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Original research article

Bacteriologic profile and anti-biogram of blood culture isolates from blood stream infections in tertiary care hospital guntur

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Abstract

Introduction: Blood stream infections (BSIs) are one of the most common healthcare-associated infections and are associated with significant morbidity and mortality. Numerous bacteria have been associated with causation of Blood Stream Infections including Gram-negative bacteria and Gram-positive bacteria. He present study was undertaken to know the bacteriological etiology of BSIs and the antibiotic susceptibility pattern of the isolated strains in patients attending to Guntur Government Hospital Guntur, and study was performed in Department of Microbiology Guntur Medical College Guntur.

Methods: Retrospective observational study was conducted in Department of Microbiology Guntur Medical college, Guntur to know the bacteriological etiology of BSIs and the antibiotic susceptibility pattern of the isolated strains. RESULTS: Out of the 2133 total cultures 422 (19.78%) were culture positive. Gram positive organisms were MRSA, MSSA, CONS and Enterococci and the Gram negative organisms were *K. pneumoniae, K. oxytoca, Pseudomonas, E. coli, Citrobacter* and *Acinetobacter* isolated. AST report showed gram positive bacteria were sensitive to vancomycin and Linezolid. Gram negative bacteria were sensitive to Piperacillin and tazobactam and Imipenem. CONCLUSION: Regular surveillance and isolation of causative agents of BSI's and their antibiogram should be done in the hospital regularly to decrease the morbidity and mortality.

Keywords: Blood stream infections, antibiogram

Introduction

Blood stream infections (BSIs) are one of the most common healthcare-associated infections and are associated with significant morbidity and mortality [2]. Clinical manifestations range from transient asymptomatic bacteremia to fulminant septic shock with high mortality. Early diagnosis and management of BSIs is life-saving.

The important risk factors for BSIs include the use of healthcare devices such as: peripheral and central venous catheters on patients is the most common and premorbid medical conditions of patients, such as diabetes mellitus, malignancies, renal failure, burns and prior hospitalization are also contribute for BSIs.

The mortality rate from bloodstream infections ranges from 4.0% to 41.5% depending on severity, age, sex, and other risk factors. Infections due to antibiotic-resistant strains of bacteria present with a significantly higher morbidity and mortality which are emerging and alarm to early detection and treatment.

In Children the Blood stream infections present with fever, difficulty in breathing, tachycardia, malaise, refusal of feeds or lethargy. May lead to serious consequences like shock, multiple organ failure, disseminated intravascular coagulation, etc.

Thus, the blood stream infections constitute one of the most serious conditions which needs timely detection and identification of blood stream pathogen is important.

Clinical assessment using a combination of symptoms and signs is a useful guide for the provisional diagnosis of septicemia. But bacteriologic culture to isolate the causative pathogen and its antibiotic sensitivity testing is remains the mainstay of definitive diagnosis of septicemia.

The prevalence and susceptibility patterns of microorganism vary according to geography and even within the same hospital with time. Hence, regular surveillance of BSI etiology is important in

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monitoring the spectrum of bacterial pathogens and their sensitivity pattern in a particular area. Such data help clinicians to start effective empirical treatment, and also prevents irrational use of antibiotics which can prevents spread of antibiotic resistance.

Numerous bacteria have been associated with causation of Blood Stream Infections including Gram-negative bacteria like *Escherichia coli, Pseudomonas species* (spp.), *Klebsiella* spp., *Serratia* spp., *Salmonella* spp. And *Enterobacter* spp. and Gram-positive bacteria: *Staphylococcus* spp., *Streptococcus* spp. And *Enterococcus* spp. Etc.

However, recent findings suggested there is increase in multidrug-resistant bacteria, including the members of the Enterobacteriaceae family and other Gram-negative bacteria, such as *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp. and *Citrobacter* spp., most of which are extended spectrum beta-lactamase (ESBL) producers and also some Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and the vancomycin-resistant enterococci.

The present study was undertaken to know the bacteriological etiology of BSIs and the antibiotic susceptibility pattern of the isolated strains in patients attending to Guntur Government Hospital, Guntur and study was performed in Department of Microbiology Guntur Medical College Guntur.

The Aims and Objectives of this study are:

1. To isolate and identify different bacterial causes of Blood Stream Infections.
2. To determine the antibiotic susceptibility patterns of isolated bacteria and to suggest the best empirical treatment of BSIs in different scenarios in the hospital.

Materials and Methods

Study design

Retrospective observational study was conducted in the Department of Microbiology, Guntur Medical College, Guntur.

Duration of study: May 2022 to June 2023.

Study setting: Department of Microbiology, Guntur Medical College, Guntur. Approval was taken from the Ethical Committee of Guntur Government Hospital and College, Guntur on 07.09.2023.

Study population

Blood samples of all inpatients from May 2022 to June 2023 were included in the study.

Materials and Methods

From 2133 suspected cases of sepsis, Blood samples were received in Brain Heart Infustion broth (BHI) in (1:10 dilution) with strict aseptic precautions. The collected bottles were incubated in an incubator at 37 °C for 24 hrs aerobically and after overnight incubation, subcultures were done onto Mac Conkey's agar, blood agar and chocolate agar. These plates are incubated overnight in the incubator at 37 °C, if growth was obtained it was identified by Colony morphology, Grams staining, motility and capsular staining and different biochemical reactions.

The subcultures are done on day 3, day 4 and finally on day 7, after that only NO growth was reported.

The standard disk diffusion test for susceptibility to routine antibiotics was done by modified Kirby-Bauer disc diffusion method. Zone sizes were measured and interpreted according to CLSI standards and drug resistant strains in primary screening were further processed for extended spectrum beta lactamases (ESBL) status by combination disk method and methicillin resistant *Staphylococcus aureus* (MRSA) was identified by Cefoxitin disk diffusion method (MRSA).

Detection of MRSA was done by Cefoxitin disk diffusion method by placing 1 µg Cefoxitin disk on the bacterial lawn culture of *S. aureus*. After overnight incubation, the zone of inhibition was measured. An inhibition zone of diameter less than or equal to 10 mm indicates MRSA.

S.aureus ATCC 25923 was used as quality control for Cefoxitin susceptibility.

ESBL producers were detected by combination disk method using cefotaxime (30 µg) and cefotaxime/clavulanate (30/10 µg) (Hi media Mumbi, India). An increase of 5 mm in the zone of inhibition in a disk containing clavulanate compared to the drug alone was considered as positive for ESBL producers.

Results

Out of the 2133 total cultures 422 (19.78%) were culture positive.

Table 1: Total number of Cultures

Total	Positives	Negatives
2133	422 (19.78%)	1711 (80.22%)

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Table 2: No of Culture positives

Total culture positives	Gram negative bacilli	Gram positive cocci
422	157 (37.2%)	265 (62.7%)

Out of 422 culture positives, 157 (37.2%) of the culture isolates were Gram negative bacilli, 265 (62.7%) of the culture isolates were Gram positive cocci, Out of 265Gram positive cocci 147 strains are Staphylococcus aureus. Out of 147 strains of *S. aureus*, 42 strains were detected as MRSA.

Table 3: Shows gender wise positivity

S. No.	Gender	No. of Patients	Percentage
1	MALES	68	16.11%
2	FEMALES	45	10.66%
3	MCH	208	49.2%
4	FCH	101	23.9%

Out of 422 (19.78%), culture positives 68 (16.11%) were males and 45 (10.66%) were females, 208 (49.2%) were male child and 101(23.9%) were female child.

Table 4: Age wise Distribution in Relation to Gender

Age Group	Male		Female		Total	
	N	%	N	%	N	%
<1 Mon	170	61.59%	75	51.36%	245	58.05%
1 Mon to 5 Years	21	7.6%	7	4.79%	28	6.63%
6 Years to 17 Years	17	6.15%	19	13.01%	36	8.53%
18 Years to 45 Years	22	7.97%	13	8.90%	35	8.29%
>45 Years	46	16.6%	32	21.91%	78	18.48%

Out of 68 (16.8%) males, 46 (67.64%) were above 45 years of age, and Out of 45 (10.66%) females 32 (71.11%) of culture positives obtained in above 45 years. Out of 208(49.2%) male child 170 (82.2%) are belongs less than 1 month of age. Out of 101(23.9%) female child 75 (74.25%) culture positives were less than 1 month of age group.

Table 5: Organism Wise Prevalence

S. No.	Organism	Number of Samples	Percentage
1	MSSA	105	24.88%
2	MRSA	42	9.95%
3	CONS	106	25.11%
4	Enterococcus	12	2.84%
5	Klebsiella pneumoniae	68	16.11%
6	Klebsiella oxytoca	33	7.81%
7	Escherichia coli	8	1.89%
8	Pseudomonas aeroginosa	24	5.68%
9	*Citrobacter* spp.	9	2.13%
10	Acenetobacter	15	

Out of 422 culture positives, 157 (37.2%) of the culture isolates were Gram negative bacilli, 265 (62.7%) of the culture isolates were Gram positive cocci. Out of 265 gram positive cocci 42 (9.95%) were Methicillin resistant *Staphylococcus aureus*, 105 (24.88%) were Methicillin sensitive *Staphylococcus aureus*, 106 (25.11%) were coagulase negative *Staphylococcus* and 12 (2.84%) were enterococcus. Out of 157 gram negative bacilli, 68(16.11%) were *Klebsiella pneumoniae*, 33(7.81%) were *Klebsiella oxytoca*, 24 (5.68%) were *Pseudomonas aeroginosa*, 9(2.13%) were *citrobacter* spp and 8 (1.89%) were *Escherichia coli*.

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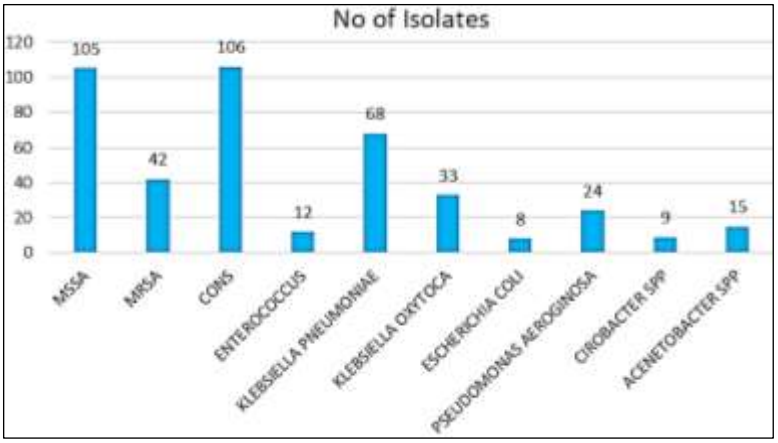

Fig 1: No of isolates

Table 6: Frequency of Isolates in Relation to Age

Isolates	<1 Mon		1 Mon to 5 Years		6 Years to 17 Years		18 to 45 Years		>45 Years	
	N	%	N	%	N	%	N	%	N	%
MSSA	68	64.76%	16	15.23%	9	8.57%	7	6.6%	5	4.76%
MRSA	17	59.52%	4	9.52%	6	14.28%	8	19.04%	7	16.6%
CONS	56	52.83%	15	14.15	4	3.77%	18	16.98%	13	12.26%
Enterococci	4	33.3%	1	8.33%	3	25%	2	16.6%	2	16.6%
Klebsiella pneumoniae	25	36.76%	9	13.23%	10	14.7%	15	22.05%	9	13,23%
Klebsiella oxytoca	14	42.42%	5	15.15%	3	9.09%	6	18.18%	5	15.15%
Escherichia coli	3	37.5%	0	0	1	12.5%	2	25%	2	25%
Pseudomonas aeroginosa	9	37.5%	3	12.5%	5	20.83%	3	12.5%	4	16.6%
Citrobacter	2	22.2%	1	11.11%	1	11.11%	2	22.2%	3	33.3%
Acenetobacter	6	40%	3	20%	2	13.3%	2	13.3%	2	12.3%

Out of total42 isolates of MRSA 36(85.71%) were isolated in ≤ one month age group, out of 105 isolates of MSSA, 68 were in ≤ one month age group, and out of 105 of CONS, 56 were ≤ one month age group, Enterococcal isolates also more in ≤ one month age group, Out of 157 gram negative bacilli, 68(16.11%) were klebsiella pneumoniae, in that 35 were belongs to ≤ one month age group, out of 33(7.81%) klebsiella oxytoca isolates 12 were ≤ one month age group, out of 24(5.68%) isolates of pseudomonas aeroginosa, 9 were ≤ one month age group, out of 9 (2.13%) isolates of citrobacter spp 3 were belongs to ≥ 45 years age group, and out of 3(1.89%) isolates of escherichia coli, 2 belongs to ≥ 45 years age group, out of 15 isolates, 6 isolates were from were ≤ one month age group.

Table 6: Frequency of Isolates in Relation to Age

Isolates	<1 Mon		1 Mon to 5 Years		6 Years to 17 Years		18 to 45 Years		>45 Years	
	N	%	N	%	N	%	N	%	N	%
MSSA	68	64.76%	16	15.23%	9	8.57%	7	6.6%	5	4.76%
MRSA	17	59.52%	4	9.52%	6	14.28%	8	19.04%	7	16.6%
CONS	56	52.83%	15	14.15	4	3.77%	18	16.98%	13	12.26%
Enterococci	4	33.3%	1	8.33%	3	25%	2	16.6%	2	16.6%

Table 7: Anti-biogram of Gram Positive Bacteria

Anti-Biotics	MSSA		MRSA		CONS		Enterococcus	
	n	%	N	%	N	%	N	%
Cefoxitin	105	100	0	0	82	77.3	10	83.3
Vancomycin	100	95.2	39	92.8	99	93.4	12	100
Linezolid	100	95.2	39	92.8	98	92.4	12	100
Clindamycin	73	69.5	28	66.6	65	61.3	8	66.6
Erythromycin	41	39.4	19	45.2	38	35.8	3	25
Amoxycillin + Clavulanic Acid	38	36.2	25	59.5	45	42.4	5	41.6

(MRSA-Methicillin Resistant Staphylococcus aureus, MSSA-Methicillin Sensitive Staphylococccus aureus, CONS- Coagulase Negative Staphylococci)

Among Gram positive Bacteria, All MRSA strains are equally sensitive to Vancomycin (92.8%) and Linezolid (92.8), followed by Clindamycin (66.6%) and Amoxy clauv (59.5%) and Erythromycin (%), MSSA stains are 100% sensitive to Cefoxitin followed by Vancomycin (95.2%), Linezolid, (95.2%) Clindamycin (73%), Erythromycin (41%) and Amoxy Clauv (38%).

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93.4% of isolated CONS were sensitive to Vancomycin followed by Linezolid, Cefoxitin,, Amoxy Clauv, and Erythromycin.
Isolated Enterococci were equally sensitive to Vancomycin and Linezolid, followed by Cefoxitin, Clindamycin, Amoxy Clauv, and Erythromycin.

Table 8: Anti-biogram of Gram Negative Bacilli

Antibiotics	K. Pneumoniae		K. Oxytoca		E. Coli		P. Aeroginosa		Citrobacter		Acinetobacter	
	N	%	n	%	n	%	n	%	N	%	N	%
Piptaz	65	95.5	31	93.9	7	87.5	20	83.3	8	88.8	13	86.6
Imipenem	66	97.5	30	90.9	6	75	19	79.1	7	77.7	11	73.3
Cefipime	63	92.6	32	96.9	7	87.5	-	-	8	88.8	14	93.3
Ciprofloxacin	39	57.3	23	69.6	4	50	-	-	5	55.5	10	66.6
Cotrimoxazole	44	64.7	28	84.8	5	62.5	18	75	6	66.6	4	26.6
Cefotaxime	31	45.5	20	60.6	4	50	17	70.8	4	44.4	8	53.3
Amikacin	-	-	-	-	-	-	12	50	-	-	-	-
Gentamycin	-	-	-	-	-	-	14	58.3	-	-	-	-
Ceftazidime	-	-	-	-	-	-	18	75	-	-	-	-

In Gram negative bacilli of Klebsiella pneumoniae sensitive to Imipenem with 97.5% followed by Piperacillin and Tazobactam 95.5%, Cefipime (92.6%), Cotriamoxazole (64.7%), Ciprofloxacin (57.3%) and Cefotaxime with 45.5%.
Klebsiella oxytoca obtained highest sensitive to Cefipime of 96.9% followed by piperacillin and tazobactam of 93.9%, Imipenem with 90.9%, Cotrimoxazole 84.8%, Ciprofloxacin 69.6%, and Cefotaxime 60.6%.
Escherichia coli obtained highest sensitivity with Piperacillin and Tazobactam and Cefipime wit 87.5%, Imipenem 75%, Cotrimoxazole 62.5%, Ciprofloxacin and Cefotaxime 50%.
Pseudomonas aeruginosa obtained highest sensitivity with 83.3% of Piperacillin and Tazobactam followed by Imipenem 79.1%, Cotrimoxazole and Ceftazidime 75%, Cefotaxime 70.8%, Gentamycin 58.35 and Amikacin 50%,
Citrobacter spp. obtained highest sensitivity with 88.8% of piperacillin and Tazobactam and Cefepime followed by Imipenem 77.7%, Cotrimaxazole 66.6%, Ciprofloxacin 55.5%, Cefotaxime 44.4%, *Acinetobacter* spp. obtained with highest sensitivity 93.3% of Cefepime, followed by Piperacillin and Tazobactam 86.6%, Imipenem73.3%, Ciprofloxacin 66,6%, Cefotaxime 53.3%, Cotrimoxazole 26.6%.

Discussion

Blood stream infections are one of the leading cause for highest mortality and morbidity world wide [2]. Early diagnosis by blood culture and study of antibiotic susceptibility pattern is most important to increase the survival rate of the patients [3, 4], and by knowing the susceptibility pattern, then physicians can choose the antibiotics for empirical treatment.
This study attempts to analyze the bacterilogical profile and assess their antimicrobial patteren to formulate antibiogram and effective empirical treatment of BSIs.
Total 2133 samples were processed, in that 422 (19.78%) were culture positive. The rate of isolation is consistent with other studies from India [2].
Out of 422 (19.78%) culture positives, 68 (16.11%) were males and 45 (10.66%) were females, 208 (49.2%) were male child and 101(23.9%) were female child.Culture positives were more in males than Females in this study (17 Karunakaran R *et al*.).
In male population out of 68 (16.11%) culture positives, 46(67.64%) belongs to above 45 years of age. Out of 45(10.66%) females, 32(71.11%) Kaur and Singh [2014] were about 45 years of age (16 Prashanth H *et al*.). Out of 208 male child 170(82.2%) are less than 1 month age. Out of 101 female child 75(74.25%) are less than 1month age group.
Out of 422 culture positives, 265(62.7%) were gram positive, in that 42(9.95%) were MRSA, 105(24.88%) MSSA, 106(25.11%) were CONS and 12(2.84%) were Enterococcus.
Out of 422 culture positive 157(37.2%) were isolated gram negative bacilli, in that 68(16.11%) were Klebsiella pneumoniae, 33(7.81%) were klebsiella oxytoca, followedby 24 (5.68%) were pseudomonas aeroginosa, 9(2.13%) were *Citrobacter* spp. and 8(1.81%) were Escherichia coli. except *Citrobacter* spp. and Acenetobacter all other organisms are isolated in the highest percentage in less than one month age group, *Citrobacter* spp. and *Acenetobacter* spp. were isolated in 45 years age group.
Among Gram positive Bacteria showed sensitivity to vancomycin and Linezolid [4], In that MRSA were equally sensitive to Vancomycin (92.8%) and Linezolid (92.8), Fayyaz *et al.* [2013] followed by Clindamycin (66.6%) and Amoxy clauv (59.5%) and Erythromycin (%), MSSA stains are 100% sensitive to Cefoxitin followed by Vancomycin (95.2%), Linezolid, (95.2%) Fayyaz *et al.* [2013] Clindamycin (73%), Erythromycin (41%) and Amoxy Clauv (38%).
93.4% of isolated CONS were sensitive to Vancomycin followed by Linezolid, Cefoxitin, Amoxy Clauv, and Erythromycin. Marshall *et al.* [1998].

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Isolated Enterococci were equally sensitive to Vancomycin and Linezolid, followed by Cefoxitin, Clindamycin, AmoxyClauv and Erythromycin.
In Gram negative bacilli of Klebsiella pneumoniae sensitive to Imipenem with 97.5% Saghir *et al.* [2009] followed by Piperacillin and Tazobactam 95.5%, Cefipime (92.6%), Saghir *et al.* [2009] Cotriamoxazole (64.7%), Ciprofloxacin (57.3%) and Cefotaxime with 45.5%.
Klebsiella oxytoca obtained highest sensitive to Cefipime of 96.9% followed by piperacillin and tazobactam of 93.9%, Saghir *et al.* [2009] Imipenem with 90.9%, Cotrimoxazole 84.8%,Ciprofloxacin 69.6%, and Cefotaxime 60.6%.
Escherichia coli obtained highest sensitivity with Piperacillin and Tazobactam and Cefipime with 87.5%, Saghir *et al.* [2009] Imipenem 75%, Cotrimoxazole 62.5%, Ciprofloxacin and Cefotaxime 50%.
Pseudomonas aeruginosa obtained highest sensitivity with 83.3% of Piperacillin and Tazobactam Fayyaz *et al.* [2013], followed by Imipenem 79.1%, Cotrimoxazole and Ceftazidime 75%, Cefotaxime 70.8%, Gentamycin 58.35% and Amikacin 50%,
Citrobacter spp. obtained highest sensitivity with 88.8% of piperacillin and Tazobactam and Cefepime followed by Imipenem 77.7%, Cotrimaxazole 66.6%, Ciprofloxacin 55.5%, Jyothi *et al.* [2013] Cefotaxime 44.4%, *Acinetobacter* spp. obtained with highest sensitivity 93.3% of Cefepime, followed by Piperacillin and Tazobactam 86.6%, Imipenem 73.3%, Ciprofloxacin 66,6%, Cefotaxime 53.3%, Cotrimoxazole 26.6%.

Conclusion

The present study provides prevalent Bacterial pathogens causing Blood Stream Infections, and their Antibiotic susceptibility pattern at tertiary care center, GGH Guntur.
Successful treatment of Blood Stream Infections depends on early diagnosis and knowledge about prevalent bacteria present in our tertiary care hospital and proper antimicrobial susceptibility pattern of the isolates.
Knowledge about antibiotic susceptibility pattern, and following antibiotic stewardship of the Infection control Committee of the hospital can reduce the arising of multi drug resistant strains in the hospital environment.
Local knowledge of bacteriological profile and antimicrobial sensitivity patterns helps rationalize empiric treatment strategies.
Strict hospital infection control measures and correct antibiotic policy for the hospital is important need of the present situation.

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