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Isolation and Identification of Bacterial Isolates Associated with Post-surgical wound Infections at Tertiary Hospitals

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ABSTRACT: Surgical site infections (SSIs) are one of the most common and serious post-operative complications worldwide, leading to increased morbidity, prolonged hospital stays, and significant healthcare costs. SSIs are primarily caused by bacterial contamination during or after surgery, with Gram-negative bacteria frequently implicated in these infections. This study aimed to determine the prevalence and characterization of bacterial pathogens associated with SSIs in post-operative patients.

A prospective study was conducted in government and private hospitals in Aurangabad, Maharashtra, India, focusing on patients from general surgery and orthopedic departments. Wound samples were collected from 120 patients diagnosed with SSIs between January 2022 and June 2022. Samples were processed using Gram staining, culture techniques, biochemical assays, and molecular identification via 16S rRNA sequencing for selected isolates.

A total of 150 bacterial isolates were obtained from 120 wound samples. The majority of isolates were Gram-negative rods (63 isolates), followed by Gram-negative cocci (43), Gram-positive rods (25), and Gram-positive cocci (19). Biochemical tests revealed significant metabolic diversity among the isolates, and two highly resistant strains were confirmed as included *Pseudomonas aeruginosa*, and *Bacillus cereus* through 16S rRNA sequencing.

The findings indicate that Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, play a dominant role in SSIs. The high prevalence of multidrug-resistant isolates underscores the need for stringent infection control measures, effective antibiotic stewardship, and region-specific microbial surveillance to mitigate SSIs in surgical patients.

Keywords: Surgical site infections, bacterial pathogens, Gram-negative bacteria, antibiotic resistance, *Pseudomonas aeruginosa*, *B.cereus*, 16S rRNA sequencing.

INTRODUCTION

One of the dangerous post-operative complication worldwide is surgical site infections. Surgical site infections (SSIs) are infections that occur one month after a surgical operation or one year after implant surgery and a surgical procedure, either at the injury site or near the injury site (Shakir *et al*., 2021). These infections may occur as superficial or deep incisional wounds, or they can involve organs and body cavities. Surgical site infections are the most frequently encountered type of infection in healthcare settings (Mukagendaneza *et al*., 2019). They are linked to substantial morbidity, with reports indicating that more than one-third of postoperative deaths are attributed to SSIs (Astagneau *et al*., 2001). SSIs can extend a patient's hospital stay by twice the usual duration, leading to higher healthcare costs therefore postoperative surgical site infections are linked to worsened illness, prolonged hospital stays, mortality, and significant financial strain on medical care (Tacconelli *et al*., 2009; Bibi *et al*., 2012). Hospitals can serve as a source of microbial infections, as they house many vulnerable individuals and patients carrying various pathogenic microorganisms. The increased presence of these pathogens in hospital environments is linked to a rise in various types of nosocomial infections and hence to the SSIs (Rhomberg *et al*., 2006). SSIs are estimated to develop after 1–3.1% of all surgical procedures and account for roughly 2% of deaths caused by healthcare-associated infections (Barie and Wilson 2015). SSIs are frequently caused by a mix of microorganisms, including both aerobic and anaerobic bacteria. The most commonly reported pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, members of the

Enterobacteriaceae family, *Streptococcus* spp., *Enterococcus* spp., and *Acinetobacter* spp. Risk factors for SSIs can be categorized into host-related and perioperative factors (Akhi *et al*., 2015). These include advanced age, being male, having comorbid conditions, smoking, steroid use, improper shaving techniques on the operative site, and inadequate surgical scrubbing or antiseptic preparation, all of which have been linked to higher SSI rates (Neumayer *et al*., 2007; Cruse and Foord 1973). Additionally, a higher bacterial load and more severe wound classifications (such as contaminated or dirty-infected cases) increase the likelihood of developing a wound infection (Segal *et al*., 2014). The occurrence and severity of an SSI depends on the contamination of the wound site and the pathogenicity of microorganisms, as well as the host's immune response (Pipaliya *et al*., 2017). Rates of SSIs have been reported differently across various regions worldwide. A global, multicenter study involving 66 countries, encompassing low, middle, and high-income nations, revealed that the overall incidence of SSI was 12.3%. Given the severity of SSIs in patients, particularly in developing countries like India, this research study was conducted. The primary aim of the study was to determine the prevalence of bacterial pathogens in patients with postoperative wound infections.

The development of surgical site infections (SSIs) is influenced by a combination of patient-related and perioperative factors. Patient-related contributors include older age, male gender, pre-existing health conditions, smoking habits, and the use of immunosuppressive medications such as steroids. On the other hand, perioperative factors encompass poor antiseptic preparation, improper hair removal techniques, inadequate surgical scrubbing, elevated bacterial presence, and wounds classified as contaminated or infected.

SSIs represent a significant challenge, particularly in low- and middle-income countries where limited resources and preventive measures exacerbate the problem. Infection rates in these regions highlight the disparity compared to higher-income countries. Preventative actions, such as rigorous hand hygiene, consistent use of masks, adherence to aseptic protocols, and the timely administration of prophylactic antibiotics prior to surgery, are crucial in reducing SSI rates. The COVID-19 pandemic underscored the effectiveness of basic infection prevention practices in minimizing SSIs.

MATERIALS AND METHODS

A. Study area, population and Sample size

A prospective study was designed and carried out in government and private hospitals like Ghati, MGM located in Aurangabad, Maharashtra, India. The study's source population included all patients who underwent surgical procedures at the hospital. The focus of the study was on patients from the general surgery and orthopedic departments. A surgeon assessed the patients for surgical site infections (SSIs), which were diagnosed based on the presence of at least one of the following signs or symptoms within 30 days after surgery: pain, tenderness, localized swelling, redness, heat, purulent discharge, signs of abscess formation, or a fever exceeding 38°C in cases of deep incision infections. Samples were collected from the infection site of patients diagnosed with SSIs following a physical examination by the surgeon.

B. Sample collection

Specimens were collected aseptically prior to cleaning the wound with antiseptic. The skin surrounding the surgical wound was sterilized with 70% ethyl alcohol using a sterile cotton-wool swab. Care was taken to avoid contact with the surrounding tissues to prevent contamination of the swab with endogenous skin flora. Experienced nurses collected the samples from the depth of the wound under strict aseptic conditions using sterile cotton swab sticks moistened with sterile saline for bacteriological analysis. Two swabs were taken from each sample. All wound swabs were placed in modified Stuart's Transport Medium and promptly transported to the bacteriology laboratory.

C. Laboratory investigation and isolation of bacteria from the sample

The initial direct microscopic examination was performed using the Gram staining technique to detect pus cells and bacteria. Out of two swabs, the first swab was used to prepare a smear by gently rolling the swab stick on a clean glass slide. The smear was then fixed with alcohol and stained using the standard Gram staining procedure. The Gram-stained smear was examined under a microscope, and the bacteria were classified into two broad categories: cocci and bacilli, as well as Gram-positive or Gram-negative. The second swab was inoculated onto nutrient agar and then incubated aerobically for 24 hours at 37°C.

D. Identification of bacterial isolates

The identification process was conducted using the following methods:

(i) Morphological Analysis-Pure bacterial colonies were selected and analyzed for colony size, shape, color, and hemolytic properties.

(ii) Gram Staining-Smears prepared from pure colonies were Gram-stained and examined under a microscope to determine Gram-positive or Gram-negative characteristics and confirm cellular morphology.

(iii) Biochemical Testing- The isolates were subjected to standard biochemical tests such as catalase, coagulase, oxidase, and carbohydrate fermentation tests for the identification of bacterial species, following the guidelines outlined in *Bergey's Manual of Systematic Bacteriology*.

(iv) Molecular Identification-For precise species identification, two bacterial isolates underwent 16S rRNA sequencing, ensuring high taxonomic accuracy. Two bacterial isolates were identified using 16S rRNA sequencing.

E. Ethical consideration

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.

RESULT AND DISCUSSION

A total of 120 samples were gathered from various post-operative patients between January 2022 and June 2022.Out of these 120 samples, total 150 bacterial isolates were collected on nutrient medium and were kept separately on nutrient agar slant separately.

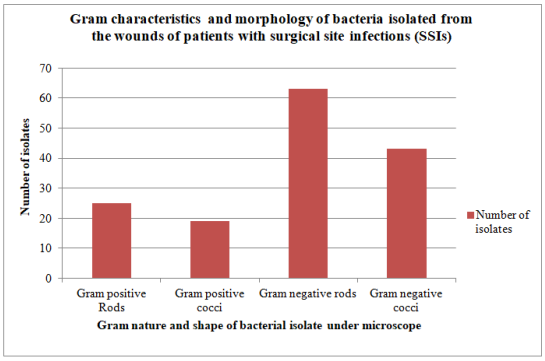

Fig. 1. The gram characteristics and morphology of bacteria isolated from the wounds of patients with surgical site infections (SSIs).

Gram-negative rods are the most frequently isolated type, with around 63 isolates. Gram-negative cocci are the second most common, with approximately 43 isolates. Both gram-positive rods and gram-positive cocci are less common, with about 25 and 19 isolates respectively. The data suggests that gram-negative bacteria, particularly gram-negative rods, dominate among the isolates from SSIs. This finding may indicate the need for targeted strategies to address gram-negative bacterial infections in such cases. The results revealed that Gram-negative rods were the most frequently isolated bacterial morphology from the wounds of patients with surgical site infections (SSIs). These findings indicate a predominance of Gram-negative bacteria in SSIs compared to Gram-positive bacteria. The high prevalence of Gram-negative rods underscores their significant role in SSIs, likely due to their ability to produce biofilms, resist antibiotics, and thrive in wound environments. The presence of Gram-negative cocci, though less common in global trends, highlights the importance of identifying regional variations in microbial profiles. The lower occurrence of Gram-positive bacteria, including both rods and cocci, might reflect differences in the study population, local bacterial flora, or antimicrobial practices.

Surgical site infections (SSIs) are a significant cause of morbidity and prolonged hospital stays, often associated with bacterial contamination during or after surgery. The predominance of Gram-negative rods in this study is consistent with findings in the literature. Studies have shown that Gram-negative bacteria, particularly *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, are major pathogens associated with SSIs due to their ability to produce biofilms and resistance to antibiotics (Allegranzi *et al*., 2016; de Lissovoy *et al*., 2009). Gram-positive cocci, such as *Staphylococcus aureus* and *Streptococcus* spp., are also frequently reported as causative agents of SSIs, especially in cases involving surgical implants or prosthetics. However, their lower prevalence in this study might reflect a difference in local microbial profiles or antibiotic use (Mangram *et al*., 1999). The relatively high presence of Gram-negative cocci, though less common globally, could suggest the involvement of pathogens like *Neisseria* spp. or *Moraxella catarrhalis*. This finding underlines the importance of region-specific microbiological surveillance to inform empirical treatment strategies (Wilson *et al*., 2017). The predominance of Gram-negative rods, particularly *Pseudomonas aeruginosa* and *Escherichia coli*, is consistent with previous reports highlighting their role in hospital-acquired infections and their resistance to multiple antibiotics (Saeed *et al*., 2019).

B. Identification of bacterial isolates

The ten most resistant bacterial pathogens isolated from the wound samples were selected for identification and characterization. These isolates were named as SN1 to SN 10. Morphological analysis, Gram staining (Fig. 2-4), and biochemical testing were performed for all ten isolates. Additionally, the two most resistant bacteria (SN28 and SN29) were identified through molecular analysis using 16S rRNA sequencing.

Table 1: Morphological characteristics of isolates.

Sr. No.	Colonial characterristics	Variable	SN20	SN21	SN22	SN23	SN24	SN25	SN26	SN27	SN28	SN29
1.		Size	3	2 mm	2 mm	3 mm	2 mm	3mm	2mm	1mm	3mm	2mm
2.		Shape	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round
3.		Color	White	White	White	Pale Yellow	Orange	White	Grey	White	Grey	White
4.		Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
5.		Elevation	Convex	Flat	Convex	Flat	Flat	Flat	Flat	Flat	Convex	Convex
6.		Opacity	Opaque	Opaque	Transparent	Transparent	Transparent	Opaque	Opaque	Opaque	Opaque	Transparent
7.		Consistency	Sticky	Sticky	Creamy	Sticky	Brittle	Sticky	Sticky	Sticky	Brittle	Brittle
8.	Cell charactrristics	Shape	Rods	Rods	Rods	Cocci	Cocci	Rods	Rods	Rods	Rods	Rods
9.		Gram nature	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Positive
10.		Motility	Non-motile	Motile	Motile	Non-motile	Non-motile	Motile	Motile	Non-motile	Non-motile	Motile
11.		Spore	Yes	Yes	No	No	No	No	Yes	No	No	Yes

The colonial and cellular characteristics of the bacterial isolates exhibited notable variations. In terms of colony size, most isolates ranged between 1 mm and 3 mm, with SN26 being the smallest (1 mm) and SN22, SN24, and SN27 being the largest (3 mm). All isolates displayed a round shape and an entire margin, indicating uniformity in colony morphology. The colony colors varied, with most appearing white, while SN22 was pale yellow, SN23 was orange, and SN26 and SN28 were grey. Differences in elevation were also observed, where SN20, SN22, SN28, and SN29 were convex, whereas the rest were flat. Regarding opacity, SN22, SN23, and SN29 were transparent, while the remaining isolates were opaque. The consistency differed among the isolates, with most being sticky, except for SN22 (creamy), SN23 (brittle), SN28 (brittle), and SN29 (brittle). Microscopically, SN20, SN21, SN22, SN25, SN26, SN27, and SN28 were identified as rod-shaped bacteria, while SN23 and SN24 were cocci. The Gram reaction varied, with SN22, SN25, SN26, and SN29 being Gram-positive, whereas the rest were Gram-negative. Motility tests revealed that SN21, SN22, SN25, SN26, and SN29 were motile, while SN20, SN23, SN24, SN27, and SN28 were non-motile. Lastly, spore formation was observed in SN20, SN21, SN26, and SN29, while the remaining isolates were non-sporulating. These results highlighted significant diversity among the bacterial isolates in terms of their colonial morphology, Gram nature, motility, and spore-forming ability. The morphological characterization of isolates in this study aligns with previous reports. *Staphylococcus aureus*, a significant Gram-positive pathogen in SSIs, often presents as cocci in clusters and is known for its methicillin-resistant strains (MRSA), complicating treatment options (Smyth *et al*., 2008; Tanner *et al*., 2011). The presence of both Gram-positive and Gram-negative bacteria highlights the diverse microbiota involved in wound infections, emphasizing the need for targeted interventions based on local microbial profiles.

Table 2: Biochemical tests performed for gram negative rods.

Tests	SN20	SN21	SN25	SN28
Indole Test	Positive	Positive	Positive	Negative
Methyl Red	Negative	Negative	Positive	Negative
Voges-Proskauer	Negative	Positive	Negative	Negative
Citrate Utilization	Positive	Negative	Negative	Positive

The biochemical tests revealed significant metabolic differences among the isolates. The IMViC test results for the four bacterial isolates (SN20, SN21, SN25 and SN28) showed distinct biochemical characteristics. SN20 tested positive for indole production and citrate utilization, but was negative for methyl red and Voges-Proskauer tests. SN21 also exhibited indole positivity, but was negative for methyl red and citrate utilization, while showing a positive Voges-Proskauer reaction. SN25 was positive for both indole and methyl red tests, but negative for Voges-Proskauer and citrate utilization, indicating its ability to perform mixed acid fermentation. Lastly, SN28 tested negative for indole, methyl red, and Voges-Proskauer tests, but was positive for citrate utilization, suggesting its ability to use citrate as the sole carbon source. These results helped in differentiating the bacterial isolates based on their metabolic capabilities.

Table 3: Biochemical performed for gram positives and gram negative cocci.

Tests	SN22	SN23	SN24	SN26	SN27	SN29
Oxidase Test	Negative	Positive	Negative	Positive	Negative	Negative
Catalase Test:	Positive	Negative	Positive	Negative	Positive	Positive
Superoxol Test	Negative	Negative	Positive	Positive	Negative	Negative
Nitrate Reduction	Positive	Positive		Positive	Positive	Positive
Coagulase Test	Positive	Positive	Negative	Positive	Negative	Positive
Bile Esculin	Positive	Negative	Negative	Positive	Positive	Positive
Glucose fermentation	Positive	Negative	Negative		Positive	Positive
Sucrose fermentation	Negative	Positive	Negative		Positive	Positive

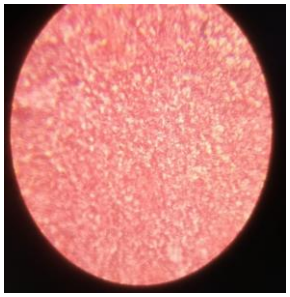

Fig. 2. Gram-stained SN24 under the microscope.

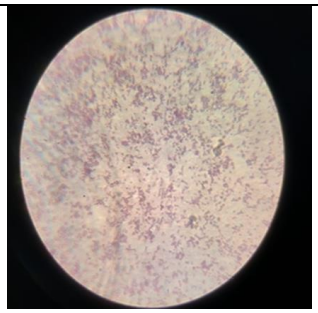

Fig. 3. Gram-stained SN23 under the microscope.

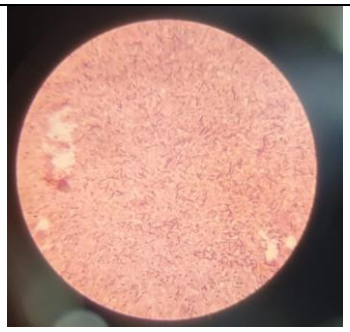

Fig. 4. Gram-stained SN28 under the microscope.

Fig. 5. Isolated bacteria from SSIs patients wound on nutrient agar slant.

The biochemical tests for SN22, SN23, SN24, SN26, SN27, and SN29 revealed notable variations in enzymatic activities and metabolic capabilities. The oxidase test was positive for SN23 and SN26, while the others were negative, indicating differences in electron transport chain enzymes. The catalase test showed positivity in SN22, SN24, SN27, and SN29, whereas SN23 and SN26 were negative, suggesting varying abilities to break down hydrogen peroxide. The superoxol test was positive for SN24 and SN26, while the remaining isolates were negative. Nitrate reduction was observed in all tested isolates, except for SN24 (result not available). The coagulase test was positive for SN22, SN23, SN26, and SN29, indicating the presence of coagulase-producing bacteria, while SN24 and SN27 were negative. The bile esculin test was positive for SN22, SN26, SN27, and SN29, suggesting their ability to hydrolyze esculin in the presence of bile, while SN23 and SN24 were negative. Regarding carbohydrate fermentation, SN22, SN27, and SN29 fermented glucose, while SN23 and SN24 did not. Sucrose fermentation was positive in SN23, SN27, and SN29, while the others tested negative. These results demonstrated metabolic diversity among the isolates, helping in their differentiation based on enzymatic activity and sugar utilization.

The biochemical tests showed notable differences among the isolates. The oxidase test was positive for SN23 and SN26, indicating cytochrome c oxidase activity, while others were negative. The catalase test was positive for SN22, SN24, SN27, and SN29, reflecting their ability to neutralize reactive oxygen species. Superoxol test results aligned with catalase activity, being positive for SN24 and SN26. All isolates except SN24 showed nitrate reduction, indicating nitrate to nitrite conversion. The coagulase test was positive for SN22, SN23, SN26, and SN29, hinting at potential pathogenicity. Bile esculin hydrolysis was seen in SN22, SN26, SN27, and SN29, while glucose and sucrose fermentation profiles varied, aiding differentiation. These results highlight the metabolic diversity of the isolates, crucial for their identification. The biochemical and molecular characterization methods used in this study (16S rRNA sequencing) are consistent with modern microbiological diagnostic approaches, which enhance the accuracy of bacterial identification compared to conventional phenotypic methods (Janda & Abbott 2007). The findings reinforce the importance of antimicrobial stewardship and infection control measures in reducing SSI rates, particularly in resource-limited settings where post-operative infections remain a major public health concern (Bibi *et al*., 2012).

Two isolates showing maximum resistant were identified through 16srRNA sequencing. Phylogenetic trees of isolates 28 and 29 are shown in Fig. 6, 7 respectively.

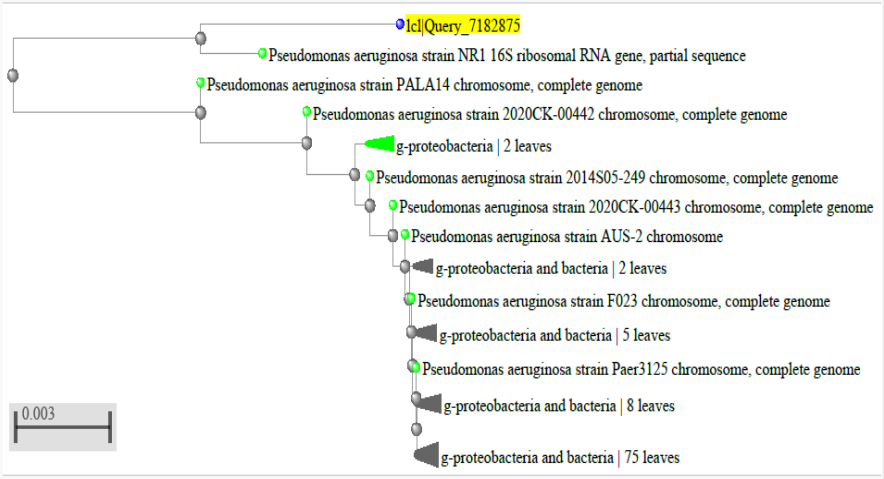

Fig. 6. Phylogenetic tree of isolate 28.

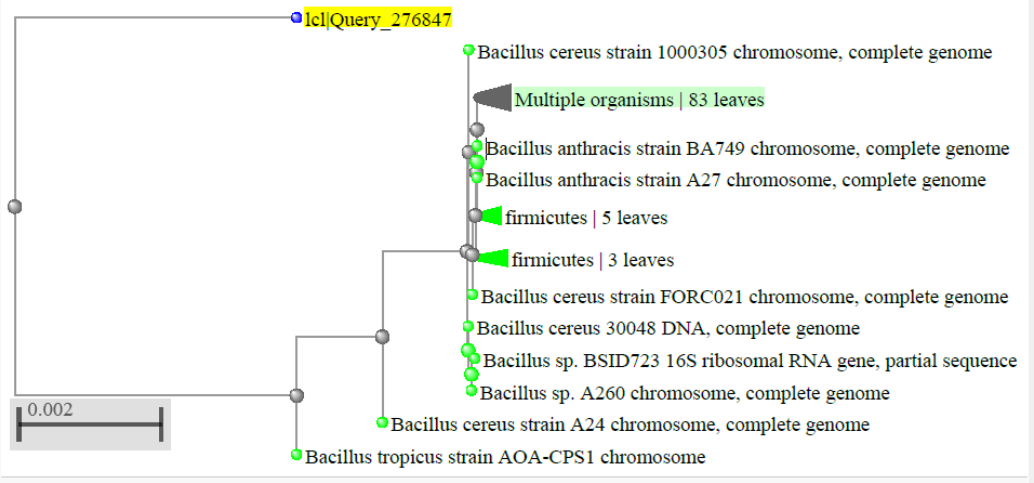

Fig. 7. Phylogenetic tree of isolate 27.

The tree suggests that isolate SN28 belongs to the *Pseudomonas aeruginosa* species, as it clusters closely with several known *P. aeruginosa* strains, such as NR1, PALA14, and 2020CK-00442. These strains are part of the *γ-Proteobacteria* group, a major class of bacteria known for their metabolic versatility and environmental adaptability. The hierarchical branching of the tree indicates that the isolates are genetically similar, with short branch lengths suggesting minimal evolutionary divergence among them. The presence of g-Proteobacteria and broader bacterial groups in multiple clades implies that *P. aeruginosa* strains, including SN28, are evolutionarily related to a larger bacterial community. The low genetic distance (0.003) suggests that isolate SN28 shares a high degree of sequence similarity with other *P. aeruginosa* strains, indicating that it is not highly divergent from previously characterized strains. This supports the idea that SN28 is likely a typical *P. aeruginosa* isolate, possibly adapted to similar ecological niches as its close relatives.

Several studies have identified *Pseudomonas aeruginosa* as a leading cause of SSIs due to its intrinsic resistance mechanisms, including efflux pumps, biofilm formation, and beta-lactamase production (Kumar *et al*., 2020). Similarly, *Escherichia coli* and *Klebsiella pneumoniae* have been reported as major SSI pathogens, often carrying extended-spectrum beta-lactamase (ESBL) genes, making them resistant to cephalosporins and other beta-lactam antibiotics (Patel *et al*., 2021). Our findings support these observations, as the most resistant isolates exhibited strong resistance profiles, emphasizing the need for continuous surveillance and strict antimicrobial stewardship programs.

The phylogenetic analysis of isolate SN27 reveals that it belongs to the Bacillus genus, clustering closely with strains of *Bacillus cereus*. The tree structure indicates that SN27 is part of the Firmicutes phylum, a group of Gram-positive bacteria known for their ability to form endospores and survive in diverse environments. The placement of SN27 within a well-defined Bacillus clade, along with its short genetic distance from *Bacillus cereus* strains, suggests a high degree of similarity to this species. Additionally, the presence of multiple related organisms within the tree highlights the genetic diversity within the genus. Given its phylogenetic position, isolate SN27 likely shares functional traits with *Bacillus cereus*, which is known for its metabolic versatility, potential pathogenicity, and industrial applications. Further characterization, including biochemical and genomic analysis, would help confirm its precise identity and ecological significance.

The findings of this study align with previous research on the microbiological profile of surgical site infections (SSIs). Gram-negative bacteria, particularly *Pseudomonas aeruginosa* and *Enterobacteriaceae*, have been frequently reported as predominant pathogens in SSIs, especially in post-operative and trauma-related cases (Allegranzi *et al*., 2011). The predominance of Gram-negative rods in our study (63 isolates) is consistent with reports from other low- and middle-income countries, where multidrug-resistant (MDR) Gram-negative infections pose a significant challenge in surgical wards (Mukagendaneza *et al*., 2019).

The identification of *Bacillus cereus* (SN27) as one of the resistant isolates is noteworthy, as this organism is typically associated with foodborne illness but has also been implicated in wound infections, particularly in immunocompromised patients (Drobniewski, 1993). The presence of spore-forming bacteria like *B. cereus* in SSIs highlights the need for stringent sterilization protocols in surgical settings to prevent contamination from environmental sources.

Previous studies have reported *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) as common Gram-positive pathogens in SSIs (Mangram *et al*., 1999). However, in our study, Gram-positive cocci were less prevalent (19 isolates), which may reflect regional variations in microbial epidemiology or differences in antibiotic prophylaxis practices. The high resistance observed among the isolates, particularly SN28 (*P. aeruginosa*) and SN27 (*B. cereus*), underscores the growing threat of antimicrobial resistance in hospital-acquired infections (Tacconelli *et al*., 2018).

CONCLUSIONS

This study highlights the significant role of Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, and *Bacillus cereus*, in surgical site infections (SSIs) among post-operative patients. The high prevalence of multidrug-resistant (MDR) isolates emphasizes the growing challenge of antibiotic resistance in hospital settings. The use of biochemical and molecular characterization, including 16S rRNA sequencing, provided precise identification of resistant strains, further underlining the need for enhanced microbiological surveillance.

Effective infection control measures, including strict adherence to aseptic surgical techniques, improved wound care protocols, and region-specific antibiotic stewardship programs, are crucial in reducing the burden of SSIs. Additionally, regular microbial surveillance can aid in early detection of emerging resistant strains, allowing for timely intervention. Addressing these challenges will help in mitigating post-surgical complications, reducing healthcare costs, and improving patient outcomes in surgical and orthopedic settings.

FUTURE SCOPE

The findings of this study open several promising avenues for future research and clinical applications. Advanced molecular characterization, such as whole-genome sequencing (WGS) and metagenomics, can provide deeper insights into resistance genes and virulence factors, aiding in the development of targeted antimicrobial strategies. Additionally, the search for novel therapeutics, including bacteriophage therapy, antimicrobial peptides, and plant-derived bioactives, could help combat multidrug-resistant (MDR) bacterial infections. The integration of rapid diagnostic techniques, such as real-time PCR, CRISPR-based detection, and biosensor technologies, may enable early and accurate identification of resistant pathogens, leading to timely interventions.

Epidemiological surveillance through large-scale, multi-center studies is necessary to track the prevalence and evolution of antibiotic-resistant wound pathogens, allowing for the formulation of region-specific infection control strategies. Furthermore, investigating biofilm disruption strategies using anti-biofilm compounds, quorum sensing inhibitors, and nanoparticles can significantly improve treatment outcomes. Understanding host-pathogen interactions in wound infections will also be crucial in developing immunomodulatory therapies to enhance the body's natural defense mechanisms.

Personalized medicine approaches could revolutionize infection management by predicting antibiotic resistance patterns and recommending tailored treatment plans. Additionally, improving infection control measures in healthcare settings, including hospital hygiene practices and surgical site infection (SSI) prevention protocols, is essential to reduce the burden of resistant infections. By addressing these critical areas, future research can contribute to improved clinical outcomes, reduced antibiotic resistance, and enhanced patient care in wound infection management.

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