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BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF BLOOD CULTURE ISOLATES IN A TERTIARY CARE HOSPITAL IN SOUTH INDIA - A RETROSPECTIVE STUDY

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ABSTRACT

Blood stream infections range from self-limiting illnesses to life threatening sepsis that require prompt and aggressive antimicrobial treatment. The present study was undertaken to provide a base-line knowledge of the micro-organisms prevailing to a particular area and their antibiotic sensitivity pattern so as to prevent the injudicious usage of antibiotics. **Objectives:** To describe the profile and determine the antibiogram of bacteriological isolates causing blood stream infections. **Materials and methods:** A retrospective study was done on the blood samples received for culture over a period of one year (January-December 2017). The bacterial isolates from positive cultures were identified by standard protocols and antimicrobial sensitivity patterns determined by CLSI guidelines. **Results**: 389 (10.1%) samples were positive for culture, out of which 376 yielded bacterial isolates. 53.3% were Gram-positive and 47.7 % were Gram-negative bacteria. Staphylococcus aureus was the predominant organism-127 (32.8%). All the Gram positive isolates were uniformly sensitive to vancomycin. Total Multi drug resistant isolates observed in our study were 75.65%. **Conclusion**: The present study provides valuable information to clinicians in initiating empirical antibiotic therapy and necessitates rational antibiotic usage in the hospital to prevent the emergence of antimicrobial resistance.

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INTRODUCTION

Blood stream infections are one of the most common healthcare associated infections and constitute an important cause of morbidity and mortality.[1] The clinical manifestations range from self-limiting illnesses to life threatening sepsis that require prompt and aggressive antimicrobial treatment.[2] The mortality rate may reach upto 100% in inappropriately treated patients or those with a history of granulocytopenia. Moreover, case fatality rate is higher among patients infected with Gram-negative bacilli compared to those who have Gram-positive cocci as causative agents of their septicaemia.[3-6], 20–50% of the children in developing countries suffer from blood stream infections.[7,8] Polymicrobial sepsis can occur in high risk patients associated with catheters, gastrointestinal diseases, neutropenia or malignancy.[9] As blood stream infections constitute one of the most serious situations, timely detection and identification of the pathogen is important. The wide variation in spectrum of micro-organisms involved in blood stream infections is subject to geographical alteration.[10,11] The provisional diagnosis of septicaemia is based on the assessment of clinical signs and symptoms. But bacteriologic culture still remains the mainstay of definitive diagnosis of septicaemia.[7]

Objectives

1. To describe the profile of bacteriological isolates causing blood stream infections in suspected cases of bacteraemia
2. To determine the antibiotic susceptibility pattern of the above isolates.

MATERIALS AND METHODS

Study Design- Descriptive record based study

Duration of study- 1 year (January 2017 to December 2017)

Study setting- Department of Microbiology, Govt.T.D. Medical College, Alappuzha

Study population- Blood samples of all patients received in the Department of Microbiology, Govt.T.D. Medical College, Alappuzha during the period January to December 2017 were included in the study. Cultures which yielded contaminants and mixed bacterial growth were excluded.

Sample size – Total number of blood samples = 3820
Culture positive samples = 350

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Data Collection Procedure

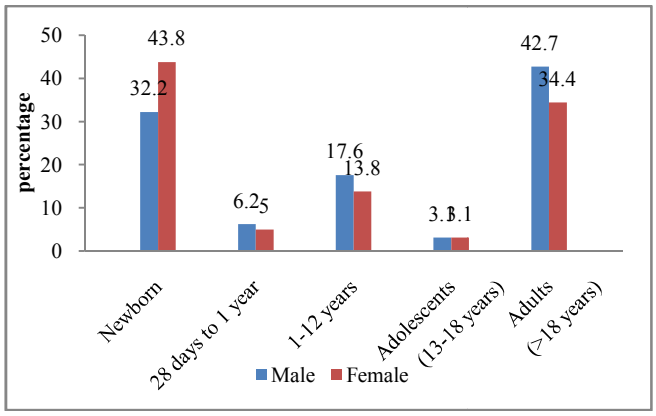
A retrospective analysis was done on the blood samples received for culture over a period of one year from January 2017 to December 2017 in the Department of Microbiology, Govt. T.D. Medical College, Alappuzha. Blood samples from clinically suspected cases of septicaemia were received and processed. 10 ml of blood was collected from adults and inoculated into 50 ml of 'Brain Heart Infusion' (BHI) broth and in paediatric cases, 1-3 ml of blood was collected into 5 - 10 ml of BHI broth. Blood culture bottles inoculated with the sample were incubated at 37°C aerobically, and periodic subcultures were done on Blood agar and Mac Conkey's agar on day 2 and day 7 respectively and in between if the broth appeared visibly turbid. The growth obtained was identified by colony morphology, Gram stain of the isolated colonies and standard biochemical identification tests. [12]

Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method and the results were recorded. For Gram positive bacteria, Penicillin (10U), Erythromycin (15µg), Clindamycin (2µg),Vancomycin (30µg), Cefoxitin (30µg), Trimethoprim/Sulphamethoxazole (1.25/ 23.75 µg) discs were used. For Gram negative bacteria, Ampicillin (10µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Cefotaxime (30µg), Cefepime (30 µg), Ceftriaxone (30 µg), Trimethoprim/Sulphamethoxazole (1.25/ 23.75 µg), Ceftazidime (30 µg), Amikacin (30 µg), Piperacillin-Tazobactam (100/10 µg), Meropenem (30 µg) were tested. The susceptibility and resistance were interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines.[13]*Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* ATCC 29212 were used as reference strains for culture and susceptibility testing.

Data Analysis-Data was entered in Microsoft Excel and analysed using SPSS software. Qualitative variables were summarised using percentage and proportions.

RESULTS

3836 blood samples received during the study period were processed for aerobic culture and 389 (10.1%) of these samples yielded growth. Graph 1 shows the age and sex-wise distribution of positive blood culture samples. Out of the 389 culture positive samples, 227 (58.5 %) were males and 162 (41.5 %) were females. The patients were in the age group ranging from 1 day to 87 years.



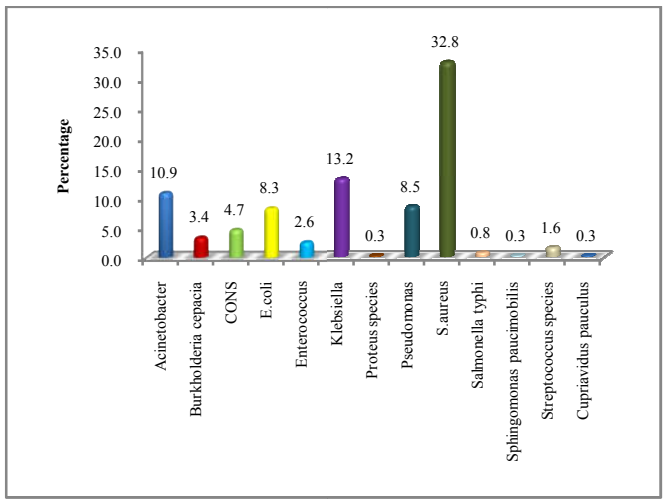
Graph I Age and sex- wise distribution of bacterial isolates

Table 1 describes the ICU and ward wise distribution of isolates. Of the total isolates from intensive care units, neonatal ICU showed maximum culture positivity- 142 isolates (36.7%), followed by paediatric ICU (30 isolates- 7.8%). In ward wise distribution, maximum number of isolates were obtained from medical wards (59 isolates- 15.2 %) followed by paediatric wards (51 isolates- 13.2%).

Table 1 Distribution of isolates in ICUs and wards

Place of admit	Number	Percentage
Neonatal ICU	142	36.7
Pediatric ICU	30	7.8
Medicine ICU	17	4.4
Other ICU	27	7.0
Pediatric Ward	51	13.2
Medicine Ward	59	15.2
Surgery Ward	14	3.6
OBG Ward	7	1.8
Other Wards	40	10.3

The distribution of bacterial isolates is shown in Graph 2. Among 389 positive blood culture samples, 376 (96.9%) yielded bacterial isolates and 13 (3.1%) were Candida species. All the infections were monomicrobial.



Graph 2 Distribution of bacterial isolates from positive blood cultures

Out of the total 376 bacterial isolates, Gram positive, Gram negative bacteria and non-fermenters contributed to 41.7%, 22.6% and 32.6% respectively. The predominant organism was Staphylococcus aureus -127 (32.8%). The other Gram positive cocci were Coagulase Negative Staphylococci (CONS), Enterococcus spp. -10 (2.6%) and Streptococcus spp. Methicillin Resistant Staphylococcus aureus (MRSA) was found in 22.3% of the total S.aureus isolates whereas Methicillin Resistant CONS was found in 14.2% of the total CONS isolates. The most commonly isolated Gram-negative bacteria among Enterobacteriaceae was Klebsiella spp. -51 (13.2 %) followed by E.coli, Salmonella typhi and Proteus spp. 3)Among the non-fermenters, Acinetobacter spp-42 (10.9%) was the commonest isolate followed by Pseudomonas spp., Burkholderia cepacia, Cupriavidus pauculus and Sphingomonas paucimobilis.

Antibacterial resistance pattern of the Gram positive, Gram negative and non-fermenter blood stream isolates are shown in Tables 2, 3 and 4 respectively. All the Gram positive isolates were uniformly sensitive to Vancomycin. The Gram-positive isolates showed high degree of resistance to Penicillin and

Erythromycin. Enterobacteriaceae showed maximum resistance to Cefotaxime and Cefepime followed by Ciprofloxacin and Gentamicin.

Table 2 Antibiotic resistance pattern of Gram positive isolates

Antibiotic	Isolate			
	S.aureus	CONS	Enterococcus	Streptococcus species
Penicillin	98 (77.2)	12 (66.7)	5 (55.6)	0 (0)
Erythromycin	67 (52.8)	6 (33.3)	5 (55.6)	0 (0)
Clindamycin	21 (16.5)	4 (22.2)	1 (50)	0 (0)
Cotrimoxazole	36 (28.3)	3 (16.7)	NT	1 (100)
Cefazolin	0 (0)	2 (11.1)	NT	NT
Cloxacillin	0 (0)	1 (5.6)	NT	NT
Ampicillin	0 (0)	0 (0)	6 (60)	0 (0)
Ciprofloxacin	33 (27)	4 (22.2)	6 (66.7)	1 (50)
Ceftriaxone	0 (0)	0 (0)	2 (50)	0 (0)
Gentamicin	21 (16.9)	4 (22.2)	0 (0)	NT

(NT-NOT TESTED)

Table 3 Antibiotic resistance pattern of Gram negative isolates

Antibiotic	Isolate			
	E.coli	Klebsiella spp.	Proteus spp.	S.typhi
Cotrimoxazole	2 (50)	9 (75)	0 (0)	0 (0)
Ampicillin	28 (87.5)	0 (0)	0 (0)	0 (0)
Amikacin	3 (9.4)	27 (52.9)	0 (0)	NT
Cefotaxime	18 (56.3)	44 (86.3)	1 (100)	0 (0)
Cefepime	12 (37.5)	35 (68.6)	1 (100)	0 (0)
Ciprofloxacin	15 (46.9)	38 (74.5)	1 (100)	0 (0)
Cefoperazone-Sulbactam	5 (15.6)	21 (41.2)	0 (0)	NT
Gentamicin	7 (21.9)	31 (60.8)	1 (100)	0 (0)
Meropenem	2 (6.3)	3 (5.9)	0 (0)	NT
Piperacillin-Tazobactam	2 (6.3)	22 (43.1)	0 (0)	NT

(NT-NOT TESTED)

Table 4 Antibiotic resistance pattern of non-fermenters

Antibiotic	Isolate				
	Acinetobacter spp.	Pseudomonas spp	Burkholderia cepacia	Cupriavidus pauculus	Sphingomona spaucimobilis
Cotrimoxazole	6 (37.5)	NT	0 (0)	0 (0)	0 (0)
Amikacin	16 (38.1)	6 (18.2)	0 (0)	1 (100)	0 (0)
Cefotaxime	28 (66.7)	NT	0 (0)	1 (100)	0 (0)
Ceftazidime	0 (0)	9 (27.3)	13 (100)	0 (0)	0 (0)
Cefepime	26 (61.9)	7 (21.2)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	21 (50)	6 (18.2)	0 (0)	1 (100)	1 (100)
Cefoperazone-Sulbactam	13 (31.7)	NT	13 (100)	0 (0)	0 (0)
Gentamicin	20 (48.8)	9 (28.1)	13 (100)	1 (100)	0 (0)
Meropenem	10 (23.8)	2 (6.3)	0(0)	0 (0)	0 (0)
Piperacillin-Tazobactam	13 (31)	2 (6.1)	13 (100)	0 (0)	0 (0)

(NT-NOT TESTED)

There was an outbreak of Burkholderiacepacia sepsis in neonatal icu in July 2017. A total of 13 cases were identified-2 babies succumbed to death. All the isolates were uniformly susceptible to Meropenem and Ciprofloxacin. Despite extensive sampling, the exact source could not be identified. However, the outbreak was controlled by itself.

DISCUSSION

In the present study, the blood culture positivity rate in clinically suspected cases of septicaemia was 10.1%. Studies by Usha and Pushpa 2007 (10%); Shalini s *et al* 2010 (11.2%) and Iran, HamedGhadhiri *et al* 2012 (10.8%) showed similar rates.[14,15,16] Certain other studies from India and other countries have showed high frequency of positive blood culture ranging from 18 to 45%.[17] The wide variation in blood culture positivity rates could be due to difference in geographical location, nature of patient population and epidemiological difference of the etiological agents. The low rate of isolation in our study could also be explained by the fact that most of the patients would have already received some kind of primary treatment at peripheral health centres before reaching a tertiary care hospital.

Out of the 389 culture positive samples, 227 (58.5%) were males and 162 (41.5%) were females. Overall proportion of males and females was found to be insignificant. (p value calculated was 0.1 ie. > 0.05). Among ICUs, the isolation rate was found to be high in neonatal ICU (36.7%) and among wards, maximum number of isolates were obtained from medical wards (15.2%). In our study, Gram positive and Gram negative bacteria constituted 41.7 % and 22.6 % respectively. This finding was in accordance with the studies of China and Gupta, Kamga *et al*., Anbumani *et al*., and Karlowsky *et al*.[18,19,20,21]In contrast, studies like Mehta *et al*., Mehdinejad *et al*., Barati *et al*. and Ayobola *et al*. showed predominant Gram-negative organisms.[22,23,24,25] The present finding also points to the fact that infections by Gram-positive organisms pose a major threat to septicaemia in our locale. The most common Gram‑positive bacterium isolated in our study was *S. aureus* (32.8%) followed by CONS (4.7%). The isolation of S.aureus is consistent with the study of Arora and Devi, Roy *et al*., and Karlowsky *et al*. where the reported isolation of the organism was 27.3%, 14% and 16.5% respectively.[26,27,21] Some studies have reported CONS as the most common Gram‑positive organism isolated from blood culture specimens.[21] Similar rates of isolation of *S.aureus* and low isolation rate of CONS in has been reported in other studies.[20]

Among Enterobacteriaceae, *Klebsiella* (13.2%) and *E. coli* (8.3%) were the predominant isolates similar to findings reported in earlier studies.[28,29] A high prevalence of nonfermenters- *Acinetobacter* spp. (10.9 %)and *Pseudomonas* spp. (8.5%) was found in our study as reported by Chhina and Gupta and Vanitha *et al*.[18,29] This finding is of major concern in the hospital settings, as these organisms are associated with a high degree of antimicrobial resistance. S. typhi was isolated in 0.8% cases; a finding concordant to the study by Jadhav *et al*. (1.5%).[30] Several other studies have reported prevalence of S.typhi between 12% and 15%.[23,31]

S. aureus was frequently found to be penicillin resistant (77.2%). Antimicrobial resistance to erythromycin, gentamicin, ciprofloxacin were 52.8%, 16.9% and 27% respectively but none of the strains showed resistance to vancomycin. Similar results have been reported by other workers.[32] Among Gram‑positive isolates, MRSA was found in significant frequency (22.3 %), almost similar to the findings in other Indian studies.[28,29] Rate as high as 35% has been reported by Ahmadey and Mohammed.[33]Multidrug resistance was found in more than 50 % of the Enterococcal isolates which was similar to the finding of Jain *et al*. (54%).[34] 2.7% of Enterococcal isolates were found to be moderately sensitive to vancomycin in the same study whereas no vancomycin‑resistant isolates were present in our study. In another study 50-60 % enterococcal isolates were resistant to all the antibiotics tested.[35]

The resistance percentage of Enterobacteriaceae to various drugs were as follows- Cefotaxime (72.4%), Cefepime (55.2%), Ciprofloxacin (50.6%) and Gentamicin (44.8%), Amikacin (36.1%) and Trimethoprim-Sulphamethoxazole

(13.25%). Majority of the Enterobacteriaceae were sensitive to Meropenem and Piperacillin-Tazobactam with a resistance rate of less than 1 % and the susceptibility to Cefoperazone-Sulbactam combination was 70%. Amikacin (73.9%), Meropenem (71.6%) and Cefoperazone-Sulbactam (70.46%) were found to be highly susceptible for nonfermenter isolates. Some other studies also show similar findings.[22,30]

The overall antibiotic susceptibility pattern suggests a high prevalence of Multi Drug Resistant organisms among Gram-negative isolates in our hospital. Total MDR isolates observed in our study were 75.65%. Multidrug resistance among Gram-negative isolates were 62.7% among Enterobacteriaceae and 50% among nonfermenters. Several other studies also have reported high frequency of Gram-negative isolates as MDR.[26,31] Indiscriminate use of antibiotics and failure to comply with the antibiotic policy in the hospital could be the main reasons for this existing problem.

In the present study, we found 30 out of the total Gram-negative isolates to be carbapenem-resistant with 25 (28.4%) and 5 (6.02 %) isolates among nonfermenters and Enterobacteriaceae, respectively. Most of the infections caused by MDR and carbapenem resistant strains are difficult to treat because of the limited options of the antibiotics available. This has also contributed to the increase in mortality.

CONCLUSION

Increasing antimicrobial resistance in both out-patients and hospitalised patients with septicaemia is a worldwide concern. Routine surveillance of blood stream etiology is essential because such data provides valuable information in formulating hospital antibiograms and selecting appropriate antibiotic therapy in the management of sepsis in a particular area. There are only a few newer antimicrobials in the research pipeline. It is foreseen that if the same kind of injudicious prescription of antibiotics continues, we are most likely to face the condition of so-called pan-drug resistance in near future. This emphasizes the urgent need for rational use of antibiotics and implementation of effective infection control practices.

The present study was therefore undertaken to describe the antibiotic resistance of blood culture isolates as it may be a useful guide to the clinicians to be aware of the emerging resistant strains of pathogens that are a threat to the community and also enables them to initiate effective empirical therapy. Also, knowledge of the baseline antimicrobial resistance specific to a hospital prevents irrational use of antibiotics in that hospital so that we are able to progress a step forward in the prevention of spread of antibiotic resistance.

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