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Social network analysis of *Staphylococcus aureus* carriage in a general youth population

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ABSTRACT

Objectives: *Staphylococcus aureus* carriage increases the risk of infection. We used social network analysis to evaluate whether contacts have the same *S. aureus* genotype indicating direct transmission or whether contagiousness is an indirect effect of contacts sharing the same lifestyle or characteristics.
Methods: The Fit Futures 1 study collected data on social contact among 1038 high school students. *S. aureus* carriage was determined from two nasal swab cultures and the genotype was determined by spa-typing of positive throat swabs.
Results: *S. aureus* carriage and *spa*-type were transmitted in the social network ($P < 0.001$). The probability of carriage increased by 5% for each *S. aureus* positive contact. Male sex was associated with a 15% lower risk of transmission compared to the female sex, although the carriage prevalence was higher for men (36% vs 24%). Students with medium physical activity levels, medium/high alcohol use, or normal weight had a higher number of contacts and an increased risk of transmission ($P < 0.002$).
Conclusion: We demonstrated the direct social transmission of *S. aureus*. Lifestyle factors are associated with the risk of transmission, suggesting the effects of indirect social groups on *S. aureus* carriage, such as friends having more similar environmental exposures. The male predominance in the carriage is determined by sex-specific predisposing host characteristics as the social transmission is less frequent in males than females. Information on social networks may add to a better understanding of *S. aureus* epidemiology.

Introduction

Nasal carriage of *Staphylococcus aureus* has a prevalence of 20-30% in the general adult population (Olsen *et al.*, 2012; Stensen *et al.*, 2021) and 40-50 % in older children and adolescents (Stensen *et al.*, 2019) and is more common among men than women (Olsen *et al.*, 2012). Carriers have an increased risk of autoinfection (Bode *et al.*, 2010; Wertheim *et al.*, 2005). Therefore, prevention of nasal carriage may reduce the disease burden of *S. aureus* (Bode *et al.*, 2010). Epidemiologic studies have searched for modifiable risk factors for *S. aureus* nasal carriage as potential targets for interventions, including body mass index (BMI), serum glucose and vitamin D, exogenous and endogenous hormones, and smoking (Johannessen *et al.*, 2012; Olsen *et al.*, 2012; Olsen *et al.*, 2013;

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Sangvik *et al., 2011*; Stensen *et al.*, 2019; Wertheim *et al.*, 2005). However, these studies did not adjust for social contact.

Direct transmission of *S. aureus* is primarily through physical contact (Knox *et al.*, 2015); however, no other study has evaluated the direct social transmission of *S. aureus* carriage in young populations. In studies that involve transmissible pathogens, an extensive problem with identifying the risk factors is the lack of adjustment for social contact. Biological host risk factors for *S. aureus* carriage may also be determinants of friendship, thereby producing an association by confounding. Predisposing lifestyle risk factors may be contagious with the consequence of researchers incorrectly assuming the transmission of the pathogen. Prevention of *S. aureus* carriage is dependent on identifying key transmission pathways and causal risk factors to correctly evaluate targets for interventions.

Infectious diseases like tuberculosis, HIV infection, and sexually transmitted diseases have been strongly connected to social networks (Jolly and Wylie, 2002; Klovdahl *et al.*, 2001; Rothenberg *et al.*, 1998). These studies demonstrate that the degree and type of contact between individuals play a significant role in disease incidence. One study showed that the introduction of *S. aureus* into a social network of active drug users created a reservoir for the bacteria linked to the general population (Gwizdala *et al.*, 2011). A recent case-control study used network analysis to reveal the transmission of methicillin-resistant *S. aureus* (MRSA) through social network in healthcare (Moldovan *et al.*, 2019). Social group effects also occur in humans, as unrelated individuals living in the same household are found to have more similar microbiota than relatives living in different households (Song *et al.*, 2013). Transmission of *S. aureus* has also been observed within households (Miller *et al.*, 2009).

Therefore, the aim of this study is to estimate the extent to which *S. aureus* carriage follows friendship ties and whether the data support the concept of direct social transmission. We also aim to identify host risk factors for *S. aureus* carriage and differentiate between the risk attributable to social contact among similar individuals compared to biologic or lifestyle-related risk.

Methods

Population and study design

The Fit Futures 1 study (FF1) was a youth health survey conducted from September 2010 to April 2011, inviting all first-year students registered at 8 high schools in the municipalities of Tromsø and Balsfjord, North Norway. Altogether, 508 female and 530 male students participated in the survey (93% participation) (Winther *et al.*, 2014). Participants visited the Clinical Research Unit of the University Hospital of North Norway (UNN) for interviews, questionnaires, clinical examinations, and microbiological samples. Trained research nurses performed all the procedures according to a standard protocol.

Host risk factors

Participants wore light clothing and no shoes. Height (cm) and weight (kg) were measured on an electronic scale with participants wearing light clothing and no shoes, and BMI (kgm^2) was calculated. The participants reported their sex, age, study program, tobacco use, alcohol use, and recreational physical activity through a web-based questionnaire.

The interview covered current hormonal contraceptive use. We categorized hormonal contraceptive use into progestin-only contraceptives and combination contraceptives with high or low ethinylestradiol dosage (Stensen *et al.*, 2019).

Assessment of S. aureus carriage

The research nurses took a first set of nasal and throat swab samples at the hospital, and a second set at school after a mean interval of 17 days. Nasal vestibules were sampled using the same 0.9 % NaCl-moistened sterile rayon-tipped swab, and both tonsillar regions were sampled with another swab. The swabs were immediately placed in a transport medium (Amies Copan, Brescia, Italy) and stored at 4°C for a maximum of 3 days. All samples were analyzed at the Department of Microbiology and Infection Control, UNN, both by direct culture (Olsen *et al.*, 2012) and enrichment culture (Stensen *et al.*, 2019) (Bacto Staphylococcus medium broth, Difco Laboratories, Sparks, Maryland, USA), using blood agar for growth control (Oxoid, UK) and chromID-plates for *S. aureus* detection (SAID, bioMérieux, Marcy I'Etoile, France and MRSA agar plates SmithMed AS/Microbiological media production, Department of Microbiology and Infection Control, UNN). The growth of any bacterial colonies on agar plates was registered as a valid sample. The dominating *S. aureus* colony type was frozen at - 70°C in glycerol-containing liquid medium after confirmation by Staphaurex plus agglutination test (bioMérieux, Marcy I'Etoile, France).

We used *S. aureus* persistent nasal carriage as the main outcome variable in the present analysis as this has been the major phenotype of interest in infection control and epidemiologic studies (van Belkum *et al.*, 2009). We defined persistent carriage as having either two positive direct cultures or two positive enrichment cultures (Supplementary Figure 1). All *S. aureus* isolates from the first throat swab sample taken at the hospital were *spa*-typed (staphylococcal protein A) as part of another study by Sangvik *et al.* (2011).

Social network

We constructed the social network based on the interview question: “Which first level high school students have you had most contact with the last week? Name up to five students at your own school or other schools in Tromsø and Balsfjord.” Reciprocity in the nomination was not mandatory. For each nomination, five “yes/no” questions assessed the type of contact: “Did you have physical contact?”, “Have you been together at school?”, “Have you been together at sports?”, “Have you been together at home?”, “Have you been together at other places?”. This gave five social networks depending on the setting: “physical contact”, “school”, “sport”, “home, and “other” networks. Adding all the relationships together formed a sixth network that was called the “overall” network. To evaluate if the friends mentioned were representative for the participants´social network, the following question was asked: “To what degree does this list of friends give an overview of your social network? Please indicate on a scale from zero (small degree) to ten (high degree).” We excluded 134 nominated friends that did not participate in FF1.

Statistical analysis

We used R version 3.6.3 and R Studio 1.3.1093 for the statistical analysis. To evaluate univariable associations between host factors and *S. aureus* persistent carriage we used Student's *t*-test and chi-square test, with Yates's correction for 2 × 2 tables and Fisher's exact test, when applicable.

In the social network analysis, nodes refer to participants in the network while edges refer to lines representing relationships between participants. To evaluate transmission of *S. aureus* through the social network, we analyzed edges between nodes using Exponential Random Graph Models or additive and multiplicative effects models. We analyzed patterns of connections (non-carriers

Table 1
Characteristics of the study population by Staphylococcus aureus persistent nasal carriage determined by direct and enrichment culture. The Fit Futures 1 study (N = 1038).

	Direct culture			Enrichment culture		
	Positive[c]	Negative[c]	Prevalence	Positive[c]	Negative[c]	Prevalence
Sex			**< 0.001**			**< 0.001**
Male	193	337	36.4 %	255	275	48.1 %
Female	122	386	24.0 %	187	321	36.8 %
Study program			0.99			0.08
General	118	272	30.3 %	163	227	41.8 %
Sports	31	73	29.8 %	55	49	52.9 %
Vocational	166	378	30.5 %	224	320	41.2 %
Smoking			0.93			0.48
Daily	14	34	29.2 %	24	24	50.0 %
Sometimes	59	129	31.4 %	76	112	40.4 %
Never	236	546	30.2 %	333	449	42.6 %
Snuff use			0.79			0.30
Daily	73	172	29.8 %	107	138	43.7 %
Sometimes	43	88	32.8 %	63	68	48.1 %
Never	192	450	29.9 %	263	379	41.0 %
Body mass index category			0.21			0.22
< 18.5 kg/m^2	35	75	31.8 %	55	55	50.0 %
18.5-<25 kg/m^2	201	509	28.3 %	289	421	40.7 %
25-<30 kg/m^2	54	93	36.7 %	68	79	46.3 %
≥30 kg/m^2	22	45	32.8 %	27	40	40.3 %
Physical activity[a]			0.15			0.07
None	80	149	34.9 %	107	122	46.7 %
Light	99	239	29.3 %	129	209	38.2 %
Medium	67	192	25.9 %	105	154	40.5 %
Hard	63	131	32.5 %	93	101	47.9 %
Alcohol intake			0.32			0.780
Never	88	192	31.4 %	115	165	41.1 %
<= 1 Month	134	286	31.9 %	183	237	43.6 %
≥2 Month	86	232	27.0 %	134	184	42.1 %
Hormonal contraceptives[b]			0.76			0.68
Non-user	78	249	23.9 %	121	206	37.2 %
Progestin-only	3	17	15.0 %	5	15	25.0 %
Combination contraceptives, low estradiol	12	38	24.0%	19	31	38.0 %
Combination contraceptives, high estradiol	26	73	27.1 %	39	60	39.4 %

[a] Physical activity in leisure time: None = reading, watching TV, or other sedentary activity; Low level = walking, cycling, or other forms of exercise at least 4 hours a week; Medium level = participation in recreational sports, heavy outdoor activities with minimum duration of 4 hours a week; High level = Participation in heavy training or sports competitions regularly several times a week.

[b] Hormonal contraceptives: Non-user = No current use of hormonal contraceptives (women only); Progestin-only = Use of hormonal contraceptives with progestin (Cerazette, Nexplanon, Depo-provera, Implanon); Combination contraceptives, low estradiol = Use of hormonal contraceptives with progestin and ethinyl estradiol less than or equal to 20µg (Mercilon, Yasminelle, Loette 28, Nuvaring). Combination contraceptives, high estradiol = Use of hormonal contraceptives with progestin and ethinyl estradiol greater than or equal to 30µg (Marvelon, Yasmin, Microgynon, Oralcon, Diane, Synfase, Evra, Zyrona). Women taking contraceptives, but who were unable to recognize the brand were removed from the analysis.

[c] Positive = two consecutive nasal swab cultures positive for *S. aureus* Negative = one or none of two consecutive nasal swab cultures positive for *S. aureus*.

connected to non-carriers, non-carriers connected to carriers, carriers connected to carriers) using the autocorrelation model Simulation Investigation for Empirical Network Analysis (O'Malley and Marsden, 2008). In further analysis, we used bootstrapping of simulated networks against the observed network, descriptive analysis, and logistic regression to evaluate the effect of host risk factors. The statistical background for our methods is described in Supplementary material.

Results

Transmission of S. aureus carriage in a general population

In the general population with a mean age of 16.4 years (SD = 1.24, range 15-28), the prevalence of *S. aureus* persistent nasal carriage determined by direct culture was 30.3%, compared with 42.6% when using enrichment culture. No MRSA isolates were detected. Prevalence of persistent carriage was higher in male compared to female participants; 36.4% versus 24.0% for direct culture, and 48.1% versus 36.8% for enrichment culture. We found no other significant differences in carrier prevalence between groups according to host characteristics (Table 1).

We first evaluated the FF1 social network structure based on all relationships between students in the five subnetworks (Supplementary Figure 2) and information about relationships and persistent carriage status of nodes in the "overall" network diagram (Figure 1). As the population was recruited from two neighboring municipalities, there were two distinct clusters of students. The number of edges within the high school cluster was higher than outside the cluster demonstrating high school as a strong driver of friendship (homophily of 87.8) (Figure 2). Likewise, participants tended to bond with similar students with respect to sex and lifestyle factors (Supplementary Table 1 and Supplementary Figure 3).

To evaluate the effect of the social network on transmission, we assessed relationships that shared the same *S. aureus spa*-type in

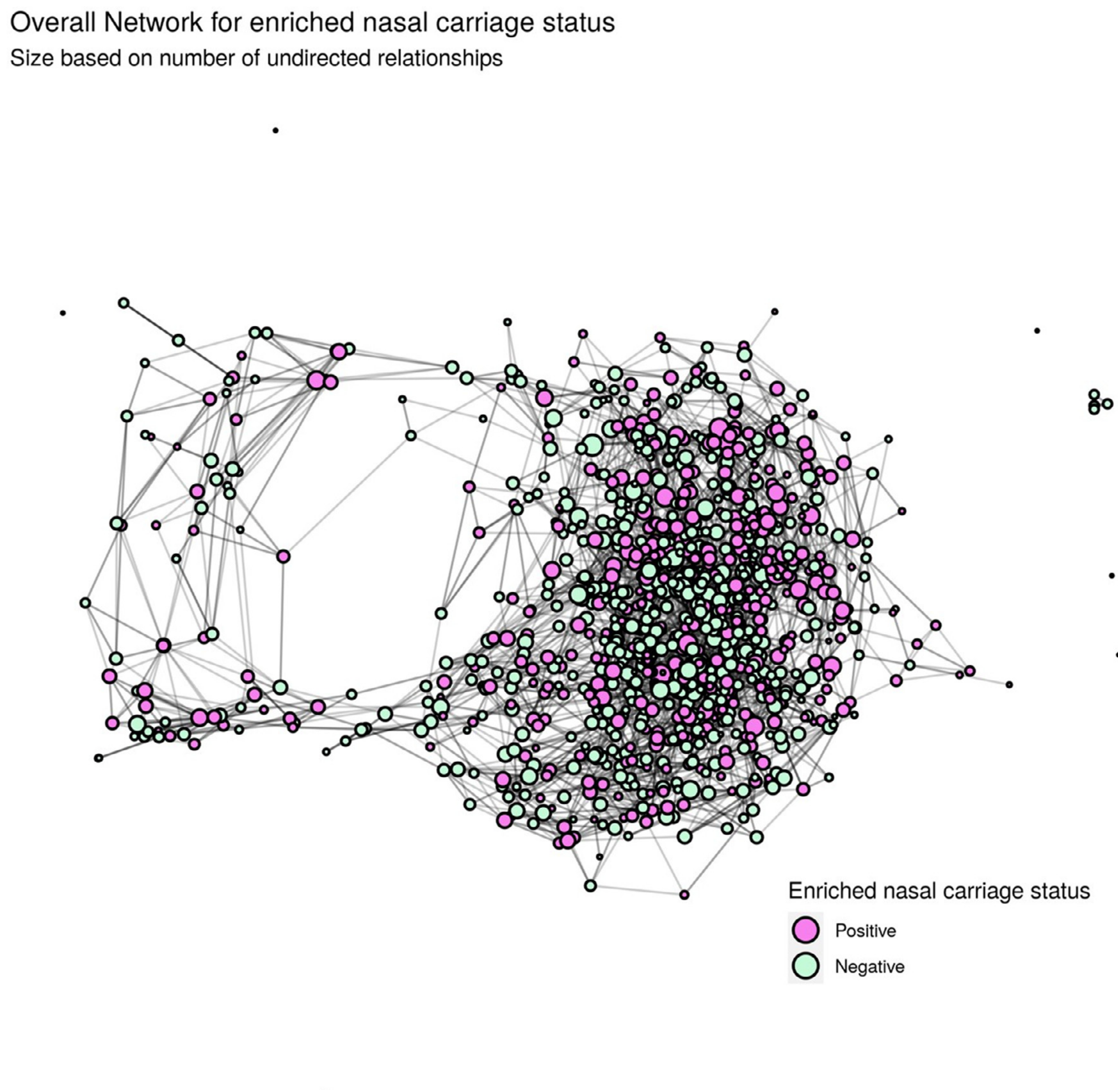


Figure 1. Overall network. The Fit Futures 1 study (N = 1038). Staphylococcus aureus persistent nasal carriage status determined by enrichment culture is highlighted for each student (Positive = *S. aureus* detected in two nasal swab samples; Negative = *S. aureus* detected in one or none of two nasal swab samples). Node size is proportional to the number of connections (undirected friendship).

the "overall" network. We registered 212 unique *spa* types among *S. aureus* throat isolates from 746 students. The 15 most prevalent *spa types* are listed in Table 2. The network analysis demonstrates that each high school has a unique distribution of *spa types*. Overall 126 edges of *S. aureus* carriers shared the same *spa type*. A total of 105 edges (83.3%) connected within the same high school, while 21 edges (16.7%) connected across different high schools (Figure 3). This suggests that *spa*-type is associated with friendship. The inclusion of non-typeable *S. aureus* strains did not affect the results and was therefore excluded from the analysis.

To test if there was a statistically significant influence of social networks on the transmission of *S. aureus*, we performed simulations of the current population for the six networks (Table 3). In the "overall" network, the transmission of *S. aureus* could be demonstrated for the persistent carriage determined by enrichment culture ($P = 0.02$). Transmission could also be demonstrated in the "school" network (direct culture: $P = 0.02$; enrichment culture: $P = 0.01$) and in the "physical contact" network (direct culture: $P = 0.06$; enrichment culture: $P = 0.04$). The same simulation-based analysis for *spa*-types showed transmission of *S. aureus* genotypes in all six social networks ($P < 0.001$).

The role of host risk factors in S. aureus transmission

In the logistic regression analysis, female participants had the highest risk of being exposed to *S. aureus* through their social interaction (Table 4). Men had a relatively low risk of transmission compared to women (0.85, 95% CI = 0.805-0.884). Also, students using alcohol twiceor more per month had a higher risk of transmission of *S. aureus* compared to students using alcohol once per month or less ($P = 0.035$; direct culture). There was a higher probability of transmission among participants doing medium-level physical activity ($P = 0.008$) compared with the light physical activity group.

The mean number of friends for female students was 3.46, which was significantly higher than the average of 3.46 friends among male students ($P = 0.008$) (Supplementary Table 3). Students consuming alcohol more than twice a month had a higher number of friends compared to those consuming less or no alcohol ($P < 0.001$).

We demonstrate that students with a higher number of friends being persistent carriers were more likely to be persistent carriers themselves. This was significant for persistent carriage defined

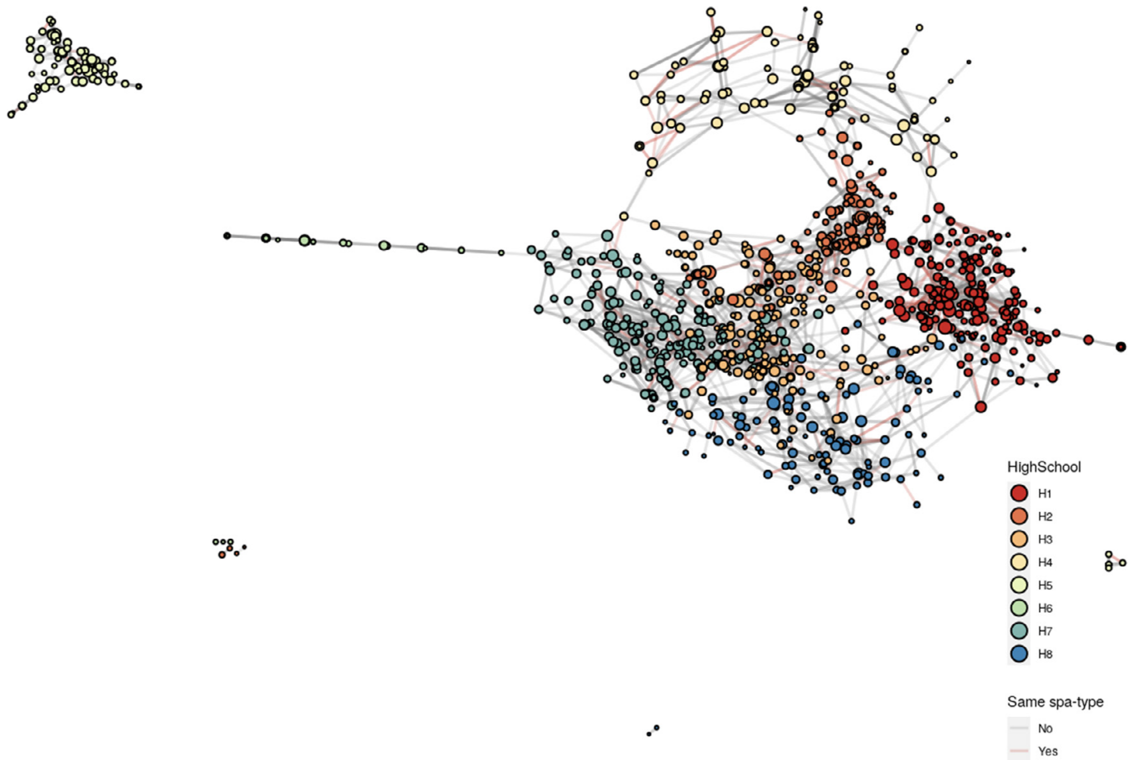
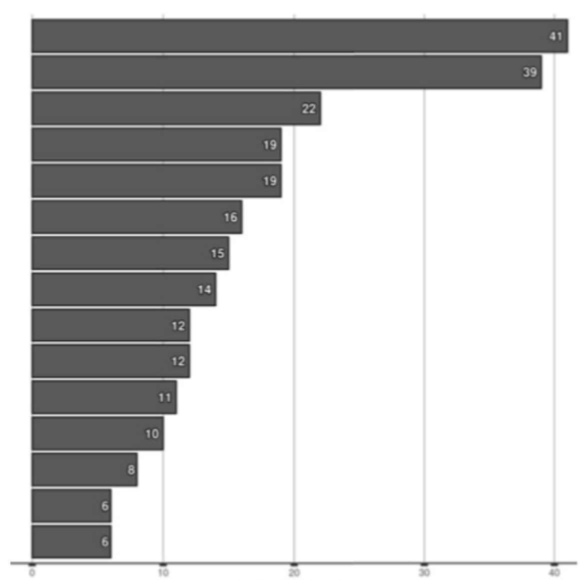


Figure 2. The overall social network within and between high schools, with a multidimensional scaling layout. The Fit Futures 1 study. Edges (lines) connecting nodes (students) with the same Staphylococcus aureus spa-type in throat culture are drawn in red. Edges connecting nodes with different spa-type are drawn in gray. High school ID (H1-H8) represents the eight high schools included in Fit Futures 1. H5 represents students at the high school in Balsfjord municipality (isolated cluster, upper left). All other high schools (H1-H4, H6-H8) are in Tromsø municipality. Only students with *S. aureus* isolated by direct or enrichment culture from the first throat swab sample are shown (N = 746). Unconnected students are not included (N = 21).

Table 2
The most prevalent *spa*-types for *Staphylococcus aureus* throat carriage. The Fit Futures 1 study (N = 746). Only persistent carriers are shown. The plots are the results for enrichment culture.

Modality	Count	Relative	Cumulative
t024	41	0.08	0.08
t084	39	0.08	0.16
t012	22	0.05	0.21
t2524	19	0.04	0.25
t065	19	0.04	0.29
t015	16	0.03	0.32
t002	15	0.03	0.35
t026	14	0.03	0.38
t267	12	0.02	0.4
t160	12	0.02	0.43
NT	11	0.02	0.45
t346	10	0.02	0.47
t021	8	0.02	0.49
T803	6	0.01	0.5
T706	6	0.01	0.51



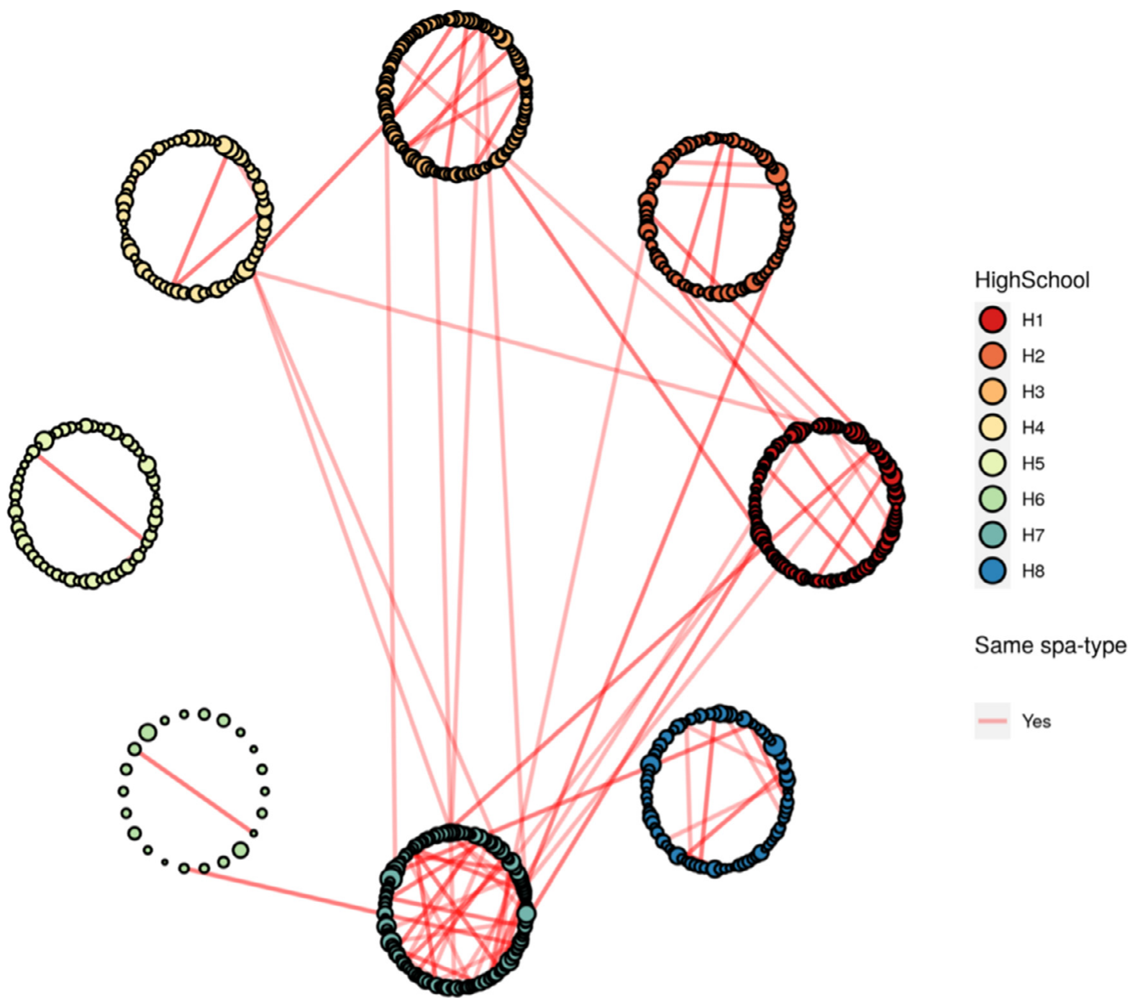


Figure 3. The overall social network with students grouped by high school. The Fit Futures 1 study. Edges (lines) connecting nodes (students) with the same Staphylococcus aureus spa-type in throat culture are drawn in red. Edges connecting nodes with different spa types are hidden. High school ID (H1-H8) represents the eight high schools included in the Tromsø Study Fit Futures 1. H5 represents students at the high school in Balsfjord municipality. All other high schools (H1-H4, H6-H8) are in Tromsø municipality. Only students with *S. aureus* isolated by direct or enrichment culture from the first throat swab sample are shown (N = 746). Unconnected students are not included (N = 21).

Table 3
Summary of 1000 simulations for each type of social network with respect to Staphylococcus aureus persistent nasal carriage and *S. aureus* spa-type. The Fit Futures 1 study. A detailed summary of this analysis is presented in Supplementary Table 4.

Network	*S. aureus* persistent nasal carriage		*S. aureus*throat colonization
	Direct culture (N = 1038)	Enrichment culture (N = 1038)	*Spa*-type (n= 746)
Overall	0.07	**0.02**	**< 0.001**
Physical	0.06	**0.04**	**< 0.001**
School	**0.02**	**0.01**	**< 0.001**
Sports	0.12	0.29	**< 0.001**
Home	0.34	0.39	**< 0.001**
Other	0.08	0.06	**< 0.001**

Numbers represent *P*-values from *t*-tests. Statistically significant values highlighted in bold.

by both direct culture (P = 0.002) and enrichment culture (P < 0.001) (Figure 3). The probability of being a carrier increased by 3.7% (95% CI = 3.52-3.94; univariable logistic regression, result not presented) on increasing the friend circle by one *S. aureus* positive friend (defined by direct culture), as illustrated in Figure 4 and Supplementary Table 4. Similarly, the probability increased by 3.4% (95% CI = 3.33-3.45) for persistent carriage defined by enrichment culture (result not presented).

An adapted linear autocorrelation analysis gave results comparable to the logistic regression analysis (Table 5). The probability of persistent carriage increased by 4.8% (P < 0.001) for each additional *S. aureus* positive friend defined by direct culture after adjusting for host risk factors. A similar increase of 6.0% (P < 0.001) was observed for the enrichment culture. The autocorrelation model also assessed the risk factors that made the participantsfriends significantly more contagious. For direct culture there was an association between sex, BMI and physical activity (P = 0.001-0.008), and for enrichment culture there was an association between study program, BMI, and physical activity (P < 0.001 for all). Because of the assumptions of the autocorrelation model,

Table 4
Associations between host risk factors and transmission of Staphylococcus aureus persistent nasal carriage in the overall social network. Results from two different regression analyses producing "P-value" for the social effect of each characteristic and Relative risk for the comparison of risk of transmission between groups. Persistent nasal carriage determined by both direct culture and enrichment culture. The Fit Futures 1 study (N = 1038).

Risk factor (categories)		Direct culture			Enrichment culture		
		P-value	Relative risk	95% CI	*P*-value	Relative risk	95% CI
Sex	Female	**0.0002**	1		**0.027**	1	
	Male	0.999	**0.845**	**0.805 - 0.884**	0.843	**0.937**	**0.900 - 0.974**
Study program	Vocational	0.548	1		0.353	1	
	General	0.510	1.002	0.950 - 1.054	0.410	0.996	0.954 - 1.038
	Sport	0.403	1.009	0.960 - 1.059	0.811	0.974	0.935 - 1.013
BMI[a]	Underweight	0.793	0.953	0.905 - 1.001	0.841	0.968	0.928 - 1.008
	Healthy	0.150	1		0.301	1	
	Overweight	1	**0.901**	**0.860 - 0.943**	0.763	0.974	0.935 - 1.012
	Obese	0.914	**0.941**	**0.896 - 0.987**	0.246	1.003	0.959 - 1.047
Smoke	Daily	0.294	1		0.855	1	
	Never	0.503	0.986	0.937 - 1.034	0.486	1.022	0.978 - 1.066
	Sometimes	0.723	0.971	0.922 - 1.020	0.262	1.036	0.991 - 1.081
Snuff	Daily	0.406	1		0.596	1	
	Never	0.414	0.999	0.949 - 1.049	0.310	1.017	0.973 - 1.060
	Sometimes	0.900	0.962	0.915 - 1.010	0.831	0.986	0.947 - 1.025
Alcohol	≥ 2 per month	**0.035**	1		0.434	1	
	≤1 month	0.806	**0.933**	**0.885 - 0.980**	0.602	0.991	0.948 - 1.034
	Never	0.739	**0.938**	**0.890 - 0.986**	0.323	1.006	0.964 - 1.049
Physical activity[b]	Light	0.301	1		0.089	1	
	None	0.994	**0.930**	**0.886 - 0.975**	0.803	**0.952**	**0.914 - 0.990**
	Medium	**0.008**	1.053	0.999 - 1.107	0.267	0.982	0.941 - 1.023
	Hard	0.883	**0.959**	**0.913 - 1.005**	0.817	**0.951**	**0.913 - 0.989**
Hormonal contra-ceptives[c]	Non-user	0.444	1		0.494	1	
	Progestin	0.369	1.126	-0.230 - 2.482	0.392	1.239	-0.576 - 3.054
	Low Estrogen	0.430	1.024	-0.414 - 2.264	0.475	1.046	-0.840 - 2.932
	High Estrogen	0.476	0.940	-0.546 - 2.425	0.483	1.027	-0.862 - 2.916

P-values from comparison between random network against a random network with only that particular category. Participants with missing values are excluded from the analysis. Statistically significant values highlighted in bold.
Relative risk and 95% confidence interval (95% CI) from univariable logistic regression analysis.
[a] BMI by kg/m^2. Underweight = <18.5; Healthy = 18.5-24.9; Overweight = 25.0-29.9; ≥ 30.0
[b] Physical activity: None = reading, watching TV, or other sedentary activity; Low level = walking, cycling, or other forms of exercise at least 4 hours a week; Medium level = participation in recreational sports, heavy outdoor activities with minimum duration of 4 hours a week; High level = Participation in heavy training or sports competitions regularly several times a week.
[c] Hormonal contraceptives: Non-user = No current use of hormonal contraceptives (women only); Progestin-only = Use of hormonal contraceptives with progestin (Cerazette, Nexplanon, Depo-provera, Implanon); Combination contraceptives low estradiol = Use of hormonal contraceptives with progestin and ethinyl estradiol less than or equal to 20μg (Mercilon, Yasminelle, Loette 28, Nuvaring). Combination contraceptives high estradiol = Use of hormonal contraceptives with progestin and ethinyl estradiol greater than or equal to 30μg (Marvelon, Yasmin, Microgynon, Oralcon, Diane, Synfase, Evra, Zyrona). Women taking contraceptives, but who were unable to recognize the brand were removed from the analysisBMI, body mass index.

Table 5
Correlation between host risk factors and *Staphylococcus aureus* carrier status. Fit Futures 1 (N = 1038). Adapted multivariable linear autocorrelation model.

	Estimate[a]	SE	*P*-value
Direct culture			
ρ	0.048	0.011	**<0.001**
Sex	-0.048	0.028	**0.0016**
Study program	0.043	0.022	0.0542
BMI[b]	0.107	0.018	**<0.001**
Smoke	-0.012	0.027	0.650
Snuff	-0.001	0.020	0.968
Alcohol	0.038	0.021	0.066
Physical activity	0.033	0.012	**0.008**
Enrichment culture			
ρ	0.060	0.010	**<0.001**
Sex	-0.017	0.030	0.578
Study program	0.087	0.024	**<0.001**
Body mass index	0.085	0.019	**<0.001**
Smoke	-0.019	0.029	0.517
Snuff	0.001	0.022	0.950
Alcohol	0.070	0.022	**0.002**
Physical activity	0.046	0.014	**<0.001**

Significant values highlighted in bold.
[a] Only estimates for the total model are valid. Beta estimates for individual host factors cannot be interpreted.
[b] BMI = body mass index.

beta estimates for individual host factors could not be interpreted and sex-specific host factors (hormonal contraceptives) could not be included in the model. Females tended to have more relationships than males, which was also true for participants with normal BMI and participants with both medium and hard-level physical activity (Supplementary Table 3).

Discussion

In the present study, we demonstrated that social network is associated with *S. aureus* persistent-carrier status and *spa* type in a young population. This is, to our knowledge, the first study to analyze the transmission of *S. aureus* using social network analysis in a young population. We demonstrated that the probability of being a persistent carrier correlates with the number of close friends colonized with *S. aureus*. The autocorrelation analysis showed a 5-6% increased probability of *S. aureus* carriage with each additional *S. aureus* carrier friend. We also showed that friends tend to have the same *spa types*, indicating that the social network effect is partly driven by direct transmission of *S. aureus*. Our results coincide with former research that demonstrated comparable results in different cohorts (Gwizdala *et al.*, 2011; Moldovan *et al.*, 2019).

We analyzed different types of networks and found an association between transmission of *S. aureus* in the social network

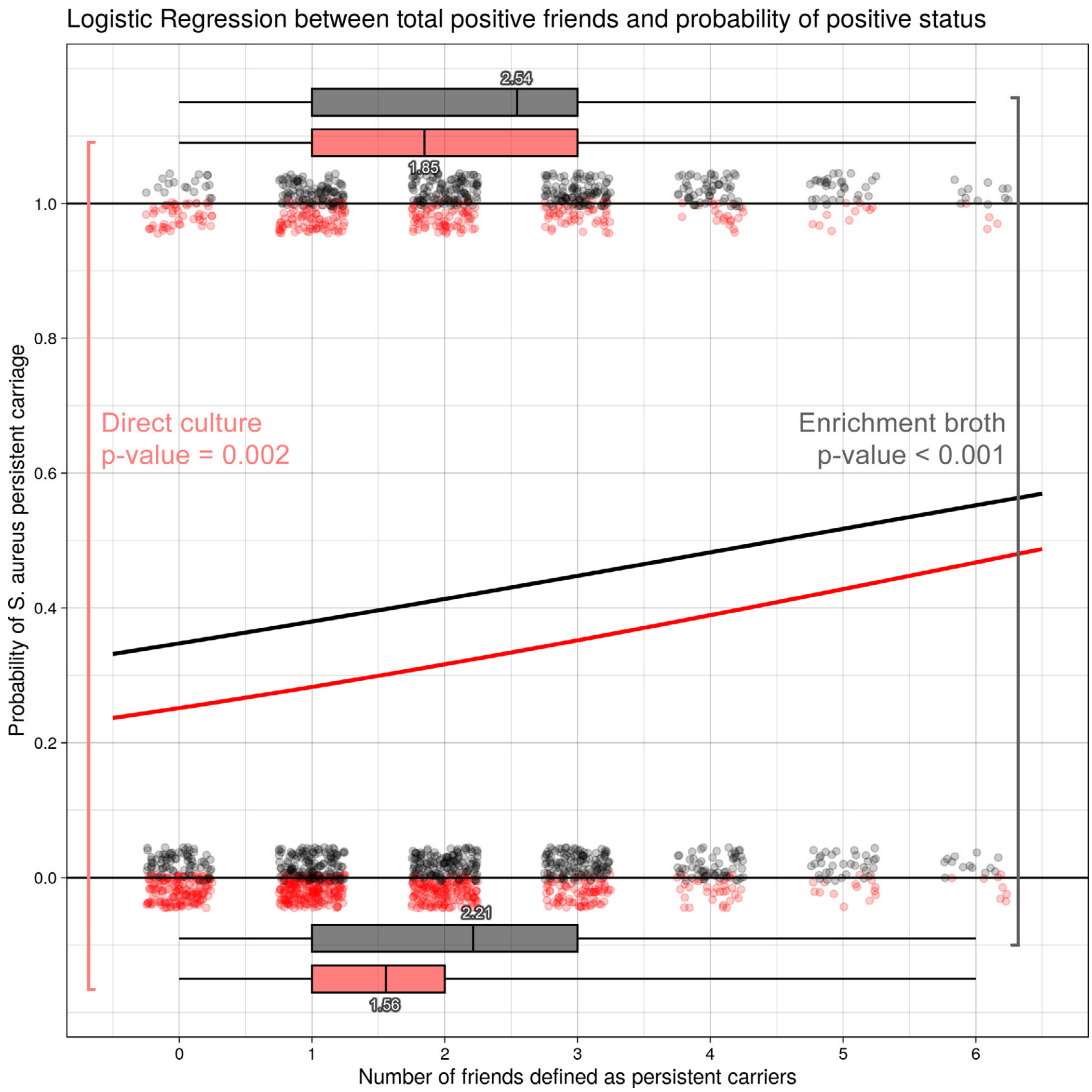


Figure 4. Probability of Staphylococcus aureus persistent nasal carriage with respect to friends' carrier status. Univariable logistic regression analysis. Fit Futures 1 (n=1038). Red color represents persistent nasal carriage defined by direct culture. Black color represents persistent nasal carriage defined by enrichment culture.
The scatterplots show the distribution of persistent nasal carriers (along Y = 1) and non-carriers (along Y = 0) for sub-populations of students having from 0 to 6 *S. aureus* positive friends.
Boxplots show the mean (middle line) and interquartile range (box limits) of *S. aureus* positive friends for persistent nasal carriers (at the top of the diagram) and non-carriers (at the bottom of the diagram). Outliers with more than 6 *S. aureus* positive friends are excluded from the figure, but did not affect the result (N = 3). For more information see Supplementary Tables 6 and 7.

where participants confirmed they had physical contact (direct culture: $P = 0.07$; enrichment culture: $P = 0.04$). This might indicate that social contact is a key pathway for the spread of *S. aureus* in the community (Hogea *et al.*, 2014), which is in line with former studies on transmission of *S. aureus* through a social network (Moldovan *et al.*, 2019). Being together at school was also significantly associated with *S. aureus* transmission. We did not find any significant spread of *S. aureus* among students who were at home or participated in sports together.

There is also a substantial social dimension for several of the known host risk factors for *S. aureus* carriage, which suggests that social network effects may have contributed to associations observed in former studies. In a univariable logistic regression model, the risk of being a persistent carrier increased by 3.4-3.7% on increasing the friend circle by one *S. aureus* positive friend. A similar analysis (autocorrelation model) adjusted for host risk factors, showed an increase of 4.8-6.0%. The difference between the logistic regression model and the autocorrelation model may partly represent the effect of the individual risk factors.

In our study, males had a higher prevalence of *S. aureus* persistent carriage compared to females which corresponds with previous studies (Knox *et al.*, 2015; Mascaro *et al.*, 2019). The social network analysis demonstrates that the female sex is the predominant social risk factor for carriage because of more relationships among females. This may substantiate the hypothesis of sex as a true biological risk factor for *S. aureus* carriage, as the male population has a higher prevalence of carriage while the relative risk of transmission is lower compared to the female population.

We also demonstrated increased transmission of *S. aureus* among students engaged in medium level physical activity in leisure time compared to those with sedentary leisure time. A former study showed an increased risk of *S. aureus* carriage in athletes doing contact sports (Mascaro *et al.*, 2019). Many of the physical activities in youth are contact sports or close-counter training. In our population a higher percentage of women engaged in medium physical activity compared to men (women = 27% and men = 23%). The increased risk of transmission related to medium physical activity could therefore be partly attributed to the observed sex differences.

The use of alcohol more than twice a month was a social factor associated with the carriage of *S. aureus* by direct culture. This may reflect increased social contact with multiple friends at parties and social gatherings. Participants consuming alcohol more than twice a month had a higher number of friends than participants consuming less or no alcohol. We do not have information about the amount of alcohol consumed, and the alcohol variable is therefore lacking some precision. We also have some outliers that may have affected the results. We found no association between alcohol use and carriage defined by enrichment culture, this may be a result of a large number of statistical tests and the more homogenous variable with a high prevalence of *S. aureus* carriage for enrichment culture.

The autoregression model indicates an association between BMI and transmission of *S. aureus* in addition to sex, alcohol use, physical activity, and study program. The effect of friendship density might be partially related to body size, as students with normal BMI had more friends.

Excluding older outliers above 20 years (n = 36) from the network analysis did not affect the results, therefore all participants were included in the analysis. None of the interview questions on social networks provide information about the type or amount of physical contact. We also lack information on the total social network of the participants, including family and other social interactions outside school. Our model also lacks animal contacts and pets, that are known to influence transmission (Loeffler and Lloyd, 2010).

This will give a bias of unknown magnitude and direction. The social networks were constructed by self-reported information on social contacts one week before the study, and this could be misrepresenting of the participants' social contact over long periods of time. We therefore asked all participants to score the representativeness of the nominated friends, and 76 % of the participants claimed a score of five or above (on a scale from one to ten). We therefore believe the representativeness of the nominations to be high (Supplementary Figure 4).

We had complete *spa*-type data only from throat isolates, while nasal carriage is generally considered as the most clinically relevant phenotype. In a validation study of 100 participants with *S. aureus* isolated from cultures of two nasal and two throat swabs in FF1, 82 participants had the same *spa* type (data not shown). Therefore, we believe that our findings from social network analysis based on *spa* type of throat isolates also represent the transmission of nasal *S. aureus*. Another limitation is that we had 10 invalid nasal samples from the first swab and 51 invalid samples from the second swab. These were re-classified as negative for *S. aureus*. Because of the analysis of social networks, we believe that it would have introduced a larger bias in excluding parts of the social network compared with the bias of including potentially misclassified samples. The study was conducted between 2010-2011, and there may be some unknown bias in comparing results to present day. Although we believe that the prevalence of *S. aureus* is quite stable in the general population.

One limitation of defining the outcome of persistent nasal carriage is the number of swabs taken and use of Staphaurex plus agglutination test for *S. aureus* confirmation. Although the Staphaurex plus agglutination test could give false positive reactions for other staphylococci (van Griethuysen *et al.*, 2001), we believe the combination of *S. aureus* selective CHROMagars and agglutination test increase sensitivity and specificity. Nouwen *et al.* proposed a "culture rule" that concludes that two nasal swabs taken at a week interval can accurately classify *S. aureus* nasal carriage (Nouwen *et al.*, 2004), but van Belkum *et al.* demonstrated a median survival of *S. aureus* of more than 154 days among persistent carriers, compared to 14 days among intermittent carriers and 4 days among non-carriers (van Belkum *et al.*, 2009). Thus, it is likely that some participants were misclassified. Both limitations are classified as non-differential bias and therefore are more likely to give underestimations of results.

We reported results using both direct culture and enrichment. When enrichment broth is used, low bacterial loads are also detected, thereby giving an increased prevalence of *S. aureus* positive tests (Antri *et al.*, 2018). In studies of decolonization, enrichment is recommended to prevent possible eradication failure (Diekema *et al.*, 2011). The relevance of low bacterial load carriage in *S. aureus* epidemiology is not known, and most studies have used only direct culture. In this study, the results were similar for both definitions and might demonstrate the robustness of the findings.

Our analysis was modeled by using one time point, while interviews with the different participants were conducted at multiple time points. Most participants nominated friends who had the same attendance date as themselves, e.g., from their own school class (Supplementary Table 5). Furthermore, persistent nasal carriage is a relatively stable phenotype (van Belkum *et al.*, 2009), and we therefore assume that time will not affect the present analysis.

In summary, our data from a general youth population supports social effects on *S. aureus* carriage and these result from both direct social transmission and shared lifestyle risk factors for carriage among friends. We demonstrated relationships between different social networks (i.e., overall, physical contact, school) and *S. aureus* persistent carriage and specific *spa*-types. We also showed that risk of transmission differs by host lifestyle factors. The male predominance in carriage is determined by sex-specific predisposing host characteristics, as social interactions among men are weak drivers of transmission compared with women. More studies are needed to further evaluate the interplay between the social environment and host risk factors in *S. aureus* carriage and should include household transmission and contact with animals.

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Ethical approval statement

Each participant signed a declaration of consent. Participants younger than 16 years had to bring written consent from a parent or guardian. FF1 was approved by The Regional Committee of Medical and Health Research Ethics North Norway (REK North, reference 2009/1282) and the Norwegian Data Protection Authority. The present study was approved by REK North (reference 2011/1710) and was conducted in accordance with the Declaration of Helsinki and national and institutional standards.

Author contributions

Anne-Sofie Furberg, Christopher Sievert Nielsen, Gunnar Skov Simonsen and Lars Ailo Bongo contributed with the conceptualization and design of the work. Anne-Sofie Furberg and Lars Ailo Bongo supervised the work. Johanna UE Sollid performed microbiological analysis of nasal and throat samples. Rafael A. Nozal Cañadas contributed with statistical analysis and statistical methods. Karina Olsen, Lars Småbrekke, Kristian Svendsen, Dina Stensen and Anne Merethe Hanssen contributed in interpretation of data. Dina B. Stensen and Rafael A. Nozal Canadas wrote the original draft. All authors reviewed and approved the final manuscript. Rafael A. Nozal Canadas and Lars Ailo Bongo verify the underlying data.

Data availability

The data that support the findings of this study are available from The Fit Futures study but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon request and with permission of The Fit Futures study. Proposals for data should be directed to fitfutures@uit.no. Statistical analysis and consent form will be available on request. Proposals should be directed to dina.b.stensen@uit.no.

Declaration of Competing Interest

The authors have no competing interests to declare

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.08.018.

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