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# Bacterial profile, their anti biogram and a light on emerging multi drug resistant microorganisms from sterile body fluids in a northern tertiary care hospital in India

## Abstract

Sterile body sites, if infected by micro-organisms then it can lead to severe morbidity and mortality. Infections caused by multidrug resistant (MDR) bacteria remain a public health threat for patients and health care workers. Therefore early diagnosis and prompt initiation of empiric treatment is necessary. This study was aimed to assess bacterial profiles and their antimicrobial susceptibility patterns (AST) from body fluids as well as looking for multidrug-resistant microorganisms.

**Methods:** A retrospective study was conducted from Jan 2017 to Dec 2017 by recruiting 1800 study samples. Different body fluids were collected and cultured on blood agar, MacConkey agar and chocolate agar and incubated aerobically and micro-aerobically. Moreover, gram staining and white blood cell count (WBC) were performed for all collected body fluid samples. Bacterial identification was made using colony morphology, gram stain and biochemical tests. Antimicrobial susceptibility testing was performed on Muller-Hinton agar using disk diffusion method.

**Results:** Overall 15% (n=270/1800) of the body fluids had bacterial growth. Most bacteria were isolated from Cerebrospinal Fluid (CSF) 56.4% and pleural fluids 34.3%. Most frequent bacterial isolates were K. pneumoniae 66.7% followed by, alpha *Streptococcus* 30% (n=8/54) and *E.coli*

Gram-negative and gram-positive bacteria showed highest resistance for Gentamycin (76%) and Erythromycin (59%) respectively. The MDR level recorded at 62.9%.

**Conclusion:** Significant numbers of bacteria with high MDR level were isolated from body fluids that call all health care workers and policy makers for concerted efforts for prudent antibiotic use, and limit the transmission of MDR bacteria in hospital and community settings. Regular monitoring of antimicrobial resistance patterns is essential. Lower rates of culture positivity may be occurred due to the presence of anaerobic; fastidious organisms with lack of enrichment techniques & prior antibiotic administrations.

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## Introduction

Body fluids are important in transporting nutrients as well as waste products, regulating body temperature and assessing respiration process.[1] Generally and normal circumstances, body fluids like cerebrospinal fluid (CSF), pleural, peritoneal, synovial and pericardial fluids are naturally free of microorganisms.[2] However, under infectious condition of central nervous system, peritoneum, joints and other sterile sites, could be inhabited by different species of bacteria, fungi, virus and parasites changing the physicochemical nature of the body fluids.[3] Body fluids like ascitic, pleural, synovial fluids, cerebrospinal and hydrocele are frequently sources of samples in microbiology for tracing the infections. These infections are associated with considerable morbidity and mortality. Number of positive cultures is low due to lesser number of pathogens as well as prior administration of empirical antibiotics in these samples.[4] For potentially pathogenic microorganism, even a single colony may be significant.[5] Different species of pathogenic bacteria belonging to Enterobacteriaceae, as well as Streptococcus pneumoniae, Neisseria meningitides, Group B Streptococci, Listeria monocytogenes, Haemophilus influenzae, Staphylococcus aureus, Acinetobacter and Pseudomonas spp. can invade the various organs and be present in the body fluids.[6–11] Body fluids invaded by such bacteria are characterized by having increased WBC count and protein concentration as well as decreased glucose concentration.[12] Though isolation and identification of bacterial etiologies are critical for patient management.[13] Developing resistance against commonly used antibiotics is becoming a challenge for treatment success.[14] Thus, the present study was undertaken to evaluate aerobic bacteriological profile along with their antibiogram with a focus on prevalence of Multi drug resistant (MDR) microorganisms in sterile body fluid.

## Materials and methods

This study was done on a retrospective basis for a period of one year from Jan 2017 to December 2017 in Department of Microbiology of a tertiary care hospital, New Delhi. A total of 1800 samples were analyzed. Pleural, peritoneal, cerebrospinal fluid (CSF), synovial and pericardial fluids were drawn using proper aseptic precautions and sent to Department of Microbiology, Within 2 hours of collection

different body fluids were cultured on Blood agar, MacConkey agar and chocolate agar and incubated aerobically and micro-aerobically (in a candle jar to provide 5-10% $CO_2$ concentration in order to give chance of growth for microareophilic fastidious bacteria). Moreover, gram staining and white blood cell count (WBC) were performed for all collected body fluids sample. Plates were examined daily for the growth of bacteria and identifications of bacterial isolates were performed using colony morphology, gram stain and conventional biochemical tests. Microorganisms isolated were identified by standard identification procedures[15] and their antimicrobial susceptibility were tested through Kirby Bauer's Disk Diffusion method and interpreted as per Clinical Laboratory Standard Institution (CLSI) guidelines.[16] The cultures were declared sterile if there was no growth on the plates after 48 hours of incubation as per the CLSI guidelines

### Drugs for gram positive cocci (GPC) pathogen

The antibiotics which were tested for GPC were Cefoxitin (30mcg), Ciprofloxacin (5mcg), Tetracycline (30mcg), Erythromycin (15mcg), Trimethoprim-sulfamethoxazole (1.25/23.75mcg), Vancomycin (30mcg) and Linezolid (30mcg).

### Drugs for gram negative bacilli (GNB) pathogen

Antibiotic and their quantities used for GNB included Ampicillin (10 mcg), Pipracillin/tazobactam (100/10mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefepime (30mcg), Ceftazidime/clavulanic acid (75/30mcg), Amikacin (30mcg), Gentamicin (10mcg), Netilmicin (30mcg), Ciprofloxacin (5mcg), Imipenem (10mcg), Meropenem (10mcg), Colistin (10mcg), Trimethoprimsulfamethaxazole (1.25/23.75mcg).

### Drugs for *Pseudomonas aeruginosa* pathogen

Antibiotics used for *Pseudomonas aeruginosa* were Piperacillin (100mcg), Piperacillin/tazobactam (100/10mcg), Ceftazidime (30mcg), Cefepime (30mcg), Amikacin (10mcg), Gentamicin (10mcg), Ciprofloxacin (5mcg), Imipenem (10mcg), Meropenem (10mcg), Netilmicin (30mcg), Colistin (10mcg). Oxacillin (methicillin) susceptibility of Staphylococcus aureus and Coagulase negative Staphylococci was interpreted using 30 µg cefoxitin as a surrogate test for Multidrug resistant Staphylococcus species.

### Quality control and quality assurance

Standard Operating Procedures (SOPs) were strictly followed verifying that media meet expiration date and quality control parameters. Visual inspections of cracks on media or plastic petridishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were checked. Quality control was performed to check the quality of medium. Each new lot was checked before use by testing *Escherichia coli* ATCC 25922 and/or *Staphylococcus aureus* ATCC 25923 standard control strains.

## Results

A total of 1800 samples were collected during the study period (Jan2017-Dec 2017). out of which 15% (270/1800) were shown to be source of bacterial growth and 85% were sterile after 48 hours of incubation. CSF= Cerebrospinal fluid, PL= Pleural fluid, AF= Ascitic fluid, SYNO= Synovial fluid

## Discussion

Normally sterile body sites such as pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid etc. can be infected by various pathogens. In this study 15 percent of samples provided positive result, through microbial culture. In comparison other studies conducted on similar lines, resulted at 31% and 24% .[16,17] Present study correlates with the study conducted by Paul Baurbeau[18] at 16.68%. Overall, from 1800 samples, 270 fluids samples provided growth of microorganisms with isolation rate of 15% (Figure 1). Out of 270 samples in our study, most commonly received fluid was Pleural (44.60%) followed by ascitic fluid (30.80%), cerebrospinal fluid (18.80%) and synovial fluid (5.70%) (Figure 2). Association of gender, age, patient types with culture results provided male predominance (50.7%) vs. female with only 35.6% positive culture. The most common age group showing culture positivity was <20 years. 70% culture positive samples were received from different wards (Table 1). Isolates from different fluids included *E.coli, Acinetobacter* spp., *Klebsiella* spp., *S. aureus, Enterococcus* spp., *Pseudomonas* spp. and *Citrobacter* spp, CONS etc. (Table 2). Antibiotic sensitivity pattern of different isolates is described in Table 3 & 4. In our study, the predominant Gram negative microorganisms were *E.coli* (67), *Klebseilla* spp (45), *Pseudomonas spp (31) Acinetobacter* spp. (24), Citrobacter spp.(16) and in Gram positives *S. aureus* (26), α Sterptococcus (26), Enterococcus spp. (13), *Pneumococcus pneumonia* (12) and CONS (10). In our study *E. coli* was the most common Gram negative microorganism isolated from body samples while in other studies conducted by Sujatha et al.[16,19] *E. coli* and *Klebsiella* spp. and *S.aureus* were the most common isolate respectively. Among the Gram negative isolates, *E.coli* was the most common isolate (n=67) followed by *Klebsiella spp.* (n=45). Similarly in several other studies *E.coli* was found to be the most common cause of infection in body fluids.[20,21] Detection of Coagulase negative Staphylococci & Acinetobacter spp. may be associated with a tendency of these pathogens to cause nosocomial infections, poor infection control practice in hospital, lack of standard facilities, poor sterilization of all gowns and equipment. It is known that gram-negative aerobic Entero bacteriaceae from the intestinal lumen can pass to mesenteric lymph nodes or other extra-intestinal sites across the intestinal-mucosal barrier and could appear in body fluids.[22] Among Staphylococcus aureus 49% were MRSA. Our study showed that gram negative isolates were mostly sensitive to Carbepenems (75-90%), Colistin (100%). *E.coli* isolates showed highest resistance to Fluoroquinolones, Cephalosporins, and moderate resistance to beta-lactambeta- lactamase inhibitors. *E.coli* isolates were highly resistant (>80) to Cephalosporins and Fluoroquinolones.[23] In the study reported by Tullu et al.[24] majority of the isolates were highly resistant (66%-100%) to Cephalosporins. We found that acinetobacter is coming up as the most resistant pathogens to many antibiotics as seen in some other studies.[25] In our study, gram positive bacteria were found to be highly resistant to erythromycin, cotrimoxa, cotrimoxazole and ciprofloxacin. The study also showed that *S. aureus* was found to be highly sensitive to vancomycin, linezolid. About 49% of *S. aureus* isolates in our study were MRSA, which is much similar to other studies performed in India.[26,27] The prevalence of MRSA continues to be increased worldwide, sometimes accounting for approximately 40-60% of all hospital acquired strains.[28] No vancomycin resistant (VRSA) or Vancomycin-intermediate resistant *S.aureus* (VISA) isolate was detected in our study. Our setup being a tertiary care hospital,

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the critical patients already have prior exposure to antibiotics, which could have resulted in high antimicrobial resistance. The high level of MDR resistance (50-75%) in this study in respect to different drugs is in agreement with studies conducted in India and outside India.[29] This high MDR level may be due to inappropriate use of commonly prescribed antibiotics.

**Table 1** Demographic data corresponding with collected samples

| Variable | Culture results (270/1800) | |
|---|---|---|
| | Positive no (%) | Negative no (%) |
| Gender | | |
| Male | 50.7 | 49.3 |
| Female | 35.6 | 64.4 |
| Age in years | | |
| <20 | 60 | 40 |
| 21-40 | 45 | 55 |
| 41-60 | 23 | 77 |
| >61 | 10 | 90 |
| Patient Type | | |
| Inpatient | 70 | 30 |
| Outpatient | 40 | 60 |

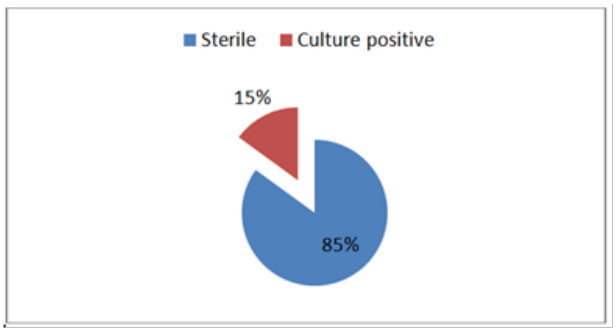


**Figure 1** Prevalence of positive samples in the study population.

**Table 2** Distribution of different microorganisms isolated from different samples

| Organisms | Total | Pleural fluid | Ascitic fluid | CSF | Synovial fluid |
|---|---|---|---|---|---|
| E.coli | 67 | 23 | 21 | 14 | 9 |
| Klebsiella spp. | 45 | 18 | 16 | 8 | 3 |
| Pseudomonas spp. | 31 | 15 | 10 | 3 | 3 |
| Acinetobacer spp. | 24 | 11 | 8 | 3 | 2 |
| Citrobacter spp. | 16 | 8 | 3 | 3 | 2 |
| S.auerus | 26 | 10 | 10 | 4 | 2 |
| Enterococcus spp. | 13 | 5 | 5 | 3 | 1 |
| CONS | 10 | 4 | 3 | 2 | 0 |
| Pneumococcus pneumoniae | 12 | 9 | 3 | 0 | 0 |
| α Streptococcus | 26 | 15 | 6 | 4 | 2 |

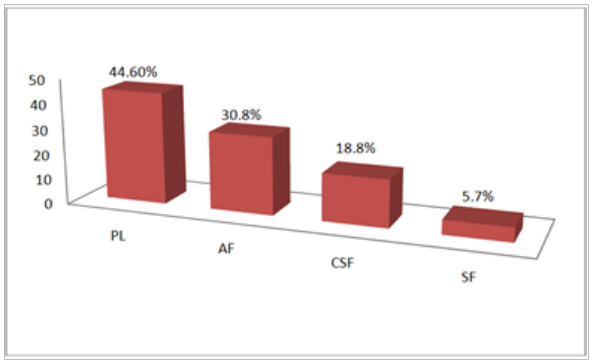


**Figure 2** Sample Profile & Rate of Positive Culture from Different Samples.

**Table 3** Rate of wise antimicrobial resistance pattern in gram-negative bacteria isolates (n=270) from body fluids samples

| Bacterial isolate (no.) | GN | CIP | CTX | CRO | CTZ | CPR | AMK | IMP | MRP | CFS | PIT | COL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E.coli (67) | 60 | 78 | 56 | 40 | 33 | 68 | 20 | 18 | 15 | 10 | 9 | 0 |
| Klebsiella spp (45). | 55 | 82 | 58 | 42 | 23 | 78 | 35 | 20 | 19 | 22 | 25 | 0 |
| Pseudomonas spp. (31) | 78 | 46 | 89 | 70 | 45 | 23 | 25 | 21 | 11 | 15 | 20 | 0 |
| Acinetobacer spp. (24) | 66 | 58 | 45 | 55 | 32 | 45 | 20 | 14 | 10 | 7 | 9 | 0 |
| Citrobacter spp. (16) | 78 | 66 | 88 | 54 | 49 | 78 | 38 | 23 | 21 | 17 | 11 | 0 |

**Table 4** Rate of wise antimicrobial resistance pattern ingram-positive bacterial isolates from body fluids samples

| Bacterial isolate (no.) | AM | P | CIP | ERY | CLN | CPR | COT | OX | C | VA | LZ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S. aureus (26) | 70 | 90 | 78 | 56 | 76 | 56 | 43 | 49 | - | 0 | 0 |
| Enterococcus spp. (13) | 13 | - | 65 | 75 | 66 | 34 | - | - | 41 | 0 | 0 |
| CONS(10) | 28 | 59 | 46 | 55 | 47 | 29 | 33 | - | - | 0 | 0 |
| Pneumococcus pneumonia(12) | 0 | 0 | 2 | 7 | 4 | 2 | 15 | - | - | 0 | 0 |
| α Streptococcus (26) | 11 | 10 | 12 | 19 | 14 | 23 | 28 | - | - | 0 | 0 |

AMP, Ampicillin; P, Penicillin; ERT, Erythromycin; CLN, Clindamycin; COT, Cotrimoxazole; OX, Oxacillin; C, Chloramphenicol; VA, Vancomycin; LZ, Linezolid

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## Conclusion

In conclusion, the yield of body fluids cultures is usually very low. Low culture positivity may be due to presence of anaerobic or fastidious organisms with lack of enrichment techniques & prior antibiotic administration. Regular monitoring of prevalent pathogenic organisms and their sensitivities will aid the clinicians in appropriate selection of antibiotic therapy in absence of a culture report and further prevent the development of antibiotic resistance. Surveillance of the incidence, microbial profile and antibiotic resistance pattern of sterile body fluids infections in a particular population is an essential part for the selection of the most appropriate empiric antibiotic regimen and to prevent selective pressure as well as further development of resistance in these pathogens.

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## Conflict of interest

Author declares that there is no conflict of interest.

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