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Rapid Identification of Clinically Important Aerobic Microorganisms by Automated Blood Culture System and their Antimicrobial Resistance Pattern at Tertiary Care Hospital at Western Rajasthan India

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Abstract

The rapid identification of clinically important microorganisms from positive blood culture provides important diagnostic and therapeutic information. Blood stream infections can lead to life threatening sepsis, require rapid diagnosis and rapid antimicrobial treatment. Aim of our study was isolation and identification of aerobic pathogens causing bacteremia & septicemias and their antibiotic resistance patterns in a tertiary care hospital western Rajasthan. 628 blood samples collected from clinically suspected cases of bacteremia and septicemias were studied by (BacT/ALERT) automated blood culture system. The isolates were identified by standard phenotypic methods and antimicrobial resistance patterns were determined by CLSI guidelines. Positive blood cultures were obtained in 182 (28.9%) of cases. Among gram positive Staph aureus (39.5%) whereas gram negative Klebsiella (16.48%) were predominant isolates, and 3.8% were Candida species. Among staph aureus methicilin among klebsiella cefixime and among pseudomonas ticarcilline was the most resistance drug. The prevalence of MRSA,VRSA and VRE were 86.1%, 9.7% and 50% respectively. Overall high positive rates of blood culture were observed. Rapid isolation of pathogens by automated blood culture system provide early and appropriate treatment to seriously ill patients leading to reduce mortality and duration of hospital stay.

Keywords: *Antibiotics resistance, Automated Blood culture, Bacterial isolate, ICUs, Rajasthan.*

Introduction

Diagnosis of infectious diseases is concerned with the isolation and identification of etiological agents. Blood culture is the single most important procedure to detect systemic infection due to bacteria. Bacteraemia is often life-threatening and Blood culture can provide bacteriological diagnosis as fatal condition that necessitates immediate treatment. Blood culture provides valuable information for the management of febrile, acutely ill patients with or without localizing symptoms and signs. In addition to its diagnostic significance, recovery of an infectious agent from the blood provides invaluable aid in determining antimicrobial therapy [1].

Rapid identification and antimicrobial susceptibility testing (AST) of the causative agent(s) of bloodstream infections are among the most important tasks of the clinical microbiology laboratory. This information is essential for clinicians to select the most appropriate antimicrobial therapy for patients with bloodstream infections [2].

There are several parameters that impact the success of blood culture, including blood collection time, blood sample amount (most important factor), number of blood collection sets, and skin disinfection [3,4].

In response to the rising incidence and increasing mortality from septicemia, numerous studies have attempted to qualify the laboratory's role in the diagnosis and identification of blood stream pathogens and in guiding appropriate therapeutic interventions [5].

Initiation of prompt and appropriate antimicrobial therapy in patients at risk for sepsis is a clinical goal [6].

Increasing the blood volume yielded significantly more pathogens [7].

Candida species are the fourth most common cause of nosocomial bloodstream infections worldwide [8].

Paul et al study that 3 days of incubation may be sufficient for the detection of routine bacteria and yeast when utilizing BacT/ALERT FA and FN blood culture bottles [9].

Conventional blood culture method was more time consuming (5-7 day) and less accurate so rapid isolation and identification of blood pathogen by automated blood culture system were less time consuming (24-72 hr) and more accurate.

Aims & Objective

Isolation and identification aerobic pathogens causing bacteremia & septicemias by automated 3D BacT/ALERT blood culture system and their antibiotic resistance patterns in a tertiary care hospital western Rajasthan.

Material & Methods

This Prospective Study was conducted in the Microbiology department Mathura das mathur (MDM) Hospital, Dr. S. N. Medical College, Jodhpur from January 2015 to April 2015 (4 months period).

During the study period 628 blood samples collected from all age group IPD patients suspected bacteremia and septicemias. All samples were collected in BacT/ALERT FA plus and BacT/ ALERT PF plus bottle (BIOMERIEUX, USA) irrespective of antibiotics administration. Quantity of blood sample from adult and children was 5-10 ml and 1-5 ml respectively collected with all aseptic precautions. Samples were incubated in the automated BacT/ALERT 3D system (BIOMERIEUX, USA) for 7 day. The negative results were followed up to 7 days and final report was issue. The preliminary signal of bacterial growth in BacT/ALERT bottle was detected and displayed on the 3D monitor of BacT/ALERT system mentioning the detection time. Further identification of all blood culture positive samples was accomplished by sub-culture on Blood agar, and MacConkey agar media and direct Gram's staning from positive blood culture. Inoculated Blood agar and MacConkeys agar plates were incubated aerobically at 37 C and examined after 18-24 hours. Final identification was done by colony charactertics, Gram's staining, Motility testing (Hanging drop method) and specific

biochemical testing (Catalage, Coagulage, Indole , Methyl red, Citrate , Urease , Triple sugar iron, PPA , and Oxidase testing) . Fungal isolate identified by Grams staining showing gram positive budding yeast cells and germ tube testing. Antimicrobial susceptibility testing of bacterial isolates was done by the Kirby-Bauer disc diffusion method using Muller Hinton agar media as per CLSI guidelines [10].

Result

During the study period, 628 blood cultures were analyzed of which 182 (28.9%) were positive, out of which 175 were bacterial isolates and 7 were fungal isolate, that is *Candida albicans*. Among bacterial isolate Staph aureus 72 (39.5%) was commonest followed by Klebsiella 30 (16.4%), Pseudomonas aeruginosa 21 (11.5%), Escherichia coli 15 (8.2%), Coagulase Negative Staph 11 (6.04%), Acinetobacter 7 (3.8%), Enterococcus 6 (3.2%), Micrococcus 4 (2.1%) and gram positive bacilli in 9 (4.9%). Among Gram Positive bacteria Staph Aureus is Commonest whereas in Gram Negative bacteria klebsiella was commonest organism isolated from positive blood culture. Coagulase negative staph, micrococcus and gram positive bacilli probably skin contaminants.

The distribution and percentage of various bacterial and fungal isolates are shown in Table 1 & figure 1.The Majority of the patients 410 (65.2%) were male and 218 (34.8%) were female. In our study maximum patients (18.7%) were 21-30 yrs age group followed by (15.6%) 0–10 yrs age groups. Detail of age distribution shown in table 2.

Table.1: organism isolated from positive blood culture listed below:

Organism isolated	Number (%)
Staph aureus	72 (39.5%)
Coagulase negative staph	11 (6.04%)
Enterococcus spp.	6 (3.29%)
Micrococcus spp.	4 (2.19%)
Klebsiella spp.	30 (16.48%)
Pseudomonas aeruginosa	21 (11.5%)
Escherichia coli	15 (8.2%)
Acinetobacter spp.	7 (3.8%)
Gram positive bacilli	9 (4.9%)
Candida spp.	7 (3.8%)
Total isolate	182

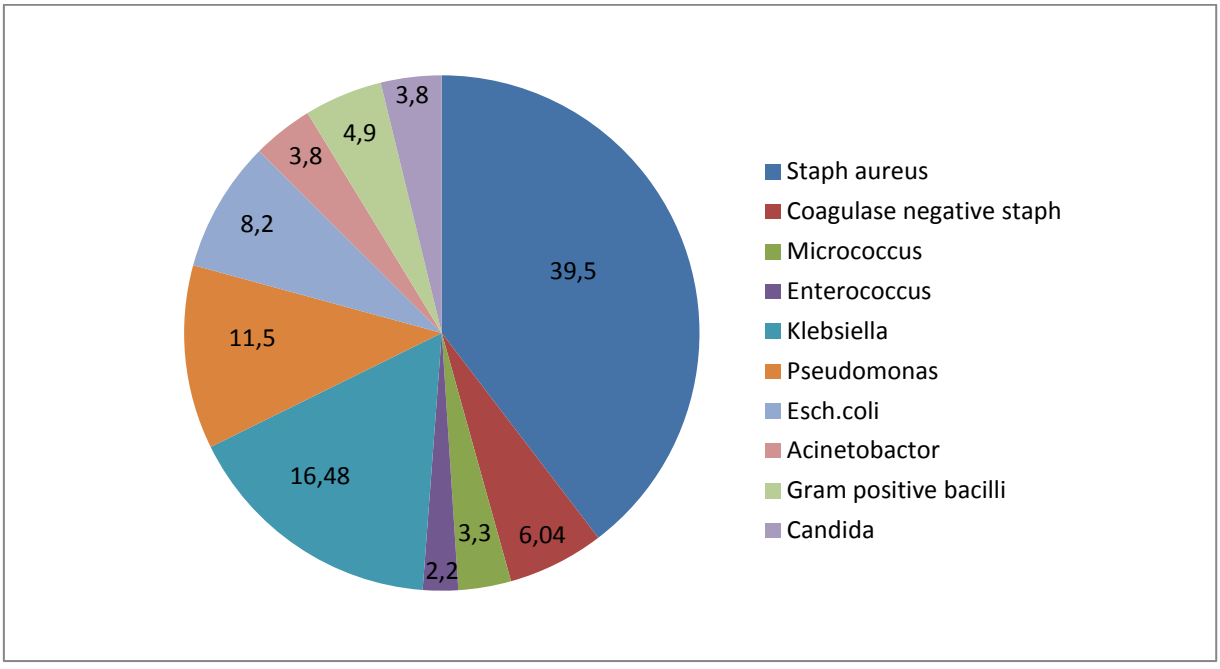

Figure 1: showing organism isolated from blood culture

Table 2: Age distribution

Age range	Patients (%)
0 – 10 years	98 (15.6%)
11 – 20 years	78 (12.4%)
21 – 30 years	118 (18.7%)
31-40 years	74 (11.7%)
41-50 years	68 (10.8%)
51-60 years	53 (8.4%)
61-70 years	81 (12.8%)
>70 years	58 (9.2%)

In our study maximum positive blood culture cases came from Trauma ICU 41/182 (22.5%) followed by MICU (18.6%), detail discription of positive blood culture cases department wise shown in **Figure.2**

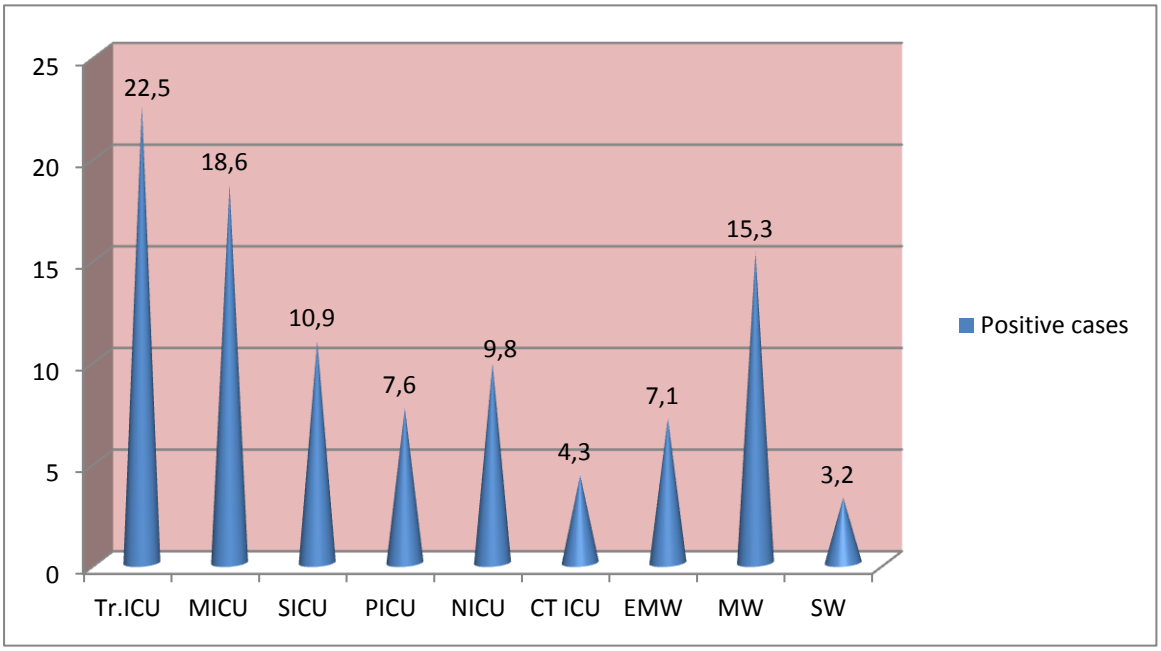

Figure 2: Showing positive blood culture cases from different ICUs and ward in Percentage (%).

(Tr-trauma, CT-cardiothorosic, EMW-emergency medical ward, MW-medicine ward, SW-surgical ward).

Gram-positive isolate showed maximum resistance to Methicillin and minimum resistance to Linezolid. Staphylococcus aureus shows methicillin resistance in 86.1% cases and Vancomycin resistance in 9.7% cases. Enterococci were 50% resistant to vancomycin. details of resistance pattern of gram positive organism shown in Table.3 & Figure.3.

Gram-negative isolates were mainly resistance to Cefixime & ticarcilline and least resistance to Imipenem. Klebsiella spp. were 66.6% resistance to cefixime and 10% resistant to imipenem. Detail description of resistance pattern of gram negative isolates shown in Table.4 & Figure 4.

Table 3: Resistance pattern of Gram positive isolates in percentage (%)

Organism	Lz	Va	Gen	Cip	Cz	Azm	Amx	Amc	Cfm	Met
Staph aureus	5.5	9.7	15.2	20.8	26.3	45.8	38.8	55.5	69.4	86.1
CONS	9.0	18.1	27.2	9.0	18.1	36.3	27.2	36.3	54.5	72.7
Enterococcus	0	50	50	16.6	33.3	50	33.3	50	66.6	83.3
Micrococcus	0	0	25	0	25	25	25	50	75	75

Lz:linazolid,**Va**:vancomycin,**Gen**:gentamycin,**Cip**:ciprofloxacin,**Cz**:cefazolin,**Azm**:azithromycin, **Amx**:amoxycilin,**Amc**:amoxicillin clavulanic acid,**Cfm**:cefixime

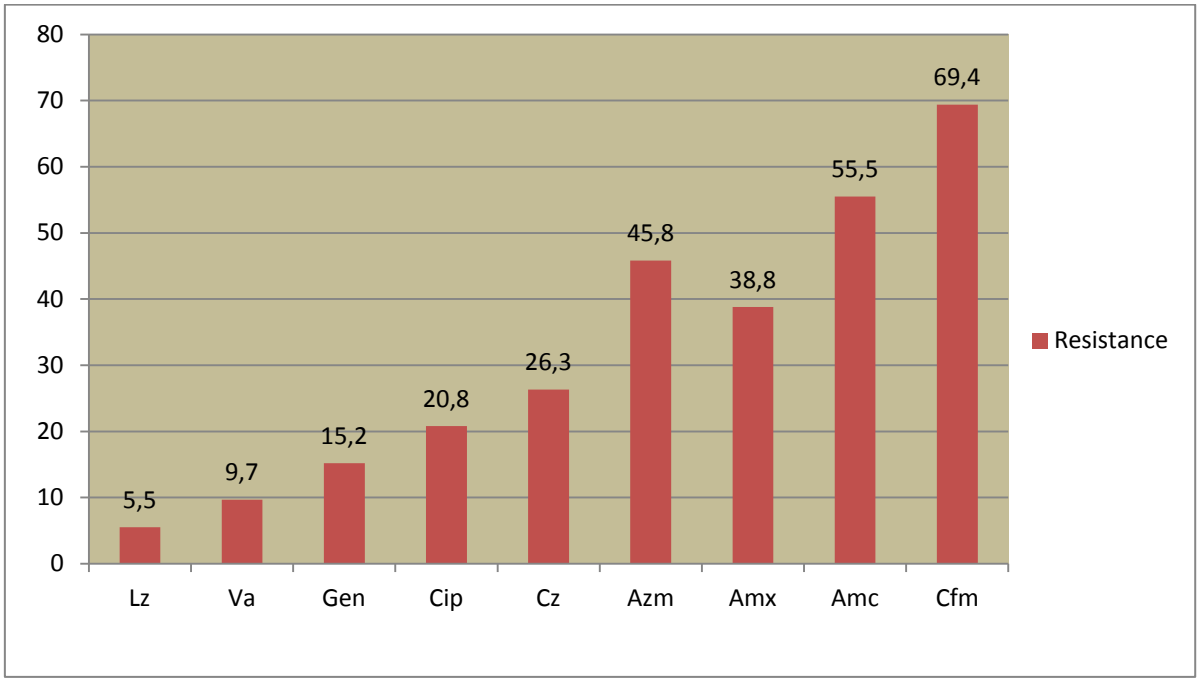

Figure 3: Showing resistance pattern of Staph aureus in percentage (%).

Table 4: Resistance pattern of gram negative isolates in percentage (%)

Organism	Ipm	Ak	At	Ctr	Cpz	cfm	Of	Pi	Ti	Amc
Klebsiella spp.	10	56.6	26.6	23.3	33.3	66.6	46.6	13.3	60	63.3
Pseudomonas spp.	19	47.6	28.5	23.8	38	61.9	42.8	47.6	71.4	52.3
Eschirichia Coli	6.6	20	13.3	26.6	20	53.3	33.3	13.3	26.5	46.7
Acinetobacter spp.	14.2	57.1	28.5	42.8	57.1	85.7	28.5	42.8	71.4	57.1

Ipm:imipenam,**Ak**:amikacin,**At**:aztreonem,**Ctr**:ceftrioxone,**Cpz**:cefoperazone,**Cfm**:cefixime,**Of**:ofloxacin **pi**: piperacilline,**Ti**:ticarcillin,**Amc**:amoxycilin-clavulanic acid.

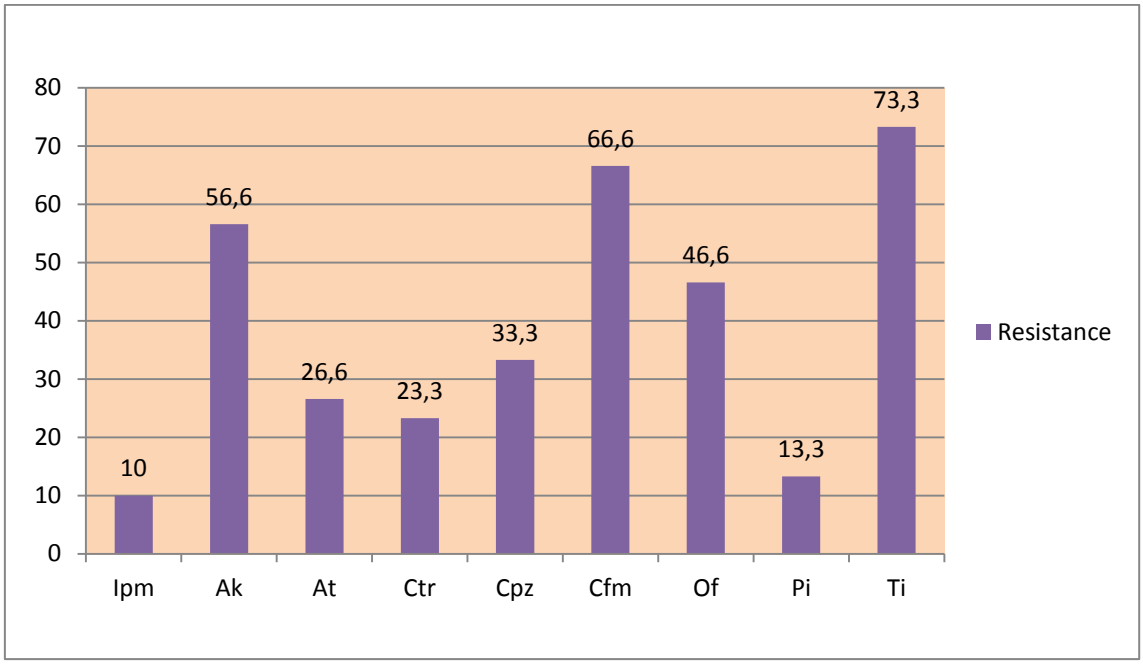

Figure 4: Showing resistance pattern of klebsiella in percentage (%).

Discussion

Sepsis is one of the leading causes of deaths, and rapid identification of blood stream infection is mandatory to perform adequate antibiotic therapy.

In the present study blood culture positivity was seen in 182 of 628 (28.9%) cases which is quite similar to Garg et al [11] and Samuel et al [12] but quite lower to other studies of Kavitha et al. [13] and Maimoona et al. [14]. Maximum patients (18.7%) were 21-30 years age groups followed by (15.6%) of 0-10 years age groups. Maximum blood culture positive cases came from trauma ICU.

In our study the incidence of Gram-positive cocci was 93/182 (51.0%) while Gram-negative bacilli was 73/182 (40.1%) which was quite similar to kalpesh et al. [15] and china et al. [16], but in most of the studies like Maimoona et al. [14] and Ayobola et al. [17] Gram-negative organisms have taken over Gram-positive organisms in hospital settings. This indicates that infections by Gram-positive organisms constitute a significant threat to bacteremia and septicemia in our hospital setup and the spectrum of organisms is subject to geographical alterations. Staphylococcus aureus was commonest (39.5%) isolate among Gram positive quite similar to kalpesh et al [16] and Anbumani et al. [18] while klebsiella was commonest (16.4%) isolate among Gram negative organism Enterococcus was isolated in 3.29% of cases similar to kalpesh et al [15].Staphylococcus aureus isolates in our study show methicillin resistance in 86.1% which is quite similar to Garg et al. [11] and quite high from the studies of Kamga et al. [19] and Karlowsky et al [20].4Vancomycin resistance in our Staphylococcus isolates was 9.7%

Vancomycin resistant Enterococcus (VRE) in our study is 50% (3/6) which is in accordance with the studies like kalpesh et al.[15] reported 40% VRE. Among the Gram-negative isolates in our study showed maximum resistance to ticarcilline and minimum resistance to imipenem. Pseudomonas spp. Show maximum resistance to cefixime.

Conclusion

Blood culture is a well-established procedure of the standard diagnostic workup for many infectious diseases. Staphylococcus aureus and organisms belonging to Enterobacteriaceae family are the leading causes of septicemia. Increasing incidence of drug resistant organisms like MRSA and VRE producers raises serious concerns about antibiotic resistance and mandates strict antibiotic policy to prevent emergence and spread of antibiotic resistance. Rapid isolation and

identification of pathogens by automated blood culture system provide early and appropriate treatment to the seriously ill patients leading to reduce mortality and reduce duration of hospitals.

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