IJPSR (2024), Volume 15, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 May 2024; received in revised form, 01 June 2024; accepted, 09 July 2024; published 01 October 2024

BACTERIAL PROFILE AND ANTIBIOGRAM OF BODY FLUIDS ISOLATED FROM VARIOUS IPDS PATIENTS

Mahesh Chandra * and Ansar Ahmad Paray

Department of Microbiology, Adesh Institute of Medical Science & Research Bathinda - 151101, Punjab, India.

Keywords:

Antimicrobial resistance, Gram-positive bacteria, Gram-negative bacteria, *Klebsiella pneumoniae*, *Staphylococcus aureus*

Correspondence to Author:
Mahesh Chandra

Demonstrator,
Department of Microbiology,
Adesh Institute of Medical Science & Research Bathinda - 151101, Punjab, India.

E-mail: mahesh0231997@yahoo.com

ABSTRACT: Introduction: Pathogenic infections place a significant burden on the healthcare system since they are linked to high rates of morbidity and mortality. Successful therapy of these illnesses depends on an early diagnosis and a good antimicrobial resistance testing. Therefore, the goal of the current investigation was to assess aerobic bacteriological profile and antibiogram from a variety of sterile fluids. **Material and Method:** This observational study was carried out in the Department of Microbiology, Adesh Institute of Medical Science & Research Bathinda, Punjab from 20 September 2023 to 15 February 2024 obtaining the approval from Ethics Committee of Biomedical & Health Research, Adesh University. All the Body fluid samples were received in Bacteriology Laboratory from various departments. All body fluid samples are processed by Bact alert system. Positive samples were further subculture on blood agar and MacConkey agar. Gram Staining was done to identify gram-positive bacteria and gram-negative Bactria, further antimicrobial susceptibility was done by Vitek 2 compact system as per CLSI guidelines 2023. **Results:** A total of 288 body fluid samples were received, from which Pus & Swab 102 (38.05%) were highest in number. Among the 157 isolates, the most predominant organism was *Escherichia Coli* 37 (23.56%) followed by *Staphylococcus aureus* 29 (18.47%), *Klebsiella pneumoniae* 25 (15.9%). The most sensitive drugs for gram-positive isolates were Daptomycin, Teicoplanin, Vancomycin, Doxycycline, Minocycline and for gram negative isolates were Amikacin, Minocycline, Tigecycline, Fosfomycin. **Conclusion:** The study concluded that multidrug-resistant bacteria are mostly responsible for infections of sterile body fluids and their antibiotic resistance varies greatly across various geographic areas and healthcare settings. Therefore, empirical treatment and antimicrobial testing crucial for effective treatment.

INTRODUCTION: Pathogenic infections place a significant burden on the healthcare system since they are linked to high rates of morbidity and mortality [1]. With sterile bodily fluids (SBF), there are no bacteria that would typically be present in the flora. Microorganisms, however, can enter sterile bodily sites [2, 3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(10).3084-89

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(10).3084-89

Leading to significant invasive illnesses and catastrophic phenomena [4, 5]. Fluids may build up in any body cavity because of infection, leading to invasive disorders such Bacteremia, bacterial meningitis, sepsis, bacterial peritonitis, and other problems [6].

Because un-treated infections at sterile body sites can result in severe, potentially fatal infections elsewhere in the body, these infections are critical, urgent conditions that need to be treated right once [7]. Particularly, infections brought on by resistant microorganisms continue to be a major global cause of severe infections, with rising rates of morbidity and mortality [8, 9].

It is largely more common in developing nations where there are fewer health care services, fewer resources, inadequate sanitation and hygiene, irrational antibiotic usage, and poverty are some of the main factors contributing to the formation of antibiotic-resistant microorganisms [10, 11]. The present study aim was to find the prevalence of bacteremia from body fluid samples and performed their antibiotic sensitivity pattern.

MATERIAL AND METHOD: The study was carried out in the Department of Microbiology, Adesh Institute of Medical Science & Research Bathinda, Punjab from 20 September 2023 to 15 February 2024 after obtaining the approval from Ethics Committee of Biomedical & Health Research, Adesh University (reference no. AU/EC_BHR/2K23/486). Body fluid and swab samples were received from various departments. The sample size was calculated by Daniel's formula with 5% of margin error [12]. Sample size was 288 body fluid samples such as ascitic fluid, pleural fluid, cerebral fluid, pericardial fluid, bronchial wash fluid, drain fluid, Synovial fluid, and pus samples. The sample size was calculated using the following formula calculated using the following formula:

$$n = Z \times P \times q / e^2$$

$$(1.96)^2 \times (0.25) \times (0.75) / (0.05)^2 = 0.72 / 0.0025 = 288$$

n = Samplesize, Z = the standard deviate, usually 1.96 which corresponds to 95% confidence P = Expected average Prevalence. According to Previous studies (25%), e = Margin of error (0.05).

The sample received in the laboratory in proper conditions was immediately processed for fluid culture by Bact alert systems, the positive samples as indicated by Bact alert systems were sub-cultured on blood agar and MacConkey agar. Swab/pus samples were directly inoculated on Blood agar and MacConkey agar and incubated at 37°C.

Identification of the Bacterial Isolates: After bact alert system indicating growth of the organism, Subculture was done on Blood agar and MacConkey agar from the Bact alert culture bottles. Swab/Pus samples were directly inoculated on blood agar and MacConkey agar using directly streak culture method. The culture plate were incubated at 37 °C for 18-24 hours in the incubator. Primary identification was done on the basis of colony characteristics, gram- staining and gram staining followed by final identification using Vitek 2 compact automated systems using Gram negative (GN) cards for gram negative and Gram positive (GP) cards for gram positive isolates.

Antimicrobial Susceptibility Test (AST): AST was performed by automated method using Vitek 2 compact system using AST cards P628 for gram positive bacteria and N405 & N406 for gram-negative bacteria as per Clinical Laboratory standard Institute (CLSI) guidelines 2023 [13]. Statical Analysis was done by using Microsoft world and Microsoft excel software.

RESULTS: During the study period between 20 September 2023 to 15 February 2024 total 288 randomly non-repetitive body fluid and Swab/pus samples obtained from various departments of hospital. Culture positivity was seen in 157 (54.51%) samples and 131 (45.48%) samples showed no growth.

Out of the 157 samples, 106 (67.50%) were of males and 51 (32.48%) females. The positive samples belong to maximum from age group 61-70 years as compared to other age groups **Fig. 1**. Out of 157 positive cultures 123 (78.34%) were gram negative bacteria and 34 (21.66 %) gram positive bacteria. Among the 157 isolates, the most predominant organism was *Escherichia Coli* 37 (23.56%) followed by *Staphylococcus aureus* 29 (18.47%), *Klebsiella pneumonia* (15.9%) as shown in **Fig. 2.**

TABLE 1: SAMPLES WISE DISTRIBUTION OF BACTERIAL PREVENANCE

Fluid name (sample)	Total samples	Growth	No growth	Prevenance
Pus & Swab	(38.19%) 110	92	18	83.63%
Bal (Bronchial wash)	(29.16%) 84	44	40	52.38%
Pleural	(12.50%) 36	08	28	22.22%
CSF	(10.41%) 30	04	26	13.33%
Drain	(4.51%) 13	07	06	53.84%
Ascitic	(3.47) 10	01	09	10.00%

Synovial	(1.73) 05	01	04	20.00%
Total	288	157	131	-

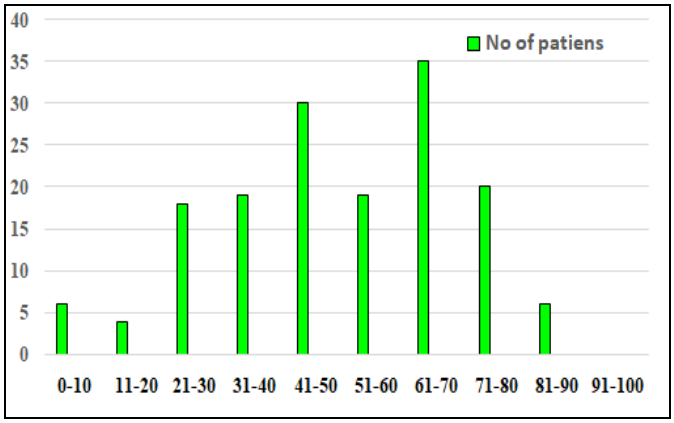

FIG. 1: AGE WISE DISTRIBUTION OF BACTERIAL SPECIES

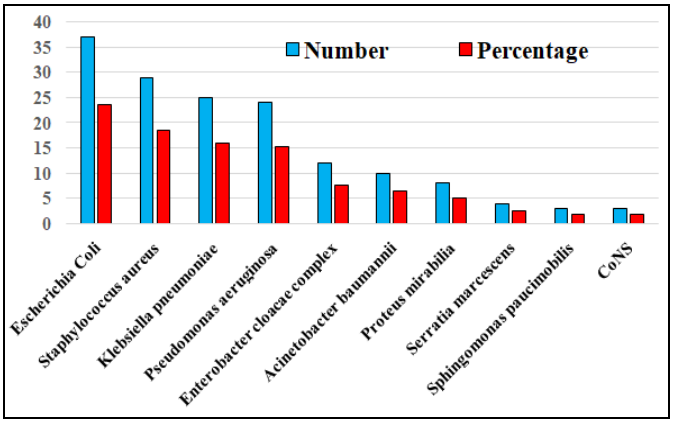

FIG. 2: DISTRIBUTION OF BACTERIAL ISOLATED

In the present study, *S. aureus* show (100%) sensitivity to Daptomycin, Teicoplanin, Vancomycin, Doxycycline, Minocycline followed by Rifampicin (96.50%), Linezolid (96.03 %), Tetracycline (95.00%) Gentamicin (79.40%) & Erythromycin (51.85%). *E. coli* shows (86.15%) sensitivity to Fosfomycin and (75.65%) to Amikacin followed by Ertapenem (68.40%), Imipenem (56.70%), Meropenem (54.00%), Cefoperazone / Sulbactam (51.35%) & Minocycline (50.00%). Klebsiella pneumoniae shows highly sensitive for Amikacin (92.00%) followed by Tigecycline (60.00%), Gentamicin (48.00%), Levofloxacin & Minocycline (42.80%). The antibiotic sensitivity patterns result of gram-positive cocci are show in **Table 2** and those are gram negative show in **Table 3.**

TABLE 2: ANTIMICROBIAL SENSITIVITY (%) PATTERN OF GRAM-POSITIVE COCCI

Antibiotics	*S. aureus*	CoNS	*Enterococcus faecium*
Benzylpenicillin	00	00	50.00
Oxacillin	21.51	00	NT
Gentamicin	79.40	00	NT
Ciprofloxacin	6.91	00	00
Levofloxacin	6.91	00	00
Erythromycin	51.85	00	00
Clindamycin	70.00	00	NT
Linezolid	96.03	100	50.00
Daptomycin	100	50.08	100
Teicoplanin	100	100	50.00
Vancomycin	100	100	50.00
Doxycycline	100	100	00
Minocycline	100	100	00
Tetracycline	96.56	50.40	00
Tigecycline	100	100	100
Nitrofurantoin	100	100	50.00
Rifampicin	96.50	100	NT
Trimethoprim/Sulfamethoxazole	55.18	00	NT

NT- Not tested.

TABLE 3: ANTIMICROBIAL SENSITIVITY (%) PATTERN OF GRAM-NEGATIVE BACILLI

Antibiotics	*E. coli*	*K. pneumoniae*	*S. aeruginosa*	*P. mirabilis*	*Enterobacter cloacae complex*	*A. baumannii*	*Serratia marcescens*	*Sphingomonas*
Amoxicillin/Clavulanic Acid	15.70	35.75	NT	NT	00	NT	00	NT
Piperacillin/Tazobactam	40.56	40.00	73.30	87.58	66.66	00	NT	100
Cefuroxime	00	21.40	NT	NT	12.56	NT	00	NT

Cefuroxime Axetil	00	21.48	NT	NT	12.58	NT	00	NT
Cefotaxime	00	21.40	NT	NT	12.55	NT	00	NT
Ceftriaxone	00	21.44	NT	NT	25.00	75.00	00	NT
Cefoperazone / Sulbactam	51.35	40.00	63.65	87.50	58.30	40.00	75.00	100
Cefepime	16.25	36.00	58.30	87.50	58.30	00	50.00	100
Ceftazidime	22.25	33.34	55.50	NT	58.35	00	50.00	100
Ertapenem	68.40	31.25	NT	NT	62.50	NT	50.00	NT
Imipenem	56.70	39.15	77.20	62.55	58.30	50.00	50.00	100
Meropenem	54.00	44.05	83.30	87.50	66.60	50.00	50.00	100
Amikacin	75.65	92.00	90.90	100	91.60	30.0	100	66.60
Gentamicin	51.30	48.0	81.80	62.55	58.30	10.0	25.0	100
Aztreonam	27.70	20.0	NT	NT	33.30	NT	50.0	00
Ciprofloxacin	17.10	20.0	72.70	25.0	41.60	30.0	00	100
Levofloxacin	11.15	42.80	69.50	37.50	33.3	00	50.0	100
Minocycline	50.00	42.85	NT	00	66.60	100	100	100
Tigecycline	11.10	60.00	100	00	91.60	NT	66.60	00
Fosfomycin	86.15	45.00	NT	62.50	75.00	NT	66.60	00

NT- Not tested.

DISCUSSION: Normally body fluids like Cerebral spinal, ascitic, pleural, cerebral, pericardial, synovial fluids and pus samples are usually sterile but can be infected by various pathogens. In the study the isolation rate of body fluid showing growth positive was 157 (54.51%) which correlates with studies conducted by Duggal *et al*.,[14], Mita *et al*.,[15] and Deboral *et al*.,[16]. They had also reported culture positivity rate of 93%, 85% and 72% respectively. The low rate of isolation may be explained by the fact of the patient probably received antibiotic therapy before culture sensitivity testing.

In the present study the maximum bacterial isolates were isolated from Pus/ swab (83.63%) samples as followed by Drain (53.4%), Bal (Bronchial wash) (52.38%), Synovial samples (20.00%), Pleural (22.22%), CSF (13.33%) and Ascitic (10.00%). Studies done by Shume *et al*., [17] and Singh *et al*.,[18] had also reported prevalence from Pleural fluid (29.8%), (14%) respectively. Sadhna *et at.,* had reported prevalence from CSF (34.4%), drain fluid (13.11%), BAL fluid (6.55%), ascitic fluid (4.91%), pleural fluid (3.27%), synovial fluid, (1.63%) [19]. The results of their studies correlated with this present study. Whereas studies by Shume *et al*.,[17] and Shrestha *et al*., [20] reported low rates of prevalence from body fluid culture accounting for 16.7% and 10.68% respectively.

The present study showed males patients had high culture positivity as compared with females' patients. i.e. 106 (67.50%) males and 51 (32.50%) were females. The result was compared with the study done by Singh *et al*., [19] and Shume *et al*., [17] they are showing 57.30%, 63.70% in males and 42.70%, 32.40% in females respectively. The reason for some difference is because of gender Bais or high admission rate of males.

In the present study, the isolation of gram-negative bacteria (78.30%) is higher than gram positive bacteria (21.70%). Which is consistent with the previous studies conducted by Shume *et al*., and Madigubba *et al*., showing gram-negative bacteria 70.60%, 83.20%. and Gram-positive bacteria 29.40%, 16.35% respectively [17, 21]. Another similar study done by Rouf *et al*., shows Gram-negative bacteria 70.00% as compared to gram positive bacteria (30%) [22].

In our study, the most Common organisms were *Escherichia Coli* 37 (23.5%) followed by *Staphylococcus aureus* 29 (18.55%), *Klebsiella pneumoniae* (15.95%), *Pseudomonas aeruginosa* (15.90%), *Enterobacter cloacae* complex (7.64%), *Acinetobacter baumannii* (6.36%), *Proteus mirabilia* (5.10%), *Serratia marcescens* (2.60%), *Sphingomonas paucimobilis* (1.91%), CoNS (1.91%), *Enterococcus faecium* (1.27%).This finding correlates with study done by Madigubba *et al*., study, showing most Common organisms were *Escherichia coli* (29.80%), *Klebsiella pneumoniae* (14.70%), *Enterobacter spp*. (5.00%), *Acinetobacter baumannii* (13.7%), *Pseudomonas aeruginosa* (13.10%), *Staphylococcus aureus* (4.50%) [21]. Another similar study done by Harshika *et al*., they are showing predominant organism isolated was *Escherichia coli* (23.2%), followed by

Pseudomonas (14%), *Klebsiella* (13.4%), *Staphylococcus aureus* (10.50%). Less commonly isolated were *Enterococcus species* (2.00%), *Enterobacter* (1.40%), and *Streptococcus pyogenes* (0.70%) [23]. One more study done by Rouf and Nazir, showing *E. coli* was most in gram-negative organism [22].

In the present study, antimicrobial susceptibility pattern among the Gram-negative organisms showed that *E. coli* was highly sensitive to Fosfomycin (86.15%) and Amikacin (75.65%) followed by Ertapenem (68.40%), Imipenem (56.70%), Meropenem (54.00%), Cefoperazone / Sulbactam (51.35%) & Minocycline (50.00%). Similar study done by Madigubba, *et al*., [21] they are showing highly sensitive to amikacin (83.00%), meropenem (81.00%), Cefoperazone-sulbactam (62.70%) and piperacillin-tazobactam (57.30%). Other antibiotics show similar sensitive to Ceftazidime and Ceftriaxone (20.80%), (16.10%) respectively. *E. coli* showing less sensitive to Gentamicin (25.00%). Another study done by Harshika *et al*., they are showing highly sensitive to Amoxyclav, Ciprofloxacin (44.00%), (62.00%) respectively. Amikacin showed highly resistance (38.00%) [23].

In the present study, *Klebsiella pneumoniae* shows sensitive for Amikacin (92.00%) followed by Tigecycline (60.00%), Gentamicin (48.00%), Levofloxacin & Minocycline (42.85%). According to another study by Harshika *et al.*, [23] showing Gentamicin (78.45%), Amoxyclav (44.00%), Ceftriaxone (38.00%) and Cefotaxime (34.00%). They are showing less sensitive to Amikacin (84.00%). One more study done by Madigubba *et al*., showing lesser sensitive to Amikacin (59.80%), Gentamicin (21.80%). Ciprofloxacin, Ceftazidime, Ceftriaxone were lesser resistant (65.50%), (72.40%), (74.50%) respectively [21].

In the present study, *S. aureus* showed (100%) sensitivity to Daptomycin, Teicoplanin, Vancomycin, Doxycycline, Minocycline followed by Rifampicin (96.50%), Linezolid (96.50 %), Tetracycline (95.00%), Gentamicin (79.40%) & Erythromycin (51.80%). The present study corallites with Rouf and Nazir, they are showing were highly sensitive to Vancomycin, Linezolid (100%), Tigecycline (60%-70%), Teicoplanin (50%-60%) [22]. Similar study done by Harshika *et al*., showing 100% sensitive to Amikacin, Gentamicin, Vancomycin, followed by Ciprofloxacin, Levofloxacin, Cefepime (92.00%), Amoxyclav (84.00%), Clindamycin (75.00%), Erythromycin (67.00%), Tetracycline (56.00%) [23].

CONCLUSION: In the present study *Escherichia coli,* followed by *Staphylococcus aureus* and *Klebsiella pneumonia* were the predominant isolates from various body fluids. Most of the Gram-positive isolates were resistant to Benzylpenicillin, Ciprofloxacin, Levofloxacin Oxacillin. Gram negative isolates were highly resistant to Amoxicillin/Clavulanic Acid, Cefuroxime, Cefuroxime Axetil, Cefotaxime, Ceftriaxone. Moreover, the rates of multiple drug-resistant isolates are alarmingly. Therefore, the study recommends hospitals to have strict antibiotics utilization policies and to support clinicians for rational choice of antibiotic therapy and to be updated with the present antimicrobial patterns of locality.

ACKNOWLEDGEMENT: Nil

Funding/Grant declared: Nil

Authors' Contribution: All authors contributed equally to the study.

Ethical Considerations: Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by authors.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Deb A, Mudshingkar S, Dohe V and Bharadwaj R: Bacteriology of body fluids with an evaluation of enrichment technique to increase culture–positivity. Journal of Evolution of Medical and Dental Sciences 2014; 3(72): 15230-15238. DOI:10.14260/jemds/2014/4050
2. Kitada K and Nishiyama A: Revisiting blood pressure and body fluid status. Clin Sci (Lond) 2023; 137(9): 755-767. doi: 10.1042/CS20220500.
3. Brinkman JE, Dorius B and Sharma S: Physiology, Body Fluids. In: StatPearls. Treasure Island (FL): StatPearls Publishing 2023; 27,
4. Grif K, Heller I, Prodinger WM, Lechleitner K, Lass-Flörl C and Orth D: Improvement of detection of bacterial pathogens in normally sterile body sites with a focus on orthopedic samples by use of a commercial 16S rRNA broad-range PCR and sequence analysis. J Clin Microbiol 2012; 50(7): 2250-4. doi: 10.1128/JCM.00362-12.

5. Badiee P: Evaluation of human body fluids for the diagnosis of fungal infections. Biomed Res Int 2013; 2013: 698325. doi: 10.1155/2013/698325. PMID: 23984401; PMCID: PMC3747334.
6. Dellinger RP, Levy MM and Rhodes A: Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013; 39(2): 165-228. doi:10.1007/s00134-012-2769-8
7. Shreekant T, Nanda M, Pattanaik S, Prasad A and Kar A: Bacteriological profile and antibiotic susceptibility pattern in sterile body fluids from a Tertiary Care Hospital in Bhubaneswar, Odisha, India: A Cross-sectional Study. JCDR. Journal of Clinical and Diagnostic Research 2023; 17(11): 20-23.
8. Sodani S and Hawaldar R: Bacteriological profile, and antibiotic sensitivity pattern in various body fluids–A retrospective study. Indian J Micro biol Res 2020; 7(1): 1–58. https://doi.org/10.18231/j.ijmr.2020.012
9. Marston HD, Dixon DM, Knisely JM, Palmore TN and Fauci AS: Antimicrobial Resistance. JAMA 2016; 316(11): 1193-1204. doi: 10.1001/jama.2016.11764. PMID: 27654605.
10. Iskandar K, Molinier L and Hallit S: Drivers of antibiotic resistance transmission in low- and middle-income countries from a "one health" perspective a review. Antibiotics 2020; 9(7): 372-375.
11. Dugassa J and Shukuri N: Review on Antibiotic Resistance, and its Mechanism of Development. JHMN [Internet]. 2017 1(3): 1-17. Available from:https://www.iprjb.org/journals/index.php/JHMN/article/view/560
12. Bujang MA: A step by step process on sample size determination for medical research. Malays J Med Sci 2021; 28(2): 15-27. doi: 10.21315/mjms2021.28.2.2. Epub 2021 Apr 21.
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 30th informational supplement, CLSI document M100. Wayne PA. Vol. S30; 2023
14. Deb A, Mudshingkar S, Dohe V and Bharadwaj R: Bacteriology of body fluids with an evaluation of enrichment technique to increase culture–positivity. Journal of Evolution of Medical and Dental Sciences 2014; 3(72): 15230-15238. DOI:10.14260/JEMDS/2014/4050
15. Mita D Wadekar MD, Sathish JV, Ayashree and Pooja C: Bacteriological profile of pus samples and their antibiotic susceptibility pattern. Indian Journal of Microbiology Research 2020; 7(1): 43–47.
16. Deboral A, Bhosale NK and Umadevi S: Aerobic Bacteriological and Antibiotic Susceptibility Profile of Pus Isolates from A Tertiary Care Hospital, Puducherry. J Pure Appl Microbiol 2020; 14(3): 1961-1966. doi: 10.22207/JPAM.14.3.35
17. Shume T, Tesfa T, Mekonnen S, Asmerom H, Tebeje F and Weldegebreal F: Aerobic bacterial profile and their antibiotic susceptibility patterns of sterile body fluids among patients at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia. Infect Drug Resist 2022; 15: 581-593. doi: 10.2147/IDR.S351961.
18. Singh P, Pandey A and Bisht AS: Sterile body fluids infections: Profile of bacterial and there antimicrobial resistance pattern in a tertiary care hospital from uttar Pradesh. Indian J Med Sci 2023; 75: 161-167. doi:10.25259/IJMS_63_2023
19. Sodani S and Hawaldar R: Bacteriological profile, and antibiotic sensitivity pattern in various body fluids –A retrospective study. Indian J Microbiol Res 2020; 7(1): 51-58. https://doi.org/10.18231/j.ijmr.2020.012
20. Shrestha LB, Bhattarai NR and Khanal B: Bacteriological profile and antimicrobial susceptibility pattern among isolates obtained from body fluids. J Nepal Health Res Counc 2019; 17(2): 173-177. doi: 10.33314/jnhrc.v0i0.1656. PMID: 31455930.
21. Madigubba H, Deepashree R, Sivaradjy M, Gopichand P and Sastry AS: Bacteriological profile and antimicrobial susceptibility pattern in sterile body fluid specimen from a tertiary care hospital, South India. J Curr Res Sci Med 2020; 1-8. DOI:10.4103/jcrsm.jcrsm_10_20
22. Rouf and Nazir: Aerobic Bacteriological Profile and Antimicrobial Sensitivity Pattern of Bacteria Isolated from Sterile Body Fluids: A Study from a Tertiary Care Hospital in North India. MRJI 2019; 28(1): 1-10.
23. Harshika YK, Shobha MKR, Patil AB and Smita NR: A study on 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. https://doi.org/10.18231/2394-5478.2018.0107

How to cite this article:
Chandra M and Paray AA: Bacterial profile and antibiogram of body fluids isolated from various IPDS patients. Int J Pharm Sci & Res 2024; 15(10): 3084-89. doi: 10.13040/IJPSR.0975-8232.15(10).3084-89.