

Spectrum of Bacterial Pathogens Isolated from Pus Samples and Their Antibiotic Sensitivity Pattern

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Abstract:

Background: Pyogenic infections are an issue of widespread morbidity, and the increasing antimicrobial resistance requires a regular review of causative agents and their resistance trends.

Aim: To identify the range of bacterial pathogens in pus samples and evaluate them in terms of antibiotic sensitivity profile.

Methodology: A cross-sectional study, which was done in Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India was carried out in a period of six months. One hundred and fifty (150) pus samples were put on Blood and MacConkey agar. The identification was done using standard biochemical tests and antimicrobial susceptibility testing was done using the Kirby Bauer disc diffusion according to CLSI guidelines. Cefoxitin disc and ESBL by double disc synergy test were used to detect MRSA.

Result: The culture positive stood at 88% (132/150). The most common isolate was *Staphylococcus aureus* (27.3%), *Pseudomonas aeruginosa* (21.2%), *E. coli* (13.6% and *Klebsiella* spp. 12.1%). MRSA constituted 36.1% of *S. aureus*. Production of ESBL was observed in 24.4% of Gram-negative isolates with the highest occurrence being *E. coli* (38.9%). The species were gram-positive cocci which were 100 per cent sensitive to vancomycin and 88 per cent sensitive to doxycycline but highly resistant to penicillin (76 per cent). The gram-negative bacilli were very resistant to ampicillin (88.9%) and completely sensitive to imipenem; aminoglycosides were active.

Conclusion: There is a high level of multi drug resistance in pus isolates; the most effective are carbapenems and vancomycin. Educated therapy should be a routine that is culture-guided to guarantee the rational use of antibiotics.

Keywords: Pyogenic Infections, Pus Culture, Antibiotic Susceptibility, MRSA, ESBL, Multidrug Resistance.

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Introduction

Infectious diseases are still one of the highest morbidity and mortality diseases in the world, especially in the developing countries that are characterized by overcrowding, poor hygiene, poor sanitation, malnutrition, and lack of access to healthcare services which promote the spread of diseases. Pyogenic infections form a large percentage of the hospital and community-acquired infections of the many infectious diseases experienced in the clinical practice. These infections are typified by localized and occasionally generalized inflammatory responses typically containing the development of pus. Pus depicts the existence of an active expression of the host immune system in response to the invading microorganisms and shows the interplay between pathogenic bacteria and the body defense system.

The pyogenic infections can be endogenous and exogenous. Endogenous infections are caused by normal microflora of the human body in case of the violation of natural barriers, and exogenous infections are caused by the introduction of microorganisms into the body by the outside factors associated with trauma, surgery, burns, and invasive procedures [1]. The skin is itself a major obstacle to microbial entry and any disruption in the skin-surface, whether through cuts, abrasions, surgical wounds, ulcers or burns serves as an entry-point to surface bacteria. After microorganisms have access to the underlying tissues, they multiply locally and cause inflammatory reactions. Host defense system reacts by attracting immune cells, neutrophils, and macrophages to the damaged area as they seek to destroy the possessing pathogens. Dead leukocytes, bacterial

debris, and tissue exudates accumulation ultimately results in the formation of pus which is a thick whitish fluid that characterizes suppurative infections [2].

Pus samples thus are a valuable clinical sample that can be used to investigate microbiology. Microbiological examination of pus assists in the identification of causative microorganisms that cause infections including abscesses, cellulitis, infected wounds, diabetic foot infections, postoperative infections and burn wound infections. These infections not only lead to an extend of hospital stay as well as cost escalation of treatment, but they also cause serious complications such as septicemia, organ failures and even death in case of failure to treat on time. The timely detection of pathogens and identification of their antimicrobial susceptibility pattern is hence important in case the patient is managed appropriately.

The range of bacteria recovered in pus differs in relation to geographic location, hospital setting, patients, as well as the underlying risk factors, including diabetes mellitus, immunosuppression, trauma, and surgical procedures. In the past, *Staphylococcus aureus* was thought to be the most frequent cause of pyogenic infections, with Gram-negative bacilli (*Escherichia coli*, *Klebsiella* species, *Proteus* species, and *Pseudomonas aeruginosa*) coming next. Nevertheless, over the last years, there has been a significant change in the bacteriological profile whereby Gram-negative organisms are increasingly becoming predominant in most healthcare facilities. This dynamic trend requires that bacterial isolates and their resistance profile be monitored periodically so that effective treatment measures are in place.

The treatment of pyogenic infections is mainly found on antimicrobial therapy and surgical drainage in case of necessity. Nevertheless, the widespread and unnecessary application of antimicrobial agents has led to the occurrence of antibiotic resistance because of the formation of resistant genes in most organisms [3]. These problems in antibiotic use, including self-medication, incomplete treatment regimes, inappropriate empirical therapy, and excessive prophylactic use, have increased the time rate of selection pressure on bacteria, allowing them to develop a variety of resistance mechanisms. They involve the synthesis of enzymes, which neutralize the effects of antibiotics, the modification of the target of drugs, the reduced cell membrane permeability, and the active efflux pumps that help tubester the bacteria to expel drugs.

One of the most frightening trends in recent decades has been the appearance and dissemination of multidrug-resistant organisms (MDROs). These include Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta-Lactamase

(ESBL) producing Gram-negative bacilli who have become a significant therapeutic concern within the hospital and community setting [4]. MRSA strains are resistant to beta-lactam antibiotics such as penicillins and cephalosporins, whereas ESBL producing organisms can hydrolyse a broad spectrum of beta-lactam antibiotics, which otherwise greatly restrict treatment. The development of these resistant strains give resistance to the widely used drugs; this is a major issue in terms of making decisions on empiric therapy [5].

The effects of antimicrobial resistance are enormous. The infected patients with resistant organisms are also likely to take long stays in hospital, have more costly and toxic medicines and might show increased cases of morbidity and mortality. Moreover, resistant organisms may easily spread in the healthcare facilities, causing outbreaks and burdening the healthcare systems. Thus, antimicrobial susceptibility testing may be considered one of the staples of clinical microbiology laboratories. It can assist clinicians in making the right choice of antibiotics, avoid the use of ineffective medicines, and contribute to the reduction of the resistance formation and dissemination.

Due to the fact that the flora of bacteria and the susceptibility to antibiotics is considered varied with time and region, it is possible that the use of standard treatment rules is not always effective. Initiation of empirical therapy can happen before the laboratory results are obtained, especially in severe infections but the empirical regimens should be found on the local epidemiological data. Constant monitoring of bacterial pathogens and their resistance trends to antibiotics are needed to track new trends of resistance, as well as update institutional antibiotic policies. These kinds of surveillance are beneficial in preventing the uninformed use of antimicrobial agents and avoid unproductive empirical treatment.

Moreover, awareness of local antibiograms can help clinicians to choose narrow-spectrum antibiotics in situations where they can be used, and therefore, they use broad-spectrum drugs when dealing with resistant infections. It also helps in infection control in the hospital environment by determining common resistant strains. This is of particular importance in such surgical wards, orthopedic units, burn units, and intensive care unit where wound infections are prevalent and antibiotic resistance is high.

The problem of the growing incidence of multidrug resistant pathogens highlights the importance of routine monitoring and revising of antimicrobial therapy guidelines. In the absence of precise evidence through laboratory testing, clinicians can opt to use inappropriate combinations of antibiotics, which complicate the situation even more. In this way, microbiological analysis of pus samples is not only useful in the work with patients but also a significant

contributor to the health of the population as it helps to develop the programs of antibiotic stewardship.

It is against this backdrop that the current study has been conducted to examine the range of bacteria pathogens that have been isolated in pus samples and identify the antibiotic sensitivity profile of the bacteria pathogens. The results will be useful in terms of information that can shed light on common isolations of bacteria and the resistance profiles that could help clinicians select adequate treatment and lead to responsible use of antibiotics.

Methodology

Study Design: The cross-sectional descriptive study was a hospital-based study done to identify the range of bacterial pathogens that could be isolated in pus samples and to establish the antimicrobial susceptibility profile of these pathogens.

Study Area: The current investigation was conducted at the Department of Microbiology, Darbhanga Medical College and Hospital (DMCH), Laheriasarai, Darbhanga, Bihar, India from March 2025 to August 2025.

Study Duration: A six-month period was used to carry out the study.

Sample Size: 150 pus samples that were submitted to the microbiology laboratory by different clinical departments during the study period were used in the study.

Study Population: The study population was all the patients that attend an outpatient department and those that were admitted in the wards and intensive care departments with clinically suspected pyogenic infections of all ages and both sexes. Surgical wounds, abscess, burns, diabetic foot ulcers, traumatic wounds and post-operative wound infections were used as the sources of the samples.

Data Collection: Pus samples were taken under aseptic conditions using sterile cotton swab or sterile syringe aspiration, the latter method being the most preferred. All of the samples were delivered to the microbiology lab as quickly as possible and processed immediately. All the specimens were inoculated on Blood agar and MacConkey agar plates and then incubated aerobically at 37C for 18-24 hours. Standard microbiological procedures were done to identify the bacterial isolates based on the morphology of the colony, Gram staining patterns and routine biochemical tests such as catalase test, coagulase test, oxidase test, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test (IMViC), hydrogen sulphide production, urease test, nitrate reduction test and sugar fermentation tests.

Antimicrobial Susceptibility Testing: The isolates were tested on antibiotic susceptibility by Kirby-Bauer disc diffusion on Mueller-Hinton agar as per Clinical and Laboratory Standards Institute (CLSI)

guidelines. Antibiotics that were tested comprised of amikacin, ampicillin, cefotaxime, co-trimoxazole, doxycycline, gentamicin, imipenem, piperacillin, ciprofloxacin, vancomycin, penicillin and erythromycin. The detection of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase negative *Staphylococci* (MRCONS) was done by using cefoxitin disc. The areas of inhibition were recorded and discussed as sensitive or intermediate or resistant according to CLSI.

Detection of ESBL Producers: The production of extended spectrum beta-lactamase Gram negative isolates was identified using double disc synergy test. Ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg) discs were positioned with 20 mm of distance on the Mueller-Hinton agar with the test organism inoculated. A zone of inhibition increase of ≥ 5 mm when using the combination disc in comparison to ceftazidime alone was regarded as a response to ESBL.

Inclusion Criteria

- All pus samples received in microbiology laboratory during study period
- Patients clinically suspected of bacterial pyogenic infection
- Samples collected before initiation of antibiotic therapy (whenever possible)
- Both indoor and outdoor patients

Exclusion Criteria

- Improperly collected samples
- Dry swabs
- Duplicate samples from same infection site
- Samples with mixed growth of commensals suggesting contamination
- Patients already on prolonged antibiotic therapy (>72 hrs) without clinical details

Procedure: The samples were received in the laboratory and cultured on suitable media, incubated and analyzed to determine the growth of bacteria. Standard microbiological tests were used to identify the isolates obtained and they were subjected to antibiotic susceptibility tests. The presence of MRSA and ESBL was investigated by the recommended phenotypic procedures. The systematic recording of all the findings was done in a pre-designed data sheet.

Statistical Analysis: All the data collected was recorded and analyzed in Microsoft Excel with the help of statistical software. Results were indicated in terms of frequency and percentage. The relation between bacterial isolates and the antibiotic resistance pattern was determined using Chi-square test and a p-value below 0.05 was taken to be statistically significant.

Result

Table 1 shows the culture positivity rate among 150 samples. Out of the total samples, 132 (88.00%)

were culture positive, while only 18 (12.00%) showed no bacterial growth, indicating a very high prevalence of detectable infection in the studied specimens.

Total Samples	Culture Positive	Percentage	No Growth	Percentage
150	132	88.00%	18	12.00%

Table 2 presents the sex-wise distribution of culture-positive cases (n = 132). A higher proportion of cases were observed in males, with 78 patients (59.10%), compared to females who accounted for

54 cases (40.90%). Thus, culture-positive infections were notably more common among male patients than female patients in this study population.

Sex	Number of Cases	Percentage
Male	78	59.10%
Female	54	40.90%
Total	132	100%

Table 3 shows the spectrum of bacterial isolates from pus samples (n = 132). The most common organism isolated was *Staphylococcus aureus* accounting for 36 cases (27.30%), followed by *Pseudomonas aeruginosa* with 28 isolates (21.20%). Among Gram-negative organisms, *Escherichia coli* constituted 18 isolates (13.60%) and *Klebsiella* spp.

16 isolates (12.10%). MRCONS was identified in 14 cases (10.60%), while less frequent organisms included *Proteus* spp. 8 (6.10%), *Acinetobacter* spp. 7 (5.30%), and *Citrobacter* spp. 5 (3.80%). Overall, *Staphylococcus aureus* was the predominant pathogen, though Gram-negative bacilli collectively formed a substantial proportion of infections.

Organism	Number of Isolates	Percentage
<i>Staphylococcus aureus</i>	36	27.30%
MRCONS	14	10.60%
<i>Pseudomonas aeruginosa</i>	28	21.20%
<i>Escherichia coli</i>	18	13.60%
<i>Klebsiella</i> spp.	16	12.10%
<i>Proteus</i> spp.	8	6.10%
<i>Acinetobacter</i> spp.	7	5.30%
<i>Citrobacter</i> spp.	5	3.80%
Total	132	100%

Table 4 presents ESBL production among Gram-negative isolates (n = 82). Overall, 20 isolates (24.40%) were ESBL producers. The highest proportion was observed in *E. coli* with 7 out of 18 isolates (38.90%), closely followed by *Klebsiella* spp. with 6 of 16 isolates (37.50%). Moderate ESBL production was seen in *Proteus* spp. (2/8; 25.00%) and

Citrobacter spp. (1/5; 20.00%), while lower rates were noted in *Acinetobacter* spp. (1/7; 14.30%) and *Pseudomonas* spp. (3/28; 10.70%). These findings indicate that ESBL production was most common among Enterobacteriaceae, particularly *E. coli* and *Klebsiella*, highlighting their important role in antimicrobial resistance.

Organism	Total isolates	ESBL Positive	% ESBL
<i>E. coli</i>	18	7	38.90%
<i>Klebsiella</i> spp.	16	6	37.50%
<i>Proteus</i> spp.	8	2	25.00%
<i>Citrobacter</i> spp.	5	1	20.00%
<i>Acinetobacter</i> spp.	7	1	14.30%
<i>Pseudomonas</i> spp.	28	3	10.70%
Total	82	20	24.40%

Table 5 shows the antibiotic susceptibility pattern of Gram-positive cocci (n = 50). Vancomycin demonstrated complete sensitivity (100%) with no resistant isolates, making it the most effective drug. High sensitivity was also observed with doxycycline (88%), followed by ceftazidime (64%) and ciprofloxacin (60%). Erythromycin showed moderate activity

(52%), while co-trimoxazole had lower effectiveness (44%). Penicillin was the least effective antibiotic, with only 24% sensitivity and 76% resistance. Overall, the results indicate considerable resistance to commonly used antibiotics, whereas vancomycin and doxycycline remain the most reliable therapeutic options against Gram-positive cocci.

Antibiotics	Sensitive	%	Resistant	%
Penicillin	12	24%	38	76%
Ceftazidime	32	64%	18	36%
Erythromycin	26	52%	24	48%
Ciprofloxacin	30	60%	20	40%
Co-trimoxazole	22	44%	28	56%
Doxycycline	44	88%	6	12%
Vancomycin	50	100%	0	0%

Table 6 illustrates the antibiotic susceptibility pattern of Enterobacteriaceae and Acinetobacter isolates (n = 54). Imipenem showed complete effectiveness with 100% sensitivity and no resistant isolates, making it the most reliable drug in this group. High susceptibility was also observed with amikacin (81.5%) and gentamicin (74.1%), indicating good efficacy of aminoglycosides. Cefotaxime

demonstrated moderate sensitivity (63%), whereas co-trimoxazole (44.4%) and ciprofloxacin (37%) showed considerable resistance. Ampicillin was the least effective antibiotic, with only 11.1% sensitivity and 88.9% resistance. Overall, the findings suggest significant resistance to commonly used first-line antibiotics, while carbapenem and aminoglycosides remain more effective therapeutic options.

Antibiotics	Sensitive	%	Resistant	%
Ampicillin	6	11.10%	48	88.90%
Cefotaxime	34	63.00%	20	37.00%
Co-trimoxazole	24	44.40%	30	55.60%
Gentamicin	40	74.10%	14	25.90%
Amikacin	44	81.50%	10	18.50%
Ciprofloxacin	20	37.00%	34	63.00%
Imipenem	54	100%	0	0%

Table 7 presents the antibiotic susceptibility pattern of Pseudomonas spp. isolates (n = 28). Imipenem demonstrated complete efficacy, with 100% sensitivity and no resistant strains detected. High sensitivity was also observed with gentamicin (78.6%) and amikacin (71.4%). Ciprofloxacin showed moderate effectiveness (67.9% sensitivity), while

piperacillin (57.1%) and ceftazidime (50%) exhibited comparatively lower susceptibility rates, indicating emerging resistance to commonly used β -lactam antibiotics. Overall, carbapenem (imipenem) remained the most reliable therapeutic option against Pseudomonas spp. in this study.

Antibiotics	Sensitive	%	Resistant	%
Amikacin	20	71.40%	8	28.60%
Gentamicin	22	78.60%	6	21.40%
Ciprofloxacin	19	67.90%	9	32.10%
Piperacillin	16	57.10%	12	42.90%
Ceftazidime	14	50.00%	14	50.00%
Imipenem	28	100%	0	0%

Table 8 shows methicillin resistance among Staphylococcus aureus isolates (n = 36). Out of the total isolates, 13 (36.1%) were identified as Methicillin-Resistant Staphylococcus aureus (MRSA), while the remaining 23 (63.9%) were Methicillin-Sensitive

Staphylococcus aureus (MSSA). Thus, although MSSA constituted the majority of isolates, over one-third of the strains were methicillin resistant, indicating a considerable burden of antibiotic resistance in the study population.

Table 8: MRSA Detection Among Staphylococcus aureus (n = 36)

Category	Number	Percentage
MRSA	13	36.10%
MSSA	23	63.90%

Discussion

The current investigation showed that the culture positivity rate of pus samples was high (88), which showed that bacteria were actively involved in most of the suppurative infections. The above finding is similar to the previous finding where 90.49% of samples produced growth indicating that there is generally good microbiological yield of pus specimen in pyogenic infections. The low percentage of sterile samples of the two studies may be explained by the previous antibiotic treatment or insufficient bacteria load. Our study had also similar results with the previous studies, where males were noted to be 59.1% similar to the previous studies where males were identified to be 56-57.66% (Duggal et al., 2015) [6] due to the higher exposure to traumas, outdoor activities, and occupational hazards.”

Staphylococcus aureus (27.3) predominated followed by *Pseudomonas aeruginosa* (21.2), *Escherichia coli* (13.6) and *Klebsiella* spp. (12.1) in the current study. The same trend was reported in earlier research in which *S. aureus* and *Pseudomonas* spp. constituted 27.65 and 21.27 percent of the isolates, respectively, and justified the overall predominance of these organisms in pyogenic infections (Rao et al., 2014; Duggal et al., 2015) [7,6]. This resemblance validates that *S. aureus* remains the major etiological agent in pus-forming lesions in various geographical locations. Other researchers however identified *E. coli* as dominating Gram negative isolate over *Pseudomonas*, which suggests the variation due to the hospital setting, usage of antibiotics and number of patients (Kaup and Sankarankutty, 2014) [8]. The presence of coagulase-negative staphylococci, *Proteus*, *Acinetobacter* and *Citrobacter* in lesser proportion in our research is also associated with earlier studies of polymicrobial tendencies of wound infection (Sowmya et al., 2014) [9].

Our research found that 36.1% of the isolates contained Methicillin-resistant *S. aureus* (MRSA), which is a little higher but similar to the previous prevalence of 28.84% (Kaup & Sankarankutty, 2014) [8]. This increase could be an indication of the growing pressure of antibiotics and the spread within hospitals. Other Indian studies have also focused on indicating similar observations with the clinical importance of MRSA on skin and soft tissue infections and have stressed on its increasing epidemiological importance (Shenoy et al., 2010) [4]. The occurrence of the methicillin-resistant coagulase-negative staphylococci (10.6) in our research also

confirms the development of the resistant Gram-positive cocci in hospitals.

In our study, Gram-negative isolates showed production of ESBL in 24.4% of cases, which is a little greater and slightly more compared to the reported 21.21% prevalence. As observed in both studies, *E. coli* had the highest rate of ESBL resistance (38.9% vs 37.05%) compared to *Klebsiella* spp. (37.5% vs 28.57%), which is why the family of Enterobacteriaceae is considered as the primary reservoir of ESBL enzymes. The reduced ESBL production of the *Pseudomonas* and *Acinetobacter* species was also in line with previous results (Afroz et al., 2015) [10]. The similarity indicates a steady resistance pattern of Gram-negative pathogens, even though the low increment in our study can have an indication of slow growth of resistant strains.

The pattern of antibiotic sensitivity of Gram-positive cocci in our study gave universal sensitivity to vancomycin (100) and high sensitivity to doxycycline (88), which was synonymous to previous studies that discovered the reliability of vancomycin as the most effective drug against staphylococci (Rao et al., 2014) [7]. Nevertheless, high resistance to penicillin (76%), erythromycin and cotrimoxazole which we have documented in our study mark a steady downward trend of the usefulness of the popular oral antibiotics. The same resistance patterns were also observed by other researchers in the past, though some studies have recorded relatively low resistance to erythromycin, which is an indication that the practice of prescribing varies locally (Duggal et al., 2015) [6].

In our study, gram-negative bacilli were more sensitive to imipenem (100%) than amikacin (81.5%), and gentamicin (74.1%). These results are quite similar to previous ones in which imipenem has been found to be 100 percent active, and aminoglycosides have a susceptibility value of around 82.6 percent (Rao et al., 2014; Duggal et al., 2015) [7,6]. However, the other investigation found ciprofloxacin being the second most effective drug to imipenem as opposed to our results where the resistance was relatively high (63) against ciprofloxacin (Sowmya et al., 2014) [9]. Such difference could be attributed to the extensive use of empirical fluoroquinolone resulting in the selection pressure and the development of resistance.

The current research also revealed that *Pseudomonas aeruginosa* isolates were also completely sensitive to imipenem and showed a good response to the aminoglycosides, which were comparable to

previous studies where imipenem and gentamicin were the most active ones (Rao et al., 2014) [7]. Our study revealed moderate susceptibility to piperacillin and ceftazidime, which indicates that there is a potential development of resistance to antipseudomonal beta-lactams that might be owing to the common use in hospitals. The current carbapenem sensitivity demonstrates that the agents are still the reserve agents in case of severe infections, but their stewardship is vital in order to avoid future resistance.

On the whole, the current research confirms that *Staphylococcus aureus* and *Pseudomonas aeruginosa* are still the most frequent pathogens in pus samples whose antimicrobial resistance becomes higher and higher, in particular, MRSA and ESBL-producing Enterobacteriaceae. The pattern of antibiotic resistance is very similar to the past studies conducted in India and supports the idea of keeping a close eye on the situation and rational policy towards antibiotics to keep the proliferation of resistant organisms under control.

Conclusion

The current research shows that the culture positivity rate in pus samples is high with a high number of male patients. Bacteriological profile showed that the most common isolate is *Staphylococcus aureus* with *Pseudomonas aeruginosa* and Enterobacteriaceae family following it and non-fermenters and other gram-negative bacilli were less common. A significant percentage of *Staphylococcus aureus* were resistant to methicillin and there was production of extended spectrum beta-lactamase in a number of the gram-negative organisms signifying emergence of multidrug resistance. Gram-positive cocci were not very vulnerable to penicillin but are fully susceptible to vancomycin and responsive to doxycycline, whereas gram-negative isolates are very resistant to commonly used agents such as ampicillin and fluoroquinolones but extremely sensitive to amino glycoside and carbapenems. The isolates of *Pseudomonas* also responded better to carbapenems as opposed to other agents. Altogether, the results indicate the changing range of pathogens of wounds and the significance of a regular culture and antibiotic susceptibility testing to inform the right empirical treatment and avoid antimicrobial resistance.

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