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Identification of Bacterial Isolates and Their Antimicrobial Susceptibility Pattern from Wound/Pus Sample in a Tertiary Care Hospital, Gwalior, India

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Abstract

Objective The goal of this investigation was to look at the frequency and dispersal of bacteria isolated from pus/wound, as well as their susceptibility patterns.

Materials and Methods A study was conducted on 175 patients who provided pus and/or wound discharge samples in different wards (outpatient department or inpatient department). MacConkey agar and blood agar plates were immediately inoculated with samples and incubated at 37°C for 24 hours. The Gram stain and biochemical tests were used to identify all isolates after incubation. Kirby–Bauer's disc diffusion method was used to perform sensitivity tests on Mueller–Hinton agar plates.

Results This study covered 175 patients, with a bacterial isolation rate of 102 (58.28%). Males outnumbered females in the samples (M:F = 1.8:1), with a median age of 45 years as majority were in the age group of 40 to 60 years which was 41 (40.20%). Total 90.1% samples showed monomicrobial infection, whereas 9.8% showed polymicrobial infection, and total 112 bacterial strains were isolated.

Conclusion *Escherichia coli* was the most prevalent isolate in present investigation, followed by *Pseudomonas aeruginosa*. Chloramphenicol is the only antibiotic which is effective for both gram-negative bacilli and gram-positive cocci. This report's susceptibility statistic may be worth considering for developing empiric treatment regimens for pyogenic infections.

Keywords
- pus sample
- wound infection
- antimicrobial susceptibility
- pyogenic
- multidrug resistant
- GNB
- GPC

Introduction

A wound is a break in the skin or tissues integrity, which can result in structural and functional disturbances.[1] Infection of the wound can be pyogenic (pus forming) or nonpyogenic, depending on the causative organism. The majority of the organisms in wounds are aerobes, includes gram-positive cocci (GPC) such as *Enterococci*, *Staphylococcus epidermis*, *S. aureus*, *Streptococcus pyogenes*, and gram-negative bacilli (GNB) such as *Pseudomonas aeruginosa*, *Klebsiella*

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pneumoniae, *Escherichia coli*, *Proteus*.[2] A wound infection is more likely to occur in situations with higher wound class (dirty-infected wound) and higher bacterial load.[3] Microbes are the unseen adversaries of humans, wreaking havoc on the human body as well as other living organisms.[4,5] Bacterial illnesses continue to be the predominant factor in morbidity and mortality.[6] Various bacterial species reside on human skin, in the nasopharynx, in the gastrointestinal system, and other areas of the body, but they have a lower risk of causing disease due to the body's first line of defense.[7] Microbial pathogens cause human skin and soft tissue infections (SSTIs) during or after trauma, burns, bites, abrasions, minor cuts, lacerations, crush injuries, gunshot injury, and surgical procedures. Compromising in front line of defense leads to bacterial contamination, resulting in the generation of pus, a white or yellow fluid containing dead leukocytes, cellular detritus, and devitalized tissue.[8,9] Infection can be either endogenous or exogenous.[10] The loss of skin integrity due to a variety of reasons creates an environment conducive to the colonization and proliferation of microorganisms.[11] Humidity, heat, and nutrition in the wound attract pathogen from the cutaneous surface, environment, or the patient's own flora, which grow and release various virulence factors, resulting in wound infection.[12] Immune cells are recruited to the infection site by the body's defense mechanism to fight pathogens.[13] Pyogenic infection results from the build-up of these cells inhibits wound healing and can lead to complications such as wound dehiscence or wound disintegration.[14] Fungus, in addition to bacteria, can induce wound infection, and they might coincide with more than one bacteria in a single lesion.[15] Infectious diseases are a major threat to human health and life.[16] Antimicrobial agents or medications are substances that have the ability to kill bacteria or stop them from multiplying.[17] Knowing the susceptibility of a certain bacteria to an antibiotic helps you to treat the patient empirically until the culture report is generated. The choice of antibiotics is then determined by the results of the culture.[13] Inadvertent and inappropriate antibiotics use results in the establishment of a drug-resistant bacteria, which leads to a lengthy hospital stay, a significant financial loss, and serious medical complications.[18] During a prolonged hospital stay, a patient may spread drug-resistant microorganisms to other patients, family, or even health care workers.[19] The antibiotics susceptibility of these organisms in a given environment change over time as bacteria evolve and as antibiotic use or misuse patterns change.[20] The rise of antibiotic-resistant pathogenic microorganism is regarded as a severe hazard to global public health.[21]

Materials and Methods

Study Design and Sampling Process

The study was conducted in the microbiology department at Birla Institute of Medical Research (BIMR) Hospital, Gwalior from September 2021 to April 2022 for a period of 8 months. The pus samples were taken from individuals who were examined in the outpatient department and were admitted to the hospital's inpatient department, using sterile cotton swabs, a syringe, or a sealed capillary tube. It was labeled and immediately sent to microbiology laboratory. The study population consisted of all individuals who had SSTIs.

Inclusion and Exclusion Criteria

As part of standard patient treatment, nonduplicated specimen was taken and cultured. The pus sample from one location is included, unless it was taken from the other location. One patient underwent susceptibility testing only once.

The study excluded patients with missing antibiotic sensitivity results, inadequate data, prior exposure to antibiotics, or repeated culture results during the last 6 months.

Isolation and Identification

The isolation and identification of microorganisms from the sample of pus were performed by streaking sample on MacConkey agar and blood agar plates, and incubating them at 37°C for 24 to 48 hours. Following incubation, bacterial colonies showing different characteristics were chosen for further investigation. The colonies grown were identified with the help of Gram staining which differentiate gram-positive and -negative bacteria followed by biochemical test such as coagulase, catalase, indole, Voges–Proskauer, methyl red, oxidase test, urease, and citrate which were performed as per standard protocol.

Catalase enzyme estimation aids to distinguish *Streptococci* from *Staphylococci* colonies. The coagulase test distinguishes *S. aureus* (which is coagulase positive) from *S. epidermis* and *S. saprophyticus* (which is coagulase negative). Oxidase test were used to distinguish Enterobacteriaceae from other GNB.

Samples considered to be negative when no growth was observed on blood agar and MacConkey agar media only after 48 hours of incubation.

Antimicrobial Agents

GNB were tested with antibiotic discs such as amikacin (30 µg), gentamycin (10 µg), ertapenem (10 µg), meropenem (10 µg), imipenem (10 µg), ceftazidime (30 µg), cefazolin (30 µg), cefepime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), ampicillin (10 µg), piperacillin–tazobactam (10 µg), ampicillin–sulbactam (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), trimethoprim–sulfamethoxazole (25 µg), ceftazidime–avibactam (30 µg), chloramphenicol (30 µg).

GPC were tested with antibiotic discs such as cefoxitin (30 µg), cefazolin (30 µg), ampicillin (25 µg), penicillin-G (2 units), erythromycin (15 µg), fusidic acid (30 µg), vancomycin (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), mupirocin (5 µg), doxycycline (30 µg), daptomycin, quinupristin–dalfopristin (15 µg), rifampin (5 µg), chloramphenicol (30 µg), linezolid (30 µg), trimethoprim–sulfamethoxazole (25 µg).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility testing was done as per Clinical and Laboratory Standards institute (CLSI)

guidelines using Kirby–Bauer's method.[22] Inoculum was prepared for each bacterial isolate by matching the turbidity to 0.5 McFarland standard and spreading on Mueller-Hinton agar (MHA) plate. Paper disc which contains antibiotics were kept on the top of the MHA plate and incubate at 37°C for 24 hours. According to CLSI M100 Guideline 2022, the size of the zones of inhibition was classified as sensitive, moderate, or resistant to the antibiotics tested.[22]

For accurate identification of pathogen and their susceptibility pattern, automated BD Phoenix M50 machine were used as per manufacturer's instruction.

Quality Control

Pseudomonas aeruginosa American Type Culture Collection (ATCC) 27853, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922 strains are used as quality control for the identification and susceptibility test (►**Table 1**).

Results

A total of 175 pus samples were received in the department of microbiology from September 2021 to April 2022. Out of total 175 pus/wound swab samples processed, 102 (58.28%) samples were culture positive, whereas 73 (41.71%) samples

Table 1 Quality control data for antibiotics

Antimicrobial agent	Diameter of zone of inhibition in mm		
	Escherichia coli ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923
Amikacin	19–26	18–26	–
Gentamicin	19–26	17–23	–
Ertapenem	29–36	–	–
Imipenem	26–32	20–28	–
Meropenem	28–35	27–33	–
Cefazolin	21–27	–	29–35
Cefuroxime	20–26	–	–
Cefoxitin	23–29	–	23–29
Ceftazidime	25–32	22–29	–
Ceftriaxone	29–35	–	–
Cefepime	31–37	25–31	–
Ampicillin	15–22	–	27–35
Ampicillin–sulbactam	15–22	–	–
Piperacillin–tazobactam	21–25	21–25	–
Ciprofloxacin	29–38	25–33	22–30
Levofloxacin	29–37	19–26	–
Trimethoprim–sulfamethoxazole	23–29	–	24–32
Ceftazidime-avibactam	21–25	21–25	–
Chloramphenicol	21–27	–	19–26
Penicillin-G	–	–	26–37
Vancomycin	–	–	17–21
Clindamycin	–	–	24–30
Erythromycin	–	–	22–30
Moxifloxacin	–	–	28–35
Doxycycline	–	–	23–29
Quinupristin–dalfopristin	–	–	21–28
Fusidic acid	–	–	24–32
Linezolid	–	–	25–32
Mupirocin	–	–	18–24
Rifampin	–	–	26–34

Abbreviation: ATCC, American Type Culture Collection.

Table 2 Age- and gender-wise distribution of bacterial growth from pus/wound sample

Age group	No. of male (%)	No. of female (%)	Frequency (%) ($n = 102$)
< 20 y	7 (6.86)	3 (2.94)	10 (9.80)
20–40 y	22 (21.56)	8 (7.84)	30 (29.41)
40–60 y	27 (26.47)	14 (13.72)	41 (40.20)
> 60 y	10 (9.80)	11 (10.78)	21 (20.59)
Total	66 (64.70)	36 (35.29)	102 (100)

were negative for growth. Out of 102 positive samples, monomicrobial infections were seen in 92 (90.19%) samples, whereas polymicrobial infections with growth of two pathogens in 10 (9.80%) samples, and total 112 bacterial strains were isolated. Among 112 isolates, 83 (74.10%) were GNB, 23 (20.53%) were GPC, and 6 (5.35%) were *Candida*. Among 102 (58.28%) culture positive, mostly in the age of 40 to 60 years, it was 41 (40.20%) cases, subsequently 20 to 40 years, >60 years and then <20 years which was 30 (29.41%), 21 (20.59%) and 10 (9.80%) instances, respectively (►**Table 2**).

Discussion

Infection of the wound is the common cause of patient's impairment and if it is not cured in early stage, then it increases the hospital stays. Severe wound infections can lead to sepsis, which can be fatal, especially if the bacteria are multidrug resistant. Any wound has the potential to get infected as infection of the wound becomes commonest hospital-acquired infection. In the present study, pus samples from a tertiary care hospital were analyzed to determine the etiological agents and their pattern of antibiotic susceptibility.

The majority (58.28%) of the samples in this study revealed positive growth. This is due to the fact that suppurative infections of the eye, ear, and skin are frequently seen in both inpatient and outpatient departments. Furthermore, among surgical patients, wound infection is the most prevalent hospital-acquired infection. It has been linked to more trauma care, longer hospitals stay, and treatment. The results revealed 58.28% positivity rate of total sample that correlate with the studies of Rai et al[23] (59%), Trojan et al[8] (60.1%), and Khanam et al[21] (61.8%); however, it exceeded a study conducted by Singh et al[13] (52.73%) and less than a research conducted by Muluye et al[7] (70.2%) and Batra et al[24] (85.02%).

According to sex, the predominance of males (64.70%) is higher than females (35.29%) in the present study (►**Table 2**). It is most likely related to increased exposure to the environment and the increased risk of accidents when earning a living, as well as social behavior in which males are treated as superior to female and are given preferential biased treatment when compared with females.

Table 3 Frequency/percentage of the isolates (monomicrobial) after aerobic culture from pus/wound sample

Isolated organisms	Frequency ($n = 86$)	Percentage
Escherichia coli	20	23.25
Pseudomonas aeruginosa	18	20.93
Staphylococcus aureus	15	17.44
Klebsiella spp.	12	13.95
Acinetobacter spp.	10	11.62
Enterobacter cloacae	2	2.32
Morganella morganii	2	2.32
CoNS	2	2.32
Micrococcus	2	2.32
Enterococcus spp.	1	1.16
Burkholderia spp.	1	1.16
Stenotrophomonas maltophilia	1	1.16
Total	86	100

Abbreviation: CoNS, coagulase-negative staphylococci.

In the present study, monomicrobial infections predominated (90.19%), while polymicrobial infections were observed (9.80%) (►**Tables 3** and **4**). The study by Sudhaharan et al[11] found that monomicrobial infection was 93.2% and polymicrobial infection was 6.8%; this result is consistent with our findings.

In the present study, GNB were the predominant isolates which was 74.10% compared with GPC which was 20.53% and *Candida* which was 5.35%. A research done by Bankar et al[25] also recorded predominance of GNB which was 51.97%, whereas GPC was 47.36% and *Candida* was 0.65%.

In the present study, the most common isolates were *E. coli* (GNB) and *S. aureus* (GPC) (►**Table 3**). The present findings correlate with the research done by Trojan et al,[8]

Table 4 Frequency/percentage of mixed isolates (polymicrobial) after aerobic culture from pus/wound sample

Mixed isolated organisms	Frequency	Percentage
Escherichia coli + *Pseudomonas aeruginosa*	3	30
Escherichia coli + *Proteus mirabilis*	2	20
Staphylococcus aureus + *Klebsiella* spp.	1	10
Staphylococcus aureus + *Pseudomonas aeruginosa*	1	10
Enterococcus spp. + *Klebsiella* spp.	1	10
Pseudomonas aeruginosa + Klebsiella spp.	1	10
Pseudomonas aeruginosa + *Morganella morganii*	1	10
Total	10	100

Table 5 Frequency/percentage of antibiotic sensitivity pattern of GNB isolated from pus/wound sample

Antibiotics	*Acinetobacter* spp. ($n=10$)	*Burkholderia* ($n=1$)	*Enterobacter cloacae* ($n=2$)	*Escherichia coli* ($n=25$)	*Klebsiella* spp. ($n=15$)	*Morganella morganii* ($n=3$)	*Proteus mirabilis* ($n=2$)	*Pseudomonas aeruginosa* ($n=24$)	*Stenotrophomonas maltophilia* ($n=1$)
Amikacin	0 (0%)	0 (0%)	2 (100%)	22 (88%	3 (20%)	3 (100%)	2 (100%)	11 (45.83%)	IR
Gentamicin	0 (0%)	0 (0%)	1 (50%)	15 (60%)	2 (13.33%)	2 (66.66%)	2 (100%)	12 (50%)	IR
Ertapenem	IR	IR	0 (0%)	14 (56%)	2 (13.33%)	2 (66.66%)	2 (100%)	IR	IR
Imipenem	0 (0%)	0 (0%)	0 (0%)	16 (64%)	2 (13.33%)	0 (0%)	NA	11 (45.83%)	IR
Meropenem	0 (0%)	0 (0%)	0 (0%)	18 (72%)	2 (13.33%)	2 (66.66%)	2 (100%)	12 (50%)	IR
Cefazolin	IR	IR	0 (0%)	3 (12%)	0 (0%)	0 (0%)	2 (100%)	IR	IR
Cefuroxime	IR	IR	0 (0%)	3 (12%)	0 (0%)	0 (0%)	2 (100%)	IR	IR
Cefoxitin	IR	IR	0 (0%)	10 (40%)	0 (0%)	3 (100%)	2 (100%)	IR	IR
Ceftazidime	0 (0%)	0 (0%)	0 (0%)	4 (16%)	2 (13.33%)	2 (66.66%)	2 (100%)	11 (45.83%)	0 (0%)
Ceftriaxone	0 (0%)	0 (0%)	0 (0%)	4 (16%)	2 (13.33%)	1 (33.33%)	2 (100%)	IR	IR
Cefepime	0 (0%)	0 (0%)	0 (0%)	3 (12%)	2 (13.33%)	1 (33.33%)	2 (100%)	7 (29.16%)	0 (0%)
Ampicillin	IR	IR	0 (0%)	3 (12%)	0 (0%)	0 (0%)	2 (100%)	IR	IR
Ampicillin–sulbactam	0 (0%)	IR	0 (0%)	5 (20%)	0 (0%)	0 (0%)	2 (100%)	IR	IR
Piperacillin–tazobactam	0 (0%)	0 (0%)	0 (0%)	11 (44%)	2 (13.33%)	3 (100%)	2 (100%)	12 (50%)	IR
Ciprofloxacin	0 (0%)	0 (0%)	0 (0%)	3 (12%)	1 (6.66%)	0 (0%)	0 (0%)	7 (29.16%)	0 (0%)
Levofloxacin	0 (0%)	0 (0%)	1 (50%)	5 (20%)	1 (6.66%)	0 (0%)	0 (0%)	8 (33.33%)	1 (100%)
Trimethoprim–sulfamethoxazole	2 (20%)	1 (100%)	0 (0%)	11 (44%)	2 (13.33%)	1 (33.33%)	1 (50%)	IR	1 (100%)
Ceftazidime–avibactam	0 (0%)	0 (0%)	0 (0%)	18 (72%)	5 (33.33%)	3 (100%)	2 (100%)	16 (66.66%)	0 (0%)
Chloramphenicol	0 (0%)	0 (0%)	2 (100%)	25 (100%)	5 (33.33%)	2 (66.66%)	1 (50%)	IR	1 (100%)

Abbreviations: GNB, gram-negative bacilli; IR, intrinsic resistant; NA, not available.

Bankar et al,[25] Sudhaharan et al,[11] and Singh et al,[13] in which *E. coli* (GNB) and *S. aureus* (GPC) were the highly prevalent bacterial isolates in the cases of wound infection.

According to present research, chloramphenicol (100%) was the most effective antibiotic against *E. coli*, followed by amikacin (88%), meropenem and ceftazidime–avibactam (72%) (►**Table 5**). Meropenem sensitivity was comparable to research conducted by Trojan et al[8] (68%); however, the results were not in synchronization with the studies of Khanam et al[21] (50%). *Pseudomonas aeruginosa* showed higher sensitivity to gentamicin, meropenem, and piperacillin–tazobactam (50%), *Klebsiella* showed higher sensitivity to ceftazidime–avibactam and chloramphenicol (33.33%). Amikacin, cefoxitin, and piperacillin–tazobactam (100%) were highly sensitive against *Morganella morganii*. Amikacin and chloramphenicol (100%) were highly sensitive against *Enterobacter cloacae*.

In the present study, *Acinetobacter* spp. and *Burkholderia* spp. are 100% resistant to multiple antibiotic except trimethoprim–sulfamethoxazole which shows 20 and 100% sensitivity, respectively (►**Table 5**). It is due to the evolution of bacteria with the passage of time.

The antibiotic sensitivity pattern of *S. aureus* in the present study shows 100% sensitivity to vancomycin, doxycycline, and linezolid (►**Table 6**). It was equivalent to the study conducted by Batra et al[24] and Trojan et al[8] which shows 100% sensitivity to linezolid and vancomycin, but it was inconsistent with the study done by Khanam et al[21] which shows 31.2 and 18.5% sensitivity to linezolid and vancomycin, respectively.

In the present study, vancomycin, doxycycline, and linezolid were 100% sensitive to *S. aureus*, coagulase-negative staphylococci (CoNS), and *Enterococcus*. Daptomycin are 100% sensitive to CoNS and *Enterococcus* (►**Table 6**). The current results are consistent with Batra et al[24] who recorded linezolid and vancomycin were 100% sensitive for *S. aureus* and CoNS.

The incidence of pyogenic isolates of bacteria and their patterns of antibiotic resistance vary widely depending on geographic location and atmospheric conditions. Due to the rising incidence of isolates that are resistant to multiple drugs-resistant bacteria, it is more prevalent in wound infections. Thus, the present study indicates to patient neglect, inadequate treatment plans, antibiotic usage, self- and mis-prescription, a lack of regional antibiogram data, and clinician's weak understanding of multidrug-resistant isolates and antimicrobial resistance. Controlling antibiotic overuse and implementing infection–prevention measures from primary to tertiary care would aid in the prevention of infections caused by resistant bacteria. Antibiotics should be used rationally, at the appropriate dose and duration.

Table 6 Frequency/percentage of antibiotic sensitivity pattern of GPC isolated from pus/wound sample

Antibiotics	*Staphylococcus aureus* ($n = 17$)	*Enterococcus* ($n = 2$)	CoNS ($n = 2$)
Cefazolin	6 (35.29%)	IR	0 (0%)
Cefoxitin	6 (35.29%)	IR	0 (0%)
Ampicillin	2 (11.76%)	0 (0%)	0 (0%)
Penicillin-g	2 (11.76%)	2 (100%)	0 (0%)
Vancomycin	17 (100%)	2(100%)	2 (100%)
Clindamycin	11 (64.70%)	IR	0 (0%)
Erythromycin	4 (23.52%)	1 (50%)	0 (0%)
Ciprofloxacin	1 (5.88%)	1 (50%)	0 (0%)
Moxifloxacin	14 (82.35%)	0 (0%)	2 (100%)
Doxycycline	17 (100%)	2 (100%)	2 (100%)
Daptomycin	16 (94.11%)	2 (100%)	2 (100%)
Trimethoprim–sulfamethoxazole	6 (35.29%)	IR	0 (0%)
Quinupristin–dalfopristin	15 (88.23%)	1 (50%)	2 (100%)
Chloramphenicol	15 (88.23%)	2 (100%)	2 (100%)
Fusidic acid	16 (94.11%)	IR	2 (100%)
Linezolid	17 (100%)	2 (100%)	2 (100%)
Mupirocin	15 (88.23%)	2 (100%)	1 (50%)
Rifampin	14 (82.35%)	NA	0 (0%)

Abbreviation: CoNS, coagulase-negative staphylococci; GPC, gram-positive cocci; IR, intrinsic resistant; NA, not available.

Conclusion

The current research highlights that GNB are the most frequent microorganisms which cause the infection of wound, and it is due to the fact that the organisms causing wound infections are frequently present in hospital environments. For GNB, amikacin, gentamicin, and chloramphenicol are the most effective antibiotics, whereas for GPC, doxycycline, linezolid, and chloramphenicol are the most effective antibiotics. Thus, the present study exhibited that increase in bacterial resistance as compared with other studies is due to the modification or evolution of bacteria with the time or irrational use of antibiotics.[26]

Ethical Approval

The study was approved by the head of the institute where study was done.

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None.

Conflict of Interest

None declared.

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