST188	CC1
8	61010701	t5234	1	NA	ST12	CC12
9	61010771	t2573	1	NA	ST42	Singleton
10	61011501	t082	2	NA	ST395	CC395

NA, These isolates did not belong to a *spa* clonal complex. MLST clonal complexes are based on eBURST on the entire public MLST database (January 2011) (Sangvik *et al.*, 2011).

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Comparative genomic hybridization (CGH)

The prevalence of genes encoding surface proteins and proteins involved in immune evasion (listed in Table 2) was assessed by microarrays. Eight isolates belonging to seven different MLST clonal complexes were selected.

The microarrays (*S. aureus*, version 9) were obtained from the Pathogen Functional Genomics Resource Center (PFGRC) at the J. Craig Venter Institute (JCVI). Genomic DNA was isolated from overnight cultures grown in BHI using the Genomic-tip 100/G column (QIAGEN). 5-μg genomic DNA was labeled and purified using the Bio-Prime Array CGH Genomic labeling System (Invitrogen) and the Cyanine Smart Pack dUTP (PerkinElmer Life Sciences), according to the manufacturer's protocol. The hybridization (with dye-swap), scanning, and analysis of fluorescent intensities/spot morphologies were performed as previously described (Solheim *et al.*, 2009).

Standard methods in the LIMMA package (Smyth & Speed, 2003) in R (http://www.r-project.org/), available from the Bioconductor website (http://www.bioconductor.org), were employed for preprocessing and normalization. Within-array normalization was first conducted by subtracting the median from the log ratios for each array. Standard loess normalization was then performed, where smoothing was based only on spots with abs (log ratio) < 2.0 to avoid biases due to extreme skewness in the log ratio distribution. For the determination of present and divergent genes, we used the approach outlined in the study by Snipen *et al.* (2009). This method requires the BLASTing of probe sequences to one or several known genome sequences, and these genomes must also be hybridized to the arrays as a calibration step. For this purpose, we used the genome sequence of *S. aureus* strain N315. A threshold of 0.7 was used to classify a gene as present or absent. The microarray data have been deposited in the ArrayExpress database with the series accession number E-MTAB-1672.

Table 2 *Staphylococcus aureus* genes evaluated by microarray in this study

Encoded function	Gene symbol	Protein full name (Protein symbol)
Surface protein	*clfB*	Clumping factor B (ClfB)
	isdA	Iron-regulated surface determinants A (IsdA)
	fnb	Fibronectin-binding protein A (FnBPA)
	fnbB	Fibronectin-binding protein B (FnBPB)
	sdrC	Serine aspartate repeat protein C (SdrC)
	sdrD	Serine aspartate repeat protein D (SdrD)
	sasG	*Staphylococcus aureus* protein G (SasG)
	sasH	*Staphylococcus aureus* protein H (SasH)
Immune evasion protein	*chp*	Chemotaxis inhibitory protein of *Staphylococcus aureus* (CHIPS)
	flr	FPR-like 1 inhibitory protein (FLIPr)
	fll	FPR-like 1 inhibitory protein like (FLIPr-like)
	oatA	O-acetyltransferase (OatA)
	sak	Staphylokinase
	scn	Staphylococcal complement inhibitor (SCIN)

Measurement of bacterial growth

Bacterial growth was quantified by viable count and optical density ($OD_{600\ nm}$) data. Overnight cultures were diluted 1 : 100 in fresh BHI broth media (brain–heart infusion broth, Sigma Aldrich), and $OD_{600\ nm}$ was measured by spectrophotometer at $t = 0$, 30, 60, 90, 120, 150, 180, and 210 min after inoculation. Serial dilutions were plated on blood agar to determine bacterial viable counts. The specific growth rate constant (μ) was calculated by the following formula:

$$\mu(\mathrm{h}^{-1}) = ((\log_{10} \mathrm{N} - \log_{10} \mathrm{N}_0)\, 2.303)/(\mathrm{t} - \mathrm{t}_0)$$

Biofilm formation

Semi-quantitative determination of biofilm formation by different *S. aureus* strains was carried out by the microtitre plate method as described previously (Flemming *et al.*, 2009). The biofilm-forming capacities of all isolates were tested using Trypticase soy broth supplemented with 5% glucose and 3% NaCl. Briefly, strains were inoculated from overnight cultures, distributed into 96-well plates, incubated at 37 °C overnight, washed, and stained. *S. aureus* PIA90 and PIA9 were used as negative and positive controls, respectively. Cutoff value was calculated principally as described previously (Christensen *et al.*, 1985).

Cell stimulation practices

An overnight culture of *S. aureus* was diluted 1 : 100 in BHI (brain–heart infusion broth, Sigma Aldrich) and incubated at 37 °C, 220 r.p.m. Bacterial growth was monitored by optical density at 600 nm ($OD_{600\ nm}$). The bacteria were pelleted, washed twice in 1× PBS (Biochrom, Germany; 37 °C), and diluted to the selected CFU mL^{-1} in DMEM with 10% FBS depending on the experimental set-up.

Internalization and adhesion assay

Internalization and adhesion assays were carried out 3 times in triplicate as previously described (35). Briefly, HaCaT cells were seeded into 24-well plates at confluent concentration between 1.5 and 1.7×10^5 cells per well in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin with 10% FBS. The day after, the cells were washed, and fresh antibiotic-free DMEM supplemented with 10% FBS was added. Approximately 5×10^7 CFU of the indicated *S. aureus* isolate (Table 1), suspended in DMEM, was added to the HaCaT cells, and the plates were incubated for 90 min at 37 °C in a 5% CO_2–95% air atmosphere (Kintarak *et al.*, 2004). Adhered and internalized bacteria were quantified after washing off nonadhered bacteria. Extracellular bacteria were killed by replacing of the culture medium by DMEM containing 50 μg mL^{-1} gentamicin (Sigma Aldrich) and 20 μg mL^{-1} lysostaphin

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(Sigma Aldrich) (Agerer *et al.*, 2003). The cells were then incubated for 1 h at 37 °C in a 5% CO_2–95% air atmosphere. This procedure is essential for the internalization assay, where only internalized bacteria remain viable and can be assessed. The determination of bacterial viable counts was carried out through serial dilution after trypsinizing and lysing of HaCaT cells with trypsin–EDTA (Sigma Aldrich) and 0.1% Triton X-100 (Sigma Aldrich) (Kintarak *et al.*, 2004). The number of attached bacteria represents bacteria attached to HaCaT cells plus the viable internalized bacteria, while the number for internalization represents viable intracellular bacteria when all the extracellular bacteria were killed by gentamicin and lysostaphin treatments.

Cytokine assay

HaCaT cells were seeded in six-well plates (Corning) at confluent concentration, 1.2–1.5 × 10^6 cells per well in DMEM supplemented with 10% FBS. The cells were left untreated or added 3.5 × 10^7 *S. aureus* cells of the indicated *S. aureus* isolate (Table 1). After 8- or 24-h incubation, the culture supernatants were collected and centrifuged at 4 °C 13 000 ***g*** for 7 min to pellet cellular debris. The supernatant containing secreted cytokines was transferred to a new Eppendorf tube. Secretion of IL-1α, IL-1β, TNF-α, IL-6, IL-8, CXCL-1, IL-10, and IL-1RA in the culture supernatants were measured using MILLIPLEX® MAP kit (Millipore Corp.) based on Luminex technology, according to the manufacturer's instructions. Each sample was assayed in duplicate. The previously described *S. aureus* strains COL, MSSA476, and NCTCT8325 were also included in the experiment as controls.

Ethical approval

The Tromsø Staph and Skin Study was approved by the Regional Committee for Medical Research Ethics, North Norway (Ref. 200605174-12/IAY/400), and the Norwegian Data Inspectorate (Ref. 07/00886-2/CAO) and is in the Biobank Registry (Ref. 2397).

Statistical analysis

Numerical data are presented as mean ± standard deviations. The validity of the results in the experiments was assured via repeating each experiment at least three times with standard independent parallels. Statistical analysis was performed using ANOVA and the nonparametric Mann–Whitney *U*-test. Correlation analysis was performed using Pearson's correlation coefficients. P values of < 0.05 were considered significant.

Results

Prevalence of genes encoding proteins involved in adhesion or immune evasion

Within an adult healthy population, some genotypes of *S. aureus* were more common than others (Sangvik *et al.*, 2011). To find any putative genetic traits associated with the relative abundance in the population, the prevalence of selected genes encoding proteins involved in adhesion and immune evasion was investigated by comparative genomics of eight selected isolates. However, no specific pattern was identified for either common or rare *spa* types of *S. aureus* (Table 3). Among genes linked to adhesive properties, all 8 isolates had *clfB, isdA, sasH, sdrD,* and *sdrC* genes, seven had *fnb* and/or *fnbB*, while the *sasG* gene was only present in isolate no 6 (Table 3). From the genes encoding immune evasive proteins, *oatA* was present among all tested isolates. Six and five of eight isolates possessed genes encoding *scn* and *sak*, respectively. The *chp* and *fll* genes were only detected in a few isolates, while none of them contained the *flr* gene (Table 3).

Table 3 Gene prevalence detected by microarray in eight selected isolates

Strain no. MLST clonal complex		1 CC30	4 CC30	5 CC45	6 CC88	7 CC1	8 CC12	9 Singleton	10 CC395
Gene	Identifier								
chp	SA1755	■	□	■	□	□	□	■	□
clfB	SACOL2652, SAR2709, SA2423	■	■	■	■	■	■	■	■
isdA	SA0977, SAR1103	■	■	■	■	■	■	■	■
fll	SAS1089	□	□	□	□	□	■	□	□
flr	SA1001	□	□	□	□	□	□	□	□
fnb	SA2291, SACOL2511, SAR2580	■	■	□	□	□	■	■	□
fnbB	SA2290, SACOL2509, SAS2387	□	■	■	□	■	■	□	■
oatA	SA2354	■	■	■	■	■	■	■	■
sak	SA1758	■	□	■	■	■	■	□	□
sasG	SACOL2505	□	□	□	■	□	□	□	□
sasH	SACOL0024	■	■	■	■	■	■	■	■
scn	SA1754	■	□	■	■	■	■	■	□
sdrC	SACOL0608, SA0519, SAR0566	■	■	■	■	■	■	■	■
sdrD	SACOL0609, SA0520, MW0517	■	■	■	■	■	■	■	■

Genes are listed alphabetically, and their annotated gene number (identifier) that was used as the template on microarray is given. Presence and absence of a gene was shown by ■ and □, respectively.

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Table 4 Growth rate measurement and biofilm formation ability of the 10 selected *Staphylococcus aureus* isolates

Isolate no.	μ (h^{-1})*	Cell concentration at 1.0 $OD_{600\ nm}$ (CFU mL^{-1})*	Biofilm formation**
1	1.44 ± 0.04	$1.66 \times 10^9 \pm 1.7 \times 10^6$	0.18 ± 0.02
2	1.17 ± 0.14	$2.34 \times 10^9 \pm 2.1 \times 10^6$	0.12 ± 0.01
3	1.65 ± 0.05	$1.66 \times 10^9 \pm 1.5 \times 10^6$	0.13 ± 0.00
4	1.48 ± 0.05	$1.36 \times 10^9 \pm 1.1 \times 10^6$	0.21 ± 0.01
5	1.44 ± 0.38	$1.55 \times 10^9 \pm 1.1 \times 10^6$	0.15 ± 0.01
6	1.66 ± 0.08	$1.70 \times 10^9 \pm 1.7 \times 10^6$	0.21 ± 0.04
7	1.48 ± 0.00	$1.92 \times 10^9 \pm 2.2 \times 10^6$	0.17 ± 0.02
8	1.61 ± 0.18	$1.74 \times 10^9 \pm 1.7 \times 10^6$	0.14 ± 0.01
9	1.40 ± 0.47	$1.39 \times 10^9 \pm 1.1 \times 10^6$	0.14 ± 0.01
10	1.29 ± 0.06	$1.65 \times 10^9 \pm 1.7 \times 10^6$	0.44 ± 0.06

*Mean ± SD of two replicates of one representative experiment for growth rate measurement.

**Mean ± SD of three independent experiments for biofilm analysis. Isolates were considered as biofilm positive (+) and biofilm negative (−) if they had an $OD_{570\ nm}$ of > 0.28 and $OD_{570\ nm}$ of < 0.28 in the assay, respectively. $OD_{570\ nm}$ in PIA9 and PIA90 was 2.59 and 0.28, respectively, in our experimental condition.

Similar growth rates among common and rare *spa* types isolated from healthy adults

To determine the difference in growth rates among the selected *S. aureus* isolates, the specific growth rate constant (μ) was calculated (Table 4). The maximum and minimum specific growth rate constants were 1.66 (± 0.08) and 1.17 (± 0.14), respectively (Table 4). However, no significant difference was identified between the common spa types (isolates 1–5) and the rare spa types (isolates 6–10) in distribution of growth rates in BHI media.

Most of the selected *S. aureus* isolates did not produce biofilm *in vitro*

A previous study has indicated that there is a correlation between the ability to form a biofilm and nasal colonization (Iwase *et al.*, 2010). The biofilm formation ability of the ten selected *S. aureus* isolates was tested. From the semi-quantitative determination, 1 of 10 isolates (isolate no 10) was found to produce biofilm *in vitro*. However, the biofilm-positive isolate produced only weak biofilm as the measured $OD_{570\ nm}$ was slightly above the cutoff value set to 0.28 (Table 4).

Internalization and adhesion assay

Staphylococcus aureus can be internalized by various cell types (Garzoni & Kelley, 2009). Whether the ability to attach to and be internalized in keratinocytes was common for all 10 selected isolates was unknown and was therefore investigated.

Adhesion and internalization assays were performed in HaCaT cells as described previously (Kintarak *et al.*, 2004). The number of attached bacteria was significantly higher than that of internalized bacteria ($P < 0.01$). Even though the same number of bacteria was seeded out on host cells, the number of attached bacteria was in the range 0.6×10^6–63×10^6 CFU mL^{-1} (i.e. 100-fold difference), and the number of viable internalized bacteria was in the range 0.13×10^3–160×10^3 CFU mL^{-1} (i.e. 1000-fold difference; Table 5). These results clearly show variations in the attachment and internalization ability among isolates from a healthy population. The numerical results of adhered and internalized bacteria were higher in isolate no 1, which belonged to the most commonly found *spa* type (Table 5).

Table 5 Attachment and internalization of the 10 selected *Staphylococcus aureus* strains to the HaCaT cells

Isolate no.	Attachment ($\times 10^6$ CFU mL^{-1})	Internalization ($\times 10^3$ CFU mL^{-1})
1	63 ± 3.5	160 ± 2.5
2	6.8 ± 0.57	18 ± 2.3
3	31 ± 8	1.3 ± 0.24
4	25 ± 1.3	1.4 ± 0.15
5	0.6 ± 0.25	0.7 ± 0.01
6	17 ± 0.071	19 ± 0.77
7	35 ± 5.5	2 ± 0.14
8	27 ± 2.8	0.14 ± 0.007
9	12 ± 2.8	0.17 ± 0.24
10	4 ± 1.2	0.13 ± 0.03

Mean ± SD of three replicates of one representative experiment.

Cytokine profiles from HaCaT stimulated with selected isolates of *S. aureus*

Cytokines are secreted by mammalian cells in response to stimuli such as LTA from *S. aureus* (Ellingsen *et al.*, 2002). Some clinical *S. aureus* isolates differ greatly in their ability to induce inflammatory response in endothelial cells (Grundmeier *et al.*, 2010). This led us to investigate whether isolates from healthy individuals differ in their ability to induce production of cytokines from keratinocytes.

All isolates were able to induce the release of proinflammatory cytokines, including IL-1α, TNF-α, IL-6, IL-8, and CXCL-1, and with minor exceptions; the overall pattern was the same after 8 and 24 h for the ten different isolates

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(Fig. 1 and results not shown). However, the magnitude of induction was strain dependent and showed wide ranges of values (Fig. 1 and results not shown). Furthermore, IL-1β was not induced in some of the studied isolates including isolate nos 2, 5, and 8.

Isolate no 6 induced the highest secretion of all the studied proinflammatory cytokines, except for IL-1β (Fig. 1 and results not shown). Interestingly, this particular isolate could induce a higher level of proinflammatory cytokines than the commercial strains *S. aureus* COL, NCTC8325, and MSSA476 except in the case of IL-1β. The highest IL-1β was induced by the presence of COL (Fig. 1).

The ability of HaCaT cells to produce IL-10 (as an anti-inflammatory cytokine) and interleukin-1 receptor antagonist (IL1-RA) responses was also strain dependent, and the magnitude of induction varied between different isolates. The overall pattern of IL-10 expression was the same for all isolates in both time points and included a narrow range of values except for *S. aureus* COL (Fig. 2 and results not shown). However, higher IL1-RA level was found after prolonged stimulation of HaCaT cells with *S. aureus* isolates and included a wide range of values ($P < 0.01$). Furthermore, fluctuation in IL-1α levels was significantly correlated with IL1-RA induction ($r = 0.88$, $P < 0.01$).

Discussion

The potential of *S. aureus* to colonize its host is the result of a complex set of interactions, as well as overcoming host defenses and adhering to nasal or throat surfaces (Edwards *et al.*, 2012; Weidenmaier *et al.*, 2012). In this study, several phenotypic traits among 10 different nasal *S. aureus* isolates were investigated. These isolates represented the five most common (nos 1–5) and five rare *spa* types (nos 6–10) from a healthy population.

Changes in *S. aureus* growth rate influence opsonization and neutrophil–staphylococcal interactions through changes in surface hydrophobicity (Domingue *et al.*, 1996). The results of our study showed that there was no significant difference in the growth rate (Table 4) that could explain the observed success for the isolates with more common *spa* types. However, *S. aureus* growth rate is dependent on nutritional and other conditions and can be highly variable between *in vitro* and *in vivo* situations.

Several mechanisms have been reported for the regulation of biofilm formation in *S. aureus*, including *agr* quorum sensing, phenol-soluble peptides, autoinducing peptides, several surface proteins, DNase, protease, cis-2-decenoic acid, D-amino acids, and pH changes (Boles & Horswill, 2011). The potential of *S. aureus* to form biofilm on host

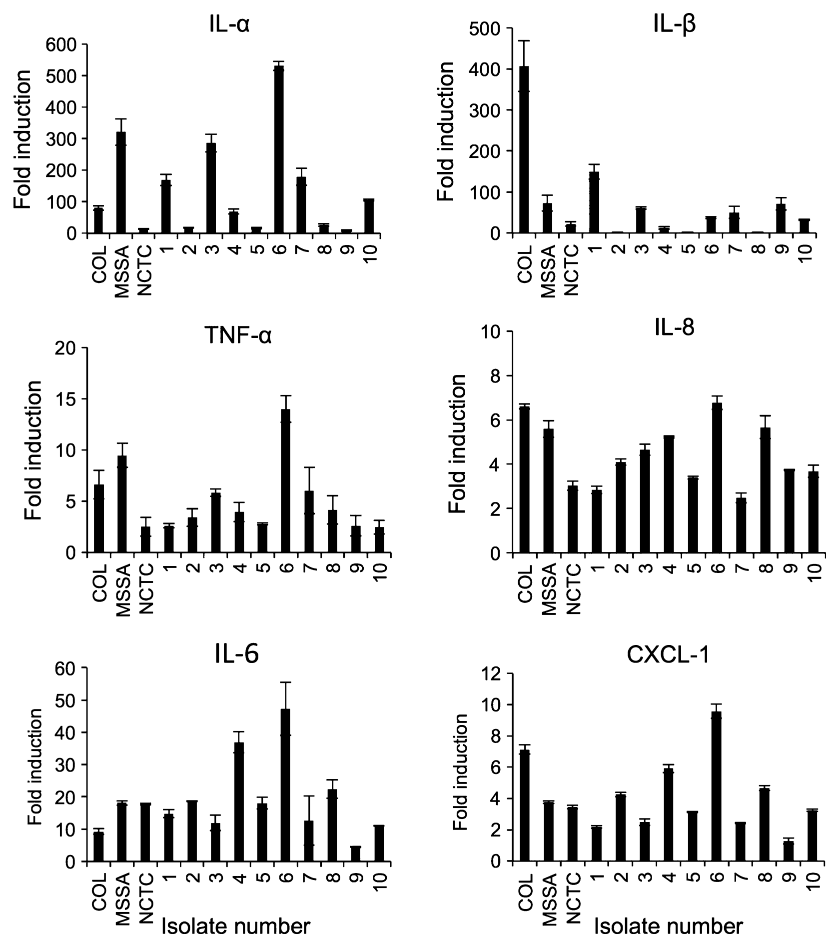

Fig. 1 Proinflammatory cytokines secreted by HaCaT cells after stimulation with *Staphylococcus aureus* for 24 h. The HaCaT cells were left untreated or stimulated with 3.5×10^7 CFU mL^{-1} of *S. aureus* in DMEM. The untreated control cells were arbitrarily set as 1, and secretion of cytokine in the treated cells is presented as fold induction. The data are expressed as mean ± SD of three independent pooled experiments.

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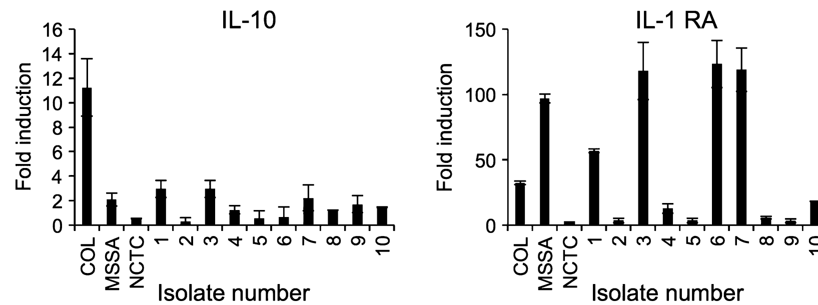

Fig. 2 IL-10 and IL-1RA secreted by HaCaT cells stimulated with *Staphylococcus aureus* for 24 h. The cells were left untreated or stimulated with 3.5×10^7 CFU mL^{-1} of *S. aureus* in DMEM. The untreated control cells were arbitrarily set as 1, and secretion of cytokine in the treated cells is presented as fold induction. The data are expressed as mean ± SD of three independent pooled experiments.

tissues and medical devices is thought to support chronic infections (Boles & Horswill, 2008). A recent study revealed that secretion of a protease called Esp by *S. epidermidis* inhibits *S. aureus* biofilm formation and nasal colonization (Iwase *et al.*, 2010). However, *S. aureus* growth during nasal colonization seems to be dispersed rather than biofilm-associated (Krismer & Peschel, 2011). Our results showed that only one of ten selected nasal isolates (no 10) could produce biofilm under our experimental conditions, and the biofilm was also considered to be weak (Table 4).

Studies of cytokine profiles contribute to understanding of the pathophysiology of infectious diseases. Our data showed that all selected isolates had the capacity to induce expression of proinflammatory cytokines in HaCaT cells, including IL-1α, TNF-α, IL-6, IL-8, and CXCL-1 (Fig. 1). Cytokines enhance leukocyte recruitment to the site of infection (Ozaki & Leonard, 2002; Strindhall *et al.*, 2005). Moreover, cytokines can ligate to their cognate receptor, thereby resulting in an increased host response (Nestle *et al.*, 2009). The magnitude of proinflammatory cytokine secretion varied after induction by different isolates (Fig. 1), and this is in agreement with previous studies. Indeed, comparisons between so-called carrier and noncarrier strains of *S. aureus* showed that they varied in their ability to interfere with expression of TLR2, β-defensin 3, IL-1α, IL-1β, and IL-1Ra in nasal epithelial cell culture (Quinn & Cole, 2007; Quinn *et al.*, 2009). Moreover, great variability in the expression of IL-6, IL-8, CXCL-1, and G-CSF by HUVEC has been reported previously among different clinical *S. aureus* strains (Strindhall *et al.*, 2005).

A crucial step in the nasal colonization establishment is attachment/adhesion of *S. aureus* to the epithelial cells in the nasal cavity (Weidenmaier *et al.*, 2012) and on ciliated epithelial cells deeper inside the vestibulum nasi (Clement *et al.*, 2005; Weidenmaier & Peschel, 2008). The surface proteins ClfB, IsdA, SdrD, and SdrC mediate *S. aureus* attachment to the squamous epithelial cells *in vitro* (O'Brien *et al.*, 2002; Clarke *et al.*, 2006; Corrigan *et al.*, 2009), and some of them can promote nasal colonization in mice (Clarke *et al.*, 2006; Schaffer *et al.*, 2006) and humans (Wertheim *et al.*, 2008). The results of the microarray studies showed the presence of *clfB, isdA, sdrD,* and *sdrC* in the genomes of all the selected isolates, with the exception of *sasG* (Table 3). This is in agreement with previous reports that *sasG* was present in eight of twenty studied lineages (McCarthy & Lindsay, 2010). Interestingly, the ability to adhere to the HaCaT cells varied 100-fold between isolates. This may show that different *S. aureus* isolates may encode variant adhesins that have diverse binding properties and/or expression levels. However, this remains to be investigated.

Staphylococcus aureus is able to internalize into different types of host cells, including human keratinocyte cells (Kintarak *et al.*, 2004). Here, we found that the ability to internalize HaCaT cells varies a 1000-fold between isolates. But, regardless of the numbers, all isolates could internalize into the HaCaT cells (Table 5). The essential role of *S. aureus* internalization in cytokine secretion was previously observed in HUVEC (Yao *et al.*, 1995, 2000). However, in our study, no direct correlation was found in the bacterial ability to internalize and the magnitude of cytokine secretion from HaCaT cells.

Taken together, we have compared *S. aureus* isolates from the nasal cavity of healthy adults. This study clearly demonstrates that *S. aureus* isolates with different *spa* types show great variability in several traits. These variations among isolates may be of importance in their fitness in specific nasal carriers. Thus, these variations should be considered before generalizing experimental results based on single *S. aureus* and merit further investigations.

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References

Agerer F, Michel A, Ohlsen K & Hauck CR (2003) Integrin-mediated invasion of *Staphylococcus aureus* into human cells requires Src family protein-tyrosine kinases. *J Biol Chem* 278: 42524–42531.

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Ammerlaan HS, Kluytmans JA, Wertheim HF, Nouwen JL & Bonten MJ (2009) Eradication of methicillin-resistant *Staphylococcus aureus* carriage: a systematic review. *Clin Infect Dis* 48: 922–930.
Bardoel BW, Vos R, Bouman T *et al.* (2012) Evasion of Toll-like receptor 2 activation by staphylococcal superantigen-like protein 3. *J Mol Med (Berl)* 90: 1109–1120.
Boles BR & Horswill AR (2008) Agr-mediated dispersal of *Staphylococcus aureus* biofilms. *PLoS Pathog* 4: e1000052.
Boles BR & Horswill AR (2011) Staphylococcal biofilm disassembly. *Trends Microbiol* 19: 449–455.
Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM & Beachey EH (1985) Adherence of coagulase-negative staphylococci to plastic tissue-culture plates - a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol* 22: 996–1006.
Clarke SR, Brummell KJ, Horsburgh MJ *et al.* (2006) Identification of *in vivo*-expressed antigens of *Staphylococcus aureus* and their use in vaccinations for protection against nasal carriage. *J Infect Dis* 193: 1098–1108.
Clement S, Vaudaux P, Francois P *et al.* (2005) Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis. *J Infect Dis* 192: 1023–1028.
Cole AM, Tahk S, Oren A, Yoshioka D, Kim YH, Park A & Ganz T (2001) Determinants of *Staphylococcus aureus* nasal carriage. *Clin Diagn Lab Immunol* 8: 1064–1069.
Corrigan RM, Miajlovic H & Foster TJ (2009) Surface proteins that promote adherence of *Staphylococcus aureus* to human desquamated nasal epithelial cells. *BMC Microbiol* 9: 22.
Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI & Knight R (2009) Bacterial community variation in human body habitats across space and time. *Science* 326: 1694–1697.
DeLeo FR, Otto M, Kreiswirth BN & Chambers HF (2010) Community-associated meticillin-resistant *Staphylococcus aureus* Reply. *Lancet* 375: 1557–1568.
Domingue G, Costerton JW & Brown MRW (1996) Bacterial doubling time modulates the effects of opsonisation and available iron upon interactions between *Staphylococcus aureus* and human neutrophils. *FEMS Immunol Med Microbiol* 16: 223–228.
Dreisbach A, Hempel K, Buist G, Hecker M, Becher D & van Dijl JM (2010) Profiling the surfacome of *Staphylococcus aureus*. *Proteomics* 10: 3082–3096.
Edwards AM, Massey RC & Clarke SR (2012) Molecular mechanisms of *Staphylococcus aureus* nasopharyngeal colonization. *Mol Oral Microbiol* 27: 1–10.
Ellingsen E, Morath S, Flo T *et al.* (2002) Induction of cytokine production in human T cells and monocytes by highly purified lipoteichoic acid: involvement of Toll-like receptors and CD14. *Med Sci Monit* 8: BR149–BR156.
Flemming K, Klingenberg C, Cavanagh JP, Sletteng M, Stensen W, Svendsen JS & Flaegstad T (2009) High *in vitro* antimicrobial activity of synthetic antimicrobial peptidomimetics against staphylococcal biofilms. *J Antimicrob Chemother* 63: 136–145.
Foster TJ (2004) Nasal colonization by *Staphylococcus aureus*. *Nat Med* 10: 447; author reply 447.
Fowler VG Jr, Olsen MK, Corey GR *et al.* (2003) Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. *Arch Intern Med* 163: 2066–2072.
Fowler VG Jr, Miro JM, Hoen B *et al.* (2005) *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA* 293: 3012–3021.
Garzoni C & Kelley WL (2009) *Staphylococcus aureus*: new evidence for intracellular persistence. *Trends Microbiol* 17: 59–65.
Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW & European Staphylococcal Reference Laboratory Working Group (2010) Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Med* 7: e1000215.
Grundmeier M, Tuchscherr L, Bruck M *et al.* (2010) Staphylococcal strains vary greatly in their ability to induce an inflammatory response in endothelial cells. *J Infect Dis* 201: 871–880.
Iwase T, Uehara Y, Shinji H *et al.* (2010) *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* 465: 346–349.
Johannessen M, Sollid JE & Hanssen AM (2012) Host- and microbe determinants that may influence the success of *S. aureus* colonization. *Front Cell Infect Microbiol* 2: 56.
Kintarak S, Whawell SA, Speight PM, Packer S & Nair SP (2004) Internalization of *Staphylococcus aureus* by human keratinocytes. *Infect Immun* 72: 5668–5675.
Kluytmans J, van Belkum A & Verbrugh H (1997) Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 10: 505–520.
Krismer B & Peschel A (2011) Does *Staphylococcus aureus* nasal colonization involve biofilm formation? *Future Microbiol* 6: 489–493.
Lamers RP, Stinnett JW, Muthukrishnan G, Parkinson CL & Cole AM (2011) Evolutionary analyses of *Staphylococcus aureus* identify genetic relationships between nasal carriage and clinical isolates. *PLoS One* 6: e16426.
Lindsay JA (2010) Genomic variation and evolution of *Staphylococcus aureus*. *Int J Med Microbiol* 300: 98–103.
McCarthy AJ & Lindsay JA (2010) Genetic variation in *Staphylococcus aureus* surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. *BMC Microbiol* 10: 173.
McCarthy AJ & Lindsay JA (2012) The distribution of plasmids that carry virulence and resistance genes in *Staphylococcus aureus* is lineage associated. *BMC Microbiol* 12: 104.
Melles DC, Gorkink RF, Boelens HA *et al.* (2004) Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. *J Clin Invest* 114: 1732–1740.
Naber CK (2009) *Staphylococcus aureus* bacteremia: epidemiology, pathophysiology, and management strategies. *Clin Infect Dis* 48(suppl 4): S231–S237.
Nestle FO, Di Meglio P, Qin JZ & Nickoloff BJ (2009) Skin immune sentinels in health and disease. *Nat Rev Immunol* 9: 679–691.
Nish S & Medzhitov R (2011) Host defense pathways: role of redundancy and compensation in infectious disease phenotypes. *Immunity* 34: 629–636.
O'Brien LM, Walsh EJ, Massey RC, Peacock SJ & Foster TJ (2002) *Staphylococcus aureus* clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. *Cell Microbiol* 4: 759–770.
Ozaki K & Leonard WJ (2002) Cytokine and cytokine receptor pleiotropy and redundancy. *J Biol Chem* 277: 29355–29358.
Piechowicz L, Garbacz K, Wisniewska K & Dabrowska-Szponar M (2011) Screening of *Staphylococcus aureus* nasal strains isolated from medical students for toxin genes. *Folia Microbiol (Praha)* 56: 225–229.
Quinn GA & Cole AM (2007) Suppression of innate immunity by a nasal carriage strain of *Staphylococcus aureus* increases its colonization on nasal epithelium. *Immunology* 122: 80–89.
Quinn GA, Tarwater PM & Cole AM (2009) Subversion of interleukin-1-mediated host defence by a nasal carrier strain of *Staphylococcus aureus*. *Immunology* 128: e222–e229.

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Sangvik M, Olsen RS, Olsen K, Simonsen GS, Furberg AS & Sollid JU (2011) Age- and gender-associated *Staphylococcus aureus* spa types found among nasal carriers in a general population: the Tromsø Staph and Skin Study. *J Clin Microbiol* 49: 4213–4218.

Schaffer AC, Solinga RM, Cocchiaro J *et al.* (2006) Immunization with *Staphylococcus aureus* clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. *Infect Immun* 74: 2145–2153.

Sivaraman K, Venkataraman N & Cole AM (2009) *Staphylococcus aureus* nasal carriage and its contributing factors. *Future Microbiol* 4: 999–1008.

Smyth GK & Speed T (2003) Normalization of cDNA microarray data. *Methods* 31: 265–273.

Snipen L, Nyquist OL, Solheim M, Aakra A & Nes IF (2009) Improved analysis of bacterial CGH data beyond the log-ratio paradigm. *BMC Bioinformatics* 10: 91.

Solheim M, Aakra A, Snipen LG, Brede DA & Nes IF (2009) Comparative genomics of *Enterococcus faecalis* from healthy Norwegian infants. *BMC Genomics* 10: 194.

Strindhall J, Lindgren PE, Lofgren S & Kihlstrom E (2005) Clinical isolates of *Staphylococcus aureus* vary in ability to stimulate cytokine expression in human endothelial cells. *Scand J Immunol* 61: 57–62.

van Belkum A, Melles DC, Nouwen J *et al.* (2009a) Co-evolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. *Infect Genet Evol* 9: 32–47.

van Belkum A, Verkaik NJ, de Vogel CP *et al.* (2009b) Reclassification of *Staphylococcus aureus* nasal carriage types. *J Infect Dis* 199: 1820–1826.

Vaudaux P & Schrenzel J (2004) Nosocomial bacteraemia caused by *Staphylococcus aureus*. *Lancet* 364: 644–645.

von Eiff C, Becker K, Metze D, Lubritz G, Hockmann J, Schwarz T & Peters G (2001) Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with Darier's disease. *Clin Infect Dis* 32: 1643–1647.

Weidenmaier C & Peschel A (2008) Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nat Rev Microbiol* 6: 276–287.

Weidenmaier C, Goerke C & Wolz C (2012) *Staphylococcus aureus* determinants for nasal colonization. *Trends Microbiol* 20: 243–250.

Wertheim HF, Walsh E, Choudhurry R *et al.* (2008) Key role for clumping factor B in *Staphylococcus aureus* nasal colonization of humans. *PLoS Med* 5: e17.

Yao L, Bengualid V, Lowy FD, Gibbons JJ, Hatcher VB & Berman JW (1995) Internalization of *Staphylococcus aureus* by endothelial cells induces cytokine gene expression. *Infect Immun* 63: 1835–1839.

Yao L, Bengualid V, Berman JW & Lowy FD (2000) Prevention of endothelial cell cytokine induction by a *Staphylococcus aureus* lipoprotein. *FEMS Immunol Med Microbiol* 28: 301–305.

Zautner AE, Krause M, Stropahl G *et al.* (2010) Intracellular persisting *Staphylococcus aureus* is the major pathogen in recurrent tonsillitis. *PLoS One* 5: e9452.

Ziebandt AK, Kusch H, Degner M *et al.* (2010) Proteomics uncovers extreme heterogeneity in the *Staphylococcus aureus* exoproteome due to genomic plasticity and variant gene regulation. *Proteomics* 10: 1634–1644.

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