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Original Article

Sterile body fluids infections: Profile of bacteria and their antimicrobial resistance pattern in a tertiary care hospital from Uttar Pradesh

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ABSTRACT

Objectives: Infections of sterile body fluids are important and significant causes of mortality and morbidity, especially healthcare-associated infections. Species-level identification and antimicrobial resistance profile of bacteria are important determinants while selecting appropriate antimicrobials for empirical and targeted therapy. We conducted this study to observe the distribution of various bacteria and their antimicrobial resistance profile isolated from sterile body fluids.

Materials and Methods: We conducted this study in a tertiary care teaching hospital from western Uttar Pradesh for a period of 2 years. All sterile body fluid samples were processed by conventional aerobic bacterial culture followed by their identification up to species level by conventional biochemicals following standard microbiological procedures. The antimicrobial susceptibility of the bacterial pathogens grown in culture was tested by Kirby–Bauer disk diffusion method and interpretation of susceptibility testing was done according to CLSI guidelines 2020.

Results: A total of 1980 sterile body fluid samples were collected during the study period and 192 samples were found positive on culture for bacterial pathogens. Gram-negative bacilli (GNB) were predominantly isolated, comprising 83.33% in comparison to 16.67 % of Gram-positive cocci. Among *Staphylococcus aureus* isolates, 75% were methicillin-resistant *S. aureus*. All *S. aureus* isolates were sensitive against vancomycin and linezolid. Among GNB, 25% were extended-spectrum beta-lactamase producers while 62.5% were carbapenemase producers. All GNBs were sensitive to colistin.

Conclusion: From this study, we concluded that the pathogenic bacteria implicated in infections of sterile body fluids are predominantly multidrug-resistant. There is a huge variation in data on the distribution of bacterial species isolated from sterile body fluids and their antimicrobial resistance patterns from different geographical locations and healthcare settings. Thus, data from a particular healthcare setting are important for empirical treatment in that healthcare setting.

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

INTRODUCTION

The various body cavities are sterile sites and these body cavities are filled with sterile body fluids. The purpose of these body fluids is to bathe the organs and membranes for the protection of vital organs, transportation of nutrients, regulation of body temperature, and reducing friction.[1,2] The body fluids are generally sterile; however, different types of microorganisms can invade the body cavities leading to infection and physicochemical changes in these body fluids. Early identification of the organisms causing sterile body fluid infection along with antimicrobial susceptibility can help clinicians to initiate early and targeted antimicrobial therapy.[3] The data on bacterial profile and their antimicrobial susceptibility pattern isolated from sterile body fluids can help to develop a local antibiogram of a particular healthcare setting. The knowledge of commonly isolated organisms from various sterile body fluids along with their antimicrobial susceptibility testing (AST) pattern in the form of antibiogram can help in the selection of appropriate empirical antibiotics.[4]

The culture positivity rate in sterile body fluid infections is comparatively low varying from 10% to 30%.[4] Moreover, the patients empirically treated with antibiotics before sample collection hinder the recovery of pathogenic bacteria on culture. However, multidrug-resistant organisms' (MDROs) emergence is also becoming an important challenge for treating clinicians and there is an urgent need for judicious use of antibiotics which can significantly reduce morbidity,

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hospital stay, and mortality, ultimately leading to reduced cost of treatment among patients with these infections.[5]

Rapidly increasing multidrug resistance (MDR) against commonly used antimicrobials is an important public health issue of concern worldwide. The distribution profile of bacteria isolated from sterile body fluids and their drug resistance patterns needs to be collected from various tertiary care institutions located in various regions which can further guide us in the implementation of antimicrobial stewardship program nationally.

Therefore, we planned this study to know the distribution profile of various bacterial isolates implicated in sterile body fluid infections, along with their AST patterns which can help us while selecting appropriate empirical antimicrobial therapy at our institution for better patient care.

MATERIALS AND METHODS

This prospective hospital-based study was done at a tertiary care medical teaching hospital in western Uttar Pradesh, for a period of 2 years. The clinical sterile body fluid specimens from patients were collected following exclusion and inclusion criteria.

Inclusion criteria

The following criteria were included in the study:
1. All sterile body fluid samples
2. Patients of all age groups
3. Patients of all genders.

Exclusion criteria

The following criteria were excluded from the study:
1. Patients with a prior history of antibiotic therapy before sample collection
2. Samples other than sterile body fluids
3. Blood samples were excluded from the study.

Sample processing

Collected clinical samples of various sterile body fluids were processed by standard microbiological procedures including:

a) Direct microscopy of Gram-stained smear from the specimens

b) Culture: Samples were subjected to aerobic bacterial culture by inoculating onto chocolate agar, blood agar, and MacConkey agar plates. The clinical specimens were also subjected to enrichment by inoculating into brain heart infusion (BHI) broth. The inoculated agar plates and broth were placed in an aerobic incubator at 37°C for 48 h. Culture plates and BHI broth were examined initially after 24 h and finally after 48 h for the appearance of any growth. The bacterial pathogens grown in culture media were identified using standard microbiological procedures and conventional biochemical tests.

c) AST: The isolated bacterial pathogens were subjected to AST performed by Kirby–Bauer disk diffusion method and interpretation as per CLSI 2020 guidelines.[6] The panel of antibiotics along with their disk content for Gram-positive cocci (GPC) and Gram-negative bacilli (GNB) is depicted in [Tables 1 and 2], respectively.

Table 1: Antibiotics tested against Gram-positive cocci.

Antibiotic	Strength
Penicillin-G	10 µg
Ampicillin	10 µg
Cefoxitin	30 µg
Cotrimoxazole	1.25/232.75 µg
Tetracycline	30 µg
Erythromycin	15 µg
Clindamycin	2 µg
Moxifloxacin	5 µg
Chloramphenicol	30 µg
Gentamicin	10 µg
Ofloxacin	5 µg
Doxycycline	30 µg
Teicoplanin	30 µg
Linezolid	30 µg
Vancomycin	30 µg
High-level gentamicin	120 µg
High-level streptomycin	300 µg

Table 2: Antibiotics tested against Gram-negative bacilli.

Antibiotics	Strength
Ampicillin	10 µg
Piperacillin	100 µg
Piperacillin-Tazobactam	100/10 µg
Amoxicillin-Clavulanic acid	20/10 µg
Ampicillin-Sulbactam	10/10 µg
Cotrimoxazole	1.25/232.75 µg
Tetracycline	30 µg
Chloramphenicol	30 µg
Gentamicin	10 µg
Ciprofloxacin	5 µg
Cefixime	5 µg
Ceftazidime	30 µg
Ceftriaxone	30 µg
Aztreonam	30 µg
Cefepime	30 µg
Amikacin	30 µg
Tobramycin	10 µg
Ertapenem	10 µg
Meropenem	10 µg
Imipenem	30 µg
Colistin	10 µg
Cefotaxime-clavulanic acid	30/10 µg
Cefotaxime	30 µg
Ceftazidime-clavulanic acid	30/10 µg

AST of colistin against GNB was performed using Minimum inhibitory concentration (MIC) by broth microdilution and AST of vancomycin against Staphylococci was performed using MIC by E test.

Among GNB, extended-spectrum beta-lactamase (ESBL) was detected by phenotypic methods using cephalosporin/clavulanate combination discs (cefotaxime 30 µg and ceftazidime 30 µg with and without clavulanate 10 µg). The modified Hodge test was used for phenotypic detection of carbapenemase production.

RESULTS

During the 2 years of the study period, 1980 sterile body fluid samples collected for culture and AST were processed. The pathogenic bacteria grown in 192 samples and culture positivity rate were found to be 9.7%. The culture positivity rate of various sterile body fluid samples is shown in [Figure 1]. The maximum culture positivity rate of 14.28% was observed in synovial fluid samples.

The culture positivity among males and females was found to be 57.37% and 42.63%, respectively. Age-wise distribution of patients with culture-positive sterile body fluids along with culture positivity rate is shown in [Table 3]. Department-wise distribution of the isolates is depicted in [Figure 2].

The distribution of various pathogenic bacterial isolates isolated from positive sterile body fluids cultures is shown in [Figure 3]. Among all bacterial isolates, GPC was 32 (16.67%) while GNB was 160 (83.33%).

Table 3: Distribution of total samples, culture-positive samples, and culture positivity according to age of the patients.

Age group	Total samples (*n*=1980)	Culture-positive samples (*n*=192)	Culture positivity (%)
<10 years	364	24	6.59
11–20 years	196	12	6.12
21–30 years	216	20	9.26
31–40 years	216	48	22.22
41–50 years	252	36	14.29
51–60 years	320	32	10
61–70 years	264	8	3.03
>70 years	152	12	7.89

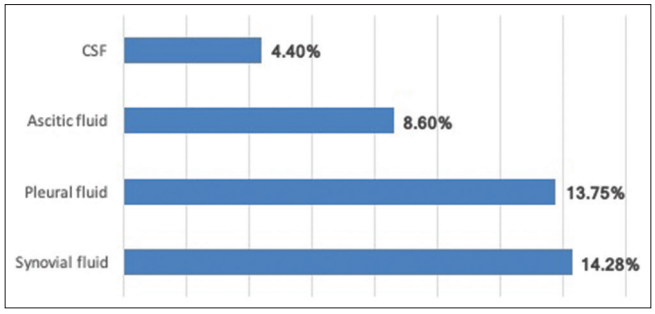

Figure 1: Positivity rate of various sterile body fluid samples.

AST pattern of GPC

The AST pattern of *Staphylococcus aureus* is depicted in [Table 4]. Among all 24 isolates of *S. aureus,* 16 (75%) were methicillin-resistant *S. aureus* (MRSA) in comparison to 8 (25 %) of methicillin-sensitive *S. aureus* (MSSA). All of the *S. aureus* isolates were found susceptible against vancomycin and linezolid.

AST results and distribution of various GNB along with ESBL enzyme producer as well as carbapenemase enzyme producer screening results are shown in [Tables 5 and 6].

DISCUSSION

Sterile body fluid infections contribute as important and significant causes of serious morbidity and mortality. These

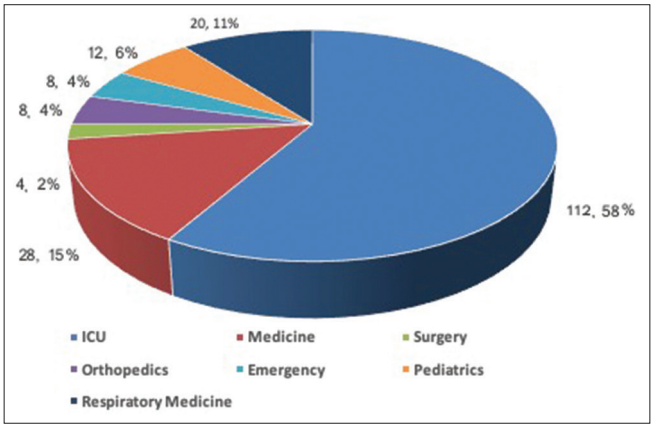

Figure 2: Department-wise distribution of isolates.

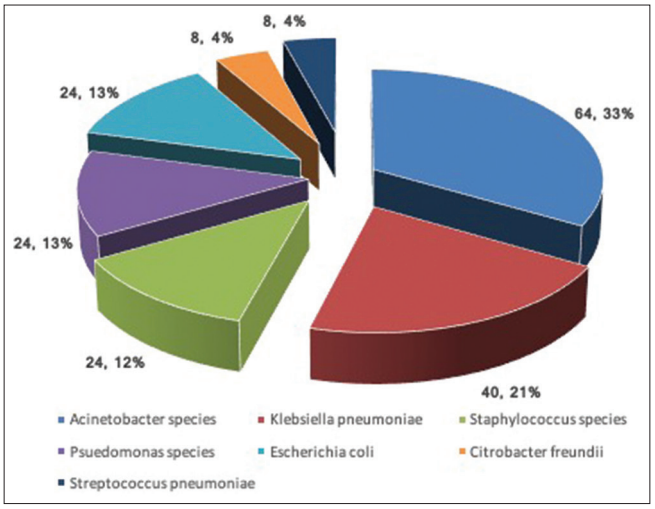

Figure 3: Distribution of all bacterial isolates isolated from sterile body fluids.

infections are among the common healthcare-associated infections (HCAIs). The condition can be life-threatening among patients in critical conditions especially those admitted to intensive care and high-dependency units of the hospitals.

The culture positivity rate of sterile body fluids was found to be 9.69% in our study. Highly variable culture positivity rates were observed in various studies ranging from less than 10% to more than 50%. A higher positivity rate of 30% was observed by Sharma *et al*. in a study done in 2017 from north India[7] and 16.70% of positivity rate was reported by Shume *et al*. in 2022.[8] Around 15% of the culture positivity rate was reported by Sharma *et al*. in 2017, Sujatha *et al*. in 2015, and Vishalakshi *et al*. in 2016.[5,9,10] The culture positivity depends on so many factors related to clinicians, sample collection personnel, microbiologists, and factors related to the processing of the samples in the laboratory.

Table 4: AST pattern of *Staphylococcus aureus* (*n*=24).

Antibiotic	Sensitivity of MRSA (*n*=16) (%)	Sensitivity of MSSA (*n*=8) (%)
Penicillin	0 (0)	8 (100)
Erythromycin	8 (50)	8 (100)
Clindamycin	8 (50)	4 (50)
Cotrimoxazole	4 (25)	4 (50)
Tetracycline	8 (50)	8 (100)
Ciprofloxacin	8 (50)	8 (100)
Moxifloxacin	4 (25)	4 (50)
Chloramphenicol	4 (25)	8 (100)
Gentamicin	8 (50)	8 (100)
Linezolid	16 (100)	8 (100)
Vancomycin	16 (100)	8 (100)
Cefoxitin	0 (0)	8 (100)

AST: Antimicrobial susceptibility testing, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin sensitive *Staphylococcus aureus*

The clinically significant isolates were predominantly isolated from male patients (57.37%) as compared to female patients (42.63%) and the male-female ratio was found to be 1.3:1 in this study. More or less similar findings were observed from various studies in the previous years including studies done by Sharma *et al*. in 2018 and Teklehymanot *et al*. in 2017.[5,11]

Age-wise distribution of patients with culture-positive sterile body fluids revealed the higher culture positivity rate among economically productive age groups such as the age group of 31–40 years, followed by 41–50 years comprising 22.22% and 14.29%, respectively. The predominance in this age group could be due to more activities leading to more exposure to infections.

A higher proportion of the isolates (58%) were grown in the sterile body fluids samples collected from the patients admitted to the intensive care unit (ICU) as compared to other wards as ICU patients are critically ill and associated

Table 5: AST results and distribution of GNB isolates (*n*=160).

Antibiotics	*Acinetobacter* (*n*=64)		*Klebsiella pneumonia* (*n*=40)		*Escherichia coli* (*n*=24)		*Pseudomonas* (*n*=24)		*Citrobacter* (*n*=8)	
	R	S	R	S	R	S	R	S	R	S
Ampicillin	52	12	28	12	8	16	NT	NT	4	4
Piperacillin	52	12	24	16	8	16	8	16	4	4
Amoxicillin-Clavulanic acid	44	20	20	20	12	12	NT	NT	4	4
Ampicillin-Sulbactam	28	36	32	8	8	16	NT	NT	0	8
Piperacillin/Tazobactam	48	16	12	28	8	16	8	16	0	8
Tetracycline	44	20	16	24	16	8	NT	NT	4	4
Cotrimoxazole	36	28	32	8	20	4	NT	NT	0	8
Ciprofloxacin	32	32	32	8	12	12	8	16	0	8
Cefixime	56	8	28	12	12	12	NT	NT	0	8
Ceftazidime	64	0	28	12	12	12	4	16	0	8
Ceftriaxone	64	0	28	12	12	12	NT	NT	0	8
Aztreonam	64	0	24	16	8	16	8	16	0	8
Cefepime	60	4	36	4	20	4	8	16	0	8
Gentamicin	24	40	28	12	4	20	8	16	0	8
Amikacin	16	48	24	16	4	20	8	16	0	8
Tobramycin	8	56	24	16	4	20	8	16	0	8
Ertapenem	56	8	28	12	8	16	NT	NT	0	8
Meropenem	56	8	28	12	8	16	8	16	0	8
Imipenem	52	12	24	16	8	16	8	16	4	4
Colistin	0	64	0	40	0	24	0	24	0	8

NT: Not tested, AST: Antimicrobial susceptibility testing, GNB: Gram-negative bacilli

Table 6: ESBL and carbapenemase producers among Gram-negative bacilli.

Isolated organism	ESBL screening		Carbapenemase screening	
	Positive	Negative	Positive	Negative
Acinetobacter spp. (*n*=64)	-	-	52	12
K. pneumoniae (*n*=40)	28	12	28	12
Escherichia coli (*n*=24)	8	16	8	16
Pseudomonas spp. (*n*=24)	-	-	8	16
Citrobacter spp. (*n*=8)	4	4	4	4
Total (*n*=160)	40	32	100	60

ESBL: Extended-spectrum beta-lactamase

with various comorbidities and immunocompromised states hence more prone to various HCAIs.

All the patients with sterile body fluid infections were having invasive devices, but the association of invasive devices with these infections could not be commented on as most of the patients were admitted to ICUs and almost all of them were having invasive devices.

In our study, there was a predominance of GNB. We observed 83.34% of GNB as compared to only 16.66% of GPC among pathogens isolated from sterile body fluids. Similarly, many studies reported predominant isolation of GNB over GPC including studies by Sharma *et al*., Shume *et al*., Sandhya *et al*., and Ebrahim *et al*. documenting 90%, 70.6%, 71%, and 74.6% of GNB in comparison to GPC isolated from sterile body fluid samples, respectively.[7,8,12,13] In a study by Bourbeau *et al*., no predominance of either GPCs or GNBs was observed.[14] In contrast, there are studies showing the predominance of GPC over GNB including studies by Vishalakshi *et al*., Çetin *et al*., and Pal *et al*.[10,15,16] This variation in the predominance of either group could be due to variation in hospital flora leading to variation among HCAIs, different hospital infection prevention and control measures followed, different sample sizes, and different geographical areas studied.[17,18]

Among the Gram-negative bacterial isolates, *Acinetobacter* spp. was found to be most predominant comprising 40%, followed by *Klebsiella pneumoniae* (25%), *Escherichia coli* (15%), *Pseudomonas* spp. (15%), and *Citrobacter* spp. (5%). Almost similar findings were reported in many studies including a study by Madigubba *et al*. with *Escherichia coli* at 40.10%, followed by *Acinetobacter* spp. 22.60%, *Pseudomonas* spp. 18.20%, and *K. pneumoniae* (14.80%).[19]

Among GPCs, *S. aureus* was found to be the most frequently isolated GPC comprising 75% of all GPCs.

In this study, 75% of *S. aureus* isolates were found to be MRSA. The reported data from the previous studies on MRSA show a wide range of variability ranging from 30% to 100% of MRSA.[8,10,15,19,20] The various factors responsible for differences in the proportion of MRSA could be geographical variations, variations in treatments followed, infection control practices and patient related factors.[4]

In this study, all *S. aureus* isolates were found susceptible against vancomycin and linezolid which are considered as last resort of antibiotics for the treatment of MRSA. The sensitivity pattern of *S. aureus* observed against other antibiotics including 50% against gentamicin, ciprofloxacin, tetracycline, clindamycin, and erythromycin each was sensitive, while 25% against chloramphenicol, moxifloxacin, and cotrimoxazole each were sensitive.

Among Gram-negative isolates, isolates 25% were ESBL producers while 62.50% were carbapenemase producers on screening while none of the isolates were resistant against colistin.

Majority of the bacterial isolates were MDROs with higher resistance against beta-lactams including cephalosporins up to third generation, aminoglycosides, and fluoroquinolones. The predominance of MDR organisms isolated from sterile body fluids was also reported in various studies including studies done by Shume *et al*. in 2022, Ebrahim *et al*. in 2020, and Tsegay *et al*. in 2019 documenting 76.4%, 75%, and 90% of isolates as MDROs.[8,13,21] A low proportion of 30% of isolates as MDR were documented in a study by Shrestha *et al*. in 2019.[20] Most of the recent studies reported majority of the pathogenic bacteria implicated in sterile body fluid infections as MDROs and our findings correlate with these recent studies.[8,13,21,22] Being a tertiary care medical teaching and referral hospital patients are referred after taking treatment at multiple hospitals in the form of antimicrobials, which can be another important reason for higher proportion of MDROs in our hospital.

The variation in the distribution of MDR organisms and their AST patterns could be due to different bacterial strains confined to that particular hospital environment, geographical variations, awareness of patients toward antibiotic usage, easy over the counter availability of antibiotics, difference in antimicrobial prescribing policies, different hospital infection control practices, and indiscriminately using antimicrobials consequently resulting in emergence and transmission of resistance against antimicrobials.[8] The higher isolation rate of MDR organisms from sterile body fluids as compared to other samples as documented by Li *et al*. indicates the inclusion of comparative data during the preparation of antibiogram.[22]

Limitations of the study

Anaerobic bacteria are important etiological agents associated with sterile body fluid infections. The molecular characterizations can also serve as an important modality for confirmation of bacterial identification as well as for genetic mechanisms associated with antibiotic resistance. We did not perform anaerobic culture of sterile body fluids and molecular testing for confirmation of isolates and detection of genetic mechanisms involved in antibiotic resistance due to limited resources.

CONCLUSION

From this study, we concluded that the pathogenic bacteria implicated in infections of sterile body fluids are predominantly MDR. There is huge variation in data on distribution of bacterial species isolated from sterile body fluids and their antimicrobial resistance patterns from different geographical locations and healthcare settings. Thus, data from a particular healthcare setting are important for empirical treatment in that healthcare setting.

The increasing trends of MDR and emergence of resistance against high-end antimicrobials are an alarming situation. It is the time for strict implementation of antibiotic stewardship and hospital infection control measures to prevent antimicrobial resistance.

Ethical clearance

Prior approval from the Institutional Ethics Committee was taken.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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