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TITLE

Sterile Set up Table in The Operating Room is Not So Sterile

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ABSTRACT

Introduction: One of the important factors for SSI prevention is implementation of an ultraclean operating room (OR). This study was designed to evaluate back table sterility during total joint arthroplasty (TJA).

Methods: This prospective study includes 52 patients undergoing primary TJA between November 2021 and January 2022. A total of 4 swabs (two air swabs and two table swabs) were obtained for each case, at the conclusion of surgery and prior to take down of drapes. One swab from each set was sent for culture and the other swab was sent for NGS analysis.

Results: Among 104 swabs sampling back table, a total of 13 (12.5%) isolated organisms. Of these, 7 isolated by culture and 6 by NGS. No microorganisms isolated by both culture and NGS from back table swabs. Among 104 swabs sampling the air, a total of 11 (10.6%) isolated organisms. Of these, 6 isolated by culture and 5 by NGS. In 4/104 swabs both culture and NGS isolated organisms from air swabs. 13/104 (12.5%) back table and air swabs were culture positive. While more than one pathogen was identified in two air swabs, all back table swabs were monomicrobial by culture. Pathogens were identified from 11/104 (10.6%) swabs by NGS, more than one pathogen was identified in four swabs (2 air and 2 back table).

Discussion: The findings of this study raise an important issue in that surgical field, including sterile table set-up for instruments is not "sterile" and can harbor pathogens.

INTRODUCTION

Infection is the leading cause of failure after total knee arthroplasty (TKA) and total hip arthroplasty (THA) [1,2]. In fact, the most recent data from the American Joint Replacement Registry (AJRR) showed that infection was the reason for 25.2% and 20.1% of the knee and hip revision surgeries, respectively [2].

Many modifiable and nonmodifiable risk factors for surgical site infection (SSI) and periprosthetic joint infection (PJI) have been identified [3]. One of the important factors for prevention of SSI is ultraclean operating room [4]. Even the early pioneers of total joint arthroplasty (TJA) have recognized the importance of operating room air and its influence on subsequent infection [5,6].

The air in the operating room is an important vehicle for transfer of pathogens to the surgical site [5,7,8]. Many studies have found significant correlation between Colony Forming Units (CFUs) in the operating room environment and the incidence of SSI [5,6,9,10]. Likewise, the International Consensus Meeting (ICM), based on available data, has also stated that there is a direct correlation between the number of particles in the operating room and the risk for subsequent SSI [11]. In order to minimize the number of particles, including pathogens, in the operating room positive ventilation systems and laminar flows are usually employed in the surgical theatres [4,12,13].

Despite all efforts, it is recognized that operating room cannot be made particle free and bacteria and other pathogens can be detected in the air [14–17]. A recent study from our institution found that 15% of swabs that were moved in the air for a period of 30 seconds and then cultured were positive for bacteria [18].

This prospective study was designed to evaluate the "sterility" of instrument set up table that is used during total joint arthroplasty. Our hypothesis was that back table, because of being out of the view of the surgeon for the most part, and potentially being outside effective ventilation, may be contaminated during surgical procedures.

MATERIALS & METHODS

This prospective study was conducted in the operating room of two hospitals, Thomas Jefferson University Hospital (TJUH) and Rothman Orthopaedic Specialty Hospital (ROSH), where primary total joint arthroplasty was being performed. The study includes fifty-two primary total hip or total knee replacements between November 2021 and January 2022. The average age of the patients was 67.6 ± 10.2 years, and 29/52 (55.8%) patients were women. Thirty-two cases (61.5%) were primary hip replacements, and the remaining 20 cases (38.5%) were primary knee replacements. Four out of 52 cases were bilateral. The operating room is fitted with horizontal laminar flow in one of the hospitals (TJUH) and positive ventilation system without laminar flow in the other hospital (ROSH).

Swab and culture protocol

Two trained research fellows, while wearing sterile gloves, collected swabs from the instrument set up table. One swab (ESwab, COPANTM) was sent for culture and another swab (CaptiSwab, CaptiGenTM) was sent for Next Generation Sequencing (NGS). Back table swabs were obtained by stroking the surface five times in a linear fashion at the center and four corners of the table. For each surgical case, the air was also sampled by waving two swabs at a 45° angle to the ceiling, for 30 seconds while walking around the operating room. The back table and air swabs were obtained at the conclusion of the surgery, as contamination at the end of surgical procedure is believed to be higher, and prior to take down of drapes, to minimize the influence of turbulence within the surgical field that can arise during drape take down. The aseptic conditions required for the surgical procedure were not compromised in any case. In addition, two set of sterile swabs, that were still in their package, were sent for processing and used as negative controls.

All COPANTM ESwabs were sealed and immediately transported to the laboratory for processing. Swabs were cultured in Trypticase soy agar (TSA) plates and incubated for 72 h at 37°C with aerobic conditions. Then, plates were cultured at room temperature for 7 days more. Growth was carefully assessed every 24 h or until positive. Upon growth, colonies were counted, plates were sealed and sent overnight for identification by NGS analysis (MicroGen Dx, Lubbock, TX).

Next Generation Sequencing

We performed NGS analysis in all the swabs collected, due to its known potential to detect fastidious microorganisms, compared to culture [19,20]. All CaptiSwabs were shipped overnight for NGS analysis (MicroGen Dx, Lubbock, TX). Samples were processed for DNA extraction, bacterial burden detection and identification by NGS, as previously described [18]. Briefly, after DNA extraction, a quantitative PCR was performed to assess the number of DNA copies within the sample. Then a PCR reaction was used to amplify the 16S rRNA [21] and internal transcribed spacer (ITS) [22]. These regions were used to identify bacteria and fungi, respectively. DNA samples were sequenced on the Ion Torrent Personal Genome Machine (PGM) system sequencing platform (ThermoFisher Scientific, Waltham, MA). Retrieved data was cured by removing short sequences [23]. After that, sequence reads were aligned against genomes in a NIH/GenBank database. The comparison with the database was performed using USearch 7, and an agreement of at least 90% between the sequence reads and the database was necessary [24].

Statistical Analysis

Descriptive statistics are presented as absolute and relative frequencies. To compare categorical data, we used the Fisher's exact test and to compare quantitative variables we used the Mann-Whitney U test.

RESULTS

We did not find any significant differences in the rate of positive swabs between the two hospitals (19/164 vs. 5/44, p>0.1) and hence the results are presented in unison. Among the 104 swabs sampling the table, a total of thirteen (12.5%) isolated organisms. Of these, seven (6.7%) were isolated by culture and six (5.8%) were isolated by NGS. No microorganisms were isolated by both the culture and NGS from the table swabs (Table 1).

Among the 104 swabs sampling the air, a total of 11 (10.6%) isolated organisms, more than one pathogen was identified in two air swabs. Of these, six (5.8%) were isolated by culture and five (4.8%) were isolated by NGS. In 4/104 (3.8%) both the culture and NGS isolated organisms from the air swabs (Table 1).

Thirteen table and air swabs out of 104 (12.5%) were culture positive; bacterial growth was detected within the first 24 h of incubation in almost half of these samples (6/13, 46.2%). More than one pathogen was identified in two air swabs: one with two different species of gram-positive bacilli and the other with gram-positive cocci and fungi. All back table swabs were monomicrobial by culture. We found a positive culture of *Staphylococcus aureus* from a back table swab, meanwhile three cultures were positive for coagulase-negative *Staphylococcus* species (two air swabs and one back table).

Likewise, pathogens were identified from 11/104 swabs (10.6%) by NGS; more than one pathogen was identified in four swabs (2 air swabs and 2 back table swabs). *Cutibacterium acnes* was detected in eight of these swabs (5 air swabs and 3 back table swabs). Gram-negative bacteria and fungi were found in three swabs (1 air swab and 3 back table swabs) and one back table swab, accordingly. *Staphylococcus* and *Streptococcus* species were detected (one back table and two swabs [air and back table swab], respectively), as well.

We also collected data on details of the surgical procedure in the supplementary Material (Figure 1 and Table 1). This study was not powered to detect difference in SSI/PJI. None of the patients in this study, at the latest follow-up, had developed infection.

DISCUSSION

Periprosthetic joint infection (PJI) is a serious complication that is associated with immense morbidity, mortality and high costs [25,26]. Thus, any effort to prevent such a devastating complication is warranted. As the conceptual formula of the Center for Disease Control (CDC) states, surgical site infection arises when bioburden exceeds the immune threshold of a host [27].

Bioburden on the surgical site may stem from the patient and/or healthcare personnel present in the operating room, droplets in the air, or as a result of contamination of the surgical field by instruments, gloves, or implants [28–30]. Reduction of particles in the room air, aiming for ultra clean air, has been a priority for orthopedic surgeons, dating back to the initial days of arthroplasty [5,6]. The implementation of ventilation systems that remove the particles, some of which are obviously live pathogens, is an essential part of designing an operating room [12]. Numerous studies, including a study from our institution, have shown that although laminar flow may not be necessary for the reduction of particles in the room air, an effective positive ventilation system is necessary to provide clean air [12,31]. A number of factors influence the particle count in the operating room, most important of which related to the number of people and the door openings in the operating rooms fitted with positive ventilation system [7,16]. In a prior simulated study, we were able to track and control clinical variables and demonstrated that there was a direct correlation between the number of people in the operating room and the number of particles detected in the operating room [7]. Further, studies have shown that the higher number of particles in the room air corresponds to a higher number of colony forming units [32,33]. In our study, we did not find a significant difference when comparing the number of positive swabs by operating room, surgeon, or time of surgery ($p \ge 0.20$). We attribute this lack of association to the heterogeneity of the operating rooms sampled for this study.

Despite all efforts, however, it is improbable to have an operating room that is free of all particles [17,33]. According to international standards [17], a cleanroom is achieved by controlling the number of particles within the room: a maximum of 352,000 particles $\geq 0.5 \ \mu m/m^3$, 83,200 particles/m³ if they are $\geq 1 \ \mu m$ or 2,930 particles/m³ if they are $\geq 5 \ \mu m$. Touching the implants and other orthopaedic devices during the surgery may introduce even higher sterility requirements. The particles in the operating room may find their way into the surgical field and the surgical incision, and if high in quantity, may result in a subsequent infection. The findings of this study are

somewhat worrisome. It appears that "sterile" set up table, or so called the back table, that accommodates instruments can be contaminated in a relatively large number of cases. It is fair to state that the table is not "sterile" by any means. A recent study has, indeed, identified a higher burden of microbial contaminants at the back table area than the sterile field [33].

The clinical relevance of the findings of this study, particularly organisms isolated by next generation sequencing (NGS), should be discussed. Although NGS can detect organisms in non-infected samples, organisms that are part of the microbiome profile, NGS is known to have a high specificity for isolation of pathogens in culture negative infections [20]. In fact, majority of the organisms isolated by culture and/or NGS in this study are known pathogens that can cause SSI or PJI [34]. *S. aureus* and coagulase-negative species of *Staphylococcus* were found in air and back table swabs by both methodologies. This finding is clinically important as these organisms associated with SSI [36]. We also detected aerobic Gram-negative bacilli, a group of bacteria with a growing concern among specialists due to their associated antimicrobial resistance patterns [37]. *Streptococcus* species were also identified by both culture and NGS. Surprisingly, PJI by this genre of bacteria are frequently described as a result of hematogenous seeding rather than perioperative contamination [38]. In addition, *P. acnes*, a notorious and slow growing pathogen, was the most frequent anaerobic microorganisms detected [18].

Corynebacterium and *Micrococcus* species were also detected frequently. Both genres of bacteria are considered contaminants; however, recent studies have shown *Corynebacterium* species might actually play a role in polymicrobial PJI, which can be as high as 15% of the total PJI [35]. We also detected members of the vaginal (*L. iners*) [39] and gut microbiome (*R. timonensis*) [40], which have not been reported as pathogens in PJI to date. Fungi species were also detected by NGS and culture. In this regard, the species found are related to the bioethanol industry (*S. cerevisiae*) [41] and the skin microbiome (*M. sympodialis*) [42], rather than SSI or PJI.

Although none of the patients in this study developed infection, it is important to acknowledge that the study was not powered to investigate the effect of back table contamination on subsequent infection. Back table is a particularly important area in joint replacement. The implants used during joint replacement is almost always dropped on the back table and assembled in this area. Any implants coming into contact with this surface can potentially be contaminated and carry the risk of subsequent infection. As stated above, there is a complex interplay between the immune system of the host and bioburden [27]. It appears that the bioburden in these patients did not reach an adequate threshold to result in a subsequent infection. It is important to point out that the standard of care at our institution requires the scrub technicians to avoid touching the implants and assemble the implants in their packaging, as much as possible. The latter practice is based on numerous studies from our institution and other studies that demonstrate pathogens lurk in the room air and can be detected by culture and/or NGS [18].

Although there was no direct correlation between the organisms detected in the air and the back table, the fact that live organisms exist in the room air should provide ample reason for surgeons to implement strategies that minimize the opportunity for pathogens to reach the surgical site. Besides avoiding contact between implants and the back table or gloves, execution of the surgical procedure in an expeditious manner is also important. The strategies mentioned are even more important in other types of joint reconstruction surgery, such as revisions or conversions, which require more hardware, more set-up tables, and are more prolonged. The issue of operative time and the risk for subsequent surgical site infection was also discussed during the International Consensus Meeting (ICM) with 98% delegates agreeing that there is a direct correlation between the number of particles in the room air and the risk of subsequent infection [11].

Another question that arises is what can be done to reduce the number of particles in the room and the contamination of the back table. Individual bacteria measure $\geq 1 \ \mu m$; however, they usually cluster together in airborne particles between 4 to 20 μm [43]. Thus, rooms with even higher sterility requirements pre-filter the upcoming air with high-efficiency particulate air (HEPA) filters and perform a final filtering step with ultra-low particulate air (ULPA) filters that remove 99.99% of $0.12 \geq \mu m$ particles. Other strategies include the increase of the air change per hour, raised floors among others [17]. A study by Jennings et al [44], demonstrated that application of UV-LED may reduce back table contamination in the operating room. The same study, incidentally, found a positive correlation between the number of door openings and the number of CFUs [44]. Another strategy to reduce back table contamination may involve fitting the back table inside a "ventilation rich" zone [7,12]. At minimum, surgeons need to be aware of the issue that back table is not sterile and should not be treated as such. Thus, effort should be made to avoid allowing implants, for example, coming into contact with the back table.

This study has several strengths. We designed this study such that it minimized the effect of numerous confounding variables that could result in detection of pathogens by these swabs. We included primary arthroplasty cases only, as revision cases may last much longer and are heterogenous in nature. The same trained personnel obtained the swab samples from all cases and at the same juncture in surgery. The samples were all processed using the same protocol.

There are also some limitations to this study. One such limitation is the relatively small sample size. The processing of the samples and the conduct of this prospective study was costly and the limited institutional grant that we had did not allow us to include a higher number of patients and/or areas to sample. However, the inclusion of a larger sample size is unlikely to alter the message of the study. Another perceived limitation may be that inclusion of a molecular technique, namely NGS, may have resulted in a high percentage of "false positive" findings. The critics of molecular techniques posit that DNA of live or dead bacteria is amplified by molecular techniques, minimizing their clinical relevance. It is important to note that NGS is not the same as polymerase chain reaction (PCR). A recent study from our center showed that DNA of pathogens detected by NGS does in fact correspond to the presence of RNA by the same organisms in the same samples, indicating that these organisms are indeed alive [45]. The fact that neither culture nor NGS detected any organisms in the negative control samples provides an additional reassurance that NGS is not "too sensitive" in picking up DNA.

We also have to consider that NGS enables the detection of fastidious microorganisms difficult to recover or grow with culture, like anaerobes, slow-growing bacteria, and fungi [19,20]. In fact, our study detected the presence of anaerobic bacteria and fungi mainly by NGS. In addition, the turnover time for NGS analysis was faster than culture and it allowed us to detect polymicrobial populations in both, air and back table swabs.

We did not perform antimicrobial susceptibility profiles; however, NGS did not detect any genes associated with antimicrobial resistance among our isolates. Likewise, we did not perform genotyping of the bacteria. We consider this approach would have brought limited value to our findings given the heterogenicity in microorganisms detected here.

CONCLUSION

The findings of this study raise an important issue in that surgical field, including the sterile table set-up for instruments is not "sterile" and can harbor pathogens. Contamination of the surgical field and the presence of organisms in the operating room air should always be considered when executing a surgical procedure and efforts should be made to minimize their entry into the surgical site.

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| Samples | Culture | NGS | Culture and NGS |
|--------------------|-----------------------------------|---|-----------------------------------|
| Back Table (n=104) | Positive (n= 7 [6.7%]) | <i>Positive</i> (<i>n</i> =6 [5.8%]) | Positive (n=0) |
| | | | |
| | | tetradius (n=1) Peptoniphilus harei (n=1) | |
| | | Malassezia sympodialis (n=1) | |
| Air (n=104) | <i>Positive</i> $(n = 6 [5.8\%])$ | <i>Positive</i> $(n = 5 [4.8\%])$ | <i>Positive</i> $(n = 4 [3.8\%])$ |

| Staphylococcus | Stenotrophomonas | Staphylococcus |
|--------------------------|---------------------|--------------------|
| epidermidis (n=1) | maltophilia (n=1) | hominis (n=1) |
| Staphylococcus | Streptococcus | Micrococcus luteus |
| <i>hominis</i> (n=1) | parasanguinis (n=1) | (n=1) |
| Streptococcus | Cutibacterium acnes | Cutibacterium |
| sanguinis (n=1) | (n=5) | acnes (n=2) |
| Saccharomyces | Romboutsia | |
| cerevisiae (n=1) | timonensis (n=1) | |
| Corynebacterium | | |
| genitalium (n=1) | | |
| Corynebacterium | | |
| <i>mucifaciens</i> (n=1) | | |
| Micrococcus luteus | | |
| (n=2) | | |