Undetected carriage explains apparent Staphylococcus aureus acquisition in a non-outbreak healthcare setting


This version is available from Sussex Research Online: http://sro.sussex.ac.uk/id/eprint/100841/

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the URL above for details on accessing the published version.

Copyright and reuse:
Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

http://sro.sussex.ac.uk
Undetected carriage explains apparent *Staphylococcus aureus* acquisition in a non-outbreak healthcare setting

James R Price\(^a,1\), Maho Yokoyama\(^a\), Kevin Cole\(^b\), Jonathan Sweetman\(^c\), Laura Behar\(^c\), Simon Stoneham\(^d\), Daire Cantillon\(^e\), Simon J Waddell\(^d\), Jonathan Hyde\(^d\), Ruhina Alam\(^f\), Derrick Crook\(^g\), John Paul\(^e\), Martin J Llewelyn\(^a\)

\(^a\) Department of Global Health and Infection, Brighton and Sussex Medical School, University of Sussex, Falmer, Brighton, BN1 9PS, United Kingdom
\(^b\) Public Health England, Royal Sussex County Hospital, Brighton, BN2 5BE, United Kingdom
\(^c\) Clinical Investigation Research Unit, Brighton and Sussex University Hospital NHS Trust, Brighton, BN2 5BE, United Kingdom
\(^d\) Department of Experimental Medicine, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom
\(^e\) Public Health England, Royal Sussex County Hospital, Brighton, BN2 5BE, United Kingdom

**A R T I C L E  I N F O**

Article history:
Accepted 19 July 2021
Available online 23 July 2021

Keywords:
*Staphylococcus aureus*
Concealed carriage
Acquisition

**S U M M A R Y**

**Objectives:** Previous studies have been unable to identify patient or staff reservoirs for the majority of the nosocomial *S. aureus* acquisitions which occur in the presence of good infection control practice. We set out to establish the extent to which undetected pre-existing carriage explains apparent nosocomial *S. aureus* acquisition.

**Methods:** Over two years elective cardiothoracic admissions were screened for *S. aureus* carriage before and during hospital admission. Routine screening (nose/goin/wound sampling), was supplemented by sampling additional body sites (axilla/throat/rectum) and culture-based methods optimised to detect fastidious phenotypes (small colony variants, cell wall deficient variants) and molecular identification by PCR.

**Results:** 35% of participants (53/151) were *S. aureus* carriers according to routine pre-healthcare screening; increasing to 42% (63/151) when additional body sites and enhanced cultures were employed. 71% (57/79) of apparent acquisitions were explained by pre-existing carriage using augmented measures. Enhanced culture identified a minority of colonised individuals (3/151 including 1 MRSA carrier) who were undetected by routine and additional screening cultures. 4/14 (29%) participants who became culture-negative during admission had *S. aureus* genomic material detected at discharge.

**Conclusions:** Conventional sampling underestimates carriage of *S. aureus* and this explains the majority of apparent *S. aureus* acquisitions among elective cardiothoracic patients.

© 2021 The Authors. Published by Elsevier Ltd on behalf of The British Infection Association. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)

**Background**

*Staphylococcus aureus* is a common commensal of the human nose and throat but also a major human pathogen. In the UK, healthcare-associated methicillin-resistant *S. aureus* (MRSA) has waned but community-onset *S. aureus* disease and nosocomial methicillin-susceptible *S. aureus* (MSSA) infections remain important in the UK and globally. *S. aureus* is the most commonly isolated single pathogen associated with nosocomial infection. In the UK measures to reduce the impact of invasive *S. aureus* infection have been partially successful. Between 2007 and 2014 laboratory-reported cases of *S. aureus* bacteraemia fell by 33.4%; this reduc-
tion was most prominent in infections caused by MRSA. Since 2014 MSSA bloodstream infections having increased for five consecutive years resulting in currently observed S. aureus bacteraemia rates in the UK at 21.3 per 100,000 population.9

Carriage of S. aureus is common and person-to-person transmission occurs during close contact in hospitals and the community. Acquisition of new strains is associated with greatest risk of invasive disease.4 In hospitals efforts are made to decolonise MRSA carriers to reduce infection risk and prevent person-to-person transmission. We have recently undertaken detailed studies of S. aureus transmission in critical care. Surprisingly, neither patients5 nor healthcare workers5 could be implicated as sources for the majority of acquisitions.

Conventional screening methods may fail to detect S. aureus carriage (i) at unsampled sites such as the gastrointestinal tract,7,8 (ii) present below the threshold for detection in particular if within tissues or cells9 and (iii) as fastidious growth phenotypes (small colony variants (SCVs) and cell wall deficient variants (CWD)).10,11 These concealed carriage states at admission may mean some patients falsely appear to acquire S. aureus while in hospital. In this study we aim to apply enhanced detection methods in order to describe patterns of S. aureus carriage in patients before and during healthcare admission and to investigate the possibility that undetected carriage might explain instances of apparent nosocomial acquisition.

Methods

Setting and participants

Between April 2017 and March 2019 adult patients undergoing elective cardiothoracic surgery procedures at a large teaching hospital on the south coast of England were eligible to participate in the study. Routine infection prevention and control measures employed during the study period are depicted in Supplementary Table 1.

Participants were recruited when they attended a pre-admission clinic. For all patients who consented to participate data were collected on demographics (age, gender identity, smoking status), pre-morbid conditions associated with S. aureus carriage (atopy, diabetes mellitus, cancer, renal disease), healthcare exposure (employment, hospital admission, procedures), antibiotic exposures, MRSA carriage status and presence of skin wounds (see Supplementary Fig. 1).

Table 1

Participant sampling during the study. Sampling (x) comprises swabs taken as part of routine clinical practice (Routine) and swabs taken as part of the study including additional site sampling (Additional) and enhanced culture (Enhanced) for small colony variants and cell wall deficient variants. *Clinical samples are taken when infection is suspected and hence may not be taken at all sampling time-points or may include > 1 sample.

<table>
<thead>
<tr>
<th></th>
<th>Pre-healthcare</th>
<th>Post-operative</th>
<th>Weekly</th>
<th>Discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groin</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound (if present)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical samples*</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Additional</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groin</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound (if present)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axilla</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enhanced</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sampling

Patient samples were taken before and during hospital stay, using augmented sampling and culture to maximise recovery of isolates and apply molecular techniques (16S rRNA PCR) to categorically identify new acquisition events in patients with pre-existing S. aureus colonisation and carriage loss with persisting concealed carriage.

Table 1 depicts participant sampling for S. aureus. All elective cardiothoracic patients are routinely screened for MRSA carriage (nose and groin plus any wound) at pre-assessment and admission to the ward. All routine screens were retrieved and cultured for all types of S. aureus. In order to determine S. aureus carriage at additional sites enhanced screening was performed of throat, axilla, and rectum prior to healthcare exposure. To determine the extent of carrying cryptic forms such as SCV and CWD, which can reside outside and within human epithelial cells, additional nose and throat samples were taken to capture epithelial cells as well as extra-cellular bacteria.12

Definitions

The following definitions were used:

**Routine screen**: MRSA screening swabs (nose plus groin and any wound) taken as part routine clinical practice at pre-admission assessment, on admission to hospital and weekly during admission. Routine screening swabs were cultured for all types of S. aureus.

**Clinical sample**: any patient sample taken as part of routine clinical practice when an infection was suspected.

**Additional screening**: additional (non-routine) body site swabbing including throat, axillae, rectum, plus nose, groin and wound swabs when routine screening is not performed at the same time.

**Enhanced culture**: additional nose and throat samples obtained to detect SCV and CWD organisms.

**Augmented screen**: additional screening plus enhanced culture.

**Pre-healthcare screen**: all samples taken prior to receipt of healthcare (i.e. those taken at pre-assessment and on admission to ward).

**Acquisition**: identification of S. aureus in a participant following a negative screen.

**Carriage loss**: negative screen after a positive screen.

**Continuously detected carriage**: any participant who has at least one swab yielding S. aureus on every set of screens taken at each time point.
**Discontinuously detected carriage:** any participant who yields *S. aureus* on at least one swab but does not fit the definition for continuous detection.

**No carriage detected:** *S. aureus* is not cultured from any swab taken from a participant.

**Microbiology:** All samples collected during routine and additional screening were collected with rayon-tipped swabs with Amies transport medium (Copan, California, United States) Routine screening swabs underwent broth enrichment in 7.0% salt broth (selective for *Staphylococcus* spp.) at 35 °C for 24 h prior to agar culture. Broth enrichment improves the sensitivity of detection.\(^{13,14}\) Following enrichment 10 µl broth was inoculated onto Columbia Blood agar (CBA) and selective *S. aureus* ID agar (SAID; bioMerieux, France) and incubated at 35 °C in air. Plates were read daily for two days and any presumptive *Staphylococcus* spp. were identified using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) and slide coagulase agglutination. Isolates were considered *S. aureus* if they exhibiting a positive slide coagulase test and were identified to species level on MALDI-TOF. All isolates had susceptibility determined to commonly used antibiotics using standard disc diffusion methods. Nose and throat swabs taken for enhanced culture were assessed for the presence of extra- and intra-cellular SCV\(^{15}\) and CWD\(^{16}\) (see **Supplementary Materials**). All organisms, swabs and broth enrichment fluid were archived in 15% glycerol at −80 °C. Selected samples from participants exhibiting discontinuous detection were evaluated using 16S PCR for the presence of *S. aureus* genomic sequences (see **Supplementary Materials**).

**Ethics**

Ethical approval for this study was gained from London-Harrow Research Ethics Committee (16/LO/2111).

**Statistical analysis**

Data were analysed using STATA 15.0. Associations between carriage profiles and the variables described were univariately assessed using the Fisher’s exact test for categorical data and the Kruskal-Wallis test for nonparametric continuous and categorical variables. Probability values of ≤0.05 were considered significant.

**Results**

151 participants were recruited to the study; 80% male, median age 70 years (IQR 65–74) (**Supplementary Table 2**). 26 (17%) participants suffered from chronic skin conditions (eczema or psoriasis), 47 (31%) were diabetic and 12 (8%) had a current skin wound. 57 (38%) participants did not have any co-morbidities. 106 (70%) participants had a history of smoking including 10 (7%) who currently smoked. Eight (5%) participants worked in a healthcare setting. Within the two years prior to recruitment 54 (36%) participants were previously admitted to hospital and 50 (34%) had received surgery. 142 (95%) participants previously received antibiotics; 32 (21%) within the last six months. Three (2%) were known to be MRSA colonised prior to the study.

**Pre-healthcare carriage**

53/151 (35%) yielded *S. aureus* on at least one routine pre-healthcare screening sample (Participants 1–53 in **Fig. 1**). All participants underwent additional screening and enhanced culture prior to healthcare exposure; 51/151 (34%) declined rectal sampling. Taken together, all forms of additional screening and enhanced culture identified *S. aureus* in an additional 10 participants.
culture-positive carriage). Seven accrued single culture-negative samples of throat, axilla, rectum (Additional) and enhanced culture (Enhanced) for small colony variants and cell wall deficient variants.

![Venn diagram depicting sites of pre-admission *S. aureus* carriage in 63 culture-positive participants. Sites include routine nose, groin and any wound (Routine), additional samples of throat, axilla, rectum (Additional) and enhanced culture (Enhanced) for small colony variants and cell wall deficient variants.](image)

(Participants 54–63 in Fig. 1) with the largest contributions being from throat sampling (six participants) especially when combined with enhanced culture for intracellular SCVs (eight participants) (Fig. 2). Of note, each of the methods failed to identify the presence of *S. aureus* in at least one participant compared with all other methods. Colonies grown from enhanced culture were phenotypically wild-type; no samples yielded phenotypic SCV or CWD variants. In addition to the two cases of MSSA carriage detected by enhanced culture alone (Participants 62 and 63 in Fig. 1), wild-type MRSA was detected by enhanced methods in one patient who only had MSSA detected by other methods (Patient 30 in Fig. 1). These figures equate to an increased incidence of pre-healthcare *S. aureus* culture-based detection of 19% (Supplementary Table 3) and a sensitivity of routine screening methods to detect *S. aureus* carriage of 84% (95% CI 73–92%) (Supplementary Table 4).

86/151 (58%) participants were electively admitted to hospital during the study period; 24 had a single further screen performed, 45 had two and 17 three further screens. 65 participants were not admitted as their procedures were cancelled or postponed outside the study period. No differences in patient demographics, co-morbidities or pre-healthcare sampling results were observed between those participants who were serially screened and those who only received pre-healthcare samples (Supplementary Table 5). Of 86 participants serially screened by routine methods, 17 (20%) yielded *S. aureus* from every serial screen (continuously detected carriage). 50 (58%) patients were culture-negative for *S. aureus* on every screen, and 19 (22%) patients intermittently yielded *S. aureus* during serial screening (discontinuously detected carriage).

**Acquisitions**

Seven acquisitions were identified where participants were culture-negative on pre-admission routine screening who subsequent sampling during admission was culture-positive for *S. aureus*. Of these, two yielded *S. aureus* from augmented screening of pre-healthcare samples; one through additional throat sampling (Patient 20 in Fig. 3C) and one through enhanced culture (Patient 16 in Fig. 3A). Culture-negative samples from the remaining five (Participants 1–5 in Fig. 3C) underwent analysis by 16S RNA PCR; pre-admission samples from these participants had *S. aureus* detected. Overall, of seven acquisitions detected by routine sampling five had evidence of preceding undetected carriage.

**Carriage loss**

14 participants were culture-positive on at least one routine pre-healthcare screening sample and were culture-negative on final sample taken during admission, which met our culture-based definition of carriage loss (Participants 6–19 in Fig. 3C). Of these nine had *S. aureus* carriage detected by routine screening and five by augmented sampling (two by throat alone, one by throat and rectum, one by axilla and rectum, and one by enhanced culture). Culture-negative samples from these participants were analysed by 16S RNA PCR; four had *S. aureus* detected on discharge sample (Patients 6, 8, 18 & 19 in Fig. 3C).

**Antibiotic exposure**

81/86 (94%) serially screened participants received antibiotics prior to recruitment. Patient characteristics between three carriage profiles identified by augmented screening did not significantly differ from those from routine screening (Supplementary Table 2). When comparing previous antibiotic exposure, patients exhibiting continuous detection (identified by augmented screening) tended to have received antibiotics in the 2 months preceding the study and those with no detected carriage received antibiotics over 6 months prior to the study. No trends with chronic skin wounds were observed.

Overall we identified:

- seven apparent acquisitions among 86 serially screened participants among which five had *S. aureus* identified prior to healthcare exposure (one by additional screening, one by enhanced culture and three by 16S RNA PCR), indicating pre-existing carriage.
- 10 instances of *S. aureus* carriage prior to admission which were missed by routine screening including one of MRSA carriage: eight by additional site sampling and two by enhanced culture methods.
- 14 of 89 participants screened throughout admission met our culture-based definition of *S. aureus* carriage loss and, of these, four had molecular evidence of *S. aureus* identified at discharge.

**Discussion**

The aims of our study were to describe *S. aureus* carriage patterns in patients before and during healthcare admission and evaluate whether undetected carriage explains apparent *S. aureus* acquisitions during healthcare exposure. Our work reveals five key findings:

- Five of seven apparent acquisitions identified during hospital admission can be explained by pre-existing carriage.
- Augmented screening increases detection of pre-healthcare *S. aureus* carriage by 19% and one in five people who are continuously culture negative by culture-based screening of nose, groin and any wound carry *S. aureus* undetected.
- Enhanced culture did not detect difficult-to-culture organisms but identifies a minority of colonised individuals (3/151 including one case of MRSA) who are undetected by routine and comprehensive detection methods. 
Molecular testing alone detected *S. aureus* carriage undetected by routine or augmented measures in 3 patients exhibiting discontinuously detected carriage.

Nearly one third of patients who become culture-negative during admission have *S. aureus* genomic material detected at discharge.

Healthcare-acquired *S. aureus* has implications for patients and healthcare services. First, there is an association between carriage and disease. Wertheim et al. revealed that colonised patients are three times more likely to develop nosocomial *S. aureus* infection compared to non-carriers, although all-cause mortality appears higher in patients becoming newly culture positive. Second, acquisitions frequently result in infection prevention and control interventions. Third, identification of *S. aureus* acquisitions in healthcare settings has a financial and reputational impact. A clear understanding of apparent acquisitions is therefore key as there is the potential to develop targeted management programmes, limit financial and reputational repercussions, and optimise infection prevention and control measures in healthcare settings.

The true burden of healthcare-acquired *S. aureus* in unknown. We previously report that the incidence of culture-based *S. aureus* acquisition in hospitalised patients in critical care is 6–8%. Studies are confounded by differences between carriage and infection, focus on MRSA (in turn dependent on local prevalence rates), variation in definitions, sensitivity of screening tests, use of culture to determine presence or absence of organism, focus on extracellular organisms. It is plausible that we are underestimating true incidence of *S. aureus* acquisition. We conducted a study of patients in elective cardiothoracic surgery setting and identified 8% (7/86) patients who were culture-negative on pre-admission screens subsequently yielded *S. aureus* from screens taken during admission. Many infection control practitioners would consider these new acquisitions. Of these seven apparent acquisitions five could be explained by pre-existing detection of *S. aureus* through augmented measures, equating to a 71% reduction. These results have a number of implications. First, a review of current screening methodologies is required to determine the value of expanding sampling sites to support detection of currently undetected *S. aureus* carriage. Second, with observations of clinical outcomes differences in patients who become newly culture-positive for *S. aureus* further work is required to understand the pathophysiology and clinical implication. Third, an understanding of the implications (financial, operational and reputational) to healthcare institutes.

**Impact of augmented screening**

Whilst the nose is reported to be the dominant reservoir of *S. aureus* carriage it has been cultured from multiple body sites. The sensitivity of a single nasal swab to detect *S. aureus* carriage is reported as 70%, increasing to c.89% when coupled with an extra-nasal swab. Common clinical practice is to perform dual swab testing for *S. aureus* screening. These performance measures are established on culture-based results. The results of the molecular work undertaken in our study suggests that culture-based sampling may under-represent the true rates of carriage.

Cryptic *S. aureus* variants, in particular SCVs, have been implicated in chronic bone and respiratory infections. To our kno-
edge no other studies have investigated their role in undetected carriage. Through employing enhanced culture measures we identified three cases of pre-healthcare carriage undetected by routine and comprehensive methods; one case of MRSA carriage and two cases of MSSA undetected. All isolates yielded from enhanced testing exhibited a wild-type phenotype, even though the culturing protocol successfully detected these variants in control experiments. It is plausible that SCV or CWD variants reverted back to wild-type prior to reviewing cultures. While this is an interesting observation, as these instances only represent a minority of cases our data suggests that SCV and CWD variants of S. aureus do not contributing significantly to undetected S. aureus carriage. These are inherently difficult to culture organisms and that itself lies a limitation – just because we didn’t find them doesn’t mean they weren’t there.

We employed methodologies specifically to aid identification of intracellular S. aureus detection (Supplementary Materials). Whilst there were three instances where S. aureus was cultured from samples optimised to detect intracellular cryptic variant and not by samples optimised to detect extracellular cryptic variants, there were no cases of carriage detected by intracellular carriage alone. Although swabbing the anterior nares with cotton-tip swabs has been shown to effectively capture nasal epithelial cells it is plausible that cellular capture from deep or other sites may contribute towards undetected carriage. Further work is required.

**Role of molecular testing**

Selected samples assessed using 16S RNA PCR revealed seven instances of culture-negative samples containing genomic signatures of S. aureus (three refuting to apparent acquisitions and four refuting apparent losses). As a pilot study this work was not designed to interrogate all samples with molecular testing and in turn there will be selection bias. Our work does suggest that molecular testing may play a role in determining undetected carriage and further investigation is required.

**Carriage profiles**

We investigated in detail 86 patients who were serially screened during the course of a healthcare admission. Serial culture-based methods of nose and groin sampling revealed 20% were culture-positive on every sample, 58% culture-negative on every sample, and 22% intermittently culturing S. aureus. This is broadly consistent with traditional culture-based carriage profiles. In our study, augmented screening revealed 10 additional carriers, equating to a 19% increase in identification of S. aureus carriage compared with routine screening. Continuous and discontinuous detection increased by 6% and 21%, respectively, resulting in a 10% reduction of those who had no carriage detected. These data suggest that one in five people who are continuously culture-negative by routine sampling are actually colonised by S. aureus at least intermittently. Furthermore, 29% (4/14) participants who appeared to loose S. aureus carriage had molecular evidence of S. aureus identified at discharge, suggesting undetected persisting carriage. Our data were collected over short time scales and only include augmenting testing in pre-assessment carriage. In turn it is plausible that our results underestimate S. aureus carriage.

**Antibiotics may impact carriage profiles**

Whilst prior receipt of antibiotics was high, there was a trend for patients with continuous detection to have received antibiotics in the last two months. It is plausible that antibiotics interrupt the microbiome resulting in a founder effect, permitting S. aureus to thrive. This observation is based on small numbers and hence further work is required.

**Limitations**

Our study has some limitations. First, 42% patients were not serially evaluated due to clinical reasons. Comparison of baseline data reveals that there were no significant differences in patient demographics, co-morbidities and prevalence of S. aureus carriage. In turn this suggests that the serially screened cohort was an unbiased representation of participants. Second, trend observed between carriage profiles, impact of previous antibiotics, previous MRSA colonisation and current skin wounds are based on small numbers. Further work is required to determine the true impact. Third, 34% (51/151) of participants did not undertake rectal testing however, of the 100 participants who undertook rectal screening, none had S. aureus carriage detected through rectal screening alone. In turn this suggests that we are unlikely to have missed a large proportion of rectal carriage that would not be detected by other routine and augmented sampling methods. Fourth, molecular testing is more sensitive the culture-based methods for detecting S. aureus. Performance measures were derived from culture-based results and, in turn, may overestimate the true sensitivity as detection may increase further if molecular tests were used. Fifth, augmented screening was not undertaken at each sample time during admission, so it is plausible that carriage at additional sites was missed. In turn this suggest that our results may under-represent apparent acquisitions being explained by undetected carriage. Sixth, it is plausible that we do not detect all difficult-to-culture organisms. S. aureus SCVs can produce negative catalase, slide coagulase and MALDI-TOF results. Most S. aureus SCVs produce a positive tube coagulase result after 18 h incubation. Whilst it is possible that some were missed all colonies grown had a negative slide coagulase result and were identified as non-S. aureus using MALDI-TOF with values reflecting high confidence in species identification. In the event of strong suspicion and negative biochemical tests molecular analysis could have been performed, including 16S RNA. Seventh, as isolates were not typed it is plausible that acquisition of different strains occurred in colonised participants, potentially underestimating the incidence of acquisition.

In conclusion, as S. aureus carriage precedes infection and new acquisition linked to higher disease-associated mortality early implementation of measures to terminate transmission chains could have significant impact on patient outcomes and healthcare resources. In turn, reliable identification of S. aureus acquisition in healthcare settings underpins effective infection prevention and control. We reveal that up to 71% of apparent S. aureus acquisitions within a cardiothoracic surgery setting can be explained by pre-existing undetected carriage. In turn, this could have a direct impact on inpatient screening methodologies. If S. aureus is present at a screen site but is undetected by current methods, then understanding of the reasons behind this has important implications on understanding the biology of S. aureus and impact on infection prevention and control.

**Declaration of Competing Interest**

All authors have completed the Unified Competing Interest form (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no authors received personal fees in the past three years outside the submitted work; no other relationships or activities that could appear to have influenced the submitted work.
CRediT authorship contribution statement

James R Price: Conceptualization, Visualization, Data curation, Funding acquisition, Formal analysis, Writing – original draft, Writing – review & editing, Project administration, Software. Maho Yokoyama: Data curation, Formal analysis, Writing – review & editing, Project administration, Software. Kevin Cole: Data curation, Writing – review & editing, Project administration, Software. Jonathan Sweetman: Data curation, Project administration, Software. Laura Behar: Data curation, Project administration, Software. Simon Stoneham: Writing – review & editing, Project administration, Software. Simon J Waddell: Project administration, Software. Jonathan Hyde: Project administration, Software. Ruhina Alam: Project administration, Software. Derrick Cook: Conceptualization, Visualization, Writing – review & editing, Project administration, Software. John Paul: Conceptualization, Visualization, Formal analysis, Writing – review & editing. Martin J Llewelyn: Conceptualization, Visualization, Funding acquisition, Formal analysis, Writing – original draft, Writing – review & editing.

Acknowledgments

We would like to acknowledge the British Infection Association for funding and the Cardiothoracic and Microbiology departments at Brighton and Sussex University Hospitals NHS Trust (Ref: 203931) for their help and support.

Ethics

Brighton and Sussex University Hospital NHS Trust was the study sponsor (Ref: 203931). The study was approved by London and Harrow Research Ethics Committee (Ref: 16/LO/2111) for patient sampling and data collection with individual consent. Routine clinical samples were collected and analysed as part of routine infection control response and, as such, was considered exempt from needing research ethics approval.

Transparency statement

The lead author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned (and, if relevant, registered) have been explained.

Data sharing

All anonymised data related to this work will be shared on request.

Public and patient involvement

The study was designed and developed with input from clinical stakeholders within the cardiothoracic surgery department at Brighton and Sussex University Hospital NHS Trust and without patient or public involvement.

Role of funding source

This work was supported by the British Infection Association [Small Project Research Grant].

Supplementary materials


References