SPECTRUM OF MICROORGANISMS ISOLATED FROM BLOOD CULTURE AND THEIR RESISTANCE PATTERN

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ABSTRACT

The aim of the present study was to determine the spectrum of microorganisms isolated from blood culture and their resistance pattern.This study was carried out between January 2014 and December 2014 in the Department of Microbiology , Era's Lucknow Medical college & Hospital, Lucknow . A total of 2278 blood culture samples of patients with suspected sepsis were included in the study. BHI broth and BACTEC aerobic & anaerobic culture bottles were used for taking cultures.All bottles were subcultured onto Blood and MacConkey's agar. In case of any growth , it was identified and antibiotic susceptibility test was done according to standard procedures.Cultures were positive in 278 samples (12.2%). Spectrum revealed that Coagulase Negative Staphylococci accounted for 34.89%,*Staphylococcus aureus*17.98% and *Klebsiella spp*9.35% . *Staphylococcus* strains showed Methicillin resistance in 10% of isolates. Extended spectrum beta lactamases constituted 26.92% each for *Klebsiella* and *Escherichia coli* strains and 40% of *Citrobacter* strains isolated .Vancomycin resistant *Enterococcus* strains accounted for 10% of strains isolated .A large proportion of patients presenting with sepsis at a tertiary care hospital, are already treated with antibiotics elsewhere. This leads to a low positive yield in blood culture.The present study highlights the increasing resistance in microorganisms causing sepsis Their early detection and resistance pattern will definitely help in modifying the treatments. Thus early blood culture report increases therapeutic compliance.

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INTRODUCTION

Bacteraemia, is the presence of bacteria in the blood. An episode of the presence of bacterial colonization in the blood circulation is usually not a life threatening condition. This is because several physiological conditions give rise to transient bacteraemia without any obvious clinical sequelae. Septicaemia on the other hand is obviously a medical emergency (1). Microorganisms present in circulating blood whether continuously or intermittently are a threat to every organ in the body. Approximately 200,000 cases of bacteraemia and fungemia occur annually with mortality rates ranging from 20-50% (2).Since early 1950s, there is striking increase in incidence of bacteraemia caused by members of enterobacteriaceae and other gram negative bacteria .*Escherichia coli* which was reported to be common in the past is being replaced by other multidrug resistant(MDR) bacteria like *Klebsiella, Enterobacter, Salmonella, Citrobacter, Pseudomonas, Acinetobacter* etc(2).Increasing antimicrobial resistance is a worldwide concern. Individuals with bacteraemia may develop septicaemia, a life threatening condition in which multiplying bacteria

release toxins into the bloodstream and trigger the release of cytokines, causing fever, chills, malaise and lethargy with difficulty in breathing especially in children.This makes septicaemia arising from various causes; a disease of serious clinical importance, and the diagnosis of other non-septicaemic bacterial ailments by recovering such bacteria from blood make blood cultures very useful tools for diagnosing several bacterial infections (1). The prevalence of resistance of blood borne isolates is increasing and it also varies in accordance with geographical and regional location. 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 (3). Differences in bacterial spectrum of different centres demonstrate the importance of knowing the etiological pathogens of each centre for better management. Additionally, with the growing incidence of antimicrobial resistance, knowledge about the pattern and antibiotic sensitivity of causative microorganisms of each region and centre is vital. It should be mentioned that the type and severity of immunosuppression has a great effect on the interpretation of the results of laboratory diagnostic tests (4). The present study was carried out to determine the pattern of microbiological agents responsible for bacteraemia in a tertiary care hospital, Lucknow and to get an updated knowledge about their antibiotic susceptibility pattern.This may help clinicians in selecting the appropriate antibiotics for empirical therapy until the results of culture / sensitivity are known.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology, at Era's Lucknow Medical college & Hospital, Lucknow. Permission from Institutional Ethical Committee & Institutional Review Board was taken. A total of 2278 blood culture samples of patients with suspected sepsis(5) admitted in different wards and intensive care units were included in the study. Blood samples were collected aseptically from each patient before the start of antimicrobial therapy; in adults 8ml and in children 3ml blood was added to each of brain heart infusion (BHI) broth (Oxoid, UK) and automated BACTEC 9050 (BD Diagnostics) culture bottles (6). Manual and automated BACTEC 9050 blood culture systems were used for blood culture. For the manual method, BHI bottles were incubated at 37°C after shaking and subcultured as per standard protocol on blood and MacConkey agar . Gram staining was performed on smears from the broth of positive culture bottles. Growth indicated by the automated system was sub-cultured on blood and MacConkey agar followed by colony morphology, Gram staining, motility testing, rapid tests like catalase, coagulase, oxidase and other requisite biochemical tests (7). Specimens yielding yeast were identified through colony morphology followed by speciation with Candida chromagar (Oxoid, UK) and Kirby-Bauer technique was used for antimicrobial susceptibility according to the guidelines of Clinical and Laboratory Standards Institute (CLSI 2012) and zones of inhibition were interpreted accordingly. Control strains were used as per requirement: ATCC 25923 *Staphylococcus aureus,* ATCC 27853 *Pseudomonas aeruginosa,* ATCC 25922 *Escherichia coli,* ATCC 19606 *Acinetobacter baumannii,* ATCC 10231 *Candida albicans* (8).

RESULTS

During the one year period a total of 2278 blood cultures processed and cultures were positive in 278 samples (12.2%). Out of 278 patients who tested positive for blood culture, 70 belonged to paediatric age group and 208 to adult age group ;168 were males and 110 were females [Table 3].

Microbiological spectrum revealed that *Coagulase negative Staphylococci* accounted for 34.89%, *Staphylococcus* aureus 17.98% and *Klebsiella spp* 9.35%.*Staphylococcus* strains showed Methcillin resistance in 10% of isolates. Extended spectrum beta lactamases constituted 26.92% each for *Klebsiella* and *Escherichia coli* strains and 40% of *Citrobacter* strains isolated.[Table 1]Vancomycin resistant *Enterococcus* strains accounted for 10% of strains isolated (7.19%). [Table 2]

DISCUSSION

Microbiological profile and the antimicrobial susceptibility are constantly evolving. Study of bacteriological profile with antibiotic susceptibility pattern plays an important role in effective management of bacteraemia cases. Many studies have been undertaken to determine the organisms responsible for blood stream infections all over the world. Results have varied in different centres and different parts of the world. Among 2278 blood cultures processed, cultures were positive in 278 samples (12.2%) which is quite similar to Gohel et al.(3) ,Mehta et al. (8)and China et al.(9).

The variability in prevalence rate is dependent on the regional location, season, infrastructural facilities and most importantly on inclusion criteria. In present study, the inclusion criteria was much relaxed, thereby the number of suspects were higher and positivity rates were relatively lower. The studies using similar criteria have observed similar prevalence rates as mentioned above.Out of 278 patients who tested positive for blood

culture, 70 belonged to paediatric age group and 208 to adult age group ;168 were males and 110 were females which coorelated with the study of Vanitha et al (2) .62.58% Gram positive bacteria and 30.93% Gram negative bacteria were isolated in present study which was similar to that reported by Prakash et al.(10)

In the present study Candida spps accounted for 6.47% which was in agreement with the observations made by Nayak et al.(11)Among different gram positive isolates CONS,*Staphylococcus aureus* and among gram negative organisms belonging to Enterobacteriaceae family are the leading causes of septicaemia which have also been reported by Karlowsky et al.(12) MRSA & MR-CONS accounted to about 10% and 9.28%respectively .This coorelated with the study of Roy et al. (13)

In present study ESBL in Enterobacteriaceae was 26.92% which was similar to that reported by Nayak et al.(11) Carbapenem Resistant *Pseudomonas* species (5.26%), Carbapenem Resistant *Acinetobacter* species (16.66%) and Carbapenem Resistant Enterobacteriaceae (5.45%) were also isolated in our study which was similar to that obtained by Litzow et al.(13) In our study, 10%Vancomycin resistant Enterococci (VRE) were isolated which was higher as compared to Vanitha et al. (2) The present study provided much needed information on the prevalence of bacterial pathogens in blood stream infections and their resistance patterns. This study identified both gram positive and gram negative bacteria which were responsible for blood stream infections and most of them were multi drug resistant. The main forces driving the increase in antimicrobial resistant bacteria are poor infection control practices and inappropriate use of antibiotics. Specific antibiotic utilization strategies like antibiotic restriction, combination therapy and antibiotic recycling may help to decrease or prevent the emergence of resistance and antibiotic usage according to the standard antimicrobial susceptibility testing. Their early detection and resistance pattern will definitely help in modifying the treatment. Thus early blood culture report increases therapeutic compliance.

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Table 1

S.NO	SPECTRUM
1.	CONS 97 (34.89%)
2.	*Staphylococcus aureus* 50 (17.98%)
3.	*Klebsiella species* 26 (9.35%)
4.	*Enterococcus species* 20 (7.19%)
5.	*Pseudomonas species* 19 (6.83%)
6.	*Escherichia coli* 18 (6.47%)
7.	*Candida species* 18 (6.47%)
8.	*Acinetobacter species* 12 (4.32%)
9.	*Streptococcus species* 7 (2.52%)
10.	*Citrobacter species* 5 (1.8%)
11.	*Salmonella* Typhi 4 (1.44%)
12.	*Proteus species* 2 (0.72%)

Table 2

S.NO	RESISTANCE PATTERN
1.	Methicillin Resistant CONS (MR- CONS) 9.28%
2.	Methicillin Resistant *Staphylococcus* Aureus (MRSA) 10 %
3.	Extended spectrum β-lactamase(ESBL) in Enterobacteriaceae 26.92%
4.	Vancomycin resistant *Enterococci* (VRE) 10%
5.	Carbapenem Resistant *Pseudomonas* species 5.26%
6.	Carbapenem Resistant *Acinetobacter* species 16.66%
7.	Carbapenem Resistant Enterobacteriaceae 5.45%

Table 3

TOTAL (278)	MALES (168)	FEMALES (110)
Paediatricage group (70)	43	27
Adults (208)	125	83

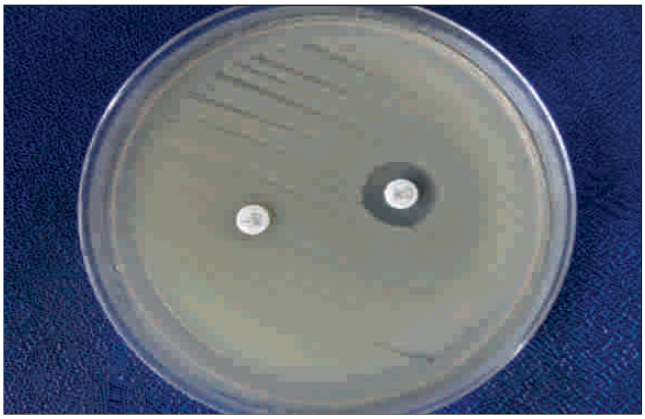

Fig.1(a)- Strain of *Staphylococcus aureus* showing resistance to oxacillin & cefoxitin

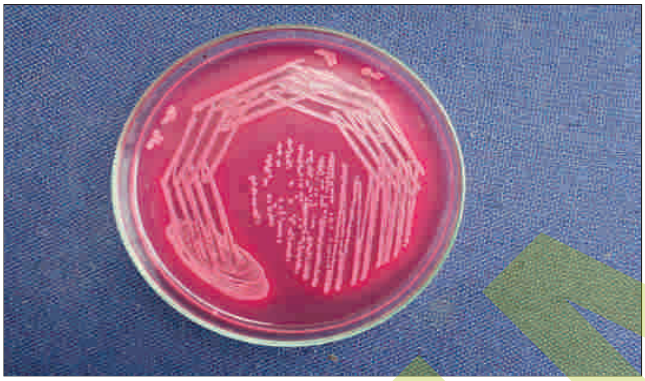

Fig.2- Lactose fermenting colonies of *E.coli* on MacConkey agar

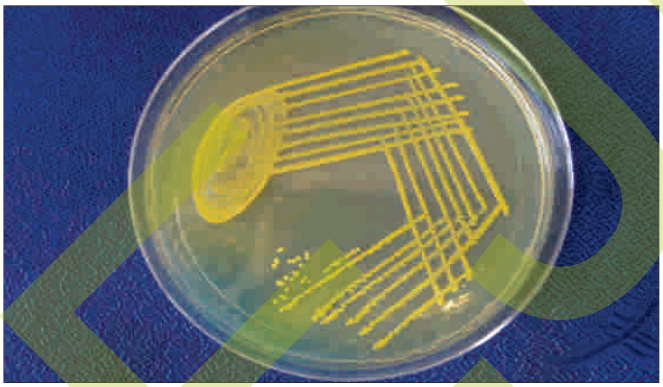

Fig.3- Golden yellow pigmented colonies of *Staphylococcus aureus* on nutrient agar

Fig.4- *Candida spps* on Hi chrome agar

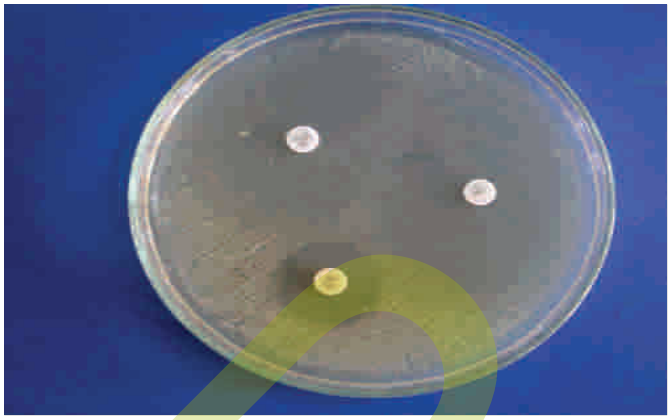

Fig.5- Antibiotic susceptibility of Candida spps on Mueller Hinton agar supplemented with dextrose & methylene blue

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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