UiT The Arctic University of Norway

Faculty of Health Sciences- The Department of Community Medicine

# *Staphylococcus aureus* nasal carriage in relation to iron status in a Norwegian adolescent population

The Tromsø Study: Fit Futures 1

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# Abbreviations

| | |
|---|---|
| BMI | Body mass index |
| CI | Confidence interval |
| cm | centimeter |
| CRP | C-reactive protein (CRP-sensitive) |
| DPA | Norwegian Data Protection Authority |
| DPIA | Data Protection Impact Assessment |
| DPU | Data and publication committee |
| TFF1 | Tromsø Study Fit Futures 1 |
| Hb | Haemoglobin |
| HbA1c | Glycated haemoglobin |
| 25-hydroxyvitamin D [25(OH)D] | Vitamin D |
| Kg | Kilogram |
| OR | Odds Ratio |
| Q | Quintile |
| REK | Regional Committee for Medical and Health Research Ethics for Northern Norway (REK-Nord) |
| *S. aureus* | *Staphylococcus aureus* |
| SD | Standard deviation |
| SPSS | Statistical Package for the Social Science |
| UNN | University hospital of North Norway |
| WHO | World Health Organisation |

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# Abstract

**Background:** *Staphylococcus aureus* (*S. aureus*) needs iron to survive and replicate and has evolved systems to harvest iron from its host. In humans, excess iron could potentially increase the risk of *S. aureus* carriage and infections. Moreover, iron plays a vital role in the development and function of the host immune system. Adolescence is a growth spurt period with increased iron requirements. The aim of this study was to examine whether iron status is associated with persistent *S. aureus* nasal carriage in a general adolescent population.

**Methods:** *S. aureus* carriage was assessed by repeated nasal swab cultures among 375 females and 401 males, aged 15-19 years, in the population based Tromsø Study Fit Futures 1 in 2010-2011. Iron, haemoglobin, and ferritin in blood were measured. Levels of iron biomarkers according to *S. aureus* nasal carriage status were examined by descriptive analysis. Separate binary multivariable logistic regression models including age, measured BMI, serum vitamin D, snuff use, atopic eczema and hormonal contraceptive use (female only), were applied to test whether level of each iron biomarker (stratified by quintiles) was associated with *S. aureus* nasal carriage.

**Results:** We found statistically significant associations between iron biomarkers and *S. aureus* carriage in males. Males with [12,16) versus [16-19) µmol/L quintile of serum iron; <12.51 versus [13.21,14.11) g/dL quintile of EDTA-blood haemoglobin and <19 versus [30, 44) µg/L quintile of serum ferritin had 2.05 times, 2.27 times and 2.48 times higher odds of carriage, respectively. There were only minor changes in the estimates when suggested outliers were removed in regression analysis. Our data did not demonstrate any relationship between iron biomarkers and *S. aureus* carriage in females.

**Conclusion:** The current study supports an association between low iron status and persistent *S. aureus* nasal carriage in males. The relationship between iron-status and *S. aureus* carriage should be addressed in future prospective studies and among other age-groups.



**Keywords:** *Staphylococcus aureus*, nasal carriage, iron-status, adolescent, cross-sectional study.

# 1 Introduction

## 1.1 *Staphylococcus aureus*

*Staphylococcus aureus* (*S .aureus*) is an important cause of morbidity and mortality in human populations worldwide (1). These gram-positive bacteria colonise human skin and mucosal surfaces, including nose in healthy individuals. *S. aureus* colonises both upper and lower layers of the nasal epithelium. Inside the cells, *S. aureus* is protected from the host immune responses and antibiotics (2). The vestibulum nasi is the major *S. aureus* reservoir associated with transmission to multiple body sites, auto-infections and transmission to others (3).

Traditionally, *S. aureus* nasal carriage status has been categorised into three groups: non-carriage, intermittent and persistent carriage (3). Harvesting two nasal culture swabs one week apart permits differentiating between persistent and intermittent carriage status (4). Since intermittent and non-carriers share related *S. aureus* nasal elimination kinetics and anti-*staphylococcus* antibody profile, Van Belkum et al. suggested a reclassification of the two carriage groups: persistent and others (non-or intermittent carriage) (5).

The prevalence of *S. aureus* nasal carriage varies with age; in young children, 20-30 %, which increase to 40-50 % in older children (6). In late adolescence, prevalence stays at 50 %, after which the prevalence drops to 20-30 % in the adult population (3,7). Males have higher *S. aureus* prevalence compared to females. Colonisation and carriage have been associated with environmental factors and host factors: family size, contact with the health care services, occupation, pets, sports involving physical contact, tobacco use, underlying diseases (i.e. atopic eczema, atopic disease, diabetes mellitus, obesity), hormonal levels, ethnicity, genetic factors, immunity and antibiotic use (8–12).

Persistent *S. aureus* nasal carriers are at risk of autoinfection in connection with hospitalisation and surgery. In a study among patients in hospital in the Netherlands, more than 80 % of *S. aureus* infections were endogenous infections (13). *S. aureus* produces enzymes and toxins that cause tissue damage and local skin infections. Moreover, *S. aureus* is a leading cause of bacteraemia and more severe infections like pneumonia, meningitis, endocarditis and sepsis (14).

*S. aureus* has shown an extraordinary capacity to develop resistance to conventional antibiotics (15). Methicillin-resistance *S. aureus* (MRSA) is a significant cause of nosocomial- and community-associated infections, and older people and new-borns are at particular risk of MRSA infection and death (16). MRSA complicates treatment and causes additional in-hospital expenses (17,18).

## 1.2 Iron status

Iron is an essential micronutrition for humans and iron absorption is regulated according to the body's needs (19). Iron up-take occurs mainly in duodenum, and some absorption in small intestine. In the diet, iron appears as hem-iron and non-hem iron, and main sources are animal products (meat, poultry and fish) and vegetable products, respectively (20).

Iron as a vital component in haemoglobin, a protein in red blood cells that transfers oxygen from the lungs to the tissues. Also, iron is an important component in another essential protein, myoglobin. Myoglobin delivers oxygen to the muscles and thus supports muscle metabolism and a healthy connective tissue. Moreover, iron is needed for neurological development, cell growth and differentiation, gene regulation, and protein synthesis (21). On the other side, overload of iron may damage tissues by producing toxic oxygen radicals (22).

Transferrin delivers iron to haemoglobin synthesis and transports excess iron to storage in hepatic parenchyma and the reticuloendothelial system (23). On the cell surface, transferrin receptor (TfR) receives iron from transferrin and transfer iron into the cells. In storage, the iron is bound to ferritin and hemosiderin (23). TfR and ferritin are inversely correlated- at low iron levels, TfR increase when ferritin decreases (24,25).

Free iron, haemoglobin, ferritin and TfR are recommended parameters to state iron-status (26). The respective analysis are performed in serum or blood by biochemical methods (27). Measurement of haemoglobin concentration can be used as a primary screening indicator. Secondary, ferritin and haemoglobin are preferred to assess for iron deficiency (28).

Prevalence of anaemia is high in low-income countries, but also a significant nutritional disorder in industrialized countries (29,30)

Infants, children, adolescents, and females in fertile age have high requirements for iron and are therefore at particular risk of developing anaemia and iron deficiency (31). Puberty and youth are a growth spurt period with increased iron requirements, expansion of blood volume and increased muscle mass. In female, the onset of menstruation also leads to iron losses (32).

Also, old age is associated with iron deficiency and iron overload, primarily caused by disturbed iron metabolism (33).

## 1.3 Iron status and *S. aureus* colonisation and carriage

Among many factors, bacteria need iron to survive and replicate and have, therefore evolved systems to *steal* iron from its human host (34–36). Thus, excess iron could potentially increase the risk of *S. aureus* carriage and infections (37).

On the other hand, experimental evidence shows that iron plays a fundamental role in the development and function of the host immune system (38). Moreover, when iron-binding proteins like transferrin are overloaded with iron, they lose their bacteriostatic effect and thereby increase the risk of infections (39). The human body produces iron-binding proteins to reduce the availability of free iron to be consumed by pathogens (40). Based on recent findings from a murine model, it was hypothesised that targeting excess iron may provide a new therapeutic target to prevent infections in human patients (41).

In the second Nord-Trøndelag Health Survey (HUNT2, 1995-1997), the researchers observed an association between severe iron deficiency and increased risk of future blood stream infections (42).

Based on existing knowledge, Dragesmith and Prentice, suggested a U-shaped relationship between iron status and susceptibility to infection. Hence, the risk of infections increase with iron deficiency and iron-overload; the lowest risk at the optimal iron metabolism (37).

During puberty and early adult life physiological and biological changes, in addition to a change in lifestyle, may change and shape the immune system and contribute to the individual's *S. aureus* carriage status (43). Knowledge about factors that influence *S. aureus*

carriage in an adolescent population may contribute to targeted prevention of transmission and infections, and thereby reduce morbidity and mortality.

To our knowledge, population-based studies of iron status and *S. aureus* carriage are mainly lacking. Thus, the aim of this cross-sectional study was to investigate if there is an association between iron status and persistent *S. aureus* nasal carriage among a large sample of adolescents from a general population. The data was collected in the Tromsø Study Fit Futures 1 (2010-2011) from the municipalities of Tromsø and Balsfjord.

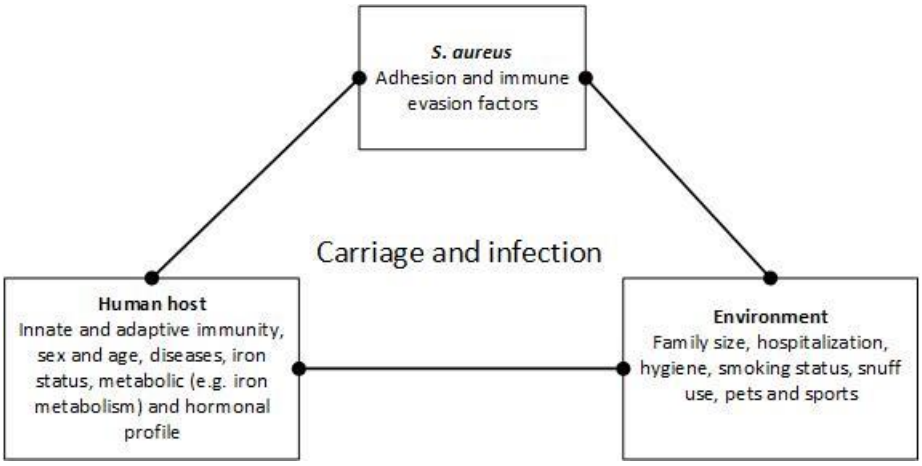


**Figure 1. Host-microbe-environment interplay.** Known and suggested interactions between microbial, host and environmental risk factors involved in *S. aureus* carriage and infection. Based on an illustration from the Tromsø Staph and Skin Study (44).

## 1.4 Research question

Primary objective:

- To investigate whether iron status is associated with persistent *S. aureus* nasal carriage in a population of adolescents in the Tromsø Study: Fit Futures 1.

Secondary objective:

- To evaluate possible sex differences in the association between iron status and *S. aureus* status.
- To examine the association between *S. aureus* carriage and each of the iron biomarkers.

# 2 Materials and Methods

## 2.1 Study population: Fit Futures

The Tromsø Study Fit Futures 1 (TFF1) is a cross-sectional study investigating lifestyles and health among upper-secondary school students in the Norwegian municipalities of Tromsø and Balsfjord.

TFF1 belongs to a population-based longitudinal study with repeated health surveys among adolescents in Northern Norway and is an expansion of the Tromsø Study (45). The study investigates family background, social networking and lifestyle affect physical and mental health, dental health, and school dropout etc. In 2010-2011, all first-year students ($n = 1117$) in Tromsø and Balsfjord municipalities attending upper-secondary schools were invited to TFF1(46). A total of 1038 students (92.9%) participated (47). Among these students, 961 were in the age group from 15 to 17 years (born 1992–1994).


Figure 2. The study population. The Tromsø Study Fit Futures 1.

*Missing blood sample due to abort sampling (n=14); refusal blood sampling (n=68); medical events (decrease in blood pressure, fainting) n=13).

## 2.2 Data Collection

All participants came to the Clinical Research Unit, University Hospital of North Norway (UNN) for a half-day visit (46). The examination of the students included: self-administered digital questionnaires, clinical interview, clinical examinations, blood samples and microbiological swab samples. All examinations were done by trained research nurses according to standardised procedures.

The self-administrated questionnaires collected data on lifestyles (e.g. snuff tobacco and alcohol), health and chronic diseases (e.g. allergic diseases) (48). The interviews included information on the use of hormonal contraceptives for the females and the use of antibiotics last 24 hours.

Body height was measured in centimetres (cm) and weight in kilograms (kg) to closest 0.1 unit with lightweight clothing and no shoes, using an electronic scale. Body mass index (BMI) was collected as weight divided by height squared ($kg/m^2$).

Non-fasting blood samples were taken from an antecubital vein and transported to the hospital laboratory or frozen within a specified time.

Microbiological samples were taken from both anterior nares with a sterile rayon-tipped swab moistened with sterile NaCl (0.9 %). The nasal swab was instantly collected and transferred into an Amies charcoal transport medium (Copan, Brescia, Italy) and stored at 4 ℃ for 3-7 days. One nasal swab was taken at the visit at the Clinical Research Unit, and a second nasal swab was collected at school one week later.

## 2.3 Measurement

### 2.3.1 Laboratory analysis

The department of Medical Biochemistry at the University Hospital of North Norway (UNN) analysed serum-iron and ferrous molecules (serum-ferritin and EDTA-blood haemoglobin), EDTA-blood glycated haemoglobin (HbA1c) and serum-CRP-sensitive (CRP). Information regarding the methods for the respective analysis can be found in the Laboratory handbook at UNN (27).

Serum-25-hydroxyvitamin D [25(OH)D] (vitamin D) samples were analysed at the Haukeland University Hospital, the Hormone Laboratory. The vitamin D method has been described previously (45).

Analysis of the nasal swab samples was done at the microbiological laboratory at the Department of Medical Biology, Faculty of Health Sciences, UiT The Arctic University of Norway (27). At the laboratory, the rayon-tipped swab in the Amies charcoal transport medium was placed in an enriched Bacto Staphylococcus medium broth (Difco Laboratories, Sparks, MD, USA). The enrichment broth was incubated for 18-24 hours at 37 ℃.

After incubation, one drop of broth was spread on blood agar (Oxoid, UK) and a selective *S. aureus* agar (CROMagar-plates, SmithMed AS). After one day of aerobe incubation, pink colonies were spread on blood agar and incubated to the next day. Isolation of *S. aureus* was confirmed by *Staphaurex plus* agglutination test (Remel, USA). Nasal *S. aureus* carriage was defined as negative or positive based on the *Staphaurex plus* agglutination test result.

## 2.4 Inclusion/Exclusion Criteria

All students attending upper-secondary school in the Norwegian municipalities of Tromsø and Balsfjord were invited to participate in TFF1 (49).

The World Health Organization (WHO) define individuals in the 10-19 age group as adolescents (50). Therefore, this research study excluded participants over 19 years. Also, individuals with missing nasal swab and blood sample, invalid results and antibiotic use targeting *S. aureus* within 24 hours were excluded. Participants missing results on iron, haemoglobin and ferritin were also excluded. Since inflammation rises ferritin levels and lowers free iron, individuals with CRP ≥5 or missing CRP-results, were excluded from the statistical analysis (51).

### 2.4.1 Dependent and independent variables

Participants' *S. aureus* nasal carriage status were categorised into *non-carriage* (two negative swabs), *intermittent carriage* (one positive swab) and *persistent carriage* (two positive

swabs) status. In the statistical analysis, participants were classified as *non- or intermittent S. aureus carriers* (non-carriers) and *persistent S. aureus carriers* (carriers) (5)

Levels of iron, haemoglobin and ferritin were used to investigate the relationship between the participants' iron-status and *S. aureus* carriage status. The iron biomarkers were used both as continuous and categorical variables defined by quintiles.

## 2.5 Statistical Analysis

All statistical analysis was two-sided and performed in SPSS (Statistical Package of Social Sciences) version 25 at the statistical significance level $p<0.05$ and 95% Confidence Interval (95%CI).

Descriptive analysis and binary logistic regression models investigated the association between iron status and *S. aureus* nasal carriage. All statistical analyses were stratified by sex.

The STROBE Checklist for Cross-sectional studies was used to ensure proper reporting (52).

### 2.5.1 Descriptive statistics – Characteristics of the study population

Characteristics of the study population by *S. aureus* nasal carriage were explored by descriptive analysis and presented as means (standard deviation, SD) or frequencies. The following variables were used in the descriptive analysis: age, BMI, serum vitamin D, HbA1c, HC and HC-type (female only), daily snuff use, alcohol use, self-rated health, physical activity, allergic rhinitis and atopic eczema.

Chi-square test and independent t-test were used to test for statistical significance for categorical and continuous variables, respectively.

### 2.5.2 Distribution of iron biomarkers

Box plots were used for visual inspection of the distribution of the independent variables iron, haemoglobin and ferritin among non-carriers and carriers of *S. aureus* and mean (SD) or median were calculated. The independent t-test was used to test for statistically significant differences in levels of iron and haemoglobin between groups. Ferritin was not normally distributed. Thus, the Mann-Whitney U-test was used to test for statistically differences in levels of ferritin between groups.

### 2.5.3 Association between iron status and *S. aureus* carriage

The iron biomarkers were stratified into quintiles by using rank cases and the ntiles method in SPSS. Visual binning was used to determine quintile limits for each of the iron status variables. Histograms were used to examine prevalence of *S. aureus* carriage by quintiles of each iron biomarker, and visual analysis of possible patterns and thresholds.

Age-adjusted and multivariable adjusted binary logistic regression analysis (second model) were used to investigate the odds for *S. aureus* nasal carriage in association to quintiles of iron, haemoglobin, and ferritin. Based on analysis of the bar charts and from a clinical perspective, the 3rd quintile was used as reference category (ref) in the main regression analysis for the respective iron biomarkers.

The statistical analysis was adjusted for known risk factors for *S. aureus* carriage. The second model included age, BMI, atopic eczema, serum vitamin D, snuff tobacco (*never, sometimes,* and *daily*), and use of hormonal contraceptive (HC) and type of HC in female. The HC was classified as a *combination* (estrogen and progestin), *progestin-only* and *non-user*.

## 2.6 Ethical perspectives and approvals

All participants were given both oral and written information about the study and signed a consent form at the Clinical Research Unit. Individuals younger than 16 years ensured written consent from parents or legal guardians,

This study does not present any direct benefits to the participants. However, the study does provide an opportunity to gain a better understanding of the association between iron status and *S. aureus* carriage in an adolescent population.

Data safety monitoring was according to the claims in Data Protection Impact Assessment (DPIA) and Regional Committee for Medical and Health Research Ethics for Northern Norway (REK-Nord). There were no conflicts of interests.

The Data and Publication committee for the Tromsø Survey (DPU) approved access to the data for the study with reference 48/19. REK-Nord approved the present study with reference 2019/30304 and Data Protection Officer at UNN, reference 2019/6596.

# 3 Results

The study population consisted of 375 females and 401 males. The mean age was 16.2 years (range:15-19).

Among the 776 participants included in the present study, 56 individuals had missing HbA1c, one female had missing serum Vitamin D, 15 individuals did not answer the atopic eczema question (seven females and eight males), and 7 females did not answer the HC question. 69 % of the female reported no current use of HC; 114 female used HC, and most of them used combination HC (27.2 %) (Table 1).

The persistent *S. aureus* nasal carriage prevalence for the total study population, TFF1, was 45.1% (350/776); for female 36.8 % (138/375) and male 52.9 % (212/401).

| Table 1. Characteristics of the study population. The Tromsø Study - Fit Futures 1 (N=776) | | |
|---|---|---|
| **Variables*** | **Females, N=375 **** | **Males, N=401 **** |
| Age, years (mean, SD) | 16.2 (0.6) | 16.1 (0.6) |
| BMI, kg/m$^2$ (mean, SD) | 22.2 (4.0) | 22.2 (3.9) |
| HbA1c (mean, SD) | 5.2 (0.3) | 5.3 (0.3) |
| Vitamin D (mean, SD) | 53.5 (22.9) | 40.8 (20.8) |
| CRP (mean, SD) | 0.9 (1.0) | 0.8 (0.9) |
| **Self-rated health** | | |
| Excellent | 85 (23.0%) | 116 (29.3%) |
| Good | 203 (54.9%) | 172 (43.4%) |
| Neither good nor bad | 66 (17.8%) | 89 (22.5%) |
| Bad | 16 (4.4%) | 19 (4.8%) |
| **Hormonal contraceptive use (female only)** | | - |
| Non-user | 254 (69.0%) | |
| Progestin-only [a] | 14 (3.8%) | |
| Combination [b] | 100 (27.2%) | |
| **Snuff use** | | |
| No, never | 257 (68.5%) | 249 (62.1%) |
| Sometimes | 48 (12.0%) | 48 (12.0%) |
| Daily | 62 (16.5%) | 98 (24.4%) |
| **Alcohol use** | | |
| Never | 96 (25.8%) | 122 (31.0%) |
| Once per month or less | 163 (43.8%) | 151 (38.3%) |
| Two or more times per month | 113 (30.4%) | 121 (30.7%) |
| **Physical activity [c]** | | |
| Yes | 321 (86.3%) | 281 (71.0%) |
| No | 51 (13.7%) | 115 (29.0%) |
| **Atopic eczema [d]** | | |
| Yes | 67 (18.2%) | 44 (11.2%) |
| No | 301 (81.8%) | 349 (88.8%) |
| **Allergic rhinitis [e]** | | |
| Yes | 35 (9.5%) | 35 (9.0%) |
| No | 332 (90.5%) | 355 (91.0%) |

* Known or suggested risk factors for *S. aureus* carriage status.

**Number may vary due to missing information.

Values are the number of subjects (%) if not otherwise stated.

N= numbers; BMI=body mass index (kg/m$^2$); HbA1c, glycated haemoglobin (mmol/L); Vitamin D, 25-hydroxyvitamin D [25(OH)D] (nmol/L)

[a] Progestin-only: Cerazette®, Implanon® or Depo-provera®

[b] Combination contraceptive: Mercilon®, Yasminelle®, Loette 28®, Yasmin®, Microgynon®, Zyrona®, Synfase®, Evra® or Nuvaring®

[c] Recreational physical activity: Low level= reading, watching TV, or other sedentary activity; Medium level= Walking, cycling, or other forms of exercise at least 4 hours a week; High level= Participation in recreational sports, heavy outdoor activities with a minimum duration of 4 hours a week, or participation in heavy training or sports competitions regularly several times a week. Medium and high= Yes and Low level=No.

[d] Have a doctor ever said that you have children's eczema or atopic eczema? No, and Do not know=No.

[e] Have a doctor ever said that you have hay-fever or allergic rhinitis? No, and Do not know=No.

| **Table 2. Levels of iron biomarkers by *S. aureus* nasal carriage status. The Tromsø Study - Fit Futures 1 (N=776)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Iron markers** | **Females** | | | | **Males** | | | |
| | **Total N=375** | **Non-carrier [a] N=237** | **Carrier [b] N=138** | **P-value** | **Total N=401** | **Non-carrier [a] N=189** | **Carrier [b] N=212** | **P-value** |
| Iron, µmol/L (mean, SD) | 15.4 (7.3) | 15.2 (6.7) | 15.8 (8.2) | 0.49 [c] | 18.8 (6.9) | 18.8 (7.2) | 18.8 (6.7) | 0.99 [c] |
| Haemoglobin, g/dL (mean, SD) | 12.6 (1.0) | 12.6 (1.0) | 12.7 (0.9) | 0.74 [c] | 14.58 (0.88) | 14.60 (0.80) | 14.56 (0.95) | 0.58 [c] |
| Ferritin, µg/L (median) | 25.0 | 24.0 | 25.0 | 0.34 [d] | 48.0 | 52.0 | 45.0 | **0.025** [d] |

N= numbers; SD=Standard deviation.

[a] Observations with two negative or one negative and one positive *S. aureus* nasal swab cultures defined as non-carrier.

[b] Observations with two *S. aureus* positive nasal swab cultures defined as persistent carriers.

[c] Independent Sample test

[d] Mann-Whitney U-test.

Table 2 shows the mean for levels of iron and haemoglobin and median for ferritin for both sexes, according to *S. aureus* carriage status. All iron biomarkers had higher mean concentrations in males than in females.

The mean iron level for females was 15.4 µmol/L (SD: ±7.3; observed range: 0-59.0 µmol/L) (the 0 µmol/L belonged to one female with low iron-status) and for males 18.8 µmol/L (SD: ±6.9; observed range: 3.0-57.0 µmol/L). The mean haemoglobin level was 12.6 g/dL (SD: ±1.0; observed range: 8.1-14.7 g/dL) and 14.6 g/dL (SD: ± 0.9; observed range: 11.0-17.4 g/dL) for females and males, respectively. The median ferritin for females was 25.0 µg/L (observed range: 2-144 µg/L) and for males 48.0 µg/L (observed range: 5-212 µg/L) (observed range not in tables).

Mean levels of the iron biomarkers did not differ by *S. aureus* nasal carriage status in women. In men, mean levels of iron and haemoglobin did not differ by carriage status. Levels of ferritin was statistically significantly lower (p-value= 0.025) in carriers (median: 52.0 µg/L) than in non-carriers (median: 45.0 µg/L).

| **Table 3. Associations between potential risk factors and *S. aureus* nasal carriage. The Tromsø Study-Fit Futures** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variables * | Female ** | | | | Male ** | | | |
| **Total population**<br><br>**N=776** | **Non-carrier** [a]<br><br>**N=237** | **Carrier** [b]<br><br>**N=138** | **P-value** [c] | **OR 95%CI** [d] | **Non-carrier** [a]<br><br>**N=189** | **Carrier** [b]<br><br>**N=212** | **P-value** [c] | **OR 95%CI** [d] |
| **Age** (mean, SD) | 16.2 (0.6) | 16.2 (0.7) | 0.94 | 1.01 (0.71-1.44) | 16.1 (0.6) | 16.1 (0.6) | 0.66 | 1.08 (0.77-1.50) |
| **BMI** (mean, SD) | 22.5 (4.3) | 21.8 (3.6) | 0.13 | 0.96 (0.91-1.01) | 21.9 (3.6) | 22.5 (4.0) | 0.11 | 1.04 (0.99-1.10) |
| **Hb1Ac** (mean, SD) | 5.29 (0.3) | 5.26 (0.3) | 0.33 | 0.71 (0.36-1.42) | 5.25 (0.3) | 5.32 (0.3) | **0.014** | **2.61 (1.21-5.66)** |
| **Vitamin D** (mean, SD) | 53.3 (22.5) | 53.9 (23.5) | 0.81 | 1.00 (0.99-1.01) | 39.1 (19.5) | 42.4 (21.8) | 0.12 | 1.01 (1.00-1.02) |
| **Self-rated health** | | | **0.024** | | | | 0.83 | |
| Excellent | 44 (51.8) | 41 (48.2) | | 1.0 (ref) | 52 (44.8) | 64 (55.2) | | 1.0 (ref) |
| Good | 131 (64.5) | 72 (35.5) | | **0.59 (0.35-0.99)** | 82 (47.7) | 90 (52.3) | | 0.89 (0.56-1.43) |
| Neither good or bad | 50 (75.8) | 16 (24.2) | | **0.34 (0.17-0.69)** | 45 (50.6) | 44 (49.4) | | 0.79 (0.46-1.38) |
| Bad | 10 (62.5) | 6 (37.5) | | 0.64 (0.22-1.93) | 8 (42.1) | 11 (57.9) | | 1.12 (0.42-3.00) |
| **Hormonal contraceptive use (female-only)** | | | 0.75 | | | | | |
| Non-user | 160 (63.0) | 94 (37.0) | | 1.0 (ref) | | | | |
| Progestin-only [e] | 10 (74.1) | 4 (28.6) | | 0.68 (0.21-2.23) | | | | |
| Combination [f] | 61 (61.0) | 39 (39.0) | | 1.09 (0.68-1.75) | | | | |
| **Snuff use** | | | 0.11 | | | | 0.39 | |
| No, never | 169 (65.8) | 88 (34.2) | | 1.0 (ref) | 122 (49.0) | 127 (51.0) | | 1.0 (ref) |
| Sometimes or daily | 65 (57.0) | 49 (43.0) | | | 65 (44.5) | 81 (55.5) | | |
| **Alcohol use** | | | 0.18 | | | | 0.89 | |
| Never | 68 (70.8) | 28 (29.2) | | 1.0 (ref) | 60 (49.2) | 62 (50.8) | | 1.0 (ref) |
| Once per month or less | 97 (59.5) | 66 (40.5) | | 1.65 (0.96-2.84) | 70 (46.4) | 81 (53.6) | | 1.12 (0.69-1.81) |
| Two or more times per month | 70 (61.9) | 43 (38.1) | | 1.49 (0.83-2.67) | 57 (47.1) | 64 (52.9) | | 1.09 (0.66-1.80) |
| **Physical activity** [g] | | | 0.49 | | | | 0.88 | |
| No | 30 (58.8) | 21 (41.2) | | 1.0 (ref) | 55 (47.8) | 60 (52.2) | | 1.0 (ref) |
| Yes | 205 (63.9) | 116 (36.1) | | 0.81 (0.44-1.48) | 132 (47.0) | 149 (53.0) | | 1.04 (0.67-1.60) |
| **Atopic eczema** [h] | | | **0.025** | | | | **0.026** | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| No | 199 (66.1) | 102 (33.9) | | 1.0 (ref) | 172 (49.3) | 177 (50.7) | | 1.0 (ref) |
| Yes | 34 (50.7) | 33 (49.3) | | **1.89 (1.11-3.23)** | 14 (31.8) | 30 (68.2) | | **2.08 (1.07-4.06)** |
| **Allergic rhinitis** [i] | | | 0.44 | | | | **0.002** | |
| No | 212 (63.9) | 120 (36.1) | | 1.0 (ref) | 176 (49.6) | 179 (50.4) | | 1.0 (ref) |
| Yes | 20 (57.1) | 15 (42.9) | | 1.33 (0.65-2.68) | 8 (22.9) | 27 (77.1) | | **3.32 (1.47-7.50)** |

N= numbers; BMI=body mass index (kg/m$^2$); HbA1c, glycated haemoglobin (mmol/L); Vitamin D, 25-hydroxyvitamin D [25(OH)D] (nmol/L); HC use=Hormonal contraceptive use.
*Known or suggested risk factors for *S. aureus* carriage status.
**Values are the number of subjects (%) if not otherwise stated; total number of subjects may vary due to missing information.
[a] Observations with two negative, or one negative and one positive *S. aureus* nasal swab cultures defined as non-carrier
[b] Observations with two *S. aureus* positive nasal cultures defined as carriers
[c] Independent t-test for continuous variables and Chi-square test for categorical variables.
[d] Univariable Binary logistic regression
[e] Progestin-only: Cerazette®, Implanon® or Depo-provera®
[f] Combination contraceptive: Mercilon®, Yasminelle®, Loette 28®, Yasmin®, Microgynon®, Zyrona®, Synfase®, Evra® or Nuvaring®
[g] Recreational physical activity: Low level= reading, watching TV, or other sedentary activity; Medium level= Walking, cycling, or other forms of exercise at least 4 hours a week; High level= Participation in recreational sports, heavy outdoor activities with a minimum duration of 4 hours a week, or participation in heavy training or sports competitions regularly several times a week. Medium and high= Yes and Low level=No.
[h] Have a doctor ever said that you have children's eczema or atopic eczema? No, and do not know=No.
[i] Have a doctor ever said that you have hay-fever or allergic rhinitis? No, and do not know=No.

Table 3 gives an overview of associations between potential risk factors and *S. aureus* nasal carriage. In males, higher levels of HbA1c were statistically significantly associated with higher prevalence of *S. aureus* nasal carriage OR = 2.61 per 0.3 mmol/L change in HbA1c. In females, those who reported excellent health had higher prevalence of carriage than those who reported "Good" or "Neither good or bad" health.

Atopic eczema was statistically significantly associated with higher prevalence of *S. aureus* nasal carriage in both female and males. Among male participants with allergic rhinitis, 77.1% were carriers compared to 22.9% among non-carriers (p=0.002).

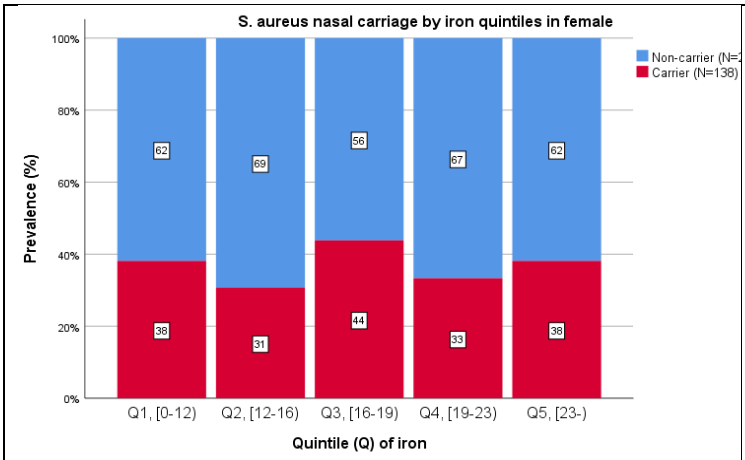


Fig. 3. Prevalence of *S. aureus* carriage by iron quintiles in female.

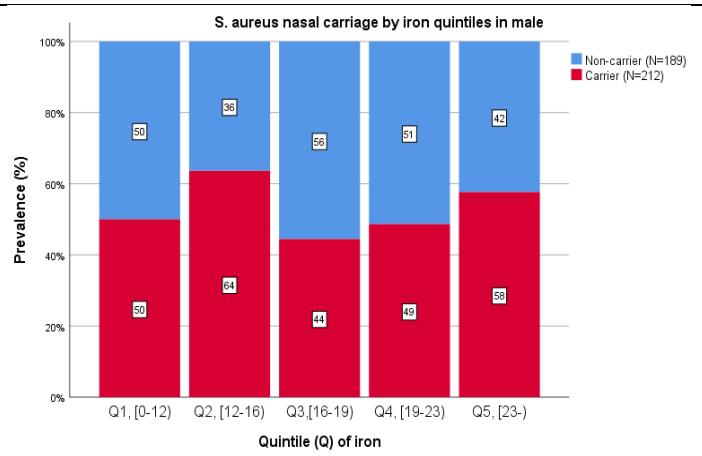


Fig. 4. Prevalence of *S. aureus* carriage by iron quintiles in male.

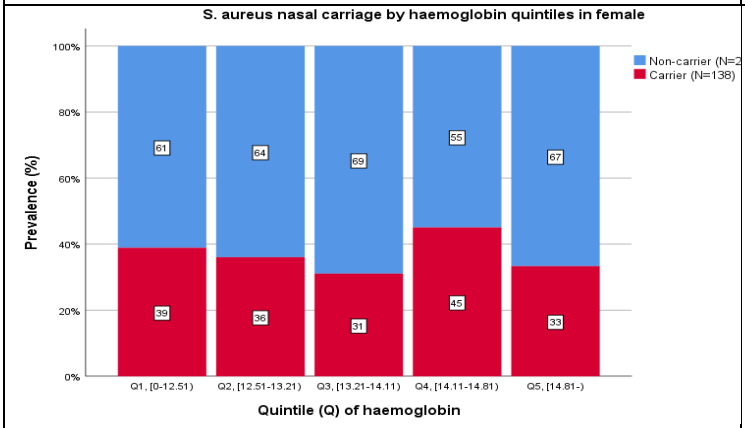


Fig. 5. Prevalence of *S. aureus* carriage by haemoglobin quintiles in female.

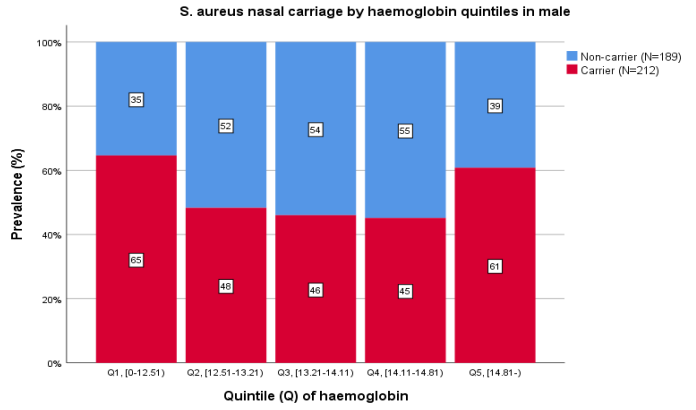


Fig. 6. Prevalence of *S. aureus* carriage by iron quintiles in male.

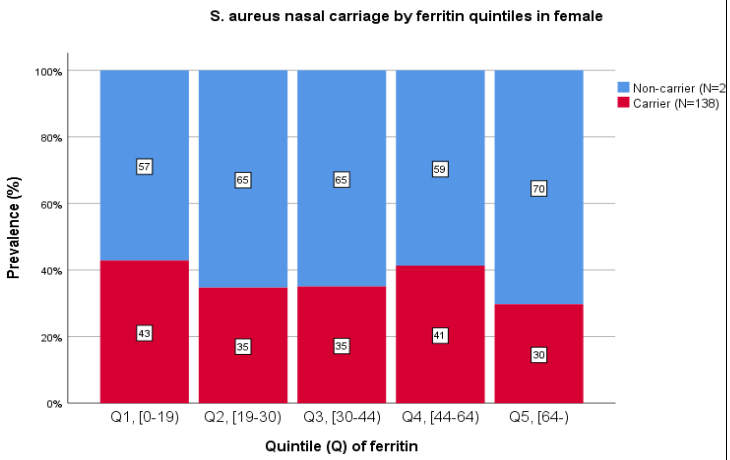


Fig. 7. Prevalence of *S. aureus* carriage by ferritin quintiles in female.

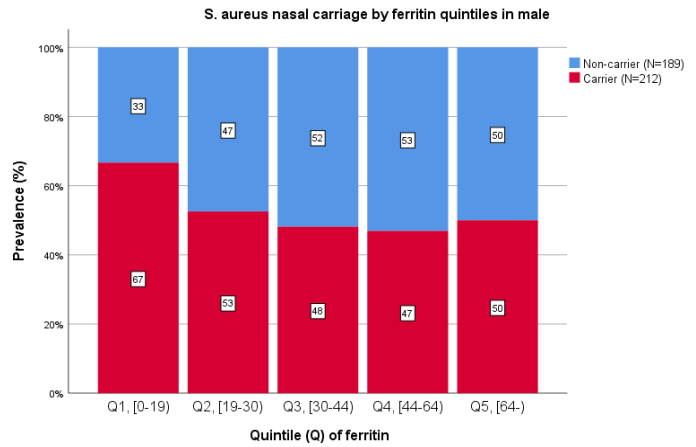


Fig. 8. Prevalence of *S. aureus* carriage by ferritin quintiles in male.

The histograms in figure 3-8 illustrate prevalence of *S. aureus* nasal carriage (x-axis) by iron (µmol/L), haemoglobin (g/dL) and ferritin (µg/L) quintiles (y-axis) in female and male. Each histogram was stratified into iron biomarkers by 1st quintile-5th quintile concentrations intervals.

Males' iron and haemoglobin histograms suggested a U-shaped prevalence distribution in *S. aureus* nasal carriage (figure 4 and 6). The 3rd and 4th quintiles of iron showed about 20% and 15% lower *S. aureus* prevalence compared to 2nd quintile, and 14% and 9% lower frequency compared to 5th iron quintile. Moreover, the 2nd, 3rd and 4th quintile of haemoglobin showed about 17% and 13%; 19% and 15%; 20% and 16% lower *S. aureus* prevalence compared to 1st and 5th quintile of haemoglobin, respectively.

Females iron and haemoglobin histograms (figure 3 and 5) did not show a similar prevalence distribution for *S. aureus* carriage compared to males. The adolescent's quintiles of ferritin distribution indicated a threshold at <19µg/L between 1st and 2nd quintiles (figure 7 and 8). In males, the 2nd, 3rd, 4th, and 5th quintile of ferritin showed 14%, 19%, 20% and 17% lower *S. aureus* nasal carriage prevalence versus 1st quintile, respectively.

Table 4. Association between level of iron and *S. aureus* nasal carriage. Odds ratio (OR) and 95 % confidence intervals (95 % CI) from a binary logistic regression analysis. Observations with two *S. aureus* positive nasal cultures defined as carriers. The Tromsø Study Fit Futures 1.

| | **Female (N=375)** | | **Male (N=401)** | |
|---|---|---|---|---|
| | **OR[a] (95%CI)** | **OR[b] (95%CI)** | **OR[a] (95%CI)** | **OR[b] (95%CI)** |
| **Iron,** µmol/L | | | | |
| 1st quintile, <12) | 0.79 (0.41-1.52) | 0.92 (0.46-1.85) | 1.25 (0.68-2.31) | 1.17 (0.62-2.20) |
| 2nd quintile, [12,16) | 0.57 (0.29-1.11) | 0.64 (0.32-1.31) | **2.20 (1.16-4.17)** | **2.05 (1.06-3.95)** |
| 3rd quintile, [16,19) | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 4th quintile, [19,23) | 0.64 (0.32-1.26) | 0.58 (0.28-1.19) | 1.17 (0.62-2.20) | 1.18 (0.61-2.27) |
| 5th quintile, ≥23 | 0.79 (0 .41-1.52) | 0.87 (0.44-1.72) | 1.69 (0.92-3.13) | 1.49 (0.79-2.84) |
| **Age** | 1.12 (0.72-1.46) | 1.20 (0.80-1.78) | 1.10 (0.79-1.54) | 1.02 (0.72-1.44) |
| **BMI** | | 0.95 (0.90-1.01) | | 1.03 (0.98-1.09) |
| **Vitamin D** | | 1.00 (0.99-1.01) | | 1.01 (1.00-1.02) |
| **Atopic eczema [c]** | | | | |
| No | | 1.0 (ref) | | 1.0 (ref) |
| Yes | | **2.03 (1.16-3.53)** | | 1.90 (0.96-3.75) |
| **Snuff use** | | | | |
| No | | 1.0 (ref) | | 1.0 (ref) |
| Yes | | **1.79 (1.07-2.99)** | | 1.22 (0.79-1.88) |
| **Hormonal contraceptive (female only)** | | | | |
| No-user | | 1.0 (ref) | | |
| Progestin-only [d] | | 0.61 (0.17-2.11) | | |
| Combination [e] | | 0.90 (0.53-1.54) | | |

N=numbers; BMI=body mass index (kg/m$^2$); Vitamin D, 25-hydroxyvitamin D [25(OH)D] (nmol/L)

[a] age-adjusted binary logistic regression; [b] binary logistic regression; OR= Odds ratios; CI= 95% confidence intervals.

[c] Have a doctor ever said that you have children's eczema or atopic eczema? No, and do not know=No.

[d] Progestin-only: Cerazette®, Implanon®or Depo-provera®

[e] Combination contraceptive: Mercilon®, Yasminelle®, Loette 28®, Yasmin®, Microgynon®, Zyrona®, Synfase®, Evra® or Nuvaring®

Each iron biomarkers were investigated in a sex-stratified binary logistic regression model. One regression model was age-adjusted, and the second model also adjusted for known risk factors for *S. aureus* carriage: BMI, vitamin D, atopic eczema, snuff use and HC (female only). In the following text on results estimates from the second model are used.

Males in the 2nd [12,15) µmol/L versus the 3rd quintile (reference: [16,19) µmol/L) of iron had a 2.05 times higher odds of *S. aureus* nasal carriage (OR= 2.05; 95%CI [1.06, 3.95]; p=0.033) (p-value not in table). There was no similar evidence for differences in odds in *S. aureus* carriage between females in different iron quintiles using logistic regression.

In a sensitivity analysis, iron outliers observed in SPSS box plot (figure 9-10), were excluded from the logistic regression analysis for both sexes (female: n=6; observed range 33-59 µmol/L and males: n=10; observed range 35-57 µmol/L). Removing iron outliers did not change the quintile limits significantly. Thus, produced similar associations between iron quintiles and nasal carriage as the original analysis: males in 2$^{nd}$ iron quintile had 2.08 times higher odds of *S. aureus* nasal carriage (OR=2.08; 95% CI [1.07, 4.01]; p=0.030) versus the 3$^{rd}$ quintile (results not shown in tables).

Table 5. Association between level of haemoglobin and *S. aureus* nasal carriage. Odds ratio (OR) and 95 % confidence intervals (95 % CI) from a binary logistic regression analysis. Observations with two *S. aureus* positive nasal cultures defined as carriers. The Tromsø Study Fit Futures 1.

| | **Female (N=375)** | | **Male (N=401)** | |
|---|---|---|---|---|
| | **OR[a] (95%CI)** | **OR[b] (95%CI)** | **OR[a] (95%CI)** | **OR[b] (95%CI)** |
| **Haemoglobin,** g/dL | | | | |
| 1st quintile, [0-12.51) | 1.41 (0.71-2.80) | 1.42 (0.69-2.90) | **2.15 (1.10-4.21)** | **2.27 (1.14-4.53)** |
| 2nd quintile [12.51,13.21) | 1.25 (0.65-2.42) | 1.06 (0.53-2.12) | 1.09 (0.57-2.9) | 1.16 (0.59-2.26) |
| 3rd quintile [13.21,14.11) | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 4th quintile [14.11,14.81) | 1.82 (0.92-3.60) | 1.81 (0.89-3.67) | 0.96 (0.50-1.82) | 1.03 (0.53-2.00) |
| 5th quintile ≥14.81 | 1.11 (0.55-2.23) | 1.08 (0.51-2.27) | 1.78 (0.89-3.54) | 1.80 (0.89-3.66) |
| **Age** | 1.02 (0.72-1.46) | 1.17 (0.79-1.71) | 1.08 (0.77-1.52) | 1.01 (0.71-1.44) |
| **BMI** | | 0.96 (0.90-1.01) | | 1.05 (0.99-1.10) |
| **Vitamin D** | | 1.00 (0.99-1.01) | | 1.01 (1.00-1.02) |
| **Atopic eczema [c]** | | | | |
| No | | 1.0 (ref) | | 1.0 (ref) |
| Yes | | **2.03 (1.16-3.54)** | | 1.86 (0.94-3.71) |
| **Snuff use** | | | | |
| No | | 1.0 (ref) | | 1.0 (ref) |
| Yes | | **1.69 (1.02-2.82)** | | 1.18 (0.77-1.83) |
| **Hormonal contraceptive (female only)** | | | | |
| No-user | | 1.0 (ref) | | |
| Progestin-only [d] | | 0.59 (0.17-2.08) | | |
| Combination [e] | | 0.93 (0.54-1.60) | | |

N= Numbers; BMI=body mass index (kg/m$^2$); Vitamin D, 25-hydroxyvitamin D [25(OH)D] (nmol/L)

[a] age-adjusted binary logistic regression; [b] binary logistic regression; OR= Odds ratios; CI= 95% confidence intervals.

[c] Have a doctor ever said that you have children's eczema or atopic eczema? No, and Do not know=No.

[d] Progestin-only: Cerazette®, Implanon® or Depo-provera®

[e] Combination contraceptive: Mercilon®, Yasminelle®, Loette 28®, Yasmin®, Microgynon®, Zyrona®, Synfase®, Evra® or Nuvaring®

Males in the 1st (<12.51 g/dL) versus the 3rd quintile of haemoglobin (reference: [13.21,14.11) g/dL) of haemoglobin had a 2.27 times higher odds of *S. aureus* nasal carriage (OR= 2.27; 95%CI [1.14, 4.53]; p=0.020) (p-value not in table). In females, there was no similar evidence for differences in odds in *S. aureus* carriage between different haemoglobin quintiles.

Haemoglobin outliers' impact on the estimates was investigated by excluding observed outliers in SPSS box plot (figure 11-12) (female: n=8; observed range 8.10-10.2 g/dL; males; n=11; observed range 11.00-12.80 g/dL). Removing haemoglobin outliers did not change the quintile limits significantly. The sensitivity analysis provided similar associations between haemoglobin and *S. aureus* nasal carriage compared to the original analysis. Males in the 1st versus the 3rd haemoglobin quintile had 2.32 times higher odds of nasal carriage (OR=2.32; 95%CI [1.13, 4.73]; p=0.021) (results not shown in tables).

In an additional regression analysis, including all observations, 1st haemoglobin quintile was selected as a reference group. Among males in the 2nd ([12.51, 13.21) g/dL; OR=0.51, 95%CI [0.27, 0.96]; 3rd ([13.21, 14.11) g/dL; OR=0.44, 95%CI [0.22, 0.88] and 4thquintiles ([14.11, 14.81) g/dL; OR=0.45, 95% CI [0.24, 0.85]) were associated with 49%, 56% and 55% lower odds of *S. aureus* nasal carriage versus the 1st haemoglobin quintile. In females, no statistically significant differences in odds of *S. aureus* nasal carriage were observed between different haemoglobin quintiles (results not shown in tables).

Table 6. Association between level of ferritin and *S. aureus* nasal carriage. Odds ratio (OR) and 95 % confidence intervals (95 % CI) from a binary logistic regression analysis. Observations with two *S. aureus* positive nasal cultures defined as carriers. The Tromsø Study Fit Futures 1.

| | **Female (N= 375)** | | **Male (N=401)** | |
|---|---|---|---|---|
| | **OR[a] (95%CI)** | **OR[b] (95%CI)** | **OR[a] (95%CI)** | **OR[b] (95%CI)** |
| **Ferritin,** µg/L | | | | |
| 1st quintile, [0,19) | 1.39 (0.72-2.66) | 1.44 (0.73-2.85) | **2.17 (1.15-4.11)** | **2.48 (1.27-4.84)** |
| 2nd quintile, [19,30) | 0.99 (0.50-1.94) | 0.94 (0.46-1.90) | 1.21 (0.65-2.27) | 1.25 (0.66-2.38) |
| 3rd quintile, [30,44) | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 4th quintile, [44,64) | 1.31 (0.68 (2.52) | 1.29 (0.65-2.58) | 0.93 (0.50-1.73) | 0.96 (0.51-1.81) |
| 5th quintile, ≥64 | 0.78 (0.39-1.55) | 0.77 (0.38-1.59) | 1.05 (0.56-1.95) | 1.16 (0.61-2.20) |
| **Age** | 1.04 (0.73-1.48) | 1.17 (0.79-1.74) | 1.11 (0.79-1.57) | 1.05 (0.74-1.50) |
| **BMI** | | 0.95 (0.90-1.01) | | 1.05 (0.99-1.11) |
| **Vitamin D** | | 1.00 (0.99-1.01) | | 1.01 (1.00-1.02) |
| **Atopic eczema [c]** | | | | |
| No | | 1.0 (ref) | | 1.0 (ref) |
| Yes | | **1.98 (1.14-3.44)** | | 1.83 (0.92-3.61) |
| **Snuff use** | | | | |
| No | | 1.0 (ref) | | 1.0 (ref) |
| Yes | | **1.72 (1.04-2.87)** | | 1.30 (0.84-2.00) |
| **Hormonal contraceptive (female only)** | | | | |
| No-user | | 1.0 (ref) | | |
| Progestin-only[d] | | 0.68 (0.19-2.39) | | |
| Combination [e] | | 0.94 (0.55-1.61) | | |

N=Numbers; BMI=body mass index (kg/m$^2$); Vitamin D, 25-hydroxyvitamin D [25(OH)D] (nmol/L)

[a] age-adjusted binary logistic regression; [b] binary logistic regression; OR= Odds ratio; CI= 95% confidence intervals.

[c] Have a doctor ever said that you have children's eczema or atopic eczema? No, and do not know=No.

[d] Progestin-only: Cerazette®, Implanon® or Depo-provera®

[e] Combination contraceptive: Mercilon®, Yasminelle®, Loette 28®, Yasmin®, Microgynon®, Zyrona®, Synfase®, Evra® or Nuvaring®

Males in the 1st (<19 µg/L) versus the 3rd ferritin quintile (reference: [30,44) µg/L) had a 2.48 times higher odds of *S. aureus* nasal carriage (OR= 2.48; 95%CI[1.27, 4.84]; p=0.008) (p-value not in table). Between females' ferritin quintiles, there was no evidence for a difference in odds in *S. aureus* carriage.

Ferritin outliers' impact on the estimates was investigated by excluding observed outliers in SPSS box plot (figure 13-14) (female: n=15; observed range: 81-144 µg/L; males; n=10; observed range: 137-212 µg/L). Removing ferritin outliers did not change the quintile limits significantly. The sensitivity analysis provided similar associations between ferritin and *S. aureus* nasal carriage compared to the original analysis. Males in the 1st versus the 3rd ferritin quintile had 2.18 times higher odds of nasal carriage (OR=2.18; 95% CI [1.15, 4.11]; p=0.017) (results not shown in tables).

In an additional regression analysis, including all observations, 1st quintile of ferritin was selected as a reference group. Among males, in the 2nd ([19,30) µg/L; OR=0.51, 95%CI [0.26, 0.99]; 3rd ([30, 44) µg/L; OR=0.40, 95%CI [0.21, 0.79]; 4th ([44, 64) µg/L; OR=0.39, 95%CI [0.20, 0.76] and 5th quintiles of ferritin (≥64 µg/L; OR=0.47, 95%CI [0.24, 0.91]) were associated with 49%, 60%, 61% and 53% lower odds of *S. aureus* nasal carriage versus the 1st quintile of ferritin. In females, no statistically significant differences in odds of *S. aureus* nasal carriage were observed between different ferritin quintiles (results not shown in tables).

## 3.1 Discussion

To our knowledge, this is the first study to investigate an association between iron biomarkers and persistent *S. aureus* nasal carriage in a general adolescent population. The analysis showed a relationship between iron biomarkers and the odds for nasal *S. aureus* carriage in men. Similar relationships were not detected in females. The results will be discussed in the next paragraphs.

## 3.2 Main results

### 3.2.1 Iron status and *S. aureus* nasal carriage

According to existing knowledge, iron is a vital micronutrition and absorption is regulated to the human body's needs. As described previously, essential human in-vivo life processes are dependent on iron, e.g. to maintain a healthy immune system. However, microbial pathogens also require iron for survival. Thus, iron is in the centre of a nutrition battle between humans and bacteria (37).

Adolescents is a period with increased needs with the expansion of blood volume and increase in muscle mass. In young female, menstruation also leads to iron losses (32). Physiological and biological changes, in addition to a change in lifestyle, may change and shape the immune system and contribute to the individual's *S. aureus* carriage status. Adolescents may be a time were life stressors or events disrupt immune development (43).

Our data suggest a U-shaped *S. aureus* prevalence distribution between quintiles of iron and haemoglobin in males (figure 4 and 6). The observed U-shaped distribution is in accordance with Dragesmith and Prentice suggested relationship between iron status and the susceptibility to infections (37).

The prevalence of *S. aureus* nasal persistent carriage was 36.8 % (138/375) in females and 52.9 % (212/401) in males. Young males with [12,16) versus [16,19) µmol/L quintile of serum iron; <12.51 versus [13.21, 14.11) g/dL quintile of haemoglobin, and <19 versus [30-44) µg/L quintile of ferritin had 2.05 times, 2.27 times and 2.48 times higher odds of carriage, respectively. Among females, no such association was detected between the different quintiles for the respective iron biomarkers.

Removing suggested outliers for the respective iron biomarkers did not have any significant influence in the observed results in males and females versus the original regression analysis. Given the above, this suggests that our data is internally reproducible and consistent. We did not find similar population studies to compare the current results. However, our data indicate an advantage for males to have iron and haemoglobin concentrations around the mean and ferritin around the median values- thus associated with reduced odds for persistent carriage (see figure 4,6 and 8).

The risk of infections increases with low iron levels; in some cases, morbidity and mortality could be predicted based on iron deficiency (53). Globally, iron deficiency is the most common nutritional disorder (54). Since adolescence is a time with increased iron needs, this group should be given special attention to ensure iron requirements are met to reduce the susceptibility to *S. aureus* carriage.

In our study, the observed association between low iron, haemoglobin and ferritin levels and increased odds of *S. aureus* nasal carriage was restricted to men. In general, females show a significantly reduced risk of *S. aureus* carriage and developing infectious diseases compared to men (55,56). Thus, the sex-dependent association between iron status and *S. aureus* carriage in our study may partly be explained by differences in immune responses between men and women.

One cause of this difference between *S. aureus* persistent carriage may lie on the X chromosomes. One X chromosome consists of about 1000 genes, and several of these genes play an essential role in the immune response. For comparison, the Y chromosome consists of only 100 genes (57). Moreover, partly inactivation of one of the X-chromosomes in females gives them a double expression of immune-related genes. Thereby, females are provided with a stronger immune system and an advantage compared to men (58).

Besides, reproductive hormones like testosterone, progesterone and estrogen influence the functioning of immune cells. In general, estrogen and progesterone exist in higher concentrations in females. Likewise, testosterone appears in raised concentrations in men compared to females. In vivo, sex steroids bind to their respective receptors that are expressed

in most immune cells and therefore, included in essentials immunological pathways. Because estrogen enhances immune functions while androgens exercise suppressive effects on both innate and adaptive immune response, females, in general, are better protected against infections (59,60). Influence of sex hormones may, therefore, explain the gender difference in our results (61).

Also, environmental factors play a role in the transmission of *S. aureus.* Males may be more prone to risk-seeking behaviour and have poorer hand-hygiene behaviour compared to females (62). These aspects, together with the biological factors discussed above, may make males more susceptible to persistent *S. aureus* carriage.

## 3.3 Covariates

### 3.3.1 Age

Previous studies confirmed an association between young age and nasal *S. aureus* carriage (6,63). Kovacs and colleagues observed 40-50% *S. aureus* carriers in schoolchildren at 7-14 years of age (63). In TFF1, the mean age of the study population was 16.2 years; the nasal *S. aureus* carriage prevalence was 36.8% and 52.9% for girls and boys, respectively. The corresponding prevalence in a non-smoking adult population in the Tromsø Study (2007-2008), was lower 21.3% and 34.1% (64). The reasons for the higher nasal *S. aureus* carriage among adolescents is not clear.

### 3.3.2 BMI

Olsen and colleagues found a positive relationship between BMI and nasal *S. aureus* colonisation in young and premenopausal females in a general population in the Tromsø Study (2007-2008). For each 2.5 kg/m$^2$ increase in BMI, a 7 % higher odds of nasal colonisation was detected (65). Moreover, in a recent Danish Blood donor cohort, the researchers observed that *S. aureus* nasal carriage also increased with obesity (66). Obesity is linked to increased serum glucose levels and type 2 diabetes; elevated glucose levels may increase the susceptibility to *S. aureus* carriage (67). The current study did not find any increased odds for *S. aureus* carriage with higher BMI. However, since BMI is a known risk factor for *S. aureus* carriage, BMI was adjusted for in the regression analysis.

### 3.3.3 Vitamin D

The Tromsø Staph and Skin Study observed an inverse association between serum vitamin D and *S. aureus* carriage in non-smoking middle-aged males. However, the study did not find a similar relationship in female, younger and older men (68). In the Tromsø Study Fit futures 2 (TFF2), an increased odds for *S. aureus* nasal carriage was discovered in young females in an insufficiency vitamin D- group versus a normal group (7). The current study did not divide the vitamin D variable into different vitamin D groups. Though, since low vitamin D levels are a known risk factor for *S. aureus* carriage, the variable was included as a covariate in the regression models.

### 3.3.4 Atopic eczema

In a systematic review, Totte and colleagues discovered an increased risk of *S. aureus* nasal colonisation in individuals with atopic eczema compared to healthy controls (69). Moreover, atopic eczema was associated with *S. aureus* carriage in adolescents above the Arctic Circle in Norway (reference). However, it is not clear whether the association is due to reverse causation: atopic eczema might make the skin and mucosa more susceptible to *S. aureus* carriage (10). In the current study, atopic eczema was associated with doubled odds for *S. aureus* nasal carriage, and this established risk factor was included in the regression models for both women and men.

### 3.3.5 Snuff use

In our study, females using snuff regularly had increased odds for *S. aureus* nasal carriage compared to those not using snuff. However, the present study did not show a similar association in males. In the TFF2 study which examined hormonal contraceptive use and *S. aureus* nasal carriage; the researchers discovered 1.39 times increased odds for nasal *S. aureus* carrier in snuff using females, but the association was not significant (7). On the other hand, another study detected contamination of *S. aureus* in some snuff products (70). Moreover, snuff use may change the oral cavity and oral mucosa. Thus, snuff use might make the oral cavity more accessible for carriage and permits transmitting of *S. aureus* to the nasal cavity.

### 3.3.6 Hormonal contraceptives

Stensen and colleagues reported 2.14 and 2.44 times higher risk of *S. aureus* nasal carriage in young females using combination hormonal contraceptives with low and high estrogen, respectively (7). The current hormonal contraceptive variable was not divided into low and high estrogen. Thus, this might explain why the present study did not show an increased risk of *S. aureus* nasal carriage in adolescent's female

## 3.4 Strengths and limitations

The advantages of epidemiological observational studies using cross-sectional design are to study associations and creating a hypothesis. However, the cross-sectional design has several limitations. For instance, this design does not give a clear answer to the causal association between the adolescent's iron-status and persistent *S. aureus* nasal carriage. In the following paragraphs, the current study's internal and external validity are discussed.

### 3.4.1 Internal validity

The internal validity assesses whether the assumed hypothesis can explain the current observations and if the results are correct and valid for the population.

TFF1 was based on a population-based study with a high attendance rate (92.9 %). The high attendance rate reduced the risk of selection bias. The population-based design provided essential information regarding adolescents. Moreover, the design allowed adjusting for known and suggested risk factors/confounders, and thereby reduced the risk of incorrect estimation between the respective iron biomarkers and *S. aureus* carriage. However, the observed associations might still be due to another unknown confounder (s).

The cross-sectional design determines the exposure of iron and ferrous molecules and *S. aureus* at the same time. To reduce random errors, the Fit Futures administration developed strict protocols; blood- and nasal sampling was performed by trained health personnel. Also, repeated nasal swabs harvested at the Research Unit and school one week later reduced the risk of detection bias. Unfortunately, the bias in sampling can never be completely ruled out.

Laboratory analysis was carried out by trained personnel according to internally validated methods. The latter reduced the risk of performance and detection bias. Excluded cases with no growth in control blood agar were assumed to be due to incorrect performance of

sampling, or errors in sampling equipment. Some students were excluded due to the refusal of blood sampling and other medical events. However, a large study-population reduced the effect of random errors.

The TFF1 study participants' mean age was representative of the group of adolescents. Admittedly, the study questionnaire was based on the Tromsø 6 questionnaire and not validated among adolescents. Some variables investigated in the questionnaire (ex. alcohol, snuff tobacco, atopic eczema, allergic rhinitis), and interview (HC and antibiotic) might be both underreported and overreported. Hence, wrong reporting may contribute to information bias. For example, individuals tend to underreport factors contributing to health risks like snuff tobacco and alcohol. Besides, the Norwegian tobacco and alcohol law is strict; it is illegal to sell to individuals younger than 18 years old. Also, sensitive information concerning sex behavioural (use of HC), could be underreported (71).

### 3.4.2 External validity

The external validity assesses whether the current observations also apply to other populations.

The Follow-up service in Troms county reported 919 adolescents not employed or in education in 2011/2012 (72). Unfortunately, it was unknown how many of these youths living in Tromsø municipality. However, a high attendance rate indicates that the current study was representative of adolescents both locally and nationally.

Also, the study was probably representative of other youths living in similar socioeconomic standards in the Nordic countries. Given the above, supplementary research will be needed to verify if the thesis results correspond to other age groups. For instance, the follow-up study TFF2 and the Tromsø fit Futures 3 (TFF3) which is in the planning phase (73).

# 4 Conclusion

The current study supports an association between low iron-status and persistent nasal *S. aureus* carriage in males. Our data did not detect similar relationship in young females. Efforts to identify and address low iron levels to prevent *S. aureus* carriage could play a role to reduce morbidity and mortality worldwide.

The relationship between iron-status and *S. aureus* carriage in other age-groups should be addressed in future prospective studies of *S. aureus* carriage.

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# Appendix

| Table 7. List of variables. The Tromsø Study-Fit Futures | | | | | |
|---|---|---|---|---|---|
| **Variable*** | Material | Unit | Type | Descriptive measurement** | Analytic measurements |
| Sex | | Male/female | Categorical | Proportions | Regression |
| Age | | Years | Continuous | Mean, SD | Regression |
| Height | | cm | Continuous | | |
| Weight | | kg | Continuous | | |
| BMI | | $Kg/m^2$ | Continuous | Mean, SD | Regression |
| Iron | Serum | µmol/L | Continuous | Mean, SD | Regression |
| Haemoglobin | EDTA-blood | g/dL | Continuous | Mean, SD | Regression |
| Ferritin | Serum | µg/L | Continuous | Median | Regression |
| Vitamin D | Serum | nmol/L | Continuous | Mean, SD | Regression |
| HbA1c | EDTA-blood | mmol/L | Continuous | Mean, SD | |
| Isolated nasal *S. aureus* | | Yes/no | Categorical | Proportions | Regression |
| Antibiotic use last 24 hours | | Yes/no | Categorical | | |
| Hormonal contraceptive use (girls only) | | Yes/no | Categorical | Proportions | Regression |
| Hormonal contraceptive type | | | Categorical | Proportions | Regression |
| Snuff use | | Yes/no | Categorical | Proportions | Regression |
| Alcohol use | | Yes/no | Categorical | Proportions | |
| Self-rated health | | | Categorical | Proportions | |
| Physical activity | | | Categorical | Proportions | |
| Allergic rhinitis | | Yes/no | Categorical | Proportions | |
| Atopic eczema | | Yes/no | Categorical | | Regression |

*Known or suggested risk factors for *S. aureus* carriage status.

**SD=Standard deviation

BMI=body mass index ($kg/m^2$); HbA1c, glycated haemoglobin (mmol/L); Vitamin D, 25-hydroxyvitamin D [25(OH)D] (nmol/L).

[f] Recreational physical activity: Low level= reading, watching TV, or other sedentary activity; Medium level= Walking, cycling, or other forms of exercise at least 4 hours a week; High level= Participation in recreational sports, heavy outdoor activities with a minimum duration of 4 hours a week, or participation in heavy training or sports competitions regularly several times a week. Medium and high= Yes and Low level=No.

[g] Have a doctor ever said that you have children's eczema or atopic eczema? No, and do not know=No.

[h] Have a doctor ever said that you have hay-fever or allergic rhinitis? No, and do not know=No.

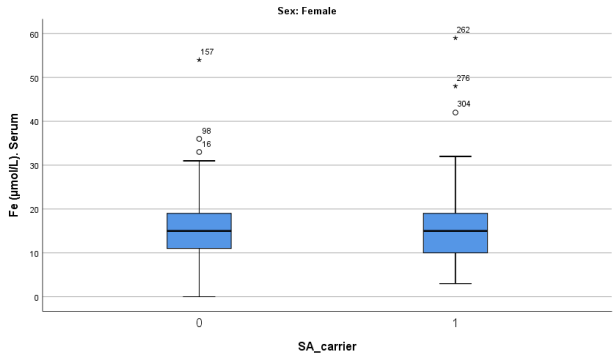


Figure 9. Iron boxplot for female *S. aureus* non-carriers (group 0; n=237) and carriers (group 1; n=138).

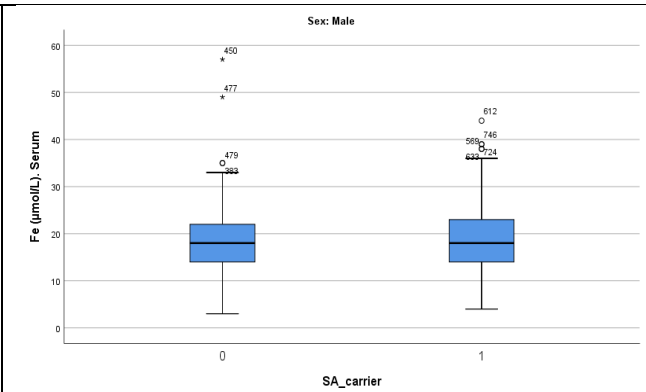


*Figure 10. Iron boxplot for male S. aureus non-carriers (group 0; n=189) and carriers (group 1; n=212).*

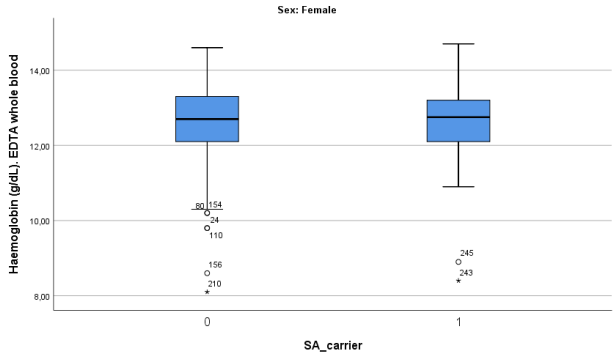


Figure 11. Haemoglobin boxplot for female *S. aureus* non-carriers (group 0; n=237) and carriers (group 1; n=138).

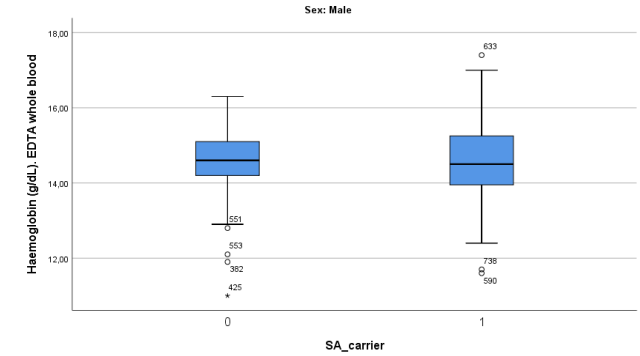


Figure 12. Haemoglobin boxplot for male *S. aureus* non-carriers (group 0; n=189) and carriers (group 1; n=212).

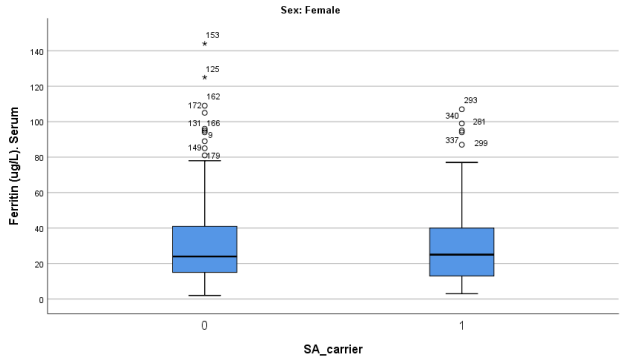


Figure 13. Ferritin boxplot for female *S. aureus* non-carriers (group 0; n=237) and carriers (group 1; n=138).

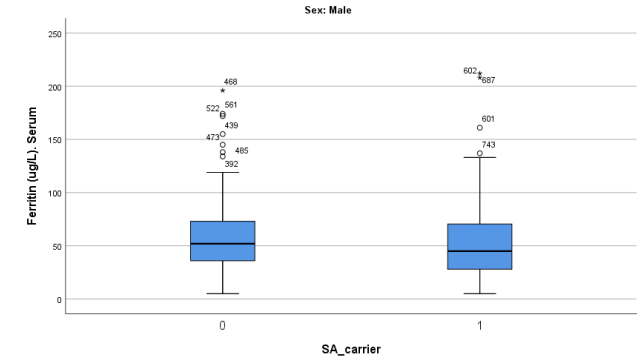


Figure 14. Ferritin boxplot for male S. aureus non-carriers (group 0; n=189) and carriers (group 1; n=212).

The boxplots in figure 9-14, illustrates the distribution of iron, haemoglobin and ferritin for female and male *S. aureus* non-carriers and carriers.
0=non-carriers, 1=carriers; o= outliers, *=extreme values.