Journal Pre-proof

Staphylococcus aureus nasal colonization among dental health care workers in Northern Germany (StaphDent study)

Nadine Lerche, Silva Holtfreter, Birgit Walther, Torsten Semmler, Fawaz Al'Sholui, Stephanie J. Dancer, Georg Daeschlein, Nils-Olaf Hübner, Barbara M. Bröker, Roald Papke, Thomas Kohlmann, Romy Baguhl, Ulrike Seifert, Axel Kramer

Staphylococcus aureus nasal colonization among dental health care workers in Northern Germany (StaphDent study)

Running title: *S. aureus* colonization in dental health care workers

Nadine Lerche[1*], Silva Holtfreter[2*,**], Birgit Walther[3], Torsten Semmler[4], Fawaz Al'Sholui[2], Stephanie J. Dancer[5,6], Georg Daeschlein[7], Nils-Olaf Hübner[1,8], Barbara M. Bröker[2], Roald Papke[1], Thomas Kohlmann[9], Romy Baguhl[1], Ulrike Seifert[10], Axel Kramer[1]

[1]Institute of Hygiene and Environmental Medicine, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, DE-17475 Greifswald, Germany; lerche.nadine@googlemail.com, Nils.Huebner@med.uni-greifswald.de, Roald.Papke@sana-kl.de, R.Baguhl@gmx.de, axel.kramer@med.uni-greifswald.de

[2]Department of Immunology, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, DE-17475 Greifswald, Germany; silva.holtfreter@med.uni-greifswald.de, Fawaz.AlSholui@med.uni-greifswald.de, broeker@uni-greifswald.de

[3]ZBS 4, Advanced Light and Electron Microscopy, Robert Koch Institute, Seestraße 10, DE-13353 Berlin, Germany; WaltherB@rki.de

[4]Microbial Genomics (NG1), Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany; SemmlerT@rki.de

[5]Department of Microbiology, Hairmyres Hospital, NHS Lanarkshire G75 8RG, UK; Stephanie.Dancer@lanarkshire.scot.nhs.uk

[6]School of Applied Sciences, Edinburgh Napier University, Edinburgh EH11 4BN, UK

[7]Department of Dermatology, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, DE-17475 Greifswald, Germany; g.daeschlein@gmx.de

[8]Central Unit for Infection Prevention and Control, University Medicine Greifswald, 17475 Greifswald, Germany; Nils.Huebner@med.uni-greifswald.de

[9]Institute for Community Medicine, Methodical Subdivision, University Medicine Greifswald, Walther-Rathenau-Str. 48, DE-17475 Greifswald, Germany; Thomas.Kohlmann@uni-greifswald.de

[10]Friedrich Loeffler Institute of Medical Microbiology, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, DE-17475 Greifswald, Germany; Ulrike.Seifert@med.uni-greifswald.de

* These authors have contributed equally to this work and share first authorship

**Corresponding author. Silva Holtfreter, Institute of Immunology and Transfusion Medicine, Department of Immunology, Ferdinand-Sauerbruchstraße, 17475 Greifswald, Germany
E-mail address: silva.holtfreter@med.uni-greifswald.de

Summary

Methicillin-resistant *Staphylococcus aureus* (MRSA) can colonize dental patients and students, however, studies on the prevalence of MRSA and methicillin-susceptible *S. aureus* (MSSA) among dental health care workers (DHCW) including use of personal protective equipment (PPE) are scarce. We conducted an observational study (StaphDent study) to (I) determine the prevalence of MRSA and MSSA colonization in DHCW in the region of Mecklenburg Western-Pomerania, Germany, (II) resolve the MSSA population structure to gain hints on possible transmission events between co-workers, and (III) clarify use of PPE. Nasal swabs were obtained from dentists (n=149), dental assistants (n=297) and other dental practice staff (n=38). Clonal relatedness of MSSA isolates was investigated using *spa* typing and, in some cases, whole genome sequencing (WGS). PPE use was assessed by questionnaire. While 22.3% (108/485) of the participants were colonized with MSSA, MRSA was not detected. MSSA prevalence was not associated with size of dental practices, gender, age, or duration of employment. The identified 61 *spa* types grouped into 17 clonal complexes and four sequence types. Most *spa* types (n=51) were identified only once. In ten dental practices one *spa* type occurred twice. WGS data analysis confirmed a close clonal relationship for 4/10 isolate pairs. PPE was regularly used by most dentists and assistants. To conclude, the failure to recover MRSA from DHCW reflects the low MRSA prevalence in this region. Widespread PPE use suggests adherence to routine hygiene protocols. Compared to other regional HCW MRSA rates the consequent usage of PPE seems to be protective.

Abbreviations: DHCP, dental health care professionals; DHCW, dental health care workers; HCP, health care professionals; HCW, health care workers; MSSA, methicllin-sensitive *Staphylococcus aureus*; MRSA, methicllin-resistant *Staphylococcus aureus*; *S. aureus*, *Staphylococcus aureus*; PPE, personal protective equipment

Key words: *Staphylococcus aureus*, MRSA, dental practices, dentist, *spa* typing, epidemiology

Introduction

The worldwide spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals, nursing homes and in the general population is of particular concern (Aslam et al., 2018; Köck et al., 2011; Tong et al., 2015). While the proportion of MRSA among all *S. aureus* isolates carried by the general population is around 1% (Holtfreter et al., 2016; Köck et al., 2016; Mehraj et al., 2014), it accounts for 13.3% of all *S. aureus* isolates in German hospitals (Layer et al., 2019). Other European countries have reported MRSA prevalences between 0.0% (Sweden) and 2.1% (Belgium) in the general population (den Heijer et al., 2013). In the hospital setting, steady or decreasing prevalences have been observed in a number of European countries, including Germany, since the early 2000s (Layer et al., 2019; Lee et al., 2018).

In contrast to MRSA, MSSA colonization is very common. For Northern Germany, *S. aureus* colonization rates between 21 and 41% have been reported for the general population (Holtfreter et al., 2016; Köck et al., 2016; Mehraj et al., 2014). Staphylococcal carriage is a risk for endogenous infections (Datta and Huang, 2008; Eiff et al., 2001; Wertheim et al., 2004). Moreover, persistent carriers spread the bacteria to other persons by direct contact from wounds or colonized skin, droplets, shared equipment or surfaces, and pets (Knox et al., 2012; Mork et al., 2020; Sakr et al., 2018). Hence, professional groups with close and frequent contact to MRSA carriers, i.e. hospital staff, ambulance personnel, and nursing home staff, have an increased risk of acquiring MRSA (Albrich and Harbarth, 2008; Price et al., 2017). Risk factors for MRSA acquisition outside the hospital settings include direct contact to persons from countries with high MRSA rates and intensive husbandry (Buenaventura-Alcazaren et al., 2020; Köck et al., 2016; van Dulm et al., 2020). MRSA may also be carried by dental healthcare workers (DHCWs), students and patients, and can be recovered from dental surfaces. However, studies on the prevalence of MRSA and methicillin-susceptible *S. aureus* (MSSA) among DHCW are scarce (Albrich and Harbarth, 2008; Petti and Polimeni, 2011; Reddy, 2010).

Pathogen transmission in healthcare settings is prevented by hand hygiene and near-patient surface disinfection, along with use of PPE (Albrich and Harbarth, 2008; Pittet et al., 2000). Dentists in Western-Pomerania, Northern Germany, have demonstrated high compliance with these basic measures, as recommended by the Commission of Hospital Hygiene and Infection Prevention at the Robert Koch-Institute (Hübner et al., 2012; Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO) beim Robert Koch-Institut, 2006).

We conducted an observational study among DHCWs (StaphDent study), firstly to determine the prevalence of MRSA and MSSA colonization among DHCWs in the region of Mecklenburg Western-Pomerania, Germany; secondly, to examine strain types in this occupational group for hints on possible transmission events, and thirdly, to record the use of basic PPE in dental practice.

Materials and Methods

Study design and recruitment. The prevalence of MRSA and MSSA carriage among dentists and their staff was determined between April and September 2016 in Mecklenburg Western Pomerania, Germany. The primary objective was to detect the prevalence of MRSA. The secondary objectives were the prevalence of MSSA, their genetic diversity, identification of factors that may promote

staphylococcal colonization and self-assessment of basic hygiene measures in dental practices. Selection of dental practices was randomized with voluntary anonymous participation. The study protocol was approved by the local ethics committee of the University of Greifswald (registration no. BB 051/16).

Out of 1017 registered practices in the 6 districts of Mecklenburg Western-Pomerania, 455 were randomly selected, informed by phone about the study and asked to participate. To ensure commitment, participants' information, declaration of consent, questionnaire and swab were sent by mail. All participants had to give written consent as prerequisite for inclusion. To ensure anonymization, participant names were not documented and only an incomplete postcode lacking the last digit was recorded. If a copy of the final microbiological report was requested, and the name was transmitted for this purpose, anonymization was performed according to the same procedure following sample analysis in the laboratory. This protects the identification of the participating person. Members of a practice were given the same number to enable referring findings to a practice while maintaining anonymity.

A questionnaire was used to record the profession, age, sex and duration of employment for each participant as well as the number of employees in their practice. Moreover, frequency of use of gloves, mouth- nose mask, protective goggles and protective clothing over work clothing was self-reported by each participant. Finally, risk factors for colonization with *S. aureus* or MRSA were assessed (Table S1).

Microbiological identification of *S. aureus*. Nose swabs were taken by the study participants themselves with sterile cotton swabs (Transystem® with Amies agar gel medium, HAIN Lifescience, Nehren, Germany). They were given detailed written instructions on how to swab the nasal vestibule and on the proper packaging and shipping of the samples. The cotton swab was transferred to the laboratory within 24 hours and processed within 1 hour upon arrival. Swabs were streaked onto Mannitol Salt Agar (Becton Dickinson, Heidelberg, Germany) to enable selective growth of *S. aureus* as well as on MRSA CHROMagar (MAST Diagnostica, Reinfeld, Germany) to identify of MRSA Afterwards, the swab was transferred into CASO broth (Carl Roth, Karlsruhe, Germany) and incubated for 24 h at 36 ± 1°C. 100 µl of these cultures were streaked onto Mannitol Salt Agar and MRSA CHROMagar. To identify suspected colonies of *S. aureus,* two coagulase tests (Pastorex™ Staph-Plus, BioRad, Feldkirchen, Germany and Slidex Staph Plus®, Biomérieux, Nürtingen, Germany) were performed.

***Spa* genotyping.** *Spa* genotyping was conducted according to published protocols using the primers spa-1113f and spa-1514r (Harmsen et al., 2003; Holtfreter et al., 2016). Closely related *spa* types (costs ≤ 3) were grouped into *spa* clonal complexes (*spa* CCs) using the BURP algorithm (Mellmann et al., 2007). Afterwards, *spa* CCs were allocated to multilocus sequence type (MLST) CCs using the SpaServer database (www.spaserver.ridom.de), experimental assessment of MLST in a subset of samples (see below), and/or the scientific literature (Grumann et al., 2011; Holtfreter et al., 2007; Holtfreter et al., 2016).

MLST. MLST analysis was performed on a subset of 14 MSSA isolates as previously reported (Enright et al., 2000), and STs were identified using the MLST database https://pubmlst.org/saureus/ and

Ridom SeqSphere software. MLST was conducted to validate *spa* type singletons (n=11) and to group strains with short *spa* repeat sequences (n=3).

Minimum spanning tree. *Spa* types were clustered using the minimum spanning tree (MST) algorithm of the *spa* typing plug-in of the BioNumerics software (version 7.1; Applied Maths, Ghent, Belgium) with default settings.

Whole genome sequencing (WGS). Whole genome sequences of 20 selected MSSA isolates were generated by de novo assembly of Illumina MiSeq reads as previously reported (Trübe et al., 2019). Whole genome alignment was generated based on the maximum common genome of all 20 strains using Roary v3.12.0 software. Pairs differing in <30 nucleotides were subjected to pair-wise alignments using Geneious 10.0.5 (Biomatters Ltd., Australia). Virulence, resistance and biocide gene screening was carried out using resources from the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services) and an in-house database (Malberg Tetzschner et al., 2020; Zankari et al., 2012). This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank (see Table S2 for accession numbers).

Statistical analysis. Proportions are reported as descriptive statistics for categorical data. Associations between categorical variables were analyzed using Pearson Chi-Square Test.

Results

Cohort description. Out of 455 contacted dental practices in Mecklenburg Western Pomerania, Germany, 127 practices agreed to participate. However, only 105 practices actually participated (response rate 23.1%). Nasal swabs were obtained a total of 454 participants, including dentists (30.7%), dental assistants (61.2%), dental receptionists (4.7%) and dental laboratory staff (3.1%). Average age was 43.6 ± 12.6 years for all occupational groups without significant differences between the professional groups. 87.4% of participants were female. With one exception (Asian), all participants were Caucasian.

Prevalence of MRSA and MSSA. MRSA were not isolated from any of the 485 participants. MSSA isolates were detected in 108 participants (22.3%) (Table 1). MSSA prevalence tended to be higher in dentists (23.5%) and their assistants (23.6%) than among employees at patient reception (8.7%) or in the laboratory (6.7%). After grouping medical staff (dentists and dental assistants) and non-medical staff (receptionists and dental technicians), this difference became significant (Chi-Square test, p≤0.05).

MSSA prevalence did not correlate with practice size, i.e. the number of medical staff in the dental practice (p = 0.339, Table 1), even if only practices in which all employees participated in the screening were included (Table S3). However, there was a trend for a higher prevalence in smaller practices. Indeed, after grouping practices into those with up to 5 employees versus those with more than 5 employees the trend became significant (31.5 vs. 18.0%; p = 0.015, Table S3). In this study, MSSA colonization did not correlate with sex (21.9% in women, 24.6% in men) or age; neither was

MSSA prevalence influenced by length of employment. We also stratified the data by the geographical region (county) of the practices, but again observed no major differences (Table 1).

Apart from practice-related parameters, all study participants were assessed for risk factors for MSSA/MRSA colonization. Direct contact with persons working in outpatient care, persons requiring dialysis, persons being in chronically need of care, and persons having direct professional contact with animals for meat production were identified as risk factors for MSSA colonization (Table S1).

Genotyping of MSSA isolates. A total of 105 MSSA strains were *spa* typed; the remaining three isolates were not available for analyses. The *spa* typing revealed a diverse MSSA population among dental staff. A total of 61 *spa* types were detected, most of them (n=47) only once (Figure 1A). The most prevalent *spa* types in the StaphDent cohort were t091 (12.4%), t012 (6.7%), t021 (5.7%), t084 (5.7%), and t015 (4.8%). *Spa* types grouped into 17 clonal complexes (CC) and 4 sequence types (STs) (Figure 1B). The diversity as well as the clonal linkage of the *spa* types within the respective CC was visualized with a minimum spanning tree (Figure 1C). The lineages CC45, CC30 and CC8 contained the largest number of different *spa* types (12, 11, and 6, respectively). In contrast, 13 out of 15 CC7 isolates belonged to *spa* type t091.

To estimate whether certain *spa* types or CCs are more common amongst dental staff, we compared our findings with the SHIP-TREND-0 cohort, a large-scale population-based study in Western Pomerania (Holtfreter et al., 2016). The *spa* type prevalence was highly concordant in both studies, except for *spa* type t091 which was more prevalent in the StaphDent cohort (12.4 vs. 5.2%, Chi Square test, p ≤ 0.01) (Figure 2A). Moreover, several usually rare *spa* types (t9519, t2598, t13835, t17549) occurred twice among dental staff. As expected, there was also a strong overlap in the most common MSSA CCs for both cohorts (Figure 2B). CC7, which is dominated by t091, was again more prevalent in the StaphDent study. An in-depth analysis of the genome sequence of 2 CC7 isolate pairs (see below) revealed a combination of virulence and immune evasion factors that is typical for this lineage (Holtfreter et al., 2016; Monecke et al., 2009). They harbored the phage-encoded immune evasion cluster (*sak*, *scn*, *sep*), genes encoding pore-forming toxins (*lukDE*, *hlgABC*), and several extracellular proteases (aureolysin, *splA*, *splB*, *splE*). Genes encoding multidrug efflux pumps involved in biocide resistance were commonly found, while genes conferring resistance towards antibiotics beyond *bla* were scarce (table S4).

Prevalence of *spa* types in individual practices. Due to their high diversity, most *spa* types (51/61) occurred just once in a single dental practice. The remaining 10 *spa* types were found in multiple practices (Table S5). The most frequently represented *spa* type t091 was detected in 11 practices, followed by t012 in 6 practices, as well as t015, t021, and t084 in five practices each. Notably, in ten out of 105 (9.5%) practices, one *spa* type each appeared twice, suggesting a tight clonal relationship of these isolates (Table S5). This included both common *spa* types, such as t012, t021, t084, and t091, as well as rare *spa* types, i.e. t2598, t9519, t13835, and t17549. This was unexpected since the likelihood for detecting even the most prevalent *spa* type t091 twice is below 3% (considering a colonization rate of 22.3% and a t091 prevalence of 12.4%).

Clonal relationship of *S. aureus* isolates. To investigate whether isolates with identical *spa* type from the same practice were considered as closely clonally related, WGS data were used to initially screen the 20 isolates with respect to their maximum common genome (MCG) (Table S6). Based on the MCG

analysis, six strain pairs which seemed closely related were subjected to a closer inspection using individual comparative whole genome alignments of the pairs, revealing a rate of ≤13 SNPs in four cases, implying clonality (Table S7).

Use of PPE. Finally, compliance with PPE use was evaluated. A total of 80% of dentists wore gloves for every treatment, with a further 14% for the majority of treatments (Figure 3A). Only 51% of dentists wore mouth-nose protection at each treatment, 24% for most treatments and 22% in cases of suspected infection. Three participants (2%) always worked without mouth-nose protection. Dental assistants were more likely to use gloves and mouth-nose protection than physicians (Figure 3B).

Discussion

Epidemiological data on the prevalence of MRSA and MSSA among DHCW are currently scarce (Petti and Polimeni, 2011). The StaphDent study is to our best knowledge first published study to analyze *S. aureus* carriage rates and strain types among DHCW in Europe.

Prevalence of MRSA. None of the 485 participants in the study were colonized with MRSA, resulting in an MRSA prevalence ≤ 0.2%. Thus it can be assumed that MRSA does not have significant epidemiological impact for this occupational group in this region. Unfortunately, our findings can hardly be placed into a global perspective due to a lack of comprehensive studies (Petti and Polimeni, 2011).MRSA was not detected in any of 277 dentists and dental students in Denmark (Kjerulf et al., 2009). In contrast, MRSA was found in 4.2% of dentists and 1.6% of dental hygienists from the United States (Reddy, 2010) . Reported MRSA colonization rates among dental students ranged from 3.1 in South Korea to roughly 20% in Mexico and the United States (Baek et al., 2016; Martínez-Ruíz et al., 2014; Roberts et al., 2011). These variable MRSA rates among DHCP and dental students from different geographical areas might be explained by varying MRSA prevalence in the community and different hygiene standards in healthcare institutions (Albrich and Harbarth, 2008; Petti and Polimeni, 2011). Moreover, each of these studies used different MRSA detection methods, which hampers a direct comparison with our data.

Our reported MRSA carriage rate among DHCPs (≤ 0.2%) was considerably lower than those among HCWs and hospital patients but similar to the rates observed for the general population in the same geographical region with 0.34% (Holtfreter et al., 2016). In a risk-adapted admission screening of patients (n = 3375) at the Greifswald University Medical Center, the MRSA prevalence after cultural diagnosis was 3.7% on average across all medical disciplines (Hübner et al., 2009). HCW in Western-Pomerania in the HICARE project had the following prevalence rates for MRSA nasal carriage: Outpatient care services 4.0% (sample size n = 150), nursing homes 2.1% (sample size n = 1383), rehabilitation clinics 0.6% (sample size n = 84; unpublished data).

High MRSA rates among HCWs were also frequently reported for other geographical regions (Albrich and Harbarth, 2008). For instance, the MRSA prevalence among HCW in non-outbreak situations was 4.6% in the Dutch-German EUREGIO (Sassmannshausen et al., 2016). Generally, higher rates are observed among nursing staff than medical staff, presumably due to the close care contacts (Dulon et al., 2014). In contrast, the MRSA prevalence among HCWs and patients was comparable to the general population in some surveys, probably depending on compliance with basic hygiene measures and use of PPE (Chen et al., 2015; Wu et al., 2019). Similarly, in German rehabilitation centers the

MRSA prevalence among HCW was with 0.4% only slightly higher (Schubert et al., 2019) than the general population (Holtfreter et al., 2016).

The relatively high prevalence of MRSA for HCWs, especially in outpatient settings, is probably explained by the frequent and close contact with patients in this setting, where the colonization status is not always known. The longer and sometimes unprotected exposure to high-risk groups may explain the higher MRSA prevalence in HCW compared with dental staff. In addition to the lower contact frequency with MRSA carriers, and the use of PPE in dental practices might also be a factor in avoiding MRSA transmission (Dancer, 2009; Gorwitz et al., 2008; Pittet et al., 2000).

Prevalence of MSSA. The observed MSSA prevalence of 22.3% in the StaphDent cohort is lower than the previously reported prevalence in the SHIP-TREND cohort (27.2%) (Holtfreter et al., 2016). Several factors may have influenced the comparably low MSSA prevalence. Firstly, our cohort was restricted to DHCW, which differ in several aspects from the general population. Secondly, mannitol salt agar has lower sensitivity to detect MSSA than other selective agars (Han et al., 2007) Finally, the self-swabbing might not have been taken thoroughly enough. Regardless, the MSSA prevalence in our StaphDent cohortwas similar to carriage rates reported by Mehraj et al. for Northern Germany, while a higher colonization rate was reported by Köck et al. (Köck et al., 2016; Mehraj et al., 2014). On an international scale, similar rates were for instance reported for the UK (25.4%) and France (21.1%), lower rates for Hungary (12.1%) and Austria (15.7%) (den Heijer et al., 2013).

Several studies have demonstrated that - similar to MRSA - the prevalence of MSSA increases in patients or professional workers in health care settings. For instance, residents in nursing homes in central Germany had an MSSA carriage rate of 36.6% (Daeschlein et al., 2006). Notably, regular nose swabs from physicians in the University Medical Center Greifswald revealed colonization rates of up to 100% after operating on open chronic wounds. Moreover, these physicians demonstrated repeated and recalcitrant strong nasal colonization with changing clones (up to four different clones/year) (Daeschlein et al. unpublished). These data clearly show that repeated and prolonged exposure to MSSA can lead to an increased colonization rate.

High diversity of the _S. aureus_ population in DHCW. _Spa_ typing of the _S. aureus_ isolates revealed a highly diverse population structure consisting of 61 _spa_ types clustering into 21 CCs/STs, but with some clustering of _spa_ types in certain practices. The StaphDent cohort (sampled in 2016) strongly resembled the _S. aureus_ population found in the general population in the same area (sampled in 2008-2012) with some fluctuations likely due to the smaller sample size in StaphDent. In line with this, highly similar prevalences of _spa_ types and corresponding CCs have been reported in two other German studies (Becker et al., 2017; Mehraj et al., 2014). Moreover, similar _S. aureus_ population structures were also found in several European and US studies (Muthukrishnan et al., 2013; Ruimy et al., 2009; Sakwinska et al., 2009; Sangvik et al., 2011).

Spa type t091 and the corresponding CC7 were significantly overrepresented in the StaphDent cohort. This _spa_ type was detected in 11 practices, in two of them twice. Whether this is due to a recent expansion of this _spa_ type in the general population of the study region (and hence also in the StaphDent cohort) or to a selection advantage of this clone in DHCWs or their working environment remains to be clarified. An in-depth analysis of the genome sequence of 2 CC7 provided no hints towards genetic features that might explain the high prevalence of CC7 in this study. The isolate pairs

revealed a combination of virulence and immune evasion factors that is commonly found in this lineage (Holtfreter et al., 2016; Monecke et al., 2009).

Prevalence of *spa* types in individual practices. We detected the same *spa* type twice in 10 out of 105 practices. WGS data analysis of these strains indicated a close clonal relationship for four pairs, which exhibited a repertoire of 3-13 SNPs during pairwise genomic alignment. Since up to 30 SNPs were reported for strains isolated from a single person over a 13-month period, these pairs were judged as descendants from a common progenitor (Young et al., 2012). We suggest two scenarios. Firstly, transmission events could occur between co-workers within the same practice, either by direct contact, airborne inhalation or via contaminated surfaces. Secondly, transmission events could occur due to intense social contacts of co-workers. It is possible that spouses or children may work within the same practice, but due to the anonymization this could not be clarified for the ten practices concerned. In line with this, in 18-28% of households 2 or more members are colonized with closely related MSSA isolates (Busato et al., 1998; Knox et al., 2012; Stone et al., 2009).

Study limitations. Because swabs were taken only from the nasal cavity, it is possible that additional carriage sites such as skin, pharynx or perineum, which are less frequently colonized than the nose, might have been colonized with MSSA or even MRSA. Thus, the real prevalence of MSSA/MRSA could have been higher (Dancer and Noble, 1991). Nasal swabs were taken by volunteers themselves. Since it is known that the bacterial yield is influenced by swabbing technique and can be strongly increased upon theoretical teaching (Warnke et al., 2014), all subjects were instructed that swabs are to be obtained by repeating concentric brushing with slight pressure. Nevertheless, the observed lower colonization rate in non-medical staff vs. medical staff could have been influenced by differences in the swabbing technique. Moreover, sampling was done only once which does not allow differentiation between intermittent and permanent carriers. Since only one isolate per sample was processed, probands carrying multiple strains were not identified in this study. Finally, MRSA detection might have been improved by direct streaking on selective MRSA agar.

Conclusions

The MSSA colonization rate in the StaphDent cohort was slightly lower than in the local general population. The observed high diversity of the *S. aureus* population in DHCW argues against the same *spa* types spreading within dental practices and probably reflects the distribution within the general population. MRSA nasal carriage was not detected, resulting in an MRSA prevalence ≤0.2%. This is considerably less than rates among HCPs and hospital patients but similar to the rates observed for the general population in the same geographical region. That could be a hint, that regular use of PPE in dental practices may prevent the acquisition of MRSA. The suspicion that PPEs prevent the acquisition of MRSA is supported by the fact that prevalence of MRSA in outpatient care service almost reached prevalence in hospitalized patients. Especially in out-patient care, the consistent use of personal protective equipment and hand disinfection is much more difficult than in the dental practice. During the COVID-19 pandemic the PPE has even gained importance to protect dental health care workers against aerogenically transmissible pathogens (Matuschek et al., 2020).

Declarations of interest: none

References

Albrich, W.C., Harbarth, S., 2008. Health-care workers: source, vector, or victim of MRSA? The Lancet Infectious Diseases 8 (5), 289–301.

Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., Nisar, M.A., Alvi, R.F., Aslam, M.A., Qamar, M.U., Salamat, M.K.F., Baloch, Z., 2018. Antibiotic resistance: a rundown of a global crisis. Infection and Drug Resistance 11, 1645–1658.

Baek, Y.S., Baek, S.-H., Yoo, Y.-J., 2016. Higher nasal carriage rate of methicillin-resistant Staphylococcus aureus among dental students who have clinical experience. Journal of the American Dental Association (1939) 147 (5), 348–353.

Becker, K., Schaumburg, F., Fegeler, C., Friedrich, A.W., Köck, R., 2017. Staphylococcus aureus from the German general population is highly diverse. International journal of medical microbiology : IJMM 307 (1), 21–27.

Buenaventura-Alcazaren, F.A., Dela Tonga, A., Ong-Lim, A., Destura, R.V., 2020. Prevalence and molecular characteristics of MRSA nasal carriage among hospital care workers in a tertiary hospital in the Philippines. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi 53 (5), 739–745.

Busato, Carneiro, L.M.T., Gabardo, 1998. Staphylococcus aureus Nasopharyngeal Carriage Rates and Antimicrobial Susceptibility Patterns Among Health Care Workers and Their Household Contacts. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases 2 (2), 78–84.

Chen, B., Dai, X., He, B., Pan, K., Li, H., Liu, X., Bao, Y., Lao, W., Wu, X., Yao, Y., Huang, S., 2015. Differences in Staphylococcus aureus nasal carriage and molecular characteristics among community residents and healthcare workers at Sun Yat-Sen University, Guangzhou, Southern China. BMC Infectious Diseases 15.

Daeschlein, G., Assadian, O., Rangous, I., Kramer, A., 2006. Risk factors for Staphylococcus aureus nasal carriage in residents of three nursing homes in Germany. The Journal of hospital infection 63 (2), 216–220.

Dancer, S.J., 2009. The role of environmental cleaning in the control of hospital-acquired infection. The Journal of hospital infection 73 (4), 378–385.

Dancer, S.J., Noble, W.C., 1991. Nasal, axillary, and perineal carriage of Staphylococcus aureus among women: identification of strains producing epidermolytic toxin. Journal of Clinical Pathology 44 (8), 681–684.

Datta, R., Huang, S.S., 2008. Risk of infection and death due to methicillin-resistant Staphylococcus aureus in long-term carriers. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 47 (2), 176–181.

den Heijer, C.D.J., van Bijnen, E.M.E., Paget, W.J., Pringle, M., Goossens, H., Bruggeman, C.A., Schellevis, F.G., Stobberingh, E.E., 2013. Prevalence and resistance of commensal Staphylococcus

aureus, including meticillin-resistant S. aureus, in nine European countries: a cross-sectional study. The Lancet. Infectious diseases 13 (5), 409–415.

Dulon, M., Peters, C., Schablon, A., Nienhaus, A., 2014. MRSA carriage among healthcare workers in non-outbreak settings in Europe and the United States: a systematic review. BMC Infectious Diseases 14, 363.

Eiff, C. von, Becker, K., Machka, K., Stammer, H., Peters, G., 2001. Nasal carriage as a source of Staphylococcus aureus bacteremia. Study Group. The New England journal of medicine 344 (1), 11–16.

Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. Journal of clinical microbiology 38 (3), 1008–1015.

Gorwitz, R.J., Kruszon-Moran, D., McAllister, S.K., McQuillan, G., McDougal, L.K., Fosheim, G.E., Jensen, B.J., Killgore, G., Tenover, F.C., Kuehnert, M.J., 2008. Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001-2004. The Journal of infectious diseases 197 (9), 1226–1234.

Grumann, D., Ruotsalainen, E., Kolata, J., Kuusela, P., Järvinen, A., Kontinen, V.P., Bröker, B.M., Holtfreter, S., 2011. Characterization of infecting strains and superantigen-neutralizing antibodies in Staphylococcus aureus bacteremia. Clinical and vaccine immunology : CVI 18 (3), 487–493.

Han, Z., Lautenbach, E., Fishman, N., Nachamkin, I., 2007. Evaluation of mannitol salt agar, CHROMagar Staph aureus and CHROMagar MRSA for detection of meticillin-resistant Staphylococcus aureus from nasal swab specimens. Journal of medical microbiology 56 (Pt 1), 43–46.

Harmsen, D., Claus, H., Witte, W., Rothgänger, J., Claus, H., Turnwald, D., Vogel, U., 2003. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. Journal of clinical microbiology 41 (12), 5442–5448.

Holtfreter, S., Grumann, D., Balau, V., Barwich, A., Kolata, J., Goehler, A., Weiss, S., Holtfreter, B., Bauerfeind, S.S., Döring, P., Friebe, E., Haasler, N., Henselin, K., Kühn, K., Nowotny, S., Radke, D., Schulz, K., Schulz, S.R., Trübe, P., Vu, C.H., Walther, B., Westphal, S., Cuny, C., Witte, W., Völzke, H., Grabe, H.J., Kocher, T., Steinmetz, I., Bröker, B.M., 2016. Molecular Epidemiology of Staphylococcus aureus in the General Population in Northeast Germany: Results of the Study of Health in Pomerania (SHIP-TREND-0). Journal of clinical microbiology 54 (11), 2774–2785.

Holtfreter, S., Grumann, D., Schmudde, M., Nguyen, H.T.T., Eichler, P., Strommenger, B., Kopron, K., Kolata, J., Giedrys-Kalemba, S., Steinmetz, I., Witte, W., Bröker, B.M., 2007. Clonal Distribution of Superantigen Genes in Clinical Staphylococcus aureus Isolates. Journal of clinical microbiology 45 (8), 2669–2680.

Hübner, N.-O., Handrup, S., Meyer, G., Kramer, A., 2012. Impact of the "Guidelines for infection prevention in dentistry" (2006) by the Commission of Hospital Hygiene and Infection Prevention at the Robert Koch-Institute (KRINKO) on hygiene management in dental practices - analysis of a survey from 2009. GMS Krankenhaushygiene interdisziplinar 7 (1), Doc14.

Hübner, N.-O., Kramer, A., Steinmetz, I., Bartels, Claus, 2009. Das Greifswalder Modell der MRSA-Prävention - Maßnahmen zur Kontrolle multiresistenter Erreger. Klinikarzt 38 (4), 192–196.

Kjerulf, A., Skov, R.L., Forsberg, C., Larsen, T., Fiehn, N.-E., 2009. The prevalence of carriers of methicillin resistant Staphylococcus aureus in Copenhagen. Statens Serum Institut and School of Dentistry, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark, May 2009, Helsinki, Finland.

Knox, J., Uhlemann, A.-C., Miller, M., Hafer, C., Vasquez, G., Vavagiakis, P., Shi, Q., Lowy, F.D., 2012. Environmental contamination as a risk factor for intra-household Staphylococcus aureus transmission. PloS one 7 (11), e49900.

Köck, R., Mellmann, A., Schaumburg, F., Friedrich, A.W., Kipp, F., Becker, K., 2011. The epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) in Germany. Deutsches Arzteblatt international 108 (45), 761–767.

Köck, R., Werner, P., Friedrich, A.W., Fegeler, C., Becker, K., 2016. Persistence of nasal colonization with human pathogenic bacteria and associated antimicrobial resistance in the German general population. New microbes and new infections 9, 24–34.

Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO) beim Robert Koch-Institut, 2006. Infektionsprävention in der Zahnheilkunde - Anforderungen an die Hygiene. Bundesgesundheitsbl - Gesundheitsforsch - Gesundheitsschutz (49), 375–394.

Layer, F., Strommenger, B., Cuny, C., Noll, I., Klingeberg, A., Werner, G., 2019. Häufigkeit, Eigenschaften und Verbreitung von MRSA in Deutschland – Zur Situation 2017/2018. Epid Bull 2019 (42), 427–442.

Lee, A.S., Lencastre, H. de, Garau, J., Kluytmans, J., Malhotra-Kumar, S., Peschel, A., Harbarth, S., 2018. Methicillin-resistant Staphylococcus aureus. Nature reviews. Disease primers 4, 18033.

Malberg Tetzschner, A.M., Johnson, J.R., Johnston, B.D., Lund, O., Scheutz, F., 2020. In Silico Genotyping of Escherichia coli Isolates for Extraintestinal Virulence Genes by Use of Whole-Genome Sequencing Data. Journal of clinical microbiology 58 (10).

Martínez-Ruíz, F.J., Carrillo-Espíndola, T.Y., Bustos-Martínez, J., Hamdan-Partida, A., Sánchez-Pérez, L., Acosta-Gío, A.E., 2014. Higher prevalence of meticillin-resistant Staphylococcus aureus among dental students. The Journal of hospital infection 86 (3), 216–218.

Matuschek, C., Moll, F., Fangerau, H., Fischer, J.C., Zänker, K., van Griensven, M., Schneider, M., Kindgen-Milles, D., Knoefel, W.T., Lichtenberg, A., Tamaskovics, B., Djiepmo-Njanang, F.J., Budach, W., Corradini, S., Häussinger, D., Feldt, T., Jensen, B., Pelka, R., Orth, K., Peiper, M., Grebe, O., Maas, K., Gerber, P.A., Pedoto, A., Bölke, E., Haussmann, J., 2020. Face masks: benefits and risks during the COVID-19 crisis. European journal of medical research 25 (1), 32.

Mehraj, J., Akmatov, M.K., Strömpl, J., Gatzemeier, A., Layer, F., Werner, G., Pieper, D.H., Medina, E., Witte, W., Pessler, F., Krause, G., 2014. Methicillin-sensitive and methicillin-resistant Staphylococcus aureus nasal carriage in a random sample of non-hospitalized adult population in northern Germany. PloS one 9 (9), e107937.

Mellmann, A., Weniger, T., Berssenbrügge, C., Rothgänger, J., Sammeth, M., Stoye, J., Harmsen, D., 2007. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of Staphylococcus aureus populations based on spa polymorphisms. BMC microbiology 7, 98.

Monecke, S., Luedicke, C., Slickers, P., Ehricht, R., 2009. Molecular epidemiology of Staphylococcus aureus in asymptomatic carriers. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology 28 (9), 1159–1165.

Mork, R.L., Hogan, P.G., Muenks, C.E., Boyle, M.G., Thompson, R.M., Sullivan, M.L., Morelli, J.J., Seigel, J., Orscheln, R.C., Bubeck Wardenburg, J., Gehlert, S.J., Burnham, C.-A.D., Rzhetsky, A., Fritz, S.A., 2020. Longitudinal, strain-specific Staphylococcus aureus introduction and transmission events in households of children with community-associated meticillin-resistant S. aureus skin and soft tissue infection: a prospective cohort study. The Lancet. Infectious diseases 20 (2), 188–198.

Muthukrishnan, G., Lamers, R.P., Ellis, A., Paramanandam, V., Persaud, A.B., Tafur, S., Parkinson, C.L., Cole, A.M., 2013. Longitudinal genetic analyses of Staphylococcus aureus nasal carriage dynamics in a diverse population. BMC Infectious Diseases 13, 221.

Petti, S., Polimeni, A., 2011. Risk of methicillin-resistant Staphylococcus aureus transmission in the dental healthcare setting: a narrative review. Infection control and hospital epidemiology 32 (11), 1109–1115.

Pittet, D., Hugonnet, S., Harbarth, S., Mourouga, P., Sauvan, V., Touveneau, S., Perneger, T.V., 2000. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Infection Control Programme. Lancet (London, England) 356 (9238), 1307–1312.

Price, J.R., Cole, K., Bexley, A., Kostiou, V., Eyre, D.W., Golubchik, T., Wilson, D.J., Crook, D.W., Walker, A.S., Peto, T.E.A., Llewelyn, M.J., Paul, J., 2017. Transmission of Staphylococcus aureus between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. The Lancet. Infectious diseases 17 (2), 207–214.

Reddy, Y., 2010. Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) nasal colonization among dental professionals. Texas Medical Center Dissertations (via ProQuest) AAI1479775.

Roberts, M.C., Soge, O.O., Horst, J.A., Ly, K.A., Milgrom, P., 2011. Methicillin-resistant Staphylococcus aureus from dental school clinic surfaces and students. American journal of infection control 39 (8), 628–632.

Ruimy, R., Armand-Lefevre, L., Barbier, F., Ruppé, E., Cocojaru, R., Mesli, Y., Maiga, A., Benkalfat, M., Benchouk, S., Hassaine, H., Dufourcq, J.-B., Nareth, C., Sarthou, J.-L., Andremont, A., Feil, E.J., 2009. Comparisons between geographically diverse samples of carried Staphylococcus aureus. Journal of bacteriology 191 (18), 5577–5583.

Sakr, A., Brégeon, F., Mège, J.-L., Rolain, J.-M., Blin, O., 2018. Staphylococcus aureus Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. Frontiers in microbiology 9.

Sakwinska, O., Kuhn, G., Balmelli, C., Francioli, P., Giddey, M., Perreten, V., Riesen, A., Zysset, F., Blanc, D.S., Moreillon, P., 2009. Genetic diversity and ecological success of Staphylococcus aureus strains colonizing humans. Applied and environmental microbiology 75 (1), 175–183.

Sangvik, M., Olsen, R.S., Olsen, K., Simonsen, G.S., Furberg, A.-S., Sollid, J.U.E., 2011. Age- and gender-associated Staphylococcus aureus spa types found among nasal carriers in a general population: the Tromso Staph and Skin Study. Journal of clinical microbiology 49 (12), 4213–4218.

Sassmannshausen, R., Deurenberg, R.H., Köck, R., Hendrix, R., Jurke, A., Rossen, J.W.A., Friedrich, A.W., 2016. MRSA Prevalence and Associated Risk Factors among Health-Care Workers in Non-outbreak Situations in the Dutch-German EUREGIO. Frontiers in microbiology 7, 1273.

Schubert, M., Kämpf, D., Jatzwauk, L., Kynast, F., Stein, A., Strasser, R., Dulon, M., Nienhaus, A., Seidler, A., 2019. Prevalence and predictors of MRSA carriage among employees in a non-outbreak setting: a cross-sectional study in an acute care hospital. Journal of Occupational Medicine and Toxicology (London, England) 14.

Stone, A., Quittell, L., Zhou, J., Alba, L., Bhat, M., DeCelie-Germana, J., Rajan, S., Bonitz, L., Welter, J.J., Dozor, A.J., Gherson, I., Lowy, F.D., Saiman, L., 2009. Staphylococcus aureus nasal colonization among pediatric cystic fibrosis patients and their household contacts. The Pediatric infectious disease journal 28 (10), 895–899.

Tong, S.Y.C., Davis, J.S., Eichenberger, E., Holland, T.L., Fowler, V.G., 2015. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews 28 (3), 603–661.

Trübe, P., Hertlein, T., Mrochen, D.M., Schulz, D., Jorde, I., Krause, B., Zeun, J., Fischer, S., Wolf, S.A., Walther, B., Semmler, T., Bröker, B.M., Ulrich, R.G., Ohlsen, K., Holtfreter, S., 2019. Bringing together what belongs together: Optimizing murine infection models by using mouse-adapted Staphylococcus aureus strains. International journal of medical microbiology : IJMM 309 (1), 26–38.

van Dulm, E., Klok, S., Boyd, A., Joore, I.K., Prins, M., van Dam, A.P., Tramper-Stranders, G.A., van Duijnhoven, Y.T.H.P., 2020. Nasal carriage of methicillin-resistant Staphylococcus aureus (MRSA) among undocumented migrants and uninsured legal residents in Amsterdam, the Netherlands: a cross-sectional study. Antimicrobial resistance and infection control 9 (1), 118.

Warnke, P., Harnack, T., Ottl, P., Kundt, G., Podbielski, A., 2014. Nasal screening for Staphylococcus aureus - daily routine with improvement potentials. PloS one 9 (2), e89667.

Wertheim, H.F.L., Vos, M.C., Ott, A., van Belkum, A., Voss, A., Kluytmans, J.A.J.W., van Keulen, P.H.J., Vandenbroucke-Grauls, C.M.J.E., Meester, M.H.M., Verbrugh, H.A., 2004. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. Lancet (London, England) 364 (9435), 703–705.

Wu, T.-H., Lee, C.-Y., Yang, H.-J., Fang, Y.-P., Chang, Y.-F., Tzeng, S.-L., Lu, M.-C., 2019. Prevalence and molecular characteristics of methicillin-resistant Staphylococcus aureus among nasal carriage strains isolated from emergency department patients and healthcare workers in central Taiwan. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi 52 (2), 248–254.

Young, B.C., Golubchik, T., Batty, E.M., Fung, R., Larner-Svensson, H., Votintseva, A.A., Miller, R.R., Godwin, H., Knox, K., Everitt, R.G., Iqbal, Z., Rimmer, A.J., Cule, M., Ip, C.L.C., Didelot, X., Harding, R.M., Donnelly, P., Peto, T.E., Crook, D.W., Bowden, R., Wilson, D.J., 2012. Evolutionary dynamics of

Staphylococcus aureus during progression from carriage to disease. Proceedings of the National Academy of Sciences of the United States of America 109 (12), 4550–4555.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. The Journal of antimicrobial chemotherapy 67 (11), 2640–2644.

Figure legends

Figure 1: The *S. aureus* population in the StaphDent cohort is highly diverse. A) The phylogenetic relationship of all *S. aureus* isolates from the StaphDent cohort was determined by *spa* typing. *Spa* types are colour-coded according to their respective CC. B) Allocation of *spa* types to CCs was based on the BURP algorithm implemented in the Ridom StaphType software as well as the Ridom SpaServer database, and aided by MLST sequencing of selected strains. MLST CC nomenclature was deduced from *spa* CCs using the Ridom SpaServer database. Overall, a total of 61 *spa* types were grouped into 17 CCs. C) A minimum spanning tree was generated from *spa* typing data using the BioNumerics software. Each node represents a unique *spa* type. The number of *S. aureus* isolates per *spa* type is reflected by the size of each node, while the genetic distance between the two bordering *spa* types is reflected by the distance between two nodes (maximum neighbor distance, 1.00). Nodes are colour-coded according to the associated CC.
*others, *spa* types occuring only once in the StaphDent cohort (t004, t024, t026, t090, t097, t190, t216, t246, t267, t306, t318, t495, t550, t840, t878, t888, t942, t1081, t1119, t1137, t1171, t1248, t1412, t1442, t1451, t1460, t1515, t1641, t1649, t1857, t1867, t1991, t2292, t2816, t3225, t3414, t3905, t4539, t5797, t6091, t11519, t11956, t12876, t16578, t17551, t17552, t17589)
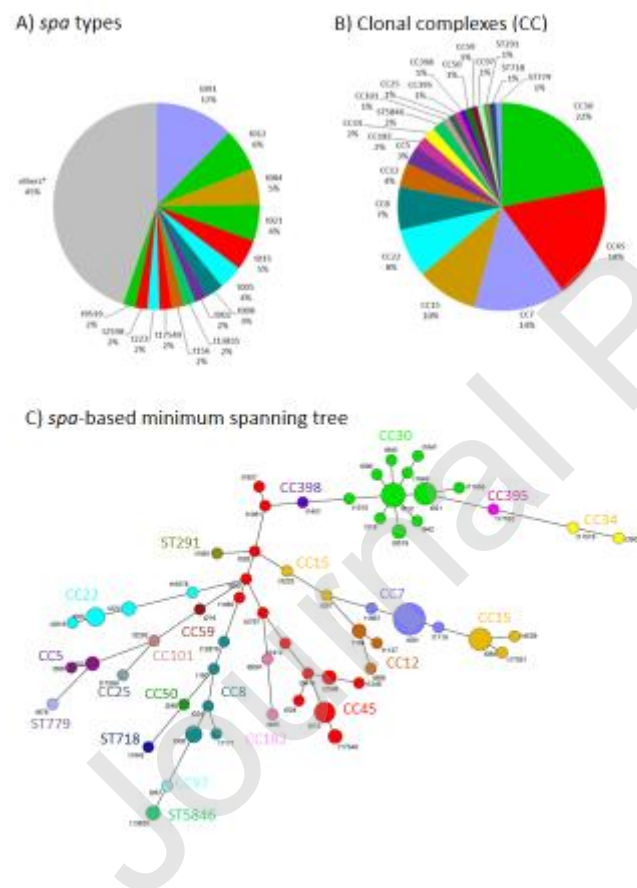

Figure 1: The S. aureus population in the StaphDent cohort is highly diverse.

Figure 2: Similar prevalence of the most common *spa* types and CCs in StaphDent and the population-based study SHIP-TREND-0. SHIP-TREND-0 is a large-scale population-based study in Western Pomerania (Germany), and represents a sub-region of the StaphDent recruitment area. Nasal *S. aureus* isolates were obtained from 1024 probands between 2008 and 2012 (Holtfreter et

al., 2016). A) This graph depicts all *spa* types with n≥2 in the StaphDent cohort (n=14) and the 14 most common *spa* types in the SHIP-TREND-0 study. B) This graph displays all CCs with a prevalence ≥ 1% in either StaphDent or the SHIP-TREND-1 cohort. Overall, we observed a strong overlap in the most common *spa* types and CCs between both cohorts. Statistics: The prevalence of individual *spa* types or CCs in both cohorts was compared using the Chi Square test. *, p ≤ 0.05. **, p ≤ 0.01. **, p ≤ 0.001.

A) *spa* types
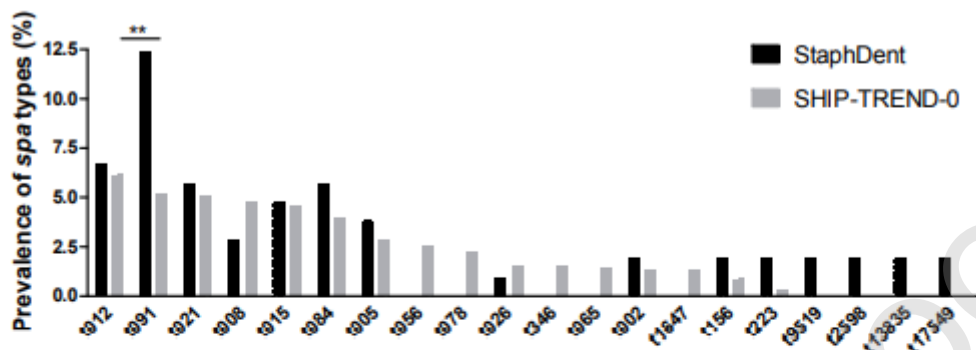
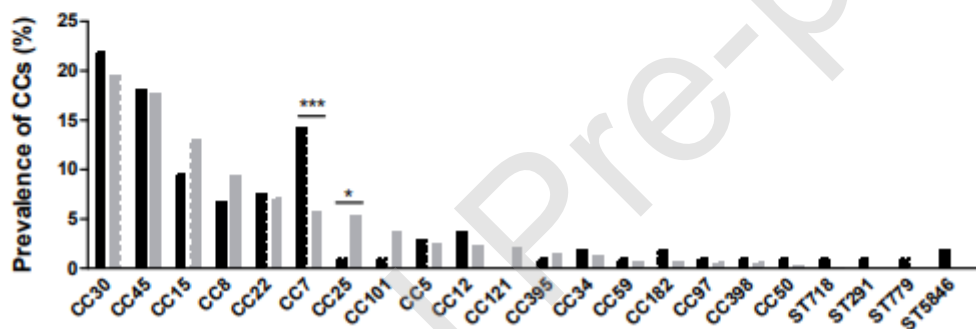

B) Clonal complexes

Figure 3: Use of personal protective equipment by dentists and dental assistants. The use of personal protective equipment was self-reported by dentists (A) and dental assistants (B) using a questionnaire. Study participants report whether they use gloves, face mask, safety glasses and protective clothing at each treatment, in the majority of treatments, only if infection is suspected or never.

A) Dentists
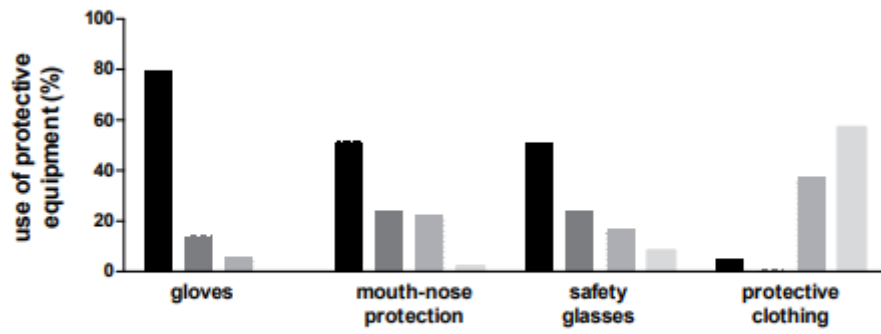
B) Dental assistants
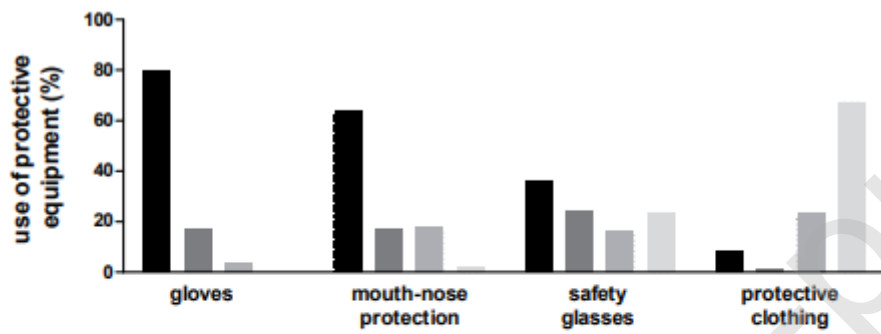
■ at each treatment
■ in the majority of treatments
■ only if infection is suspected
■ never

Table 1

Prevalence of *S. aureus* in DHCW in the StaphDent study

Variable	% (No *S. aureus*-positive/total No)		p value[1]
Total	22.3	(108/485)	
Occupation[2]			0.174
Dentist	23.5	(35/149)	
Dental assistant	23.6	(70/297)	
Dental receptionist	8.7	(2/23)	
Dental technician	6.7	(1/15)	
Employees per practice[3]			0.339
2	36.4	(4/11)	
3	27.5	(22/80)	
4	20.3	(14/69)	
5	26.3	(21/80)	
6	17.7	(11/62)	
7	19.1	(9/47)	
8-10	19.0	(12/63)	
11-15	15.5	(9/58)	
16-25	40.0	(6/15)	
≤5	25.4	(61/240)	0.099
>5	19.2	(47/245)	
Sex			0.641
Female	21.9	(93/424)	
Male	24.6	(15/61)	
Age (yrs)			0.599
16-19	37.5	(3/8)	
20-29	28.2	(20/71)	
30-39	21.7	(25/115)	
40-49	21.3	(23/108)	
50-59	22.5	(29/129)	
60-69	15.4	(8/52)	
>69	0.0	(0/2)	
Length of employment (yrs)[4]			0.327
1-3	25.8	(25/97)	
4-10	25.0	(21/84)	
> 10	19.6	(57/291)	
Geographical region (Counties)			0.097
Mecklenburg lake district	24.0	(36/150)	
Western Pomerania - Greifswald	17.5	(20/114)	
Rostock	18.0	(23/128)	
North-West Mecklenburg	28.6	(16/56)	
Western Pomerania - Rügen	38.7	(12/31)	
Ludwigslust - Parchim	16.7	(1/6)	

[1] Pearson Chi-Square Test

[2] One participant did not report his/her profession.

[3] In some practices not all employees participated in the study.

[4] Length of employment within the same practice (seniority); 13 participants did not report their length of employment.