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# Bacteriological profile of blood culture isolates from Suspected septicemia in paediatric age group

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**Abstract**
Microorganisms cause septicaemia, systemic disease due to their multiplication and toxins in the blood. These bloodstream infections constitutes a significant public health problems and are a major cause of morbidity and mortality in the hospitalised patients and require rapid antimicrobial treatment. Most children die of septicaemia infections as a result of inappropriate antimicrobial therapy.
**Aim:** 1. To know the occurrence of blood stream infections.
2. To isolate and identify the bacterial agent from blood sample.
3. To determine the antibiotic susceptibility pattern of the isolates.
**Materials and Methods:** A total number of 300 paediatric blood culture samples were processed and identified in the Department of Microbiology from December 2014 to July 2016 in tertiary care centre according to CLSI guidelines. Drug resistant strains in primary screening were further processed for ESBL and MRSA by standard guidelines
**Results:** Out of 300 patients, 137(46%) developed septicaemia with the positive blood culture. Of the 137 positive culture, 87(63.5%) were gram negative bacilli, 50(36.4%) were gram positive cocci. Most common isolates were *Klebsiella pneumoniae* (51.4%) among gram negative isolates and *Staphylococcus aureus* (50%) among gram positive isolates. Maximum isolated strains showed high resistance towards penicillin, cephalosporins and fluoroquinolone, ESBL producers among the *Klebsiella* and *E. coli* isolates were 67.7% while 64% were MRSA among Gram positive cocci.
**Conclusion:** The present study provides much needed information on the prevalence of bacterial pathogens in blood stream infections which highlights the need for periodic surveillance of etiologic agents, their antibacterial susceptibility pattern, also changing trends in the distribution of *isolates* to prevent further emergence and spread of resistant pathogens.

**Keywords:** Paediatric septicaemia, Blood culture, antibiotic susceptibility pattern, extended spectrum β-Lactamases (ESBL), methicillin resistant *Staphylococcus aureus* (MRSA)

## Introduction
Bloodstream infection (BSI) caused by bacteria is one of the most important causes of morbidity and mortality throughout the world [1]. The disease may be short and self-limiting or may result in death or serious morbidity including admission to intensive care or prolonged hospital stay [2]. Continuous or transient presence of microorganism within the blood stream is Bacteraemia while its dissemination throughout the body with evidence of systemic responses towards microorganism with variable severity is Septicemia [3]. Illness associated with bloodstream infections range from self-limiting in fections to life threatening sepsis, multiple organ failure, disseminated intravascular coagulation that requires rapid and aggressive antimicrobial treatment [4]. Organisms like *Escherichia coli, Klebsiella pneumoniae,*
*Staphylococcus aureus, Coagulase negative staphylococci (CONS), Pseudomonas* species, *Salmonella* species and *Acinetobacter* species are potential pathogens in bacteraemia because of their frequent isolation [5-7]. Respiratory, genitourinary tract and intra-abdominal foci are identifiable sources of blood stream infections [8, 9]. There is striking increase in incidence of bacteraemia caused by members of Enterobacteriaceae since early 1950s and *Escherichia coli* which was reported to be the commonest in the past is being replaced by other multidrug resistant bacteria like *Klebsiella, Enterobacter, Salmonella, Citrobacter,* etc [10].

Blood is normally a sterile site, a blood culture have a high positive predictive value and is a key component for an accurate diagnosis of bloodstream infections and an appropriate choice of antibiotics that is important for subsequent management of sepsis in new-borns and children [11]. Most children die of septicaemia infections as a result of inappropriate antimicrobial therapy. Some of these deaths could have been prevented with the appropriate antimicrobial therapy. For selecting empiric therapy, many factors should be considered including the kind of pathogen that is most probably the cause of the infection according to age, risk factors and antibiotic susceptibility pattern. Thus, rapid detection and identification of clinically relevant microorganisms by blood cultures is very essential and determination of antimicrobial susceptibility pattern for rapid administration of antibiotics.

Hence the aim of this study was to diagnose the bacterial septicaemia by conventional blood culture techniques and to determine antimicrobial resistance of the bacterial isolates among children suspected of septicaemia in tertiary care centre.

## Aims and Objective

1. To know the occurrence of blood stream infections.
2. To isolate the bacterial agent from blood sample.
3. To identify the bacterial agent isolated from the blood samples.
4. To determine the antibiotic susceptibility pattern of the isolates

## Material and Methods

A descriptive study was conducted in the department of Microbiology in a tertiary care hospital A total of 300 blood culture samples were analysed. All children of either sex admitted in Paediatric ward and PICU during the period of December 2014 to July 2016 who were clinically diagnosed to have septicaemia were included in the study. Fully informed and voluntary consents were obtained from the parents or attendants. Detailed history and complete physical examinations of each paediatric child was carried out and recorded Blood sample was collected for culture with proper aseptic precautions. The local site was cleansed with 70% alcohol and povidone iodine (1%), followed by 70% alcohol again before initiating antibiotic therapy. Approximately 1-3 ml of blood was collected in sterile bottle containing 1% glucose broth and incubated at 37 °C. Blind subculture were made on Blood agar, Chocolate agar and MacConkey agar after 24 hours, 48 hours, 72 hours and 7 days, If no growth was observed on plates after 7th day, the sample was reported as negative. Isolate was identified by their characteristic appearance on their respective media, Gram staining and confirmed by the pattern of biochemical reactions [10] Members of the family Enterobacteriaceae were identified by Indole production, $H_2S$ production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests, and other tests. For Gram-positive bacteria, coagulase, catalase, bacitracin and optochin susceptibility tests and other tests were used. Blood culture broth that showed no microbial growth within seven days was reported as culture negative, only after result of routine subculture on Blood, MacConkey, and chocolate agar [10]. Antimicrobial susceptibility testing was performed for all blood culture isolates by Kirby–Bauer disc diffusion method according to CLSI guidelines 2014 [11].

Methicillin resistant Staphylococcus aureus (MRSA) detection was done according to CLSI guidelines 2014 using cefoxitin disc (30g). The ESBL detection in case of *E. coli and K. pneumoniae* isolates was done using phenotypic confirmatory method as per CLSI guidelines 2014 [11]. An isolate was considered as ESBL producer when zone diameter of ceftazidime/Clavulanic acid disc (30/10 µg) was 5 mm than the diameter of ceftazidime (30µg)

The drugs for disc diffusion testing were in the following concentrations: Ampicillin (10 µg), amoxiclav (20/10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), penicillin (10 units), co-trimoxazole (1·25 µg trimethoprim/23·75 µgsulfamethoxazole), amikacin (30 µg), ceftriaxone, ceftazidime + clavulanic acid, imepenum. (10ug) The discs were obtained from Himedia (India) Laboratories

## Results

Blood culture samples were taken from 300 paediatric patient suspecting of having septicaemia in present study.

**Table 1:** Age wise distribution of paediatric children

| Age Group | Number of Patients |
|---|---|
| <28 days | 80(26.67%) |
| <1 yr | 140(46.67%) |
| <5 yrs | 70(23.33%) |
| <12 yrs | 10(3.33%) |
| Total | 300(100%) |

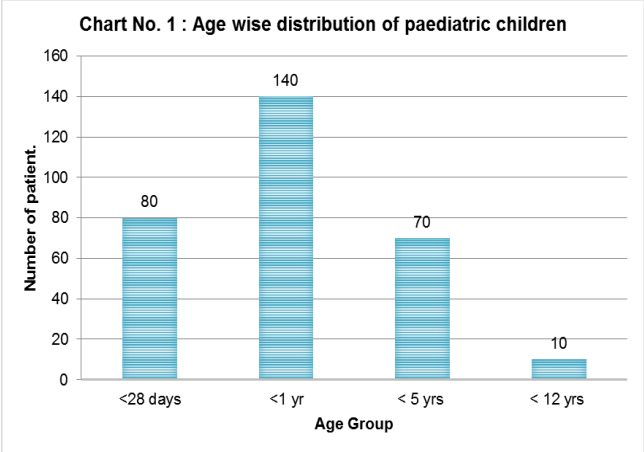
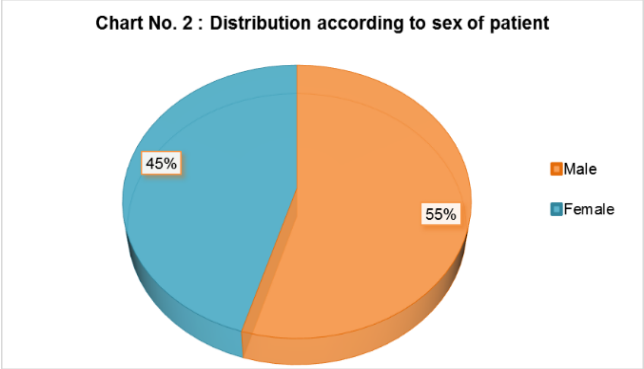
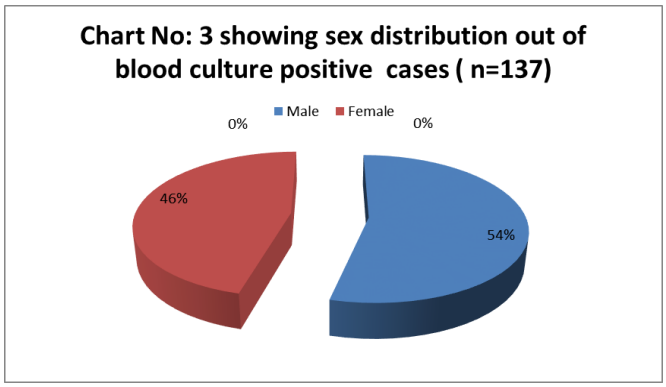



**Table 2:** Distribution according to sex

| Sex | Total Number | Percentage (%) |
|---|---|---|
| Male | 164 | 55% |
| Female | 136 | 45% |





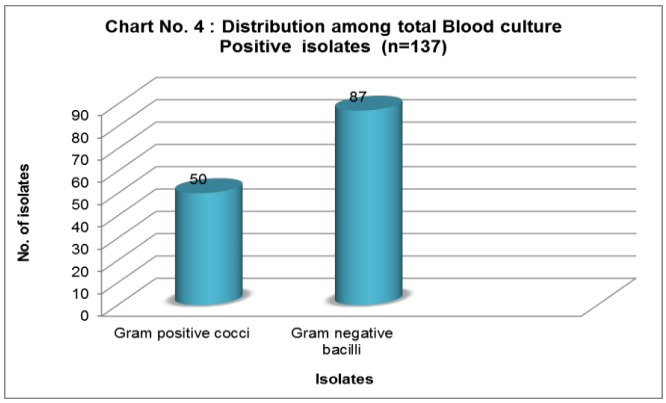

It was seen in Chart 3 that out of 137 culture positive patients, 74 (54%) were males and 63 (46%) were females.

**Table 3:** Showing Distribution of blood culture positivity

| Number of patients according to age | Blood Culture Positive | Blood Culture Negative | Total |
|---|---|---|---|
| <28 days | 40 (50%) | 40 (50%) | 80 |
| < 1yr | 80 (57.14%) | 60 (42.85%) | 140 |
| < 5 yrs | 14 (25%) | 56 (98.21%) | 70 |
| <12 yrs | 3 (30%) | 7 (70%) | 10 |
| Total | 137(45.6%) | 163 (54.4%) | 300 |

**Table 4:** Bacterial isolates (gram positive and gram negative isolates) in suspected septicaemia in paediatric patients

| Isolates | Total isolates (n = 137) | Percentage |
|---|---|---|
| Gram positive cocci | 50 | 36.4% |
| Gram negative bacilli | 87 | 63.5% |




Gram negative bacilli (isolates) were predominantly isolated from suspected septicaemia patients as compared to gram positive cocci (isolates)

**Table 5:** Frequency of Bacterial isolates causing septicaemia

| Sr No | Organism | Number | Percentage |
|---|---|---|---|
| A | Gram Positive Isolates | 50 | 36.4% |
| 1 | *Staphylococcus aureus* | 25 | 50% |
| 2 | *CONS* | 23 | 46% |
| 3 | *Enterococcus* | 2 | 4% |
| B | Gram Negative Isolates | 87 | 63.50 |
| 1 | *Klebsiella pneumoniae* | 45 | 51.72 % |
| 2 | *Escherichia coli* | 20 | 22.% |
| 3 | *Acinetobacter baumannii* | 10 | 11.4% |
| 4 | *Pseudomonas aeruginosa* | 7 | 8% |
| 5 | *Citrobacter species* | 5 | 6.08% |
| | Total | 137 | 100% |

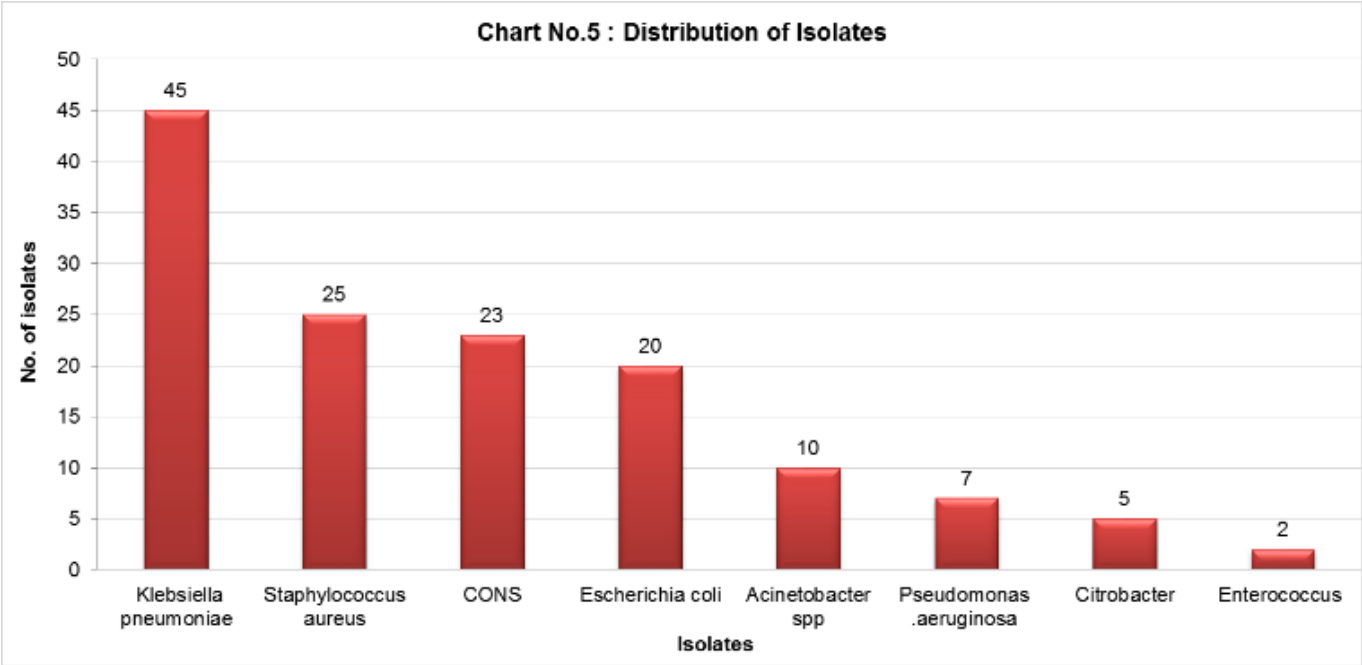



**Table 6:** Gram negative isolates showing ESBL Production (n = 65)

| Isolates | ESBL Producers | NON ESBL Producers | Total |
|---|---|---|---|
| *Klebsiella* | 32 | 13 | 45 |
| *Escherichia coli* | 12 | 8 | 20 |
| Total with percentage | 44(67.70%) | 21(32.30%) | 65(100%) |

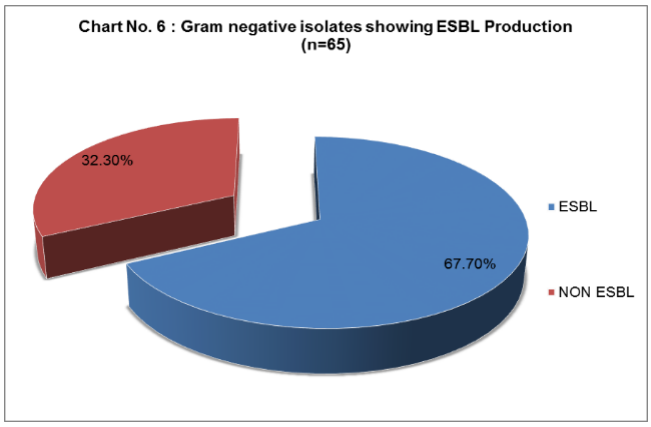



**Table 7:** Gram negative isolates showing MBL Production (n = 17)

| Isolates | MBL Producers | NON MBL Producers | Total |
|---|---|---|---|
| Pseudomonas aeruginosa | 3 | 4 | 7 |
| Acinetobacter baumannii | 3 | 7 | 10 |
| Total with Percentage | 6(35.29%) | 11(64.71%) | 17(100%) |

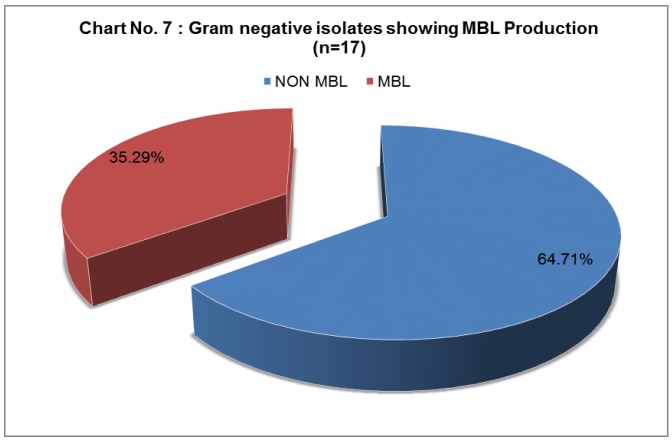
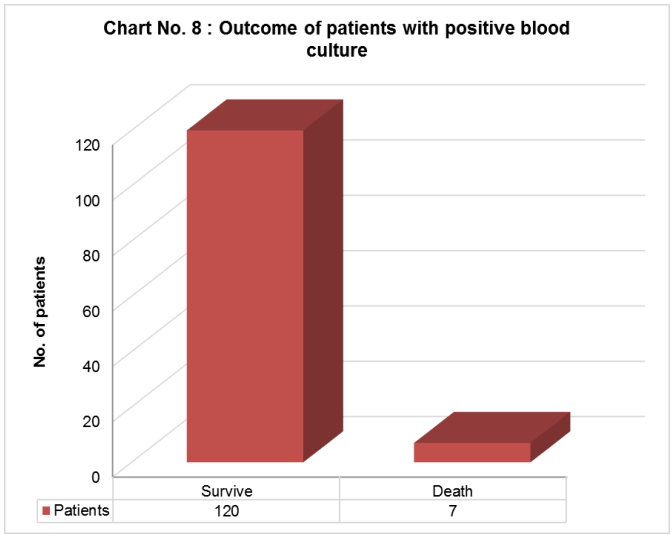
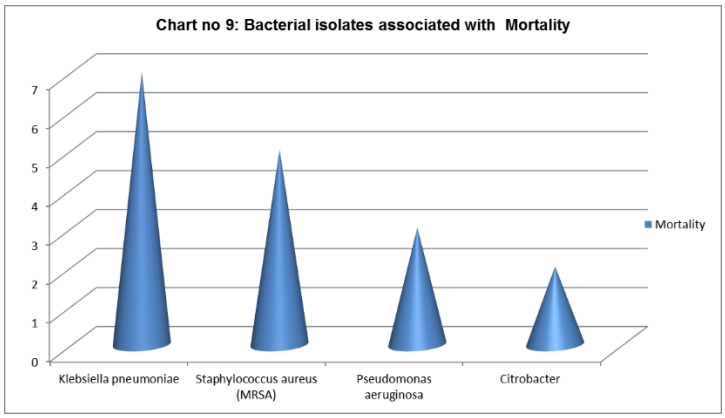



**Table 8:** Blood culture positivity and mortality

| Outcome | Number of Blood culture Positive Patients | Percentage (%) |
|---|---|---|
| Survival | 120 | 87.59% |
| Mortality | 17 | 12.4% |
| Total | 137 | 100 % |




## Discussion

Paediatric septicaemia with its high mortality rate still remains a diagnostic and treatment challenge for the paediatric clinicians. An early diagnosis of paediatric septicaemia helps the clinician in instituting antibiotic therapy at the earliest, thereby reducing the mortality rates in the children.

In the present study an attempt has been made to know the occurrence of blood stream infections, considering blood culture as a gold standard for diagnosis of paediatric septicaemia. This study also tried to isolates the various etiological agents, identify them and to determine their antibiotic susceptibility patterns which are responsible for paediatric septicaemia

The most common age group affected in present study was in children of Less than 1 year followed by neonates (Table 1 and 3, Chart 1). Similar results were seen in studies conducted by Jumah DS *et al.* [14], Kashibu E [12]

Culture positivity rate is highly variable from place to place and positive blood cultures reportedly range from 8% to 73 %. [10]. In present study the overall isolation rate was 45%. Similar results were found as 45.9% in studies of Prabhu K *et al.* [13]

In the present study out of 137 culture positive cases, 74(54%) were males and 63 (45.9%) were females. These Findings were similar with studies of Ansari *et al.* [14], Dagnew *et al.* [15], Negussie *et al.* [16] Male preponderance was observed in the present study which is in concordance with the above referred studies. The possible reason for the male preponderance is that the factors regulating synthesis of gamma globulins are probably situated on X chromosome and presence of one X chromosomes in male thus confers less immunological protection compared to female counter part [6].

Out of 137 cultures positive cases, 50(36.4%) were gram positive isolates and 87 (63.5%) were gram negative isolates. These findings were similar with studies like and Ansari. S and *et al.* [14]. In the present study amongst Gram negative isolates *Klebsiella pneumoniae* (51.4%) was the most common causative agent of paediatric septicaemia followed by *E. coli* (22%). which are similar to studies like Dagnew *et al.* [15]. Among gram positive isolates *Staphylococcus aureus* (50%) was most common isolate followed by Coagulase Negative Staphylococcus aureus (*CONS)* (46%). which was similar to findings seen in Prabhu K *et al.* [13] The finding of predominance of isolation of Gram negative isolates and *Klebsiella pneumoniae* as a predominant pathogen correlates well with the above referred studies and present study However the findings of present study were consistent with studies done by Negussie

*et al*. [16] where they reported the overall predominance of gram negative organisms with *Klebsiella pneumoniae* as a predominant pathogen in, 51.8% cases of paediatric septicaemia respectively. This shows that there is a marked geographical variation in the microbiological spectrum of paediatric septicaemia and it differs from hospital to hospital Septicaemia was caused by only one organism (monomicrobial) in our study similar to other studies [8]. Septicaemia of polymicrobial etiology observed in other studies [8-12] is significant, some microbiologist considers polymicrobial growth as a contamination, but sepsis should be clinically correlated

In the present study, about 16 out of 25 isolated *Staphylococcus aureus* (64%) of them were *Methicillin Resistant Staphylococcus aureus* (MRSA). Findings similar to present study were closer to studies done by Karthikeyan *et al*. [8] from Chennai, 66% of Staphylococcus aureus isolated from cases of paediatric septicaemia were methicillin resistant. So screening for MRSA in every *Staphylococcus aureus* isolate will be of immense value for providing efficient patient care. The high resistance rates found may be associated with frequent use of antimicrobial drugs for both prophylactic and therapeutic treatment of hospitalized children.

The high resistance of all these organisms to third generation cephalosporins can be attributed to frequent production of extended spectrum beta lactamases (ESBL) by these organisms

In the present study, out of 45 isolates of *Klebsiella pneumoniae* of 32 (71.11%) and out of 20 isolates of *Escherichia coli* 12 (60%). shows ESBL production followed various authors have given different observations on ESBL production in their study on paediatric septicaemia

Paediatric septicaemia is associated with high morbidity and mortality. In the present study mortality rate was 12.4% in blood culture positive cases there was significant statistical association between mortality and blood culture positivity.

The present study provides information on the prevalence of bacterial pathogens causing blood stream infections along with their antibiotic susceptibility profile. The study identified both Gram‑positive and Gram‑negative isolates to be responsible for blood stream infections, Vancomycin and Linezolid were found to be most effective for Gram positive isolates whereas Imipenem and Amikacin were found to be most effective for Gram negative isolates. The increase in the prevalence of Drug resistant bacteria emphasize the urgent need for rational use of antibiotics, formulation of antibiotic policy, and implementation of infection control practices for the effective management and prevention of drug resistance.

## Conclusion

The periodic surveillance of etiological agents and their susceptibility pattern should be done as the patterns of bacterial organisms are changing constantly with time and place in order to use better choice of antibiotics. Routine detection of ESBL and MBL producing microorganisms and Methicillin Resistant Staphylococcus aureus (MRSA) is required since most of these are multidrug resistant, the therapeutic strategies to control infections in children has to be carefully formulated. Implementation of infection control measures, restricting the use of broad spectrum antibiotics, rotation of antibiotics and rationalizing the use of antibiotics can decrease antibiotic resistance. Early diagnosis and specific treatment can reduce children mortality and morbidity.

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