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Profile and antimicrobial susceptibility pattern of bacteria isolated from pus samples in a tertiary care hospital

Namrata Pawar[1], Dr.Kalpana Sadawarte[2], Dr.Tukaram Prabhu K,[3]
Department of Microbiology PCMS & RC Bhopal
Department of Microbiology PCMS & RC Bhopal

Abstract:
Background*: Pus, which is white to yellow fluid usually consist of dead neutrophils, necrotic tissue, cellular waste and the infecting microorganism. It is usually formed when the pathogenic microorganism causes infections in skin and soft tissues, may be due to trauma, burn or surgical procedures. It is usually confined in an abscess which may develop anywhere in the body. Aerobic as well as anaerobic, Gram-positive and Gram-negative bacteria have been isolated for pus samples.*
Materials and Methods*: This is a retrospective study conducted in Department of Microbiology of a tertiary care hospital from Jan 2024 to Dec 2024. A total of 268 pus samples submitted to the Department during the period of study were included in the study. All the samples were aseptically collected and inoculated on to blood agar and Mac Conkey agar. Plates were incubated at 37^0C for up to 48 hours. All the positive cultures with characteristic colony feature were further identified and confirmed with the help of standard biochemical tests. Antimicrobial susceptibility as per CLSI guidelines 2023*
Results*: A total of 268 pus samples received in the Department of Microbiology were included in the study and out of these, 158 (58.9%) yielded growth of bacteria. Of the 158 positive samples, 89 (56.33%) were from male patients and 69 (43.67%) from female patients. Among the 158 isolates, Staphylococcus aureus was the predominant organism isolated 26 (36.08%), followed by Klebsiella pneumoniae 18 (20.89%), Escherichia coli 17 (18.35%) and Pseudomonas aeruginosa (14.56%). The distribution of isolates showed a nearly equal proportion from IPD (48.73%) and OPD (51.27%). However, antimicrobial resistance was significantly higher among IPD isolates compared to OPD isolate.*
Conclusion: *These findings emphasize the importance of regular surveillance of local antibiograms, rational antibiotic use, and strict infection-control measures to guide appropriate empirical therapy and limit the emergence of antimicrobial resistance.*
Key Word*: Pus Sample, Staphylococcus aureus, Klebsiella pneumoniae.*

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I.Introduction

Pus, which is white to yellow fluid usually consist of dead neutrophils, necrotic tissue, cellular waste and the infecting microorganism. It is usually formed when the pathogenic microorganism causes infections in skin and soft tissues, may be due to trauma, burn or surgical procedures. These various injuries result in development of wounds which bring the patients to the health care facilities. It is usually confined in an abscess which may develop anywhere in the body. [1,2]

Aerobic as well as anaerobic, Gram-positive and Gram-negative bacteria have been isolated for pus samples. Among the Gram positive bacteria, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes and Enterococcus spp are commonly implicated in pyogenic infections while among the Gram negative Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus spp are common.[3, 4 5]

Wound infections that are hospital acquired can lead to severe morbidity and extended hospital stay with an increase in economic burden as well as higher risk of death due to disease. [1,6] Increase hospital stay may also contribute to the spread of pathogen to other patients and also to the care givers.[7]

These organisms also tend to be more antibiotic resistant, bacteria like Acinetobacter baumannii, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Methicillin resistant Staphylococcus aureus are known pathogens with resistance to multiple drugs.

The emergence of multidrug resistant bacteria is a threat worldwide and factors like self-medication, improper compliance to antibiotic regimens and empirical treatment with higher antibiotics have been responsible for this.[8,9,] .A continuous surveillance and update of antibiotic resistance pattern of micro-organisms is essential for infection control programs and accurate antibiotic treatment in the burnt patients.[10]

Even though the type of bacteria causing the pyogenic infection may be similar in different geographical regions, the antibiogram of these organisms do vary. And across the globe these studies have been consistent in helping the physician in initiating empirical treatment while they await the culture reports. So continuous surveillance is necessary to document the changing trends. The aim of this study was to identify the bacteria found in pus samples and determine their antibiogram in a tertiary care hospital of Central India.

II. Material And Methods

This is a retrospective study conducted in Department of Microbiology of a tertiary care hospital from Jan 2024 to Dec 2024. A total of 268 pus samples submitted to the Department during the period of study were included in the study. All the samples were aseptically collected and inoculated on to blood agar and Mac Conkey agar. Plates were incubated at 37^0C for up to 48 hours. All the positive cultures with characteristic colony feature were further identified and confirmed with the help of standard biochemical tests.[5]

Antimicrobial susceptibility of all the isolates was performed by Kirby Bauer technique and the results interpreted as per CLSI guidelines 2023 [11]. Briefly, the turbidity of the bacterial growth was compared with Mc Farland 0.5 standard and was spread on Mueller Hinton agar to give a lawn culture.

Following antibiotics were tested for Gram negative bacilli– Amikacin (30 µg), Amoxycillin-clavulanic acid (20//10 µg), Cefepime (30 µg), Cotrimoxazole (1.25/23.75 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Meropenem (10 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg) and Piperacillin-tazobactam.

For Gram positive cocci, Ampicillin (10 µg), Azithromycin (15 µg), Cefoxitin (30 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Cotrimoxazole (1.25/23.75 µg), Doxycycline (30 µg), Gentamicin (10 µg), Linezolid (30 µg) and Penicillin (10 units) were used.

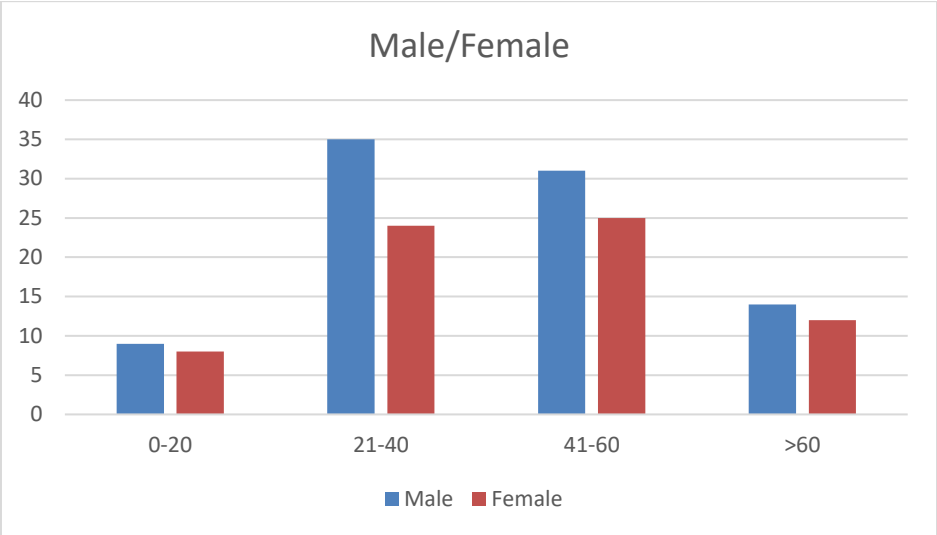
Amikacin (30 µg), Aztreonam (30 µg), Cefepime (30 µg), Cefoperazone (75 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Meropenem (10 µg), Piperacillin-tazobactam (100/10 µg) and Tobramycin (10 µg) were used for Pseudomonas spp.

III. Result

A total of 268 pus samples received in the Department of Microbiology were included in the study and out of these, 158 (58.9%) yielded growth of bacteria. Of the 158 positive samples, 89 (56.33%) were from male patients and 69 (43.67%) from female patients. There was no significant difference between the two genders. Among the 158 positive samples, 77 (48.73%) were from inpatients while 81 (51.27%) were from out-patients.

Table 1: Shows the age wise distribution of positive samples among male and female patients.

Age group	Male	Female
0-20	9	8
21-40	35	24
41-60	31	25
>60	14	12
Total	89	69



In our study, of the 158 isolates, 57 (36.08%) were Staphylococcus aureus, followed by 33 (20.89%) Klebsiella pneumoniae, 29 (18.35%) Escherichia coli and 23 (14.56%) Pseudomonas aeruginosa. Acinetobacter baumanii and Proteus mirabilis were isolated in 7 (4.43%) and 9 (5.70%) samples respectively.

Table 2: Shows the distribution of bacteria isolated in IPD & OPD

Bacteria	**IPD**	**OPD**	**Total**	**%**
Escherichia coli	17	12	29	18.35
Klebsiella pneumoniae	18	15	33	20.89
Acinetobacter	7	0	7	4.43
Proteus	0	9	9	5.70
Staphylococcus aureus	26	31	57	36.08
Pseudomonas aeroginosa	9	14	23	14.56
Total	77	81	158	

Among the Klebsiella isolated from IPD patients, percentage of resistance to Co-trimoxazole was 77.8% followed by 61.1% to Gentamicin and 50% to Amoxycillin-clavulanic acid. None of the strains were resistant to Meropenem, and resistance to cephalosporins was in the range of 11-33% as shown in the table . In case of Escherichia coli, 70.6% of the isolates were found to be resistant to Cefuroxime, Cotrimoxazole, and Gentamicin followed by 64.7% to Amikacin. Resistance to Amoxycillin-clavulanic acid, cefepime and meropenem was the least at 11.8%. Among Acinetobacter baumanii, 100% resistance was exhibited to Amoxycillin-clavulanic acid while all were susceptible to ciprofloxacin. Also, 85.7% of isolates were resistant to Cotrimoxazole followed by 57.1% each to cefuroxime and Gentamicin.

Table 3 : Shows the distribution of bacteria isolated in IPD

IPD	Escherichia coli (n=17)	Klebsiella pneumonia (n=18)	Acinetobacter (n=9)
Amikacin	64.7	61.1	57.1
Amoxycillin-clavulanic acid	11.8	50.0	100.0
Cefepime	11.8	16.7	28.6
Cefoxitin	23.5	22.2	28.6
Ceftazidime	23.5	33.3	42.9

Ceftriaxone	29.4	11.1	42.9
Cefuroxime	70.6	22.2	57.1
Ciprofloxacin	29.4	22.2	0.0
Cotrimoxazole	70.6	77.8	85.7
Gentamicin	70.6	38.9	57.1
Meropenem	11.8	0.0	42.9
Piperacillin-tazobactam	29.4	11.1	42.9

The level of resistance seen in the OPD patients was considerably lower than those seen in IPD patients. Among E.coli, 50% of the isolates were resistant to Ceftriaxone and Cefuroxime, while 41.7% were resistant to Ciprofloxacin and Gentamicin. Among Klebsiella isolates, 80% were resistant to Co-trimoxazole and 33.3% to Amikacin and Amoxycillin-clavulanic acid. Among Proteus isolates, 33.3% were resistant to Amoxycillin-clavulanic acid and Cotrimoxazole.

Table 4 : Shows the distribution of bacteria isolated in OPD

OPD	Escherichia coli (n=12)	Klebsiella pneumonia (n=15)	Proteus (n=7)
Amikacin	25.0	33.3	11.1
Amoxycillin-clavulanic acid	8.3	33.3	33.3
Cefepime	8.3	13.3	22.2
Cefoxitin	16.7	13.3	22.2
Ceftazidime	8.3	20.0	22.2
Ceftriaxone	50.0	0.0	0.0
Cefuroxime	50.0	20.0	0.0
Ciprofloxacin	41.7	0.0	22.2
Cotrimoxazole	25.0	80.0	33.3
Gentamicin	41.7	20.0	11.1
Meropenem	8.3	0.0	0.0
Piperacillin-tazobactum	16.7	0.0	22.2

Among Pseudomonas strains isolated from IPD patients, 55.6% were resistant to Tobramycin and 44.4% strains were resistant to Amikacin, Cefoperazone-sulbactam, Ceftazidime and Piperacillin-tazobactam. Among the OPD stains, 50% were resistant to Ciprofloxacin and 35.7 to Tobramycin.

Table 5 : Shows the Pseudomonas isolated in IPD and OPD

Pseudomonas aeroginosa	IPD (n=9)	OPD (n= 14)
Amikacin	44.4	28.6
Aztreonam	33.3	14.3
Cefepime	33.3	21.4
Cefoperazone sulbactum	44.4	21.4
Ceftazidime	44.4	28.6
Ciprofloxacin	33.3	50.0
Imipenem	33.3	14.3
Meropenem	22.2	14.3
Piperacillin-tazobactum	44.4	21.4
Tobramicin	55.6	35.7

In case of Staphylococcus aureus isolated from IPD patients, 57.5% were resistant to Penicillin followed by 53.8% to Ampicillin and 38.5% to Gentamicin, while among the OPD patients, 38.7% were resistant to Penicillin followed by 35.5% to Ampicillin and Gentamicin.

Table 6: Shows the Staphylococcus aureus isolated in IPD and OPD

Staphylococcus aureus n= (36)	IPD (26)	OPD (31)
Ampicillin	53.8	35.5
Azithromycin	23.1	22.6
Cefoxitin	23.1	19.4
Ciprofloxacin	23.1	19.4
Clindamycin	26.9	29.0
Cotrimoxazole	19.2	16.1
Doxycycline	15.4	12.9
Gentamicin	38.5	35.5
Linezolid	7.7	6.5
Penicillin	57.5	38.7

IV. Discussion

This study was conducted in department of Microbiology in a tertiary care hospital. A total of 268 Pus samples received from different departments of the hospital were processed, 158 (58.9%) showed bacterial growth, which is comparable to other studies by Rajput K, Johri S et.al (58.28% samples were culture positive) [12] and Hanumanthappa P et.al (56% of positive aerobic growth) [13]. Among 158 culture positive cases maximum number were males with male to female ratio of 1.25:1. Similar observation was done by Rajput K, et.al [12]. Maximum number of samples were from patients in the age group of 21-40 years.

Among the 158 isolates, Staphylococcus aureus was the predominant organism isolated 26 (36.08%), followed by Klebsiella pneumoniae 18 (20.89%), Escherichia coli 17 (18.35%) and Pseudomonas aeruginosa (14.56%). By Hanumanthappa P et.al, Roy S et al, Jain V et.al .[13,14 ,15] have shown similar findings. This pattern is consistent with previous reports where Staphylococcus aureus remains a leading cause of skin and soft tissue infections, while Gram-negative bacilli such as Klebsiella and Escherichia coli are commonly implicated in hospital-acquired infections.

The distribution of isolates showed a nearly equal proportion from IPD (48.73%) and OPD (51.27%). However, antimicrobial resistance was significantly higher among IPD isolates compared to OPD isolates, suggesting the role of nosocomial pathogens and selective pressure in the hospital environment.

Among Gram-negative bacteria, Klebsiella spp. from IPD showed high resistance to Cotrimoxazole (77.8%), Gentamicin (61.1%) and Amoxycillin-clavulanic acid (50%), while none of the isolates were resistant to Meropenem. Our study correlates with study done by Jain et al.[8] Among isolated Enterobacteriaceae group most effective antibiotic was Meropenem. Escherichia coli isolates from IPD demonstrated 70.6% resistance to Cefuroxime, Cotrimoxazole and Gentamicin, but only 11.8% resistance to Amoxycillin-clavulanic acid, Cefepime and Meropenem. Acinetobacter baumannii exhibited 100% resistance to Amoxycillin-clavulanic acid and 85.7% resistance to Cotrimoxazole, yet all isolates were susceptible to Ciprofloxacin. For Pseudomonas spp., IPD isolates showed 55.6% resistance to Tobramycin and 44.4% resistance to Amikacin, Cefoperazone-sulbactam, Ceftazidime and Piperacillin-tazobactam. In contrast, OPD isolates had lower resistance rates, with 50% resistance to Ciprofloxacin being the highest - this is similar to study by Jain V et.al.[15] whereas Roy S et.al.[14]. in their study showed Amikacin as most effective followed by Polymyxin B for pseudomonas.

Among Gram-positive cocci, Staphylococcus aureus was the most common isolate. Resistance to Penicillin was 57.5% in IPD and 38.7% in OPD, while resistance to Ampicillin and Gentamicin was also higher in IPD. Similar findings were found in other studies like Jain V et.al, Rajput K et.al,Roy S et.al. [15,12,14] whereas Hanumanthappa P et.al [13]. Notably, resistance to Linezolid remained low at 7.7% in IPD and 6.5% in OPD. The higher resistance observed in IPD patients can be attributed to prolonged hospital stay, empirical use of broad-spectrum antibiotics. The emergence of multidrug-resistant organisms such as Acinetobacter, Klebsiella, Pseudomonas and MRSA is a matter of concern and poses a therapeutic challenge.

Although the spectrum of organisms causing pyogenic infections may be similar across regions, their antibiogram shows considerable variation. Therefore, continuous surveillance of local bacterial profiles and their resistance patterns is essential to guide empirical therapy and to formulate effective antibiotic policies.

V. Conclusion

Pyogenic infections showed a predominance of *Staphylococcus aureus*, followed by Gram-negative bacilli such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Antimicrobial resistance was notably higher among IPD isolates than OPD isolates, with multidrug-resistant organisms posing a significant therapeutic challenge. Meropenem remained highly effective against Enterobacteriaceae, while resistance patterns varied considerably among other pathogens. These findings emphasize the importance of regular surveillance of local antibiograms, rational antibiotic use, and strict infection-control measures to guide appropriate empirical therapy and limit the emergence of antimicrobial resistance

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