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MICROBIOLOGICAL PROFILE AND ANTIMICROBIAL SENSITIVITY PATTERN OF BLOOD CULTURE ISOLATES OF HOSPITALIZED PATIENTS IN A TERTIARY CARE HOSPITAL OF GUWAHATI, ASSAM.

Dr Melody Baruah* Consultant microbiologist, Healthcity hospital, Guwahati, Assam *Corresponding Author

ABSTRACT **Introduction:** Blood stream infections range from self-limiting infections to life threatening sepsis that requires rapid and aggressive antimicrobial treatment. Knowledge about the bacteriological profile and antimicrobial susceptibility patterns in the local unit helps the clinician in rationalizing the empirical treatment protocols and optimizing the duration of therapy. **Aims and Objectives of the study:** This study was aimed to study the profile of organisms causing bacteremia and understand antibiotic sensitivity patterns at our hospital. **Materials and Methods:** 995 blood samples collected over a period of 14 months from clinically suspected cases of bacteremia were studied. The isolates which flagged positive in Bact Alert system were subcultured and were thereby identified and antimicrobial susceptibility performed using VITEK-2 systems. **Results:** Positive blood cultures were obtained in 25.7% (256 out of 995) of cases of which Gram-positive bacteria accounted for 35.9% of cases with Staphylococcus aureus predominance; gram negative bacteria accounted for 57.03 % with Klebsiella pneumonia predominance; and 7.03 % were Candida isolates. Antibiotic sensitivity test report showed all gram positive bacteria were sensitive to tigecycline and 75-100% were sensitive to daptomycin, 88-93% to linezolid and 82-97 % to teicoplanin. The prevalence of MRSA and vancomycin resistance was 25 % and 7 %, respectively. The most sensitive drugs for Gram-negative were colistin (82-93%), tigecycline (56-100%), aminoglycosides (58-96%), and carbapenems (52-91%). **Conclusions:** The present study emphasizes the need for continuous scrutiny and surveillance for the most common pathogens isolated in patients with blood stream infections along with antibiotic sensitivity patterns for formulating rationalized antibiotic treatment protocols and infection control strategies for prevention of septicaemia in hospitalized patients.

KEYWORDS : Antimicrobial Susceptibility, Microbiological Profile, Blood Culture

INTRODUCTION

Blood stream infections range from self-limiting infections to life threatening sepsis that requires rapid and aggressive antimicrobial treatment. [1] Bloodstream infections (BSI) are a major cause of morbidity and mortality worldwide. Approximately 200,000 cases of bacteraemia occur annually with mortality rates ranging from 20-50% worldwide. [2] Organism isolated from blood culture vary according to geographical distribution and development of multidrug resistant organism is of great concern as they prolong hospital stay. Increased cost of treatment can be a cause of high mortality.

[3] These infections require rapid identification and also antibiotic susceptibility testing of the causative agent in order to facilitate specific antimicrobial therapy. [4] So, regular surveillance of blood culture isolates and their antibiogram in the hospital seems essential for determining empiric antibiotic therapy and also for alerting clinicians to emerging pathogens that may pose a threat to the community. [5,6,7] Keeping in view that knowledge about the bacteriological profile and antimicrobial susceptibility patterns in the local unit guides the clinician in rationalizing the empirical treatment protocols and optimizing the duration of therapy, this study was aimed to study the profile of organisms causing bacteremia in indoor patients and thereby to understand the antibiotic susceptibility patterns at our hospital.

MATERIALS AND METHODS

All blood samples received for culture and sensitivity at Department of Laboratory Medicine of Healthcity Hospital during January 2020 to February 2021 (14 months duration) were included and processed in the study. Blood samples were collected after thorough cleaning of the venous site with 70% alcohol and subsequently followed by povidone iodine. Sample was collected from 2 different sites (5- 8 mL per site) in every patient and inoculated immediately into BacT/ALERT FA and FN blood culture bottles. In pediatric cases 1-2 mL of blood was inoculated in BacT/ALERT PF bottles. After collection these bottles were immediately incubated in BacT/ALERT 3D system for detection of growth. The negative results were followed up to 7 days and final report was issued. While, in case of a positive growth, the BacT/ALERT automatically gives an alert. The positive bottles were then subcultured on Sheep blood agar, Mac Conkey agar and Chocolate agar.

Inoculated plates were incubated aerobically at 37□ C and examined after 18-24 hours of incubation. Isolates were identified by Vitek-2 Compact (Biomerieux) using gram negative, gram positive, yeast identification and AST cards for antimicrobial susceptibility testing. The results were interpreted according to CLSI 2020 criteria. [8]

RESULTS

During the study period, 995 blood cultures were analyzed of which 256 samples were positive. 238 were bacterial isolates and 18 were fungal (Candida) isolates. The positivity rate in our study was 25.7% (256 out of 995). Among positive blood culture isolates gram-negative and gram-positive bacteria constituted 57.03% (142/256) and 35.9% (92/256) respectively and 7.03 % (18/256) were Candida isolates. The commonest gram negative bacteria was Klebsiella pneumonia, followed by Acinetobacter species and E coli. CONS and Candida albicans respectively were the most common gram positive bacteria and yeast isolated in our study.

Table: 1- Species distribution of Gram negative bacilli, gram positive cocci and Candida:

Sl No.	Type	Organism	Total isolates	Percentage
1	GRAM NEGATIVE BACILLI	Klebsiella pneumoniae	53	20.7%
		Acinetobacter species	30	11.7%
		E coli	30	11.7%
		Enterobacter cloacae	10	3.9%
		Pseudomonas aeruginosa	9	3.5%
		Serratia marcescens	5	1.9%
		S typhi	3	1.1%
		Proteus mirabilis	2	0.7%
		Burkholderia cepecia	2	0.7%
		Cryseobacterium indolens	2	0.7%
2	GRAM POSITIVE COCCI	E faecalis	6	2.3%
		E faecium	14	5.4%
		E gallinarium	4	1.5%
		S aureus	28	10.9%
		CONS	30	11.7%
		Streptococcus species	11	4.2%
3	CANDIDA	Candida albicans	14	5.4%
		Candida tropicalis	2	0.7%
		Candida parapsilosis	1	0.3%
		Candida guilliermondi	1	0.3%

Among the gram negative isolates Klebsiella and Acinetobacter showed higher resistance as compared to Escherichia. The most sensitive drugs for Gram-negative were, tigecycline (56-100%), aminoglycosides (58-96%), and carbapenems (49-91%).

82-93% of colistin were intermediate sensitive in VITEK-2 with MIC (mean inhibitory concentration) $\leq$ 0.5. The gram-negative isolates were least sensitive to Ampicillin (15- 62%), Amoxicillin-clavulanic acid (50-82%), Ceftriaxone (50-74%), Ciprofloxacin (48-66%) and Piperacillin Tazobactam (38-85%).

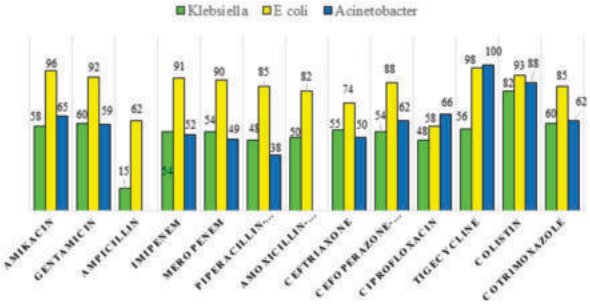

Figure 1: Comparison of sensitivity percentages of Klebsiella, E coli and Acinetobacter isolates:

Antibiotic sensitivity test report showed all gram positive bacteria were sensitive to tigecycline and 75-100% were sensitive to daptomycin, 78-100% to rifampicin, 88-93% to linezolid, 82-97 % to teicoplanin and 75-93 % of them were sensitive to vancomycin. The prevalence of MRSA and vancomycin resistance was 25 % and 7 %, respectively. All the Staphylococcus aureus showed resistance to Penicillin (100%).The least susceptible antibiotics amongst Staphylococcus aureus were Erythromycin (42%) Quinolones (52%), Trimethoprim-sulphamethoxazole (66%) and Oxacillin (75%), and Enterococcus were least susceptible to Penicillin (60%), Quinolones (49%), Erythromycin (22%), High level Gentamicin (75%) and Tetracycline (59%).

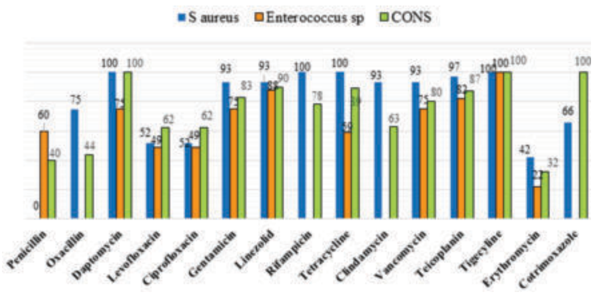

Figure 2 : Comparison of sensitivity percentage of Gram positive isolates:

Out of the Candida isolates majority was Candida albicans (77.7%) and 22.3% was non- albicans Candida. Fluconazole (88%) and Amphotericin B (88%) was the most sensitive drug followed by Flucytosine (83%). Voriconazole (70%) was the least sensitive amongst the Candida isolates.

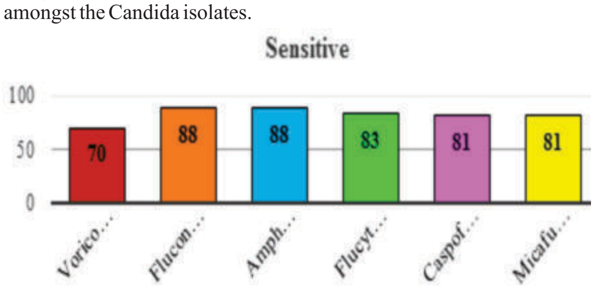

Figure 3: Sensitivity of Candida isolates:

DISCUSSION:

The present study provides information on the microbiological profile and antibiotic susceptibility pattern of isolates causing bloodstream infections which plays a crucial role in effective management of septicemia cases. In our study a high prevalence rate of 25.7% (256 out of 995) was seen. Gram negative organism was predominant and isolates were resistant to most commonly used antibiotics. The prevalence rate of 25.7% in the present study is approximately similar to Nazir A et al., (25.3%) [9], Nidhi pal et al., (22.3%) [10] and Arora et al., (20.2%) [11]. However some other studies showed lower positivity rates of 10.3% (Laxmi Kant et al.)

[12] and 10.29% (Jyoti P et al.) [13]. Such differences in prevalence of BSI across various studies could be due to the difference in blood culture system, sample size, the study design, geographical location, nature of patient population, epidemiological difference and differences in the infection control policies in different organizations. [13-16]

In our study, Gram negative and Gram positive bacteria constituted 57.03% and 35.9% respectively. Similar to the present study, in most of the studies, gram negative bacilli have taken over the gram-positive organisms, especially in hospital settings. This finding was in accordance with other studies. [10, 13, 18-22]

In this study, the predominant isolates were Klebsiella pneumoniae 53 (20.7%) followed by Acinetobacter spp. 30 (11.7%), E.coli 30 (11.7%), CONS (11.7%) and S aureus 28 (10.9%) which was in accordance with other studies [10, 17, 23]. E. coli and Klebsiella spp. were the predominant gram negative isolates in our study which is in accordance with other studies of Jyoti P et al., Nidhi P et al., and Mehta et al. [10, 13, 18]. The high occurrence of non-lactose fermenters especially Acinetobacter spp., which has emerged as important nosocomial pathogens, is of concern and is associated with a high degree of resistance to antibiotics and high morbidity and mortality. Multidrug resistant bacterial strains are emerging as a major problem in the management of sepsis leaving clinicians with fewer treatment options. [13, 17, 19, and 23].

Among gram positive pathogen CoNS (11.7 %) and S.aureus (10.9%) were most common isolates similar to Nidhi P et al., [10] Vanitha et al., [23] and Rao et al., [25]. Similarly, CoNS were considered as contaminants in the past but nowadays they have become one of the leading cause of blood stream infections due to increasing use of medical devices such as prosthetic heart valves, vascular grafts, indwelling catheters etc. [23, 26] CoNS was also one of the important organism related to blood stream infection (10.8%) in another study by Lakshmi K et al. [12]. Such low isolation of Gram-positive pathogens over gram negative suggest an increasing emergence of Gram-negative isolates with Multi-drug resistance and ESBL production, so a strict antibiotic policy should be implemented with emphasis on local susceptibility findings. [13, 27]. In our study the incidence of Candidemia was 7.03% and was mainly due to C.albicans. Another study by Mehta et al [18] showed much higher (20.29%) prevalence of Candida with C. albicans predominance. However in the study by Nidhi P et al., [10] 11% of positive blood culture gave growth of non-albicans candida spp. Septicaemia was caused by only one organism in our study similar to other studies.[10, 28]

Table 2: Comparison of sensitivity percentage patterns of gram negative isolates:

	Our study			Jyoti P. Sonawane et l.[13]			Sarangi KK et al. [29]		Nazir A et al. [9]		
	Kleb (I)	Ecoli (I)	Acineto (I)	Kleb	E coli	Acineto	E coli	Acineto	Eleb	Ecoli	Acineto
Amikacin	58	96	65	48	59	42	87	100	46	70	30
Gentamicin	60	92	59	37	53	32	50	50	22	42	9
Ampicillin	15	62	-	0	3	0	25	0	-	-	-
Imipenem/ Meropen	54	91	52	95	100	88	100	0	53-55	62-64	15-17
Piperacillin- tazo	48	85	38	39	43	54	75	0	24	57	11
Ceftriaxone	55	74	50	7	3	22	62	0	13	27	5
Cefo-sulbacta	54	88	62	-	-	-	-	-	19	59	10
Ciprofloxacin	48	58	66	35	12	40	75	50	8	12	5
Tigecycline	56	98	100	-	-	-	100	100	90	100	85
Colistin (intermidiate)	82	93	88	100	100	92	-	-	100	100	100
Cotrimoxazole	60	85	62	70	12	38	50	50	-	-	-

In this study E coli had a higher susceptibility as compared to Klebsiella and Acinetobacter which is similar to the findings by Jyoti P et al., [13], Sarangi KK et al., [29] and Nazir A et al. [9] Similar to our study all the above three studies shows higher susceptibility only to colistin and tigecycline. This showed none of the commonly used antibiotics were sufficiently active against gram negative bacterial isolates from blood stream infections. This increasing rate of drug resistance to commonly used antibiotics alarms clinicians and microbiologists for need of other effective antibiotics against infections caused by these drug resistant organisms.[12] Another important issue is the lack of regulation regarding prescription of antibiotics, which are widely used, even for minor illnesses such as rhinitis. Whereas no study has assessed this phenomenon, it is worth noting that a recent study documented a high level of irrational prescription of antibiotics by healthcare profession associated with a steady increase of antimicrobial drug resistance. [13] The increase in the prevalence of Multidrug-resistant bacteria emphasize the immediate need for rational use of antibiotics, formulation of antibiotic policy, and implementation of infection control practices for the effective management and prevention of drug resistance. [13]

Table 3: Comparison of sensitivity percentage patterns of gram positive isolates:

	Our stuy		Jyoti P. Sonawane et al. [13]			Sarangi KK et al. [29]		Nazir A et al. [9]		
	S.aureus	Entero	CONS	S.aureus	Entero	S.aureus	ONS	S.aureus	Entero	CONS
Penicillin	0	60	40	0	0	16	0-16	15	1	5
Oxacillin	75	-	44	90	-	66	41-66	80	0	69
Levo/ Ciprofloxacin	52	49	62	50	13	32-66	33	33	31	28
Gentamicin	93	75	83	50	13	17-41	16	57	0	44
Linezolid	93	88	90	100	100	100	100	100	100	100
Clindamycin	93	-	63	-	-	50	50-66	58	0	32
Vancomycin	93	75	80	100	100	100	100	100	85	100
Teicoplanin	97	82	87	-	-	-	-	100	85	100
Tigecycline	100	100	100	-	-	100	100			
Erythromycin	42	22	32	25	0	66	57-66	18	0	29
Cotrimoxazole	66	-	100	30	-	83	66-100	46	54	44

Amongst the gram positive isolates Tigecycline, Vancomycin, Teicoplanin and Linezolid had higher susceptibility and Penicillin, Oxacillin, Quinolones and Erythromycin had lower susceptibility similar to Jyoti P et al., [13] Sarangi KK et al., [29] and Nazir A et al. [9] However our study showed higher susceptibility (75-93%) to Gentamicin unlike the above studies. The reason could be due to lesser use of gentamicin as drug of choice for empirical therapy at our institute.

Table 4: Comparison of sensitivity percentage patterns of Candida isolates:

	Our study	Chakraborty et al. [30]	G Marshall Lyon et al. [31]	Sahar Mohammed Khairat et.[32]	Supram Hosuru Subramanya [33]
Micafungin	81	-	99.8	-	-
Caspofungin	81	-	99.8	99	85-100
Flucytosine	83	92	-	-	-
Amphotericin B	88	67-92	-	97	95-100
Fluconazole	88	94	97.4	64	33-56
Voriconazole	70	-	-	-	77-92

Anti-fungal testing showed higher susceptibility to Fluconazole and Amphotericin B which is similar to a study by Chakraborty et al. [30]. However this is in contrast to other studies by G Marshall Lyon et al. [31] and Sahar Mohammed Khairat et al.[32] where echinocandins showed higher susceptibility. In another study by Supram Hosuru Subramanya [33] et al., Amphotericin B had higher susceptibility but Fluconazole had lower sensitivity as compared to our study. However unlike the other studies our study showed sensitivity below 90% for all the tested anti- fungal drugs. This could be due to the impact of COVID -19 in recent times when the study was conducted and the re-emergence of Candida as opportunistic infection amongst COVID patients and thereby increasing anti-fungal resistance. The other studies showing higher antifungal susceptibility was undertaken in the pre- COVID era.

CONCLUSIONS:

The present study emphasizes the need for continuous scrutiny and surveillance for the most common pathogens isolated in patients with blood stream infections along with antibiotic sensitivity patterns for formulating rationalized antibiotic treatment protocols and infection control strategies for prevention of septicemia in hospitalized patients. Clinicians should also exercise caution in their use of restricted antibiotics in order to preserve and prolong their therapeutic usefulness. Increasing incidence of drug resistant organisms like MRSA, VRE, and ESBL producers raises serious concerns about antibiotic resistance and mandates strict antibiotic policy on a large scale.

Conflict of interest: There is no conflict of interest.

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