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BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN FROM VARIOUS BODY FLUIDS OF PATIENTS ATTENDING RAMA MEDICAL COLLEGE HOSPITAL, KANPUR

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ABSTRACT
Sterile body fluids like pleural, peritoneal, cerebrospinal, synovial or pericardial fluids can be invaded and infected with microorganisms, especially bacteria which may result in severe morbidity and mortality. This study was undertaken to determine the antibiotic sensitivity pattern and their etiological agents of various bacterial infections in the body fluids. A total of 100 samples from patients who visited Rama Medical College Hospital & research center, Kanpur were analysed for bacteriological culture and antibiotic sensitivity over a period of one year. Clinical specimens (body fluids) were processed for bacterial culture according to the standard procedures and antimicrobial susceptibility test for isolated organisms was done using agar disk diffusion method. In 100 samples of various body fluids culture was positive in 31%. . Growth was most commonly seen in Pleural fluid (21%), peritoneal fluid (6%) and CSF (4%) respectively. There were no growth from synovial fluid and pericardial fluid. The most predominant isolates were *E.coli* (38.7%), *S.aureus* (22.5%), *S.pneumoniae* (19.35%), *Klebsiella spp*. (12.9%) and *Pseudomonas spp*. (6.45%). E.*coli, Klebsiella spp*. and *Pseudomonas* are most commonly sensitive to Imipenem, Meropenem, Colistin, Polymixin B; S.aureus to, clindamycin, Linezolid, Teicoplanin and Vancomycin; Pseudomonas to Piperacillin + Tazobactum. Drug resistance was high in the isolated microorganisms. The clinical implication warrant further study of antibiotic sensitivity in the treatment of above infected body fluids. It is necessary to treat the body fluid infections by the empirical use of antimicrobial drugs as soon as possible to reduce the morbidity and mortality, this is based on the knowledge of epidemiology of bacterial susceptibility pattern in each area.

INTRODUCTION

Body fluids like Pleural, Peritoneal, Cerebrospinal, Synovial, and Pericardial are usually sterile. Microorganisms like bacteria, fungi, virus and parasites may invade and infect the body fluids and results in severe morbidity and mortality. Even one colony of a potentially pathogenic microorganism may be significant [1]. There are certain common pathogenic bacteria like *E.coli, H.influenza, N. meningitides, S.aureus, S.pneumoniae, Klebsiella spp*. that infect the major body site. A pleural effusion is an abnormal excessive collection of this fluid which may be caused by bacterial infection like Haemophilus influenzae, Mycobacterium tuberculosis, Streptococcus pneumoniae etc. The peritoneal cavity is the fluid-filled gap between the wall of the abdomen and the organs contained within the abdomen. Problems with the peritoneal cavity can occur when there is an increase in

fluid accumulation. Peritonitis is a significant cause of mortality in patients or chronic ambulatory peritoneal dialysis (CAPD). Normal skin flora including *S.epidermidis*, are the most common aetiological agents in CAPD peritonitis.[2]Cerebrospinal fluid (CSF) is collected in case of Central nervous system (CNS) infections like meningitis. Meningitis is a medical emergency that require urgent rational antibiotics therapy.Despite of availability of potent newer antibiotics, the mortality rate due to acute bacterial meningitis remains significantly higher in India and other developing countries, ranging from 16-32% [3,4].

Common pathogens that infect CNS are Neisseria meningitidis & Streptococcus pneumococci, Haemophilus influenzae B, haemolytic streptococcus (Streptococcus agalactiae) and Escherichia coli. Synovial fluid analysis may be ordered to help diagnose the cause of joint inflammation, pain, swelling, and fluid accumulation. Septic arthritis remains an important cause of joint destruction. The clinical diagnosis of septic arthritis may be supported by laboratory diagnosis by different synovial fluid in patients with suspected septic arthritis is frequently disappointing and causative organism is only recover in one two third of cases [5].

Pericarditis is caused by a wide range of microorganisms. The most frequent etiological agents are viruses. Bacterial pericarditis is most frequent caused by *S.pneumoniae* or *S.aureus*. [6]. It is necessary to treat the body fluid infections by the empirical use of antimicrobial drugs as soon as possible to reduce the morbidity and mortality, this is based on the knowledge of epidemiology of bacterial susceptibility pattern in each area. This study was done for identifying the bacterial pathogens and their antimicrobial susceptibility pattern in the patients visiting Rama Medical College Hospital & research center, Kanpur.

MATERIAL AND METHODS

This study was conducted in the Rama Medical College Hospital and Research Center, Kanpur, from October 2013 to September 2014 in Departments of Microbiology. A total of 100 samples were analysed. Pleural, Peritoneal, Cerebrospinal (CSF), Synovial and pericardial fluids were drawn using proper aseptic precautions and sent to Department of Microbiology, in appropriate transport medium within the time limit, were included in this study.

Exclusion criteria

Contaminated, delayed and sample transported in inappropriate medium were excluded from the study.

Sample Processing

Pleural fluid, CSF, Peritoneal fluid, Synovial fluid and pericardial fluid carried to laboratory according to inclusion criteria were processed in laboratory using standard procedure. Blood agar, Mac-Conkey agar, Thioglycollate medium (Himedia, Mumbai, India) were used for culture with the purpose of obtaining isolated colonies.

Thus obtained isolated colonies were used for gram's staining for the differentiation of organism and for the confirmation whether the organism isolated were gram positive or gram negative using microscope. Thus differentiated and confirmed gram positive and gram negative colonies were then identified using biochemical tests and after identification antibiotic pattern was determined following Kirby Bauer's method according to CLSI guidelines [7], Ethical clearance from the Institute was obtained.

Drugs for GPC pathogen

The antibiotics which were tested for GPC were ampicillin (10mcg), oxacillin (1mcg), cefoxitin (30mcg), amikacin (10mcg), gentamycin (10mcg), ciprofloxacin (5mcg), tetracycline (30mcg), imipenem (10mcg), mero penem (10mcg), clindamycin (2mcg), erythromycin (15mcg), vancomycin (30mc), teicoplanin (30mcg). The company manufactured these drugs was Himedia (Mumbai, India).

Drugs for GNB pathogen

For GNB ampicillin(10mcg), amoxicillin(30mcg), pipracillin/tazobactam (100/10mcg), oxacillin(1mcg), cefoxitin (30mcg), ceftazidime (30mcg), ceftriaxone (30mcg), cefotaxime (30mcg), cefepime (30mcg), cefe perazone/ sulbactam (75/30mcg), amikacin (10mcg), gentamycin (10mcg), netilmycin (30mcg), ciprofloxacin (5mcg), tetracycline (30mcg) ,imipenem(10mcg), meropenem (10mcg), aztreonam (30mcg), polymyxin B(300mcg), colistin(10mcg), tigicycline(15mcg) antibiotics were used.

Drugs for *Pseudomonas auerogenosa* pathogen

Antibiotics used for *Pseudomonas aerogenosa* were ampicillin(10mcg), amoxycillin (30mcg), piperacillin/tazobactam (100/10mcg), carbanecillin (100mcg), cefoxitin (30mcg), ceftazidime (30mcg), ceftriaxone (30mcg), cefotaxime (30mcg), cefepime (30mcg), cefeperazone/sulbactam (75/30mcg), amikacin (10mcg), gentamycin (10mcg), netillmycin (30mcg), ciprofloxacin (5mcg), tetracycline (30mcg), imipenem (10mcg), meropenem (10mcg), aztreonam (30mcg), polymyxin B (300mcg), colistin (10mcg), teicoplanin (30mcg), tigicycline (15mcg).

RESULTS

A total 100 different body fluid were collected from patients, which included Pleural fluid, Peritoneal fluid, Cerebrospinal fluid, Synovial fluid and Pericardial fluid. Among 100 samples 31 fluids showed growth [Table 1] and the age and gender profile of the patients showed in [Table 2] isolates from different fluids were *E.coli*, *S.aureus*, *S.pneumoneae*, *Klebsiella spp*. and *Pseudomonas spp*. [Table 3]. Antibiotic sensitivity pattern of different isolates were showed in [Table 4].

Table 1. Different type of samples

Samples	Total no. of samples	Growth	No growth
Pleural fluid	75	21	54
Peritoneal fluid	14	6	8
Cerebrospinal fluid	8	4	4
Synovial fluid	2	0	2
Pericardial fluid	1	0	1
Total	100	31	69

Table 2. Ages and sex wise distribution of samples

Age	Total Samples	Growth	No Growth
<20	19	3(15.78%)	16(84.21%)
21-40	23	5(21.73%)	18(78.26%)
41-60	41	15(36.58%)	26(63.41%)
61-80	15	7(46.66%)	8(53.33%)
>80	2	1(50%)	1(50%)
Gender			
Male	69	23	46
Female	31	8	23

Table 3. Different organisms isolated from different samples

Organisms	Total	Pleural Fluid	CSF	Peritoneal Fluid	Synovial Fluid	Pericardial Fluid
E.Coli	12(38.7%)	6(28.5%)	1(25%)	5(83.3%)	0	0
S.Auerus	7(22.5%)	5(23.8%)	2(50 %)	0	0	0
S.Pneumoneae	6(19.35%)	6(28.5%)	0	0	0	0
Klebsiella Spp.	4(12.9%)	2(9.5%)	1(25%)	1(16.6%)	0	0
Pseudomonas Spp.	2(6.45%)	2(9.5%)	0	0	0	0
Total	31	21	4	6	0	0

Table 4. Antibiotic sensitivity patterns of isolates

Drugs	*E.coli* (%)	*Staphylococci aureus* (%)	*Streptococcus pnemoniae* (%)	*Klebsiella sp.* (%)	*Pseudomonas sp.* (%)
AMP	33.3	28.5	50.0	25.0	50.0
AMC	41.6	ND	66.6	25.0	50.0
CB	ND	ND	ND	ND	100
PIT	91.6	ND	83.3	75.0	100
OX	ND	71.4	66.6	ND	ND
CX	75.0	60.0	66.6	75.0	50.0
CAZ	75.0	ND	ND	75.0	100
CTX	75.0	ND	83.3	75.0	50.0
CTR	75.0	ND	ND	75.0	50.0
CPM	75.0	ND	ND	75.0	100
CFS	100	ND	ND	100	100
AK	91.6	85.7	66.6	100	50.0
GEN	83.3	85.7	50.0	100	50.0
NET	91.6	ND	ND	100	100
CIP	66.6	42.8	83.3	75.0	100
TE	83.3	71.4	83.3	75.0	50.0
IPM	100	100	ND	100	100
MRP	100	100	ND	100	100
CD	ND	100	100	ND	ND
E	ND	85.7	83.3	ND	ND
VA	ND	85.7	83.3	ND	ND
AT	83.3	ND	ND	75.0	50.0

LZ	ND	100	83.3	ND	ND
PB	100	ND	ND	100	100
CL	100	ND	ND	100	100
TEI	ND	100	83.3	ND	ND
TGC	100	ND	ND	100	100

DISCUSSION

Normally body fluids are sterile but in abnormal condition organisms can infect, which can be isolated from different body fluids such as pleural fluid, peritoneal fluid, cerebrospinal fluid pericardial fluid synovial fluid etc. In this study 31% samples give culture positive result, while other studies also conducted and found 48.4% and 24% positive results [8,9]. In the study out of 100 samples studied, pleural fluid was (75%), CSF (8%), peritoneal fluid (14%), synovial fluid (2%) and pericardial fluid (1%) respectively. Sorlin P [8] also studied on peritoneal fluid (50%), CSF (8%), pleural fluid (8%), synovial fluid (12%) and amniotic fluid (2%) but predominant organisms were *Enterobacter, S.aureus, CONS, Enterococci & Streptococci spp*. but in our study *E.coli* (38.8%) was the predominant organism followed by *S. aureus* (22.5%), *Streptococcus pneumoneae* (19.3%), Klebseilla spp. (12.9%) and *Pseudomonas spp* (6.45%).

Ahmed et al [10] and Liang et al [11] observed streptococcus spp. and Dorob et al [2] found pseudomonas spp. was the most frequent microorganism isolated from pleural fluid while in our study *E.coli* (28.5%) was the most common isolate from pleural fluid. Similar to our study, Ramakrishnaih et al [12] also found *E.coli* and *Klebsiella spp*. were the predominant isolates while Evan et al [13] got *S.aureus* was the most common isolate.

The microorganisms more commonly isolated from cases of SPO (*Spontaneous peritonitis*) are *Escherichia coli* (~70%), *Klebsiella* species (~10%), *Proteus* species, *Enterococcus faecalis* (~4% each), *Pseudomonas* species (~2%) and others (~6%) was observed by Arroyo et al [14], which was similar to our study which showed E.coli (83.3%), Klebsiella (16.6%), though the clinical diagnosis was not known in our study. According to Bhagawati G et al [15] found the most common bacterial isolate from CSF was S. aureus, (29.41%). Other recent studies have reported S. pneumoniae and K. pneumoniae as the most common cause of meningitis 35.29% [16,17].

A study from Gambia [18] reported *H. influenzae* (20%), *N. meningitidis* (1%), *S. pneumonia* (20%), *Salmonella sp.* (2%), E. coli (2%), Molyneux et al [19] observed commonest isolate as *S.pnemoniae* (27%) followed by *H. influenzae* (21%). In our study *S.aureus* (50%) was the commonest isolate followed by *E.coli* (25%) and *Klebsiella spp*. (25%) from CSF. On synovial fluid, there were many studies conducted by authors like Ahmed lafi S et al [20] and Nutt L et al [5] that found *S.auerus* was the most predominant isolates from synovial fluid 55% and 30% respectively. And H.Reuter et al [21] diagnose pericardial fluid and found *S.aureus* and *Salmonella spp* while Kambiz Mozaffari et al found *S.epidermidis* (73.3%). In the present study, an important finding was that there was no organism isolated from synovial fluids and pericardial fluids. This could be due to the reason that we have used conventional culture methods to isolate the bacteria due to cost constraints.

In our study all gram negative isolates (*E.coli, Klebsiella, and Pseudomonas spp*.) were sensitive to all Imipenem, Meropenem, Colitin, Polymixin B and Tegicycline. Cefeperazone salbactum was sensitive to most of isolates which is consistent to finding of Zoveri R.[21] According to Barai L et al [23] *E.coli* isolates were highly resistant (>80) to Cephalosporins and Fluoroquinolones. Tullu MS et al [24] study too, majority of the isolates were highly resistant (66%-100) to Ampicillin and Cephazolin. Similarly in our study *E.coli* showed low sensitivity to antibiotics: 25% to Ceftazidime, 25% to Cefotaxime, 33.3% to ampicillin and 41.6% to amoxyclav. Auranzeb et al [25] in one study reveal 75% resistence to Ampicillin similarly our study showed only 28.5% of sensitivity to ampicillin for Staphylococcus aureus. All *Staphylococcus aureus* strains were susceptible to Linezolid, Teicoplanin and Vancomycin in our study. Other studied also showed good sensitivity to Linezolid and Amikacin [26]. Nwadioha SI et al [2] found *S. pnemoniae* was susceptible to ciprofloxacin (87%), ceftriaxone (87%), clavulinate-amoxycillin (78%) and also Anagaw B et al [27] showed 11% of resistance to ciprofloxacin which is inconsistence with other studies in Nigeria (20%) [28] and Ethiopia (16%) [29]. In this study all strains of *Pseudomonas aeruginosa* was sensitive to Ciprofloxacin and Piperacillin + Tazobactum while Dorobat OM et al [2] found 67.8% resistance to Ciprofloxacin and D Narayanappa et al [26] showed Pseudomonas was sensitive to ceftazidime, cefotaxime, amikacin, ciprofloxacin and resistant to tetracycline.

CONCLUSION

Drug resistance was high in the isolated microorganisms. The clinical implication warrant further study of antibiotic sensitivity in the treatment of above infected body fluids It is necessary to treat the body fluid infections by the empirical use of antimicrobial drugs as soon as possible to reduce the morbidity and mortality, this is based on the knowledge of epidemiology of bacterial susceptibility pattern in each area.

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