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Tracking *Staphylococcus aureus* in the intensive care unit using whole-genome sequencing

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SUMMARY

Background: *Staphylococcus aureus* remains an important bacterial pathogen worldwide. This study utilized known staphylococcal epidemiology to track *S. aureus* between different ecological reservoirs in one 10-bed intensive care unit (ICU).

Methods: Selected hand-touch surfaces, staff hands and air were screened systematically 10 times during 10 months, with patients screened throughout the study. *S. aureus* isolates were subjected to *spa* typing and epidemiological analyses, followed by whole-genome sequencing to provide single nucleotide polymorphism (SNP) data.

Results: Multiple transmission pathways between patients and reservoirs were investigated. There were 34 transmission events, of which 29 were highly related (<25 SNPs) and five were possibly related (<50 SNPs). Twenty (59%) transmission events occurred between colonized patients and their own body sites (i.e. autogenous spread); four (12%) were associated with cross-transmission between patients; four (12%) occurred between patients and hand-touch sites (bedrails and intravenous pump); four (12%) linked airborne *S. aureus* with staff hands and bedrail; and two (6%) linked bed tables, bedrail and cardiac monitor.

Conclusion: Colonized patients are responsible for repeated introduction of new *S. aureus* into the ICU, whereupon a proportion spread to hand-touch sites in (or near) the patient zone. This short-term reservoir for *S. aureus* imposes a colonization/infection risk for subsequent patients. More than half of ICU-acquired *S. aureus* infection originated from the patients' own flora, while staff hands and air were rarely implicated in onward transmission. Control of staphylococcal infection in the ICU is best served by patient screening, systematic cleaning of hand-touch surfaces and continued emphasis on hand hygiene.

Introduction

There is continued interest in preventing healthcare-associated infection (HCAI), especially for patients in critical care. The mechanism by which patients acquire infection,

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including the role of the air, is still not fully understood, so more studies exploring pathogen transmission in healthcare environments are required [1,2]. Meticillin-susceptible and -resistant *Staphylococcus aureus* (MSSA and MRSA) provide useful markers for transmission as they frequently colonize patients, visitors and staff, and contaminate the environment including air. Dynamic transmission of staphylococci between patients, air and hand-touch surfaces, from direct contact and/or hand carriage, facilitates acquisition of *S. aureus* by patients [3,4]. The objective of this study was to establish the reservoirs and potential spread of specific strains of MSSA and MRSA in one intensive care unit (ICU) using molecular techniques including whole-genome sequencing (WGS) [5–7]. This study complements previous work establishing the most frequent hand-touch sites in this ICU, with systematic sampling of surfaces, patients, staff hands and air on designated sampling days over a 10-month period [8,9]. It was hoped to identify the most likely transmission pathways between key reservoirs in order to prioritize infection prevention in critical care.

Methods

Setting

The study was undertaken in a 10-bed adult ICU in a semi-rural Scottish district general hospital, as described previously [8,9]. The unit is broadly representative of critical care in Scotland, and receives >600 admissions each year, admitting patients with multiple trauma, acute sepsis and poisoning, cardiac events, pneumonia, and those who require support following surgery. The ICU is mechanically ventilated with filtered and tempered air at 22.6±1.9°C with no humidification. Ventilation rates are maintained at 10 air changes/h.

Sampling days

Ten sampling days were chosen according to ICU bed occupancy (≥50%) and staffing. Sampling began at 10 am (Monday–Saturday) and was completed by 12 noon. Five near-patient hand-touch sites (intravenous pump, cardiac monitor, bed table, and right and left bedrails) were screened systematically, followed by active and passive air sampling at four ICU sites [8,9]. Sampling was repeated 10 times over a 10-month study period, with a minimum of two weeks and maximum of six weeks between sampling episodes.

Patient screening

All ICU patients are screened routinely for MSSA/MRSA on admission, at discharge and twice weekly by sampling nose, perineum, urine and any wounds. This study sought to establish carrier patients (transient or persistent), chart duration of staphylococcal carriage, estimate colonization pressure on sampling days, and confirm acquisition incidents [9].

Colonized patients were identified 48 h after admission, and managed with mupirocin or neomycin nasal creams and chlorhexidine body washes. Staphylococcal infection confirmed >48 h after admission was documented as ICU-acquired using national criteria (http://www.nipcm.scot.nhs.uk). Patients with staphylococcal infection were prescribed flucloxacillin or vancomycin ± gentamicin according to antimicrobial susceptibility testing. Patients discharged from the ICU within 48 h of sampling days were followed-up for acquisition of MSSA/MRSA for a further two weeks, whether they stayed in hospital (weekly screening) or went home [general practitioner (GP) samples].

Sampling

Dipslides coated with nutrient and staphylococcal selective agars (Hygiena Ltd, Watford, UK) were used for sampling cardiac monitor, intravenous pump, right and left bedrails, and bed table. These provided quantitative (colony-forming units/cm^2) and qualitative (MSSA/MRSA) data from hand-touch surfaces [8–11]. Settle plates containing the same agars were placed on pre-cleaned 1-m-high trolleys positioned >1 m away from walls for 1 h at four sites [9,12]. MSSA/MRSA per m^3 of ICU air were captured using an automated air sampler [9]. Ten staff voluntarily placed thumb and finger tips of the dominant hand on to blood agar plates on each of the 10 sampling days. Different staff were screened on each occasion.

All agar plates and dipslides were processed in an NHS-accredited on-site microbiology laboratory. Coagulase-positive staphylococci were identified according to standard operating procedures from one representative colony-forming unit/plate or slide. MSSA/MRSA isolates from patients, staff hands, air and environment were characterized, documented and stored on beads at -20°C pending further characterization. Laboratory data were retained in accordance with health board policies.

Strain characterization

Study isolates were referred to the Staphylococcal Reference Laboratory [National Infection Service, Public Health England (PHE)] for analysis. Isolates were anonymized and included cultures from hand-touch surfaces, air (passive and active air sampling), staff hands and clinical samples throughout the 10-month study. Where *S. aureus* was recovered on multiple occasions from one patient, only the first isolates from either carriage and/or infection sites were characterized. All isolates were subjected to *spa* typing, and multi-locus sequence typing (MLST) clonal complex (CC) assignments were inferred by reference to the *spa* server (http://spa.ridom.de/mlst.shtml), MLST database (http://saureus.mlst.net) and in-house PHE database [13].

Isolates with related *spa* types and/or epidemiological links were investigated further by WGS [14]. This involved highlighting similar isolates from patients, staff hands and the environment collected on the same ward within the same week. Patient GP and postcodes were included if necessary. The matching exercise could be further refined using bed space location, specimen collection time and time periods between presumed contacts. All possible transmission events were subject to robust assessment in order to select isolates for WGS. Following selection, genomic DNA was extracted using the QIA symphony instrument. DNA libraries were prepared with Nextera XT kit and sequenced on Illumina HiSeq 2500 instrument generating 100bp paired end sequence fragments (Illumina, Cambridge, UK). MLST data were derived from sequence reads using MOST [15].

The phylogenetic relationship between isolates was determined at the core genome level by single nucleotide

polymorphism (SNP) analysis using an in-house pipeline (https://github.com/phe-bioinformatics/PHEnix). Isolates exhibiting <50 SNPs between them were highlighted for further exploration [16]. Sequence reads were mapped on to an MLST-matched genome reference sequence (ST5: BA000018; ST8: CP000253; ST15: CP000253; ST22: HE681097; ST30: CP002388; ST45: CP006044) using BWA software, and SNPs were called and filtered using GATK2 (ad_ratio: 0.9, min_depth: 10, qual_score: 40, mq_score: 30 and mq0_ratio: 0.1), and concatenated allowing 20% of Ns and gaps. Maximum likelihood analyses were performed using RaxML (GTR model, bootstrap: N=100). The best maximum likelihood tree was drawn using FigTree (data not shown). The pairwise distance matrix was calculated from the alignment excluding Ns and gaps.

Ethics

This project received ethical permission from NHS Lanarkshire R&D.

Results

Two hundred isolates of *S. aureus* were recovered during the course of this study. Of these, five were lost and 15 were duplicates, leaving 180 (169 MSSA, 11 MRSA) available for further characterization. These included isolates from carrier sites, clinical isolates, near-patient surfaces, air and staff hands. The total number of patient isolates was 156; of these, 146 were MSSA and 10 were MRSA. No patient had both MRSA and MSSA during the study. There were 24 environmental isolates, of which 10 (one MRSA) came from surfaces, four MSSA from air, and 10 MSSA from staff hands.

All available isolates (N=180) received initial *spa* typing and epidemiological assessment to highlight candidates for WGS; of these, 144 isolates were assigned to one of 14 major lineages (CC1, 5, 7, 8, 9, 15, 22, 25, 30, 45, 59, 97, 121 and 182). This facilitated selection of strains that might be closely related. Ultimately, 140 strains underwent WGS-based analyses, with 34 clusters <50 SNPs found to link patients and reservoirs. There were a further four pairs demonstrating strong epidemiological and phenotypic similarities, for which genotypic identity could not be confirmed due to non-survival of isolates.

Table I details the SNP differences observed between all epidemiologically linked strains. Twenty of 34 (59%) pairs were highly related (<25 SNPs), with a carried strain paired with the strain causing acquired infection, i.e. autogenous transmission (Table II, Figure 1) [17]. Most were ventilator-associated lower respiratory tract infections (13/20); there were also four wound infections, two central-line infections, one abscess and one intra-abdominal infection. The time scale between identification of a colonized patient and first recovery of a later isolate causing infection ranged from zero to eight days (average 2.7 days).

There were four (12%) cross-transmission episodes (<25 SNPs) between four pairs of patients with time intervals from two to three days to several months (Table I, Figure 1). Two pairs were thought to be possibly related, occurring after two- and four-day intervals. The relationship between the other two pairs is uncertain, despite differences of <25 SNPs, because these were EMRSA-15 and potentially endemic in the hospital [16,18]. One MRSA pair was separated by an interval of five months but involved two patients who had been together on the same ward: one before ICU admission and the other afterwards. The second MRSA pair was separated by an interval of four days with both patients resident on the ICU at the same time. There were no other patients with MRSA in the ICU during admission periods for either of these patients.

There were four pairs of *S. aureus* between patients and hand-touch sites, three of which were highly related (<5 SNPs), and linked the patient and sites within their own, or adjoining, bedspaces (Table I and Figure 1). The fourth episode involved isolates from a patient and adjacent bedrail, which differed by 49 SNPs and was therefore classified as uncertain [19]. There was a four-day interval between these isolates, whereas the other three pairs were recovered in one to three days. There was a highly related pair of isolates from the cardiac monitor and bed table in adjoining bedspaces (0 SNPs), and an uncertain association between two isolates from a bedrail and bed table three bedspaces apart (<25 SNPs), despite collection on the same day.

Airborne *S. aureus* were linked with isolates from staff hands on three occasions; two pairs were collected over 40 days apart but were highly related (<5 SNPs). The third pair, from staff hands and a settle plate, differed by <25 SNPs and were recovered 50 days apart, making the relationship uncertain. It is possible that these three pairs were due to individual staff carriers, as patients rarely stayed on the ICU for this length of time. There were no pairs linking staff hands and patients, or airborne strains and patients. One further transmission episode involving air occurred between airborne MSSA and an isolate from a bedrail on the same day (<5 SNPs). The airborne strain was collected using the air sampler beside Beds 5–7, while its partner was isolated from the left bedrail adjoining Bed 7.

Contaminated bedrails were implicated in five transmission episodes, four of these involving the left bedrail. Bedrails are known to be one of the most frequently handled sites, with staff predominantly touching the bedrail on the patient's right and visitors more often approaching the patient's left [8]. Bedtable strains were implicated in two linked pairs, and the cardiac monitor and intravenous pump were each involved in two separate transmission episodes.

There were an additional four episodes suggesting both autogenous and cross-infection (Figure 1, Table I). These pairs showed strong epidemiological and phenotypic similarities, but genomic confirmation was denied due to missing isolates. The isolates in each pair had identical antibiograms based on minimum inhibitory concentration data from VITEK. Three pairs, including two patients previously identified with ICU-acquired MSSA infection (Patients 5 and 8), demonstrated possible autogenous infection (Table II) [9]. The fourth involved two patients (Patients 6 and 9) who were assigned the same bedspace on the ICU three weeks apart. Patient 9, who had eczema, acquired a lower respiratory tract infection with a strain illustrating a unique antibiogram within the study collection (MSSA resistant to penicillin, fusidic acid, clarithromycin, clindamycin, trimethoprim and doxycycline). This differed from his nasal strain but closely resembled all strains from Patient 6, who had been resident on the ICU for 25 days before discharge (Figure 1).

This paper is the third of a series of papers reporting data from one study. Eleven patients with ICU-acquired MSSA or MRSA occurring within 72 h of a study sampling day are

Table I
Whole-genome sequencing (WGS) categories and pathways, lineage, sites, intervals (days) and single nucleotide polymorphism (SNP) differences of meticillin-susceptible *Staphylococcus aureus*/meticillin-resistant *S. aureus* (MSSA/MRSA) clusters in a 10-bed intensive care unit during a 10-month study

WGS category	Transmission pathway	Lineage (MLST-CC)	Patients and sites involved	Days between clusters	No. SNP differences
Highly likely [16]	1. Autogenous	8	Nose and Resp	2	<5
	2. Pt ↔ fomite (touch site)	5	Pt 2 Resp, Bed 3 → IVP, Bed 3	3	<5
	3. Pt ↔ fomite (touch site)	5	Pt 2 Resp, Bed 3 ↔ R/rail, Bed 3	3	<5
	4. Autogenous	15	Nose and Resp	5	<5
	5. Autogenous	15	Nose ↔ CLT	5	<25
	6. Autogenous	22 (MRSA)	Pt 4 Per and Pt 4 DRF	2	<5
	7. Autogenous	22 (MRSA)	Nose and Resp	2	0
	8. Autogenous	22	Nose and Resp	1	<5
	9. Pt ↔ fomite (touch site)	22 (MRSA)	L/rail ↔ Pt 4 Per and Pt 4 DRF	1	<5
	10. Autogenous	30	Resp and Nose	4	<5
	11. Autogenous	30	Nose and Resp	2	<5
	12. Autogenous	30	Pt 7 Nose and Pt 7 Per/Wound	5	<5
	13. Autogenous	30	Nose and Wound	1	<5
	14. Autogenous	45	Nose ↔ Resp	1	<25
	15. Autogenous	45	Nose ↔ Resp	2	<5
	16. Autogenous	45	Resp ↔ Nose	2	<25
	17. Autogenous	45	Pt 3 Per ↔ Pt 3 Wound	3	<5
	18. Air ↔ fomite	45	Air, Beds 5–7 ↔ L/rail, bed 7	0	<5
	19. Fomite ↔ fomite	45	Table ↔ CM	0	0
	20. Autogenous	7	Pt 6 nose ↔ Pt 6 CLT	8	<10
	21. Autogenous	34	Nose ↔ Resp ↔ Thr	2	<25
	22. Autogenous	59	Nose ↔ Resp	5	<25
	23. Autogenous	59	Nose ↔ Resp	0	<25
	24. Autogenous	188	Resp ↔ Nose	0	<10
	25. Autogenous	121	Abscess ↔ Nose	2	<10
	26. Staff hand ↔ air	25	Hand ↔ Air, Beds 5–7	43	<5
	27. Staff hand ↔ air	25	Hand ↔ Air, Beds 8–10	43	<5
Possible	28. Pt ↔ Pt cross-infection	59	Wound ↔ Nose and Resp	2	<25
	29. Pt ↔ Pt cross-infection	1	Nose ↔ Nose	4	<25
Uncertain [18,19]	30. Pt ↔ fomite (touch site)	5	Resp, Bed 2 ↔ L/rail, Bed 2	4	<50
	31. Staff hand ↔ air	5	Hand ↔ Settle plate	50	<25
	32. Pt ↔ Pt cross-infection	22 (MRSA)	Per ↔ Nose	161	<25
	33. Pt ↔ Pt cross-infection	22 (MRSA)	Nose ↔ Nose	3	<25
	34. Fomite ↔ fomite	30	L/rail, Bed 4 ↔ Table, Bed 7	0	<25
Presumed (phenotypic and epidemiologic relationships only)	1. Autogenous[a]	30	Pt 5 Nose → Pt 5 Resp, matching antibiograms	1	N/A
	2. Autogenous[a]	45	Pt 8 Nose→ Pt 8 Wound, matching antibiograms	4	N/A
	3. Autogenous[a]	1	Nose → Wound, matching antibiograms	0	N/A
	4. Pt ↔ Pt cross-infection[a,b]	7	Pt 6 Nose/CLT → Pt 9 Resp	48	N/A

MLST, multi-locus sequence typing; CC, clonal complex; ICU, intensive care unit; Pt, patient; Resp, respiratory secretions; DRF, drain fluid; IVP, intravenous pump; CTL, central line site; Per, perineum; L/R rail, left/right bedrail; CM, cardiac monitor; Thr, throat; N/A, unavailable for *spa* typing or WGS.

[a] Matching antibiograms included minimum inhibitory concentrations performed using VITEK2.

[b] These patients were allocated the same bedspace on the ICU three weeks apart. All *Staphylococcus aureus* from Pt 6 (including sputum) had matching antibiograms, which were unique within the study. Pt 6 stayed in the ICU for 25 days. Pt 9 had eczema and carried an unrelated nasal *S. aureus*.

Table II
Details of patients with intensive care unit (ICU)-acquired meticillin-susceptible *Staphylococcus aureus* (MSSA) or meticillin-resistant *S. aureus* (MRSA) infection during 10 sampling days [9]

Patient	Date of admission	Staph screen	No. of days to infection	No. of days in ICU	Age/sex	Admission diagnosis	Clinical infection	Site of isolation	Origin of infective strain	Onward transmission
1	4/2/15	Neg	4	7	52/M	Pancreatitis	MSSA VAP	Sputum; BLC	No matches	No matches
2	15/4/15	Neg	2	10	60/M	Colectomy for colon cancer	MSSA VAP	Sputum	No matches	**Spread to intravenous pump and bedrail**
3	2/5/15	Pos MSSA	5	21	74/M	Ruptured AA	MSSA SSI	Wound swab	**Autogenous**	No matches
4	10/5/15	Neg	6	8	57/M	Colitis	MRSA SSI	Drain fluid	**Bedrail**	Cross-infection outside ICU
5	22/6/15	Pos MSSA	2	12	72/F	Necrotizing fasciitis	MSSA VAP	Nasal swab; sputum	Autogenous	No matches
6	11/7/15	Pos MSSA	8	25	85/F	Ruptured AA	MSSA LI	CVL site; sputum	**Autogenous**	Possible spread to Patient 9
7	22/7/15	Pos MSSA	5	7	61/F	APR for rectal cancer	MSSA SSI	Wound swab	**Autogenous**	No matches
8	23/7/15	Pos MSSA	4	5	63/F	Sigmoid volvolus	MSSA cellulitis	Wound swab	Autogenous	No matches
9[a]	1/9/15	Pos MSSA[b]	4	4	20/M	Overdose	MSSA VAP	Sputum	Cross-infection inside ICU[b]	No matches
10[a]	5/10/15	Neg	8	4	73/M	EVAR	MSSA SSI	Wound swab	NT	NT
11[a]	8/10/15	Neg	2	2	46/F	Amputation ischaemic toes	MSSA LI	Arterial line site	NT	NT

BLC, blood cultures; AA, aortic aneurysm; CVL, central venous line; APR, abdominoperineal resection; EVAR, endovascular aneurysm repair; VAP, ventilator-associated pneumonia; SSI, surgical site infection; LI, line infection; NT, not typed.
Patients were diagnosed with ICU-acquired staphylococcal infection according to national criteria [9]. All patients were resident in the ICU during study sampling days. Bold text indicates matches established by whole-genome sequencing.
[a] Diagnosis made after ICU discharge.
[b] Different strain causing infection to original colonizing strain.

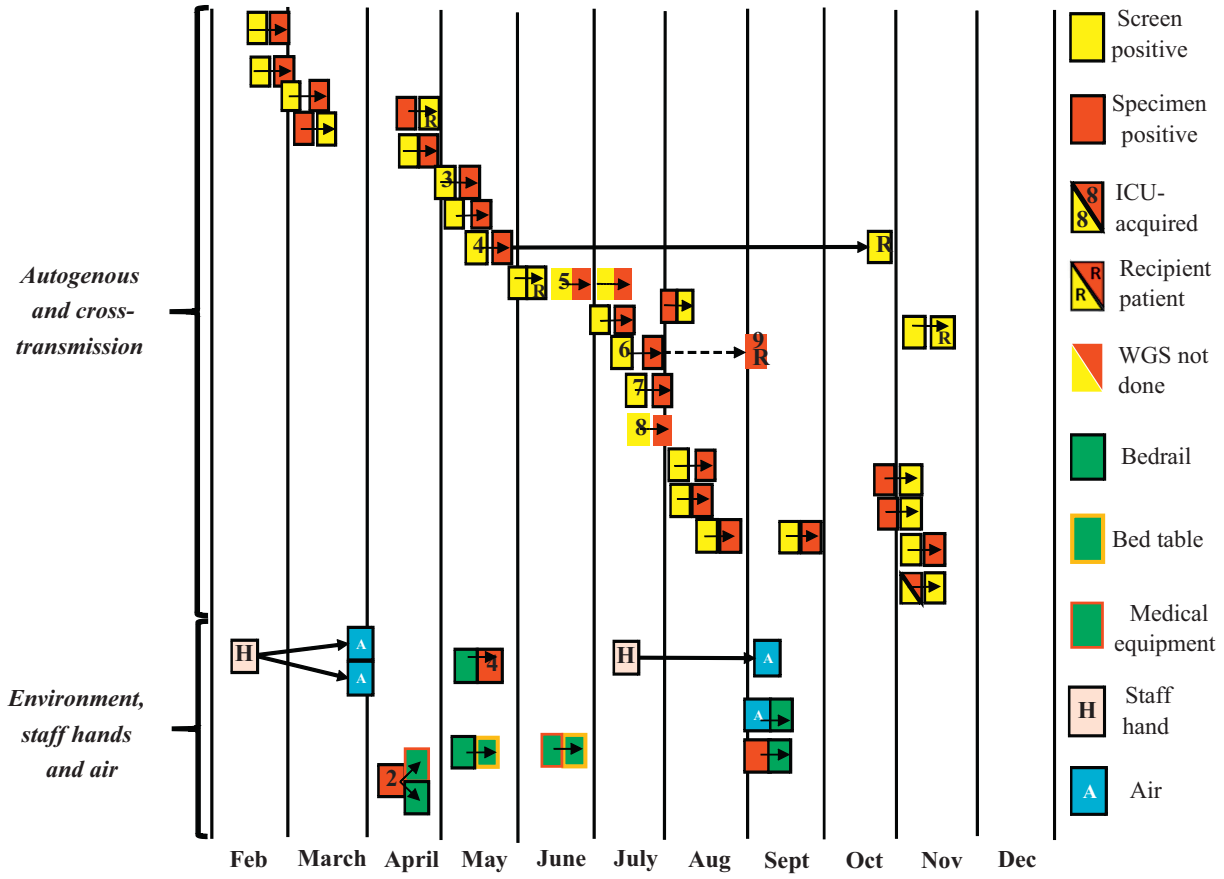

Figure 1. Timeline of epidemiologically ± whole-genome-sequencing (WGS)-related meticillin-susceptible *Staphylococcus aureus*/meticillin-resistant *S. aureus* pairs from patients, near-patient environment, medical equipment, staff hands and air during a 10-month study. ICU, intensive care unit. Arrows indicate genomic identity; dashed arrows indicate phenotypic and epidemiological relationships only; numbers indicate specific patients as detailed in Table II.

described in the second paper. WGS helped determine the origin or spread of MSSA/MRSA for five of these patients (Tables I and II, Figure 1) [9]. Patients 3, 6 and 7 were likely autogenous, as the colonizing strain was indistinguishable to that from the infection site (<10 SNPs). Patient 4 was admitted to the ICU six days before MRSA was recovered from a bedrail in the adjoining bedspace; indistinguishable MRSA strains (<5 SNPs) were isolated from this patient one day later. Patient 2 directly or indirectly contaminated an intravenous pump and bedrail in his bedspace (<5 SNPs). A non-WGS link between Patients 6 and 9 has been mentioned above, as well as possible autogenous infections for Patients 5 and 8. The origin of acquired strains for the remaining screen-negative patients is unknown.

Discussion

The combined genomic and epidemiological links in this study provide a valuable snapshot of MSSA/MRSA transmission pathways during routine care in the ICU. The greater granularity afforded by WGS is increasingly used to investigate and define links between patients and the healthcare environment [20]. Although there were just 10 sampling days in this study, 34 presumptive transmission events involving strains that were highly, possibly or tentatively genotypically related were identified. Over half of ICU-acquired *S. aureus* infections originated from the patients' own flora. Most were confirmed to be ventilator-associated pneumonia, already reported as a major risk for colonized patients [21].

There was also evidence for cross-infection in the ICU, with four pairs of related isolates between different patients (Table I, Figure 1). One of these pairs was separated by 161 days, suggesting prolonged survival of strains on unsampled surfaces and/or staff carriage. Staff were not screened apart from their hands, and this illustrates one of the main confounders of this study. A previous study did include staff screening but reported infrequent transmission to patients [3]. It should also be acknowledged that not all study isolates were genotyped due to selection methods, non-survival and finite study resources. More environmental screening occasions might have provided additional transmission events.

Further limitation concerns the impact of transient carriage for patients with ICU-acquired *S. aureus*, as an initial 'negative' screen depended upon swabbing and laboratory protocol. Critical care patients often receive broad-spectrum antibiotics, and as these encourage proliferation of endogenous organisms, detection of low-level carriage in individuals staying more than a few days would seem plausible. Patients might also have carried more than one strain or a metapopulation ('cloud of diversity'), so the number of transmission

events could be an underestimate [22]. Further confounders include the role of visitors, not screened, who probably contributed towards the transmission network on the ICU. Certainly, the left bedrail was implicated in more transmission episodes than the other sites, and visitors touch the left bedrail more than the right bedrail in this ICU [8].

Other than bedrails, only three additional hand-touch sites were screened around patient beds. There are numerous other hand-touch sites in the ICU, with frequently touched sites some distance from the patients [7]. A recent study screened mobile phones, departmental phones and ICU keyboards for multidrug-resistant organisms (MDROs), and compared these against patient isolates [23]. There were no genotypic matches, prompting the conclusion that phones and keyboards are unlikely to contribute to ICU-acquired MDROs in a low endemic setting. MDROs may have survived elsewhere, however, specifically ventilation ducts, filters and grilles [24]. This study found four airborne *S. aureus*, all of which were implicated in transmission links involving staff hands and a bedrail. Previous work has confirmed airborne microbes as a subset of surface contamination, and the single episode of genotypic identity between air and bedrail isolates supports this [8,25].

The absence of any links between staff hands and patient isolates is interesting because HCAI is usually attributed to contaminated staff hands [6]. While the number of sampling events was small (100 hand screens), the absence of confirmed transmission between staff hands and patients suggests that hand hygiene compliance is adequate in this unit. This is supported by good compliance rates from regular hand hygiene audits. Staff were not told when sampling was planned, and were screened during routine duties. Certainly, the two episodes linking airborne staphylococci and staff hands show that hands can be contaminated with *S. aureus* but this does not necessarily result in transfer to the patient.

MSSA/MRSA were recovered from near-patient sites on nine occasions. Patient 3 acquired MRSA three days after a genomically matched strain was isolated from a bedrail [9]. Patient 2, originally screen-negative, developed *S. aureus* lower respiratory tract infection, and an identical strain was recovered from an intravenous pump and right bedrail one day later. In a review of 1022 nosocomial outbreaks, Gastmeier *et al.* found that two of the top three sources of infection were associated with the environment (11.6%) or medical equipment (11.9%) [26]. Only patients themselves (25.7%) were a more common source of traceable outbreaks. The current study was performed during routine care, not during an outbreak, but six of 34 (18%) transmission episodes involved the surface environment, including clinical equipment. Similar findings were reported from a WGS study of vancomycin-resistant enterococci in an Australian ICU [20]. While cleaning in the study ICU is comprehensive, it may still be compromised by release of planktonic organisms from enmeshed biofilm [8,27—30].

The association between surface bioburden and ICU-acquired infection has been reported previously in this, and other, ICUs [9,10,31]. Higher levels of near-patient surface contamination are assumed to increase the risk of infection [32]. This study found qualitative evidence for this association, since there were genomic links established between near-patient hand-touch sites and patients themselves. While this supports the need for regular cleaning of these sites, the daily frequency required for the ICU as opposed to general wards (once daily) remains unconfirmed [11,33]. A previous study monitored the effect of cleaning on MRSA in an ICU, and showed how rapidly sites became recontaminated [34]. A similar study with concurrent monitoring of patient acquisition using genomic analyses would help establish a risk-based hypothesis for best cleaning practices in the critical care environment.

In conclusion, this study systematically collected MSSA and MRSA from patients, staff hands and environmental reservoirs in one ICU, and demonstrated links between them using WGS. New strains and lineages are introduced into the ICU constantly from colonized patients, which pose an infection risk for other patients via contamination of near-patient surfaces. This study could not demonstrate transmission involving staff hands, nor was there any evidence for links between airborne *S. aureus* and patients. While this single study cannot comprehensively define transmission hierarchies, the results support admission screening of patients for MSSA/MRSA, as well as regular cleaning of hand-touch sites and continued emphasis towards hand hygiene for both staff and visitors.

Conflict of interest statement
None declared.

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References

[1] Spagnolo F, Cristofari P, Tatonetti NP, Ginzburg LR, Dykhuizen DE. Pathogen population structure can explain hospital outbreaks. ISME J 2018;12:2835—43.
[2] Dancer SJ. Infection control; evidence-based common sense. Infect Dis Health 2016;21:147—53.
[3] Price JR, Cole K, Bexley A, Kostiou V, Eyre DW, Golubchik T, et al. Transmission of *Staphylococcus aureus* between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. Lancet Infect Dis 2017;17:207—14.
[4] Dancer SJ. Importance of the environment in MRSA acquisition: the case for hospital cleaning. Lancet Infect Dis 2008;8:101—13.
[5] McAdam PR, Templeton KE, Edwards GF, Holden MT, Feil EJ, Aanensen DM, et al. Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant *Staphylococcus aureus*. PNAS 2012;109:9107—12.
[6] Price JR, Golubchik T, Cole K, Wilson DJ, Crook DW, Thwaites GE, et al. Whole-genome sequencing shows that patient-to-patient transmission rarely accounts for acquisition of *Staphylococcus aureus* in an intensive care unit. Clin Infect Dis 2014;58:609—18.
[7] Wille I, Mayr A, Kreidl P, Brühwasser C, Hinterberger G, Fritz A, et al. A cross sectional point prevalence survey to study the environmental contamination of nosocomial pathogens in intensive care units under real life. J Hosp Infect 2018;98:90—5.
[8] Adams CE, Smith J, Robertson C, Watson V, Dancer SJ. Examining the relationship between surface bioburden and frequently touched sites in intensive care. J Hosp Infect 2017;95:76—80.
[9] Smith J, Adams CE, King M-F, Robertson C, Noakes C, Dancer SJ. Is there a relationship between airborne and surface microbes in the critical care environment? J Hosp Infect 2018;100:e123—9.
[10] White L, Dancer SJ, Robertson C, MacDonald J. Are hygiene standards useful in assessing infection risk? Am J Infect Control 2008;36:381—4.
[11] Bogusz A, Stewart M, Hunter J, Yip B, Reid D, Robertson C, et al. How quickly do hospital surfaces become contaminated after detergent cleaning? Healthcare Infect 2013;18:3—9.

[12] Pasquarella C, Pitzurra O, Svino A. The index of microbial air contamination. J Hosp Infect 2000;46:241—56.
[13] Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 2003;41:5442—8.
[14] Lahuerta-Marin A, Guelbenzu-Gonzalo M, Pichon B, Allen A, Doumith M, Lavery JF, et al. First report of lukM-positive livestock-associated methicillin-resistant *Staphylococcus aureus* CC30 from fattening pigs in Northern Ireland. Vet Microbiol 2016;182:131—4.
[15] Tewolde R, Dallman T, Schaefer U, Sheppard CL, Ashton P, Pichon B, et al. MOST: a modified MLST typing tool based on short read sequencing. Peer J 2016;4:e2308.
[16] Coll F, Harrison EM, Toleman MS, Reuter S, Raven KE, Blane B, et al. Longitudinal genomic surveillance of MRSA in the UK reveals transmission patterns in hospitals and the community. Sci Transl Med 2017;9. pii: eaak9745.
[17] Kluytmans JA, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505—20.
[18] Toleman MS, Watkins ER, Williams T, Blane B, Sadler B, Harrison EM, et al. Investigation of a cluster of sequence type 22 methicillin-resistant *Staphylococcus aureus* transmission in a community setting. Clin Infect Dis 2017;65:2069—77.
[19] Harrison EM, Ludden C, Brodrick HJ, Blane B, Brennan G, Morris D, et al. Transmission of methicillin-resistant *Staphylococcus aureus* in long-term care facilities and their related healthcare networks. Genome Med 2016;8:102.
[20] Lee AS, White E, Monahan LG, Jensen SO, Chan R, van Hal SJ. Defining the role of the environment in the emergence and persistence of vanA vancomycin-resistant enterococcus (VRE) in an intensive care unit: a molecular epidemiological study. Infect Control Hosp Epidemiol 2018;39:668—75.
[21] Paling FP, Wolkewitz M, Bode LGM, Klein Klouwenberg PMC, Ong DSY, Depuydt P, et al. *Staphylococcus aureus* colonization at ICU admission as a risk factor for developing S. aureus ICU pneumonia. Clin Microbiol Infect 2017;23:49.e9—49.e14.
[22] Nouwen JL, Ott A, Kluytmans-Vandenbergh MF, Boelens HA, Hofman A, van Belkum A, et al. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a "culture rule''. Clin Infect Dis 2004;39:806—11.
[23] Smibert O, Aung AK, Woolnough E, Carter GP, Schultz MB, Howden BP, et al. Mobile phones and computer keyboards: unlikely reservoirs of multidrug-resistant organisms in the tertiary intensive care unit. J Hosp Infect 2018;99:295—8.
[24] Wagenvoort JHT, Davies BI, Westermann EJA, Werink TJ, Toenbreker HMJ. MRSA from air-exhaust channels. Lancet 1993;341:840—1.
[25] Creamer E, Shore AC, Deasy EC, Galvin S, Dolan A, Walley N, et al. Air and surface contamination patterns of meticillin-resistant *Staphylococcus aureus* on eight acute hospital wards. J Hosp Infect 2014;86:201—8.
[26] Gastmeier P, Stamm-Balderjahn S, Hansen S, Nitzschke-Tiemann F, Zuschneid I, Groneberg K, et al. How outbreaks can contribute to prevention of nosocomial infection: analysis of 1,022 outbreaks. Infect Control Hosp Epidemiol 2005;26:357—61.
[27] Lei H, Jones RM, Li Y. Exploring surface cleaning strategies in hospital to prevent contact transmission of methicillin-resistant *Staphylococcus aureus*. BMC Infect Dis 2017;17:85.
[28] Russotto V, Cortegiani A, Fasciana T, Iozzo P, Raineri SM, Gregoretti C, et al. What healthcare workers should know about environmental bacterial contamination in the intensive care unit. BioMed Research Int 2017:6905450. Available at: https://doi.org/10.1155/2017/6905450 [last accessed January 2019].
[29] Vickery K, Deva A, Jacombs A, Allan J, Valente P, Gosbell IB. Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. J Hosp Infect 2012;80:52—5.
[30] Ledowitz K, Dancer SJ, Otter JA, Kerr K, Roposte D, Rushton L, et al. Beware biofilm! Dry biofilms containing bacterial pathogens on multiple healthcare surfaces; a multi-centre study. J Hosp Infect 2018;100:e47—56.
[31] Salgado CD, Sepkowitz KA, John JF, Cantey R, Attaway HH, Freeman KD, et al. Copper surfaces reduce the rate of healthcare-acquired infections in the intensive care unit. Infect Control Hosp Epidemiol 2013;34:479—86.
[32] Dancer SJ. Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination. Clin Microbiol Rev 2014;27:665—90.
[33] Stewart M, Bogusz A, Hunter J, Devanny I, Yip B, Reid D, et al. Microbiological effect of cleaning near-patient sites with electrolysed water. Infect Control Hospital Epidemiol 2014;35:1505—10.
[34] Aldeyab MA, McElnay JC, Elshibly SM, Hughes CM, McDowell DA, McMahon MA, et al. Evaluation of the efficacy of a conventional cleaning regimen in removing methicillin-resistant *Staphylococcus aureus* from contaminated surfaces in an intensive care unit. Infect Control Hosp Epidemiol 2009;30:304—6.