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.[1,2]

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.[1,2]

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.

Materials and Methods

This retrospective observational study was conducted between January 2017 to December 2017 in the department of Microbiology KIMS Hubli.

A total of 635 samples were analysed. Body fluid samples like Pleural, Peritoneal, Synovial, Pericardial and CSF were collected under proper aseptic precautions and processed within 2 hour of collection.

Inclusion Criteria: 1) All the samples received from patients with body fluid infections, admitted in KIMS hospital Hubli, irrespective of age and sex were included.

Exclusion Criteria: 1) Blood samples 2) Patients with history of antibiotics within the last 2 weeks 3) Contaminated samples 4) Delayed body fluids for more than 2 hours.

Sample Processing: The samples collected were processed in our laboratory using Standard microbiological procedures. The samples were subjected for Gram Stain and Culture. The culture media used were Chocolate Agar, Mac Conkey Agar and Thioglycollate Medium (Hi-Media, Mumbai India) to obtain isolated colonies. The isolated colonies were then identified by Gram Stain and Standard Biochemical tests.

Antimicrobial Susceptibility Test: The Antimicrobial sensitivity test was performed for isolated organisms by Kirby Bauer Disc Diffusion method according to CLSI guidelines.[7]

Drugs for Gram Positive Cocci: Cefoxitin, Ciprofloxacin, Gentamicin, Cefotaxime, Cefepime, Tetracycline, Erythromycin, Clindamycin, Co trimoxazole, Amoxycillin Clavulanate, Linezolid and Levofloxacin, Vancomycin.

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

Drugs for *Pseudomonas:* Piperacillin, Piperacillin tazobactum, Ceftazidime, Cefepime, Cefpodoxime, Cefeperazone, 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 Amoxyclav, 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 Piperacillin tazobactam (92%).

Table 1: Growth pattern of various body fluids

	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	Cerebro Spinal 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%
Amoxyclav	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%
Ceftriaxone	18%	38%	58%	58%	0%

Cefepime	44%	72%	82%	78%	0%
Cotrimoxazole	44%	34%	60%	42%	0%
Imipenem	100%	100%	100%	100%	100%

Table 4: Antibiotic sensitivity pattern of Gram positive isolates (n=28)

Drugs	Staphylococcus aureus	CONS (Coagulase negative Staphylococcus)	Enterococcus Species	Streptococcus pyogenes
Ampicillin	16%	33%	0%	0%
Amoxyclav	84%	78%	100%	87%
Amikacin	100%	78%	100%	92%
Gentamicin	100%	89%	100%	96%
Ciprofloxacin	92%	78%	100%	90%
Levofloxacin	92%	67%	100%	86%
Cefepime	92%	89%	100%	94%
Cotrimoxazole	75%	78%	100%	84%
Erythromycin	67%	67%	100%	78%
Clindamycin	75%	67%	100%	80%
Vancomycin	100%	100%	100%	100%
Tetracycline	56%	78%	100%	76%

Table 5: Antibiotic sensitivity pattern of Pseudomonas aeruginosa. (n=20)

Drugs	Ak	Gen	Cip	Le	CAZ	CPD	CPM	CPZ	IPM	AT	PTZ
Pseudo	76%	50%	50%	32%	12%	8%	60%	22%	96%	50%	92%

Discussion

The Microbial pathogens 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.[4]

In our study, 22% of samples gave culture positive result, which is in comparison to other studies, which were 30%, 31% positive results.[5,6]

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[5] where predominant organism was *E-coli* followed by *Acinetobacter*. Our results are in contrast to studies of Sujatha et al[6] and Evan et al[11] 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 (88.4%),[8] Gupta S K et al (84.6%),[9] Mohanty et al (86.4%).[10] Prior to the availability of antibiotics, *S.pneumoniae* and *S.pyogenes* accounted for most of the empyema cases. After the discovery and widespread use of antibiotics in the 1940s, *Staphlococcus aureus* succeeded *S.pneumoniae and S.pyogenes* as the major cause of empyema. Since the advent of beta-lactamase resistant semi-synthetic penicillins in the 1960s, the incidence of staphylococcal empyema has decreased and infections due to aerobic GNB as the predominant pathogen has increased markedly. The isolation of aerobic GNB or multiple pathogens from pleural fluid is associated with a poor prognosis and indicates a more aggressive antimicrobial chemotherapy in contrast to the empyema caused by Gram positive pathogens.

Gram negative bacteria were more commonly isolated from Ascitic fluid than Gram positive bacteria. Among Gram negative bacteria *NFGNB* (13) was the most common isolate followed by *E-coli* (9), *Klebsiella* (9), *Pseudomonas* (9), which is in contrast to the study of Sujatha R et al,[6] Arroyo et al[12] and Chawla P[13] 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 noscomial 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 much more likely to form purulent effusions and to 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[5] 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.[6]

Pseudomonas species were highly sensitive to Imipenem (96%) and Piperacillin 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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