

PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIAL ISOLATES FROM SURGICAL WOUND INFECTIONS IN A TERTIARY CARE HOSPITAL: A CROSS-SECTIONAL STUDY

Haider Khan¹, Dr. Irfan Saleem², Sakina Bibi Silachi³, Rabica Faryal⁴, Hafiz Yaseen Khan⁵, Shah Alam Khan⁶, Imran Ali⁷

¹BS Surgical Technologist, Khyber Medical University, Peshawar, Pakistan

²Deputy Medical Superintendent (DMS), Sir Ganga Ram Hospital, Lahore, Pakistan

³Assistant Professor, Department of Botany, Government Girls Degree College, Sibi, Balochistan, Pakistan

⁴Physiotherapist, Jinnah Hospital, Karachi, Pakistan

⁵Department of Allied Health Sciences, City University of Science and Information Technology (CUSIT), Peshawar, Pakistan

⁶MSPT, Riphah International University, Pakistan

⁷Department of Allied Health Sciences, City University of Science and Information Technology (CUSIT), Peshawar, Pakistan

¹haiderkhan786.aa@gmail.com, ²irfansaleem610@yahoo.com, ³sakinasilachi@gmail.com, ⁵dryaseenkhan11@gmail.com, ⁶alamshah977@gmail.com

Corresponding Author: *

Irfan Saleem

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ABSTRACT

Background: Surgical site infections (SSIs) remain among the most frequent healthcare-associated infections worldwide and are increasingly complicated by antimicrobial resistance (AMR), particularly in resource-limited tertiary care settings. This study aimed to determine the prevalence of culture-positive surgical wound infections and characterize the antimicrobial resistance profiles of the causative bacterial isolates.

Methods: A hospital-based, prospective cross-sectional study was conducted over a 12-month period (January–December 2025) at the Department of Surgery and Microbiology, a 500-bed tertiary care teaching hospital Peshawar. Wound swabs/pus aspirates were collected aseptically from patients with clinically suspected SSI following the Centers for Disease Control and Prevention (CDC) surveillance definitions. Bacterial identification was performed using standard microbiological and biochemical methods (and/or an automated VITEK-2 system), and antimicrobial susceptibility testing (AST) was performed by the Kirby–Bauer disc diffusion method interpreted per current Clinical and Laboratory Standards Institute (CLSI) breakpoints. Multidrug resistance (MDR), extensive drug resistance (XDR), and pandrug resistance (PDR) were classified according to the Magiorakos et al. international consensus definitions. Data were analyzed using SPSS (version 26.0); categorical variables were expressed as frequencies/percentages, and associations were tested using the chi-square test, with $p < 0.05$ considered statistically significant.

Results: Of 850 surgical patients screened, 210 (24.7%) developed clinical signs of SSI, of which 158 (75.2%; 95% CI: 68.9–80.7%) were culture-positive, yielding 172 bacterial isolates (7.9% polymicrobial). Gram-negative bacteria predominated (108/172, 62.8%), led by *Escherichia coli*

(19.8%), *Klebsiella pneumoniae* (16.3%), and *Pseudomonas aeruginosa* (14.0%); *Staphylococcus aureus* was the leading Gram-positive isolate (22.1%), of which 57.9% were methicillin-resistant (MRSA). High resistance was observed among Gram-negative isolates to ampicillin (88.9%), amoxicillin-clavulanate (74.1%), and ceftriaxone (65.7%), with comparatively preserved susceptibility to meropenem (91.7%) and colistin (97.2%). Gram-positive isolates showed high resistance to penicillin (90.6%) but retained near-universal susceptibility to vancomycin (98.4%) and linezolid (100%). Overall, 46.5% (80/172) of isolates met criteria for multidrug resistance, and 8.1% (14/172) were extensively drug-resistant.

Conclusion: Gram-negative organisms, particularly Enterobacterales and non-fermenters, are the predominant cause of surgical wound infections at this tertiary center, with a substantial burden of multidrug resistance. Carbapenems, colistin, vancomycin, and linezolid remain the most reliable empirical options, but their reserved use should be safeguarded through structured antimicrobial stewardship, periodic antibiogram updates, and strengthened infection-prevention practices.

Keywords: surgical site infection; antimicrobial resistance; multidrug resistance; antibiogram; tertiary care hospital; nosocomial infection

1. Introduction

Surgical site infection (SSI) is one of the most common and expensive types of healthcare-associated infection (HAI), affecting 2-5% of clean surgical procedures in high-income countries and higher proportions (typically 10-20% or more) in low- to middle-income countries (LMICs), where infection-prevention infrastructure, sterile practices and perioperative antibiotic stewardship are often limited. In addition to the direct medical consequences of delayed wound healing, longer hospital stays, reoperation, and sepsis, SSIs also have a significant economic impact on individuals and health care systems and are an independent risk factor for postoperative death.

The landscape of etiological causes of SSIs has changed significantly over the last 20 years. Although *Staphylococcus aureus* has been considered the prototypical wound pathogen, modern surveillance data from tertiary hospitals in South Asia, the Middle East and Sub-Saharan Africa reveal that Gram-negative Enterobacterales (mainly *Escherichia coli* and *Klebsiella pneumoniae*), along with non-fermenting bacilli like *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are emerging as predominant or co-dominant culprits. This change has important therapeutic implications, as Gram-negative organisms are over-represented in hospital settings with regard to the production of extended-spectrum beta-lactamase (ESBL), AmpC

hyperproduction and the production of carbapenemases.

According to the World Health Organisation (WHO), antimicrobial resistance (AMR) is one of the top ten global public health threats, and surgical wards are identified as hot spots for the emergence of multidrug-resistant organisms (MDROs), especially in the context of high patient numbers, frequent invasive procedures and long duration of antibiotic therapy. In general, clinical antibiotic choice for an SSI is based on local antibiograms, but resistance to antibiotics can differ significantly between hospitals, regions, and times, and so imported or old data on antibiotic susceptibilities are of limited value for the treatment of SSI. Even so, many tertiary centres in resource-limited areas still use "empirical" regimens that have not been re-validated and re-susceptibility-tested with respect to the local resistance problem, leading to mistreatment, treatment failure and additional selection pressure for resistance.

In light of this background, the periodic and institution-specific monitoring of SSI microbiology and antimicrobial susceptibility is crucial for guidance on rational empirical antibiotic therapy and revision of surgical antibiotic prophylaxis guidelines and for the implementation of antimicrobial stewardship programmes. This study was therefore aimed at (i) assessing (i) the incidence of culture-positive surgical wound infections in patients undergoing

surgical procedures at a tertiary care hospital, (ii) describing the spectrum of microorganisms isolated from the surgical wounds and (iii) describing the antimicrobial resistance of the isolated microorganisms to provide an updated and locally relevant antibiogram of surgical wound pathogens.

2. Materials and Methods

2.1 Study Design, Setting, and Duration

The study was a hospital-based, prospective, cross-sectional (descriptive) study conducted in the Department of General Surgery in association with the Department of Microbiology at a 500-bedded tertiary care teaching and referral hospital catering to the urban and rural population of the area. This study lasted for 12 months from January 2025 to December 2025. The manuscript is reported according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for cross-sectional studies.

2.2 Study Population, Sample Size, and Eligibility Criteria

Patients included in this study were all surgical patients who developed one or more clinical features that met CDC/National Healthcare Safety Network (NHSN) surveillance case definitions for SSI, with the symptoms of purulent drainage, localised erythema, warmth, tenderness, wound dehiscence, or fever, within 30 days of surgery (or 90 days if an implant was placed).

Exclusion criteria: patients who were not in the clinical case definition of SSI, who did not provide informed consent (or informed assent for minors), and who had either had a dirty surgical procedure or were undergoing a surgical procedure that was not clean, clean-contaminated, or contaminated. Exclusion criteria were patients who had been prescribed antibiotics for another reason within the previous 48 hours (without documentation), wounds that were not sampled properly (e.g., swabbing intact skin without any discharge), and patients who refused to participate.

The required sample size was calculated using Cochran's formula for estimating a single population proportion:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

where $Z = 1.96$ for a 95% confidence level, P represents the anticipated prevalence of surgical site infection based on previous regional literature, and $d = 0.05$ (5% margin of error). After accounting for possible incomplete records and specimen losses, the calculated minimum sample size was increased by 10%. During the study period, all eligible patients meeting the inclusion criteria were enrolled consecutively until the required sample size was exceeded, resulting in the inclusion of 210 clinically suspected SSI cases.

2.3 Specimen Collection, Transport, and Bacterial Identification

Wounds were swabbed (or aspirated, if a fluctuant collection was present) under strict sterile precautions with sterile cotton-tipped applicators or syringe aspiration after cleaning the wound margin with sterile normal saline to reduce contaminating skin flora. The specimens were collected and sent to the microbiology lab in Amies transport medium within 2 hours. Samples were plated on blood agar, MacConkey agar and (when appropriate) chocolate agar and were incubated aerobically at 37°C for 18–24 hours, with a 48-hour incubation if no growth was observed after 24 hours. Bacterial identification was done by standard colony morphology, Gram stain and a panel of biochemical tests (catalase, coagulase, oxidase, indole, citrate utilisation, urease, triple sugar iron, and related tests) and/or by the automated VITEK-2 Compact system, bioMérieux.

2.4 Antimicrobial Susceptibility Testing

All confirmed isolates were tested for antimicrobial susceptibility (AST) by the disc diffusion method on Mueller-Hinton agar, as per year-specific Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial susceptibility testing (AST). Isolates were tested against a standardised antibiotic panel as recommended by the CLSI: against a Gram-negative panel of antibiotics (ampicillin, amoxicillin-clavulanate, ceftriaxone, ceftazidime, cefepime, piperacillin-tazobactam, ciprofloxacin,

gentamicin, amikacin, imipenem, meropenem, and colistin) and a Gram-positive panel (penicillin, cefoxitin (surrogate for oxacillin), ciprofloxacin, clindamycin, erythromycin, gentamicin, linezolid, vancomycin, and teicoplanin). The cefoxitin disc diffusion method was used to confirm MRSA and coagulase-negative staphylococci resistance. Each batch of testing included quality control organisms *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923.

2.5 Definitions of Multidrug, Extensive-Drug, and Pandrug Resistance

The MDR, XDR and PDR classifications were based on the internationally agreed definitions of Magiorakos et al. (2012): MDR was defined as resistance to at least one agent in three or more antimicrobial categories, XDR as resistance to all agents in at least two but not three antimicrobial categories and PDR as resistance to all agents in all antimicrobial categories tested.

2.6 Data Collection and Statistical Analysis

The sociodemographic (age, sex, occupation) and clinical variables (type of surgery; wound class according to the wound classification system of the CDC; hospital stay; previous antibiotic usage; and comorbidities like diabetes mellitus) were obtained in a pre-designed structured proforma. The data was entered into Microsoft Excel and analysed in IBM SPSS Statistics (version 26.0). Prevalence, distribution of organisms, and

resistance distribution were summarised using descriptive statistics (frequencies, percentages, and means $\pm$ standard deviation). The chi-square test or Fisher's exact test was used to determine the associations between categorical variables (e.g., organism type and MDR status, wound class and culture positivity), and a p-value < 0.05 was considered statistically significant (two-tailed).

2.7 Ethical Considerations

Written informed consent was signed by all participants (patients under 18 years of age signed by their legal guardian). The data from patients were anonymised and coded for confidentiality, and the study adhered to the principles of the Helsinki Declaration.

3. Results

3.1 Sociodemographic and Clinical Characteristics

Overall, 850 patients who had undergone at least one surgical procedure were screened, while 210 (24.7%) developed clinical signs and symptoms compatible with SSI and were included. The average age of the patients enrolled was 42.6 ± 16.3 years (range 4 – 81 years), and 56.7% were male. Of the total number of cases, 38.1% were emergency surgeries and 41.4% had contaminated/dirty wounds. The prevalence of diabetes mellitus in participants was 22.9% and significantly correlated with culture positivity ($p = 0.011$).

Table 1. Sociodemographic and clinical characteristics of study participants (N = 210)

Characteristic	Category	n	%
Age group (years)	≤ 20	22	10.5
	21–40	78	37.1
	41–60	76	36.2
	> 60	34	16.2
Sex	Male	119	56.7
	Female	91	43.3
Type of surgery	Elective	130	61.9
	Emergency	80	38.1

Characteristic	Category	n	%
Wound class (CDC)	Clean	28	13.3
	Clean-contaminated	95	45.3
	Contaminated	62	29.5
	Dirty/infected	25	11.9
Diabetes mellitus	Present	48	22.9
	Absent	162	77.1

CDC, Centers for Disease Control and Prevention wound classification.

3.2 Prevalence of Culture-Positive Surgical Site Infection

Overall, 158/210 (75.2% (95% CI: 68.9–80.7)) of clinically suspected specimens of SSI were culture positive. Prior antibiotic use was also documented in 61.5% of the culture-negative specimens and was a factor in the lack of growth in 52 specimens (24.8%). Of the 12 specimens (7.6% of culture-positive) with polymicrobial growth (i.e., ≥ 2 organisms from a single specimen), a total of 172 bacterial isolates were analysed.

3.3 Distribution of Bacterial Isolates

The majority of the bacteria were Gram-negative (108/172; 62.8%), and Gram-positive cocci were 64/172 (37.2%). The predominant organism overall was *Escherichia coli* (19.8%), followed by *Staphylococcus aureus* (22.1% of MSSA/MRSA), *Klebsiella pneumoniae* (16.3%) and *Pseudomonas aeruginosa* (14.0%).

Table 2. Distribution of bacterial isolates from surgical wound infections (n = 172)

Organism	Frequency (n)	Percentage (%)
<i>Escherichia coli</i>	34	19.8
<i>Staphylococcus aureus</i> (MSSA + MRSA)	38	22.1
– of which MRSA	22	57.9 of <i>S. aureus</i>
<i>Klebsiella pneumoniae</i>	28	16.3
<i>Pseudomonas aeruginosa</i>	24	14.0
<i>Acinetobacter baumannii</i>	14	8.1
Coagulase-negative <i>Staphylococci</i>	16	9.3
<i>Proteus mirabilis</i>	8	4.7
<i>Enterococcus</i> species	10	5.8
Total	172	100.0

3.4 Antimicrobial Resistance Pattern of Gram-Negative Isolates

The gram-negative isolates were highly resistant to the first-line and second-line agents which are commonly used. However, resistance to the antibiotics ampicillin (88.9%), amoxicillin-

clavulanate (74.1%) and ceftriaxone (65.7%) was relatively high and indicative of a high level of the ESBL-producing Enterobacterales (EPE). The rate of resistance to the fluoroquinolone, ciprofloxacin, was 55.6% of the isolates. In contrast, meropenem (91.7% susceptible),

imipenem (88.9% susceptible), and colistin (97.2% susceptible) were the most reliable choices

for the treatment of severe or resistant Gram-negative SSI.

Table 3. Antimicrobial resistance pattern of Gram-negative bacterial isolates (n = 108)

Antibiotic	Resistant n (%)	Sensitive n (%)
Ampicillin	96 (88.9)	12 (11.1)
Amoxicillin-clavulanate	80 (74.1)	28 (25.9)
Ceftriaxone	71 (65.7)	37 (34.3)
Ceftazidime	64 (59.3)	44 (40.7)
Cefepime	58 (53.7)	50 (46.3)
Piperacillin-tazobactam	39 (36.1)	69 (63.9)
Ciprofloxacin	60 (55.6)	48 (44.4)
Gentamicin	46 (42.6)	62 (57.4)
Amikacin	28 (25.9)	80 (74.1)
Imipenem	12 (11.1)	96 (88.9)
Meropenem	9 (8.3)	99 (91.7)
Colistin	3 (2.8)	105 (97.2)

3.5 Antimicrobial Resistance Pattern of Gram-Positive Isolates

Resistance to penicillin was virtually universal (90.6%) among Gram-positive isolates (n = 64), and 57.9% of *S. aureus* isolates were cefoxitin-resistant, evidence of methicillin resistance (MRSA). Resistance to erythromycin and clindamycin was also high (56.3% and 43.8%,

respectively), and the extent of inducible clindamycin resistance among the macrolide-resistant isolates needs to be carefully observed. Susceptibility to glycopeptides vancomycin (98.4%) and teicoplanin (96.9%) continued to be excellent, and to linezolid (100%), which confirms that they retain their role in resistant Gram-positive SSI and confirmed MRSA.

Table 4. Antimicrobial resistance pattern of Gram-positive bacterial isolates (n = 64)

Antibiotic	Resistant n (%)	Sensitive n (%)
Penicillin	58 (90.6)	6 (9.4)
Cefoxitin (methicillin surrogate)	24 (37.5)*	40 (62.5)
Ciprofloxacin	28 (43.8)	36 (56.3)
Erythromycin	36 (56.3)	28 (43.8)
Clindamycin	28 (43.8)	36 (56.3)
Gentamicin	22 (34.4)	42 (65.6)
Vancomycin	1 (1.6)	63 (98.4)
Teicoplanin	2 (3.1)	62 (96.9)

Antibiotic	Resistant n (%)	Sensitive n (%)
Linezolid	0 (0.0)	64 (100.0)

*Cefoxitin resistance calculated across all Gram-positive isolates tested; among *S. aureus* alone (n = 38), 22/38 (57.9%) were MRSA.

3.6 Burden of Multidrug Resistance

In total, 46.5% (80/172) of the isolates were multidrug resistant (MDR), 8.1% (14/172) of isolates were extensively drug-resistant (XDR), and no pandrug-resistant (PDR) isolate was detected. The prevalence of MDR was higher among Gram-negative (52/108, 48.1%) compared to Gram-

positive (28/64, 43.8%) isolates, but this was not statistically significant ($p = 0.19$). Among Gram-negative organisms, *Klebsiella pneumoniae* had the highest proportion of MDR (57.1%), and *Acinetobacter baumannii* had the highest proportion of MDR (64.3%), and the majority of MDR Gram-positive organisms were MRSA.

Table 5. Prevalence of multidrug resistance (MDR) by organism

Organism	Isolates tested (n)	MDR n (%)
<i>Escherichia coli</i>	34	14 (41.2)
<i>Klebsiella pneumoniae</i>	28	16 (57.1)
<i>Pseudomonas aeruginosa</i>	24	11 (45.8)
<i>Acinetobacter baumannii</i>	14	9 (64.3)
<i>Proteus mirabilis</i>	8	2 (25.0)
<i>Staphylococcus aureus</i>	38	22 (57.9)
Coagulase-negative <i>Staphylococci</i>	16	5 (31.3)
<i>Enterococcus</i> species	10	1 (10.0)
Overall	172	80 (46.5)

4. Discussion

A 75.2% rate of culture-positive surgical site infection was revealed in this study, and the majority of the isolates were Gram-negative organisms, nearly two-thirds. The distribution is comparable to recent regional surveillance, as a tertiary hospital study in Northeast India identified *Escherichia coli* and *Klebsiella* spp. as the most common isolates in surgical site infections while also reporting high levels of ampicillin and cefazolin resistance in Gram-negative bacteria and high levels of ceftriaxone and cefepime resistance. A tertiary-centre study in western India also found Gram-negative organisms as the major cause of SSIs, in which *Klebsiella pneumoniae* and *E. coli* were found to predominate, and a significant number of

carbapenem-resistant Gram-negative organisms. In conjunction with the present results, this confirms the already well-established transition of SSI epidemiology from a traditionally Gram-positive-dominated pattern to Enterobacterales and NF-GNB in many tertiary hospital environments. This proportion of MRSA (57.9%) falls within the range of other South Asian tertiary hospital studies where proportions of MRSA among *S. aureus* isolates from wound samples have been reported to be from one-fifth to more than half depending on the study design and infection control measures. The high level of vancomycin, teicoplanin and linezolid susceptibility found in the vast majority of isolates in this study is consistent with similar studies indicating that *S. aureus* and MRSA remained highly susceptible to

the glycopeptides, even though they showed a high level of resistance to beta-lactam and aminoglycoside antibiotics. This uniformity helps to facilitate the prudent use of glycopeptides and oxazolidinones as reserve drugs for MRSA wound infection, and they should not be used as first-line drugs on a blind basis, as this could promote the emergence of resistance.

The overall MDR burden was 46.5%, which is similar to that reported by another tertiary hospital in Uganda for cancer patients who had SSI (48%), but slightly higher than the 43.9% MDR rate from a teaching hospital in southern Ethiopia. These are likely due to differences in the case mix of patients (immunocompromised vs. general surgical patients) as well as the intensity of local antibiotic prescribing practices, previous hospitalisation or antibiotic exposure, and the intensity of interventions to control infection between institutions. The relative susceptibility of Gram-negative isolates to meropenem, imipenem and colistin in the current study is reassuring but should by no means be seen as an excuse to use these last-line agents in the wild. The number of carbapenem-resistant Enterobacterales (CRE) and colistin-resistant organisms is gradually rising in analogous tertiary-care units, and unrestricted use of last-line agents will jeopardise this residual susceptibility.

These findings have a number of clinical and public health implications. First, the fact that Gram-negative organisms are predominant and are high on the list of organisms with resistance to empirical antibiotic agents (ceftriaxone and ciprofloxacin) indicates that the current surgical antibiotic prophylaxis and empirical treatment regime in this hospital may warrant review with regard to the updated local antibiogram. Secondly, the high burden of MDR in *K. pneumoniae* and *A. baumannii* highlights the importance of implementing enhanced contact precautions, environmental cleaning and hand hygiene measures on surgical wards where these organisms are often nosocomially transmitted. Third, susceptibility to carbapenems, colistin, vancomycin and linezolid is largely maintained, emphasising the need for antimicrobial stewardship programmes to limit the use of these

agents to culture-confirmed indications to preserve their usefulness.

5. Limitations

There are a number of limitations to this study that need to be taken into account when looking at the results. The findings from this single center, cross-sectional study may not be applicable to other centers with different patient populations, surgical case-mix, or infection control measures. No anaerobic culture or molecular characterisation of resistance mechanisms (such as specific ESBL or carbapenemase genes) was conducted and this meant that the observed phenotypic resistance was less easily interpreted in terms of mechanisms of resistance. Furthermore, data on previous antibiotic use (although collected) might have affected culture positivity and resistance patterns recorded, and residual confounding cannot be ruled out. To confirm and expand on these results, longitudinal multiple center studies with molecular resistance characterization are recommended.

6. Conclusion

In this tertiary health care centre, the main pathogens isolated from surgical wound infections are Gram-negative bacteria especially *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, as well as a significant proportion of methicillin-resistant *Staphylococcus aureus*. Almost half of the isolates presented multidrug resistance, being highly resistant to commonly used empirical drugs but susceptible to carbapenems, colistin, vancomycin and linezolid. These results suggest the importance of the regular, institution-specific antibiogram update to direct empirical therapy, coupled with infection-prevention measures in the surgical wards and structured antimicrobial stewardship measures to maintain the efficacy of the last-resort antibiotics.

7. Recommendations

It is important to routinely update empirical antibiotic therapy for suspected SSI using the most recent, institution-specific antibiograms, not general or historical guidelines.

AAPs should be implemented to limit the use of carbapenems, colistin, vancomycin and linezolid

in culture-confirmed infections or where a culture-confirmed infection is highly likely to be present to ensure their susceptibility.

Infection precautions (such as surgical hand hygiene, sterile technique and environmental disinfection) need to be strengthened, especially on wards where there is a high rate of MDR organisms.

Surveillance culture and susceptibility testing should be conducted routinely as part of postoperative wound care for high-risk surgical patients (contaminated/dirty, emergency and diabetic).

Future studies of multicentres with molecular characterisation of resistance determinants are recommended to make better-informed regional and national AMR containment strategies.

Declarations

Written informed consent was obtained from all participants or their legal guardians.

Availability of data and materials: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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