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A STUDY ON AEROBIC BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM IN STERILE BODY FLUIDS FROM A TERTIARY CARE HOSPITAL IN NORTHEAST INDIA

Microbiology

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ABSTRACT

For better management of patients and framing the antibiotic policy, the knowledge of likely prevalent strains along with their antimicrobial resistance pattern is essential. So, the present study was undertaken to evaluate aerobic bacteriological profile along with their antibiogram from various sterile fluids excluding Blood and CSF. A retrospective observational study was done in a tertiary care hospital between January 2016 to December 2016. Identification was done by standard protocols and the antimicrobial susceptibility testing by Kirby Bauer's Disk Diffusion method was performed. Total of 1269 isolates was obtained. Ascitic fluid was the most frequently encountered body fluids accounting 47.20% followed by Pleural fluid 45.39%, Bile (2.76%), Synovial fluid (2.44%) and Pericardial fluid (2.21%). Organisms isolation rate was 18.36% of which Gram negative organism isolation rate (71.24%) was more than gram positive isolates (28.76%). *Escherichia coli* (30.90%) and *Enterococcus species* (12.45%) was the most commonly isolated Gram negative and Gram positive organisms. Amongst the commonly isolated GNB, *Klebsiella pneumoniae* and *Acinetobacter baumanii* were the most resistant one. They showed higher degree of resistance not only to beta lactam antibiotics, Cephalosporins, fluoroquinolones, Aminoglycosides but also higher combination antibiotics and Carbapenems. Other gram negative isolates showed increasing pattern of resistance to the beta lactam antibiotics, Cephalosporins, fluoroquinolones and other first line drugs but they were sensitive to higher combination antibiotics and Carbapenems. In our study total *Staphylococcus aureus* isolated were 14 and 28.57% was Methicillin resistant. Out of 29 Enterococcus species, 13 was HLAR *Enterococcus species*. Though Enterococcus isolates showed increased resistance to Penicillin, Ampicillin, Tetracycline, Ciprofloxacin but they were highly sensitive to Vanvomycin, Linezolid and Teicoplanin. Regular monitoring of prevalent pathogenic organisms and their sensitivities are essential as this will help in formulating the hospital antibiotic policy and aid the clinicians in appropriate selection of antibiotic therapy in absence of a culture report thereby preventing indiscriminate use of unnecessary antibiotics and the development of antibiotic resistance.

KEYWORDS

Sterile Body Fluids, Aerobic Bacteriological Profile, Antibiogram

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 [1]. Early detection and rapid identification of microorganisms are crucial for the appropriate management. As availability of such early information helps the clinician to initiate early and more specific treatment and reduced lengths of stay of the patients in the hospital with less adverse effects [2]. In developing nations, including India, infection and antimicrobial resistance are global concern. Surveillance of antimicrobial susceptibility is necessary to combat the emergence of resistance. For empirical treatment, awareness of local antimicrobial susceptibility pattern and causative bacteria is essential. Moreover for better management of patients and framing the antibiotic policy, the knowledge of likely prevalent strains along with their antimicrobial resistance pattern is essential [3]. So, the present study was undertaken to evaluate aerobic bacteriological profile along with their antibiogram from various sterile fluids in a tertiary care hospital in North East India.

MATERIALS AND METHODS

SOURCE OF DATA

Patients samples from the different speciality wards of the hospital and the outpatient department of NEIGRIHMS, Tertiary hospital, Shillong.

STUDY TYPE AND DURATION

A retrospective observational study was done between January 2016 to December 2016 in Microbiology department.

Inclusion criteria

All sterile body fluids received for aerobic culture and sensitivity from different IPDs & OPDs

Exclusion criteria

All blood samples and Cerebrospinal fluids samples

METHODS

Processing of samples

Gram staining was done directly from the sample. Then all these samples were processed for culture and sensitivity by standard methods [4]. Media used for culture were Blood agar, Chocolate agar, Mac-Conkey agar, Brain Heart Infusion broth (Himedia, Mumbai, India) .

All significant isolates were identified by standard procedures and their antimicrobial susceptibility was tested by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI, 2015) recommendations[5] .

The routine antimicrobial sensitivity tests were put for the following antibiotics:

Drugs for GPC pathogen

Penicillin(10 units), Ampicillin(10µg), Cefoxitin(30µg), Ciprofloxacin(5µg), Gentamicin(10µg), Cefotaxime(30µg), Chloramphenicol(30µg), Gentamicin(120µg), Erythromycin(15µg), Clindamycin(2µg), Vancomycin(30µg), Tetracycline(30µg), Linezolid(10µg), Teicoplanin(30µg), Ofloxacin(5µg). (HIMEDIA, Mumbai, India)

Drugs for GNB pathogens

Piperacillin (100µg), Ampicillin (10µg), Ciprofloxacin(5µg), Amikacin (30µg), Gentamicin(10µg), Cefotaxime(30µg), Cefoperazone(75µg), Chloramphenicol(30µg), Cotrimoxazole 25 µg (1.25/23.75µg), Imipenem (10µg), Meropenem (10µg), Cefoperazone+Sulbactam (75µg /30 µg), Piperacillin+ Tazobactam(100 µg /10 µg), Ofloxacin (5µg), Ceftazidime(30 µg). (HIMEDIA, Mumbai, India)

RESULTS

A total of 1269 body fluids samples was collected of which 793 samples was from male patients (62.49%) and 37.5% (476) was female. Majority of samples was from Inpatient department .

The age range of the study participants was in between 0 to 95 years. Majority of the patients were in the age group of 21-60 years (Fig 1)

1269 samples included Pleural fluid, Ascitic fluid, Bile, Synovial fluid and Pericardial fluid. Of all these body fluids, Ascitic fluid was the most frequently encountered body fluids accounting for 47.20% followed by Pleural fluid 45.39%, Bile (2.76%), Synovial fluid (2.44%) and Pericardial fluid (2.21%). (Fig 2)

Out of 1269 samples, 233 fluids samples showed growth of organisms with an isolation rate of 18.36%(Fig 3) of which Gram negative organism had an isolation rate of 71.24% as compared to gram positive isolates (28.76%). Amongst the Gram negative the most common was *Escherichia coli* (30.90%) and amongst Gram positive the most common pathogenic organism was *Enterococcus species* including HLAR *Enterococcus species* (12.45%). (Fig 4)

Fig 1:Age wise distribution of samples (n= 1269)

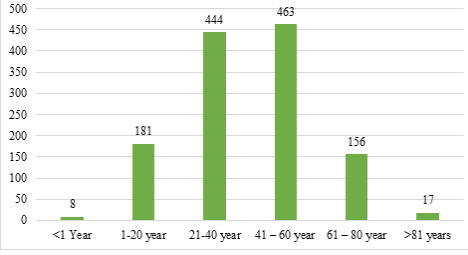


FIG 2: Percentage Distribution Of Various Samples

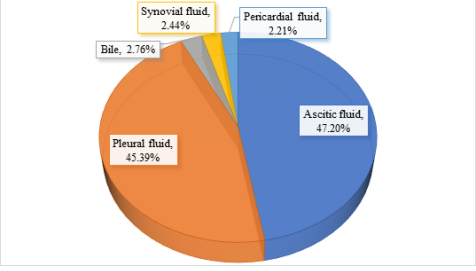


Fig 3: Percentage Distribution Of Growth In Various Samples

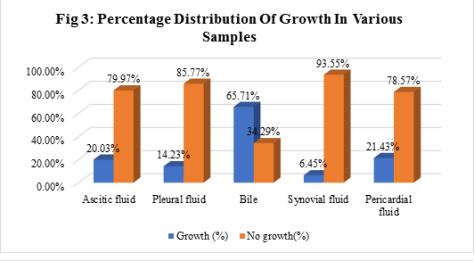


Fig 4 : Isolation rate of organisms

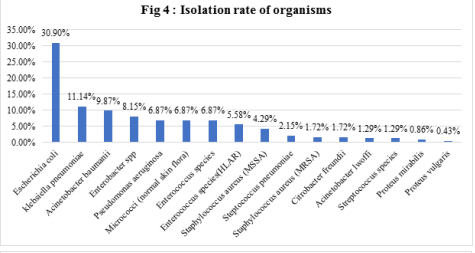


Fig 5: Percentage Distribution Of Gram Positive Cocci In Various Samples

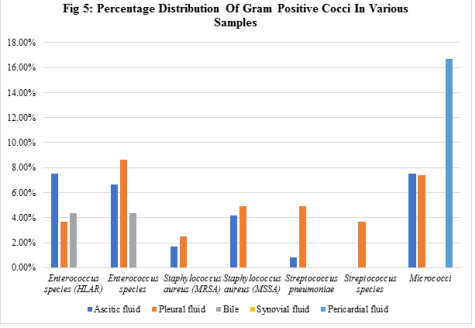


FIG 6, 7, 8 , 9 AND 10 shows Percentage Distribution of gram negative bacilli in various samples

Fig 6: Percentage Distribution Of Gram Negative Bacilli In **Ascitic Fluid**

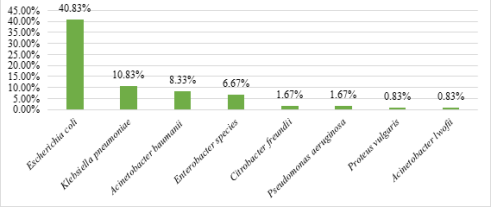


Fig 7 : Percentage Distribution Of Gram Negative Bacilli In **Pleural Fluid**

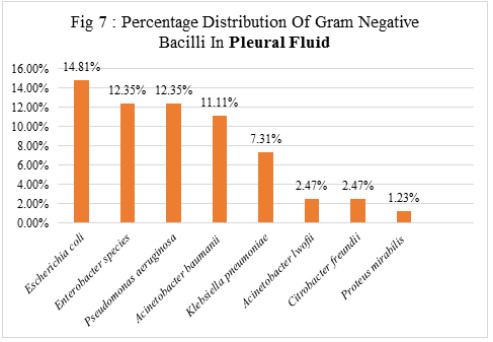


Fig 8 : Percentage Distribution Of Gram Negative Bacilli In **Bile**

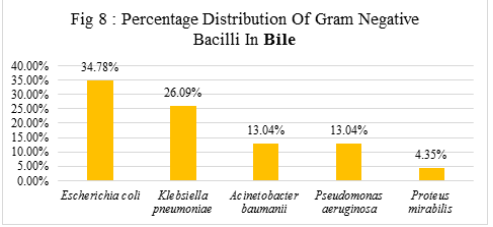


Fig 9: Percentage Distribution Of Gram Negative Bacilli In **Synovial Fluid**

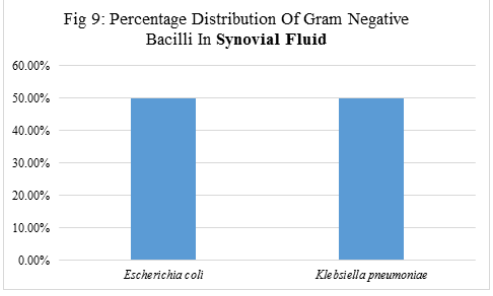


Fig 10 : Percentage Distribution Of Gram Negative Bacilli In **Peritoneal Fluid**

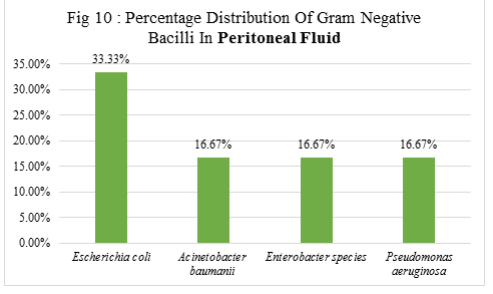
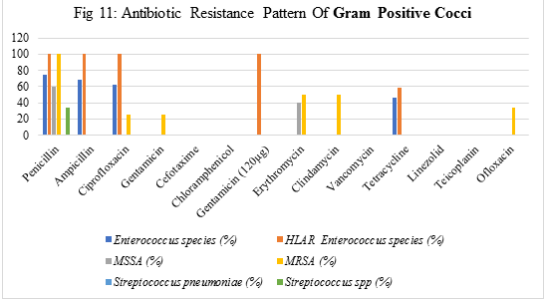
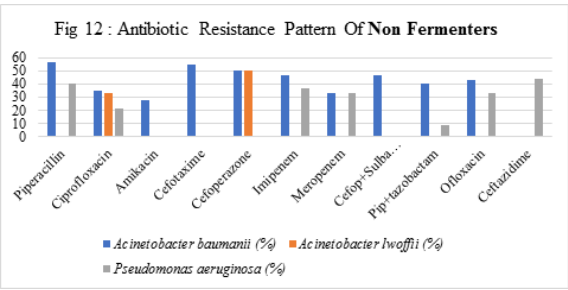


FIG 11, 12 AND 13 shows Antimicrobial resistance pattern of Gram positive cocci, Non Fermenters and Enterobacteriaceae.

Fig 11: Antibiotic Resistance Pattern Of **Gram Positive Cocci**





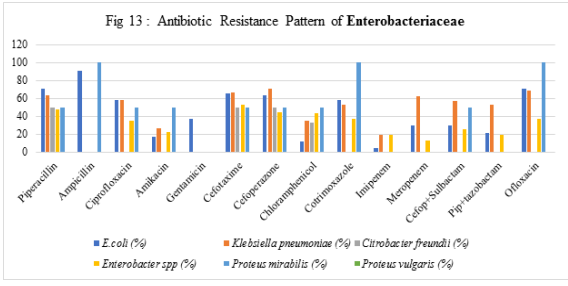


FIG 14: Anitbiotics Sensitivity And Resistance Pattern Of Gram Positive Cocci

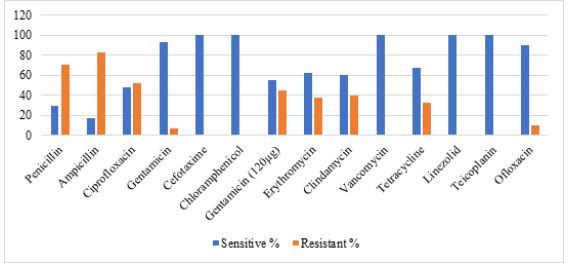
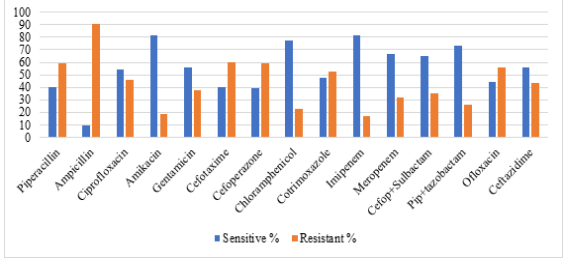


FIG 15: Antibiotic Sensitivity And Resistance Pattern Of Gram Negative Bacilli



DISCUSSION

In this study isolation rate was 18.36% which was quite low compared to other Indian studies which showed higher isolation rate of 30% [6]and 31%[1]

A lower positive culture rates similar to this study has been observed in other Indian studies like that of Deb A *et al* [7] and Kasana D et al [3]which showed isolation rate of 14.41% and 14.8% respectively. Western studies like that of Teklehymanot F et al. [8] too showed lower culture positive rates of about 14.1%. This might be due to over diagnosing, prior exposure to antibiotics and emergence of non-infectious conditions like malignancy [8]

The reported spectrum of microorganisms responsible for body fluid infection is varied, and is modified by introduction of antibiotics, patient specific factors such as surgical procedures, trauma or underlying conditions or by methodological factors namely the proper specimen collection, transport and culture. For these reasons, several studies have found discordant results in the spectrum of pathogens causing these infections [3]. In our study, Gram positive isolation rate was 28.76% whereas 71.24% of the samples showed growth of gram negative bacilli, the most common being those belonging to the Enterobactereaceae family (124 isolates) followed by other Gram negative bacilli belonging to the non-fermenter group (42 isolates). This finding was similar to Deb A et al study [7]. The Overall predominant pathogenic organisms were *Escherichia coli* followed by other Gram negative bacilli like *klebsiella pneumoniae, Acinetobacter baumanii, Enterobacter spp.*, *Pseudomonas aeruginosa*. Most common gram positive isolates was *Enterococcus species* followed by HLAR *Enterococcus species*, *Methicillin sensitive Staphylococcus species*, *Streptococcus pneumoniae* and others.

In case of Ascitic fluid and Pleural fluid, Gram negative organisms was isolated more compared to gram positive organisms similar to some studies [3,7]. This is in contrast to few other studies where gram positive organisms accounted for maximum number of cases and *Staph aureus* (70%) was the most common pathogen isolated followed by CONS and others [2]. In our study in Ascitic fluid, *E.coli* (40.83%) and *klebsiella pneumoniae* (10.83%) were the commonest organism followed by *Acinetobacter baumanii* (8.33%), similar to other studies in literature [1,3,6]. Amongst Gram positive isolates *Enterococcus species*(6.67%) and *HLAR Enterococcus species* (7.50%) was the most common which is in agreement with few studies[6,7]. In pleural fluid *Escherichia coli, Pseudomonas aeruginosa, Enterobacter species* and *Acinetobacter baumanii* was the most common isolates similar to findings of Teklehymanot F et al. and Deb A et al [8].

In bile Gram negative isolation rate was more compared to Gram positive in which *E. coli* (34.78%) isolation rate was maximum followed by *Klebsiella pneumoniae* (26.09%) as similar to findings of Suna et al [9].

In case of synovial fluid we found *E. coli* (50%) and *Klebsiella pneumoniae*(50%) as the only isolates whereas other studies conducted isolated *S.aureus*, *Klebsiella spp*., *Pseudomonas species* and *Entercoccus spp*[6].

In a study from South Africa,[10] *S.aureus and Salmonella spp.* were commonly isolated from pericardial fluid samples. In the study from India by Sharma et al [6], *Klebsiella spp.* was isolated in 12% of pericardial fluid samples. Whereas In our study *E.coli*, (3.33%), *Acinetobacter baumanii* (16.67%), *Pseudomonas aeruginosa* (16.67%)and *Enterobacter spp* (16.67%)was isolated . This low isolation of organism from Synovial fluid and Pericardial fluid could be possibly due to the reason that we have used conventional culture methods to isolate the bacteria due to cost constraints.

Amongst the commonly isolated GNB, it was *Klebsiella pneumoniae* and *Acinetobacter baumanii* which was the most resistant one. They showed higher degree of resistance to most of the antibiotics. This high resistance level may be due to inappropriate use of commonly prescribed antibiotics as described in some studies. [6,8]. As our hospital is a tertiary care hospital so most of the patients who are admitted here are already exposed to antibiotics. Similar problem was encountered by B. Vishalakshi et al[2].

Other gram negative isolates showed increasing pattern of resistance to the beta lactam antibiotics, Cephalosporins, fluoroquinolones and other first line drugs but they were sensitive to higher combination antibiotics and Carbapenems. This have been reported across the globe[7]. We found that *Acinetobacter baumanii* was the most resistant pathogens to many antibiotics as seen in some other studies [6]. *Acinetobacter baumanii* is an important public health problem, especially in patients on broad spectrum antimicrobial therapy and requiring life support. Most of the samples in our study was from indoor patients requiring broad spectrum antimicrobial therapy and some required life support as seen in ICU patient. Highly resistant Acinetobacter are common in such groups[6,11].

In our study Total *Staphylococcus aureus* isolated were 14, out of which four was *MRSA* which accounted for 28.57% of *Staphylococcus aureus* isolates and 1.72% of the total isolates (233). Some studies reported bit higher isolation rate about 38.5% & 51% of *MRSA* isolates [6,12]. Whereas in some studies almost all isolates of *Staphylococcus aureus* was Methicillin sensitive [2,7]. This variation might be because of variation in antibiotic usage and infection control practices in different places or variation in patient and clinical specimens. No vancomycin resistant (VRSA) or Vancomycin-intermediate resistant S.aureus (VISA) isolates were detected in our study. This may be due to judicious and controlled use of Vancomycin in our hospital. However, very few studies reported vancomycin resistance (VRSA) in MRSA strains [13,14]. Except Penicillin and Erythromycin MSSA was highly sensitive to almost all the antibiotics tested whereas MRSA apart from

Penicillin and Erythromycin it showed reduced susceptibility to Clindamycin, Ciprofloxacin and Gentamicin. Similar antibiogram of *Staphylococcus aureus* was seen in study done by Kasana et al [3].

Regarded as nosocomial pathogen of the 1990s, *Enterococci* have become increasingly important not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. Serious *Enterococcal* infections are often refractory to treatment and the mortality is high. High level gentamicin resistance (HLGR) was first reported in *E.faecalis* in 1979[15] and in recent years, there has been a rapid increase in the incidence of infection and colonisation of patients with vancomycin-resistant enterococci (VRE)[16] but no VRE was detected in our study. Out of 29 *Enterococcus species*, 13 was HLAR *Enterococcus species* with an isolation rate of 44.82% among all *Enterococcal isolates* and 5.59% of total isolates. Not much information could be gathered regarding HLAR isolation rate in sterile fluids study. Most of the study mentioning HLAR was done not solely in sterile fluids but also in non sterile fluids. The knowledge of HLAR prevalence is important because if this information is available, clinicians can prescribe the various drug combination (cell wall inhibitor + aminoglycosides) at the very beginning of treatment avoiding the unnecessary usage of other antimicrobials [17]. Though *Enterococcus* isolates showed increased resistance to Penicillin, Ampicillin, Tetracycline, Ciprofloxacin but they were highly sensitive to higher drugs like Vancomycin, Linezolid and teicoplanin similar to some studies [6,7].

Thus, we observed an overall increasing trend of resistance in both gram negative and gram positive isolates, which warrants regular surveillance studies. Surveillance of the incidence, microbial profile and antibiotic resistance pattern of sterile body fluids infections 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.[6].

CONCLUSION

Culture positivity rate was quite low and this may be due to prior exposure to antibiotics. Absence of anaerobic culture and molecular methods which has been shown to be more sensitive method[18] could also be responsible for low isolation rate. Infections of sterile body fluids are usually associated with high morbidity and risk of sequelae and this can be prevented by early intiation of appropriate therapy. Significant numbers of both gram negative and gram-positive bacteria were isolated from various body fluids samples. The antibiogram observed urgently call for concerted and immediate attention of health care workers and policy makers for prudent antibiotic use. Regular monitoring of prevalent pathogenic organisms and their sensitivities are essential as this will help in formulating the hospital antibiotic policy and aid the clinicians in appropriate selection of antibiotic therapy in absence of a culture report thereby preventing indiscriminate use of unnecessary antibiotics and the development of antibiotic resistance.

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