Journal of Hospital Infection xxx (xxxx) xxx

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Genetic relationship between bacteria isolated from intraoperative air samples and surgical site infections at a major teaching hospital in Ghana

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ARTICLE INFO

Article history:
Received 7 July 2019
Accepted 11 November 2019
Available online xxx

Keywords:
Surgical site infections
Airborne bacteria
Low- and middle-income countries
Staphylococcus aureus
Whole genome sequencing
Metagenomic

SUMMARY

Background: In low- and middle-income countries (LMICs) the rate of surgical site infections (SSI) is high, leading to negative patient outcomes and excess healthcare costs. A causal relationship between airborne bacteria in the operating room and SSI has not been established, at a molecular or genetic level. We studied the relationship between intraoperative airborne bacteria and bacteria causing SSI in an LMIC.
Methods: Active air sampling using a portable impactor was performed during clean or clean-contaminated elective surgical procedures. Active patient follow-up consisting of phone calls and clinical examinations was performed 3, 14 and 30 days after surgery. Bacterial isolates recovered from SSI and air samples were compared by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) identification, ribotyping, whole genome sequencing (WGS), and metagenomic analysis.
Results: Of 128 included patients, 116 (91%) completed follow-up and 11 (9%) developed SSI. Known pathogenic bacteria were isolated from intraoperative air samples in all cases with SSI. A match between air and SSI isolates was found by MALDI-TOF in eight cases. Matching ribotypes were found in six cases and in one case both WGS and metagenomic analysis showed identity between air- and SSI-isolates.
Conclusion: The study showed high levels of intraoperative airborne bacteria, an SSI-rate of 9% and a genetic link between intraoperative airborne bacteria and bacteria

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https://doi.org/10.1016/j.jhin.2019.11.007
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isolated from SSIs. This indicates the need for awareness of intraoperative air quality in LMICs.

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Introduction

Surgical site infections (SSI) are among the most common surgical complications leading to negative patient outcomes and excess costs [1,2]. Studies are needed to document the burden and the causes of SSI in low- and middle-income countries (LMICs), in order to guide preventive interventions and prioritize scarce funds [3,4]. Previous studies have identified several risk factors for SSI such as overweight, smoking, diabetes, health-status, and duration of procedure [1,2]. Intraoperative airborne bacteria represent a possible source of SSI, but international recommendations in many cases do not consider air quality important for reduction of SSI in LMICs [1,2]. We have recently found high levels of airborne bacteria in operating rooms at a major teaching hospital in Ghana [5]. Despite the established association between intraoperative colony-forming units per cubic metre of air (cfu/m^3) and SSI risk, the aetiological role of these bacteria has not been formally demonstrated [6–8]. In this study, we assessed predictors for SSI in an LMIC and used whole-genome sequencing (WGS) and metagenomic analysis to assess the relationship between intraoperative airborne bacteria and SSI. To our knowledge, this is the first attempt to prove causality between environmental intraoperative airborne bacteria and SSI with the use of genetic methods.

Methods

Setting

The study adds to our previously published work from the General Surgery Unit, Korle-Bu Teaching Hospital [5]. Operating rooms were equipped with non-laminar ventilation with highly efficient particulate air (HEPA) filters. Both air intake and exhaust are located at the ceiling. Operating rooms are not constructed to obtain positive pressure [5]. No routine maintenance services were in place at the time of study. No details of air change per hour were available. Staff wore institutional clothing, shoes, disposable hoods and facemasks [5]. The scrub team further wore sterile gowns and gloves [5]. Incision sites were disinfected three times before incision with disinfection liquid consisting of 0.15% chlorhexidine, 1.5% cetrimide, 34% alcohol (95% ethanol and 5% methanol), and water. Skin disinfectant was produced at the hospital's central pharmacy. No routine sterility test of the ingredients or final mixture was made at the hospital pharmacy or at the surgical unit. Non-disinfected skin was covered with sterile cotton sheets. Instruments were sterilised by steam autoclaving for 55 min at 121°C 2 atm for plastic items, and 45 min at 134°C 3 atm for remaining items. Correct sterilization was controlled with heat-sensitive tape. As previously described a high level of human activity was seen during surgery and both the number of individuals and the number of door openings were predictive factors of cfu counts in the operating room [5]. Prophylactic antibiotics where administered prior to first incision. No local protocol for antibiotic prophylaxis existed at the time of the study. Between each surgery, surfaces, kickbuckets and floor were cleaned from cleanest to dirtiest, and top to bottom with a 10% chlorine solution. Operating rooms were equipped with temperature control and ranged between 16 and 25°C. Remaining parts of the hospital had no temperature control.

Selection of patients

Patients undergoing surgery were included consecutively if: age ≥18 years; American Society of Anaesthesiologists physical classification score (ASA-score) ≤III; elective non-implant procedure; and wounds classified as clean or clean-contaminated according to the Centre for Disease Control (CDC) classification [9,10].

Observation and patient characteristics

We piloted and used standardized questionnaires to record patient age, height, weight, sex, smoking habits, diabetes status, ASA-score, ICD-10-PCS procedure code, date and duration of procedure, peri-operative antibiotics, number of blood transfusions, and surgeon's assessment of the CDC wound classification [11].

Air samples

Air samples were collected during surgery as part of our previously published study [5]. In brief, air samples were obtained on 5% blood agar using a portable impactor (MAS-100; Merck, Darmstadt, Germany) operating 5 min at a flowrate of 100 L/min every 20 min from the time of the first incision to final wound closure, shifting between a position 30–60 cm from the wound and a position opposite the entrance 1.5 m from the wall [5]. Plates were incubated at 37°C for 48 h and cfu were thereafter counted [5]. After cfu count, each plate was scraped with a sterile loop and biological material transferred to a sterile broth-based medium with 10% glycerol, mixed and frozen at −80°C before transfer to Denmark on dry ice.

Follow-up

Patients were followed by surgeons with phone calls 3, 14 and 30 days after surgery, or until SSI diagnosis. Patients were asked whether they had received wound treatment and interviewed for SSI symptoms according to CDC criteria [12]. If SSI were suspected, patients were invited for a clinical examination. If SSI was clinically confirmed, a sample was obtained using E-Swabs™ (COPAN ITALIA S.P.A, Brescia, Italy). Sampling was performed by trained surgeons. Samples were taken by rotating a sterile swab in the infected wound carefully touching only the wound. Swabs were frozen at −80°C in sterile broth-based freezing medium with 10% glycerol until transport to Denmark.

Please cite this article as: Stauning MA et al., Genetic relationship between bacteria isolated from intraoperative air samples and surgical site infections at a major teaching hospital in Ghana, Journal of Hospital Infection, https://doi.org/10.1016/j.jhin.2019.11.007

Identification of bacterial isolates from air and wound samples

A sample from each vial containing material from air samples obtained during procedures with subsequent SSI as well as wound swabs were thawed and plated on 5% blood agar, blue agar (a selective medium for large Gram-negative rods), and 7.5% NaCl plates (SSI Diagnostica, Hillerød, Denmark). After incubation for 48 h at 37°C, morphologically different colonies were sub-cultured on 5% blood agar, incubated 24 h and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF, Microflex, Bruker, Bremen, Germany).

WGS of SSI isolates

DNA was prepared with DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). Purification of DNA was conducted with lysozyme treatment according to MALDI-TOF identification. Illumina Miseq was used for sequencing, generating 250-base pair paired-end reads using a multiplexed Nextera XT protocol (Illumina, Hilden, Germany). Sequence reads were error-corrected using ALLPATHS-LG's stand-alone error correction tool and de-novo-assembled using de Bruijn graph based assembler Velvet v.1.2.10 with Velvet Optimizer v. 2.2.5 [13].

Comparison of air and SSI isolates

Air and SSI isolates were paired with respect to operating room and time of surgery. Paired isolates matching at species level after MALDI-TOF identification were compared by ribotyping (RipoPrinter system®, DuPont Qualicon, Geneva, Switzerland) according to manufacturers' instructions [14]. Air isolates in the same ribogroup or with >85% similarity to a paired SSI isolate were selected for WGS following the same protocols as SSI isolates. We used BacDist with Snippy v.4.1.0 to call SNPs in each of the isolates relative to a relevant National Center for Biotechnology Information (NCBI) reference genome and compare shared sequences [15,16]. Only SNPs at positions covered by at least 10 reads in both isolates with one isolate showing >80% non-reference reads were included. SNP distances were computed with snp-dists v.0.6.3 [17]. Approximately maximum-likelihood phylogenetic trees were constructed with Parsnp and visualized in iTOL-v3 [18,19].

Metagenomic sequencing of air samples

Metagenomic sequencing was performed on DNA extracted directly from each vial containing material from air samples obtained during procedures with subsequent SSI. DNA purification was conducted with lysozyme treatment to extract DNA from both Gram-positive and -negative bacteria. Metagenomic taxonomic classification was performed with Kaiju in Greedy-1 mode with s = 100 [20]. Only species with >5000 classified reads/metagenome were included in the results.

Comparison of metagenomes from air samples and whole genomes of SSI isolates

SNPs unique to each SSI isolate (ID-SNPs) were identified by Parsnp comparison with complete NBCI genomes [19]. To check for the presence of ID-SNPs in the metagenomes, we mapped reads of each metagenome to the genome of the SSI isolate with the respective ID-SNPs using bwa-mem [21]. Metagenomic readcounts for each base at each ID-SNP position were counted using bam-readcount to determine whether the ID-SNP was present. Only mapped reads with a minimal quality >30 were included in read counts to determine the number of reads that contained the respective ID-SNP. To control for location-specific ID-SNPs not present in the NCBI database, *Staphylococcus aureus* results were further compared with 15 WGS results from two Ghanaian hospitals obtained by Donker *et al*. [22]. The full details and pipeline used for the metagenomic comparison can be found at GitHub [23].

Statistics

Odds ratios were provided by logistic regression between SSI, cfu/m^3 and possible confounders. Variables were first analysed by univariate regression and entered in a multivariable model if $P<0.1$. In the multivariable model backwards elimination was applied and variables were kept if $P<0.05$. All non-significant variables were heretafter re-entered one by one and kept for the final model if $P<0.05$. Differences in cfu/m^3 between surgeries with and without subsequent SSI were assessed by Wilcoxon-rank-sum-test. Analyses were performed with R v.3.4.1 and the epitools-package [24,25]. Data is reported with 95% confidence intervals (CIs). *P*-values <0.05 were considered statistically significant.

Ethics

Informed consent was mandatory for inclusion. Sampling did not alter the surgical procedure. For all SSI, an additional swab was obtained and processed independently of the study. The study was reviewed and granted approval from Korle-Bu Teaching Hospital Institutional Review Board (ref. KBTH-IRB/0004/2016), the Danish National Committee on Health Research Ethics (ref. 1610254) and the Danish Data Protection Agency (ref. 2012-58-0004).

Results

SSI and air sampling

A total of 214 patients were found eligible for inclusion, 128 were included and 116 completed follow-up (91%, Supplementary Figure S1). SSI was suspected for 13 patients and clinically confirmed for 11 patients (9.5%, 11/116). For SSI rate according to type of surgery, see Table I. Most infections were identified post-discharge ($N = 8$, 73%). Air samples were available for 124 of 128 cases, and for all cases with SSI (Supplementary Figure S1). As previously described, mean cfu/m^3 in empty operating rooms were 39 cfu/m^3 (95% CI: 36, 42; range: 12, 81) after 1 h of ventilation [5].

Risk factors associated with SSI

There were significantly higher cfu/m^3 during procedures on patients that developed SSI than the remaining procedures

Table I
Surgical environment and infection rate according to type of surgery

	Number of procedures	Completed follow-up (*N*)	SSI detected (*N*)	SSI rate
General anaesthesia				
Thyroidectomy and parathyroidectomy	26	25	0	0%
Non-cosmetic mammary surgery	30	26	5	19%
Excision of lipomas or subcutaneous tissue	3	3	0	0%
Controlled abdominal surgery	8	7	4	57%
Local anaesthesia				
Repair of Inguinal hernia	28	25	1	4%
Non-cosmetic mammary surgery	19	18	0	0%
Excision of lipomas or subcutaneous tissue	14	10	1	10%

For type of surgery, ICD-10-PCS procedure codes were collected, grouped according to similarity, and reported in our previous study [5]. Non-cosmetic mammary surgery in general anaesthesia were mastectomies and wide local resections (ICD-10-PCS; 0HBT0ZX ($N = 6$), 0HBT0ZZ ($N = 1$), 0HBU0ZX ($N = 6$), 0HBU0ZZ ($N = 1$), 0HTT0ZZ ($N = 8$), 0HTU0ZZ ($N = 7$) and 0WB80ZZ ($N = 1$)). Non-cosmetic mammary surgery in local anesthesia were excision biopsies (ICD-10-PCS; 0HBT0ZX ($N = 5$), 0HBU0ZX ($N = 13$), and 0HTT0ZZ ($N = 1$)). Controlled abdominal surgery was defined as procedures with surgical entry through the peritoneum, with no leak of intestinal fluid (ICD-10-PCS; 0FT40ZZ ($N = 5$), 0GT20ZZ ($N = 1$), and 0WQF0ZZ ($N = 2$)). Excision of lipomas or subcutaneous tissue in general anesthesia include ICD-10-PCS; 07B60ZX ($N = 1$), 0JBF0ZZ ($N = 1$), and 0YB80ZZ ($N = 1$). Excision of lipomas or subcutaneous tissue in local anesthesia include ICD-10-PCS; 07B20ZX ($N = 1$), 0JB10ZZ ($N = 1$), 0JB50ZZ ($N = 2$), 0JB70ZZ ($N = 2$), 0JBF0ZZ ($N = 1$), 0JBL0ZZ ($N = 1$), 0QBM0ZZ ($N = 1$), 0VQ60ZZ ($N = 2$), 0WBK0ZZ ($N = 1$), 0XB30ZX ($N = 1$), and 0YBG0ZZ ($N = 1$). Thyroidectomies include ICD-10-PCS; 0GTK0ZZ (N=24). Parathyroidectomies include ICD-10-PCS; 0GTQ0ZZ (N=1) and 0CT90ZZ (N=1). Repair of inguinal hernia include ICD-10-PCS; 0YQ50ZZ ($N = 21$) and 0YQ60ZZ ($N = 7$). Surgical site infection (SSI) rate is the percentage of patients who completed follow-up that developed SSI. For a detailed description on human activity and level of intraoperative air contamination see Ref. [5]. All controlled abdominal cases were classified as clean contaminated wounds by the surgeons. All other cases were classified as clean.

(P=0.03). In eight of 11 SSI cases, mean cfu/m^3 of air samples taken during the individual procedures was >360 cfu/m^3 (Figure 1, Table II). Surgeons classified wounds as clean-contaminated in five cases and three of these developed SSI (Table II). ASA-score >1, clean-contaminated wounds and levels of airborne bacteria >360 cfu/m^3 were significantly associated with SSI in the multivariate logistic regression (Table II).

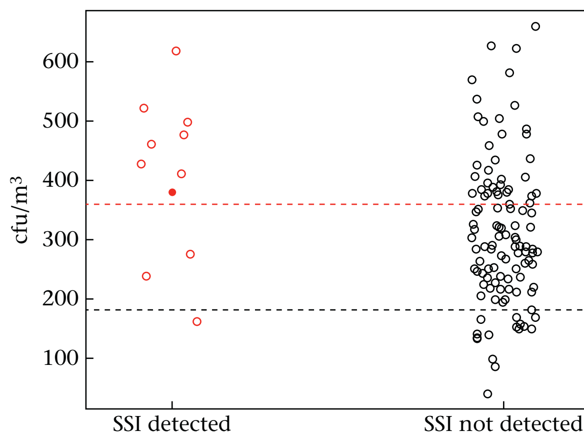

Figure 1. Average air contamination during surgery for each procedure. The density of airborne bacteria was significantly higher during procedures that were followed by a surgical site infection (SSI, red circles) than those that were not (black circles), P=0.03. The filled red circle indicates the case where whole-genome sequencing confirmed a match between bacteria isolated from air and wound sample. The line represents the Healthcare Infection Society's recommended maximum level of 180 cfu/m^3 of air [32]. The red line represents 360 cfu/m^3 of air, which is chosen as a level of gross contamination.

Identification of bacteria in SSI and air isolates

Seventeen species of bacteria were identified in 11 SSI samples (Table III). More than one species was isolated from the majority of SSI. Thirty-nine different bacteria were identified in air, sampled during cases with subsequent SSI. Most were non-pathogenic environmental and skin bacteria, but also pathogens and/or potential reservoirs of multidrug resistance such as *S. aureus*, *Klebsiella* spp. and *Acinetobacter* spp. were found (Supplementary Table S1).

Comparison of air and SSI isolates

In eight of 11 cases a match could be found at species level by MALDI-TOF between one or more bacteria isolated from SSI and the corresponding air isolates (Table III). Six pairs of SSI and air isolates showed close similarity after ribotyping and were successfully whole-genome sequenced. A median of 797,888 (range 241,932 to 1,569,838) read pairs were generated per sequenced isolate. Median coverage depths of Velvet assemblies were 25–116 reads. In one case (ID 98, *S. aureus*) the match was confirmed by WGS (Table III, Figure 2).

Metagenomic analysis

A median of 2,819,836 (range 294,597 to 4,721,524) read pairs/sample were generated by metagenomic analysis. The analysis identified 82 species with >5000 identified reads/metagenome in air sampled during cases with SSI (Supplementary Table S1). Most identified bacteria were non-pathogenic skin and environmental bacteria, but also pathogens such as *S. aureus*, *Klebsiella spp.*, *Enterobacter spp.* and *Acinetobacter spp.* were found (Supplementary Table S1). For bacteria with >50 complete NCBI-genomes available, analyses showed high likelihood of identical bacteria in paired SSI and air samples for ID 98 and clear negative results in the remaining

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Table II

Patient characteristics and factors associated with surgical site infections

Variable	Overall ($N = 128$)	Completed follow-up SSI, not detected ($N = 105$)	Completed follow-up, SSI detected ($N = 11$)	Loss to follow-up ($N = 12$)	Unadjusted OR	95% CI	*P*	Adjusted OR	95% CI	*P*
Average mean air contamination[a]	318 cfu/m^3 95% CI: 296, 340 Range: 38, 659	314 cfu/m^3 95% CI: (291, 337) Range: 83, 626	405 cfu/m^3 95% CI: 314, 495 Range: 160, 615	272 cfu/m^3 95% CI: 175, 370 Range: 38, 659						
cfu/m^3 >180	107 (84%)	89 (85%)	10 (90%)	8 (67%)	1.34	0.23, 25.8	0.79	0.45	0.05, 9.8	0.51
cfu/m^3 >360	43 (34%)	34 (32%)	8 (73%)	1 (8%)	5.25	1.42, 25.2	**0.02**	4.68	1.1, 24.7	**0.045**
General anaesthesia	67 (52%)	53 (50%)	9 (82%)	5 (42%)	4.58	1.12, 31.0	0.06	1.1	1.51, 9.9	0.95
Preoperative antibiotics	9 (7%)	5 (5%)	3 (27%)	1 (8%)	7.5	1.35, 36.9	**0.014**	2.54	0.2, 21.0	0.41
ASA >1	47 (37%)	36 (34%)	9 (81%)	2 (16%)	8.63	2.09, 58.6	**0.008**	8.17	1.77, 61.1	**0.02**
Male sex[b]	46 (36%)	36 (34%)	4 (36%)	6 (50%)	1.10	0.27, 3.88	0.89	1.18	0.18, 6.1	0.85
Wound classified clean contaminated[c]	5 (4%)	2 (2%)	3 (27%)	0 (0%)	19.3	2.83, 164.2	**0.003**	1.87	1.49, 371	**0.03**
BMI >30	35 (27%)	25 (24%)	5 (45%)	5 (42%)	2.6	0.71, 960	0.13	1.18	0.22, 6.20	0.84
Duration of surgery >60 min	67 (52%)	54 (51%)	8 (73%)	5 (42%)	2.52	0.68, 12.0	0.19	1.35	0.25, 7.1	0.68
Diabetes[d]	9 (7%)	8 (8%)	1 (9%)	0 (0%)	1.21	0.06, 7.6	0.86	0.88	0.04, 7.51	0.91
Smokers[d]	1 (1%)	1 (1%)	0 (0%)	0 (0%)	—	—	—	—	—	—
Blood transfusion given	1 (1%)	0 (0%)	1 (9%)	0 (0%)	—	—	—	—	—	—

Unadjusted odds ratio (OR) for surgical site infection (SSI) ($N = 11$) for each variable tested independently. Adjusted OR is controlled for cfu/m^3 >360, wound classified as clean-contaminated and American Society of Anaesthesiologists physical classification score (ASA) >1. Adjusted OR for >180 cfu/m^3 is only controlled for ASA >1 and wound classified as clean-contaminated. BMI, body mass index; CI, confidence interval.

P less than 0.05 is considered significant and highligthed in bold.

[a] For each surgery mean cfu/m^{3}of the obtained air samples was calculated. Average air contamination is the average of these values for all surgeries in a given group. Values of cfu/m^3 were gathered as part of our previous study [5]. cfu/m^3 is missing from four cases due to technical problems during sampling. These four cases completed follow-up and none developed SSI.

[b] Classified according to biological sex.

[c] Surgeon's assessment at closure. Only wounds classified as clean- or clean-contaminated were included.

[d] Self-reported by patients.

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Table III
Comparison of bacteria isolated from surgical site infections and airborne bacteria during surgery

Patient and SSI data					Comparison by MALDI-TOF, ribotyping and WGS				Comparison by metagenomic analysis				
Case ID.	Infection type	Type of surgery	SSI Isolate	Species determined by WGS	Match found at species level by MALDI TOF	Match found by Riboprinting	NCBI reference genome used in whole genome comparison	Match found by whole genome comparison	Number of available complete reference genomes from NCBI	No. of SNPs in core genome that are unique to SSI isolate (ID-SNPs)	No. of positions with ID-SNPs that are covered by metagenomic reads	Fraction of ID-SNPs that were identified in at least one sequence read in the matched metagenome	Interpretation of meta-genomic analysis
4	Deep	Non-cosmetic mammary surgery	4-S-O-b	*Serratia marcescens*	No	No	—	No	32	3194	328	4,26%	Low likelihood of match
4	Deep	Non-cosmetic mammary surgery	4-S-aa-a	*Pseudomonas aeruginosa*	No	No	—	No	145	547	177	7,34%	Low likelihood of match
15	Deep	Non-cosmetic mammary surgery	15-S-O-c1	*Acinetobacter Baumani*	No	No	—	No	113	266	43	13,95%	Low likelihood of match
15	Deep	Non-cosmetic mammary surgery	15-S-O-A1	*Bacillus thuringiensis*	Yes	Yes (89% similarity)	—	No (different species, 71% shared genome)	42	569	266	6,01%	Low likelihood of match
15	Deep	Non-cosmetic mammary surgery	15-S-O-d1	*Corynebacterium jeikeium*	No	No	—	No	3	219	206	53,88%	Low likelihood of match
17	Super-ficial	Controlled abdominal surgery	17-S-O-A	*Staphylococcus epidermidis*	Yes	No	—	No	11	549	549	31%	Low likelihood of match
17	Super-ficial	Controlled abdominal surgery	17-S-O-B	*Staphylococcus haemolyticus*	Yes	No	—	No	5	860	860	77%	Possible match, but not clear due to few reference genomes
25	Deep	Controlled abdominal surgery	S-25	*Staphylococcus hominis*	Yes	No	—	No	2	31275	730	17,397%	Low likelihood of match
33	Superficial	Non-cosmetic mammary surgery	S-33	*Staphylococcus aureus*	Yes	Yes (87% similarity)	NC_007795.1	No (12344 snp difference)	342	131	55	10,9%	Low likelihood of match
40	Superficial	Controlled abdominal surgery	40-S-O-A1	*Staphylococcus haemolyticus*	Yes	Yes	NC_007168.1	No (2967 SNP difference)	5	1870	1116	33,15%	Low likelihood of match
40	Superficial	Controlled abdominal surgery	40-S-O-b	*Achromobactecr xylosoxidans*	No	No	—	No	7	25436	4440	19,52%	Low likelihood of match
49	Deep	Non-cosmetic mammary surgery	S-49-A	*Enterobacter cloacae*	No	No	—	No	43	34	3	66,66%	Possible match, but not clear due to few reference genomes and low cover
49	Deep	Non-cosmetic mammary surgery	S-49-b	*Pseudomonas aeruginosa*	No	No	—	No	145	508	192	14%	Low likelihood of match
63	Organ space	Controlled abdominal surgery	S-63	*Staphylococcus epidermidis*	Yes	Yes (same RiboGroup)	NC_004461.1	No (23232SNP difference)	11	1291	1161	66,58%	Possible match, but not clear due to few reference genomes
79	Deep	Non-cosmetic mammary surgery	S-79-A	*Staphylococcus epidermidis*	Yes	No	—	No	14	9441	9312	53,84%	Low likelihood of match

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79	Deep	Non-cosmetic mammary surgery	S-79-BD	*Staphylococcus haemolyticus*	Yes	No	—	No	5	6196	6082	81%	Possible match, but not clear due to few reference genomes
79	Deep	Non-cosmetic mammary surgery	S-79-C	*Staphylococcus arlettae*	Yes	Yes (same RiboGroup)	NZ_ALWK01000001.1	No (7831 SNP difference)	0	—	—	—	Not analysed
79	Deep	Non-cosmetic mammary surgery	S-79-FH	*Staphylococcus saprophyticus*	Yes	Yes	NC_007350.1	No (2082 SNP difference)	6	11973	9833	82,42%	Possible match, but not clear due to few reference genomes
79	Deep	Non-cosmetic mammary surgery	S-79-G	*Staphylococcus warneri*	Yes	No	—	No	3	111682	100544	66,00%	Low likelihood of match
98	Superficial	Repair of Inguinal hernia	S-98	*Staphylococcus aureus*	Yes	Yes (same RiboGroup)	NC_007795.1	Yes (0 SNP difference)	342	66	56	94,64%	High likelihood of match
98	Superficial	Repair of Inguinal hernia	S-98-AF	*Corynebacterium jeikeium*	No	No	—	No	3	215	58	17,24%	Low likelihood of match
98	Superficial	Repair of Inguinal hernia	S-98-r2	*Pseudomonas aeruginosa*	No	No	—	No	145	102	10	0%	Low likelihood of match
99	Deep	Excision of lipomas or subcutaneous tissue	S-99-a	*Corynebacterium glaucum*	No	No	—	No	0	—	—	—	Not analysed
99	Deep	Excision of lipomas or subcutaneous tissue	S-99-b	*Neisseria* sp.	No	No	—	No	3	906	38	0%	Low likelihood of match

Comparison of bacterial isolates from surgical site infection (SSI) and air samples and matched metagenomes. Morphologically different colonies from pooled biomass of air and SSI samples were subcultured before, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) identification, ribotyping screening and whole-genome comparison. For whole-genome comparison we used BacDist with Snippy v.4.1.0 to call single nucleotide polymorphisms (SNPs) in each of the isolates relative to a relevant National Center for Biotechnology Information (NCBI) reference genome and compare all homologous sequences shared by isolates. Only SNPs at positions covered by at least 10 reads in both isolates with one isolate showing >80% non-reference reads were included. Metagenomic methods require no subculture after air sampling and biological material is sampled directly from pooled homogenized biomass in air samples obtained during surgeries with SSI.

*Both air and SSI isolates were initially identified as *Bacillus cereus* by MALDI-TOF and therefore included in the riboprint analysis. Whole genome sequencing (WGS) subsequently identified the air sample as *B. cereus* and the SSI sample as *B. thuringiensis*. For type of surgery, see Table I.

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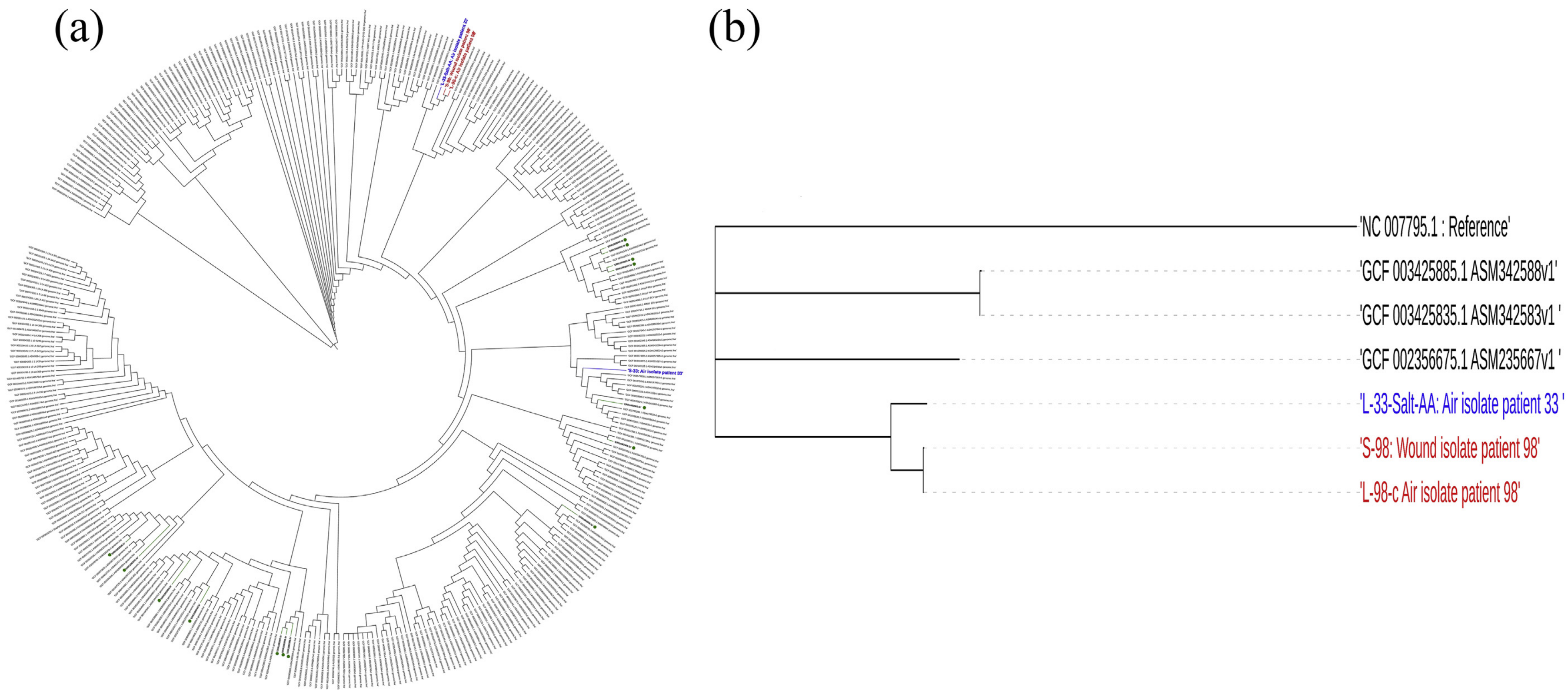

Figure 2. Whole genome-based phylogeny of *Staphylococcus aureus*. (a) Phylogenetic tree base on the core genome of wound isolates (S-98 and S-33), and air isolates (L-33-Salt-AA and L-98-c), together with 342 available complete genomes in NCBI and 15 extra genomes collected from Ghana (Donkor *et al*. [22], green dots). Bacteria from the same surgery are coloured in the same colours (red or blue). The branch lengths are not represented. (b) Second tree representation of the S-98's clade. Branch lengths are represented. Colour indicates surgery origin.

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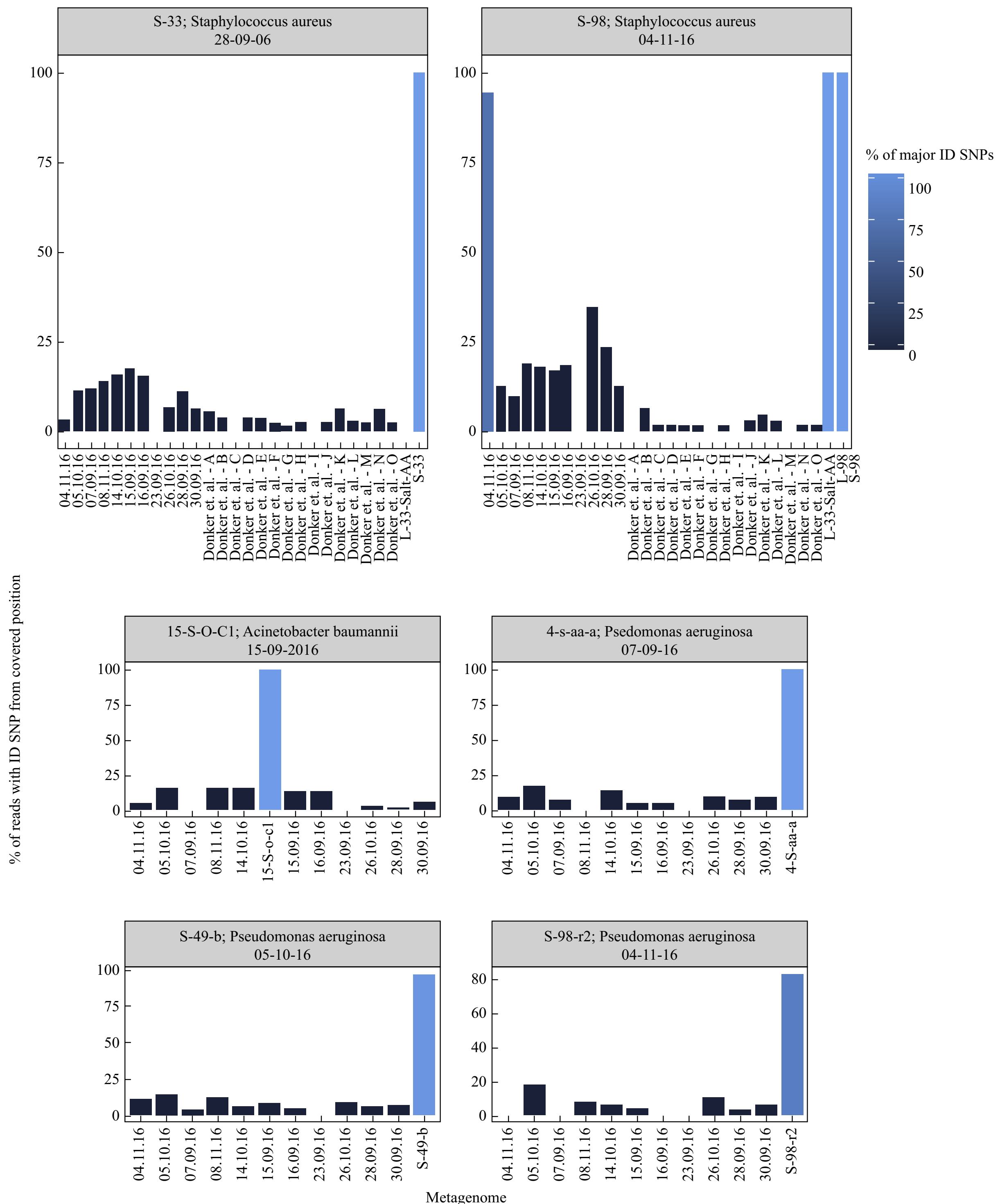

Figure 3. Metagenomic comparison of bacteria with >50 reference NBCI-genomes available. Bars show fraction of SNPs unique to each surgical site infection (SSI) isolate (ID-SNPs) that were identified in at least one sequence read in each of the metagenomes. Only positions with mapped reads are used to determine the fraction, i.e. the presence or absence of ID-SNPs in positions with no mapped reads is not included in the count. The colour of the bar indicates the percentage of covered positions in which the ID-SNP constitutes the majority of mapped reads. Metagenomes are represented by date of surgery, and genome of SSI isolates is represented by sample name. Supplemental Ghanaian *Staphylococcus aureus* strains were obtained from Donkor *et al.* [22]. Surgical dates were: case 4 (07.09.16), case 15 (15.09.16), case 17 (16.09.16), case 25 (23.09.16), case 33 (28.09.16), case 40 (30.09.16), case 49 (05.10.16), case 63 (14.10.16), case 79 (26.10.16), case 98 (04.11.16) and case 99 (08.11.16).

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cases (Figure 3, Table III). For bacteria with <50 NCBI-genomes a match could be suspected for ID 49 and 79 (Table III, Supplementary Figure S2). Furthermore, results for ID 25, 79 and 99 suggested a match between SSI isolates and air samples obtained at procedures prior to the date of surgery (Supplementary Figure S2).

Discussion

We found an SSI rate of 9.5 % after clean and clean-contaminated surgery in this tertiary-level hospital in Ghana. There was significantly increased risk of SSI when bacterial counts in the air exceeded 360 cfu/m^3 and, in one case (9%, 1/11) both WGS and metagenomic analysis showed a match between air- and SSI-isolates. The association between air contamination and SSI risk remained significant when adjusting for wound-class and ASA-score. To our knowledge, no previous studies have examined the association between intraoperative airborne bacteria and SSI in an LMIC.

No routine system for SSI monitoring is yet implemented in Ghana. A recent multicentre point-prevalence survey at 10 Ghanaian Hospitals found SSI to be the most common hospital-acquired infection (HAI) [26]. Our result is slightly lower than the World Health Organization's (WHO's) estimated 14.1% (95% CI: 11.6–16.8) pooled incidence of SSI in general surgery in LMIC countries, but the WHO estimate is based on scarce data and includes both elective and acute procedures [4].

A strength of our study was the active follow-up. With a scattered population and no register to gather data from out-patient clinics, the combination of phone calls and clinical examinations gave a good balance between logistically feasible and clinically reliable methods. All included patients could provide one or more telephone numbers and 91% completed follow-up. A recent review found similar follow-up rates and feasibility of telephone follow-up to detect SSI in LMICs [27]. Studies testing sensitivity and specificity of phone calls for detection of SSI in LMIC settings will be of high value.

We found more than one species of bacteria in most infected wounds. Some of the organisms may have been skin contaminants. Conversely, mixed infections could arise due to exposure to high bacterial loads and diversity in the environment both on admission and after discharge. We have not found collected reports on the aetiologies of SSI in low- and middle-income countries and further studies of this will be valuable.

Given the association between air contamination and SSI, we aimed to genetically compare air- and SSI isolates. This approach has previously been used to analyse the global outbreak of *Mycobacterium chimaera* infections, associated with aerosols formed in contaminated extra corporal circulation systems [28]. We could demonstrate a match at whole-genome resolution for such paired isolates in one of 11 cases. Air sampling and wound swabs are not able to capture all organisms, and individual bacteria may die during transport or be missed when selecting colonies for subculture. In particular, the use of only blood agar for recovery of airborne organisms could miss fungi and bacteria that require more complex media. Furthermore, the methodology used for detection of bacteria in the wounds may have missed slow-growing and biofilm-associated organisms. Finally, the study was restricted to a 3-month period (September–November) with relatively average weather. We did not observe any major changes in cfu counts over the period [5]. We cannot rule out that cfu counts could rise during the dusty Harmattan period around January. The proportion of SSI caused by airborne bacteria should thus be considered a conservative estimate. As a novel approach to find additional matches, we used metagenomic analysis. This did not show additional matches, but correctly identified and confirmed the result of WGS comparison. An advantage of metagenomic analysis is high-throughput taxa detection and classification. To reduce risk of misclassification, Kaiju settings only allowed one mismatch at amino acid level to the NCBI database and only species with >5000 classified reads/metagenome were included. This results in a high specificity but reduced sensitivity [20]. Even so, metagenomic analysis identified 82 species compared to the 39 identified by sub-culture and MALDI-TOF (Supplementary Table S1). For bacteria with <50 NCBI-genomes, we are less able to identify true ID-SNP's and suggested matches should therefore be interpreted with caution. No generally accepted method for comparing metagenomes is yet available. A reference-free approach has been suggested by Brooks *et al.*, basing comparison on de novo assembly of metagenomic reads [29]. With the vast biomaterial in our air samples we generated few reads/bacteria and did not find an assembly-based strategy suitable. De novo assembly from metagenomes is a novel method, lacking standardization and benchmarking [30,31].

Based on studies from high-income countries as well as expert opinions, the Healthcare Infection Society recommends a maximum of 180 cfu/m^3 in the air during non-implant surgery [32]. However no internationally accepted standard exists and, among others, Scandinavian health authorities set a maximum as low as 100 cfu/m^3 [33,34]. Our results indicate that even when such low levels of cfu/m^3 cannot be reached, a reduction to <360 cfu/m^3 may lead to reduction in SSI risk. Especially in high risk areas such as operating rooms a reduction of pathogenic bioaerosols may be valuable. This emphasizes the importance of considering intraoperative air quality in guidelines and interventions to prevent SSI in LMIC. We have previously suggested that air quality may be improved by reducing human activity in operating rooms [5]. This challenges the recently published comprehensive WHO *Global Guidelines for Prevention of Surgical Site Infections* that lacks recommendations on staff behaviour [1]. Improved ventilation, mobile air filtering devices and improved staff clothing are other strategies to consider [8,32,35]. Active air sampling is a valuable tool to create awareness of air quality and can be carried out with limited investment in equipment and basic laboratory facilities. Interventional studies are needed to test the clinical effects of improved air quality in LMICs.

Acknowledgements

The authors would like to thank all involved staff and patients at the Korle-Bu Teaching Hospital. Special thanks go to Dr. Nii Armah Adu-Aryee, Professor Mercy J. Newman, Post doc Enid Owusu and laboratory technician Amos Akumwena for support during the study. Special thanks are further given to Dr. Maame Araba Buadu for assistance with follow-up and Marlene Høg for assistance with sample preparation and handling.

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Conflict of interest statement

The authors declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Funding sources

DANIDA (ref. A30025 and 16-P01-GHA), Augustinusfonden (ref. 17-0408), Rigshospitalets Forskningsfond, University of Copenhagen (ref. A5287), Dansk Tennisfond (ref.13.02.90), Knud Højgaards fond (ref. 16-01-0991), Nordea Fonden (ref. 01-2016-001569) and Oticon Fonden (ref. 16-1716). R.L.M. is supported by the Danish National Research Foundation (grant number 126). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2019.11.007.

WGS and Metagenome sequences are uploaded to the NCBI Reference Sequence Database. Bioproject reference RJNA592050 Release date 31/12/2019 https://www.ncbi.nlm.nih.gov/refseq/

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