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Molecular epidemiology of *Staphyloccocus aureus* colonization in the Old Order of Amish of Lancaster county, Pennsylvania, USA

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SUMMARY

Transmission of *Staphylococcus aureus* colonization in community-based populations is not well understood. We sought to describe the molecular epidemiology of *S. aureus* colonization in the Old Order Amish. The study was a prospective, observational study of healthy adults and their same-sex siblings who were cultured from the anterior nares twice. *S. aureus* isolates were characterized using *spa* typing. Overall, 40% (159/398) of the study population was colonized with *S. aureus*. There were 84 *spa* types with the most abundant *spa* types being t012 (13%) and t021 (7%). There was no clustering of *spa* types within sibling groups; however, there was clustering within households. There were 111 *S. aureus*-colonized participant pairs living within the same household. Of these, 47% had concordant *spa* types. The diversity of *spa* types across a relatively isolated, genetically homogenous population with a similar lifestyle is striking. Taken together this suggests that *S. aureus* transmission is a local phenomenon limited to very close contact.

Key words: Epidemiology, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is an opportunistic bacterial pathogen and a common cause of both community-acquired and hospital-acquired infections. Colonization plays a key role in development of *S. aureus* infections. Nasal colonization with *S. aureus* is common and most often precedes *S. aureus* infection. Rates of infection are threefold higher in nasal carriers [1]. Colonized individuals also serve as a source of transmission to others.

Nasal colonization can therefore be looked at as a reservoir for *S. aureus* that exists within the community. It is estimated that 29% of adults in the USA are colonized with *S. aureus* with 1·5% carrying methicillin-resistant *S. aureus* (MRSA) [2]. *S. aureus* colonization status is influenced by multiple factors including host factors such as age, sex, ethnicity, socioeconomic status, antibiotic use, and underlying diseases such as upper respiratory inflammation affect colonization [3, 4]. Environmental factors such as exposure to a heavily colonized individual in the household or hospital affect transmission of *S. aureus* to others; however, the transmission of *S. aureus* in community-based populations is not well understood.

In a familial aggregation study, we recently reported that host genetic factors are not a strong determinant of persistent *S. aureus* colonization in the Old Order Amish of Lancaster county

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Pennsylvania [5]. The Old Order Amish live in a rural, community-oriented environment with uniform socio-economic status and lifestyle. In general, they are not socially integrated with the non-Amish population. Our earlier finding implies that other factors determine *S. aureus* colonization. The objective of this analysis was to describe the molecular epidemiology of *S. aureus* colonization in this unique community-based population in order to determine risk factors for colonization and transmission based on colonization with matching genotypes.

METHODS

Clinical protocol methods

This study population was derived from a prospective, observational study of healthy, Old Order Amish adults and their same-sex siblings [5]. The protocol was approved by the University of Maryland, Baltimore IRB. Written informed consent was obtained from all participants. Briefly, we started by recruiting a convenience sample of healthy, Old Order Amish adults ($n=166$) and then recruited their siblings who lived in different households and were of the same sex. We did not systematically sample household members of this cohort; household members were included when eligible for the original study population and most often were spouses. All recruitment was performed between March 2008 and October 2009. A different street address was required for each same-sex sibling. Individuals were not eligible for the study if they were aged <18 years, had active skin conditions, diabetes, end-stage renal disease, or had taken antibiotics in the last 30 days. Demographic and risk-factor information was obtained by interviewing study participants.

Specimen collection and microbiology methods

A trained research nurse obtained two cultures of the anterior nares from each participant. Swabs were aseptically inserted into the front of the nostril and rotated twice. Using the same swab, the process was repeated in the other nostril. Two cultures were obtained between 1 and 6 weeks apart. All samples were tested for *S. aureus* by use of standard microbiological procedures [6]. Participants with *S. aureus* from either the first or second culture were considered *S. aureus* colonized for the objective of this analysis.

Molecular typing

Each *S. aureus* isolate was characterized by use of DNA amplification and DNA sequencing of the protein A (*spa*) gene hypervariable region [7]. *Spa*-type data were then tested for clustering within the assigned groups using Ridom Staphtype software [8–11]. We limited the cost between different strains in a cluster to four to achieve a discriminatory index of 0·966 (95% CI 0·956–0·982) for *spa* type and 0·919 (95% CI 0·889–0·943) for clonal clusters using the Hunter–Gatson Discriminatory Index (HGDI) method [12, 13].

Statistical methods

The association between *S. aureus* colonization and potential predictors was measured using the χ^2 test or Fisher's exact test for categorical variables and Student's t test for normally distributed continuous variables.

RESULTS

Epidemiology of *S. aureus* colonization

Overall, 40% (159/398) of the study population was *S. aureus* colonized (see Table 1); only two subjects had MRSA. The average age was 46 years and they were predominantly female (73%). More than half of participants reported routinely handling some type of animal (62%). Participants who were *S. aureus* colonized and those who were not colonized did not significantly differ in the routine handling of animals ($P=0·19$). There were also no significant differences by colonization status in the type of animal handled (data not shown).

Molecular epidemiology of *S. aureus* colonization

Overall, there was great variability in *S. aureus* strains found in the Old Order Amish population. Of the 159 colonized subjects, 99 were positive on both cultures. Eighty-three of these 99 had the same or a closely related *spa* type. Thus, we performed the remaining analysis using the last isolate collected from the first two cultures for each individual. There were a total of 159 isolates that clustered into 84 *spa* types of which 71 were previously known. The most abundant *spa* types were t012 and t021 at 13% and 7%, respectively. The 71 previously known *spa* types were grouped using BURP clustering in Ridom Staphtype software with

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Table 1. *Description of study population and characteristics associated with* Staphylococcus aureus *colonization in healthy, adult Old Order Amish in Lancaster county, PA, 2008–2009*

		S. aureus colonization status*		
Characteristic	All participants	Colonized	Non-colonized	*P* value†
Number	398	159	235	—
Households sampled	305	145	196	—
Age (years)	46·2 ± 15·2	46·7 ± 15·7	45·6 ± 14·9	0·48
Female sex	289 (73)	119 (75)	168 (72)	0·46
Adults in household	2·0 ± 1·4	2·1 ± 1·4	2·0 ± 1·3	0·33
Children in household	3·3 ± 2·8	3·2 ± 2·8	3·3 ± 2·7	0·78
Routinely handle animals‡				
Yes	247 (62)	93 (58)	152 (65)	0·19
No	150 (38)	66 (42)	82 (35)	
History of skin boils or lesions	6 (2)	2 (1)	4 (2)	1·00
Surgery in last year	14 (4)	4 (3)	9 (4)	0·47
Hospitalized in last year	20 (5)	8 (5)	11 (5)	0·87
Taken antibiotics in last year	55 (14)	20 (13)	34 (15)	0·59

Values given are mean±S.D. or *n* (%)
* Colonization status unknown for four study participants.
† *P* values are from χ^2 test, Fisher's exact test, or *t* test as appropriate.
‡ Status for routine handling of animals was unknown for one study participant.

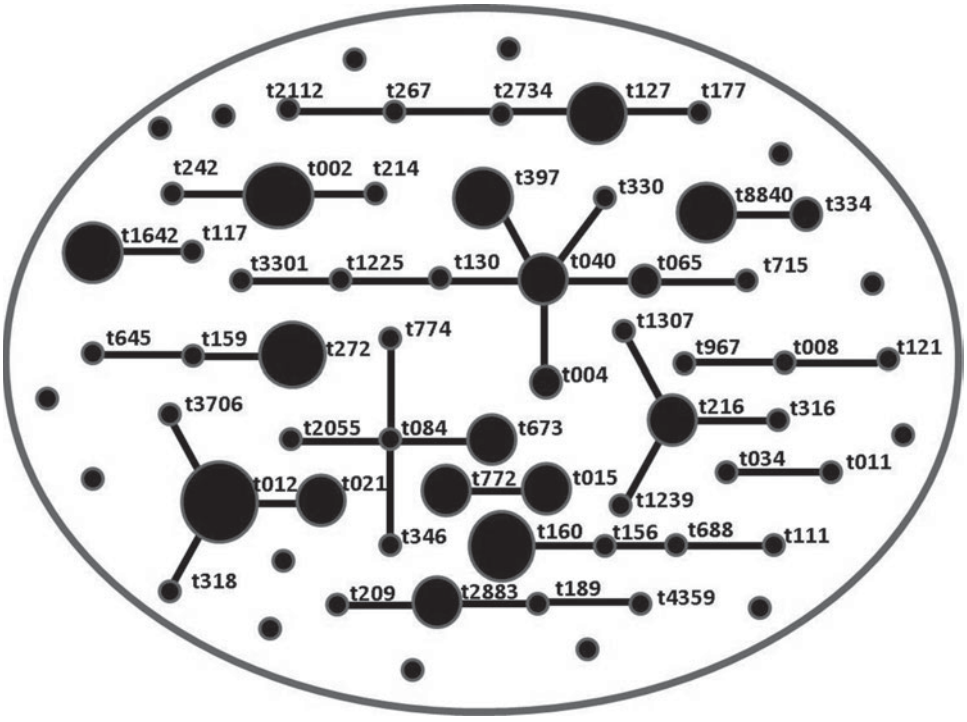

Fig. 1. Diagram of relatedness of 71 *spa* types identified in 145 isolates from Old Order Amish individuals. Relatedness was calculated in BURP which uses cost differences of <4 to group *spa* types into clusters. The size of circle is proportional to the number of isolates with that *spa* type. The lines connect *spa* types with minimum cost. There are 15 clusters and 14 singletons represented as single dots. The spacing between clusters does not have any evolutionary bearing.

the following results: five *spa* types were excluded from clustering because they had less than four repeats rendering them evolutionarily uninformative, 14 *spa* types were singletons (>4 differences between *spa* types in clonal complexes), and 52 *spa* types were clustered into 15 clonal complexes (see Fig. 1).

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We grouped *spa*-type concordance according to household and sibling groups. In total, there were 111 pairs living within the same household that were *S. aureus* colonized on at least one culture. Of these, 47% had concordant *spa* types. There were 24 sibling groups from the familial aggregation study with two or more members colonized with *S. aureus*. These sibling groups lived in different households. Only one (4%) of these 24 sibling groups had concordant *spa* types, one-tenth of the concordance rate within households.

DISCUSSION

Studying *S. aureus* colonization in the Old Order Amish presents a unique opportunity because the population is socially isolated and homogenous in terms of genetic background and lifestyle, reducing the influence of confounding factors. In this setting, we did not identify any host-related risk factors for *S. aureus* colonization. Our molecular typing demonstrated that individuals with more than one positive culture were likely to have the same or related *spa* type. At the population level, there were 52 *spa* types which clustered into 15 clonal clusters. We found evidence for clustering in households, but not in siblings who lived in different households.

We did not see a higher risk of *S. aureus* colonization in men compared to women as others have reported [14, 15]. We did not detect a difference in *S. aureus* colonization by age [4, 14], and saw no association with animal handling. Our failure to detect a difference may reflect our relatively small sample size. In addition, our sampling framework was not designed to be representative of the Amish population as a whole and only included adults with no known risk factors for *S. aureus* colonization.

Our molecular typing results are consistent with other community-based studies. At the population level, there were 52 *spa* types which clustered into 15 clonal clusters; this is consistent with other community-based studies [4, 16–18]. The most common *spa* types, t012 and t021, are closely related with only one repeat difference and have been associated with multilocus sequence types 30 and 33. These *spa* types have been associated with methicillin-susceptible *S. aureus* (MSSA) colonization in other community-based samples [16, 18, 19]. We found very little t011 and t034 (one isolate each), *spa* types associated with multilocus sequence type 398, livestock-associated MRSA. We found evidence for clustering in households. This is also consistent with household transmission studies. Household transmission studies, which have focused mainly on MRSA, have shown that transmission from MRSA-colonized patients or healthcare workers occurs in 15–29% of household contacts [20, 21].

The main limitation of our study is that the data was collected as part of a familial aggregation study of healthy Old Order Amish adults. The relatively small study population was recruited as a convenience sample in sibling groups and the siblings were required to live in different households. Thus, we have little information about *S. aureus* colonization status of other household members that were not in the original study. We were also limited to the information that was collected during the primary study. Therefore we did not have detailed information on other environmental exposures.

Given our sampling framework, the diversity of *spa* types across a relatively socially isolated, genetically homogenous population with a similar lifestyle is even more striking. Taken together this suggests that *S. aureus* transmission is a local phenomenon limited to very close contact such as might be seen in a household, hospital or athletics field.

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DECLARATION OF INTEREST

None.

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