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BACTERIOLOGICAL PROFILE AND DRUG RESISTANCE OF SURGICAL SITE INFECTION IN A TERTIARY CARE HOSPITAL – A PROSPECTIVE STUDY

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ABSTRACT **Background–** Surgical site infections are an important cause of health care associated infections among surgical patients and needs special attention. Thus, the purpose of this study is to determine the prevalence of Surgical site Infection in surgery ward and determine the bacteriological profile and antimicrobial susceptibility pattern of the isolate. **Materials & Methods:** A prospective observational study was conducted from January 2021 to June 2022. During study period, 375 specimens received from surgical site infections were processed for isolation and identification of bacterial pathogens according to the standard microbiological techniques. **Results:** Surgical site infections rate in surgery ward was found to be 26.04%. It was observed that maximum number of patients were from 41-60 years age group and male, out of 375 suspected samples, 184 of them were culture positive for pathogenic organisms. Methicillin resistant Staphylococcus aureus was the most common isolate followed by Escherichia coli. **Conclusion:** Methicillin resistant Staphylococcus aureus was the most common isolate followed by Escherichia coli. Proper infection control practices, appropriate pre-operative preparation of the patient and antimicrobial prophylaxis can reduce SSIs.

KEYWORDS : Surgical site infection, Prevalence, Antibiotics Susceptibility, Bacterial isolates, Multi-drug resistance

INTRODUCTION

Surgical Site Infections (SSIs) are known to be one of the most common causes of health care associated infections worldwide specially in low- and middle-income countries like India1. Globally, surgical site infection rates have been reported ranging from 2.5% to 41.9%.2 In India, the rate of surgical site infections ranges from 4 to 30%.[3]

Surgical site infections are defined as "infections that develop at the surgical site within 30 days of surgery (within 90 days for breast, cardiac and joint surgeries)." They are categorized into 3 levels - superficial incisional, deep incisional, and organ or space infection.[4] The most frequently identified pathogens are *Staphylococcus aureus,* Coagulase-negative *Staphylococci, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa*.[5]

Surgical site infections due to multi drug resistant isolates are at heightened risk for mortality, morbidity, longer hospital stays, and catastrophic health expenditures. Over the past decade, Gram-positive bacteria, such as methicillin resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci have attracted attention in the battle against multi drug resistant (MDR) microorganisms. In addition, MDR Gram-negative bacilli such as extended spectrum-β-lactamases (ESBL) and metallo-β-lactamases (MBLs) -producing Enterobacterales have become a growing problem.[4]

METHODOLOGY– This prospective observational study was conducted in the microbiology department of Gajra Raja Medical College, Gwalior in association with the surgery department after prior approval from the institutional ethics committee. All suspected SSI cases with clean and clean contaminated wound admitted in post operative ward at surgery department, Gajra Raja Medical College during 18 months study period (January2021 - June 2022) were included in the study after taking informed consent from the patients. Non-surgical wounds, stitch abscesses, burn wounds and contaminated dirty wounds were excluded from the study. A predesigned and semi-structured questionnaire was used for collection of sociodemographic and clinical characteristics of the patients.

Sample collection and microbiological processing

Two pus samples using sterile swab sticks: one for Gram staining and the other for culture. The Gram-stained smears were examined under oil immersion on a microscope to determine the presence, type, and relative number of bacteria. The samples were then inoculated on blood agar, MacConkey agar, and nutrient agar, and incubated aerobically at 37°C for 24 hours. The plates were checked the next day, and if there was no bacterial growth, incubation was extended to 48 hours. Isolated colonies were identified based on colony morphology, Gram staining, and standard biochemical tests.[6]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by the Kirby- Bauer disc diffusion method on Muller Hinton agar plates and the interpretation of results was done as per the recommendations of the Clinical and Laboratory Standards Institute (CLSI) Guidelines.[7] The bacterial isolates which were found to be resistant to any agent from three or more classes of antibiotics were considered as multidrug resistant.

Data analysis Data was entered in to Microsoft excel spread sheet and analysed by SPSS (trial version). Frequencies and cross-tabulations were used to summarize descriptive statistics. Appropriate test of significance was applied wherever necessary.

RESULTS-

A total number of 1440 surgeries were conducted during the study period. Out of these 375 cases developed surgical site infection clinically. The prevalence of surgical site infections in surgery ward was found to be 26.04%.

Table -1 Sociodemographic and clinical data of study participants

Variable		Bacterial Isolate			
		Positive(n=184)	Negative(n=191)	Total(n=375)	
Age	< 20	4 (2.17%)	12 (6.28%)	16 (4.26%)	0.001
	21-40	24 (13.04%)	75 (39.26%)	99 (26.4%)	
	41-60	145 (78.80%)	84 (43.97%)	229 (61.06%)	
	61-80	10 (5.43%)	18 (9.42%)	28 (7.46%)	
	>80	1 (0.54%)	2 (1.04%)	3 (0.8%)	
Gender	Male	131 (71.19%)	128 (67.01%)	259 (69.06)	0.38
	Female	53 (28.80%)	63 (32.98%)	116 (30.93%)	

Co-morbidities	Present	154 (83.69%)	96 (50.26%)	250 (66.66%)	0.0001
	Absent	30 (16.30%)	95 (49.73%)	125 (33.33%)	
Wound	Clean	49 (26.63%)	120 (62.82%)	169 (45.06%)	0.0001
	Clean contaminated	135 (73.36%)	71 (36.97%)	206 (54.9%)	
Type of surgery	Elective	63 (34.2%)	95 (49.7%)	158 (42.1%)	0.023
	Emergency	121 (65.76%)	96 (50.2%)	217 (57.8%)	

Sociodemographic and clinical data

As per table 1, in this study it was observed that maximum number of patients were males and were from the age group of 41-60 years. Mean age of the participants was 44.62±11.30 years. Many patients were suffering from some kind of co-morbidity (66.6%) at the time of their surgery. In the present study, SSIs were more commonly seen in emergency surgeries accounting for 57.8% of the total cases.Out of 375, 184(49.06%) were culture positive, while the rest, 191(50.9%), were culture negative. Out of a total of 184 bacterial isolates, 124(67.39%) were Gram-negative, 90(32.61%) were Gram-positive bacteria.

Bacteriological Profile of SSIs

As shown in figure 1 out of 184 positive samples maximum number of isolates were Methicillin Resistant Staphylococcus aureus that is 54(29.35%), followed by Escherichia coli 43(23.37), Klebsiella pneumoniae 31(16.85%) and Pseudomonas aeruginosa 22(11.96%) respectively. Least number of samples showed growth of Enterobacter spp and Streptococcus spp.

Table No 02 Antibiotic resistance profile of Gram-positive bacteria isolates from surgical sites infection

Antibiotics tested	Gram Positive Bacteria		
	Methicillin Resistant Staphylococcus aureus(n=54)	Methicillin Sensitive Staphylococcus aureus(n=5)	Streptococcus spp(n=1)
Amoxicillin Clavulanate	31(57.41%)	3(60%)	0(0%)
Erythromycin	25(46.30%)	1(20%)	0(0%)
Levofloxacin	14(25.93%)	2(40%)	0(0%)
Cotrimoxazole	11(20.37%)	1(20%)	0(0%)
Doxycycline	13(24.07%)	0(0%)	0(0%)
Vancomycin	0(0%)	0(0%)	0(0%)
Cefoxitin	54(100%)	0(0%)	1(100%)
Cephalexin	20(20.37%)	1(20%)	0(0%)
Clindamycin	19(35.19%)	1(20%)	0(0%)

Table no -2 shows antibiotic resistance profile of gram-positive bacterial isolates from surgical sites infections and it was observed that maximum in vitro resistance was observed against amoxicillin clavulanate that is 57.41% (n=31) for Methicillin Resistant Staphylococcus aureus and 60% (n=3) for Methicillin Sensitive Staphylococcus aureus. Vancomycin was observed to be the most effective antimicrobial agent in vitro against all gram-positive isolates.

Table No 03 Antibiotic resistance profile of gram-negative bacteria isolates from Surgical Site Infections

Antibiotics tested	Gram Negative Bacteria						
	Escherichia coli (n=43)	Klebsiella pneumoniae (n=31)	Pseudomonas aeruginosa (n=22)	Citrobacter freundii (n=18)	Proteus mirabilis(n=5)	Acinetobacter baumanii S(n=3)	Enterobacter spp(n=2)
Amoxicillin Clavulanate	31 (72.09%)	22 (70.97%)	19 (86.36%)	9 (50%)	3 (60%)	3 (100%)	0 (0%)
Levofloxacin	9 (20.93%)	9 (29.03%)	8 (36.36%)	5 (27.78%)	3 (60%)	1 (33.3%)	0 (0%)
Cotrimoxazole	8 (18.60%)	5 (16.13%)	8 (36.36%)	3 (16.67%)	3 (60%)	1 (33.3%)	0 (0%)
Doxycycline	4 (9.30%)	5 (16.13%)	1 (4.55%)	3 (16.67%)	2 (40%)	1 (33.3%)	1 (50%)
Amikacin	4 (9.30%)	1 (3.23%)	0 (0%)	7 (38.89%)	1 (20%)	1 (33.3%)	0 (0%)
Imipenem	9 (20.93%)	8 (25.81%)	4 (18.18%)	3 (16.67%)	0 (0%)	0 (0%)	0 (0%)
Ceftriaxone	17 (39.5%)	13 (41.9%)	8 (36.3%)	8 (44.4%)	1 (20%)	1 (33.3%)	0 (0%)
Piperacillin Tazobactam	13 (30.23%)	9 (29.03%)	6 (27.27%)	5 (27.78%)	2 (40%)	0 (0%)	0 (0%)

As per table no 3 Antibiotic Resistance profile of Gram-negative bacterial isolates from surgical sites infections, it was observed that maximum resistance was observed against Amoxicillin Clavulanate i.e. 72.09% (n=31) for E. coli, 70.97% (n=22) for Klebsiella pneumoniae and 86.36% (n=19) for Pseudomonas aeruginosa. Least resistance was observed against Doxycycline and Amikacin.

Table No. 04 Multidrug resistance pattern of bacterial isolates

Bacterial Isolates	Resistance Pattern						
	R0	R1	R2	R3	R4	>R5	Total
GRAM POSITIVE BACTERIAL ISOLATES							
Methicillin Sensitive Staphylococcus aureus	2 (40%)	2 (40%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	5 (100%)
Methicillin Resistant Staphylococcus aureus	0 (0%)	10 (18.5%)	36 (66.6%)	6 (11.1%)	1 (1.85%)	1 (1.85%)	54 (100%)
Streptococcus spp	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Grand Total	2	13	37	6	1	1	60
GRAM NEGATIVE BACTERIAL ISOLATES							
Escherichia coli	4 (9.3%)	16 (37.2%)	18 (41.8%)	3 (6.9%)	1 (2.3%)	1 (2.3%)	43 (100%)
Klebsiella pneumoniae	1 (3.23%)	10 (32.2%)	15 (48.3%)	4 (12.9%)	0 (0%)	1 (3.2%)	31 (100%)
Pseudomonas aeruginosa	1 (4.55%)	7 (31.8%)	7 (31	4 (18.18%)	3 (13	0	22 (100%)
Citrobacter freundii	0 (0%)	5 (27.7%)	5 (27.7%)	5 (27.7%)	2 (11.1%)	1 (5.5%)	18 (100%)
Proteus mirabilis	1 (20%)	1 (20%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	5 (100%)
Acinetobacter baumanii	0 (0%)	0 (0%)	2 (66.6%)	0 (0%)	0 (0%)	1 (33.3%)	3 (100%)
Enterobacter spp	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
Grand Total	7	41	50	16	6	4	124

*R*0: no resistance, *R*1: resistance to one drug, *R*2: resistance to two drugs, *R*3: resistance to three drugs, *R*4: resistance to four drugs, ≥*R*5: resistance to five or more drugs As per table 5 multiple drug resistance (resistance ≥ 3 drugs) was observed in 18.4% bacterial isolates. More than 75% MRSA isolates demonstrated evidences of resistance to two or more than two antibiotics. Two isolates of MSSA were susceptible

to all the antibiotics tested. Overall, more than 50% Gram negative isolates demonstrated evidence of resistance to two or more than two antibiotics. Pan resistance was observed in one bacterial isolate of *E. coli*.

DISCUSSION

Postoperative SSIs is one of the most significant sources of morbidity among patients who have undergone surgery. These patients have higher hospital expenditures due to longer stay in hospital and and are in need of more nursing and wound care. So,the identification of bacterial pathogens and the selection of appropriate antibiotics that are effective against the organism is crucial for the effective management of surgical site infections. In current study prevalence of surgical site infections in surgery ward was 26.04% which is similar to studies done in chhatisgarh and ethiopia while contrast to studies done in West India and Ranchi.[8,9,10,11] Difference in magnitude of SSIs can be due to difference in types of surgeries, infection control practices, hand hygiene habits and other contributory social and environmental factors. As earlier studies, SSIs is predominantly reported in males and 41-60 years age group.[10, 12,13,14] In current study it was observed that prevalence of surgical site infections was more in emergency surgeries 217 (57.8%) which reflects on the fact that the type of surgery is a significant factor in development of SSIs.[13,15] Higher SSI rate in emergency surgery can be caused by a confluence of factors including poor general health, insufficient time for preparation, and operations performed on contaminated areas.

In this study 49.06% bacterial culture positivity was observed. The isolation rate of Gram-negative bacteria 124(67.39%) was more than Gram positive bacteria 60(32.61%) which is like a study of Ethiopia and in contrast to a study conducted in Nepal.[9, 13] The possible reason for disparity in the studies could be attributed to variances in the populations investigated, diversity of surgical procedures performed on the study participants, as well as timing of specimen collections.

In many studies *Staphylococcus aureus* has been found to be the leading pathogen in post-surgical wound infections followed by the members of the enterobacteriaceae [12,16,17,18,19] which was also seen in the bacteriological profile of current study i.e. Methicillin Resistant *Staphylococcus aureus*(29.35%) followed by *E.coli*(23.27%), *Klebsiella pneumonia*(16.85%) and *Pseudomonas aeroginosa*(11.96%), *Citrobacter freundii*(9.78%), *Proteus mirabilis*(2.7%), MSSA (2,7%), *Acinetobacter baumannii*(1.6%), *Enterobacter spp* (1.09%) and *Streptococcus* spp (0.54%).

In present study, all gram-positive isolates (*MRSA, MSSA, Streptococcus spp*) were showing in vitro susceptibility to vancomycin. *Staphylococcus aureus* exhibited marked resistance to amoxicillin (57.4%) and erythromycin (46.3%), which might be due to the fact that these antibiotics are widely prescribed for the treatment of various infections in our region while least resistance were reported for doxycycline and cotrimoxazole. Similar findings were also seen in previous studies[12,19,20]

Resistance to Amoxicillin Clavulanate was observed in Gram-negative bacterial isolates like *Klebsiella pneumoniae* (86.36%), *Pseudomonas aeroginosa*(72.09%) and *Escherichia. coli*, (70.97%) which was also observed by Mengistu Abayneh et al in Southwest Ethiopia.[21] In current study least resistance observed for Doxycycline, Imipenem and Amikacin which is similar to study done in Ethiopia and North –East India,[15,19] while contrast to Mengistu Abayneh et al study.[21] Another matter of concern is the emergence of non-fermenters like *P.aeruginosa*, *Acinetobacter baumanii* as causative agents of SSI as they are intrinsically resistant to many antibiotics. In this study, these isolates showed complete in vitroresistance to Amoxycillin-clavulanic acid and azithromycin. One out of three isolates of *Acinetobacter baumanii* resistant to all antibiotics except Imipenem and Piperacillin and Tazobactam.

The work in this study has reiterated the need for management strategies designed for specific group of patients with certain co-morbidities and infections in order to improve therapeutic benefits, cost-effectiveness, and reduction in the incidence of antibiotic resistance Appropriate prophylactic use of suitable antibiotics and aseptic wound management along with proper infection prevention practices will reduce the chances of surgical site infections. Regular monitoring and rational use of antimicrobial drugs are necessary to ensure effective therapy for wound infections and to decrease or stop the emergence of multidrug-resistant bacteria.

CONCLUSION

The incidence of SSIs was more common in emergency surgeries and in males of middle to late adulthood. Among the culture positive cases Gram negative bacteria were more common than Gram positive bacteria. Methicillin Resistant *Staphylococcus aureus* was the most common Gram-positive organism isolated and *Escherichia coli* was the most common Gram-negative organism isolated. Many of the bacterial isolates were multi drug resistant. This study recommends performing continued surveillance of SSIs and generation of empirical treatment and prophylaxis protocols based on common microorganisms and their resistance patterns. Implementation and meticulous adherence to infection control practices and rational use of antibiotics is the need of hour for reduction of surgical site infections rates and associated complications.

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