

Antibiotic Susceptibility and Bacterial Profiles of Sterile Body Fluids in a Tertiary Hospital in India**Mitesh Kamothi¹, Ritapa Ghosh², Nisarg Trivedi³**¹Associate Professor, Department of Microbiology, GMERS Medical College, Gotri, Vadodara, India^{2,3}Assistant Professor, Department of Microbiology, GMERS Medical College, Gotri, Vadodara, India

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Abstract**Background:** Infections of sterile body fluids are associated with high morbidity and mortality, particularly in the presence of multidrug resistant organisms. Surveillance of bacterial spectrum and drug susceptibility patterns is crucial for guiding effective empirical therapy.**Material and Methods:** This prospective observational study included 120 sterile body fluid samples collected from outpatient and inpatient departments of a tertiary care hospital in India. Samples were cultured and isolates were identified using standard microbiological techniques. Antimicrobial susceptibility testing was performed according to CLSI M100 (34th edition, 2024) guidelines. Data were analyzed statistically, with $p < 0.05$ considered significant.**Results:** Gram-negative bacilli were predominant, with *E. coli*, *K. pneumoniae*, *Acinetobacter* spp., and *Pseudomonas* spp. constituting the majority of isolates. Colistin, tigecycline, and minocycline showed the highest activity against multidrug resistant Gram-negatives. Gram-positive cocci, including coagulase-positive and coagulase-negative staphylococci, demonstrated high sensitivity to vancomycin and linezolid, while *Enterococcus* showed reduced susceptibility to ampicillin and vancomycin. Malignancy was the only comorbidity significantly associated with multidrug resistant infections.**Conclusion:** The study highlights the predominance of multidrug resistant Gram-negative organisms in sterile body fluid infections and underscores the importance of continuous surveillance and antimicrobial stewardship to optimize therapy.**Keywords:** sterile body fluids, multidrug resistance, antimicrobial susceptibility, tertiary care hospital.This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

Infections involving sterile body fluids such as cerebrospinal, pleural, peritoneal, synovial, and pericardial fluids are clinically significant due to their potential for rapid progression to life-threatening conditions if not managed promptly [1]. Although these compartments are normally devoid of microorganisms, breach by pathogenic bacteria leads to altered fluid characteristics, impaired host defenses, and increased morbidity and mortality [1]. Emerging antimicrobial resistance (AMR) among bacteria isolated from these sterile sites represents a growing challenge across healthcare systems worldwide. The WHO GLASS 2025 report highlights escalating rates of resistance to key antibiotics, particularly among Gram-negative organisms, underscoring an urgent need for localized AMR surveillance [2].

In India, the ICMR AMR Surveillance Network's 2023 report reveals troubling trends in resistance among clinical isolates—particularly in hospital

settings—underscoring that empirical therapy without local antibiogram support may lead to treatment failure, prolonged hospital stays, and poorer outcomes [3]. Recent tertiary care-based studies offer direct insights into the prevalence and resistance patterns among sterile body fluid pathogens. Patel et al. (2024) reported a 17.8% culture positivity rate from sterile fluid specimens, with Gram-negative bacteria accounting for 84% of isolates; *E. coli* showed high sensitivity to gentamicin and fosfomycin but pronounced resistance to cefoperazone-sulbactam [4].

Khatiyani et al. (2025) described that among 495 sterile body fluid samples, 48 (9.7%) were culture-positive, with *Acinetobacter* (35%), *Klebsiella* (23%), and *E. coli* (13%) being predominant. Crucially, Gram-negatives were universally sensitive to colistin, followed by amikacin and gentamicin, while Gram-positives were entirely sensitive to vancomycin and linezolid [5].

Similarly, Singh et al. (2023), working in Uttar Pradesh, found a culture positivity rate of 9.7% (192 of 1,980 samples). Gram-negative bacilli comprised 83.3% of isolates; notably, 75% of *Staphylococcus aureus* isolates were MRSA, all remaining susceptible to vancomycin and linezolid, while 25% of GNB were ESBL-producers and 62.5% were carbapenemase-producers, though all GNB were sensitive to colistin [6].

Studies from other regions add weight to these findings. A study in Eastern Ethiopia (Shume et al. 2022) recorded a 16.7% culture positivity, with 70.6% of isolates being Gram-negative; remarkably, 76.5% displayed multidrug resistance, reinforcing the global nature of this problem [7].

Microbiological methodology plays a pivotal role in generating reliable data. The CLSI M100-2024 standards remain the gold-standard guides for antimicrobial susceptibility testing (AST) globally, offering updated breakpoints and protocol clarity [8].

From an epidemiological perspective, Admas et al. (2024) emphasized that the prevalence of *Enterobacter cloacae* in peritoneal and cerebrospinal specimens may represent emerging nosocomial threats, with variable resistance profiles, emphasizing the need for periodic surveillance of pathogen dynamics and resistance mechanisms as local epidemiology evolves [9].

More recently, Mahato et al. (2021) demonstrated the variability in resistance patterns among bacterial isolates from pleural and peritoneal fluids in an Indian tertiary center, stressing that inappropriate empirical therapy without hospital-based antibiogram support significantly increases morbidity and mortality [10].

Taken together, these findings underscore the critical importance of conducting region-specific surveillance on sterile body fluid pathogens and their antimicrobial susceptibility patterns. This data is indispensable for guiding empirical therapy, shaping antibiotic policy, informing stewardship efforts, and improving patient outcomes in Indian tertiary care settings.

Material and Methods

This prospective observational study was conducted in the Department of Microbiology at a tertiary care hospital in India over a period of twelve months. A total of 120 sterile body fluid samples were included in the study.

These samples were collected from patients attending both the outpatient department (OPD) and those admitted to the inpatient department (IPD), who were clinically suspected of harboring infections in sterile body sites such as cerebrospinal, pleural, peritoneal, synovial, and

pericardial cavities. The inclusion criteria comprised all patients irrespective of age and gender presenting with clinical indications for sterile fluid aspiration and subsequent culture. Samples showing evidence of contamination or insufficient volume were excluded from the analysis to ensure reliability of the microbiological results.

All specimens were collected under strict aseptic precautions by trained clinicians following institutional protocols. Cerebrospinal fluid samples were obtained via lumbar puncture, pleural and peritoneal fluids through thoracentesis and paracentesis respectively, synovial fluid by joint aspiration, and pericardial fluid through pericardiocentesis. Each specimen was immediately transferred into sterile, leak-proof containers, labeled properly, and transported without delay to the microbiology laboratory. Processing of samples was carried out within one hour of collection to avoid overgrowth of contaminants and loss of viability of fastidious organisms.

Direct microscopic examination was performed using Gram staining to provide preliminary information on the presence and morphology of bacteria. Culture was carried out by inoculating samples onto blood agar, MacConkey agar, and chocolate agar plates, which were incubated aerobically at 37°C for 24–48 hours. Growth was examined daily, and isolates were identified based on colony morphology, Gram reaction, and a battery of standard biochemical tests. For confirmation, automated identification systems were used wherever required.

Antimicrobial susceptibility testing (AST) of all bacterial isolates was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines, 34th edition, 2024.

A panel of commonly used antibiotics covering both Gram-positive and Gram-negative organisms was tested, including penicillins, cephalosporins, aminoglycosides, fluoroquinolones, carbapenems, glycopeptides, and colistin. For isolates suspected of being methicillin-resistant *Staphylococcus aureus* (MRSA), cefoxitin disk diffusion was employed, while extended-spectrum beta-lactamase (ESBL) production among Gram-negative bacilli was detected using the combination disk method. Carbapenemase production was confirmed using the modified carbapenem inactivation method (mCIM). Quality control for susceptibility testing was ensured by using standard ATCC strains including *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. All findings were systematically recorded and analyzed. The

distribution of isolates, their antimicrobial susceptibility patterns, and resistance mechanisms were compiled and compared across OPD and IPD patients. Statistical analysis was performed using SPSS version 25.0, and results were expressed as frequencies and percentages. Associations between clinical variables and bacterial isolates were tested using chi-square analysis, with a p-value of less than 0.05 considered statistically significant.

Results

The demographic characteristics and risk factors of patients with and without chronic liver disease (CLD) are summarized in Table 1. Out of the 120 patients included, 58 had underlying CLD and 62 did not. The mean age was similar between groups, and although males predominated in both categories, the difference in male-to-female ratio was statistically significant. Hypertension and encephalopathy were also significantly more frequent in patients with CLD. Other comorbidities such as diabetes mellitus, chronic kidney disease, heart disease, pleural effusion, chronic obstructive pulmonary disease (COPD), and malignancy did not differ significantly between the groups. Parameters including length of hospital stay, total leukocyte count, and serum-ascites albumin gradient (SAAG) ratio were not significantly different between patients with or without CLD. Mortality rates were also similar in both groups.

The demographic profile and risk factors associated with multidrug resistant (MDR) isolates in patients with purulent infections are outlined in Table 2. Among 120 patients, 54 (45%) yielded MDR organisms. The mean age of patients with MDR isolates was 44.7 years, with a predominance of males, although gender difference was not

statistically significant. None of the comorbidities, including diabetes, CLD, CKD, hypertension, or COPD, showed significant associations with MDR infections, except malignancy which demonstrated a strong correlation. Other variables such as length of hospital stay, total leukocyte count, and SAAG ratio were not significantly different. Mortality was higher among MDR cases but did not reach statistical significance.

The antimicrobial sensitivity pattern of Gram-negative bacilli is shown in Table 3. *E. coli* demonstrated the highest susceptibility to colistin, tigecycline, and minocycline, while showing negligible sensitivity to cephalosporins and fluoroquinolones. *Acinetobacter* species retained moderate sensitivity to colistin and minocycline but were largely resistant to carbapenems and beta-lactam-beta-lactamase inhibitor combinations. *K. pneumoniae* exhibited near-complete resistance to most first- and second-line drugs but retained full sensitivity to colistin and good sensitivity to tigecycline. *Pseudomonas* species showed variable resistance, with relatively higher susceptibility to colistin, minocycline, and piperacillin-tazobactam, though carbapenem sensitivity was low. The Gram-positive cocci isolates are detailed in Table 4. Coagulase-positive *Staphylococcus aureus* were uniformly sensitive to vancomycin, teicoplanin, doxycycline, and amikacin, but highly resistant to erythromycin and gentamicin. Coagulase-negative staphylococci also demonstrated preserved susceptibility to vancomycin, teicoplanin, and amikacin, with moderate sensitivity to doxycycline. *Enterococcus* species showed strong sensitivity to linezolid, doxycycline, and minocycline, though their susceptibility to ampicillin and vancomycin was reduced.

Table 1: Demographic characteristics of patients and risk factors for bacterial body fluid infections with and without chronic liver disease (CLD) (N=120)

Parameters	With CLD (n=58)	Without CLD (n=62)	P-value
Age, years, mean (SD)	42.12 ± 20.45	41.98 ± 21.10	0.922
Gender, male/female	45:13	34:28	0.006*
Diabetes mellitus, %	15	12	0.392
Chronic kidney disease, %	18	25	0.241
Heart disease, %	8	14	0.166
Hypertension, %	24	14	0.028*
Pleural effusion, %	17	27	0.076
COPD, %	5	8	0.377
Malignancy, %	7	12	0.228
Encephalopathy, %	22	6	<0.001*
Organ transplant, %	4	1	0.149
Post-operative patients, %	18	21	0.674
Anemia, %	43	45	0.941
Length of hospital stay, mean (SD)	23.11 ± 15.90	25.36 ± 18.94	0.399
Total leukocyte count, mean (SD)	15,482 ± 10,264	14,890 ± 9,318	0.474
SAAG ratio, mean (SD)	1.81 ± 0.892	1.53 ± 0.962	0.091
Death, %	17	19	0.812

Table 2: Demographic characteristics of patients and risk factors for isolation of multidrug resistant microorganisms in patients with purulent infections (N=120)

Parameters	MDR microorganisms (n=54/120, 45.0%)	P-value	95% CI
Age, years, mean (SD)	44.72 ± 18.28	0.261	39.45 – 50.00
Gender, male/female %	63.0/37.0	0.569	1.22 – 1.50
Diabetes mellitus, %	15 (27.8%)	0.208	1.58 – 1.83
Chronic liver disease, %	24 (44.4%)	0.482	1.39 – 1.68
Chronic kidney disease, %	22 (40.7%)	0.319	1.44 – 1.71
Heart disease, %	11 (20.4%)	0.319	1.65 – 1.88
Hypertension, %	17 (31.5%)	0.911	1.54 – 1.81
Pleural effusion, %	15 (27.8%)	0.118	1.57 – 1.83
COPD, %	6 (11.1%)	0.498	1.78 – 1.96
Malignancy, %	12 (22.2%)	0.021*	1.63 – 1.88
Encephalopathy, %	11 (20.4%)	0.733	1.65 – 1.88
Organ transplant, %	1 (1.9%)	0.239	1.93 – 1.99
Post-operative patients, %	21 (38.9%)	0.176	1.46 – 1.74
Anemia, %	41 (75.9%)	0.581	1.09 – 1.32
Length of hospital stay, mean (SD)	25.47 ± 19.40	0.419	19.85 – 30.62
Total leukocyte count, mean (SD)	16,318 ± 10,285	0.133	13,410 – 19,226
SAAG ratio, mean (SD)	1.60 ± 1.105	0.437	1.26 – 1.89
Death, %	20 (37.0%)	0.127	1.23 – 1.50

Table 3: Percentage sensitivity pattern for first- and second-line drugs in most commonly isolated Gram-negative bacilli

Antibiotics	E. coli %	Acinetobacter %	K. pneumoniae %	Pseudomonas %
Amikacin	73.91	50.00	15.38	37.50
Ceftazidime	8.69	16.67	0.00	25.00
Ceftriaxone	0.00	16.67	0.00	-
Ciprofloxacin	8.69	33.33	0.00	-
Levofloxacin	-	-	-	37.50
Cefoperazone-Sulbactam	34.78	16.67	0.00	31.25
Imipenem	60.87	33.33	0.00	31.25
Meropenem	60.87	33.33	0.00	31.25
Ertapenem	52.17	-	0.00	-
Colistin	100.00	83.33	100.00	93.75
Tigecycline	100.00	83.33	69.23	-
Minocycline	95.65	83.33	69.23	75.00
Aztreonam	-	-	-	18.75
Piperacillin-Tazobactam	-	-	-	37.50

Table 4: Percentage sensitivity pattern for first- and second-line drugs in most commonly isolated Gram-positive cocci

Antibiotics	Coagulase-positive Staphylococcus %	Coagulase-negative Staphylococcus %	Enterococcus %
Ampicillin	-	-	25
Ampicillin-Sulbactam	66.67	47.83	41.67
Amikacin	100	82.60	-
Clindamycin	33.33	60.87	-
Cefoxitin	33.33	56.52	-
Doxycycline	100	73.91	83.33
Erythromycin	0	13.04	-
Gentamicin	0	0	33.33
Levofloxacin	0	26.08	16.67
Vancomycin	100	100	41.67
Teicoplanin	100	91.30	41.67
Linezolid	-	-	91.67
Minocycline	-	-	83.33

Discussion

The present study highlights the distribution of bacterial isolates and their antimicrobial susceptibility patterns from sterile body fluids, providing important insight into resistance dynamics within a tertiary care hospital in India. Our findings are consistent with recent literature demonstrating that Gram-negative bacilli, particularly *E. coli*, *K. pneumoniae*, *Acinetobacter* spp., and *Pseudomonas* spp., remain the predominant pathogens responsible for serious body fluid infections. The high prevalence of multidrug resistant organisms underscores the ongoing threat of antimicrobial resistance in critical care settings. Recent work by Ranjan et al. (2023) emphasized that the emergence of carbapenem-resistant *K. pneumoniae* and *Acinetobacter* poses significant challenges in India, with mortality risk amplified when appropriate therapy is delayed [11].

Our results corroborate this pattern, showing poor sensitivity to carbapenems and beta-lactam/beta-lactamase inhibitor combinations, while colistin remains one of the few reliably effective agents. A multicenter Indian surveillance study by Bansal et al. (2022) similarly reported colistin as retaining more than 90% sensitivity against multidrug resistant Gram-negatives, although concerns over nephrotoxicity and emerging resistance limit its routine use [12].

The emergence of tigecycline and minocycline as effective alternatives against multidrug resistant isolates, as shown in our data, has been reported elsewhere. Verma et al. (2021) described high susceptibility of carbapenem-resistant Enterobacteriaceae to tigecycline, supporting its potential role in salvage therapy for severe infections [13]. However, variability in pharmacokinetics and limited evidence in bloodstream infections call for cautious interpretation. Our Gram-positive cocci results also align with reports by Chakraborty et al. (2022), who demonstrated universal susceptibility of MRSA and coagulase-negative staphylococci to vancomycin and linezolid, highlighting that these remain the mainstay of therapy against resistant staphylococcal infections [14]. Importantly, the persistence of vancomycin-susceptible enterococci, albeit with reduced sensitivity compared to linezolid, indicates an evolving resistance landscape that requires continuous monitoring.

Global data continue to emphasize the need for hospital-specific antibiograms to guide empirical therapy. According to O'Neill et al. (2024), development of local antimicrobial stewardship programs tailored to individual hospital epidemiology is crucial in addressing inappropriate antibiotic use and minimizing resistance selection [15]. Our findings reinforce this recommendation,

demonstrating distinct susceptibility profiles that, if integrated into local treatment protocols, could improve clinical outcomes. Collectively, the results of this study emphasize the urgent need for antimicrobial stewardship, judicious antibiotic use, and robust infection prevention measures in tertiary care centers.

Conclusion

This study demonstrates that Gram-negative bacilli constitute the majority of pathogens isolated from sterile body fluids, with high rates of multidrug resistance posing therapeutic challenges. Colistin, tigecycline, and minocycline remain the most effective agents against multidrug resistant Gram-negatives, while vancomycin and linezolid retain activity against resistant Gram-positive cocci. The findings highlight the necessity for continuous surveillance, hospital-specific antibiogram development, and the implementation of antimicrobial stewardship strategies to optimize treatment outcomes and curb the spread of resistance.

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