

Original Research Article

Bacterial Isolates and Drug Susceptibility Pattern of Sterile Body Fluids from Tertiary Hospital in Saurashtra Region: A One-Year Retrospective Study

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ABSTRACT

Background:

Body fluids obtained from sterile body sites are often expected to be devoid of any pathogenic or commensal microorganisms. The cause of infected body fluids could be any pathological agents or skin contaminants that may be harbored in intensive care units. This study identifies commonly isolated bacteria from the body fluid samples along with their antibiotic sensitivity pattern.

Methods:

All body fluid samples (except blood, and CSF) which were received in the Bacteriology section of the Department of Microbiology in our tertiary care center of Saurashtra region between June 2021 to June 2022 were included in the study. All microorganisms obtained from bacterial culture were subjected to identification by using conventional methods and standard biochemical tests were done to aid identification. Antibiotic sensitivity testing by Kirby Bauer disc diffusion test and the results were analyzed.

Results:

Out of 250 samples of body fluids, 72 (28.80%) showed bacterial growth on culture. Male predominance among patients was seen. Patients falling between the age group of 41-50 show maximum positivity and the ratio of prevalence of infection in the young and old patients seems to be nearly similar (52% in patients less than 50 years of age and 48% in patients elder than 50 years) *Escherichia coli* was the most commonly isolated bacteria (27.77%) which was followed by *Pseudomonas* (25 %) and *Klebsiella pneumonia* (22.2 %).

Conclusion:

This study shows the spectrum of bacterial isolates observed from body fluid samples received in our laboratory and helps in the empirical treatment of patients based on antibiotic susceptibility patterns. It also highlights the importance of adhering to antibiotic sensitivity results in reducing the empirical broad-spectrum antibiotics abuse and infection control practices to prevent the spread of multidrug-resistant infection in the hospital environment.

Keywords: Body fluids, Kirby Bauer disc diffusion test, Antibiotic sensitivity pattern.

INTRODUCTION

Body sites are called sterile when they have no bacteria or other microorganisms as commensals in

healthy condition. The infections caused in these sites could be either due to any pathological agents

or skin contaminants harbored from intensive care units.¹

The body fluids obtained from sterile body sites are similarly expected to be devoid of any pathogenic or commensal microorganisms.

Sterile body fluids include cerebrospinal fluid (CSF), synovial fluid, pericardial fluid, pleural fluid and peritoneal dialysate fluid.

Most common causative agents of pathogenic microorganisms infecting the sterile body fluids are lactose fermenting gram-negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* species, and *Citrobacter* species.

Non-lactose fermenting gram-negative bacteria like *Acinetobacter* species, *Burkholderia cepacia*, *Pseudomonas* species, and gram-positive organisms like Methicillin resistant *Staphylococcus aureus* and *Enterococcus* species which lead to morbid infections with poor outcome.^{2,3}

The morbidity and ability to cause life-threatening infections have rendered these cases a medical emergency that demands early diagnosis and suitable treatment. Due to low inoculums of pathogenic bacteria and early administration of empirical antibiotics there were fewer chances of retrieving positive cultures.⁴

The difficulty in diagnosing the causative microorganism of body fluid infections and the need for effectively managing the patients admitted to the intensive care unit has made it the need of the hour to frame an antibiotic policy and to know the common pathogenic microorganisms with appropriate antibiotic sensitivity pattern.⁵

As far as we know, there is limited knowledge regarding the microbiological characteristics, bacterial spectrum, and antimicrobial resistance in our geographical setting.

The knowledge of the common pathogenic bacteria and their antibiotic susceptibility pattern is crucial for clinicians to combat a range of infections and administer adequate antibiotics. So, this study conducted at a tertiary care centre in Northern India

sheds light on the spectrum of bacterial isolates from the body fluid samples collected from outpatient department (OPD) and inpatient department (IPD) patients at our center along with their antibiotic susceptibility patterns.

MATERIAL AND METHODS

This retrospective, observational, single-center study was conducted from June 2021 to June 2022 in the Bacteriology section of the Department of Microbiology at a tertiary care center.

A total of 250 non-repeat samples of clinically suspected infected body fluids were collected aseptically and the samples included in the study consist of pleural fluid (127, 50.80 %), peritoneal fluid (60, 24.0 %), ascitic fluid (43, 17.20 %) and other fluids (20, 8%) from other sterile areas of the body.

Inclusion criteria:

All the samples received from patients with clinically suspected body fluid infections, from the OPD and IPD patients at our centre, irrespective of age and gender were included.

Exclusion criteria:

- 1) Blood samples,
- 2) Cerebrospinal fluid samples,
- 3) Contaminated samples,
- 4) Body fluids with delay in transportation for more than 2 hours.

Sample processing:

The samples were collected at a combined receiving station, sent to the bacteriology section of the Department of Microbiology, and processed in our laboratory according to the standard protocols. The samples were subjected to Gram's stain and culture. The culture media used were Blood agar, MacConkey agar, and Robertson's cooked meat broth (RCM). Isolated colonies were observed on the Blood and MacConkey agar plates and turbidity was observed in the RCM and the isolates were

identified by Grams' stain and standard biochemical tests.

Antimicrobial susceptibility testing:

The antibiotic sensitivity testing was performed for each of the bacterial isolates by the Kirby Bauer Disc Diffusion method according to the guidelines of CLSI [6]. Antibiotic discs : AS=Ampicillin/Sulbactam, BA=Cotrimoxazole, PR=Cephalexin, TE=Tetracyclin, CF=Cefotaxime, RC=Ciprofloxacin, QB=Levofloxacin, LZ=Linezolid, CX=Cloxacillin, AT=Roxithromycin, LM=Lincomycin, GM=Gentamycin, PC=Piperacillin, CH=Chloramphenicol, CI=Ceftizoxime, TE=Tetracyclin, ZN=Ofloxacin, AK=Amikacin, GF=Gatifloxacin, PT=Piperacillin+Tazobactam, IPM=Imipenem were used in this test.

Briefly, inoculums were prepared for each bacterial isolate by adjusting the turbidity to 0.5 McFarland standard and spread on Muller-Hinton agar plates. Antibiotic discs were placed on the agar plates and incubated overnight at 37°C for 24 h. The zones of inhibition were measured and the isolates were classified as sensitive, intermediate, and resistant according to CLSI tables and guidelines.⁶

Microbiological characteristics and drug resistance patterns were analyzed for all the samples included in the study.^{7,8}

RESULT

Demographic Characteristics of the Study Subjects.

During the study period, a total of 250 patients were investigated. Majority of the study participants, 145(58%), were males, whereas 105 (42%) were females. The age of the study subjects ranged from 1 day of life with majority 221 (88.4%) in the age group of 10 years and above.

Prevalence of Bacterial Infection:

Out of 250 cultured sterile body fluid specimens, the overall bacterial isolates were 72 (28.8 %). From the patients investigated for bacterial infection 37 (51.3 %) were from pleural fluid , 20 (27.7%) from peritoneal fluid, and 10 (13.8 %) from acitic fluid and 5(6.94 %)other sterile body fluids (Synovial, Perinephric fluid and Broncho-Alveolar Lavage(BAL) being infected. Of the total 72 isolates, gram negative bacteria were prevalent, 62 (86.1 %) than gram positive bacteria, 10 (13.9 %). The predominantly isolated bacteria were *E. coli* 20 (27.70 %) followed by *P.aeruginosa* 18 (25.0 %) *K.pneumoniae* 16 (22.2 %), among the gram negatives and *S. aureus* 8(11.1%), which was among the gram positive bacteria (Table 3).

Antimicrobial Susceptibility Testing:

Among the gram positive bacteria (n=10), 9 (90%) out of 10 isolates were sensitive to Cloxacillin and 8 (80%) of the isolates were sensitive to Ampicillin/Sulbactam, Cephalexin Tetracyclin, Linezolid, respectively. The resistance pattern of these isolates ranges from 30% for ceftriaxone to 60 % for ciprofloxacin respectively . *S. aureus* which were the predominant isolates among gram positive bacteria 8(11.1%), show susceptibility pattern of 8 (100%), 7 (87.5 %), and 4 (50%) to cloxacillin , secondly to Ampicillin/Sulbactam Cephalexin,, Tetracycline ,Cefotaxime ,Linezolid ,Lincomycin ,Gentamycin, respectively (Table 2). In gram negative bacterial isolates (n=62) showed a resistance rate of 74.19 % &71.0% to cefotaxime and trimethoprim/sulfamethoxazole. Resistance against gentamycin, norfloxacin, chloramphenicol, nitrofurantoin, and amoxicillin-clavulanic acid was observed in the range of 20-70 %. However, all gram negative bacterial isolates showed low level resistance against piperacillin tazobactam and imepenem (Table 3).

Table-1: Sample distribution based on positivity

Samples tested	Results	Percentage
178	Negative	71.2
72	Positive	28.8
250	Total	100%

Table-2: Distribution according to Gender of patient

Gender	Number of patients	Percentage
Male	145	58%
Female	105	42%
TOTAL	250	100%

Table 2 shows the distribution of patients who tested positive for bacterial culture according to their gender.

Table-3: Distribution according to Age group

AGE GROUP (in years)	NUMBER OF PATIENTS	PERCENTAGE
0 to 10	29	11.6%
11 to 20	32	12.7%
21 to 30	28	11.7%
31 to 40	42	16.7%
41 to 50	47	18.7%
51 to 60	42	16.7%
>=60	30	12%
TOTAL	250	100%

Table 3 shows the distribution of patients who tested positive for bacterial culture according to their age.

Table-4: Distribution of bacterial etiologic agents from body fluids

Isolated Bacteria	Pleural Fluid	Peritoneal Fluid	Ascitic Fluid	Other Body Fluids	Total
Gram Positive	3	2	2	3	10
Staphylococcus aureus	3	2	0	3	8
Enterococcus spp.	0	0	1	0	1
CONS	0	0	1	0	1
Gram Negative	34	18	8	2	62
Escherichia coli	7	10	3	0	20
Klebsiella pneumonia	8	5	2	1	16
Acinetobacter baumannii	5	1	1	0	7
Pseudomonas aeruginosa	13	2	2	1	18
Proteus vulgaris	1	0	0	0	1
Total	37	20	10	5	72

CONS-Coagulase Negative Staphylococcus.

Other body fluids includes Synovial, Perinephric fluid and Broncho-Alveolar Lavage (BAL)

**Table-5: Antimicrobial susceptibility pattern
Gram-positive bacteria (n=10) isolated from body fluids**

Bacterial Isolates	Pattern	AS	BA	PR	TE	CF	RC	QB	LZ	CX	AT	LM	GM
CONS	R:	0	1(100)	1(100)	0	1(100)	1(100)	0	1(100)	0	0	1(100)	1(100)
	S:	1(100)	0	0	1(100)	0	0	1(100)	0	1(100)	1(100)	0	0
Enterococcus spp.	R:	1(100)	0	1(100)	1(100)	1(100)	1(100)	1(100)	0	1(100)	1(100)	1(100)	1(100)
	S:	0	1(100)	0	0	0	0	0	1(100)	0	0	0	0
S. aureus	R:	1(12.5)	2(25)	1(12.5)	1(12.5)	1(12.5)	4(50)	2(25)	1(12.5)	0	2(25)	1(12.5)	1(100)
	S:	7(87.5)	6(75)	7(87.5)	7(87.5)	7(87.5)	4(50)	6(75)	7(87.5)	8(100)	6(75)	7(87.5)	7(87.5)
Total	R:	2(20)	3(30)	2(20)	2(20)	3(30)	6(60)	3(30)	2(20)	1(10)	3(30)	3(30)	3(30)
	S:	8(80)	7(70)	8(80)	8(80)	7(70)	4(40)	7(70)	8(80)	9(90)	7(70)	7(70)	7(70)

Table-6: Antimicrobial susceptibility pattern of gram-negative bacteria (n=62) isolated from body fluids

Bacterial Isolates	Pattern	AS	BA	CF	PC	CH	RC	CI	TE	ZN	GM	AK	GF	PT	IPM
E. coli	R:	11 (55)	13 (65)	16 (80)	6 (30)	2 (10)	16 (80)	14 (70)	15 (75)	15 (75)	11 (55)	4 (20)	13 (65)	0	0
	S:	9 (45)	7 (35)	4 (20)	14 (70)	18 (90)	4 (20)	6 (30)	5 (25)	5 (25)	9 (45)	16 (80)	7 (35)	20 (100)	20 (100)
K.pneumoniae	R:	6 (37.5)	7 (43.7)	9 (56.2)	1 (6.25)	2 (12.5)	6 (37.5)	9 (56.2)	5 (31.2)	4 (25)	2 (12.5)	1 (6.25)	5 (31.2)	0	0
	S:	10 (62.5)	9 (56.2)	7 (43.7)	15 (93.7)	14 (87.5)	10 (62.5)	7 (43.7)	11 (68.5)	12 (75)	14 (87.5)	15 (93.7)	11 (68.7)	16 (100)	16 (100)
A. baumannii	R:	6 (85.7)	5 (71.4)	3 (42.8)	3 (42.8)	7 (100)	4 (57.1)	4 (57.1)	3(42.86)	3(42.86)	3(42.86)	3(42.86)	2(28.57)	0	0
	S:	1(14.29)	2(28.57)	4(57.14)	4(57.14)	0	3(42.86)	3(42.86)	4(57.14)	4(57.14)	4(57.14)	4(57.14)	5(71.43)	7(100)	7(100)
P. aeruginosa	R:	18(100)	18(100)	17(94.44)	2(11.11)	18(100)	5(27.78)	10(55.55)	17(94.44)	4(22.22)	4(22.22)	3(16.67)	3(16.67)	0	2(11.11)
	S:	0	0	1 (5.56)	16 (88.8)	0	13 (72.2)	8 (44.4)	1 (5.56)	14	14 (77.7)	15 (83.3)	15 (83.3)	18 (100)	16 (88.8)
P.vulgaris	R:	1	1	1	0	0	1	1	1	1	1	0	1	0	0
	S:	0	0	0	1	1	0	0	0	0	0	1	0	1	1
Total	R:	42 (67.7)	44 (70.9)	46 (74.1)	12 (19.3)	29 (46.7)	32 (51.6)	38 (61.2)	41 (66.1)	27 (43.5)	21 (33.8)	11 (17.7)	24 (38.7)	0	2 (3.22)
	S:	20 (32.2)	18 (29.0)	16 (25.8)	50 (80.6)	33 (53.2)	30 (48.3)	24 (38.7)	21 (33.8)	35 (56.4)	41 (66.1)	51 (82.2)	38 (61.3)	62 (100)	60 (96.7)

AS=Ampicillin/Sulbactam, BA= Cotrimoxazole, CF=Cefotaxime, PC=Piperacillin, CH=Chloramphenicol, RC=Ciprofloxacin, CI=Ceftizoxime, TE=Tetracycline, ZN=Ofloxacin, GM=Gentamicin, AK=Amikacin, GF=Gatifloxacin, PT=Piperacillin Tazobactam, IPM=Imipenem

DISCUSSION

In this study that comprising 218 (peritoneal , pleural, ascitic and other sterile fluids like synovial body fluid) samples received in the microbiology laboratory, the percentage of positive cultures was 28.8% which has higher finding than studies done in India, 14.79% and 14.78%. The reason for this wide disparity in positivity rates of sterile fluids was attributed to differences in techniques, antibiotic use, or the prevalence of effusions caused by infective processes. Some variations are likely explained by the differences in the study population.

In our present study, 13.90 % of infections were caused by gram positive bacteria and 86.10 % by gram negative bacteria. The similar predominance of gram negative bacteria has been observed in previous studies conducted in the northern part of India in the state of Uttar Pradesh.⁹ The most common organism isolated from pleural fluid was pseudomonas aeruginosa (13) and these findings are in contrast with the findings of studies by Sharma et al, Sujatha et al and Evans et al where the most common causative agent of pleural fluid infections was Escherichia coli.¹⁰⁻¹²

This study represents the ability of the aerobic gram negative bacteria to predominantly cause purulent pleural fluid infections. In studies by Jain et al, Gupta et al and Mohanty et al a similar finding was observed that the gram negative bacteria were more commonly isolated from the purulent infections of pleural fluid.¹³⁻¹⁵

The gram positive microorganisms isolated from pleural fluid infections consist mainly of Staphylococcus aureus. The isolation of aerobic gram negative bacteria or isolation of multiple pathogenic bacteria holds poor prognosis and thus arises need of rigorous antimicrobial therapy.¹⁶

The gram negative bacteria most commonly isolated from ascitic fluid samples was Escherichia coli (3), followed by Klebsiella pneumoniae (2) and Pseudomonas spp (2) which is correlates with the studies conducted by Arroyo et al and Chawla et al which showed Escherichia coli as the most common microorganism causing peritonitis.^{17,18} In a study by

Harshika et al, non lactose fermenting bacteria were the most commonly isolated microorganisms from the ascitic fluid samples disagrees with the findings of this study but dissimilarity in the spectrum of pathogenic bacteria causing peritonitis also depends upon the geographical area of isolation.¹⁶

The gram negative bacteria were almost completely susceptible to drug of last resort, Imepenem and Piperacillin tazobactam, followed by good sensitivity for drugs like Amikacin And Gentamicin. A good susceptibility to carbapenems seen in a studies conducted by Harshika et al.¹⁶ and Sharma et al.¹⁰ which correlates with our study.

The isolates in were relatively resistant to Ceftizoxime, Ceftriaxzone, Ciprofloxacin and Ampicillin Sulbactam, which corroborates with studies conducted by Tullu et al.¹⁹ The Pseudomonas species isolates were highly sensitive , Imepenem and Piperacillin tazobactam. Only most of isolates of Pseudomonas species were susceptible to Piperacillin-Tazobactam which coordinates with the study conduct by Harshika et al.¹⁶

Gram positive bacteria isolated were highly sensitive to Linezolid which is in agreement with the study by Sujatha et al.¹¹ but disagrees with the complete resistance to Gentamicin shown in among our isolates.

Thus, evaluation of the results of antimicrobial resistance to the isolates is suggestive of rapid emergence of multidrug resistance. Ability to attain the resistant genes from other resistant microorganisms has lead to rise of multidrug resistance through the years. Before seeking treatment at a tertiary care centre, most patients are subjected to unnecessary antimicrobial therapy that renders them resistant to most first line antibiotics given to treat a specific microorganism. Lack of proper antimicrobial stewardship program and no adherence to the antibiotic susceptibility testing has lead to increased antimicrobial resistance.

CONCLUSIONS

This study shows spectrum of bacterial isolates observed from the body fluid samples received in our laboratory and helps in guiding the empirical treatment of patients based on antibiotic susceptibility patterns. The increase in the incidence of multidrug resistance also points out the need to implement strict adherence to the antibiotic sensitivity. The spread of multidrug resistant and extensively drug resistant microorganisms can also be curtailed by strict adherence to infection control practices, educating the health care workers and patients about hand hygiene and make them aware about the morbidity of suffering from a multidrug resistant infection.

REFERENCES

1. Badiie P. Evaluation of human body fluids for the diagnosis of fungal infections. *BioMed Res Int*. 2013;:698325.
2. Hughes JG, Vetter EA, Patel R, Schleck CD, Harmsen S. Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. *J Clin Microbiol*. 2001;39(12):4468-4471.
3. Daur AV, Klimak F, Cogo LL, Botao GD, Monteiro CL. Enrichment methodology to increase the positivity of cultures from body fluids. *Braz J Infect Dis*. 2006;10(6):372-373.
4. Deb A, Mudshingkar S, Dohe V, Bharadwaj R. Bacteriology of body fluids with an evaluation of enrichment technique to increase culture positivity. *J Evol Med Dent Sci*. 2014;3(72):15230-15238.
5. Harshika YK, Medegar KR, Patil AB, Smita NR. Bacteriological profile and antimicrobial resistance pattern from various body fluids of patients attending a tertiary care hospital. *Indian J Microbiol Res*. 2018;5(4):530-534.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-sixth informational supplement. CLSI Document M100-S26. Wayne, PA: CLSI; 2016.
7. Fariñas MC, Martínez-Martínez L. Multiresistant Gram-negative bacterial infections: Enterobacteria, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and other non-fermenting Gram-negative bacilli. *Enferm Infecc Microbiol Clin*. 2013;31(7):402-409.
8. Cantón R, Ruiz-Garbajosa P. Infections caused by multi-resistant Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus* spp.). *Enferm Infecc Microbiol Clin*. 2013;31(9):543-551.
9. Kar M, Chaudhary R, Sahu C, Dubey A, Patel SS. Bacterial isolates and drug susceptibility pattern of sterile body fluids at a tertiary care hospital in Northern India.
10. Sharma R, Anuradha, Nandini D. Bacteriological profile and antimicrobial sensitivity pattern in sterile body fluids from a tertiary care hospital. *J Appl Microbiol Biochem*. 2017;1(1):1.
11. Sujatha R, Pal N, Arunagiri D, Narendram D. Bacteriological profile and antibiotic sensitivity pattern from various body fluids of patients attending Rama Medical College Hospital, Kanpur. *Int J Adv Case Rep*. 2015;2(4):119-124.
12. Evans LT, Kim WR, Poterucha JJ, Kamath PS. Spontaneous bacterial peritonitis in asymptomatic outpatients with cirrhotic ascites. *Hepatology*. 2003;37(4):897-901.
13. Jain S, Banavaliker JN. Empyema thoracis: Bacteriological analysis of pleural fluid. *IOSR J Dent Med Sci*. 2013;3(6):46-51.
14. Gupta SK, Kishan J, Singh SP. Review of one hundred cases of empyema thoracis. *Indian J Chest Dis Allied Sci*. 1989;3(1):15-20.
15. Mohanty S, Kapil A, Das BK. Bacteriology of parapneumonic pleural effusions in an Indian hospital. *Trop Doct*. 2007;37(4):228-229.

16. Harshika YK, Medegar KR, Patil AB, Smita NR. Bacteriological profile and antimicrobial resistance pattern from various body fluids of patients attending the tertiary care hospital, KIMS, Hubli. *Indian J Microbiol Res.* 2018;5(4):530-534.
17. Mohanty S, Kapil A, Das BK. Bacteriology of parapneumonic pleural effusions in an Indian hospital. *Trop Doct.* 2007;37(4):228-229.
18. Arroyo V, Bataller R, Gines P. Spontaneous bacterial peritonitis. *Comprehensive Clinical Hepatology.* Barcelona: Mosby; 2000. p. 10-7.
19. Tullu MS, Deshmukh CT, Baveja SM. Bacterial profile and antimicrobial susceptibility pattern in catheter-related nosocomial infections. *J Postgrad Med.* 1998;44(1):7-13.

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