Original Article

Bacteriological profile with phenotypic detection of MDR isolates in surgical site infections of Nishtar hospital, Multan

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ABSTRACT

Objective: To determine bacteriological profile with phenotypic detection of MDR isolates in surgical site infections.

Materials and Methods: This cross-sectional descriptive study determined the frequency of bacteria causing surgical site infections in patients admitted at Nishtar Hospital, Multan. A total of 175 wound samples were collected and processed in the laboratory. All bacterial strains were characterized, and multidrug resistant strains were identified by an antibiotic susceptibility test. Moreover, modified carbapenem inactivation method, combine disc diffusion, and double disc synergy methods were employed to identify carbapenemases, metallo beta-lactamases, and extended spectrum beta-lactamases production among gram negative bacilli, respectively. Likewise, Cefoxitin-disc diffusion method was employed to identify *S. aureus* strains as *methicillin-resistant staphylococcus aureus*.

Results: In this study, *P. aeruginosa* (40%), *E. coli* (19.4%), *Proteus* spp. (8.6%), *K. pneumoniae* (6.3%), *Enterobacter* (2.9%), and *A. baumannii* (2.2%) made up the majority of the detected Gram-Negative Bacilli, while *S. aureus* (20.6%) was the only isolated Gram-Positive Cocci. A significant proportion of Gram-Negative Bacilli showed resistance to amoxicillin/clavulanic acid, ampicillin, ceftriaxone, trimethoprim/sulfamethoxazole, ciprofloxacin, amikacin, piperacillin/tazobactam, and meropenem, while Gram Positive Cocci showed resistance to ampicillin, amoxicillin/clavulanic acid, cefoxitin, and ceftriaxone. In this study, among 139 identified Gram-Negative Bacilli, 111 (79.9%) strains were CP (+), 122 (87.8%) strains were MBL (+), and 62 (44.6%) strains were ESBL (+). Likewise, 36 isolated strains of *S. aureus* were analyzed, out of which 30 (83.3%) were *Methicillin-Resistant Staphylococcus Aureus* (+).

Conclusion: our study will help in surveillance of resistance patterns of antibiotics and provide a cornerstone for the appropriate therapeutic strategy against multidrug-resistant infection.

Keywords: Carbapenemases, ESBLs, MBLs, *methicillin-resistant staphylococcus aureus*, surgical site infections

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INTRODUCTION

The Centers for Disease Control and Prevention (CDC) defines surgical site infection as an infection of the wound that happens within 30 days of an operation or after a year (if the infection is suspected to be connected to surgery) [1]. surgical site infection is the second most prevalent hospital-acquired infection (HAIs) with a probability between 2 and 11% for all surgical procedures [2]. surgical site infection typically occurs because of microbes present in the environment of the operating room and contaminated surgical tools [3]. Surgical site infection can be avoided, but it is associated with significant mortality among patients, lengthy hospital stays, and increased expenses [4]. The opportunistic and commonly isolated Gram-positive bacteria (GPB) and gram-negative bacilli from surgical site infection are

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Acinetobacter baumannii, *Enterobacter* spp., *Enterobacteriaceae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus* spp., and *Staphylococcus aureus* [5-7]. *Staphylococcus aureus* is the most common cause of surgical site infection in the skin microbiome. Besides that, *Enterococcus* species and *Escherichia coli* from the gut microbiome cause the most surgical site infection [8].

The majority of the time, foreign and/or microbiome bacteria penetrate surgical wounds (SWs) either during the procedure (primary infection) or right after the procedure (secondary infection) [9]. The common symptoms of infected SWs are pain, discomfort, inflammation, swelling, and discharge from infection sites. The primary infections appear within five to seven days after surgery. The majority of surgical site infections are not complicated because they just infect the skin and tissue underneath. Numerous patient-specific factors (old age, food habits, concomitant illness, subpar surgical methods, and insufficient sterilization of surgical tools) might have a significant impact on the incidence of surgical site infection. In addition to these, the virulence and intrusiveness of microorganisms, the integrity of the immune system, and the condition of the surrounding tissues also play a major role [9, 10].

Intracavitary, moderate incisional, and deep incisional are different kinds of surgical site infection. Deep incisional surgical site infection usually involves more extensive debridement following surgery and frequent prophylactic antibiotic therapy than moderate surgical site infection [11]. Surgical site infection can be classified as either acute (lasting less than 30 days) or chronic (lasting longer than 30 days) wound infections. In current study patients were included with surgical site infection irrespective of duration of infection. The free-floating bacteria that cause acute wound infections tend to proceed with accelerated destruction of tissue and appearance, although they often recover. However, chronic infections are persistently undulating with many flare-ups, and they respond only partially to systematic antibiotics [11, 12].

The incorporation of antibiotics into clinical trials was a critical component in the development of the contemporary hospital system [13]. However, over time, the rise of multidrug-resistant bacteria (MDR) has become a global health concern. It further makes it difficult for medical professionals to establish the best treatment alternatives. The negative impacts of MDR bacteria spread are most severe in developing countries[14]. The resistance to antibiotics is one of the top ten health risks worldwide. According to the World Health Organization (WHO), approximately fifty percent of cases caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* exhibited resistance to many potent antibiotics [7].

The hospitals in Pakistan lack a well-organized surveillance program to track surgical site infection daily. The occurrence of surgical site infection in hospitals can be reduced if there is more published literature on the proper diagnosis of surgical site infection, the ascending problem of MDR bacteria, and the appropriate use of antibiotics. Therefore, the current study was carried out to determine the causative pathogens with their antimicrobial resistance in patients suffering from surgical site infection in different surgical wards of Nishtar Hospital, Multan. Furthermore, standard phenotypic methods were employed to determine the frequency of metallo-β-lactamases (MBLs), extended-spectrum β-lactamases (ESBLs), carbapenemase (CRs) producers, and *methicillin-resistant staphylococcus aureus* (MRSA) among isolated MDR bacteria.

MATERIAL AND METHODS

The current cross-sectional descriptive study was conducted in the microbiology laboratory of the Pathology Department, Nishtar Hospital Multan, after receiving approval from the Ethical Review Committee. A sample size of 175 was calculated by using the WHO sample size calculator after taking the confidence level (95%), the margin of error (5%), and an anticipated MDR frequency of 13 from a

previous study [15]. The patients with surgical wounds representing symptoms of swelling, pain, redness, a foul smell, and discharge from wounds were selected for the current study. However, the patients with known infections or taking antibiotic treatment were excluded from the study. A written consent was obtained from every patient to use their samples for the current study.

The sterile cotton-tipped applicators were used to collect samples from patient with surgical site infection. Firstly, adequate pressure was applied around the wounds to cause the purulent exudates to express themselves. After that, each cotton-tipped applicator was gently circulated over a 1 cm^2 area of wound for 5 seconds. Following that, each wound underwent two swabs. Finally, all collected samples were transferred to the microbiological laboratory under aseptic conditions. Gram staining was performed for the first swab, while the second swab was inoculated into blood and MacConkey agar plates. The agar plates were incubated at 37°C overnight aerobically. The bacterial isolates were further identified and characterized by microscopy, colony morphology, and standard biochemical tests. Gram negative bacilli were characterized by oxidase, TSI, SIM, urease, and citrate tests, whereas GPC were characterized by catalase, coagulase, and DNase tests.

The antibiotic susceptibility testing (AST) was performed by employing the Kirby-Bauer disc diffusion method on Muller Hinton agar (MHA) plates, and the zones of inhibition (ZOIs) were interpreted following Clinical Laboratory Standards Institute (CLSI) guidelines for 2022. In this method, 0.5 McFarland suspensions of test and control bacteria were separately prepared in 5 mL of normal saline. These suspensions were aseptically inoculated onto the MHA plates. After 20 minutes, antibiotic discs were subsequently placed onto the inoculated plates, and the plates were incubated at 37 °C for 20 hours.

For modified carbapenem inactivation (mCIM) method, 1 µL loopful of strain (*Enterobacteriaceae*) or 10 µL loopful of *P. aeruginosa* or *A. baumannii* from agar plates were emulsified in 2 mL of trypticase soy broth. A meropenem disc was then immersed in the suspension and incubated for 4 hours at 35 °C. A 0.5 McFarland suspension of *E. coli* ATCC 25922 was prepared in 5 mL of saline using the direct colony suspension method. The MHA plate was inoculated with *E. coli* ATCC 25922 using the routine disc diffusion procedure. The meropenem disc was removed from the TSB and placed on the MHA plate previously inoculated with the *E. coli* ATCC 25922 indicator strain. This plate was incubated at 35 °C in ambient air for 18−24 h. No ZOI or colonies within a 16-18 mm zone was considered a positive result, while a ZOI of ≥ 19 mm was considered a negative result.

The MBL producers were identified by the combined disc diffusion method. For this experiment, imipenem (IPM-10 µg) was soaked in a test tube containing 10 µl of 0.5 M EDTA for 10-20 seconds. The MHA plates were inoculated with bacterial suspensions (0.5 McFarland). The IPM-10+EDTA and IPM-10 discs were placed on these inoculated MHA plates at appropriate distances. The agar plates were incubated at 37 °C overnight. The next day, if the increase in ZOI with the IPM-10+EDTA disc was 7 mm or more than that of the IPM-10 disc alone, then it was considered a positive result.

The ESBL producers were detected by the double disc synergy test. For this experiment, ceftazidime (CAZ-30 µg) and clavulanic acid (CLA-10 µg) antibiotic discs were placed on MHA plates previously inoculated with bacterial suspensions (0.5 McFarland). The inoculated plates were incubated at 37 °C overnight. The next day, a ≥ 5 mm increase in the ZOI for CAZ-30 in combination with CLA-10 was considered a positive result.

All *methicillin-resistant staphylococcus aureus* strains were determined by a disc diffusion test using a cefoxitin (FOX-30 µg) disc on an MHA plate. In 4 mL of saline, an over-night grown *S. aureus* culture was suspended, and turbidity was compared to standard 0.5 MacFarland. The MHA plate was inoculated with this suspension. After that, FOX-30 was positioned on the agar plate. This plate was incubated at 33-35°C for 24 hours. A ZOI ≤ 21

mm was considered a positive result for *methicillin-resistant staphylococcus aureus* strains.

The collected data were entered into the computer and analyzed using SPSS (Statistical Package for Social Sciences) version 25. All variables (age, gender, bacterial isolates, and resistant patterns) were represented in the form of frequencies and percentages in this cross-sectional descriptive study.

RESULTS

In the current study, 175 patients were included. Among them, 53 (30.3%), 50 (28.6%), 37 (21.1%), 23 (13.1%), and 12 (6.9%) patients belonged to age groups of <15 years, 16-30 years, 31-45 years, 46-60 years, and >60 years, respectively. Similarly, 98 (56.0%) patients were male and 77 (44.0%) were female. In this study, out of 175 patients 139 (79.4%) strains were gram negative bacilli, and 36 (20.5%) were gram positive cocci. The identified gram-negative bacilli were *P. aeruginosa* (70), *E. coli* (34), *Proteus* spp. (15), *K. pneumoniae* (11), *Enterobacter* spp. (5), and *A. baumannii* (4). The only identified Gram-positive bacterium was *S. aureus* (36) (Figure-I).

In the current study, the screening of CPs, ESBLs, and MBLs production was observed in isolated gram-negative bacilli. According to the results, 111 (79.9%) strains were CP (+), while 28 (20.1%) strains were CP (-). Out of these positive strains, 3 (75%), 5 (100%), 27 (79.4%), 8 (72.7%), 12 (80%), and 56 (80%) were *A. baumannii*, *Enterobacter* spp., *E. coli*, *K. pneumoniae*, *Proteus* spp., and *P. aeruginosa*, respectively (Figure-IIa). Similarly, 122 (87.8%) strains were MBL (+), while 17 (12.2%) strains were MBL (-). Out of these positive strains, 4 (100%), 4 (80%), 31 (91.2%), 10 (90.9%), 11 (73.3%), and 62 (88.6%) were *A. baumannii*, *Enterobacter* spp., *E. coli*, *K. pneumoniae*, *Proteus* spp., and *P. aeruginosa*, respectively (Figure-IIb).

Likewise, 62 (44.6%) strains were ESBL (+), while 77 (55.4%) strains were ESBL (-). Out of these positive strains, 2 (50%), 1 (20%), 24 (70.6%), 3 (27.3%), 8 (53.3%), and 24 (34.3%) were *A. baumannii*, *Enterobacter* spp., *E. coli*, *K. pneumoniae*, *Proteus* spp., and *P. aeruginosa*, respectively (Figure-IIIa). In the current study, 30 (83.3%) isolated strains of *S. aureus* were identified as *methicillin-resistant staphylococcus aureus* (Figure-IIIb).

Table-I: Antibiogram of isolated gram-negative bacilli.

Bacteria	AMP		AMC		AK		CRO		CIP		MEM		SXT		TZP	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
A. baumannii	4	-	4	-	4	-	4	-	4	-	4	-	4	-	2	2
Enterobacter spp.	4	1	5	-	5	-	2	3	3	2	5	-	5	-	5	-
E. coli	13	21	24	10	11	23	12	22	19	15	31	3	34	-	29	5
K. pneumoniae	7	4	10	1	4	7	7	4	7	4	10	1	3	8	9	2
Proteus spp.	7	8	18	7	4	11	9	6	6	9	15	-	8	7	10	5
P. aeruginosa	-	-	61	9	33	37	47	23	38	32	60	10	67	3	57	13

*AMP (Ampicillin), AMC (Amoxicillin-Clavulanic Acid), AK (Amikacin), CRO (Ceftriaxone), CIP (Ciprofloxacin), MEM (Meropenem), SXT (Sulfamethoxazole-Trimethoprim), and TZP (Piperacillin-Tazobactam), R (resistant), S (sensitive)

Table-II: Antibiogram of isolated Gram-positive bacilli.

Bacteria	AMP		AMC		CRO		DA		FOX		LNZ		VA	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
S. aureus	32	4	31	5	30	6	8	28	30	6	6	30	8	28

*AMP (Ampicillin), AMC (Amoxicillin-Clavulanic Acid), CRO (Ceftriaxone), DA (Daptomycin), FOX (Cefoxitin), LNZ (Linezolid), and VA (Vancomycin), R (resistant), S (sensitive).

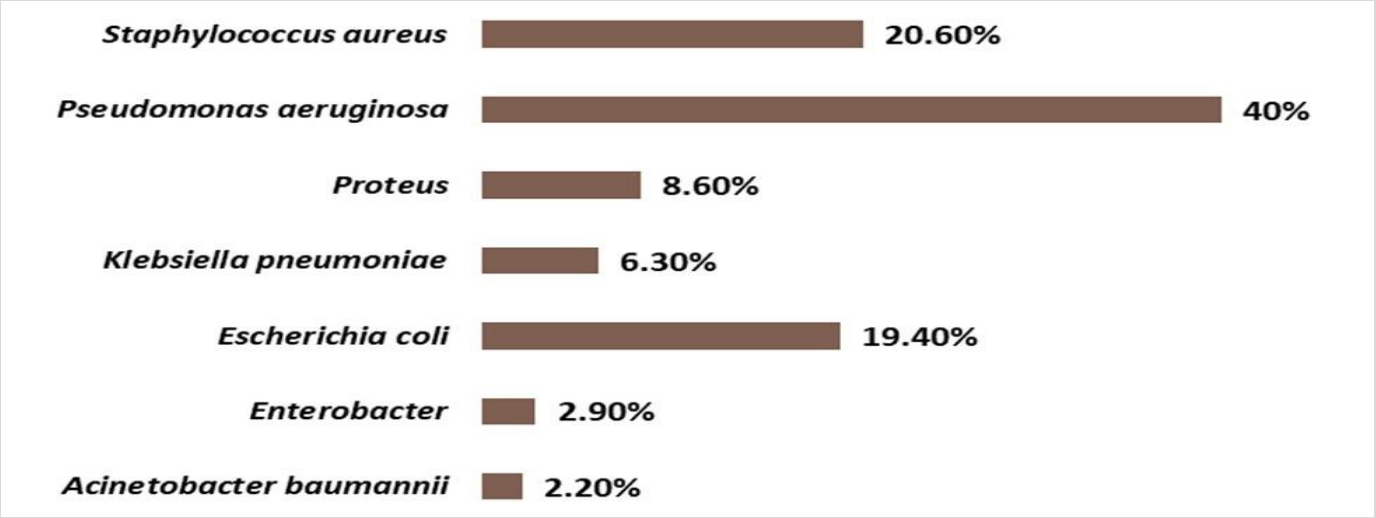

Figure-I: Bacterial strain distribution among all patients (n=175).

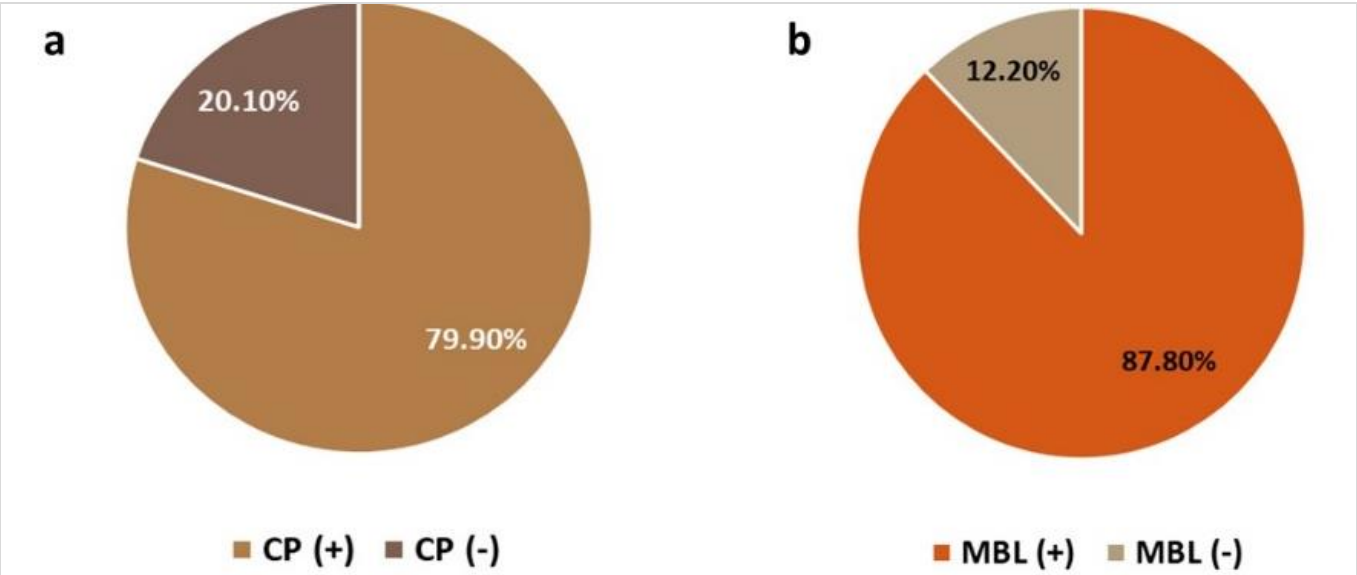

Figure-II: Pie graphs show percentages of CP producers or non-producers (a) and proportions of MBL producers or non-producers (b).

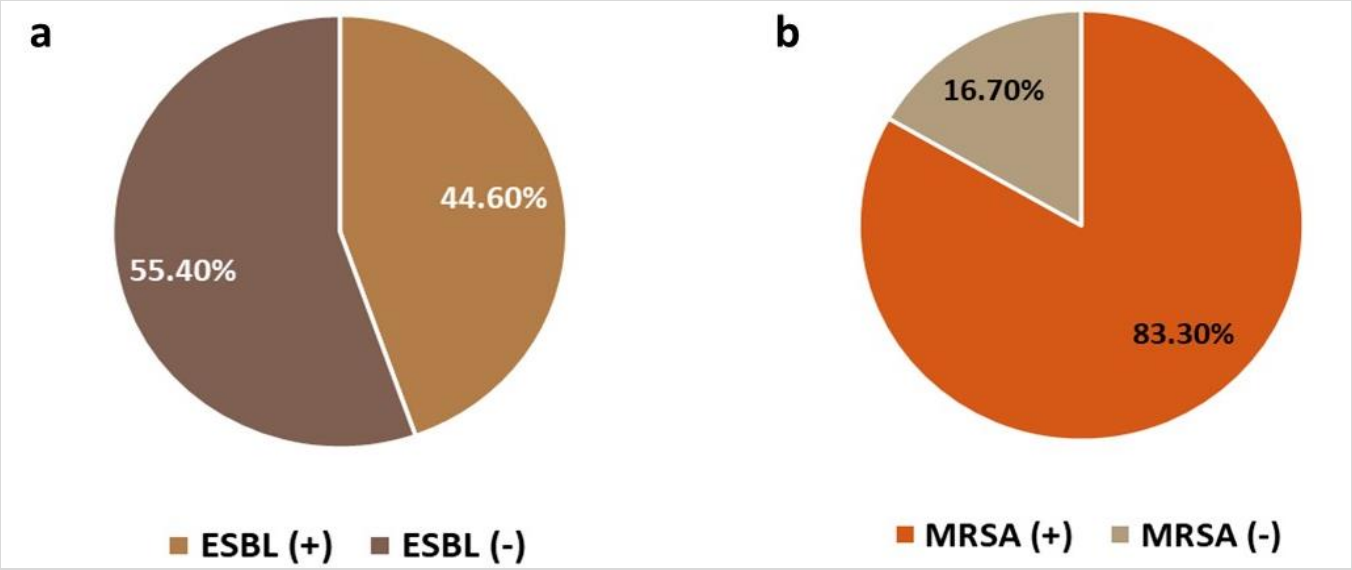

Figure-III: Pie graphs show percentages of ESBL producers or non-producers (a) proportions of *methicillin-resistant staphylococcus aureus* among isolated strains of *S. aureus* (b).

DISCUSSION

Surgical site infection is among the most often reported nosocomial infections that develop following an invasive surgical procedure [16]. Surgical site infection may result in a protracted hospital stay, a high rate of readmissions increased patient morbidity and death, reoperations, and increased medical expenses [17]. Drug resistance occurs due to the misuse and mismanagement of antibiotics is a major threat to humans. Another important dimension to the problem of surgical site infection is the recent spread of MDR bacterial pathogens [18].

In our study, a total of 175 isolated strains were isolated and further investigated, and 79.4% (139) of them were gram negative bacilli, and 20.5% (36) were GPB. The percentages of identified Gram-negative rods were *P. aeruginosa* (40%), *E. coli* (19.4%), *Proteus* spp. (8.6%), *K. pneumoniae* (6.3%), *Enterobacter* (2.9%), and *A. baumannii* (2.2%). The isolated GNB & GPC in our study were consistent with those of recent investigations performed by various researchers [19-21]. Similarly, types of causative organisms including various GNB & GPC are consistent with a previous study the main causative agents of surgical site infection are gram negative bacilli, for example, *Pseudomonas* spp., *E. coli*, *Enterobacter* spp., *A. baumannii*, *Proteus* spp., and *Klebsiella* spp. Besides that, *S. aureus* surgical site infection is common among hospitalized patients [22]

In our study, 25.2% (35), 80.6% (112), 80.6% (112), 89.9% (125), 58.3% (81), 55.4% (77), 43.9% (61), and 87.1% (121) isolated strains of Gram-negative rods showed resistance to AMP, AMC, TZP, MEM, CRO, CIP, AK, and SXT, respectively. Various studies have indicated variable resistance of Gram-negative and Gram-positive bacterial isolates to different antimicrobial agents. Other studies, similar to ours, have generally indicated that resistance patterns for Gram-negative rods are usually very high to a wide range of antibiotics. Researcher quoted resistance rates of 23% to ampicillin (AMP), 82% to amoxicillin-clavulanic acid (AMC), 79% to piperacillin-tazobactam (TZP), and 87% to meropenem (MEM), which shows quite a close pattern to our analysis. In another investigation, Jones et al. (2021) recorded resistance rates to ceftriaxone and ciprofloxacin at 60% and 56%, respectively, comparable to our findings, which revealed 58.3% and 55.4%, respectively. [23]. In addition researcher showed the following rates of resistance among Gram-negative strains: aminoglycosides (AK)-45% and sulfamethoxazole-trimethoprim (SXT)-85%, similar to our rates of 43.9% and 87.1%, respectively [23].

66.7% (24), 83.3% (30), 86.1% (31), and 69.4% (25) isolated strains of Gram-positive cocci showed resistance to AMP, AMC, FOX, and CRO, respectively, while 77.8% (28), 83.3% (30), and 77.8% (28) showed susceptibility to VA, LNZ, and DA, respectively. Some previous findings agreed with our observations in Gram-positive bacteria to a certain extent. Saka et al. described 65% of Gram-positive isolates as resistant to AMP and 80% resistant to AMC. In our study, the same flora had been resisted at rates of 66.7% and 83.3%, respectively. Moreover, they reported 70% and 74% resistance from Gram-positive strains to FOX and CRO, respectively, which agreed with our rates of resistance, 69.4% and 83.3%, respectively. On the other hand, several studies reported susceptibility rates ranging from 80% to 90% for Gram-positive strains to VA, LNZ, and DA, which agreed with our susceptibility rates of 77.8% [24].

In this study, the screening of CPs, ESBLs, and MBLs production was observed in isolated gram-negative bacilli. According to the results, the CP producers' percentages were *A. baumannii* (75%), *Enterobacter* (100%), *E. coli* (79.4%), *K. pneumoniae* (72.7%), *Proteus* (80%), and *P. aeruginosa* (80%). However, the MBL producers' percentages were *A. baumannii* (100%), *Enterobacter* (80%), *E. coli* (91.2%), *K. pneumoniae* (90.9%), *Proteus* (73.3%), and *P. aeruginosa* (88.6%). On the other hand, the ESBL producers' percentages were *A. baumannii* (50%), *Enterobacter* (20%), *E. coli* (70.6%), *K. pneumoniae* (27.3%), *Proteus* (53.3%), and *P. aeruginosa* (34.3%). In this study, 83.3% (30) isolated strains of *S. aureus*

were identified as *methicillin-resistant staphylococcus aureus*. The findings of this study are consistent with those of recent investigations that came before it [24-26].

Gram negative bacilli can acquire multi-drug or extensive drug resistance (XDR) through a variety of resistance mechanisms, such as the production of β-lactamases (ESBLS, MBLs, and AmpCs) and carbapenemases [27]. *Methicillin-resistant staphylococcus aureus* are aggressive pathogenic biovars of *S. aureus* that meet particular requirements for methicillin and cefoxitin resistance [28].

CONCLUSION

The study had a total of 175 patients. There was a very interesting distribution of age groups: <15 years constituted 30.3%, 16-30 years 28.6%, 31-45 years 21.1%, 46-60 years 13.1%, and >60 years 6.9%. The subjects were predominantly males 56.0% as opposed to females 44.0%. The microbiological examination revealed that 79.4% of the isolated strains were gram-negative bacilli, the most representative species being *Pseudomonas aeruginosa*, 40.0%, followed by *Escherichia coli*, 18.9%. Gram-positive bacteria accounted for 20.5%, with only *Staphylococcus aureus* being in the same percentage. The antibiotic susceptibility testing showed remarkable resistance among gram-negative bacteria studied. Accordingly, strains of *Pseudomonas aeruginosa* showed high resistance rates to Ceftriaxone (CRO) with a total of 61 strains and to Ciprofloxacin (CIP) with a total of 67 strains. Likewise, *Escherichia coli* revealed high resistive potential, especially to Ampicillin (AMP), with 13 resistant's versus 21 sensitive, and to Ciprofloxacin (CIP), with 31 resistant's versus 3 sensitive. Among the Gram-positive isolates, *Staphylococcus aureus* showed remarkable resistance to the commonly used antibiotics, particularly to Ampicillin: 32 resistant and 4 sensitive, Ceftriaxone: 30 resistant, 6 sensitive. Notably, 83.3% of the strains were *methicillin-resistant S. aureus*. Resistance was highly prevalent among gram-negative bacilli, including 79.9% of strains producing CPs, 87.8% producing MBLs, and 44.6% producing ESBLs. Among these, the leading proportion of CP and MBL producers was *Pseudomonas aeruginosa* and *Escherichia coli*, respectively. These data underscore the urgent need for ongoing surveillance of resistance patterns of antibiotics and provide a cornerstone for the appropriate therapeutic strategy against multidrug-resistant infection.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Sumera Malik: Conception, manuscript writing, data collection, accountable for all aspects of the work

Blossom Neelam: Data collection, study design, interpretation of the work, accountable for all aspects of the work

Qurat Ul Ain Ayaz: Critical review, revisions, accountable for all aspects of the work

Abdul Wahab Majid: Data collection, analysis, investigation, accountable for all aspects of the work

Syed Muhammad Abbas Naqvi: Study design, proofreads, accountable for all aspects of the work

Javairia Saeed: Revisions, proofreads, accountable for all aspects of the work

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