

Original Article**Study of Aerobic Bacteria from Surgical Site Infection and their Antibiotic Susceptibility Pattern at a Tertiary Care Centre in Eastern Nepal**Kumari Ragani Yadav¹, Kewal Shrestha¹, Rekha Shah², Bikram Yadav³, Saibijaya Rijal⁴, Prabhat Kumar¹¹Department of Microbiology, Nobel Medical College Teaching Hospital, Biratnagar, Nepal,²Department of Pharmacology, Birat Medical College and Teaching Hospital, Biratnagar, Nepal,³Minimally Invasive Diagnostic and Therapeutic Hospital, Lagankhel Kathmandu, Nepal ⁴School of Medicine and Allied Health Science, Manmohan Technical University, Budhiganga, NepalArticle Received: 12th November, 2023; Accepted: 16th March, 2024; Published: 30th June, 2024DOI: <https://doi.org/10.3126/jonmc.v13i1.68108>**Abstract****Background**

The surgical site infections are the third most common nosocomial infection. The most common organism is *Staphylococcus aureus*. Nepal has a 4-7% overall prevalence rate for surgical site infection. The study objective was to find out the aerobic bacteria from surgical site infection and their antibiotic susceptibility pattern in a tertiary care hospital in Nepal.

Materials and Methods

A descriptive cross-sectional study was conducted at a tertiary care center with effect from September 2022 to September 2023 approval from the Institutional Review Committee. Pus samples were taken from the postoperative wound infection site and submitted to the microbiological laboratory for culture and sensitivity. Gram-positive aerobic bacteria and gram-negative aerobic bacteria were isolated and identified by standard microbiological procedures. An antimicrobial susceptibility test was done on Muller-Hinton agar using the Kirby Bauer disc diffusion method. The result was interpreted according to clinical and laboratory standard institute guidelines.


Results

The rate of surgical site infection was 22.24% and the most common organisms isolated were *Staphylococcus aureus* followed by *Escherichia coli* and *Pseudomonas aeruginosa*. All the gram-positive cocci were sensitive to linezolid, teicoplanin, and vancomycin and gram-negative bacteria were sensitive to meropenem, piperacillin/tazobactam, cefoperazone/sulbactam and cefepime

Conclusion

The present study concluded that the most common aerobic bacteria isolated from surgical site infection in our setting was found to be *Staphylococcus aureus*. This study also showed that the gram-positive aerobic bacteria were most susceptible to linezolid, and teicoplanin, and gram-negative aerobic bacteria were sensitive to meropenem, piperacillin/tazobactam, and cefoperazone/sulbactam.

Keywords: Antimicrobial susceptibility, *Escherichia coli*, Surgical site infection, *Staphylococcus aureus*

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Citation

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Introduction

Surgical site infection (SSI) is a postoperative infection that develops at the surgical site within 30 days of surgery and within 1 year if any prosthetic material is implanted at the surgical site [1]. The third most common nosocomial infection and it is the major global problem among the operated cases [2, 3]. Clean wounds are due to the skin flora of the surgery team or the environmental organism, the most common being *Staphylococcus aureus* [4-6]. Despite advancements in operating room procedures, instrument sterilization techniques, and improving infection prevention strategies, surgical site infections continue to be a leading cause of hospital-acquired infections [7].

The prevalence of SSIs gradually increased and documentation of different rates of SSIs by different authors 4.7%, 18.7% and 16.92% in Nepal [8-10]. It is important to identify the most frequent organisms that cause SSIs in our setting. Therefore, identifying agents of infection and their susceptibility to certain antibiotics would help clinicians in deciding which antibiotics they can use empirically before the results from antibiotic sensitivity tests are out.

This study is carried out to find the prevalence of surgical site infection due to aerobic bacteria and their antibiotic susceptibility pattern in a tertiary care hospital in Nepal.

Materials and Methods

This descriptive cross-sectional study was conducted in the department of Microbiology at Nobel Medical College Teaching Hospital, Biratnagar, Nepal with effect from September 2022 to September 2023 after approval from the institutional review committee (Ref: IRC-NMCTH650/2022) of the college. Patients of both sexes and of all age groups who had surgical wound pus discharge, seropurulent discharge, and concurrent signs of sepsis were included. Patients with suture abscesses, pre-contaminated wounds, and dirty wounds were not included in the study. The patient's history record sheet that was provided with the samples used in our study allowed us to acquire extensive information about the patient's age, sex, illness type, diagnosis, type of surgery and length of surgery performed, antibiotic prophylaxis, and any co-morbid diseases that were present. Convenient sampling was done and sample size (n) was calculated as:

$$n = z^2 pq / e^2$$

$$n = (1.96)^2 \times 0.1692 \times 1 - 0.1692 / (0.05)^2 = 216$$

Where, z = 1.96 at a 95% confidence interval

n = Sample size

p = Prevalence of surgical site infection 16.92% [10]

q = 1-p = 0.8308

e = margin error, 5% = 0.05

The sample size was calculated to be 216. However, a total of 989 pus samples were collected from post-operative wound infection from OPD and IPD were enrolled. Under aseptic conditions, 989 pus samples from SSI were obtained using two sterile swabs without contaminating them with skin commensals. Using the first swab, a smear was made immediately for gram staining. The second swab was used for the culture and all samples were aerobically inoculated onto Blood, Mac-Conkey, and Chocolate agar, and then incubated for 18-24 hours at 37°C. Organism isolates were identified by gram staining, colony characteristics, and biochemical tests.

All the bacterial isolates were subjected to antimicrobial susceptibility testing on Muller-Hinton agar (MHH) using the Kirby Bauer disc diffusion method. With the help of straight inoculating wire, 1-2 well-isolated colonies were picked and inoculated into a tube containing 5ml peptone water and incubated into the incubator at 37°C for 2-4 hours and then compared with the turbidity of 0.5 McFarland standard solutions. After the turbidity was maintained, a sterile cotton swab was dipped into the peptone water containing bacterial solution and any excess solution was removed by pressing the swab on the inside wall of the tube. Followed by lawn culture on MHA and there after antimicrobial discs were placed onto the surface of an inoculated agar plate and the plate was incubated in the incubator at 37°C for 18-24 hr. The zone of inhibition was measured by using a Vernier caliper and the result was interpreted according to clinical and laboratory standard institute (CLSI) guidelines [11].

The antibiotic discs and concentration used for both gram-positive and gram-negative aerobic bacteria were as follows: penicillin (P) 10 units, erythromycin (E) 15µg, cefoxitin (CX) 30µg, clindamycin (CD) 2µg, cotrimoxazole (COT) 5µg, chloramphenicol (C) 30µg, linezolid (LZ) 30µg, teicoplanin (TEI) 30µg, high-level gentamycin (HLG) 120µg, vancomycin (VA) 30µg, ceftriaxone (CTR) 30µg, high-level streptomycin (HLS) 10µg, ampicillin (AMP) 10µg, cefuroxime (CXM) 30µg, ceftazidime (CAZ) 30µg, cefotaxime (CTX) 30µg.

According to CLSI guidelines, [11] the organisms (*Escherichia coli*, *Citrobacter* species, *Acinetobacter* species, *Enterobacter aerogenes*, and



Klebsiella species) were screened for ESBL production using ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg). The organisms showing reduced susceptibility to at least one of these antibiotics with a zone of inhibition on Mueller Hinton Agar for ceftazidime (30µg) ≤22mm, ≤25mm with ceftriaxone (30µg), and ≤27mm with cefotaxime (30µg) were considered as potential ESBL producing organisms and selected for phenotypic confirmatory test for ESBL [12].

The collected data were entered in Microsoft Excel and data was analyzed statistical package for the social science for Windows using SPSS version 20.

Results

Out of a total of 989 pus samples collected from the post-operative wound infection site, 220 (22.24%) samples showed significant bacterial growth by culture. Among these SSI was found to be higher in females (64.1%) and the rate of SSI was higher in age groups between 20-40 years. The significantly higher rate of SSIs in emergency surgery (61.4%). In high proportions of participants, 100 (45.5%) were identified in the contaminated wound. Cases of SSI were more in Smokers (76%). A total of 220 cases of SSIs, 85 (38.6%) patients having co-morbid conditions. The SSI was higher in IPD -71.4% shown in Table 1. Compared to other organisms, *Staphylococcus aureus* and *Escherichia coli* were the most prevalent isolates, making up 43.2% and 27.7%, respectively. (Table 2)

Table 1: Baseline characteristics of the study population

Characteristics	Categories	No of patients	Percentage
Age in years	= 20	33	15.0
	21 – 40	119	54.0
	41 – 60	45	20.5
	>60	23	10.5
Gender	Female	141	64.1
	Male	79	35.9
Types of surgery	Elective	85	38.6
	Emergency	135	61.4
Types of SSI	Clean	68	30.9
	Clean	52	23.6
	Contaminated	100	45.5
Duration of surgery in hours	= 2	77	35.0
	>2	143	65.0
Antibiotic prophylaxis received	No	43	19.5
	Yes	177	80.5
Smoking habit	Non-smoker	167	75.9
	Smoker	53	24.1
Co-morbidity present	No	135	61.4
	Yes	85	38.6
Type of Co-morbidity (n=85)	Anemia	21	24.7
	Diabetic mellitus	48	56.5
	Hypertensive	16	18.8
Sample from	IPD	157	71.4
	OPD	63	28.6
	Total	220	100.0

Table 2: Aerobic gram-positive and gram-negative bacteria isolated from SSIs

Name of Organisms	No of sample	Percentage
<i>Staphylococcus aureus</i>	95	43.2
<i>Escherichia coli</i>	61	27.7
<i>Pseudomonas aeruginosa</i>	16	7.3
<i>Klebsiella pneumoniae</i>	11	5
Coagulase-negative staphylococcus (CONS)	9	4.09
<i>Streptococcus pyogenes</i>	6	2.7
<i>Enterococcus</i> species	5	2.3
<i>Citrobacter koseri</i>	5	2.3
<i>Enterobacter aerogenes</i>	4	1.8
<i>Acinetobacter anitratus</i>	3	1.4
<i>Acinetobacter baumannii</i>	2	0.9
<i>Edwardsiella tarda</i>	1	0.5
<i>Klebsiella oxytoca</i>	1	0.5
<i>Proteus mirabilis</i>	1	0.5
Total	220	100.0

Table 3 showed almost all the *Staphylococcus aureus*, CONS, and *Enterococcus* species were 100% sensitive towards linezolid, teicoplanin, vancomycin, and resistance to penicillin and erythromycin. All the *Streptococcus pyogenes* showed 100% sensitivity to penicillin, erythromycin, linezolid, clindamycin, and vancomycin.

Table 3: Antimicrobial susceptibility pattern of gram-positive aerobic bacteria isolated from SSI

	Organisms			
	<i>Staphylococcus aureus</i> (95)	CONS (9)	<i>Enterococcus</i> species (5)	<i>Streptococcus pyogenes</i> (6)
Antimicrobial	S/R	S/R	S/R	S/R
Cefoxitin	65/30	8/1	NT	NT
Penicillin	12/83	1/8	1/4	6/0
Erythromycin	44/51	4/5	2/3	6/0
Clindamycin	66/29	7/2	NT	6/0
Cotrimoxazole	73/22	7/2	NT	4/2
Ciprofloxacin	57/38	5/4	NT	NT
Chloramphenicol	90/5	7/2	4/0	NT
Linezolid	95/0	9/0	5/0	5/0
Teicoplanin	95/0	9/0	5/0	NT
Vancomycin	92/3	9/0	5/0	6/0
Amikacin	68/27	5/4	NT	NT
Ciprofloxacin	69/26	8/1	NT	NT
Streptomycin	NT	NT	4/1	NT

Non- ESBL producing *Pseudomonas aeruginosa* showed the highest sensitivity towards tobramycin, ceftazidime, and amikacin whereas ESBL-producing *Pseudomonas aeruginosa* showed the highest sensitivity to meropenem and Pepracillin/tazobactam. The gram-negative aerobic bacteria other than *Pseudomonas* were sensitive to gentamycin, cephalosporins and



resistant to ampicillin. ESBL-producing gram-negative aerobes were sensitive to meropenem, piperacillin/tazobactam, cefoperazone/sulbactam and cefepime and least sensitive to ampicillin and cephalosporins (Table 4-6).

Table 4: Antimicrobial susceptibility of Non-ESBL and ESBL-producing *Pseudomonas aeruginosa*

Antibiotics	Organism <i>Pseudomonas aeruginosa</i>			
	Non-ESBL(16)		ESBL(2)	
	Sensitive	Resistance	Sensitive	Resistance
Ceftazidime	14	2	0	2
Ciprofloxacin	12	4	0	2
Gentamycin	11	5	0	2
Levofloxacin	13	3	2	0
Amikacin	13	3	0	2
Cefepime	12	4	0	2
Tobramycin	15	1	1	1
Aztreonam	NT	NT	0	2
Meropenem	NT	NT	2	0
Pepracillin/tazobactam	NT	NT	2	0

Table 5: Antimicrobial susceptibility of gram-negative aerobic bacilli isolated from SSI

Antibiotics	Organisms			
	<i>Escherichia coli</i> (20)	<i>Citrobacter</i> species (2)	<i>Klebsiella pneumoniae</i> (7)	<i>Edwardsiella tarda</i> (1)
	S/R	S/R	S/R	S/R
Ampicillin	4/16	0/2	0/7	0/1
Cefixime	17/3	2/0	4/3	1/0
Cefotaxime	19/1	2/0	7/0	1/0
Ceftazidime	18/2	2/0	7/0	1/0
Gentamycin	18/2	2/0	7/0	1/0
Ciprofloxacin	17/3	2/0	5/2	1/0

Table 6: Antimicrobial susceptibility of ESBL producing gram-negative aerobic bacilli isolated from SSI

Antimicrobial	Organisms					
	<i>Citrobacter</i> species (3)	<i>Klebsiella pneumoniae</i> (5)	<i>Acinetobacter</i> species (5)	<i>Edwardsiella aerogenes</i> (4)	<i>Proteus mirabilis</i> (1)	<i>E. coli</i> (41)
	S/R	S/R	S/R	S/R	S/R	S/R
Ampicillin/sulbactam	1/2	4/1	2/3	2/2	1/0	33/8
Cefoperazone/sulbactam	2/1	5/0	4/1	3/1	1/0	40/1
Amikacin	1/2	5/0	2/3	4/0	1/0	36/5
Levofloxacin	2/2	4/1	1/4	4/0	1/0	21/20
Cotrimoxazole	2/2	3/2	1/4	2/2	1/0	16/25
Meropenem	3/0	5/0	5/0	4/0	1/0	41/10
Cefepime	2/1	4/1	4/1	3/1	1/0	31/10

Discussion

In the present study, the overall infection rate of SSIs was 22.24% which compares well with a study conducted by Giri S et al the infection rate was 23.0% in the year 2013 [4]. The rate of SSIs

in our study was higher in comparison with infection rates of 4.7%, 9.6%, and 12.6% in Nepal conducted by Sutariya PK et al, Tuladhar NR et al and Shrestha S et al respectively [8,13,14]. The rate of SSIs in our study was 22.24% which was similar to the study conducted by Misha G et al (21.0%) in year 2021 [15]. In our study, the infection rate was higher in patients undergoing emergency surgery as compared to routine elective surgery which was also reported in several other studies [4, 14, 16]. The effect of emergency surgery on the rate of SSI is likely to be due to a lack of pre-operative preparation which increased the rate of SSI.

In our study, the SSIs infection was more in the age group between 20-40 years which was similar to the study done by Shrestha S et al [9]. SSIs were more prevalent in females 64% as compared to males 35.9% which was not similar to the study done by Ghimire P et al [10]. The rate of SSIs varies from hospital to hospital within a country and in comparison to other countries like the USA it is 2.8% and in European countries, it is 2-5% which is quite low compared to our country like Nepal [3]. This is mainly due to not maintaining proper hand hygiene, lack of infection control practices, and prolonged hospital stays can be major factors for the development of SSI infection. The rate of infection was highest in the contaminated type of wounds (45.5%), followed by clean contaminated wounds (30.9%) and least in clean wounds (23.6%) which was similar to another study by Shahane V et al found the rate of SSIs in the contaminated type of wounds (12.3%), followed by clean contaminated wounds (8.0%) and least in clean wounds (4.6%) [17].

We observed in our study that a longer duration of surgery was significantly increased with SSI than a shorter duration of surgery. A similar finding was documented in various studies [8, 17, 18].

The percentage of patients receiving antibiotic prophylaxis was 80.5% which was well correlated with 98% and 90% reported from Turkey and Israel [19, 20]. The overall SSI rate was 30.7% and 5.4% for clean, 35.5% for clean-contaminated, and 77.8% for contaminated wounds [21] which was almost similar to our finding where the rate of SSI was 22.24% and clean wounds (23.6%), clean contaminated (30.9%) and contaminated wound (45.5%).

Our study revealed that the rate of SSI was higher in smokers (75.9%) as compared to non-smokers (24.1%) which was similar to the study conducted by Kamet US et al, Arabshahi KS et al



and Sorensen LT et al [21, 22, 23]. Smokers were 2.3 times more likely to develop SSI than non-smokers. This is due to tissue hypoxia brought on by local and systemic vasoconstriction, which delays the healing of initial wounds. Our finding was not similar to the study done by Sutariya PK et al and Movic JM et al mentioned in their study that smoking had not any association with SSI [8, 24]. Compared to other organisms, in our study *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were the most prevalent isolates, making up 43.2%, 27.7%, and 7.3% respectively and this finding was not similar to a study done by Kollef MH et al [25], Shahane V et al [17], and Amare B et al [26] has demonstrated *Escherichia coli* was the most common bacterial isolate, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and this finding was not similar to our study finding.

However, our result was consistent with numerous other investigations. *Staphylococcus aureus* has been identified by the authors of this research as the most prevalent pathogen causing SSI in their studies [27, 28, 29]. In our study, almost all the *Staphylococcus aureus*, *CONS*, and *Enterococcus* species isolated from SSIs showed 100% sensitivity towards linezolid, teicoplanin, vancomycin, and resistance to penicillin and erythromycin. This was similar to a study done by Negi V et al in India [3]. All the *Streptococcus pyogenes* isolated from SSIs showed 100% sensitivity towards penicillin, erythromycin, linezolid, clindamycin, and vancomycin in the present study, and a similar finding was observed in a study conducted by Patel P et al [30].

The majority of ESBL-producing gram-negative isolates in our study were sensitive to meropenem, piperacillin/tazobactam, cefoperazone/sulbactam, cefepime and least sensitive to ampicillin and cephalosporins. This finding was well in agreement with a study conducted by Biradar A et al, Bhumbra U et al and Trojan R et al [31, 32, 33]. The resistance in gram-negative isolates is due to not following the antibiotic policy by clinicians in hospitals, indiscriminate use of empirical antibiotics, and insufficient treatment duration. In our study increased number of ESBL producers was *E. coli* (41) followed by *Klebsiella* species (5) and *Acinetobacter* species (5) which was similar to the study done by Bhandari P et al where the commonest ESBL producer was *E. coli* followed by *K. oxytoca* [34].

Conclusion

This study emphasizes how crucial it is to regularly monitor SSIs and the patterns of antibiotic

resistance they exhibit. The most common aerobic bacteria isolated from surgical site infections, according to the present study, was *Staphylococcus aureus*. Gram-positive aerobic bacteria are more susceptible to teicoplanin and linezolid, whereas gram-negative bacteria are more sensitive to meropenem, piperacillin/tazobactam, and cefoperazone/sulbactam. Strict adherence to infection control procedures and customized antibiotic strategies based on local resistance data are necessary to effectively manage and prevent SSIs.

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Conflict of interest: None

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