

Original Research Article

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## Bacteriologic Profile and Antimicrobial Resistance of Blood Culture Isolates of Septicemic Patients from Various Intensive Care Units in a Teaching Tertiary Care Institute of Haryana, India

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### ABSTRACT

The objective of the study was to describe the pattern of bacterial isolates from the blood cultures in intensive care units of our institute and to determine their antibiotic sensitivity, so that the study can provide guidelines for choosing an effective antibiotic therapy in cases of septicemia. In this prospective study 462 blood samples collected from clinically suspected cases of septicemia received over a period of one year. The isolates were identified by standard biochemical tests and antimicrobial susceptibility testing determined by National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Positive cultures were obtained in 266 (57.6%) cases. Among culture positive isolates, 63.1% were Gram-positive and 29.7% by Gram negative bacilli and remaining 7.1% by *Candida* spp. Among Gram-positive isolates, *Staphylococcus aureus* was the predominant isolate followed by *CONS* and *Enterococcus faecalis*. *Pseudomonas* spp. was the most common Gram negative isolate followed by *Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella typhi*, *Proteus*, *E.coli* and *Acinetobacter* spp. Incidence of MRSA and ESBL was 48% and 18.6% respectively Percentage sensitivity of isolates to individual antibiotic was also noted. This study provides information on antibiotic sensitivity of blood isolates in our set up. It may be a useful guide for physicians for initiating empiric therapy and will help in formulation of antibiotic policy in this part of the country.

### Keywords

Septicaemia,  
Bacteraemia,  
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## Introduction

Infection rates in intensive care units (ICUs) have been found to be the highest among all hospital acquired infections in various multicentre studies carried out in the United States of America (USA) and Europe (Vincent JL *et al.*, 1995) and (Jarvis WR *et al.*, 1991). This occurs due to the use of large numbers of invasive monitoring devices, endotracheal and tracheostomy

tubes along with the patient factors including extremes of age, immunocompromise state, malnutrition and severe underlying disease. Also a high incidence of cross infection is seen in ICUs (Weber DJ *et al.*, 1999). These ICU infections are costly to treat, lead to prolong ICU stay and increased mortality rates (Eggimann, *et al.*, 2001).

Sepsis is a common and threatening infection in the intensive care unit, where up to 35% of patients develop such a condition at some point during their stay. The associated mortality is 27% but exceeds 50% in cases of septic shock (Vincent, *et al.*, 2006).

Blood cultures represent an important diagnostic tool, although they detect bacteremia in only about 50% of patients clinically suspected of having sepsis (Brun-Buisson, *et al.*, 1995), with an even lower rate of positivity when drawn in the presence of ongoing antibiotic therapy (Bergmans, *et al.*, 1997). The presence of a blood pathogen represents a negative prognostic factor, but the isolation of such pathogen is important for verifying the appropriateness of antibiotic therapy, which in turn is known to reduce morbidity and mortality (Grace, *et al.*, 2001). Also, false-positive results due to bacterial contaminants may lead to unnecessary antibiotic therapy, longer hospital stays and selection of resistant microorganisms (Bates, *et al.*, 1991).

There is wide diversity between institutions in the prevalence of pathogens and in their antimicrobial susceptibility. There is also variation in the frequency and types of infections among different subsets of patients within the same ICU. To produce effective empirical antibiotic protocols for individual ICUs, knowledge of common organisms and their sensitivity patterns is essential. The aim of this study was to determine the rate of positive cultures and to identify the common organisms and their susceptibility patterns from bacteremic patients in the Intensive Care Units, of our institute over a one-year period. Knowing the most frequent pathogenic microorganisms that colonise or infect such type of patient allow for effective empirical

antibiotic treatment taking into account microorganisms' susceptibility and resistance patterns. A general increase in bacterial resistance rates has been reported around the world, giving rise to multi resistant strains within hospitals and thereby generating serious problems for health-care institutions.

## **Materials and Methods**

This study was carried out from January 2014 to December 2014 in a tertiary care institute. Consecutive blood samples received from various ICUs were included, clinical details of patients were noted. Processing of samples was done immediately after receiving in department of microbiology. 5 ml of blood was collected from each adult patient using strict aseptic precautions, and inoculated immediately into 50 ml of 'Brain Heart Infusion' (BHI) broth with 0.025% of sodium polyanthol sulphonate as anticoagulant (HI media). In paediatric cases 1 - 2 ml of blood was inoculated in 5 - 10 ml of BHI broth. The broths were subcultured on blood agar and MacConkey agar after overnight incubation. A negative result was followed-up by examining the broth daily and doing a final subculture at the end of seventh day. Positive growth was identified by Gram staining, colony characteristics, and standard biochemical tests.

The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines and classified as sensitive and resistant (NCCLS, 2002.). Standard antibiotics such as ampicillin (30 µg), amoxyclav (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), gentamicin (10 µg and 120 µg), imipenem (10 µg), cefoxitin (30 µg), linezolid (30 µg), vancomycin (30 µg), piperacillin (100 µg),

chloramphenicol (30 µg), Ciprofloxacin (5µg), Erythromycin (10 µg), Netilmicin(30 µg), Colistin(10 µg), Teicoplanin(30 µg), Amikacin (30 µg), Piperacillin Tazobactam(100/10 µg), Tigecyclin(30 µg), Polymyxin B(10 µg) Clindamycin (2µg), Cotrimoxazole (Trimethoprim1.25µg/Sulfomethoxazole 23.75µg) were tested. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were used for quality control. All the culture media, biochemical media and antibiotics used were obtained from Hi Media.

Detection of Methicillin resistant *Staphylococcus aureus* (MRSA) was done with Cefoxitin (30 µg) disc. Extended spectrum betalactamase (ESBL) production was detected with the help of Ceftazidime (30 µg) and Ceftazidime + Clavulanic acid (30/10 µg). Results obtained were analyzed by counts and percentages using MS Excel, 2007 version.

## Results and Discussion

A total of 462 samples were received during the study period out of which 266 (57.6%) were culture positive. Out of these 266 positive samples 226 (84.9%) had C reactive protein (CRP) value more than 6.4 mg/dl. Most infections were due to a single organism, while blood of two neonates grew *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* both. The breakup of 462 samples received from various ICUs is shown in Figure 1, among which Paediatric ICU with 246 samples (53.2%) was the major contributor followed by Neonatal ICU with 190 samples (41.1%), Medical ICU with 21 samples (4.5%) and Surgical ICU with 5 samples (1.2%). The various bacterial isolates obtained were compared among paediatric patients versus adults which is depicted in Figure 2 and Table 2.

A total of 266 organisms were isolated. The commonest organism was *Staphylococcus aureus* (33.5%) followed by Coagulase Negative *Staphylococcus spp* i.e CONS (25.9%), *Enterobacteriaceae* (18.4%), *Pseudomonas* (9.4%), non albicans *Candida* (6.8%), *Enterococcus* (3.4%), *Acinetobacter* (1.9%) and *Candida albicans* and *Meningococcus* both (0.04%) as shown in Figure 3.

In Neonatal ICU from total 190 samples the positivity rate was 66.3% (126/190). Similarly, in Paediatric ICU from total 246 samples the positivity rate was 50.4% (124/246). The sample positivity rate from MICU and SICU was 58.3% (14/21) and 40.0% (2/5) respectively. The most predominant bacteria isolated from all the ICUs was *Staphylococcus aureus* followed by CONS, *Enterobacteriaceae* and *Pseudomonas*. Non albicans *Candida* and *Candida albicans* were mainly isolated only from patients of NICU and PICU. The incidence and distribution of isolates from various ICUs is shown in Table 1.

The most predominant gram positive bacterium isolated was *Staphylococcus aureus* from all ICUs followed by CONS and Enterococci. Though *Enterobacteriaceae* was the most predominant family isolated among gram negative bacteria (GNB), *Pseudomonas* was the most predominant single Gram negative organism from all ICUs except NICU where *Klebsiella pneumoniae* was the most common GNB isolated. *Enterobacteriaceae* isolated were *Klebsiella pneumoniae*, *E.coli*, *Serratia marcescens*, *Proteus mirabilis*, *Citrobacter spp*, *Enterobacter spp* and *Salmonella typhi*.

The distribution of pathogens in PICUs differed from that we have observed in adults, and within the PICUs and changed with age. Isolation of CONS was

significantly higher in children when compared to adults ( $p \leq 0.05$ ). Similarly, *E. fecalis* was isolated only from children. Incidence of *S. aureus* was similar in adults and children. Similarly, Gram-negative bacteria isolated were also comparable. Fungal pathogens were reported more commonly in bloodstream infections in children, but much less frequently in adult ICUs (7% vs 0) (Table II).

The antibiogram of gram positive cocci revealed that resistance to Vancomycin, Linezolid and Teicoplanin is yet to be seen in our set up. *S. aureus* shows very high resistance to Amoxycillin, Amoxy Clav, Cotrimoxazole and Ciprofloxacin (sensitivity ranging from 10-30%). *S. aureus* was fairly sensitive to Amino glycoside group (resistance ranging from 25-35%). Also *S. aureus* was sensitive to Macrolide group (resistance 30-40%). *Enterococcus* was highly resistant to Penicillins, Cotrimoxazole and Macrolides (resistance ranging from 70-80%). Sensitivity to Gentamicin was good in *Enterococcus*. (Table III)

Gram negative bacteria of Enterobacteriaceae except *Salmonella typhi* were most susceptible to Imipenem (sensitivity 90-100%), only two isolates of *Klebsiella spp* showed resistance. Enterobacteriaceae showed very high resistance to Ampicillin, Amoxy Clav and Ciprofloxacin (sensitivity ranging from 5-30%). Resistance to Cephalosporins ranged from 30- 50% in these isolates. Sensitivity to polymixin B and Colistin was 100% as all Enterobacteriaceae was sensitive (Table IV).

In *Salmonella typhi* Ceftriaxone and ciprofloxacin were very effective with no resistance as none of the 4 isolates showed resistance. Also no resistance was seen against Chloramphenicol in our institute. All

the four isolates were resistant to Ampicillin (100% resistance) and 3/4 isolates were resistant to Cotrimoxazole (75% resistance) as shown in (Table IV).

No resistance against Imipenem, Polymixin B and Colistin was reported in *Pseudomonas* and *Acinetobacter spp* isolated from blood (Table IV). Piperacillin and Netilmicin are no more effective against *Pseudomonas* as resistance is high being 60-75%, Piperacillin Tazobactam combination being slightly better (resistance 56%). Resistance to Ceftazidime is also on the rise in our set up being 72%. Sensitivity to Amikacin and Gentamicin is still there in *Pseudomonas* being 60 and 64% respectively.

Out of 19 *Candida spp*, 18 were non albicans *Candida* and one was *Candida albicans*. The antifungal susceptibility testing was not done. One *Meningococcus* isolated was sensitive to all antibiotics tested.

Incidence of MRSA and ESBL was 48% and 18.6% respectively.

Sepsis, a systemic inflammatory response to infection, is an important cause of morbidity and mortality in critically ill patients, resulting in frequent diagnostic testing, greater prescription of antibiotics, and increased length of hospitalization. Bacteremia (including fungemia) in critically ill patients has an average attributable mortality of 26%, (Pittet, *et al.*, 1994). However, the microbiologic diagnosis of bacteremia acquired after admission is often difficult in the ICU, complicated by the fact that many critically ill patients receive empiric antibiotic therapy. In fact, antibiotic consumption is 10 times greater in ICUs than on general hospital wards (Bergmans, *et al.*, 1997). Pediatric intensive care units (PICUs) differ

from adult intensive care units (ICUs) in a number of ways, apart from the age of their patients. First, they are usually multidisciplinary, because there are too few patients to justify separate medical and surgical units. Second, they frequently lack the physical barriers between patients now commonly present in adult ICUs. Third, fewer children than adults in ICUs have chronic or degenerative organ system disorders and probably the majority of children in PICUs will, if successfully treated, return to a normal productive life.

Our analysis suggests that the epidemiology of nosocomial infections in PICUs and NICU differs from that seen in adult critical care units. The distribution of infection sites lies between what we have previously reported in neonatal ICUs and adult medical ICUs (Gaynes *et al.*, 1996). First, primary bloodstream infections were the most

common sites of infection, followed by pneumonia and UTIs. In adult medical ICUs, UTIs were most frequently reported. In neonatal ICUs, bloodstream infections were an even greater proportion of all infections than in pediatric units. We have also observed high rate of positive blood culture in children compared to adults (50-66% in children vs 40-58% in adults) though this difference was not statistically significant ( $p \geq 0.05$ ).

The distribution of pathogens in PICUs differed from that we have observed in adults, and within the PICUs and changed with age. These differences in pathogen distribution seem likely to be, in part, an effect of age but also to reflect other patient-related factors, including underlying medical and surgical conditions, previous hospital stay, and antibiotic exposure.

**Table.1** Incidence and distribution of microorganisms isolated from blood cultures from various ICUs.

Isolate	NICU n=126	PICU n=124	MICU n=14	SICU n=2	TOTAL n=266
CONS	30(23.8)	37(29.8)	2(14.3)	0	69(25.9)
<i>S.aureus</i>	33(26.2)	50(40.3)	5(35.7)	1	89(33.4)
<i>Enterococcus</i>	6(4.8)	3(2.4)	0	0	9(3.4)
<i>Pseudomonas</i>	10(7.9)	12(9.7)	3(21.4)	0	25(9.4)
<i>Acinetobacter</i>	2(1.5)	3(2.4)	0	0	5(2.0)
<i>Salmonella spp.</i>	0	2(1.6)	2(14.3)	0	4(1.5)
<i>E. coli</i>	1(0.7)	2(1.6)	0	1(50)	4(1.5)
<i>Klebsiella spp.</i>	15(11.9)	3(2.4)	0	0	18(6.8)
<i>Citrobacter</i>	2(1.5)	6(4.8)	2(14.3)	0	10(3.8)
<i>Serratia marcescens</i>	4(3.2)	0	0	0	4(1.5)
<i>Enterobacter spp</i>	6(4.8)	3(2.4)	0	0	9(3.4)
<i>C. albicans</i>	1(0.8)	0	0	0	1(0.4)
Non albicans <i>Candida</i>	16(12.7)	2(1.6)	0	0	18(6.8)
Meningococcus	0	1(0.8)	0	0	1(0.4)

\*Number in Parenthesis denotes Percentage



**Table.2** Comparison of isolates b/w children and adults

	NEONATES/CHILDREN 250	ADULTS 16
CONS	67(26.8)	2(12.5)
S.aureus	83(33.2)	6(37.5)
Enterococcus	9(3.6)	0
Pseudomonas	22(8.8)	3(18.7)
Acinetobacter	5(2.0)	0
Enterobacteriaceae	44(17.6)	5(31.2)
C. albicans	1(0.4)	0
Non albicans Candida	18(7.2)	0
Meningococcus	1(0.4)	0

\*Number in Parenthesis denotes Percentage

**Table.3** Resistance pattern of Gram-positive isolates.

Antibiotic	<i>Staphylococcus</i> spp N= 89	<i>Enterococcus</i> spp N=9
Penicillin	78(87.6)	7(77.8)
Amoxicillin	79(88.7)	0(100)
Amoxy Clav	70(78.6)	0(100)
Cefoxitin	52(58.4)	-
Erythromycin	39(43.8)	4(44.4)
Gentamicin	32(35.9)	-
Amikacin	25(28.1)	-
Ciprofloxacin	46(51.6)	-
Clindamycin	36(40.4)	3(33.3)
Gentamicin 120	21(23.6)	2(22.2)
Vancomycin	0(0)	0(0)
Linezolid	0(0)	0(0)
Teicoplanin	0(0)	0(0)

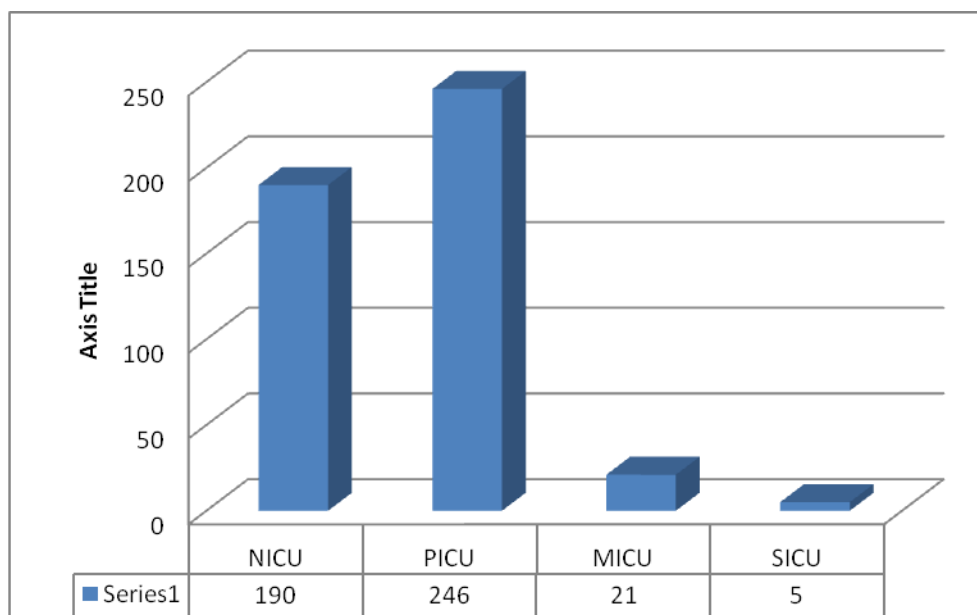
\*Number in Parenthesis denotes Percentage

**Table.4** Resistance pattern of Gram-negative isolates.

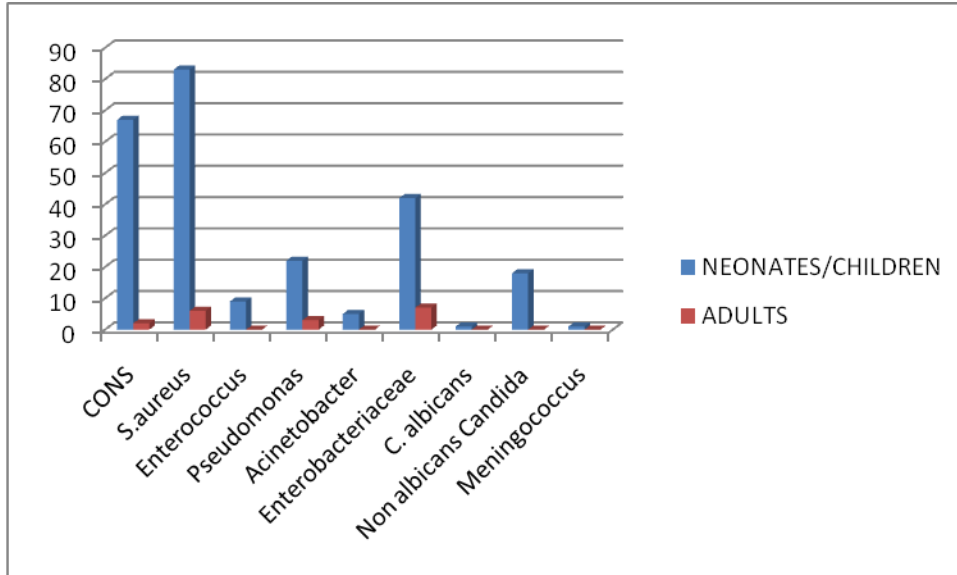
Antibiotic	Enterobacteriaceae except <i>Salmonella typhi</i> N=45	<i>Salmonella typhi</i> N=4	<i>Pseudomonas</i> N=25	<i>Acinetobacter</i> N=5
Ampicillin	43(95.6)	4(100)	-	5(100)
Ceftriaxone	24(53.3)	0(0)	-	3(60)
Ceftazidime	27(60)	-	18(72)	2(40)
Gentamicin	14(31.1)	-	9(36)	1(20)
Amikacin	10(22.2)	-	10(40)	1(20)
Ciprofloxacin	37(82.2)	0(0)	17(68)	4(80)
Cotrimoxazole	39(86.7)	3(75)	-	-
Chloramphenicol	-	0(0)	-	-
Piperacillin	-	-	19(76)	-
Netilmicin	-	-	16(64)	-
Piperacillin + tazobactam	-	-	14(56)	-
Imipenem	0(0)	-	0(0)	0(0)
Polymyxin B	0(0)	-	0(0)	-
Tigecyclin	0(0)	-	0(0)	2(40)
Colistin	0(0)	-	0(0)	0(0)

\*Number in Parenthesis denotes Percentage

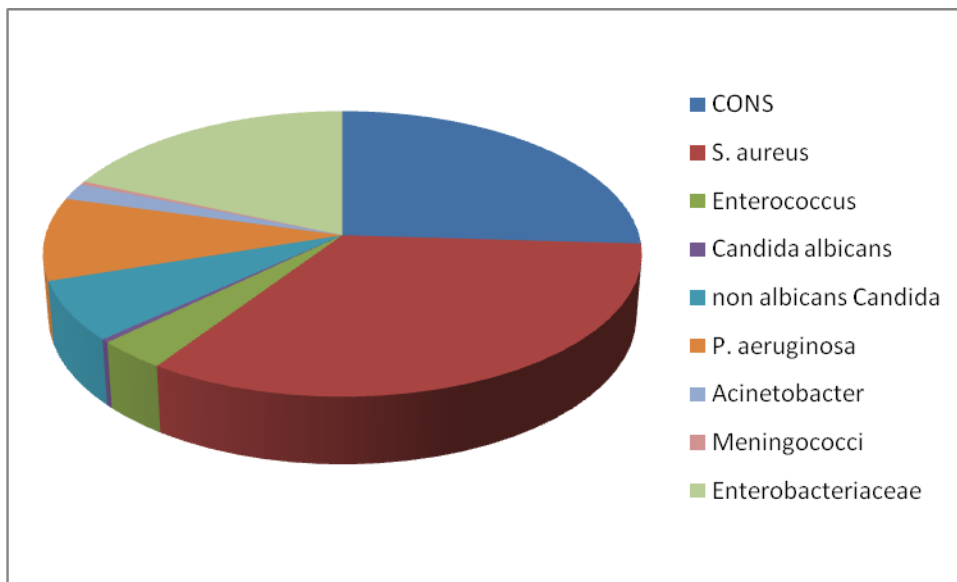
**Fig.1** Sample Distribution from various ICUs.



**Fig.2** Comparison of isolates obtained in Neonates/ Children Vs Adults



**Fig.3** Distribution of various isolates.



The common isolates in blood culture in our study were *S. aureus* (33.5%) followed by GNBs (29.7%), coagulase negative staphylococci (25.9%), NAC (7.2%), *E.fecalis* (3.6%) and *C. albicans* (0.4%). The findings are consistent with those of the previous studies conducted by (Karlowsky JA *et al.*, 2004) and (Ben, *et al.*, 2004). The results of our study demonstrate that blood

culture Positivity rate in clinically suspected septicaemia cases was 57.6%. Overall, 63.1% of septicaemia was caused by Gram-positive and 29.7% by Gram negative bacilli and remaining 7.1% by *Candida* spp. this was in accordance with (Dagneu, Mulat *et al.*, 2013) (M S Alam *et al.*, 2011) and (Mamishi S *et al.*, 2005)



Like many other studies Coagulase-negative Staphylococcus were among the major blood culture isolates; however, given that CONS isolated from blood are often contaminants (> 85% are clinically insignificant) their antibiotic susceptibility was not determined. (Roy, *et al.*, 2002)

In conclusion, Knowledge of the causative agents of bacteremia is useful in the selection of antimicrobials for empiric therapy. As *S. aureus* was the most common organism isolated, it suggests that good hygiene practices by the patient, their attendants and the health care providers is very crucial especially in neonatal and paediatric ICUs. Also fairly high rate of isolating *Pseudomonas spp* needs attention. The susceptibility data suggests that multidrug resistance is not a severe problem in local area yet as we are a new set up. But rational use of antibiotics should be practiced to avoid emergence of multi drug resistant. Reserved drugs need to be kept in reserved inventory to prevent their misuse and emergence of resistance. The in vivo susceptibility of the antimicrobials agents correlates well with the in vitro susceptibility reports and in our majority of the paediatric patients as a good therapeutic response was achieved. A changing trend in antibiotic sensitivity profile of the isolates need to be monitored as there is limited availability of newer drugs and the emergence of resistant bacteria far exceeds the rate of new drug development.

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