MICROBIOLOGICAL ANALYSIS AND IDENTIFICATION OF PATHOGENS IN ORTHOPEDIC THEATRES: AL-NASSIRIYAH CITY'S STUDY

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Bacteria found in the operating room can lead to surgical site and hospital-acquired infections. This study was conducted in Imam Hussein Hospital in Nasiriyah to investigate the contamination levels in the operating rooms. The main goal of the research was to identify the bacteria responsible for contamination and the factors contributing to it. The study also aimed to map these microorganisms' distribution across different operating room areas and their antibiotic resistance pattern using microbiological standards. We gathered 1358 samples for analysis from surfaces and objects in the operating room. The results showed that 3.1% tested positive for bacteria, and 96.9% were negative cultures. Six types of pathogenic bacteria have been identified; Coagulase-negative staphylococci 14.3%, *Staphylococcus aureus* 11.9%, *Pseudomonas aeruginosa* 19.1%, *E. coli* 21.4%, *Bacillus spp.* 11.9%, and Enterobacter spp. 21.4%. We observed moderate to high resistance pattern to amoxicillin and ampicillin, Cefaclor, Cefuroxime, Cefadroxil, Erythromycin. The highest resistance pattern was detected in *P. aeruginosa* isolates followed by *E. coli*, it showed different resistance patterns to 14 antibiotics showing susceptibility to Amikacin only. Conclusion: the study at Imam Hussein Hospital found a generally low but notable level of bacterial contamination in orthopedic operating theaters, with specific pathogens posing risks to patients. These findings align with global data, underscoring common challenges in maintaining sterile surgical environments. Identifying contamination hotspots and patterns over time highlights the need for targeted interventions and continuous monitoring.

Keywords: *Pseudomonas aeruginosa*, operating room areas, antibiotic resistance

Compliance with ethical standards: the study was conducted in accordance with the standards of good clinical practice and evidence-based medicine.

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Received: 01.06.2025 **Accepted:** 06.07.2025 **Published online:** 15.07.2025

DOI: 10.24075/brsmu.2025.034

МИКРОБИОЛОГИЧЕСКИЙ АНАЛИЗ И ИДЕНТИФИКАЦИЯ ПАТОГЕНОВ ВО ВРЕМЯ ОРТОПЕДИЧЕСКИХ ОПЕРАЦИЙ: ИССЛЕДОВАНИЕ В Г. ЭН-НАСИРИЯ

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Бактерии, встречающиеся в операционных, могут приводить к хирургическим и внутрибольничным инфекциям. Целью работы было идентифицировать бактерии, ответственные за обсеменение, определить факторы, способствующие обсеменению, а также картировать распределение этих микроорганизмов по различным зонам операционного блока и определить характер их устойчивости к противомикробным средствам с использованием микробиологических стандартов. Было отобрано 1358 образцов с поверхностей и предметов в операционной. В 3,1% случаев результаты анализа на наличие бактерий были положительными, а в 96,9% случаев имело место отсутствие роста бактерий. Идентифицированы патогенные бактерии шести типов: коагулазонегативные стафилококки (14,3%), *Staphylococcus aureus* (11,9%), *Pseudomonas aeruginosa* (19,1%), *E. coli* (21,4%), *Bacillus spp*. (11,9%) и *Enterobacter spp*. (21,4%). Выявлена умеренная и сильная устойчивость к амоксициллину и ампициллину, цефаклору, цефуроксиму, цефадроксилу, эритромицину. Самая высокая устойчивость выявлена у изолятов *P. aeruginosa*. На втором месте — *E. coli*, продемонстрировавшая разную устойчивость к 14 противомикробным средствам и чувствительность только к амикацину. Таким образом, исследование, проведенное в Больнице Имама Хусейна, выявило в целом низкий, но заметный уровень обсеменения ортопедических операционных опасными для пациентов специфическими патогенами. Полученные результаты согласуются с глобальными данными, что говорит об универсальности проблемы обеспечения стерильности операционных. Постепенное выявление очагов и закономерностей обсеменения подчеркивает необходимость применения целенаправленных мер и постоянного наблюдения.

Ключевые слова: *Pseudomonas aeruginosa*, зоны операционного блока, устойчивость к противомикробным средствам

Соблюдение этических стандартов: исследование проведено в соответствии со стандартами добросовестной клинической практики и доказательной медицины.

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Статья получена: 01.06.2025 **Статья принята к печати:** 06.07.2025 **Опубликована онлайн:** 15.07.2025

DOI: 10.24075/vrgmu.2025.034

A critical factor in the occurrence of hospital-acquired infections (HAI) is the existence of germs in the room. The clinical implications of this finding are significant, not only for the patient but also for the surgical team that is caring for them [1, 2]. According to statistics from the Centers for Disease Control and Prevention (CDC), nosocomial infections impact around 1.7 million people annually. They are responsible for 99.000 fatalities [3]. Orthopedic surgery, especially elective treatments such as joint arthroplasty and hand surgery, reduces the risk of postoperative infection [4]. Orthopaedic surgery implements more stringent sterile protocols compared to other surgical procedures. Although the measurement technique used to assess the NI risk index may vary, the incidence might fluctuate greatly [5].

On the other hand, NI continues to be a source of worry because of the significant morbidity that results from it and the

extended hospital stays, readmissions, and revision operations that lead to a rise in medical treatment expenses [6, 7]. According to the data, due to bacterial NI, approximately 1718 total operations were performed in Nasiriyah during the past two years (2021–2023) for hip, knee, internal fixation, surgery, hip, and hand surgery. It should come as no surprise that these concerning figures have prompted several attempts by medical experts to identify and minimize NI origins and associated risks. Research has revealed many sources linked to the spread of pathogenic microorganisms, such as air, hospital areas, nitrogen gas freezers, staff clothing, and even keeping sterile plates open for an extended period [8, 9].

It has been determined that the operating room pollution results from several distinct reservoirs. These storage containers include unprocessed air, ventilated spaces, and antiseptic fluids; drainage of wounds; patient and gathering bag travel; surgical group; degree of inside traffic; theatre dress; foot wares; gloves and hands; use of inadequately sterilized supplies; polluted surroundings; and grossly polluted surfaces [10, 11]. By appropriately implementing infection control procedures, it is possible to avoid microbial contamination in the operating room. Reducing airborne bacteria in the operating room by about thirteen times, for instance, would result in a 50% reduction in wound contamination this mostly depends on the frequent fumigation of operation theater (OT) and improving cleaning and disinfection procedures.[12, 13].

In addition, research has shown that mobile devices, which medical professionals use throughout the hospital, are a prominent origin of pollution inside the operating room. Several studies have shown that disease-causing microbes, such as coagulase-negative staphylococci (CoNS), *S. aureus*, and *Acetinobacter*, utilize mobile phones [14, 15].

Several preventable causes of NI have been identified. If the proper treatments are implemented, the incidence of NI may be decreased. There are many primary focus areas of concern, including patients, surgeons, nurses, the atmosphere of the operating room, and the equipment. Although several methods have been established to reduce the risk of infection after implant surgery, infection is still a potential. To reduce the number of infections that arise in the operating room, engaging in practices such as practicing hand hygiene, providing prophylactic antibiotics in an acceptable manner and at the proper dose, donning surgical clothing, and reducing staff mobility is important. Surgical site infections are the third most frequently reported nosocomial infection (about 14–16%) . Given that antibiotic resistance has become a worldwide issue with corresponding consequences, the operating rooms serve as both a breeding ground and a hub for the spread of multidrug-resistant (MDR) microorganisms across the hospital setting. In developed and developing nations, the prevalence of nosocomial infections was linked directly or indirectly to bacterial contamination has been rising despite the high degree of relative hygiene and cleanliness as well as basic understanding of infection control with preventive techniques [16, 17].

It becomes essential to conduct surveillance within the unit to produce the vastly needed epidemiological data, which serves as a model for the creation of infection control and prevention policies. Bone and joint infection are considered a major cause of morbidity ' emotional stress, and enhanced mortality, in addition to significant economic loss ,and so one can imagine the magnitude of the problem. The purpose of this study was to examine the degree of contamination in the operating rooms at Imam Hussein Hospital in Nasiriyah. Finding the bacteria causing contamination and the related factors was the primary objective of the study. Using microbiological criteria, the study also sought to map the spread of these microbes throughout several operating room locations and their pattern of antibiotic resistance. We made the decision to evaluate the degree of bacterial contamination in our hospital's Orthopedic Theatres rooms in light of this information.

METHODS

Imam Hussein Hospital, located in the city of Nasiriyah under study, contained three distinct operating rooms, distributed as three rooms designated for general surgery, two for urology, and three rooms for orthopedic operation theatres between February 2022 and May 2024. We assessed the microbial contamination at orthopedic theatres, within the hospital approximately four to five times per month. To perform the swab, sterile swabs were soaked in sterile normal saline, then instantly & severally rolled onto floor surfaces, doors, telephone hands, walls, washbasins, and medical tools in the operating room and the intensive care unit (ICU) before and after surgery, then the samples were properly labeled before transportation to the to be processed in Laboratory. After sample collection, all swabs were immediately cultured using the sectioning technique on enriched medium blood agar and CLED agar [18] (Fig. 1).

In accordance with CSLI guidelines on Mueller Hinton agar, antibiotic susceptibility testing was conducted using the disc diffusion technique (Fig. 2). The 15 tested antibiotic discs were Ampicillin (AM), Amoxicillin (AMX), Amoxicillin/Clavulate (AMC), Cefaclor (CEC), Cefadroxil (CFR), Cefuroxime (CXM), Ceftriaxon (CRO), Ceftazidime (CAZ), Cefotaxim (CTX), Cefixim (CFM), Erythromycin (ERY), Ciprofloxacin (CIP), Levofuxacin (LE), Gentamycin (GM) Amikacin (AK) (Fig. 3). Following the guidelines, the zone of growth inhibition diameter of the bacterial isolates was measured and interpreted [19].

Statistical analysis

Values expressed as frequencies and percentages were analyzed using the statistical software SPSS version 17.0. Statistical analysis of the data was done considering an alpha error of 0.05 with a 95% confidence interval. Data are presented by mean ± SD. Frequency and percentage are used to describe demographic and baseline factors. Chi-square test was used for the above comparisons when the test assumptions were met, otherwise T- test method test was used.

RESULTS

For microbiological analysis, one thousand three hundred fifty-eight swabs were taken from the orthopedic surgery rooms between February 2022 and May 2024. Following the inoculation and cultivation of the swabs under aerobic and anaerobic conditions, it was discovered that 42 (3.09%) of the swabs had positive growth, while the negative culture was 1316 (96.90%).

The temporal distribution of positive culture shows a high pattern in the months (February, March, April), while there was generally a decrease in May.

A high incidence of positive cultures was detected in those swabs obtained from the couch of operating room 12 (28.57%), floor 11 (26.19%), and wall 9 (21.43%). A low incidence of positive cultures was recorded in those swabs taken from section parts 5 (11.91%), antiseptic solutions 3 (7.14%), and anesthetic trollies 2(4.76%), swabs from gauze

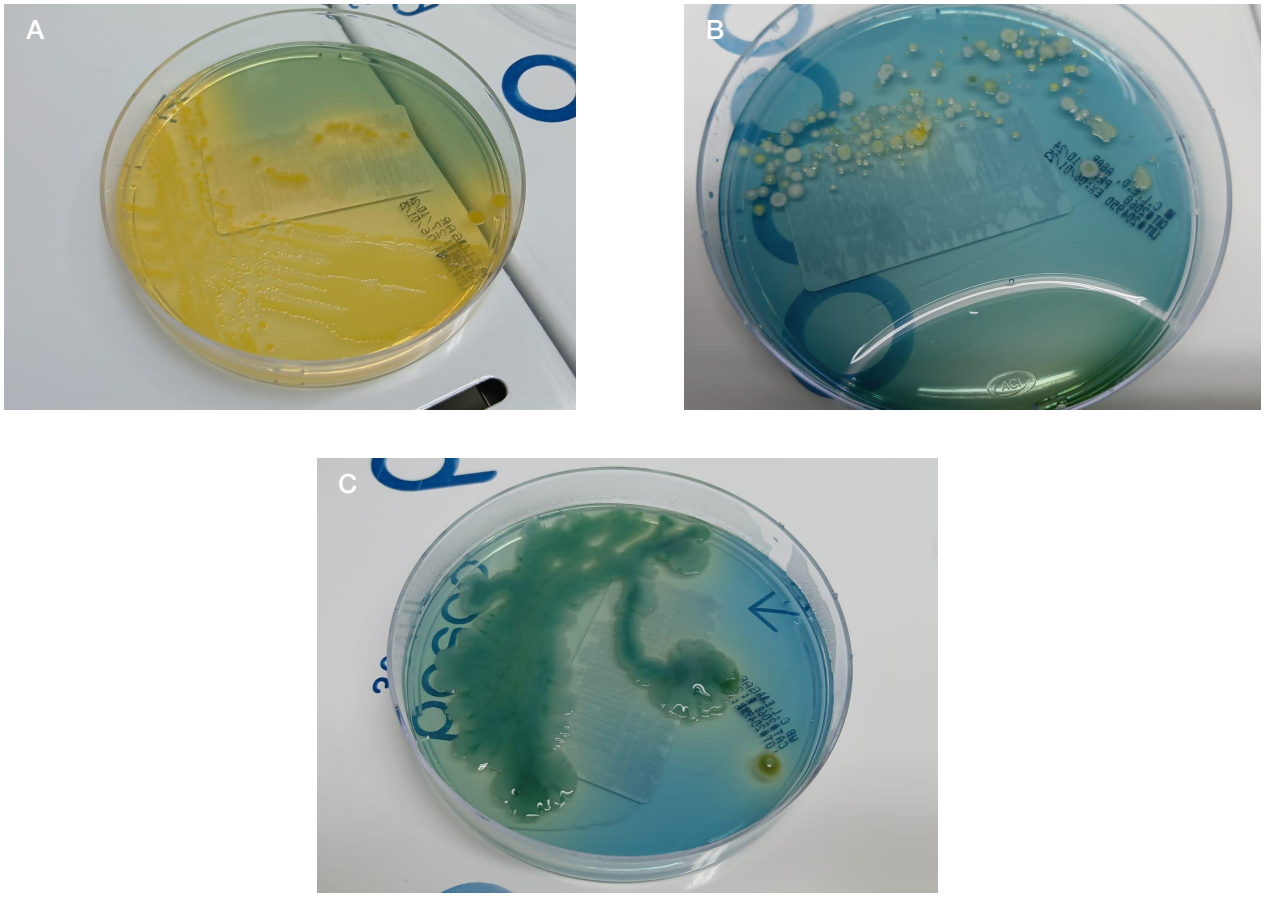

Fig. 1. A. Gram –ve bacteria appearance on CLED media. **B**. Gram +ve bacteria appetence on CLED media. **C**. Characteristic appetence of *P. aeruginosa* isolates

(0%) and surgical instruments (0%) show no growth of bacteria %) (Fig. 3). A highly significant difference (*P*-value < 0.001) was detected in bacterial contamination rates across different swab sources, where the contamination in couch, floor, and wall was significantly more contaminated than other areas.

Six types of pathogenic bacteria have been identified in orthopedic operating rooms as follows: CoNS 6 (14.29%), *S. aureus* 5 (11.90%), *P. aeruginosa* 8 (19.05%), *E. coli* 9 (21.43%), *Bacillus spp*. 5 (11.90%), and *Enterobacter spp*. 9 (21.43%). There is no statistically significant difference in the frequency distribution of the bacterial species (Table 1).

We observed moderate to high resistance pattern (Table 2) to amoxicillin and ampicillin, Cefaclor, Cefuroxime, Cefadroxil, Erythromycin. All the detected organisms showed highest resistances to both amoxicillin and ampicillin, this resistance pattern were decreased upon adding clavulate to amoxicillin.

S. aureus demonstrated the highest resistance to Ampicillin and amoxicillin (*n* = 5), followed by Amoxicillin/ Clavulate and Erythromycin (*n* = 3), Cefaclor (*n* = 2), only one isolate was resistant to Cefadroxil, Ceftazidime, Cefotaxim, Cefixim, and Gentamycin, While all the isolates were susceptible to the rest of the tested antibiotics. The highest resistance pattern was detected in *P. aeruginosa* isolates followed by *E. coli*, it shoed different resistance patterns to 14 antibiotics showing susceptibility to Amikacin only. The lowest resistant patterns were detected among CoNS, *Enterobacter spp.* & *Bacillus spp*. Which showed resistance to (4, 5, 4) antibiotics, respectively. Resistance rates differ significantly (*P*-value < 0.05) across antibiotics with the highest resistance battern to AM, AMX, ERY, while the least resistance to LE, AK, CFM. Also, the resuts shoed that, *E. coli* and *P. aeruginosa* were significantly resistance to antibiotics than other bacteria (*P*-value < 0.05).

DISCUSSION

It is possible for patients and their families to face severe consequences as a result of postoperative infections that are caused by microbial contamination in operating rooms. Investigating any suspected case of hospital-acquired infection (HAI) involves the collection of cultures from several regions of the patient's body, as well as from other patients, staff members, and the surrounding environment [20]. When trying to gather useful data, it is vital to pick the specimens that will be cultivated with great care. Infections are responsible for extending hospital admissions, causing long-term impairment, increasing resistance to antimicrobials, causing avoidable deaths, and representing a large additional cost burden for

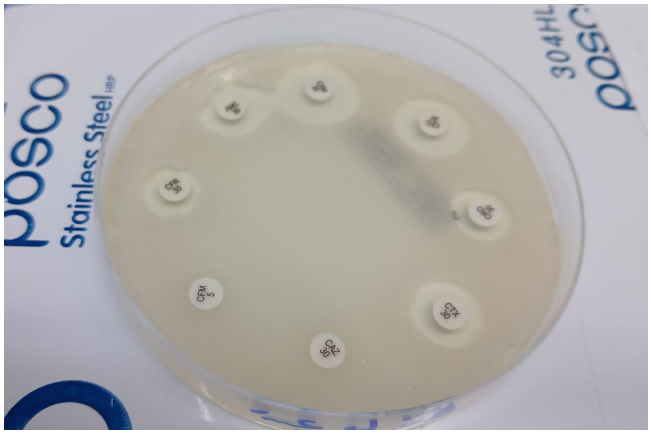

Fig. 2. Bacterial culture showing resistance to both Ceftazidime (CAZ), Cefixim(CFM)

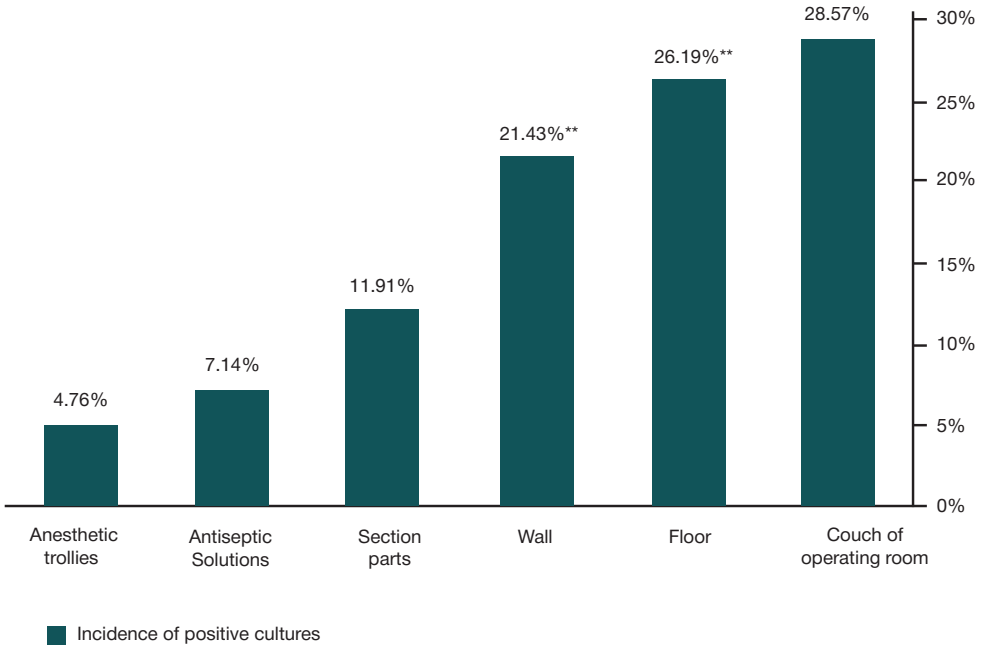

Fig. 3. Visualization of the Frequency of Positive Microbial Cultures in Operating Rooms Broken Down by Sample Source. ** — indicates statically significant difference, *P*-value < 0.001

health systems. Therefore, the solution is a well-implemented infection control program that may increase staff education and accountability.

Additionally, research should be conducted to adapt and validate surveillance procedures based on developing nations' realities to attain acceptable performance. This can potentially minimize the number of HAIs by approximately one-third [21]. The environmental disinfection and instrument sterilization procedures undoubtedly demand the most stringent monitoring out of all the procedures and regulations.

The study identified six types of pathogenic bacteria in the positive swabs: CoNS (14.29%), *S. aureus* (11.90%), *P. aeruginosa* (19.05%), *E. coli* (21.43%), *Bacillus spp*. (11.90%), and *Enterobacter spp*. (21.43%). These pathogens are well-known culprits in hospital-acquired infections, and their presence in operating theaters is a matter of concern. *S. aureus* is notorious for its role in postoperative wound infections. It can be challenging to treat due to its resistance to multiple antibiotics. The relatively high incidence of *E. coli* and *Enterobacter spp.*, which accounted for 21.43% of the identified pathogens, highlights the potential for contamination from sources like surgical instruments or healthcare workers' hands, emphasizing the need for strict sterilization protocols.

The distribution of positive cultures within the orthopedic operating rooms shows a concerning pattern, with the highest contamination rates observed on the couch (28.57%), floor (26.19%), and walls (21.43%). This suggests that these surfaces, which are frequently in contact with patients, staff, and equipment, are critical contamination points. The presence of pathogens on the couch, where patients lie during surgery, directly threatens patient safety, potentially leading to surgical site infections. The significant contamination of the floor and walls further emphasizes the need for rigorous cleaning protocols and the regular disinfection of all surfaces within the operating room. In contrast, a lower incidence of positive cultures were found on section parts (11.91%), antiseptic solutions (7.14%), and anesthetic trollies (4.76%). At the same time, no bacterial growth was detected on gauze or surgical instruments. The absence of contamination on surgical instruments and gauze is a positive finding, indicating that sterilization procedures for these critical items effectively prevent the transmission of pathogens. This aligns with best infection control practices, prioritizing the sterility of instruments used in invasive procedures. A study cited by [22] showed that operating rooms harbor bacteria, S.aureus, also found in this study with a prevalence rate of around 15% in surgical settings — slightly higher than the 10.34% detected in AL Nassiriyah.

Variations in how hospitals handle sanitation and infection control measures in different regions could explain the differences observed.

A relevant study [23] focused on contamination in clean orthopedic procedures, highlighting Coagulase *Staphylococci* and *Enterobacter spp,* as the types of bacteria. These findings align with the study emphasizing the prevalence of

Table 1. Distribution of bacterial isolates in operation surgical rooms

Species isolated	No. of isolated	*n* (%)
CoNS	6	–14.29%
S. aureus	5	–11.90%
P. aeruginosa	8	–19.05%
E. coli	9	–21.43%
Bacillus spp.	5	–11.90%
Enterobacter spp.	9	–21.43%
Total	42	100%
P-value		0.766

Table 2. Antibiotic resistance patterns of the detected bacterial isolates

Enterobacter spp.	*Bacillus spp.*	*E. coli*	*P. aeruginosa*	*S. aureus*	CoNS	Antibiotic
4	3	5	8	5	4	AM
2	2	6	8	5	3	AMX
1	0	3	6	3	0	AMC
0	1	5	6	2	0	CEC
0	0	6	7	1	0	CFR
0	1	4	7	0	1	CXM
0	0	1	3	0	0	CRO
0	0	1	2	1	0	CAZ
0	0	1	3	1	0	CTX
0	0	0	1	1	0	CFM
1	1	3	6	3	2	ERY
0	0	1	2	0	0	CIP
0	0	0	1	0	0	LE
0	0	2	2	1	0	GM
0	0	1	0	0	0	AK

Note: mpicillin (AM), Amoxicillin (AMX), Amoxicillin/Clavulate (AMC), Cefaclor (CEC), Cefadroxil (CFR), Cefuroxime (CXM), Ceftriaxon (CRO), Ceftazidime (CAZ), Cefotaxim (CTX), Cefixim (CFM), Erythromycin (ERY), Ciprofloxacin (CIP), Levofuxacin (LE), Gentamycin (GM), Amikacin (AK).

Coagulase *Staphylococci* and *Enterobacter* species. The study emphasized the role of laminar ventilation systems in preventing contamination and the impact of bacteria, indicating improvements in operating rooms in AL Nassiriyah.

Escherichia coli and *P. aeruginosa* raise concerns about procedure environment sterility. *P. aeruginosa,* known for its resistance and link to hospital-acquired infections, was found in 17.24% of samples. This contrasts with a study [24] reporting *Pseudomonas* prevalence in surgical settings, highlighting a notable increase.

This suggests that the methods used to control infections in AL Nassiriyah might not have been effective. Likewise, *Escherichia coli* was found in 24.14% of the samples, which is much higher than in studies like the one referenced by [25], where *E. coli* was found in more than 10% of samples. This difference highlights the need for hygiene and sterilization practices to prevent contaminations. The impact of these contaminants on patients cannot be overstated. Infections after surgery can lead to procedure complications, resulting in longer hospital stays, more surgeries, and higher medical costs. A study [26] aimed to measure the effects of surgical site infections. Found that infected patients had their hospital stay doubled, and readmission rates increased fivefold. Orthopedic patients are at risk for surface and deep infections due to the bacteria identified in the AL Nassiriyah research, which can significantly hinder their recovery and overall health outcomes.

Comparing these results with other studies, it is evident that the contamination rates and types of pathogens identified are consistent with findings from similar investigations in orthopedic and other surgical settings. For example, a study conducted in a tertiary care hospital reported that the most common contaminants in operating theaters were S. aureus and *P. aeruginosa*, with contamination rates ranging from 2% to 5% [27], similar to the 3.09% contamination rate observed in this study.

Another study from a hospital in Nigeria found that *Escherichia coli* and *Enterobacter spp*. were among the most frequently isolated bacteria in operating rooms [28], supporting the present study's findings. These comparisons suggest that the types of bacteria identified in the Imam Hussein Hospital's orthopedic theaters are typical of those found in surgical environments globally, underscoring the universal challenge of maintaining sterility in these settings.

The bacterial highest resistance was detected against amoxicillin and ampicillin, flowed by Cefaclor, Cefuroxime, Cefadroxil, and Erythromycin. Amoxicillin resistance pattern were decreased upon adding clavulate to amoxicillin. S.aureus demonstrated the highest resistance to Ampicillin and amoxicillin (5 isolates), followed by Amoxicillin/ Clavulate and Erythromycin (3 isolates), Cefaclor (2 isolates), only one isolate was resistant to Cefadroxil, Ceftazidime, Cefotaxim, Cefixim, and Gentamycin, While all the isolates were susceptible to the rest of the tested antibiotics. The highest resistance pattern was detected in *P. aeruginosa* isolates followed by *E. coli,* it shoed different resistance patterns to 14 antibiotics showing susceptibility to Amikacin only. The lowest resistant patterns were detected among Coagulase-negative *Staphylococci, Enterobacter spp*. & *Bacillus spp*. Which showed resistance to (4, 5, 4) antibiotics, respectively. Evaluation of the pathogens' antibiogram and contamination rate typically acts as an early warning indicator for timely intervention. Substantial resistance to amoxicillin, and ampicillin was found in this investigation; this trend was consistent with research done in underdeveloped nations [29].

However, in a study conducted by Mohammed et al., isolates showed resistance to two additional medications: Erythomycin & Gentamycin. These should raise concerns because they are among the most widely used and least expensive antibiotics. Antibiotic use may be the cause of variations in resistance patterns, which emphasizes the necessity of monitoring antimicrobial programs[30].

The temporal distribution of positive cultures, which showed higher contamination rates in February (28.57%), March (38.10%), and April (23.81%), with a decrease in May (9.52%), may be indicative of seasonal variations in microbial load or fluctuations in operating room usage and staff activity levels. This pattern could reflect the increased patient load and surgical procedures during the earlier months, potentially leading to higher contamination risks. Alternatively, it may suggest lapses in cleaning protocols during busier periods. The drop in contamination in May could be attributed to improved

cleaning practices, reduced surgical activity, or changes in environmental conditions. These findings highlight the need for continuous monitoring and adjustment of infection control measures throughout the year to address potential seasonal variations in contamination risk.

The study's findings significantly affect infection control practices in orthopedic operating rooms. Identifying specific contamination hotspots, such as the couch, floor, and walls, provides valuable information for targeted interventions to reduce microbial load in these areas. Regular and thorough cleaning of these surfaces and using effective disinfectants are essential to minimize the risk of patient exposure to harmful pathogens. Additionally, the study underscores the importance of maintaining strict sterility of surgical instruments and other critical items used in the operating room, as evidenced by the absence of bacterial growth on gauze and instruments. The results also suggest that ongoing staff education and training in infection control practices, particularly in the handling and cleaning high-risk areas, are crucial to sustaining low contamination rates.

CONCLUSIONS

The microbiological analysis of orthopedic operating theaters in Imam Hussein Hospital reveals a generally low but significant level of bacterial contamination, with specific pathogens posing a risk to patient safety. The study's findings are consistent with similar worldwide investigations, highlighting the universal challenges of maintaining sterile environments in surgical settings. Identifying contamination hotspots provides a basis for targeted interventions. At the same time, the temporal distribution of positive cultures suggests the need for continuous monitoring and adjustment of infection control measures. Ultimately, the study emphasizes the critical importance of rigorous infection control practices in preventing hospital-acquired infections and ensuring the safety of patients undergoing orthopedic surgery.

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