Bacteriological Profile and Antimicrobial Sensitivity pattern in Sterile Body Fluids from a Tertiary Care Hospital

Abstract

Background: Sterile body sites, if infected by micro-organisms than it can lead to severe morbidity and mortality. Therefore early diagnosis and prompt initiation of empiric treatment is necessary.

Aim: This study was done to evaluate causative organisms of sterile body site infections and their antimicrobial sensitivity pattern in a tertiary care hospital, New Delhi.

Settings and design: Prospective study over a period of one year from January 2015 to December 2015.

Material and methods: Sterile body fluid specimens were processed for bacterial culture according to the standard procedures and antimicrobial susceptibility test for isolated organisms was done using agar disk diffusion method.

Results: Amongst 405 samples, 122 fluids samples showed growth of organisms with an isolation rate of 30%. Isolates from different fluids were E. coli (28.6%), Acinetobacter spp. (27%), Klebsiella spp. (19.6%), Staphylococcus aureus (10.6%), Enterococcus spp. (7.3%), Pseudomonas spp. (4.9%) and Citrobacter spp. (1.6%). Gram negative isolates were mostly sensitive to carbepenems, colistin and polymyxin B (100%) and gram positive isolates were highly sensitive to vancomycin (100%), linezolid (100%) and ciprofloxacin (70%). Acinetobacter was the most resistant pathogens to many antibiotics. About 38.5% of S. aureus isolates in our study were MRSA.

Conclusion: Therefore, knowledge of bacteriological and antimicrobial profile of sterile body fluids is important so that such life threatening infections can be treated effectively on an urgent basis.

Keywords: Acinetobacter; Antimicrobial resistance; MRSA; Sterile body fluids

Introduction

Infections of the sterile body sites typically have greater clinical urgency and these infections could be life-threatening [1,2]. Different types of microorganisms like bacteria, fungi, virus and parasites may invade and infect the body fluids resulting in severe morbidity and mortality. For potentially pathogenic microorganism, even a single colony may be significant [3]. The common pathogenic bacteria of concern are Escherichia coli, Acinetobacter spp., Klebsiella spp., Staphylococcus aureus and Enterococcus spp. Therefore, it is necessary to monitor the epidemiology of bacterial susceptibility pattern in each area, so that such infections must be treated by the empirical use of antimicrobial drugs as soon as possible to reduce the morbidity and mortality. This study was done for identifying the bacterial pathogens and their antimicrobial susceptibility pattern in the patients admitted in a tertiary care Hospital, New Delhi.

Materials and Methods

This study was done on a prospective basis for a period of one year from Jan 2015 to December 2015 in Department of Microbiology...
of a tertiary care hospital, New Delhi. A total of 405 samples were analyzed. Pleural, peritoneal, synovial and pericardial fluids were drawn using proper aseptic precautions and sent to Department of Microbiology, within 2 hours of collection.

Sample Processing

Pleural fluid, peritoneal fluid, synovial fluid and pericardial fluid were processed in laboratory using standard microbiological procedures. Blood agar, Mac-Conkey agar and chocolate agar (HiMedia, Mumbai, India) were used for culture with the purpose of obtaining isolated colonies. The isolated colonies were then identified using standard biochemical tests [3].

Antimicrobial susceptibility test: The antimicrobial susceptibility test was performed for isolated organisms by Kirby Bauer’s disk diffusion method according to clinical and laboratory standard institute (CLSI, 2014) guidelines [4]. The routine antimicrobial sensitivity tests were put for the following antibiotics:

**Drugs for GPC pathogen**

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

**Drugs for GNB pathogen**

For GNB Ampicillin (10 mcg), Piperacillin/tazobactam (100/10 mcg), Ceftazidime (30 mcg), Ceftriaxone (30 mcg), Cefepime (30 mcg), Ceftazidime/clavulanic acid (75/30 mcg), Amikacin (30 mcg), Gentamicin (10 mcg), Netilmicin (30 mcg), Ciprofloxacin (5 mcg), Imipenem (10 mcg), Meropenem (10 mcg), Aztreonam (30 mcg), Polymyxin B (300 mcg), Colistin (10 mcg), Trimethoprim-sulfamethaxazole (1.25/23.75 mcg).

**Drugs for Pseudomonas aeruginosa pathogen**

Antibiotics used for *Pseudomonas aeruginosa* were Piperacillin (100 mcg), Piperacillin/tazobactam (100/10 mcg), Ceftazidime (30 mcg), Cefepime (30 mcg), Amikacin (10 mcg), Gentamicin (10 mcg), Ciprofloxacin (5 mcg), Imipenem (10 mcg), Meropenem (10 mcg), Netilmicin (30 mcg), Aztreonam (30 mcg), Polymyxin B (300 mcg), Colistin (10 mcg).

**Results**

A total 405 different body fluid were collected from suspected patients, which included pleural fluid, peritoneal fluid, synovial fluid and pericardial fluid. Amongst 405 samples, 122 fluids samples showed growth of organisms with an isolation rate of 30% (Table 1). Isolates from different fluids were *E.coli*, *Acinetobacter* spp., *Klebsiella* spp., *S. aureus*, *Enterococcus* spp., *Pseudomonas* spp. and *Citrobacter* spp. (Table 2). Antibiotic sensitivity pattern of different isolates is shown in (Table 3). Gram negative isolates were mostly sensitive to Carbapenems, Colistin and Polymyxin B (100%). *E.coli* isolates showed highest resistance to Cephapelorins, Fluoroquinolones and moderate resistance to beta-lactam-beta-lactamase inhibitors. According to Barai L et al. [13] *E.coli* isolates were highly resistant (>80) to Cephapelorins and Fluoroquinolones. In Tullu et al. [14] study too, majority of the isolates were highly resistant (66%-100%) to Cephapelorins. *Klebsiella* spp. showed least resistance to Carbapenems, Tigecycline and moderate resistance to aminoglycosides, and beta-lactam beta-lactamase inhibitor combination. In our study, more than 95% of *Pseudomonas* isolates were sensitive to Piperacillin-tazobactam and more than 80% of the isolates were sensitive to Meropenem, Imipenem and Ticarcillin.

**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 30% samples give culture positive result, which is in comparison to other studies conducted on similar lines, were 31% and 24% positive results [5,6]. A total of 405 samples were studied out of which, 156 were peritoneal fluids, 140 were pleural fluids, 93 were synovial fluids and 16 were pericardial fluid samples. In our study, the predominant organisms were *E.coli* (28.6%) and *Acinetobacter* spp. (27%), followed by *Klebsiella* spp. (19.6%), *S. aureus* (10.6%), *Enterococcus* spp. (7.3%), *Pseudomonas* spp. (4.9%) and *Citrobacter* spp. (1.6%). In our study *Acinetobacter* spp. and *E. coli* were the commonest organisms isolated from pleural effusion samples while other studies done by Sujatha et al. [5] and Evan et al. [7] found *E. coli* and *Klebsiella* spp. and *S.aureus* as the most common isolate respectively.

Gram negative organisms (90%) were more commonly isolated from ascitic fluids than Gram positive organisms (9%). Among the Gram negative isolates, *E.coli* was most common isolate (35%) followed by *Acinetobacter* spp. (26.8%) and *Klebsiella* spp. (21.9%). Similarly in several other studies *E.coli* was found to be the most common cause of ascitic fluid infection [5,8,9].

On synovial fluid, there were many studies conducted by authors [10,11] that found *S.aureus* as the most predominant isolates 55% and 30% respectively. We found *S.aureus*, *Klebsiella* spp. and *Enterococcus* spp. with the isolation rate of 68.7%, 18.7% and 12.5% respectively. In a study from South Africa, [12] *S.aureus* and *Salmonella* spp. were commonly isolated from pericardial fluid samples. In the present study, *Klebsiella* spp. was isolated in 12% of pericardial fluid samples.

In our study showed that gram negative isolates were mostly sensitive to Carbapenems, Colistin and Polymyxin B (100%). *E.coli* isolates showed highest resistance to Cephapelorins, Fluoroquinolones and moderate resistance to beta-lactam-beta-lactamase inhibitors. According to Barai L et al. [13] *E.coli* isolates were highly resistant (>80) to Cephapelorins and Fluoroquinolones. In Tullu et al. [14] study too, majority of the isolates were highly resistant (66%-100%) to Cephapelorins. *Klebsiella* spp. showed least resistance to Carbapenems, Tigecycline and moderate resistance to aminoglycosides, and beta-lactam beta-lactamase inhibitor combination. In our study, more than 95% of *Pseudomonas* isolates were sensitive to Piperacillin-tazobactam and more than 80% of the isolates were sensitive to Meropenem, Imipenem and Ticarcillin.

**Table 1 Different type of samples.**

| Samples          | Total no. of samples | Growth | No growth |
|------------------|----------------------|--------|----------|
| Pleural fluid    | 140                  | 22     | 106      |
| Peritoneal fluid | 156                  | 82     | 86       |
| Synovial fluid   | 93                   | 16     | 77       |
| Pericardial fluid| 16                   | 2      | 14       |
| Total            | 405                  | 122    | 283      |
Table 2 Different organisms isolated from different samples.

| Organisms       | Total | Pleural Fluid- 140 (22) | Peritoneal Fluid- 156 (82) | Synovial Fluid 93 (16) | Pericardial Fluid 16 (2) |
|-----------------|-------|-------------------------|----------------------------|------------------------|--------------------------|
| E.coli          | 35    | 6                       | 29                         | -                      | -                        |
| Klebsiella spp. | 24    | 2                       | 18                         | 3                      | 2                        |
| Pseudomonas spp.| 6     | 2                       | 4                          | -                      | -                        |
| Acinetobacter spp. | 33  | 11                      | 22                         | -                      | -                        |
| Citrobacter spp.| 2     | -                       | 1                          | -                      | -                        |
| S.aureus        | 13    | 1                       | 1                          | 11                     | -                        |
| Enterococcus spp.| 9   | -                       | 7                          | 2                      | -                        |

Table 3 Antibiotic sensitivity patterns of isolates *MIC determination by E-test.

| Drugs | Acinetobacter | Klebsiella | E.coli | Pseudomonas | S. aureus | Enterococcus |
|-------|---------------|------------|--------|-------------|-----------|--------------|
| AK    | 75            | 68         | 63     | 100         | ND        | ND           |
| GEN   | 78            | 63         | 67     | 80          | ND        | 64           |
| CAZ   | 40            | 55         | 47     | 80          | ND        | ND           |
| CIP   | 68            | 71         | 61     | 76          | 79        | 68           |
| COT   | 64            | 54         | 49     | ND          | 65        | ND           |
| PT    | 68            | 81         | 72     | 95          | ND        | ND           |
| IMP   | 90            | 89         | 79     | 100         | ND        | ND           |
| CPM   | 89            | 83         | 81     | ND          | ND        | ND           |
| NET   | 87            | 79         | 82     | 94          | ND        | ND           |
| CL    | 100           | 100        | 100    | 100         | ND        | ND           |
| PB    | 100           | 100        | 100    | 100         | ND        | ND           |
| AT    | ND            | 71         | 69     | 63          | ND        | ND           |
| CX    | ND            | 78         | 75     | ND          | 61.5      | ND           |
| E     | ND            | ND         | ND     | ND          | 56        | ND           |
| T     | ND            | ND         | ND     | ND          | 61        | 23.7         |
| VA*   | ND            | ND         | ND     | ND          | 100       | 100          |
| LZ    | ND            | ND         | ND     | ND          | 100       | 100          |

We found that acinetobacter was the most resistant pathogens to many antibiotics as seen in some other studies [15]. Acinetobacter is an important public health problem, especially in patients on broad spectrum antimicrobial therapy and requiring life support [16,17]. A study has shown that multi drug resistant Acinetobacter isolates are commonly seen in ICUs and may cause severe infections with a high mortality rate [18].

In our study, gram positive organisms were found to be highly resistant to erythromycin and tetracycline. The study also showed that S. aureus was found to be highly sensitive to vancomycin, linezolid and ciprofloxacin. About 38.5% of S. aureus isolates in our study were MRSA, which is much similar to other studies performed in India [19,20]. The present study shows antibiotic resistance pattern within India, and has been conducted specifically on sterile body fluids, whereas others were done on a wide variety of sterile and non-sterile clinical specimens [21,22]. Therefore, the present findings can serve as an index of actual antibiotic resistance specifically in sterile body fluids.

The prevalence of MRSA continues to increase worldwide, sometimes accounting for approximately 40-60% of all hospital acquired strains [23]. No vancomycin resistant (VRSA) or Vancomycin-intermediate resistant S.aureus (VISA) isolates were detected in our study. There could be many explanations for such differences, like effective infection control measures, antibiotic prophylaxis and treatments policy in hospital. While there are reports around the world indicating a tendency toward decreasing susceptibility to Vancomycin in S.aureus, [24] we had no VRSA or VISA isolates. This may be due to judicious and controlled use of Vancomycin in our hospital.

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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