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Identification Of Bacterial Isolates and Antimicrobial Susceptibility Pattern Among Patients Diagnosed with Surgical Site Infections

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Author`s Contribution

ME, SH Conception and design, ME, SH Collection and assembly of data, SM, MF, MA Analysis and interpretation of the data, Statistical expertise.

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A B S T R A C T

Background: Despite the use of prophylactic antibiotics and preventive measures, surgical site infections (SSIs) continue to be a common postoperative complication, attributed mostly to increasing antimicrobial resistance.

Objective: To investigate the bacterial profile and antimicrobial susceptibility patterns of isolates from patients diagnosed with SSIs at Jinnah Hospital Lahore

Methodology: A cross-sectional study design was used for sampling, and 65 surgical wound samples were collected from admitted patients. All specimen (Pus and wound swabs) were collected for bacterial isolation, identification and antimicrobial testing using standard microbiological techniques. SPSS software was used for statistical analysis and P value (<0.05) was considered as statistically significant.

Results: This research investigated that SSIs were found in 24 (37%) of patients who belonged to 16-30 years of age group. More males 41(63%) were affected as compared to females. SSIs were more common in lower extremities 43 (66%). Majority of patients 30(46%) were diagnosed to have double microbial infections at SSIs. More males 14 (22%) developed SSIs due to *E-Coli* (P<0.05) and double microbial infection was also common in males (p<0.05). *MRSA* was found resistant to antimicrobial drugs while *S. aureus* was found sensitive to antimicrobial drugs. (P value<0.05).

Conclusion: In our study SSIs were found prevalent in lower extremities and mostly due to double microbial infections. *E-Coli* was found to cause SSIs in males.

Key words: SSIs, surgical wounds, bacterial isolates, antimicrobial susceptibility

Introduction

Surgical site infections are defined as infections that occur near or at the surgical site following invasive surgical procedure, usually develop within 1 month after surgery, or within 1 year in cases where an implant is left in place. These infections may involve superficial incision, deep soft tissues, or organs at the surgical site.[1] SSIs are among the most frequently occurring healthcare-associated infection worldwide and are significant contributors to increased patient morbidity, mortality, prolonged hospital stay, and healthcare costs. They also impose a significant economic burden on healthcare systems, particularly in developing countries where infection control resources may be limited.[2]

Nearly 20% of all healthcare-associated infections and approximately 5% of patients undergoing surgery develop an SSI.[15] Reported incidence of SSIs among hospitalized surgical patients ranges from 2–5%; however, the actual burden may be higher, as nearly half of these infections become apparent only after hospital discharge.[14]

Bacterial contamination in surgical wounds can originate from either internal or external sources. Internal (endogenous) sources include the patient's natural microbiota present at the surgery site or other body regions. External (exogenous) sources involve factors like the surgical team, the environment, and the materials or instruments used in the procedure. SSIs incidence varies according to type of surgical procedure, patient-related factors, and the effectiveness of hospital infection control practices.[3, 16]

Factors such as diabetes mellitus, obesity, malnutrition, prolonged hospital stay, immunosuppression, advanced age, and poor surgical aseptic techniques have been reported as important contributing factors associated with the development of SSIs.[4, 12] Age and sex are the non-modifiable patient related risk factors contributing for SSIs. Individuals with advanced age are often at higher risk due to reduced immune function and the presence of comorbid conditions that can complicate both surgery and recovery, thereby increasing the likelihood of surgical site infections (SSIs). In addition, sex-specific differences may influence the types and frequency of surgical procedures performed, which can also affect the overall risk of developing SSIs.[13, 17]

Most SSIs are caused by pathogens from the patient's own flora[5]. Surgical wound infections (SWIs) can be caused by various microorganisms, including bacteria, fungi, and viruses. The most commonly implicated bacterial pathogens include *P. aeruginosa, E. coli, Klebsiella species, Enterobacter species, Proteus species, Acinetobacter species, Serratia species* and *Staphylococcus aureus*. Prevalence of antibiotic-resistant strains, particularly methicillin-resistant *Staphylococcus aureus (MRSA)* is also increasing notably.[1,7] Receiving the advised antibiotic prophylaxis to prevent surgical site infections may be compromised by a reported penicillin allergy.[6]

Healthcare providers can substantially lower the frequency of SSI's by addressing these risk factors, which can in turn lead to shorter hospital stay, better patient outcomes and cut down healthcare cost. A clear risk awareness of these factors is crucial for developing targeted protective strategies and optimizing surgical outcomes.[13]

Even with advances in medical technology and the implementation of various preventive measures, such as air-conditioned operating rooms, antibacterial drapes, and perioperative antibiotic prophylaxis, surgical site infections (SSIs) remain a challenging issue to address. This study aimed to investigate the types of bacteria and their antimicrobial resistance patterns isolated from patients with SSIs.

Materials and Methods

Data Collection

Sixty-five samples of wound swab and relevant clinical detail were obtained from the diagnosed cases of surgical site infection (SSI) at Jinnah Hospital Lahore.

Culture of Specimen

Under aseptic conditions clinical specimen was collected and transported within 30 min to laboratory for further processing and each swab was placed in sterile tube containing normal saline. For bacterial isolation samples were cultured on MacConkey, Blood, and Chocolate agar media. MacConkey and Blood agar plates were incubated aerobically, while Chocolate agar plates were incubated at 37°C in a 5–10% CO_2 environment for 24–48 hours.

Bacterial Pathogen Identification

Bacterial cultures were characterized by their morphology on selective and differential media. Motility and biochemical tests were conducted based on standard methods.

Automated testing system

For additional testing, an automated system like VITEK 2, along with the Kirby-Bauer disk diffusion method, was utilized. VITEK 2 is a fully automated platform designed for bacterial identification and for assessing antibiotic susceptibility while Kirby-Bauer disk diffusion method was used to determine the susceptibility/resistance of pathogenic bacteria to a range of antimicrobial agents, providing essential guidance for physicians in selecting effective treatment options for patients.

Statistical Analysis

SPSS software version 26 was used for the statistical analysis of data. Mean ± standard deviation was used for quantitative variables, while frequency and percentages were used to express qualitative variables. The Chi-square test was used to find out differences between the variables and p value <0.05 was considered statistically significant.

Results

Present study included 65 patients diagnosed with surgical site infections (SSIs). The ages of patient ranges from <15 to >60 years. In this study SSIs were found in 24 (37%) of patients who belonged to 16-30 years of age group. More males were found to diagnose with SSI's 41(63%) as compared to females. Surgical site infections were common in lower extremities 43 (66%) as compared to other parts of body as shown in Table 1.

Table 1: Demographic data of study participants

Age Groups	Frequency (%age) (N=65)
<15	10 (15%)
16-30	24 (37%)
31-45	10 (15%)
46-60	5 (8%)
>60	16 (25%)
Gender	
Male	41(63%)
Female	24(37%)
Location of Wounds	
Abdomen	7 (11%)
Upper Extremities	12 (18%)
Lower Extremities	43 (66%)
Others	3 (5%)

Out of 65 samples processed for bacteriologic evaluation, 40% were isolated as mixed gram-negative organisms. Gram negatives and gram positives organisms were found to be 29% and 25% respectively. The predominant isolated was *E-Coli* followed by *MRSA, S. Aureus* & *K-Pneumonae* at a frequency of 15(23%), 12 (18.5%), 4 (6%) and 4 (6%) respectively. Moreover, the proportion of *E-Coli* co-growth with *P. miribilis* and *Klebsella* was found at 25% as shown in Table 2.

Table 2: Bacteriologic profile of SSI patients	
Organisms Isolated	**Frequency (%age) (n=65)**
Gram Positive	16 (25%)
Gram Negative	19(29%)
Mixed Gram-Negative Isolates	26 (40%)
Mixed Gram Positive and Gram-Negative Isolates	4 (6%)
Gram positive Bacteria	
S. Aureus	4 (6%)
MRSA	12 (18.5%)
Gram Negative Bacteria	
E-coli	15(23%)
Klebsiella-Pneumonae	4(6%)
Mixed Gram-Negative Isolates:	
E. coli-P.miribilis	8(12.3%)
E. coli-P.seudomonas	2(3.1%)
E. coli- A.baumini	4 (6.2%)
E. coli-Klebsella	8 (12.3%)
K.pneumonae- C.koseri	2(3.1%)
K pneumonae- M morgani	2(3.1%)
Mixed Gram Positive and Gram-Negative Isolates:	
S.Aureus-E. coli	2 (3.1%)
S.Aureus-P.miribilis	2(3.1%)

Effect of gender on location of wounds

In this study no association was found between gender and location of wounds (p>0.05). However, in both males (47%) and females (18%) infections were common in lower extremities as compared to other sites. (Figure 1).

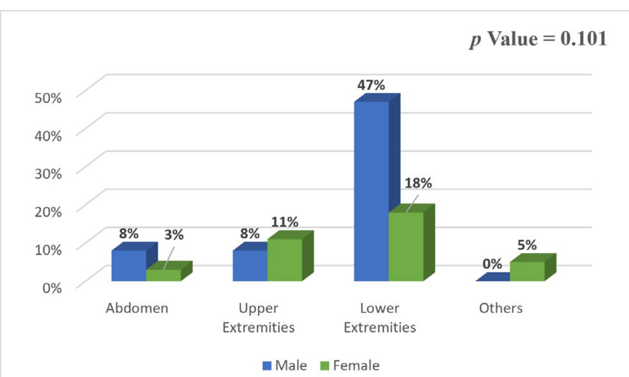

Figure 1: Effect of Gender on Location of Wounds.

Effect of gender on organisms isolated

In this study no effect has been found between gender and organisms isolated however, more males (63%) were affected with SSI as compared to females (37%). Table 3 showed *MRSA* effects more males 8 (12%) as compared to females 4 (6%). Table 3 also showed a positive association between gram negative organism *E-Coli* with males to cause SSIs. (P value <0.05). Double microbial infections were found frequent in both males and females. Positive association (p value=<0.05) was found between gender and double microbial infections to cause SSIs as more males (24%) were affected than females (21%) as shown in Table 3.

Table 3: Effect of gender on organisms isolated			
Organisms	**Gender (n=65)**		
	Male	**Female**	***P-Value***
Gram Positive	10 (15%)	6 (9%)	0.194
Gram Negative	15 (23%)	4 (6%)	
Double microbial Infections	16 (25%)	14 (22%)	
Gram Positive			
S-aureus	2 (3%)	2 (3%)	0.551
MRSA	8 (12%)	4 (6%)	
Gram Negative			
E-Coli	14 (22%)	2 (3%)	0.04
Klebsiella Pneumoniae	1 (2%)	2 (3%)	
Mixed Gram-Negative Isolates:			
E-coli - Proteus Mirabilis	6 (9%)	2 (3%)	0.03
E- coli - Pseudomonas Aeruginosa	2 (3%)	0 (0%)	
E-coli - Acinetobacter Baumannii	0 (0%)	4 (6%)	
E-coli - klebsiella Pneumoniae	4 (6%)	4 (6%)	
klebsiella Pneumoniae - Citrobacter koseri	2 (3%)	0 (0%)	
klebsiella Pneumoniae - Morganella morganii	0 (0%)	2 (3%)	
Mixed Gram Positive and Gram-Negative Isolates			
S aureus - E coli	2 (3%)	0 (0%)	0.04
S aureus - Proteus Mirabilis	0 (0%)	2 (3%)	

Effect of location of wounds on organisms isolated

Figure (2) showed no association was found between location of wounds and organisms isolated. However, 25% of mixed gram negatives are isolated, 20% gram negative, and 15%-gram positive organisms caused SSIs in lower extremities as compared to other parts of body.

Distribution of Bacterial Isolates According to Wound Location

Figure 3 showed 11% *MRSA* and 4% *S-Aureus* caused SSI in lower extremities. Only 4% MRSA was found to cause SSI in upper extremities (Figure 3). In our study a strong association (p =0.002) was found between gram negative organisms to cause SSIs in lower extremities. As 22% of E-Coli cause SSIs in Lower extremities.

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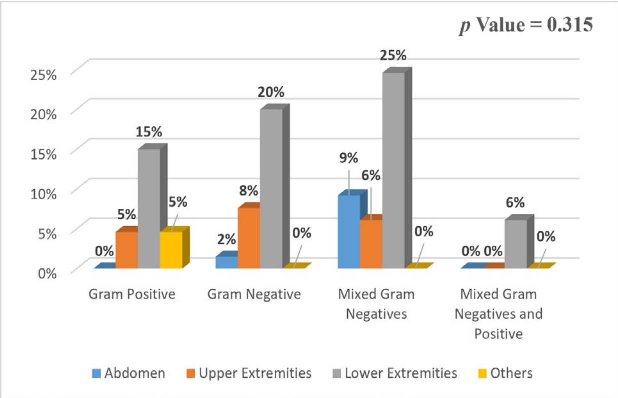

Figure 2: Effect of location of wounds on organisms isolated.

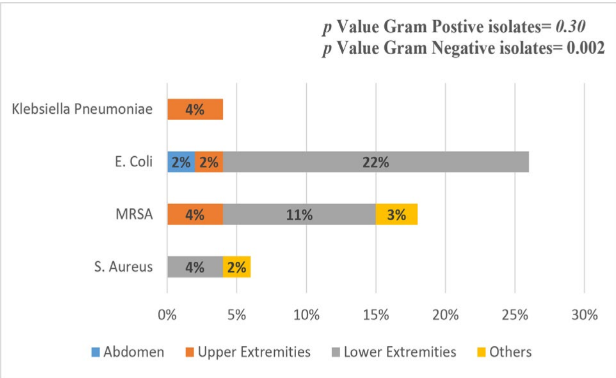

Figure 3: Distribution of Bacterial Isolates According to Wound Location.

In our study out of 65 patients 30(45%) were found to have double microbial infections at surgical site infections. Figure 4 showed positive association (p=0.01) was found between double microbial infections to cause SSIs in lower extremities. In eight specimens (12%) *E-coli* and *P. Miribilis* mixed growth was found to cause SSIs in lower extremities.

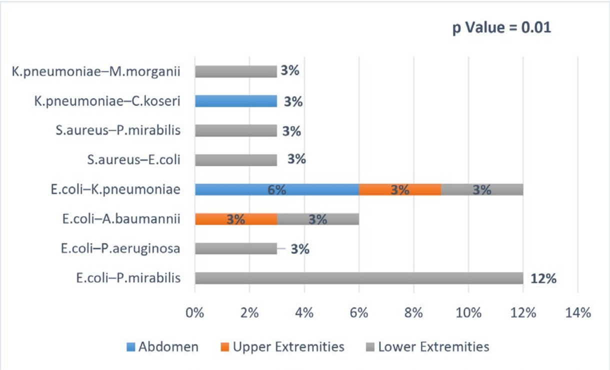

Figure 4: Distribution of Double Microbial Infections According to Wound Location.

Table 4 demonstrates the organism-wise antibiotic resistance patterns among bacterial isolates. The heat map showed higher resistance frequencies among *MRSA* and *E. coli* isolates. *MRSA* exhibited marked resistance to Penicillin (n=11), Oxacillin (n=10), and Amoxicillin, Ampicillin, Co-Amoxi, and Pipracil (n=9 each). Similarly, *E. coli* showed high resistance against Amoxicillin (n=14), Ampicillin (n=13), and Ticarcillin/Cotrimoxazole (n=10 each). In contrast, lower resistance rates were observed for Meropenem, Ertapenem, and Ceftriaxone.

Table 4: Heat Map of Organism-wise Antibiotic Resistance

Antibiotic	S. Aureus (n=4)	MRSA (n=12)	E. Coli (n=15)	Klebsiella (n=4)
Penicillin	4	11	0	0
Erythromycin	3	6	0	0
Oxacillin	0	10	0	0
Cephradine	0	4	0	0
Cephalexin	0	4	0	0
Meropenem	0	0	2	1
Ertapenem	0	0	3	1
Ticarcillin	0	0	10	1
Ampicillinsul	0	0	2	2
Cotrimoxazole	4	5	10	2
Amoxicillin	4	9	14	0
Cefotaxim	0	6	8	1
Ampicillin	4	9	13	0
Co-Amoxi	0	9	7	3
Ceftazidime	0	1	7	3
Ceftriaxone	0	3	1	0
Cefuroxime	0	7	2	1
Ciprofloxacin	3	7	8	2
Gentamycin	0	1	4	2
Imipenem	0	8	2	1
Pipracil	0	9	8	2

Light Color: Low Resistance.

Dark Red: High Resistance.

Figure 5 illustrates the overall percentage resistance against tested antibiotics. Penicillin showed the highest resistance rate

(94%), followed by Amoxicillin (77%) and Ampicillin (74%). The lowest resistance rates were observed for Ceftriaxone (11%), Meropenem (16%), and Gentamycin (20%), indicating relatively better antimicrobial effectiveness of these agents.

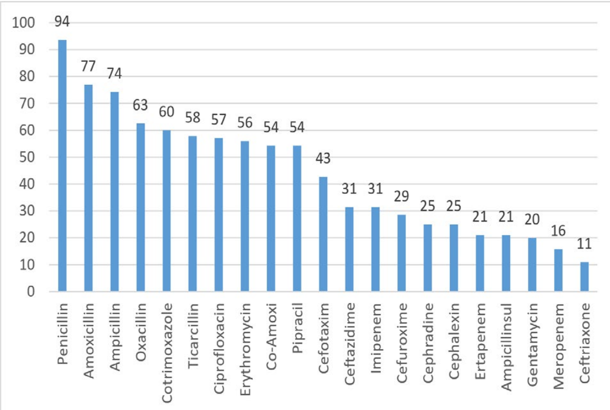

Figure 5: Overall percentage resistance against tested antibiotics.

Discussion

Postoperative surgical site infections remain a leading cause of morbidity among surgical patients. Affected individuals often experience higher healthcare costs due to prolonged hospitalization, intensive care, advanced wound care, possible readmissions, and, in some cases, the need for additional surgical procedures. Identifying the bacterial pathogens involved and choosing an effective antibiotic treatment are crucial steps in managing these infections successfully. The present study addressed the prevalence of SSI, bacterial etiologies and antibiotic susceptibility patterns of bacterial isolates in post-operative patients.

In the present study, surgical site infections were more common among patients aged 16–30 years. Similar findings were reported in a 2021 study, in which 87 (52.7%) of the 165 study participants with surgical site infections belonged to the 16–40 years age group, indicating a higher frequency of SSI among younger adults.[7] However, contradictory findings have also been documented, reporting a significantly higher risk was found in the 76–80 years age group due to reduced immunity, multiple comorbidities, and delayed wound healing.[18]

In the present research we conducted, males had a higher SSI rate (63%) than females (37%), which is consistent with data from other studies that showed males have 59.4% and 57.5%.[7,8] This could be attributable to a variety of factors, including males being more exposed to risk factors such as cigarette smoking, which has been linked to an increased SSI rate. However, a previous study conducted in 2023 in which females (74.3%) reported to have higher incidence of SSI's as compared to males (25.7%).[9]

Samples from sixty-five patients (54%) had monomicrobial growth while the remaining 46% had polymicrobial growth, which is consistent with the study conducted in Ethopia reported single microbial infection was observed in 82.9% of the samples, while the remaining 17.1% showed mixed bacterial growth.[9] A recent study by Kalladi et al. reported a substantially higher frequency of monomicrobial isolates (53.4%) compared to polymicrobial isolates (12.5%) among surgical site infection samples.[19]

On the gram stain analysis 46% of SSI's were reported due to double microbial organisms, gram negative 29% and gram positive 24%. It contradicts with the study done in Ethiopia where the gram-negative rate was comparatively high 57% and gram-positive rate was 42% and double microbial were 26%.[1]

In the present study *E-Coli* accounted for 23% followed by *MRSA* 18.5%. A previous study conducted in 2021 by Misha et al., showed consistent results.[1] However, a study conducted by scientists showed inconsistent results reported *Klebsiella species, E-coli, S aureus,* and *Pseudomonas aeruginosa* as predominant isolates.[10]

In the present research SSIs were more common in lower extremities 43 (66%) followed by upper 12 (18%), abdomen (11%) and others 5%. Previous study conducted in 2012 showed consistent findings as lower limb surgeries had higher SSI occurrence (6.8%) compared to upper limb procedures (4.4%), with most SSIs occurring in lower extremity operations due to higher exposure to contamination and soft tissue injury.[20] A previous reported contradicted results 60.3% of the bacterial isolates were recovered from the abdomen and 29.5% from lower limbs while the only 6.4% and 3.8% contribution was found from other body sites and arms respectively.[11]

In present study 24.6% of mixed gram negatives isolates, 20% gram negative, and 15%-gram positive organisms caused SSIs in lower extremities as compared to other parts of body. A similar study conducted in 2020 reported consistent results that 57.7% of gram-negative rods and 42.3% of gram-positive bacteria was found to cause SSIs in lower extremities.[11]

Conclusion

Present study emphasizes the importance of identifying bacterial pathogens and antimicrobial susceptibility/resistant patterns in SSIs patients. SSIs were more commonly observed in younger age groups, particularly males, and most frequently involved the lower extremities. *E-coli* was the most prevalent organism isolated, followed by methicillin-resistant

Staphylococcus aureus (MRSA), S aureus, and *K pneumoniae.* A notable association was observed between male patients and Gram-negative bacterial infections, with *E. coli* predominantly affecting the lower limbs. Double microbial infections were also more common in males than in females.

Antibiotic susceptibility patterns showed high resistance among Gram-positive bacteria to penicillin, oxacillin, and erythromycin, while Gram-negative isolates demonstrated marked resistance to ticarcillin, ampicillin, and other beta-lactam antibiotics. These findings highlight the importance of effective infection prevention and control strategies and strong antimicrobial stewardship programs to improve SSI management and combat the rising threat of antimicrobial resistance.

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