Is there a relationship between airborne and surface microbes in the critical care environment?

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Running title: Air and surface bacteria in critical care

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Abstract

Objective: This study attempted firstly to correlate environmental contamination of air and surfaces in the intensive care unit (ICU); and secondly, to examine any association between environmental contamination and ICU-acquired staphylococcal infection.

Design: We screened patients, air and surfaces on 10 sampling days in a mechanically ventilated 10-bed ICU during 10 months.

Methods: Near-patient hand-touch sites (n=500) and air (n=80) were screened for total colony count and *Staphylococcus aureus* using dipslides, settle plates (passive air sampling) and an MAS-100 slit-sampler (active air sampling). Air counts were compared with surface counts according to proposed standards for air and surface bioburden. Patients were monitored for ICU-acquired staphylococcal infection throughout.

Results: Overall, 235 of 500 (47%) surfaces failed the standard for aerobic counts (≤2.5 cfu/cm^2). Half of passive air samples (20 of 40: 50%) failed the 'Index of Microbial Air' contamination (2 cfu/9cm plate/hr), and 15/40 (37.5%) active air samples failed the clean air standard (<10 cfu/m^3). Settle plate data was closer to the pass/fail proportion from surfaces and also provided the best agreement between air parameters and surfaces when evaluating surface benchmark values between 0-20 cfu/cm^2. The surface standard most likely to reflect hygiene pass/fail results compared with air was 5 cfu/cm^2. Rates of ICU-acquired staphylococcal infection were associated with surface counts/bed during 72 hours encompassing sampling days (p=0.012).

Conclusion: Passive air sampling provides quantitative data analogous to that obtained from surfaces. Settle plates could serve as a proxy for routine environmental screening to determine the infection risk in ICU.

Introduction

While the role of the air in hospital-acquired infection (HAI) has been investigated in operating theatres and immunocompromised units, there are few data and no accepted standards for air quality elsewhere in the hospital. [1-3] This includes the Intensive Care Unit (ICU), which accommodates particularly vulnerable patients. Any relationship between airborne pathogens and HAI risk in the ICU remains largely unknown.

An 'index of microbial air contamination' (IMA) was proposed in 2000, which specifies a standard for aerobic colony forming units (cfu) on 9cm settle plates placed 1 metre above the ground, 1 metre away from wall for 1 hour (1x1x1 rule).[4] The IMA has not been compared with environmental counts or infection rates among patients outside operating theatres. Another standard for active air sampling specifies <10 cfu/m^3 air during theatre commissioning in the UK.[5,6] . There are also proposed standards for hospital surfaces, comprising cfu/cm^2 and specific pathogens at hand-touch sites.[7] The latter have been used to compare surface bioburden with cleaning activities and HAI incidence.[8-14]

The aim of this study was to investigate any association between air and surface counts in the ICU, and model against ICU-acquired infection rates. Systematic collection of colony counts from hand-touch sites and air would allow data sets to be compared using proposed standards for surfaces and air. We chose coagulase-positive staphylococci as indicator pathogens, since these organisms represent a useful marker of hospital hygiene. Methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S.aureus* (MRSA)

contaminate air and surfaces and colonise staff, patients and visitors.[15,16] For this reason, all patients were monitored for ICU-acquired staphylococcal infection during the study.

Methods

Study ICU: The study was performed in a ten bed adult ICU in a Scottish hospital (Figure 1). The unit receives >600 admissions each year and serves a largely rural community. It 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/hour as recommended for critical care.[5] Each ventilated patient is nursed on a 1:1 basis with highly dependent patients receiving 1:2 nursing care. Bed occupancy ranges from 50-100%, with daily turnover of 1-5 patients. Case-mix includes pneumonia, trauma, poisoning, sepsis and post-operative support.

Domestic and nursing staff share routine cleaning, with domestics cleaning bathrooms and general surfaces once daily. Near-patient sites are cleaned by nurses twice daily at 7am and 7 pm. Cleaning is detergent-based, using wipes (Vernacare Tuffie™ wipes) and detergent (Hospec™) for general surfaces. Bed-spaces of patients colonised or infected with hospital pathogens are cleaned with bleach (Actichlor Plus™). Terminal cleaning of the bed-space is performed following discharge.

Study days: Ten study days within a 10 month period were selected for sampling according to bed occupancy (>50%). There was a minimum of two weeks and maximum of six weeks between study days in order to allay any Hawthorne effect from staff and allow a complete change of patients. Sampling took place between 10-12am (Mon-Sat). Five hand-touch sites

around each bed were systematically screened from bed 1 (side-room) to bed 10 (Fig 1). Two 9cm agar settle plates were placed on one metre high trolleys in the side-room and three other sites with the lids removed for one hour (Sites 1-4A: Fig 1).[4] Trolley sites corresponded with nearby beds, so that site 1 sampled air in the side-room; site 2 sampled air beside beds 2-4; site 3 sampled air beside beds 5-7; and site 4 sampled air beside beds 8-10. Active air sampling was performed in the side-room and main ICU at sites 1-4A. People-traffic was crudely assessed by auditing the number of people passing the nurses' station in 5 mins, repeated three times 30 mins apart.

Study sites: Prior audit of hand-touch events established five commonly touched sites: over-bed table, bedrails, infusion pump and cardiac monitor.[17] The number of times a site is handled corresponds with the level of microbial soil recovered from that site.[17] The current report used this data to compare with air counts collected at the same time.

Surface screening: Surface counts were categorised as previously described.[17] Screening was performed using double-sided dipslides (Hygiena Int., Watford, UK), coated with nutrient and staphylococcal selective agars. Each slide was systematically placed on each site for 10 seconds at a pressure of 25 g/cm^2 with no overlap between the different agars.[18] Dipslides were loosely capped and incubated at 35°C in CO_2 for 48-72 hours.

Microbiology: Growth on nutrient agar supplied aerobic colony counts (ACC) per cm^2 (no growth (NG); scanty growth (SG) <2.5 cfu/cm^2; light growth (LG) ≥2.5-12 cfu/cm^2; moderate growth (MG) >12-40 cfu/cm^2; heavy growth (HG)>40 cfu/cm^2).[17] Selective agar highlighted

potential staphylococci, which were sub-cultured on to *S.aureus* Identification (SAID) agar (Oxoid Ltd, UK), followed by automated susceptibility testing (VITEK).[11,12]

Air sampling: Settle plates (nutrient and staphylococcal selective agars) were used for passive air sampling (cfu/9cm plate/hr). Active air sampling was performed using an MAS-100 slit sampler (Merk; Germany), based on the Andersen impactor principle and calibrated according to manufacturer's instructions. Air was directed onto a 9cm Petri dish at 116 litres/min for 10x1 min at each site. ACC and staphylococci per m^3 of air were cultured using the same agars and processed as for dipslides.

ICU-acquired infection: ICU patients are routinely screened for MSSA/MRSA on admission and twice weekly thereafter unless discharged within 4 hours. Staphylococcal infection confirmed >48 hours after admission was documented as ICU-acquired using national criteria (http://www.nipcm.scot.nhs.uk). The number of patients with ICU-acquired MSSA/MRSA infection occurring within a 72hr period encompassing the sampling day (one day before, until one day after, screening) were compared with meteorological parameters, bed-occupancy, staphylococcal colonization pressure, people-traffic and surface and air data recovered on sampling days. These infections were adjusted for bed occupancy over the same 72 hr period by dividing the number of confirmed infections by % ICU bed occupancy.

Confounding parameters

Potential confounders were temperature (inside/outside ICU); outside humidity and air pressure; bed occupancy; staffing; people-traffic, including visitors; seasonal influences;

weather; building work; ward geography; staphylococcal carriers (patients only); cleaning practices; patient bed movements; and meal times.[16] External meteorological conditions were monitored because there were windows which could be opened, and the main exit was adjacent to a main hospital entrance. This ICU regularly undergoes both hand hygiene and environmental audits every 2-3 months, with data posted at the main entrance.

Statistics

Air data was compared against surface bioburden for 10 sampling days. Data from the side-room (one bed) and main ICU (nine beds) were analysed together and separately. Staphylococci were compared with surface counts, bed occupancy and people-traffic. All measured variables were compared with ICU-acquired MSSA/MRSA infection. Analysis of variance was used to assess ACC levels over time. Non-parametric statistical tools were used throughout and confidence intervals (CI) given where appropriate. Significance levels were set at 5% for all reported calculations. Linear and logistic regression was conducted using R (3.2.1) to investigate any correlation between ACC and MSSA/MRSA.

Results

Five hundred near-patient sites yielded counts from 0->40cfu/cm^2 (Table I).[17] There was a 47% failure rate using <2.5cfu/cm^2 as benchmark.[13] Pass and fail proportions were then compared with data from both air sampling methods (Table II). Passive air sampling ranged from 0-40 cfu/plate/hr, with >2cfu/plate/hr recovered from 20/40 plates. The IMA proposes ≤2cfu/plate/hr, which gave a failure rate of 50%.[4] The active air sampling standard is <10

cfu/m^3.[5,6] We obtained 0-40 cfu/m^3 from active air sampling, with 15/40 samples giving >10 cfu/m^3 (failure rate: 37.5%). Thus, proportionate fails from passive air sampling (50%) more closely resembled surface failure rate (47%) than from active sampling (37.5%). Quantitative data was examined on a site-by-site basis for each sampling day (Appendix 1). Beds were categorised based on their proximity to sampling sites as previously described (Fig 1). The pass/fail status from air sampling methods was compared with the pass/fail status for surface sampling (≤2.5cfu/cm^2). Only 19/40 (47.5%) pairs agreed for active air data and surface bioburden. There was a closer alignment between passive air data and surface counts (26/40: 65%).

The comparison above depends on using 2.5cfu/cm^2 as surface benchmark. We wondered whether pass/fail proportions for air counts would show similar agreement with surface data if another standard was chosen. Consequently, pass/fail agreement between active and passive air data was compared with surface standards from 0-20 cfu/cm^2. Figure 2 shows percentage pass/fail agreement between air parameters and different surface standards. The highest percentage agreements between air and surface standards occur with passive air counts for surface standards between 0.5-6 cfu/cm^2; there is similar proportionate agreement for both active and passive air sampling if surface standards are 7-8 cfu/cm^2; and surface standards from 9-17.5 cfu/cm^2 show closer agreement with active air pass/fail proportions. The best agreement (70%) between any air parameter and specific surface standard occurs at 5cfu/cm^2 for passive air counts. Five cfu/cm^2 is a recognised benchmark for food industry surfaces and has already been proposed for hospitals.[7]

There was a positive correlation between MSSA/MRSA isolation and quantitative count from the same sites (p=0.0007; 95% CI=1.02-1.12) but not for air (p=0.8, 95% CI=0.89-1.11). Surfaces with the highest contact (bedrails, tables) were more likely to host MSSA/MRSA compared with other sites. No staphylococci were recovered from surfaces or air within the side-room. Recovery of MSSA/MRSA was predictably low, with four MSSA isolates from air and ten staphylococcal isolates (including one MRSA) from surfaces (Tables I, II; Appendix 1). Only once were MSSA or MRSA detected both on surfaces and air (sampling day 9). There were no relationships between the likelihood of finding MSSA/MRSA from surfaces and air on any day, nor were there any between surface MSSA/MRSA and the likelihood of pass/fail outcome for air counts. While staphylococcal isolation intimates a hygiene 'fail', adding these fails to those already obtained did not change overall findings.

As expected, bed occupancy was associated with people-traffic, but surface contamination was found to decrease slightly with increasing footfall, which is unexpected (p=0.00485) (Appendix 2). Passive air data and people-traffic were not associated (p=0.54) but active air sampling was correlated with higher traffic (p=0.09). No relationship was found between either bed occupancy or people-traffic and detection of MSSA/MRSA, although the number of patients with MSSA/MRSA had a statistically significant effect on colony counts at the 90% (instead of 95%) level (p=0.08) (Appendix 1). Eleven patients acquired staphylococcal infections during the 72hr period encompassing sampling days (Appendix 3). The number of infections was adjusted for %bed occupancy and plotted against total surface count/bed for Beds 2-10, since these patients were accommodated in the main ICU, none in the side-room (Bed 1) (Fig 3). Rate adjusted ICU-acquired staphylococcal infection was associated with average surface count for beds 2-10 (p=0.012) (Appendices). There was no indication that

external meteorological conditions influenced any microbiological findings in ICU on sampling days.

Discussion

There continues to be a strong focus on HAI in the UK's NHS. We still know little about the transmission of infection, particularly the role of the air.[19] This study attempts to link air and surface bioburden in a controlled environment in order to compare and contrast quantitative and qualitative values using proposed microbiological criteria.

Air and surface counts at near-patient sites agreed on pass or fail just one third of the time (15/40) (Appendix 1). Most disagreements occurred where there was a fail on allied surfaces and a pass from air; only 3/40 showed a pass from the surface with fails from air (beds 5-7, study days 1 and 2). This suggests that surface counts are a combination of air deposition and contact routes, while air samples represent a proportion of total surface contamination. Thus, passive air sampling could be used as a routine monitoring strategy, while outbreak investigation should combine both passive air and surface sampling. Surface sampling offers a more accurate risk assessment since it is less likely to give a false positive. A measure of the air is included in surface data and this provides assurance that air quality is acceptable. Air sampling alone cannot detect surface contamination from other routes.

On 10 of 40 occasions, either MSSA or MRSA or both were recovered from surfaces or air; for these 10 occasions, nine showed surface hygiene failures from bed sites adjacent to a specific sampling point. This reflects previous work that noted the association of

MSSA/MRSA with higher surface counts.[20] The more microbial soil in the vicinity, the more likely it is that a pathogen can be isolated.[20]

Surfaces in the side-room were cleaner than the rest of ICU although the data varied ($p=0.001$). This was attributed to the fact that the door was kept shut when the room was occupied and the room itself was often left unused. More people-traffic and positive correlation with active air sampling ($p=0.04$) at higher bed occupancy is also unsurprising. However, there was no association between surface counts and people-traffic, nor passive air data and people-traffic. This may have been due to the method used for auditing footfall in ICU. People-traffic was measured beside the nurses' station, which is situated away from beds and sampling points (Fig 1). Furthermore, air samples were collected in the morning, which illustrates a major limitation of the study. A previous study in a naturally ventilated ward showed that airborne bioburden fluctuated significantly with activity over a day and yielded values that were considerably higher than this study.[16]

There are additional limitations. These include the fact that the study was performed in a single ICU only; there were just 10 sampling days in 10 months; patient demographics were not reported (other than patients with ICU-acquired staphylococcal infection: Appendix 3); and there was no data on other factors, such as the effectiveness of environmental cleaning; or whether patients were isolated when indicated along with compliance with contact precautions, etc. It is also possible that some staphylococcal carriers were unscreened, due to short (<4 hours) admission periods or fatal outcome.

At present, there is no reliable method for assessing infection risk from the environment. Visual inspections cannot accurately determine HAI risk for patients.[15] Monitoring cleanliness using microbiological screening is resource dependent, and ATP bioluminescence is expensive and monitors organic soil, not presence of pathogens.[21] Previous work suggests that surface counts and HAI risk are related, in that the higher the surface soil, the more likely it is that patients will suffer HAI.[13,14] This study supports that relationship, since average count/bed was associated with ICU-acquired MSSA/MRSA. Given the association between settle plate and surface data, perhaps settle plates could be utilised as a proxy for routine screening. Passive air sampling is easy to do, inexpensive, and would not require microbiological interpretation other than counting colonies.[4] Future work should consider a long term study that investigates passive air sampling against HAI in order to explore this.

In conclusion, this study systematically screened near-patient hand-touch sites and air using both active and passive air sampling over 10 months in an ICU. There may be an association between surface counts and settle plate data, provided that ACCs are interpreted according to given benchmark standards. The surface standard gaining the best alignment between passive air sampling and surface counts in this ICU was 5cfu/cm^2.

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Conflicts of interest

None reported.

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Figure Legends

Figure 1: Intensive Care Unit (ICU) layout.

Figure 2: Agreement between active and passive air sampling and surface bioburden using a range of surface standards from 0-20 cfu/cm^2. The X axis shows the percentage pass or fail agreement between active and passive air data for each bioburden standard; the Y axis shows the surface bioburden value in cfu/cm^2.

Figure 3: Total bioburden (5 sites)/bed (cfu/cm^2) plotted against % ICU-acquired MSSA/MRSA infection (adjusted for bed occupancy) for Beds 2-10 on 10 sampling days**.**

Table I: Microbial soil categories for five hand-touch sites on ICU

Site	No Growth	Scanty Growth <2.5 cfu/cm^2	Light Growth ≥2.5-12 cfu/cm^2	Moderate Growth >12-40 cfu/cm^2	Heavy Growth >40 cfu/cm^2	No. of Hygiene fails (>2.5 cfu/cm^2)
Infusion Pump	16	47 MSSA	22	13 MSSA	2	37/100: 37%
Cardiac Monitor	45	28	16 MSSA	9	2	27/100: 27%
Right Bedrail	6	38	17	27	12 MSSA	56/100: 56%
Over-bed Table	13	35	33 MSSA	16 MSSA	3	52/100: 52%
Left Bedrail	6	31	26	25 MSSA x2	12 MSSA & MRSA	63/100: 63%

MSSA: methicillin-susceptible *S. aureus* and MRSA: methicillin-resistant *S.aureus* isolated on one or two occasions only.

Hygiene standard for surfaces: <2.5 cfu/cm^2(ref 6)

Average surface fail = **47%** (range: 27-63%)

Table II: Microbial burden categories for air (active and passive sampling) and hygiene fails according to standards

Passive air sampling N=40	No Growth	Scanty Growth 0-2 cfu/plate	Light Growth >2-10 cfu/plate	Moderate Growth >10-40 cfu/plate	Heavy Growth >40 cfu/plate	No. of Hygiene fails (>2 cfu/plate/hr)
Air settle cfu/plate/hr	1	19 MSSA	18	2	0	20/40 = 50%
Active air sampling N=40	**No Growth**	**Scanty Growth 0-2 cfu/m³**	**Light Growth >2-10 cfu/m³**	**Moderate Growth >10-40 cfu/m³**	**Heavy Growth >40 cfu/m³**	**No. of Hygiene fails (>10 cfu/m³)**
Air sampler cfu/m³	1	6	18 MSSAx2	15 MSSA	0	15/40 = 37.5%

MSSA: methicillin-susceptible *S. aureus* and MRSA: methicillin-resistant *S.aureus* isolated on one or two occasions only.

Hygiene standard for air (passive) [4]: ≤2 cfu/9cm² plate/hr

Hygiene standard for air (active) [5]: <10 cfu/m³

Overall, 50% passive air samples fail standards; 37.5% active air samples fail standards.

Table

APPENDIX 1

Surface and air bioburden (cfu) assigned pass/fail according to proposed standards for surfaces and air sampling (active & passive) in ICU

STUDY DAY No. of MSSA MRSA patients	Bed 1 (5 sites)		Beds 2-4 (15 sites)		Beds 5-7 (15 sites)		Beds 8-10 (15 sites)	
	Surface bioburden	Air Active Passive	Surface bioburden	Air Active Passive	Surface bioburden	Air Active Passive	Surface bioburden	Air Active Passive
1 **MSSAx2** **MRSAx1**	NGx2 SGx3	**A=1 P** **S=1 P**	NGx3 SGx11 MGx1	**A=1 P** **S=1 P**	NGx2 SGx12 MGx1	**A=26 F** **S=1 P**	NGx2 SGx11 MGx2	**A=1 P** **S=1 P**
Total cfu **Av site cfu**	**3** **0.6 P**		**37** **2.5 P**		**38** **2.5 P**		**63** **4.2 F**	
2 **MSSAx2**	MGx4 HGx1	**A=5 P** **S=1 P**	NGx2 SGx6 LGx5 MGx2	**A=5 P** **S=5 F**	NGx8 SGx6 LGx1	**A=26 F** **MSSA** **S=26 F**	NGx5 SGx8 HGx2	**A=5 P** **MSSA** **S=5 F**
Total cfu **Av site cfu**	**144** **28.8 F**		**93** **6.2 F**		**11** **0.73 P**		**88** **5.9 F**	
3 **MSSAx3**	NGx5	**A=1 P** **S=1 P**	NGx4 SGx10 LGx1	**A=5 P** **S=0 P**	NGx4 SGx3 LGx5 MGx3	**A=26 F** **S=1 P**	NGx3 SGx7 **MSSA** LGx2 MGx2 HGx1	**A=5 P** **S=5 F**
Total cfu **Av site cfu**	**0** **0 P**		**15** **1.0 P**		**106** **7.1 F**		**109** **7.3 F**	
4 **MSSAx3**	MGx4 HGx1	**A=0 P** **S=1 P**	NGx2 SGx3 LGx2 MGx4**MSSA** HGx4**MSSA**	**A=26 F** **S=1 P**	NGx1 SGx7 LGx4 MGx3	**A=5 P** **S=5 F**	NGx3 SGx1 LGx5 MGx5 HGx1	**A=5 P** **S=1 P**
Total cfu **Av site cfu**	**144** **28.8 F**		**277** **18.5 F**		**105** **7.0 F**		**196** **13.1 F**	
5 **MSSAx1** **MRSAx1**	NGx2 SGx2 MGx1	**A=1 P** **S=1 P**	NGx1 SGx3 LGx3 MGx4**MSSA** HGx4 **MRSA**	**A=5 P** **S=26 F**	SGx2 LGx3 MGx9**MSSA** HGx1	**A=5 P** **S=5 F**	NGx1 SGx2 LGx11 MGx1	**A=5 P** **S=5 F**
Total cfu **Av site cfu**	**28** **5.6 F**		**282** **18.8 F**		**291** **19.4 F**		**83** **5.5 F**	

APPENDIX 1

Surface and air bioburden (cfu) assigned pass/fail according to proposed standards for surfaces and air sampling (active & passive) in ICU

6 **MSSA** **X1**	LGx2 HGx3	**A=1 P** **S=1 P**	SGx1 LGx8 MGx6	**A=5 P** **S=1 P**	SGx5 LGx8**MSSA** **MSSA** MGx2	**A=26 F** **S=5 F**	SGx6 LGx6 MGx2 HGx1	**A=26 F** **S=5 F**
Total cfu **Av site cfu**	**130** **26.0 F**		**197** **13.1 F**		**97** **6.5 F**		**128** **8.5 F**	
7 **MSSA** **X4**	NGx3 SGx1 LGx1	**A=5 P** **S=1 P**	NGx3 SGx7 LGx2 MGx1 HGx2	**A=5 P** **S=5 F**	NGx1 SGx6 LGx4 MGx2 HGx2	**A=26 F** **S=5 F**	NGx2 SGx7 LGx2 MGx4	**A=5 P** **S=5 F**
Total cfu **Av site cfu**	**6** **1.2 P**		**123** **8.2 F**		**158** **10.5 F**		**121** **8.1 F**	
8 **MSSA** **x2**	NGx3 SGx1 LGx1	**A=5 P** **S=1 P**	NGx2 SGx7 LGx4 MGx1 HGx1	**A=26 F** **S=5 F**	NGx2 SGx3 LGx4 MGx3 HGx3	**A=26 F** **S=5 F**	NGx6 SGx5 LGx1 MGx1 HGx2	**A=26 F** **S=5 F**
Total cfu **Av site cfu**	**6** **1.2 P**		**93** **6.2 F**		**221** **14.7 F**		**116** **7.7 F**	
9 **MSSA** **X6**	NGx1 SGx2 LGx1 MGx1	**A=5 P** **S=1 P**	NGx1 SGx7 LGx4 MGx3**MSSA**	**A=26 F** **S=1 P**	NGx3 SGx5 LGx1 MGx6**MSSA**	**A=5 P** **MSSA** **S=1 P**	NGx2 SGx7 LGx4 MGx2	**A=26 F** **S=1 P** **MSSA**
Total cfu **Av site cfu**	**38** **7.6 F**		**105** **7.0 F**		**171** **11.4 F**		**79** **5.3 F**	
10 **MSSA** **x2**	NGx3 SGx1 MGx1	**A=26 F** **S=5 F**	NGx1 SGx2 LGx6 MGx4 HGx2	**A=26 F** **S=5 F**	NGx1 SGx7 LGx5 MGx2	**A=5 P** **S=5 F**	NGx2 SGx2 LGx8 MGx3	**A=26 F** **S=5 F**
Total cfu **Av site cfu**	**27** **5.4 F**		**216** **14.4 F**		**84** **5.6 F**		**120** **8.0 F**	

Surface bioburden mid category in cfu/cm^2: NG=0; SG=1; LG=5; MG=26; HG=40

Surface bioburden fails if the standard is >2.5 cfu/cm^2 at hand touch site; P=pass; F=fail

Passive (S) air standards fail if >2 cfu/plate/hour; Active (A) air standards fail if >10 cfu/m^3

MSSA: methicillin-susceptible *S.aureus*; MRSA: methicillin-resistant *S.aureus*; T = Total cfu/cm^2; Av = Average cfu/cm^2 (for all hand touch sites).

Table

Appendix 2: Weather and temperature variables, people-traffic, staphylococcal infections, bed-occupancy and bioburden in air and on surfaces for ten sampling days in a 10-bed ICU

STUDY DAY N=10	Temp°C Inside ICU Outside ICU	Humidity (%) Pressure (mb) Weather	Bed Occupancy (%) N=10 (100%)	People Traffic* (av.3 values)	Average bioburden (cfu/cm^2) per site (all beds) n=50/day	Average bioburden (cfu/cm^2) per bed (beds2-10) n=9/day	No. & rate % of ICU-acquired MSSA/MRSA infection#	Average bioburden: active air sampling (cfu/m^3) n=4/day	Average bioburden: passive air sampling (cfu/plate/hr) n=4/day
1	22 6	81 1032 Dry: cloudy	90	14.67	2.82	15.33	X1 MSSA 11.1	7.25	1.00
2	22 7	55 1010 Dry: light cloud	90	14.67	6.72	21.33	0 0	10.25	9.25
3	22 9	67 1032 Dry: sunny	50	9.00	4.6	25.55	0 0	9.25	1.75
4	22 10	60 1032 Dry: some sun	70	11.00	14.44	64.22	x1 MSSA 14.3	9.00	2.00
5	22 9	60 1020 Rain: some cloud	60	5.33	13.68	72.88	x1 MSSA x1 MRSA 33.3	4.00	9.25
6	22 15	61 1017 Dry: light cloud	50	12.33	11.04	46.88	x1 MSSA 20.0	14.50	3.00
7	22 13	52 1020 Sunny intervals	70	14.33	8.16	44.66	x1 MSSA 14.3	10.25	4.00
8	22 15	58 1013 Rain: scattered clouds	70	12.67	8.72	47.77	x2 MSSA 28.6	20.75	4.00

9	**22** **12**	**71** **1017** **Sunny intervals**	**80**	**12.00**	**7.86**	**39.44**	**x1 MSSA** **12.5**	**15.50**	**1.00**
10	**22** **7**	**87** **1013** **Sun; some cloud**	**80**	**16.30**	**8.94**	**46.66**	**x2 MSSA** **25.0**	**20.75**	**5.00**

NB: *People traffic estimates as number of people passing nursing station in 5 mins; repeated three times 30 mins apart during 2 hr sampling period.

#Staphylococcal acquired infection rate adjusted according to Bed Occupancy.

MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S.aureus*

Table

Appendix 3: Details of patients with confirmed ICU-acquired staphylococcal infection during ten sampling periods

Patient	Date of admission	No. of days to infection	Age/ sex	Diagnosis	Type of infection	Positive specimens	Site of infection
1	4/2/15	4	52/M	Pancreatitis	MSSA	Sputum; BLC	Chest
2	15/4/15	2	60/M	Colectomy for Ca colon	MSSA	Sputum	Chest
3	2/5/15	5	74/M	Ruptured aortic aneurysm	MSSA	Wound swab	Abdominal wound
4	10/5/15	6	57/M	Colitis	MRSA	Drain fluid	Peritoneal collection
5	22/6/15	2	72/F	Necrotising fasciitis	MSSA	Sputum	Chest
6	11/7/15	8	85/F	Ruptured aortic aneurysm	MSSA	CVL site; sputum	Line site (neck)
7	22/7/15	5	61/F	APR for Ca rectum	MSSA	Wound swab	Perineal wound
8	23/7/15	4	63/F	Sigmoid volvulus	MSSA	Wound swab	Cellulitis arm
9*	1/9/15	4	20/M	Overdose	MSSA	Sputum	Chest
10*	5/10/15	8	73/M	EVAR	MSSA	Wound swab	Groin
11	8/10/15	2	46/F	Amputation ischaemic toes	MSSA	Arterial line site	Line site (arm)

Key: MSSA: Methicillin-susceptible *S.aureus*, MRSA: Methicillin-resistant *S.aureus*, ICU: Intensive Care Unit, BLC; Blood cultures, CVL: Central Venous Line, APR: Abdominoperineal Resection, Ca: Cancer, EVAR: Endovascular Aneurysm Repair

*denotes diagnosis after ICU discharge.

NB. Patients were diagnosed with ICU-acquired staphylococcal infection according to national criteria, >48 hrs following admission and within 72 hrs of study sampling days. The average time to acquired staphylococcal infection was 4.5 days.

Figure

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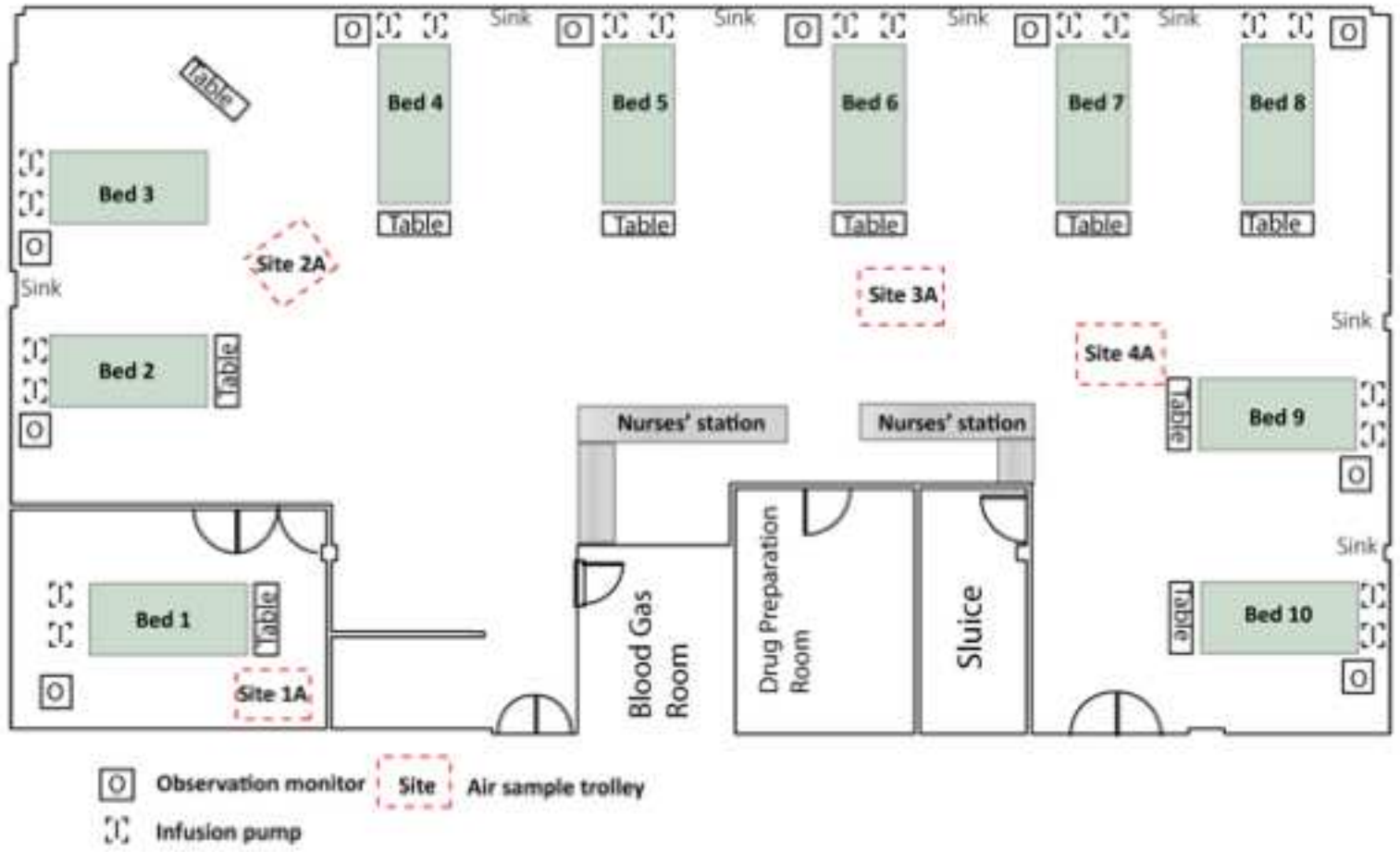

Figure

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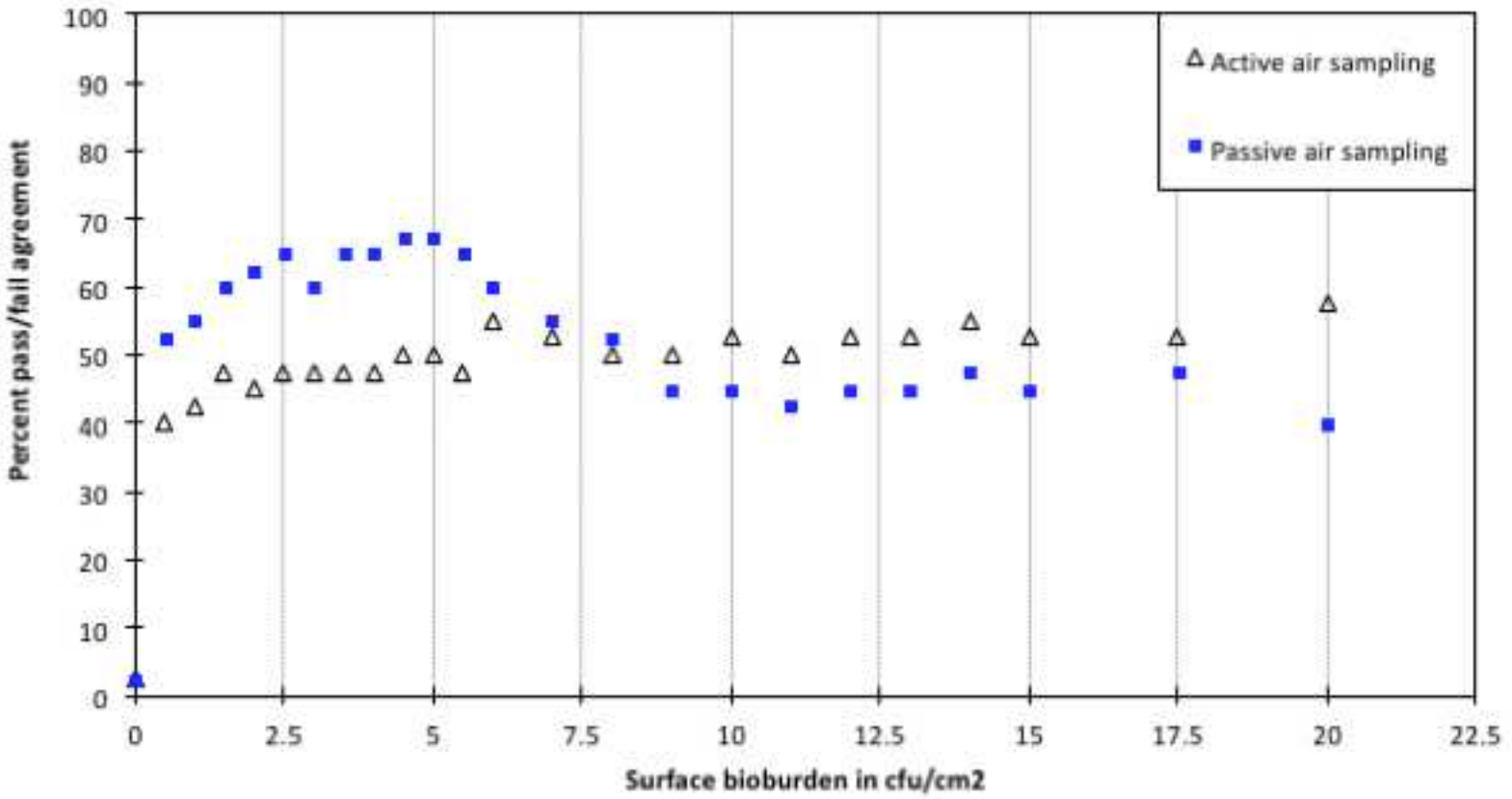

Figure

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