

ABSTRACT

Title: Prevalence and Antimicrobial Susceptibility Pattern of Bacterial Isolates from Blood Cultures of Hospitalized Patients in a Rural Tertiary Care Hospital: A 10 years Study.

Authors: Dr. Sandhya Kulkarni,

Institution:- Department of Microbiology, MIMER Medical College, Talegaon (Dabhade), Pune

Abstract:-

Background- Bloodstream infections (BSI) are associated with significant mortality and morbidity worldwide.

Aims- i) To investigate bacteria isolated from blood cultures.

ii) To determine the antimicrobial susceptibility pattern of these bacterial isolates.

This is a retrospective study of blood samples received in the department of microbiology over a period of 10 years.

Materials and Methods- The bacterial isolates were identified by standard microbiological techniques and antimicrobial susceptibility testing was done by Kirby –Bauer method.

Results- During the study period, 2063 blood culture samples were analyzed. 720 (34.9%) samples were culture positive. From 720 culture positive samples, 745 isolates were obtained of which 452(60.67%) were Gram-positive and 293(39.33%) were Gram-negative bacteria.

Most of the culture positive samples were of mono-microbial etiology (97.36%) and only from 19(2.64%) samples more than one organism were isolated.

The most predominant organism we isolated was Staph. aureus (55.43%).

More than 75% of Gram positive isolates were sensitive to Linezolid (80.12%), Levofloxacin (78.5%), and Ampicillin-Sulbactam (76.64%). Among the group of Gram-negative organisms, the best overall sensitivity was

noted to Gatifloxacin (70.67%) and the highest resistance was noted against Cefuroxime (95.45%), Cefadroxil (90.38%) and Amoxyclav (87.5%).

Conclusion- Staph. aureus was the most predominant isolate. Most of the isolates showed a variable degree of resistance to commonly tested antibiotics. The periodic evaluation of bacterial isolates and their antimicrobial susceptibility testing will help in selecting antibiotics to treat septicemia patients. This study seems to be helpful in providing guidelines to choose an effective antibiotic in cases of septicemia.

Key words- Septicemia, Bacterial isolates, Antimicrobial susceptibility

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INTRODUCTION:-

Bloodstream infections (BSIs) cause significant morbidity and mortality worldwide and are among the most common healthcare-associated infections ^[1,2]. Blood culture is the essential investigation for the management of sepsis. The clinical microbiology laboratory plays a significant role in the management of patients of bloodstream infections. Culturing a pathogenic microorganism from blood is highly specific indicator of bloodstream infections and performance of antimicrobial susceptibility testing (AST) may assist in the appropriate use of microbial therapy for patients with bloodstream infections ^[3,4,5]. Increasing rates of antimicrobial resistance, changing pattern of antimicrobial usage, wide application of new medical technologies- change the epidemiology and outcome of bloodstream infections ^[1]. In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available ^[6]. Early initiation of appropriate antimicrobial treatment is critical in decreasing morbidity and mortality among patients with bloodstream infections ^[7] so a right choice of empiric therapy is utmost important.

There are surveillance study reports available from developed countries but less data is available from developing countries, hence the present study was undertaken to know the prevalence and antimicrobial susceptibility pattern of bacterial isolates from blood of hospitalized patients in a tertiary care rural hospital.

Thus, the present study was undertaken

- (i) To investigate the bacterial isolates from blood
- (ii) To determine their antimicrobial susceptibility

MATERIALS AND METHODS:-

In the present study, a total of 2063 blood samples from various clinical departments were reviewed over a period of 10yrs from Jan.2004 to Dec.2013.

All the samples were collected at Dr. Bhausaheb Sardesai Talegaon Rural Hospital, Talegaon (Dabhade), Pune (M.S., India), a tertiary care hospital

These samples were processed in the department of Microbiology, MIMER Medical College, Talegaon (Dabhade), Pune (M.S., India). The samples were received in the bottle containing 30ml of tryptose phosphate broth. On receiving in the laboratory, the samples were incubated at 37°C. The broths were sub-cultured on Blood agar and MacConkey's agar after overnight incubation. A negative sample (no growth) sample was followed upto 7days with two more subcultures. Organisms from positive blood culture were identified by using standard microbiological methods and antimicrobial sensitivity was performed by Kirby-Bauer's disk diffusion method on Muller-Hinton agar plates [8]. Anaerobic and fungal blood cultures were not done.

The antibiotic disc used were- Ampicillin/Sulbactam (10mcg), Gentamycin (30mcg), Co-trimoxazole (25mcg), Piperacillin (100mcg), Erythromycin (15mcg), Amikacin (30mcg), Amoxicillin-Clavulanate (30mcg), Ciprofloxacin (5mcg), Levofloxacin (5mcg), Ofloxacin (5mcg), Gatifloxacin (5mcg), Cephalexin (30mcg), Cefotaxime (30mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefuroxime (30mcg), Linezolid (30mcg), Oxacillin (1mcg), Cefoxitin (30mcg).

All collected data was later on statistically analyzed.

RESULTS:-

During the 10years study period, 2063 blood culture samples were analyzed. 720 (34.9%) blood samples were culture positive. Of these 720, 141(19.58%) were from pediatric ward, 258(35.84%) were from NICU and remaining 321(44.58%) were from other wards.

From 720 culture positive samples, 745 isolates were obtained of which 452(60.67%) were Gram-positive and 293(39.33%) were Gram-negative bacteria.

Most of the culture positive samples were of mono-microbial etiology (97.36%) and only from 19(2.64%) samples more than one organism were isolated.

Staph. aureus was the most predominantly (55.43%) isolated organism.

TABLE-1:- Depicts the distribution of Bacterial Isolates from Blood Culture-

Isolate	Total No. of isolate (N=745)	%
Staph. Aureus	413	55.43
Coagulase Neg. Staph.	39	5.23
Unclassified GNB	84	11.27
Klebsiella spp.	56	7.51
E.coli	43	5.8
Pseudomonas spp.	43	5.8
Salmonella spp.	41	5.5
Citrobacter spp.	18	2.41
Proteus spp.	3	0.4
Enterobacter spp.	2	0.26
Morgagnella spp.	2	0.26
Serratia spp.	1	0.13
Total	745	100

TABLE-2:- Depicts the ward wise distribution of Bacterial Isolates

Isolates/ Wards	1	2	3	4	5	6	7	8	9	10	11	12	Total
S.aureus	97	141	53	36	53	3	3	13	7	1	1	5	413
CoNS	8	13	10	4	1	2	1						39
Klebsiella spp.	8	35	3	4	5			1					56
E.coli	6	10	4	8	8	2	1	2	1			1	43
Pseudomonas spp.	8	25	1	2	5			1				1	43
Salmonella spp.	13	1	24	2	1								41
Citrobacter spp.	2	10	1		5								18
Proteus spp.		1	1					1					3
Enterobacter spp.		1	1										2
Morgenella spp.		2											2
Serratia spp.		1											1
Unclassified GNB	19	25	20	6	7	1	2	2	1			1	84
Total	161	265	118	62	85	8	7	20	9	1	1	8	745

Isolates/ Wards	1	2	3	4	5	6	7	8	9	10
S.aureus	97	141	53	36	53	3	3	13	7	1
CoNS	8	13	10	4	1	2	1			
Klebsiella spp.	8	35	3	4	5			1		
E.coli	6	10	4	8	8	2	1	2	1	
Pseudomonas spp.	8	25	1	2	5			1		
Salmonella spp.	13	1	24	2	1					
Citrobacter spp.	2	10	1		5					
Proteus spp.		1	1					1		

Enterobacter spp.		1	1							
Morgenella spp.		2								
Serratia spp.		1								
Unclassified GNB	19	25	20	6	7	1	2	2	1	
Total	161	265	118	62	85	8	7	20	9	1

1. Pediatrics Ward

2. Neonatal Intensive Care Unit

3. Male Medicine Ward

4. Female Medicine Ward

5. Intensive Care Unit

6. Surgery Ward

7. Orthopedics Ward

8. Obstretsic and Gynecology

9. Tuberculosis and Chest Ward

10. Skin and VD Ward

11. Ophthalmology Ward

12. Out Patient Department

TABLE-3:- Depicts the Percentage of Antimicrobial Resistance of Bacterial Isolates

Antibiotic	GPC	S.aureus	GNB	E.coli	Klebsiella spp.	Salmonella spp.	Pseudomonas spp.
Ampi./Sulbact	23.36	23.22	51.45	53.84	70.58	15	85.29
Gentamycin	43.4	44.6	65.74	76.74	89.28	34.34	61.53
Amikacin	32	31.37	41.66	23.25	51.78	31.7	42.1
Clindamycin	34.17	34.6					
Erythromycin	58.22	58.97					
Oxacillin	61.11	61.61					
Linezolid	19.88	19.25					
Cefoxitin	50.9	51.11					
Co-trimaxazole	46.53	45.5	53.84	68.75	76.19	8.1	77.27
Chlorampheni			61.32	68.75	88.1	10.81	66.66
Piperacillin			61.87	68.75	88.1	10.81	66.66
Amoxyclave	74.68	74.35	87.5	93.33	66.66		100
Ciprofloxacin	42.92	43.42	49.14	55.81	67.85	34.14	41.86
Gatifloxacin			29.33	21.42	25.71	25.8	41.17
Ofloxacin	53.8	53.33	50.67	57.56	61.36	37.5	46.42
Levofloxacin	21.5	20.49					
Cephalexin	51.65	49.5					
Cefotaxime	48.32	50	61.53	69.76	89.28	9.75	72.22
Ceftazidime			66.12	54.16	80	25	68
Cefuroxime	69	68.88	95.45	100	100	100	100
Ceftriaxone	62	61.53	67.34	66.66	66.66	33.33	75

GPC- Gram positive cocci, GNB- Gram negative bacilli

Gram positive Isolates:- More than 75% of Gram positive isolates were sensitive to Linezolid (80.12%), Levofloxacin (78.5%), and Ampicillin-Sulbactam (76.64%). The highest resistance was noted against Amoxicillin-Clavulanic acid (74.68%).

Gram-negative Isolates:- Among the Gram-negative isolates, the highest resistance was noted against Cefuroxime (95.45%), and Augmentin (87.5%). Most of the isolates showed 50-60% resistance against tested antimicrobials. Among the group of Gram-negative organisms, the best overall sensitivity was noted to Gatifloxacin (70.67%).

DISCUSSION:-

Study of bacteriological profile with antimicrobial susceptibility pattern plays an important role in effective management of bacteremia ^[9].

Various studies have been undertaken to investigate organisms causing septicemia all over world. The results of these studies are varied at different parts of the world. Many factors play role in explaining this difference e.g. socio-economic, geographic, use of ventilators, administration of different antibiotics etc. ^[9].

The purpose of this study was to investigate the bacterial isolates from blood and to determine their antimicrobial susceptibility. *S. aureus* was the predominant (55.43%) organism isolated and the least frequent isolate, was *Serratia* spp. (0.13%).

The present study was carried out at a tertiary care teaching hospital over a period of 10 years (Jan.2004 to Dec.2013). During the 10years study period, 2063 blood culture samples were analyzed. 720 (34.9%) blood samples were culture positive. This rate is high as compared with the studies done in India and abroad ^[6, 9-16]. But it is comparable with the study done in Islamabad, Pakistan (42%) ^[17], study done by Jain et al (44%) ^[18], and a study done in Nigeria (44.9%) ^[19].

The poly-microbial BSIs have been reported by various workers with an incidence ranging from 4.7 to 18.7%, most of which are hospital acquired ^[20]. However we reported that 97.36% of blood cultures were of mono-microbial etiology which is comparable with other studies ^[6,21,22,23].

Most of the studies reported Gram-negative organisms are responsible for septicemia in hospitalized patients ^[6, 9-14]. However, we reported Gram-positive organisms are responsible for septicemia (60.67%). This finding is supported by other studies ^[1,2,15,21,23].

Surveillance studies have documented both an increase in antimicrobial resistance rates and a shift in organism distribution among important blood stream pathogens, both in the hospitals and community settings. E.g. in the hospital setting, there has been shift from a predominance of gram negative organisms in the late 1970s to the present day primacy of gram positive organisms as cause of nosocomial BSIs ^[24].

We isolated *Staph. aureus* as the most common agent (55.43%) of bloodstream infections. Our results are correlating with the reports of Tambekar et al (64.54%)^[25], DJ Diekema (20%)^[11], Ambumani et al (36.4%)^[9], Usha Arora and Pushpa Devi (27.37%)^[15], Liliana Vargas et al (23.4%)^[26], Erik M (20%)^[27] have also reported *Staph. aureus* as the common causative agent of BSIs.

There are studies reporting Coagulase-negative Staphylococci (CONS) as the predominant Gram-positive organisms causing sepsis^[2,6,13-14,21,23]. Thylefors JD et al reviewed the increasing importance of CONS bacteremia^[28]. However CONS remains the most frequent contaminant of positive blood culture^[22]. James A Karlowsky et al^[2] in their surveillance study reported 42% of all isolates as CONS. In our study, we reported 5.23% of isolates as CONS which is correlating with the findings of Mehdinejad^[10], and Sobhani et al^[16].

Among the group of gram-negative organisms, we reported the highest rate of isolation as unclassified gram negative bacilli (11.27%). This was followed by *Klebsiella* spp. (7.51%), *S.typhi* (6.17%), *E.coli* and *Pseudomonas* spp. (5.8%), and other *Salmonella* spp. (5.5%), *Citrobacter* spp (2.41%). We have also reported small number of other gram negative isolates as *Enterobacter*, *Proteus*, *Moraxella* and *Serratia* spp. [**Table-1**].

There are studies reporting *Klebsiella* as the predominant causative agent of bloodstream infections^[10-12] & some are with low percentage of *Klebsiella* isolates from blood cultures^[2,14,25]. Some of the study reports *Klebsiella* and *E.coli* as the predominant isolates from blood^[10-12]. *Pseudomonas* as the most common cause of septicemia has been reported by Mehata et al^[11]. Vijaya Devi et al (29.78%)^[14] and Sobhani et al (22.2%)^[16] reported *S.typhi* as the most common isolate from blood culture.

The selection of antimicrobials to be used for empiric therapy should be based on the local rates of susceptibility and on the site of infection^[29]. The initiation of empiric therapy requires knowledge of the likely pathogen(s) and their usual antimicrobial susceptibility pattern^[29,30]. Combinations of antimicrobial agents are often prescribed as empiric therapy for suspected or laboratory confirmed BSIs^[29]. The combination therapy is recommended to cover the broad range of possible pathogens, polymicrobial infections. Combinations may have additive/ synergistic antimicrobial activity and may prevent the emergence of resistance^[2].

In the present study, we tested susceptibility for ampicillin-sulbactam combination and 62.69% of all isolates showed in-vitro susceptibility to it. 76.64% of gram positive and 48.55% of gram negative organisms were sensitive to ampicillin-sulbactam combination.

Among the group of Gram-negative organisms isolated from blood, the highest resistance was noted against Cefuroxime (95.45%), and the least resistance was noted against Gatifloxacin (29.33%). **[Table-2]**

Among the group of Gram-positive isolates, the best sensitivity was noted to Linezolid (80.12%), Levofloxacin (78.5%), and Ampicillin-Sulbactam (76.64%) & the highest resistance was noted against augmentin (74.68%). **[Table-3]**

In the present study, the oxacillin resistance rate of S.aureus was 61.11% which is high as compared to other studies e.g. 49.3% ^[2], 22-57% ^[21], 42.8% ^[23], and 41.8% ^[26]. But Atul Garg et al ^[6] have reported much high rate of oxacillin resistance (75.65). As cefoxitin is a surrogate marker for methicillin resistance S.aureus (MRSA), we have tested for it and we report 51.11% resistance to cefoxitin.

S.aureus showed least resistance against linezolid (19.25%), levofloxacin (20.5%), and to ampicillin-sulbactam combination (23.22%), and the highest resistance was noted against augmentin (74.35%). More than 40% S.aureus isolates were resistant to ciprofloxacin (43.42%), gentamicin (44.6%), co-trimaxazole (45.5%), cephalixin (49.5%), cefotaxime (50%), ofloxacin (53.33%), erythromycin (58.97%), ceftriaxone (61.53%), cefuroxime (68.88%).

In case of Pseudomonas isolates, more than 40% were resistant to amikacin, ofloxacin, ciprofloxacin and gatifloxacin. More than 60% isolates were resistant to gentamicin, ceftazidime, piperacillin and cefotaxime. We have reported 100% sensitivity to meropenem.

The results of the antimicrobial susceptibility testing are different with the various studies. But similar to other studies we have also reported multi-drug resistant bacteria isolated from blood cultures ^[6, 9, 11, 12, 25].

CONCLUSION:-

From the present study, it is observed that both gram-positive and gram-negative organisms are responsible for bloodstream infections. Most of these isolates were multi-drug resistant.

Staph. aureus was the most predominant isolate from blood cultures.

The Gram-positive strains showed best sensitivity to Linezolid, Levofloxacin and Ampicillin-Sulbactam combination.

Gatifloxacin was noted as the most sensitive drug for Gram-negative isolates.

Most of the bacteria of BSIs are acquired in primarily from the hospital. Thus, there is a need for stringent caution on the part of hospital management as well as care givers to bring down the incidence of nosocomial infections^[9]. It is important to understand the proper nature of BSIs and its causative agent to implement empiric therapy.

To update clinicians with current data concerning the efficacy of commonly used antimicrobials, a periodic evaluation of bacterial isolates and their antimicrobial susceptibility testing is required. This will help to modify the selection of antibiotics for the best result among the septicemia patients.

A regular epidemiological study of blood culture isolates and determination of their susceptibility to antibiotics is necessary to improve empirical therapy^[13]. To reduce the incidence of BSIs, rational and discrete use of antibiotics according to the standard antimicrobial susceptibility is essential^[9-10, 14].

This study has helped us to have an insight into the incidence of bloodstream infections and the antibiotic sensitivity pattern of these isolates. Also this will help us to formulate management guidelines and antibiotic policy for effective management and proper antibiotic therapy of BSIs in our set up.

Thus to conclude, the antimicrobial resistance surveillance programs should be carried out regularly^[21, 26]

- i) To define the species distribution
- ii) To define the resistance pattern of pathogens causing BSIs
- iii) To provide the basis for appropriate empiric antimicrobial therapy
- iv) To design the programs to control antimicrobial resistance, and

- v) To develop infection control measures to avoid cross transmission of multi-drug resistant organisms.

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- Names of all contributors- Dr. Sandhya S. Kulkarni
M.D. (Microbiology), Professor and Head of the Department Microbiology,
MIMER Medical College, Talegaon (Dabhade), Pune (MS), India

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- Address for correspondence - Dr. Sandhya S. Kulkarni

Professor and Head of the Department Microbiology, MIMER Medical

College, Talegaon (Dabhade), Pune (MS), India.

M-9850053426

Sandhyak69@yahoo.co.in

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