Pure Appl. Biol.,13(3):311-327, September, 2024
http://dx.doi.org/10.19045/bspab.2024.130028

Research Article

Antibiogram and molecular screening of resistance genes in bacteria isolated from post-surgical wounds infections in Peshawar

Yasin Ahmad[1*ϯ], Ashraf Ali[1ϯ], Mubashir Hussain[1], Hafsa Shahzadi[2], Jabal Noor[1], Shams Ur Rahman[1], Shahzad Jamil[1], Islam Ullah[1], Muhammad Danish Khan[1], Abdul Qadeer Khan[1] and Muhammad Asim[3]

1. Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar, Pakistan
2. Institute of Microbiology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan
3. Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan
ϯ. These two authors contributed equally
**Corresponding author's email:* ahmadyasin9312@gmail.com

Citation
Yasin Ahmad, Ashraf Ali, Mubashir Hussain, Hafsa Shahzadi, Jabal Noor, Shams Ur Rahman, Shahzad Jamil, Islam Ullah, Muhammad Danish Khan, Abdul Qadeer Khan and Muhammad Asim. Antibiogram and molecular screening of resistance genes in bacteria isolated from post-surgical wounds infections in Peshawar. Pure and Applied Biology. Vol. 13, Issue 3, pp311-327. http://dx.doi.org/10.19045/bspab.2024.130028

Received: 29/11/2023 Revised: 12/01/2024 Accepted: 20/01/2024 Online First: 26/01/2024

Abstract

Postoperative wound infections are infections at the site of the operation within thirty days of surgery. Postoperative infections are a major problem worldwide, leading to increased morbidity and mortality. This study was conducted to determine the prevalence of bacterial pathogens that cause wounds infection in operating theater and antimicrobial susceptibility patterns of isolated bacteria. This experimental study was conducted among patients admitted in different surgical ward rooms at a Tertiary Care Hospital, Peshawar. A total of 110 patients were included in the study using the non-probability adjusted sampling technique. The data was collected through a structured questionnaire from February to June 2022. Out of 110 pus samples 64 pus samples were positive. Out of these 64 positive samples, 51 (46.36%) yielded gram-negative and 13 (11.81%) yielded gram-negative positive bacteria. In gram negative bacterial isolates, the most common isolates were *E. coli*, followed by *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter*, and in gram positive isolates the coagulase negative *staphylococci* were 8 (61.53%) and coagulase positive *Staphylococci* were 5 (38.46%). The most effective antibiotics against gram-negative strains were found to be Tobramycin (96.29%) followed by Gentamicin (50.89%). In the case of gram-positive strains, Teicoplanin and Vancomycin exhibited 100% effectiveness. In conclusion, our study highlights gram-negative bacteria, especially *E. coli*, in Peshawar's post-surgical wound infections. Tobramycin's efficacy and ESBL gene presence underscore the need for targeted treatments.

Keywords: Antibiotic resistance; Multi-Drug Resistant; Peshawar; Resistance genes; Surgical wounds

Introduction

Postoperative wound infections are operative site infections within 30 days of a surgical procedure involving a surgical incision made in the skin, flesh, or deeper tissues at the surgical site mostly between the fifth and tenth day after surgery [1]. Postoperative infections are a major problem worldwide leading to increased morbidity and mortality. One of the most prevalent hospitals acquired infections is surgical wound infection, which is a significant cause of morbidity and accounts for 70–80% of death [2, 3].

Surgical Site Infection (SSI) is a form of healthcare-associated infection that occurs in hospitals when an individual is admitted for reasons unrelated to the infection itself. Healthcare-associated infections are those acquired within medical facilities, including hospitals and other healthcare settings [4]. Worldwide, 23% of patients develop surgical site infections among all surgeries annually. SSIs pose significant challenges to surgeons and are recognized as a critical issue in infection control worldwide. In the United States, these infections affect approximately 2-5% of patients annually, leading to at least 500,000 infections, causing an additional 3.7 million hospital days, and incurring approximately $1.6 billion in extra hospital expenses [5, 6].

The majority of post-operative wound infections are acquired in hospitals and can differ not only between different hospitals but also within the same hospital. These infections are linked to higher levels of illness and death. The infection site may be confined to the suture line or can spread extensively within the surgical site. The specific microorganisms causing the infection vary depending on the type and location of the surgery and the antimicrobial treatments administered to the patient [7].

The incidence of SSIs is higher in developing countries compared to developed nations. SSIs make up more than 20% of all healthcare-associated infections in patients undergoing surgery [8]. Bacteriological studies have shown that both gram-positive and gram-negative bacteria contribute to the infection of surgical wounds. Among these bacteria, *Staphylococcus aureus* is the most common, followed by *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii*, and *Proteus mirabilis* [9]. In numerous developing nations, including Pakistan, the absence of a well-structured surveillance system to document routine SSIs rates poses a significant challenge. Several factors contribute to this shortfall, making the establishment of an organized surveillance mechanism a complex undertaking [6].

The infection rate is influenced by several factors, including skin preparation, wound contamination, the duration of pre-operative hospital stays, wound drainage, patient age, surgery duration, and the skill and technique of the surgeon. Senior surgeons tend to have a higher infection rate, which can be attributed to the complexity and length of the surgeries they perform. On the other hand, medical officers have a lower infection rate, mainly because they handle simpler and less complicated surgeries [10].

Surgeons face significant challenges due to the prevalence of multi-drug-resistant bacteria among these pathogens. The ongoing struggle between bacteria and their susceptibility to drugs remains a significant concern for the general public, researchers, physicians, and pharmaceutical companies who are searching for effective solutions. To combat this issue, various measures have been proposed, including the development of new antimicrobial drugs, the enhancement of infection control programs, and the more judicious use of existing antimicrobial agents. Different investigators have put forth various recommendations regarding the susceptibility of microorganisms to drugs. This multifaceted problem requires a collaborative effort from various stakeholders to address and find effective solutions [11, 12].

In some cases, wounds can get infected with methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to commonly used antibiotics. However, vancomycin and linezolid have shown susceptibility against all gram-positive bacteria. On the other hand, gram-negative bacteria exhibited substantial resistance to the majority of antibiotics, with amikacin being the most effective against these types of bacteria. This highlights the importance of judicious use of antibiotics and the need for continued research to develop new and effective antimicrobial strategies to combat antibiotic-resistant bacteria [13-15].

Materials AND Methods

Study location and period

The present study was carried out in the MLT Laboratory of Medical Laboratory Technology Department, Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Technology, Peshawar Khyber Pakhtunkhwa, from February to June 2022.

Samples collection and isolation of bacteria

Clinical samples were collected from surgical ward of a Tertiary Care Hospital, Peshawar using commercially available sterile cotton swabs. A total 110 samples were collected through sterile swabs from suspected patients with apparent signs and symptoms of surgical wound infection. The swabs were removed from the tube and the sample was collected from surgical wound by rotating the swabs 180 degree back and forth. After swabbing, the swabs were inserted back into the tubes and samples were labeled properly. The cotton swabs that were collected were evenly spread onto Blood agar, MacConkey agar, and Mannitol Salt Agar (MSA) within a controlled laboratory environment. Subsequently, these agar plates were subjected to an incubation period at a temperature of 37°C for a duration ranging between 15 to 24 hours. The results after incubation were meticulously documented, and the same procedural steps were systematically repeated for each of the collected swabs.

Biochemical identification of the bacterial isolates

In order to identify and classify the bacterial isolates, a set of biochemical tests involving microscopic examination and various assays were conducted. These included the Gram staining, Catalase test, Oxidase test, Coagulase test, Citrate test, and the utilization of Triple Sugar Iron agar. These tests were systematically employed to ascertain the presence or absence of specific enzymes within the isolated bacterial specimens.

Antibiotics sensitivity testing

To determine the antibiotic susceptibility patterns of the bacterial isolates, the Kirby-Bauer disc diffusion method was employed, assessing their response to a selection of antibiotics. Cultures incubated between 18 to 24 hours, initially grown in nutrient broth, were compared with a standard reference of 0.5 McFarland's. In cases where the culture appeared turbid, sterile normal saline was employed for sample dilution. The resulting culture suspension was then evenly spread onto sterile Muller-Hinton Agar plates, and antibiotic discs were placed onto the agar surface. Subsequently, all plates were subjected to an incubation period at 37°C for 24 hours. The day after inhibition zones were measured in millimeters (mm) using a calibrated scale. The results were interpreted in accordance with the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI, 2022), classifying antibiotic susceptibility as sensitive, intermediate, or resistant for each individual antibiotic. Specific antibiotics for both gram-negative and gram-positive bacteria are detailed in (Table 1).

Multiple Antibiotic Resistance Index (MARI)

The MAR (Multiple Antibiotic Resistance) index serves as a reliable, valid, and straightforward approach for gauging the prevalence of drug resistance within a collection of bacterial isolates. Determination of MAR index followed the procedure, in which the number of antibiotics an isolate is resistant to (a) is divided by the total number of the antibiotics used in the study (b). The calculating formula is shown below:

MDR Index = a/b

If the MAR index exceeds 0.2, it indicates that the primary source of contamination risk is in environments where antibiotics are frequently employed.

Detection of ESBL resistant genes

Polymerase Chain Reaction was used to detect antibiotic resistance genes in isolates at the Medical Laboratory Technology Laboratory, SIAHS, SUIT, Peshawar, Khyber Pakhtunkhwa. For the detection of bla_{CTX-M1}, bla_{SHV} and bla_{TEM} DNA was extracted with the help of centrifugation and the extracted DNA was used as a template. PCR was performed under optimized PCR conditions. PCR reaction mixture (25µl) was prepared containing Master Mix 5 µl, forward and reverse primers 1µl each, Nuclease-free water 13µl and template DNA 5µl. An identified positive strain was used as positive control. While PCR reaction lacking template DNA was used as negative control. The PCR reaction was performed in a Thermal cycler (Bio-Rad T100, California, USA). Each PCR run was followed by agarose gel electrophoresis (Invitrogen, UK). In all the experimental works, a 1.5% agarose gel prepared in 1xTAE was used. Gel electrophoresis was performed for 45 min at 100 V. 100bp DNA ladder (Fermentas, Lithuania) was loaded together with the PCR products as a size marker. The primer sequences are presented in (Table 2).

Results

Overall prevalence of positive samples

A total of 110 samples were collected from suspected patients. In 110 pus samples, 64 (58.18%) resulted in microbial growth, while in 46 pus samples (41.81%) no growth was observed.

Demographic sketches of patients, comorbid conditions, polymicrobial status and prevalence of bacteria in post-surgical wound infections

In our study, the majority of the study population were females 78 (70.90%) while 32 (29.09%) were males. Male to female ratio was recorded to be 16:39. The age of patients ranged from 14 years to 80 years in which the infection rate was comparatively high i.e., 40.62% in the age group of 21-40 followed by 26.56% in 41-60 years of age group as presented in (Table 3). Higher number of patients having surgical wounds infections (54.68%) were found to have diabetes while 46.31% were non-diabetic.

Out of 110 pus samples, 64 (58.18%) resulted in microbial growth, while in 46 pus samples (41.81%) no growth was observed. Out of 64 positive samples 13 (20.23%) showed more than one type of colony on blood and MacConkey agar suggestive of microbial growth of pathogens. Total 64 pus samples were positive. Out of the 64 positive samples, 51 (46.36%) were gram-negative and 13 (11.811%) were gram positive. Wound culture yielded of gram-negative *E. coli* 64 (50.39%), followed by *Acinetobacter baumannii* 21 (16.53%), *Klebsiella pneumoniae* 18 (14.17%), *Pseudomonas aeruginosa*. 9 (7.08%), *Citrobacter* 2 (1.57%). Among gram-positive bacteria, coagulase-negative *staphylococci* accounted for 8 cases (61.53%), while coagulase-positive *staphylococci* were identified in 5 cases (38.46%).

Table 1. Breakpoints of Antibiotics used for gram positive and gram-negative bacterial isolates

Antibiotics	Breakpoints of antibiotics used for gram positive strains				
	Abbreviations	Potencies	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Tetracycline	TE	30 µg	≤14	15-18	≥19
Teicoplanin	TEC	30 µg	≤2	NA	≥2
Vancomycin	VA	30 µg	NA	NA	NA
Chloramphenicol	C	30 µg	≤12	13-17	≥18
Linezolid	LZD	30 µg	≤20	NA	≥21
Cefoxitin	FOX	30 µg	≤21	NA	≥22
Clindamycin	DA	2 µg	≤14	15-20	≥21
Clarithromycin	CLR	15 µg	≤13	14-17	≥18
	Breakpoints of antibiotics used for gram positive strains				
Ciprofloxacin	CIP	5 µg	≤21	22-25	≥26
Ceftriaxone	CRO	30 µg	≤19	20-22	≥23
Cotrimoxazole	SXT	25 µg	≤10	11-15	≥16
Meropenem	MEM	10 µg	≤19	20-22	≥23
Doxycycline	DO	30 µg	≤10	12-13	≥14
Pipracillin-tazobactam	TZP	110 µg	≤17	18-20	≥21
Amoxicillin	AMC	30 µg	≤13	14-17	≥18
Ampicillin	AMP	10 µg	≤13	14-16	≥17
Gentamicin	CN	10 µg	≤12	13-14	≥15

Table 2: **Sequence of the primers used for resistance genes amplification through PCR**

S. No	Primers	Sequences	No of primer nucleotides	Amplicon size (bp)	Annealing Tm	Ref.
1	*bla*$_{CTX-M}$	F: 5'-AAAAATCACTGCGCCAGTTC-3' R: 5'-AGCTTATTCATCGCCACGTT-3'	20 20	415	60°C	[16]
2	*bla*$_{SHV}$	F: 5′-AAGCGAAAGCCAGCTGTCG-3′. R: 5′-TTCGCTCCAGCTGTTCGTC-3′	19 19	178	54°C	[17]
3	*bla*$_{TEM}$	F; 5′-TCCGCTCATGAGACAATAACC-3′ R; 5′-TAATACCGCACCACATAGCAG-3′	21 22	296	54°C	[18]

Table 3: Age and Sex distribution of patients with SSIs

Age in years	Male N (%)	Female N (%)	Overall (n=64)
0-20	4 (6.25%)	2 (3.12%)	6 (9.37%)
21-40	10 (15.62%)	16 (25.00%)	26 (40.62%)
41-60	4 (6.25%)	13 (20.31%)	17 (26.56%)
61-80	2 (3.12%)	13 (20.31%)	15 (23.43%)
Total	20 (31.25%)	44 (68.75%)	64 (100%)

The antibiotic susceptibility profiles of gram-negative bacterial isolates from post-surgical wound infections

The susceptibility pattern of the pathogens to the different antibiotics differed with respect to the isolates, *E. coli* (n=63), *Klebsiella pneumoniae* (n=18), *Pseudomonas aeruginosa* (n=9), *Acinetobacter baumannii* (n=21) and *Citrobacter spp* (n=2) isolated from surgical site infection were being subjected to antimicrobial susceptibility testing.

Tobramycin demonstrated remarkable effectiveness against most of the *E. coli* isolates, with a susceptibility rate of 73.01%, followed by Meropenem (61.90%), Gentamicin (52.38%), Pipracillin-Tazobactam (34.92%), and Doxycycline (31.72%). Conversely, Ampicillin exhibited a significantly high resistance rate (100%) among the *E. coli* isolates, followed closely by Ceftriaxone (96.82%), Cefazolin/Cefotaxime (93.65%), Ciprofloxacin (85.71%), Cotrimoxazole (71.81%), and Ceftazidime/Amoxicillin (71.42%). Notably, a portion of the *E. coli* isolates showed intermediate resistance to Amoxicillin (14.28%) as presented in (Table 4 & Fig. 1).

In the case of *Klebsiella pneumoniae* isolates, Tobramycin displayed exceptional efficacy, boasting an 88.88% susceptibility rate, followed by Gentamicin (72.22%), Ceftriaxone, Meropenem (50%), and Cefotaxime and Ceftazidime (38.88%). Conversely, Cotrimoxazole, Ampicillin, and Cefazolin exhibited a high resistance rate (100%) against *Klebsiella pneumoniae* isolates, followed by Ciprofloxacin, Doxycycline, and Pipracillin-Tazobactam (72.22%), as well as Cefotaxime and Ceftazidime (61.11%). Interestingly, some *Klebsiella pneumoniae* isolates displayed intermediate resistance to Tobramycin (11.11%) as presented in (Table 4 & Fig. 1).

Moving on to *Pseudomonas aeruginosa* isolates, Tobramycin demonstrated notable effectiveness, with a susceptibility rate of 77.77%, followed by Pipracillin-Tazobactam (55.55%). Conversely, Ciprofloxacin, Amoxicillin, and Ampicillin exhibited a high resistance rate (100%) against *Pseudomonas aeruginosa* isolates, followed by Ceftriaxone, Cotrimoxazole, Meropenem, Doxycycline, Gentamicin, Cefazolin, Cefotaxime, and Ceftazidime (77.77%). Pipracillin-tazobactam showed intermediate resistance (44.44%), while Tobramycin demonstrated the least resistance against *Pseudomonas aeruginosa* isolates as presented in (Table 4 & Fig. 1).

For *Acinetobacter baumannii* isolates, Doxycycline displayed significant effectiveness (90.04%), followed by Tobramycin (71.42%) and Gentamicin (33.33%). In contrast, Ciprofloxacin, Meropenem, Ampicillin, Cefazolin, and Ceftazidime exhibited a high resistance rate (100%) against *Acinetobacter baumannii* isolates, followed by Ceftriaxone, Cotrimoxazole, Pipracillin-tazobactam, and Cefotaxime (90.47%), as well as Gentamicin (57.14%). Some *Acinetobacter baumannii* isolates displayed intermediate resistance to Pipracillin-tazobactam and Gentamicin (9.52%) as presented in (Table 4).

Lastly, Gentamicin proved highly effective (100%) against all *Citrobacter spp*. isolates. Conversely, Ciprofloxacin, Ceftriaxone, Cotrimoxazole, Meropenem, Doxycycline, Pipracillin-tazobactam, Amoxicillin, Ampicillin, Tobramycin, Cefazolin, Cefotaxime, and Ceftazidime all exhibited a high resistance rate (100%) against Citrobacter isolates. The (Table 4) shows antibiotic sensitivity profiles of all isolated gram-negative bacterial isolates from wounds.

Multi-Antibiotic Resistant (MAR) Index for gram negative bacterial isolates

The Multi-Antibiotic Resistant (MAR) Index serves as a valuable tool for evaluating antibiotic resistance patterns within bacterial populations. In our study, the results revealed that 03 bacterial isolates exhibited a MAR Index (MARI) of 1, indicating a high level of resistance to multiple antibiotics. Additionally, 04 isolate displayed a MARI of 0.92, highlighting a substantial degree of antibiotic resistance, albeit slightly lower than the first group. These findings underscore the presence of diverse antibiotic resistance phenotypes among the bacterial isolates in our investigation. MAR Index for gram negative bacterial isolates as presented in (Table 5).

The antibiotic susceptibility profile of gram-Positive bacterial isolate from post-surgical wound infection.

All *Staphylococcus aureus* isolates were resistant Cefoxitin confirming them to be MRSA. The Teicoplanin and Vancomycin (100%) were very active against most of the *Staphylococcus aureus* isolates followed by Linezolid, Tetracycline (84.61%), Chloramphenicol (69.23%) and Clindamycin (69.23%). Clarithromycin (15.23%) showed least activity against *Staphylococcus aureus* isolates. Some *Staphylococcus aureus* isolates showed intermediate resistance to Clarithromycin (15.23%) as shown in (Table 6 & Fig. 2).

PCR based molecular screening of resistance genes in bacterial isolates

PCR based molecular screening of beta lactamase resistance genes was carried out in 24 randomly selected bacterial isolates due to limited molecular resources. A total of 03 beta-lactam resistance genes were screened including *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX\text{-}M1}$. The most prevalent gene was found to be *bla*$_{CTX\text{-}M1}$ (41.66%) followed by *bla*$_{TEM}$ (33.33%) as shown in (Fig. 3). The least prevalent gene was *bla*$_{SHV}$ whose prevalence was found to be 25%. The prevalence data of beta lactamase resistance genes is given in (Fig. 3). (Fig. 4-6) show gel electrophoresis diagrams of *bla*$_{CTX\text{-}M1}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ respectively.

Table 4: Antibiotic sensitivity profiles of all gram-negative bacterial isolates

Antibiotics	*E. coli* (N = 64)			*K. pneumonia* (N = 18)			*P. aeruginosa* (N = 09)			*A. baumannii* (N = 21)			*Citrobacter spp* (N = 02)		
	R %	I %	S %	R %	I %	S %	R %	I %	S %	R %	I %	S %	R %	I %	S %
CIP	85.71	0.0	14.28	72.22	0.0	27.78	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
CRO	96.82	0.0	3.17	50.00	0.0	50.00	77.77	0.0	22.22	90.47	0.0	9.52	100	0.0	0.0
SXT	71.82	0.0	28.57	100	0.0	0.0	77.77	0.0	22.22	90.47	0.0	9.52	100	0.0	0.0
MEM	38.09	0.0	61.90	50.00	0.0	50.00	77.77	0.0	22.22	100	0.0	0.0	100	0.0	0.0
DO	68.25	0.0	31.74	72.22	0.0	27.78	77.77	0.0	22.22	9.52	0.0	90.04	100	0.0	0.0
TZP	65.07	0.0	34.92	72.22	0.0	27.78	44.44	0.0	55.55	90.47	9.52	0.0	100	0.0	0.0
AMC	71.42	14.28	14.28	100	0.0	0.0	100	0.0	0.0	80.95	0.0	19.04	100	0.0	0.0
CN	47.61	0.0	52.38	27.78	0.0	72.22	77.77	0.0	22.22	57.14	9.52	33.33	0.0	0.0	100
TOB	26.98	8.51	73.01	0.0	11.11	88.88	22.22	0.0	77.77	28.57	0.0	71.42	100	0.0	0.0
KZ	93.65	0.0	6.34	100	0.0	0.0	77.77	0.0	22.22	100	0.0	0.0	100	0.0	0.0
AMP	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
CTX	93.65	0.0	6.34	61.11	0.0	38.88	77.77	0.0	22.22	90.47	0.0	9.52	100	0.0	0.0
CAZ	71.42	7.94	20.63	61.11	0.0	38.88	77.77	0.0	22.22	100	0.0	0.0	100	0.0	0.0

Table 5: Multi-Antibiotic Resistant (MAR) Index for gram-negative bacterial isolates

Antibiotics	No of isolates	MARI
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, MEM, DO, CN, TOB	03	1
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, MEM, DO, TOB	01	0.92
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, MEM, CN, TOB	02	0.92
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, MEM, DO, CN,	01	0.92
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, MEM, TOB	01	0.84
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, MEM, DO,	02	0.84
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, MEM, CN,	03	0.84
AMP, KZ, CIP, CTX, CRO, AMC, CAZ, TZP, MEM, DO, TOB	01	0.84
AMP, KZ, CIP, CTX, CRO, SXT, CAZ, TZP, MEM, CN, TOB	01	0.84
AMP, KZ, CIP, CTX, CRO, SXT, AMC, TZP, MEM, DO, TOB	01	0.84
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, CN, TOB	01	0.84
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, DO, TOB,	01	0.84
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, DO, CN	01	0.84
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, MEM,	04	0.76
AMP, KZ, CIP, CTX, CRO, AMC, CAZ, TZP, MEM, DO,	01	0.76
AMP, KZ, CIP, CTX, CRO, SXT, AMC, CAZ, TZP, DO,	01	0.76
AMP, KZ, CTX, SXT, AMC, CAZ, TZP, MEM, DO, CN,	01	0.76
AMP, KZ, CIP, CRO, SXT, CAZ, TZP, MEM, DO, CN,	01	0.76
AMP, KZ, CIP, CTX, CRO, SXT, TZP, DO, CN, TOB	01	0.76
AMP, CIP, CRO, SXT, DO, CN, KZ, AMP, CTX, CAZ	01	0.76
AMP, KZ, CIP, CTX, CRO, AMC, CAZ, TZP, MEM,	02	0.69
AMP, KZ, CIP, CTX, CRO, SXT, AMC, DO, CN,	01	0.69
AMP, KZ, CIP, CTX, CRO, SXT, CAZ, TZP, CN,	01	0.69
AMP, KZ, CIP, CTX, CRO, CAZ, TZP, DO, CN,	01	0.69
AMP, KZ, CIP, CTX, SXT, AMC, CAZ, DO, CN,	01	0.69
AMP, KZ, CTX, CRO, SXT, AMC, CAZ, TZP,	01	0.61
AMP, KZ, CIP, CTX, CRO, AMC, CAZ, TZP,	01	0.61
AMP, KZ, CIP, CTX, CRO, SXT, AMC, DO,	01	0.61
AMP, KZ, CIP, SXT, AMC, TZP, MEM, DO,	01	0.61
AMP, KZ, CTX, CRO, SXT, AMC, DO, CN,	01	0.61
AMP, KZ, CTX, CRO, AMC, CAZ, TZP,	01	0.53
AMP, KZ, CIP, CRO, SXT, AMC, CAZ,	01	0.53
AMP, KZ, CIP, CTX, CRO, SXT, CAZ,	01	0.53
AMP, KZ, CTX, SXT, CAZ, MEM, CN,	01	0.53
AMP, CIP, CTX, CRO, AMC, DO, CN,	01	0.53
AMP, KZ, CIP, CTX, CRO, SXT, DO	01	0.53
AMP, KZ, CIP, CRO, AMC, MEM,	01	0.46
AMP, KZ, CIP, SXT, AMC, DO,	01	0.46
AMP, KZ, CIP, CTX, CAZ,	01	0.38
AMP, KZ, SXT, AMC, DO,	01	0.38
AMP, SXT, AMC, DO,	01	0.30

Table 6: Antibiotic sensitivity profile of gram-positive bacterial isolates

	No	Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)
Gram positive Bacteria	13	TEC	0.0	0.0	100
		VA	0.0	0.0	100
		C	30.76	0.0	69.23
		LZD	15.38	0.0	84.61
		TE	15.38	0.0	84.61
		FOX	100	0.0	0.0
		DA	30.76	0.0	69.23
		CLR	69.23	15.23	15.38

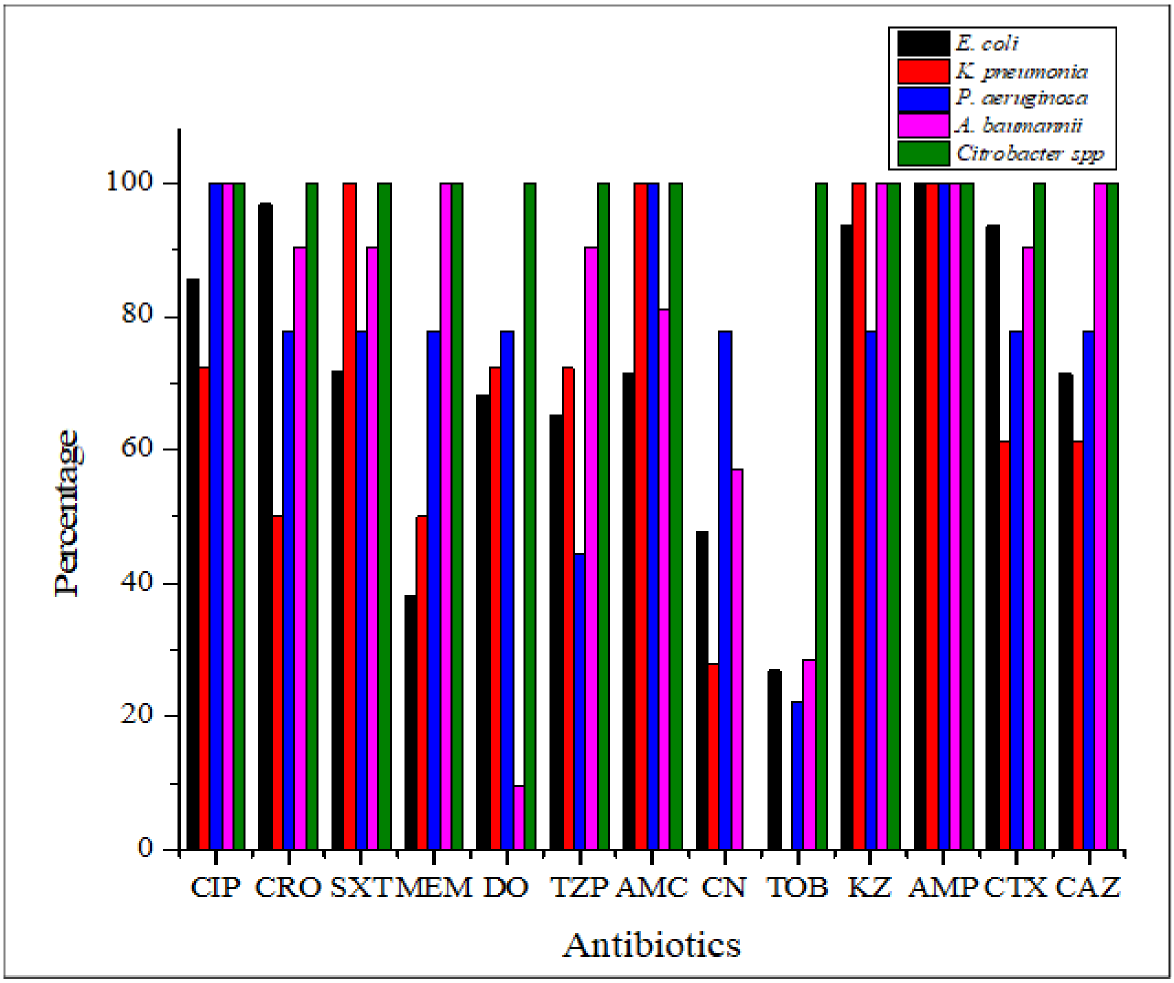

Figure 1: Percentage sensitivities of gram-negative bacterial isolates from post-surgical wound infections

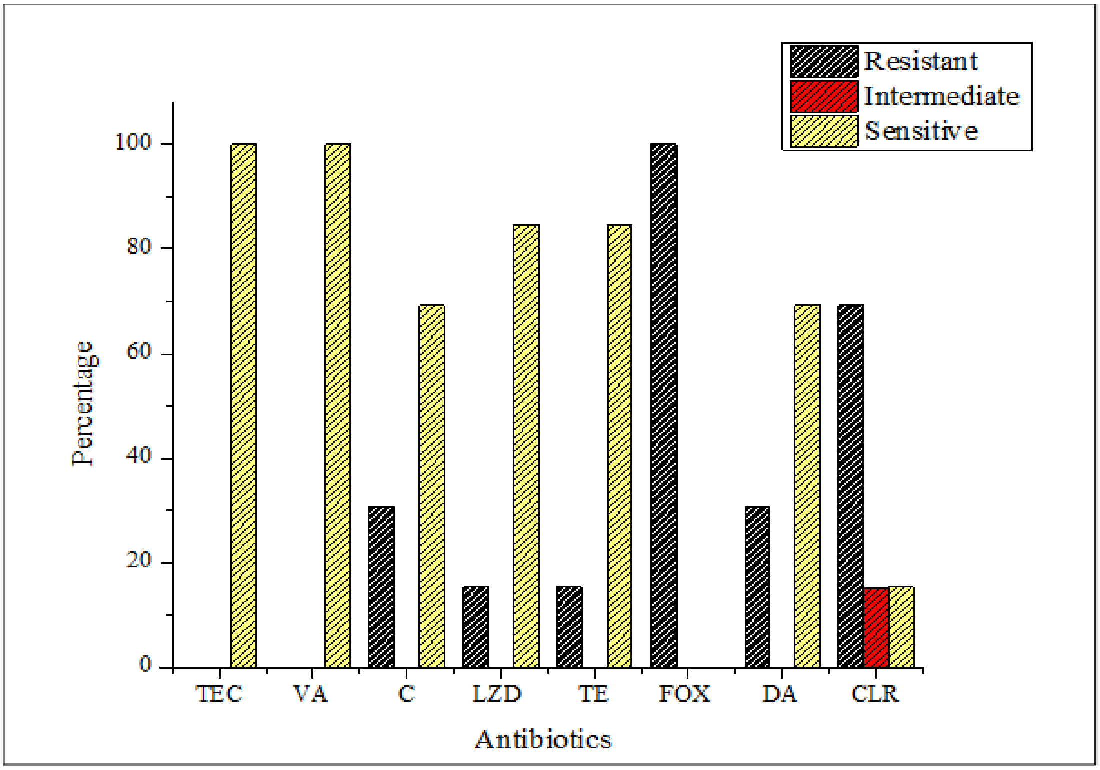

Figure 2: Diagrammatic representation of antibiotic sensitivity profiles of gram-positive bacterial isolates

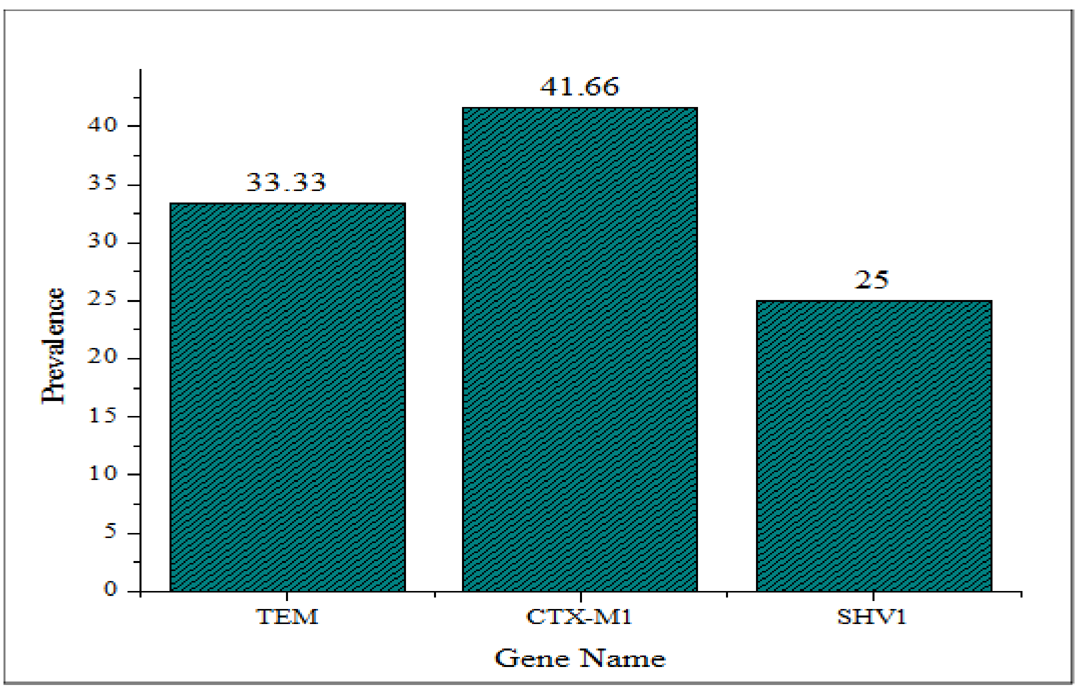

Figure 3: Percentage Prevalence of TEM, SHV, and CTXM in gram-negative bacterial isolates from post-surgical wound infections

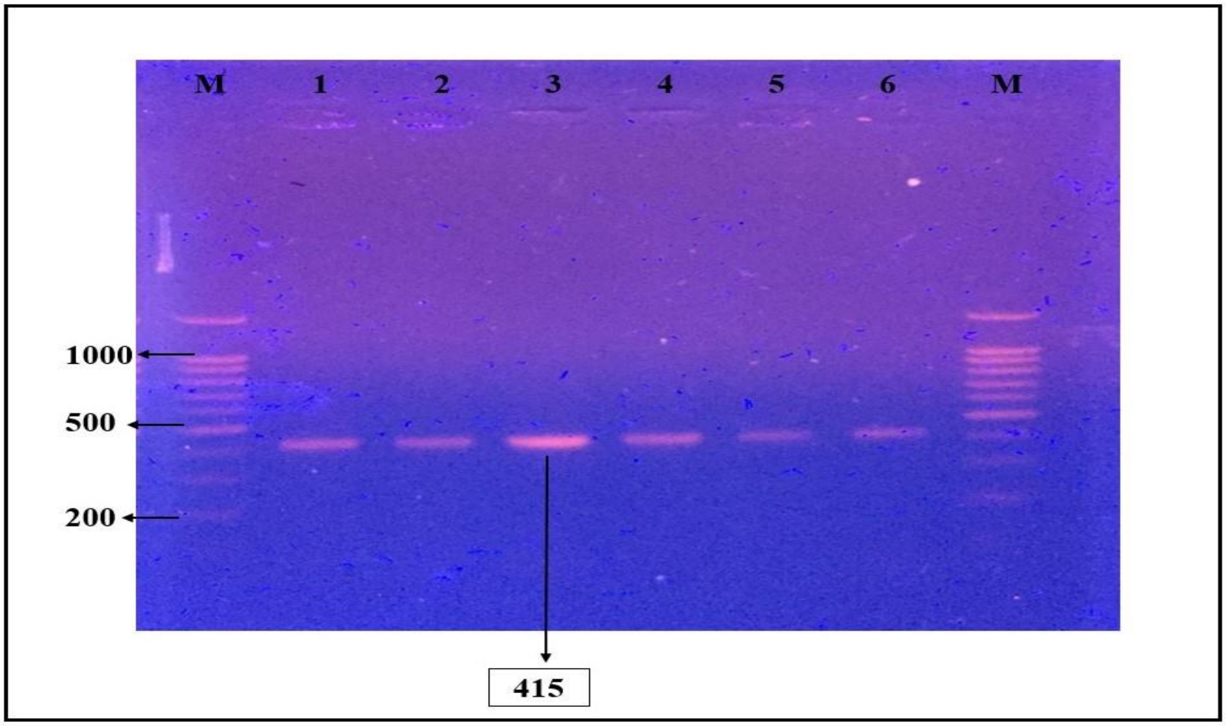

Figure 4: Gel electrophoresis diagram of *bla*$_{CTX\text{-}M1}$ gene. M shows 100bp ladder while Lane 3 shows 415bp PCR product of *bla*$_{CTX\text{-}M1}$ gene

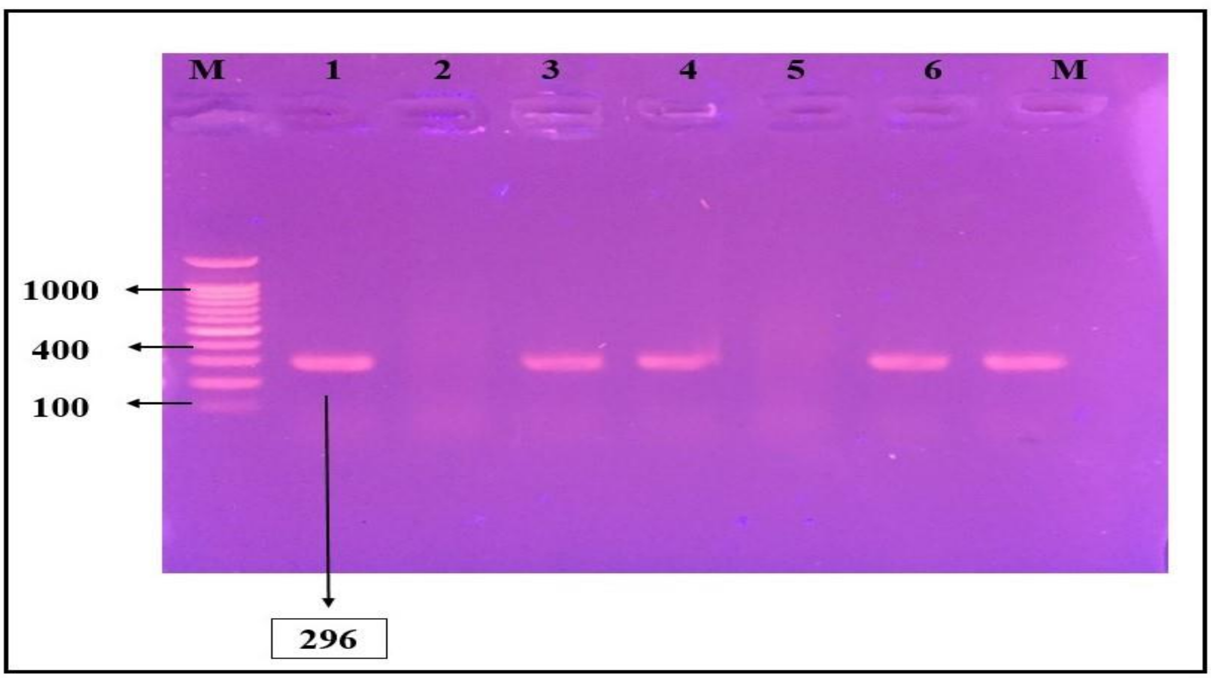

Figure 5: Gel electrophoresis diagram of *bla*$_{TEM}$ gene. M shows 100bp ladder while Lane 1 shows 296bp PCR product of *bla*$_{TEM}$ gene

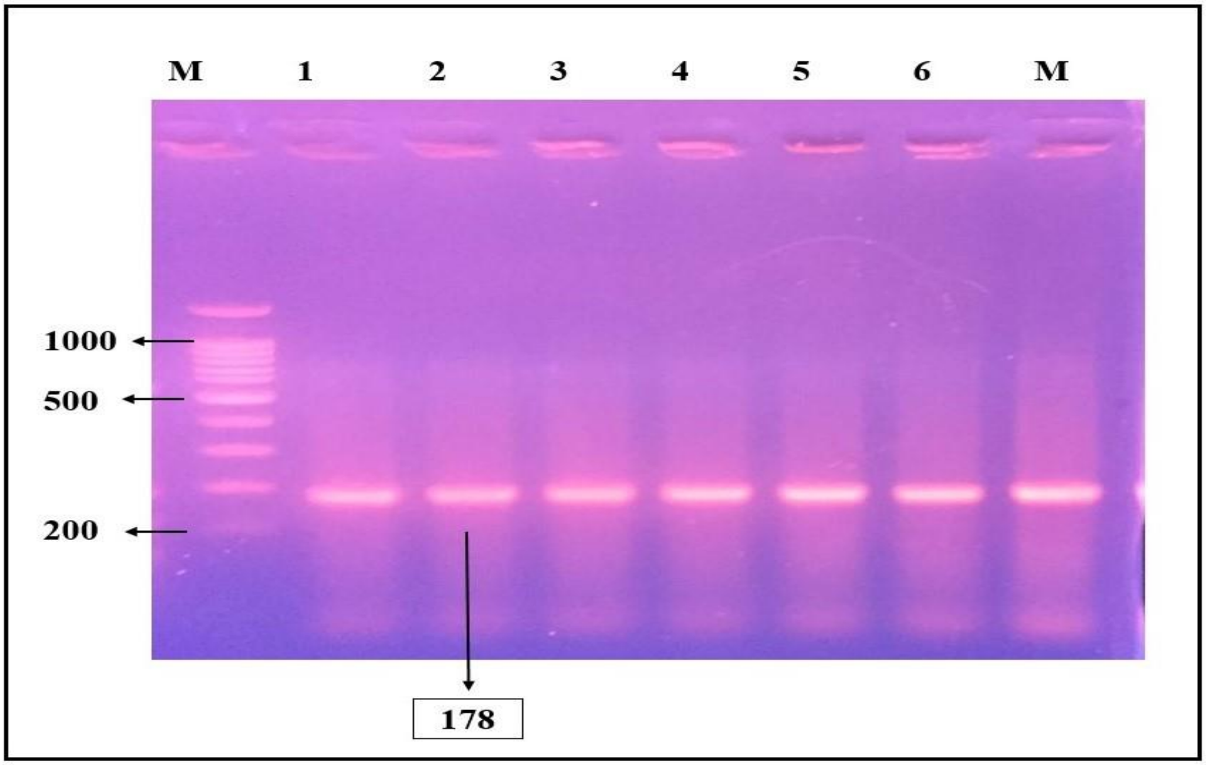

Figure 6: Gel electrophoresis diagram of *bla$_{SHV}$* gene. M shows 100bp ladder while Lane 2 shows 178bp PCR product of *bla$_{SHV}$* gene

Discussion

Wound infections are a continuing problem for many patients in both developing and developed countries. Infected wounds can cause great distress in terms of associated morbidity and mortality, increased length of hospital admission, delayed wound healing and increased discomfort and have long been known to increase healthcare cost significantly [19]. This study was conducted with the aim to find prevalence and antibiotic sensitivity data of bacterial isolates commonly detected in post-surgical wound infections in Peshawar region. In this study, a total of 110 samples were collected and studied. Out of 110, 64 (58.18%) resulted in growth over suitable culture media while 46 (41.79%) were negative for any growth. Similar results have been reported by Giri et al., 2008 reporting a prevalence of 56.8% [20]. The study conducted by Misha et al., 2021 in Ethiopia reported 71.7% prevalence which is much higher as compared to our results [21]. A research study conducted in Tertiary Care Hospital in Abbottabad reported 33.38% prevalence of bacterial isolates in postsurgical wound infections which is lower than our results [6]. The high prevalence in our study might be due to inefficient infection control practice in the hospital and improper environmental hygiene.

Consistent with the findings of Hubab et al., 2018, our current study observed a higher frequency of gram-negative organisms compared to gram-positive ones [22]. The prevalence of gram-negative bacteria, accounting for 57.9% of the 127 isolated microbial species from infected wounds, aligns with similar trends reported in other studies [23, 24]. This dominance of gram-negative bacteria emphasizes the clinical significance of these pathogens in post-surgical wound infections. Hubab et al.'s 2018 study [22] and our current findings collectively underscore a notable shift toward gram-negative bacterial prevalence in wound infections. This trend may reflect evolving microbial patterns or variations in healthcare settings, highlighting the importance of ongoing surveillance and understanding regional epidemiology for effective infection control strategies. In our investigation, the higher proportion of gram-negative bacteria (57.9%) compared to gram-positive bacteria (36.6%) reaffirms the broader consensus observed in the literature [23, 24]. The prevalence of gram-negative organisms in wounds exhibiting signs of infection suggests potential challenges in managing and treating these cases, considering the inherent resistance mechanisms associated with many gram-negative pathogens.

In our study, *E. coli* were the most frequently isolated pathogens with a prevalence of (50.30%), followed by *Acinetobacter baumannii* (16.53%), *Klebsiella pneumoniae* (14.17%), *Pseudomonas spp*. (7.08%), *Citrobacter spp* (1.57%). Similar results were also recorded by [25]. In another study *E. coli* (35.7%) was the most common pathogen isolated followed by *Staphylococcus aureus* (21.4%), *Pseudomonas aeruginosa* (14.3%), and *Klebsiella pneumoniae* (14.3%). *E. coli* invasion of the wound is a clear case of poor hospital hygiene [19, 26]. The difference in the report might be explained by the difference in the setting, study population, hospital environments and poor hospital hygiene. Most of the surgeries performed were abdominal surgeries and most cuts were contaminated which had leakage from the gastrointestinal tract.

In current study, pus samples were collected from patients ranging in age from 14 years to 80 years old. Out of 64 culture positive samples, highest number of samples was from the age group (21-40) years (40.62%), followed by the age group 41-60 (26.56%) and 61-80 (23.43%). The prevalence of wound infection was not significantly affected by age. Similar results were obtained in a study carried out by Kumari, [27] in Bir Hospital in which highest prevalence was found in age group (21-30 years) Raza *et al*. [26] also reported high prevalence in age group (21-40 years) in Nepal. Studies suggest that aging is associated with an increased risk of developing some chronic conditions and delayed recovery.

Out of the total pus samples 64 samples showed growth, of these, (79.68%) cases resulted in single type of isolates and (20.23%) cases showed polymicrobial growth. The monomicrobial growth was higher than polymicrobial growth in pus swabs. The result are in agreement with the study carried out by Kumari, [27] where single isolates were observed in (78.3%) of the cases.

All gram-positive bacterial isolates were resistant to ampicillin. The reason behind this could be the irrational use of ampicillin, which was one of the most used antibiotics for empiric prophylaxis [28]. The study conducted in Tertiary Care Hospital of Northeast India Deka *et al*. [29] also reported very high resistance to ampicillin (95.7%) which is in line with our results.

E. coli isolates were 100% resistant to Ampicillin. Similar result for Ampicillin were reported from Ethiopia [30]. *E. coli* isolates were also resistant to Ceftriaxone (96.82%), and Cotrimoxazole, Cefazolin (93.65%) and Tobramycin (73.01%) followed by Meropenem (61.90%) which clearly indicates the irregular use of these antibiotics in clinical settings as community practices. In contrast, a study in Uganda by Anguzu and Olila [31] reported that *E. coli* species had 87.05% sensitivity to Gentamycin. The difference in antibiotics susceptibility pattern is due to differences in geography and sample size of the study. This data demonstrate that these groups of drugs can still be recommended for the treatment of *E. coli* in SSIs.

Klebsiella pneumoniae isolates showed high resistance to Cotrimoxazole, Amoxicillin, Ampicillin and Cefazolin (100%) while Tobramycin was found to be the drug of choice against *Klebsiella pneumoniae* followed by Gentamycin (72.22%). The results are in agreement with studies conducted in Iraq **[32]** in which the least sensitive antibiotic was Amoxicillin (94.72%) Various research studies have reported sensitivity *Klebsiella pneumoniae* isolates to Meropenem **[32, 33]**.

Most of the *Pseudomonas aeruginosa* strains were highly sensitive to Tobramycin (77.77%) followed by Pipracillin-tazobactam (55.55%). The Ciprofloxacin, Amoxicillin and Ampicillin showed 100% resistance to *Pseudomonas aeruginosa*. In other study conducted by Blomberg *et al*. **[34]** reported that Meropenem showed (100%) sensitivity followed by Tobramycin and Gentamicin (86%) and the Ampicillin and Doxycycline showed 100% resistance. The difference in antibiotics susceptibility pattern is due in region and sample size. This data demonstrate that these groups of drugs can still be recommended for the treatment of *Pseudomonas* in SSIs.

All *Acinetobacter baumannii* isolates in our study were highly resistant to majority of antimicrobial agents tested. *Acinetobacter baumannii* showed high (100%) resistance to Meropenem, Ciprofloxacin and Ampicillin followed by Ceftriaxone, Cotrimoxazole. Cefotaxime showed 90.47% resistance. In our study we found that for SSIs caused by *Acinetobacter baumannii*, the drug of choice is Doxycycline (90.04%) followed by Tobramycin (71.42%). Similarly results have been reported by Blomberg *et al*. **[34]** in which Ampicillin and Cotrimoxazole showed (100%) resistant.

In our study *Citrobacter* showed (100%) resistance to all the antibiotic except Gentamycin. The 100% multi drug resistance of *Citrobacter* has been reported both in Ethiopia and Pakistan **[35, 36]**. The reason of high resistance to all the antibiotic is due to smaller number of isolates of *Citrobacter* bacteria in our study.

In this study, among gram positive isolates, the most sensitive antibiotic was Tetracycline and Vancomycin (100%) followed by Linezolid and Teicoplanin (84.61%), Chloramphenicol and Clindamycin (69.23%). The most resistance antibiotic was found to be Cefoxitin (100%) Clarithromycin (69.23%). Hossain *et al*., 2017 also reported that Vancomycin and Teicoplanin, was found to be most effective (100%) against *S. aureus* while isolates were resistant to Cefoxitin **[37]**.

Bacteria that produce extended-spectrum β-lactamase enzymes (ESBLs) are clinically important pathogens. An increasing number of ESBL-producing bacterial strains showing multidrug resistance have been observed **[38]**. In our study, the prevalence of bla_{TEM} was (33.33%), bla_{SHV} (25%), and $bla_{CTX\text{-}M1}$ (41.66%) genes among gram-negative bacterial isolates. bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M1}$ genes are the most common β-lactamases often found in *Enterobacteriaceae* **[39]**. Similar results have been reported in Switzerland by **[40]** detecting bla_{SHV} and $bla_{CTX\text{-}M1}$ in 23% and 39.2% isolates respectively. On contrary, the prevalence of bla_{TEM} was high (71.3%) as compared to our findings. These observations contribute to the knowledge of the epidemiology of bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M1}$ producing gram-negative isolates that have now become endemic in major hospitals in Peshawar. Continuous monitoring, proper infection control programs, and surveillance and prevention practices will limit the further spread of these infections within these hospitals and clinical settings.

Conclusion

This study reported high prevalence rate of surgical wound infection by gram-negative bacteria predominated by *E. coli, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Citrobacter spp* and in gram positive *Staphylococcus aureus* was the mostly common isolated. Tobramycin, Gentamycin, Meropenem, and Doxycycline were effective against gram negative bacteria, whereas Ampicillin, Cefazolin, Cefotaxime and Ciprofloxacin were the least effective against gram negative bacterial isolates. All gram-positive isolates were MRSA. The best effective antibiotic for MRSA in our study was Tetracycline and Vancomycin. Therefore, treatment of wound infections has to be made based on the culture and susceptibility test results. In addition, to address the high rate of MDR, health education must be strengthened and illicit drug handling practices and irrational prescription of drugs must be discontinued in health facilities.

Authors' contributions

Designed and conceived the study: Y Ahmad, A Ali & M Hussain, Collected and processed the samples: Y Ahmad, SU Rahman, J Noor & I Ullah, Analyzed the data: Y Ahmad, H Shahzadi, AQ Khan & MD Khan, Contributed to the provision of chemicals and tools: Y Ahmad, S Jamil & M Asim: Wrote the manuscript: Y Ahmad.

References

1. Zabaglo M & Sharman T (2023). Postoperative Wound Infection. *StatPearls Treasure Island (FL)*
2. Bellows CF, Mills KT, Kelly TN, & Gagliardi G (2011). Combination of oral non-absorbable and intravenous antibiotics versus intravenous antibiotics alone in the prevention of surgical site infections after colorectal surgery: a meta-analysis of randomized controlled trials. *Tech Coloproct* 15(4): 385-395.
3. Haque M, Sartelli M, McKimm J, & Abu Bakar M (2018). Health care-associated infections - an overview. *Infect Drug Resist* 11: 2321-2333.
4. McFee RB (2009). Nosocomial or hospital-acquired infections: an overview. *Dis Mon* 55(7): 422-438.
5. Awoke N, Arba A & Girma A (2019). Magnitude of surgical site infection and its associated factors among patients who underwent a surgical procedure at Wolaita Sodo University Teaching and Referral Hospital, South Ethiopia. *PLoS One* 14(12): e0226140.
6. Sattar F, Sattar Z, Zaman M, & Akbar S (2019). Frequency of Post-operative Surgical Site Infections in a Tertiary Care Hospital in Abbottabad, Pakistan. *Cureus* 11(3): e4243.
7. Mezemir R, Seid A, Gishu T, Demas T, & Gize A (2020). Prevalence and root causes of surgical site infections at an academic trauma and burn center in Ethiopia: a cross-sectional study. *Patient Saf Surg* 14: 3.
8. Leaper D, McBain AJ, Kramer A, Assadian O, Sanchez JL, Lumio J, & Kiernan M (2010). Healthcare associated infection: novel strategies and antimicrobial implants to prevent surgical site infection. *Ann R Coll Surg Engl* 92(6): 453-458.
9. Guggenheim M, Zbinden R, Handschin AE, Gohritz A, Altintas MA, & Giovanoli P (2009). Changes in bacterial isolates from burn wounds and their antibiograms: a 20-year study (1986-2005). *Burns* 35(4): 553-560.
10. Andersen BM (2019). Prevention of Postoperative Wound Infections. *Springer Inter Publ* 377-437
11. Uddin TM, Chakraborty AJ, Khusro A, Zidan BRM, Mitra S & Emran TB (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic

strategies and future prospects. *J Infect Pub Health* 14(12): 1750-1766.

12. Ventola CL (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharm and Therapeut* 40(4): 277-283.
13. Bessa LJ, Fazii P, Di Giulio M, & Cellini L (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. *Inter Wound J* 12(1): 47-52.
14. Negut I, Grumezescu V, & Grumezescu AM (2018). Treatment Strategies for Infected Wounds. *Mol* 23(9): 2392.
15. Okwu MU, Olley M, Akpoka AO, & Izevbuwa OE (2019). Methicillin-resistant *Staphylococcus aureus* (MRSA) and anti-MRSA activities of extracts of some medicinal plants: A brief review. *AIMS Microbiol* 5(2): 117-137.
16. Woodford N, Fagan EJ, & Ellington MJ (2006). Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. *J Antimicrob Chemother* 57(1): 154-155.
17. Meyer L, Labuschagne CDJ, Ehlers MM, Dove MG, & Weldhagen GF (2007). Diversity of bla-type genes in extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolated during 2003 — 2004 at Pretoria Academic Hospital. *SAJID* 22(1): 5-7.
18. Ghorbani-Dalini S, Kargar M, Doosti A, Abbasi P, & Sarshar M (2015). Molecular Epidemiology of ESBL Genes and Multi-Drug Resistance in Diarrheagenic *Escherichia Coli* Strains Isolated from Adults in Iran. *Iran J Pharm Res* 14(4): 1257-1262.
19. Shimekaw M, Tigabu A, & Tessema B (2022). Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Risk Factors Among Patients With Wound Infections at Debre Markos Referral Hospital, Northwest, Ethiopia. *Int J Low Extrem Wounds* 21(2): 182-192.
20. Giri BR, Pant HP, Shankar PR, Sreeramareddy CT, & Sen PK (2008). Surgical site infection and antibiotics use pattern in a tertiary care hospital in Nepal. *J Pak Med Assoc* 58(3): 148-151.
21. Misha G, Chelkeba L, & Melaku T (2021). Bacterial profile and antimicrobial susceptibility patterns of isolates among patients diagnosed with surgical site infection at a tertiary teaching hospital in Ethiopia: a prospective cohort study. *Ann Clin Microbiol Antimicrob* 20: 33.
22. Hubab M, Ullah O, Hayat A, Rehman MU, & Sultana N (2018). Antibiotic susceptibility profile of bacterial isolates from post-surgical wounds of patients in tertiary care hospitals of Peshawar, Pakistan. *J Pak Med Assoc* 68: 1517-1521.
23. Guan H, Dong W, Lu Y, Jiang M, Zhang D & Aobuliaximu Y (2021). Distribution and Antibiotic Resistance Patterns of Pathogenic Bacteria in Patients With Chronic Cutaneous Wounds in China. *Front Med (Lausanne)* 8: 609584.
24. Puca V, Marulli RZ, Grande R, Vitale I, Niro A & Molinaro G (2021). Microbial Species Isolated from Infected Wounds and Antimicrobial Resistance Analysis: Data Emerging from a Three-Years Retrospective Study. *Antibiotics (Basel)* 10(10): 1162.
25. Hamdy Mohammed el S, Elsadek Fakhr A, Mohammed El Sayed H, Al Johery SA, & Abdel Ghani Hassanein W (2016). Spread of TEM, VIM, SHV, and CTX-M beta-Lactamases in Imipenem-Resistant Gram-Negative Bacilli Isolated from Egyptian Hospitals. *Inter J Microbiol* 8382605.
26. Raza MS, Chander A, & Ranabhat A (2013). Antimicrobial Susceptibility Patterns of the Bacterial Isolates in Post-Operative Wound Infections in a Tertiary Care Hospital, Kathmandu, Nepal. *Open*

J Med Microbiol 03: 159-163.
27. Kumari K (2008). Pattern of bacterial isolates and antibiogram fromopen wound infection among the indoor patients ofbir hospital. MSc Thesis. Central Department of Microbiology, Tribhuvan University.
28. Hope D, Ampaire L, Oyet C, Muwanguzi E, Twizerimana H, & Apecu RO (2019). Antimicrobial resistance in pathogenic aerobic bacteria causing surgical site infections in Mbarara regional referral hospital, Southwestern Uganda. *Sci Rep* 9(1): 17299.
29. Deka S, Kalita D, Mahanta P, & Baruah D (2020). High Prevalence of Antibiotic-Resistant Gram-Negative Bacteria Causing Surgical Site Infection in a Tertiary Care Hospital of Northeast India. *Cureus* 12(2): e12208.
30. Mengesha RE, Kasa BG-S, Saravanan M, Berhe DF, & Wasihun AG (2014). Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. *BMC Res Notes* 7(1): 575.
31. Anguzu JR & Olila D (2007). Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *Afr Health Sci* 7(3): 148-154.
32. Ali KM & Al-Jaff BM (2021). Source and antibiotic susceptibility of gram-negative bacteria causing superficial incisional surgical site infections. *Int J Surg Open* 30: 100318.
33. Mahmood A (2000). Bacteriology of surgical site infections and antibiotic susceptibility pattern of the isolates at a tertiary care hospital in Karachi. *J Pak Med Ass* 50(8): 256-259.
34. Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DS & Jureen R (2007). Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. *BMC Infect Dis* 7: 43.
35. Godebo G, Kibru G, & Tassew H (2013). Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. *Ann Clin Microbiol Antimicrob* 12: 17.
36. Dilnawaz S, Shaikh M, Shaikh M, Naqvi BS, Shaikh MR & Shoaib MH. 2003. Post surgical wound infections: a study on threats of emerging resistance. *Pak J of Pharma* 20(1): 31-41.
37. Hossain MZ, Naher A, Hasan P, Mozazfia KTE, Tasnim H & Ferdush Z (2017). Prevalent Bacteria and Their Sensitivity and Resistance Pattern to Antibiotics: A Study in Dhaka Medical College Hospital. *J Dhaka Med Coll* 26(1): 52-64.
38. Ojdana D, Sacha P, Wieczorek P, Czaban S, Michalska A & Jaworowska J (2014). The Occurrence of blaCTX-M, blaSHV, and blaTEM Genes in Extended-Spectrum β-Lactamase-Positive Strains of *Klebsiella pneumoniae*, Escherichia coli, and Proteus mirabilis in Poland. *Int J Antibiot* 2014: 1-7.
39. Shah AA, Hasan F, Ahmed S, & Hameed A (2004). Characteristics, epidemiology and clinical importance of emerging strains of Gram-negative bacilli producing extended-spectrum beta-lactamases. *Res Microbiol* 155(6): 409-421.
40. Zurfluh K, Hachler H, Nuesch-Inderbinen M, & Stephan R (2013). Characteristics of extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae Isolates from rivers and lakes in Switzerland. *Appl Environ Microbiol* 79(9): 3021-3026.