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Original Research Article

Assessment of Causative Bacterial Agents and Their Antimicrobial Susceptibility Pattern to Surgical Site Infections

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Conflict of interest: Nil

Abstract:

Background: Surgical site infections (SSIs) are infections that occur at or near a surgical incision site. The present study was conducted to assess causative bacterial agents and their antimicrobial susceptibility patterns to surgical site infections.

Materials & Methods: During the period of two years in current study, total Surgeries done in institute as Emergency and Elective surgery was 4504 Major cases and 7092 minor cases of both genders. Minor cases were excluded from study as they were discharged within 24 hours. From those who developed SSI during 30 days of follow up specimen was collected for bacteriological analysis. 878 cases (out of 4504 Major cases) with clinically diagnosed SSIs were taken into consideration in the current study. Samples were collected using sterile cotton swabs from all patients. Antimicrobial susceptibility testing was done using modified Kirby-Bauer disc diffusion method.

Results: Age of patients range 20-70 years (mean age = 41.5± 12 years). The mean age of the patients was 41.5± 12 years (range 20 to 70 years) and the peak incidence of SSI was observed in age group <50 years (57.14%). Out of 878 suspected samples, 430/878 (48.98%) were gram stain positive, culture positive and 378/878 (43.05%) were gram stain negative, culture negative. The remaining 70/878 (7.97%) samples showed organisms in Gram stain but no growth on aerobic culture which might be due to prior antibiotic use or anaerobic and non-fastidious infectious aetiology.

Conclusion: The majority of the isolates from surgical sites in the study area were gram-negative bacteria. Escherichia coli were the most frequent Gram-negative bacteria that caused surgical site infections.

Keywords: Antimicrobial, *Escherichia coli*, Surgical Site Infections.

Introduction

Surgical site infections (SSIs) are infections that occur at or near a surgical incision site. These infections can involve the skin, underlying tissues, or organs and can occur after any type of surgery. SSIs can lead to complications, prolonged hospital stays, and increased healthcare costs [1].

They bear the blame for elevated treatment expenses, prolonged hospital stays, and notable rates of morbidity and mortality [2]. Even at hospitals with the most up-to-date amenities, surgical site infections (SSIs) remain a serious issue despite technological advancements in infection control and surgical techniques. Exogenous and/or endogenous microorganisms that infiltrate the surgical site during the procedure (primary infection) or afterwards (secondary infection) are typically the source of these illnesses. Primary infections typically manifest five to seven days after surgery and are more dangerous [3].

The most common microorganism cultured from SSIs is *Staphylococcus aureus*. When a viscus, such as the large bowel, is opened, tissues are likely to be contaminated by numerous organisms [4]. For example, Enterobacteriaceae and anaerobes can cause SSI after colorectal surgery. The presence of a foreign body from prosthetic surgery reduces the number of pathogenic organisms required to cause SSI [5].

Aims and Objectives: The present study was conducted to assess causative bacterial agents and their antimicrobial susceptibility pattern to surgical site infections.

Materials and Methods

Study Area and Period: The present cross-sectional observational study was conducted at the Department of General Surgery in collaboration with the Department of Microbiology at Nalanda Medical College & Hospital, Patna, Bihar, India, for a period of two years (July 2021–June 2023). The Institutional Ethics Committee gave the study its approval.

Study Design and Population: During the period of two years in current study, total Surgeries done in institute as Emergency and Elective surgery was 4504 Major cases and 7092 minor cases of both genders. Minor cases were excluded from study as they were discharged within 24 hours. All the eligible patients were followed for 30 days for the occurrence of SSI. From those who developed SSI during 30 days of follow up specimen was collected for bacteriological analysis. 878 cases (out of 4504 Major cases) with clinically diagnosed SSIs were taken into consideration in the current study. Pus samples were collected using two sterile cotton swabs from 878 clinically suspected cases having SSIs and were processed as per standard microbiological techniques i.e. gram stain, aerobic culture and antimicrobial susceptibility testing.

Antimicrobial susceptibility testing was done using Kirby-Bauer disc diffusion method and following CLSI guidelines.

Inclusion Criteria

- Patients of both sex, age ≥18 years, who had surgical wound pus discharge, with serous or ser
- opurulent discharge and with signs of sepsis present concurrently (warmth, erythema, induration, tenderness, pain, raised local temperature).
- Exudate and swab samples of patients received with suspected Surgical Site Infection were included.

Exclusion Criteria

- Patients who had suture abscesses, wounds with cellulitis and no drainage were excluded from the study.
- Infected Burns.

Sampling Size Determination and Sampling Technique: The following simple formula would be used for calculating the adequate sample size in prevalence study

$N= Z^2 P (1-P)/d^2$

N= sample size, Z= level of confidence, P= prevalence, d= Absolute error or precision

Z = Is standard normal variate (at 5% type 1 error ($P< 0.05$) it is 1.96 and at 1% type 1 error ($P<0.01$) it is 2.58). As in majority of studies P values are considered significant below 0.05 hence 1.96 is used in formula. p = Expected proportion in population based on previous studies or pilot studies.

d = Absolute error or precision

The sample size was calculated using a single population proportion formula, by considering, 95% confidence level, a 5% margin of error, and a 17.8% estimated proportion of overall rate of surgical site infections among patients who underwent surgery in Uttarakhand State, India [6];

Sample size = $1.96^2 \times 0.178\ (1-0.178)/0.05^2$

=226.83

Considering 10% non-response rate, the total minimum sample size for study was 250 patients.

In present study, we included 878 clinically suspected cases of having SSIs in the surgical wards.

A detailed history regarding age, sex, type of illness, diagnosis, type and duration of surgery performed, antibiotic therapy and the associated co-morbid diseases was obtained from the patients. Data such as name, age, gender, type of illness, diagnosis, type and duration of surgery performed, antibiotic therapy and the associated co-morbid diseases was obtained from the patients, was recorded. Samples were collected using sterile cotton swabs from all patients having clinically suspected SSIs and were processed as per standard microbiological techniques. All the pus samples or wound swabs of clinically suspected SSIs cases were received in the Department of Microbiology, Nalanda Medical College and Hospital, Patna, Bihar. These samples were subjected to direct microscopic examination by Gram stain and inoculated onto nutrient agar, 5% sheep blood agar and Mac Conkey agar using a sterile bacteriological loop. Plates were incubated aerobically at 37°C for 24 hours and if there was no growth, they were incubated for another 48 hours. Antimicrobial susceptibility testing was done using modified Kirby-Bauer disc diffusion method.

Statistical Analysis: statistical analysis was performed on the obtained data by using SPSS version 22.0 (IBM Corp., 2016), and Microsoft 16. P value < 0.05 was considered significant.

Results

A total of 4,504 surgeries were performed during the study period in the department of General Surgery. Out of these 878 clinically suspected SSIs samples were taken and sent to the Microbiology laboratory for Bacterial identification and Antibiotic susceptibility testing. Age of patients range 20-70 years (mean age = 41.5± 12 years). The mean age of the patients was 41.5± 12 years (range 20 to 70 years) and the peak incidence of SSI was observed in age group <50 years (57.14%).

Out of 878 suspected samples, 430/878 (48.98%) were gram stain positive, culture positive and 378/878 (43.05%) were gram stain negative, culture negative. The remaining 70/878 (7.97%) samples showed organisms in Gram stain but no growth on aerobic culture which might be due to prior antibiotic use or anaerobic and non-fastidious infectious aetiology.

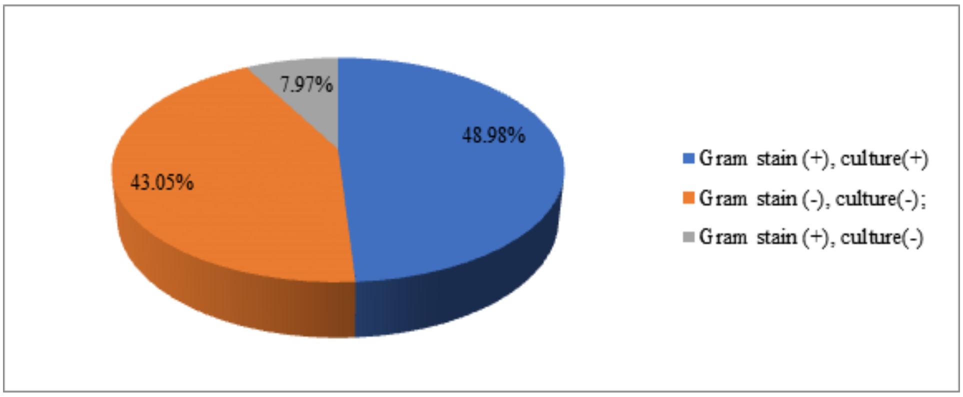


Figure 1: Correlation of Gram stain and Culture

Incidence of SSIs: In the present study the overall rate of clinically suspected cases of having SSI was 19.5%.

Table 1: Overall rate of SSIs

Variables	No. of patients (%), n=4504
Clinically suspected SSIs cases	878 (19.5%)
Without SSI	3626 (80.5%)

Table 2: Socio-demographic characteristics of study participants

Variable	Category	SSIs (n=878)	Without SSIs (n=3,626)	P value
Gender	Male	544 (61.96%)	1,695 (46.75%)	0.02
	Female	24(38.04%)	1,931 (53.25%)	
Age (Years)	<50	376 (42.82%)	1,236 (34.09%)	0.83
	≥50	502 (57.18%)	2,390 (65.91%)	
Residence	Rural	613 (69.82%)	2,709 (74.71%)	0.41
	Urban	265 (30.18%)	917 (25.29%)	
ASA score	<3	753(85.76%)	3,556(98.07%)	0.001
	≥3	125 (14.24%)	70 (1.93%)	
Comorbidity	Yes	153 (17.43%)	722 (19.91%)	0.03
	No	725 (82.57%)	2,904 (80.09%)	
Received antibiotic prophylaxis	yes	711 (80.98%)	3,056 (84.28%)	0.50
	No	167 (19.02%)	570 (15.72%)	
Duration of surgery	<1 hour	376 (42.82%)	1,445 (39.85%)	<0.001
	≥1 hour	502 (57.18%)	2,181 (60.15%)	
Urgency of surgery	Routine	154 (17.54%)	1,459 (40.24%)	0.001
	Emergency	724 (82.46%)	2,167 (59.76%)	

ASA: American Society of Anaesthesiologists

The significant factors for SSI development were sex, ASA Score, duration of surgery, timing of surgery while age, residence and antibiotic prophylaxis were not significantly associated with the development of differences. Nearly three-fourths of patients were from rural areas. More than two-thirds of surgical procedures were emergent. 17.43% of patients were presented with one or more co-morbidities, as shown in Table 2.

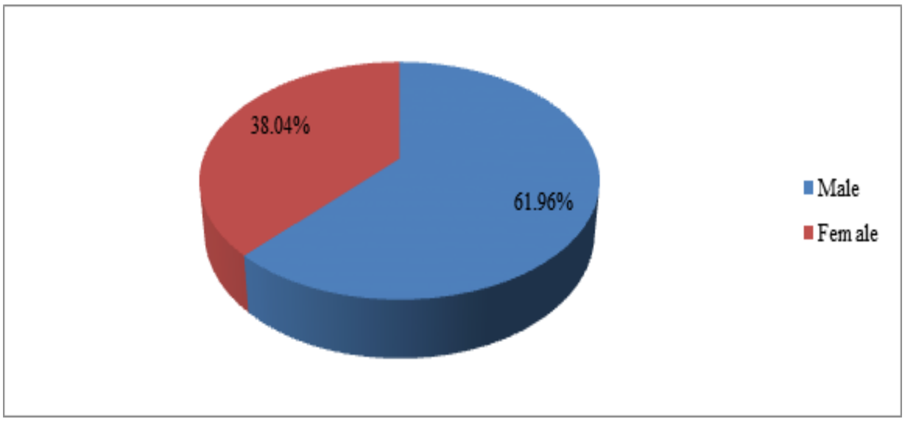


Figure 2: Gender wise distribution of SSIs

Table 3: Bacterial pathogens from patients with SSIs having gram stain (+), culture (+); (n=430/878)

Bacteria isolates (n=430)	Organisms	Frequency (%)	P value
Gram-positive isolates 115/430 (26.74%)	*Staphylococcus aureus*	62 (14.42%)	0.01
	Coagulase-negative Staphylococcus	37 (8.60%)	
	Enterococcus species	16 (3.72%)	
Gram-negative isolates 315/430 (73.26%)	*Escherichia coli*	120 (27.91%)	
	Pseudomonas aeruginosa	70 (16.28%)	
	Klebsiella species	58 (13.49%)	
	Proteus species	35 (8.14%)	
	Enterobacter species	19 (4.42%)	
	Citrobacter species	13 (3.02%)	

Identified bacterial isolates from patients who developed SSI (n = 878), wound swabs, or pus aspirates were collected. Out of 878 suspected samples, 430/878 (48.98%) were Gram stain positive and culture positive. Out of these, 430 bacterial isolates, 115/430 (26.74%) were gram-positive bacteria isolates, while 315/430 (73.26%) were gram-negative bacteria isolates. Among the types of bacteria identified, Escherichia coli accounted for 120 (27.91%), followed by Pseudomonas aeruginosa 70 (16.28%), Staphylococcus aureus 62 (14.42%), Klebsiella species 58 (13.49%), Coagulase-negative S. aureus (CoNS) 37 (8.60%), Proteus species spp. 35 (8.14%), Enterobacter spp. 19 (4.42%), and Enterococcus species 16 (3.72%), and Citrobacter spp. 13 (3.02%) (Table 3).

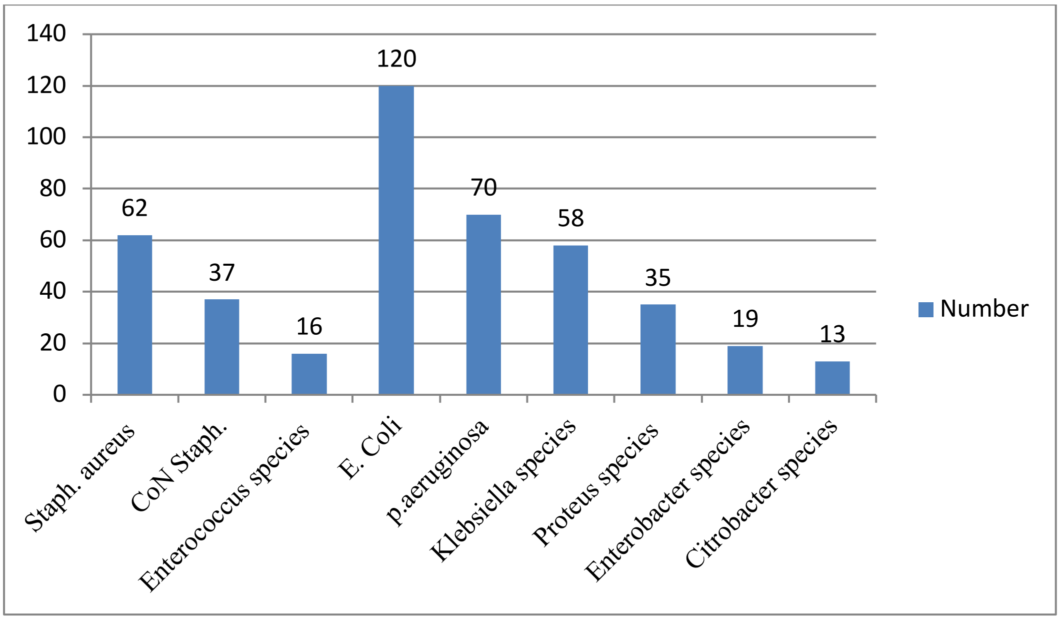


Figure 3: Different type of Bacteria isolated from patients

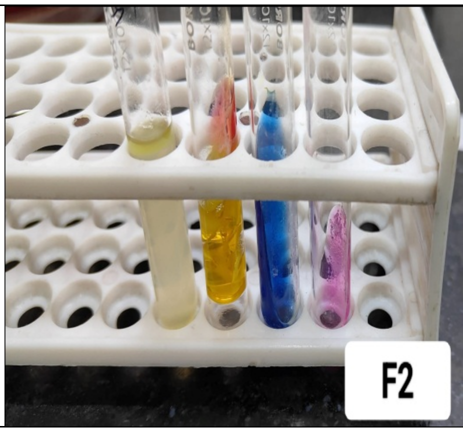
Table 4: Antibiotic sensitivity pattern (percentage wise) of aerobic bacterial isolates in surgical site infections

Antibiotics	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Acinetobacter species***	***Citrobacter species***	***Klebsiella species***
AMC	32.7	100	100	83.4	100	100
AMP	85.2	98	96.2	92.5	94.5	100
AMK	7.3	15.7	49.2	24.7	22	0
AZT	NT	99	98	98.6	100	100
CFP	NT	96.4	100	99.1	98	100
CFT	26.5	97.2	93.4	100	97.2	NT
CTR	NT	99.6	90.5	97.2	94.6	54.2
PTZ	NT	12.4	18.5	14.6	10.3	25.1

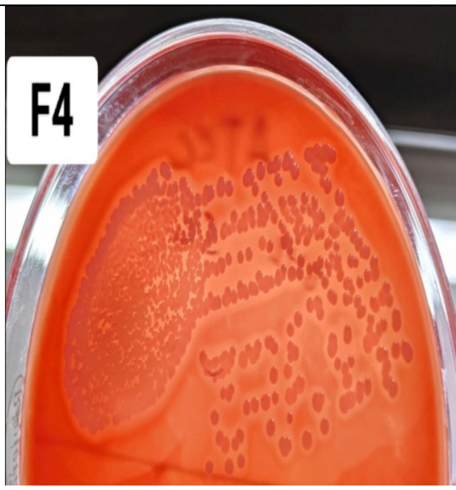
Antibiotic sensitivity pattern of aerobic bacterial isolates in surgical site infections. *Sensitivity pattern shown in the table is the percentage of isolates resistant to the antibiotic. Intermediately sensitive isolates were considered as resistant.

AMC: amoxicillin-clavulanate; AMP: ampicillin; AMK: amikacin; AZT: aztreonam; CFP: cefotaxime; CFT: ceftazidime; CTR: cotrimoxazole; PTZ: piperacillin-tazobactam; NT: not tested

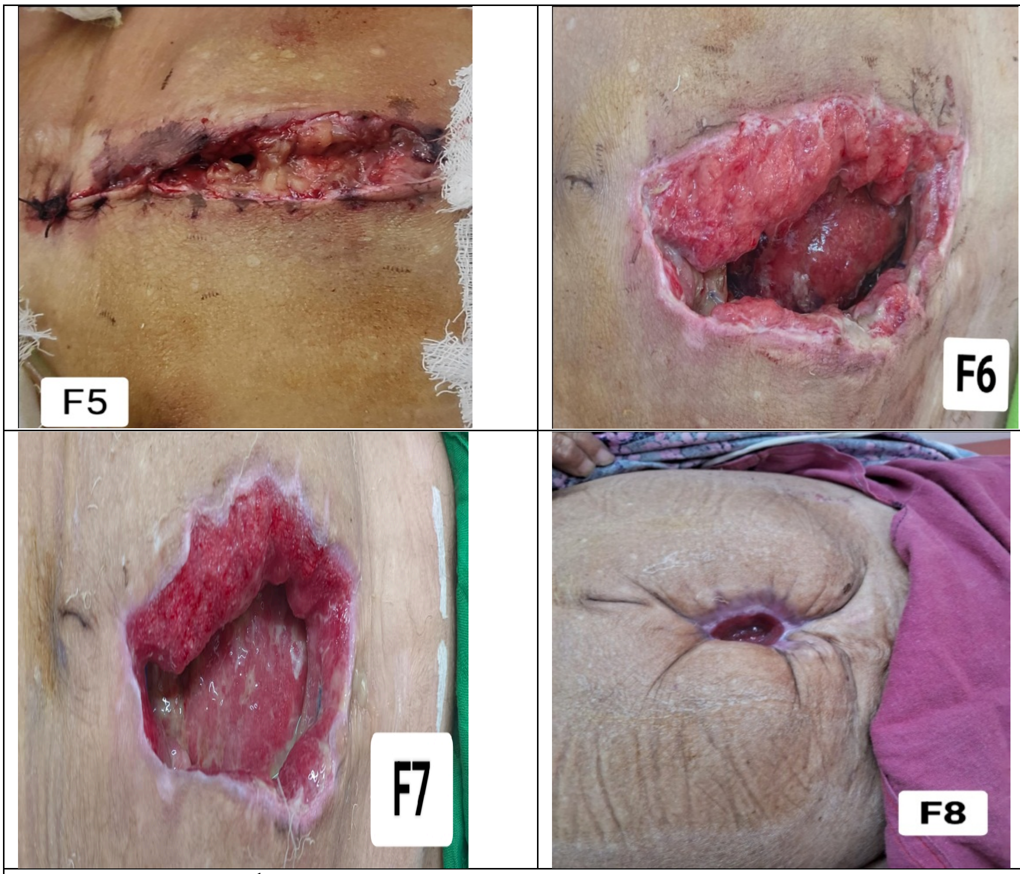
Table 4, shows that the maximum antibiotic susceptibility of *Staphylococcus aureus* was seen against AMP (85.2%). *E. coli* showed against AMC (100%), *Pseudomonas aeruginosa* showed maximum against AMC and CFP each (100%), *Acinetobacter species* against CFT (100%), *Citrobacter species* against AMC, and AZT each (100%), and *Klebsiella species* against AMC, AMP, AZT, CFP (100%) each.



F1: Colony Of *Klebsiella Pneumoniae* On Mac Conkey Agar Plate
F2: Test tube showing biochemical test of *Klebsiella Pneumoniae*



F3: Colony Of *Escherichia coli* On Mac Conkey Agar Plate
F4: Colony Of *Staphylococcus aureus* On Blood Agar Plate



F5: Picture showing 8th day of post-operative case of mesh hernioplasty with SSIs
F6 & F7: Picture showing 11th &15th day of post-operative case of mesh hernioplasty with SSIs
F8: Picture showing healed wound of incisional hernioplasty after SSIs

Discussion

Nosocomial infections become prominent in surgical wards because of surgical intervention and operative procedures. Most of the surgical site infections (SSIs) are hospital acquired and vary from hospital to hospital. Postoperative SSI remains one of the most significant causes of morbidity among surgically treated patients. These patients incur higher costs due to longer hospitalizations, more nursing care, additional wound care, potential hospital admissions, and further surgical procedures. Identification of bacterial pathogens and the selection of an effective antibiotic against the organism are essential in successful management of bacterial infection.

In the present study the overall rate of SSI was 19.5% which was in concordance with the study conducted by Negi Vikrant et al., who reported the overall rate of SSI as 17.8% in their study [6]. Various other studies from India have shown the rate of SSIs to vary from 6.1% to 38.7% [7-9]. However, in comparison to the Indian hospitals the rate of infection reported from other countries is quite low, for instance in USA it is 2.8% and in European countries it is reported to be 2-5% [8]. The lack of attention towards the infection control measures, inappropriate hand hygiene practices and overcrowded hospitals can be the major contributory factors for high infection rate in Indian hospitals.

The difference in surgical site infection's magnitude may be due to the type of procedures, surgical setup, and environmental factors. Bacterial isolates were examined for the determination of its categories and antibacterial sensitivity patterns. The lack of attention towards the infection control measures, inappropriate hand hygiene practices and overcrowded hospitals can be the major contributory factors for high infection rate in Indian hospitals.

The predominance of male patients was seen in present study with male: female ratio of 1.14:1and this finding was in contrast to the other studies where a much higher number of female patients have been reported [10,11]. The patients with age ≥50 years had a higher incidence of SSI (57.18%) in comparison to an incidence of 42.82% among the patients who were <50 years of age. Advancing age is an important factor for the development of SSIs, as in old age patients there is low healing rate, low immunity, increased catabolic processes and presence of co-morbid illness like diabetes, hypertension, etc [12]. Regarding the duration of the operation a prolonged time was found to be a significant risk factor for SSI and it was observed that as the order and the duration of surgery increased, the rate of infection also increased.

Out of 878 suspected samples, 48.98% were gram stain (+), culture (+) and 43.05% were gram stain (-), culture (-). The remaining 7.97% samples showed organisms in Gram stain (+) but no growth on aerobic culture which might be due to prior antibiotic use or anaerobic and no-nfastidious infectious aetiology. Our finding on culture positivity is similar to the study reported by Kokate et al., [13] and Anbuchezzian B [14].

In the current study, the overall culture positivity rate from patients with surgical site infection was 48.98 %, which was lower than results previously reported by Shabnum M. [15] from India (68%) and by Mengesha RE, et al. [16] from Mekelle (75%), but lower than a report by Mohammed A et al. [17] from Nigeria (82%).

The isolation rate of Gram-negative bacteria was 43.05%, lesser than Gram-positive bacteria (56.95%) in present study. This, in similar, to study done by Khanam RA, et al. [18] from Bangladesh, and by Maharjan N et al. [19] from Nepal. The prevalence of mixed infections in the current study (10%) was lower than previous study by Azene MK et al. [20] from Dessie (18.5%).

In present study most common bacteria isolated was *Escherichia coli* accounted for 27.91%, similar to previous study carried out by S.M. Patel et al. [21], and Kakati B et al. [22].

S.M. Patel et al. [21] demonstrated that *Escherichia coli* (35.7%) was the most common pathogenic isolate followed by *Staphylococcus aureus* (21.4%), *Pseudomonas aeruginosa* (14.3%), and *Klebsiella species* (14.3%). Kakati B et al.[22] from India, *Escherichia coli* (41.17%) was reported as the most common bacterial isolates, followed by *Staphylococcus aureus* (13.72%), *Klebsiella pneumonia (*9.80%), *Pseudomonas aeruginosa* (7.84%).

We found that the maximum antibiotic susceptibility of *Staphylococcus aureus* was seen against AMP (85.2%). *E. coli* showed against AMC (100%), *Pseudomonas aeruginosa* showed maximum against AMC and CFP each (100%), *Acinetobacter species* against CFT (100%), *Citrobacter species* against AMC, and AZT each (100%), and *Klebsiella species* against AMC, AMP, AZT, CFP (100%) each.

Antibiotic susceptibility results revealed that a high degree of resistance was seen for majority of the bacterial isolates. For gram positive bacteria Ampicillin, Amoxicillin-clavulanate was found to be the most effective antibiotics. The degree of resistance was even higher among the gram-negative bacteria and the commonly used drugs were found to be more resistant with an average resistance range from 50% to 100%. Amoxicillin-clavulanate, piperacillin-tazobactam, and amikacin were found to be the most effective antimicrobial agents whereas ampicillin, amoxicillin-clavulanate and cefotaxime were among the most resistant drugs. *Pseudomonas aeruginosa* showed maximum against AMC and CFP each (100%) in comparison to the previous studies by Masaadeh HA et al.[23].

Limitations of the study: This was a single-centre retrospective study, and the results might not be generalizable. A population-based or large-scale study is required to clarify the association between genotypes, resistance spectrum, phenotypes, and clones of microbes isolated from patients with SSIs.

Conclusion

The majority of the isolates from surgical sites in the study area were gram-negative bacteria. Escherichia coli were the most frequent Gram-negative bacteria that caused surgical site infections.

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