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ORIGINAL ARTICLE

Contaminations of laboratory surfaces with *Staphylococcus aureus* are affected by the carrier status of laboratory staff

M. Schmidlin, M. Alt, G. Vogel, U. Voegeli, P. Brodmann and C. Bagutti

State Laboratory Basel City, Biosafety Laboratory, Kannenfeldstrasse 2, Basel, Switzerland

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Correspondence
Claudia Bagutti, State Laboratory Basel City, Kannenfeldstrasse 2, CH-4012 Basel, Switzerland. E-mail: claudia.bagutti@bs.ch

Present address
G. Vogel, Mabritec AG, Lörracherstrasse 50, CH-4125 Riehen, Switzerland.

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Abstract

Aim: As a biosafety laboratory, we take samples from surfaces in microbiological laboratories to survey the handling of micro-organisms. Whereas contaminations with other micro-organisms were rare, *Staphylococcus aureus* was found in the working environment of many laboratories. As 20–60% of the healthy population are carriers of *S. aureus* we wanted to asses the effect of carriers on our sampling results.

Methods and Results: Nasal swabs of staff members in nonmicrobiological laboratories and offices as well as surface samples from their personal work environment were taken and analysed for *S. aureus* DNA. In addition *S. aureus* strains were isolated using *S. aureus*-specific agar plates and analysed by randomly amplified polymorphic DNA (RAPD)–PCR and multilocus sequence typing (MLST). Our data show that contaminations with *S. aureus* in nonmicrobiological environments are common with 29% of the surface samples containing *S. aureus* DNA. In the working environment of carriers, the number of contaminations was significantly increased compared to the environment of noncarriers.

Conclusion: The carrier status of staff members significantly affects the number of contaminations on laboratory surfaces. Therefore, even in the absence of intentional handling of *S. aureus*, contaminations can be detected on a substantial amount of surfaces.

Significance and Impact of the Study: Sampling procedures need to be adapted based on these results with respect to the locations where samples are taken and the threshold for significant contaminations. Because of its wide distribution, *S. aureus* can serve as a marker for hygienic standards in laboratories.

Introduction

Compliance with guidelines for the work in enclosed systems and good laboratory practice are prerequisites for safe handling of pathogenic and genetically modified micro-organisms. As an enforcement authority, we survey biotech companies and research institutes subjected to the Swiss Containment Ordinance (1999). We take samples from laboratory surfaces to check for contaminations with intentionally used and potentially harmful micro-organisms. In this context, we perform sampling campaigns focusing on specific locations (e.g. centrifuges) or organisms to compare the results between different laboratories.

Staphylococcus aureus is a frequently handled pathogen in diagnostic laboratories and is one of the most common causes of community- and hospital-acquired infections. The emergence of multidrug-resistant strains has made these infections more difficult to treat posing a major challenge in hospitals (Chambers 2001). In a large fraction of the population, *S. aureus* colonizes the human skin, throat and most frequently the anterior nares without causing any harm to the carriers. However, nasal carriage has been reported to increase the risk of *S. aureus* infections (Kluytmans *et al.* 1997; von Eiff *et al.* 2001; Wertheim *et al.* 2005). Twenty to sixty per cent of the healthy population are carriers of *S. aureus* (Kluytmans *et al.* 1997; Vandenbergh *et al.* 1999). A large study

including about ten thousand US citizens showed a mean carriage rate of 37·2% in the population (Graham *et al.* 2006; Kuehnert *et al.* 2006). The different rates found in different studies can be explained by the fact that there are two types of carriers: Approximately 20% of the population are persistent carriers, whereas 60% carry *S. aureus* intermittently. Twenty per cent never carry *S. aureus* at all (Kluytmans *et al.* 1997; Vandenbergh *et al.* 1999). The highest number of colony forming units can be isolated from the nares of persistent carriers (Vandenbergh *et al.* 1999). The factors affecting nasal carriage rate are not clearly defined but seem to involve host-specific (Cole *et al.* 2001; Nouwen *et al.* 2004; van den Akker *et al.* 2006; van Belkum *et al.* 2007) as well as bacterial-specific factors (Weidenmaier *et al.* 2004). No hyper-virulent strains have been described (Feil *et al.* 2003), and large numbers of unique genotypes can be identified among carriers with one strain per person in most cases (Sakwinska *et al.* 2009). Ethnicity, age and sex (Bischoff *et al.* 2004; Choi *et al.* 2006; Graham *et al.* 2006; Kuehnert *et al.* 2006) as well as behavioural factors (Wertheim *et al.* 2006) have been shown to influence the carriage state, but these data are not consistent.

Staphylococcus aureus is transmitted by direct contact or airborne dispersal (Bassetti *et al.* 2005; Bischoff *et al.* 2006). Surface contaminations are a common source of nosocomial infections as *S. aureus* is able to survive for months on dry surfaces (Kramer *et al.* 2006) and despite regular decontamination inanimate surfaces in hospitals are frequently contaminated with this pathogen (Boyce *et al.* 1997; French *et al.* 2004; Lemmen *et al.* 2004). Therefore, careful disinfection after handling *S. aureus* is necessary in microbiological laboratories.

In this work, we present data we collected since 2002 by swabbing surfaces in different laboratories in Switzerland. The swabs were analysed for the presence of *S. aureus* and other micro-organisms (*Pseudomonas aeruginosa, Salmonella enterica* and *Streptococcus pneumoniae*) handled in these laboratories. Different contamination rates with these micro-organisms might be expected because of their differing ability to adhere and survive on surfaces. In particular, the common nasal carriage of *S. aureus* may affect the amount of contaminated surfaces. Therefore, we wanted to study the background load we detect with our method in an environment similar to the microbiological laboratories but with no experimental contact to *S. aureus*. We analysed whether a correlation between the carrier status of staff members and contaminations in the personal work environment of these people can be found. For this purpose, the anterior nares of volunteers at the State Laboratory of Basel City and different locations in the personal work environment of these people were sampled. The load of *S. aureus* was determined, and the results were compared.

Materials and methods

Micro-organisms and cultures

Positive control *S. aureus* ATCC 25923 (DSMZ, Braunschweig, Germany) and swab samples tested for *S. aureus* were cultured at 37°C under aerobic conditions on blood agar plates (Columbia agar with sheep blood, BA; Oxoid, Pratteln, Switzerland) or in tryptone soy broth (TSB; Oxoid). In addition, *S. aureus* samples were cultured on CHROMAgar™ *S. aureus* plates (CSA; Becton Dickinson, Allschwil, Switzerland). *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus pneumoniae* DSMZ 11967 and samples tested for these micro-organisms were transported in TSB, whereas samples tested for *Salmonella enterica* and the respective positive control NZL 66125-89 were placed in peptone water (Oxoid).

Sample collection

Nasal swabs from each nostril of 40 employees at the State Laboratory of Basel City were taken with a sterile, PBS-moistened cotton swab. The swab was placed in 3-ml growth medium on ice for transport. In addition, a sample was taken from the desk of the volunteers by wiping *c.* 100 cm^2 of the surface with PBS-moistened cotton swabs before placing the sample into 3 ml of TSB on ice. At latest 2 h after swabbing, 200 μl aliquots of the samples were taken for DNA extraction (direct sample). After an overnight incubation step, another 200 μl aliquot was taken for DNA extraction (enriched sample) and 100 μl were streaked on CSA plates for strain isolation. The CSA plates were incubated for 1 day. Positive colonies (*S. aureus* cells grow as mauve-coloured colonies on CSA plates) were verified using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Further swab samples were also taken from the computer mouse and the personal telephone of the volunteers, and DNA was extracted directly and after overnight enrichment. For positive controls, overnight cultures of the respective bacteria were streaked onto empty plastic Petri dishes and air-dried for 4 h before swabbing. Swabbing efficiency from Petri dishes was similar to laboratory surfaces ranging from 20 to 70% depending on the organism. The samples from the microbiological laboratories were wiped as described earlier and placed in 5 ml of the appropriate growth medium on ice for transport prior to DNA extraction.

DNA extraction

DNA was extracted using QIAamp® DNA Mini Kit (Qiagen, Basel, Switzerland) according to the manufacturer's instructions.

TaqMan® quantitative real-time PCR

Primers and probes used for the detection of *S. aureus* and *S. epidermidis* 23S rRNA gene copies in the swab samples from the State Laboratory have been described by Ludwig and Schleifer (2000). In a 25 μl reaction, 100 nmol l^{-1} primers, 100 nmol l^{-1} probe, 1× TaqMan® Universal PCR Master Mix or Fast Universal PCR Master Mix (Applied Biosystems, Rotkreuz, Switzerland) and 5-μl DNA extracts were mixed. Amplification and analysis were performed either on the 7500 Fast System (Applied Biosystems) with an initial heating to 95°C for 10 min before performing 45 cycles of 95°C for 15 s and 65°C for 60 s or on the StepOne™Plus System (Applied Biosystems) with an initial heating to 95°C for 20 s before performing 45 cycles of 95°C for 3 s and 65°C for 30 s (Fast Protocol). Both systems delivered comparable results. Primers for *S. enterica* and *S. pneumoniae* detection have been described by Rahn *et al.* (1992) and Greiner *et al.* (2001), respectively. For detection of the *P. aeruginosa*, the following primers and probe were used: P.aerug_oprL_F (5′-CGA TGT GCG CGA GCC TT-3′), P.aerug_oprL_R (5′-CGA ATT TCA GCA TTT CCA TCA TG -3′) and P.aerug_oprL_VIC_MGB (5′-VIC-ATA AGT TTG TAA CCG TTG GCG AC-MGB-3′). All primers and probes were tested for specificity and cross-reactivity (data not shown). For quantification, PCR products of the respective sequences were cloned into pGEM-T Vector using the pGEM®-T System II (Promega, Wallisellen, Switzerland), and standard dilutions of the plasmid with known gene copies were amplified. These dilutions as well as swabbing experiments of known amounts of dried bacteria were used to determine the limit of quantification, which was at ten copies per PCR (10^4 copies per sample for the microbiological laboratories and 6×10^3 for surfaces in a nonmicrobiological environment). Amplification was performed in duplicates, and only results with two values above the limit of quantification (LOQ) were considered positive. For nasal swabs, the mean value of the two nostrils was calculated. The measurement error of the combined swabbing and quantification method is less than 35% (data not shown).

MALDI-TOF MS

Colonies from CSA plates were picked and streaked for single colonies on BA plates. After overnight incubation, a small amount of cell material was picked and placed onto a stainless steel target plate. The sample was mixed on the plate with 0·3 μl of 2·5-dihydroxybenzoic acid matrix solution (2·5-DHB; AnagnosTec, Potsdam-Golm, Germany) and co-crystallized by air-drying at room temperature prior to analysis. Mass spectra were acquired using a Voyager-DE™ PRO Biospectrometry Workstation (Applied Biosystems) in the linear mode. The mass range of spectra was from 2000 to 20 000 m/z. *E. coli* DH5α samples were used for external calibration. From each sample, six spectra with 75 laser shots were accumulated. MALDI-TOF MS creates mass spectral fingerprints of micro-organisms predominantly from the desorbed cell wall (Claydon *et al.* 1996; Edwards-Jones *et al.* 2000; Lay 2001). The obtained mass spectra were compared with spectra of known micro-organisms using the SARAMIS™ software (Spectral Archiving and Microbial Identification System; AnagnosTec) containing a database of over one thousand species. At least four different colonies were analysed for each sample.

Randomly amplified polymorphic DNA-PCR

Three different primers were used to amplify polymorphic regions of the *S. aureus* genome in three independent standard PCRs as described elsewhere (van Belkum *et al.* 1995; van Leeuwen *et al.* 1996): 5 ng (in the AP-1 and AP-7 reaction) or 25 ng (in the ERIC-2 reaction) of genomic DNA from overnight cultures were used as templates together with 1× PCR Gold buffer (Applied Biosystems), 2·5 mmol l^{-1} $MgCl_2$, 0·2 units AmpliTaq Gold® DNA Polymerase (Applied Biosystems) 0·2 mmol l^{-1} dNTPs and 1 μmol l^{-1} Primer (AP-1, AP-7 or ERIC-2) in a 50-μl reaction. Forty cycles of 1 min 94°C, 1 min 40°C, 2 min 72°C were run and the amplified fragments were separated on a ethidium bromide-stained 1·5% agarose gel and analysed under UV light. As an internal control, PCR products from genomic DNA of a reference strain were run on each gel.

Multilocus sequence typing

MLST was performed as described by Enright *et al.* (2000). Sequences of fragments of the housekeeping genes such as carbamate kinase (arcC), shikimate dehydrogenase (aroE), glycerol kinase (glp), guanylate kinase (gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi) and acetyl coenzyme A acetyltransferase (yqiL) were compared. PCRs were carried out in a 50 μl reaction containing 5 ng chromosomal DNA, primers at a final concentration of 1 μmol l^{-1}, 1 U AmpliTaq Gold® DNA Polymerase (Applied Biosystems), 0·2 mmol l^{-1} dNTPs and 2·5 mmol l^{-1} $MgCl_2$ in 1× PCR Gold buffer (Applied Biosystems). The PCR was performed in a Eppendorf Mastercycler gradient (Vaudaux-Eppendorf, Schönenbuch, Switzerland), with an initial 4 min denaturation at 95°C, followed by 30 cycles of 1 min 95°C, 1 min 55°C and 1 min 72°C and a final extension step of 5 min at 72°C. The PCR product

was analysed on an agarose gel and purified using the QIAquick® PCR purification kit (Qiagen). One microlitre of the purified PCR product and 3·2 pmol of the primers used in the initial amplification were used for the sequencing reaction with the BigDye® Terminator v3·1 kit (Applied Biosystems) according to the manufacturer's instructions. The products were precipitated with 70% ethanol, washed with 100% ethanol and resuspended in formamide. Sequences were determined on the ABI Prism 3100 Genetic Analyser (Applied Biosystems). Sequences of at least five different loci were compared for each strain.

Ethical statement

This study was approved by the Basel ethics committee (EKBB).

Results

Staphylococcus aureus contaminations in diagnostic laboratories

During biosafety inspections in diagnostic laboratories in Switzerland, samples were taken and tested for the presence of DNA from different micro-organisms, including *S. aureus, S. pneumoniae, S. enterica* and *P. aeruginosa*. All samples taken since 2002 in centrifuges or at places, where no micro-organisms are handled (outside the primary containment), are summarized in Fig. 1a. The data are arranged in order of increasing amounts of gene copies. This allows a direct comparison of the amount of contaminations found for a particular micro-organism per spot. As not the same number of samples were taken for the different bacteria at the indicated locations, the

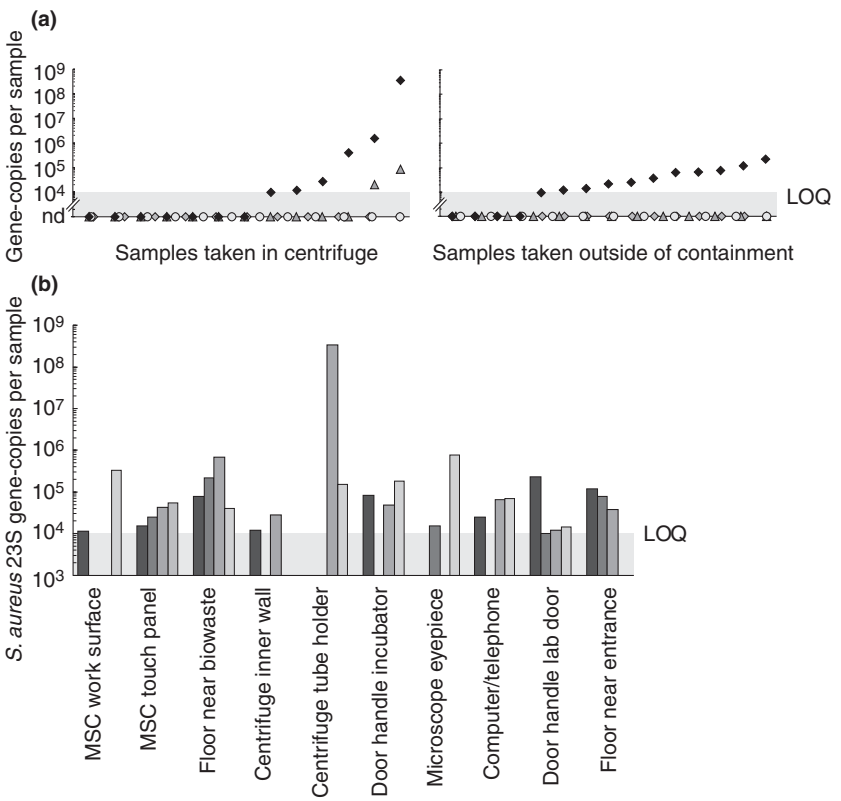

Figure 1 *Staphylococcus aureus* contaminations on laboratory surfaces in microbiological laboratories. (a) The numbers of organism-specific gene-copies per wipe-sample detected by real-time PCR from all samples analysed for *S. aureus*, *P. aeruginosa*, *S. pneumoniae* and *S. enterica* since 2002 are summarized. Each symbol represents a sample taken in a centrifuge or outside the containment (places where no bacteria are handled). For each microorganism, the data are arranged according to the number of gene-copies in the sample (samples for each organism were equally distributed on the *x*-axis with the lowest number on the left and highest on the right). The grey box represents the limit of quantification (10 000 gene copies/sample; LOQ). *S. aureus*, (◆); *P. aeruginosa*, (◈); *S. pneumoniae*, (▲) and *S. enterica* (○). (b) Levels of *S. aureus*-specific 23S rRNA gene-copies per wipe-sample detected by real-time PCR found on comparable surfaces in four different diagnostic laboratories are shown. One sample was taken from each indicated surface. MSC, Microbiological Safety Cabinet. (■) Lab A; (■) Lab B; (■) Lab C; (□) Lab D.

data points are equally distributed on the *x*-axis for each of the four tested micro-organisms. The data show that *S. aureus* contaminations are much more frequent than contaminations with other organisms. Whereas in centrifuges contaminations with *S. pneumoniae* could be found (Fig. 1a, graph on the left), the number of *S. aureus*-specific gene copies detected was much higher. Outside of the primary containment, only significant contaminations with *S. aureus* could be detected (Fig. 1a, graph on the right). Interestingly, the contaminations found in the centrifuge are fewer but more severe than the contaminations found outside of the containment.

Focusing on results from four diagnostic laboratories, where identical locations were wiped, shows that *S. aureus*-specific DNA could be detected in all four laboratories (Fig. 1b). Again, no significant contaminations with other micro-organisms handled in these laboratories were detected (data not shown). In 28 out of 40 samples (70%), *S. aureus* DNA was found with levels significantly surpassing the limit of quantification (grey box, Fig. 1b). Analysis of the samples after overnight enrichment showed increased levels of *S. aureus* in 4 of these 28 samples indicating the presence of living organisms (data not shown). A similar distribution of *S. aureus* DNA could be observed in all four laboratories with contaminations at 'spillage hotspots' as well as at locations outside the containment.

Staphylococcus aureus contaminations in a nonmicrobiological environment

To investigate the background levels of *S. aureus* in a laboratory environment, we found a suitable environment in the State Laboratory of Basel City. In this building, many nonmicrobiological laboratories (chemical laboratories) and offices that are spatially separated from biological laboratories could be sampled. Forty persons volunteered to participate in this study. Three surfaces in the personal working environment of each volunteer were swabbed (telephone, computer-mouse and desk in the laboratory for laboratory staff or in the office for people working primarily in the office). We concentrated on spots that were exclusively used by one of the participants to be able to assign the contaminations to the individuals. Fifteen per cent of the direct samples of all locations (18 out of 120) contained *S. aureus* with up to $2{\cdot}7 \times 10^5$ gene copies per sample (Fig. 2a, bars). These values significantly surpassed the limit of quantification. After an overnight enrichment step, 29% of all samples (35 out of 120) contained quantifiable levels of *S. aureus* 23S rRNA gene copies (Fig. 2b, bars). The contaminations were evenly distributed among the different locations with no preference to a certain spot. Seventeen of the positive samples in the enriched fraction contained *S. aureus* concentrations higher than 10^8 23S rRNA gene copies per sample. Because of the significant increase in specific DNA, these samples were considered to contain living bacteria. In the working environment of nine persons, all three surfaces tested contained *S. aureus* (Fig. 2b), whereas in 24 cases no significant contaminations were found on the three surfaces tested.

Carrier status of laboratory staff

In parallel to the surface samples, nasal swabs from all volunteers were taken, one in each nostril. Again, DNA was extracted from direct and enriched samples. As a positive control, the presence of *Staphylococcus epidermidis* 23S rRNA gene copies was investigated in the direct samples. *S. epidermidis* colonizes the nares of all people, and as expected, all volunteers tested positive for *S. epidermidis* indicating that the process of sampling and DNA extraction was successful (data not shown). Fifty per cent (20 out of 40) of the persons tested carried *S. aureus* in their anterior nares. Fourteen of these carriers could already be identified from the direct sample, whereas six additional carriers were revealed after the overnight incubation step. The maximal number of 23S rRNA gene copies found in the direct nasal sample was $5{\cdot}9 \times 10^6$ (mean value of the two nostrils, Fig. 2a, diamonds).

Correlation between carrier status and contaminations

To find out whether the number and severity of the contaminations found in the working area correlate with the carrier status of the staff members, we compared the data obtained from the nasal swabs and from the surface wipes. Interestingly, no significant contamination could be found in the direct samples of noncarriers (Fig. 2a, right half of graph). Among the enriched samples, only four contaminations could be found at the working place of noncarriers (Fig. 2b, volunteers 4, 21, 24 and 34). Of those contaminations, three might be assigned to carriers working in the same laboratory or office (person 21 works with person 37, person 24 with person 23 and person 34 with person 35). The six persons with the highest amount of *S. aureus* bacteria in the nares had significant contaminations on all three surfaces tested. The other carriers had variable numbers of contaminated surfaces although still more than the noncarriers. Among the carriers, a mean contamination rate of 1·6 contaminations (out of 3 possible contaminations) per person was found, whereas only an average rate of 0·2 was found among noncarriers (Student's *t*-test $P < 0{\cdot}0001$). These data strongly suggest that the detected contaminations stem from the carriers working at the respective places and that

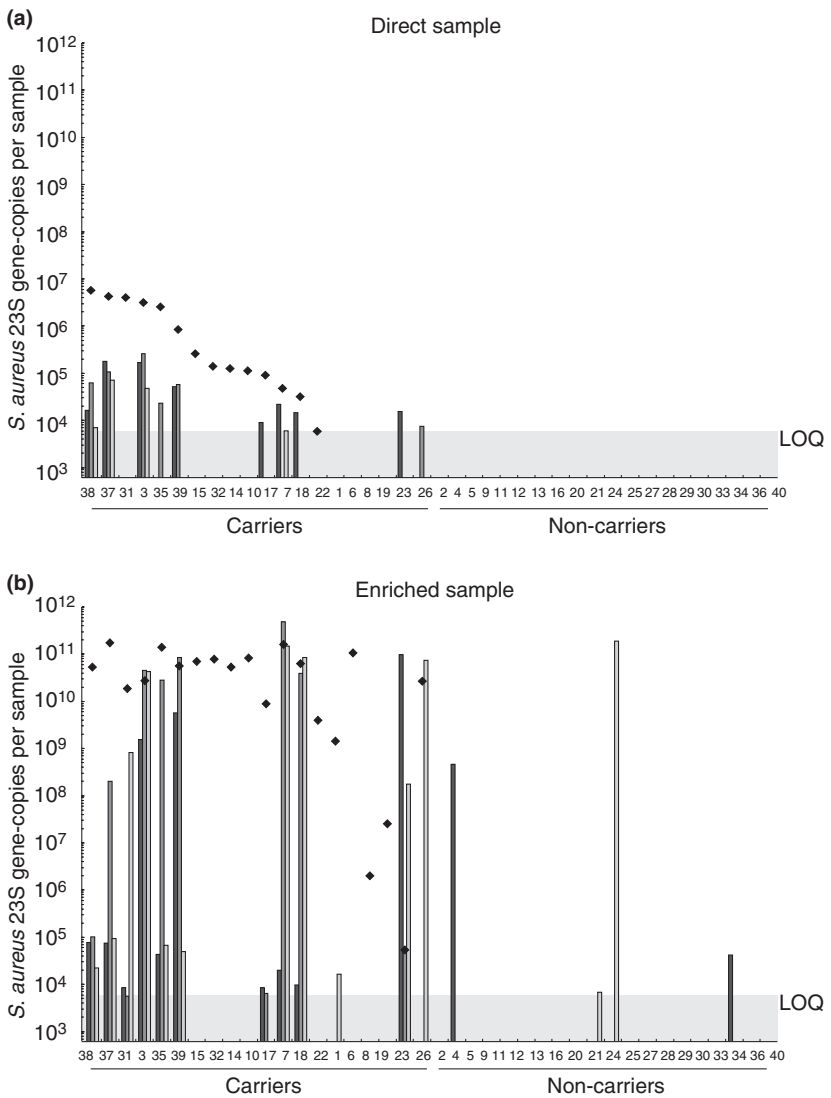

Figure 2 Correlation between carrier-status and contaminations in the personal working environment. *Staphylococcus aureus* 23S rRNA gene-copies/sample were measured by quantitative real time PCR on surfaces (bars) and in nasal samples (mean values of the two nostrils are shown as diamonds). The volunteers are numbered from 1 to 40. DNA was extracted either directly after sampling (a) or after overnight incubation (b). The data are arranged according to the number of gene-copies in the direct sample (nasal sample with the highest number on the left and the non-carriers on the right). The grey box represents the limit of quantification (6000 gene copies/sample; LOQ). (■) telephone; (▩) computer; (□) desk; (◆) nose.

the carriage load has an influence on the spreading of the bacteria on surfaces. The detailed data are summarized in Table 1.

Typing of *Staphylococcus aureus* strains

To investigate whether the *S. aureus* strains found on the surfaces are identical to the strains found in the nares of the carriers, we analysed the strains isolated from CSA plates. The identity of the colonies was confirmed using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Where available, corresponding samples from nose and desk were typed using randomly amplified polymorphic DNA (RAPD)–PCR. Comparing the patterns of amplified DNA fragments by gel electrophoresis allows the identification of identical strains. Three independent reactions using three different primers were run (Fig. 3). The ERIC-2 primer had the least distinctive power with eight different patterns being detected (Table 2). With the other two primers, ten different patterns could be observed. Seven samples from the nose could be directly compared to samples from the desk: Four times identical *S. aureus* strains could be found in the nares and on the respective desk (Table 2, persons 3, 7, 18, 31). These strains were exclusively found in the vicinity of the corresponding carrier. In two of the remaining cases, where the patterns did not match, the volunteers only carried minor amounts of *S. aureus* in their nose and could only be identified as carriers after enrichment of the nasal sample (persons 23, 26). Interestingly, the strain found at these locations (Table 2, pattern 5 in AP-1, AP-7 primed reactions, pattern 4 in ERIC-2 primed reaction) appeared also on a desks of a noncarrier (person 24). It could not be assigned to a strain found in

Table 1 Contaminations found among carriers and noncarriers of *Staphylococcus aureus*

Swabbed surfaces	No. of samples	No. of positives (%)	
		Direct sample	Enriched sample
All surfaces	120	18 (15·0)	35 (29·2)
Surfaces at work environment of carriers (DS)*	42	16 (38·1)	26 (61·9)
On telephone	14	7 (50·0)	9 (64·3)
On PC	14	5 (35·7)	9 (64·3)
On desk	14	4 (28·6)	8 (57·1)
Surfaces at work environment of carriers (ES)*	18	2 (11·1)	5 (27·8)
On telephone	6	1 (16·7)	1 (16·7)
On PC	6	1 (16·7)	1 (16·7)
On desk	6	0 (0·0)	3 (50,0)
Surfaces at work environment of non-carriers	60	0 (0·0)	4 (6·7)
On telephone	20	0 (0·0)	2 (10·0)
On PC	20	0 (0·0)	1 (5·0)
On desk	20	0 (0·0)	1 (5·0)

*Carriers were identified in direct sample (DS) or enriched sample (ES).

the nose of a staff member. However, two of the persons having this strain on their desks work in the same laboratory and the other person periodically visits this laboratory. Only in one case (Table 2, person 39), *S. aureus* could be detected in the direct sample of the nasal swab and the isolated strains from desk and nose did not match. The results of the RAPD–PCR analysis were verified using MLST (Table 2). Seven loci were sequenced and compared. Between three (pta locus) and seven (tpi locus), different sequences were obtained per locus. Two times the isolated colonies turned out not to be *S. aureus*; therefore, no MLST was performed on these bacteria. At least 5 different loci were compared for each of the remaining strains, and the results obtained by RAPD–PCR typing were confirmed: Four times *S. aureus* strains with identical sequences were discovered on the desk and in the nares of the respective staff members (Table 2, persons 3, 7, 18 and 31), and one strain with unknown origin could be detected on three desks including one from a noncarrier (Table 2, persons 23, 24 and 26). The two remaining *S. aureus* strains isolated from the nares of two carriers were not found on any surfaces (Table 2, persons 26 and 39).

Discussion

As an enforcement authority, we survey laboratories for their compliance with the guidelines for the work in closed systems and 'good laboratory practice' listed in the Swiss Containment Ordinance (1999) by sampling laboratory surfaces. The sampling comprises wiping of different spots. As we do not monitor contaminations in laboratories on a regular basis, our samplings are snapshots of the situation in the laboratories. Because of practical reasons, it is not possible to wipe the same spot twice for statistical analysis. Therefore, at least ten different locations are sampled per laboratory to be able to draw qualitative conclusions. To achieve a higher detection rate, we include an enrichment step, allowing us a sensitive detection of relevant contaminations. A systematic analysis of all samples taken by our laboratory since 2002 demonstrates that, compared to other micro-organisms, *S. aureus* contaminations are very common. *Staphylococcus aureus* may be used more frequently in laboratories and may survive longer on surfaces than some of the

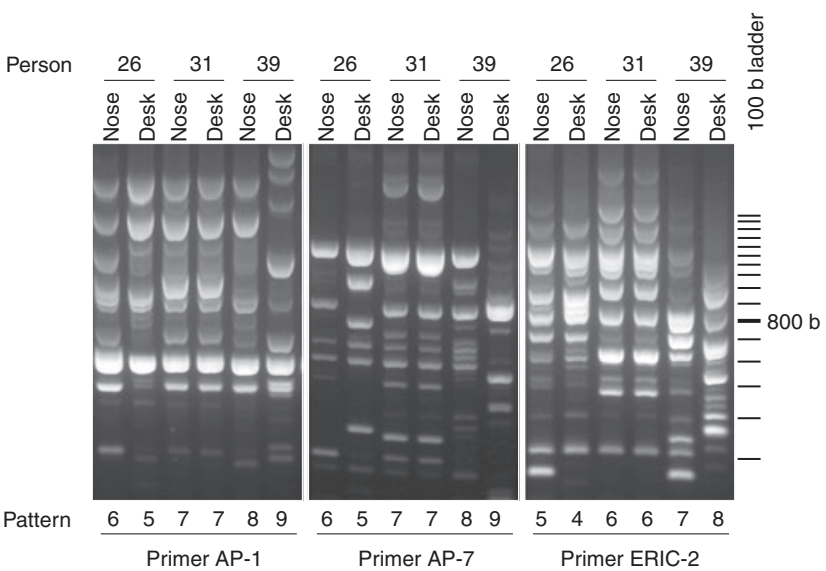

Figure 3 RAPD-PCR typing: Representative examples of RAPD-PCR typing are shown. The volunteer number and the location where the sample was taken are indicated on top. Three individual reactions using primers AP-1, AP-7 or ERIC-2 are shown and numbers were assigned to each pattern (see also Table 2).

Table 2 Different *Staphylococcus aureus* strains detected by RAPD–PCR typing and MLST

		RAPD pattern			MLST sequence							
Sample		AP-1	AP-7	ERIC-2	arcC	aroE	glpF	gmk	pta	tpi	yqil	Remark*
Person 3	Nose	1	1	1	1	1	1	–	1	–	1	*S. aureus* in DS
	Desk	1	1	1	1	1	1	1	1	1	1	
Person 7	Nose	2	2	2	2	2	2	2	2	2	2	*S. aureus* in DS
	Desk	2	2	?	2	2	2	2	2	2	2	
Person 18	Nose	3	3	2	3	3	2	3	2	3	2	*S. aureus* in DS
	Desk	3	3	2	3	3	2	3	2	3	2	
person 23	Nose†	4	4	3	ND	ND	ND	ND	ND	ND	ND	*S. aureus* in ES
	Desk	5	5	4	4	4	1	3	1	–	3	
Person 24	Desk	5	5	4	4	4	1	3	1	4	3	non-carrier
Person 26	Nose	6	6	5	1	5	3	4	1	5	4	*S. aureus* in ES
	Desk	5	5	4	4	4	1	3	1	4	3	
Person 31	Nose	7	7	6	5	3	2	3	2	6	5	*S. aureus* in DS
	Desk	7	7	6	5	3	2	3	2	6	5	
Person 39	Nose	8	8	7	–	6	4	5	3	7	–	*S. aureus* in DS
	Desk†	9	9	8	ND	ND	ND	ND	ND	ND	ND	
Control		10	10	2	ND	ND	ND	ND	ND	ND	ND	ATCC25923

Numbers were assigned to each pattern detected with RAPD–PCR and to each sequence obtained by MLST. ND, not determined; –, no sequence obtained.

*DS, direct sample; ES, enriched sample.

†Isolated colony turned out not to be *S. aureus*.

other organisms tested (*S. enterica* and *S. pneumoniae*). Interestingly, contaminations are not restricted to surfaces, where the micro-organism is handled intentionally. The places where *S. aureus* DNA was found (such as door handles, telephones) and the fact that contaminations at these places are very common but not severe suggest that at least some of these contaminations do not come from the handling of the micro-organism but from the carriers among the staff members. The subsequent study, aimed at mimicking our routine sampling procedures, showed that in nonmicrobiological laboratories and offices, 15% of the samples taken contained *S. aureus* DNA in the direct sample with levels of up to $2{\cdot}7 \times 10^5$ 23S rRNA gene copies per sample. Many of the contaminations exhibited lower levels and, therefore, are likely to originate from the people frequenting the laboratories. However, the rate of contamination was considerably higher than the observed background load in a nonmicrobiological environment. If we take into account that in the background study offices were included, where generally surfaces are less frequently cleaned and where no gloves are used, our experimental set-up represents a 'worst-case scenario' with respect to *S. aureus* contaminations originating from people. This strongly suggests that still a considerable amount of the contaminations found in the microbiological environment were because of the handling of *S. aureus*. Furthermore, the location and the level of these contaminations (such as those in centrifuges) are strengthening this hypothesis.

Our data showed a nasal carriage rate of 50% among the staff of the State Laboratory. Because some carriers can be identified by throat swabs only (Mertz *et al.* 2007), the actual number of carriers is probably even higher. However, when *S. aureus* only colonizes the throat, a distribution of the bacteria onto surfaces is less likely. We therefore concentrated on nasal carriers. This study was designed to reveal potentially existing correlations between work environment contaminations and carrier status of the staff at the date of swabbing. Therefore, we tested the participants of this study only once, being aware of the fact that a proportion of the carriers are intermittent carriers. This snapshot approach mimics the sampling situation in laboratories. Although similar experiments on the distribution of *S. aureus* have been performed in the hospital environment, to our knowledge, this is the first study focusing on the laboratory environment and correlating the carrier status of persons with the distribution of the micro-organism. Our results demonstrate a clear correlation between the carrier status of a person and the number of contaminations found at their working environment. Although some behavioural differences (nose picking, personal hygiene) are likely to have an effect on the spreading of the bacteria, the amount of *S. aureus* in the nose plays an important role. In addition, identical strains could be assigned to nasal samples and the respective environmental swab by RAPD–PCR typing and MLST. Only persons with low levels of *S. aureus* in the nasal sample had additional or

different strains on their desk surfaces. These contaminations could either originate from undetected strains carried by laboratory workers or brought into the laboratory from outside the building.

Taken together, the presented data show that substantial contaminations with *S. aureus* can be found in the nonmicrobiological work environment of carriers. The fact that the volunteers do not frequent microbiological laboratories, the results from the typing and the correlation between surface contaminations and carrier status strongly argue that the contaminations are mainly brought into the laboratories and offices by the carriers. This poses the question whether compliance with biosafety guidelines can be measured by testing for *S. aureus* contaminations. A clear identification of the source of contaminations would only be possible by typing the laboratory strains and comparing it to isolated strains from the swab samples. However, the presence of *S. aureus* strains from carriers in these same samples could interfere with the identification of laboratory strains. Because of ethical reasons in the supervised laboratories, the carrier status of staff members cannot be tested by taking nasal samples from the laboratory staff.

Surface contaminations are a common source of nosocomial infections (Kramer *et al.* 2006). Therefore, contaminations of surfaces should be minimized by regular disinfection. Although the main risk for infections comes from the endogenous strains already present in the carriers (von Eiff *et al.* 2001; Wertheim *et al.* 2004), contaminations with high titres of living cells originating from the laboratory work can be an additional source of infections that should be eliminated. Irrespective of the source, contaminations with living bacteria should not be found at working places that are regularly disinfected (e.g. microbial safety cabinet or centrifuges). Further, the usage of gloves at such places should eliminate any contaminations with bacteria from carriers. According to our data, $2{\cdot}7 \times 10^5$ 23S rRNA gene copies/sample was the highest amount found in a nonmicrobiological environment. If the levels exceed a limit of 1×10^6 23S rRNA gene copies/sample, the contamination is almost certainly caused by the intentional handling of the micro-organism. However, lower sample counts may be caused by *S. aureus* naturally carried by the staff. Therefore, for further sampling campaigns, the threshold for significant contaminations should be raised to 1×10^6 23S rRNA gene copies/sample. With this new threshold, none of the contaminations with *S. aureus* detected on the personal equipment since 2002 is significant and the number of contaminations in centrifuges is similar to the one detected with other organisms.

As *S. aureus* contaminations are present in high numbers, testing for these contaminations at selected places could be indicative for the efficiency of decontamination and cleaning routines and for the compliance with good laboratory practice. Thus, regular testing might be helpful to identify safety problems arising with other organisms that are not easily detectable and may therefore be missed by occasional swabbing campaigns.

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