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A STUDY ON BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF STERILE BODY SITE INFECTIONS IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL

Medical Microbiology

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ABSTRACT

Body fluids like cerebrospinal fluid, ascitic fluid, pleural fluid, bile, synovial fluids are free of microorganisms. If infection of the sterile body compartment occurs, it can be life threatening which mandates early detection and antibiogram of organism. This study was aimed to evaluate the bacterial aetiology and antimicrobial susceptibility pattern of isolates obtained from body fluids. This is a prospective study conducted in the Department of Microbiology, MMC and RI. A total of 215 body fluid samples were studied. Samples were processed for aerobic bacterial culture and identification was done according to standard procedures and antimicrobial susceptibility test was done using Kirby Bauer disc diffusion method. Out of 215 samples, 43(20%) showed growth of organisms, of which 67.4% were Gram-negative and 32.6% gram-positive. Amongst the Gram-negative isolates, Escherichia coli (34.5%;10/29) was the commonest and Staphylococcus aureus (42.8%;6/14) was the commonest among gram positive organisms. All the gram-negative isolates were susceptible to colistin, followed by Carbapenems (48.3%;14/29), aminoglycosides (37.9%;11/29) and monobactams (37.9%;11/29). 50% of the Staphylococcus aureus isolated were Methicillin resistant and all isolates were susceptible to Linezolid and Vancomycin, in Enterococcus species isolated, 1 was Vancomycin resistant and 2 High level aminoglycosides resistant. In this study, high resistance was observed with significant number of isolates which may lead to high morbidity and mortality, and this mandates regular monitoring of prevalent pathogens and their antibiogram.

KEYWORDS

Sterile body fluids, antimicrobial susceptibility, Gram negative bacteria, Gram positive bacteria.

INTRODUCTION

Body fluids from sterile body sites like peritoneal fluid, pleural fluid, synovial fluid, cerebrospinal fluid are generally free of microbes in healthy individuals. Infections of sterile body sites like meningitis, peritonitis, pleural effusion, septic arthritis are comparatively less common but associated with considerable morbidity and mortality.[1] Worldwide studies done by Wunrow et. al. has shown that there were 2.5 million cases of meningitis which led to 236,000 deaths. Although effective antimicrobials are available which can cross the blood brain barrier, meningitis poses a serious threat to public health in developing nations. Likewise, bacterial peritonitis has a prevalence of 10-30%, of which approximately 15% of infections occur in post-operative period. Similarly, prevalence of bacterial pleural infection is 14.9% globally, while that of septic arthritis is two cases per 100000 people per year.[2] This data reveals that there are high rates of infections of sterile body sites which needs to be evaluated at the right time for better management of the patients. The commonly associated pathogens are *Escherichia coli, Klebsiella species, Pseudomonas species, Acinetobacter species, Staphylococcus aureus, Enterobacter species* and *Enterococcus species*.[3]

In today's world, antimicrobial resistance is one of the most urgent public health problems which is associated with longer hospital stays, higher treatment cost and increased mortality. Moreover, in the infections of sterile body sites it becomes more challenging as sample has to be collected from invasive locations for identification of aetiological agent because of which doctors have to start empirical therapy which may further contribute to higher antibiotic resistance. It is important to detect and identify microorganisms rapidly and inform clinicians as soon as possible because availability of early information helps the clinician to initiate specific treatment and reduced lengths of stay of the patients in the hospital with less adverse effects. In the present scenario of multidrug resistant bacterial infections, treating these patients can be difficult which contributes to increased rate of mortality. Therefore, periodic analysis of the local geographical bacteriological profile and antibiogram of organisms isolated is necessary to monitor the epidemiology of bacterial susceptibility pattern in each area, so that such infections are treated by the empirical use of antimicrobial drugs as soon as possible to reduce the morbidity and mortality.[4] Thus, this study was done to assess the bacteriological profile and antibiogram of organisms isolated from the body fluids obtained from the individuals attending our hospital.

MATERIALS AND METHODS

This is prospective study done in the Department of Microbiology, MMC and RI, over a period of 6 months from July 2023 to December 2023. Ethical clearance was obtained from the Institutional Ethics Committee.

Samples:

A total of 215 body fluid samples from sterile body sites received for aerobic culture and antimicrobial susceptibility from different in-patient departments were included in the study.

Sample Processing:

All the samples were subjected to Gram staining and then processed for aerobic culture using Blood agar, Chocolate agar, Mac-Conkey agar. Blood agar and MacConkey agar were incubated aerobically at 35-37°C and chocolate agar was incubated in candle jar (5-10% CO_2) at 37°C. All the isolates were identified by standard biochemical tests and their antimicrobial susceptibility was tested, according to the organism identified and site of infection, by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute recommendations (M100 2023).[5].

RESULTS:

A total of 215 body fluids samples were collected, of which 60.5% samples were from male patients (130/215) and 39.5% (85/215) were from females, which consisted of ascitic fluid (42.8%), pleural fluid (32.6%), synovial fluid (14.4%), CSF (9.3%) and bile (0.9%). (Fig 1)

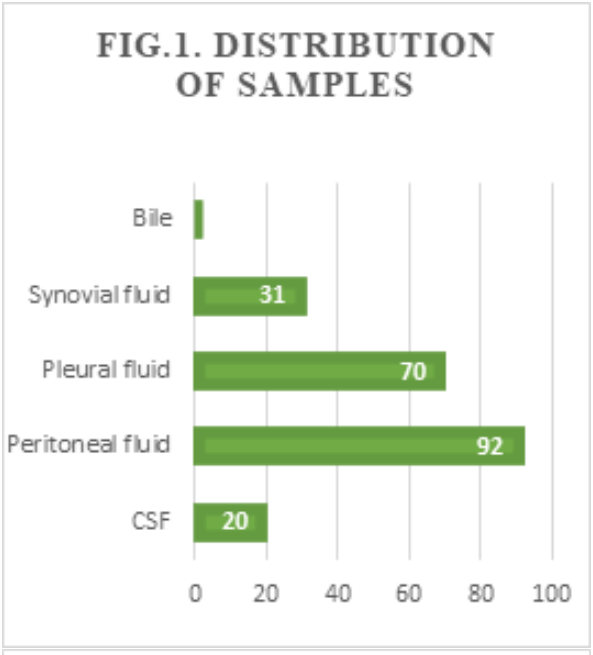
Out of 215 samples, 43(20%) showed growth of organisms (Fig 2), of which Gram-negative organism had an isolation rate of 67.4% (29/43) and 32.6% (14/43) gram-positive organisms were isolated (Fig 3).

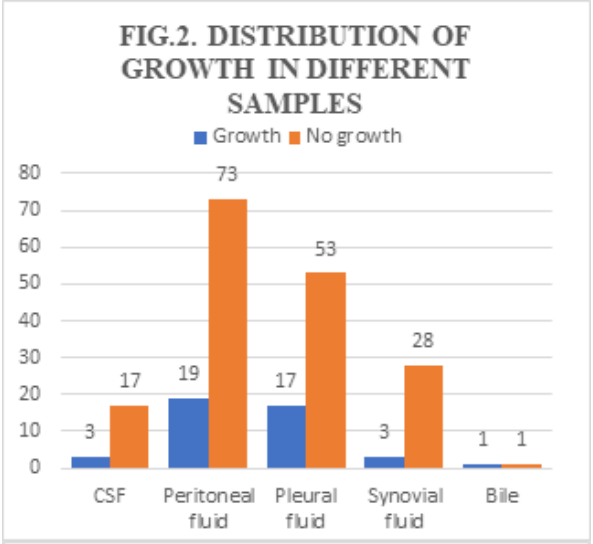
Among Gram-negative isolates, *Escherichia coli* (34.5%;10/29) was the commonest, followed by *Klebsiella spp.* (27.6%;8/29), *Pseudomonas spp.* (17.2%;5/29), *Acinetobacter spp.* (17.2%;5/29) and *Stenotrophomonas maltophilia* (3.4%;1/29). *Staphylococcus aureus* (42.8%;6/14) was the commonest among gram positive isolates, followed by *Enterococcus spp.* (28.6%;4/14), *Streptococcus pneumoniae* (21.4%;3/14) and *Streptococcus spp.* (7.1%;1/14). (Fig 4). Table 1 demonstrates the pathogens isolated from different samples.

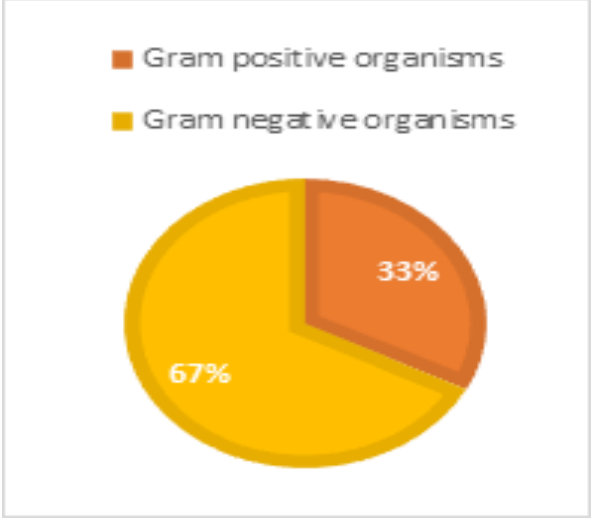
Among Gram-negative organisms, highest sensitivity was recorded for Carbapenems (48.3%;14/29), followed by aminoglycosides (37.9%;11/29) and monobactams (37.9%;11/29) (Fig.5).

Among gram-positive organisms, 50% of the *Staphylococcus aureus* isolated were Methicillin resistant, and all the isolates were susceptible to Linezolid and Vancomycin. Out of 4 *Enterococcus spp.*, 1 was

Vancomycin resistant and 2 High level Gentamicin resistant. All 3 *Streptococcus pneumoniae* were susceptible to Penicillin, Linezolid, Clindamycin and Cotrimoxazole, but resistance was noted with Erythromycin and Doxycycline. 1 *Streptococcus spp.* was susceptible to Erythromycin, Linezolid, Clindamycin and Doxycycline, but resistance to Penicillin and Cotrimoxazole (Fig. 6).







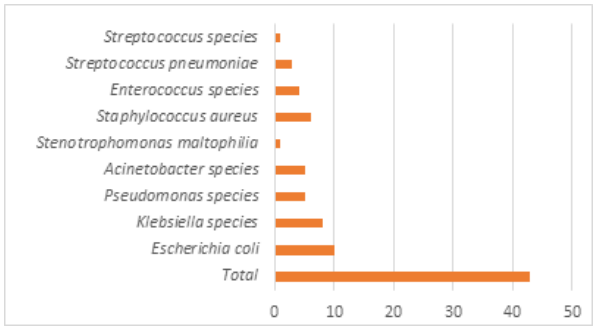


Fig.3 & 4. Distribution Of Organisms Isolated

Table 1. Sample-wise Sistribution Of Organisms

ORGANISMS ISOLATED	SAMPLES					Total
	Peritoneal fluid	Pleural fluid	Synovial fluid	Cerebrospinal fluid	Bile	
Gram positive						
Staphylococcus aureus	3	1	2	0	0	6
Enterococcus species	4	0	0	0	0	4
Streptococcus species	0	0	0	0	1	1
Streptococcus pneumoniae	0	2	0	1	0	3
Gram negative						
Escherichia coli	6	3	0	1	0	10
Klebsiella species	3	4	0	1	0	8
Pseudomonas species	1	4	0	0	0	5
Acinetobacter species	2	2	1	0	0	5
Stenotrophomonas maltophilia	0	1	0	0	0	1
Total	19	17	3	3	1	43

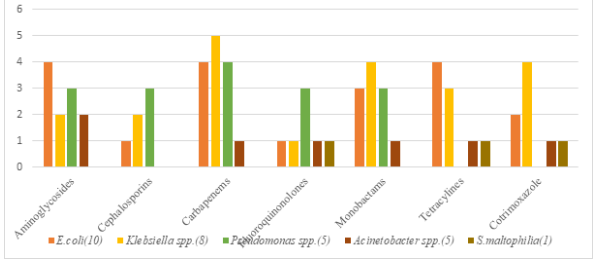


Fig.5. Antibiogram Of Gram-negative Isolates

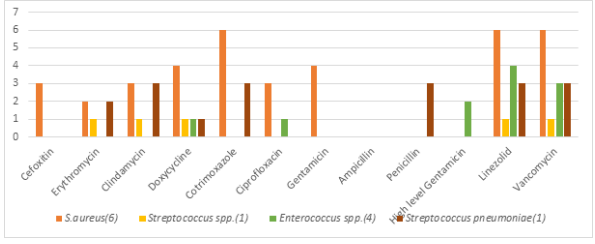


Fig.6. Antibiogram Of Gram-positive Isolates

DISCUSSION:

In this study, culture positivity was seen in 20% of the samples, which is similar to study done by Ramadanu et. al. [6] that showed a positivity rate of 18.36%, whereas, studies done by Sujatha et. al. [7] and Sharma et. al. [8] showed higher positivity rates at 31% and 30%, respectively. We observed a low culture positivity rate which may be due to administration of antibiotic before sample collection or other causes of infection like viral, fungal or mycobacterial infection in the patients.

Out of the 43 culture positive samples, 67.4% showed growth of gram-negative organisms and 32.6% had gram-positive organisms, which is in concordance with study done by Ramadanu et. al. [6] and Sultana et. al. [3] who isolated 71.24% gram-negative and 28.76% gram-positive organis; and 72.7% gram negative and 27.3% gram-positive

organisms, respectively.

In peritoneal fluid, gram negative isolates were more compared to gram positive isolates which is similar to study done by Ramadanu et.al.[6], whereas, study done by Pal et.al.[9], showed a higher proportion of gram-positive organisms compared to gram negatives. In our study, *E. coli* (31.6%;6/19) was the commonest organism isolated from peritoneal fluid, which is similar to study done by Kasana et.al. [10], while Vishalakshmi et.al.[11] demonstrated only *Staphylococcus species* in ascitic fluid. *Enterococcus species* was the most commonly isolated gram-positive cocci in ascitic fluid which is similar to study done by Deb et.al.[12]and Ramadanu et.al.[6]

Our study showed more *Klebsiella species* and *Pseudomonas species* in pleural fluid, whereas, study done by Teklehymanot et.al.[13] showed a greater number of *E. coli* in pleural fluid.

We found more gram-negative bacilli in CSF, while, study done by Deb A. et.al.[12] showed prevalence of gram-positive cocci in CSF infection. In synovial fluid, *Staphylococcus aureus* was the most common organism isolated which is similar to the study done by Sharma et.al.[8]

1 out of 2 bile samples from post-operative cases of cholecystitis showed growth of *Streptococcus species*. The organisms most commonly associated with biliary tract infections are *Escherichia coli* (25.0%), *Enterococcus spp.* (13.4%). *Klebsiella spp.* (11.1%), *Pseudomonas spp.* (11.1%), and *coagulase-negative Staphylococcus* (9.7%).[15] *Streptococcus species* is known to cause endocarditis, meningitis, sepsis and colorectal cancers, but cases of biliary tract infection is very rare. *Streptococcus species* is found as colonizer in 10% healthy individuals; thus, operative procedures can lead to lodgement of these organisms leading to infection of the biliary tract.[16] Lee et. al. [17], in their study, evaluated 37 cases of bacteraemia with *Streptococcus species* in which they found 14 were associated with biliary tract disease.

The spectrum of microbes causing infection of sterile body compartments in different studies is varied which may be due to lesser number of samples included in our study, different precipitating factors like trauma or surgery, usage of antimicrobials, age of patients, method of sample collection, maintenance of sterility during sample collection and transportation. Moreover, this hospital being a tertiary care centre, there are chances of acquiring nosocomial infections with highly resistant strains due to overcrowding and inadequate infection control practices.

In our study, 100% gram-negative isolates were susceptible to Colistin, followed by Carbapenems (48.3%) and Aminoglycosides (40%), which is similar to Tullu MS et.al.[18] *Acinetobacter* was the most resistant pathogen which was also observed in studies done by Parez et.al.[19] and Rouf et.al.[4] *Acinetobacter* is one of the most important public health problems showing multidrug resistance which is very difficult to treat leading to severe infections with very high mortality rate.[20] Most of the isolates were resistant to Cephalosporins and Fluoroquinolones which may have occurred due to inappropriate usage of antibiotics.

Among gram-positive isolates, 50% (3/6) of the *Staphylococcus aureus* were Methicillin resistant which is similar to the study done by Kulshreshtha et. al. [21] but study done by Ramadanu et.al. [6]and Sharma et.al. [8] showed a lower isolation rate of 28.57% and 38.5%, respectively, which may be due to variation in antibiotic usage and different hospital infection control protocols. All the isolates were susceptible to Linezolid and Vancomycin, but reduced susceptibility was noted for Erythromycin, Clindamycin and Ciprofloxacin, which is in concordance with the study done by Kasana et.al.[10]

All 3 *Streptococcus pneumoniae* isolates were susceptible to Penicillin, Erythromycin, Clindamycin, Linezolid and Vancomycin, while 1 *Streptococcus spp.* isolated from bile was resistant to Penicillin and Erythromycin.

Enterococcus is an important nosocomial pathogen which shows resistance to many antibiotics and can cause serious infections which are often refractory to treatment.[6] High level aminoglycoside resistance (HLAR) was first reported in 1979.[22]In recent times, there is an increase in the incidence of infection and colonisation with HLAR and *Vancomycin resistant enterococci* (VRE) [23]. In this study, we isolated 1 VRE and 2 HLAR. All 4isolates were resistant to Erythromycin and Clindamycin.

We observed an increasing trend of antimicrobial resistance which may have occurred because of prolonged antibiotic usage in patients in chronically ill patients or in ICUs, frequent usage of antimicrobials in cases where it is not indicated, availability of over-the-counter drugs, self-medication. Moreover, there are mobile genetic elements in the microbes which can be transferred horizontally from one organism to the other giving rise to infection with multidrug resistant strains.

CONCLUSION:

Infection of sterile body compartments is associated with high morbidity and the increasing trend of resistance makes it very difficult to treat the infections which may lead to high mortality. In this study, we observed a lower isolation rate on culture as the sample size was small and only aerobic bacterial culture was done, because of which we may have missed anaerobic organisms and for this reason anaerobic culture should be done along with aerobic culture. Moreover, we observed an increased resistance to antimicrobials among the commonly isolated organisms which mandates cautious usage of broad-spectrum antibiotics and requires surveillance at regular intervals to look for the prevalent organisms and their antibiogram so that proper antibiotic policies can be prepared to limit the increasing trend of antibiotic resistance.

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