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Aerobic Bacteriological Profile and Antimicrobial Sensitivity Pattern of Bacteria Isolated from Sterile Body Fluids: A Study from a Tertiary Care Hospital in North India

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Authors' contributions

This work was carried out in collaboration between both authors. Author MR designed the study, performed the statistical analysis, wrote the protocol, and. Author AN wrote the first draft of the manuscript and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to isolate and identify the aerobic bacterial pathogens of sterile body fluids and to determine their susceptibility to various antibacterial agents.
Study Design: This study was a retrospective observational study conducted in a tertiary care hospital.
Place and Duration of Study: This study was conducted in the Department of Microbiology, SMHS hospital, Srinagar. A total of 814 samples were analysed for bacteriological culture and antibiotic sensitivity over a period of one year, from April 2018 to March 2019.
Methodology: Clinical specimens (pleural fluid, ascitic fluid, cerebrospinal fluid, Synovial fluid, pericardial fluid and bile) were processed for bacterial culture according to standard procedures and antimicrobial susceptibility test for isolated organisms was done using Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) recommendations.

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Results: In 814 samples of various body fluids, 88 samples showed growth of organism with an isolation rate of 10.81%. growth was most commonly seen in CSF (34.09%) followed by Ascitic fluid (23.86%, Bile (20.45), Pleural fluid (15.90%) and Synovial fluid (5.68%). No growth was obtained from pericardial fluid. The most predominant isolates were *E. coli* (23.86%), *Pseudomonas sp* (15.90%), *Acinetobacter* (14.77%), *Klebsiella sp* (7.95%), *Staphylococcus aureus* (11.36%), CONS (12.5%) and *Enterococcus sp* (4.54%). *E. coli* and *Klebsiella* were sensitive to imipenem, meropenem. colistin, amikacin and gentamicin. *Staph. aureus* and *CONS* were mostly sensitive to vancomycin, linezolid, and teicoplanin. *Pseudomonas* was sensitive to imipenem, meropenem, colistin and piperacillin/tazobactam. *Acinetobacter, E. coli and Klebsiella sp* were the most resistant organisms.

Conclusion: In our study significant numbers of multidrug resistant bacteria were isolated from body fluids which calls for regular monitoring of prevalent pathogenic organisms and their sensitivities to avoid indiscriminate use of unnecessary antibiotics and the development of antibiotic resistance.

Keywords: Body fluids; sterile; antibiotic sensitivity; resistance; E. coli; Staphylococcus aureus.

1. INTRODUCTION

Sterile body fluids are those in which no bacteria or microbes exist as commensals when in a healthy state. Body fluids like Pleural, Peritoneal, Cerebrospinal, Synovial and Pericardial are usually sterile. Infections of the sterile body sites typically have greater clinical urgency and these infections could be life-threatening [1,2]. Microorganisms like bacteria, fungi, virus and parasites may invade and infect the body fluids and results in severe morbidity and mortality [3]. The common pathogenic bacteria of concern are *Escherichia coli, Acinetobacter* spp., *Klebsiella* spp*., Staphylococcus aureus* and *Enterococcus* spp.

A pleural effusion is an abnormal excessive collection of fluid in pleural cavity which may be caused by bacteria like *Streptococcus pneumoniae* and *Haemophilus influenza*e Mycobacterium tuberculosis. Cerebrospinal fluid (CSF) is present in Central nervous system (CNS). CSF is collected in case of infections of CNS like meningitis. Common pathogens that infect CNS are *Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae B, haemolytic streptococcus* and *Escherichia coli*. Meningitis is a life-threatening condition and requires urgent antibiotics therapy. Despite of availability of newer antibiotics, the mortality rate due to acute bacterial meningitis remains significantly higher in India and other developing countries, ranging from 16-32% [4,5]. The peritoneal cavity is the fluid-filled gap between the wall of the abdomen and the organs contained within the abdomen. Infection of peritoneal cavity can occur when there is increased fluid accumulation. Peritonitis is a significant cause of mortality in patients who are on chronic ambulatory peritoneal dialysis (CAPD). Normal skin flora including *S. epidermidis*, are the most common aetiological agents in CAPD peritonitis [6].

Synovial fluid analysis is done to help diagnose the cause of joint inflammation, pain, swelling, and fluid accumulation. Septic arthritis is one of important cause of joint destruction. The clinical diagnosis of septic arthritis may be supported by laboratory. 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*. [7].

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 [8,9,10].

There is a need of periodic analysis of the local geographical bacteriological profile and antibiotic susceptibility pattern of organisms isolated and the results need to be communicated to the clinician. It is necessary to monitor the epidemiology of bacterial susceptibility pattern in each area, so that such infections must be treated by the empirical use of antimicrobial drugs as soon as possible to reduce the morbidity and mortality. 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.

2. MATERIALS AND METHODS

Samples: This retrospective observational study was conducted in a tertiary care hospital, in Srinagar for a period of one year from April 2018 to March 2019 in Department of Microbiology. A total of 819 samples were analyzed. Pleural, peritoneal, cerebrospinal fluid (CSF), synovial, pericardial fluids and bile were drawn using proper aseptic precautions and sent to Department of Microbiology.

Processing of Samples: Samples were subjected to Gram stain for provisional report and then processed using standard microbiological procedures [11]. Blood agar, Mac-Conkey agar and chocolate agar (Himedia, Mumbai, India) were used for culture. Inoculated plates were incubated at 37ºC overnight. Culture plates were checked for the bacterial growth next day. All bacterial isolates were examined for colony characteristics, Gram staining, motility and biochemical tests. Biochemical tests employed were oxidase, catalase, nitrate, urea hydrolysis, citrate utilization, sugar fermentation, indole production test and H2S production on TSI agar. Any sample was considered sterile only after 48 hours of incubation.

Antibiotic Susceptibility Testing: Antimicrobial susceptibility was tested by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) recommendations [12]. Briefly, 3-5 pure colonies of bacteria were picked from blood agar for Gram positives bacteria, from MacConkey agar for Gram-negative bacteria then emulsified in sterile nutrient broth using sterile wire loop. In order to make standardized inoculums size, the bacterial suspension was adjusted to 0.5 McFarland standard and the suspension was swabbed on to Muller-Hinton agar Appropriate control strains were used for quality control.

The antibiotics used for Gram positive organisms included cefoxitin (30 µg), benzyl penicillin (10 units), oxacillin(1 mcg), gentamicin(10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), linezolid (10 µg), teicoplanin (30 µg), vancomycin (30 µg), tetracycline (30 µg), tigecycline(15 mcg), cotrimoxazole (25 µg).

The antibiotics used for Gram negative lactose fermenting organisms included ampicillin (10 µg), piperacillin-tazobactam Tazobactam(100 µg /10 µg), ceftriaxone (30 µg), cefoperazone-sulbactam (75 µg /30 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), gentamicin (10µg), nalidixic acid, ciprofloxacin (5µg), tigecycline (15 mcg), colistin (10 mcg), cotrimoxazole 25 µg (1.25/23.75 µg).

The antibiotics used for Gram negative Non lactose fermenting organisms included piperacillin-tazobactam(100 µg /10 µg), ceftazidime (30 mcg), cefoperazone-sulbactam (75 µg /30 µg), cefepime(30 mcg), aztreonam(30 mcg), imipenem(10 µg), meropenem(10 µg), amikacin(30 µg), gentamicin(10 µg), ciprofloxacin(5µg), tigecycline(15 µg), colistin(10 µg), cotrimoxazole 25 µg (1.25/23.75 µg). All discs used were from HIMEDIA Laboratories Pvt. Ltd., Mumbai, India.

3. RESULTS

A total of 814 body fluid samples were included in the study out of which CSF samples (387) constituted 47.54%, pleural fluid samples (194) 23.83%, peritoneal fluid (138) 16.95%, bile (33) 4.05% and synovial fluid (63) 7.73% as shown in Table 1 and Fig. 1.

Out of 814 samples, 88 fluid samples showed growth of organisms with an isolation rate of 10.86% of which Gram-negative organism had an isolation rate of 70% as compared to gram positive isolates (30%).as shown in Fig. 2.

Amongst the Gram negative the most common was *E. coli* (23.86%) and amongst Gram positive the most common pathogenic organism was *Staphylococcus aureus* (12.5%) as shown in Fig. 3.

Table 1. Growth pattern seen in various body fluids

Samples	Total number of samples	Growth	No Growth
Pleural Fluid	194	14	180
Cerebrospinal fluid	387	30	357
Ascitic Fluid	137	21	116
Synovial Fluid	63	5	58
Bile	33	18	15
Total	814	88	726

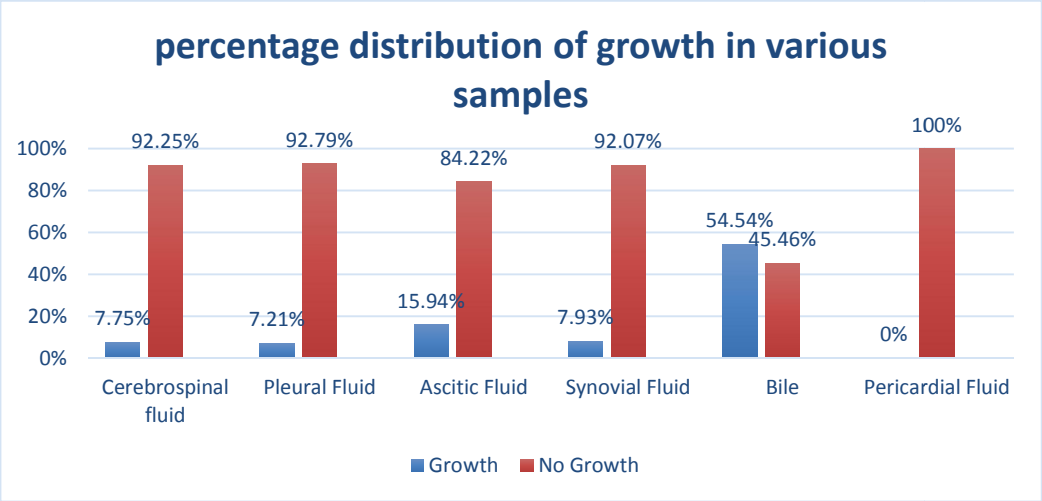

Fig. 1. Percentage distribution of various samples

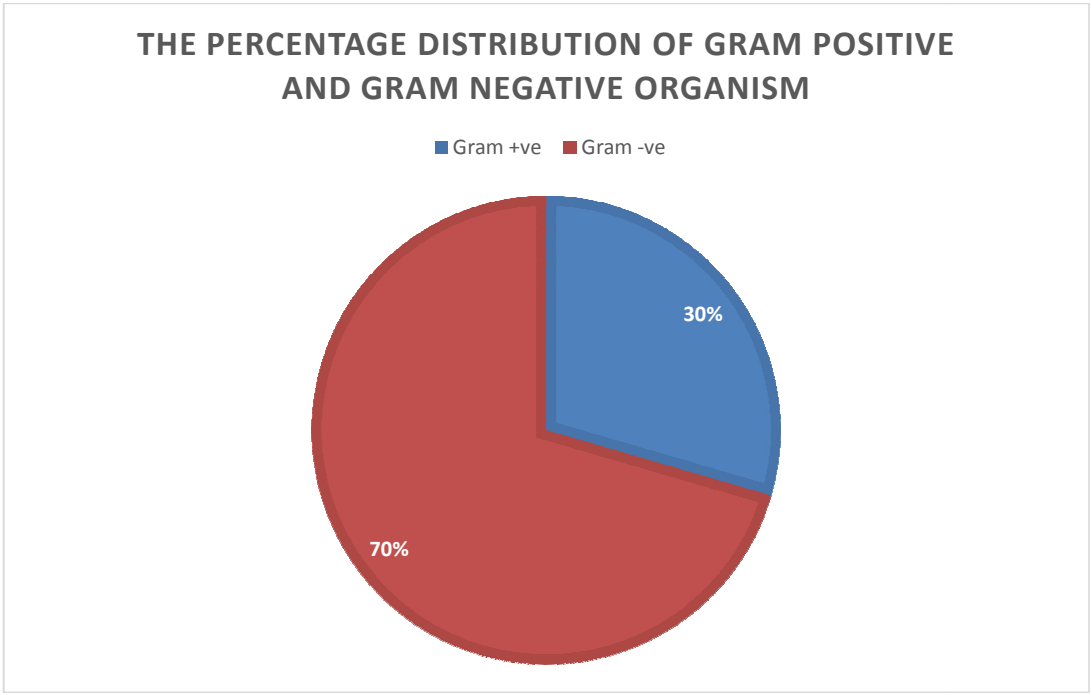

Fig. 2. Percentage distribution of gram positive and gram-negetive organism

The percentage of various gram-positive organisms obtained from different body fluids is shown in Fig. 4. As is evident from the figure the most commonly isolated Gram positive organism was *Staphylococcus aureus* followed by *Coagulase Negative Staphylococci* and *Enterococcus* sp.

As shown in Fig. 5 *E. coli* was the most commonly isolated gram-negative organism *E.* followed b *Pseudomonas sp*, *Acinetobacter*, and *Klebsiella sp*.

The antibiotic sensitivity pattern of different isolates is shown in Figs. 6,7 and 8. Colistin (100%) was the most effective drug for both lactose fermenting and non-lactose fermenting organisms followed by Carbapenems (50%-60%), Amikacin (50%) and Gentamicin (50%). Gram positive isolates were highly sensitive to

Vancomycin (100%), Linezolid (100%), Tigecycline (60%-70%), Teicoplanin (50%-60%). About 36.6% of isolates in our study were MRSA and 36.6% of isolates were MR-CONS. Also, multidrug resistance was seen among Gram negative organisms.

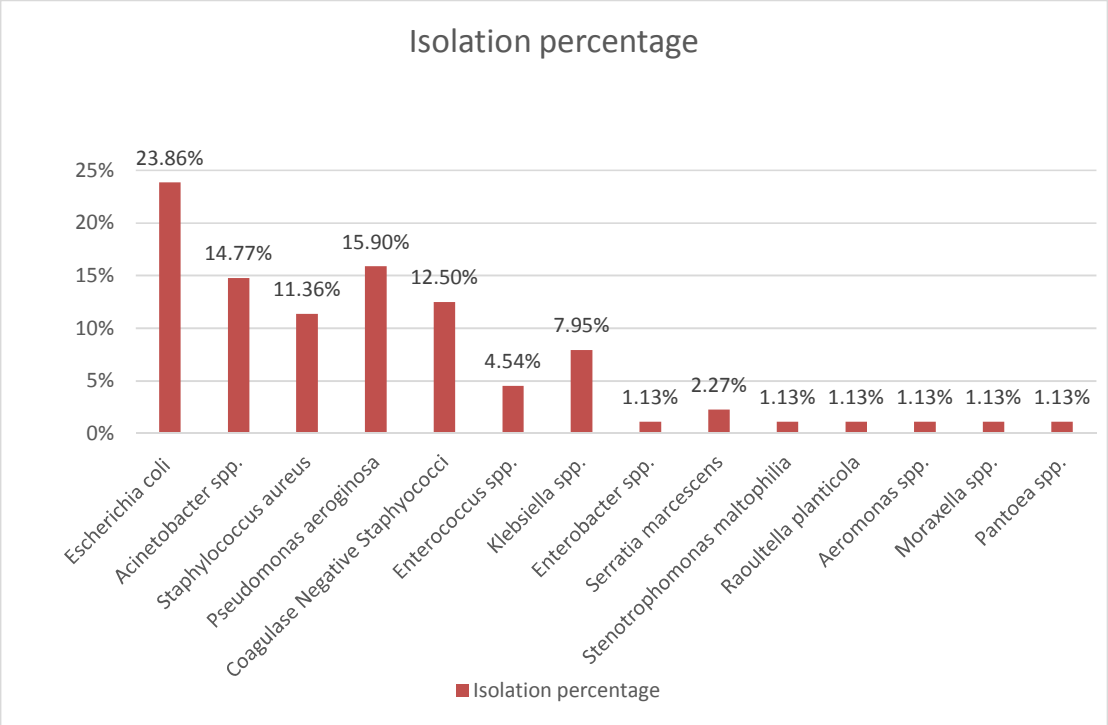

Fig. 3. Rate of isolation of various organisms

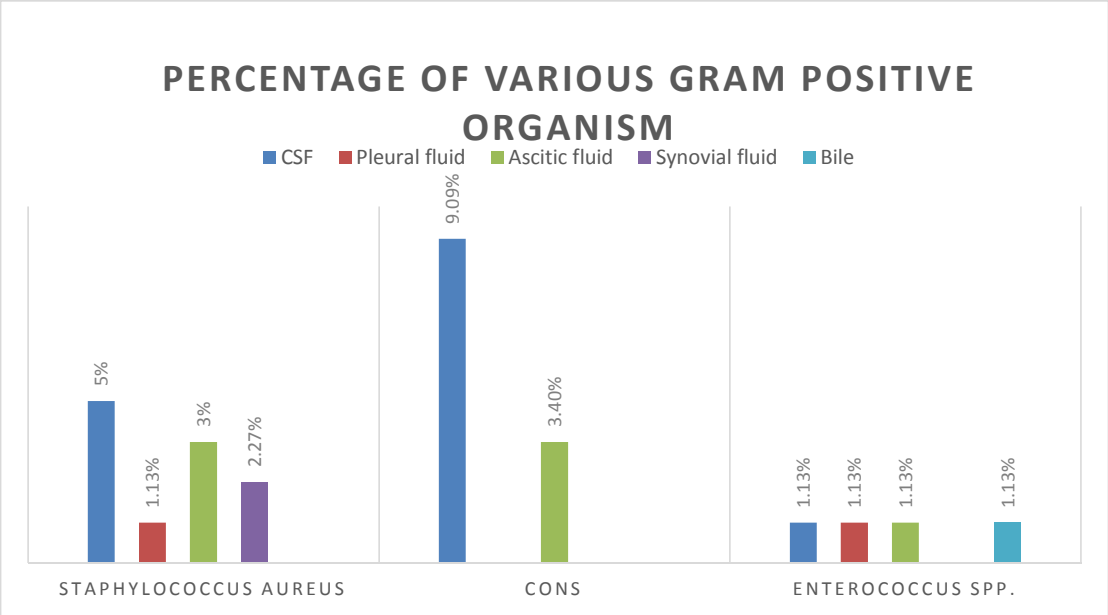

Fig. 4. Percentage of various gram-positive organism obtained from different body fluids

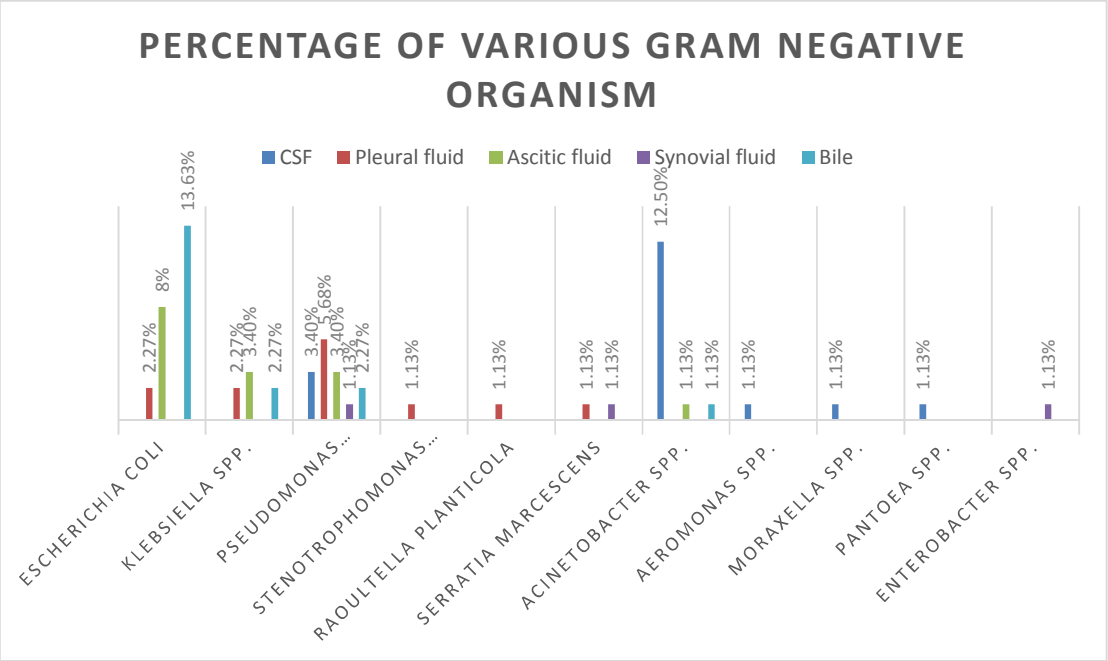

Fig. 5. Percentage of various gram-negative organism obtained from different body fluids

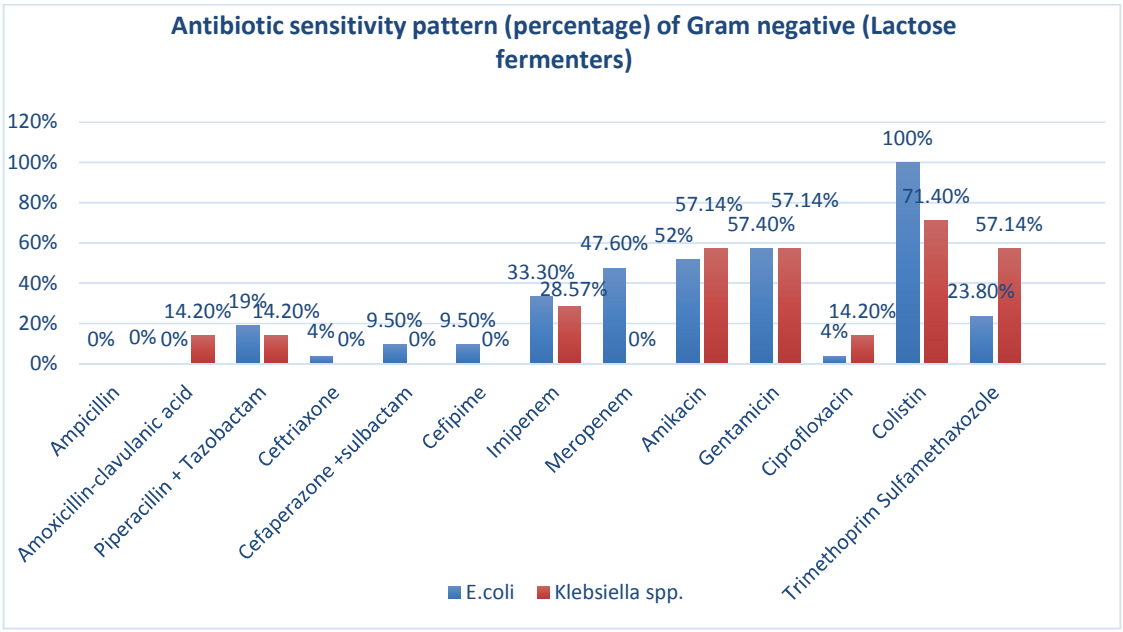

Fig. 6. Antibiotic sensitivity pattern (percentage) of gram negative (Lactose fermenters)

4. DISCUSSION

Sterile sites are those in which no bacteria or microbes exist as commensals when in a healthy state. As such any growth isolated from these sites is considered significant and can either be due to pathogenic microorganisms or contaminants.

In our study, the culture positivity rate was 10.81%. This correlates with the findings of Deb A et al. [13] and Kasana et al. [3] which showed

isolation rate of 14.41% and 14.8% respectively. Other studies like that of Teklehymanot F et al [14] too showed lower culture positive rates of about 14.1%.

This is in contrast to other studies conducted on similar lines where the positive results were 31% and 24% respectively. [15,16] This might be due to over diagnosing, prior exposure to antibiotics and emergence of non-infectious conditions like malignancy. [14,15] Some of the variations are likely explained by the differences in the study population [17].

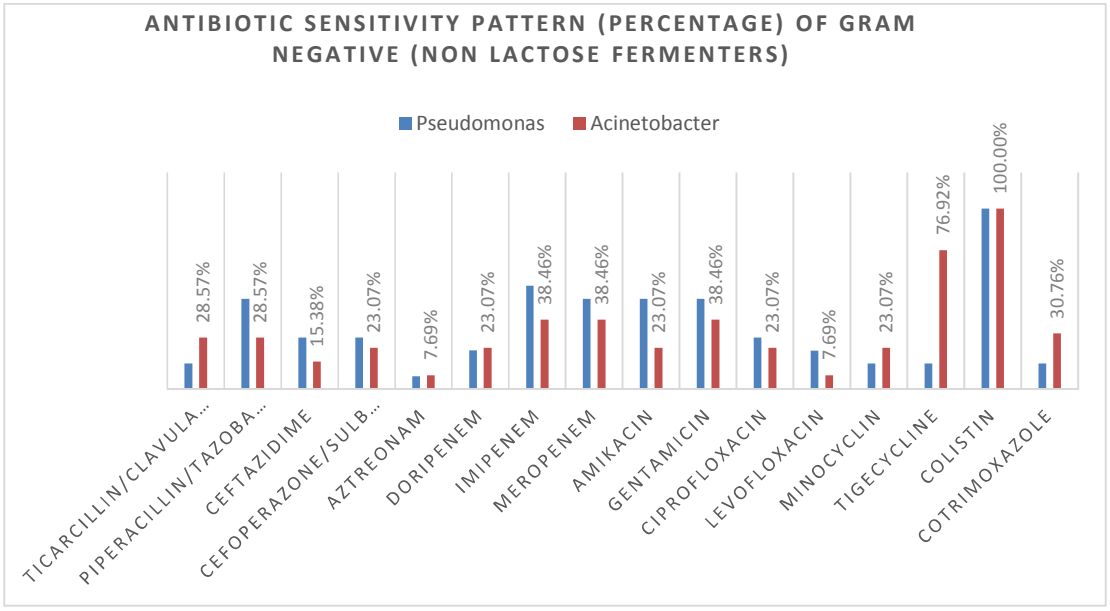

Fig. 7. Antibiotic sensitivity pattern (percentage) of gram negative (Non lactose fermenters)

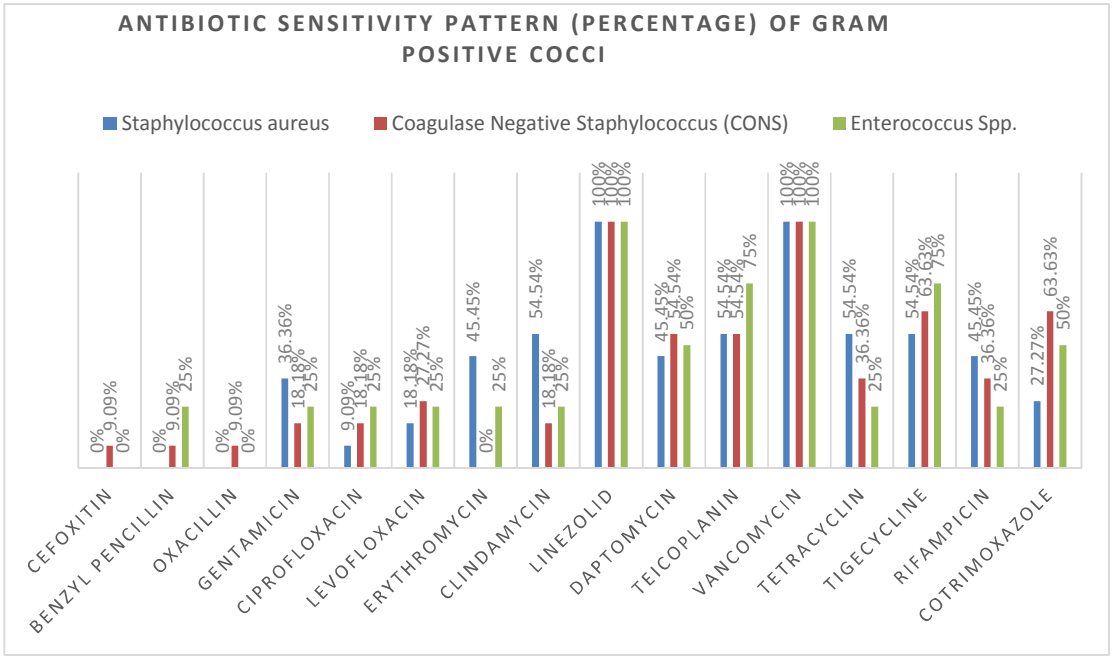

Fig. 8. Antibiotic sensitivity pattern (percentage) of gram positive cocci

A total of 814 body fluid samples were included in the study out of which CSF samples (387) constituted 47.25%, pleural fluid samples (194) 23.68%, peritoneal fluid (133) 16.23%, bile (33) 4.02%, synovial fluid (63) 7.69% and pericardial fluid (4) 49%.

In our study, Gram-negative organisms had an isolation rate of 70% as compared to gram positive isolates (30%). Amongst the Gram negative the most common was Escherichia coli (23.86%) and amongst Gram positive the most common pathogenic organism was Staphylococcus aureus (12.5%). This finding was similar to Deb A et al. study [13] and Harshika et al study [18]. However, this is in contrast to the study conducted by Sharma et al [19] where *Acinetobacter spp.* was the main isolate.

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.

Gram negative bacteria were more commonly isolated from Ascitic fluid than Gram positive bacteria. Among Gram negative bacteria *E. coli* (7) was the most common isolate followed by Non Fermenter Gram Negative bacilli (NFGNB) (4), and *Staphylococcus aureus* (4) which is in similar to the study of Sujatha R et al. [15], Arroyo et al. [20] and Chawla [21] which also showed *E-coli* as the commonest organism. Some studies have shown NFGNB to be the most important organisms isolated from Ascitic fluid [18].

In pleural fluid, Gram negative organisms were isolated more compared to gram positive organisms similar to some studies. [3,13] This is in contrast to few other studies where gram positive organisms accounted for maximum number of cases and Staphylococcus aureus (70%) was the most common pathogen isolated followed by CONS. [22] The isolation of aerobic Gram Negative Bacilli 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. In bile Gram negative isolation rate was more compared to Gram positive in which *E. coli* (66.66%) isolation rate was maximum, similar to findings of Suna et al. [23].

In case of synovial fluid, we found *Staphylococcus aureus* (40%) *Pseudomonas sp* (20%) and Enterobacter sp (20%) and *Serratia marcescens* (20%) as the only isolates whereas other studies conducted isolated *S. aureus, Klebsiella spp., Pseudomonas species and Enterococcus spp* [19].

In our study the most effective antibiotic against Gram negative organisms was colistin followed by carbapenems, amikacin and gentamicin. These findings are in agreement with the findings of Tullu MS et al. [24] and Sharma et al [19] which also showed 100% sensitivity to colistin. Also, in our study *Acinetobacter* was the most resistant pathogens to many antibiotics which was also seen in some other studies [19] [25]. Acinetobacter is an important public health problem, especially in ICUs and may cause severe infections with a high mortality rate [26].

Gram positive isolates were 100% sensitive to Vancomycin and Linezolid. About 36.36% of *S. aureus* isolates in our study were MRSA, which is much similar to other studies performed in India [27].

As is evident from our study, we observed an overall increasing trend of resistance in both gram negative and gram-positive isolates, which warrants regular surveillance studies. Judicious use of antibiotics, along with strict adherence of hospital infection control may result in significant decline in morbidity and mortality among patients.

5. CONCLUSION

Infections of sterile body fluids are usually associated with high morbidity and risk of sequelae and this can be prevented by early initiation of appropriate therapy. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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