The Culturome of the Human Nose Habitats Reveals Individual Bacterial Fingerprint Patterns

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Summary

The complex anatomy of the human nose might offer distinct microbial niches. Microbiota composition may affect nose inflammatory diseases and *Staphylococcus aureus* carriage. Considering different nasal cavity locations, microbial colonisation was analysed across individuals exhibiting chronic nasal inflammatory diseases (n=18) and those without local inflammation signs (n=16). Samples were collected systematically during surgery and examined by an extensive culture-based approach and, for a subset, by 16S rRNA gene community profiling. Cultivation yielded 141 taxa with members of *Staphylococcus*, *Corynebacterium* and *Propionibacterium* as most common isolates comprising the nasal core culturome together with *Finegoldia magna*. *S.aureus* was most frequently found in association with *Staphylococcus epidermidis* and *Propionibacterium acnes* and the posterior vestibules were redefined as *S.aureus*' principle habitat. Culturome analysis revealed host-specific bacterial "fingerprints" irrespective of host-driven factors or intranasal sites. Comparisons between cultivable and molecular fingerprints demonstrated that only a small fraction of phylotypes (6.2%) was correlated. While the total number of different phylotypes was higher in the molecular data set, the total number of identifications down to the species level was higher in the culturomic approach. To determine host-specific microbiomes, the advantages of molecular approaches should be combined with the resolution and reliability of species identification by culturomic analyses.

Introduction

Mammalian body surfaces provide diverse habitats for a large number of microbial species and numerous studies have described the diversity and complexity of human microbial ecosystems (Grice and Segre, 2012; The Human Microbiome Project Consortium, 2012) and their impact for health and disease (Kosiewicz et al., 2011; Pflughoeft and Versalovic, 2012). In contrast to the orogastrointestinal tract, the surfaces of the human nasal cavity represent a less studied ecosystem. First culture-independent analyses revealed a densely populated habitat for many bacterial species (Frank et al., 2010; Wos-Oxley et al., 2010; Camarinha-Silva et al., 2012) where age was shown to significantly shape the composition of the microbiota (Camarinha-Silva et al., 2014).

Besides questions concerning etiopathogenic links between the nasal microbiota and chronic nasal disease entities such as rhinosinusitis and polyposis, the human nose as principle habitat of the major opportunistic pathogen *Staphylococcus aureus* warrants studies on the composition of nasal microbiota in healthy and diseased individuals. *S. aureus* permanently colonises approximately 20-30% of the human population (van Belkum et al., 2009; Sollid et al., 2014). Though the species mostly acts as an asymptomatic commensal in the nose, its colonisation can be source and risk factor for subsequent infections (Kluytmans et al., 1997; von Eiff et al., 2001; Wertheim et al., 2004). The global burden by healthcare-, community- and livestock-associated methicillin-resistant *S. aureus* (MRSA) clonal lineages aggravates its clinical and socioeconomic impact by drastically limiting our therapeutic armamentarium (Köck et al., 2010, 2013; Mediavilla et al., 2012; Dantes et al., 2013). Colonisation of *S. aureus* in the nasal cavity happens under the influence of numerous co-occurring species (Frank et al., 2010; Wos-Oxley et al., 2010) that have been assumed to may have beneficial, mutual or antagonistic effects on *S. aureus* regarding its establishment and permanent residence in the nose (Uehara et al., 2000; Lina et al., 2003; Frank et al., 2010; Yan et al., 2013). Anyway, most microbiome studies are exclusively based on molecular data (Frank et al., 2010; Lemon et al., 2010; Yan et al., 2013), without validating results by comparison to a different method.

The human nose may be referred to as a connection site between the lumen inside of the human body and its outer ecological environment. Filtering, warming and humidifying the inhaled air,

it functions as a passage to the lungs and the paranasal sinuses and exhibits a high diversity of anatomical sites on a narrow space (Geurkink, 1983; Wagenmann and Naclerio, 1992). Skin-like squamous epithelium containing vibrissae and sebaceous or sweat glands can be found in the anterior vestibules whereas the deeper nasal cavity is characterised by pseudostratified columnar ciliated epithelium (respiratory epithelium) being covered with a mucus blanket that is in constant movement (Geurkink, 1983; Cheesman and Burdett, 2011). In this posterior part of the nasal cavity, three nasal meatuses (superior, middle and inferior meatus) are found located beneath each of the corresponding nasal conchae. The knowledge about these entirely different anatomical structures appearing close to each other inevitably leads to the hypothesis of defined ecological niches and microbial structures. However, this factor has been widely disregarded hitherto (Glück, 2001) and studies of the human nasal microbiome either focused on one anatomical site (Feazel et al., 2012) or neglected mentioning the exact site of sampling (Gittelman et al., 1991; Frank et al., 2010; Alvarez et al., 2013). Moreover, especially in literature concerning *S. aureus* colonisation, the anterior nares are often equated to the complete nasal vestibule. In fact, the nasal vestibule (vestibulum nasi) comprises the area between the nares (atrium) and the limen nasi as gradient zone to the respiratory epithelium behind the vestibules.

Here, we present the first study based on an in depth cultivation approach to catalogue the nasal microbiota that considers the different anatomical sites of the nasal cavity as possible distinct ecological niches with different microbial communities. We further elucidated the influence of chronic inflammatory conditions of nasal epithelial surfaces on the bacterial composition. Members of the culturome were identified by molecular and mass spectrometric techniques down to the species level. For comparison, a subset of samples was analysed by barcoded 16S rRNA gene Illumina paired-end sequencing.

Results

Study group and collection of specimens

A total of 34 ENT patients (22 males and 12 females; 19 to 78 years) were enrolled comprising a first group with 16 individuals without any signs of nasal and systemic inflammation and a second group with 18 patients suffering from chronic inflammatory diseases. Of the second group, 10 patients were diagnosed with chronic sinusitis (CS), 1 showed a chronic polyposis (CP) and 7 suffered from both. None of the patients had been taking antibiotics for at least 6 weeks prior to surgery, but 10 of them had frequently been using nose sprays that also contained cortisone in 7 of the cases. Further information on patient metadata is given in Supporting Information Table S1.

Bacterial diversity of the culturome

By cultivation, a total of 141 different bacterial taxa were identified including all patients and nasal habitats tested (Table S2). Of these, 139 (98.6%) could be differentiated down to the species level and two to the genus level. Per patient, 15.2 different taxa were identified on average (Table 1).

Most prevalent species were *Staphylococcus epidermidis* and the facultative anaerobe *Propionibacterium acnes*, both found in 97.1% of patients followed by *Corynebacterium accolens* and *Corynebacterium tuberculostrictum* each colonising 64.7% of the individuals. Twenty of the 34 (58.8%) patients were colonised by *S. aureus* that was characterised as methicillin-resistant *S. aureus* (MRSA) in two of the patients. *spa*-types are given in Supporting Information Table S1. *Propionibacterium avidum* and *Propionibacterium granulosum*, were present in 58.8% and 50.0% of patients, respectively. These seven species were categorised as part of the cultivable core microbiome. All other species cultivated were found in less than 50% of patients.

Moreover, apart from *S. epidermidis*, other coagulase-negative staphylococci (CoNS) were identified. Amongst those, *Staphylococcus haemolyticus* (44.1% of patients), *Staphylococcus capitis* and *Staphylococcus hominis* (41.2% of patients, respectively), *Staphylococcus warneri* (32.4% of patients) and *Staphylococcus lugdunensis* (26.5% of patients) were the most prevalent.

The highest variability of species within a genus was observed for *Corynebacteria*. There were 23 different species distributed among 33/34 patients. Besides *C. accolens* and *C. tuberculostrictum*, most prevalent species were *Corynebacterium amycolatum* (20.6% of patients), *Corynebacterium aurimucosum* and *Corynebacterium propinquum* (17.6% of patients, respectively).

The *Streptococcus* genus was represented by eleven different species with the most prevalent members *Streptococcus mitis/oralis* (17.6% of patients) and *Streptococcus parasanguinis* (11.8% of patients) both belonging to the viridans group of streptococci (VGS). *Streptococcus pneumonia* appeared in 4 individuals (11.8%), all without chronic local inflammation of the nose.

Twenty different anaerobic species belonging to ten different genera were cultivated and differentiated in this study. As stated above, three different species of the moderate anaerobic genus of *Propionibacterium* were found in great extend throughout all patients. Moreover, strictly anaerobes comprised members of the genera *Clostridium*, *Anaerococcus*, *Finegoldia*, *Parvimonas*, *Peptoniphilus*, *Veillonella* and *Fusobacterium*. Among those, *Finegoldia magna* was the most common isolate (38.2% of patients).

Overall, the majority (80.9%, n = 114) of all cultivable bacterial taxa were detected in only less than 5 patients. Of these, the highest fraction (48.9%) was found in no more than one patient, 16.3% were isolated from two, 9.2% from three and 6.4% from four different patients (Supporting Information Table S2).

Culturome analysis of the different nasal habitats

When analysed by culture, all four nasal habitats contained similar numbers of bacterial taxa, ranging from 8.1 (inferior meatus) to 8.7 (middle meatus) different species/genera per habitat on average (Table 1). This was further confirmed when distinguishing between the patient groups. No significant variations in the number of different taxa were apparent throughout the nasal habitats if groups were stratified according to gender or *S. aureus* carriage (Table 1, Supporting Information Table S3).

Nearly all species/genera that had been isolated from at least five different patients could also be found throughout all nasal habitats. Of these, only *Acinetobacter lwoffii* and *Corynebacterium simulans* were not found in all four habitats (Fig. 1A).

S. aureus also showed an even distribution throughout the different nasal habitats in most of its carriers. In 14 of the 20 individuals (70.0%) colonised by *S. aureus*, this species was present in all nasal sites; one patient showed carriage in the posterior vestibule and both nasal meatuses, one patient was colonised in the anterior and posterior vestibule and in four patients, *S. aureus* was exclusively isolated from the posterior vestibules (Supporting Information Table S4). Thus, the posterior vestibules were colonised in 100% of individuals who were categorised as *S. aureus* carriers.

In fact, microbial fingerprints were generally more similar between sites within the same host, as can be seen in the nMDS plot, where there is a high similarity between the different nasal habitats by revealing visible clusters being built by all samples within each individual (Fig. 2). When samples in the same plot were marked according to the nasal site where they had been collected from, no significant pattern was observed (Supporting Information Fig. S1A).

Analysis of the co-existence of cultivable members of the nasal microbiota

In order to investigate the co-occurrence patterns of species, cluster analysis was used to determine how often (across samples) these groups of species co-inhabit together (Field et al., 1982). When species cluster together on the dendrogram at a high percent similarity, this indicates that these species are almost always present together in the same samples. The most prevalent species of the nasal cavities, *S. epidermidis*, *P. acnes*, *C. accolens*, *C. tuberculostrictum*, *S. aureus*, *P. avidum* and *P. granulosum*, formed the core community of the cultivable part of the nasal microbiota together with the anaerobic species *F. magna*, showing a similarity level of > 50% (Fig. 3). The species *S. epidermidis* and *P. acnes* appeared together with *S. aureus* at a similarity level of 68.4%. Notable simultaneous colonisation was also observed for distinct pairs of species: *Dolosigranulum pigrum* and *Corynebacterium propinquum* (66.7%), *Staphylococcus capitis* and *Micrococcus luteus* (64.0%) and *Streptococcus mitis/oralis* and *Actinomyces odontolyticus* (54.6%). However, these species were found to be present together with the core members at a similarity level of less than 50% (Fig. 3).

The nasal cultivable microbiome in health and disease

In the cultivable-fraction of the microbial community, samples from patients with chronic nasal inflammation did not show clear differences to those of individuals without signs of inflammation when being arranged in a nMDS plot (Supporting Information Fig. S1B). This was confirmed by an ANOSIM test showing a global R of 0.375 and thus arguing for strong overlapping of bacterial communities from the two groups ($p = 0.029$). Further comparison of the presence of distinct taxa demonstrated that all species showing frequent presence throughout patients (detected in ≥ 5 patients) were also always detected in both patient groups (Fig. 1B). Thus, none of these frequently occurring species was exclusively connected to a distinct patient group. Overall, patients with and without nasal inflammatory complaints presented similar presence of members from the three main phyla of *Actinobacteria*, *Firmicutes* and *Proteobacteria* (Supporting Information Table S4).

The individual nasal cultivable microbiome

There was a visible similarity between the microbial communities of single individual samples at the distinct nasal sites (Fig. 2). Variation in communities between different hosts appeared to be stronger than intraindividual differences in the nasal microbiome at different sites, an observation confirmed by ANOSIM (global $R=0.842$, $p=0.001$). Other factors like *S. aureus* carriage, gender, age, allergies, use of nose sprays and smoking habits did not show any clear influence on the arrangement of samples in non-metric multidimensional scaling (data not shown).

Cultivable fingerprint vs. molecular fingerprint

For a subset of five patients each with and without signs of inflammation, a third sampling set was collected during surgery for molecular fingerprinting via Illumina 16S rRNA gene paired end sequencing (Supporting Information Table S6). The comparative analysis of the culturomic and the molecular data revealed that only a small amount of phylotypes/isolates ($n = 11$) matched in both identification methods (Table 2, Fig. 4, Supporting Information Table S7). Higher numbers were identified exclusively in either the culturome ($n = 53$) or the molecular data set ($n = 113$) (Table 2). Analysing the resolution of both approaches, the high fidelity of species identification by cultivation was demonstrated as expected. Of the 64 different taxa identified via cultivation, 62 (96.9%) could be

199 tracked down to their species level. While the molecular approach yielded a higher number of different
200 phylotypes (n = 124), only 33 (26.6%) were identified to species level (Table 3). However,
201 considering that only 160 bases of the 16S rRNA gene were used in this analysis, the sensitivity of the
202 method needs to be appreciated. Moreover, as shown independently for the culturome, the molecular
203 approach also yielded an individual pattern for each patient included (data not shown).

Discussion

There is growing interest in understanding the human body surfaces as an exceptionally complex and highly varying microbiological environment. Also for the nose, efforts have been made to elucidate its microbial diversity (Wos-Oxley et al., 2010; Yan et al., 2013) as well as interspecific interactions of its core members (Uehara et al., 2000; Lina et al., 2003) and other factors (Cole et al., 2001; Johannessen et al., 2012) that might play a role, especially in connection with *S. aureus* colonisation.

Here, we compared the nasal microbiota of two distinct groups of patients who were admitted to an ENT department: (i) people suffering from chronic nasal inflammatory diseases and (ii) people without any signs of inflammation. Using an extensive culturomic approach, we were able to isolate more than five times the number of species that can usually be detected via standard routine cultivation (Feazel et al., 2012) and more than four times the number obtained by prior cultivation studies (Alvarez et al., 2013). Composition of the most prevalent species comprising the core community in our study is in agreement with previous data reported in both, culture-based and molecular, microbiome studies (Frank et al., 2010; Wos-Oxley et al., 2010; Feazel et al., 2012; Alvarez et al., 2013; Yan et al., 2013).

Since the human nostrils are easily accessible for specimen sampling, most studies for determination of nasal *S. aureus* carriage were restricted to the more or less precisely defined “anterior nares” (Gillespie, 1939). Thus, the “anterior nares” have been considered as the principal habitat of *S. aureus* and swabbing of this region has become a main part of MRSA screening procedures. Here, a surgical approach has been chosen (i) to reach posterior areas of the nasal cavity and (ii) to avoid any contamination with the skin around the nasal ostium. Cole et al. (2001) showed that *S. aureus* was localised primarily to moist squamous epithelium on the septum adjacent to the nasal ostium. Also in our study, *S. aureus* was always detected in the vestibule, however, only 15/20 (75.0%) of the individuals tested *S. aureus*-positive in at least one of the four sampling sites showed colonisation of the anterior region of the vestibule while all were *S. aureus*-positive for the posterior vestibule. Consequently, the principle habitat definition for *S. aureus* should be extended to the complete vestibule area. Furthermore, *S. aureus* nasal eradication strategies should also cover posterior regions

of the complete nasal cavity (vestibulum nasi and nasal meatuses) by the use of appropriate formulations of the topical agent applied. Our finding that *S. aureus* could be found throughout all nasal sites tested is supported by findings of recent investigations that also consider the different anatomical parts (Yan et al., 2013). Compared to current literature, the incidence of *S. aureus* carriers in the study group was higher than expected (Sollid et al., 2014). Data in the literature are often based on simple swabbing techniques of a single nasal site (Wertheim et al., 2006; Mertz et al., 2009). Here, for the first time, different parts of the human nose were precisely analyzed with a standardised surgical approach applying extensive culture. The *S. aureus* clonal structure was very diverse (24 isolates with 21 *spa* types) and three novel *spa* types were detected (t14141, t14142, and t14143). Of interest, one of the two MRSA isolates detected (*spa* type t034), belonged to the ST398 clonal lineage which comprises livestock-associated MRSA known to occur on pig farms in Northwestern Germany and found to enter the hospitals in this region (Köck et al., 2009, 2013).

Amongst nasal specimen analysed by culture, 11 CoNS species were detected with most prevalent species being in accordance with prior findings (Becker et al., 2006; Piette and Verschraegen, 2009). Noteworthy, a relatively high presence of *S. lugdunensis* was observed. Despite being a member of the CoNS, similar to *S. aureus*, *S. lugdunensis* shows a high potential to cause aggressive and fast proceeding infections (Seifert et al., 2005; Piette and Verschraegen, 2009; Liu et al., 2010). Both species also share the ability to express a clumping factor and to produce a thermostable DNase (Becker et al., 2014). Hitherto, it has been assumed, that the principal habitat of *S. lugdunensis* is largely restricted to the inguinal or pelvic area (Bellamy and Barkham, 2002; van der Mee-Marquet et al., 2003; Hellbacher et al., 2006). Considering the high prevalence of *S. lugdunensis* in our samples, the human nose may be taken into consideration as a natural habitat of equal value. Of more recently described novel staphylococcal species, only *S. pettenkoferi* was found in one nasal location of one individual (Trülzsch et al., 2007).

For *S. pneumoniae* it has been shown, that the highest rate of nasopharyngeal colonisation takes place in an early stage of life (Bogaert et al., 2004; Regev-Yochay et al., 2004). This corresponds to the low number of *S. pneumoniae* carriers in our study, as only adult individuals were sampled. However, other members of the *Streptococcus* genus, mainly VGS, were isolated from more than a

third of individuals in this group. As VGS contribute to the common flora to the upper respiratory tract (Hardie and Whiley, 1997; Aas et al., 2005; Nakajima et al., 2013), a regular nasal presence is plausible.

As expected, *M. luteus*, a coloniser of the human skin, was the most prevalent “micrococcal” species found in 11/34 (32.4%) patients by culture. Newly established species of the *Micrococcaceae* and *Dermaococcaceae* families (suborder *Micrococcineae*), namely *Kocuria koreensis*, *Kocuria palustris*, *Kocuria rhizophila* and *Kytococcus schroeteri* were found in a much less extend below 10%. Of interest, these “micrococcal” species have been originally associated with an occurrence in the environment or fermented food, however, human infections have been recently described for *K. rhizophila* and *K. schroeteri* (Kovács et al., 1999; Becker et al., 2008; Park et al., 2010; Moissenet et al., 2012).

The *Corynebacteria* genus was the most species-rich taxon in this study, comprising 23 different species and being present in almost every patient sampled. Only one individual did not show colonisation in any of the nasal habitats. *Corynebacteria* are being considered as part of the normal skin flora, but are highly present especially in moist regions (Grice and Segre, 2011).

Interestingly, despite the absolute number of isolates per enterobacterial species was low, more than half of the number of the individuals harboured enterobacterial species of the genera *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pantoea*, *Proteus* and *Serratia*. Thus, the nasal cavity may represent a so far neglected reservoir for the occurrence of *Enterobacteriaceae*, which might be of importance for the surveillance of isolates expressing extended spectrum beta lactamases (ESBL) and carbapenemases. Nonfermenter of hospital hygienic and therapeutic concern, such as *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, occurred in only one and two patients, respectively.

Though the nasal cavity is well ventilated, there were considerable amounts of moderate as well as strictly anaerobic species vital throughout all nasal sites. Whilst former studies failed to detect strictly anaerobic species like *F. magna* (Feazel et al., 2012; Alvarez et al., 2013), known to be common members of the nasal microbiome (Wos-Oxley et al., 2010), with this approach, those species were frequently detected.

Our data did not provide any evidence of a distinct species distribution or deviating microbial diversity influenced by epidermal surface constitutions. Neither the variety of the different nasal habitats nor chronic inflammation had a visible impact on the microbial composition. Whereas there was no clear prove of habitat- and/or inflammation-associated differences, individual host-specific patterns of microbiota composition were revealed (Fig. 2). Most recently, such host-specific microbial structures have been shown to be already present at a very early stage of life (Sakwinska et al., 2014). Of note, other aspects such as ethnic origin, age, allergies or medical use should still be taken into account as probable drivers for these individual microbial “fingerprints” of the nasal microbiota and should be investigated in a higher resolution in following studies.

While metagenomic data emerge increasingly, there is an obvious lack of comparative analysis of this method with other techniques. In this study, we were able to cultivate species that could not be found via next generation sequencing and to identify a higher number of isolates down to their species level (Table 3). These results are generally in line with former comparative studies about different techniques to analyse the human gut microbiome (Lagier et al., 2012). Indeed, it should be noted that molecular data can give a qualitative analysis of the composition of the microbiome, and typically characterise the community structure down to a certain level of abundance, whereas culture-based studies more easily identify low abundant but easy to culture members of the community.

Applying state-of-the-art highly specific identification tools, an in-depth inventory of the bacterial species occurring on the surfaces of the human nasal cavity was done by culture and, in part, by 16S rRNA gene community profiling. For the first time, a standardised surgical approach was chosen for that purpose to avoid any contamination with the microbiota of the skin outside of the nasal cavity. The culturomic data revealed fingerprint-like, individual host-specific nasal microbiota that are characterised by a consistent species distribution throughout the different nasal habitats analysed. These bacterial communities appeared irrespective of local inflammation status, gender and *S. aureus* carrier state. By cultivation, numerous species were detected that were not identified by molecular analysis at the chosen resolution level. It was thus demonstrated that an extensive cultural approach in combination with molecular analysis is indispensable for a detailed analysis of microbial structures of a given habitat. Thorough analysis of the composition of the nasal microbiota is precondition for

317 studies investigating the interactions of their members and their putative influence on the colonisation
318 with the major human pathogen *S. aureus*. The definition of the principle habitat for *S. aureus* should
319 be extended to the complete vestibule area particularly including the posterior regions.
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Experimental procedures

Study design

The study was conducted from October 2011 to April 2013 with full written consent of patients (file number 2010-468-f-S) admitted to the Department of Otorhinolaryngology Head and Neck Surgery (ENT) of the University Hospital Münster. Nasal swab samples from two patient groups comprising (i) 18 individuals with signs of chronic inflammation of nasal sites and (ii) those that were categorised as non-inflamed ($n = 16$) were systematically collected during surgery. The last group included patients without any signs of acute or chronic local or systemic inflammation intended for surgical corrections of innate or traumatic nasal deformations. Each dual swab sample (Transwab® Amies Duo Plain MW169P, MWE, Corsham Wiltshire, United Kingdom) was taken precisely from four defined locations in the nasal cavity: the anterior and posterior vestibules comprising skin-like squamous epithelium as well as the inferior and middle meatuses lined by ciliated mucosa. From a randomly chosen subset of ten patients, a third set of swab samples (SK-2S, Isohelix, Harrietsham, United Kingdom) was taken and used for molecular fingerprinting.

Cultivation techniques

After sampling, dual swabs were divided and immediately placed into either BHI medium (Becton Dickinson, Franklin Lakes, NJ) supplied with 0.2% TWEEN 80 (Merck, Darmstadt, Germany) for aerobic incubation or into thioglycollate medium (Heipha Dr. Müller GmbH, Eppelheim, Germany) for anaerobic cultivation. Transport tubes were sealed gas-tight and kept at room temperature (max. 1 h) until they were further processed. Samples were plated onto Columbia blood agar (Becton Dickinson) with 5% sheep blood (Oxoid, Wesel, Germany), chocolate agar (Becton Dickinson) with 10% sheep blood (Oxoid), Schaedler agar with 5% sheep blood (Becton Dickinson) and Schaedler agar supplied with Kanamycin, Vancomycin and 5% horse blood (Oxoid). All agar plates were incubated at 37°C either under aerobic, CO₂ enriched (5%) or anaerobic conditions. Swabs were also suspended in either BHI medium supplied with 0.2% TWEEN 80 for aerobic incubation overnight or thioglycollate medium for anaerobic incubation for 48 h. After incubation, 100 µl aliquots of the liquid

cultures were serially diluted up to 10^{-5} - 10^{-7} and inoculated onto Columbia blood agar with 5% sheep blood or Schaedler agar with 5% sheep blood. These plates were incubated at 37°C under aerobic or anaerobic conditions.

All agar plates were read after 24 h, 48 h, 5 days, 7 days and 14 days according to the respective cultivation condition. Different phenotypes were isolated and further grown as pure cultures (Supporting Information Fig. S2). Isolates were kept at -80°C in cryo tubes (Cryobank™ System, Mast Diagnostica, Rheinfeld, Germany) for long-term storage.

Identification

For identification of isolates down to the species level, matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry (MALDI-TOF MS) was applied as described elsewhere (Idelevich et al., 2014). Briefly, single colonies of freshly grown pure cultures were harvested with a sterilised toothpick and smeared on a ground steel target plate (Bruker, Daltonics, Billerica, MS) in two replicates. Samples were covered with 1 µl of HCCA matrix solution (alpha-cyano-4-hydroxycinnamic acid solved in 50.0% acetonitrile and 2.5% trifluoroacetic acid). After co-crystallisation they were processed in the MALDI-TOF mass spectrometer (Microflex, Bruker Daltonics) using the flexControl software (flexControl 3.3, Bruker Daltonics) according to manufacturer's instructions. Spectra were analysed by MALDI Biotyper 4.0 (Bruker Daltonics) in the m/z range of 4000-10000 Da. Isolates were considered as explicitly identified on the species level when yielding a score of ≥ 2.0 .

Members of *Fusobacterium* sp., *Klebsiella* sp., *Escherichia* sp. and all streptococci performing α -hemolysis were also characterised biochemically by VITEK®2 (bioMérieux, Marcy l'Etoile, France) applying VITEK®2 GP ID cards or VITEK®2 GN ID cards (bioMérieux), respectively. Streptococci were screened for their resistance to optochin to distinguish pneumococci. Determination of methicillin resistance (*mecA*, *mecC*) of *S. aureus* isolates were verified by respective PCR procedures as published (Becker et al., 2006; Kriegeskorte et al., 2012). *spa* typing was done as described elsewhere (Mellmann et al., 2006).

In the case of ambiguous MALDI-TOF MS and biochemical identification results, respectively, 16S rRNA gene sequencing was performed as described before (Becker et al., 2004; Breitkopf et al., 2005). Briefly, total genomic DNA was extracted using the QiaAmp MiniKit according to manufacturer's instructions (Qiagen, Venlo, Netherlands). Primers 29f and 907r(m) were used to target the V1-V4 hypervariable regions of the 16S rRNA gene (Breitkopf et al., 2005; Lane, 1991). PCR products were also sequenced with these primers (MWG Eurofins, Ebersberg, Germany) and the obtained 16S rRNA gene sequences were aligned with SeqMan ProTM (DNASTAR, Madison, WI). Comparison of sequences was carried out in the RDP-II database (Wang et al., 2007) using the Seqmatch program against sequences obtained from validly described type strains and isolates. Sequences were assigned on species level at a similarity score of $\geq 98\%$.

Molecular fingerprinting via 16S rRNA gene Illumina paired-end sequencing

A subset of nasal samples from ten patients was analysed by barcoded 16S rRNA gene Illumina paired-end sequencing. For this, the dry swab was extracted for DNA, the bacterial 16S rRNA gene amplified, amplicon libraries prepared, and bioinformatic analysis carried out as previously described (Camarinha-Silva et al., 2014). In brief, primers 27F and 338R were used to target the V1-2 regions of the 16S rRNA gene, where in addition the forward primer contains a 6 nucleotide (nt) barcode and a 2 nt CA linker, and both primers comprise sequences complementary to the Illumina specific adaptors to the 5'-ends. Each sample is amplified with a forward primer containing a unique barcode, so that derived sequences can be sorted into their respective samples bioinformatically in downstream analysis. For evaluation of sequence quality and possible sequencing errors, positive control samples containing equimolar concentrations of established 16S rRNA gene sequences from *S. aureus* (DSM3463), *C. accolens* (GU074966), *M. nonliquefaciens* (JF927886) and *S. epidermidis* (JF927883) were used, as described in Camarinha-Silva et al., 2014. These additional samples were treated together with the other nasal samples and pooled in the final library. DNA libraries were sequenced on an Illumina MiSeq platform (Illumina Inc., San Diego, CA) using 250 nt paired-end sequencing chemistries, although all sequences were trimmed to 80 nt to account for sequence deterioration giving 160 nt of paired end sequence information for downstream analysis. In total, 477205 sequence reads

were obtained. Sequences were discarded if they returned a low Phred quality score, comprised a N character in their sequence, any mismatches within the primers and barcodes or more than 10 homopolymer stretches (Camarinha-Silva et al. 2014). After filtering, samples contained a mean of 7192 sequence reads (min of 877, max of 36355).

Statistical analysis

In order to compare the microbial fingerprints of each individual person across conditions and sites, non-parametric multivariate statistical analysis was conducted with PRIMER v6 (Clarke, 1993; Clarke and Gorley, 2006). For comparison of the microbial fingerprints across samples within predefined groups (individual hosts or inflammation status), a sample-similarity matrix was generated using the Bray-Curtis coefficient (Bray and Curtis, 1957). Samples were then ordinated using non-metric multidimensional scaling (nMDS) with 50 restarts. Predefined groups of samples were tested for differences via an Analysis of Similarity (ANOSIM) test using 999 permutations. Samples were considered to be significantly different when the accompanying p-value was < 0.05 . The associated R statistic ranging from 0 to 1 gave further information pertaining to the degree of separation between groups of samples (Clarke and Warwick, 2001). In order to assess the co-occurrence nature of species across these samples, a species-similarity matrix was generated using the Bray-Curtis coefficient. Most prevalent species (present in ≥ 5 patients) were clustered using group-average agglomerative hierarchical clustering (Field et al., 1982; Bryan, 2010; Arantes et al., 2009). When the cultivable-fingerprinting method was analysed, the data matrix comprised presence/absence data (a binary matrix).

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Non-metric multi-dimensional scaling plot (nMDS) demonstrating similarities of the cultivable part of microbial communities between samples from (A) distinct nasal locations and (B) patients with and without chronic nasal inflammation. No significant differences were demonstrated for the respective groups via ANOSIM (Clarke and Warwick, 2001). Kruskal 2D Stress: 0.28.

Fig. S2. Scheme of sampling sites from the human nasal cavity chosen for cultivation and molecular analysis and cultivation protocol for clinical samples collected during surgery. Exemplary design for one set of swab samples obtained from one single nasal site. Swabs were first plated on agar then inoculated in liquid media for overnight cultivation. On the next day liquid cultures were plated onto agar directly and in dilution steps 10^{-5} - 10^{-7} . AV, anterior vestibules; PV, posterior vestibules; MM, middle meatus; IM, inferior meatus. *For a subset of samples from 10 patients a third set of swab samples was collected and next generation sequencing was conducted in parallel to the culturome

Table S1. Overview of patients' metadata and nasal colonisation by *S. aureus* as detected by culture

Table S2. List of all species/genera isolated by culture in the group of 34 individuals and their presence in every single individual analysed

Table S3. Absolute numbers of different cultivable species/genera isolated from the different nasal habitats and patients included in this study

Table S4. Distribution of *S. aureus* throughout patient groups and nasal habitats as found by culture

657 Table S5. List of all species/genera isolated by culture in the group of 34 individuals and their
658 appearance in the different patient groups and nasal habitats

659

660 Table S6. List of all phylotypes detected by 16S rRNA gene community profiling in the subset of
661 samples from 10 individuals and their presence throughout all nasal samples analysed

662

663 Table S7. Comparison of species reliably identified in the subset of samples from 10 individuals via
664 cultivation and 16S rRNA gene community profiling

665

Figure legends

Fig. 1. Prevalence of the most common cultivable nasal species (isolated from ≥ 5 patients) in (A) the different nasal habitats and (B) individuals with and without chronic inflammatory nasal diseases.

A: bars represent the proportion of patients carrying the respective species when being grouped into individuals suffering from chronic nasal inflammation ($n = 18$) and those without inflammatory complaints ($n = 16$). B: bars represent the fraction of all participants ($n = 34$) carrying the respective species in either of the nasal habitats analysed. *Bacillus cereus* group includes isolates identified as *Bacillus cereus*, *Bacillus mycoides*, *Bacillus thuringiensis* and *Bacillus weihenstephanensis* via MALDI-TOF MS.

Fig. 2. Non-metric multi-dimensional scaling plot (nMDS) visualising the individual specific similarities of the different nasal habitats' cultivable microbiota.

Shown is an overview of all samples from the 34 patients (big box) and the individual-related samples singled out from the overview (small boxes). Samples from one individual (different habitats) are marked in the same colour. Triangles indicate patients suffering from chronic inflammatory nasal diseases, circles represent individuals without inflammation. Kruskal 2D Stress: 0.28. Analysis of similarities (ANOSIM) on single individual's nasal microbiota showed significant differences of the interindividual nasal communities (Global $R = 0.842$; $p = 0.001$). AV, anterior vestibule; PV, posterior vestibule; MM, middle meatus; IM, inferior meatus.

Fig. 3. Cluster diagram of the most prevalent species/genera (detected in ≥ 5 patients) illustrating their relative co-occurrence on patient level.

Position of nodes represents the relative frequency of the different species/genera appearing simultaneously in the same patient. From presence/absence data on patient level of the different species, a similarity matrix was generated using the Bray-Curtis coefficient. The dendrogram was calculated via group-average agglomerative hierarchical clustering. *Bacillus cereus* group includes

isolates identified as *Bacillus cereus*, *Bacillus mycoides*, *Bacillus thuringiensis* and *Bacillus weihenstephanensis* via MALDI-TOF MS.

Fig. 4. Prevalence of isolates identified via culturomic analysis and 16S rRNA gene community profiling onto species level in the comparative subset of samples from 10 patients. Bars represent the relative number of nasal samples tested positive for the respective species via each of the methods. The complete list of identifications down to species level in the subset analysed via culture dependent and independent methods is given in Table S7.

702 **Tables**

703

704 Table 1. Absolute numbers of cultivated species/genera observed on average per patient and nasal habitat and stratified according to inflammatory state of
705 nasal sites, gender and *S. aureus* carriage state.

Nasal habitat ^a	Inflammatory state ^b		Gender		<i>S. aureus</i> carriage ^c		Total patients
	CI	NI	Male	Female	positive	negative	
AV	7.8 ±2.2	8.5 ±4.1	8.6 ±3.7	7.3 ±1.8	7.8 ±3.2	8.6 ±3.2	8.1 ±3.2
PV	8.6 ±3.0	7.9 ±3.0	8.2 ±2.8	8.5 ±3.4	8.4 ±3.3	8.2 ±2.4	8.3 ±3.0
MM	8.3 ±3.3	9.3 ±4.6	9.4 ±4.0	7.5 ±3.7	8.5 ±3.7	9.1 ±4.3	8.7 ±3.9
IM	9.2 ±3.4	6.9 ±3.1	8.5 ±2.7	7.4 ±4.6	8.4 ±3.8	7.7 ±3.0	8.1 ±3.4
All habitats	15.7 ±6.0	14.5 ±6.7	15.2 ±5.5	15.0 ±7.6	14.8 ±6.5	15.6 ±6.1	15.2 ±6.2

706 a. Nasal sites chosen for sampling in his study. AV, anterior vestibule; PV, posterior vestibule; MM, middle meatus; IM, inferior meatus.

707 b. Inflammatory nasal diseases such as chronic sinusitis, chronic polyposis or both. CI, individuals with chronic nasal inflammation; NI, individuals without
708 chronic nasal inflammation.

709 c. Nasal *S. aureus* carriage as detected by culture.

710

711 Table 2. Comparison of cultivation and molecular analysis regarding their potential to exclusively identify distinct phylotypes/species^a.

Patient number	7	10	15	26	29	4	14	19	22	30	Total ^b	% ^c
Isolates/phylotypes exclusively identified via:												
Culturome	9	10	9	10	14	15	12	9	6	16	53	29.9
Molecular fingerprinting	30	52	40	25	25	48	21	15	22	32	113	63.8
Both methods	6	4	4	6	1	4	2	5	3	2	11	6.2
Total (isolates/phylotypes)	45	66	53	41	40	67	35	29	31	50	177	100.0
Species exclusively identified via:												
Culturome	8	10	8	9	13	15	11	8	6	16	51	60.7
Molecular fingerprinting	8	15	14	3	8	14	7	3	8	9	22	26.2
Both methods	6	4	4	6	1	4	2	5	3	2	11	13.1
Total (species)	22	29	26	18	22	33	20	16	17	27	84	100.0

712 a. Given are the total numbers of isolates/phylotypes and species identified via either cultivation or molecular analysis and those identified via both methods

713 in each of the 10 individuals where molecular analyses have been performed.

714 b. Total amount of different isolates/phylotypes or species found in the complete subset of patients.

715 c. Relative amount of different isolates/phylotypes or species found in the complete subset of patients.

716

717 Table 3. Comparison of quantity and resolution of species identification via cultivation and molecular analysis^a.

Patient number	7	10	15	26	29	4	14	19	22	30	Total ^b	% ^c
Different isolates in culturome identified on:												
Species level	14	14	12	16	15	19	13	13	9	18	62	96.9
Genus level	1	0	1	0	0	0	1	1	0	0	2	3.1
Family level	0	0	0	0	0	0	0	0	0	0	0	0.0
No distinct level	0	0	0	0	0	0	0	0	0	0	0	0.0
Total (isolates)	15	14	13	16	15	19	14	14	9	18	64	100.0
Different phylotypes in molecular data set identified on:												
Species level	14	19	18	9	9	17	9	8	11	11	33	26.6
Genus level	19	31	22	12	12	30	8	9	12	13	68	54.8
Family level	2	4	3	3	4	2	4	2	2	7	11	8.9
No distinct level	1	2	1	7	1	3	2	1	0	3	12	9.7
Total (phylotypes)	36	56	44	31	26	52	23	20	25	34	124	100.0

718 a. Listed are the absolute numbers of isolates/phylotypes that were identified in each of the 10 individuals where molecular analyses have been performed, as
719 well as the level of differentiation.

720 b. Total amount of different isolates/phylotypes found in the complete subset of patients.

721 c. Relative amount of different isolates/phylotypes found in the complete subset of patients.