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Environmental Microbial Contamination in Operating Rooms at Jalo Central Hospital: A Cross-Sectional Study

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Abstract

This study was conducted at (JCH) to assess microbial contamination in operating rooms (OR) and the operating support room (OSR). A total of 25 environmental samples were collected from various surfaces, including drawer handles, instrument tables, surgical instruments, door handles, and the hands of medical staff. Swabs were obtained under aseptic conditions and immediately transported to the laboratory for culture on selective and general media. Bacterial cultures were incubated at 37 °C for 18–24 hours, while fungal cultures were incubated at 25 °C for 4–7 days. The overall microbial contamination rate was 8%, with fungal growth observed in 20% of samples from the OR. No bacterial growth was detected in any of the samples (0%). The absence of bacterial contamination reflects the effectiveness of sterilization procedures, hygiene practices, and the use of disinfectants in these highly controlled environments Cross-sectional observational study. Sampling method: Surface swabbing of predefined areas in the OR and OSR.Key limitations: Limited sample size (25 samples) due to the availability of active operating rooms and personnel constraints, and the absence of air sampling due to resource limitations. These findings highlight the importance of continuous environmental monitoring and strict adherence to infection control protocols to minimize microbial contamination in critical healthcare settings.

1. Introduction

Operating rooms stand at the frontline of modern healthcare, where even the slightest lapse in sterility can mean the difference between a successful surgical outcome and a life-threatening infection, making microbial contamination control an absolute clinical priority rather than merely a procedural necessity.

Operating rooms (ORs) represent one of the most critical and highly controlled environments within healthcare facilities, where strict sterilization and infection control measures are essential due to their direct involvement in surgical procedures. These environments are particularly vulnerable to microbial contamination, which is a major contributing factor to surgical site infections (SSIs). Such infections not only increase patient morbidity and mortality but also impose a significant economic burden on healthcare systems. Therefore, maintaining a sterile operating environment is fundamental to ensuring patient safety and improving surgical outcomes.

Microbial contamination in operating rooms originates from multiple sources, including airborne particles, contaminated surfaces, surgical instruments, healthcare personnel, and movement within the operating area. Human activity, in particular, plays a crucial role in increasing microbial load through the shedding of skin particles and associated microorganisms. Despite the implementation of rigorous cleaning, disinfection, and sterilization protocols, the complete elimination of microorganisms remains challenging, especially when infection control measures are inconsistently applied or when environmental conditions favor microbial persistence.

The issue of microbial contamination is not limited to operating rooms but has been widely reported across various indoor and controlled environments. Studies have demonstrated that indoor

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settings, such as university buildings, exhibit variable microbial loads influenced by occupancy and environmental conditions, with higher bacterial contamination often observed in densely populated areas [1]. The application of structured hygiene systems, such as Hazard Analysis and Critical Control Points (HACCP), has been shown to significantly reduce microbial counts and improve safety outcomes compared to unregulated systems [2]. These findings highlight the importance of systematic infection control approaches that can be adapted to operating room settings.

In healthcare environments, including operating rooms, contaminated surfaces, reusable equipment, curtains, and even sanitation systems have been identified as potential reservoirs and transmission pathways for pathogenic microorganisms [3–5]. Airborne contamination also plays a critical role, as bioaerosols can carry bacteria and fungi that may settle on sterile surfaces or directly enter surgical sites. Advanced monitoring techniques, including bioaerosol sampling, flow cytometry, hyperspectral imaging, and molecular diagnostic methods, have enhanced the detection and characterization of microbial contamination, although certain limitations in sensitivity and standardization remain [6–8].

To address these challenges, various control strategies have been implemented in operating rooms. Engineering controls, including positive air pressure systems, high-efficiency particulate air (HEPA) filtration, and optimized ventilation systems, are widely used to reduce airborne contamination. In addition, targeted disinfection practices, antimicrobial surface coatings, and strict adherence to aseptic techniques have demonstrated effectiveness in minimizing microbial load and reducing infection risks [9–11]. Regular environmental monitoring and validation of sterilization procedures are also essential components of infection prevention programs.

Recent research emphasizes the importance of integrated and data-driven approaches in controlling microbial contamination. Combining environmental monitoring with microbial source tracking and community-level analysis allows for a better understanding of contamination sources and transmission pathways [12,13]. Furthermore, continuous training of healthcare personnel, the implementation of standardized protocols, and the use of automated and advanced disinfection technologies have been shown to enhance compliance with infection control measures and reduce contamination levels in clinical settings [14].

Overall, microbial contamination in operating rooms is a multifactorial issue influenced by environmental conditions, human activity, microbial characteristics, and the effectiveness of infection control practices. Addressing this challenge requires a comprehensive approach that integrates strict sterilization procedures, continuous environmental monitoring, advanced detection technologies, and adherence to standardized hygiene protocols. Such strategies are essential to minimize infection risks, improve surgical safety, and protect both patients and healthcare workers in these highly sensitive environments [15].

2. Methods

2.1. *The materials used*

Plastic Petri dishes (9 and 6 cm in diameter), isolation needle, cotton, cellophane paper, sterile swabs, nutrient media: nutrient agar, Blood agar, Cystine Lactose Electrolyte Deficient (CLED) agar, Salmonella - Shigella agar, Sabouraud Dextrose Agar (SDA), Lactophenol cotton blue stain, Distilled water, parafilm, microscope slides and glass coverslips.

2.2. The devices used

Sensitive balance, cooler, incubator, water bath, isolation chamber, microscope, Bunsen burner, Heat sterilizer Autoclave, and Refrigerator.Description of the study area; The study area is the municipality of Jalo, located in south-central Libya, as shown in the figure. The population of Jalo municipality is 31,000 (as of 2025), and the main economic activity is agriculture. This study was conducted in the surgical and orthopedic surgery departments of Jalo Central Hospital (JCH).

2.3. Sample Collection

Samples were collected on 12/10/2025 at (JCH). In this study, 25 samples were collected from two operating rooms (OR) in two departments: the surgical surgery department and the orthopedic surgery department. The samples included 11 swabs from the surgical surgery room (SSR), and 14 swabs from the orthopedic surgery room (OSR). The samples were taken from various locations in the rooms, such as door handles, drawer handles, etc. Each location was assigned a unique number. The samples were collected using sterile cotton swabs.

2.4. Sample Collection Locations

Swab samples were collected from two operating areas at (JCH): the operating room (OR) and the operating support room (OSR), with each room assigned a specific code. A total of eleven swabs were obtained from the OR and fourteen from the OSR. The samples were collected from a variety of locations, including drawer handles, light switches, door handles, sterilization trays, instrument tables, instrument trolleys, beds, cameras, surgical instruments, sinks, the hands of medical staff, medicine cabinets, anesthesia machines, X-ray machines, light bulbs, oxygen cylinders, device stands, floors, and corridor walls. Each sampling location was assigned a numerical code in addition to the corresponding room code for accurate identification and data organization.

2.5. Procedure

Samples were collected under strict aseptic conditions following hand sanitization and the use of gloves and face masks. A standardized area of 10 cm × 10 cm was marked at each sampling site. Sterile cotton swabs were used to collect samples, which were then immediately transported to the laboratory in appropriate sterile containers to maintain sample integrity and prevent contamination.

The samples were cultured on different types of culture media, including nutrient agar, blood agar, cystine lactose electrolyte-deficient (CLED) agar, and Salmonella–Shigella agar. For bacterial isolation, all inoculated Petri dishes were incubated at 37 °C for 18–24 hours.

For fungal isolation, swabs collected from the same sampling sites were cultured on Sabouraud dextrose agar (SDA). The inoculated fungal plates were incubated at 25 °C for 4–7 days. Each sample was properly labeled using a standardized coding system, indicating the room name and the exact sampling location. The Petri dishes were inverted and placed in the incubator according to their required incubation temperature and duration.

2.6. Bacterial Culture

An appropriate amount of culture medium was prepared by accurately weighing the required quantity for each type. The medium was dissolved in distilled water and mixed thoroughly to ensure complete homogenization. It was then gently heated over a Bunsen burner, ensuring that the container was not tightly sealed to prevent pressure buildup, and subsequently sterilized using an autoclave.

In the case of blood agar preparation, the sterilized medium was allowed to cool under running water until it reached approximately 45 °C, after which 5% sterile blood was aseptically added and mixed gently. All prepared culture media were then poured into sterile Petri dishes under aseptic conditions and allowed to solidify.

Following solidification, the samples were inoculated onto the culture media to isolate and cultivate bacteria of medical importance. The inoculated Petri dishes were properly labeled using appropriate identification codes and incubated at 37 °C for 24 hours.

Examples for bacterial isolated from samples *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli.*

2.7. Fungal Culture

Sabouraud dextrose agar (SDA), used for fungal growth and isolation, was prepared by dissolving 65 g of agar powder in 1000 mL of distilled water, according to the manufacturer's instructions. The medium was accurately weighed using a sensitive balance, thoroughly mixed, and then heated in a water bath for 35–40 minutes until completely dissolved and a clear solution was obtained.

The prepared medium was then distributed into 250 mL flasks to facilitate handling and pouring. These flasks were sterilized in an autoclave for 15–20 minutes. After sterilization, the medium was allowed to cool slightly and then aseptically poured into sterile Petri dishes, where it was left to solidify.

Subsequently, the samples were inoculated onto the prepared SDA plates. The inoculated plates were incubated at 25 °C for 4–7 days to allow fungal growth. Each Petri dish was properly labeled with the corresponding sample code, including the room designation and sampling location.
No fungi were found in the isolated samples.

2.8. Diagnosis of Isolates (Colonies)

Bacterial and fungal isolates (colonies) were identified based on their morphological characteristics, including shape, color, texture, and odor. Since no bacterial growth was observed, no further bacterial identification procedures were performed.

For fungal identification, a small portion of the fungal growth was aseptically isolated from the culture media using a sterile inoculating needle. The isolate was then prepared as a smear on a clean glass slide, to which a drop of lactophenol cotton blue stain was added, and covered with a coverslip for microscopic examination.

The prepared slides were examined under a light microscope at different magnifications (10×, 40×, and 100×). The observed macroscopic and microscopic features were compared with standard identification references and previously characterized isolates, and the results were recorded accordingly.

2.9. Study Limitations

2.9.1. Sample Size Limitation:

A total of 25 environmental samples were collected in this study. This limited sample size was due to the restricted availability of active operating rooms where surgical procedures are performed, which is attributed to a shortage of medical staff and a low number of conducted operations.

2.9.2. Absence of Air Sampling:

Due to resource limitations and the scope of the study, only surface samples were collected, and no air sampling was performed.

3. Results

This study was conducted at (JCH), where swab samples were collected from the operating room (OR) and the operating support room (OSR) and cultured on sterile media. A total of 25 environmental samples were taken from various enclosed surfaces within the OR and OSR, and the overall microbial contamination rate was 8%. These findings are consistent with the study by [16], which reported a contamination rate of 7.89%, and with [17]. However, the results differ from those reported by [18] and [19], which found contamination rates of 78.6% and 75.5%, respectively.

In the present study, fungal growth was detected in the OR at a rate of 20% (Table 1, Figure 1). No bacterial growth was observed in any of the samples, representing 0% bacterial contamination. These results align with those of [16] and [20], who also reported no bacterial growth (0%), suggesting a low level of microbial contamination in the hospital environment. However, this could also be influenced by the limited sampling area or timing of sample collection (e.g., before or after cleaning).

The absence of bacterial contamination can be attributed to the effectiveness of implemented sterilization and hygiene procedures. Additionally, bacteria are generally sensitive to commonly used disinfectants, sterilizers, and some antibiotics, which further explains their absence in the tested environments. Nevertheless, certain specific areas may still require enhanced microbial control measures to ensure complete sterility.

Table 1. Number and Proportion of Samples Collected in the Study

Room/Samples	Total	Positive	Negative	Contamination%	non-contamination%
Operating room	11	2	9	%20	%80
Orthopedic surgery room	14	0	14	%0	%100

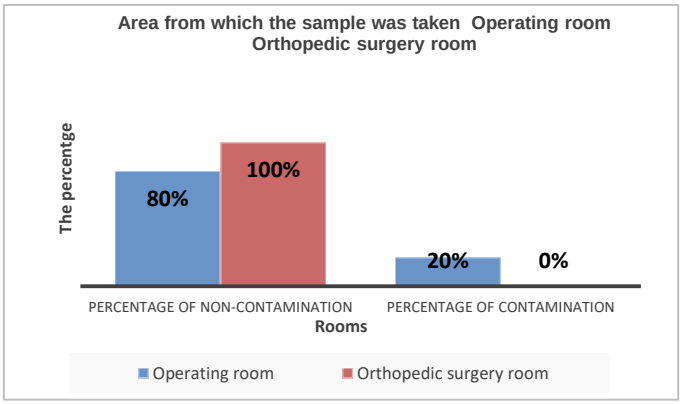

Figure 1. Proportion of Contaminated and Uncontaminated Samples in the Study at (JCH)

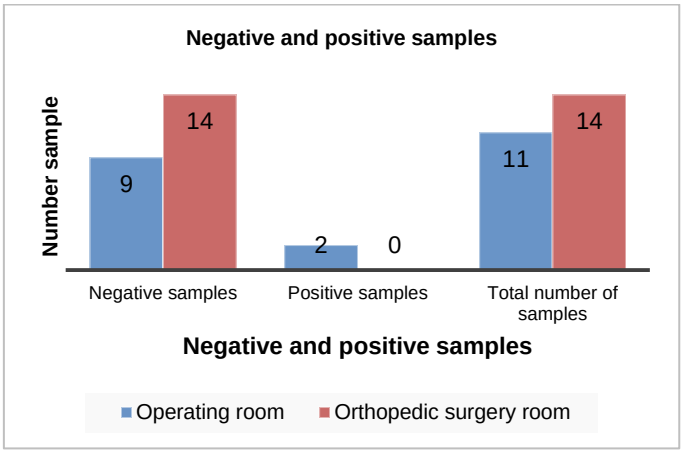

Figure 2. Distribution of Positive and Negative Samples at (JCH)

The results obtained from various locations within the operating room (OR) and the operating support room (OSR) at (JCH) showed that most samples tested negative for bacterial growth. Fungal growth, however, was detected in only two samples from the OR, with the highest contamination rate (8%) observed on the hands of medical staff and the medicine cabinet (Table 2). These findings are consistent with the study conducted by [16], which reported a contamination rate of 11% on healthcare workers' hands, as well as with the study by [21], which documented a contamination rate of 10% in similar settings.

In contrast, our findings differ from those reported in [22], where bacterial growth was detected on surfaces such as light bulbs, sinks, and door handles at a rate of 4.25%. In the present study, no bacterial growth was observed on these surfaces. Similarly, our results contradict the findings of [23], who isolated bacterial species from operating tables that had been in contact with patients. In this study, however, no bacterial or fungal growth was detected on such surfaces (Table 3).

Furthermore, no contamination was identified on door handles or drawer handles, which is inconsistent with the findings of [24], where contamination was reported in these locations. Additionally, no microbial contamination associated with surgical instruments was observed in the current study, as neither bacterial nor fungal growth was detected. This can be attributed to the effectiveness of sterilization procedures, particularly the use of autoclaving.

The predominance of negative samples in the operating room indicates a contamination-free surgical environment, reflecting a high level of sterilization and strict adherence to infection control protocols. The autoclave is considered one of the most effective methods for ensuring complete sterilization of surgical instruments, as it eliminates all microorganisms, including bacteria and fungi, through the use of high-pressure saturated steam at elevated temperatures for a specified duration. This process significantly reduces the risk of surgical site infections by preventing the transmission of pathogens from contaminated instruments or surfaces to patients during surgical procedures.

Therefore, the absence of microbial growth in most samples can be attributed to the efficiency of autoclave sterilization, in combination with other aseptic techniques and infection control practices implemented during surgical procedures.

3.1. Statistical analysis

The study used descriptive for statistical analysis.

Table 2. Sampling Locations, Culture Media, and Microbial Growth Results in the OR. Bacterial Media: Nutrient Agar, Blood Agar, CLED Agar, Salmonella–Shigella (S.S) Agar; Fungal Medium: Sabouraud Dextrose Agar (SDA)

No	Location for taking swabs	Bacterial	Fungal
1	Sterilization Case	Negative	Negative
2	Camera device	Negative	Negative
3	The hands of the medical staff	Negative	Positive
4	Medicine Cabinet	Negative	Positive
5	Lighting lamps	Negative	Negative
6	Oxygen Tubes	Negative	Negative
7	Light switches	Negative	Negative
8	Tool Table	Negative	Negative
9	Device Holder	Negative	Negative
10	Tool Cart	Negative	Negative
11	Bed	Negative	Negative

Table 3. Sampling Locations, Culture Media, and Microbial Growth Results in the OSR. Bacterial Media: Nutrient Agar, Blood Agar, CLED Agar, Salmonella–Shigella (S.S) Agar; Fungal Medium: Sabouraud Dextrose Agar (SDA)

No.	Location for taking swabs	Bacterial growth	Fungal growth
1	Drawer Handles	Negative	Negative
2	Light knobs	Negative	Negative
3	Doorknobs	Negative	Negative
4	Tool Table	Negative	Negative
5	Tool Cart	Negative	Negative
6	Bed	Negative	Negative
7	Surgical Instruments	Negative	Negative
8	Wash basin	Negative	Negative
9	Anesthesia Machine	Negative	Negative
10	Radiology	Negative	Negative
11	Floor	Negative	Negative
12	Device Holder	Negative	Negative
13	Corridor wall	Negative	Negative
14	The hands of the medical staff	Negative	Negative

4. Conclusion

This study provides insight into microbial contamination in operating rooms (OR) and the operating support room (OSR) at (JCH), focusing on surface sampling and standard microbiological culture methods. The findings indicate a low overall bacterial contamination rate (0%) and limited fungal growth (20% in the OR), suggesting that current sterilization and hygiene practices are largely effective in maintaining a relatively safe surgical environment.

Strengths of the study include:

- Addressing an important infection control issue.
- Providing local data from a hospital environment.
- Employing standardized microbiological culture techniques.

Limitations of the study include:

- A relatively small sample size (25 environmental samples), due to limited availability of active operating rooms and personnel constraints.
- Absence of air sampling, which may have overlooked airborne microorganisms.
- Sampling was limited to specific surfaces and time points, which may not fully represent overall microbial contamination.

Overall, while the results suggest effective infection control measures in the studied areas, caution is warranted in generalizing these findings. Further studies with larger sample sizes, inclusion of air sampling, and repeated measures over time are recommended to provide a more comprehensive assessment of microbial contamination in hospital operating environments.

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التلوث الميكروبي البيئي في غرف العمليات بمستشفى جالو المركزي: دراسة مقطعية

الملخص

أُجريت هذه الدراسة في مستشفى (JCH) لتقييم التلوث الميكروبي في غرف العمليات وغرفة دعم العمليات. جُمع ما مجموعه 25 عينة بيئية من أسطح مختلفة، بما في ذلك مقابض الأدراج، وطاولات الأدوات، والأدوات الجراحية، ومقابض الأبواب، وأيدي الطاقم الطبي. أُخذت المسحات في ظروف معقمة ونُقلت فورًا إلى المختبر لزراعتها على أوساط انتقائية وعامة. حُضنت مزارع البكتيريا عند درجة حرارة 37 درجة مئوية لمدة 18-24 ساعة، بينما حُضنت مزارع الفطريات عند درجة حرارة 25 درجة مئوية لمدة 4-7 أيام. بلغ معدل التلوث الميكروبي الإجمالي 8%، مع ملاحظة نمو فطري في 20% من عينات غرف العمليات. لم يُرصد أي نمو بكتيري في أي من العينات (0%). يعكس غياب التلوث البكتيري فعالية إجراءات التعقيم، وممارسات النظافة، واستخدام المطهرات في هذه البيئات الخاضعة لرقابة مشددة. دراسة رصدية مقطعية. طريقة أخذ العينات: مسح الأسطح في مناطق محددة مسبقًا في غرفة العمليات وغرفة العمليات الجراحية. القيود الرئيسية: صغر حجم العينة (25 عينة) نظرًا لمحدودية غرف العمليات المتاحة وعدد الموظفين، وعدم إمكانية أخذ عينات من الهواء بسبب محدودية الموارد. تُبرز هذه النتائج أهمية المراقبة البيئية المستمرة والالتزام الصارم ببروتوكولات مكافحة العدوى للحد من التلوث الميكروبي في مرافق الرعاية الصحية الحرجة.

الكلمات المفتاحية : التلوث الميكروبي؛ غرف العمليات؛ غرف عمليات جراحة العظام؛ التلوث الفطري؛ نمو البكتيريا.