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

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Abstract

Objective: Th*is* **study attempted to correlate environmental contamination of air and surfaces with hospital-acquired infection (HAI) in the critical care environment.**

Design: We screened patients, air and surfaces in a mechanically ventilated 10-bed intensive care unit (ICU) on 10 sampling days 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, with data interpreted according to proposed standards for surfaces and air. 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. Passive air sampling also provided the best agreement between air 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 sampling was 5cfu/cm^2. There was an association between rates of ICU-acquired staphylococcal infection and surface count/bed during 72 hours encompassing sampling days.

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

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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)

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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

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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. 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 data variables 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

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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

Each study month provided a series of results of 5 surface sites of each of the 10 beds, 4 passive air collections samples and 4 active air collection samples for the 10 months. All data were subjected to statistical analyses conducted in R (3.2.1). Passive and active air sample data were modelled against surface bioburden for 10 sampling days using linear regression and the assumption of normality for intra-phase data was validated using residual plots. Data from the side-room (one bed) and main ICU (nine beds) were analysed together and separately. Staphylococcal colony counts were regressed on surface ACC, bed occupancy and people-traffic using through linear regression. All variables were compared with ICU-acquired MSSA/MRSA infection using logistic regression and confidence intervals given where appropriate. Analysis of variance was used to assess importance of ACC over time. Significance levels are set at 5% or as specified otherwise.

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

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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]

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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 implies a hygiene 'fail', adding these fails to those already obtained did not change overall findings.

As expected, bed occupancy was associated with people-traffic using linear regression, but surface contamination was found to decrease slightly with increasing footfall, which is unexpected (p=0.00485) (Appendix 2). Linear regression also showed that 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 strong 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 ACC count per bed for Beds 2-10, since these patients were accommodated in the main ICU, none in the side-room (Bed

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1) (Fig 3). Rate adjusted ICU-acquired staphylococcal infection was associated with average surface ACC 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.

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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 for this 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 periods within 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; and whether patients were isolated when indicated along with

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compliance with contact precautions, etc. It is also possible that some staphylococcal carriers remained unscreened, due to short (<4 hours) admission periods.

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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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**.**

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%

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

Cardiac Monitor	45	28	16 MSSA	9	2	27/100: 27%
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%

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

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/9cm^2 plate/hr

Hygiene standard for air (active) [5]: <10 cfu/m^3

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

Active air sampling N=40	No Growth	Scanty Growth 0-2 cfu/m^3	Light Growth >2-10 cfu/m^3	Moderate Growth >10-40 cfu/m^3	Heavy Growth >40 cfu/m^3	No. of Hygiene fails >10 cfu/m^3
Air sampler cfu/m^3	1	6	18 MSSAx2	15 MSSA	0	15/40 = 37.5%