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Original Research Article

Bacteriological Profile and Antibiogram of Isolates Causing Bloodstream Infection in Children

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Abstract

Objective*:** (1) To identify and characterize bacterial isolates causing blood stream infection in children. (2) To determine the antibiotic sensitivity pattern of isolates obtained. ***Materials And Methods*: *Study design*:** Descriptive study**. *Study population: Blood samples collected from pediatric patients aged upto 12 years who are admitted in Government Medical College Kottayam, with clinical features of blood stream infection. ***Sampling methodology***: The sample size of suspected blood stream infection is 345. All pediatric patients with clinical features suggestive of blood stream infection in the study period satisfying inclusion and exclusion criteria will be included in the study. The identification of bacterial pathogens and antimicrobial susceptibility tests were done by conventional and automated methods like VITEK system. ***Results:*** Out of 345 study sample population, 46 were culture positive, of which 21(45.7%) were Gram negative isolates and 20(43.4%) were Gram positive. Major isolates obtained were Klebsiella spp (15%), Methicillin Resistant Coagulase negative Staphylococci (15%), followed by Staphylococcus aureus (13%), Coagulase negative Staphylococci (8%) and Acinetobacter baumannii (8%). Klebsiella pneumoniae was resistant to most of the antibiotics tested except meropenem. All isolates of Coagulase negative Staphylococci and Staphylococcus aureus were susceptible to vancomycin and linezolid. ***Conclusion*:** Gram negative organisms were predominant pathogens in blood stream infections. Klebsiella pneumoniae, Coagulase negative Staphylococci, Staphylococcus aureus were the most commonly isolated pathogens. Amikacin along with the third generation cephalosporins should be used for empirical treatment of Gram negative sepsis. Vancomycin and linezolid can be used for Gram positive pathogens.

Key words: Blood stream infection, children.

INTRODUCTION

Sepsis is defined as a life-threatening condition that occurs when the body's response to an infection becomes dysregulated, leading to organ dysfunction. It is a serious medical emergency that requires immediate attention and treatment [1]. In the pediatric population, sepsis is a significant cause of mortality worldwide with an estimated 7.5 million deaths occurring annually. Among children who develop sepsis worldwide, 49% have a comorbid condition that leaves them vulnerable to infection. Children with septicemia present with fever, difficulty in breathing, tachycardia, malaise, refusal of feeds or lethargy. It is a medical emergency that requires urgent rational antibiotics therapy. The gold standard for diagnosis of septicemia is the isolation of bacterial agent from blood culture. Bacteriological culture is done to isolate the offending pathogens and to know about the sensitivity pattern of the isolates. It remains the mainstay of definitive diagnosis and the management of BSIs. A variety of organisms are isolated in BSIs like Staphylococci, Enterococci and Enterobacteriaceae etc. There are several factors that can contribute to the increasing incidence of sepsis. Indwelling catheters, such as those used for intravenous access or urinary catheterization, can provide a pathway for bacteria to enter the body and cause infections [6].

Sepsis in neonates is broadly categorized into early and late onset sepsis depending upon the postnatal day of presentation. Early-onset neonatal sepsis (EONS) occurs within first 72 h of life, while the late-onset neonatal sepsis (LONS) occurs between 72 h to 90 days of life. The bacterial agents implicated in early-onset sepsis include Group B Streptococcus (GBS), Escherichia coli, Coagulase-negative Staphylococcus,

Haemophilus influenzae and Listeria monocytogenes. The organisms commonly associated with late-onset sepsis include Coagulase-negative Staphylococci (CONS), Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Enterobacter spp., Pseudomonas aeruginosa and Acinetobacter species. As delay in the treatment of neonatal sepsis is associated with increased mortality, empirical therapy is the cornerstone in the management of neonatal sepsis. A combination of ampicillin or third generation cephalosporins with an aminoglycoside (gentamicin) is the commonly used empirical regimen [3]. However, the appropriateness of this empirical therapy is being challenged in the present era of changing bacteriological profile and increasing antimicrobial resistance. Hence, there is a need for surveillance to understand the trends in pathogens causing sepsis in children and the antibiotic susceptibility profile of those pathogens.

This study is to determine the common bacterial agents associated with sepsis in children and their antibiotic susceptibility pattern.

METHODOLOGY

Study Design: Descriptive study.

Study Setting: Department of Microbiology Government Medical College Kottayam.

Duration Of Study: 12 months

Study Population: Blood samples collected from pediatric patients (aged upto 12 years) who are admitted in Government Medical College Kottayam, with clinical features of bloodstream infection.

Sample Size

Using the formula $4pq/d^2$, taking p as 22.58, q as 77.42 and d^2 as 20.25 based on a study conducted in Gujarat by Hetal G Vaghela, Bithika Duttaroy and Khyati C Prajapati. Bacteriological profile and antibiogram of blood isolates from pediatric patients with special reference to ESBL and MRSA in a tertiary care centre [2].

Sample size = $4pq/d^2$

P is 22.5 Q is 77.42 d is 20% of p

$4pq/d^2=4\times22.58\times77.42/4.5\times4.5$

$=345$

Inclusion Criteria: Pediatric patients (less than or equal to 12 years, admitted in wards and ICUs with clinical features suggestive of bloodstream infection.

Exclusion Criteria: Repeat isolate obtained from same patient

Sampling Methodology: All pediatric patients with clinical features suggestive of blood stream infection in the study period satisfying inclusion & exclusion criteria will be included in the study.

Study Tools

A proforma, pediatric blood culture bottle (conventional or BacT/ ALERT), Gram's staining, Blood agar, MacConkey agar, Mannitol salt agar, Mueller Hinton Agar for antibiotic sensitivity testing, antibiotic discs, Biochemical reactions for identification of isolate- indole reagent, oxidase reagent, xylene, nitrate reagent, Christensen's urease agar, Simmon's citrate agar, Triple sugar Iron Agar, Mannitol motility media, sugar fermentation media, Hugh-Leifson 's oxidation-fermentation media, decarboxylase media, arginine dihydrolase media. Automated techniques like VITEK system.

RESULT

Age Distribution of Study Sample Population.

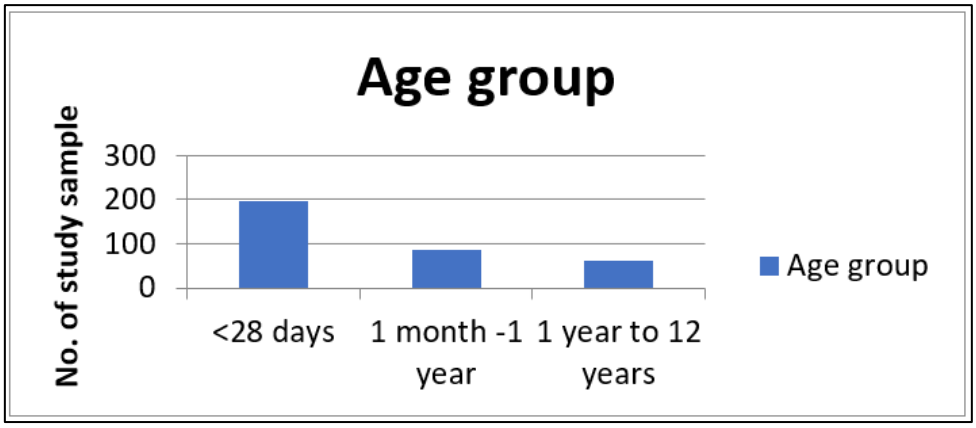

Figure 4: Bar diagram showing distribution of study sample according to Age

Table 9: Distribution of study sample according to Age

Age group	Frequency	Percentage
<28 days	198	57.4
1 month -1 year	86	24.9
1 year to 12 years	61	17.7
Total	345	100.0

Out of 345 samples collected, 198 samples were from children aged <28 days (57.4%), followed by 86 from age group 1month- 1year (24.9) and 61 from age group 1- 12 year (17.7%).

Gender Distribution in Study Sample Population

Table 10: Distribution of study sample according to gender

Gender	Frequency	Percentage
Male	217	62.9
Female	128	37.1
Total	345	100.0

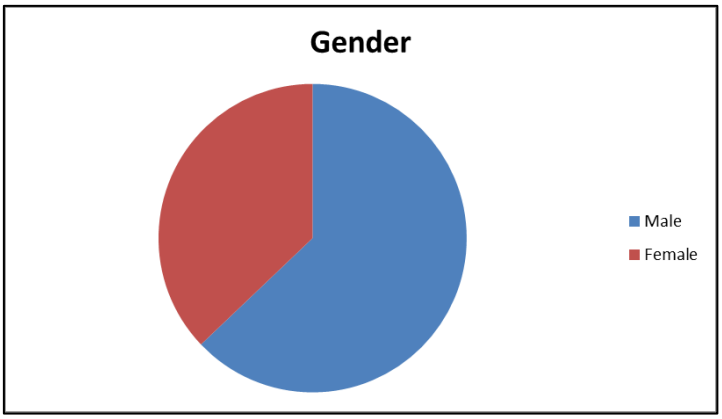

Figure 5: Pie chart showing distribution of study sample according to gender

Gender distribution among study samples was 217 males and 128 females.

Culture Status of Study Sample Population

Out of 345 study samples collected, 46 samples showed growth on blood culture with culture positivity rate of 13.3%.

Table 11: Distribution of study sample according to growth in culture

Culture growth	Frequency	Percentage
Present	46	13.3
Absent	299	86.7
Total	345	100.0

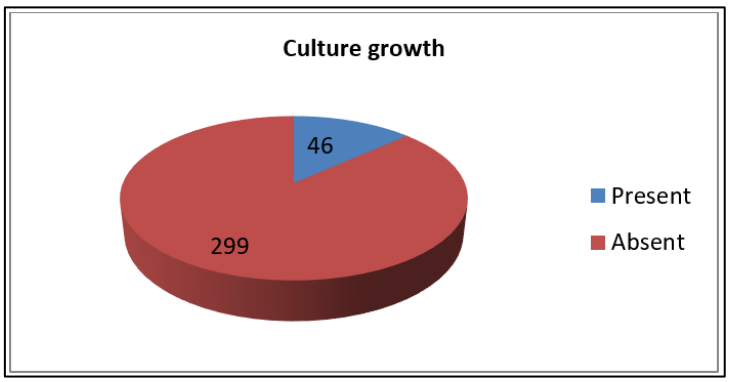

Figure 6: Pie chart showing distribution of positive sample according to growth in culture

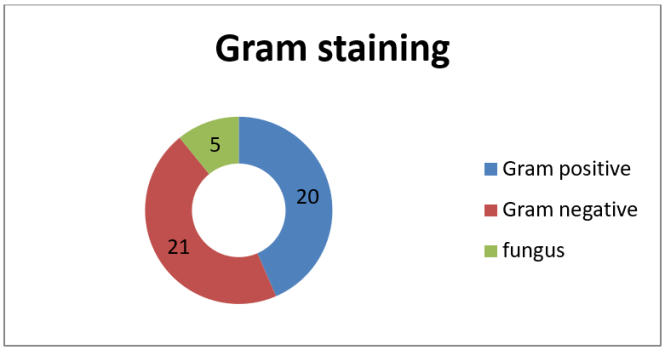

Figure 7: Distribution of study sample according to gram staining property of isolates

Table 12: Distribution of study sample according to gram staining property of isolates

Gram staining	Frequency	Percentage
Gram positive	20	43.4
Gram negative	21	45.7
Fungus	5	10.9
Total	46	100

Among various pathogens isolated, 21 were Gram negative bacilli (45.7%) followed by 20 Gram positive cocci (43.4%) and 5 Candida species (10.9%).

Age Distribution of Culture Positive Population

Table 13: Distribution of study sample according to age group of culture positive population

Age group	Frequency	Percentage
<28 days	25	54.3
1 month -1 year	11	23.9
1 year to 12 years	10	21.7
Total	46	100.0

Out of 46 culture positive samples majority showed growth in samples collected from children aged < 28 days (54.3%) followed by 23.9% in age group of 1 month – 1 year and 21.7% in age group 1- 12 years.

Gender Distribution of Culture Positive Population

Table 14: Distribution of study sample according to gender of culture positive population

Gender	Frequency	Percentage
Male	22	47.8
Female	24	52.2
Total	46	100.0

Among 46 culture positive population, 24 (52.2%) were females and 22 (47.8%) were males.

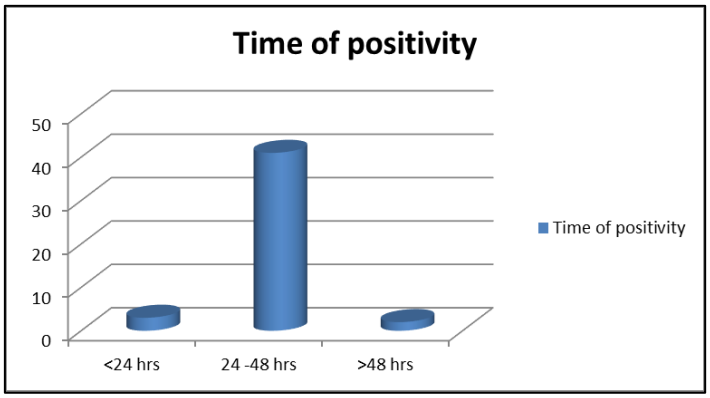

Figure 8: Bar diagram showing distribution of study sample according to time to positivity

Majority of study samples showed culture positivity during 24- 48 hours of incubation of culture samples.

Distribution of Sepsis Onset

Table 15: Distribution of study sample according to onset of sepsis of culture positive population

Onset of sepsis	Frequency	Percentage
Early onset	6	13.0
Late onset	23	50.0
NA	17	37.0
Total	46	100.0

Among the culture positives, 23 of them presented with late onset sepsis and 6 with early onset sepsis.

Table 16: Distribution of study sample according to CRP of culture positive population

CRP	Frequency	Percentage
Not elevated	4	8.7
Elevated	42	91.3
Total	46	100.0

91.3% culture positive population showed elevated CRP in serum

Table 17: Distribution of Pathogens Isolated

Organism isolated	Frequency	Percentage
Klebsiella spp	7	15
Coagulase negative Staphylococci	4	8
Citrobacter koseri	1	2
Staphylococcus aureus	6	13
Methicillin resistant coagulase negative staphylococci	7	15
MRSA	1	2
Burkholderia spp.	1	2
Pseudomonas aeruginosa	3	6
Escherichia coli	3	6
Streptococcus pneumoniae	2	4
Salmonella typhi	2	4
Acinetobacter baumannii	4	8
Candida albicans	2	4
Candida krusei	3	6
Total	46	100.0

Among 46 culture isolates, predominant organisms were Klebsiella spp (15%) and Methicillin Resistant Coagulase negative Staphylococci (15%) followed by Staphylococcus aureus (13%), Coagulase negative Staphylococci (8%), Acinetobacter baumannii (8%), Pseudomonas aeruginosa & Escherichia coli, Pneumococcus, Salmonella typhi, along with Citrobacter koseri, MRSA, Burkholderia spp.

Table 18: Etiological agents of early onset and late onset sepsis

Organisms isolated in neonates	No. of isolates in early onset sepsis	No. of isolates in late onset sepsis
CONS	2	6
Klebsiella pneumoniae	0	5
E coli	2	1
Acinetobacter spp	1	2
Staphylococcus aureus	0	2
Pseudomonas aeruginosa	0	1
MRSA	0	1
Citrobacter spp	0	1
Burkholderia spp	0	1
Candida spp	1	3
Total	6	23

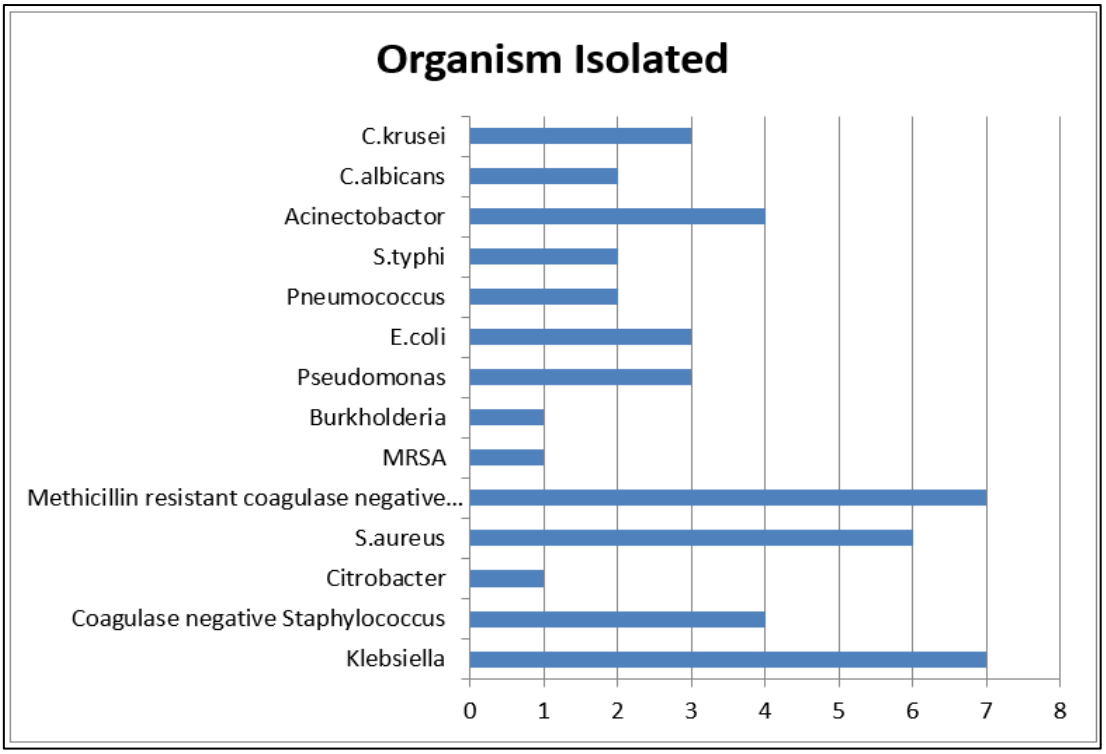

Figure 9: Bar chart showing distribution of study sample according to organism isolated in culture

Antibiotic Susceptibility Pattern in Culture Isolates

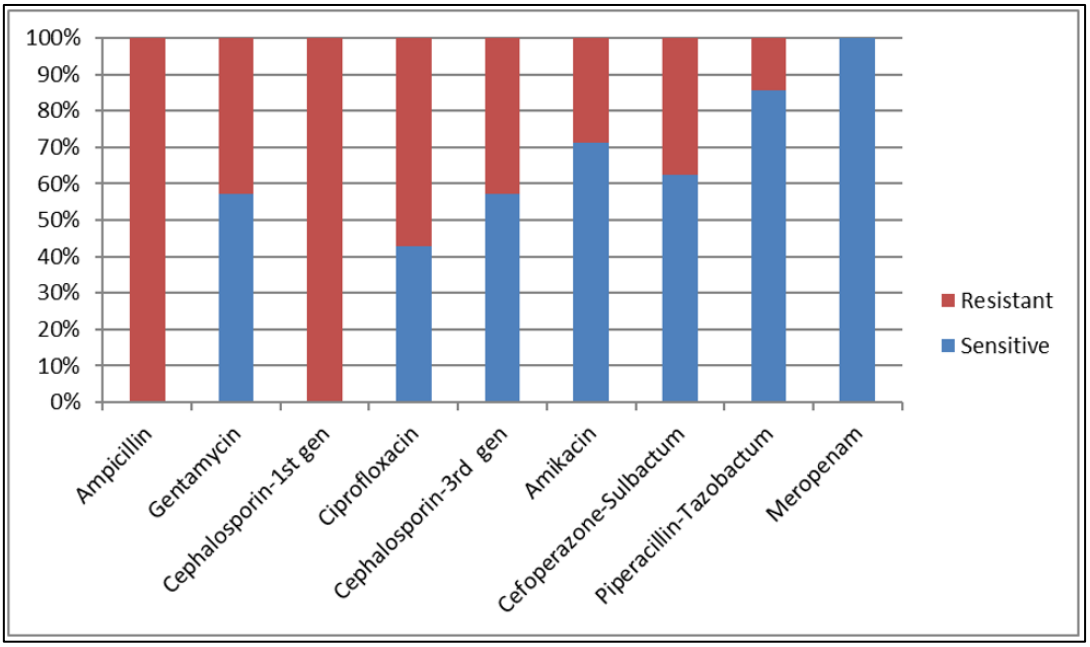

Figure 10: Susceptibility pattern of Klebsiella pneumoniae

All isolates of Klebsiella pnemoniae were resistant to Ampicillin and 1st generation Cephalosporins. 57% were susceptible to 3rd generation cephalosporin (cefotaxime). All were susceptible to meropenem.

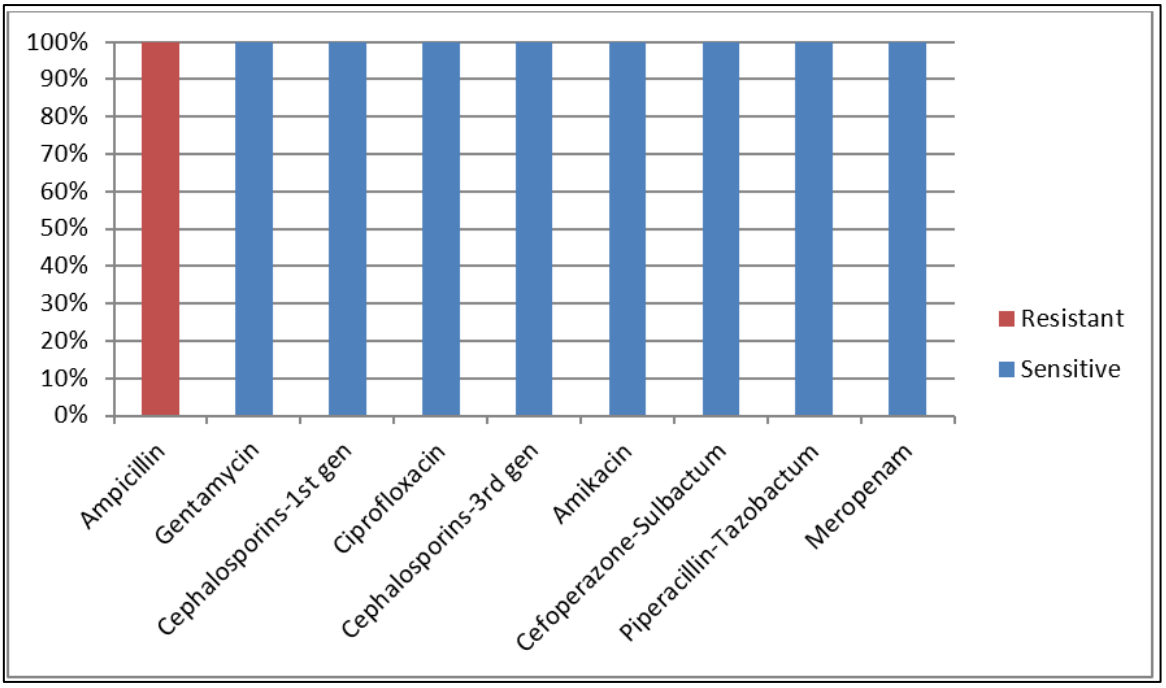

Figure 11: Susceptibility pattern of Citrobacter koseri

Citrobacter was resistant to ampicillin which indicates its intrinsic resistance.

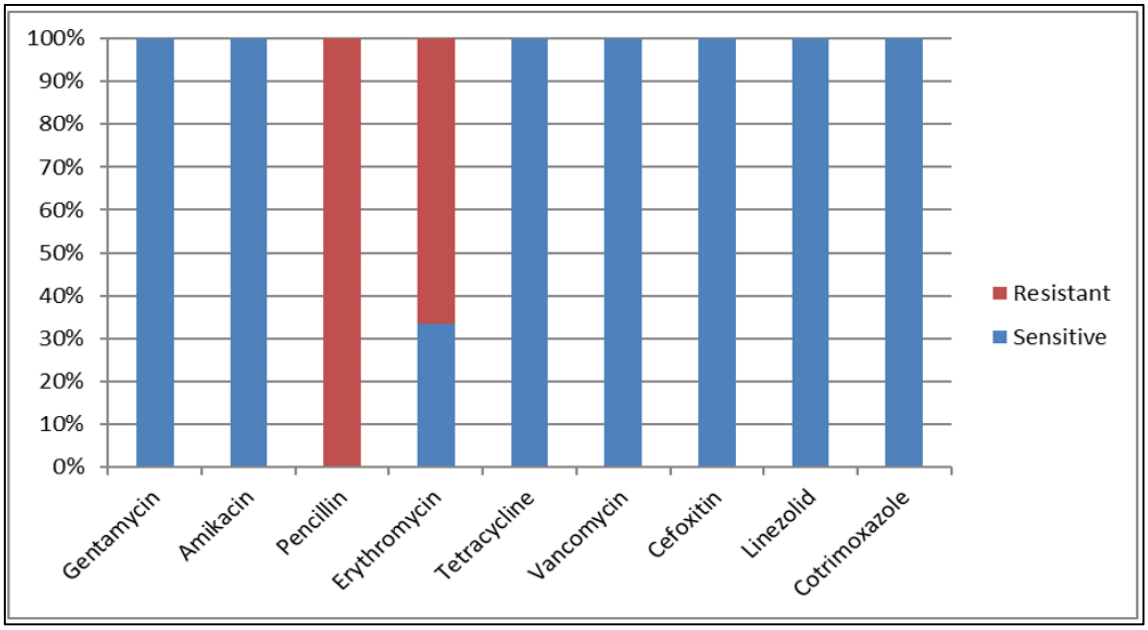

Figure 12: Susceptibility pattern of Staphylococcus aureus

All isolates were penicillin resistant. 33% of Staphylococcus aureus were susceptible to erythromycin. All isolates were susceptible to gentamicin, amikacin, vancomycin, linezolid and cotrimoxazole.

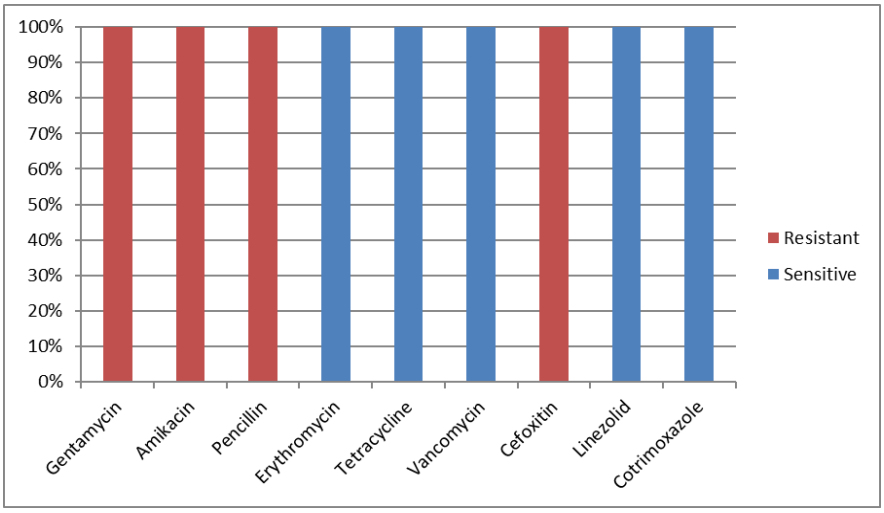

Figure 13: Susceptibility pattern of MRSA

MRSA were susceptible to erythromycin, tetracycline, vancomycin, linezolid and cotrimoxazole.

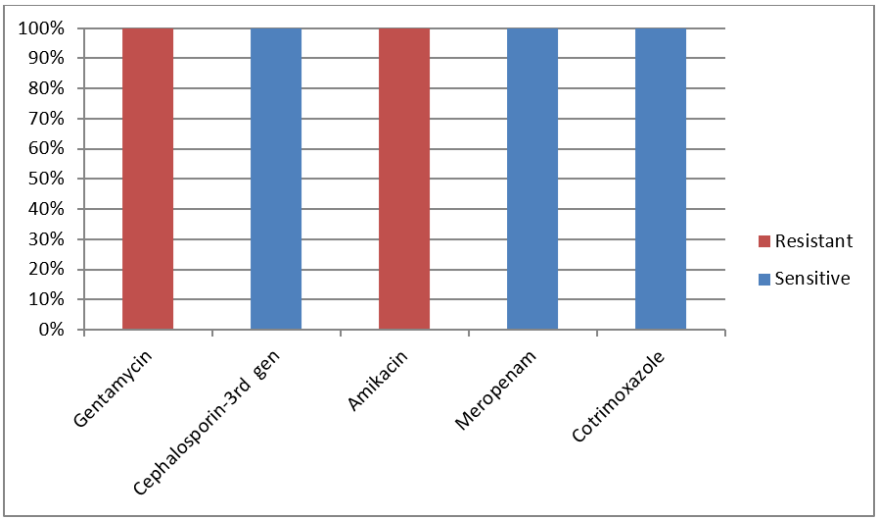

Figure 14: Susceptibility pattern of Burkholderia spp

Burkholderia spp. was susceptible to 3rd generation cephalosporin (Ceftazidime), cotrimoxazole and meropenem.

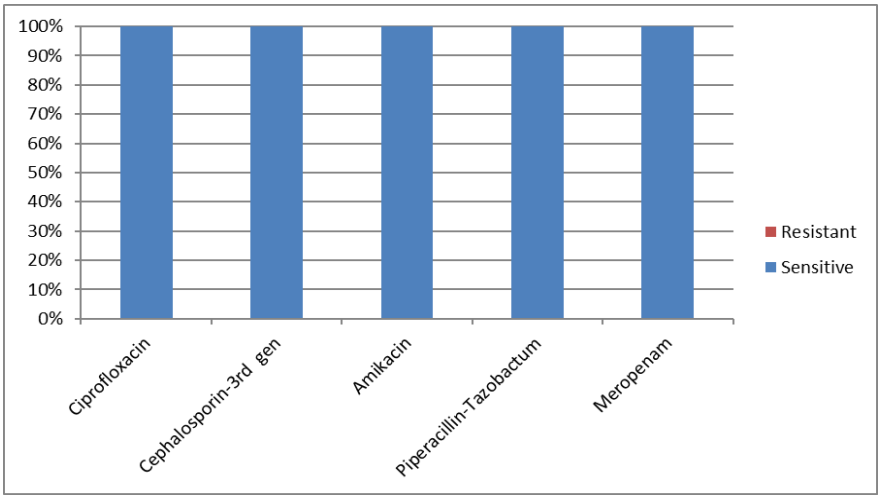

Figure 15: Susceptibility pattern of Pseudomonas aeruginosa

All Pseudomonas were susceptible to ciprofloxacin, amikacin, ceftazidime, piperacillin-tazobactam and meropenem.

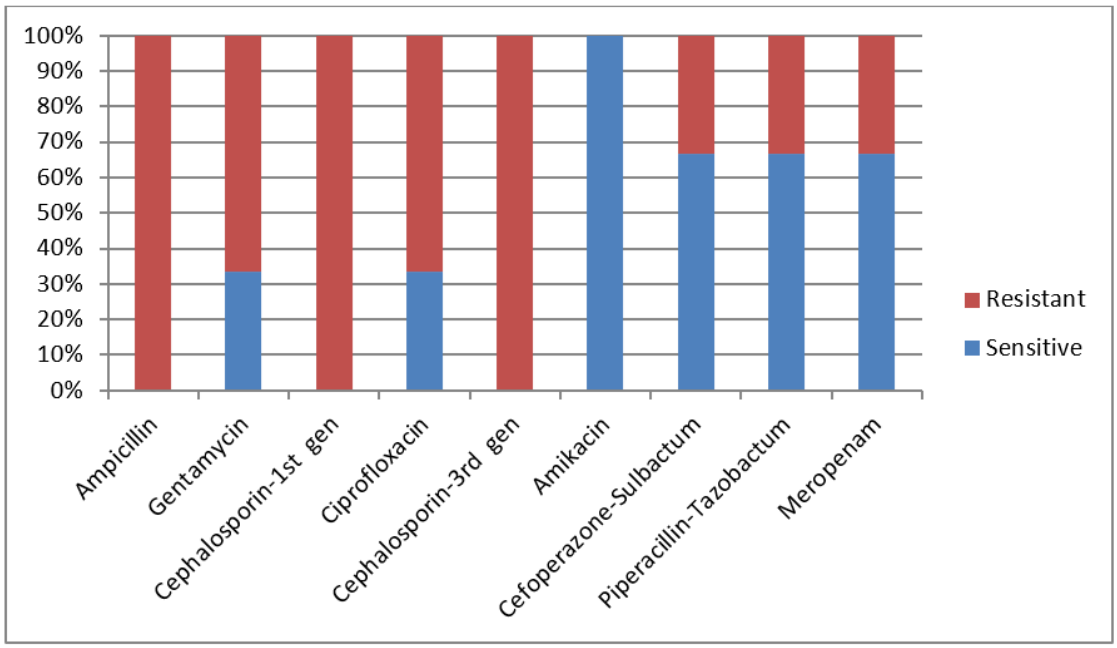

Figure 16: Susceptibility pattern of Escherichia coli

100% were resistant to ampicillin, 1st and 3rd generation cephalosporins. Only 34% of E coli were susceptible to gentamicin and ciprofloxacin. 67% were susceptible to cefoperazone-sulbactam, piperacillin-tazobactam and meropenem.

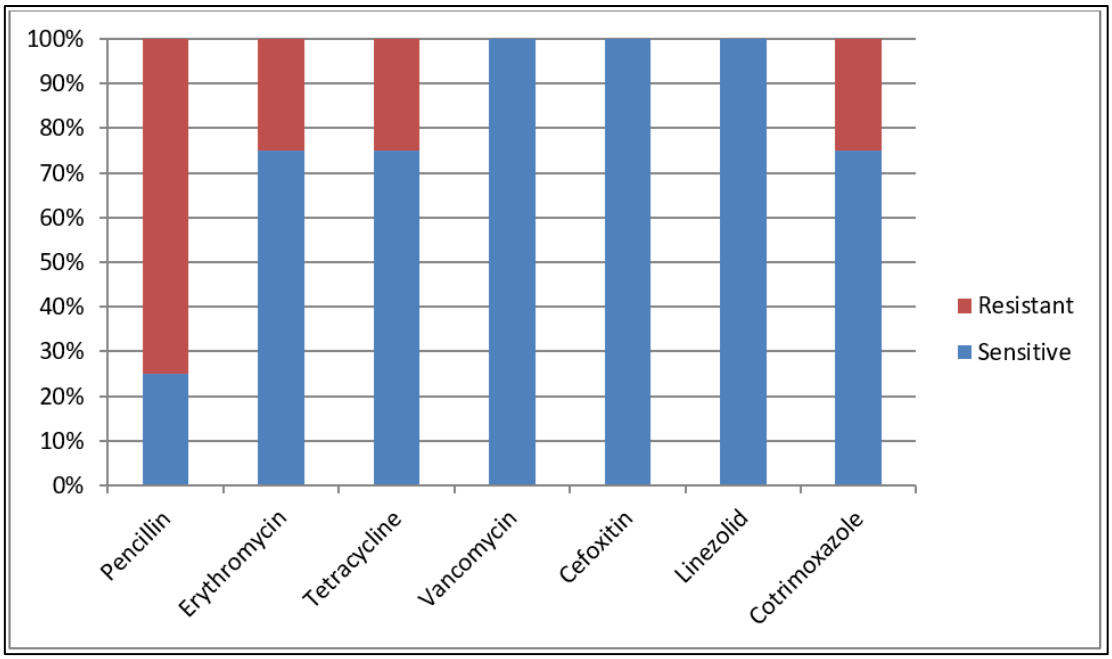

Figure 17: Susceptibility pattern of CONS

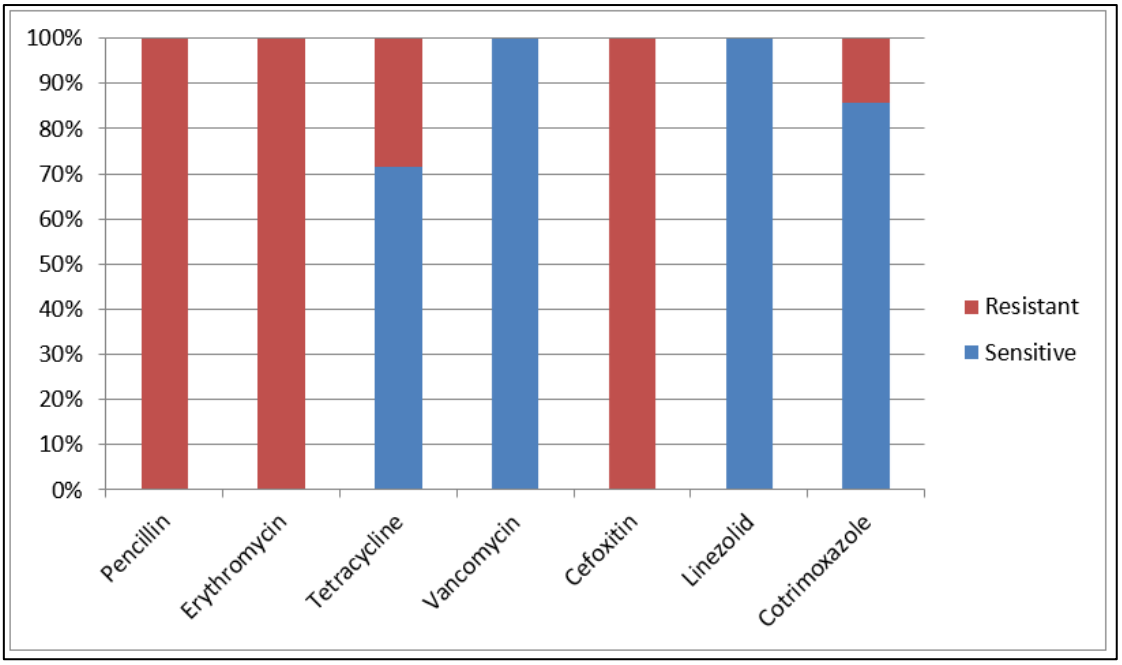

Figure 18: Susceptibility pattern in MRCONS

All isolates of methicillin resistant coagulase negative staphylococci were susceptible to vancomycin and linezolid. 85% were susceptible to cotrimoxazole.

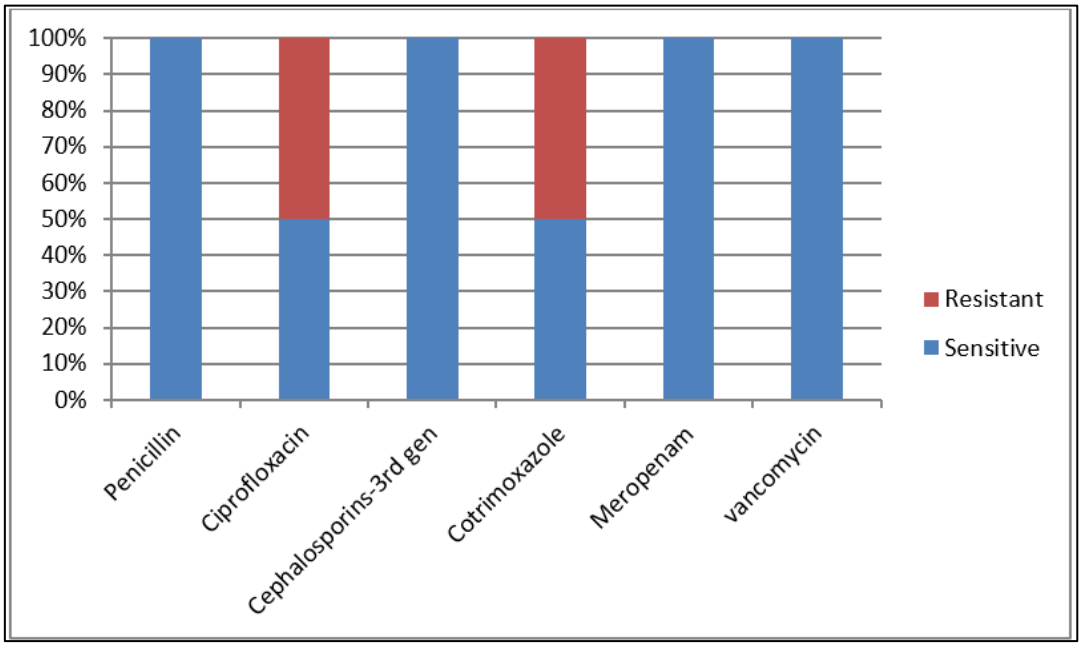

Figure 19: Susceptibility pattern of Streptococcus pneumoniae

All isolates were susceptible to penicillin, vancomycin and meropenem. 50% were susceptible to ciprofloxacin and cotrimoxazole.

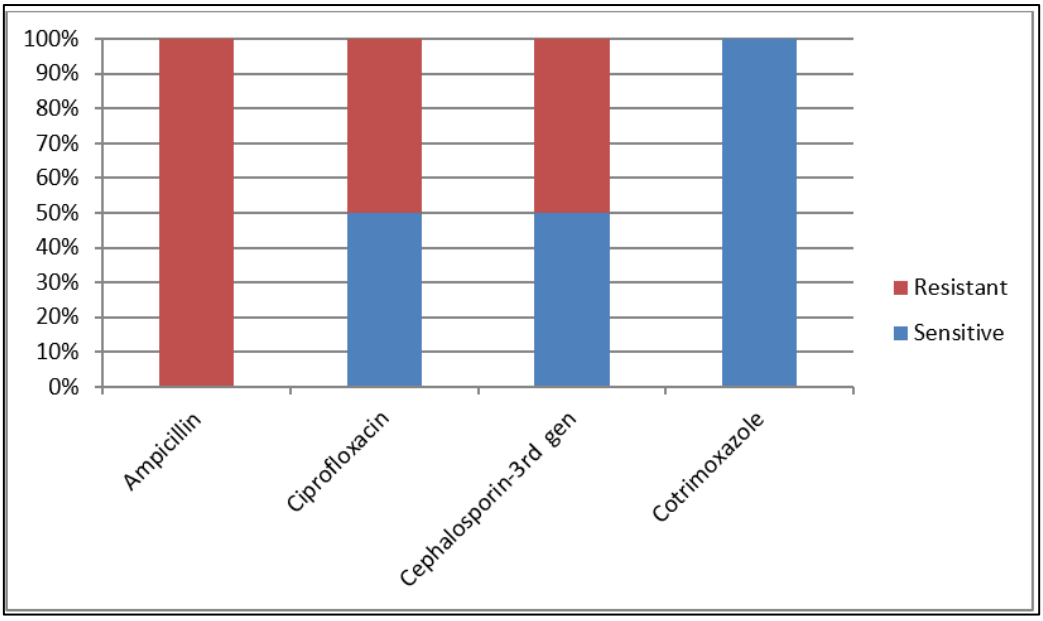

Figure 20: Susceptibility pattern of Salmonella typhi

100% are resistant to ampicillin.50% are resistant to ciprofloxacin and ceftriaxone. All are sensitive to cotrimoxazole.

DISCUSSION

This study was conducted over a period of one year to find out the bacteriological profile and antibiogram of isolates causing bloodstream infection in children (<12years) admitted in Government Medical College, Kottayam. A total of 345 samples collected from patients with clinical features suggestive of bloodstream infection were included in the study. Positive bacterial growth was observed in 46 samples showing a culture positivity of 13.3%. 1-3ml of blood sample was taken aseptically and send in automated blood culture bottle along with a properly filled proforma containing clinical history and important investigation results. When blood culture flags positive we will strictly follow up the patient.

In this study, among the 46 cases with blood culture confirmed sepsis 24 (52.2%) were females. In a study conducted by Venturini *et al.*, females (61.5%) showed higher incidence of central line associated bloodstream infection in a tertiary care children's hospital which was similar to present study. In this study the rate of isolation was found highest among newborns (54.3%) followed by children of 1mon- 1year age Group (23.9%). Similar results were reported in a study conducted by S P Pant *et al.*, in Nepal where the isolation rate was 45.8% among neonates. In another study conducted in selected hospitals in Ethiopia also the rate of isolation was highest among neonates. On statistical analysis, culture confirmed bacteremia and age were found significantly associated that means the culture positivity is dependent on age groups. Higher incidence of sepsis among them might be due to their underdeveloped immune system or lack of proper hygienic practices during delivery.

Among clinically suspected cases of sepsis in neonates, 70 were early onset and 194 were late onset sepsis. In 6 early onset cases the culture flagged positive and in 23 late onset sepsis cases pathogen was isolated. Common organisms isolated in late onset sepsis are Coagulase negative Staphylococci and Ecoli were as in late onset sepsis organisms isolated were Coagulase negative Staphylococci followed by Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter spp. The etiological agents in early onset sepsis differ from that of late onset sepsis as the mode of infection is different [3]. Early onset is acquired transplacentally or as an ascending infection from cervix or during passage of the baby through a colonized birth canal. Late onset sepsis is usually acquired from the care giving environment and Coagulase negative Staphylococci, Staphylococcus aureus, Escherichia coli and Klebsiella spp are the common agents involved. In a study conducted by Zakariya regarding neonatal sepsis, Klebsiella pneumoniae was the common agent in early onset sepsis while no Group B Streptococcus was isolated. Klebsiella pneumoniae and CONS were the agents of late onset. In present study also there was no Group B Streptococcus although this organism was considered as an important agent associated with early onset sepsis, recent studies are showing a decreasing trend in the incidence of this pathogen. Many studies are showing increasing trend of Coagulase negative Staphylococci.

Severity of infection can be associated with certain sepsis markers in serum especially CRP level. Both acute systemic Gram-positive and Gram-negative bacterial infections, as well as systemic fungal infections

cause marked CRP rises, even in immunodeficient patients. By contrast, CRP concentrations tend to be lower in most acute viral infections [1-10]. Hence we send sepsis markers for every patients with suspected blood stream infection on the same day of admission. In the present study similar association can be seen between culture positive population and serum CRP.

PATHOGENS ISOLATED

Of the total blood culture isolates the Gram negative bacteria accounts for 45.7% while Gram positive bacteria accounts for 43.4%. Among Gram negative bacterial isolates the most predominant organism were Klebsiella pneumoniae (15%) followed by Acinetobacter spp of 8%, Pseudomonas aeruginosa and Ecoli of 6% each, Salmonella typhi of 4%,2% each of Citrobacter spp and Burkholderia spp. The commonest Gram positive bacterial isolates were Methicillin Resistant Coagulase negative Staphylococci of 15% followed by Staphylococcus aureus of 13%, Coagulase negative Staphylococci of 8%, 4% of Streptococcus pneumoniae and 2% Methicillin Resistant Staphylococcus aureus. A study by Tariq conducted in a children's hospital in Kabul yielded culture positivity of 12.2%. Among Gram negative bacilli of 51.7% major isolate was Klebsiella spp followed by Enterobacter, Ecoli and Serratia. Among 44.8% of Gram positive cocci, most frequently isolated species were Coagulase negative Staphylococci followed by Staphylococcus aureus, Streptococcus and Enterococcus.

ANTIMICROBIAL SUSCEPTIBILITY TESTS

In present study we got Gram positive and Gram negative isolates. Antimicrobial susceptibility test done for each isolates. For Gram negative catalase positive oxidase negative organisms, antibiotics like ampicillin, gentamicin, amikacin, ciprofloxacin, first and third generation cephalosporins its combination with sulbactam, piperacillin-tazobactam, meropenem tested by Kirby-bauer disc diffusion method. Oxidase positive Gram negative bacilli are tested with gentamicin, amikacin, ceftazidime, ciprofloxacin, piperacillin-tazobactam, meropenem by disc diffusion method. For Salmonella spp. Ampicillin, azithromycin, cotrimoxazole, ceftriaxone, ciprofloxacin, chloramphenicol were tested. Gram positive isolates were tested with penicillin, erythromycin, tetracycline, gentamicin, amikacin, vancomycin, linezolid, cotrimoxazole.

In this study, all isolates of Klebsiella were resistant to ampicillin and first generation cephalosporins but sensitive to meropenem. About 58% were susceptible to gentamicin and 3rd generation cephalosporin. 15% were resistant to piperacillin-tazobactam. One isolate was multidrug resistant but susceptible to colistin. A study done in Ethiopia showed Klebsiella spp. were 100% resistant to commonly used empirical agents like ampicillin, gentamicin and ceftriaxone and 100% sensitive to ciprofloxacin. All isolates of Pseudomonas spp were sensitive to ciprofloxacin, ceftazidime, amikacin. For Salmonella spp 100% were resistant to ampicillin while 100% sensitive to cotrimoxazole. 50% were resistant to ciprofloxacin and ceftriaxone.

Among 20 Gram positive isolates, 11 Coagulase negative staphylococci were isolated of which 7 were resistant to methicillin. All isolates were sensitive to vancomycin and linezolid. For Staphylococcus aureus 100% were penicillin resistant but was 100% sensitive to gentamicin, vancomycin, linezolid and cotrimoxazole. There was only one MRSA isolate which was resistant to penicillin, gentamicin and amikacin in other ways it was sensitive to vancomycin and linezolid.

In the present study, 100% of Pneumococci were sensitive to penicillin, ceftriaxone, vancomycin and showed 50% resistance to ciprofloxacin and cotrimoxazole. In suspected case of pneumococcal sepsis in children common antibiotics like penicillin and 3rd generation cephalosporins seem to be reasonable choice for empiric therapy. For those with signs of meningitis cephalosporins are preferred due to penicillin resistance in one-third of meningeal isolates.

CONCLUSION

Sepsis is one of the leading causes of death worldwide. While the management of critically ill patients with sepsis is certainly better now compared to 20 years ago, sepsis-associated mortality remains unacceptably high. Antibiotic prophylaxis, immunizations, and healthcare quality improvement initiatives are crucial components in the efforts to reduce the morbidity and mortality associated with sepsis worldwide. By incorporating these strategies into a comprehensive and coordinated campaign, we can make significant strides in improving pediatric health globally. Major isolates obtained in the study were Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa among Gram negative agents followed by CONS and Staphylococcus aureus among Gram positives. These organisms remains the principal pathogens responsible for sepsis among pediatric patients.

Antibiotic prophylaxis plays a vital role in preventing sepsis by administering antibiotics to individuals at high risk of infection. This approach helps to eliminate potential pathogens, reducing the likelihood of infection and subsequent sepsis development. By enhancing healthcare practices and providing timely and appropriate care, we can improve patient outcomes, reduce the severity of sepsis, and prevent sepsis-related deaths.

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