Original Article

Bacteriological Profile and Antibiotic Susceptibility Pattern in Sterile Body Fluids from a Tertiary Care Hospital in Bhubaneswar, Odisha, India: A Cross-sectional Study

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ABSTRACT

Introduction: Sterile body sites are those where no microorganisms exist as commensals in an otherwise healthy person. Isolation of microorganisms from these sites can indicate either pathological agents or contaminants from the skin. Sterile body fluids are frequently received in microbiology laboratories for culture and sensitivity testing, as the isolation of pathogens from these sites is associated with significant mortality and morbidity.

Aim: To assess the current scenario of aerobic bacteriological profiles and their antibiotic susceptibility patterns in various sterile body fluids at a tertiary care hospital in Bhubaneswar, Odisha, India.

Materials and Methods: A hospital-based observational study was conducted in the Department of Microbiology at a Tertiary Care Hospital in Bhubaneswar, Odisha, India. The study duration was one year, from January 2022 to December 2022. A total of 450 body fluid samples were collected from 567 patients, of which 117 samples did not meet the inclusion criteria. Out of the 450 samples, 315 were from male patients and 135 were from female patients. All infected body fluids received from clinically diagnosed cases, irrespective of age and gender, were included. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method following the guidelines of the Clinical Laboratory Standards Institute (CLSI). Sterile body fluids were collected with complete aseptic precautions and processed in the Department of Microbiology using standard laboratory procedures. Statistical data analysis was conducted using MS Excel and Statistical Package for Social Sciences (SPSS) version 27.0. A p-value <0.05 was considered statistically significant.

Results: A total of 450 clinical samples were processed, of which 153 (34%) were peritoneal fluid, 92 (20.5%) were synovial fluid, 77 (17.1%) were pleural fluids, 118 (26.2%) were Cerebrospinal Fluid (CSF), and 10 (2.2%) were pericardial fluid. In the present study, 126 (28%) pathogens were isolated from the 450 processed samples, with gram negative bacilli being the predominant isolates (88/126, 69.8%), while the remaining 38/126 (30.2%) were gram-positive isolates. Among the 88 gram negative isolates, *Escherichia coli* was the most common (27, 21.4%), followed by *Klebsiella pneumoniae* (23, 18.2%), *Pseudomonas aeruginosa* (14, 11.1%), *Acinetobacter* spp. (12, 9.5%), *Enterobacter* spp. (7, 5.6%), and *Citrobacter spp*. (5, 4.0%). Similarly, among the gram-positive isolates, *Staphylococcus aureus* was the most common (20, 15.9%), followed by Coagulase-Negative Staphylococci (CONS) (16, 12.7%) and *Enterococcus spp*. (2, 1.6%). Gram negative isolates showed 100% sensitivity to colistin and polymyxin B, followed by imipenem (90%) and cefepime (80%). Gram-positive isolates exhibited 100% sensitivity to linezolid, followed by vancomycin.

Conclusion: Early identification of pathogens from these sites, along with their antibiotic susceptibility patterns, will help clinicians initiate targeted therapy. This approach can reduce hospital stays for patients and minimise the development of drug resistance.

Keywords: Antimicrobial resistance, Microorganisms, Pathological agents

INTRODUCTION

Body fluids like peritoneal fluid, pleural fluid, synovial fluid, pericardial fluid, and CSF are usually sterile. These sterile body fluids are frequently sent for bacteriological culture and sensitivity. If these sterile body fluids become infected with microorganisms, it can be life-threatening and lead to severe morbidity and mortality [1]. Therefore, infections of sterile body fluids are medical emergencies and require early diagnosis and effective management. Hence, knowledge of the prevalent strains causing infection in sterile body fluids, along with their antibiotic susceptibility patterns, is essential for antibiotic policy makers and clinicians to improve patient management [2]. Limited data are available in this geographical area regarding the bacteriological profile and antibiotic susceptibility patterns of sterile body fluids. Understanding the bacteriological profile, along with the antibiotic susceptibility patterns, is crucial for microbiologists,

physicians, infectious disease specialists, and antibiotic policy makers to ensure proper diagnosis and judicious use of antibiotics, thereby reducing morbidity and mortality [3]. It is necessary to monitor the epidemiology of bacterial susceptibility patterns in each geographical area so that infections can be treated empirically with antibiotics as soon as possible, thereby reducing morbidity and mortality [4]. Common bacterial agents causing infections in sterile body fluids include *Escherichia coli, Klebsiella pneumoniae, Acinetobacter* spp., *Pseudomonas aeruginosa, Enterobacter* spp., *Staphylococcus aureus*, CONS, and *Enterococcus* species. These infections are more prevalent in developing countries with limited healthcare services, poor hygiene and sanitation, and irrational use of antibiotics [5,6]. Therefore, the primary objective of this study was to determine the aerobic bacteriological profile of various sterile body fluids, and the secondary objective was to assess their antibiotic susceptibility patterns.

MATERIALS AND METHODS

A cross-sectional and hospital-based study was conducted in the Department of Microbiology at a Tertiary Care Hospital in Bhubaneswar, Odisha, India, from January 2022 to December 2022. The study was approved by Institutional Ethical Committee (IEC) and informed consent was obtained from the patients.

Inclusion criteria: All infected body fluids received from clinically diagnosed cases, irrespective of age and gender, were included in the study.

Exclusion criteria: Blood samples, samples from patients with a history of antibiotic intake within the last two weeks, contaminated samples, and samples delayed for more than two hours were excluded from the study.

The study included 450 body fluid samples collected from 567 patients, excluding 117 samples that did not meet the inclusion criteria. Out of the 450 samples, 315 were from male patients and 135 were from female patients.

Study Procedure

Body fluid samples, such as peritoneal fluid, pleural fluid, synovial fluid, CSF, and pericardial fluid, were collected with complete aseptic precautions. The samples were sent to the Microbiology Department for further processing within two hours of collection. Standard microbiological procedures were used to process the samples, which were then inoculated on blood agar and MacConkey agar (Himedia, Mumbai, India) plates. After overnight incubation at 37°C, colony morphology was studied, and organisms were identified up to the species level using standard biochemical tests. Antimicrobial susceptibility tests were performed on isolated pathogens using the Kirby-Bauer disc diffusion method following CLSI guidelines [7]. For gram negative bacilli, the antibiotic discs used were ampicillin, cefotaxime, ceftriaxone, cefepime, gentamicin, amikacin, levofloxacin, cotrimoxazole, imipenem, piperacillin-tazobactam, colistin, and polymyxin B. Similarly, for gram-positive isolates, the antibiotic discs used were ampicillin, amoxicillin-clavulanic acid, ciprofloxacin, levofloxacin, cotrimoxazole, erythromycin, clindamycin, gentamicin, linezolid, and vancomycin.

STATISTICAL ANALYSIS

For statistical data analysis, MS Excel and SPSS version 27.0 (Chicago, IL, USA) were used. The chi-square test was applied to compare the growth and no growth patterns.

RESULTS

A total of 450 clinical samples were collected from various suspected patients. Out of the 450 processed samples, 126 (28%) body fluids showed growth. The most common body fluid received in our laboratory was peritoneal fluid, with 153 (34%) samples, followed by CSF with 118 (26.2%), synovial fluid with 92 (20.4%), pleural fluid with 77 (17.1%), and pericardial fluid with 10 (2.2%) [Table/Fig-1].

Out of the 126 culture-positive growths, the predominant isolate was Escherichia coli with 27 (21.4%), followed by *Klebsiella pneumoniae* with 23 (18.2%), *Staphylococcus aureus* with 20 (15.8%), CONS with 16 (12.6%), *Pseudomonas aeruginosa* with 14 (11.1%), Acinetobacter species with 12 (9.5%), Enterobacter species with 7 (5.5%), Citrobacter species with 5 (3.9%), and *Enterococcus species* with 2 (1.58%) [Table/Fig-2].

The p-value was calculated to be 0.062037, indicating that $p>0.05$. Gram negative isolates showed resistance to various antibiotics. They showed 100% sensitivity to colistin and polymyxin B, followed by imipenem, cefepime, and piperacillin+tazobactam [Table/Fig-3].

Types of samples	Male	Female	Total n (%)	Growth		Total n (%)	No growth		Total n (%)
				Male	Female		Male	Female	
Pleural fluid	55	22	77 (17.1)	23	07	30 (39)	32	15	47 (61)
Peritoneal fluid	107	46	153 (34)	43	09	52 (34)	73	28	101 (66)
Synovial fluid	53	39	92 (20.4)	14	11	25 (27)	43	24	67 (73)
Pericardial fluid	08	02	10 (2.2)	02	0	02 (20)	06	02	08 (80)
Cerebrospinal Fluid (CSF)	92	26	118 (26.2)	14	03	17 (14)	77	24	101 (86)
Total	315	135	450 (100)	96	30	126 (28)	231	93	324 (72)

[Table/Fig-1]: Gender-wise classification of body fluids with growth and no growth pattern.
Chi-square value for growth is 7.686 which statistically not significant for 3d. f at 95% confidence level and provides that sex has no relation on the growth in the body fluids with p-value >0.05

Isolates	Peritoneal fluid	Pleural fluid	Synovial fluid	CSF	Pericardial fluid	Total n (%)
Escherichia coli (E. coli)	14	6	2	5	0	27 (21.4)
Klebsiella pneumoniae (K. pneumoniae)	10	8	1	3	1	23 (18.2)
Staphylococcus aureus (S. aureus)	4	3	10	3	0	20 (15.9)
Coagulase Negative Staphylococci (CONS)	5	4	6	1	0	16 (12.6)
Pseudomonas aeruginosa (P. aeruginosa)	6	4	2	2	0	14 (11.1)
Acinetobacter species	6	3	1	1	1	12 (9.5)
Enterobacter species	3	1	2	1	0	07 (5.6)
Citrobacter species	3	1	0	1	0	05 (4.0)
Enterococcus species	1	0	1	0	0	02 (1.6)
Total	52	30	25	17	02	126 (100)

[Table/Fig-2]: Bacteriological profile from different sterile body fluids sample.
One-way Analysis of Variance (ANOVA) test was applied to the above table in which the d.f.=4 for between treatments and d.f.=39 for within treatments. F.Value=2.45091 and p-value=0.062037. The result was not statistically significant at $p <0.05$

Antibiotics	*E. coli* (n=27)	*Klebsiella pneumoniae* (n=23)	*Acinetobacter* species (n=12)	*Enterobacter* species (n=7)	*Citrobacter* species (n=5)
Ampicillin	10 (37%)	9 (39.1%)	4 (33.3%)	2 (28.5%)	1 (20%)
Gentamicin	15 (55.5%)	14 (60.8%)	6 (50%)	4 (57.1%)	3 (60%)
Amikacin	17 (62.9%)	17 (73.9%)	7 (58.3%)	4 (57.1%)	3 (60%)
Levofloxacin	18 (66.6%)	17 (73.9%)	6 (50%)	3 (42.8%)	4 (80%)
Co-trimoxazole	19 (70.3%)	18 (78.2%)	4 (33.3%)	4 (57.1%)	4 (80%)

Imipenem	25 (92.5%)	21 (91.3%)	10 (83.3%)	6 (85.7)	3 (60%)
Cefotaxime	11 (40.7%)	9 (39.1%)	3 (25%)	3 (42.8%)	3 (60%)
Ceftriaxone	12 (44.4%)	10 (43.4%)	4 (33.3%)	3 (42.8%)	2 (40%)
Colistin	27 (100%)	23 (100%)	12 (100%)	7 (100%)	5 (100%)
Polymyxin B	27 (100%)	23 (100%)	12 (100%)	7 (100%)	5 (100%)
Piperacillin+tazobactam	24 (88.8%)	20 (86.9%)	9 (75%)	4 (57.1%)	4 (80%)
Cefepime	24 (88.8%)	20 (86.9%)	9 (75%)	5 (71.4%)	4 (80%)

[Table/Fig-3]: Antimicrobial susceptibility pattern of gram-negative bacterial isolates (n=74).

Gram-positive isolates showed 100% sensitivity to linezolid, followed by vancomycin, co-trimoxazole, levofloxacin, etc., [Table/Fig-4].

Antibiotics	*Staphylococcus aureus* (n=20)	Coagulase Negative Staphylococci (n=16)	*Enterococcus species* (n=2)
Ampicillin	12 (60%)	10 (62.5%)	0 (0%)
Amoxycillin+ clavulinic acid	13 (65%)	14 (87.5%)	1 (50%)
Ciprofloxacin	15 (75%)	13 (81.25%)	1 (50%)
Levofloxacin	15 (75%)	15 (93.75%)	1 (50%)
Co-trimoxazole	16 (80%)	15 (93.75%)	-
Erythromycin	13 (65%)	11 (68.75%)	2 (100%)
Clindamycin	14 (70%)	13 (81.25%)	1 (50%)
Gentamicin	16 (80%)	14 (87.5%)	2 (100%)
Linezolid	20 (100%)	16 (100%)	2 (100%)
Vancomycin	19 (95%)	16 (100%)	2 (100%)

[Table/Fig-4]: Antibiotic sensitivity pattern of gram-positive isolates (n=38).

Pseudomonas aeruginosa showed 100% sensitivity to polymyxin B, followed by cefepime, piperacillin+tazobactam, imipenem, and aztreonam [Table/Fig-5].

Antibiotics	*Pseudomonas aeruginosa* (n=14)
Gentamicin	6 (42.8%)
Amikacin	7 (50%)
Ciprofloxacin	5 (35.7%)
Levofloxacin	9 (64.2%)
Ceftazidime	10 (71.4%)
Imipenem	12 (85.7%)
Aztreonam	11 (78.5%)
Piperacillin+tazobactam	13 (92.8%)
Cefepime	13 (92.8%)
Polymyxin B	14 (100%)

[Table/Fig-5]: Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* (n=14).

DISCUSSION

Sterile body fluids are typically devoid of microorganisms, whether the individual is immunocompetent or immunocompromised. However, if any microorganism such as bacteria, viruses, fungi, or parasites are isolated from these sites, it is considered pathogenic and can be life-threatening to patients. Since the microorganisms and their susceptibility patterns can vary over time and across different locations, accurate identification of the organism and its antibiotic susceptibility pattern is necessary to initiate appropriate therapy promptly [3].

In the present study, out of the 450 samples processed, 126 (28%) were culture positive. This finding was consistent with studies conducted by Sharma S et al., who reported a 28.8% culture positive growth, Sharma R et al., who reported a 30% growth, and Sujatha R et al., who reported a 31% growth [8-10]. Mohanty S et al., found a growth rate of 15.8%, while Shrestha LB et al., reported a growth rate of 10.68% [11,12]. These variations in growth rates may be attributed to differences in microorganisms over time and across different locations. Among the 126 culture positive isolates,

88 (69.8%) were gram negative bacilli. The predominant pathogens were *E. coli* with 27 (21.4%) isolates, followed by *K. pneumoniae* with 23 (18.2%), *S. aureus* with 20 (15.9%), CONS with 16 (12.7%), and others. This finding was similar to a study conducted by Bajare B et al., where the culture positive rate was 24.34% and the majority of isolates were gram negative bacteria (96.3%) [13].

Among the gram negative isolates, *E. coli* was the most common, followed by *K. pneumoniae*. Among the gram-positive isolates, *S. aureus* was the most common pathogen, followed by CONS. This finding was well-correlated with the studies conducted by Rouf M and Nazir A [14]. Kar M et al., also found in their studies that 31.2% of samples were culture positive, and *E. coli* was the most common culprit among gram negative isolates [15].

Gram negative pathogens were most commonly isolated from peritoneal fluid in 42 (33.3%) cases out of 126, followed by pleural fluid in 23 (18.2%) out of 126 cases. Similarly, gram-positive pathogens were most commonly isolated from synovial fluid in 17 (13.4%) cases, followed by peritoneal fluid in 10 (7.9%) cases. Sharma R et al., found 74 (60.6%) cases out of 122 gram negative pathogens in peritoneal fluid, followed by 21 (17.2%) in pleural fluid. Similarly, the maximum number of gram-positive pathogens in synovial fluid (13, 10.6%) followed by pleural fluid (8, 6.5%) was reported [9]. The current study differs from Sharma R et al., because the percentage of isolation varies due to geographical differences, hospital infection control measures, socio-economic status of the patients, etc., [9].

The present study showed that gram negative pathogens were mostly sensitive to colistin and polymyxin B (100%). Gram negative isolates showed the highest resistance to ampicillin, followed by gentamicin. Similarly, gram-positive isolates showed the highest sensitivity to linezolid, followed by vancomycin, which was consistent with a study conducted by Singh P et al., and the highest resistance was seen against ampicillin and amoxicillin+clavulanic acid [16].

This was a hospital-based study, and therefore, the data may show some variations from other studies. Variations in the antibiotic susceptibility pattern have been noted in studies conducted by various authors. These variations could be attributed to factors such as the population under study, geographical differences, institution-based variations, socio-economic status of the patients, local patterns of antibiotic resistance in the area, antibiotic policies, and hospital infection control measures implemented by healthcare workers.

In a study conducted by Sharma A et al., 120 clinical samples were processed, and out of those, 42 (35%) samples showed growth. Among the 42 growths, 34 (80.9%) were gram negative bacilli, and 8 (19.1%) were *Staphylococcus aureus*. Similarly, Dutta V et al., processed 134 clinical samples, and 50 (37.3%) pathogens were isolated. Among the 50 isolates, 36 (72%) were gram positive cocci, 13 (26%) were gram negative bacilli, and 1 (2%) was *Candida species* [17,18]. These variations in the bacteriological profile and antibiotic susceptibility pattern may reflect the local trends of bacterial prevalence and antibiotic susceptibility patterns in our area. Since this was a hospital-based study, there may be multiple factors at play that should be taken into consideration. However, it is necessary to report the differences between present study and

other researchers' findings, as it may reflect recent shifts in the bacteriological profile and antibiotic susceptibility patterns, although it cannot be generalised.

Body fluids can be infected by both gram positive and gram negative bacteria. Therefore, regular monitoring and surveillance of organisms causing infections in body fluids are required to formulate an infection control policy that can guide clinicians in choosing appropriate antibiotics [19]. It is necessary to increase awareness among patients about the harmful effects of misuse and overuse of antibiotics. Empirical treatment should be encouraged, and there is a need to develop a hospital-based antibiotic policy with special reference to sterile body fluids. This policy will guide treating physicians in providing efficient and prompt treatment, ultimately reducing mortality and morbidity significantly.

Strict antibiotic stewardship programs need to be implemented to prevent the spread of antibiotic resistance.

Limitation(s)

The number of clinical samples and culture-positive isolates was relatively low in the present study. The aetiology of sterile fluid infections also includes anaerobic bacteria, viruses, and fungi, which were not included in the present study.

CONCLUSION(S)

Infections of sterile body fluids are usually life-threatening and associated with a high degree of mortality and morbidity. Therefore, the identification of the organism from these sites as early as possible, along with its antibiotic sensitivity pattern, is essential. Prompt diagnosis and initiation of treatment with appropriate antibiotics will reduce the duration of hospital stay for patients and also decrease the development of drug resistance.

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