

Utilization of the PCR technique for identifying post-surgical infections at Rzgari Hospital in Erbil

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Abstract:

Contaminated surgical wounds have emerged as a significant problem in many healthcare settings, leading to prolonged hospitalization and increased treatment costs. One of the primary purposes of this research was to analyze the types of microorganisms present in all collected samples from post-surgical wounds and to assess their antimicrobial susceptibility. PCR was applied as a reliable diagnostic tool to identify the isolated microbial agents. Specimens included 110 wound swabs collected from patients aged between 10 and 55 years who had recently undergone various surgical procedures at Rzgari Hospital in Erbil, Iraq. The bacterial isolates were identified using traditional microbiological approaches, and all isolates were subjected to antibiotic resistance testing performed on all isolated species. PCR was employed to differentiate between the microbial species. The results revealed the presence of several bacterial species in surgical wound sites, with the following prevalence rates: *E. coli* (35.9%), *S. aureus* (27.8%), *P. aeruginosa* (24.3%), *Acinetobacter* spp. (9.9%), and *Enterobacter* spp. (9.1%). The predominant isolates demonstrated Susceptibility testing to vancomycin and amikacin. ERIC-PCR analysis was applied to 9 *S. aureus* and 17 *E. coli* isolates, revealing four distinct genetic profiles, indicating considerable genetic diversity. DNA fingerprinting images were obtained for both species. The results of this study underscore the critical role of precise microbial identification and antibiotic susceptibility testing in effectively managing postoperative wound infections. The outcomes of this research could enhance infection control strategies and support improved clinical care for patients undergoing surgery.

Article Info:

Submitted:

23-06-2025

Revised:

20-07-2025

Accepted:

25-07-2025

Keywords:

surgical wound infections; nosocomial pathogens; antimicrobial susceptibility; molecular identification; ERIC-PCR typing

 <https://doi.org/10.53713/nhsj.v5i3.544>

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INTRODUCTION

Surgical site infections (SSIs) represent a significant and persistent challenge within global healthcare systems, contributing substantially to patient morbidity, prolonged hospital stays, increased healthcare costs, and elevated mortality rates (Khan et al., 2025). Despite advancements in surgical techniques and infection control protocols, SSIs remain among the most common healthcare-associated infections (HAIs), affecting millions of patients annually worldwide (Habtie et al., 2025). Their occurrence not only imposes a heavy burden on individual patients through pain, delayed healing, and potential disability but also strains healthcare resources significantly, underscoring the critical need for effective prevention and rapid, accurate diagnosis (Rezaei et al., 2025).

Mitigating the risk of SSIs necessitates a multifaceted approach grounded in evidence-based practices. Core strategies include the meticulous application of aseptic techniques during surgery, the judicious and timely administration of appropriate antibiotic prophylaxis tailored to the procedure and local resistance patterns, and the implementation of rigorous postoperative wound care protocols (Rehman et al., 2024). Furthermore, continuous education and training for all healthcare

personnel involved in the surgical pathway – from preoperative preparation to discharge – on standardized infection prevention and control (IPC) measures are paramount to sustaining effective practices and minimizing avoidable complications (Reeves & Torkington, 2021).

SSIs are frequently polymicrobial, involving various bacterial pathogens with varying virulence and resistance profiles (Bucataru et al., 2024). Common culprits include *Staphylococcus aureus* (notably methicillin-resistant *S. aureus* - MRSA), *Enterococcus species* (including vancomycin-resistant enterococci - VRE), and various *Streptococcus species* (Kaapu et al., 2023). Critically, these organisms, alongside Gram-negative pathogens such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, and *Serratia marcescens*, exhibit remarkable resilience, capable of persisting on dry hospital surfaces and equipment for weeks or even months, facilitating transmission and complicating eradication efforts within the healthcare environment (Pinchera et al., 2022).

The timely and accurate identification of causative pathogens is fundamental for targeted antimicrobial therapy and effective infection control (Wang et al., 2024). However, traditional microbiological methods, including direct microscopy of stained smears, culture-based techniques, and subsequent biochemical profiling, suffer significant drawbacks (Parasuraman et al., 2024). These approaches are inherently time-consuming, often requiring 48-72 hours or longer to yield definitive results, which delays appropriate treatment. Moreover, they lack sensitivity in detecting fastidious organisms, may fail to identify all pathogens in polymicrobial infections, and cannot readily distinguish between viable and non-viable bacteria or detect resistance genes directly (Ahmad et al., 2024).

The limitations of conventional diagnostics create a critical window where empirical antibiotic therapy is often initiated, potentially contributing to the development of antimicrobial resistance (AMR) if not precisely targeted (Hassall et al., 2024). There is an urgent need for diagnostic tools that provide rapid, sensitive, and specific identification of pathogens and their resistance mechanisms directly from clinical specimens (Rentschler et al., 2020). Such tools are essential for guiding precise antimicrobial stewardship, optimizing patient outcomes, and informing effective infection control interventions to prevent outbreaks within healthcare facilities (Schinas et al., 2023).

Polymerase Chain Reaction (PCR) technology has emerged as a powerful and indispensable tool in modern clinical microbiology, directly addressing the shortcomings of traditional culture methods (Alsharksi et al., 2023). By amplifying specific nucleic acid sequences unique to target pathogens, PCR enables the rapid (often within hours), highly sensitive, and specific detection of a broad spectrum of microorganisms, including those difficult or impossible to culture. Its ability to identify pathogens directly from complex samples, even in polymicrobial settings, and to detect specific resistance markers makes it particularly valuable for diagnosing challenging infections like SSIs (Srivastava & Prasad, 2023).

While PCR-based diagnostics are increasingly adopted in high-resource settings for SSI management, their application within the Iraqi healthcare system, particularly for routine surgical infection surveillance and diagnosis, remains largely unexplored and underutilized. There is a conspicuous lack of localized data characterizing the precise microbial etiology and resistance profiles of SSIs in Iraqi hospitals using advanced molecular techniques. This knowledge gap hinders the development of evidence-based, locally relevant prevention protocols and treatment guidelines tailored to the specific pathogen landscape encountered in Iraqi surgical patients (Marhash et al., 2025).

This research, conducted at Rzgari Hospital in Erbil, Kurdistan Region of Iraq, directly addresses this critical void. It represents the study within Iraq to systematically apply PCR technology for the detailed molecular identification of pathogens responsible for post-surgical wound infections. Moving beyond conventional culture, this study aims to provide a comprehensive and accurate profile of the microbial agents – including fastidious organisms and resistance determinants – actively contributing to SSIs in this specific regional hospital setting, offering unprecedented insights into the local epidemiology (Nasir et al., 2025).

The primary purpose of this investigation is to design and implement a targeted PCR-based approach to identify the microorganisms responsible for surgical wound infections among patients at Rzgari Hospital. By generating robust, molecular-level data on the causative pathogens and their

characteristics, this study seeks to establish a foundation for more precise diagnosis, targeted antimicrobial therapy, and enhanced infection prevention strategies (Ragothaman et al., 2022)—evaluation of Polymerase Chain Reaction in the Identification and Quantification of Clinically. The findings are expected to significantly improve postoperative care standards, reduce SSI rates, and ultimately enhance patient safety and outcomes at Rzgari Hospital. They will also potentially serve as a model for broader implementation across the Iraqi healthcare system.

METHOD

Identification and Sample Collection

Bacterial samples were collected from patients undergoing surgical procedures using sterile cotton swabs. A total of 110 isolates were obtained for further microbiological analysis. The Surgical Unit of Rzgari Hospital in Erbil operated from January 5 to April 30, 2025. Wound swabs were collected under aseptic conditions, and the samples were promptly transported to the Microbiology Laboratory at Rzgari Hospital for processing. The specimens were cultured on appropriate differential and selective media, including chromogenic media (CHROM agar), blood agar, and MacConkey agar, and incubated at 37°C for 24 to 48 hours. After incubation, colony morphology was observed, and Gram staining was performed to differentiate between Gram-positive and Gram-negative bacteria. Further bacterial identification was conducted using various biochemical assays, including the IMViC tests (Indole production, Methyl Red, Voges-Proskauer, and Citrate utilization), as well as coagulase, catalase, oxidase, arginine hydrolysis, urease activity, hydrogen sulfide (H₂S) production, and motility tests. These assays helped confirm the identity and classification of the bacterial isolates prior to molecular analysis.

Determination of Bacterial Antibiotic Resistance and Susceptibility

Antibiotic susceptibility testing was conducted employing disk diffusion, and guidelines were established based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. Muller-Hinton agar was employed as the culture medium due to its optimal composition, which facilitates the diffusion of antibiotic discs and produces clear inhibition zones. A sterile loop was used for each bacterial isolate to pick approximately 10⁵ colony-forming units (CFUs). The bacterial cells were then suspended in 2.5 mL of distilled sterile water. The swab was sterile and used to evenly spread the suspension over the entire agar plate surface of Mueller-Hinton. The antibiotic discs were carefully placed above the inoculated medium using sterile forceps. All plates and media were incubated for 19 to 24 hours at 37°C. Following incubation, all inhibition zones around each antibiotic disc were measured under reflected light with a ruler to assess the susceptibility or resistance of the bacterial isolates to the antibiotics tested.

Extraction of DNA and (PCR) Methodology

Bacterial cells were collected from nutrient agar plates using a sterile loop and prepared for PCR analysis. The harvested cells were washed twice with 2 mL of 1X Tris-EDTA buffer (10 mM Tris-HCl, pH > 7). The resulting pellet was resuspended in 0.6 mL of sterile distilled water and subjected to boiling at 100°C for 10–15 minutes. After boiling, samples were immediately chilled on ice for approximately 11 minutes, followed by centrifugation at 11,500 × g for 6 minutes. The DNA concentration in the supernatant was quantified using a spectrophotometer. Extracted DNA samples were stored at –20°C until further use. ERIC-PCR was performed using primers 5'-ATG TAA GCT CCT GGG GAT TCA C-3' and 5'-AAG TAA GTG ACT GGG GTG AGC G-3'. The PCR reaction mixture (total volume 25 µL) contained 13.5 µL of Ready Mix™ Taq PCR Mix, 0.8 µM of each primer, 3 mM MgCl₂ (Sigma), and 160–200 ng of template DNA. The final concentrations included 0.4 mM dNTPs and approximately 1.5 units of Taq DNA polymerase. Thermal cycling conditions were as follows: initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 50 seconds, annealing at 40°C for 50 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes. PCR products were resolved by electrophoresis on a 1.6% agarose gel stained with ethidium bromide. Gel images were captured for subsequent analysis. The DNA

fingerprint patterns derived from banding profiles were used to compare and differentiate bacterial isolates based on their genetic characteristics.

RESULT

The study comprised 110 patients, including 59 females (54.8%) and 51 males (46.9%), aged 10 to 55. Among the collected samples, 57 (54.4%) showed positive culture growth, whereas 53 (48.2%) tested negative. The demographic data of patients admitted to the surgical ward at Rzgari Hospital in Erbil are detailed in Tables 1 and 2.

Table 1. Distribution of Patients by Gender in the Surgical Unit with a Sample Size of 108 Patients

The Gender	The Number of Patients	Percentage
Female	59	54.8%
Male	51	46.9%
Total	110	100

Table 2. Distribution of Patients, Age at the Surgical Ward (n=108)

The Age Gap	The Frequency	The Percentage
10-14	10	9.2%
15-19	12	11.1%
20-24	12	11.1%
25-29	11	10.1%
30-34	14	12.9%
35-39	10	9.2%
40-44	13	12%
45-49	14	11.1%
50-55	14	12.9%
Total	110	100

Regarding postoperative surgical site infections (SSI), pathogens were detected in the following distribution: *E. coli* (35.9%), *Staphylococcus aureus* (27.8%), *Pseudomonas aeruginosa* (24.3%), *Acinetobacter sp.* (9.9%), and *Enterobacter sp.* (9.1%). Refer to Table 3 for a detailed summary.

Table 3. Surgical Sites and Corresponding Pathogens in SSI Patients

Harmful Species	Infection at the Surgical Site					Total
	Surgery in urology	General surgical	An appendix	Breakage	Back & abdomen	
<i>E. coli</i>	3(4.6%)	4(6.4%)	2(4.6%)	8(13.6%)	5(8.2%)	24 (35.9%)
<i>E. sp</i>	2(2.8%)	2(2.8%)	-	-	3(3.5%)	9(9.1%)
<i>P. aeruginosa</i>	4(9.8%)	4(6.4%)	2(2.8%)	2(2.7%)	3(4.6%)	15(24.3%)
<i>A. baumannii</i>	2(2.8%)	-	2(2.8%)	4(6.4%)	-	8(9.9%)
<i>S. aureus</i>	6(9.8%)	3(4.5%)	3(4.6%)	4(6.4%)	3(4.6%)	21(27.8%)

Antibiotic Sensitivity and Resistance

Patterns of the isolated pathogens were assessed with antibiotic susceptibility, with results varying based on the type of bacterial species. The data are expressed in both numbers and percentages, categorized into Gve- bacteria bacteria (e.g., *E. coli*, *A. baumannii*, *P. aeruginosa*). And Gve+ (*S. aureus*, *Enterobacter*). A summary of the findings is provided in Tables.

Table 4. Antibiotic Sensitivity and Resistance of Gram-positive Bacteria

Antibiotics	Number of resistance (%)	Number of Sensitivity (%)
Tobramycin	3 (0%)	15 (91.0%)
Vancomycin	0 (0%)	15 (11.10%)
Penicillin	16 (83.9%)	2(7.3%)
Ciprofloxacin	9 (60%)	7 (50%)
Gentamycin	4 (19.7%)	12 (72.1 %)
Erythromycin	8 (50%)	9 (60%)
Amikacin	2 (7.3%)	16 (83.9%)

Table 5. Sensitivity and Resistance Profiles of Gram-Negative Bacterial Isolates to Antibiotics

Antibiotics	The quantity of antibiotics used	Number of resistance (%)	Number of Sensitivity (%)
Vancomycin	16	0 (0%)	16 (100%)
Tobramycin	16	4 (29.0%)	10 (82.0%)
Ceftriaxone	16	8 (56.6%)	6 (43.4%)
Chloramphenicol	16	8 (56.6%)	6 (43.4%)
Aztreonam	16	11 (82%)	4 (27 %)
Pipracillin	16	10 (55.6%)	5 (34.3%)
Ciprofloxacin	16	4 (34.3%)	10 (55.7%)
Gentamycin	16	5 (28 %)	11 (62 %)
Imipenem	16	0 (0%)	16(100%)
Cefotaxime	16	8(50)	8 (50%)
Amikacin	16	0 (0%)	16 (100%)

Table 6. Antimicrobial Sensitivity Rates of Predominant Pathogens to Selected Antibiotics

Antibiotics	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>
Vancomycin	100%	100%	100%
Tobramycin	50%	50%	100%
Piperacillin	100%	12%	-
Ciprofloxacin	62.5%	34%	50%
Gentamycin	75%	75%	81.2%
Imipenem	100%	100%	-
Ceftriaxone	100%	42%	-
Amikacin	100%	100 %	93.8%

According to the data presented in the preceding tables, *Escherichia coli* and *Pseudomonas aeruginosa*, both Gram-negative bacteria, exhibited significant resistance to several antibiotics, notably cefotaxime, cephalothin, gentamicin, and co-trimoxazole. The data identified *Staphylococcus aureus*, *E. coli*, and *P. aeruginosa* as the predominant pathogens in surgical wound infections, all showing sensitivity to amikacin. Notably, *E. coli* also exhibited high sensitivity to vancomycin and imipenem, suggesting that despite its prevalence among wound infections, it can be effectively managed using these antibiotics. In contrast, *S. aureus* demonstrated substantial resistance to ceftriaxone and piperacillin, highlighting the increasing trend of antibiotic resistance in this species. A total of 9 *S. aureus* and 17 *E. coli* isolates underwent ERIC-PCR typing, as their differing resistance patterns implied potential genetic heterogeneity.

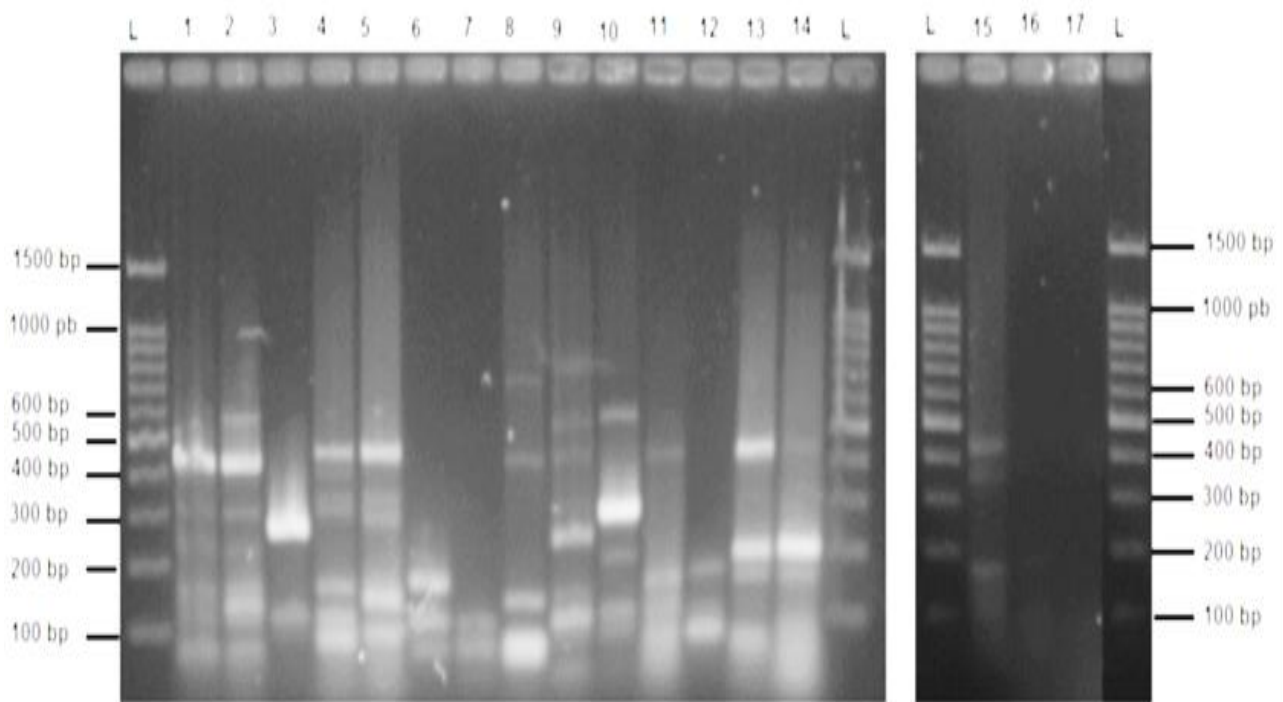


Figure 1. Genetic Fingerprint Patterns of *E. coli* Isolates derived from Surgical Wound Infections, as determined by ERIC-PCR Analysis.

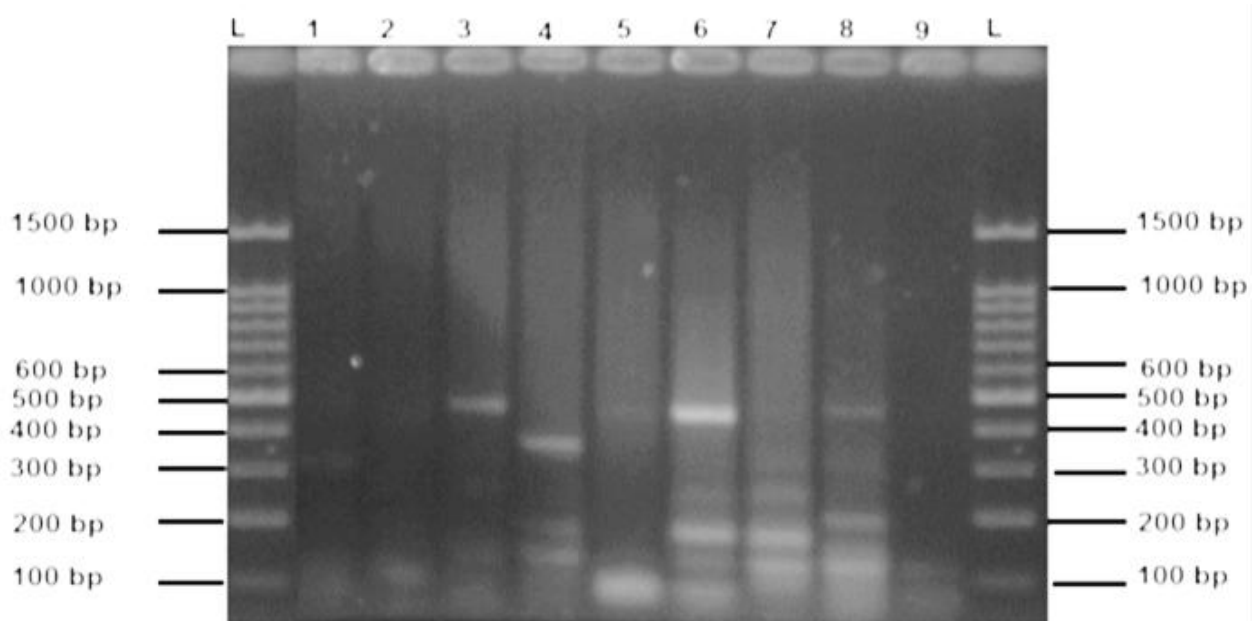


Figure 2. DNA Fingerprint Patterns of *Staphylococcus aureus* Isolates derived from Surgical Wound Infections, Produced using the ERIC-PCR Technique

DISCUSSION

The culture positivity rate of 54.4% observed in this study underscores a substantial bacterial burden in surgical wounds at Rzgari Hospital, aligning with global SSI prevalence estimates (typically 5–30%, though higher in resource-limited settings). The near-equal gender distribution (54.8%

female) and broad age range affected (10–55 years) challenge assumptions that SSIs predominantly target specific demographics, suggesting that infection risk is more strongly linked to procedural factors, comorbidities, or hospital environment than inherent patient characteristics. Critically, *Escherichia coli* emerged as the predominant pathogen (35.9%), followed by *Staphylococcus aureus* (27.8%) and *Pseudomonas aeruginosa* (24.3%). This profile diverges from high-income settings where *S. aureus* typically dominates, implicating regional factors such as higher rates of gastrointestinal/urological surgeries (where *E. coli* is endemic) or environmental contamination in Erbil's healthcare infrastructure. The prominence of Gram-negative pathogens (*E. coli*, *P. aeruginosa*) signals a pressing need for infection control protocols tailored to these resilient organisms (Elfadadny et al., 2024).

The alarming resistance profiles revealed by susceptibility testing reflect a critical threat to effective SSI management. *S. aureus* exhibited near-ubiquitous resistance to penicillin and high resistance to ceftriaxone and piperacillin—consistent with global MRSA trends—but retained universal sensitivity to vancomycin and amikacin. Similarly, *E. coli* and *P. aeruginosa* demonstrated extensive resistance to aztreonam, co-trimoxazole, and cefotaxime, corroborating reports from Northwest Ethiopia and Europe where extended-spectrum β -lactamase (ESBL) producers are prevalent. Notably, amikacin and imipenem maintained high efficacy across all three major pathogens, positioning them as vital empiric therapy options in this setting while awaiting culture results (Kalin et al., 2023). This pattern highlights a dangerous reliance on last-resort antibiotics. It underscores the urgent need for antimicrobial stewardship programs to preserve these critical drugs, particularly given the scarcity of newer agents in Iraqi healthcare (Schweiger et al., 2024).

ERIC-PCR analysis revealed significant genetic heterogeneity among *E. coli* and *S. aureus* isolates, with four distinct profiles identified at 50% similarity. Unrelated strains (e.g., *E. coli* 16/17; *S. aureus* 2/9) suggest sporadic, community-acquired, or environmentally sourced infections rather than clonal outbreaks. Conversely, identifying similar strains implies potential cross-transmission within the hospital—likely via contaminated surfaces, instruments, or healthcare worker vectors. This duality emphasizes that SSIs at Rzgari Hospital arise from both endogenous flora and exogenous transmission routes, necessitating a dual-pronged infection control strategy: enhanced environmental disinfection targeting high-touch surfaces (where *P. aeruginosa* and *Acinetobacter* persist for months) alongside strict adherence to contact precautions during wound care. Molecular typing thus proves indispensable for distinguishing outbreak scenarios from background endemicity (Schinas et al., 2023b).

The dominance of *E. coli* and multidrug-resistant Gram-negatives aligns with trends across low- and middle-income countries (LMICs), where limited access to advanced diagnostics, inconsistent sterilization practices, and antibiotic overuse drive resistance. The universal vancomycin sensitivity in *S. aureus* is reassuring but precarious; without stewardship, vancomycin-resistant strains could emerge, as seen with VRE in enterococci (Oladokun, 2023). While this study provides the first molecular characterization of SSIs in Iraq, limitations include reliance on culture-positive cases (potentially missing fastidious or anaerobic pathogens) and a single-center design restricting generalizability. Nevertheless, the data offer actionable pathways for improvement: (1) Prioritizing imipenem and amikacin for empiric therapy in high-risk surgeries; (2) Implementing routine ERIC-PCR surveillance during suspected outbreaks to identify transmission chains rapidly; (3) Strengthening environmental monitoring for *E. coli* and *P. aeruginosa* in operating theaters; and (4) Integrating PCR-based diagnostics into standard workflows to reduce the 54.4% culture-negative gap. Crucially, these measures must be paired with staff training on aseptic technique and antibiotic stewardship. As the pioneering molecular SSI study in Iraq, this work establishes a benchmark for national surveillance. It underscores that combating SSIs requires clinical vigilance and systemic investment in diagnostics and infection control infrastructure.

CONCLUSION

This study highlighted the significant role of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* as major causative agents of surgical site infections (SSIs) at Rzgari Hospital. The high culture positivity rate and notable antibiotic resistance patterns, particularly

penicillin, ceftriaxone, and aztreonam, underscore the growing concern of multidrug-resistant organisms in clinical settings. Nonetheless, the consistent sensitivity to amikacin and imipenem across isolates suggests their continued efficacy as empirical treatments. Furthermore, molecular typing using ERIC-PCR revealed considerable genetic diversity among the isolates, emphasizing the value of molecular tools in infection tracking and epidemiological surveillance. In light of these findings, it is recommended that healthcare institutions adopt molecular diagnostic methods such as PCR to enhance detection speed and accuracy. Implementing robust antibiotic stewardship programs and revising treatment protocols based on local resistance profiles are essential to combat rising antimicrobial resistance. Additionally, strict adherence to infection control practices, continuous training for healthcare staff, and increased patient awareness regarding postoperative care are vital measures to reduce the incidence and impact of SSIs.

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