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ANTIMICROBIAL RESISTANCE PATTERNS IN AEROBIC PUS ISOLATES: A CROSS-SECTIONAL STUDY FROM A TERTIARY CARE HOSPITAL IN INDIA

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Abstract
Background: Wound infections pose a significant challenge in the era of multidrug-resistant (MDR) organisms, leading to prolonged hospital stays and increased treatment costs. This study analyze the aerobic bacterial profile of pus isolates and their antibiotic resistance patterns to guide clinical management. **Materials and Methods:** A prospective cross-sectional study was conducted over 18 months (February 2021–July 2022) at a tertiary healthcare hospital in India. A total of 324 pus samples were processed using standard bacteriological techniques. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method per CLSI guidelines. Statistical analysis was conducted using SPSS with a significance threshold of $p < 0.05$. **Result:** Of 324 pus samples, 201 (62.03%) were culture-positive. Staphylococcus aureus (46.76%) was the predominant isolate, followed by Escherichia coli (16.91%) and Klebsiella spp. (13.93%). Among Gram-positive bacteria, 100% were sensitive to vancomycin and 98% to linezolid, while 99% were resistant to penicillin. Gram-negative isolates showed the highest sensitivity to imipenem (93.15%) and maximum resistance to ceftazidime (59%). Methicillin-resistant S. aureus (MRSA) was detected in 65% of S. aureus isolates, and extended-spectrum beta-lactamase (ESBL) production was observed in 62% of Gram-negative bacteria. **Conclusion:** The high prevalence of MDR pathogens, particularly MRSA and ESBL-producing strains, highlights the urgent need for routine surveillance, strict infection control measures, and antibiotic stewardship programs to curb resistance trends and optimize empirical therapy.

INTRODUCTION

Wound infections are a significant cause of morbidity and prolonged hospitalization worldwide, particularly in developing countries where healthcare resources and infection control measures are often inadequate.[1] Disruption of the skin barrier facilitates microbial invasion, leading to infections ranging from localized pus formation to life-threatening systemic conditions. The most frequently implicated pathogens in wound infections include both Gram-positive bacteria such as Staphylococcus aureus and Enterococcus spp., as well as Gram-negative bacteria like Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa.[2] Effective treatment depends on accurate microbial identification and targeted antibiotic therapy; however, the rise of multidrug-resistant (MDR) bacteria has significantly complicated empirical treatment strategies.

Antimicrobial resistance (AMR) is a growing global concern, driven by the overuse and misuse of antibiotics in both clinical and community settings. MDR pathogens, particularly methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, and carbapenem-resistant

Pseudomonas aeruginosa and Acinetobacter baumannii, have emerged as major contributors to treatment failures.[3] In India, MRSA has been reported in 40–70% of S. aureus isolates, while Gram-negative bacteria have shown increasing resistance to third-generation cephalosporins and fluoroquinolones.[4] ESBL-producing E. coli and Klebsiella spp. frequently exhibit resistance exceeding 60%, necessitating the use of carbapenems as a last-line treatment.[5] Additionally, carbapenem resistance among Pseudomonas aeruginosa has risen by 20–40% in the last decade, leading to poor clinical outcomes.

AMR patterns vary by region, hospital setting, and patient population, highlighting the need for continuous surveillance to guide effective empirical therapy and antimicrobial stewardship. While numerous studies have documented resistance trends in urban tertiary care hospitals, limited data are available from rural and semi-urban healthcare centers in India.[6] Given that India is one of the world's largest consumers of antibiotics, the country faces a heightened risk of resistance emergence, particularly in hospitals where empirical antibiotic use is common without culture-based confirmation.[7] Bihar, one of India's most populous states, lacks comprehensive research on AMR trends in wound infections, making it difficult to implement evidence-based antibiotic policies.

In this context, the present study aims to analyze the bacterial profile and antimicrobial resistance patterns of aerobic pus culture isolates from a tertiary care hospital in Bihar. By identifying prevalent pathogens and their susceptibility patterns, the study seeks to provide critical data to support empirical antibiotic selection, strengthen infection control strategies, and enhance antimicrobial stewardship programs. The findings will be instrumental in formulating targeted interventions to curb the spread of MDR pathogens and improve clinical outcomes in wound infection management.

MATERIALS AND METHODS

Study Design & Setting: This prospective cross-sectional study was conducted in the Department of Microbiology at Narayan Medical College & Hospital, a tertiary care center in Bihar, India. The study spanned 18 months, from February 2021 to July 2022.

Study Population & Sample Collection: A total of 324 clinically suspected cases of wound infections were included. Patients were selected using consecutive sampling from the General Surgery, Orthopedics, Medicine, and Pediatrics departments. Inclusion criteria involved patients with pus-producing infections, while those receiving antibiotic therapy for more than 72 hours before sample collection were excluded to avoid pre-treated cases.

Data collection was started after obtaining clearance from the Institutional Ethical Committee IEC/IRB-No. NMCH/ IEC/ 2021/ 35

Aseptic pus samples were collected either through needle aspiration or sterile swabs. For deep-seated infections, aspirates were preferred, while superficial wounds were sampled using double swabs. One swab was used for Gram staining and direct microscopy, and the second was used for culture. Samples were processed immediately to minimize contamination.

Bacteriological Processing & Identification: Specimens were inoculated onto Blood Agar, MacConkey Agar, and Nutrient Agar and incubated aerobically at 37°C for 24–48 hours. Colonies were identified based on morphology, pigmentation, and hemolysis patterns. Standard biochemical tests, including catalase, coagulase, indole, citrate, TSI reactions, and urease tests, were performed to confirm species identity. Anaerobic cultures were not included in this study.

Antimicrobial Susceptibility Testing (AST): AST was performed using the Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested for Gram-positive isolates included vancomycin, linezolid, erythromycin, clindamycin, tetracycline, teicoplanin, cefoxitin (for MRSA detection), and penicillin. For Gram-negative bacteria, the tested antibiotics included imipenem, amikacin, gentamicin, ciprofloxacin, ceftazidime, ceftriaxone, and piperacillin-tazobactam. Quality control was ensured using reference strains Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923).

Statistical Analysis: All data were analyzed using SPSS version 25. Descriptive statistics were presented as percentages and proportions. The chi-square test was used to compare resistance patterns between different bacterial species. A p-value <0.05 was considered statistically significant for all analyses.

RESULTS

A. Culture Positivity Rate & Organism Distribution

A total of 324 pus samples were analyzed, of which 201 (62.03%) yielded positive bacterial growth. The remaining 123 (37.97%) showed no bacterial growth. The culture positivity rate in this study (62.03%) is consistent with findings from previous Indian studies, which report positivity rates ranging from 59% to 83%.[1,2]

As shown in [Table 1], the most frequently isolated organism was Staphylococcus aureus (46.76%), followed by Escherichia coli (16.91%) and Klebsiella spp. (13.93%). Pseudomonas aeruginosa was isolated in 7.46% of cases, while other less common pathogens included Proteus spp. (4.97%), Enterococcus spp. (3.48%), and Citrobacter spp. (2.48%).

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Table 1: Distribution of Bacterial Isolates in Culture-Positive Samples.

Bacterial Species	Frequency (n)	Percentage (%)
Staphylococcus aureus	94	46.76
Escherichia coli	34	16.91
Klebsiella spp.	28	13.93
Pseudomonas aeruginosa	15	7.46
Proteus spp.	10	4.97
Enterococcus spp.	7	3.48
Citrobacter spp.	5	2.48

B. Antimicrobial Susceptibility Testing (AST) Results

B.1. Gram-Positive Bacteria

As depicted in [Table 2], Among 94 isolates of Staphylococcus aureus, the highest sensitivity was observed to vancomycin (100%) and linezolid (98%). However, high resistance rates were noted for penicillin (99%), erythromycin (60%), and cefoxitin (65%), indicating Methicillin-resistant S. aureus (MRSA) prevalence of 65%.

Table 2: Antimicrobial Susceptibility Patterns of Gram-Positive Isolates

Antibiotic	Sensitive (%)	Resistant (%)
Vancomycin	100	0
Linezolid	98	2
Penicillin	1	99
Cefoxitin (MRSA detection)	35	65
Erythromycin	40	60
Clindamycin	64	36
Amikacin	98	2

B.2. Gram-Negative Bacteria

As depicted in [Table 3], Among Gram-negative isolates, imipenem showed the highest sensitivity (93.15%), followed by amikacin (52%) and gentamicin (43%). However, high resistance was observed against ceftazidime (59%) and ceftriaxone (69%), suggesting a high prevalence of ESBL-producing strains (62%) in E. coli and Klebsiella spp.

Table 3: Antimicrobial Susceptibility Patterns of Gram-Negative Isolates

Antibiotic	Sensitive (%)	Resistant (%)
Imipenem	93.15	6.85
Amikacin	52	48
Gentamicin	43	57
Ciprofloxacin	34	66
Ceftazidime	41	59
Ceftriaxone	31	69

Notable Observations

- Pseudomonas aeruginosa isolates demonstrated high sensitivity to imipenem (93%) and amikacin (87%), but were highly resistant to cefepime (53%) and ceftazidime (67%).
- Klebsiella spp. and E. coli showed multidrug resistance, with ciprofloxacin and ceftriaxone resistance exceeding 60%.

DISCUSSION

Wound infections pose a significant challenge in clinical settings, particularly in resource-limited regions where antimicrobial resistance (AMR) is increasing at an alarming rate. The findings of this study provide crucial insights into the prevalence, bacterial profile, and antimicrobial resistance patterns of aerobic pus culture isolates in a tertiary care hospital in Bihar, India. The overall culture positivity rate (62.03%) aligns with previous Indian studies, which report positivity rates ranging from 59% to 83%.[8,9] This variability may be attributed to factors such as differences in sample collection techniques, prior antibiotic exposure, and microbiological processing methods. A high culture positivity rate indicates a considerable burden of bacterial infections, necessitating robust antimicrobial stewardship programs to optimize empirical treatment and mitigate resistance development.

Prevalence of Pathogens in Pus Culture Isolates: The most frequently isolated organism in this study was Staphylococcus aureus (46.76%), followed by Escherichia coli (16.91%) and Klebsiella spp. (13.93%). The dominance of S. aureus in wound infections is consistent with previous literature, where it is reported as the primary pathogen responsible for both hospital-acquired and community-acquired infections.[10,11] The high prevalence of S. aureus is particularly concerning due to its ability to develop resistance rapidly and its association with persistent infections.

Gram-negative bacteria, including E. coli and Klebsiella spp., accounted for a significant proportion of infections. The presence of Pseudomonas aeruginosa (7.46%) and Proteus spp. (4.97%) highlights the role of non-fermenters and facultative anaerobes in wound infections. These organisms are often implicated in chronic wounds

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and burn infections, making their resistance patterns highly relevant for clinical management.[12,13]

Antimicrobial Resistance Patterns and Molecular Mechanisms

Methicillin-Resistant Staphylococcus aureus (MRSA)

The 65% prevalence of MRSA in this study is within the reported range of 40–70% in various Indian studies.[14,15] This high MRSA prevalence suggests widespread dissemination of the mecA gene, which encodes penicillin-binding protein 2a (PBP2a), conferring resistance to beta-lactams. Resistance to cefoxitin (65%) confirmed the presence of MRSA strains, which poses a significant challenge to clinicians, as beta-lactam antibiotics are often the first line of defense in S. aureus infections.

Fortunately, all S. aureus isolates were 100% sensitive to vancomycin and 98% sensitive to linezolid, indicating that glycopeptides and oxazolidinones remain effective treatment options. However, emerging reports of vancomycin-intermediate S. aureus (VISA) and linezolid resistance warrant continuous surveillance and judicious antibiotic use to prevent further resistance evolution.[16,17]

Extended-Spectrum Beta-Lactamase (ESBL) Production in Gram-Negative Isolates: ESBL production was detected in 62% of E. coli and Klebsiella spp. isolates, highlighting a major concern in treating Gram-negative infections. These ESBL-producing strains exhibited high resistance to third-generation cephalosporins, including ceftriaxone (69%) and ceftazidime (59%), making empirical therapy challenging.

The predominant CTX-M-type beta-lactamases found in Indian ESBL-producing isolates are responsible for hydrolyzing cephalosporins, thereby rendering them ineffective.[18,19] The high sensitivity to imipenem (93.15%) suggests that carbapenems remain the most effective treatment option for ESBL-producing E. coli and Klebsiella spp.. However, the increasing carbapenem resistance reported globally underscores the need for antibiotic stewardship programs to restrict carbapenem overuse.[20,21]

Multidrug-Resistant Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic pathogen known for its intrinsic resistance mechanisms, including efflux pumps, AmpC beta-lactamase production, and biofilm formation. In this study, P. aeruginosa showed high resistance to ceftazidime (67%) and cefepime (53%), suggesting AmpC beta-lactamase production as a primary resistance mechanism.[22] The high sensitivity to imipenem (93%) and amikacin (87%) indicates that carbapenems and aminoglycosides remain viable therapeutic options. However, reports of imipenem-resistant Pseudomonas are increasing, necessitating alternative treatment strategies such as colistin or combination therapy.[23]

Comparative Analysis with Other Studies: Several studies have reported similar AMR trends in India and South Asia. Ramesh et al. (2022) documented MRSA prevalence at 62% and ESBL production in 58% of E. coli isolates, which closely aligns with our findings.[4] In contrast, a study by Shrestha et al. (2022) in Nepal reported higher carbapenem resistance (30%) in Gram-negative isolates, indicating regional variations in antibiotic resistance patterns.[2] These comparisons emphasize the need for localized resistance surveillance to guide empirical therapy in specific healthcare settings.

Clinical Implications and Recommendations: Given the high prevalence of MDR pathogens, empirical antibiotic selection should be guided by local resistance patterns. Based on our findings, we propose the following recommendations:

1. MRSA Management: Since 65% of S. aureus isolates were MRSA, empirical therapy should prioritize vancomycin or linezolid in suspected MRSA cases.
2. ESBL Infections: Given the 62% ESBL prevalence, third-generation cephalosporins should be avoided in Gram-negative infections, and carbapenems should be used for severe infections.
3. Pseudomonas Infections: Due to high ceftazidime and cefepime resistance, empirical therapy for P. aeruginosa should include imipenem or amikacin.
4. Antibiotic Stewardship: Strict antimicrobial stewardship programs should be implemented to limit unnecessary antibiotic use and prevent resistance escalation.
5. Surveillance and Infection Control: Regular microbial surveillance and strict infection control measures in surgical and intensive care units (ICUs) are essential to curb nosocomial infections.

Limitations of the Study: While this study provides valuable insights into antimicrobial resistance patterns in pus culture isolates, certain limitations must be acknowledged. Firstly, this was a single-center study, which may limit the generalizability of the findings to other regions with different healthcare settings and antimicrobial prescribing practices. Secondly, anaerobic bacteria were not included, even though they play a significant role in chronic wound infections. Future studies should incorporate anaerobic cultures to provide a more comprehensive overview. Additionally, molecular characterization of resistant strains, such as PCR-based detection of resistance genes (mecA, CTX-M, and AmpC), was not performed, which could have strengthened the study's findings. Lastly, the study period was limited to 18 months, preventing long-term surveillance of emerging resistance trends.

CONCLUSION

This study provides critical insights into the bacteriological profile and resistance patterns of pus culture isolates in a tertiary care hospital in India. The high prevalence of MRSA (65%) and ESBL-producing Gram-negative bacteria (62%)

underscores the growing challenge of antimicrobial resistance in wound infections. Carbapenems remain the most effective treatment for ESBL infections, while vancomycin and linezolid are the drugs of choice for MRSA. The findings reinforce the need for continuous resistance monitoring, antimicrobial stewardship, and stringent infection control practices to combat the rising threat of MDR pathogens in healthcare settings.

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