Traffic flow and microbial air contamination in operating rooms at a major teaching hospital in Ghana


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Traffic flow and microbial air contamination in operating rooms at a major teaching hospital in Ghana

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SUMMARY

Background: Current literature examining the relationship between door-opening rate, number of people present, and microbial air contamination in the operating room is limited. Studies are especially needed from low- and middle-income countries, where the risk of surgical site infections is high.

Aim: To assess microbial air contamination in operating rooms at a Ghanaian teaching hospital and the association with door-openings and number of people present. Moreover, we aimed to document reasons for door-opening.

Methods: We conducted active air-sampling using an MAS 100® portable impactor during 124 clean or clean-contaminated elective surgical procedures. The number of people present, door-opening rate and the reasons for each door-opening were recorded by direct observation using pretested structured observation forms.

Findings: During surgery, the mean number of colony-forming units (cfu) was 328 cfu/m³ air, and 429 (84%) of 510 samples exceeded a recommended level of 180 cfu/m³. Of 6717 door-openings recorded, 77% were considered unnecessary. Levels of cfu/m³ were strongly correlated with the number of people present (P = 0.001) and with the number of door-openings/h (P = 0.02). In empty operating rooms, the mean cfu count was 39 cfu/m³ after 1 h of uninterrupted ventilation and 52 (51%) of 102 samples exceeded a recommended level of 35 cfu/m³.

Conclusion: The study revealed high values of intraoperative airborne cfu exceeding recommended levels. Minimizing the number of door-openings and people present during
Introduction

Globally, few studies have examined the relationship between door-opening rate, number of people present, and microbial air contamination in the operating room [1]. In 2015 Birgand et al. conducted a systematic review and concluded that more robust scientific evidence was needed to substantiate infection control recommendations [1]. In low- and middle-income countries, the risk of surgical site infection (SSI) is high, with huge financial and human costs [2–5]. Studies are urgently needed from these settings to guide future interventions. In high-income countries, it has been shown that a high density of airborne bacteria during surgery increases SSI rates [2,6–8]. Reducing microbial air contamination during surgery may thus be a valuable intervention to prevent SSI. Knowledge of factors influencing air contamination is therefore important to develop effective strategies and interventions. The aim of this study was to examine the level of microbial air contamination and the effect of door-openings and number of people present during general surgery at a large Ghanaian teaching hospital. Furthermore, we examined reasons for door-openings, to assess possibilities for reducing traffic flow. To our knowledge, no studies on microbial air contamination and staff behaviour in operating rooms have yet been performed in a low- and middle-income country setting.

Methods

Setting

Data were collected at the General Surgery Unit, Korle-Bu Teaching hospital, Accra, Ghana, in three parallel operating rooms with similar sizes (36 m²) and equipment set-up. The hospital conducts ~2300 general surgery procedures annually. Operating rooms are equipped with non-laminar ventilation with high-efficiency particulate air (HEPA) filters. Both air intake and exhaust are located on the ceiling. Operating rooms are not constructed to provide positive pressure. All personnel wore institutional cotton or cotton/polyester short-sleeved shirts, institutional cotton or cotton/polyester trousers, surgical hoods, facemasks, and shoes, designated for the surgical unit only. The scrub team further wore non-disposable sterile long-sleeved cotton gowns and two layers of disposable sterile gloves.

Selection of surgical cases

Surgical cases were included if: age ≥18 years; American Society of Anesthesiologists score ≤ III; and if the patient underwent elective non-implant procedures with surgical wounds classified by surgeons as clean or clean-contaminated according to the Centers for Disease Control and Prevention adaptation of the American College of Surgeons wound classification scheme [9,10]. In all, 214 possible cases were identified. Informed, written consent was obtained from 206 patients and 124 were included. Of those excluded, 35 were excluded because surgery began before sampling was finished in a different operating room, 33 because surgery was postponed, cancelled, or moved to operating rooms elsewhere at the hospital, six for violation of inclusion criteria, four because sampling equipment was unavailable, and four due to technical problems.

Air sampling

Samples were obtained using a portable impactor (MAS-100; Merck, Darmstadt, Germany) operating five minutes at a flowrate of 100 L/min [11,12]. When planning the study, information on ventilation system was not available. Two sampling points were therefore chosen to examine any difference in air quality inside the operating rooms. During surgery samples were obtained 1 m above the floor every 20 min, shifting between a position 30–60 cm from the wound and a position opposite the entrance 1.5 m from the wall. Sampling was repeated in empty operating rooms after 1 h of uninterrupted ventilation, using the same sampling positions and flowrate, and a sampling time of either 5 or 10 min. Agar plates were incubated for 48 h at 37°C, and read by laboratory technicians blinded to the level of door-openings and people present. Colombia blood agar (CM0331; Thermo Scientific–Oxoid, Basingstoke, UK) with 5% defibrinated sheep blood was used for all samples. Media were prepared weekly according to manufacturer’s instructions [13]. The air sampler was cleaned with 82% ethanol and 0.5% chlorhexidine swabs before and after each sample. To control for contamination, agar plates were left overnight in the air-sampler and thereafter incubated at 37°C for a minimum of 24 h. On one occasion, a single colony was seen. The air-sampler was carefully cleaned and re-controlled before sampling.

Observational method

We used pre-tested, structured observation forms to record the number of people present and the door-opening during surgery at 20 min intervals from the time of the first incision to final wound closure. Reasons for door-openings were grouped in predefined categories according to needs to finish the surgical procedure or secure patient safety (Table I). The number of people present, excluding the patient and researcher, was noted at the start of each interval. Date, time of first incision, the specific operating room, and type of procedure according to ICD-10-PCS classification were recorded [14].

Data analysis

Microbial air contamination was expressed as cfu/m³. For each sampling interval, door-opening rate was calculated by dividing the number of door-openings in a sample interval with the duration of the interval, and for each procedure a total door-opening rate was calculated by dividing the total number of door-openings with the duration of the procedure. To examine
associations between cfu/m³ in a given sampling interval and covariables recorded prior to the air sample, a univariate analysis was conducted. Variables with $P/C20$ were stepwise entered in a multivariable linear mixed-effects model, keeping only variables that contributed significantly to the model. Differences in cfu/m³ in empty operating rooms, as well as differences in mean door-opening rate and number of people present according to kind of surgical procedure, were assessed using analysis of variance and post-hoc testing by Tukey's multiple comparisons of means. For statistical purposes ICD-10-PCS procedure codes were grouped according to similarity (Appendix A, Table A.I). All tests were two-sided with $P < 0.05$ considered significant. All analyses were performed with R version 3.4.1 in conjunction with the lme4 and lmerTest packages.

**Ethical considerations**

Sample collection did not prolong the surgical duration or alter the treatment provided. No data that revealed the identity of staff were recorded. The study was approved by 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**

**Microbial air contamination during surgery**

During 124 surgical procedures, 510 air samples were collected. Mean cfu/m³ per sample was 328 cfu/m³ (95% CI: 315–341) and mean average cfu/m³ per procedure was 318 (95% CI: 296–340). Of 510 samples, 429 (84%) exceeded 180 cfu/m³, and 186 (36%) exceeded 360 cfu/m³ (Figure 1). A total of 263 samples were collected close to the operating table and 247 samples were collected at the periphery. There was no significant difference between cfu/m³ close to the operating table and cfu/m³ at the periphery of the operating room. Three samples had too many colonies to yield reliable counts. These samples were assigned the value 401 cfu per sample corresponding to the highest measured cfu per sample $+1$. The cfu/m³ was distributed with positive skew and log-transformed to achieve near-normal distribution.

**Microbial air contamination in empty operating rooms**

During 26 different sampling days, 52 samples were collected close to the operating table and 50 samples were collected at the periphery of the operating room. The cfu/m³ was normally distributed. Mean cfu/m³ close to the operating table was 35 cfu/m³ (95% CI: 31–39) and mean cfu/m³ at the periphery was 43 cfu/m³ (95% CI: 39–47). The difference in cfu/m³ was significant (difference in means: 9 cfu/m³; 95% CI: 2–15; $P = 0.007$). In all, 52 of 102 samples (51%) exceeded 35 cfu/m³ (Figure 1). No significant difference in cfu/m³ was seen among the operating rooms, and no trend was observed in cfu/m³ during the study time.

**Traffic flow and number of people present during surgery**

During 8529 min of surgery, 6717 door-openings were recorded. Of these, 77% were considered unnecessary, 6% semi-necessary, and 17% necessary (Table I). The mean door-
opening rate per sample was 47 door-openings/h (95% CI: 45–49) (Table II). Mean door-opening rate per procedure was 46 door-openings/h (95% CI: 43–49); there were 53 door-openings/h (95% CI: 49–58) for procedures in general anaesthesia and 38 door-openings/h (95% CI: 34–42) for procedures in local anaesthesia (difference in means: 3.7; 95% CI: 3–8; P < 0.001). Median number of people present per sample was 8 (range: 2–23) (Table II). Average mean number of people present per procedure was 7.7 (95% CI: 7.1–8.2), with 8.5 (95% CI: 8.7–10) for procedures in general anaesthesia and 5.7 (95% CI: 5.1–6.4) for procedures in local anaesthesia. No significant difference in mean door-opening rate or number of people was found between the various kinds of surgical procedures in either general or local anaesthesia. Adjusting for type of surgery and use of general anaesthesia, a slight rise in door-opening rate was observed during the study period (0.25

Table II

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of procedures</th>
<th>Duration (min) mean (95% CI)</th>
<th>No. of sampling intervals</th>
<th>Samples/procedure median (range)</th>
<th>Door openings/hour mean (95% CI)</th>
<th>People present median (range)</th>
<th>cfu/m³ mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General anaesthesia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>25</td>
<td>100 (87–112)</td>
<td>131</td>
<td>5 (4–8)</td>
<td>57 (52–62)</td>
<td>10 (5–22)</td>
<td>395 (372–416)</td>
</tr>
<tr>
<td>Non-cosmetic mammary surgery</td>
<td>30</td>
<td>84 (70–98)</td>
<td>135</td>
<td>4 (2–8)</td>
<td>48 (44–52)</td>
<td>8 (3–19)</td>
<td>354 (329–380)</td>
</tr>
<tr>
<td>Excision of lipomas or subcutaneous tissue</td>
<td>2</td>
<td>82 (76–89)</td>
<td>8</td>
<td>4 (4–4)</td>
<td>46 (29–63)</td>
<td>8 (6–14)</td>
<td>358 (338–477)</td>
</tr>
<tr>
<td>Controlled abdominal surgery</td>
<td>8</td>
<td>76 (49–104)</td>
<td>33</td>
<td>4 (3–6)</td>
<td>58 (48–68)</td>
<td>9 (4–23)</td>
<td>407 (354–460)</td>
</tr>
<tr>
<td>Local anaesthesia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repair of inguinal hernia</td>
<td>27</td>
<td>60 (52–69)</td>
<td>113</td>
<td>4 (3–6)</td>
<td>36 (33–39)</td>
<td>5 (2–12)</td>
<td>254 (234–274)</td>
</tr>
<tr>
<td>Non-cosmetic mammary surgery</td>
<td>19</td>
<td>38 (29–47)</td>
<td>54</td>
<td>3 (1–5)</td>
<td>43 (44–52)</td>
<td>6 (2–16)</td>
<td>244 (218–269)</td>
</tr>
<tr>
<td>Excision of lipomas or subcutaneous tissue</td>
<td>13</td>
<td>30 (20–40)</td>
<td>34</td>
<td>3 (1–5)</td>
<td>38 (33–43)</td>
<td>5 (2–10)</td>
<td>269 (213–325)</td>
</tr>
</tbody>
</table>

Figure 1. Microbial air contamination in empty operating rooms and during surgery. Each dot represents one air sample. Green dots represent values below the Healthcare Infection Society (HIS) recommended maximum levels. Red dots represent values above the recommended maximum levels. Broken lines represent the HIS maximum levels at 180 cfu/m³ during surgery and 35 cfu/m³ in empty operating rooms.
### Table III
Associations between variables and log$_{10}$ cfu/m$^3$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>Door-openings/h</td>
<td>0.003 (0.001, 0.005)</td>
<td>&lt; 0.001</td>
<td>For each hour of surgery, a single door-opening corresponds to 0.2% rise in cfu/m$^3$ air</td>
</tr>
<tr>
<td>No. of people present</td>
<td>0.037 (0.024, 0.050)</td>
<td>&lt; 0.001</td>
<td>Each person present in the operating room corresponds to 2.5% rise in cfu/m$^3$ air</td>
</tr>
<tr>
<td>Use of general anaesthesia</td>
<td>0.43 (0.30, 0.57)</td>
<td>&lt; 0.001</td>
<td>Use of general anaesthesia corresponds to 30% rise in cfu/m$^3$ air</td>
</tr>
<tr>
<td>Time of the day for first incision (hours after 07:00)</td>
<td>-0.07 (-0.11, -0.028)</td>
<td>0.001</td>
<td>Difference in cfu/m$^3$ during the day can be explained by difference in door-opening rate, number of people present, and use of general anaesthesia</td>
</tr>
<tr>
<td>Time span of surgery (minutes after first incision)</td>
<td>-0.002 (-0.0005, 0.002)</td>
<td>0.003</td>
<td>Difference in cfu/m$^3$ during the individual procedures can be explained by difference in door-opening rate, number of people present, and use of general anaesthesia</td>
</tr>
<tr>
<td>Sample position periphery of operating room</td>
<td>0.061 (0.011, 0.11)</td>
<td>0.02</td>
<td>Air quality was uniform in the operating room during surgery</td>
</tr>
<tr>
<td>Sample position close to operating room table</td>
<td>-0.061 (-0.11, -0.01)</td>
<td>0.02</td>
<td>Air quality was uniform in the operating room during surgery</td>
</tr>
<tr>
<td>Surgery in operating room 1</td>
<td>-0.36 (-0.51, -0.22)</td>
<td>&lt; 0.001</td>
<td>Difference in cfu/m$^3$ between operating rooms can be explained by difference in door-opening rate, number of people present, and use of general anaesthesia</td>
</tr>
<tr>
<td>Surgery in operating room 2</td>
<td>0.23 (0.069, 0.39)</td>
<td>0.006</td>
<td>Difference in cfu/m$^3$ between operating rooms can be explained by difference in door-opening rate, number of people present, and use of general anaesthesia</td>
</tr>
<tr>
<td>Surgery in operating room 3</td>
<td>0.21 (0.038, 0.39)</td>
<td>0.02</td>
<td>Difference in cfu/m$^3$ between operating rooms can be explained by difference in door-opening rate, number of people present, and use of general anaesthesia</td>
</tr>
<tr>
<td>Surgery type: thyroidectomy</td>
<td>0.34 (0.16, 0.52)</td>
<td>&lt; 0.001</td>
<td>Difference in cfu/m$^3$ between thyroidectomies and other procedures can be explained by difference in door-opening rate, number of people present, and use of general anaesthesia</td>
</tr>
<tr>
<td>Surgery type: repair of inguinal hernia</td>
<td>-0.27 (-0.45, -0.09)</td>
<td>0.004</td>
<td>Difference in cfu/m$^3$ between repair of inguinal hernia and other procedures can be explained by difference in door-opening rate, number of people present, and use of general anaesthesia</td>
</tr>
<tr>
<td>Surgery type: controlled abdominal</td>
<td>0.26 (-0.044, 0.57)</td>
<td>0.1</td>
<td>No difference in cfu/m$^3$ between controlled abdominal surgery and other procedures</td>
</tr>
<tr>
<td>Surgery type: excision of lipomas or subcutaneous tissue</td>
<td>-0.20 (-0.44, 0.037)</td>
<td>0.1</td>
<td>No difference in cfu/m$^3$ between excision of lipomas or subcutaneous tissue and other procedures</td>
</tr>
<tr>
<td>Surgery type: non-cosmetic mammary surgery</td>
<td>-0.02 (-0.18, 0.14)</td>
<td>0.8</td>
<td>No difference in cfu/m$^3$ between excision of lipomas or subcutaneous tissue and other procedures</td>
</tr>
<tr>
<td>Time span of study period (days from first sampling day)</td>
<td>-0.0026 (-0.006, 0.0001)</td>
<td>0.1</td>
<td>cfu/m$^3$ was unaffected by the time span of the study</td>
</tr>
</tbody>
</table>

Univariate analysis shows parameters when the variable is tested as single variable in a linear mixed effects model. Multivariate analysis shows parameters when the variable is tested in a multivariable linear mixed effects model adjusting for door-opening rate, number of people present and type of anaesthesia.
additional door-openings/h/day; 95% CI: 0.12–0.38; \( P < 0.001 \)). No trend in the number of people present during surgery was observed (Table III).

**Predictors of microbial air contamination during surgery**

Surgical procedures with high mean cfu/m³ generally also had high mean door-opening rate and number of people present (Table IV). In the multivariate regression model, a significant correlation was seen between cfu/m³ and the number of people present (\( P = 0.001 \)), door-opening rate (\( P = 0.02 \)), and use of general anaesthesia (\( P < 0.001 \)). For each person present, there was a 2.5% rise in cfu/m³, and for each door-opening/h there was a 0.2% rise in cfu/m³ (Table III). No significant correlation was found between cfu/m³ and other recorded variables (Table III).

**Discussion**

The standards for acceptable air contamination during general surgery vary considerably, ranging from 180 cfu/m³ as suggested by the Healthcare Infection Society to 100 cfu/m³ as is widely used by the Scandinavian health authorities [18–20]. High levels of cfu/m³ have been associated with SSIs [6–8]. The cfu levels measured in the present study may therefore be regarded as a potential risk to patient safety. A strong correlation was found between staff behaviour and cfu/m³, with increasing cfu levels for each door-opening and person present in the operating room. This is in line with previous reports from high-income countries [21–25]. A large proportion of the people present during surgery were students, who observed surgery as part of their education. As suggested by other studies, it may be feasible to observe surgery and train basic skills and teamwork without direct patient contact through video transmission from the operating room and simulation-based training [26–28]. Such educational initiatives could reduce the number of people present. We recorded a high mean door-opening rate with almost 50 door-openings/h. A high proportion of door-openings were categorized as ‘unnecessary door-openings’, indicating that the door-opening rate could be reduced without negative impact on the surgical performance. Frequently, door-openings were for logistical reasons irrelevant to the ongoing surgery, or not ascribed any detectable reasons. A large reduction in door-opening rate should thus be possible if the necessary supplies were stored in piercing cabinets with access from both inside and outside the operating room, and by using telephone communication for urgent messages and questions. Moreover, door-openings for logistic reason related to the ongoing surgery could be limited if all necessary equipment is brought to the operating room before first incision. Door-opening rates and the number of people present are closely associated, and both may be reduced by a general behavioural change in operating rooms, allowing only people with an assigned purpose inside and restricting individuals from leaving or entering during surgery unless it is strongly indicated. Whereas a single door-opening or person might have limited effect on the overall cfu levels, the cumulative effect is substantial. It has been proposed that, unless there is an unusually high activity in the operating room, \( > 180 \) cfu/m³ can only be amended by improved ventilation [18]. Detailed technical information on the ventilation system was not available in the present study, but the relatively high cfu counts in empty operating rooms (Figure 1) indicate a need for improved ventilation. Possible interventions to improve ventilation could be an increase in air change per hour, regular maintenance of HEPA filters, use of smoke generators to reveal air-pattern shortcuts, as well as use of portable air-treatment devices [18,20,29]. No changes to ventilation system were made in the present study, and further studies are thus needed to test the exact impact of these interventions. However, our findings demonstrate a high level of human activity in the operating rooms during surgery, and behavioural changes alone may therefore achieve major improvements in air quality. The vast difference between empty and active operating rooms (Figure 1), as well as

<table>
<thead>
<tr>
<th>Variable</th>
<th>Average air contamination during surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;180 \text{ cfu/m}^3)</td>
<td>(180–360 \text{ cfu/m}^3)</td>
</tr>
<tr>
<td>No. of procedures</td>
<td>17</td>
</tr>
<tr>
<td>Average door-openings/h* (95% CI)</td>
<td>35 (30–40)</td>
</tr>
<tr>
<td>Average number of people present* (95% CI)</td>
<td>4.5 (3.4–5.0)</td>
</tr>
<tr>
<td>Average hours after 07:00 for first incision (95% CI)</td>
<td>5.1 (4.1–6.1)</td>
</tr>
<tr>
<td>Procedures in general anaesthetics*</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Procedures in operating room 1</td>
<td>12 (7%)</td>
</tr>
<tr>
<td>Procedures in operating room 2</td>
<td>3 (17%)</td>
</tr>
<tr>
<td>Procedures in operating room 3</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>No. of thyroidectomy procedures</td>
<td>-</td>
</tr>
<tr>
<td>No. of controlled abdominal procedures</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>No. of excision of lipomas or subcutaneous tissue procedures</td>
<td>5 (29%)</td>
</tr>
<tr>
<td>No. of non-cosmetic mammary procedures</td>
<td>4 (24%)</td>
</tr>
<tr>
<td>No. of repair of inguinal hernia procedures</td>
<td>7 (41%)</td>
</tr>
</tbody>
</table>

\(<180 \text{ cfu/m}^3\) represents the Healthcare Infection Society recommended upper limit of contamination during surgery. \(>360 \text{ cfu/m}^3\) was arbitrarily chosen as a level representing gross contamination. Door-opening rate is calculated as the total number of door-openings divided by the total duration of a procedure. Average number of people present is the average of the people present in the sampling intervals of the procedure. Significance of all variables is tested in the multivariable mixed-effect model: * indicates that a significant correlation was found between the variable and cfu/m³.
the fact that average contamination levels <180 cfu/m³ were seen with the current ventilation conditions during surgical procedures with low human activity (Table IV), supports this assumption. To our knowledge, evaluation of air quality interventions in sub-Saharan countries has not yet been published in peer-reviewed journals [30]. Based on our data and the available literature we argue that reducing the number of door-openings and personnel present during surgery would reduce intra-operative levels of airborne cfu and consequently prevent SSIs. Intervention studies are required to evaluate this hypothesis.

There is no internationally recognized standard for air sampling in operating rooms [31,32]. Choice of media, sampling volume, frequency, sampling position, and incubation time were based on recommendations from infection control staff at the Copenhagen University Hospital, Rigshospitalet, where sampling is performed on a regular basis. A sampling volume of 1000 L, suggested by Hoffman et al., was not possible since a pilot study with this volume returned all samples with too many colonies to give reliable counts [18]. To limit observational bias the observer had no prior relationship with the surgical staff. The structured observation forms were piloted in real-life settings and adjusted before the study. No changes in the number of people present during surgery, and only a slight change in door-opening rate was seen during the study period, indicating that the presence of an observer had a constant influence on staff behaviour. Reasons for door-openings were based solely on the observer’s direct judgment, and a large proportion is grouped as ‘no detectable reason’. Asking surgical staff for their reasons to enter might have re-classified a part of these entries, but might also have biased the study by inducing changes in staff behaviour. Other factors are expected to influence the level of cfu/m³, including staff clothing, and internal staff constellation and movement [32–35]. It was not possible to obtain sufficiently accurate information on these factors for incorporation into the regression model.

In conclusion, this study identified substantial microbial air contamination in operating rooms during surgery. cfu/m³ correlated strongly with the number of door-openings and people present during surgery, highlighting the need for changes in staff behaviour. We suggest that recommendations on air quality and staff behaviour during surgery should be included in future strategies aimed at preventing SSI in low- and middle-income countries.

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Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jhin.2017.12.010.

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**Statistical software:** R. Available at: https://CRAN.R-project.org/package=lmerTest [last accessed December 2017].


