Nasal colonization with methicillin-resistant *Staphylococcus aureus* associated with elevated homocysteine levels in the general US adults

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Abstract

Given the emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) as a global health threat, understanding the risk factors for MRSA infection in the community may be a reasonable strategy to prevent it. We investigated the associations between serum homocysteine levels and prevalence of nasal colonization with *S. aureus* and MRSA among United States adults. We conducted a cross-sectional analysis of a nationally representative sample of 7832 adults (20 years or older). The main outcome variables were nasal colonization with *S. aureus* and MRSA. Percentages of colonization with *S. aureus* and MRSA were calculated by the quartiles of serum homocysteine. A total of 7832 of 2051 subjects (26.2%) were culture positive for *S. aureus*, 98 (4.8%) of whom had nasal colonization with MRSA. In comparison with subjects having the lowest serum homocysteine, the odds of nasal colonization with MRSA were significantly higher in those with the highest homocysteines (odds ratio, 3.09; 95% confidence interval, 1.11–8.61) in multivariate analysis, adjusted for all confounding variables. By contrast, homocysteine elevation was not significantly associated with *S. aureus* colonization. Nasal colonization with MRSA in the general community was significantly associated with increases in serum homocysteine levels.

Abbreviations: BMI = body mass index, CA-MRSA = community-acquired MRSA, CI = confidence interval, DM = diabetes mellitus, HA-MRSA = hospital-associated MRSA, MRSA = methicillin-resistant *Staphylococcus aureus*, MSA = mannitol salt agar, NCHS = National Center for Health Statistics, NHANES = National Health and Nutrition Examination Survey, OR = odds ratio, SAH = S-adenosyl-homocysteine, SCVs = small-colony variants.

Keywords: antibiotic resistance, homocysteine, methicillin-resistant *Staphylococcus aureus*, oxidative stress, *Staphylococcus aureus*

1. Introduction

*S. aureus*, a gram-positive bacterium that typically colonizes normal skin and anterior nares of the host, is one of the most common causes of infections in humans.[1] *S. aureus* can cause many infections, including superficial skin and soft tissue infection as well as invasive infections, including bloodstream, surgical site, and bone and joint infections or pneumonia.[2] Treatment of *S. aureus* infection included administration of benzylpenicillin (penicillin G), a β-lactam antibiotic; however, by the end of the 1950s, benzylpenicillin resistant *S. aureus* strains emerged, which produced β-lactamase enzymes that rendered the antibiotics inactive. Unfortunately, history repeated itself. While this resistance encouraged the development of a novel antibiotic methicillin in 1959, methicillin-resistant *S. aureus* (MRSA) strains were isolated only 2 years thereafter.[3] In these strains, methicillin resistance was conferred by chromosomal gene mecA encoding a low-affinity penicillin-binding protein, which is located in a mobile genetic element called the staphylococcal cassette chromosome (SCC) mec.[4]

Prevalence of invasive MRSA infections in hospitals has increased worldwide.[4,5] A more formidable challenge is the spread of community-acquired MRSA (CA-MRSA) infection in healthy individuals, which is characterized by enhanced virulence and transmission and presence of different genetic MRSA strains (SCCmec type IV) from the traditional hospital-associated MRSA (HA-MRSA) strains (SCCmec types I-III).[6] CA-MRSA is perplexing because unlike HA-MRSA infection that has identifiable predisposing risk factors (i.e., surgically or nonsurgically placed indwelling medical devices), CA-MRSA infection can occur in otherwise healthy individuals.[6,7]; this fact highlights the need for improved understanding of the host- and pathogen-related factors associated with CA-MRSA.

Homocysteine is a nonessential, sulfur-containing amino acid produced during the metabolic conversion of methionine to...
cysteine. Homocysteine has been implicated in many medical conditions, including vascular and neurodegenerative diseases and cancer.\textsuperscript{[8–10]} Molecular mechanisms through which elevated homocysteine levels are associated with diseases remain incompletely understood; however, homocysteine is considered as a proinflammatory molecule that activates synthesis of several cytokines and increases production of reactive oxygen species and resultant oxidative stress, thereby mediating the pathogenesis of many health problems.\textsuperscript{[6,9,11,12]} Based on experimental evidence that MRSA infection is associated with enhanced inflammation and oxidative stress,\textsuperscript{[13,14]} increased homocysteine levels may provide additional information regarding individuals at a risk of developing MRSA infections.

The 2001 to 2004 National Health and Nutrition Examination Survey (NHANES) has launched a large national population-based survey for assessing the prevalence of nasal colonization with \textit{S aureus} and MRSA in the general population, which provided a unique opportunity to investigate many factors associated with the epidemiology of MRSA infection. Using these data, we investigated whether elevated homocysteine levels are associated with the nasal colonization with \textit{S aureus} and MRSA in a population.

2. Materials and methods

2.1. Study population

NHANES is a major research program conducted by the National Center for Health Statistics (NCHS) to assess the health and nutritional status of adults and children in the United States (US). The survey examines a representative sample of the civilian non-institutionalized US population. The survey constitutes interviews including questions related to demographic, socioeconomic, dietary, and health as well as medical, physiological, and laboratory examinations. The study protocols and all NHANES testing procedures were reviewed and approved by the NCHS Institutional Review Board. Both oral and written consent was obtained from all participants.

In the 2001 to 2004 NHANES, we initially selected a total of 9516 participants aged ≥20 years who were available for the study of colonization with \textit{S aureus} and MRSA. Of these, we excluded 371 participants who did not complete tests for total plasma homocysteine levels because these homocysteine were conducted in males and females aged between 16 and 69 years. A total of 1313 participants with missing data of other variables (ie, income, smoking status, and alcohol consumption) were further excluded. Finally, a total of 7832 adults were included in the study population.

2.2. Nasal colonization with \textit{S aureus} and with MRSA

Details for colonization with \textit{S aureus} and MRSA are available in the public domain at https://wwwn.cdc.gov/Nchs/Nhanes/2001–2002/L35_B.htm. Briefly, nasal cultures were collected from both anterior nares using a Culturette swab (Becton Dickinson Microbiology Systems, Cockeysville, MD). Culturette swabs were plated on mannitol salt agar (MSA; Becton Dickinson Microbiology Systems), and the MSA plates were incubated at 35°C for 48 hours. Yellow or gold colonies detected on the MSA plate were subcultured onto trypticase soy agar supplemented with 5% sheep blood plates (Becton Dickinson Microbiology Systems).

The plates were incubated at 35°C overnight. \textit{S aureus} was identified using colony morphology, latex agglutination test (Remel, Lenexa, KS), and tube coagulase test using ethylenediaminetetraacetic acid (Becton Dickinson Microbiology Systems).

\textit{S aureus} isolates were screened for methicillin resistance following the National Clinical and Laboratory Standards Institute disk diffusion method. Disk diffusion was performed using a 1-μg oxacillin disk with Mueller-Hinton agar. The agar plates were incubated overnight at 35°C, and zone diameters were measured and interpreted as susceptible (≥13mm), intermediate (11–12 mm), and resistant (<10 mm).

2.3. Total plasma homocysteine

Total plasma homocysteine level was measured using a fluorescence polarization immunoassay with the Abbott Homocysteine Assay and Abbott Assym system (Abbott Homocysteine Assay Package Insert; Abbott Diagnostics, Abbott Park, IL). Detailed laboratory methods for the measurement of total plasma homocysteine are available in the public domain at https://wwwn.cdc.gov/Nchs/Nhanes/2003–2004/L06MH_C.htm.

Briefly, dithiothreitol reduces homocysteine bound to albumin and other small molecules, homocysteine, and mixed disulfides to free thiol. S-adenosyl-homocysteine (SAH) hydrolase catalyzes the conversion of homocysteine to SAH in the presence of added adenosine. Subsequently, the specific monoclonal antibody and fluoresceinlabeled SAH analog tracer constitute the fluorescence polarization immunoassay detection system. Serum homocysteine concentrations were calculated by the Abbott Assym immunoassay analyzer using a machine-stored calibration curve.

2.4. Other variables of interests

We included other factors, including age (20–29, 30–39, 40–49, 50–59, or 60–69, 70–79, or ≥80 years), sex (male or female), race (White, Black, Hispanic, or Other), education (less than high school, high school graduate, or greater than high school), and family income (less than or greater than $20,000). Health behavior-related variables included self-reported smoking status (current smoker, former smoker, or never smoked) and current alcohol consumption (drinker or nondrinker), and moderate physical activity (yes or no). Body mass index (BMI) was calculated, and obesity was defined as BMI ≥25 kg/m². With regard to disease history, diabetes mellitus (DM) was defined as self-reported physician diagnosis, current use of antidiabetic medication, or fasting blood glucose of ≥126 mg/dL. Hypertension was defined based on the blood pressure of at least 140 mm Hg and diastolic blood pressure of at least 90 mm Hg, or a prior physician diagnosis of hypertension. Regarding resistance to antibiotics, the questions related to the use of prescribed antibiotics in the previous month and history of overnight hospitalizations in the past years were included (“yes” or “no”). Serum levels of folic acid and vitamin B12 were included as essential nutrients associated with elevated homocysteine levels.

2.5. Statistical analysis

Statistical differences among study population characteristics based on the presence of nasal colonization with \textit{S aureus} and MRSA were evaluated. For each variable, Chi-square test was
performed for significance testing across subjects with or without *S. aureus* and those with or without MRSA colonization. Subjects were categorized on the basis of serum homocysteine levels as the quartile: Quintile 1 (≤6.71 μmol/L), Quintile 2 (6.72 – 8.26 μmol/L), Quintile 3 (8.27–10.40 μmol/L), and Quintile 4 (≥10.41 μmol/L). Odds ratios (ORs) and respective 95% confidence intervals (CIs) were calculated and analyzed based on the risk of incurred nasal colonization with *S. aureus* and MRSA in the highest quartile of homocysteine versus risk of this outcome in the lowest quartile as a reference. Regression models were initially adjusted for age and sex and then for all confounding variables, including age, sex, race/ethnicity, family income, smoking, alcohol consumption, moderate physical activity, obesity, history of DM or hypertension, use of prescribed antibiotics during the past month, overnight hospitalization in the past year, and serum folate and Vitamin B12 levels.

Weighted estimates of population parameters were computed according to the NCHS to account for the complex sampling design. Statistical analyses were performed using the PROC SURVEY procedures in SAS 9.2 (SAS Institute, Cary, NC). Two-sided *P* < 0.05 was considered statistically significant.

### 3. Results

Table 1 summarizes the characteristics of study population based on the presence of nasal colonization with *S. aureus* and MRSA. A total of 7832 of the 2051 subjects (26.2%) were culture-positive for *S. aureus*, of which 98 (4.8%) were considered to present nasal colonization with MRSA. The prevalence of nasal colonization with *S. aureus* was significantly associated with age (higher in the 20 seconds than other age ranges, *P* = 0.0002), sex (higher in males than in females, *P* < 0.001), race/ethnicity (higher in White than other races, *P* < 0.001), smoking status (higher in never smokers than current or former smokers, *P* = .0010), and hypertension history (higher in no hypertension than hypertension groups, *P* = .0038). Among *S. aureus* carriers, participants colonized with MRSA were more likely to be older, be female, belong to the race “Black,” have lower income (<$20,000), be former smokers, be current nondrinkers, be unable to do physical activity, and be nonobese. Regarding medical condition, participants with a history of DM or hypertension, those who used prescribed antibiotics in the past month, and those who were hospitalization overnight in the past year exhibited higher prevalence of MRSA colonization. Serum vitamin B12 levels were significantly associated with nasal colonized MRSA (*P* = .0001).

Figure 1 presents mean homocysteine levels by the presence of nasal colonization with *S. aureus* and MRSA. Mean homocysteine levels were significantly higher in adults with MRSA colonization than in those without MRSA colonization (11.28 μmol/L vs 9.13 μmol/L; *P*-value < .0001). In contrast, no significant difference in homocysteine level was observed based on the presence of colonization with *S. aureus* (9.19 μmol/L vs 9.05 μmol/L; *P*-value = .2733).

Figure 2 presents the percentages (%) of participants with nasal colonization with *S. aureus* and MRSA by quartiles of serum homocysteine levels. The percentage of *S. aureus* carriers (*P* = .4761) did not significantly differ across quartiles of serum homocysteine level (*P* = .4761), being 513 (27.1%), 529 (26.9%), 507 (25.6%), and 502 (25.3%) in Quartiles 1 to Quartile 4, respectively. By contrast, participants with MRSA colonization showed significantly increased colonization with quartile increases in serum homocysteine level (*P* = < .0001), being 11 (2.1%), 19 (3.6%), 22 (4.3%), and 46 (9.2%) in Quartiles 1 to 4, respectively.

Table 2 presents the results of multivariate analysis of the probability (95%) of nasal colonization with *S. aureus* and MRSA by the quartile of serum homocysteine levels. In unadjusted and adjusted regression models, odds of *S. aureus* colonization were not associated significantly with quartile increases in serum homocysteine quartile. In contrast, odds of colonization in the highest quartiles of homocysteine (Quartile 4) were significantly increased compared with odds in the lowest homocysteine quartile (Quartile 1), which is consistent with the trend observed with unadjusted models (OR, 3.70; 95% CI, 1.91–7.17), in age and sex-adjusted models (OR, 4.46; 95% CI, 1.78–11.19), and in completely adjusted models (OR, 3.09; 95% CI, 1.11–8.61).

### 4. Discussion

The emergence of CA-MRSA has raised much attention as a public health threat.[6,7] In the US, the national rate of HA-MRSA sharply decreased from 27.7% in 2005 to 27.7% in 2011; however, CA-MRSA were only slightly decreased by 5% in 2011.[15,16] Similarly, the 2001 to 2002 and 2003 to 2004 NHANES studies demonstrated sharp increase in the prevalence of nasal colonizations with MRSA from 0.8% in 2001–2002 to 1.5% in 2003–2004, although the proportion of individuals with *S. aureus* colonization decreased from 32.4% to 28.6%.[17,18] Although many risk factors for HA-MRSA infection have been reported in the literature,[19–21] factors associated with CA-MRSA infection are not well reported. Thus, understanding risk factors for MRSA infection in the general population may be a reasonable strategy to prevent it.

In the present study, we investigated the association between serum homocysteine levels and nasal colonization with *S. aureus* and MRSA in US. Our results demonstrated that elevated homocysteine levels were significantly associated with the presence of nasal colonization with MRSA, but not with nasal colonization with *S. aureus*. In particular, compared with adults with the lowest homocysteine levels (Quartile 1), those with the highest homocysteine (Quartile 4) presented a 3.09-fold increased risk of having nasal colonization with MRSA. In contrast, there was no significant association between homocysteine level and nasal colonization with *S. aureus*. This finding is an association and not a causal link, suggesting that homocysteine elevation may be a risk factor for incidence of CA-MRSA infection.

Homocysteine elevation is considered indicative of pathogenic infection based on increased oxidative stress and activated proinflammatory factors, which are expected to be an increased risk for many clinical diseases.[8,11] The etiology of increased colonization with MRSA in individuals with high homocysteine levels remains uncertain, but this phenomenon may be explained by unconventional mechanisms of antibiotics resistance, indicating that in certain infections, antioxidant levels decrease and prooxidant levels increase the pathogen burden.[22,23] Notably, antibiotic resistance is conferred by the acquisition of resistance genes[24,25] as well as the impact of environmental stress (e.g., nutrient limitation, reactive oxygen species, heat, pH, and envelope stress) on bacteria.[23,26,27] Growing evidence has suggested that oxidative stress induced by reactive oxygen species elicits adoptive responses to protect bacteria from the stress[23,26,27] such that enhanced bacterial survival manifest physiological changes in cells, thereby affecting innate antimi...
crobial susceptibility or upregulating multidrug efflux pumps to decrease the effective intracellular concentration of antibio-
cids. S aureus can adopt to oxidative stress by altering its population characteristics. For example, S aureus small-
colony variants (SCVs) are associated with decreased expression of toxins, increased expression of adhesions, intracellular persistence, and reduced antimicrobial susceptibility, leading to therapeutic failure. A recent study by Painter et al have demonstrated that exposure of S aureus to sublethal hydrogen peroxide levels led to a specific, dose-dependent increase in the population frequency of gentamicin-resistant SCVs. Given the existing evidence, oxidative stress in the host may stimulate physiological changes in the bacterial cell as adoptive response (for example, emergency of SCVs in S aureus), possibly leading to high resistance to antibiotics, such as the emergence of MRSA. Unfortunately, our result was based on an observational study involving the general population, and identifying mechanism underlying the association between homocysteine levels and

| Characteristics                  | No. of subjects tested (n = 7832) | Prevalence of nasal colonization | S aureus (n = 2051) | MRSA (n = 98) |
|----------------------------------|----------------------------------|---------------------------------|---------------------|--------------|
| Age, yr                          |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| 20–29                            | 1449                             | 427 (29.5)                      | 10 (2.3)            | <.0001       |         |        |
| 30–39                            | 1311                             | 357 (27.2)                      | 12 (5.4)            | <.0001       |         |        |
| 40–49                            | 1348                             | 375 (27.8)                      | 11 (2.9)            | <.0001       |         |        |
| 50–59                            | 1023                             | 264 (25.8)                      | 8 (3.0)             | <.0001       |         |        |
| 60–69                            | 1199                             | 300 (25.0)                      | 27 (9.0)            | <.0001       |         |        |
| 70–79                            | 901                              | 200 (22.2)                      | 14 (7.0)            | <.0001       |         |        |
| ≥80                              | 601                              | 128 (21.3)                      | 16 (25.9)           | <.0001       |         |        |
| Sex                              |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| Male                             | 3808                             | 1089 (28.6)                     | 34 (3.1)            | <.0001       |         |        |
| Female                           | 4024                             | 962 (23.9)                      | 64 (6.7)            | <.0001       |         |        |
| Race/ethnicity                   |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| White                            | 4277                             | 1200 (28.1)                     | 65 (5.4)            | <.0001       |         |        |
| Black                            | 1406                             | 297 (21.1)                      | 20 (6.7)            | .0101        |         |        |
| Hispanic                         | 1872                             | 480 (25.6)                      | 10 (2.1)            | <.0001       |         |        |
| Others                           | 277                              | 74 (26.7)                       | 3 (4.1)             | <.0001       |         |        |
| Family income                    |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| <$20,000                         | 2462                             | 641 (26.0)                      | 44 (6.9)            | <.0001       |         |        |
| ≥$20,000                         | 5370                             | 1410 (26.3)                     | 54 (3.8)            | <.0001       |         |        |
| Cigarette smoking                |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| Current                          | 1746                             | 400 (22.9)                      | 17 (4.3)            | <.0001       |         |        |
| Former                           | 2125                             | 557 (26.2)                      | 44 (7.9)            | <.0001       |         |        |
| Never                            | 3961                             | 1094 (27.6)                     | 37 (3.4)            | <.0001       |         |        |
| Moderate activity over the past 30 d |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| Yes                              | 3844                             | 994 (25.9)                      | 35 (5.5)            | <.0001       |         |        |
| No                               | 3772                             | 994 (26.4)                      | 50 (5.0)            | <.0001       |         |        |
| Unable to do activity            | 216                              | 63 (29.2)                       | 13 (20.6)           | <.0001       |         |        |
| Obesity (BMI ≥25 kg/m²)          |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| Yes                              | 5276                             | 1424 (26.5)                     | 64 (4.5)            | <.0001       |         |        |
| No                               | 2456                             | 627 (25.5)                      | 34 (5.4)            | <.0001       |         |        |
| Diabetes history                 |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| Yes                              | 908                              | 253 (27.9)                      | 24 (9.5)            | <.0001       |         |        |
| No                               | 6924                             | 1736 (26.0)                     | 74 (4.1)            | <.0001       |         |        |
| Hypertension history             |                                  |                                 | No. (%)             | P-value      | No. (%) | P-value |
| Yes                              | 2834                             | 688 (24.3)                      | 45 (6.5)            | <.0001       |         |        |
| No                               | 4998                             | 1363 (27.3)                     | 53 (3.9)            | <.0001       |         |        |
| Taken prescribed antibiotics in past month |            | 0.3857                          | 2 (0.5)             | .0139        |         |        |
| Yes                              | 44                               | 9 (20.5)                        | 2 (22.2)            | <.0001       |         |        |
| No                               | 7788                             | 2042 (26.2)                     | 96 (4.7)            | <.0001       |         |        |
| Overnight hospital patient in last year |            | 5144                            | 26 (10.2)           | <.0001       |         |        |
| Yes                              | 946                              | 256 (27.1)                      | 26 (10.2)           | <.0001       |         |        |
| No                               | 6886                             | 1795 (26.1)                     | 72 (4.0)            | <.0001       |         |        |
| Folate, serum, ng/mL             |                                  | 14.77 (18.43)                   | 15.4 (10.9)         | <.0001       |         |        |
| Vitamin B12, serum, pg/mL        |                                  | 523.29 (507.50)                 | 0.0595              | 0.5428       |         |        |

BMI = body mass index, MRSA = methicillin-resistant Staphylococcus aureus.

*P*-value was based on the Chi-square test across subjects with S aureus colonization versus without S aureus colonization.

†P*-value was based on the Chi-square test across subjects with MRSA colonization versus without MRSA colonization.

S aureus can adopt to oxidative stress by altering its population characteristics. For example, S aureus small-colony variants (SCVs) are associated with decreased expression of toxins, increased expression of adhesions, intracellular persistence, and reduced antimicrobial susceptibility, leading to therapeutic failure. A recent study by Painter et al have demonstrated that exposure of S aureus to sublethal hydrogen peroxide levels led to a specific, dose-dependent increase in the population frequency of gentamicin-resistant SCVs. Given the existing evidence, oxidative stress in the host may stimulate physiological changes in the bacterial cell as adoptive response (for example, emergency of SCVs in S aureus), possibly leading to high resistance to antibiotics, such as the emergence of MRSA. Unfortunately, our result was based on an observational study involving the general population, and identifying mechanism underlying the association between homocysteine levels and
Figure 1. Mean homocysteine levels by the presence of nasal colonization with S. aureus and MRSA. Pink bars represent mean homocysteine levels for participants by the presence or absence of nasal colonization with MRSA, and green bars represent mean homocysteine levels for participants by the presence or absence of nasal colonization with S. aureus. MRSA = methicillin-resistant Staphylococcus aureus.

Figure 2. Percentages of subjects with nasal colonization with S. aureus and MRSA by the quartile of serum homocysteine. This chart has 2 different y-axes. The left axis presents the percentage of participants with nasal colonization with MRSA, ranging from 0% to 30%. The right axis presents the percentage of participants with nasal colonization with S. aureus, ranging from 0% to 10%. Pink bars represent the percentage of participants with nasal colonization with MRSA, and green bars represent the percentage of participants with nasal colonization with S. aureus. To compare values for different percentages of participants with nasal colonization with S. aureus and MRSA in each quartile of serum homocysteine levels, dark pink and light green lines are drawn on the top of the bars. Homocysteine (umol/L): Quintile 1 (<6.71), Quintile 2 (6.72–8.26), Quintile 3 (8.27–10.40), Quintile 4 (≥10.41). MRSA = methicillin-resistant Staphylococcus aureus.
Table 2

| Homocysteine, µmol/L | Odds for nasal colonization |
|----------------------|-----------------------------|
|                      | S aureus (95% CI) | MRSA (95% CI) |
| Unadjusted model     |                |                |
| Quintile 1 (6.71)    | Reference       | Reference       |
| Quintile 2 (6.72–8.26) | 1.08 (0.91–1.28) | 1.56 (0.84–2.89) |
| Quintile 3 (8.27–10.40) | 1.00 (0.87–1.17) | 1.94 (0.92–4.08) |
| Quintile 4 (≥10.41)  | 0.97 (0.81–1.15)  | 3.70 (1.91–7.17)  |
| P-for trend          | .6732           | .0002           |
| Age and gender adjusted model |                |                |
| Quintile 1 (6.71)    | Reference       | Reference       |
| Quintile 2 (6.72–8.26) | 1.01 (0.86–1.20) | 1.78 (0.93–3.38) |
| Quintile 3 (8.27–10.40) | 0.92 (0.77–1.10) | 2.66 (1.14–6.20) |
| Quintile 4 (≥10.41)  | 0.93 (0.76–1.14)  | 4.46 (1.78–11.19) |
| P-for trend          | .7571           | .0100           |
| Fully adjusted model |                |                |
| Quintile 1 (6.71)    | Reference       | Reference       |
| Quintile 2 (6.72–8.26) | 1.05 (0.88–1.24) | 1.47 (0.94–2.30) |
| Quintile 3 (8.27–10.40) | 0.98 (0.81–1.18) | 2.24 (0.97–5.14) |
| Quintile 4 (≥10.41)  | 1.00 (0.81–1.24)  | 3.09 (1.11–8.61)  |
| P-for trend          | .9426           | .1282           |

CI = confidence interval, MRSA = methicillin-resistant Staphylococcus aureus; OR = odds ratio.
* Adjusted for age, sex, race/ethnicity, family income, smoking, alcohol intake, moderate physical activity, obesity, a history of DM or hypertension, taken prescribed antibiotics during past month, overnight hospital patient in the last year, and serum levels of folate and Vitamin B12.

nasal colonization with MRSA is beyond the scope of the present study. Nonetheless, high homocysteine levels (ie, oxidative stress activation) may act as a cue triggering S aureus to confer antibiotic resistance, leading to nasal colonization with MRSA. Future studies should further investigate the observed association in our study and expand on the current knowledge of the occurrence of MRSA in the general population.

Our study has several important limitations. First, this study was a cross-sectional study; thus, we cannot rule out the “reverse causality” in the fact that nasal colonization with S aureus and MRSA may be a cause of homocysteine elevation. Second, nasal colonization with S aureus and MRSA was not serially cultured and was measured only at a single time point during the study period. Individuals who were only transiently or intermittently colonized may not have been detected and seasonal variation in the colonization may not have been explored. Third, prevalence of MRSA colonization was in relatively small samples; thus, statistical estimates may not meet the reliability of the observed association between homocysteine levels and MRSA colonization. Fourth, our observations of MRSA infection and homocysteine levels were based on results in the general population with nasal colonization of MRSA, rather than in patients with MRSA diagnosed in the hospitals; therefore, we cannot comment on the clinical usefulness of homocysteine levels in terms of diagnosis and treatment of MRSA. Specifically, whether homocysteine measurement is a diagnostic marker for identifying patients at a risk of acquiring MRSA infection or whether MRSA treatment affects homocysteine level in patients remains unknown. Therefore, future studies should clarify such clinical implications of homocysteine levels through hospital-based studies including patients with MRSA infection. Finally, because of the observational nature of this investigation, the possibility of residual confounding effects by unmeasured confounders and respondents’ recall bias remains. In particular, some variables affecting the colonization, for example, recent exposure to antimicrobial agents, underlying medical conditions (specifically, device use treatment), and occupation, were not analyzed in the statistical model.

Despite these limitations, the present analysis of the NHANES data outlines for the first time a possible association between MRSA nasal colonization and elevated homocysteine levels in the general population. Therefore, we suggest that homocysteine levels may be an important indicator of MRSA colonization.

Author contributions

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References

[1] Grundmann H, Aires-de-Sousa M, Boyce J, et al. Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. Lancet 2006;368:874–83.
[2] Finland M. Emergence of antibiotic-resistant bacteria. N Engl J Med 1995;333:909–22.
[3] Chambers HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin Microbiol Rev 1997;10:781–91.
[4] Far R, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Med Clin 2014;6:25–64.
[5] Stefani S, Chung DR, Lindsay JA, et al. Meticillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents 2012;39:273–82.
[6] Delo R, Otto M, Kreisworth BN, et al. Community-associated meticillin-resistant Staphylococcus aureus. Lancet 2010;375:1557–68.
[7] David MZ, Daum RS. Community-associated meticillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010;23:616–87.
[8] Ansari R, Mahta A, Mallack E, et al. Hyperhomocysteinemia and neurologic disorders: a review. J Clin Neurol 2014;10:281–8.
[9] Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J 2015;14:6.
[10] Zhang D, Wen X, Wu W, et al. Elevated homocysteine level and folate deficiency associated with increased overall risk of carcinogenesis: meta-analysis of 83 case-control studies involving 35,758 individuals. PLoS One 2015;10:e0123423.
[11] Papathodorou I, Weiss N. Vascular oxidant stress and inflammation in hyperhomocysteinemia. Antioxid Redox Signal 2007;9:1941–58.
[12] Abdel-Razik A, Eldars W, Elhelayy R, et al. Homocysteine: a new diagnostic marker in spontaneous bacterial peritonitis. Eur J Gastroenterol Hepatol 2018;30:779–85.
[13] Haunma EM, Rietveld MH, de Breij A, et al. Inflammatory and antimicrobial responses to meticillin-resistant Staphylococcus aureus in an in vitro wound infection model. PLoS One 2013;8:e82800.
[14] Tang H, Long N, Lin L, et al. Effect of MRSA on CYP450: dynamic changes of cytokines, oxidative stress, and drug-metabolizing enzymes in mice infected with MRSA. Infect Drug Resist 2018;11:229–38.
[13] Dantes R, Mu Y, Belflower R, et al. National burden of invasive methicillin-resistant Staphylococcus aureus infections, United States, 2011. JAMA Intern Med 2013;173:1970–8.

[14] Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA 2007;298:1763–71.

[15] Gorwitz RJ, Kruszon-Moran D, McAllister SK, et al. Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001-2004. J Infect Dis 2008;197:1226–34.

[16] Kuehnert MJ, Kruszon-Moran D, Hill HA, et al. Prevalence of Staphylococcus aureus nasal colonization in the United States, 2001-2002. J Infect Dis 2006;193:172–9.

[17] Carnicer-Pont D, Bailey KA, Mason BW, et al. Risk factors for hospital-acquired methicillin-resistant Staphylococcus aureus bacteraemia: a case-control study. Epidemiol Infect 2006;134:1167–73.

[18] Graffunder EM, Venema RA. Risk factors associated with nosocomial methicillin-resistant Staphylococcus aureus (MRSA) infection including previous use of antimicrobials. J Antimicrob Chemother 2002;49:999–1005.

[19] Hidron AI, Kourbatova EV, Halvosa JS, et al. Risk factors for colonization with methicillin-resistant Staphylococcus aureus (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage. Clin Infect Dis 2005;41:159–66.

[20] Al-Habib A, Al-Saleh E, Safer AM, et al. Bactericidal effect of grape seed extract on methicillin-resistant Staphylococcus aureus (MRSA). J Toxicol Sci 2010;35:337–44.

[21] Fang FC, Frawley ER, Tapscott T, et al. Bacterial stress responses during host infection. Cell Host Microbe 2016;20:133–43.

[22] Andam CP, Fournier GP, Gogarten JP. Multilevel populations and the evolution of antibiotic resistance through horizontal gene transfer. FEMS Microbiol Rev 2013;35:756–67.

[23] Wiedenbeck J, Cohan FM. Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiol Rev 2011;35:957–76.

[24] Grant SS, Hung DT. Persistent bacterial infections, antibiotic tolerance, and the oxidative stress response. Virulence 2013;4:273–83.

[25] Poole K. Bacterial stress responses as determinants of antimicrobial resistance. J Antimicrob Chemother 2012;67:2069–89.

[26] Parva CN, Bozza MT. Are reactive oxygen species always detrimental to pathogens? Antioxid Redox Signal 2014;20:1000–37.

[27] Aertsen A, Michiels CW. Stress and how bacteria cope with death and survival. Crit Rev Microbiol 2004;30:263–73.

[28] Garcia LG, Lemaire S, Kahl BC, et al. Antibiotic activity against small-colony variants of Staphylococcus aureus: review of in vitro, animal and clinical data. J Antimicrob Chemother 2013;68:1455–64.

[29] Moisan H, Brouillette E, Jacob CL, et al. Transcription of virulence factors in Staphylococcus aureus small-colony variants isolated from cystic fibrosis patients is influenced by SigB. J Bacteriol 2006;188:64–76.

[30] Proctor RA, Kriegeskorte A, Kahl BC, et al. Staphylococcus aureus small colony variants (SCVs): a road map for the metabolic pathways involved in persistent infections. Front Cell Infect Microbiol 2014;4:99.

[31] Painter KL, Strange E, Parkhill J, et al. Staphylococcus aureus adapts to oxidative stress by producing H2O2-resistant small-colony variants via the SOS response. Infect Immun 2015;83:1830–44.