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Original Article

Microbiology Section

Microbiological Profile and Antimicrobial Resistant Pattern of Blood Culture Isolates, Among Septicaemia Suspected Patients

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ABSTRACT

Introduction: Microorganisms cause septicaemia,a systemic disease due to their multiplication and toxins in the blood. These bloodstream infections constitutes significant public health problems and are a major cause of morbidity and mortality in the hospitalised patients and require rapid antimicrobial treatment. Infection by MDR organisms are more likely to increase the risk of death in these patients.

Aim: To determine the microbiological profile and antimicrobial resistant patterns among patients suspected to have septicaemia at a tertiary care hospital.

Materials and Methods: A total number of 121 patients were included in this study suspected to have septicaemia. The blood culture samples were processed and identified in the microbiology laboratory according to CLSI guidelines in RMCH&RC over a period of six months. Drug resistant strains in primary screening were further processed for ESBL and MRSA by standard guidelines.

Results: Out of 121 patients, 27(22.3%) developed septicaemia with the positive blood culture. Of the 27 positive culture, 24(88.9%) showed bacterial growth, in which 16(59.3%) were gram negative bacilli, 8(29.6%) were gram positive cocci and 3(11.1%) isolates were gram positive budding yeast cells. Most common isolates were *E.coli* (22%), *Klebsiella pneumoniae* (22%), Coagulase negative *Staphylococcus* (CONS) spp. (15%) followed by *Pseudomonas aeruginosa* (11%) *S.aureus* (11%), *Klebsiella oxytoca* (4%), Enterococcus faecalis(4%) and *Candida spp* (7% *C.glabrata* and 4% *C.krusei*) was mainly isolated in neonates. Maximum isolated strains showed high resistance towards penicillins, cephalosporins and fluoroquinolones. *S.aureus* and CONS were mostly resistant to co-trimoxazole and erythromycin. Resistance to imipenem was seen approximately in 66% in *E.coli*, *Klebsiella pneumoniae* and *Pseudomonas* spp. ESBL producers among the *E.coli* and *Klebsiella* isolates were 50% and 66.6% respectively, while 33.3% were MRSA and 50% MR-CONS among Gram positive cocci. Tigecycline and polymyxin-B were the most effective antimicrobial agents for Gram-negative bacteria, while linezolid, teicoplanin for Gram-positive bacteria.

Conclusion: The present study provides much needed information on the prevalence of bacterial pathogens in blood stream infections and also demonstrates the presence of fungaemia due to non-*Candida albicans* spp., which highlights the need for periodic surveillance of etiologic agents, their antibacterial and antifungal susceptibility pattern. Also changing trends in the distribution of *Candida* species to prevent further emergence and spread of resistant pathogens.

Keywords: Blood stream infections, Drug resistance

INTRODUCTION

Blood stream infections cause significant morbidity and mortality worldwide and among the most common health care associated infection [1]. Bacteraemia and fungaemia are one of the cause for high mortality rate (20%-50%) [2]. Septicaemia may be a transient, self limited phenomenon without clinical consequences, it frequently reflects the presence of serious infection [3]. It has been confirmed by culture that is associated with clinical manifestation and systemic response [4-6]. It is often associated with hospitalization, insertion of foreign bodies such as catheters into blood vessels, and other predisposing factors like ICU, lapses in handwashing, and non-adherence to infection control practices of medical staff. Genitourinary tract, intra-abdominal foci and respiratory tract are the common sources of blood stream infections [2,4]. Organism isolated from blood culture vary according to geographical distribution and development of multidrug resistant organism is of great concern as they prolong hospital stay. Increase cost of treatment and can be a cause of high mortality [7]. Blood

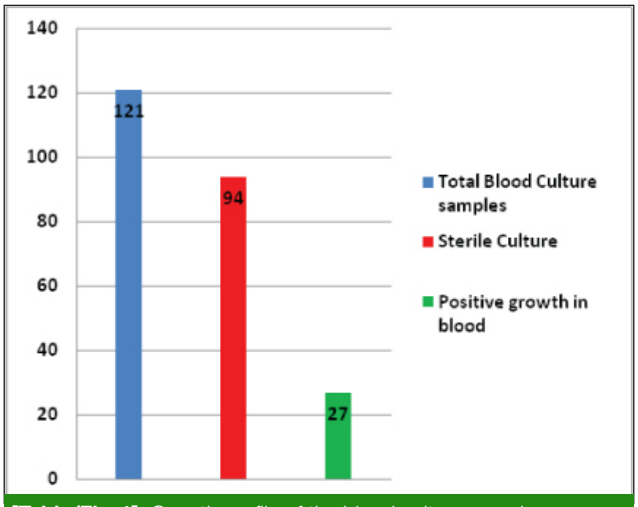
culture is the gold standard for the diagnosis [6]. The changes in the local patterns of infection and susceptibility to various antibiotic should be critically evaluated periodically [3]. Therefore, this study was aimed at finding the resistance pattern of the isolates from blood in a tertiary care centre, which can help the clinician to provide empirical treatment.

MATERIALS AND METHODS

This was a prospective study conducted in the Rama Medical College Hospital and Research Centre, Kanpur in the Department of Microbiology over a period of six months from January 2015 to June 2015. A total of 121 blood culture samples were analysed. Blood samples were collected under aseptic precautions using a sterile syringe after applying tourniquet. From an adult 10 ml, children 5 ml, and neonates 1 ml of blood into BHI Broth (Hi Media, Mumbai, India),70 ml and 20 ml bottles, from different wards and critical care units from septicaemia suspected patients was collected. All blood cultures were processed in laboratory using standard procedure by conventional method. After 24 hrs blood culture samples were sub-cultured onto Blood agar and Mac-Conkey agar to look for growth. From the obtained growth isolated colonies were used for gram's staining and biochemical tests for the differentiation of organism and antibiotic sensitivity was done by Kirby Bauer's method according to CLSI guidelines [8], and no growth plates were incubated for further 24 hours. Subcultures from blood culture bottles were done on 2nd, 4th and 6th day, Samples were reported as no growth after 7 days of aerobic incubation. Septicaemia suspected patients who were not given antibiotics were included in the study, and patients already on antibiotics before the blood culture were excluded from the study. The antibiotics which were tested were ampicillin (10mcg), piperacillin/tazobactam (100/10mcg), ofloxacin (10 mcg), oxacillin (1mcg), cefotaxime (30mcg), ceftazidime (30mcg), cefepime (30mcg), cefoperazone/sulbactum (75/30mcg), cefoxitin (30mcg), amikacin (10mcg), gentamicin (10mcg), ciprofloxacin (5mcg), co-trimoxazole(1.25/23.75mcg), tetracycline (30mcg), polymyxin B (300mcg), colistin (10mcg), tigecycline (15mcg), erythromycin (15mcg), vancomycin (30mcg), linezolid (10mcg), teicoplanin (30mcg). Drugs used were from Hi-Media (Mumbai, India). The screened strains were further processed for ESBL and MRSA according to CLSI guidelines [8]. Ethical clearance was obtained from the institute.

RESULTS

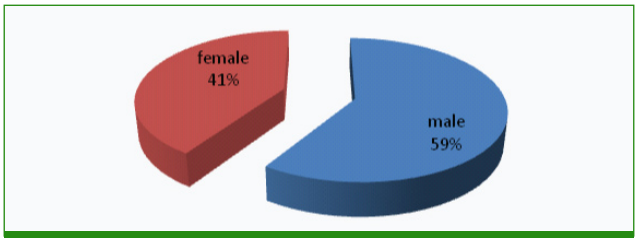
Out of 121 blood cultures, 27(22.3%) gave positive result [Table/Fig-1]. Bacteraemia was seen in 24 patients (88.9%), and fungaemia was seen in three cases (11.1%).The majority of septicaemia patients were adults (>18 yrs) (37.0%) and neonates (25.9%) [Table/Fig-2]. In positive isolates males were 16(59%) and females were 11(41%). [Table/Fig-3] Among positive blood culture isolates Gram-negative and Gram-positive bacteria constituted 59.3% and 29.6%, respectively and 11.1% were *Candida*. The most common pathogens found was *E.coli* (22%), *Klebsiella pneumonia* (22%) followed by Coagulase negative *Staphylococcus* (CONS) spp.(15%), *Pseudomonas aeruginosa* (11%), *S.aureus* (11%), *Klebsiella oxytoca* (4%), *Enterococcus foecalis* (4%) and *Candida* spp. was mainly isolated in neonates (7% *C. glabrata* and 4% *C.*



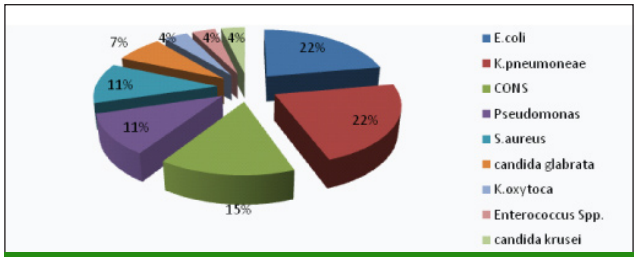
[Table/Fig-1]: Growth profile of the blood culture samples.

Age distribution	Growth	Sterile	Total
Infant (<1 yr)	7(25.9%)	30	37
Children (1-12)	6(22.2%)	15	21
Adolescent (13-18)	4(14.8%)	23	27
Adult (>18)	10(37.0%)	26	36
Total	27	94	121

[Table/Fig-2]: Age distribution of the blood culture samples.

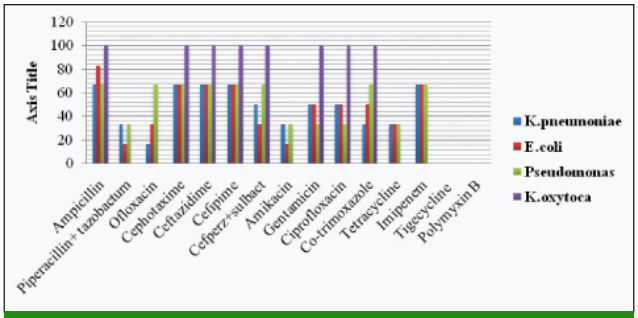


[Table/Fig-3]: Sex distribution among positive blood culture isolates.

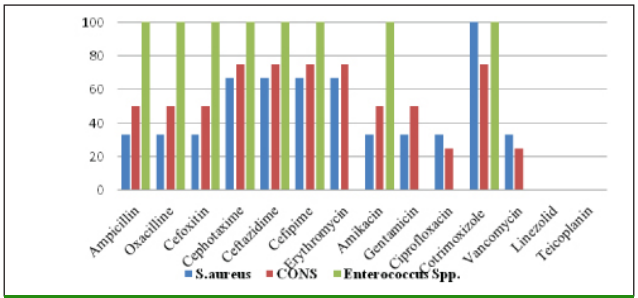


[Table/Fig-4]: Isolates from blood.

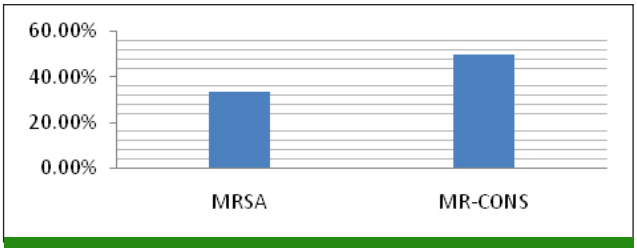
krusei) [Table/Fig-4]. Antibiotic sensitivity pattern of different isolates were showed in [Table/Fig-5-8].



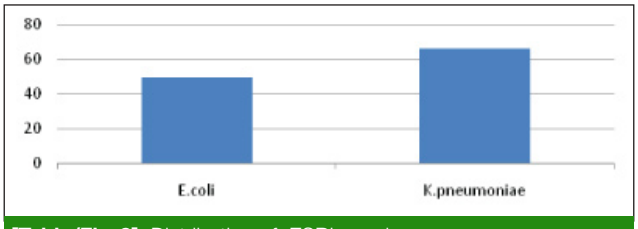
[Table/Fig-5]: Antibiotic resistance pattern of Gram negative bacilli.



[Table/Fig-6]: Antibiotic resistance pattern of Gram positive cocci.



[Table/Fig-7]: Distribution of MRSA and MR-CONS.



[Table/Fig-8]: Distribution of ESBL producers.

DISCUSSION

In our study a high prevalence rate of 27(22.3%) was seen. Gram negative organism was the predominantly seen. Isolates were resistant to most commonly used antibiotics. Multidrug resistant bacterial strains are emerging as a major problem in the management of sepsis . The amount and number of blood culture samples included in the studies could be one of the reasons for variation in the prevalence rate [9]. The prevalence rate of 22.3% in the present study, which is approximately similar to Arora et al., [10] 20.2% and lower than the studies done in India, by Khanal et al.,[11] and Sharma et al., [12], have reported high frequency of positive blood cultures accounting for 44% and 33.9% respectively. While other studies showed low prevalence by Mehdinejad M et al., [13] Vanitha et al., [14] and Mehta et al., [15] 5.6%, 8.39 and 9.94% and the variation may be due to the different methodology used and the area of study, because of regional variation known to occur.

Similar, to present study in most of the studies, gram-negative bacilli have taken over the gram-positive organisms, especially in hospital settings. In our study *E.coli*, *Klebsiella*, *pseudomonas* was most predominant gram negative isolates. Other authors also found similar findings with addition of *Acinetobacter* spp, *Enterobacter* spp and *Salmonella* spp [12,14].

Among gram positive pathogen CoNS and *S.aureus* were most common isolates similar to Vanitha et al., [14] and Rao et al., [16] though CoNS when isolated from blood cultutres are often considered as skin contaminants [7], but repeated isolation of CoNS suggests that infections by these agents constitute a significant threat.

In other studies the incidence of Candidemia was much higher (20.29%) [16] mainly due to *C.albicans* while in present study 11% of positive blood culture gave growth of non-albicans candida spp (7% *C.glabrata* and 4% *C. krusei*) similar to other studies [14,17]. Candidemia in our study was due to non-albicans species, and all the cases were seen in neonates. This could probably be due to increased use of invasive devices, and broad spectrum antibiotics in NICU and PICU [18]. Prevalence of neonatal septicaemia in our study was 25.9% which was comparatively lower than other studies which showed a rate of 56% and 36% [12,19,20], the reason for this could be the less number of sample included in the studies, or anaerobic bacteraemia or a false history from patients in use of antibiotics. High prevalence of septicaemia in other studies may be due to low socioeconomic status of their parents, poor hygiene practices, bottle feeding and high incidence of delivery at home [21].

Septicaemia was caused by only one organism in our study similar to other studies [22]. Septicaemia of polymicrobial etiology observed in other studies [23-25] is significant. Some microbiologist considers polymicrobial growth as a contamination, but sepsis should be clinically correlated [26]. The clinician should be caution, when septicaemia is due to the multiple microbes not neglecting it as contaminants, but consider it as a pathogen if it is suspected to be isolated from the hospital environment [21]. In our study males were slightly more affected than female but there is no effect of gender in blood culture positivity rate.

In our study >60% of strains were resistant to ampicillin among the gram positive cocci similar to other studies

[27,28]. Resistance to cephalosporins was also observed with *Staphylococcus aureus* and *Enterococcus faecalis* in the present study. Among all Gram positive cocci 33.3% were MRSA and 50% were MR-CONS. This could be due to the indiscriminate use of these drugs especially third generation cephalosporins in hospitals as an emergency empirical therapy, as reported by Nathisuwan et al., [29] and Vanitha R et al., [14] The study also showed that *Staphyloccocus aureus* was found to be mostly sensitive to vancomycin, Teicoplanin, linezolid and ciprofloxacin as reported by other authors also [14].

One study of Asrat D et al., [30] found only gentamicin and kanamycin were relatively effective against gram negatives and in our study approx 50% of strains were sensitive to gentamicin. Piperacillin + tazobactum, amikacin, tigecycline, polymyxin B were most effective drug. Ofloxacin were sensitive to *Klebsiella* species and *E.coli* but more than 60% *Pseudomonas* was resistance. In the present study 50% of *E.coli* and 66.6% of *Klebsiella* were ESBL producer which is higher than Kalpeshet al., [7], Kavitha et al., [31], and Arora et al., [10] who reported ESBL producers as 39.6%, 32% and 34.4% respectively found only gentamicin and kanamycin were relatively effective against gram negatives and in our study approx 50% of strains were sensitive to gentamicin.

LIMITATION

The limitations of this study is that though the fungaemia was seen due to *Candida* isolates ,antifungal susceptilibility was not done due to cost constraints.

CONCLUSION

The present study provides much needed information on the prevalence of bacterial pathogens in blood stream infections predominantly gram negative bacilli were isolated from blood and non-albicans Candida spp. was mainly isolated in neonates. This study highlights the increase in antimicrobial resistant bacteria that may be due to poor infection control practices and inappropriate use of antibiotics. The key to control of antibiotic resistant pathogens is to strictly adhere to infection control practices and mandates antibiotic policy on a large scale for every hospital.

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