COMPARATIVE STUDY OF NASAL BACTERIAL CARRIAGE IN PEDIATRIC PATIENTS FROM TWO DIFFERENT GEOGRAPHICAL REGIONS

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The aim of this study was to determine the prevalence of bacterial carriage in the anterior nares of two different patient cohorts, aged 5–15 years. By use of a sensitive enrichment broth, Gram-positive and -negative bacteria were cultured from the two cohorts of each 100 patients at the Referral Clinic in Eritrea and at a German University Hospital. In the German cohort, 27% of the patients were positive either for Gram-negative ($n=5$) or -positive bacteria, including *Staphylococcus aureus* ($n=8$; MRSA ($n=2$)), *Staphylococcus epidermidis* ($n=12$), *Corynebacterium* spp. ($n=4$), and *Streptococcus pyogenes* ($n=1$). In comparison, the Eritrean cohort revealed 33% bacterial carriers in the anterior nares. Among the identified species were *S. aureus* ($n=2$), *S. epidermidis* ($n=13$), *Streptococcus haemolyticus* ($n=9$), and Gram-negative rods including *Klebsiella pneumoniae/oxytoca* ($n=5$), *Enterobacter agglomerans* ($n=4$), *Escherichia coli* ($n=2$), and *Pseudomonas aeruginosa* ($n=1$). Noteworthy, none of the Eritrean patients were positive for MRSA. In both cohorts there was no co-occurrence of Gram-positive and -negative bacteria in the anterior nares. However, we observed in two subjects of the Eritrean cohort co-colonization with *S. epidermidis* and *S. haemolyticus*. The occurrence of Gram-negative bacteria was less significant by age in the German cohort, whereas in the Eritrean cohort Gram-negative bacteria were more frequently detected in carriers aged 5–9 years. Continued surveillance of *S. aureus* and Gram-negative bacteria carriage deserves further attention and might help to determine future trends in the characteristics of nasal carriage, subsequent incidence of infections, and the potential effectiveness of targeted population based intervention.

Keywords: nasal carriage, staphylococci, Gram-negative bacteria, East Africa, Germany

Introduction

Bacterial colonization and the subsequent infections remain a major cause of morbidity and mortality in patients with underlying diseases. Carriage of *Staphylococcus aureus*, as the major human pathogen, appears to play a key role in the epidemiology and pathogenesis of infection by this species [1]. Multiple studies have shown that nasal carriage of *S. aureus* is a risk factor for pathogenic infection [2–5]. However, in the recent time it has been observed that high risk is only evident in persistent nasal carriers whereas intermittent and non-carriers exhibit low levels of infection [6]. Aside from straightforward incidences in which endogenous strains establish pathogenic infections in their hosts, the overall extent to which nasal carriage strains are responsible for transmissible infection is currently poorly known. Approximately 30% of healthy people carry *S. aureus* in their anterior nares. *S. aureus* nasal carriage can provide an indication of a higher risk for subsequent infection, including with methicillin-resistant *S. aureus* (MRSA) [2]. Although the risk factors for and characteristics of Panton–Valentine leukocidin (PVL) gene positive MRSA colonization in the healthy pediatric popu-

lation are poorly understood, previous antibiotic therapy is associated with PVL-positive MRSA colonization [7]. PVL gene as a virulence factor is associated with necrotizing pneumonia and skin infections [8]. PVL genes may be more common among *S. aureus* isolates causing clinically apparent infection than among isolates causing asymptomatic colonization although there are conflicting data [9].

Many studies have focused on nasal *S. aureus* colonization in different age groups, but the relationship between nasal carriage of staphylococci and Gram-negative bacteria is not well understood, rather less in comparison of individuals from two different geographical areas.

Moreover, no population-based prevalence study has been conducted to measure *S. aureus* carriage in Eritrea, and national population estimates are lacking. To obtain these data, we measured the prevalence of *S. aureus* and other bacterial organisms in nasal swabs of the Eritrean and German pediatric population aged 5–15 years. The age range 5 to 15 years was preferred to fill the gap between the infants, children below 5 years and the adults, normally the cohorts which have been studied elsewhere before [2–5]. We used a sensitive culture technique to minimize errors arising from low recovery rates.

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Materials and methods

Study population

Over a 4-week period, patients aged between 5 and 15 years admitted to both hospitals in Asmara and Magdeburg were studied prospectively. These pediatric patients were screened for nasal carriage of *S. aureus* and Gram-negative bacilli, including resistant *Enterobacteriaceae* and resistant nonfermenting bacilli within 48 h of admission. We analyzed 100 nasal swabs for each enrolled patients' groups, total $n=200$. All patients had no surgical history at the time of survey, and no procedure was planned. The exclusion criteria were any antibiotic usage in the past month, treatment for allergy and immunotherapy, any systemic disease, and any active upper respiratory tract infections.

Microbiological study

Nasal specimens were obtained by five rotations of the swab (Culture Swab Transport System, Copan, Brescia, Italy) in the two anterior nares and analyzed by the bacteriology laboratory in Magdeburg. Nasal swabs samples were streaked on Chapman agar to detect *S. aureus* and on MRSA-ID screen agar (bioMérieux, Marcy l'Étoile, France) for methicillin-resistant *S. aureus* (MRSA) isolates. Additionally, swabs were streaked on MacConkey plates to detect Gram-negative bacilli. Media were incubated for 24–48 h at 37 °C. The coagulase test (Coagulase Plasma System; Difco Laboratories) was employed to identify *S. aureus* strains. In addition, methicillin-susceptible *S. aureus* (MSSA) strains were preliminarily detected by characteristic growth on mannitol salt agar and absence of growth in the presence of 6 µg/ml oxacillin. Growth on both agar plates was presumed to indicate the presence of MRSA. After morphological and microscopic examination, relevant bacteria were further identified by VITEK2®. Antimicrobial susceptibility results were evaluated by using the Clinical and Laboratory Standards Institute (CLSI) reference methods [10], and polymerase chain reaction (PCR) assays for detection of PVL gene in *S. aureus* was performed as previously [11, 12].

Results

Nasal carriage of bacteria

In comparison, different carriage rates of either Gram-positive or -negative bacteria were observed in the two study groups, 27% in German cohort and 33% in Eritrean cohort, respectively. Organisms isolated from the study subjects at the pediatric hospital in Asmara included a wide range of bacteria as shown in Table 1. The less prevalent organism was *S. aureus* ($n=2$). This organism together with the coagulase-negative staphylococci was isolated from 15% of the study subjects. Major numbers of Gram-negative or-

ganisms were isolated with highest occurrence of *Enterobacter* and *Klebsiella* spp. (Table 1). Minor numbers of streptococci comprising *Streptococcus pyogenes* ($n=1$), *Streptococcus constellatus* ($n=1$), and viridian streptococci ($n=4$) were isolated from specimens of the German children. Low rate of Gram-negative rods, comprising *Haemophilus influenzae* ($n=1$), *Klebsiella* spp. ($n=1$), *Morganella morganii* ($n=1$), *Proteus mirabilis* ($n=1$), and *Pseudomonas aeruginosa* ($n=1$) were obtained from the German children. There was no significant association of nasal bacterial colonization with sex or age, except for the Eritrean cohort with all Gram-negative rods being isolated from specimens of children aged 5–9 years.

Antimicrobial resistance of selected bacteria and PVL gene detection in *S. aureus*

All of the *S. aureus* isolates ($n=8$ in the German cohort and $n=2$ in the Eritrean cohort) were sensitive to cotrimoxazole, tetracycline, gentamicin, fusidic acid, vancomycin, and teicoplanin. The prevalence of methicillin-resistance in *S. aureus* was observed in two German isolates. Both MRSA isolates were additionally resistant towards ciprofloxacin, clindamycin, and erythromycin. All German MSSA isolates were penicillin, clindamycin, and erythromycin resistant, but two isolates, whereas the two Eritrean MSSA isolates were only penicillin resistant.

Among the streptococci no antimicrobial resistance was observed while among the coagulase-negative staphylococci in both cohort groups the resistance towards penicillin, oxacillin, cotrimoxazole, tetracycline, gentamicin, and ciprofloxacin was 100%, 100%, 48%, 48%, 100%, and 100%, respectively.

The antimicrobial resistance among the Gram-negative rod organisms was relatively low in both cohort groups; none of the isolates indicated multidrug-resistance. A resistance rate of 100% was observed for ampicillin for all the Gram-negative bacteria in both groups. In addition, *Escherichia coli* isolates were resistant towards piperacillin, amoxicillin/clavulanic acid, and tetracycline. Two *Enterobacter agglomerans* isolates showed resistance towards amoxicillin/clavulanic acid and one isolate towards piperacillin, cefuroxime, tetracycline, and cotrimoxazole. *P. aeruginosa* isolates of each cohort were resistant towards piperacillin and cotrimoxazole, and additionally towards cefotaxime and tetracycline in the Eritrean *P. aeruginosa* isolate.

The PVL gene was determined for all MRSA and MSSA isolates. Neither the German MRSA isolates nor the *S. aureus* isolates of both cohorts were positive for PVL gene.

Discussion

In this prospective study, we simultaneously examined the prevalence of *S. aureus*, other Gram-positive and Gram-

Table 1. Prevalence of bacterial species among study subjects from Eritrea (ER) and Germany (DE), total subjects each $n=100$

Bacterial organism	Prevalence in DE (n)	Prevalence in ER (n)
<i>Staphylococcus aureus</i> ,	8	2
including MRSA	2	–
<i>Staphylococcus epidermidis</i>	12	13
<i>Staphylococcus haemolyticus</i>	–	9
<i>Corynebacterium</i> spp.	4	–
<i>Streptococcus pyogenes</i>	1	–
<i>Streptococcus constellatus</i>	1	–
<i>Escherichia coli</i>	–	2
<i>Enterobacter agglomerans</i>	–	4
<i>Haemophilus influenzae</i>	1	–
<i>Klebsiella pneumoniae/oxytoca</i>	1	3
<i>Morganella morganii</i>	1	–
<i>Proteus mirabilis</i>	1	–
<i>Pseudomonas aeruginosa</i>	1	1

negative organisms from nasal swabs of pediatric patients aged 5–15 years from two different hospitals, the pediatric clinic in Asmara, Eritrea and the pediatric clinic at the Otto-von-Guericke-University in Magdeburg, Germany. Nasal carriage of methicillin-susceptible and -resistant *S. aureus* is well documented in various populations and varies in different geographical regions worldwide [2]. Because microbial consortia within the nasal cavity may be an important determinant of *S. aureus* colonization, we screened Gram-negative and -positive bacteria in the nasal cavity of children. The nasal specimens yielded a wide range of bacterial agents (Table 1) for both study groups, but for the German cohort, *S. aureus* was one of the principal organisms, besides *S. epidermidis*. Within the German cohort, the MRSA prevalence was relatively low ($n=2/100$) which reflects earlier dataset of the University Clinic in Magdeburg. All MRSA and MSSA isolates were negative for PVL gene [11–13].

Similarly as in our study, Frank et al. [14] determined the composition and dynamics of the nasal microbiota by use of culture-independent methods and correlated specific microorganisms with *S. aureus* colonization in adults. According to their study, the majority of nares rRNA sequences obtained from healthy individuals belonged to only two bacterial phyla, the *Actinobacteria* (e.g. *Corynebacteria*) and *Firmicutes* (Gram-positive organisms such as staphylococci), whereas *Proteobacteria*, *Bacteroidetes*, *Fusobacteria*, *Cyanobacteria*, *Tenericutes*, and *Deinococcus* accounted for the remainder of phylum-level diversity. Similar to healthy adults, most of the phylum-level nasal biodiversity observed in the hospitalized adults was restricted to the *Firmicutes* (71%) and *Actinobacteria* (20%). However, *Firmicutes* were significantly more abundant in hospitalized compared to healthy adults (71% vs. 27% sequence abundance, respectively). Otherwise, *Actinobacteria* were concomitantly less abundant (20% vs. 68% for hospitalized vs. healthy adults) [14]. Differences

in the abundances of *Firmicutes* between hospitalized and healthy adults were due primarily to increased abundances of just two species, *S. aureus* and *S. epidermidis*, which together accounted for >50% of the hospitalized individuals. With regard to our results, the Eritrean pediatric group carried abundantly coagulase-negative staphylococci, such as *S. epidermidis* and *S. haemolyticus*, rather less *S. aureus* ($n=2$). Thus, in the Eritrean study population *S. aureus* colonization was negatively correlated with *S. epidermidis* and *S. haemolyticus* abundance. This was reflected by the study of Frank et al. [14] which concluded that *S. epidermidis* sequences were most abundant in the non-*S. aureus* carriers. Moreover, in their study, healthy adults harbored significantly more species-rich and diverse nares microbiotas than did hospitalized individuals. Approximately twice as many species-level microbial groups were identified in healthy adult nares microbiotas compared to microbiotas of hospitalized patients. In contrast to the Eritrean cohort, *Corynebacterium* spp. ($n=4$) were detected in the German cohort; however, these species were less abundant in *S. aureus*-colonized subjects.

Our dataset for the Eritrean cohort indicated that 12 hospitalized patients were nasal carriers of Gram-negative bacteria (*Enterobacteriaceae* and *Pseudomonas aeruginosa*). Interestingly, these patients were negative for the Gram-positive bacteria, such as *S. aureus* and the other staphylococci, as might be expected given the range of morbidities that likely were associated with *Staphylococcus* spp. in hospitalized patients. In the German cohort, only 5 patients were positive for Gram-negative bacteria in their nasal cavities, and none of them was co-colonized with Gram-positive bacteria as well. These findings may explain the bacterial competition for human nasal cavity colonization. This competition could be either Gram-positive versus Gram-negative bacteria or within the same group of bacteria as was shown in an animal model by Park et al. [15]. They investigated whether the applica-

tion of commensal resident bacteria could prevent nasal colonization with MRSA in an established murine model system, and showed that mice nasally pre-colonized with *S. epidermidis* became more resistant to colonization with MRSA. They suggested that application of commensal bacteria with antibiotics could represent a more effective strategy to prevent MRSA colonization [15]. Development of *S. epidermidis*-dominated microbiota may either protect an individual patient from subsequent *S. aureus* colonization or be an intermediate stage in succession to a nasal microbiota dominated by *S. aureus*. In the latter model, loss of commensal species, such as *Propionibacterium acnes*, rather than gain of *S. epidermidis* could be the key determinant of subsequent *S. aureus* colonization [14].

In Frank et al. [14] study, a subset of hospitalized adults exhibited elevated levels of *Proteobacteria* (mainly members of the orders *Enterobacteriales* and *Pseudomonadales*) and Firmicutes other than *S. aureus* and *S. epidermidis*. Another study by Cho et al. [16] compared two culture-based and molecular detection methods in nasal samples of 19 healthy adults. *S. epidermidis* was the most frequently detected bacteria, followed by *S. aureus*. PCR-based methods revealed 23 different species. However, staphylococci and *Enterobacter aerogenes* were detected most frequently.

In the Eritrean cohort, we did not find that healthcare exposure was significantly associated with MRSA nasal carriage, a finding that has been widely reported in the previous literature [2, 5, 17, 18] and in the German cohort of the current study. Due to relatively small sample sizes in this prospective study, associations may not have been detected, particularly for MRSA isolates. In contrast, a relatively high prevalence of MRSA colonization was found (11.6%) for a cohort of healthy children aged ≤ 14 years in community settings in Taiwan over a 5-year period with increasing tendency, despite an overall decrease in the prevalence of nasal colonization by *S. aureus* [18]. Kuehnert et al. [5] concluded that the *S. aureus* colonization prevalence in the nares was highest in participants 6–11 years old in the United States, whereas MRSA colonization was associated with age ≥ 60 years and being female but not with recent health-care exposure. An earlier study by Creech et al. [17] in healthy US children stated that 36.4% were colonized with *S. aureus*, whereas 9.2% were colonized with MRSA, significantly higher than in the German cohort. Although phenotypic data suggest that *S. aureus* carriage is determined or modulated by the host, genetic studies have not provided a definitive answer [19]. To the less extent, there is no answer to the Gram-negative bacteria carriage in the anterior nares and their determining factors. Factors influencing colonization and elimination are not as yet fully understood, but adhesion to mucosal receptors and immune responses are implicated in addition to bacterial properties and colonization resistance dynamics. Some of factors for bacterial colonization may comprise genetic background and socioeconomic conditions including housing, access to health care,

hygiene habits, family size, day-care contact, etc. [20–22]. In industrialized countries, routine hygienic procedures, aimed at reducing the spread of bacteria in maternity and neonatal wards, have strongly influenced the colonization pattern of the newborn infant [22, 23].

Viridans streptococci may antagonize colonization of nasopharyngeal site by other streptococci, especially group A β -hemolytic streptococci [20].

The nares' environment is colonized by a temporally stable microbiota that is distinct from other regions of the integument. Negative association between *S. aureus*, *S. epidermidis*, and other bacterial species suggests microbial competition during colonization of the nares [14], as was seen in our present study, more significantly in the Eritrean cohort.

In conclusion, this prospective study of bacterial nasal carriage in hospitalized pediatric patients is, to our knowledge, the first of its kind to investigate the presence of Gram-negative and Gram-positive bacteria including *S. aureus* in two different geographic areas. Overall, the study data of the two cohorts indicate geographical differences among nasal colonization by staphylococci, including MSSA and MRSA, and other Gram-positive and -negative bacterial species. *S. aureus* colonization was less common in Eritrean pediatric cohort; in contrast, the occurrence of this species including low number of methicillin resistant isolates was observed more frequently in the German cohort which corresponds to the local epidemiologic prevalence and clonality of MRSA in the study region [11–13]. Controlled, prospective clinical trials in the future might be helpful to determine whether nasal carriage of Gram-negative bacteria in such patients lead to the incidence of infection with these bacterial organisms. Moreover, a comprehensive study of *S. aureus* and MRSA nasal carriage in the Eritrean pediatric patients and in other healthcare settings should be considered in the future.

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