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Post- surgical Infections and Associated Bacteria: A Study on Antibiotic Resistance Profiles of Bacterial Isolates

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ABSTRACT: Surgical site infections (SSIs) are significant postoperative complications associated with increased morbidity, prolonged hospital stays, and rising healthcare costs. This study investigated the microbial diversity and antibiotic resistance profiles of pathogens isolated from SSIs in post-operative patients between January 2022 and June 2022. A total of 90 wound samples were analyzed, yielding 150 bacterial isolates. Among these, Gram-negative rods were the most prevalent (39%), followed by Gram-negative cocci (31%). Gram-positive isolates accounting for 30%. Antibiogram analysis focused on the 10 most resistant isolates from each group. Among Gram-positive isolates, SN 81 exhibited the highest resistance, with 88% resistance to tested antibiotics. Among Gram-negative isolates, SN 123 showed the highest resistance at 94%, particularly against carbapenems and cephalosporins. These findings highlight the critical need for robust infection control practices, surveillance of resistance patterns, and the development of tailored therapeutic strategies to combat antimicrobial resistance in healthcare settings.

Keywords: Surgical site infections, antimicrobial resistance, antibiogram.

INTRODUCTION

Surgical site infection (SSI) refers to an infection occurring in the area of the body where a surgical procedure was performed, typically within 30 days of the operation, or up to one year in cases involving implants (Pal and Guhathakurta 2012). SSIs are a prevalent complication of surgery, posing significant risks to patients and healthcare systems alike. They account for approximately 1–3.1% of all surgical procedures globally and contribute to about 2% of mortality related to healthcare-associated infections (HAIs) (Barie and Wilson 2015). The World Health Organization (WHO) has identified antimicrobial resistance (AMR) as a critical global health threat, projecting it could lead to 10 million deaths annually by 2050 (O'Neill, 2014). The incidence of nosocomial infections, including SSIs, ranges from 2–20% in developed countries (Sawyer *et al*., 2018), while rates are higher in low-resource regions. In India, the incidence of SSI is reported to be between 4.04–30% (Pal and Guhathakurta 2012), emphasizing the regional variability in infection rates. Globally, a multicenter study conducted across 66 countries revealed an overall SSI incidence of 12.3% (Sawyer *et al*., 2018). Specific studies have documented varying rates: Sierra Leone at 11.5% (Lakoh *et al*., 2022), sub-Saharan Africa at 14.8% (Ngah *et al*., 2016), Ethiopia at 21.1% (Misha *et al*., 2021), and lower rates in China (Li *et al*., 2020).

The predominant causative pathogen of SSIs is *Staphylococcus aureus* (Spagnolo *et al*., 2013; Berríos *et al*., 2017), though Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, and *Proteus* species are frequently implicated (El-Saed *et al*., 2015). Infections are often polymicrobial, involving both aerobic and anaerobic organisms (Akhi *et al*., 2015; Bishnoi *et al*, 2021). Emerging multidrug-resistant strains, defined as pathogens resistant to three or more antimicrobial classes, complicate treatment outcomes (El-Kholy *et al*., 2018). Anaerobic bacteria also contribute significantly, highlighting the need for precise identification of causative microorganisms and their antimicrobial resistance profiles (Akhi *et al*., 2015).

Factors contributing to the development of SSIs can be classified as patient-related or perioperative. Patient-related factors include advanced age, male sex, smoking, comorbidities, and the use of immunosuppressive drugs like steroids (Neumayer *et al*., 2007; Cruse and Foord 1973). Perioperative factors include inadequate antiseptic preparation, improper shaving techniques, suboptimal surgical scrubbing, high bacterial load, and contaminated or infected wound classifications (Segal *et al*., 2014; Collin *et al*., 2019).

SSIs remain a major burden, particularly in low- and middle-income countries where resources and preventive measures may be limited (Mehtar *et al*., 2020; Ademuyiwa *et al*., 2021). Infection rates in these regions underscore the disparity compared to developed countries. Preventive strategies include proper hand hygiene, universal mask usage, compliance with aseptic techniques, and timely administration of antibiotic prophylaxis before incision (Tanner *et al*., 2011; Chauveaux *et al*., 2015). The COVID-19 pandemic demonstrated that adherence to basic infection prevention practices, such as hand hygiene, masking, and social distancing, can significantly reduce SSIs (Pantvaidya *et al*., 2022; Cappelli *et al*., 2022).

Effective SSI management involves mechanical or anatomic source control and often requires antimicrobial therapy (Akhi *et al*., 2015; Velin *et al*., 2021). Identifying the causative microorganisms and their antibiotic resistance profiles is critical for selecting appropriate treatments and achieving optimal outcomes. Prevention and control efforts must focus on reducing bacterial load at the surgical site and improving adherence to infection prevention protocols (Sawyer *et al*., 2018; Lubega *et al*., 2017).

SSIs, though preventable in most cases, continue to challenge healthcare systems worldwide. A coordinated effort involving healthcare providers, patients, and policymakers is essential to mitigate their impact and improve surgical outcomes (Ademuyiwa *et al*., 2021; Mukagendaneza *et al*., 2019).

MATERIALS AND METHODS

A. Specimen Collection

Surgical wound specimens were collected aseptically before any cleaning with antiseptics to ensure the preservation of original microbial flora. The skin surrounding the surgical wound was sterilized with 70% ethyl alcohol using a sterile cotton swab. Special care was taken to avoid contact with surrounding tissues to prevent contamination from endogenous skin flora.

Experienced nurses collected the wound samples from the depth of the wound using sterile cotton swabs moistened with sterile saline under strict aseptic conditions. Two swabs were collected for each wound. The swabs were immediately placed in modified Stuart's Transport Medium and transported to the bacteriology laboratory within one hour to ensure sample viability.

B. Laboratory Processing and Bacterial Isolation

(i) Microscopic Examination. One of the swabs was used to prepare smears on clean glass slides. The smears were fixed with alcohol and stained using the Gram staining technique. This procedure enabled the detection of pus cells and bacteria, which were classified, based on morphology (cocci or bacilli) and Gram characteristics (Gram-positive or Gram-negative).

(ii) Bacterial Culture. The second swab was inoculated onto several culture media, including: Blood Agar: To detect hemolysis patterns and support the growth of a broad range of bacteria. MacConkey Agar: To differentiate Gram-negative enteric bacteria based on lactose fermentation, Mannitol Salt Agar: To isolate *Staphylococcus* species, particularly *Staphylococcus aureus*, Nutrient Agar: To support the growth of non-fastidious organisms. These culture plates were incubated aerobically at 37°C for 24 hours. The presence and growth of bacterial colonies were recorded for further identification.

(iii) Bacterial Identification. The identification of the bacterial colonies was carried out by examining their morphology, Gram nature and biochemical tests. For Gram staining, smears were prepared from pure colonies then stained with Gram stain, and then subjected to microscopic examination to assist in the identification process.

C. Ethical Considerations

Ethical approval was obtained from the Ethical Review Committee of the affiliated university. Participants provided written informed consent before sample collection. All samples were collected as per standard procedures given by Standard Operating Procedure for Antimicrobial Resistance Surveillance National AMR Surveillance Network (NARS-Net) 2023, India. All data were pseudonymized and securely stored to maintain participant confidentiality. This study followed international ethical guidelines, including the Declaration of Helsinki, to ensure ethical compliance and respect for participants' rights.

D. Antibiogram test

The antibiotic susceptibility examination was done using the disc diffusion method (Kirby-Bauer Method) on Muller-Hinton agar according to the National Committee for Clinical Laboratory Standards. Antimicrobial profiling was conducted using two distinct antibiotic panels sourced from HiMedia (Mumbai, India). The first panel consisted of 32 discs targeting Gram-positive bacteria and included the following antibiotics: Cefazolin (CZ, 30 µg), Chloramphenicol (30 µg), Ciprofloxacin (CIP, 5 µg), Clindamycin (2 µg), Erythromycin (E, 15 µg), Levofloxacin (LUX, 5 µg), Oxacillin (OX, 1 µg), Penicillin (P, 10 µg), Roxithromycin (RXT), Rifampicin (RD), Tetracycline (TE), Vancomycin (VA), Ampicillin-Sulbactam (SAM), Cefdinir (CN, 5 µg), Cefuroxime (CXM, 30 µg), Clarithromycin (CLR, 15 µg), Benzylpenicillin (G), Linezolid (LZ, 30 µg), Amoxicillin (AX, 25 µg), Amoxicillin-Clavulanic Acid (AC, 20/10 µg), Cefotaxime (CT), Cefepime (CP), Cephalothin (CEP, 30 µg), Methicillin (MET, 5 µg), Teicoplanin (TEI, 30 µg), Amikacin (30 µg), Ampicillin (10 µg), Azithromycin (AZ, 15 µg), Piperacillin (PC, 100 µg), Cefuroxime Sodium (CR, 30 µg) Cephalexin (CP, 30 µg), Co-Trimoxazole (CT, 25 µg).The second panel included 31 discs targeting Gram-negative bacteria and comprised the following antibiotics: Imipenem (IPM, 10 µg), Ciprofloxacin (CIP, 5 µg), Tobramycin (TOB, 10 µg), Moxifloxacin (MO, 5 µg), Ofloxacin (OFX, 5 µg), Norfloxacin (NX, 10 µg), Sparfloxacin (SPX, 5 µg), Levofloxacin (LE, 5 µg), Co-Trimoxazole (COT, 25 µg), Colistin (CL, 10 µg), Nalidixic Acid (NA, 30 µg), Augmentin (AMC, 30

µg), Kanamycin (K, 30 µg), Gatifloxacin (GAT, 5 µg), Gentamicin (GEN, 10 µg), Amikacin (AK, 30 µg), Streptomycin (S, 25 µg), Ceftriaxone (CTR, 30 µg), Cefpodoxime (CPD, 10 µg), Ticarcillin (TI, 75 µg), Cefazolin (CZ, 30 µg), Cefuroxime (CXM, 30 µg), Chloramphenicol (C, 30 µg), Piperacillin (PIP, 100 µg), Piperacillin + Tazobactam (TZP, 100/10 µg), Cefdinir (CN, 5 µg), Lomefloxacin (LOM, 10 µg), Linezolid (LZ, 30 µg), Meropenem (MEM, 50 µg), Ampicillin + Sulbactam (20 µg), Ceftazidime + Clavulanic Acid (CAC, 40 µg).A bacterial suspension (of each isolated bacterium) was prepared in saline to achieve a turbidity equivalent to 0.5 McFarland standards. The suspension was then evenly swabbed across the surface of a Mueller-Hinton Agar (MHA) plate, which was allowed to dry briefly at room temperature. Subsequently, antibiotic disks were placed on the agar surface, and the plates were incubated at 35°C for 18–24 hours. After incubation, the plates were examined for zones of inhibition or resistance, and the observations were carefully recorded.

RESULTS AND DISCUSSIONS

A. Specimen Collection

During the study period, surgical site infections (SSIs) were identified in 120 patients. Out of these, samples were obtained from all the120 patients. In total, 150 samples were collected from different post-operative patients between January 2022 and June 2022.

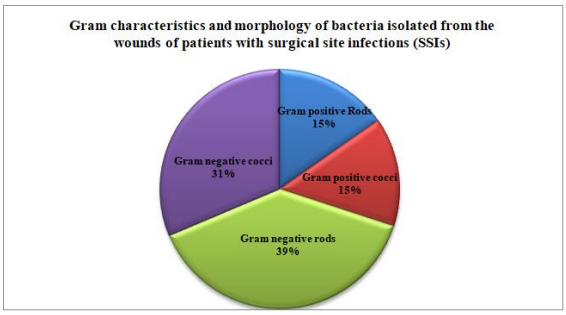

Fig. 1. Distribution of Bacterial Types in Surgical Site Infections Based on Gram Staining and Morphology.

B. Isolation and identification of bacterial isolates

A total of 150 distinct bacterial isolates were obtained from 120 samples. These isolates were sequentially labeled as SN1 to SN150. Each isolate was thoroughly examined for its microscopic characteristics. Based on their Gram-staining properties and morphological appearance under the microscope, the isolates were categorized into four groups: Gram-positive rods, Gram-positive cocci, Gram-negative rods, and Gram-negative cocci.

The pie chart (Fig. 1) illustrates the Gram characteristics and morphology of bacteria isolated from the wounds of patients with surgical site infections (SSIs). Among the 150 bacterial isolates, Gram-negative rods were the most prevalent, accounting for 39% of the isolates. This was followed by Gram-negative cocci, which constituted 31% of the isolates. Gram-positive rods and Gram-positive cocci each represented 15% of the total isolates.

Table 1: Distribution of Bacterial Isolates by Gram Staining and Morphology.

Gram nature	Number of isolates
Gram positive Rods	23
Gram positive cocci	22
Gram negative rods	58
Gram negative cocci	47

The predominance of Gram-negative rods suggests their significant role in SSIs, which aligns with previous studies highlighting pathogens like *Escherichia coli* and *Pseudomonas aeruginosa* as common Gram-negative isolates in wound infections (Sanchez *et al.*, 2013; Bediako-Bowan *et al.*, 2020). The notable presence of Gram-negative cocci also supports their association with SSIs, particularly in polymicrobial infections (Golia *et al.*, 2014). In contrast, Gram-positive isolates such as *Staphylococcus aureus* and *Enterococcus* spp. remain key contributors to SSIs, as reflected by the 30% combined prevalence of Gram-positive rods and cocci. The findings underscore the diversity of bacterial pathogens in SSIs and the importance of tailoring antimicrobial treatments based on Gram-staining results.

These results highlight the critical need for targeted antibiotic therapy to manage SSIs effectively. Understanding the distribution of Gram-positive and Gram-negative bacteria aids in optimizing empirical antibiotic choices and reducing the risk of antibiotic resistance.

B. Antibiogram test

Out of the 150 bacterial strains isolated from surgical site infection (SSI) wounds, some demonstrated sensitivity, while others exhibited resistance to specific antibiotics. Each bacterial isolate displayed a unique pattern of sensitivity and resistance to the antibiotics tested. A total of 64 antibiotics were employed in this analysis, with 32 antibiotics tested against Gram-positive isolates and 31 antibiotics tested against Gram-negative isolates. The selection of antibiotics was tailored to the Gram-staining characteristics of the isolates to ensure appropriate and effective evaluation**.** Some bacterial isolates exhibited resistance to one or two antibiotics, while others showed resistance to nearly all antibiotics tested. This pattern of antibiotic resistance was observed in both Gram-positive and Gram-negative isolates.

For further analysis, the top 10 most resistant isolates from each group—Gram-positive and Gram-negative—were selected for detailed studies. The selected Gram-negative isolates were: SN 18, SN 27, SN 69, SN 74, SN 90, SN 123, SN 133, SN 136, SN 141, and SN 142. Similarly, the top 10 Gram-positive isolates exhibiting the highest levels of resistance were: SN 9, SN 29, SN 35, SN 51, SN 62, SN 81, SN 99, SN 115, SN 129, and SN 148.

The resistance patterns of these isolates were analyzed comprehensively, and the results are presented in Fig. 2 and 3, which depict the antibiogram profiles for the selected Gram-positive and Gram-negative isolates, respectively. This detailed examination provides insights into the degree of resistance and highlights the urgent need to address antimicrobial resistance in clinical settings.

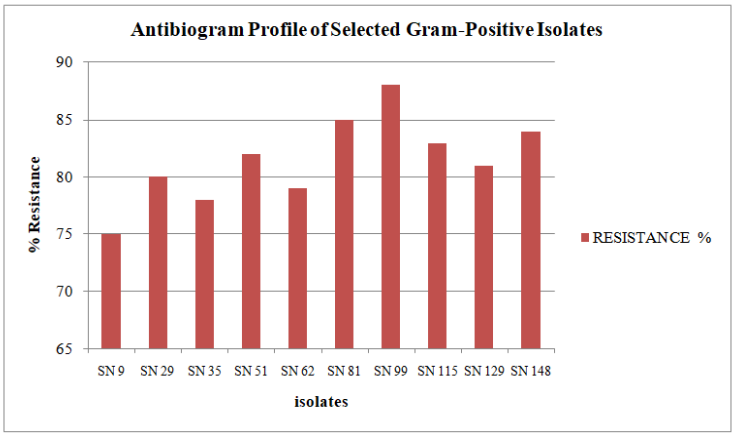

Fig. 2. Antibiotic Resistance Patterns of Selected Gram-Positive Isolates.

The graph (Fig. 2) illustrates the antibiogram profile of selected Gram-positive isolates, showcasing their percentage resistance to antibiotics. Among the isolates, SN 81 exhibited the highest resistance at approximately 88%, followed by SN 99 and SN 148, both showing resistance levels close to 85%. In contrast, SN 9 showed the lowest resistance percentage at around 72%. Isolates SN 29, SN 35, SN 51, SN 62, SN 115, and SN 129 displayed intermediate resistance levels ranging between 75% and 83%. The data highlights significant variation in resistance among the isolates, reflecting the diverse antibiotic resistance mechanisms in Gram-positive bacteria.

The results revealed concerning levels of antibiotic resistance among the selected Gram-positive isolates. High resistance levels observed in isolates such as SN 81 and SN 99 underscore the growing challenge posed by multidrug-resistant Gram-positive bacteria in clinical settings. Gram-positive bacteria, such as *Staphylococcus aureus* and *Enterococcus faecium*, are known for their ability to develop resistance through various mechanisms, including the production of beta-lactamases, modification of target sites, and efflux pump activity (Munita & Arias 2016).

The intermediate resistance levels observed in several isolates suggest the possibility of selective pressure from the overuse or misuse of antibiotics, a phenomenon that has been widely reported in both hospital and community settings (Davies & Davies 2010). Additionally, the relatively lower resistance in isolates such as SN 9 indicates that some strains may still be susceptible to available antibiotics, which could guide treatment decisions. The findings align with global reports highlighting the prevalence of antibiotic resistance in Gram-positive bacteria (WHO, 2020). Addressing this issue requires a multifaceted approach, including the prudent use of antibiotics, implementation of infection control measures, and on-going surveillance to monitor resistance trends (Pérez *et al.*, 2019).

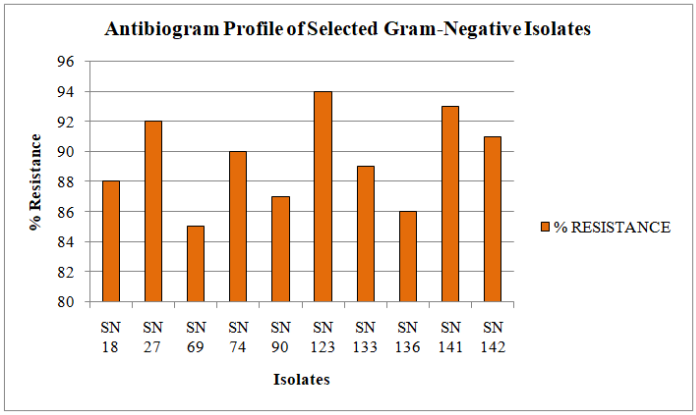

Fig. 3. Antibiotic Resistance Patterns of Selected Gram-Negative Isolates.

The presented bar graph (Fig. 3 and 4) illustrates the antibiogram profile of selected Gram-negative isolates based on their percentage resistance to tested antibiotics. Among the isolates, SN 123 exhibited the highest resistance percentage at 94%, followed by SN 141 with 92%. Isolates such as SN 27, SN 69, and SN 142 showed resistance levels exceeding 90%. Meanwhile, the isolates SN 18, SN 74, and SN 90 displayed intermediate resistance percentages, ranging between 86% and 89%. The isolates SN 133 and SN 136 recorded the lowest resistance levels, falling just above 84%. These findings highlight the variability in resistance patterns among Gram-negative isolates, indicating significant concerns regarding antibiotic efficacy.

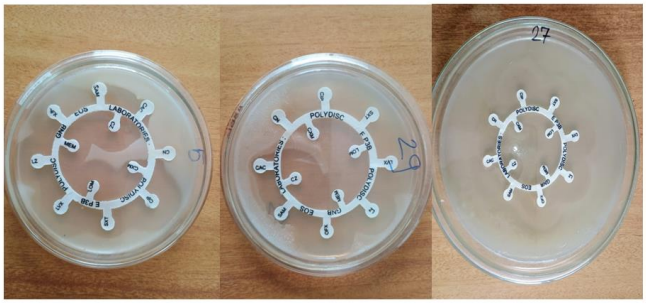

Fig. 4. Antimicrobial Susceptibility profiling of Isolates.

The results underscore the alarming prevalence of high antibiotic resistance among Gram-negative isolates. SN 123 and SN 141, with resistance rates above 92%, point to multidrug-resistant profiles that pose a severe threat to clinical treatments. Similar observations have been reported in recent studies, which attribute the high resistance in Gram-negative bacteria to the presence of robust outer membranes and efflux pumps that reduce antibiotic penetration (Chaudhary *et al.,* 2021). Moreover, the variation in resistance levels among isolates may stem from genetic differences, environmental pressures, and antibiotic exposure histories (Pérez *et al.,* 2019).

These findings align with global trends in antimicrobial resistance, particularly among Gram-negative pathogens like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (World Health Organization, 2020). Efforts to combat this issue necessitate stringent antimicrobial stewardship programs and the development of novel therapeutic strategies, including phage therapy and resistance-modifying agents. Further molecular characterization of these isolates is essential to identify resistance determinants and inform targeted interventions.

CONCLUSIONS

This study underscores the growing challenge posed by multidrug-resistant organisms in surgical site infections (SSIs). Gram-negative isolates exhibited alarming resistance rates, with SN 123 showing the highest resistance at 94%, highlighting the threat posed by extensively drug-resistant strains. Among Gram-positive isolates, SN 81 demonstrated the highest resistance at 88%, reflecting the persistent challenge of treating infections caused by pathogens like *Staphylococcus aureus*. These findings call for urgent action, including the implementation of antimicrobial stewardship programs, adherence to infection control protocols, and continued research into novel therapeutic strategies. Addressing these challenges is essential to improving surgical outcomes and mitigating the impact of antimicrobial resistance on global healthcare.

FUTURE SCOPE

The future scope of this study includes expanding the research to diverse geographic regions to assess regional variations in resistance patterns and conducting advanced molecular studies to identify resistance mechanisms. It can explore alternative therapies like phage therapy, rapid diagnostic tools for early detection, and the development of enhanced infection control protocols. Implementing antibiotic stewardship programs and aligning with global initiatives like WHO's action plan on antimicrobial resistance could amplify its impact. Long-term monitoring of resistance trends, investigating host-pathogen interactions are promising areas. These efforts can guide policy-making, improve therapeutic strategies, and enhance surgical outcomes globally.

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