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ORIGINAL ARTICLE

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

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## 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.



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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,

**TABLE 1. Characteristics of 1878 study participants**

| Description | Category | *n* | % |
|---|---|---|---|
| Participation in the cohort study | At 1 time point only | 427 | 22.7 |
| | At 2 time points | 283 | 15.1 |
| | At all 3 time points | 1168 | 62.2 |
| Sex | Female | 1096 | 58.4 |
| | Male | 782 | 41.6 |
| Age distribution | <20 years | 107 | 5.7 |
| | 21–30 years | 387 | 20.6 |
| | 31–40 years | 240 | 12.8 |
| | 41–50 years | 423 | 22.5 |
| | 51–60 years | 384 | 20.4 |
| | 61–70 years | 163 | 8.7 |
| | 71–80 years | 132 | 7.0 |
| | ≥81 years | 41 | 2.2 |
| | Unknown | 1 | 0.1 |
| Country of birth | Germany | 1544 | 82.2 |
| | Country other than Germany | 237 | 12.6 |
| | Unknown | 97 | 5.2 |
| Household size | 1 person | 307 | 16.4 |
| | 2 persons | 661 | 35.2 |
| | 3 persons | 366 | 19.5 |
| | ≥4 persons | 522 | 27.8 |
| | Unknown | 22 | 1.2 |
| Pets present in household | Yes | 738 | 39.3 |
| Employment in healthcare sector | Yes | 205 | 10.9 |
| Regular contact with medical personnel | Yes | 162 | 8.6 |
| Occupational direct contact with livestock or meat | Yes | 61 | 3.2 |
| Regular contact to persons with livestock-related employment | Yes | 80 | 4.3 |
| Smoker | Yes | 560 | 29.8 |
| Travel abroad | No | 708 | 37.7 |
| | Other European countries | 1116 | 59.4 |
| | Africa | 75 | 4.0 |
| | Asia | 70 | 3.7 |
| | Australia | 14 | 0.7 |
| | Canada | 7 | 0.4 |
| | United States | 47 | 2.5 |
| | South or Middle America | 24 | 1.3 |
| Colonization with *Staphylococcus aureus* | At 1 time point only[a] | 123 of 427 | 28.8 |
| | At 2 time points[a] | 120 of 283 | 42.4 |
| | At all 3 time points[a] | 525 of 1168 | 44.9 |
| | Total[a] | 768 of 1878 | 40.9 |
| Colonization with *Enterobacteriaceae* | At 1 time point only[a] | 98 of 427 | 23.0 |
| | At 2 time points[a] | 90 of 283 | 31.8 |
| | At all 3 time points[a] | 440 of 1168 | 37.7 |
| | Total[a] | 628 of 1878 | 33.4 |
| Colonization with nonfermenting pathogen | At 1 time point only[a] | 3 of 427 | 0.7 |
| | At 2 time points[a] | 11 of 283 | 3.9 |
| | At all 3 time points[a] | 55 of 1168 | 4.7 |
| | Total[a] | 69 of 1878 | 3.7 |

[a]Nasal colonization with (group of) microorganism(s) mentioned in at least one sample of participant; persons who participated at one time point provided only one nasal swabs; those who participated at two time points had two swabs; and those who participated at three times had three swabs.

prostheses). Moreover, information on hospitalization (within the past 12 months) was obtained. Participants were asked whether they received antibiotics or antimycotic agents (if yes, oral/intravenous or topical), oral contraceptives or medication against clinical depression or neuroleptic agents. Data on density of livestock and human population in different districts as well as geographical data were assessed from the German Regional Database (https://www.regionalstatistik.de/; codes 116-33-4, 173-21-4, 449-01-4).

Statistical analysis was performed by SPSS 22 (IBM, Armonk, NY, USA). Chi-square or Fisher's exact tests were used to calculate differences between categorical variable; *t* test was used for parametric variables. $p < 0.05$ was considered significant. For assessing independent risk factors associated with nasal colonization of different groups of bacteria, we applied multivariable logistic regression by stepwise backward selection of variables and probabilities of 0.05 for entry into and 0.01 for removal from the model (SPSS 22). All variables associated with p values of $\leq 0.2$ were entered into the initial regression model.

## Results

Overall, 1878 persons participated in the study. Among these, 1168 (62.2%) participated at all three time points, 283 (15.1%) at two time points and 427 (22.7%) only once (Table 1). The majority of participants were female (58.4%), and the median (mean) age was 45 years (45.1 years) (range, 7–97 years). Fig. 1 shows that all but six of the participants lived in a total of 38 districts in the German federal states of North Rhine–Westphalia and Lower Saxony.

Of all 1878 participants, 1203 (64.0%) were colonized with at least one of the bacterial pathogens assessed at least at one time point. The overall prevalence of nasal *S. aureus* carriage was 41.0% (*n* = 768); 33.4% of the participants were nasally colonized with *Enterobacteriaceae* (*n* = 628) and 3.7% with nonfermenters (*n* = 69) (Fig. 2). At the species level, *Klebsiella oxytoca* (6.4%), *Escherichia coli* (6.1%), *Proteus mirabilis* (6.0%), *Citrobacter koseri* (3.6%), *Pantoea agglomerans* (3.1%), *Enterobacter aerogenes* (2.6%), *Klebsiella pneumoniae* (2.4%), *Citrobacter freundii* (2.2%), *Enterobacter cloacae* (1.4%), *Acinetobacter baumannii* (1.1%), *Raoultella ornithinolytica* (1.1%) and *Serratia marcescens* (1.0%) were detected in more than 1% of the 1878 participants. Among those participants carrying enterobacteria, *n* = 515 were colonized with one enterobacterial species, 96 with two, 16 with three, and one with four different species, respectively. Of the 69 participants colonized by nonfermenters, all carried one nonfermentative species only. Fig. 2 shows a matrix of the frequency of co-colonization. Statistically significant differences in the co-colonization patterns were mainly found for *S. aureus* carriers, who were less frequently colonized with various enterobacteria and nonfermenters (Fig. 3).

Table 2 shows the persistence of nasal colonization as indicated by detection of the bacteria at the three different time points of investigation. Focusing on those participants who provided three nasal swabs at time points separated by four to six months each, the persistence of colonization, which was firstly detected at the initial time point of the study, was significantly ($p < 0.001$) different for carriers of *S. aureus* (*n* = 525; 63.8% colonized for more than four months and

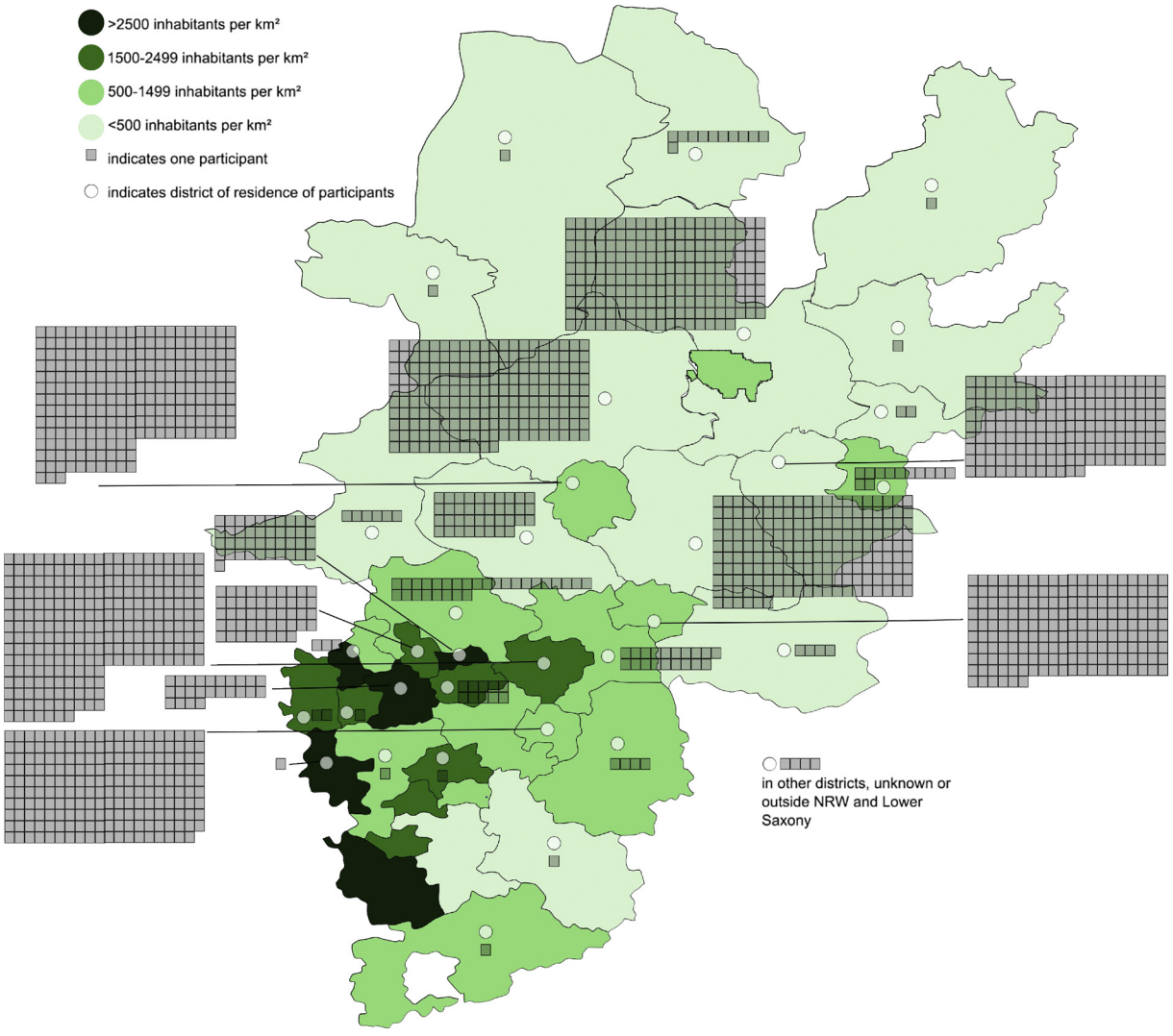


FIG. 1. Places of residence of participants in German federal states of North Rhine–Westphalia and Lower Saxony showing rural and urban districts and population density (four of overall 1878 participants of study lived outside depicted area).

39.2% for more than eight months), *Enterobacteriaceae* (*n* = 440; 54.7% and 32.1%) and nonfermenters (*n* = 55; 5.5% and 0%).

At the three time points, we obtained a total of 1378 *S. aureus* isolates (all *nuc* positive), 1169 *Enterobacteriaceae* isolates (from 28 species) and 73 nonfermenter isolates (from nine species). *Enterobacteriaceae* mostly belonged to *Proteus mirabilis* (*n* = 211 isolates; 18.0% of all 1168 *Enterobacteriaceae* isolates), *E. coli* (*n* = 180; 15.4%), *K. oxytoca* (*n* = 175; 15.0%), *C. koseri* (*n* = 138; 11.8%), *E. aerogenes* (*n* = 86; 7.4%), *C. freundii* (*n* = 64; 5.5%), *P. agglomerans* (*n* = 63; 5.4%), *K. pneumoniae* (*n* = 61; 5.2%), *E. cloacae*, *R. ornithinolytica*, *Hafnia alvei*, and *S. marcescens* (each *n* = 27; 2.3%). Among all nonfermenter isolates, the following species were detected: *A. baumannii* (*n* = 24 isolates; 32.9% of all nonfermenter isolates), *Stenotrophomonas maltophilia* (*n* = 14; 19.2%), *Pseudomonas putida* (*n* = 12; 16.4%), *Pseudomonas aeruginosa* (*n* = 8; 11.0%), *Pseudomonas fluorescens* (*n* = 6; 8.2%), *Pseudomonas stutzeri* (*n* = 5; 6.8%), *Pseudomonas spp.* (*n* = 2; 2.7%), *Pseudomonas monteilii* (*n* = 1; 1.4%) and *Achromobacter xylosoxidans* (*n* = 1; 1.4%).

The antibiotic susceptibility test results of every first isolate detected per participant are shown in Table 3; data are shown for those enterobacterial and nonfermenter species, which were detected in more than ten participants, respectively. Resistance against linezolid, vancomycin and tigecyclin was not detected among *S. aureus* isolates. Thirteen participants (0.7%) were colonized with MRSA, as defined by cefoxitin-resistant isolates harbouring *mecA*. This represents a proportion of 1.7% MRSA on all 768 *S. aureus* first isolates. In addition, *S. aureus* isolates from two participants (0.1%) were cefoxitin resistant, but neither *mecA* nor *mecC* was detectable, and

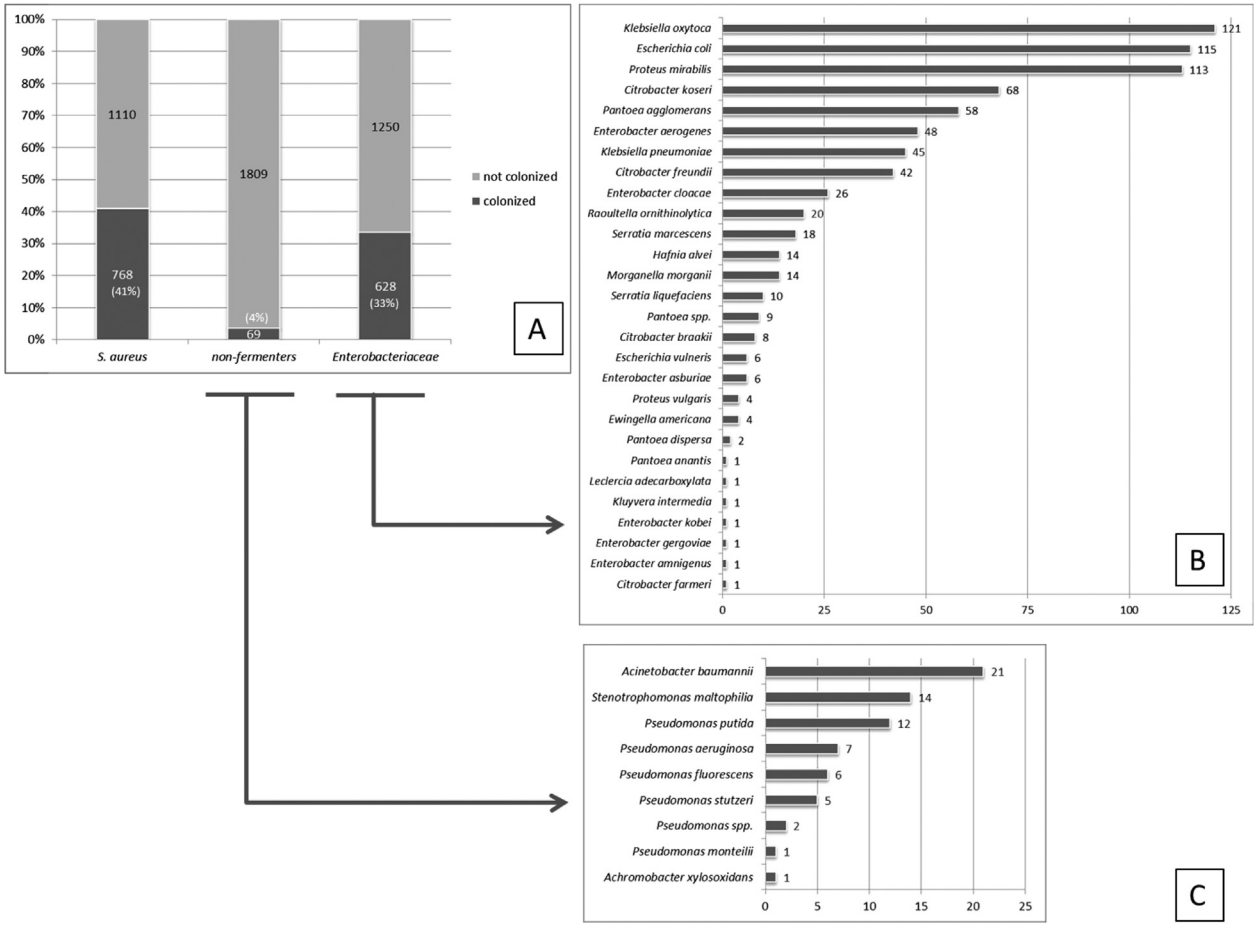


**FIG. 2.** Prevalence of nasal colonization with *Staphylococcus aureus, Enterobacteriaceae* and nonfermentative bacteria. (A) Prevalence of nasal colonization with *S. aureus, Enterobacteriaceae* and nonfermentative bacteria among 1878 participants. (B) Total number of participants colonized with different enterobacterial species. (C) Total number of participants colonized with different nonfermenter species.

hyperproduction of β-lactamases was excluded. For these isolates, the mechanism of cefoxitin resistance remained unclear. Among the enterobacterial species, the Vitek2 ESBL screening test was negative for all *E. coli* and *Klebsiella* spp. isolates. When testing meropenem, imipenem and ertapenem, all *Enterobacteriaceae* were susceptible. Overall, participants' first isolates of *Enterobacteriaceae* (all species) were resistant to ampicillin, cefuroxime, cefotaxime, ciprofloxacin, trimethoprim–sulfamethoxazole and gentamicin in 67.0, 11.9, 1.6, 0.4, 2.5 and 2.4%, respectively.

Supplementary Tables S1, S2 and S3 show the association between the risk factors assessed and nasal colonization (at any time point) with *S. aureus, Enterobacteriaceae* and non-fermenters based on univariate analysis and logistic regression. We found that household sizes of three (odds ratio (OR) 1.420, 95% confidence interval (CI) 1.109–1.818) or more than four persons (OR 1.349, 95% CI 1.083–1.682), living in regions with >100 livestock animals per km$^2$ (OR 1.251, 95% CI 1.001–1.562), atopic dermatitis (OR 1.706, 95% CI 1.025–2.841) and asthma (OR 1.501, 95% CI 1.015–2.219) were independently associated with *S. aureus* carriage, while female gender (OR 0.752, 95% CI 0.621–0.911), smoking (OR 0.726, 95% CI 0.588–0.896), colonization with *Enterobacteriaceae* (OR 0.644, 95% CI 0.525–0.789) and unreported household size (OR 0.259, 95% CI 0.075–0.886) were significantly less frequent among *S. aureus* carriers.

Performing a separate analysis for participants colonized with *mecA*-positive MRSA showed that only occupational contact with livestock animals (6/13 vs. 55/1865; p <0.001) and taking antidepressant/neuroleptic drugs (4/13 vs. 125/1865; p 0.009) were significantly associated with MRSA in univariate analysis. A regression model initially containing the risk factors associated with p values of <0.2 revealed that occupational livestock contact (OR 31.036; 95% CI 8.480–113.585), chronic renal insufficiency (OR 40.611; 95% CI 3.735–441.627), taking antidepressant/neuroleptic medication (OR 4.974; 95% CI

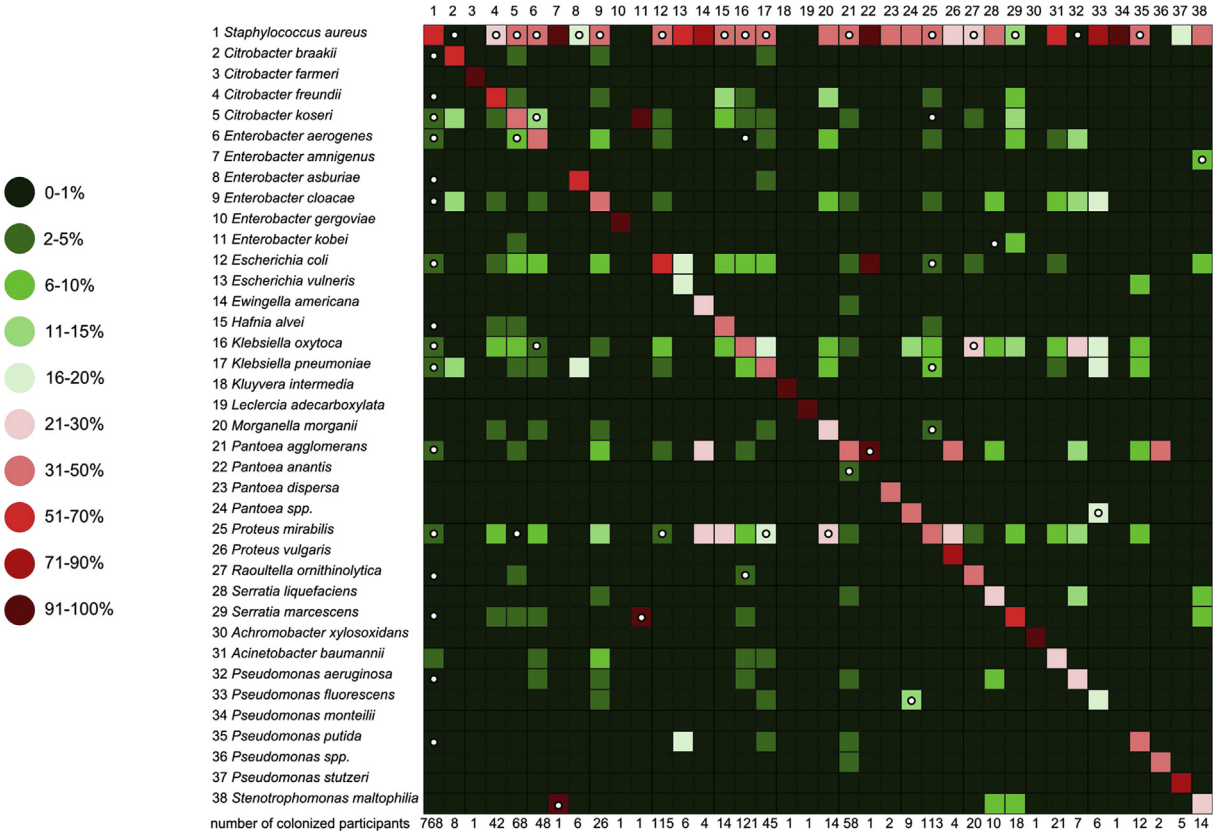


**FIG. 3.** Nasal co-colonization pattern of *Staphylococcus aureus, Enterobacteriaceae* and nonfermentative bacteria. Total number of participants colonized with each of 38 bacteria is indicated at bottom of each column. Colours indicate percentage of participants colonized with respective combination of microorganisms, except for those cells defined by identical microorganism. In these cells, colours indicate percentage of participants colonized with respective bacterium only (i.e. without co-colonization). White circles indicate statistically significant ($p < 0.05$) differences between participants colonized with respective species.

1.140–21.701) and chronic obstructive pulmonary disease (OR 28.111; 95% CI 1.808–437.103) were independent risk factors for MRSA.

Nasal colonization with *Enterobacteriaceae* (Supplementary Table S2) was associated with age of 71–80 years (OR 1.505, 95% CI 1.00–21.78), travel to the United States (OR 2.022, 95% CI 1.117–3.660) and topical antimycotic therapy (OR 2.933, 95% CI 1.585–5.428). The factors *S. aureus* carriage (OR 0.671, 95% CI 0.547–0.822), smoking (OR 0.596, 95% CI 0.475–0.748) and hemato-oncologic diseases (OR 0.099, 95% CI 0.013–0.760), living in regions with low population densities of <500 inhabitants per km$^2$ (OR 0.739, 95% CI 0.594–0.919)

**TABLE 2. Persistence of nasal colonization with *Staphylococcus aureus, Enterobacteriaceae* and nonfermenting pathogens**

| Pathogen/pathogen group | Participation at[a] | Negative at all time points | Positive at 1 time point[a] | Positive at 2 time points[a] | Positive at 3 time points[a] | Total[a] |
|---|---|---|---|---|---|---|
| *S. aureus* | 1 time point only | 304 (71.2) | 123 (28.8) | NA | NA | 427 (100) |
| | 2 time points | 163 (57.6) | 57 (20.1) | 63 (22.3) | NA | 283 (100) |
| | 3 time points | 643 (55.1) | 190 (16.3) | 129 (11.0) | 206 (17.6) | 1168 (100) |
| *Enterobacteriaceae* | 1 time point only | 329 (77.0) | 98 (23.0) | NA | NA | 427 (100) |
| | 2 time points | 193 (68.2) | 57 (20.1) | 33 (11.7) | NA | 283 (100) |
| | 3 time points | 728 (62.3) | 199 (17.0) | 100 (8.6) | 141 (12.1) | 1168 (100) |
| Nonfermenting pathogen | 1 time point only | 424 (99.3) | 3 (0.7) | NA | NA | 427 (100) |
| | 2 time points | 272 (96.1) | 11 (3.9) | 0 (0) | NA | 283 (100) |
| | 3 time points | 1113 (95.3) | 52 (4.5) | 3 (0.3) | 0 (0) | 1168 (100) |

NA, not applicable.
[a]Number (% per row) of participants in whom colonization with respective group of bacteria was detected stratified by participation at one, two or three times of participation in the study.

**TABLE 3. Antibiotic susceptibilities of *Staphylococcus aureus, Enterobacteriaceae* and nonfermenting isolates from nasal swabs**

| | | Susceptibility (%)[a] to: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | ***n*** | **PEN** | **AMP** | **OXA** | **CXM** | **CTX** | **MER** | **LEV** | **CIP** | **RIF** | **CLI** | **SXT** | **GEN** |
| *Staphylococcus aureus* | 768 | 35.7 | — | 98.0 | — | — | — | 98.4 | — | 100 | 90.0 | 99.6 | 98.8 |
| ***Enterobacteriaceae*** | | | | | | | | | | | | | |
| *Klebsiella oxytoca* | 121 | — | — | — | 98.4 | 99.2 | 100 | — | 100 | — | — | 100 | 100 |
| *Escherichia coli* | 115 | — | 80.0 | — | 98.3 | 100 | 100 | — | 99.1 | — | — | 95.7 | 98.3 |
| *Proteus mirabilis* | 113 | — | 85.8 | — | 99.1 | 100 | 100 | — | 99.1 | — | — | 92.0 | 98.2 |
| *Citrobacter koseri* | 68 | — | | — | 73.5 | 89.7 | 100 | — | 100 | — | — | 100 | 98.5 |
| *Pantoea agglomerans* | 58 | — | 69.0 | — | 91.4 | 100 | 100 | — | 100 | — | — | 100 | 100 |
| *Enterobacter aerogenes* | 48 | — | — | — | 100 | 100 | 100 | — | 100 | — | — | 100 | 100 |
| *Klebsiella pneumoniae* | 45 | — | — | — | 93.3 | 100 | 100 | — | 100 | — | — | 97.8 | 97.8 |
| *Citrobacter freundii* | 42 | — | — | — | 100 | 100 | 100 | — | 100 | — | — | 100 | 100 |
| *Enterobacter cloacae* | 26 | — | — | — | 65.4 | 100 | 100 | — | 100 | — | — | 100 | 100 |
| *Raoultella ornithinolytica* | 20 | — | — | — | 95.0 | 100 | 100 | — | 100 | — | — | 100 | 100 |
| *Serratia marcescens* | 18 | — | — | — | 11.1 | 100 | 100 | — | 100 | — | — | 100 | 88.9 |
| *Hafnia alvei* | 14 | — | — | — | 92.9 | 100 | 100 | — | 100 | — | — | 100 | 100 |
| *Morganella morganii* | 14 | — | — | — | — | 71.4 | 100 | — | 92.9 | — | — | 92.9 | 92.9 |
| *Serratia liquefaciens* | 10 | — | — | — | — | 100 | 100 | — | 100 | — | — | 100 | 10.0 |
| **Nonfermenting pathogens** | | | | | | | | | | | | | |
| *Acinetobacter baumannii* | 21 | — | — | — | — | — | 100 | — | 100 | — | — | — | 100 |
| *Stenotrophomonas maltophilia* | 14 | — | — | — | — | — | — | — | 100 | — | — | 100 | — |
| *Pseudomonas putida* | 12 | — | — | — | — | — | 50.0 | — | 100 | — | — | — | 91.7 |

AMP, ampicillin; CIP, ciprofloxacin; CLI, clindamycin; CTX, cefotaxime; CXM, cefuroxime; GEN, gentamicin; LEV, levofloxacin; MER, meropenem; OXA, oxacillin/cefoxitin; PEN, benzylpenicillin; RIF, rifampicin; SXT, trimethoprim–sulfamethoxazole.

[a]Percentage of susceptible isolates for the respective antibiotic. On species level, data are shown for those species for which the number of isolates tested was at least ten. Data are sorted in order of isolates tested for different groups of bacteria. If the same microorganism was isolated more than once from the same participant (at different study time points), only the first isolate was included in the calculation. Intermediate test results are counted as nonsusceptible. A dash indicates that antibiotic was not tested.

and with rather low livestock densities of 50–99 animals per km$^2$ (OR 0.779, 95% CI 0.628–0.966) were significantly less frequent among participants colonized with *Enterobacteriaceae*. Carriage of nonfermenters (Supplementary Table S3) was independently associated with living in areas with a livestock density of <50 animals per km$^2$ (OR 2.114, 95% CI 1.276–3.504), travel to the United States (OR 3.756, 95% CI 1.418–9.946) and oral antibiotic therapy (OR 1.729, 95% CI 1.018–2.937). For both *Enterobacteriaceae* and nonfermenters, the univariate analysis of lifestyle-related risk factors was repeated for colonization, with each of the different bacterial species included in these two groups of bacteria as the independent variable (Supplementary Table S4).

## Discussion

In this prospective study, we assessed nasal colonization with important human pathogens among a cohort of >1800 individuals from the general population. Because of the design of the study, and because the study was carried out in only two federal states, the participants involved are not fully representative for the German general population. However, our approach of recruiting volunteers resulted in the formation of a sufficiently heterogeneous cohort. Comparing the demographic data assessed with reference data for the German population (Federal Statistical Office of Germany (DESTATIS); https://www.destatis.de/; data based on 2011–2012 census; date of data retrieval, 11 September 2014), we can assume that basic parameters such as gender (female; census/cohort: 52%/58%), mean age (census/cohort: 44 years/45 years) or migration background (census: 20%; German nationality 91.7%/cohort: 12.6% birth country other than Germany) are comparable. Also, working in the healthcare sector (10.9% of all participants), which could significantly influence colonization, was representative for the German general population, where—according to DESTATIS data—about 12% of the population were employed in the healthcare sector in 2011.

Surprisingly, we found that 33.4 and 3.7% of all participants were colonized with *Enterobacteriaceae* and nonfermenters, respectively. Data on studies investigating nasal carriage with Gram-negative rods are rare because most reports focus on colonization of the intestinal tract. In Switzerland, Gram-negative rods (*Klebsiella, E. coli, E. cloacae, Citrobacter diversus*) were isolated from nasal samples obtained from 117 (38%) of 534 healthy men [21]. In a cohort of German and Eritrean children, *Enterobacteriaceae* were found in 4–8% [22]. In a recent study on the nasal culturome, more than half of the number of the individuals harboured isolates belonging to the *Enterobacteriaceae* family [23]. For *K. pneumoniae,* some studies even showed that colonization rates in the nares or in the respiratory tract were higher than in stool samples [24]. Hence, our data support that a major part of the general population is nasally colonized with *Enterobacteriaceae*.

Antimicrobial susceptibility testing revealed that among nasal *E. coli, K. pneumoniae* and *K. oxytoca* isolates there was hardly any resistance against second- (93–98% susceptible) or third-generation cephalosporins (99–100% susceptible). This finding

is interesting, as clinical isolates of these species are resistant in higher proportions according to German national surveillance data, where cefuroxime/cefotaxime were susceptible in 88.3/92.4% of *E. coli*, 81.3/90.8% of *K. pneumoniae* and 87.5/95.3% of *K. oxytoca* isolates (https://ars.rki.de/; reference data for ambulatory care, 2013). Moreover, large studies recently indicated the emergence of resistance against third-generation cephalosporins (associated with ESBL production) in *E. coli* and *Klebsiella* isolates obtained from persons in the general population. In Germany, testing of stool samples from healthy individuals and outpatients revealed ESBL colonization rates of 4.1–6.3% [25,26].

Several investigations demonstrated that rectal colonization with ESBL-producing *Enterobacteriaceae* was associated with travel. In the Netherlands, the prevalence of ESBL genes detected in stool samples of travellers increased from 0 before to 33.6% after travel (mainly to Southeast Asia) [16]. In a Scandinavian study, ESBL-producing *Enterobacteriaceae* were found in samples from 30% of travellers after return. Risk factors for ESBL carriage were travel to the Indian subcontinent (OR 24.8), Asia (OR 8.63) and Northern Africa (OR 4.94) [14]. In addition, among patients colonized with ESBL–*E. coli* identified in samples obtained <72 hours after admission to a German hospital, Asian mother tongue (OR 13.4) was a significant risk factor [27].

As we detected no ESBL production in any of the *E. coli, K. pneumoniae* and *K. oxytoca* isolates tested in this study, we assume a divergent antimicrobial resistance pattern between *Enterobacteriaceae* from the nasal and the intestinal habitats with respect to ESBL-producing organisms. The reasons for this are unclear, and it is a limitation of this study that it was not possible to analyse faecal and nasal samples in parallel, which would have enabled direct comparability of these two reservoirs. Hypothetically, the density and variability of enterobacterial species in the intestinal tract could facilitate exchange of resistance determinants. Alternatively, transmission of ESBL-producing bacteria could predominantly follow orofaecal transmission pathways, leading to colonization of the intestinal tract rather than droplet or direct smear infection or cross-transmission *via* the hands enabling nasal colonization. Nevertheless, our findings may have an impact on defining active surveillance strategies for prevention of infections with multiresistant *Enterobacteriaceae*.

There are only few truly population-based studies on the prevalence of colonization with *S. aureus*. In the United Kingdom, two studies yielded carriage of 23–28% in the general population [28–31]. This corresponds to investigations from other countries [30,31]. A review of longitudinal studies found that about 20% (range 12–30%) of individuals persistently carry *S. aureus*, while about 30% are intermittently colonized (range 16–70%), and about 50% (range 16–69%) are noncarriers [5]. Our data mostly confirm these studies. Interestingly, we found significantly lower rates of persistent nasal carriage for *Enterobacteriaceae* and for nonfermenters, although 54.5% of participants colonized with *Enterobacteriaceae* were carriers for more than 4 and 32.1% for more than 8 months. However, for drawing more exact conclusions on long-term persistence, genotyping of all isolates would be necessary in order to differentiate between repetitive short-term contamination (with several bacterial clonal lineages) and long-term colonization (with one clonal lineage).

Thirteen participants (0.7%) were colonized with *mecA*-positive MRSA. Unlike the United States, where the prevalence of MRSA carriage in the population was estimated to be 1.5% in 2004 [32], MRSA colonization in the German general population has rarely been assessed systematically. Various studies report MRSA carriage at the time of hospital admission to be about 1.5% [33]. However, these patients already represent a selected group of people. Interestingly, we detected two participants who carried cefoxitin-resistant, *nuc*-positive *S. aureus*, which did not harbour the resistance determinants *mecA* or *mecC* [34]. This might indicate the presence of genetically divergent yet unknown *mec* homologues.

Analysis of risk factors confirmed other studies indicating that *S. aureus* colonization is more frequent among male subjects [32,35,36], and some investigators hypothesized that taking hormonal contraceptives could have a preventive effect [37]. Although the latter risk factor was not significantly associated with *S. aureus* in our cohort, factors such as larger household size, atopic dermatitis and asthma were more frequent among *S. aureus* carriers compared to other participants, which is in line with previous investigations [38–41]. We found that smokers were less frequently carriers of *S. aureus*, putatively due to the bactericidal activity of the smoke, which confirms other studies [42,43]. In addition, we showed that nasal colonization with *Enterobacteriaceae* also prevented (OR 0.64) *S. aureus* carriage, which was previously described for the competitive effects of corynebacteria and coagulase-negative staphylococci [44].

The geographic area of this study (as defined by the participants' places of residences) comprises both rural and urban parts of the German federal states of North Rhine–Westphalia and Lower Saxony (Fig. 1). The northwestern areas are represented by a low density of inhabitants and a high density of livestock (especially pigs). Persons with occupational livestock contact had a 31 times higher risk for MRSA carriage compared to the rest of the cohort. It has been shown that livestock-associated MRSA plays a major role among persons in this part of Germany, where up to 29% of human MRSA carriers in hospitals were colonized with MRSA molecular types belonging to the clonal lineage CC398 as defined by multilocus sequence

typing [18,45,46], which is predominant in European livestock populations [47]. While the risk of acquiring MRSA CC398 *via* direct livestock contact has been demonstrated in various studies (reviewed in [47]), recent reports from the Netherlands indicated that MRSA CC398 is increasingly acquired independently from livestock exposure by persons in the general population living in pig-dense regions [48]. In this context, the results of this study clearly show that this phenomenon does not affect major parts of the population, as only 0.7% of the participants were colonized with MRSA.

Taking antidepressant/neuroleptic drugs was another risk factor for MRSA carriage in this study. An antimicrobial effect of psychotherapeutic drugs was reported decades ago [49,50]. This raises the question of whether or to what extent pharmaceutical agents contribute to the selection pressure on antibiotic-resistant bacteria.

We are not aware of any studies assessing risk factors for nasal colonization with *Enterobacteriaceae* or nonfermenters in the general population. In this study, carriage of both enterobacteria and nonfermenters was associated with travel to the United States. In addition, factors such as topical antimycotic therapy, age of more than 70 years and oral antibiotic therapy, as well as certain characteristics of the place of residence had an impact of colonization with enterobacteria and nonfermenters. These findings are unexplained. In contrast, our results that *S. aureus* carriage, smoking and hemato-oncologic diseases were significantly less frequent among participants colonized with *Enterobacteriaceae* could be due to previous antibiotic consumption, the bactericidal effects of smoking and bacterial competition in the nasal habitat.

Overall, this prospective cohort study provided insight into nasal colonization patterns with *S. aureus, Enterobacteriaceae* and nonfermenters in the German general population as well as in associated antimicrobial resistance profiles. We demonstrated frequent and mostly persistent nasal colonization with *S. aureus*. However, MRSA carriage was detected in only 0.7% of the participants. Although colonization rates with *Enterobacteriaceae* were unexpectedly high (overall 33%, with *K. oxytoca, E. coli, Citrobacter* spp. and *Pantoea* spp. being predominant), antimicrobial resistance of *Enterobacteriaceae* was infrequent, and colonization with ESBL-producing *Enterobacteriaceae* was not detected.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nmni.2015.11.004.

## Conflict of Interest

None declared.

## Appendix

**Prevalence of Multiresistant Microorganisms (PMM) Study Group members**

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