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Antimicrobial resistance pattern and species distribution profile of aerobic bacteria causing sterile body fluid infection from a tertiary care hospital

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ABSTRACT

Objectives: Body fluids such as pleural fluid, ascitic fluid, and cerebrospinal fluid are sterile but they can get infected by different microorganisms leading to life-threatening infections. We conducted this study to evaluate the distribution profile of various aerobic bacteria and their antimicrobial resistance pattern isolated from sterile body fluids from the patients attending a tertiary care hospital in North India.

Materials and Methods: This hospital-based cross-sectional observational study was conducted in the Department of Microbiology of a tertiary care hospital. A total of 495 sterile body fluid samples were processed for species-level identification of aerobic bacteria by conventional methods following standard laboratory procedures. The antimicrobial susceptibility testing was performed by Kirby–Bauer disc diffusion method according to clinical and laboratory standards institute guidelines 2020.

Results: Out of a total of 495 sterile body fluid samples, 48 were found positive on culture for bacterial pathogens. The predominant organisms isolated from sterile body fluids were *Acinetobacter* species (35%), followed by *Klebsiella* species (23%), *Escherichia coli* (13%), *Pseudomonas* species (12%), *Staphylococcus aureus* (13%), and *Streptococcus* species (4%). All of the Gram-negative isolates were 100% sensitive to Colistin followed by amikacin (67.50%) and gentamicin (60%). The Gram-positive isolates were 100% sensitive to vancomycin and linezolid followed by gentamicin (50%).

Conclusion: Infections of sterile body fluids can lead to high morbidity and mortality. The knowledge of the bacteriological and antimicrobial resistance profile of bacteria causing sterile body fluid infections is vital in determining appropriate antimicrobial therapy.

Keywords: Antimicrobial resistance, Extended-spectrum beta-lactamase, Multidrug resistant organisms, Methicillin-resistant *Staphylococcus aureus*, Sterile body fluid infection

INTRODUCTION

The body fluids present in different body cavities are sterile. These include cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, synovial fluid, and pericardial fluid.[1]

However, during any infection of the central nervous system, peritoneum, joints or any other sterile sites, different types of bacteria, fungi, viruses, and parasites invade and change the physicochemical nature of these body fluids and, therefore, alter their ability to perform their normal functions.[2] Often these infections have high morbidity and mortality; thus, a

timely diagnosis and immediate treatment is crucial.[1] Early identification of these organisms with antimicrobial susceptibility is necessary for the proper management of these infections. This can help clinicians initiate early and targeted antimicrobial therapy, which will reduce the hospital length of stay and reduce the risk of adverse effects.[2]

Data on antimicrobial susceptibility testing of the organisms isolated from sterile body fluids over a period of time can be used to create a local antibiogram. The knowledge of common causative organisms in various sterile body sites and their antimicrobial susceptibility pattern can help in starting appropriate empirical antibiotics.[3] The culture positivity rate in sterile fluids is comparatively low varying from 10% to 30%. Moreover, the patients are empirically treated with some antibiotics before sample collection, which hinders bacterial growth. However, the emergence of multidrug-resistant (MDR) organisms in sterile body fluids is also becoming a challenge for clinicians and there is an urgent need for judicious use of antibiotics which can significantly reduce the morbidity, length of hospital stay, and mortality among patients with such infections.[3-5]

The positive cultures are expected to be low due to less number of pathogens as well as prior administration of empirical antibiotics especially in intensive care units.[1] Recently, the World Health Organization and European Commission have recognized the importance of studying the emergence and the determinants of antibiotic resistance and the need for strategies for its control.[6] The reports from Centers for Disease Control and Prevention suggested that strains are developing resistance to many antibiotics. The tendency of increasing antibiotic resistance in today's scenario needs careful prescription and rational use of antibiotics based on antibiotic sensitivity testing results.[7]

There are only few studies available on sterile body fluid infections from this geographical region of North India including the studies by Rouf *et al*. and Sharma *et al*.[3,4] We planned this study to observe the frequency of occurrence of sterile body fluid infections, along with the species distribution and antimicrobial resistance pattern of the aerobic bacteria causing these infections.

MATERIALS AND METHODS

This cross-sectional study was conducted in a tertiary care hospital for a period of one year from January 2020 to December 2020. The clinical sterile body fluid specimens from patients were collected in accordance with the inclusion and exclusion criteria. Patients were included in the study if (1) they had all sterile body fluids collected, (2) are of any age, and (3) are of any gender. Patients were excluded if they had (1) a history of antibiotic therapy before sample collection, (2) samples other than sterile body fluids collected, and (3) pathogens other than aerobic bacteria grown on culture, and had blood samples collected.

Sample processing

Standard microbiological techniques were used for processing the clinical samples including:

a) Direct microscopy of the Gram-stained smears from the specimens
b) Culture on blood agar, chocolate agar, and MacConkey agar plates for aerobic bacterial isolation
c) Infusion of the clinical specimens into brain-heart-infusion (BHI) broth for enrichment. The BHI broth and inoculated agar plates were incubated at 37°C aerobically. The culture plates and BHI broth were examined for the presence of any growth after 24 h and then again after 48 h. Standard biochemical testing and microbiological techniques were used to identify the bacterial pathogens cultivated on culture media.

Antimicrobial susceptibility testing (AST)

The AST was performed for isolated organisms by the Kirby–Bauer disc diffusion method and interpretation was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2020.[8] The minimum inhibitory concentration based methods were used for AST of vancomycin and colistin. The antibiotic panels and disk content of antibiotics used for Gram-positive cocci (GPC) and Gram-negative bacilli (GNB) according to CLSI guidelines 2020.[8]

RESULTS

A total of 495 sterile body fluid samples received for culture during the study period were processed out of which 48 (9.69%) were found positive. The sample-wise distribution of culture positivity is shown in Figure 1.

The sterile body fluid culture positivity rate was highest for ages between 31 and 40, followed by 41–50, and 51–60 years [Table 1]. The culture positivity rate of sterile body fluids was higher in intensive care unit (ICU) patients (58%) as compared to patients from other wards (42%). The department-wise distribution of culture positivity of sterile body fluids is shown in Table 2.

There was predominance of GNB (83%%) over GPC (17%) in the sterile body fluids. The most commonly isolated GPC was *Staphylococcus aureus* (75.00%) followed by *Streptococcus* species (25.00%). Among all *S. aureus* isolates, 66.66% were methicillin resistant *S. aureus* (MRSA) while 33.34% were methicillin sensitive *S. aureus* (MSSA). Among all GNB isolates, *Acinetobacter* was predominant followed by *Klebsiella, Escherichia coli,* and *Pseudomonas*. The proportion

of various bacteria isolated from sterile body fluids is shown in Table 3.

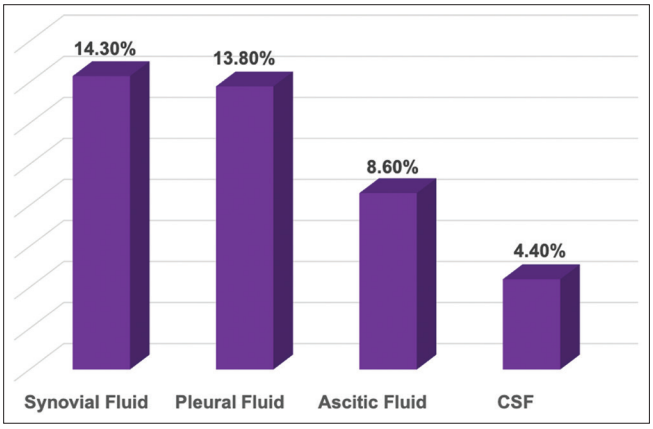

Figure 1: Sample-wise culture positivity of sterile body fluid. CSF: Cerebrospinal fluid

Table 1: Age-wise distribution of samples.

Age Group	Total samples (*n*=495)	Culture positive samples (*n*=48)	Culture positivity rate (%)
<10 Years	91	6	6.59
11–20 Years	49	3	6.12
21–30 Years	54	5	9.25
31–40 Years	54	12	22.22
41–50 Years	63	9	14.28
51–60 Years	80	8	10
61–70 Years	66	1	1.51
>70 Years	38	3	7.89

Table 2: Department-wise distribution of culture-positive samples.

Department	Number of samples	Percentage
Medicine	17	35
Respiratory medicine	12	25
Surgery	10	21
Pediatrics	5	10
Orthopedics	2	4
Emergency	2	4

Table 3: Distribution of all bacterial isolates.

Name of isolate	Number of isolate	Percentage
Acinetobacter	17	35
Klebsiella	11	24
Escherichia coli	6	12
Pseudomonas	6	12
Staphylococcus aureus	6	12
Streptococcus	2	5

The AST results showed majority of *S. aureus* isolates resistant against penicillin, cefoxitin, cotrimoxazole, and moxifloxacin comprising 66.67%. The AST patterns of GPC (*S. aureus*) and GNB isolates are shown in Tables 4 and 5, respectively.

The results of screening testing for extended spectrum beta-lactamase (ESBL) and carbapenemase producing GNBs are shown in Table 6.

DISCUSSION

Sterile body fluid infections are an important cause of serious morbidity and mortality and are considered among the most common health-care-associated infections. The condition can be life-threatening in critically ill patients. In the present study, 495 samples of various sterile body fluids were processed and analyzed by culture and AST. The sterile body fluid culture positivity rate in our hospital was 9.69%. There are variations in the culture positivity rates of sterile body fluids as documented in literature from various studies. Similar positivity rates were also reported in various other studies from same region, including the studies conducted by Rouf and Nazir in 2019 and Sharma *et al.* in 2018 from North India. They reported a culture positivity rate of 10% and 15%, respectively.[3,4] A higher positivity rate of 16.70% was observed in a study conducted by Shume *et al.* from Eastern Ethiopia.[7] The aerobic bacterial isolates from sterile body fluids were predominantly isolated from male patients (57.37%) as compared to female patients (42.63%). Similar findings were reported in a study conducted by Teklehymanot *et al.* from Ethiopia comprising 40.70% male and 59.30% female.[9] The predominance in males over females was also reported by Sharma *et al.* from North India.[4] The age-wise distribution of patients with culture positive sterile body fluids revealed that maximum number of isolates were seen in ages between 31 and 40 years, followed by ages between 41–50, and 51–60 years.

Table 4: Sensitivity pattern of *Staphylococcus aureus* (*n*=6).

Antibiotics	Sensitive isolates; Number (%)
Penicillin	2 (33.3)
Erythromycin	4 (66.7)
Clindamycin	3 (50)
Cotrimoxazole	2 (33.3)
Tetracycline	4 (66.7)
Ciprofloxacin	4 (66.7%)
Moxifloxacin	2 (33.3)
Chloramphenicol	3 (50)
Gentamicin	4 (66.7)
Linezolid	6 (100)
Vancomycin	6 (100)
Cefoxitin	2 (33.3)

Table 5: Sensitivity pattern of Gram-negative bacilli (*n*=40).

Antibiotics	*Acinetobacter* (*n*=17); Number (%)	*Klebsiella* (*n*=11); Number (%)	*Escherichia coli* (*n*=6); Number (%)	*Pseudomonas* (*n*=6); Number (%)
Ampicillin	3 (17.65)	3 (27.27)	4 (66.67)	-
Piperacillin	3 (17.65)	4 (36.36)	4 (66.67)	4 (66.67)
Amoxicillin-clavulanic acid	5 (29.41)	5 (45.45)	3 (50)	-
Ampicillin-sulbactum	9 (52.94)	2 (18.18)	4 (66.67)	-
Piperacillin-tazobactum	4 (23.53)	7 (63.63)	4 (66.67)	4 (66.67)
Tetracycline	5 (29.41)	6 (54.54)	2 (33.33)	-
Cotrimoxazole	7 (41.18)	2 (18.18)	1 (16.67)	-
Ciprofloxacin	8 (47.06)	2 (18.18)	3 (50)	4 (66.67)
Cefixime	2 (11.76)	3 (27.27)	3 (50)	-
Ceftazidime	0	3 (27.27)	3 (50)	4 (66.67)
Ceftriaxone	0	3 (27.27)	3 (50)	-
Aztreonam	0	4 (36.36)	4 (66.67)	4 (66.67)
Cefepime	1 (5.88)	1 (9.09)	1 (16.67)	4 (66.67)
Gentamicin	10 (58.82)	3 (27.27)	5 (83.33)	4 (66.67)
Amikacin	12 (70.59)	4 (36.36)	5 (83.33)	4 (66.67)
Tobramycin	14 (82.35)	4 (36.36)	5 (83.33)	4 (66.67)
Ertapenem	2 (11.76)	3 (27.27)	4 (66.67)	-
Meropenem	2 (11.76)	3 (27.27)	4 (66.67)	4 (66.67)
Imipenem	3 (17.65)	4 (36.36)	4 (66.67)	4 (66.67)
Colistin	17 (100)	11 (100)	6 (100)	6 (100)

Table 6: Distribution of ESBL and carbapenemase producing GNBs.

Organism	ESBL Screening		Carbapenemase Screening	
	Positive; Number (%)	Negative; Number (%)	Positive; Number (%)	Negative; Number (%)
Acinetobacter species (*n*=17)	-	-	14 (82)	3 (18)
Klebsiella pneumoniae (*n*=11)	8 (73)	3 (27)	8 (73)	3 (27)
Escherichia coli (*n*=6)	2 (33)	4 (67)	2 (33)	4 (67)
Pseudomonas species (*n*=6)	-	-	2 (33)	4 (67)

ESBL: Extended-spectrum beta-lactamase, GNB: Gram-negative bacilli

The differences between various age groups may be due to various factors including immune status of the patients and associated factors. We observed more cases in ICU patients comprising 28 (58.33%) as compared to patients from other wards comprising 20 (41.67%). These findings highlight the factors associated with ICU stay including patient-related comorbidities.

In our study, there was predominance of GNBs (83%) as compared to GPCs (17%). Similar findings were also reported in other studies including the studies conducted by Sharma *et al.* in 2018 from North India, Shume *et al.* in 2022 from Eastern Ethiopia, and Sandhya *et al.* in 2019 from India and they reported 90%, 70.6%, and 71% of GNBs, respectively, as compared to GPCs.[4,7,10] There is huge variation in the distribution of GNBs and GPCs as reported in various studies. There are studies in which either no predominance or predominance of GPCs over GNBs was reported such as a study by Bourbeau *et al.* from United States of America reported no predominance, while studies by Vishalakshi *et al.* and Pal *et al.* from India reported the predominance of GPCs over GNBs.[11-13]

Acinetobacter species (42.5%) were found to be most predominant, followed by *Klebsiella pneumoniae* (26.5%), *E. coli* (15%), and *Pseudomonas* species (15%) in GNB isolates. There are variations between the findings reported in various studies including the data reported by Madigubba *et al.* from South India comprising *E. coli* (40.10%), *Acinetobacter* species (22.60%), *Pseudomonas* species (18.20%), and *K. pneumoniae* (14.80%).[14] *S. aureus* was the most frequently isolated (75%) followed by *Streptococcus* species (25%) in GPCs. Antibiogram of all the bacterial isolates indicates that GNBs exhibited a higher level of antimicrobial resistance toward various antibacterial agents. All the *S. aureus* isolates were found 100% sensitive to vancomycin and linezolid. All of the GNB isolates were found sensitive to colistin.

Most of the *S. aureus* isolates were methicillin resistant (66.67%) as well as resistant to penicillin, cefoxitin, cotrimoxazole, and moxifloxacin comprising 66.67%. The increasing prevalence of MRSA infection is a serious problem in patient management as therapeutic options are limited for such resistant strains. Vancomycin is a drug of choice for the treatment of MRSA infection. However, tolerance to vancomycin in MRSA isolates is on the rise. The antibiotics such as linezolid and vancomycin are the only alternative therapeutic agents for these MDR pathogens. Linezolid is one of the few therapeutic options shown to be effective against MDR Staphylococcal infections and is available both as oral and parenteral formulations. If resistance develops to these drugs, we are left with no therapeutic options. Due to the ease of oral administration, linezolid has been misused in clinical practice. Thus, judicious use of these reserve antibiotics and strict implementation of infection control measures are the only ways to prevent spread and reduce emergence of resistance. None of the isolates was found resistant to colistin in our study which is similar to the resistance pattern reported by Tullu *et al*. from South India.[15] Colistin and tigecycline are the last resort of drugs remaining for the treatment of MDR pathogens. Among all GNBs, 73% of *K. pneumoniae* isolates were ESBL producers while 82% of *Acinetobacter* isolates and 73% of *K. pnemoniae* isolates were carbapenemase producers. The most of the studies conducted on this subject highlighted the higher rates of isolation of MDR pathogens from sterile body fluid infections globally including the studies conducted by Shume *et al*. in 2022 from Eastern Ethiopia, Ebrahim *et al*. in 2020, Tsegay *et al*. in 2019 from Northern Ethiopia, and Li *et al*. in 2022 from China.[7,16-18] These MDR pathogens frequently lead to therapy failure and higher chances of morbidity and mortality especially among critical patients admitted to ICUs with various comorbidities.

Limitations of the study

We did not perform molecular characterization of resistance genes associated with antimicrobial resistance as well as the confirmation of bacterial identification by genetic methods due to limited resources.

CONCLUSION

The majority of the bacteria implicated in sterile body fluid infections are MDR and this is the time to stress on antimicrobial stewardship. The antibiogram and identification profile of bacteria-based antibiotic treatment guidelines as well as the antibiotic policy and antibiotic restriction policy of a particular hospital should be strictly followed to prevent the emergence of antimicrobial resistance.

Ethical approval

The research/study approved by the University Ethics Committee (Medical), Swami Vivekanand Subharti University, Meerut, number SMC/UECM/2019/56/68, dated December 26, 2019.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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