

Original Research Article

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Bacteriological Profile and Antimicrobial Resistance of Blood Culture Isolates from a 350 bedded Hospital Lucknow, India

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ABSTRACT

Blood stream infections can lead to life threatening sepsis and require immediate antimicrobial treatment. Blood culture is an essential tool for the investigation of clinically suspected bacteremia or septicemia. A timely detection of organism type and antibiotic susceptibility testing is critical for the effective treatment of the patient. Illness associated with blood stream infection ranges from self-limiting infections to life-threatening sepsis that require rapid and aggressive antimicrobial treatment. The present study was to describe the profile of bacterial isolates from the blood cultures in a Polyclinic and to know their antibiotic resistance, so that the study can provide guidelines for choosing an effective antibiotic therapy in cases of septicemia. This is a retrospective study of 2452 blood samples collected from clinically suspected cases of bacteraemia reviewed over a period of one year in vevekananda polyclinic & institute of medical Sciences. 2452 blood samples collected over a year from clinically suspected cases of bacteremia were studied. The isolates were identified by standard biochemical tests and antimicrobial resistance patterns were determined by CLSI guidelines 2013. Positive cultures were obtained in 752 (30.6%) cases. Among culture positive isolates, Gram-negative bacteria accounted for 51.2% cases; most common being *Klebsiella spp* (23%) followed by *Escherichia coli* (20%) and *Acinitobacter* (04%). Of the pathogenic Gram-positive isolates, *Enterococci* (20%) was the predominant isolate followed by *Coagulase negative Staphylococci* (16%). Maximum Gram-negative isolates were sensitive to Colistin (95%) and Imipenen Meropenem (90). Daptomycin sensitivity was reported in 100% *Staph. aureus* and *CONS* and Vancomycin 90% in *Enterococcus spp*. This study provides information on antibiotic resistance of blood isolates. It may be a useful guide for physicians initiating empiric therapy and will help in formulation of antibiotic therapy strategy.

Keywords

Blood Stream Infection, Antibiotic resistant, Vancomycin Resistant Enterococci, Antimicrobial therapy, Septicemia.

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Introduction

Blood stream infections range from self-limiting infections to life threatening sepsis that requires rapid and aggressive antimicrobial treatment. A wide spectrum of organisms has been described that cause

blood stream infections and this spectrum is subject to geographical alteration. Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance of blood borne isolates is increasing and it also

varies in accordance with geographical and regional location (Kalpesh *et al.*, 2014)

Blood culture is an essential tool for investigation of clinically suspected bacteremia or septicemia, and guidance for appropriate antimicrobial management (Raghava *et al.*, 2015).

The infection caused by MDR organisms is more likely to prolong the hospital stay, increase the risk of death, and require treatment with more expensive antibiotics. In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available. Keeping in mind the high mortality and morbidity associated with septicemia, right choice of empiric therapy is of importance. Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance in hospitalized patients with septicemia is increasing, and it varies in accordance with geographical and region allocation. In almost all cases antimicrobial therapy is initiated empirically before the results of blood culture are available. Keeping in mind the high mortality and morbidity associated with septicaemia, a right choice of empiric therapy is of utmost importance.

The present study was undertaken to analyze the various organisms causing septicemia and their antibiotic resistance patterns, and also prevent the hospital infection. as it would be a useful guide for clinicians initiating the empiric anti-microbial therapy.

Materials and Methods

In this retrospective study, a total of 2,452 blood samples from the clinically suspected cases of bacteraemia were reviewed for a period of one year from January 2014 to December 2015. All the samples were collected at Vivekananda Polyclinic and

Institute of Medical sciences, 350 bedded, tertiary care, teaching Hospital providing full range of medical, surgical and super speciality facilities. Processing of samples was done at the Department of Microbiology, VPIMS, Lucknow, Uttar Pradesh, India.

Blood was collected from 2 different sites average 5 mL per site in every patient using strict aseptic precautions by nursing personnel or physician and inoculated immediately into 50 ml of 'Brain Heart Infusion' (BHI) broth with 0.025% of sodium polyanethol sulphonate as anticoagulant (Bactec Blood culture bottle).

BacT/ALERT FA plus—aerobic blood culture bottle for adult patients and in pediatric cases 1-2 mL of blood was inoculated in BacT/ALERT PF plus culture bottles. After collection these bottles were immediately incubated in BacT/ALERT 3D (manufactured by bioMerieux)—a fully automated blood culture system for detection of growth in blood culture. The negative results were followed up to 5 days and final report was issued. While, in case of a positive growth, the BacT/ALERT automatically gives an alert. After the positive indication the sample were sub cultured on 5% sheep blood agar and MacConkey agar and put for overnight incubation.

After growth the colonies on blood agar 0.5 McFarland suspension was prepared which was then subjected to identification and susceptibility testing on Vitek 2 (manufactured by bioMerieux)—which is a fully automated system for identification of organism and antimicrobial susceptibility testing as per the CLSI 2013 guidelines. The card was automatically filled by a vacuum device, sealed and inserted into the VITEK 2 reader-incubator module (incubation temperature, 35.5°C), and subjected to a kinetic fluorescence measurement every 15

min. The results were interpreted by the ID-GPC database, and final results were obtained automatically. All cards used were automatically discarded into a waste container.

The ESBL status was determined by Vitek 2 as per the CLSI guidelines and was not subjected to any further testing.

Data Analysis

Data was analyzed as per the CLSI 2013 guidelines. which were received in the clinical microbiology laboratory during the period. The patient's name, accession number, date and number of bottles received, earliest date of positive results, number of positive bottles, and final organism identification were recorded and evaluated.

Results and Discussion

During the one year study period 2452 blood cultures were cultured. 1700 (69.3%) blood culture show no any growth. 752(30.6%) positive growth were isolated from patients. Most blood stream infection were due to a single organism, while 20(2.6%) were of polymicrobial aetiology. In 11 of these episodes, two different microbes were detected while in 4 patients three microbes were isolated. All the polymicrobial infections were isolated from hospital admitted patients. Gram-negative bacteria were encountered more often 385(37.8%) than Gram-positive micro-organisms. The common Gram-negative organisms were *Klebsiella spp* 173(23%) followed by *Escherichia coli* 152(20.2%), *Acinetobacter spp* 30(4%), *Enterobacter* and *Salmonella typhi* 15(2%) and *pseudomonas* 6(0.7%).

Among the Gram-positive bacteria, *Enterococcus spp.* was the predominant isolate 157(20.8%) followed by a *Coagulase*

-negative Staphylococcus 120(16%) and *Staphylococcus aureus* 61 (8.1%). The distribution of bacterial species of the 752 microorganism isolated Table-1.

The *S. aureus* strains showed resistance to vancomycin in 4(0.5%) isolates. In *Enterococcus spp.*, resistance to vancomycin was seen in 10(1.3%) isolates.

Among Gram positive bacteria Daptomycin shows 100% sensitivity in all *staphylococci spp.* followed by vancomycin, teicoplanin and tigecyclin 96% sensitive (Table 2)

The most common bacterial isolate was *Klebsiella spp.* Colistin, Imipenem/Meropenem and ciprofloxacin followed by piperacillin tazobactam were seen very effective drug with low resistance of 8%,10%, 27% and 30% respectively. In *Escherichea coli*, Colistin, imepenem/meropenem and amikacin was seen low resistant 5%. 6% and 12 % respectively. In *Acinetobacter* imepenem/meropenem show lower resistant 10% followed by cefaparazone and clarithromycin 12 %. *Entrobacter spp* show lower resistant with Imipenem/Meropenem (8%), salmonella with Gentamycin (2%) and *Pseudomanas* in Clarithromycin 4%.

Among Gram negative bacteria Amoxicillin noted as high resistant drug and Imepenem/meropenem in some bacteria colistin was seen as a most sensitive drug (Table 3)

Fungal septicemia caused by *Candia albicans* isolated 03 % in total no of samples.

We isolated the bacteria most causing septicemia and their susceptibility pattern with the distribution of the microorganism in this retrospective study from hospital blood samples. The incubation period employed for automated continuous-monitoring blood

culture systems, when introduced, was six to seven days. The currently recommended incubation period is now commonly accepted as five days. In this study, we reviewed the time to positivity for 2452 blood cultures over a period of one year. We have shown that more than 90 percent of organisms from blood cultures were detected within three days of incubation. This is almost identical to the previous blood culture study reported by others (Doern, 2000). At least one study has shown that 97 percent of significant isolates were recovered within three days of incubation (Bourbeau *et al.*, 2001).

Only 752 organisms out of a total of 2,452 were isolated after the third day of incubation. Review of clinical information for days four and five isolates demonstrated that they were of limited clinical significance. Similar studies using different automated blood culture systems have shown that three or four days of incubation may be sufficient to detect clinically significant organisms. (Johnson *et al.*, 2000). It is in accordance with the studies of China and Gupta (2013), Kamga *et al.*, (2011), Anbumani *et al* (2008), and Karlowsky *et al* (2004) who reported similar incidences but in most of the studies like, Mehdinejad *et al* (2007), Barati *et al* (2009), Ayobola *et al* (2011) and Mehta *et al* (2005).

In view of significant vancomycin resistance of 0.5%, in staphylococcal isolates, drugs like daptomycin, tigecyclin and teicoplanin should be considered in the treatment of MRSA . Vancomycin resistant Enterococcus (VRE) in our study is 1.3% which is in accordance with the studies like Gupta *et al.*, (2015) reported 1.3 to 4%

Enterococcus spp. was the predominant isolate 20.8% cases in this present paper. The isolation of *Enterococcus* is the consist with the study with the contradictory result kalpesh *et al.*, 3.5% (2014) in this study

Coagulase-negative Staphylococcus (16%) isolated it may be due to skin contamination similar studies Sapna *et al.*, (2015) while the target rates for the contamination should ideally not exceed 2%-3%. The actual rates for the contamination vary widely from institution to institution, ranging from 0.6% to over 6% (1997). Studies which were done in Virginia (USA) and south India also showed that these organisms, particularly *CONS*, were an increasing source of true bacteraemia, especially in patients with prosthetic devices and central venous catheter. Therefore, it is extremely important that certain criteria are set for determining the contamination in the blood cultures.

Staphylococcus was isolated in 8% of cases and *CONS* in 16 % of cases in the present paper. The isolation of *Staphylococcus aureus* is consistent with the study of Arora and Devi, Roy *et al.*, (2002), and Karlowsky *et al.*, (2004) where the reported isolation of the organism was 27.3%, 14%, and 16.5%, respectively. However, reported isolation of *CONS* was 20.16%, 16.5%, and 42%, respectively, similar to our study. *CONS* isolated from blood are often skin contaminants which are clinically insignificant.

Klebsiella 23% *Escherichia coli* 20.2% was the most frequent Gram negative pathogen isolated in this present study that is similar to study of Mehta *et al.*, Karlowsky *et al.*, Kamga *et al.*, (2011), and China and Gupta (2013).

In this study also observed frequency of *Acinetobacter* as in this study that is 4% but we observed only 2% *Enterobacter*, *Salmonella* and 0.7 % *pseudomonas*. This is isolated in the frequency of 10 to 20% in other studies. Generally, *Salmonella* is community-acquired infection in general population which gains entry via feco-oral route (2004).

The most common bacterial isolate was *Klebsiella spp.* Colistin, Imipenem/ Meropenem and ciprofloxacin followed by piperacillin tazobactam were seen very effective drug with low resistance of 8%,10%, 27% and 30% respectively. High resistant *Klebsiella* with seen in trimethoprim sulfamethoxazole followed by amoxicillin-clavulanate and ceftriaxone 80%, 75% and 73 % respectively.

In *Escherichia coli*, Colistin, imipenem/ meropenem and amikacin was seen low resistant 5%. 6% and 12 % respectively. In *Acinetobacter* imipenem/ meropenem show lower resistant 10% followed by cefepime and clarithromycin 12 %. *Enterobacter spp* show lower resistant with Imipenem/Meropenem (8%), *salmonella* with Gentamycin (2%) and *Pseudomonas* in Clarithromycin 4%.

Table.1 Spectrum of microorganism in blood culture from all wards

Sn.no.	Isolated Organism	Total no of organism (n= 752)	Percent (%)
1.	<i>Klebsiella spp.</i>	173	23%
2.	<i>Enterococci</i>	157	20%
3.	<i>Escherichiae coli</i>	152	20%
4.	<i>CONS</i>	120	16%
5.	<i>Staph.aureus</i>	61	08%
6.	<i>Acinetobacter</i>	30	04%
7.	<i>Pseudomonas</i>	6	0.8%
8.	<i>Salmonella spp.</i>	15	02%
9.	<i>Enterobacter</i>	15	02%
10.	<i>Candia albicans</i>	23	03%

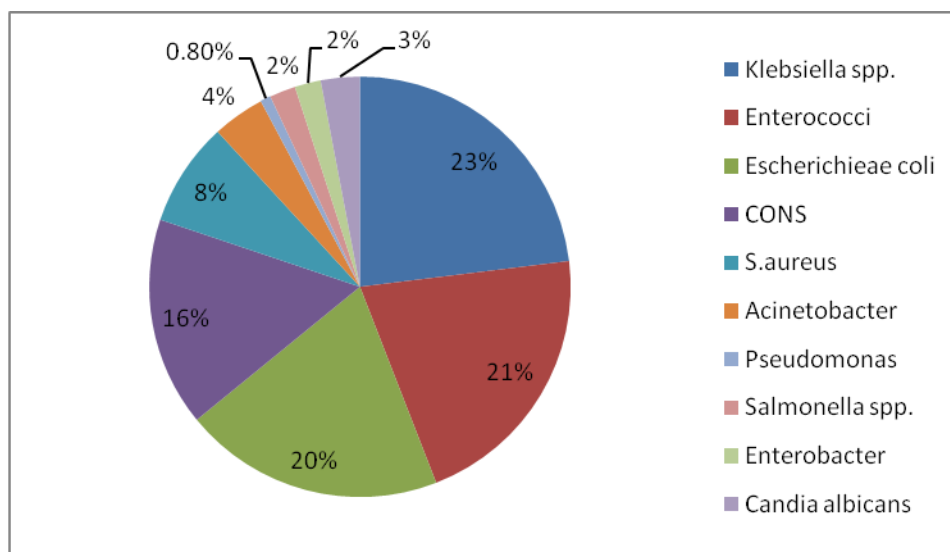


Table.2 Resistant pattern of Gram Positive organism (n=338)

Organism	Amp	Linozo	Vanco	Teico	Doxy/te	Cipr	Genta	Levo	Dap	tigcy
<i>CONS (n=120)</i>	90	5	8	7	20	25	28	-	0	8
<i>S.aureus(n=61)</i>	90	88	4	4	15	30	32	-	0	4
<i>Enterococci(n=157)</i>	90	12	10	10	38	28	87	30	-	-

Amp=Ampicillin, Lino=Linozolid, Teico=Tecoplanin, Doxy=Doxycyclin, Tetra=tertracyclin, Cipro=Ciproflox, Genta=Gentamycin, Levo=Levoflox, Dapto=Dapyomycin, Tig=Tigcyclin

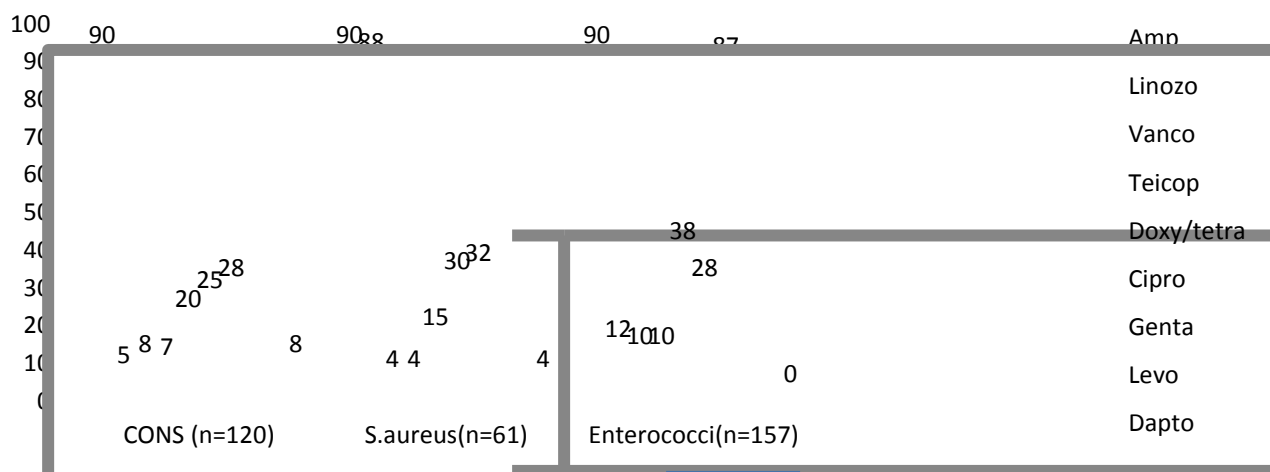


Table.3 Resistant pattern of Gram Negative organism (n=385)

	<i>Escherichea coli (n=152)</i>	<i>Klebsiella (n=173)</i>	<i>Pseudomonas N=6</i>	<i>Salmonella (n=15)</i>	<i>Acinitobactor (n=30)</i>	<i>Entnerobacter (n=15)</i>
Colistin	5 %	8%	-	-	-	-
IMP/MEM	6 %	10%	6%	0%	10%	8%
Ertapenem	48 %	55%	-	-	-	-
Pip/tazo	22%	30%	10%	-	20%	22%
CFS	35%	65%	10%	40%	12%	15%
Cipro/oflox-	40%	27%	45%	-	25%	-
Amika	12%	42%	25%	-	33%	
Ceftraxone	62%	73%	-	-	-	-
Tmp/SMX	65%	80%	-	30%		
Amoxi/clave	70%	75%	-	40%	95%	
Clarithro	-	-	4%	0%	12%	
Genta	-	-	-	20%	-	-
Ceftizidime	-	-	40%			
Azreonam			65%	-	58%	

Imp=impenem, Mem=Meropenem, Pip=Pipracyclin, Tazo=Tazobactum, CFS=Cefaparazone, Cipro=Ciproflox, Oflox=Ofloxacin, Amika=Amikacin, Tmp=Trimethoprim, Smx=sulfamethoxazole, Clarithro=Clarithromycin, Genta=Gentamycin

Fig 1 Showing Antibiotic Resistant Pattern of *Escherichia coli* and *Klebsiella* spp.

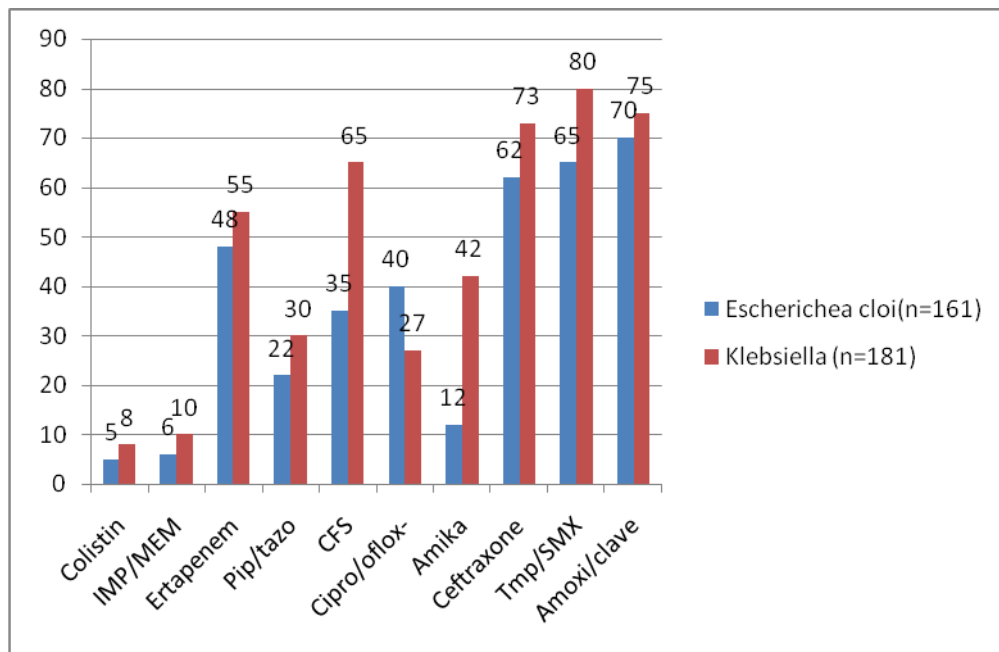


Fig 2 Showing Antibiotic Resistant Pattern of *Pseudomonas* and *Salmonella* spp

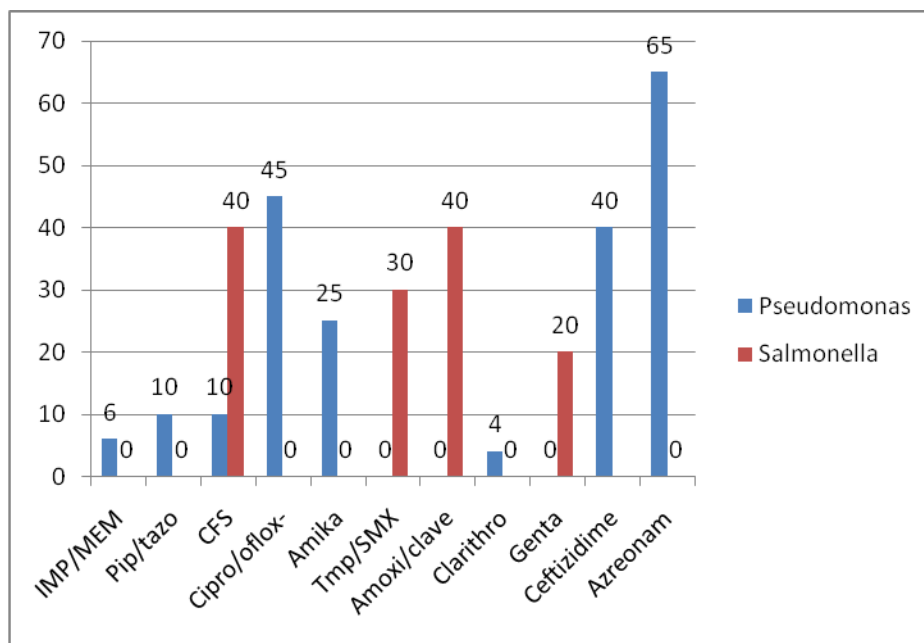
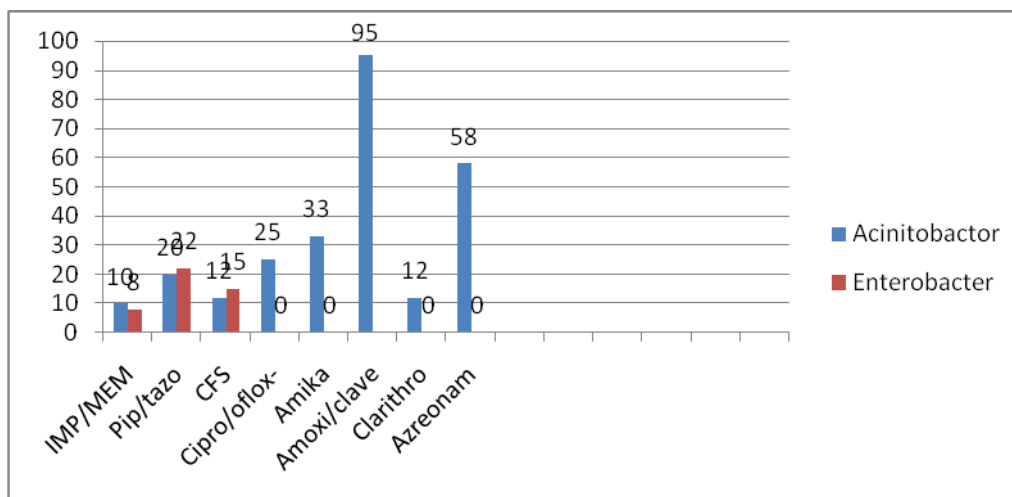


Fig 3 Showing Antibiotic Resistant Pattern of *Acinetobacter* and *Enterobacter* spp.



Among Gram negative bacteria Amoxicillin noted as high resistant drug and Imepenem/meropenem in some bacteria colistin was seen as a most sensitive drug similar to study of Kavitha Prabhu *et al.*, (2010).

The *S. aureus* strains showed resistance to Ampicillin followed by ciprofloxacin and gentamycin, 80%, 32% and 30% respectively. Vancomycin resistant found in 4(0.5%) isolates. In *Enterococcus spp.*, resistance to vancomycin was seen in 10(1.3%) isolates (Table 2)

Among Gram positive bacteria Daptomycin shows 100% sensitivity in all *staphylococci spp.* followed by vancomycin, teicoplanin and tigcyclin 96% sensitive. Similar study observed to prakash *et al.*, (2011) & Mehta *et al.*, (2005).

In this current study fungal infection caused by *Candida albicans* observed 03% that is found more than few gram negative bacteria.

In conclusion, this data provided important information on the prevalence of antimicrobial resistance amongst pathogens causing blood stream infections. BSI an important cause of morbidity and mortality

in our patients especially among chronically ill elderly adult males. Coagulase-negative *staphylococcus*, *klesiellaspp* and *E. coli* were the most important bacterial pathogens causing BSI. Prescription of Trimethoprim/ Sulphamethoxazole, Ampicillin, Cepharadine, Gentamicin and Amoxicillin/Clavulanic acid to bacteremia patients needs attention. The rise in antibiotic resistance in blood isolates emphasizes the importance of sound hospital infection control, rational prescribing policies, and the need for awareness to use antimicrobial drugs. Our results seem helpful in providing useful guidelines for choosing an effective antibiotic in cases of septicemia and for choosing salvage therapy against BSI and also to determine the source of infection and risk factors associated with BSI are further considered necessary.

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