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Original article:

Spectrum of bloodstream pathogens in paediatric patients of a tertiary care hospital from North Karnataka

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ABSTRACT:

Background: Blood stream infections (BSI) are very important cause of mortality especially among children residing in developing countries. Due to the changing trend in drug resistance, regular antimicrobial resistance surveillance of the locally isolated pathogens is necessary to start appropriate therapy. Objectives: To know the bacteria causing BSI and their antibiogram.

Methods: Blood samples from suspected BSI cases were cultured by standard method. Identification of the bacteria isolate, antimicrobial susceptibility, methicillin resistance and ESBL production were performed by standard protocol.

Results: Of the 889 blood specimens processed for culture, 17.9% yielded bacterial growth. Among 160 positive cultures, 3.8% showed the growth of Candida spp. and 58.1% yielded Gram-negative bacteria. Compared to Gram positive bacteria, higher drug resistance was noted amongst Gram negative bacteria. Most of the Gram-negative bacteria were resistant to ampicillin, ceftriaxone, ceftazidime and piperacillin-tazobactam combination; suggesting that these drugs are not effective in this area.

Conclusions: Routine surveillance studies are needed to have knowledge about the most effective empirical treatment. The study depicts pathogens responsible for BSI and their antibiotic susceptibility pattern which in turn provides valuable guidelines to clinicians in initiating empirical therapy.

Keywords: Blood stream infections (BSI), Antibiotic resistance, ESBL

Introduction:

Blood stream infections (BSI) are an imperative cause of mortality worldwide. This is especially true in children residing in developing countries like India. High frequency of mortality is being attributed to increased resistance of the pathogens to most of the commonly used antibiotics especially among the gram-negative bacilli. This is related to the production of ESBLs, carbapenemases and metallo-beta-lactamases. BSI due to carbapenem and colistin-resistant *K. pneumonia* has been reported from India.[1]

Accurate diagnosis and rapid administration of appropriate antimicrobials

can improve patient's survivability and reduce the morbidity and mortality significantly. Reports suggest that initiation of inappropriate antimicrobial therapy leads to fivefold increase in mortality in Septic Shock.[2]

In the era of increasing antimicrobial resistance across the globe, change in the trend of bacteria causing BSI and their antibiotic resistance pattern is anticipated. Hence regular antimicrobial resistance surveillance of the locally isolated pathogens is necessary to help the clinicians in appropriate management of cases and develop rational antibiotic policy. There is no much published data on BSI from this area in the recent past.

Hence the study was undertaken with the objectives to know the bacterial profile of bloodstream infections in the pediatric age group and their antibiotic resistance pattern.

Materials and methods:

Study design: A prospective study was conducted in the Department of Microbiology after obtaining the approval from the Institutional Ethical Committee and the consent from the participants/parents. The study duration was from September 2018 to August 2019.

Inclusion Criteria: Blood culture specimens of pediatric patients who gave consent (patients/parents) were included in the study.

Exclusion Criteria: Patients/ parents who did not give consent were excluded from the study. Also contaminated, duplicate and repeat specimens from the patients were excluded.

Collection of Sample and Processing: For each culture 1-2ml of blood was collected from neonates (depending upon the weight) and 5 ml from children, following skin preparation by a two-step process with 70% alcohol and povidone iodine application. The blood specimens were inoculated into Brain Heart Infusion (BHI) broth supplemented with 0.05% Sodium polyanethol sulfonate (SPS) at the blood to broth ratio of 1:10 and incubated, at 37°C. Blind subcultures were made on MacConkey's agar and Blood agar plates every alternate day for seven days. Conversely if the growth was observed further sub culturing was not done. The plates were observed for bacterial growth after overnight aerobic incubation at 37°C. Identification of the isolates was done by using standard microbiological techniques.[3] Samples were considered sterile, if no growth was observed on subculture after 7 days of aerobic incubation at 37°C.

Antimicrobial Susceptibility Testing: The susceptibility of the isolates against various ntibiotics was tested by the Kirby-Bauer disk diffusion method on Mueller Hinton agar and result interpretation of results was done as per CLSI guidelines.[4]

For Gram positive bacteria, following antibiotics were used – Erythromycin (15µg), Ciprofloxacin (5µg), Gentamicin (10 µg), Amoxyclav (30µg), Linezolid (30µg), Co-trimoxazole (25µg), Clindamycin (2µg), Ceftazidime (10 µg) and Cefoxitin (30µg).

Methicillin resistance in Staphylococcus aureus (MRSA) was tested using Muller Hinton Agar with Cefoxitin disc (30µg), by Kirby-Bauer disc diffusion method

For Gram negative bacteria following antibiotics were tested - Ampicillin (10µg), ceftriaxone (30µg), Gentamicin (10µg), Co-trimoxazole (25µg), Ciprofloxacin (5µg), Amikicin (30µg), Levofloxacin (5µg), Piperacillin + Tazobactam (100/10µg), Ceftazidime (10µg), Carbenicillin (100µg), Imipenem (10µg) and Meropenem (10µg). Antibiotic discs were procured from HiMedia Laboratories, India.

ESBL detection - Phenotypic method was used for screening for ESBL production. Isolate was considered potential ESBL producer with the zone diameter size of ≤ 22 mm for ceftazidime and ≤ 25 mm for ceftriaxone. Isolates with positive screening test were confirmed by double disc approximation test.[5]

Confirmation of ESBL production: This was done by double disc approximation test. For this a lawn culture of test organism was prepared on Muller Hinton Agar plate.

Ceftazidime (30 µg) and ceftazidime plus clavulanic acid combination (30/10 µg) discs were then placed at an appropriate distance (2.5 cm) from each other. The plates were incubated aerobically overnight at 37°C. Isolate was confirmed as ESBL producer if an increase in the zone diameter of ≥ 5 mm was noted around ceftazidime/clavulanate combination disc than that of ceftazidime disc alone. For reference E. coli ATCC 25922 was used as negative control and a known in-house ESBL producer as positive control.

Throughout the study for culture and antimicrobial susceptibility testing *S.aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used as quality control.

Detection of methicillin-resistance:

Cefoxitin disc diffusion method was used to identify MRSA and methicillin-resistant coagulase-negative staphylococci (MRCoNS) amongst staphylococcal isolates.[6]

Results:

A total of 889 blood specimens were received for culture, of which 160 (17.99%) yielded bacterial growth.

Among the culture positive samples maximum were from neonates and sex wise there was no significant difference noted with male (88) to female (72) ratio being 1.2:1 (Fig 1.).

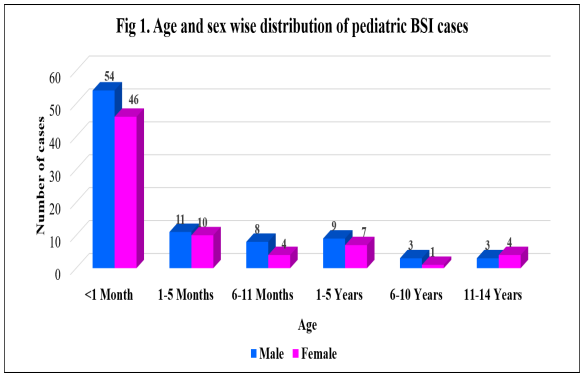
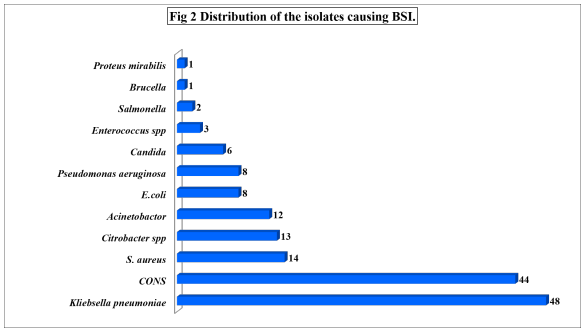


Figure 2. shows the distribution of different bacterial isolates causing BSI in paediatric age.



Antibiotic resistance pattern of the isolates is depicted in table No 1 and 2.

Table 1. Antibiotic resistance pattern of the Gram-positive isolates.

Antibiotic	*S. aureus* n = 14	*CoNS n=44*	*Enterococcus spp.* n=3
Erythromycin	35.7% (5)	45.5% (20)	100% (3)
Ciprofloxacin	78.57 (11)	65.9% (29)	100% (3)
Gentamicin	21.42% (3)	27.3% (12)	66.6% (2)
Amoxyclav	85.71 % (12)	93.2% (41)	100% (3)
Linezolid	14.3% (2)	6.8% (3)	33.3 % (1)
Co-trimoxazole	42.9% (6)	40.8% (17)	Not done
Clindamycin	28.6% (4)	34.1% (15)	100% (3)
Ceftazidime	100% (14)	93.2% (41)	100% (3)
Cefoxitin	35.7% (5)	34.1% (15)	--

S. aureus - Staphylococcus aureus, CoNS- Coagulase Negative Staphylococci

Table 2. Antibiotic resistance pattern of the Gram-negative isolates.

Antibiotic	K. pneumonia n =48	E. coli n = 8	Citrobacter spp. n = 13	Acinetobactor n=12	P. aeruginosa n =8
Ampicillin	100% (48)	87.5% (7)	76.9% (10)	83.3% (10)	--
Ceftriaxone	79.2% (38)	100% (8)	76.9% (10)	100% (12)	87.5 (7)
Gentamicin	54.2% (26)	62.5% (5)	23.1% (3)	66.7% (8)	37.5% (3)
Co-trimoxazole	33.3% (16)	25% (2)	61.5% (8)	16.7% (2)	-
Ciprofloxacin	77% (37)	87.5% (7)	84.6% (11)	83.3% (10)	37.5% (3)
Amikicin	14.58% (7)	37.5% (3)	15.4% (2)	33.3% (4)	00

Levofloxacin	47.9% (23)	87.5% (7)	61.5% (8)	83.3% (10)	75% (6)
Piperacillin - tazobactam	79.2% (38)	100% (8)	76.9% (10)	100% (12)	62.5% (5)
Ceftazidime	79.2% (38)	75% (6)	69.2% (9)	91.6 (11)	87.5 (7)
Carbenicillin	---	---	---	---	00
Imipenem	00	00	00	(8.3%) 1	00
Meropenem	00	00	00	00	00

K. pneumonia- Klebsiella pneumonia, P. aeruginosa - Pseudomonas aeruginosa

Table 3. Results of ESBL screening and confirmation test.

Organism	Total isolates	Screening test positive (%)	Confirmatory test positive (%)
E. coli	08	08 (100)	06 (75)
Klebsiella	48	38 (79.1)	23 (66.6)
Citrobacter	13	10 (76.92)	08 (62.53)
Acinetobacter	12	12 (100)	08 (66.6)
P. aeruginosa	08	07 (87.5)	05 (62.5)

Discussion:

Blood culture positivity rate in our study on BSI among pediatric group was found to be 17.99%, which is in accordance with other Indian studies.[7, 8] However, our isolation rate is low when compared to the study by Anjali Agarwal *et al.* (35.26%).[9] This may be explained by the fact that in our study some of the patients were already treated before coming to our hospital. About 25% (224) of these patients were treated by local doctors before reaching our tertiary care hospital and 2.8% (23) of them received the medications without a doctor's prescription (over the counter).

Literature reveals both lower and higher isolation rates.[7-10] Such disparity in blood culture positivity might be due to variation in the quantity of blood collected, number of blood cultures performed for each patient and type of media used.

Predominance of Gram-negative bacilli over Gram positive cocci has been noted in this study which is in agreement with the study by Gupta *et al.*[7]Among the gram-negative bacilli, 90% belonged to Enterobacteriaceae. Predominant isolates in the study were *Klebsiella* species followed by *Citrobacter*. Study by Kim *et al.* has reported isolation of *P. aeruginosa* in 38.9% of children with BSI, in the present study only five percent cases grew *pseudomonas.*[11]

In contrast to our findings some studies have shown gram positive cocci to be predominant bacteria causing BSI in pediatric age group.[8-10]

Coagulase-negative staphylococci (CoNS), earlier considered as skin contaminants or part of normal flora are now gaining importance as pathogens causing bloodstream, urinary tract, surgical sites and prosthetic devices infections. In our study, among Gram-positive bacteria Coagulase-negative *Staphylococci* were the most common followed by *S. aureus,* similar findings have been reported by Agarwal *et al*.[9] There is apprehension regarding increasing incidence of methicillin-resistance amongst the CoNS.[12] In this study 34% CoNS were methicillin resistant. Most of the Gram-positive cocci showed resistance to amoxyclav, ciprofloxacin and, ceftazidime similar to the findings of Parajuli NP *et al*.[10] While addressing the antibiotic resistance pattern, higher rate of resistance among gram negative bacteria than gram positive bacteria has been noted in the study. Also, a trend of Multi drug resistant (MDR) strains replacing sensitive bacterial strains is evident in this study. In our study more than 80% of *E. coli, Klebsiella* and *Citrobacter* isolates were resistant to ampicillin, ceftriaxone, ceftazidime and piperacillin-tazobactam combination; suggesting that these drugs are not effective in this area. Less drug resistance was noted for amikacin, co-trimoxazole and gentamicin. None of the enterobactericeae showed resistance to imipenem and meropenem. Screening test for Extended Spectrum Beta-Lactamase gave positive results in 88.7% of gram-negative bacteria and the confirmatory test was positive in 66.6% strains. BSI due to ESBL producing *E. coli* strains among children has been reported by Malande *et al.* [13]

In this study about 10% isolates were ESBL producers.

In this study *Brucella melitensis* was isolated in one boy, who had contact with infected animals and showed positive agglutination test (STAT: 1280 IU, 2ME:640 IU). Blood stream infections by *Brucella* and their further complications have been reported in pediatric age group by some authors.[14-15]

This study gives an idea regarding the type of bacteria causing blood stream infections and their antibiotic susceptibility profile in this area.

Due to over the counter availability, irrational use of antibiotics, there is upsurge of drug resistance in bacteria. For the effective management and prevention of drug resistance there is an urgent need to have an antibiotic policy, implementation of infection control practices and forbid the availability of antibiotics over the counter.

Conclusion:

As the etiology and antibiotic resistance pattern vary from time to time in a particular geographical area, for effective empirical treatment, routine antibiotic resistance surveillance is needed.

Antibiotic susceptibility pattern of blood isolates made here would work as a useful guide for clinicians while prescribing empirical antibiotics to the patient.

Limitations of the study:

Number of samples screened and time period of the study is less.

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