

## A Retrospective Study of Characterization of Various Pathological Isolates in Blood Culture Sample and its Antimicrobial Sensitivity Pattern at Tertiary Care Hospital

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Conflict of interest: Nil

### Abstract

**Context:** Blood stream infections are an important cause of mortality and morbidity and are among the most common health-care associated infections. Illness associated with blood stream infection ranges from self-limiting infections to life-threatening sepsis that require rapid and aggressive antimicrobial treatment.

**Aims:** The aims and objectives of the study is to characterize the various pathological isolates in blood culture & its antimicrobial sensitivity pattern attended tertiary hospital.

**Study Design:** This is a retrospective study in which 476 blood samples were collected over a period of one year. The clinically suspected cases were reviewed for bacteraemia.

**Material and Methods:** The isolates were identified by laboratory Standard biochemical tests and the Antimicrobial Susceptibility testing was determined by Clinical and Laboratory Standards Institute (CLSI) guidelines.

**Results:** Positive cultures were obtained in 207 (43.4%) cases out of 476 samples. Gram-positive bacteria were predominant and it accounted for 75.8% cases, Methicillin Sensitive Coagulase Negative Staphylococcus aureus (10.6%) was the predominant isolate followed by Methicillin resistant Staphylococcus aureus-MRSA (9.6%), followed by Methicillin resistant coagulase negative Staphylococcus aureus-MRCONS (8.2%), followed by Methicillin sensitive Staphylococcus aureus (4.8%), and followed by enterococcus (3.8%). Among culture positive isolates, Gram-Negative Bacteria accounted for 18.8% cases; most common being Acinetobacter spp. (5.3%), followed by Klebsiella spp. (4.8%), and E. Coli (2.4%), followed by CDC No 1 (2.4%), followed by Pseudomonas (1.9%), followed by Salmonella typhi (0.9%), followed by Enterobacter (0.4%), followed by Citrobacter (0.4%). Maximum Gram-negative isolates were sensitive to ceftazidime/clavulanic acid combination. Staph. aureus & CONS were 100% sensitive to Vancomycin drug.

**Keywords:** Septicaemia, Bacteraemia, Antibiotic resistance, Blood culture.

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### Introduction

It is observed that the presence of bacterial or fungal isolates in the blood of a patient carries with it considerable morbidity and mortality. Blood infection by pathogens indicates one of the most important complications of diagnosis, blood cultures have become critically important and frequently performed tests in clinical microbiology laboratories. To provide considerable diagnosis for bloodstream infections is an important role of clinical microbiology laboratories. Because attributable mortality for bacteraemia remains as high as 12% [1], the rapid, accurate, and reliable Identification of patients with bacteraemia or fungemia is critically important in influencing

treatment and thereby, patient outcomes. Result of blood culture gives idea about antimicrobial therapy, subsequent surgical procedures, removal of vascular access lines, and other clinical interventions. Moreover, diagnosis of bacteraemia and fungemia requires more than one blood culture drawn from different sites, identification of any Isolates recovered, antimicrobial susceptibility test of isolates, and laboratory findings. Deciding which patients have bacteraemia or fungemia, and identification of isolates and their antimicrobial susceptibility patterns, are the most essential objectives for use of blood cultures as a diagnostic test. [2]

There is strong relations between sites of infection and with isolates recovered from the bloodstream [3], observations that give critically important information to providers. [4,5]

The infection occur by Multi drug resistant pathogens are more likely to prolong the hospital stay, increase the risk of opportunistic infection, death and require treatment with more expensive antibiotics. Antimicrobial therapy is initiated after the results of blood cultures are available. [6]

### Materials and Methods

A total of 476 samples from clinically suspected cases of bacteraemia & fungemia were studied at Integral Institute of Medical Sciences & Research of Integral University for a period of one year from January 2019 to December 2019. Blood samples were collected from IPD & OPD in this hospital and was processed in the Microbiology laboratory. The sample was inoculated into BD BACTEC peds plus/ F culture vials with adherence to standard precaution. After aseptic collection, these BD BACTEC (commercial bottles) bottles were immediately incubated in BACTEC 9050 (manufactured by Becton Dickinson)—a fully automated blood culture system for detection of growth in blood culture. Samples were observed 5 days for bacterial growths and 21 days for fungal growth. BACTEC 9050 alarmed for positive sample. [7]

The positive bottles were then sub-cultured on Blood agar and Mac-Conkey agar and were incubated at 37°C in the incubator for overnight. The positive bottles which were positive for fungal element were sub-cultured on Blood agar and Sabouraud Dextrose Agar (SDA) and were incubated at 37°C and 25°C for overnight. [8]

The colonies on blood agar, Mac Conkey agar, SDA were processed for identifications and smear was prepared for provisional diagnosis and accordingly biochemical testing was subjected to identification and final diagnosis and Antimicrobial/antifungal susceptibility test was done according to Clinical Laboratory Standards Institute (CLSI) guidelines (M100 S24,) by disc diffusion method (Kirby bauer test). [9]

**Antibiotics used for sensitivity testing of Gram-positive isolates:** Oxacillin (5µgm)/cefoxitin, Ampicillin (10µgm), Cephalexin (30µgm), Ceftriaxone (30µgm), Cefotaxime (30µgm),

Ceftizoxime (30µgm), Erythromycin (5µgm), Azithromycin (15µgm), Clindamycin (2µgm), Trimethoprim/Sulfameth-oxazole (25 [1.25/23.75] µgm), Gentamicin (10µgm), Amikacin (30µgm), Vancomycin (30µgm), Ciprofloxacin (5µgm), Ofloxacin (5µgm), Linezolid and Chloramphenicol (30µgm).

### Antibiotics used for sensitivity testing of Gram-Negative isolates

Ampicillin/ Sulbactam (10/10µgm), Piperacillin/ Tazobactam (100/10µgm), Tetracycline (30µgm), Levofloxacin (5µgm), Gentamicin (10µgm), Amikacin (30µgm), Ceftriaxone (30µgm), Ceftriaxone/ Sulbactam (30µgm/10µgm), Ceftazidime (30µgm), Ceftazidime/ Clavulanic acid (30µgm/10µgm), Cefoxitin (30µgm), Cefixime/ Clavulanic acid (5µgm/10µgm), Cefepime (30µgm), Aztreonam (30µgm), Imipenem (10µgm), Doripenem (10µgm), Meropenem (10µgm), and Tigecycline (15µgm).

**Antifungal used for sensitivity testing of fungal isolates:** Fluconazole, Itraconazole, Ketoconazole, Flucytosine and Amphotericin B.

### Results

The total 476 samples 196 samples were positive for bacteremia, out of which, 11 samples were positive for fungemia and the rest of 269 samples were sterile. Out of 197 samples, 109 (47.30%) sample was positive in female and 98(47.30) were male. The 157 samples were positive for Gram positive organisms & 39 samples were positive for Gram negative organisms. Gram positive isolates were Micrococcus (34.2%), which show there was lack of proper aseptic precautions while during Methicillin resistant CONS (10.6%), Methicillin sensitive Staphylococcus aureus-MSSA (4.8%), Methicillin resistant coagulase negative Staphylococcus aureus- MRCONS (8.2%), Diphtheroids (4.3%), Enterococcus Spp. (3.8%), Methicillin Resistant Staphylococcus aureus (MRSA) (9.6%). Gram negative isolates were Acinetobacter (5.3%), Klebsiella Spp. (4.8%), CDC NO 1 (0.4%), E. Coli (2.4%), Pseudomonas (1.9%), Salmonella Typhi (0.9%), Enterobacter (0.4%), Citrobacter (0.4%). Fungal isolates in blood samples were Candida albicans (5.3%). Fungal isolates were sensitive for all antibiotics. Fungal isolate, Candida albicans were confirmed by Germ tube test.

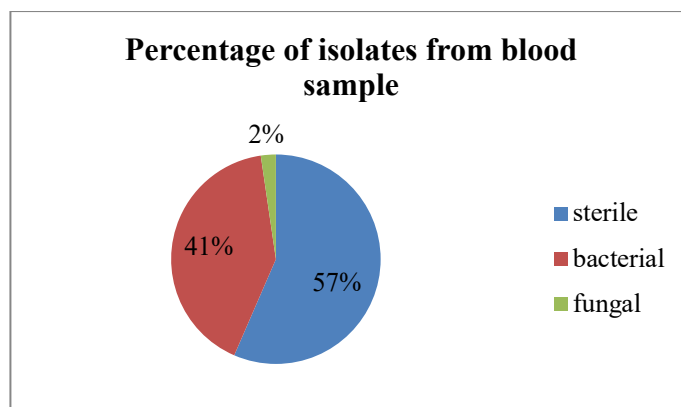


Figure1: Percentage of isolates from blood sample.

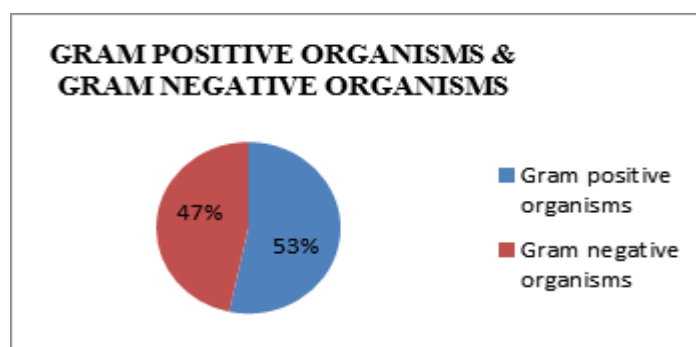


Figure 2: Gram positive organisms and Gram-Negative organisms

Table 1: Percentage of fungal isolates

Fungal isolates	Number	Percent
Candida albicans	11	2%

In this study CONS were the most predominant cause of bacteremia followed by MRSA, followed by MRCONS shown details in (Figure 3).

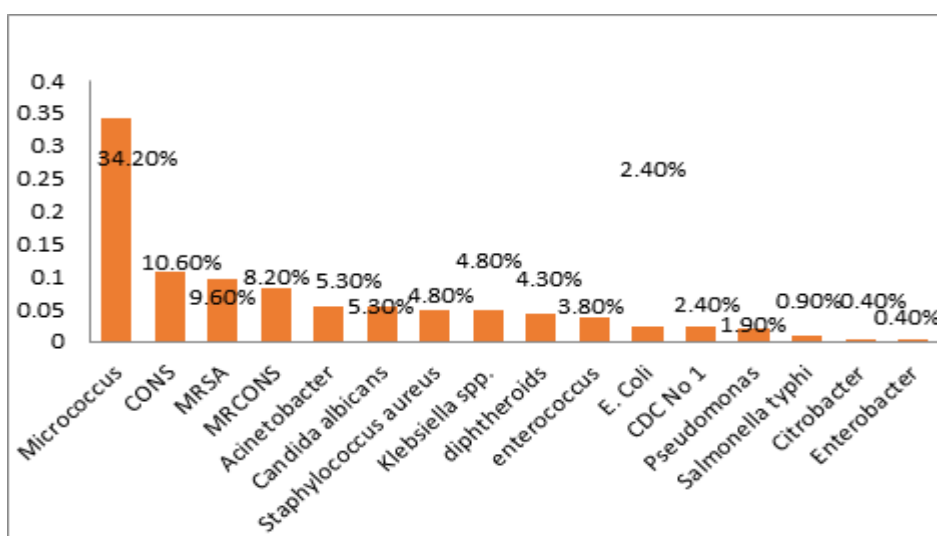


Figure 3: Percentage of various pathological isolates on the basis of biochemical reactions in blood samples

In this study population we have also studied antibiogram of various Gram positive and Gram-negative organisms in details. The Antibiotic sensitivity pattern of various organism are as

follows. 53% of organisms were Gram negative bacilli (GNB). Species identification was done by biochemical test. 26% of GNB were Klebsiella species. Klebsiella species shows highest resistance with Ceftriaxone (95%) followed by Ceftriaxone

(75%) & Ciprofloxacin (75%). Tigecycline & Amikacin shows 60% sensitive which is highest sensitive followed by Piperacillin, Tetracycline which is 50% carbapenem were only 40% sensitive in Klebsiella species positive patients. 50% of Klebsiella species were Extended Spectrum Beta Lactamase producer. Similarly, Escherichia coli was seen in 13% of the patients. Antibiotic sensitivity pattern of E. Coli in which Ceftriaxone, Ceftazidime shows 100% resistance followed by Ciprofloxacin, Levofloxacin which was 90% followed by Ceftazidime/Clavulanic acid. Amikacin shows maximum sensitivity of (90%) followed by Ampicillin, Piperacillin, Tetracycline, Gentamycin, Cefoxitin, Tigecycline. 40% of E. coli were ESBL (Extended Spectrum Beta-Lactamase) producer. The antibiogram of salmonella species shows that salmonella is 100% sensitive to Ampicillin, Tetracycline, Gentamicin, Amikacin, Doripenem, Meropenem. None of the sample were sensitive to ciprofloxacin, levofloxacin, ceftazidime. The Antibiotic sensitivity pattern of Enterobacter in show that it is sensitive to all the antibiotics on Mueller Hinton Agar. Acinetobacter species which is non fermenter shows highest resistance with ciprofloxacin (90%), followed by levofloxacin 70%. Ceftriaxone was (63%) resistant in the individual. Tigecycline shows highest sensitivity appx 85% cases followed by carbapenem groups of drugs like meropenem, imipenem which was 74% sensitive. Aminoglycosides groups of drugs like Tobramycin, Gentamycin were all also seen high sensitivity. Combination of drugs like ceftazidime-clavulanic,

ceftriaxone-Sulbactam were sensitive in 90%. MDR -multidrug resistant were seen in 25% of the cases. ESBL were present in 60% of cases. Another important non fermenter organisms Pseudomonas species Pseudomonas shows highest resistance to Ciprofloxacin (50%) followed by Amikacin (25%), Tobramycin (10%). None of the pseudomonas species were resistant to, Ceftriaxone/Sulbactam, Ceftazidime, Ceftazidime/Clavulanic acid, hence they shows highest sensitivity. The Antibiogram of Citrobacter show highest sensitivity with carbapenem group of drugs like meropenem, imipenem, doripenem (100%). Same presentation is seen with aminoglycoside groups of drugs. It is seen that 3<sup>rd</sup> generation cephalosporin were more resistant.

This table shows Antibiotic sensitivity pattern of Citrobacter in which Tetracycline, Ceftazidime, Cefoxitin, Aztreonam are resistant drugs. Ampicillin, Piperacillin, Amikacin, Doripenem, Meropenem, Tigecycline and combination of Ceftriaxone/Sulbactam are sensitive for Citrobacter.

#### Antibiogram of Gram-Positive Organisms

This table shows Antibiotic sensitivity pattern of Staphylococcus aureus in which ciprofloxacin shows highest resistance followed by Erythromycin, followed by Cefoxitin, followed by Levofloxacin, followed by COT, Teicoplanin, Linezolid shows highest sensitivity followed by Amikacin, followed by Doxycycline, followed by Gentamycin, Tobramycin.

**Table 2: Antibiotic sensitivity pattern of Staphylococcus aureus species isolated from blood sample**

Name of Antibiotics	<i>Staphylococcus aureus</i> (n-30)			
	Sensitive	Resistant	Intermediate	Percentage of sensitivity
Cefoxitin	10	20	0	33.3%
Teicoplanin	30	0	0	100%
Gentamicin	26	2	2	86.6%
Amikacin	28	0	2	93.3%
Tobramycin	26	0	4	86.6%
Erythromycin	6	22	2	20%
Clindamycin	15	9	0	50%
Tetracycline	26	2	2	86.6%
Doxycycline	27	3	0	90%
Ciprofloxacin	0	27	3	0%
Levofloxacin	24	4	0	80%
COT	21	2	1	70%
Linezolid	30	0	0	100%

From the above table it is concluded that 20 blood samples are Methicillin resistant Staphylococcus aureus (MRSA) positive out of 30 blood samples. All the Methicillin resistant Staphylococcus aureus were not methicillin sensitive to the drugs Linezolid & Teicoplanin. These 2 Antibiotics can be used as drug of choice for treatment.

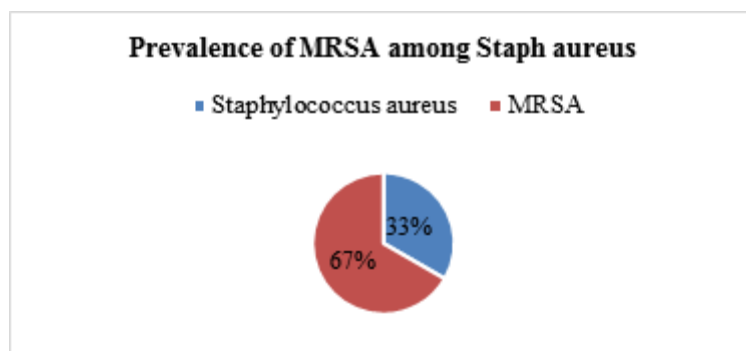


Figure no 4: Prevalence of MRSA among Staphylococcus aureus

Table3: Antibiotic sensitivity pattern of CONS

Name of Antibiotics	CONS (n-39)			
	Sensitive	Resistant	Intermediate	Percentage of Sensitivity
Cefoxitin	22	17	0	73.33%
Teicoplanin	39	0	0	100%
Gentamicin	29	6	4	74.43%
Amikacin	36	3	0	92.3%
Tobramycin	32	5	2	82.05%
Erythromycin	0	38	1	0%
Clindamycin	30	3	4	76.92%5
Tetracycline	32	3	4	82.05%
Doxycycline	37	0	2	94.87%
Ciprofloxacin	2	35	2	5.12%
Levofloxacin	0	34	2	0%
COT	0	25	0	0%
Linezolid	37	2	0	94.87%

This table shows Antibiotic sensitivity pattern of CONS in which Erythromycin is highly resistant. Out of 39 CONS 17 were Cefoxitin resistant (MRCONS) and 22 were not resistant to Cefoxitin but all these were highly sensitive to Linezolid and teicoplanin. CONS were highly sensitive to Teicoplanin, Linezolid, Doxycycline. The prevalence of MRCONS 44%.Antibiogram of Enterococcus Species shows that there were 87.5% Levofloxacin and 100% HLS resistance followed by Penicillin and Tetracycline. Enterococcus is highly sensitive to HLG 100% & Linezolid 100% Teicoplanin 100% followed by Doxycycline, Ciprofloxacin. VRE (Vancomycin resistant Enterococcus) none.

### Discussion

In this study, the total sample collected of blood about half of the patients were suffering from bacteremia & fungemia. [10] 7 isolates of Gram-positive bacteria were present in blood sample, 8 isolates of Gram-negative bacteria were present in one year of retrospective study from blood sample. CONS (Coagulase negative staphylococcus aureus) is the major causative agent of bacteremia i.e. 10.6% in blood sample which is similar to result reported by C L Obi et al. Cent Afr J Med.1996 Dec. And similar to D Asrat et al. Ethiop Med J. 2001 Apr. CONS were predominant in result

obtained by Ching Chi Lee et al. J Microbiol Immunol Infect. 2007 Oct. [11,12] Staphylococcus aureus were 5.3%, MRCONS (Methicillin resistant coagulase negative Staphylococcus aureus) were 9.6%, Enterococcus were 3.8%. Staphylococcus aureus was more sensitive to drugs (Vancomycin, Teicoplanin, & Linezolid) and more resistant drugs were (Penicillin, Cefoxitin, & Ciprofloxacin). [13,14] CONS were most sensitive to drugs (Vancomycin, Teicoplanin, tobramycin, Doxycycline, Linezolid) and more resistant to drugs were (Cefoxitin, Penicillin, Erythromycin, COT, Ciprofloxacin, Levofloxacin). [15,16] MRSA were most sensitive to (Vancomycin, Teicoplanin& Linezolid) & resistant to Cefoxitin. Methicillin resistant Staph aureus was highly sensitive to Linezolid (100%) and all methicillin resistant Staph aureus were sensitive to vancomycin. [17,18] Coagulase negative Staph aureus were all sensitive to vancomycin and linezolid which is similar to the results reported by Asutosh Ghosh et al. J Indian Med Assoc. 2009 Jan. [19,20] Enterococcus were most sensitive to HLG, Vancomycin, teicoplanin& were resistant to HLS, Levofloxacin. Enterococcus shows highest sensitivity to Vancomycin, Teicoplanin, Linezolid similar to the results reported by Khanal LK et al., [21,22] In the study VRE (Vancomycin resistant Enterococcus) was not found this is not found shows dissimilarity from

previous studies Khanal LK et al., where 6% VRE were found. [23,24]

From 30 positive samples for *Staphylococcus aureus* 20 samples are MRSA reported. It is concluded that all the MRSA and *Staphylococcus aureus* were sensitive to Vancomycin and Linezolid & all were resistant to cefoxitin. [25,26] So, for MRSA as well as *Staphylococcus aureus* drug of choice is Vancomycin & Linezolid. From the total of gram-negative isolates *Acinetobacter* spp. Were the most common cause of bacteremia after that *Klebsiella* spp., then *E. Coli* & CDC NO 1. From total isolates *Acinetobacter* were 5.3%, *Klebsiella* spp. were 4.8%, CDC NO 1 were 2.4%, *E. Coli* were 2.4%, *Pseudomonas* spp. was 1.9%, *Salmonella* spp. was 0.9% & *Citrobacter* were 0.4%. *Klebsiella* spp. were most sensitive to drugs (Tigecycline, Piperacillin, tetracycline and combination drugs of Ceftazidime/Clavulanic acid) & were most resistant to drugs (Ceftriaxone, Ceftazidime). [27,28] *Acinetobacter* spp. was most sensitive to Tigecycline, Doripenem, Ampicillin, Tetracycline & were most resistant to drugs Cefoxitin, Chloramphenicol, ciprofloxacin. Majority of the *Klebsiella* species were more resistant to Ceftazidime in comparison to Ceftriaxone which is similar to Lee S et al. *E. Coli* were most sensitive to amikacin, tobramycin, cefoxitin, tetracycline, piperacillin, ampicillin & were most resistant to drugs ciprofloxacin, levofloxacin, ceftazidime, Ceftriaxone. *Escherichia coli* was found to be more resistant from the drugs were Ceftazidime and Ceftriaxone which is similar to Lee S et al. Most of the Gram-negative isolates were resistant to Ceftazidime but susceptible to combination drugs of Ceftazidime/Clavulanic acid. More of these isolates were *Klebsiella* spp. then *E. Coli* & *Acinetobacter*. These isolates were extended spectrum beta-lactamase (ESBL) producer. [29] ESBL producer is most common in *E. Coli* & *Klebsiella* species which is similar to study reported by Diekema et al. *Salmonella* spp, were most sensitive to drugs Ampicillin, Piperacillin, Gentamycin, Amikacin, Cefoxitin, Doripenem, meropenem & more resistant to drugs were Levofloxacin, Clavulanic acid. *Pseudomonas* were most sensitive to Piperacillin, Ceftriaxone, Ceftazidime, Clavulanic acid & were most resistant to drugs were Ciprofloxacin. [30] All the 11 fungal isolates were sensitive to all the antibiotics i.e., AP, IT, FLC VRC (Fluconazole, Itraconazole, Amphotericin B, Voriconazole) which is similar to results reported by Jaiswal A et al. All fungal isolates were *Candida albicans* which were confirmed by Germ tube test. [31,32]

### Conclusion

This study was done at Integral Institute of Medical Sciences & Research, Lucknow entitled "Characterization of various pathological isolates

in blood sample and its Antimicrobial sensitivity pattern attended IIMS&R- A retrospective study" from January 2019 –December 2019 one year study. We conclude in this study that 476 patients of IIMS&R which were registered in microbiology laboratory for blood culture test. In this study 207 samples of patients were positive for bacteremia and fungemia out of 476 samples of patients. We found 476 samples were registered in IPD from microbiology laboratory. Out of 476 patients who were included in our study with 278 were females and 198 were males. 109 Females and 98 Males were detected for bacteremia and fungemia. The prevalence of bacteremia was significantly higher in females than males. It is concluded that in Gram positive isolates CONS were the most predominant isolate of bacteremia after that MRSA and then MRCONS. All these Gram-positive isolates were sensitive to Vancomycin and Linezolid and resistant to Cefoxitin. In Gram negative isolates *Acinetobacter* spp. & *Klebsiella* spp. is the major causative agent of bacteremia then *E. Coli*, CDC NO 1 & *Pseudomonas* and the least found bacterial isolate in Gram negative isolates is *Citrobacter* and *Enterobacter*. In Gram negative isolates *Acinetobacter* were most sensitive to Tigecycline drug & *Klebsiella* spp. and *E. Coli* were found to be most resistant by drugs Ceftazidime and Ceftriaxone and sensitive by combination of drugs i.e., Ceftazidime/Clavulanic acid. In ESBL (Extended spectrum beta lactamase) producer isolates combination of drugs i.e., Ceftazidime/Clavulanic acid were found susceptible and Ceftazidime and Ceftriaxone resistant. In fungal isolates all isolates are susceptible to all antibiotics. Therefore, susceptible antibiotics should be selected for right medication for right bacteremia agents.

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