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

Abstract
Sterile body sites, if infected by micro-organisms than 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. Hence, this study aimed at assessing bacterial profiles and their antimicrobial susceptibility patterns (AST) from body fluids and also looks for multidrug resistant organisms.

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 then incubated aerobically and micro-aerobically. Moreover, gram staining and White blood cell count (WBC) were performed for all collected body fluids sample. 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 streptococcus30% (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 was 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 with a focus on prevalence of anaerobic; fastidious organisms with lack of enrichment techniques & prior antibiotic administrations.

Introduction
Body fluids are important in transporting nutrients as well as waste products, regulating body temperature and assessing respiration process. Generally, body fluids like cerebrospinal fluid (CSF), pleural, peritoneal, synovial and pericardial fluids are naturally free of microorganisms under normal circumstance. However, under infectious condition of central nervous system, peritoneum, joints and other sterile sites, different types of bacteria, fungi, virus and parasites could present and change the physicochemical nature of the body fluids. Body fluids like ascitic, pleural, synovial fluids, cerebrospinal and hydrocele are frequently received samples in the microbiology laboratory for culture in suspected infections. These infections are associated with considerable morbidity and mortality. Positive cultures are low because of less number of pathogens as well as prior administration of empirical antibiotics in these samples. For potentially pathogenic microorganism, even a single colony may be significant. Different pathogenic bacteria like Enterobacteriaceae, Streptococcus pneumoniae, Neisseria meningitides, Group B Streptococci, Listeria monocytogenes, Haemophilus influenzae, Staphylococcus aureus, Acinetobacter and Pseudomonas spp. can invade the various organ and present in the body fluids. Body fluids invaded by such bacteria are characterized by having increased WBC count and protein concentration as well as decreased glucose concentration. Though isolation and identification of bacterial etiologies are critical for patient management, Developing resistance against commonly used antibiotics is becoming a challenge for treatment success. 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) organisms 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 collected and cultured on Blood agar, MacConkey agar, MacConkey agar and chocolate agar then incubated aerobically and micro-aerobically.
aggar and chocolate agar then incubated aerobically and micro-aerobically (in a candle jar to provide 5-10% CO2 concentration in order to give chance of growth for microaerophilic 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 from colonies (from culture plates) and conventional biochemical tests. Organisms isolated were identified by standard identification procedures13 and their antimicrobial susceptibility testing were done for the isolates by 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**

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

**Drugs for Pseudomonas aeruginosa pathogen**

Antibiotics used for Pseudomonas aeruginosa were Piperacillin (100mcg), Piperacillin/tazobactam (100/10mcg), Cefazidime (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 was checked. Quality control was performed to check the quality of medium. Each new lot was checked before use by verifying that media meet expiration date and quality control tests. Standard control strains were used to check the quality of media. Each new lot was checked before use by Kirby Bauer’s Disk Diffusion method and interpreted as per CLSI guidelines. The antibiotics used for GPC were Cefoxitin (30mcg), Ciprofloxacin (5mcg), Tetracycline (30mcg), Erythromycin (15mcg), Trimethoprim-sulfamethoxazole (1.25/23.75mcg), Vancomycin (30mcg) and Linezolid (30mcg).

**Results**

A total of 1800 samples were received during the study period (Jan2017-Dec 2017). Out of which 15% (270/1800) were shown 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% samples give culture positive result, which is in comparison to other studies conducted on similar lines, were 31% and 24% positive results.16,17 Present study correlates with the study conducted by Paul baureau18 with rate of 16.68%. Amongst 1800 samples, 270 fluids samples showed growth of organisms with an isolation rate of 15% (Figure 1). Out of 270 samples in our study, most commonly received fluid is as 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 showed male predominance (50.7%) while in female only 35.6% were culture positive. 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 were 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 shown in Table 3 & 4. In our study, the predominant Gram negative organisms were E.coli (67), Klebsiella spp (45), Pseudomonas spp (31) Acinetobacter spp. (24), Citrobacter spp.(16) and in Gram positive S. aureus (26), a Strepococcus (26), Enterococcus spp. (13), Pneumococcus pneumonia (12) and CONS (10). In our study E. coli was the commonest Gram negative organism isolated from body samples while other studies done by Sujatha et al.16,19 found E. coli and Klebsiella spp. and S.aureus as the most common isolate respectively. Among the Gram negative isolates, E.coli was 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 The 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 Enterobacteriaceae 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 Carbapenems (75-90%), Colistin (100%). E.coli isolates showed highest resistance to Fluoroquinolones, Cephalosporins, and moderate resistance to beta-lactam-beta- lactamase inhibitors. E.coli isolates were highly resistant (>80) to Cephalosporins and Fluoroquinolones.23 In Tullu et al.24 study too, majority of the isolates were highly resistant (66%-100%) to Cephalosporins. We found that acinetobacter is upcoming as the most resistant pathogens to many antibiotics as seen in some other studies.25 In our study, gram positive organisms were found to be highly resistant to erythromycin, cotrimoxan our study, gram positive organisms were found to be highly resistant to erythromycin, cotrimoxazol 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 increase worldwide, sometimes accounting for approximately 40-60% of all hospital acquired strains.28 No vancomycin resistant (VRSA) or Vancomycin-intermediate resistant S.aureus (VISA) isolates were detected in our study. Our setup being a...
tertiary care hospital, 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 study conducted in India and outside India. This high MDR level may be due to inappropriate use of commonly prescribed antibiotics.

Table 1: Distribution of different organisms isolated from different samples

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total</th>
<th>Pleural fluid</th>
<th>Ascitic fluid</th>
<th>CSF</th>
<th>Synovial fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>67</td>
<td>23</td>
<td>21</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>45</td>
<td>18</td>
<td>16</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>31</td>
<td>15</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>24</td>
<td>11</td>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>16</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>S.aureus</td>
<td>26</td>
<td>10</td>
<td>10</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>13</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>CONS</td>
<td>10</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pneumococcus pneumoniae</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>α Streptococcus</td>
<td>26</td>
<td>15</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 1: Prevalence of positive sample in the study population.

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

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

Table 4: Percentage wise antimicrobial resistance pattern of gram-positive bacteria isolates from body fluids samples

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

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

Citation: Sharma B, Kasana D, Ganbir S. Bacterial profile, their anti biogram and a light on emerging multi drug resistant organisms from sterile body fluids in a northern tertiary care hospital in India. J Bacteriol Mycol Open Access. 2018;6(4):249-252. DOI: 10.15406/jbmoa.2018.06.00213
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 infusions 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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None.

Conflict of interest

Author declares that there is no conflict of interest.

References


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