A study on bacteriological profile and antimicrobial resistance pattern from various body fluids of patients attending the tertiary care Hospital, KIMS, Hubli

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Abstract
Introduction: Body fluids like pleural fluid, ascitic fluid, cerebrospinal fluid etc are usually sterile, but they can get infected by different micro organisms, thus leading to life threatening infections.¹²

Aims and Objectives: 1) To isolate the causative organisms of sterile body fluid infections. 2) To determine the antimicrobial resistance pattern of the isolates.

Materials and Methods: 1) This is a retrospective observational study, conducted from January 2017 to December 2017 in the department of Microbiology KIMS Hubli. 2) Sterile body fluids were processed according to Standard laboratory Procedures. 3) Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method as per CLSI guidelines.

Results: A total of 635 samples were studied out of which 233(36.6%) were pleural fluids, 222(34.9%) were ascitic fluids, 174(27.4%) were cerebrospinal fluids and 06(0.9%) were pericardial fluid. In our study the predominant organisms were E-coli (23.23%) and NFGNB (19.01%) followed by Pseudomonas (14.08%), Klebsiellaspp (13.38%), S.aureus (10.56%) and Citrobacter spp (7.04%). Gram negative isolates were 100% sensitive to Imipenem followed by Amikacin (78%), Gentamicin (74%), Cefepime (69%). The Gram positive isolates were 100% sensitive to Vancomycin and Linezolid followed by Gentamicin (96%), Cefepime (94%), Amikacin (92%). The Pseudomonas isolates were highly sensitive to Imipenem (96%) and Piperacillin Tazobactum (92%). High resistance was observed to Ceftazidime, Cefoperazone and Levofloxacin.

Conclusion: Knowledge of bacteriological and antimicrobial profile of sterile body fluids is necessary, so that the life threatening infections can be effectively treated and thus to prevent antimicrobial resistance associated with it.

Keywords: Sterile Body fluids, Antimicrobial resistance, E-coli, NFGNB, Kirby-Bauer disk diffusion method.

Introduction
Body fluids like pleural fluid, peritoneal fluid, CSF, synovial fluid and pericardial fluid are usually sterile. There are certain common pathogenic bacteria like E.coli, Klebsiella species, Haemophilus influenza, Staphylococcus aureus, Neisseria Meningitidis, NFGNB (Non fermenting Gram Negative Bacillus), Pseudomonas, Acinetobacter, which invade and infect the sterile body fluids leading to morbidity and life threatening infections.¹²

Hence infections of sterile body fluids are a medical emergency and needs an early diagnosis and effective treatment. Moreover for the better management of patients and framing the antibiotic policy, the knowledge of prevalent strains along with their antimicrobial resistant pattern is essential.

As of now, there are very limited data on bacterial profiles and their antimicrobial susceptibility pattern from body fluids in our geographical area. Hence assessing bacterial profiles and antimicrobial sensitivity pattern from body fluids is very crucial to clinicians, Microbiologists, Pharmacists and Policy makers for proper diagnosis of different infections and for prudent antibiotic use.

So the present study was undertaken to know the current status of bacterial profile and their susceptibility patterns from various body fluids collected from patients attending our tertiary care hospital.
Drugs for Gram Positive Cocci: Cefoxitin, Ciprofloxacin, Gentamicin, Cefotaxime, Cefepime, Tetracycline, Erythromycin, Clindamycin, Cotrimoxazole, Amoxicillin Clavulanate, Linezolid and Levofloxacin, Vancomycin.

Drugs for Gram Negative Bacilli: Ampicillin, Amoxyclov, Cefotaxime, Ceftriaxone, Cefepime, Ceftazidime, Amikacin, Gentamicin, Imipenem, Levofloxacin, Cotrimoxazole.

Drugs for Pseudomonas: Piperacillin, Pipericillin tazobactum, Ceftazidime, Cefepime, Celpodoxime, Cefeperrazon, Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Imipenem, Aztreonam.

Results
A total of 635 samples were collected from suspected patients which included pleural fluid, peritoneal fluid, Cerebrospinal fluid (CSF) and pericardial fluid. Out of 635 samples processed, 142 fluids showed growth with isolation rate of 22%. The most common received fluid was pleural fluid (36.6%), followed by Ascitic fluid (34.9%), Cerebrospinal fluid (27.4%) and Pericardial fluid (0.9%).

Out of 142 culture positive samples, the predominant organism isolated was Escherichia coli (23.2%), followed by NFGNB (19%) and Pseudomonas (14%). Klebsiella (13.4%), Staphylococcus aureus (10.5%). Less commonly isolated were Enterococcus species (2%), Enterobacter (1.4%), and Streptococcus pyogenes (0.7%).

The Gram negative bacterial isolates showed multidrug resistance pattern (MDR) but they were 100% sensitive to Imipenem. They showed high resistance to Cephalosporins like ceftriaxone and cefotaxime and also to Amoxyclov, cotrimoxazole and Ampicillin.

Multi drug resistance pattern was not much observed in Gram positive isolates compared to Gram Negative isolates. They were relatively resistant to Tetracycline, Erythromycin, Clindamycin and Ampicillin. They were 100% sensitive to Vancomycin and Linezolid.

The Pseudomonas isolates showed high resistance to Cephalosporins and fluoroquinolones. They were highly sensitive to Imipenem (96%) and Pipericillin tazobactum (92%).

Table 1: Growth pattern of various body fluids

| Fluid                  | Total number of samples | Growth | No Growth |
|------------------------|-------------------------|--------|----------|
| Pleural fluid          | 233                     | 59     | 174      |
| Ascitic fluid          | 222                     | 56     | 166      |
| Cerebrospinal fluid    | 174                     | 23     | 151      |
| Pericardial fluid      | 06                      | 04     | 02       |
| Total                  | 635                     | 142    | 493      |

Table 2: Bacterial profile in different body fluid samples

| Organisms              | Total Number | Pleural fluid | Ascitic fluid | Cerebrospinal fluid | Pericardial fluid |
|------------------------|--------------|---------------|---------------|---------------------|-------------------|
| E.coli                 | 33           | 16            | 09            | 06                  | 01                |
| Klebsiella species     | 21           | 06            | 09            | 06                  | 01                |
| NFGNB                  | 27           | 10            | 13            | 03                  | 01                |
| Pseudomonas aeruginosa | 20           | 09            | 09            | 02                  | --                |
| Citrobacter species    | 13           | 06            | 05            | 01                  | --                |
| Staphylococcus aureus  | 15           | 10            | 03            | 02                  | --                |
| CONS                   | 08           | 02            | 05            | 01                  | 01                |
| Enterobacter species   | 02           | 01            | --            | --                  | --                |
| Streptococcus pyogenes | 01           | 00            | --            | --                  | --                |
| Enterococcus species   | 03           | 03            | --            | --                  | --                |

Table 3: Antimicrobial sensitivity pattern of Gram negative bacterial (GNB) isolates. (n=94)

| Drugs               | Escherichia coli | Klebsiella species | NFGNB | Citrobacter species | Enterobacter species |
|---------------------|------------------|--------------------|-------|---------------------|---------------------|
| Ampicillin          | 9%               | 0%                 | 30%   | 22%                 | 100%                |
| Amoxyclov           | 44%              | 44%                | 63%   | 64%                 | 100%                |
| Amikacin            | 62%              | 84%                | 74%   | 92%                 | 100%                |
| Gentamicin          | 58%              | 78%                | 66%   | 92%                 | 100%                |
| Ciprofloxacin       | 62%              | 56%                | 70%   | 86%                 | 100%                |
| Levofloxacin        | 56%              | 44%                | 56%   | 100%                | 100%                |
| Cefotaxime          | 18%              | 34%                | 58%   | 58%                 | 0%                  |
| Ceftraxone          | 18%              | 38%                | 58%   | 58%                 | 0%                  |
The emergence of antibiotic resistance to the study was more commonly reported in other studies, which showed a high rate of isolation of GNB from pleural fluid. Prior to the availability of antibiotics, the predominant organisms were Escherichia coli, Pseudomonas, and Enterobacter. As well as their antibiotic resistance patterns may change from time to time and place to place. The emergence of antibiotic-resistant organisms, the increase in the frequency of nosocomial infections, and increasing number of immune-compromised patients have combined to keep pleural and ascitic fluid infections a common entity.

In our study, 22% of samples gave culture positive results, which is in comparison to other studies, which were 30%, 31% positive results.

In our study the predominant organisms were Escherichia coli (33), NFGNB (27), followed by Pseudomonas (20), Klebsiella (19), Staphylococcus aureus (15), Citrobacter species (13), CONS (8), Enterococcus (4), Enterobacter (2), Streptococcus pyogenes (1).

The most common organism isolated from pleural fluid were Escherichia coli (16), NFGNB (10), Staphylococcus aureus (10), Pseudomonas (12). Our study findings correlates with the study of Rajani et al where predominant organism was E-coli followed by Acinetobacter. Our results are in contrast to studies of Sujatha R et al and Evan et al where in E-coli, Klebsiella and Staphylococcus aureus were the most common isolates.

Our study highlights the emergence of aerobic gram negative bacteria as the predominant pathogens in empyema. A similar high rate of isolation of GNB from pleural fluid cultures was reported in India by Sonali Mohanty et al, which showed E-coli as the commonest organism. Our results show that NFGNB is more prevalent in ascitic fluid samples in our geographical area. However further studies with more sample size has to be done to find the common etiological agents of ascitic fluid in our set-up.

Bacterial meningitis is being reported predominantly in adults in USA because of immunization practices adopted and also due to relative increase in frequency of nosocomial meningitis. In North America and Europe because of the vaccine related decline in H.influenzae disease, Streptococcus pneumonia and Nesseriae meningitides remain important pathogens in Children and young adults. Group B streptococcus is the most common pathogen associated with meningitis in newborns. Listeria...
monocytogenes is also recognized as a significant cause of meningitis in newborns and the elderly in the United States. As compared to western studies, the relative incidence of meningitis caused by H.influenzae, Nessieriae meningitides and listeria is less in South East Asia.

On the contrary Gram Negative bacilli such as E.coli Klebsiella pneumonia and Pseudomonas aeruginosa are increasingly being reported in cases of meningitis especially among elderly and in patients with cirrhosis, diabetes and malignancies.[14–16] Interestingly, similar findings was observed in our study where E.coli and Klebsiella species were commonly isolated from CSF followed by NFGNB and Staphylococcus aureus.

In our hospital very few pericardial fluid samples were received, only 1% compared to other body fluids. Out of 6 samples received only 4 samples showed growth. CONS, E –coli, Klebsiella, NFGNB were isolated which is in contrast to the studies of H Reuter et al[17] where Staphylococcus aureus and Salmonella species were commonly isolated. Bacterial infections of pericardium are relatively uncommon; however they are more much likely to form purulent effusions and proceed to cardiac tamponade or pericardial constriction. Purulent pericarditis is almost exclusively seen as a secondary infection in the patients with seriously underlying diseases such as AIDS and those undergoing hemodialysis, thoracic surgery, and chemotherapy. It is not typically a primary infection but rather almost exclusively a complication of an underlying infections.[18,19]

In our study, the Gram negative bacterial isolates were 100% sensitive to Carbapenems. Good sensitivity was also observed to Amikacin Gentamicin Cefepime and less sensitivity to Ciprofloxacin, Levofloxacin and Amoxyclav. They were relatively resistant to Ampicillin, Ceftriaxone, Cefotaxime and Cotrimoxazole. E.coli isolates showed highest resistance to Cephalosporins and ampicillin. Our findings are in agreement with Rajani Sharma[3] wherein gram negative isolates were 100% sensitive to Carbapenems and E.coli was highly resistant to cephalosporins and fluoroquinolones. In Tullu MS et al[20] study too, majority of the isolates were highly resistant to ampicillin and caphazolin which is in agreement with our study findings.

Gram positive isolates were 100% sensitive to Vancomycin and Linezolid. They showed good sensitivity to Gentamicin (96%), Cefepime (94%), Amikacin (92%), Ciprofloxacin (87%), Amoxyclav (87%), Levofloxacin (86%), Cotrimoxazole (84%). They were resistant to Ampicillin. Our results are in agreement with Sujatha et al.[5]

Pseudomonas species were highly sensitive to Imipenem (96%) and Pipercillin Tazobactum (92%). They showed good sensitivity to Amikacin, Cefepime. They were less sensitive to Aztreonam, Ciprofloxacin, gentamicin. They were resistant to Ceftazidime, Cefpodoxime, Cefperazone and Levofloxacin. Our results are in agreement to Rajani Sharma et al.[5] but in contrast to Sujatha R[6] wherein they showed good sensitivity to Ciprofloxacin and Ceftazidime.

Conclusion
In the present study E.coli was the most common organism isolated from various body fluids. Significant numbers of both gram negative and gram positive bacteria were isolated from various body fluid samples. The high level of MDR strains were observed among gram negative isolates which calls for immediate attention of health care workers and policy makers for the prudent antibiotic use and thus limit the transmission of MDR bacteria in the hospital and community settings.

Hence, 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 which helps the clinicians to treat effectively and thus prevent morbidity and mortality associated with these infections.

Limitations
The culture positivity of sterile body fluids was relatively less as the etiology of sterile fluid infections also includes anaerobic bacteria and Viruses, which were not included in our study.

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How to cite this article: Harshika Y K, Shobha M. K, R, Patil A B, Smita N R. 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.