ORIGINAL ARTICLE

Persistence of nasal colonization with human pathogenic bacteria and associated antimicrobial resistance in the German general population

R. Köck[1], P. Werner[2], A. W. Friedrich[3], C. Fegeler[2] and K. Becker[1], for the Prevalence of Multiresistant Microorganisms (PMM) Study Group

1 University Hospital Münster, Institute of Medical Microbiology, Münster, 2 University Heilbronn, GECKO Institute of Medicine, Informatics and Economy, Heilbronn, Germany and 3 University Hospital Groningen, Department for Medical Microbiology and Infection Control, Groningen, The Netherlands

Abstract

The nares represent an important bacterial reservoir for endogenous infections. This study aimed to assess the prevalence of nasal colonization by different important pathogens, the associated antimicrobial susceptibility and risk factors. We performed a prospective cohort study among 1878 nonhospitalized volunteers recruited from the general population in Germany. Participants provided nasal swabs at three time points (each separated by 4–6 months). *Staphylococcus aureus, Enterobacteriaceae* and important nonfermenters were cultured and subjected to susceptibility testing. Factors potentially influencing bacterial colonization patterns were assessed. The overall prevalence of *S. aureus, Enterobacteriaceae* and nonfermenters was 41.0, 33.4 and 3.7%, respectively. Thirteen participants (0.7%) were colonized with methicillin-resistant *S. aureus. Enterobacteriaceae* were mostly (>99%) susceptible against ciprofloxacin and carbapenems (100%). Extended-spectrum β-lactamase–producing isolates were not detected among *Klebsiella oxytoca, Klebsiella pneumoniae* and *Escherichia coli*. Several lifestyle- and health-related factors (e.g. household size, travel, livestock density of the residential area or occupational livestock contact, atopic dermatitis, antidepressant or anti-infective drugs) were associated with colonization by different microorganisms. This study unexpectedly demonstrated high nasal colonization rates with *Enterobacteriaceae* in the German general population, but rates of antibiotic resistance were low. Methicillin-resistant *S. aureus* carriage was rare but highly associated with occupational livestock contact.

Keywords: Community, ESBL, Germany, livestock, MRSA, nose, pigs, risk, urban
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Corresponding author: K. Becker, University Hospital Münster, Institute of Medical Microbiology, Domagkstr. 10, 48149 Münster, Germany
E-mail: kbecker@uni-muenster.de
Study group members are listed in the Appendix.

Introduction

Microorganisms colonizing the human body are involved in important immunologic processes. They prevent the establishment of potentially harmful pathogens and assist in improving the immune system [1]. On the other hand, the microbiota may promote the development of allergic diseases and is a major reservoir for endogenous infections. For bacteria in the nasal habitat, the latter has mainly been demonstrated for the role of nasal *Staphylococcus aureus* carriage in the development of nosocomial infections such as bacteraemia, sternal or orthopaedic infections [2–5].

Recently, noncultural methods have greatly increased knowledge on the communities of microorganisms colonizing different body sites [6]. Hence, analyses of 16S rRNA sequences in samples from the nares of healthy individuals demonstrated that >80% of rRNA sequences detected belonged to either *Actinobacteria* (e.g. cornyebacteria) or *Firmicutes* (e.g. staphylococci) [6]. In addition, other bacterial taxa including *Proteobacteria* (e.g. *Enterobacteriaceae* or *Pseudomonales*), *Bacteriodetes* or

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Fusobacteria were also found [6–8]. Interestingly, various studies indicated that the composition of the microbiota of different human habitats is influenced by several demographic factors such as gender, age and ethnicity, but also by health-related factors like hospitalization, intake of antibiotics, anti-pneumococcal vaccination or presence of viral infections as well as by lifestyle-associated factors like frequency of hand washing or smoking [9–13].

Recognizing the value of culture-independent methods, these techniques have two major limitations. Firstly, they cannot assess bacterial antibiotic susceptibilities, and secondly, the overload of information on predominating phyla tends to dissolve information on the presence of important human pathogens as a result of the lack of abundance of these species. However, it is important to overcome these limitations, as recent studies have indicated changes in the occurrence of antimicrobial resistant pathogens in the healthy general population. This was demonstrated for extended-spectrum β-lactamase (ESBL)- or carbapenemase-producing *Enterobacteriaceae* and methicillin-resistant *S. aureus* (MRSA), which were increasingly found as colonizers of the nares and the intestinal tract in association with travel activities, contact with or ingestion of contaminated food items or contact with livestock husbandries [14–18]. In addition, the emergence of antimicrobial resistant Gram-negative bacteria among humans has mainly been documented by studies assessing these pathogens in stool samples or rectal swabs. Data on their occurrence and persistence in the nares, which might be important for the probability of transmission to the environment and to other persons, are lacking.

Therefore, the objective of this prospective cohort study was to assess the occurrence of nasal colonization of important facultative pathogenic bacteria including *S. aureus, Enterobacteriaceae* and nonfermentative bacteria in samples from a large number of nonhospitalized volunteers. In addition, information on associated antimicrobial resistance and lifestyle conditions was obtained and correlated with the nasal bacterial colonization pattern.

Methods

Nonhospitalized adult participants were recruited in ambulatory departments of public health offices (offering, e.g., travel vaccination or health checks) or were employees of these offices; dental practices; a family physician's practice; and among students at colleges or universities. At the beginning of the study, participants were informed about the study's objectives and provided written informed consent. Ethical approval was obtained by the ethical commission of the Westphalian Wilhelms-University Münster (2006-268-f-S).

Samples were taken at three different time points (June–August 2011, January–March 2012, August–October 2012), each separated by at least 4 months. At every time point, participants were asked to provide a nasal swab and to answer a standardized questionnaire. Interviews and nasal swabs were performed by trained medical or dental students. Answers of the participants were directly assessed in a protected data-collection application on a portable USB flash drive.

From all participants, premoistened nasal swabs (FLOQSwabs™; Copan, Murrieta, CA, USA) were used to sample the anterior nares. Swabs were immediately transferred to the microbiologic laboratory. After nonselective enrichment in Mueller-Hinton broth (24 hours, 36°C), swabs were streaked onto Columbia blood agar and MacConkey agar as well as ESBL and *S. aureus* screening agars (all Oxoid, Wesel, Germany) and incubated for 24 hours at 36°C. All colonies suspicious for *S. aureus, Enterobacteriaceae* or clinically important non-fermenters (i.e. *Acinetobacter baumannii, Pseudomonas* spp., *Achromobacter* spp. and *Stenotrophomonas* spp.) were sub-cultured on Columbia blood agar.

For species identification, all isolates were tested by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Antimicrobial susceptibility testing was performed using Vitek2 automated systems (and for some nonfermenter species using agar disc diffusion) with clinical breakpoints and application of expert rules as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). All *S. aureus* isolates were analysed for the presence of the *nuc* and *mecA* genes [19]. For *S. aureus* isolates that were cefoxitin resistant, *mecC* was tested in addition as previously described [20].

The questionnaire assessed the following data for each participant: year of birth, sex, zip code for place of residence, country of birth, employment in healthcare institutions, employment with contact to livestock animals, number of other persons living in the same household, living with companion animals in the same household, being a smoker, having regular (i.e. at least weekly) contact with medical personnel, having regular (i.e. at least weekly) contact with persons with employment in livestock production, and travelling abroad (if yes, which countries; within the past 12 months). In addition, participants were asked whether they had diabetes mellitus, allergies, chronic skin diseases (e.g. psoriasis, atopic dermatitis, acne), chronic diseases of the liver or the airways (e.g. asthma, chronic obstructive pulmonary disease), chronic renal insufficiency, chronic inflammatory diseases of the paranasal sinuses or the bowel, chronic osteomyelitis, solid tumors or haemato-oncologic diseases. Participants provided information on whether they were immunocompromised (if yes, why?), whether they had implanted devices (e.g. heart valves,