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Original Research Article

Aerobic microbiological profile and antibiogram in sterile body fluids from a tertiary care centre in Kashmir Valley

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Abstract

Background: Infections involving sterile bodily fluids, particularly those linked to healthcare settings are substantial and major sources of mortality and morbidity. The identification of species-level bacteria and their antimicrobial resistance profile are crucial factors to consider when choosing the right antimicrobials for both empirical and targeted therapy.
Aim and Objective: The aim of study was to identify the bacterial isolates in the sterile body fluids and study their antimicrobial resistance pattern.
Materials and Methods: A retrospective observational study was carried in the Department of Microbiology of SKIMS Medical College, Bemina, Srinagar, a tertiary care hospital for a period of 1 year. All sterile body fluid samples received in the Microbiology department were cultured aerobically and then identified up to species level using conventional biochemicals in accordance with established microbiological methods. The antimicrobial susceptibility of bacterial pathogens produced in culture was examined using the Kirby-Bauer disk diffusion method, and results were interpreted in accordance with CLSI recommendations.
Results: A total of 650 clinical samples were processed for a period of 1 year; of them 72 showed Positive bacterial growth. 52 (72.2%) were Gram Negative Bacteria and 20 (27.7%) were Gram Positive Bacteria. CSF samples (280) constituted 43.07%, pleural fluid samples (188) 28.92%, peritoneal fluid (114) 17.5%, synovial fluid (56) 8.6% and bile (12)1.84.Escherichia coli was the most prevalent of the 52 Gram-Negative isolates (24.50%), and *Staphylococcus aureus* and CONS (12.5%) were most prevalent in Gram Positives. Most Gram Negatives showed Multi Drug resistance but were fully sensitive to colistin and polymyxin B, In Gram positives, Vancomycin and Linezolid showed 100 sensitivity.
Conclusion: This study identified emerging ESKAPE organisms. The presence of multidrug resistance patterns in bacterial isolates highlights the need for further research in various parts of the country to prevent needless antibiotic use and resistance development.

Keywords: Antimicrobial resistance, Microorganisms, Sterile body fluids, ESKAPE, Antibiotics sensitivity.

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1. Introduction

Body fluids, including peritoneal, pleural, synovial, pericardial, and cerebrospinal fluid, are normally sterile. Sterile bodily fluids are commonly sent for bacterial culture and sensitivity testing. Infection of sterile body fluids with microbes can be life-threatening, causing severe morbidity and fatality Hughes et al.[1] The hospital antibiogram summarizes the antimicrobial susceptibilities of local bacterial isolates. Clinicians use antibiograms to determine local susceptibility rates for empiric antibiotic therapy and track resistance trends over time within an institution. Monitoring predominant bacteria and their antibiotic susceptibility can inform antibiotic policies and improve patient treatment Fridkin SK.[2]

Bacteria that commonly cause infections in sterile body fluids include Escherichia coli, *Klebsiella pneumoniae*, *Acinetobacter spp*., *Pseudomonas aeruginosa*, *Enterobacter spp*., *Staphylococcus aureus*, CONS, and *Enterococcus species*. In underdeveloped countries, infections are more common due to inadequate healthcare, poor hygiene and sanitation, and excessive antibiotic use SHUME T & Ramudhamu.[3,4]

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There is limited evidence available in this domain on the bacteriological profile and antibiotic susceptibility of sterile bodily fluids. Understanding the bacteriological profile and antibiotic susceptibility patterns is essential for microbiologists, physicians, infectious disease specialists, and policymakers to ensure accurate diagnosis and use of antibiotics, reducing morbidity and mortality Harshika YK.[5] So, this study was undertaken to assess the current bacterial profile and susceptibility patterns in body fluids obtained from patients at our tertiary care hospital.

2. Materials and Methods

This retrospective observational study was carried out in a tertiary care hospital in Srinagar from January 2023 to January 2024, in the Department of Microbiology. The study included all sterile body fluids from clinically identified individuals of any age or gender but the study Excluded blood samples, samples other than sterile body fuilds, samples from individuals taking antibiotics within the past two weeks, tainted samples, and samples delayed for more than two hours.

During the time period, the study analyzed 650 body fluid samples from 721 patients, discarding 71 that did not match the inclusion criteria. Of the 650 samples, 470 came from male patients and 180 from female patients.

2.1. Sample processing, Culture & Identification

Samples were processed using standard microbiological techniques after being subjected to Gram stain for a tentative report Colle JG.[6] The culture media used were Blood Agar, Mac-Conkey Agar, and Chocolate Agar (Himedia, India). The inoculation plates were incubated for a whole night at 37ºC. The following day, the bacterial growth on the culture plates was examined. Gram staining, motility testing, colony features, and biochemical analyses were performed on each isolated bacterium. Conventional biochemical assays were utilized to further differentiate the species. A sample was deemed sterile only during a 48-hour incubation period. Appropriate control strains were used for quality control.

2.2. Antibiotic susceptibility testing

Using the Kirby-Bauer disc diffusion method, antimicrobial susceptibility tests were conducted on isolated microorganisms in accordance with CLSI standards clinical.[7] Appropriate control strains were used for quality control. Escherichia coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853). For Gram Negative organisms (GNB), the antibiotic discs used were Trimethoprim/ Sulphamethoxazole [Cotrimoxazole] (1.25/23.75µg), Ceftriaxone (30 µg), Cefotaxime (30µg), Gentamicin (10µg), Amikacin (30µg), Piperacillin/Tazobactum (100/10µg), cefoperazone sulbactam (75µg /30µg),Ciprofloxacin (5µg), Levofloxacin (5µg), Imipenem (10µg), Meropenem (10µg), tigecycline (15mcg), Tobramycin (10µg), and, colistin (10 mcg) were used. For Gram Positive Organisms (GP) Cefoxitin (30µg), Penicillin G (10 units), Clindamycin (2ug), Erythromycin (15ug), Trimethoprim/ Sulfamethoxazole (1.25/23.75ug), Vancomycin (30µg), Linezolid (30µg), Teicoplanin (30µg), tigecycline (15mcg), tetracycline (30µg), Gentamicin (10µg), and high-level Gentamicin (120µg) were used.

3. Results

650 sterile bodily fluid samples that met the inclusion criteria were gathered for processing and antibiotic sensitivity testing over the study period, out of which CSF samples (280) constituted 43.07%, pleural fluid samples (188) 28.92%, peritoneal fluid (114) 17.5%, synovial fluid (56) 8.6% and bile (12)1.84% as shown **Figure 1**.

72 samples (11.07%) showed bacterial growth, the gender wise positivity was 42 (58%) for males and 30 (42%) for females. Out of these 52 (72.2%) were Gram Negative Bacteria and 20 (27.7%) were Gram Positive Bacteria.(**Figure 2**)

Out of 280 CSF samples, 188 Pleural fluid samples, 114 Peritonial fluid samples 56 Synovial fluid samples and 12 Bile samples, Bacterial growth was seen in 20 (7.14%), 17 (9.04%), 20 (17.5%), 9 (16.07%) and 6 (50%) respectively. (**Table 1**)

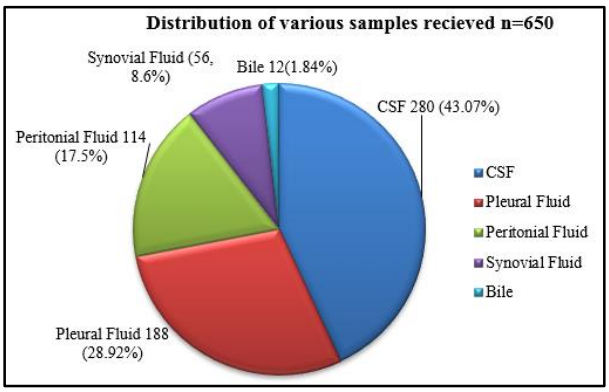

Figure 1: Distribution of various samples received.

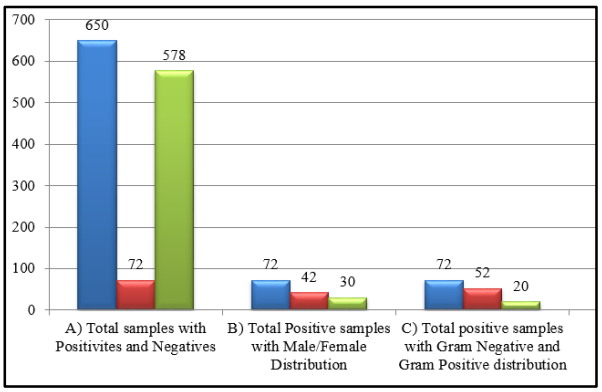

Figure 2: Distribution of various samples

A. Total samples vs Total Positives vs Total Negatives (N=650)
B. Total Positives vs Males vs Females (N= 72)
C. Total Positives vs Gram Negatives vs Gram Positives (N=72)

In this study we found that, the most prevalent organism among Gram Negative bacteria were *E. coli* (24.50%) followed by followed b *Pseudomonas sp, Acinetobacter sp*, *Klebsiella sp* etc. respectively whereas the most prevalent Gram Positive organisms were *Staphylococcus aureus* (12.5%) followed by Cogulase Negative Staphylococci (CONS), *Enterococcus sp* etc. The Isolation pattern of various organisms is shown in the **Figure 3** and the Bacteriological profile from different sterile body fluids samples in terms of Percentage is shown in **Figure 4**.

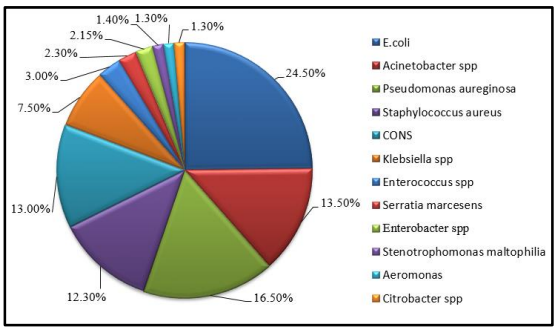

Figure 3: Percentage of various organisms isolated

The antibiotic sensitivity pattern obtained for the different organisms is shown in **Table 2** and **Table 3**. The Gram negative organisms showed multi-drug resistant pattern but were 100% sensitivity to Colistin and Polymyxin B. This was followed by Carbapenams, Gentamycin and Amikacin with near sensitivity of 60%, 50% and 50% each respectively. Among the Gram positives, 100% sensitivity was seen in case of Vancomycin and Linezolid followed by Teicoplanin, tigicycline and Cotrimoxazole (50-60%) each. In our study we found that 44 percent of *Staphylococcus aureus* and CONS were Methicillin Resistant.

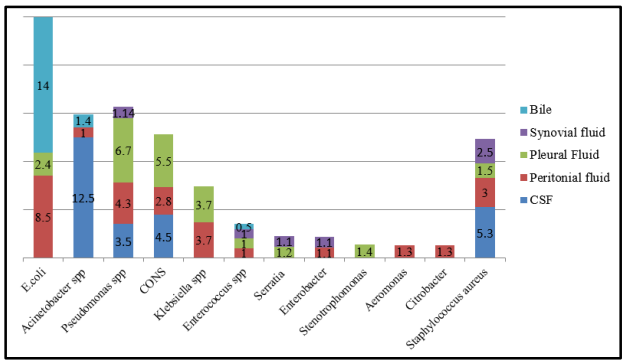

Figure 4: Bacteriological profile from different sterile body fluids sample in terms of percentage

Table 1: Distribution of growth pattern across various samples

Samples	Total No Received	Positive for Growth	Negative for Growth	Positive percentage
CSF	280	20	260	7.14%
Pleural fluid	188	17	171	9.04%
Peritonial fluid	114	20	94	17.5%
Synovial fluid	56	9	47	16.07%
Bile samples	12	6	6	50%
Total	650	72	578	

Table 2: Antimicrobial susceptibility pattern of some important gram-negative bacterial isolates

Antibiotics	***E. coli* (n=17)**	***Klebsiella pneumoniae* (n=5)**	***Acinetobacter species* (n=8)**	***Pseudomonas aeruginosa* (n=12)**	***Enterobacter species* (n=3)**	***Citrobacter species* (n=1)**
Ampicillin	6(35%)	0	3(38%)	-	1(33%)	0
Amikacin	11(63%)	3(60%)	4(50%)	5(41%)	2(66%)	1(100%)
Ceftriaxone	6(35%)	1(20%)	3(38%)	-	1(33%)	0
Co-trimoxazole	10(58%)	3(60%)	3(38%)	-	2(66%)	1(100%)
Cefotaxime	7(40%)	2(40%)	2(25%)	-	1(33%)	0
colistin	17(100%)	5(100%)	8(100%)	12(100%)	3(100%)	1(100%)
ciprofloxacin	4(24%)	1(20%)	2(25%)	4(33%)	1(33%)	0
Gentamycin	10(58%)	3(60%)	3(38%)	5(41%)	2(66%)	1(100%)
Imipenem	12(70%)	3(60%)	3(38%)	6(50%)	2(66%)	1(100%)
Meropenam	1 (70%)	3(60%)	3(38%)	6(50%)	2(66%)	1(100%)
Polymyxin B	17(100%)	5(100%)	8(100%)	12(100%)	3(100%)	1(100%)
Piperacillin+tazobactam	3(20%)	1(20%)	2(25%)	10(83%)	2(66%)	1(100%)

Table 3: Antimicrobial susceptibility pattern of gram-Positive bacterial isolates

Antibiotics	***Staphylococcus aureus* (n=9)**	**CONS (n=9)**	***Enterococcus spp* (n=2)**
Ampicillin	5(56%)	5(56%)	0
Amoxiciilin + Clavulinic acid	6(66%)	7(77%)	1(50%)
Penicillin G	0	2(22%)	0
Co-trimoxazole	3(33%)	6(66%)	-
Clindamycin	5(56%)	2(22%)	1(50%)
Levofloxacin	2(22%)	3(33%)	0
ciprofloxacin	1((11%)	2(22%)	1(50%)
Gentamycin	3(33%)	2(22%)	2(100%)
Linezolid	9(100%)	9(100%)	2(100%)
Vancomycin	9(100%)	9(100%)	2(100%)
Tigicycline	5(56%)	6(66%)	1(50%)
Daptomycin	4(44%)	1(20%)	1(50%)
Teicoplanin	5(56%)	5(56%)	2(100%)
Erythomycin	6(66%)	6(66%)	2(100%)
Cefoxitin	6(66%)	6(66%)	0

4. Discussion

Systemic sickness may arise from microbial invasion of typically sterile regions of the body. This is a potentially fatal emergency, therefore any delay in receiving care could be fatal. Furthermore, any bacteria discovered in an absence of resident microbiota must be taken seriously Vetter E.[8] More recently, as resistant germs proliferate, the efficacy of currently available antibiotics is declining globally. Consequently, infections brought on by resistance drugs are challenging and eventually untreatable Chokshi A.[9] Accurately identifying the organism and its antibiotic susceptibility pattern is essential to starting the right therapy as soon as possible because microorganisms and their susceptibility patterns can change over time and between various places Harshika.[5]

The rate of culture positive in our study was 11.07%. The results of Rouf et al.[10] and Shrestha LB et al.,[11] which reported isolation rates of 10.81% and 10.68%, respectively, are consistent with this. Lower culture positive frequencies of roughly 14.4% were also observed in other research, such as Deb A et al.[12] These little fluctuations in growth rates could be explained by variations in microorganisms over time and in various environments. In our study 52 (72.2%) of the 72 culture-positive isolates were Gram-Negative bacilli. Gram Positives were led by S. aureus and CONS (12.5%), while *E. coli* (24.50%) isolates were the most common pathogen among Gram Negatives. This result of Gram Negative organisms predominating the load was consistent with research by Sandhya Ema et al[13] (71%) and Urvashi et al[14] (77%). This, however, is not the case with the study by Sharma et al.,[15] where the primary isolate was *Acinetobacter spp*. Our study showed that most of the Possitive samples came from from Bile, followed by Peritonial fluid and synovial fluid, similar results were shown by Rouf et al[10] and Tiwari S et al[16] which showed near similar results. These results however differ from the study conducted by Sharma R et al.'s because of factors such patient socioeconomic level, hospital infection control protocols, and geographic variations in the percentage of isolation. Sharma et al.[15]

Colistin and polymyxin B proved to be the most efficient antibiotic in our investigation against Gram-negative pathogens, with carbapenems, amikacin, and gentamicin following closely behind.

These results are consistent with those of Harshika et al,[5] Sharma et al[15] and Tullu MS et al[17] who similarly reported 100% sensitivity to colistin and polymyxin B . Ampicillin resistance was greatest in gram-negative isolates, and it was followed by gentamicin resistance. In gram-positive isolates also had the maximum sensitivity to linezolid, and vancomycin. Our results matched with the studies conducted by Singh P et al,[18] Rouf et al[10] and Joshi S et al.[19]

5. Conclusion

Our work clearly shows that resistance is generally on the rise in both gram-positive and gram-negative isolates, which calls for ongoing surveillance investigations. When patients are treated with antibiotics judiciously and hospital infection control protocols are strictly followed, patient morbidity and fatality rates can significantly decrease. Consequently, it is crucial to identify the organism from these places as soon as feasible and to determine its pattern of antibiotic sensitivity. Patients' hospital stays will be shorter when they receive prompt diagnosis and treatment with the right antibiotics, which will also slow the emergence of drug resistance.

6. Conflict of Interest

Authors have declared that no competing interests exist.

7. Source of Funding

None.

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