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Stephanie J. Dancer, MD, FRCPath, Claire E. Adams, MRCP, Jennifer Smith, BSc,
Bruno Pichon, PhD, Angela Kearns, PhD, Donald Morrison, PhD



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Tracking *Staphylococcus aureus* in ICU using Whole-Genome Sequencing

Stephanie J. Dancer MD, FRCPath^{1,2*}

Claire E. Adams MRCP³

Jennifer Smith BSc¹

Bruno Pichon PhD⁴

Angela Kearns PhD⁴

Donald Morrison PhD²

Affiliation

1. Dept. of Microbiology, Hairmyres Hospital, NHS Lanarkshire, Scotland, UK
2. School of Applied Sciences, Edinburgh Napier University, Scotland, UK
3. Dept. of Critical Care, Edinburgh Royal Infirmary, NHS Lothian, Scotland, UK
4. Healthcare Associated Infections and Antimicrobial Resistance Division, National Infection Service, Public Health England, Colindale, London, UK

Corresponding author*

Professor S. J. Dancer, Dept. of Microbiology, Hairmyres Hospital, Lanarkshire G75 8RG

E-mail: stephanie.dancer@lanarkshire.scot.nhs.uk

Tel: +44-1355 584792

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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 ten-bed intensive care unit (ICU).

Methods: Selected hand-touch surfaces, staff hands and air were systematically screened ten times during ten months, with patients screened throughout the study. *S. aureus* isolates were subjected to *spa* typing and epidemiological analyses, followed by whole-genome sequencing (WGS) 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 possibly related (<50 SNPs). Twenty of 34 (59%) occurred between colonized patients and their own body sites (i.e. autogenous spread); 4 (12%) were associated with cross-transmission between patients and 4 (12%) occurred between patients and hand-touch sites (bedrails and IV pump). Four (12%) transmission events linked airborne *S. aureus* with staff hands and a bedrail and two (6%) linked bed tables, bedrail and cardiac monitor.

Conclusion: Colonized patients are responsible for repeated introduction of new *S. aureus* into 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 ICU is best served by patient screening, systematic cleaning of hand-touch surfaces and continued emphasis on hand hygiene.

248 words

Introduction

There is continued interest in preventing healthcare-associated infection (HAI), especially for patients in critical care. The mechanism by which patients acquire infection, 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 since they frequently colonise 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 The 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 ten month period.^{8,9} It was hoped to identify the most likely transmission pathways between key reservoirs in order to prioritise infection prevention in critical care.

Methods

Setting

The study was carried out in a ten bed adult ICU in a semi-rural Scottish district general hospital as previously described.^{8,9} The unit is broadly representative of critical care in Scotland and receives >600 admissions each year, admitting 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 $22.6 \pm 1.9^\circ\text{C}$ with no humidification. Ventilation rates are maintained at 10 air changes/hour.

Sampling days

Ten sampling days were chosen according to ICU bed occupancy ($\geq 50\%$) and staffing. Sampling began at 10 am (Monday-Saturday) and was completed by 12 noon. Five near-patient hand-touch sites (iv pump, cardiac monitor, bed table and right and left bed-rails) were systematically screened, followed by active and passive air sampling at four ICU sites.

^{8,9} Sampling was repeated ten times within a ten month study period, with a minimum of 2 weeks and maximum of 6 weeks between sampling episodes.

Patient screening

All ICU patients are screened routinely for MSSA/MRSA on admission, discharge and twice weekly by sampling nose, perineum, urine and any wounds. We 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 hours after admission and managed with mupirocin or neomycin nasal creams and chlorhexidine body washes. Staphylococcal infection confirmed >48 hours 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 ICU within 48 hours 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 (GP samples).

Sampling

Dipslides coated with nutrient and staphylococcal selective agars (Hygiena Ltd, Watford, UK) were used for sampling cardiac monitor, intravenous (IV) pump, right and left bed rails and bed table. These provided quantitative (cfu/cm²) and qualitative (MSSA/MRSA) data from hand-touch surfaces.⁸⁻¹¹ Settle plates containing the same agars were placed on pre-cleaned 1 metre high trolleys positioned >1 metre away from walls for one hour at four sites.^{9,12} MSSA/MRSA per m³ of ICU air were captured using an automated air sampler.⁹ Ten staff voluntarily placed thumb and finger tips of the dominant hand onto blood agar plates on each of the ten 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 cfu/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), Colindale) for analysis. Isolates were anonymised and included cultures from hand-touch surfaces, air (passive and active air sampling), staff hands and clinical samples throughout the ten 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 MLST-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 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). Multi-locus sequence type (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 onto 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 ML 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 duplicate, 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 (1 MRSA) came from surfaces; 4 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; Fig.1).¹⁷ Most were ventilator-associated lower respiratory tract infections (13 of 20); there were also four wound infections (4 of 20); 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 0-8 days (average 2.7 days).

There were four of 34 (12%) cross-transmission episodes (<25 SNPs) between four pairs of patients with time intervals from 2-3 days to several months (Table I; Fig.1). Two pairs were thought possibly related, occurring after 2 and 4 day intervals. The relationship between the other two pairs is uncertain, despite <25 SNP difference, because these were EMRSA-15 and potentially endemic in the hospital.^{16,18} One MRSA pair was separated by an interval of 5 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 4 days with both patients resident on ICU at the same time. There were no other patients with MRSA in 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 SNP) and linked the patient and sites within their own, or adjoining, bedspaces (Table I; Fig.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 1-3 days. There was a highly related pair of isolates from cardiac monitor & 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 hand 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, since patients rarely stayed on ICU for this length of time. There were no pairs linking staff hands and patients or between 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 SNP). 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 Bed-table strains were implicated in two linked pairs and the cardiac monitor and IV pump were each involved in two separate transmission episodes.

There were an additional four episodes suggesting both autogenous and cross-infection (Fig. 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 MIC data from VITEK2™. Three pairs, including two patients previously identified with ICU-acquired MSSA infection (Pts 5 & 8) demonstrated possible autogenous infection (Table II).⁹ The fourth involved two patients (Pts 6 & 9) who were assigned the same bedspace on 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 ICU for 25 days before discharge (Fig.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 hrs of a study sampling day are described in the second paper. WGS helped determine origin or spread of MSSA/MRSA for five of these patients (Tables 1 & 2; Fig. 1).⁹ Patients 3, 6 and 7 were likely autogenous, since the colonizing strain was indistinguishable to that from the infection site (<10 SNPs). Patient 4 was admitted to ICU six days before MRSA was recovered from a bedrail in the adjoining bedspace; indistinguishable MRSA strains (<5 SNP) were isolated from this patient one day later. Patient 2 directly or indirectly contaminated an IV pump and bedrail in his bedspace (<5 SNP). A non-WGS link between Patients 6 and 9 has already been mentioned, 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 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 ten sampling days in this study, we found 34 presumptive transmission events involving strains that were highly, possibly or tentatively genotypically related. Over half of ICU-acquired *S. aureus* infection originated from the patients' own flora. Most were confirmed ventilator-associated pneumonia, already reported as a major risk for colonised patients.²¹

There was also evidence for cross-infection in the ICU, with four pairs of related isolates between different patients (Table I; Fig.1). One of these pairs was separated by 161 days, suggesting prolonged survival of strains on unsampled surfaces and/or staff carriage. We did not screen staff other than 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 We should also acknowledge that not all study isolates were genotyped due 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*, since an initial 'negative' screen depended upon swabbing and laboratory protocol. Critical care patients often receive broad-spectrum antibiotics and since 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 >1 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 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 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 (MDRO) 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 MDRO in a low endemic

setting. MDRO may have survived elsewhere, however, specifically ventilation ducts, filters and grilles.²⁴ We 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 hand and patient isolates is interesting because HAI 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 IV 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 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 6 of 34 (18%) transmission episodes involved the surface environment, including clinical equipment. Similar findings were reported from a WGS study of VRE in an Australian ICU.²⁰ While the cleaning in our 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 previously reported 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 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.

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 constantly introduced into the ICU from colonised patients, which pose an infection risk for other patients via contamination of near-patient surfaces. We 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 toward hand hygiene for both staff and visitors.

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Conflicts of interest statement

None disclosed by any author.

Author contributions

SD conceived the concept, wrote the protocol, obtained funding, analysed data and wrote the paper; CA performed all sampling and constructed study database; JS performed laboratory isolation, identification, documentation and storage of staphylococci; BP and AK performed *spa* typing and genomic analyses; SD, DM, BP and AK selected isolates for WGS

based on initial *spa* typing and epidemiology and analysed transmission pathways; all authors provided critical input and approved the final manuscript.

Figure Legend

Figure 1: Timeline of epidemiologically +/- WGS related MSSA/MRSA pairs from patients, near-patient environment, medical equipment, staff hands and air during a ten month study

Footnote for Figure: Arrows indicate genomic identity; dashed arrows indicate phenotypic and epidemiological relationships only; numbers indicate specific patients as detailed in Table II; WGS, whole-genome sequencing

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Table I: Whole-Genome Sequencing (WGS) categories and pathways, lineage, sites, intervals (days) and SNP differences of MSSA/MRSA clusters in a ten-bed ICU during a ten 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 & 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 & Resp	5	<5
	5. Autogenous	15	Nose ↔ CLT	5	<25
	6. Autogenous	22 (MRSA)	Pt. 4 Per & Pt. 4 DRF	2	<5
	7. Autogenous	22 (MRSA)	Nose & Resp	2	0
	8. Autogenous	22	Nose & Resp	1	<5
	9. Pt ↔ fomite (touch site)	22 (MRSA)	L/Rail ↔ Pt. 4 Per & Pt. 4 DRF	1	<5
	10. Autogenous	30	Resp & Nose	4	<5
	11. Autogenous	30	Nose & Resp	2	<5
	12. Autogenous	30	Pt. 7 Nose & Pt. 7 Per/Wound	5	<5
	13. Autogenous	30	Nose & 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 & 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. <i>Autogenous</i> *	30	Pt. 5 Nose → Pt. 5 Resp Matching antibiograms	1	N/A
	2. <i>Autogenous</i> *	45	Pt. 8 Nose → Pt. 8 Wound Matching antibiograms	4	N/A
	3. <i>Autogenous</i> *	1	Nose → Wound Matching antibiograms	0	N/A
	4. Pt ↔ Pt Cross-infection*#	7	Pt. 6 Nose/CLT → Pt. 9 Resp	48	N/A

Key: ICU=Intensive Care Unit; SNP=Single nucleotide polymorphism; 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.

*Matching antibiograms included MICs performed using VITEK2™;

#These patients were allocated the same bedspace on ICU 3 weeks apart. All *S.aureus* from Pt 6 (including sputum) had matching antibiograms, which were unique within the study. Pt 6 stayed in ICU for 25 days. Pt 9 had eczema and carried an unrelated nasal *S.aureus*.

Table II: Details of patients with ICU-acquired MSSA or MRSA infection during ten 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 Ca colon	MSSA VAP	Sputum	No matches	Spread to iv pump & 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	Necrotising 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 Ca rectum	MSSA SSI	Wound swab	Autogenous	No Matches
8	23/7/15	Pos MSSA	4	5	63/F	Sigmoid volvulus	MSSA cellulitis	Wound swab	?Autogenous	No matches
9*	1/9/15	Pos MSSA†	4	4	20/M	Overdose	MSSA VAP	Sputum	Cross-infection inside ICU†	No matches
10*	5/10/15	Neg	8	4	73/M	EVAR	MSSA SSI	Wound swab	NT	NT
11*	8/10/15	Neg	2	2	46/F	Amputation ischaemic toes	MSSA LI	Arterial line site	NT	NT

Key: MSSA: Methicillin-susceptible *S.aureus*, MRSA: Methicillin-resistant *S.aureus*, ICU: Intensive Care Unit, BLC; Blood cultures,

AA: Aortic aneurysm, CVL: Central Venous Line, APR: Abdominoperineal Resection, Ca: Cancer, EVAR: Endovascular Aneurysm Repair;

VAP: Ventilator-associated pneumonia; SSI: Surgical Site Infection; LI: Line infection, NT: Not typed

*denotes diagnosis made after ICU discharge. † denotes different strain causing infection to original colonising strain.

NB. Patients diagnosed with ICU-acquired staphylococcal infection according to national criteria.⁹ All patients were resident in ICU during study sampling days. Red text indicates matches established by WGS.

