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Isolation, identification, and antibiotic sensitivity patterns of bacterial strains isolated from human pus samples

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Abstract

The objective of this study was to isolate, identify, and evaluate the antibiotic sensitivity patterns of bacterial strains in order to cure the infections in patients with wounds *S. aureus, Enterobacter, Citrobactor* and *E. coli, Klebsiella spp* were isolated from pus and wound samples, and they were identified based on morphology, colony, gram staining and various biochemical tests. A total of 128 samples were collected from pus and wound infections. Bacterial growth was observed in 65 of these samples. Data showed a higher infection rate among males (53%) compared to females (47%). The identified bacteria include *Escherichia coli (E. coli)* (32%), *Staphylococcus aureus (S. aureus)* (23%), and *Pseudomonas* species (23%), being the most prevalent pathogens. Gram-negative bacteria were found to be more prevalent among the isolated pathogens. The most frequently identified bacteria included *Escherichia coli* (*E. coli)*, *Staphylococcus aureus (S. aureus),* and various species of *Pseudomonas* showed high susceptibility to vancomycin and imipenem but lower susceptibility to ampicillin and tetracycline. Gram-negative bacteria showed higher sensitivity to antibiotics such as ciprofloxacin, vancomycin, ampicillin, imipenem, and levofloxacin. Our study successfully isolated and identified bacterial strains from human pus samples. Antibiotic sensitivity patterns revealed valuable information for effective treatment strategies.

Introduction

Infectious diseases cause death and have side effects on socioeconomic advancement. Several pathogens are responsible for the disorder [1]. Health problems could result from environmental pollution, frequent intake of food products polluted with pesticides and associated substances, and a lack of physical activity [2]. Abscesses form on any body part and contain pus due to a bacterial infection. In addition, wounds infected by viruses, protozoa, fungi, and occasionally, they do so concurrently with one or more bacteria in different wounds [3]. In a pus infection, germs infiltrate the skin, which serves as the host's protective layer, and disrupt its normal function [4].

Different types of bacterial pathogens can cause numerous forms of wound infections, including burn wound infections, pyogenic wound infections, leg and detritus (pressure) ulcers, acute soft tissue infections, and biting wound infections [5]. Significant health issues in children include falls, burns, and road accidents [6]. Pus production and local systemic inflammation are the marks of pyogenic infection. Pus accumulation in soft tissue infections is due to the pyogenic nature of the bacteria. Aerobic and anaerobic bacteria can cause soft tissue and wound infections, respectively. Many infections heal without the need for special treatment, but others, particularly mixed infections, can require serious synergic therapy [7]. A wound is a break in the skin that exposes subcutaneous tissue after the skin loses its integrity. This creates a wet, warm, and nutrient-rich environment that is favorable for microbial colonization and growth [8].

S. aureus is a facultative anaerobic gram-positive coccus that has catalase activity and solitary cells that cluster together. Impetigo, furunculosis, folliculitis, hidradenitis suppurativa, and staphylococcal scalded skin syndrome are only a few examples of skin lesions that can be brought on by it in humans [9]. According to a recent investigation, milk and food products can help disseminate *S. aureus* strains to various geographic locations [10]. The best option for defense against disease-causing organisms is antibiotic therapy and antibiotic susceptibility testing. Antibiotic susceptibility testing aims to identify the effectiveness or failure of antibiotic therapy and to manage certain pathogenic bacteria that are resistant to antibiotics [11].

The ability of bacteria to survive when treated with antibiotics that typically kill them or stop their growth is known as antibiotic resistance [12]. It is very important to find out the proper antibiotic and its concentration for the treatment of infection, and also point out the causative agent of infection [13]. Detection in the liquid test, which is based on changes in OD, and on solid agar plates, the inhibitory zones are identified by using the disc diffusion method [14].

The purpose of the present study was to identify the potential of microbial pathogens, microbial contamination, and the effectiveness of antimicrobial agents. The proper antibiotics can be used to treat the wounds and thereby minimize the complications that are at risk to all patients. A professional may also understand the evaluation of certain bacteria isolated from an injury as a diagnosis of a wound condition requiring the administration of an appropriate antibiotic therapy to reduce microbial resistance.

Materials and Methods

Chemicals

For bacterial growth nutrient-rich media i.e. Blood Agar (BA), MacConkey Agar (MA), Nutrient Agar (NA), MacConkey agar, and Muller Hinton agar were used.

Apparatus and Instruments

Micro streaker, autoclave, BACT/ALERT 3D, Memmert incubator (model;100-800), light microscope antibiotic disk dispenser, refrigerator, antibiotic disks, syringes, biosafety cabinet, forceps, Glass slide, Alcohol burner, gas lighter, sterile cotton swabs, test tubes, slide staining rack, test tube rack, flask, funnel, petri dishes, cotton, cover slips, disposable hand gloves.

Sample collection

A total of 128 wound and pus samples were collected from the Bahwal Victoria Hospital (BVH), Bahawalpur, Pakistan, using aseptic techniques after receiving approval from the ethical committee. The pus samples were collected from the patients attending the outdoor and admitted to the ICU wards of the hospital. The samples were collected using sterile cotton swabs and syringes. The collected samples were then transferred to the microbiology laboratory for further processing.

Sample Processing

All wound swab samples were inoculated on Blood Agar (BA) plates, MacConkey Agar (MA), and Nutrient Agar (NA), and incubated for 18 to 24 hours at 37 °C.

Gram's staining

The bacterial isolates were screened as Gram-positive or -ve using Gram's staining. To identify the bacterial isolates, traditional biochemical assays were performed on primary cultures.

Biochemical tests

Biochemical tests, including the catalase test, oxidase test, triple sugar iron (TSI), citrate utilization, and urease test, were used to identify gram-negative rods. By using the catalase, oxidase, and coagulase tests, gram-positive cocci were identified [15].

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method after the samples were inoculated on the appropriate culture media. The results were interpreted in accordance with the criteria provided by the Clinical Laboratory and Standards Institute (CLSI) [16]. Vancomycin, imipenem, ampicillin, tetracycline, ciprofloxacin, amox+clav, penicillin, cefotaxime, polymyxin, levofloxacin, and tazobactam antibiotics were used for antibiotic susceptibility testing [17].

Results

Out of 128 pus and wound samples, 65 samples were successfully cultured for further processing. The collected samples were positive for *S. aureus, E. coli, Pseudomonas, Klebsiella spp*, *Citrobacter spp*., and *Enterococcus spp*.

Antibiotic sensitivity and resistance of gram-positive bacterial isolates

Gram-positive bacteria isolated in our study were found to be highly sensitive to Vancomycin (88%) and Imepenem 14(77%) while least sensitive to Ampyciline (33%), Tetracycline (27%), Cifroxomine (55%), Amox+Clav (55%), penicillin (55%), Cefotaxime (55%), polymyxin (66%), Levofloxin (38%), and Tazobactam (16%) antibiotics **(Table 1)**. According to our findings, resistance development and the tendency toward high-class antimicrobials are increased as a result of increased antimicrobial intake frequency. Gram-negative bacterial isolates presented antibiotic resistance Tazobactam (84%), Cefotaxime (84%), Tetracycline (73%), ampicillin (67%) drugs and less resistance was observed for *S. aureus* species for polymyxin (34%), ciprofloxacin (45%), Amox+Clav (45%), penicillin (45%) and levofloxacin (62%) antibiotics **(Table 1).**

Table 1: Antibiotic sensitivity and resistance of gram-positive bacteria isolated from pus and wounds

Sr. #	Antibiotics	***S. aureus* (n=18)**	
		Antibiotic sensitivity	**Antibiotic Resistance**
01	Ampicillin (AMP)	06(33%)	12(67%)
02	Tetracycline (TGC)	05(27%)	13(73%)
03	Cifroxomine (CT)	10(55%)	08(45%)
04	Amox+Clav (AUG)	10(55%)	08(45%)
05	Penicillin (P)	10(55%)	08(45%)
06	Imipenem (IMI)	14(77%)	04(23%)
07	Vancomycin (VA)	16(88%)	02(12%)
08	Cefotaxime (CTX)	03(16%)	15(84%)
09	Polymyxin (PB)	12(66%)	06(34%)
10	Levofloxin (LEO)	07(38%)	11(62%)
11	Tazobactam (TZP)	03(16%)	15(84%)

Antibiotic sensitivity and resistance of gram-negative bacterial isolates

A total of 48 samples were positive for anaerobic bacteria. Most commonly isolated species were *E. coli*, Klebsiella spp., and Citrobacter spp., as shown in **Table 2**. All the anaerobes were tested for antimicrobial sensitivity and resistance, showing highest sensitivity for *E. coli* species against ciprofloxacin (74%), ampicillin (73%) and vancomycin (70%) drugs, whereas less sensitivity to cifroxomine (44%), levofloxacin (30%), tetracycline (21%), ceftazidime (18%), cefotaxime (14%) and cefepium (14%) antibiotics. *E. coli* was revealed with the highest resistance to ceftazidime (86%) and cefotaxime (82%), and less resistance to other drugs used. Tetracycline and ceftazidime antibiotics demonstrated 100% sensitivity to Klebsiella spp, and less sensitivity was revealed by the ampicillin (25%) drug.

Table 2: Antibiotic Resistance & Sensitivity for Enterobacteriaceae family

Sr. #	Antibiotic	***E. coli* (n=23)**		***Klebsiella spp* (n=4)**		***Citrobacter (n=03)***	
		Antibiotic Sensitivity	**Antibiotic Resistance**	**Antibiotic Sensitivity**	**Antibiotic Resistance**	**Antibiotic Sensitivity**	**Antibiotic Resistance**
01	Ampicillin (AMP)	17(73%)	06(27%)	01(25%)	03(75%)	01(33%)	02(67%)
02	Tetracycline (TGC)	05(21%)	18(79%)	04(100%)	02(50%)	02(66%)	01(34%)
03	Cifroxomine (CTX)	10(44%)	13(56%)	02(50%)	00(0%)	00(0%)	03(100%)
04	Amox+Clav (AUG)	10(44%)	13(56%)	02(50%)	02(50%)	01(33%)	02(67%)
05	Penicillin (P)	10(44%)	13(56%)	03(75%)	01(25%)	02(66%)	01(34%)
06	Imepenem (IMI)	14(61%)	09(39%)	01(75%)	03(25%)	03(100%)	00(0%)
07	Ciproflaxin (CIP)	17(74%)	06(26%)	02(50%)	02(50%)	01(33%)	02(67%0)
08	Vancomycin (VA)	16(70%)	07(30%)	03(75%)	01(25%)	00(0%)	03(100%)
09	Meropenem (MEM)	08(35%)	15(65%)	00(0%)	04(100%)	02(66%)	01(34%)
10	Cefotaxime (CTX)	03(14%)	20(86%)	03(75%)	01(75%)	03(100%)	00(0%)
11	Cefepime (FEP)	03(14%)	20(86%)	02(50%)	02(50%)	01(33%)	02(67%)
12	Levofloxin (LEO)	07(30%)	16(70%)	01(25%)	03(75%)	03(100%)	00(0%)
13	Tazobactam (TZP)	09(39%)	14(61%)	02(50%)	02(50%)	02(66%)	01(34%)
14	Ceftazidime (CAZ)	04(18%)	19(82%)	04(100%)	00(0%)	02(66%)	01(34%)

According to the sensitivity pattern, *S. aureus* was generally susceptible to azithromycin (30.4%), doxycycline (31%), and ciprofloxacin (27.1%), ciprofloxacin (27.6%), and exhibited 100% cefotaxime resistance. The Proteus spp. was resistant to cefotaxime (0%) and vancomycin (0%), while it was sensitive to penicillin (75%), tetracycline (72.2%). Pseudomonas spp. and *Escherichia coli* had 100.0% resistance to penicillin and 50.0% sensitivity to cefotaxime and piperacillin, 14.3% and 42.9%, respectively. The Klebsiella spp. exhibited 100% resistance to cotrimoxazole, penicillin, piperacillin, tetracycline, and vancomycin, and 11.5% sensitivity to cefixime.

Citrobacter spp. showed 100% sensitivity to Imipenem, cefotaxime, and levofloxin antibiotics. The highest resistance was observed to Cifroxomine and vancomycin, followed by ampicillin, tetracycline, amox+clav, penicillin, imipenem, ciproflaxin, and meropenem antibiotics resistance to Tazobactam (100%) and less resistance to other antibiotics **(Table 3)**.

Discussion

The present study was designed to isolate, identify, and evaluate the antibiotic sensitivity of bacteria isolated from pus samples. The bacterial pathogens were identified by Gram's staining and biochemical tests. A similar study conducted by Mumtaz (2022) on frequency of *P. aeruginosa* to determine the frequency of wound infections, the impact of age, gender, and other co-morbid conditions on the

Table 3: Antibiotic sensitivity and resistance of *Pseudomonas aeruginosa*

Sr. #	Antibiotic Numbers	***Pseudomonas aeruginosa* (n=10)**	
		Antibiotic sensitivity	**Antibiotic Resistance**
01	Ampicillin (AMP)	08(80%)	02(20%)
02	Tetracycline (TGC)	05(50%)	05(50%)
03	Cifroxomine (CTX)	10(100%)	00(0%)
04	Amox+Clav (AUG)	05(50%)	05(50%)
05	Penicillin (P)	04(40%)	06(60%)
06	Imipenem (IMI)	06(60%)	04(40%)
07	Cefotaxime (CTX)	07(70%)	03(30%)
08	Polymyxin (PB)	01(10%)	09(90%)
09	Tazobactam (TZP)	00(0%)	10(100%)

frequency, and their sensitivity to antimicrobial agents [18]. In our study, out of 128 pus samples processed, 65 samples showed significant growth, with single growth, 63 samples showed no growth 3 samples showed multiple growth of *E. coli*, Pseudomonas spp. 68 were from male patients with positive cases, and 65 were from female patients with positive cases. Our study results were comparable to the work of Ayesha (2022) and Jobayer (2021) [19-20]. The study conducted by M. Khan (2021) [21] showed that Staphylococcus sp. (30%), Enterococcus (22%), Proteus sp. (15%), E. coli (15%), and Pseudomonas spp. (8.43%) were the further gram-positive isolates that were most frequently found in pus samples. Furthermore, the group of pyogenic bacteria included Staphylococcus coagulase-negative. According to the results of our investigation and a previous study, a gram-negative bacterium is the primary cause of pyogenic infection.

Our results demonstrated that the highest variations were observed between growth-positive and non-growth samples. The variation in bacterial growth may be due to sampling error, diabetes, cancer, or different biochemical tests. The maximum growth rate in males may also increase the risk of trauma. A similar study was conducted by Duwadi (2020) [22] showed that the main variation in bacterial growth in males was due to cultural techniques. Growth positive and sample collection ratio showed that males were involved in many professions increased the risk of disease, whereas in females mostly due to surgery.

Our study showed that Gram-negative bacteria showed the highest resistance to drugs as compared to Gram-positive bacteria. This may be due to the composition of the cell wall. A similar study conducted by Mohanasundari (2021) [23] demonstrated that gram-negative bacteria have the highest levels of antibiotic resistance to a variety of drugs, supporting this finding. It may be due to the chemical makeup of bacterial cell walls. The resistance of bacteria showed that gram-negative bacteria have a higher amount of lipid content than gram-positive bacteria may increase their resistance to antibiotics. The development of enzymes that break down antibiotics, important 61 changes to protein present on the cell wall's outer surface, which may prevent antibiotics from binding, and crucial changes to the process of membrane permeability are only a few examples of the resistance mechanisms. These modifications may give bacteria enough protection from varied antibiotic concentrations.

Our results revealed 100% antibiotic sensitivity by Klebsiella spp. for tetracycline and ceftazidime, whereas 75% sensitivity was observed for penicillin, Imipenem, vancomycin, and ceftazidime. Levofloxacin antibiotics showed very less sensitivity (25%). Kbellsiela spp. showed high resistance (75%) to Meninopenum (MEM) and Ampicillin (AMP). A similar study conducted by Patilaya (2019) [24] also been shown that tetracycline has low resistance to *K. pneumoniae* pathogens. Similar results were reported by many researchers, indicating that the low sensitivity of tetracycline in *K. pneumoniae* is due to chromosomal mutation in the bacterial membrane.

Conclusions

In conclusion, wound and pus infections were more prevalent in the surgical ward of Bahawal Victoria Hospital, Bahawalpur, Pakistan. The isolated bacteria mostly showed the highest resistance to different second-generation antibiotics. The susceptibility data from all collected reports may be a worthy circumstance while utilizing a verifiable treatment scheme for infections. Strict health regulations should be put in place simultaneously to control the sale and distribution of ongoing monitoring and reporting of antibiotic resistance, prescription, and limiting unsupervised antibiotics. *S. aureus* was the most frequently isolated bacterium from the pus aerobic cultures. However, gram-positive cocci were found to be rarer than gram-negative bacilli. The most frequent bacterium causing wound infection among gram-negative bacilli was *E. coli.* The issue in treating these illnesses was the changing antibiotic resistance. To successfully treat these clinical problems in the future, it will be necessary to make a careful and appropriate selection of antibiotics based on antibiotic sensitivity data. Thus, our research will assist clinicians in selecting appropriate medicines that result in better patient care and avoid the development of drug resistance in susceptible patients.

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Conflict of interest

The authors declare no conflict of interest.

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