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Major article

Staphylococcus aureus dispersal from healthy volunteers

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Background: Understanding *Staphylococcus aureus* dispersal from human carriers is vital for preventing transmission and colonization of this organism in health care settings. This study investigated the *S aureus* supershedder hypothesis in relation to attributes of healthy volunteers.

Methods: Microbial aerosol generation from volunteers was quantified within a controlled environmental chamber during walking or sitting activities. Biological air samplers were used to determine numbers of total *S aureus* colony-forming units disseminated during these activities.

Results: A total of 17 volunteers was sampled on 3 occasions. Hairstyle (long hair tied up or a shaved head) was the only significant predictor of dissemination of *S aureus* (5% significance level). No other significant effects were found at the 5% level. A negative binomial distribution provides the best fit with respect to *S aureus*.

Conclusion: We found that, in the context of our small sample size, hairstyle (long hair tied up or a shaved head) statistically affected levels of bacteria shed from volunteers. However, we found no evidence for "supershedders" or "cloud adults," suggesting they are at an extreme end of a continuous distribution.

Staphylococcus aureus is a frequent cause of infections in both hospital and community health care environments. Methicillin-resistant *S aureus* is a particular cause of concern given that it is a significant cause of morbidity and mortality in many hospitals throughout the world.[1] Therefore, understanding *S aureus* dispersal from human carriers, distribution, and survival in the environment is vital in preventing transmission and colonization of this organism.

The principle route of *S aureus* transmission is via direct contact between individuals or indirect contact with contaminated materials.[2,3] However, the airborne route of transmission is also considered to be important.[4-14]

S aureus carriage is thought to be the primary determinant of bacteria released into the environment. Three different patterns of *S aureus* nasal carriage have been identified within healthy individuals: persistent carriage, intermittent carriage, and noncarriage.[15] Approximately 20% of healthy populations are persistent carriers, 60% are intermittent carriers, and 20% are noncarriers.[16] A high carriage rate is associated with higher counts of bacteria and an increased risk of acquiring *S aureus* infection.[17]

Persistent carriers are often colonized by a single strain of *S aureus* over long time periods,[12] which may imply that host characteristics substantially determine the carrier state of an individual, although these characteristics have yet to be defined.[18] Persistent carriers of *S aureus* among hospital personnel have been implicated in nosocomial transmission.[19,20] There is some evidence that a certain percentage of the population are "supershedders" or "cloud people."[19,21,22] Both these terms refer to those who shed significantly higher counts of *S aureus* than other carriers.

The aim of the study was to gather evidence for the theory that some individuals are supershedders of *S aureus* and to ascertain whether differences among individuals (eg, height, hairstyle) help to explain the quantity of *S aureus* that is shed.

MATERIALS AND METHODS

The study was undertaken within a controlled test chamber that excluded external contamination and enabled remote operation of the sampling equipment. The chamber was approximately 22 m^3 in volume, with a floor space of 2.2 m × 4 m and was accessed via a dedicated lobby. The room was supplied with high-efficiency

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particulate air filter, which operated up to 250 air changes an hour, under positive pressure, giving high levels of air cleanliness. Sampling was performed under static conditions, with the ventilation being used between tests to prevent cross contamination between each test and from the operators. Tryptone Soya agar (TSA) settle plates were used to investigate large particle deposition onto the floor and table surfaces. In addition a 6-stage Andersen[23] air sampler (TSA plates) was used to determine the particle size distribution, and 2 Sartorius air samplers (loaded with gelatine filters [0.3 μm pore size]; Sartorius Stedim UK Limited, Epsom Surrey, UK) were used to determine airborne pathogen load. Figures 1 and 2 detail the location of settle plate monitors, the 6-stage Andersen air sampler (calibrated to run at 28.3 L/min), and the 2 Sartorius air samplers (calibrated to run at 75 L/min).

Recruits were supplied with a project information sheet and asked to sign a consent form. Participants were recruited from within Public Health England's Porton Down site via site-wide e-mails. None of the recruits were known to be *S aureus* carriers prior to this investigation. Ethical approval was sought from and approved by Public Health England's Ethical Review Board and was conducted under Public Health England's research governance arrangements. Each volunteer was sampled 3 times on different days. Prior to each sampling session, volunteers were asked to complete a participant questionnaire detailing their age, height, gender, smoking status, pet-owning status, date and time of last shower, skin conditions (eczema and dandruff were included), respiratory symptoms and hairstyle (short, long loose, midlength loose, tied up long hair, or shaved head).

The first protocol that the recruit was asked to follow was to walk anticlockwise or clockwise (randomly allocated) for 2 minutes inside the walking zone. The volunteer was instructed to enter the room and wait at point X until they could hear the sample pumps turn on; they then started to walk around the room at their own pace. During the walking procedure, the observer counted the number of room rotations completed and recorded this. If the 2 minutes finished midrotation, then the volunteer was instructed to finish the rotation and then leave the room. The settle plates, Andersen plates, and Sartorius filters were changed between sampling times by operators wearing Tyvek suits, latex gloves, and shoe covers to minimize the potential for the operator to contaminate the samples. Background controls were taken to ensure that operators did not contaminate samples. The room then had a blow-down period of 5 minutes, after which the sitting experiment was conducted. The recruit was advised to enter the room and sit on the chair at the front of the table and remain there for 5 minutes (see Fig 2); the volunteer was supplied with a magazine that they could read throughout the 5 minutes sampling time. When the sampling time was over, the volunteers were instructed to leave the room, the samples were collected, and the room then had a "blow-down" period of 15 minutes prior to the start of the next recruit's sampling time. The airflow during "blow-down" periods was 250 air changes/hour. These 2 sampling activities were chosen to simulate the activities that might be undertaken by a visitor to a hospital ward, ie, walking to a relative's bedside and sitting next to the bed for a period of time.

At several time points during the course of this investigation, additional background samples were taken that measured the level of background contamination in the room. This ensured that there was no cross contamination between volunteers and that all recovery was from the volunteer being studied and not from some other unknown source.

After sample collection, the left Sartorius gelatine filter was plated directly onto a TSA plate, and the right Sartorius gelatine filter was placed into a glass Petri dish, dissolved in 15 mL of warmed phosphate-buffered saline with 0.05% Tween 80 at 37°C

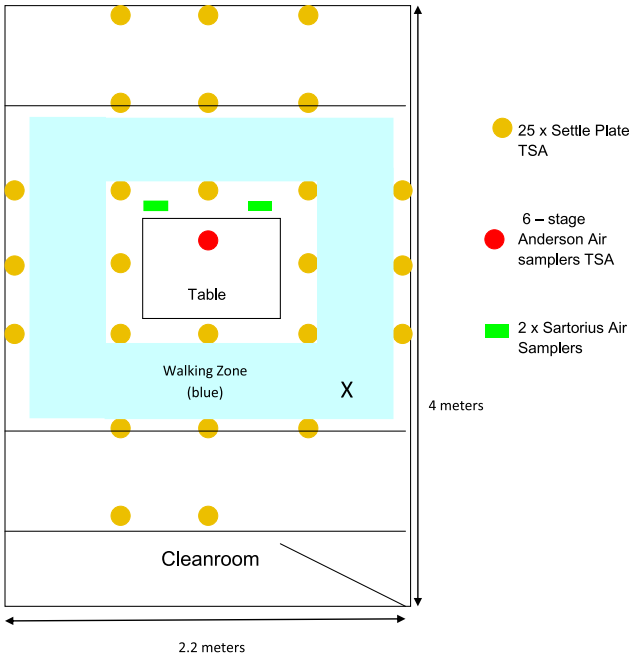

Fig 1. The clean room layout for the walking experiments. Settle plates are detailed as yellow circles, red circle donates the 6-stage Andersen air sampler, and green rectangles represent the Sartorius filters. The blue shaded areas represent the walking zone in which the walking rotations occurred. The volume of the room is 22 m^3.

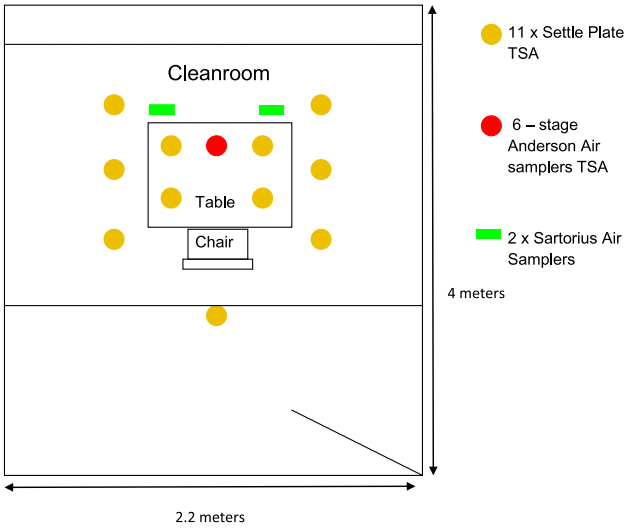

Fig 2. The clean room layout for the sitting experiments. Settle plates are detailed as yellow circles, red circle donates the 6-stage Andersen air sampler, and green rectangles represent the Sartorius filters. The volume of the room is 22 m^3.

for 30 minutes, after which the liquid was transferred to a falcon tube and vortexed for 30 seconds. Aliquots (2 × 1 mL) were then plated onto TSA agar.

All TSA plates were incubated at 37°C for 48 hours. Colonies were counted, and all potential staphylococci were colony picked and subcultured onto Baird Parker agar for 48 hours. Colonies that exhibited a zone of clearing were identified as *Staphylococcus aureus*.

Counts of *S aureus* colony-forming units (CFU) were calculated from each of the settle plates, Andersen plates, and Sartorius filters for each individual on 3 separate occasions. Counts for each air sampler were expressed as CFU/m^3/min, and counts for settle