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Aerobic bacterial profile and antibiotic susceptibility patterns of post-operative wound infections in a tertiary care hospital

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Abstract

Background: Post-operative wound infection has been a problem since surgery was started as a treatment modality and is the third most common cause of nosocomial infections with a reported incidence rate of 14-16%. This study aimed to investigate the prevalence, isolate and identify aerobic pathogenic bacteria from surgical site infections (SSI) and to determine the antibiotic susceptibility testing (AST) pattern of pathogenic bacteria.

Methods: This study was conducted at the Department of Microbiology SKIMS-Medical College, Bemina Srinagar, over a period of six months from November 2021 to April 2022. In the study, 210 samples from patients with SSI were included. Isolation, identification, and AST of the isolates were performed by standard microbiological techniques.

Results: Out of 210 SSI samples, 163 bacterial isolates were recovered and infection rate was more in 21-30 years age group (24.2%). Gram-negative bacteria were isolated in 50.4% (82/163) cases and *E. coli* was the most common organism (59.75%, 49/82). Gram-negative bacteria were sensitive to imipenem and none were resistant to polymyxin-B and colistin. *E. coli* was mostly resistant to cefoperazone, *Acinetobacter* and *Klebsiella species* were resistant to ceftazidime, *Pseudomonas* and *Citrobacter* were resistant to ceftriaxone. Gram-positive bacteria constituted 49.6% (81/163) and Methicillin-resistant *Staphylococcus aureus* (MRSA) was a frequently isolated species (66.6%, 54/81). MRSA- and methicillin-sensitive *Staphylococcus aureus (*MSSA*)* were mostly sensitive to amikacin, gentamycin, and tetracycline. Moreover, none of the Gram-positive isolates were resistant to linezolid, vancomycin, and teicoplanin. *Enterococcus spp* was mostly resistant to gentamycin.

Conclusion: This study developed an insight into post-operative wound infections and their incidence, organisms' prevalence, and their antibiogram. Culture positivity in suspected cases of SSI was high (77.6%). MRSA *and E. coli* were frequently isolated from Gram-positive and Gram-negative bacteria.

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Introduction

Post-operative wound infections, according to Centers for Disease Control and Prevention (CDC), are defined as infections related to an operative procedure that occurs at or near the surgical incision within 30 days of the procedure (Within 90 days if prosthetic material is implanted at surgery) (1).

Post-operative wound infections are a considerable universal problem in the field of surgery leading to many complications. Most post-operative wound infections are hospital-acquired and vary from one hospital to hospital (2). Post-operative wound infections can be fatal. They also cause financial distress to the patient and the hospital administration due to prolonged recuperation, prolonged post-surgical hospital stays, additional outlay, nursing care, and an unessential waste of time (3). The infected surgical wound can be marked by redness of skin, pain, warmth, swelling, tenderness and pus formation. The main risk factors responsible for post-operative wound infection are co-morbidity, substandard surgical procedures, pre-existing infection, and improper sterilization of surgical tools. Other factors include the virulence of the pathogens, physiological position of the wound, and the immune system of the patient (4). Post-operative wound infections or surgical site infections (SSIs) are the third most common cause of nosocomial infections with a reported incidence rate of 14-16% (5). Globally, post-operative wound infection rates have been reported from 2.5% to 41.9% (6). National Centre for Health Statistics and National Healthcare Safety Network suggested that 250,000 to 1 million patients develop post-operative wound infections out of 26.6 million national surgical procedures performed annually in the USA. The proportion of post-surgical wound infection differs from developed to developing countries due to differences in the quality of health set-up among the countries; for instance, in Nepal, the prevalence rate of post-operative wound infections ranged from 7.3 to 23% (4). In India, the overall incidence of wound infection ranges from 10-33% (7). Recently published papers reported the incidence of post-operative wound infections as 3.38% in Karamsad, 18.14% in Telangana, and 11.6% in Western Rajasthan (6). The estimated rate of incidence is 2-20%, even in modern hospital settings. For example, incidence rates of post-operative wound infections in different socio-economic level countries were 20.3% in Nigeria, 16% in India, and 14.7% in Japan (8). In Africa, post-surgical wound infections have been estimated to have an incidence as high as 27.9%. In Tanzania past studies have reported different rates, ranging from 35% in Muhimbili National Hospital to 19.1% in KCMC (9). The discovery of antiseptics and antibiotics plays an essential role in surgical procedures to be safe that was not believed to be possible before the discovery of antimicrobial agents. Bacteriological causes of post-operative wound infections differ according to geographical location, hospitals, wards, and type of surgical techniques (10). *Staphylococcus aureus* is the most commonly isolated bacteria in post-operative wound infections, followed by *E. coli*, *Enterococci*, coagulase-negative *Staphylococci* (CONS), *Pseudomonas spp*., multi-drug-resistant bacteria, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), metallo-beta-lactamase-producing *Pseudomonas aeruginosa*, and vancomycin-resistant *Enterococcus* (VRE). Extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* also plays a vital role in post-operative wound infections. Apart from this issue, *Acinetobacter* and methicillin-sensitive *Staphylococcus aureus* (MSSA) are also responsible for post-operative wound infections. The elevated level of morbidity and mortality rate plays an essential role in the growth of antimicrobial resistance (2,11). The patterns of resistance of microorganisms responsible for post-operative wound infections differ from country to country, depending on the region, local epidemiology reports and susceptibility testing strategy. Therefore, it is important to keep an eye on anti-microbial resistance rates continuously in medically significant pathogens for proper treatment required for multi-drug-resistant pathogens (12).

The aim of this study was to investigate the prevalence of post-operative wound infection, to isolate and identify aerobic bacteria from post-operative wound infections and to determine the antibiotic sensitivity pattern of pathogenic bacteria in a tertiary care hospital.

Methods

This study was conducted at the Department of Microbiology SKIMS-Medical College, Bemina Srinagar, over a period of six months from November 2021 to April 2022. A total of 210 clinical samples from pus swab/aspirated pus samples collected from patients suspected of surgical site infection were included in the study. Demographic and surgical ward information collected. Out of 210 samples, 163 (77.6%) were culture-positive and organisms isolated were stored in nutrient agar butts in duplicates placed at -80 ºC and ambient room temperature.

A loop full of a colony from preserved agar was inoculated into the BHI broth and incubated for 4-6 hours. The subculture from BHI broth was performed on 5% sheep blood agar (BA) and MacConkey agar (MA), and incubated at 37 ºC for 48 hours before being reported as sterile. Growth on culture Petri dishes was identified by its colony characteristics and the standard biochemical tests.

Antimicrobial sensitivity testing (AST) was carried out by the Kirby Bauer disc diffusion method where a loop full of a colony was emulsified in 0.85 w/v saline and vortex mixed. The turbidity of the suspension was adjusted to 0.5 MacFarland's solution. Using a sterile cotton swab, suspension was inoculated on Muller Hinton agar using the lawn technique. Antibiotic discs were placed as per recommendation from Clinical and Laboratory Standards Institute (CLSI) guidelines 2022 Palates were incubated at 37 ºC overnight and results were interpreted in accordance with Clinical Laboratory Standards Institute guidelines 2022. For Polymyxin B, Colistin, and Vancomycin, AST was done by microbroth dilution method in accordance with CLSI guidelines 2022 (13). Methicillin-resistance was checked by cefoxitin disc diffusion method using breakpoints reference from CLSI guidelines 2022 (13).

Results

Out of 210 samples, 163 (77.6%) were culture-positive and 47 (22.4%) were culture-negative. Among 163 culture-positive cases, 79.1%, 11.04%, 6.75%, and 3.06% of the cases were from general surgery, orthopedics, intensive care unit (ICU), and ophthalmology, respectively (Table 1). Out of 210 clinically diagnosed cases, 19 (9.04%) were infected in (0-10) age group, 28 (13.3%) were infected in (11- 20) age group, 51 (24.2%) infected cases were seen in (21-30) age group, 36 (17.1%) cases in (31-40) age group, 33 (15.7%) cases were affected in (41-50) age group, 25(11.9%) cases in (51-60) age group. Among the (61-70) age group, 13 (6.1%) infected cases were seen, and 5 (2.3%) infective cases were seen in the age group of 70 and above. Out of 210 samples, of clinically diagnosed cases, 114 (54.6%) were males and 96(45.4%) were female patients (Table 1).

Out of 210, clinically diagnosed cases, surgical site infection rate was more in 21-30 age groups (24.2%) and males were more infected by surgical site infection (54.6%).

Out of 163 bacterial isolates, post-operative wound infections in general surgery had more isolates of *E. coli* and MRSA. *Staphylococcus aureus* infections, including both MRSA and MSSA, were more common in orthopedics. *Acinetobacter spp* were frequently isolated from ICU and in ophthalmology MRSA was most commonly isolated. The isolation rate of Gram-negative bacteria (50.4%) and Gram-positive bacteria (49.6%) did not show much difference. Among all 82(50.4%) Gram-negative isolates E. *coli* was the most common 49(59.75%) followed by *Acinetobacter spp* 13(15.85%) and *Pseudomonas spp* 10(12.2%). In addition, *Klebsiella spp* were 7(8.5%), *Citrobacter* were 2(2.4%), and *Proteus mirabilis* was 1(1.22%). Out of 81(49.4%) Gram-positive organisms, MRSA was the most common organism isolated i.e., 54(66.6%) followed by MSSA 22(27.2%) and *Enterococcus spp* 5 (6.2%) (Figure 1).

Polymyxin B and Colistin showed 100% sensitivity among Gram-negative bacteria. All of the Gram-negative bacteria showed moderate to higher sensitivity to imipenem. The most common Gram-negative bacterial isolate was *E. coli,* which was resistant to cephalosporins. *Klebsiella spp* showed resistance to quinolones, cephalosporins, and ureidopenicillins. *Proteus spp* were mostly sensitive to Aminoglycosides followed by cephalosporins, carbapenems, and ureidopenicillins Pseudomonas showed susceptibility to Aminoglycosides, carbapenems, Polymyxins B, Colistin, and ureidopenicillins. Citrobacter showed susceptibility to Polymyxins B, Colistin, and ceftazidime (Table 2).

Table 1. Correlation between bacterial isolates and infected cases in various surgical units. Infections due to Gram-positive and Gram-negative organisms were more common in general surgery cases

WARDS	Cases No. (%)	Aerobic bacterial isolates		Total No. (%)
		Gram-Positive No. (%)	Gram-Negative No. (%)	
Surgery	174 (82.9)	65 (50.34)	64 (49.6)	129 (79.1)
Orthopedics	19 (9.05)	11 (61.1)	7 (38.9)	18 (11.04)
ICU	11 (5.24)	1 (9.09)	10 (90.9)	11 (6.75)
Ophthalmology	6 (2.9)	4 (80)	1 (20)	5 (3.06)
Total	210	81 (49.6)	82 (50.4)	163 (100)

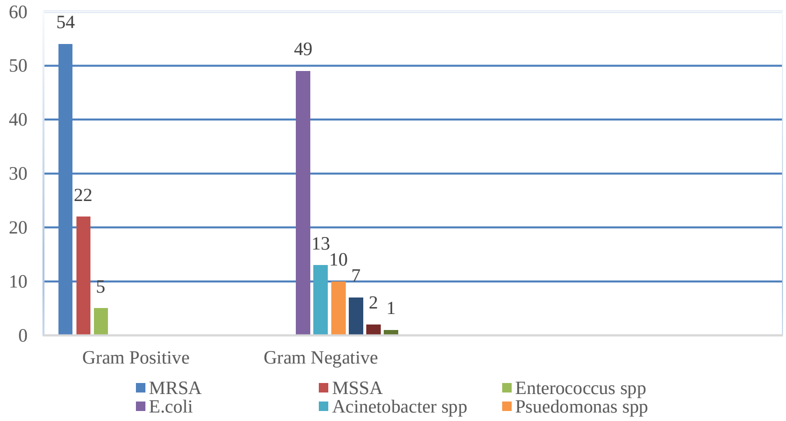

Figure 1. Distribution of Gram-positive and Gram-negative isolates

Table 2. Susceptibility pattern of Gram-negative isolates

Antibiotics	Bacteria					
	E. Coli	*Acinetobacter spp*	*Pseudomonas spp*	*Klebsiella spp*	*Citrobacter spp*	*Proteus Mirabilis*
Amikacin (AK)	90%	10%	66%	71%	50%	85%
Gentamycin (G)	83%	15%	71%	60%	50%	80%
Ciprofloxacin (CIP)	38%	10%	50%	35%	-	20%
Ceftazidime (CAZ)	45%	5%	30%	0%	70%	70%
Cefoperazone (CPZ)	8%	10%	35%	20%	-	30%
Ceftriaxone (CTR)	20%	8%	-	25%	0%	60%
Imipenem (IMP)	63%	40%	70%	66%	50%	70%
Tobramycin (TOB)	86%	16%	42%	45%	30%	26%
Polymyxin B (PB)	100%	100%	100%	100%	100%	-
Colistin (CL)	100%	100%	100%	100%	100%	-
Piperacillin Tazobactam (PIT)	60%	10%	85%	15%	-	60%

Discussion

Patients were divided into seven age groups and post-operative wound infections. In this regard, SSIs were found to be more common in 21-30 age group, which showed concordance with a similar study conducted by Gayathree Naik et al., with a higher rate of SSIs in 20-30 age group. The higher incidence rate of SSIs in this age group may be due to higher percentage of patients admitted for surgical interventions belonging to the same age group (14). Another similar study conducted by Patel Sachin et al., found higher rate of SSIs in patients above 50 years of age which were associated with various factors such as malnutrition, reduced immunity, and malabsorption (15).

While studying the SSI in various units it was found that SSI rate was higher in general surgery unit 129 (79.1%) compared to orthopedics 18 (11.04%), ICU 11 (6.75%), and ophthalmology 05 (3.06%). *Staphylococcus aureus* was the most common organism isolated in orthopedics and *E. coli* was mostly isolated from general surgery. Similar results were observed in studies conducted by Gayathree Naik et al., Brian Mawalla et al., and Saravanakumar, R et.al; who showed high rates of *Staphylococcus aureus* infections in orthopedic cases and *E. coli* from general surgery, respectively (14). The culture-positivity rate of (77.6%) showed concordance with other similar studies where positivity rate of 72% and 82.36% was recorded Gayathree Naik et al (14).and Lilani SP et al (16). The culture-negative rate of 22.4% may be due to intake of antibiotics prior to sample collection or presence of fastidious/anaerobic bacteria in the samples.

In our study, the isolation percentage of Gram-negative organisms (50.4%) and Gram-positive (49.6%) organisms were observed. Among the Gram-positive bacteria *Staphylococcus aureus* (93.8%), both MSSA and MRSA, were predominately isolated. Similar results were found in other studies also. Lilani et al. (16) Chia JYH et al. (17) Garibaldi Richard et al. (18) Jido et al. (19). Giacometti et al. (20) Shekhar pal et al. (21) and SP Chakraborty (22). Among the Gram-negative bacilli, *E. coli* (59.75%) was the most common isolated organism. The higher isolation rate of *E. coli* from general surgery showed concordance with other similar studies conducted by Brian Mawalla et al. (23). which may be attributed to laparotomy surgery done for most of the cases and a possible source could be the colonization of Enterobacteriaceae in the bowel and intestines.

All Gram-positive isolates (MRSA, MSSA and Enterococcus) were 100% sensitive to Vancomycin, Linezolid, and Teicoplanin (Table 3). A similar degree of sensitivity was seen in other studies conducted by Deboral et al. (12). Slochana Khatiwada et al. (24). Afzalunnessa Binte Lutfor et al. (25). and Ying Jain (26). All Gram-negative isolates were 100% susceptible to polymyxin-B and colistin (Table 3). With moderate to higher sensitivity for imipenem. *E. coli* showed resistance against cefoperazone. *Acinetobacter* and *Klebsiella spp* were mostly resistant to ceftazidime, *Pseudomonas,* and *Citrobacter* were the least sensitive to ceftriaxone, which correlates with the studies conducted by Slochana Khatiwada et al. (24). Deboral et al (12). Naz et al. (1). and Micheal J Trimble et.al. (27). Thus, Gram-positive organisms were mostly sensitive to Linezolid, Vancomycin, and Teicoplanin, whereas Gram-negative organisms showed greater susceptibility to Polymyxin-B and Colistin.

Table 3. MRSA and MSSA showed 100 % susceptibility to vancomycin and Linezolid. There was a significant difference in macrolides' susceptibility among MRSA and MSSA. Enterococcus species showed 80 and 85% susceptibility to doxycycline and Ampicillin

Antibiotics	Bacteria		
	MRSA	**MSSA**	***Enterococcus Spp***
Vancomycin (VA)	**100%**	**100%**	**100%**
Linezolid (LZ)	**100%**	**100%**	**100%**
Amikacin (AK)	**90%**	**100%**	**80%**
Gentamycin (GEN)	**60%**	**100%**	**50% (HLG)**
Tetracycline (TE)	**96%**	**100%**	**85%**
Teicoplanin (TEI)	**100%**	**100%**	**100%**
Amoxycillin and Clavulanic acid	**0 %**	**26 %**	**-**
Penicillin	**0**	**22 %**	**10**
Cefazolin	**0**	**82**	**-**
Clindamycin	**25**	**82**	**-**
Erythromycin	**35**	**65**	**25**
Azithromycin	**40**	**75**	**-**
Doxycycline	**55**	**85**	**80**
Ampicillin	**-**	**-**	**85**

Conclusion

This study developed an insight into post-operative wound infections and their incidence, organisms' prevalence and their antibiogram. The steps must be included in the hospital infection control policy to avoid SSIs, which include periodical training of healthcare workers about hand hygiene and other infection control measures like sanitization of operation theaters and hospital wards. Appropriate antibiotic policies and infection control measures must be imposed and supervised regularly in each hospital to prevent the emergence of antibiotic-resistant strains, which is an emerging global challenge of large proportion.

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Ethical statement

The ethical approval was not required as the study did not involve direct interaction with human participants or animals. The study is a basic life science study, which has been conducted on achieved isolates.

Conflicts of interest

All authors declare no conflicts of interest.

Author contributions

Arshi Syed conceived and designed the method. Syed Majid Ali and Azhar Shafi implemented the algorithm. Jalila Qayoom and Talat Masoodi analyzed the data. Jalila Qayoom and Syed Majid Ali wrote the manuscript. All authors read and approved the final manuscript.

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