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Microbiology

Microbiological Profile and Antimicrobial Sensitivity Pattern of Suspected Adult Septicaemic Patients at VIMSAR, Burla

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Abstract **Original Research Article**

Introduction: Microorganisms cause septicaemia, a systemic disease due to their multiplication and toxins in the blood. Microorganisms present in the circulating blood - whether continuously, intermittently or transiently are a threat to every organ of the body. These blood stream infections constitutes a significant public health problems and a major cause of morbidity and mortality in the hospitalized patients. Hence it requires rapid antimicrobial treatment. Infection by multidrug resistant (MDR) organisms are more likely to increase the risk of death in these patients. ***Aims & Objectives:*** To determine the microbiological profile in suspected adult septecamic patients admitted in ICU and different wards at VIMSAR, Burla and their antimicrobial sensitivity pattern. ***Materials and Methods:*** Study was carried out at VIMSAR, Burla with a total number of 246 admitted adult patients having suspected septicaemia. The blood culture samples were processed in Microbiology Laboratory using standard procedure by conventional method. The pathogenic microorganism were identified and antimicrobial sensitivity was done as per CLSI guidelines. (Clinical laboratory and standard institute). ***Results:*** Out of 246 suspected adult septicaemic patients, 32 (13.01%) developed septicaemia with the positive blood culture. Out of the 32 positive culture, 28 (87.5%) showed bacterial growth, in which 17 (53.13%) were gram positive cocci, 11 (34.37%) were gram negative bacilli and 4 (12.50%) isolates were gram positive budding yeast cells. Most common isolates were *Staphylococcus aureus* (31.25%), Enterococcus spp. (21.87%), Klebsiella spp. (15.62%) followed by *Pseudomonas aeruginosa* (9.37%), Candida spp. isolated were 12.50%. Antibiotic sensitivity pattern of gram positive bacteria (*Staphylococcus aureus* and Enterococcus spp.) showed high sensitivity to Vancomycin and Linezolid, 100% each. Gram negative bacteria, Enterobacteriaceae family showed a higher rate of resistance as compared to Gram positive bacteria. Imipenem resistance was seen in 12.5% Gram negative Bacteria which is an alarming sign. ***Conclusion:*** The present study provides much needed information on the prevalence of bacterial pathogens in blood stream infections (BSI) and also demonstrates the presence of fungemia due to Candida spp. The timely detection of bacteremia and fungemia followed by expeditious identification of pathogen and determination of susceptibility to antimicrobial agents can have great diagnostic and prognostic importance and thus preventing morbidity and mortality.

Keywords: BSI, MDR, CLSI, Septicaemia, Blood Culture.

INTRODUCTION

Blood stream infections (BSI) are defined as invasion of the blood stream by micro-organisms leading to most serious situations in infectious disease. Micro-organisms present in the circulating blood-whether continuously, intermittently or transiently are a threat to every organ of the body [1].

If the patient is not aware of the illness- the condition is called “silent” or “sub-clinical”. In contrast septicemia (sepsis) is a clinical syndrome characterized by fever, chills, malaise, tachycardia, hyperventilation and toxicity or prostration [2].

Septicemia results when circulating bacteria multiply at a rate that exceeds their removal by phagocytes. The symptoms are produced by microbial toxins and/or cytokines produced by inflammatory cells [2].

Blood stream infections cause significant morbidity and mortality worldwide and are among the most common health care associated infections [3]. It requires rapid and aggressive antimicrobial therapy [4].

Bacteremia and fungemia are among the most common cause for high mortality rate (20%-50%) [5]. According to CDC, incidence of BSI has doubled in past decade which is the leading cause of death in US. It is the most expensive condition to treat in hospital (20% of ICU admissions) and leading cause of non-cardiac ICU mortality [1].

MATERIALS & METHODS

The study was conducted after due approval from Institutional Review Board (VIREC) of VSSIMSAR, Burla, Dist- Sambalpur, Odisha. The study was carried out in the Department of Microbiology,VSSIMSAR, Burla from Nov. 2017 to Oct.2019. A total of 246 clinically suspected adult septicaemic patients admitted in ICU and different Wards (Medicine, Surgery, Orthopaedic, ENT and O&G) at VSSIMSAR, Burla were included in this study.

Study Tools & Techniques

1. Blood samples are to be collected from suspected adult septicaemic patients under aseptic precautions after applying tourniquet above cubital fossa; the venepuncture site is to be disinfected with 70% alcohol & 2% tincture iodine. Using a sterile syringe, 10 ml of blood was collected and injected aseptically into 50 ml of BHI Broth (Hi Media, Mumbai, India) in 100ml bottles, from different wards and critical care units [6].
2. All blood cultures are to be processed in microbiology laboratory using standard procedure by conventional method [7].

Conventional method

- After 24 hours of aerobic incubation blood culture samples were sub-cultured onto blood agar and MacConkey agar to look for growth. Isolated colonies were used for gram's staining and biochemical tests for differentiation of organism. Antibiotic sensitivity was done by Kirby Bauer's disc diffusion method according to CLSI guidelines [6].
- If there is no growth the blood culture bottles were further incubated. Subcultures from blood culture bottles were done on 4^{th} and 6^{th} day. Samples were reported as no growth after 7 days of aerobic incubation [6].
- 2 blood samples were taken from each patient; one hour apart for blood culture and sensitivity [1].

Quality control (QC): Reference strains *E.coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as a control reference strains for identifications and drug susceptibility testing [8].

The following antibiotic discs were subjected to susceptibility test for the respective organisms and were interpreted as per CLSI guidelines [9]. Discs were obtained from Himedia laboratories Pvt. Ltd., Mumbai

Gram positive organisms- Amikacin (30mcg), Gentamicin (10mcg), Ciprofloxacin (5mcg), Ampicillin/Sulbactam (10mcg/10mcg), Amoxiclav (30mcg), Erythromycin (15mcg), Vancomycin (30mcg), Cotrimoxazole (25mcg), Linezolid (30mcg), Cefoxitin (30mcg), Cefotaxime (30mcg), Lincomycin, Chloramphenicol, Lincomycin (15mcg), Chloramphenicol (10mcg).

Gram Negative organisms- Gentamicin (10mcg), Amikacin(30mcg), Ciprofloxacin(5mcg), Ampicillin/Sulbactam (10mcg/10mcg), Amoxiclav (30mcg), Piperacillin/ Tazobactam (100/10mcg), Aztreonam (30mcg), Ceftriaxone (30mcg), Cefoperazone/ Sulbactum (75/10 mcg), Imipenem(10mcg), Chloramphenicol (10 mcg), Ceftriaxone (10mcg).

Pseudomonas species - Amikacin (30mcg), Gentamicin (10mcg), Ciprofloxacin (5mcg), Ceftazidime (30mcg), Piperacillin/Tazobactam (100/10mcg), Imipenem (10mcg), Meropenem (10mcg), Cefepime (30mcg), Aztreonam (30mg)

AFST (Antifungal Susceptibility Test) - Amphotericin-B (20mcg), Fluconazole (10mcg), Voriconazole (1mcg).

RESULTS AND DISCUSSION

Blood culture samples received from 246 suspected adult septicaemic patients admitted in different wards at VIMSAR, Burla. They were processed in Microbiology Department and following observations were made.

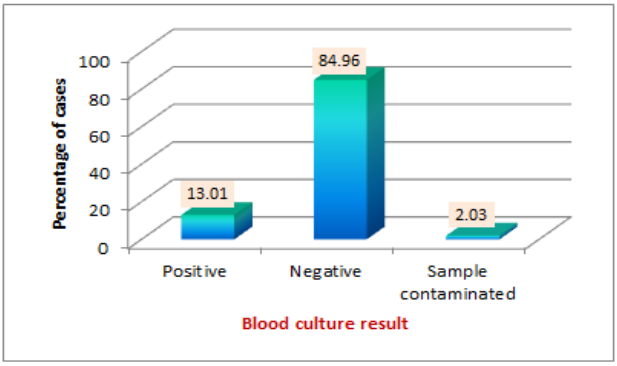

Fig-1: Chart showing total blood culture positivity in adults with septicemia

Out of 246 cases, 32 (13.01%) gave positive growth result, in 209 cases (84.96%) blood cultures were negative (no growth) and in 5 cases (2.03%) blood culture samples were contaminated.

This study was consistent with the study of Pandey S *et al.* reporting 12.6% blood culture positivity and Dash M *et al*. showing 17.2% positive growth [10, 11].

This study was in contrast to similar studies done in Iran by Mehdinejad A *et al*. which showed lower positivity rate of 5.6% [18]. Studies which were conducted in Delhi by Meheta M *et al*., in Pakistan by Choudhury I *et al*. and Latif S *et al.* showed markedly higher rates of more than 20 % positivity rate [13-15]. In eastern India studies done by Mohanty *et al*. showed markedly very higher positivity rate of 41.4% [16].

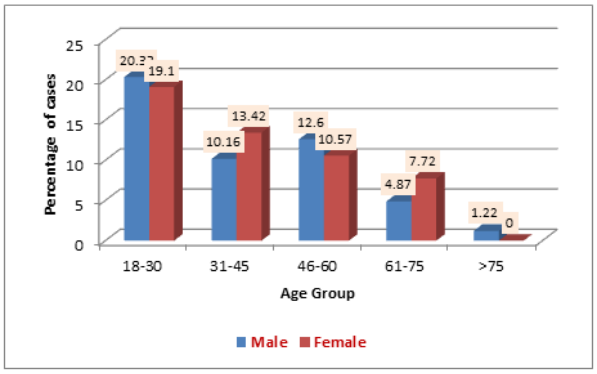

Fig-2: Chart showing age and sex distribution of blood culture received

Blood culture samples received from admitted male patients was maximum in age group of 18-30yrs, 97 cases (39.43%) and least in age >75 yrs, 3 cases (1.22%).

This is in contrast with studies of Qazi M.S *et al*. where they received maximum samples from age group 46-60 years constituting 26.7% [17].

In current study, blood culture samples received from 125(50.81%) admitted female patients and from 121 admitted male patients (49.19%) respectively.

Current study is in contrast with studies by Qazi *et al*. where blood samples received from male and female patients were 57.3% and 42.7% respectively [17].

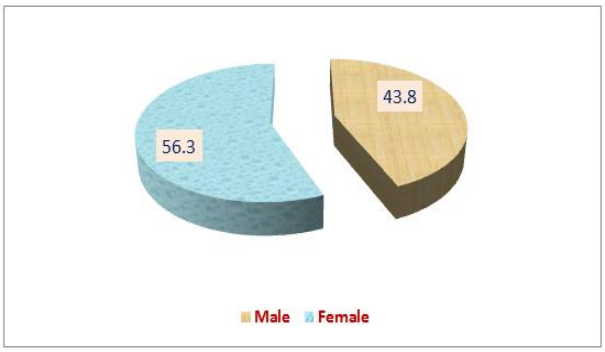

Fig-3: Chart showing sex distribution of positive growth

Out of 32 positive growth isolates, female cases were 18 (56.3%) and male cases 14 (43.8%)

This study was consistent with studies done by Rajeevan Sumita *et al*. where 52.67% of positive growth isolates were in female patients and 47.32% of positive growth in male patients respectively [18].

Current study was in contrast with studies conducted, where males were predominantly affected as observed by Qazi MS *et al*. (51%), Kante M *et al*. (61.7%) followed by other studies done by Mohanty *et al*., Divyashanthi CM *et al*. and Gohel K *et al*.[17,19,16,20,21]/

Table-1: Table showing distribution of ward (n=32)

Ward	No. of cases	Percentage
Cardiology	3	9.4
CICU	2	6.3
FMW	6	18.8
ICU	4	12.5
MMW	10	31.3
MSW	1	3.1
O&G	5	15.6
UROLOGY	1	3.1
Total	32	100

Out of 32 positive growths, maximum cases10 (31.3%) were from male medicine ward (MMW).

This study is consistent with studies conducted by Garg A *et al.* where maximum positive growth isolates was from medicine ward [22].

Table-2: showing positive growth isolates among study population. (n =32)

Type of Growth		No. of cases	Percentage (%)
Bacterial growth	Gram positive cocci	17	53.13
	Gram Negative bacilli	11	34.37
Yeast (Candida spp.)		4	12.50

Out of 32 positive growth isolates, gram positive cocci accounted for 17 cases (53.13%), gram negative bacilli for 11 cases (34.37%) and budding yeast cell for 4 cases (12.50%).

The higher isolation of gram positive organisms were in accordance with the study of Gohel K *et al.* (GPC 58.3%, GNB 40.2%), Rajeevan Sumita *et al*. (GPC 53.57% and GNB 46.4%) [18, 21]. China and Gupta, Kamga *et al*. and Anbumani *et al*. Also reported similar incidences [23, 24].

In current study 12.5% were budding yeast cells *(Candida spp.)*, which was in contrast with studies done by Gohel K *et al*., where fungal isolates (*Candida spp.)* were 1.51% & Dash M *et al.* where fungal isolates were 7.3% [11,25]. However our study was consistent with studies of Pal N *et al*. where fungal isolates (*Candida spp.)* were 11.1% [6].

This indicates that blood stream infections by Gram positive organisms constitute a significant threat in our locale and the spectrum of organisms is subject to geographical alteration [25].

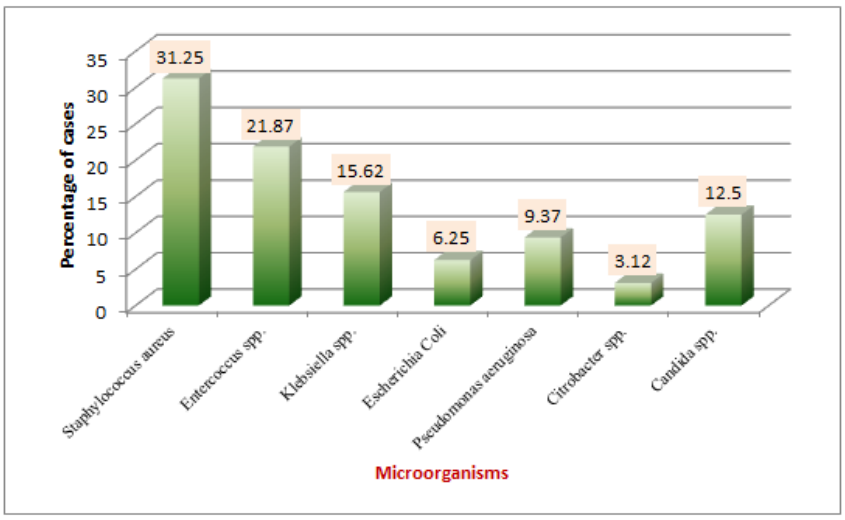

Fig-4: Chart showing microorganisms identified

The current study was consistent with studies done by Rajeevan Sumita *et al*. detecting *Staphylococcus aureus* (67%). Sahoo D *et al*. detected 58.3% *Staphylococcus aureus* and Enterobecteriaceae (40.2%)[18,26].

Table-3: showing antimicrobial sensitivity pattern of gram positive organisms (n=17)

Antibiotics	No of organisms sensitive	Percentage of organism's sensitive (%)
Vancomycin	17	100
Linezolid	17	100
Lincomycin	13	76.47
Ciprofloxacin	12	70.58
Chloramphenicol	11	67.70
Gentamicin	10	58.82
Cotrimoxazole	10	58.82
Amikacin	8	47.05
Amoxyclav	7	41.18
Cefoxitin	6	35.29
Ampicillin/Sulbactam	6	35.29
Cefotaxime	5	29.41
Erythromycin	4	23.52

High sensitivity of Gram positive organisms to Vancomycin 17(100%) and Linezolid 17(100%) followed by Lincomycin 13 (76.47%), Ciprofloxacin 12 (70.58%), Chloramphenicol 11 (67.70%) and Gentamicin 10 (58.82%).

In current study, gram positive organisms showed maximum resistance to Erythromycin 76.48% and Ampicillin/ Sulbactam 64.7% which was similar to studies done by Arora U *et al*., showing resistance 69.67% and 74.61% respectively to above drugs [27].

Table-4: Showing sensitivity pattern of pathogenic bacteria belonging to family Enterobacteriaceae (n=8)

Antibiotics	No of Organisms sensitive	Percentage of organism's sensitive (%)
Imipenem	7	87.5
Aztreonam	7	87.5
Gentamicin	6	75
Ciprofloxacin	6	75
Piperacillin Tazobactam	6	75
Cefoperazone/Sublactum	5	62.5
Ceftriaxone	5	62.5
Amikacin	4	50
Ampicillin/Sulbactam	4	50
Chloramphenicol	4	50
Amoxyclavulanic acid	3	37.5

Enterobacteriaceae were highly susceptible to Imipenem 7 (87.5%), Azteronam 7 (87.5%) followed by Gentamicin, Ciprofloxacin & Piperacillin/Tazobactam 6 (75%) each. They were least sensitive to Amoxyclavulanic acid 3 (37.5%).

Antimicrobial resistance profile of Gram negative bacteria (Enterobacteriaceae family) showed a higher rate of resistance as compared with Gram positive bacteria which was consistent with studies done by Nazir A *et al*. and other studies. In present study, Imipenem resistance was 12.5%, which is an alarming sign for the clinicians because this leaves a very limited choice of drugs in the form of Colistin and Tigecycline, which have serious side effects and toxicity. In our study high resistance, 62.5% was observed in beta-bectam antibiotics like Amoxicillin clavulanic acid. The current study was similar to studies conducted by Nazir A *et al*. and Qazi M. S *et al*. [18, 28].

Table-5: showing antimicrobial sensitivity pattern of *Pseudomonas aeruginosa* (n=3)

Antibiotics	No of organisms sensitive	Percentage of organism's sensitive (%)
Imipenem	3	100
Meropenem	3	100
Amikacin	2	66.66
Cefepime	2	66.66
Piperacillin / Tazobactam	2	66.66
Aztreonam	2	66.66
Ceftazidime	2	66.66
Gentamicin	1	33.33
Ciprofloxacin	1	33.33

Out of 3 isolates, all 3 cases showed 100% sensitivity to Imipenem and Meropenem followed by Amikacin, Cefepime, Piperacillin/Tazobactum, Aztreonam and Ceftazidime 2 cases (66.66%) each.

100% sensitivity to *Psendomonas aeruginosa* was shown by Carbapenems followed by Amikacin 66.66%. Gentamicin and Ciprofloxacin were highly resistant, 66.67% each respectively.

The current study was consistent with studies done by Mahajan *et al*., where Imipenem was 93.75%, Amikacin was 85.42% and Piperacillim/ Tazobactum were 85.42% sensitive [28].

Table-6: showing antifungal sensitivity pattern (n=4)

Antifungal agents	No of organisms sensitive	Percentage of organism's sensitive (%)
Amphotericin-B	4	100
Voriconazole	4	100
Fluconazole	2	50

Out of 4 fungal isolates, all the 4 cases showed 100% sensitivity to Amphotericin-B and Voriconazole followed by 2 cases (50%) to Fluconazole.

In our study shows that out of 4 *Candida spp*. isolated, 2 were *Candida albicans* and 2 were non-albicans *Candida*. The isolates showed 100% sensitivity to Amphotericin-B and Voriconazole and 50% sensitive to Fluconazole.

The current study was similar to studies done by Dash M *et al*, from eastern India (Odisha), where the isolates showed 100% sensitivity to Amphotericin-B and Voriconazole[9].

There is the emergence of non-albicans *Candida* and resistance to most commonly used antifungal agents have been reported in different parts of India [29, 30].

CONCLUSION

In the present study, both Gram positive and Gram negative bacteria were predominantly responsible for adult sepsis. *Staphylococcus aureus* and *Klebsiella spp.* were among the most commonly isolated Gram positive and Gram negative organisms respectively.

The timely detection of bacteremia and fungemia followed by expeditious identification of pathogen and determination of susceptibility to antimicrobial agents can have great diagnostic and prognostic importance.

Prompt initiation of appropriate antimicrobial therapy is demonstrably important for preventing morbidity and mortality.

There is an emergence of antimicrobial resistance in almost every corner of the world pointing toward active microbial surveillance in all clinical settings. Such monitoring of data regarding the prevalence of microorganisms and its resistance patterns would definitely benefit the current prescribed antimicrobial regimen, especially in resource limited countries. This also helps in improving the infection control practices by formulating policies for empirical antimicrobial therapy.

Knowledge of the distribution of blood stream infection and their drug susceptibility profiles; increases the level of understanding on Blood stream infections and the common pathogens isolated. It provides updated information on susceptibility pattern of the isolates and can be a source of information for policy makers or decision makers in this area. Health sector can design and implement preventive activities including expansion, strengthening of Blood Stream Infection (BSI) prevention and monitoring. Our study can be used as a baseline for next studies in this area.

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