Bacteriological Profile and Multidrug Resistance Patterns of Blood Culture Isolates in a Teaching Hospital in South India.

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Abstracts: Background: Blood stream infections can lead to life threatening sepsis and require rapid antimicrobial treatment . The organisms implicated in these infections vary with the geographical alteration . Further , infections caused by MDR organisms are more likely to increase the risk of death in these patients . Objectives : To study the profile of organisms causing blood stream infections and analyse their antibiotic resistance patterns in our teaching hospital.: Materials and methods : Prospective study of 524 blood samples from clinically suspected cases of bacteraemia was performed over a period of three years. The isolates were identified by standard biochemical tests and antimicrobial resistance patterns were determined by CLSI guidelines. Results : Positive blood cultures were obtained in 22.9% of cases. Among the culture positives , gram positive bacteria accounted for 61.5% of cases ; the most common isolate being Staphylococcus aureus(29.2 %) . Of the gram negative isolates , bacteria belonging to Enterobacteriaceae were the predominant isolate , Klebsiella being the commonest isolate.The most sensitive drugs for gram positive isolates were Amikacin, Erythromycin , Ofloxacin and Piperacillin –Tazobactam.And the most sensitive drugs for gram negative isolates were Amoxyclav and Piperacillin – Tazobactam.The prevalence of MRSA in our Staphylococcal isolates was 37.1% and Vancomycin resistance in these isolates was 25.7%. Vancomycin resistance in E nterococcal isolates was 33.3 % .ESBL prevalence was 32.6 %.Conclusion : Increasing incidence of Drug resistant organisms in blood stream infections calls for increased efforts by clinicians to exercise caution in use of these drugs . Vancomycin should be replaced by simpler drugs like Linizolid or Cotrimoxazole to preserve this useful antibiotic and prolong its therapeutic usefulness. [Pavani G et al NJIRM 2012; 3(3) : 55-59]

Key words: Andhra Pradesh , Bacteriological Profile , Antibiotic Resistance , Septicaemia , Blood culture.

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Introduction: Blood stream infections range from self limiting infections to life threatening sepsis,that requires rapid and aggressive antimicrobial treatment[1].A wide spectrum of organisms have been described that cause blood stream infections and this spectrum is subject to geographical alteration[2-5]. Increasing antimicrobial resistance is a world wide concern. 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 organisams are 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

septicaemia, right choice of empiric therapy is of importance.[6] Therefore the present study was undertaken to analyse the various organisms causing septicaemia and their antibiotic resistance patterns as it would be a useful guide for clinicians initiating the empiric antibiotic therapy.

Materials and Methods : A total of 524 samples from clinically suspected cases of bacteriaemia were studied for a period of three years from september2008 to August 2011. All the samples were collected from our teaching hospital. Processing of the samples was done in the Central laboratory, Mediciti Institute of medical sciences,Medchal, Andhrapradesh, India.Approval was obtained from the Institute

Research Council prior to commencement of the study.

5 ml of blood was collected from each adult patient using strict aseptic precautions and inoculated immediately into 50ml of Brain Heart Infusion Broth with 0.025% of sodium poly anethol sulphonate as anticoagulant. In paediatric cases 1-2 ml of blood was inoculated in 5-10ml of BHI broth. The broths were subcultured on 5% Sheep blood agar and Mac Conkey agar after overnight incubation. A negative result was followed up by examining the broth daily and doing a final subculture at the end of seventh day. Positive growth was identified by gram staining , colony characteristics and standard biochemical tests.Antimicrobial susceptibility testing was performed by Kirby- Bauer disc diffusion method as per CLSI guidelines.The antibiotic discs used were penicillin (10 units), erythromycin (15mcg), vancomycin (30mcg), ceftazidine (30mcg) , ceftriaxone (30mcg), gentamycin (10mcg), amikacin (30 mcg), ciprofloxacin(5mcg), chloramphenicol (30mcg), doxycycline(30mcg), cotrimaxazole (1.25/23.75mcg), ofloxacin(5mcg), cefaperazone(75mcg),imipenem(10mcg), cefaclor (30mcg),cefepime(30mcg),cefotaxime (30mcg), piperacillin –tazobactem (100/10mcg) ,amoxycillin (30mcg), amoxyclav (20/10mcg), Bacitracin (10units), oxacillin (1mcg) and ceftazidime clavulanic acid (30/10mcg).

Oxacillin screening and confirmatory tests were done on the pathogenic staphylococcal isolates using CLSI guidelines. MRSA isolates were identified and subjected to Vancomycin screening test using MHI agar with 4 mcg/ml vancomycin for screening and FDA MIC method for confirmation. Vancomycin screening and confirmatory tests were also done on enterococcal isolates as per CLSI guidelines . TheKlebsiella and Esch coli isolates were subjected to ESBL screening test with ceftazidime and confirmed by double disc potentiation test as per CLSI guidelines using ceftazidime and amoxyclav.

Results : During the three years study period, 524 blood cultures were analysed.122 microorganisms were isolated from 120 patients, out of which 121 were bacterial isolates and 1 was a fungal isolate i.e., candida albicans.All infections were due to a single organism except two (1.6%) which was of polymicrobial etiology.The distribution and percentage of various bacterial and fungal isolates is shown in Table 1 .

Table 1- Distribution of bacterial and fungal isolates in blood cultures

Bacterial isolates	No of isolates (percentage)
Gram positive isolates	**73(60.8%)**
Staphylococcus aureus	35(29.2%)
CONS	28(23.5%)
Streptococci	7(5.8%)
Enterococci	3(2.5%)
Gram negative isolates	**46(38.3%)**
Klebsiella	28(23.3%)
Citrobacter	7(5.8%)
Esch.coli	6(5%)
Acinetobacter	3(2.5%)
Proteus	1(0.8%)
Pseudomonas	1(0.8%)
Polymicrobial isolates	2(1.7%)
Coagulase negative stphylococci and Klebsiella	1(0.8%)
Staphylococcus aureus and Acinetobacter	1(0.8%)
Fungal isolates	1(0.8%)
Candida species	1(0.8%)

Among the gram positive isolates, the predominant isolate Staphylococcus aureus exhibited least resistance to Imipenem, Cefotaxime, Ceftriaxone, Chloramphenicol and Piperacillin- Tazobactem 1(2.9%). Oxacillin resistance was 37.1% in these strains. Vancomycin resistance in Staphylococcus aureus isolates was 25.7 % . In Coagulase negative staphylococcal strains, resistance was not observed with Amoxycillin, Amikacin, Erythromycin, Imipenem, Ciprofloxacin,

Ofloxacin, Cefaclor, Cefaperazone, Ceftriaxone and Chloramphenicol. Streptococcal isolates showed no resistance to pencillin, Amoxiclav, Amikacin, Bacitracin, Erythromycin, Ofloxacin and cefepime. Least resistance was observed with amoxycillin and cefotaxime . Enterococcal isolates showed no resistance to amoxycillin, amoxiclav, amikacin, Erythromycin, Imipenem, Ciprofloxacin, Ofloxacin, Cefaclor, Ceftriaxone and Doxycycline. Least resistance was seen with Pencillin, Vancomycin, Piperacillin-Tazobactem and Ceftazidime(33.5%).

Among the gram negative isolates, the predominant isolate Klebsiella showed no resistance to Amoxyclav and Piperacillin – Tazobactam. Citrobacter, the next common isolate showed no resistance to Amoxyclav , Ciprofloxacin , Ofloxacin, Cotrimoxazole, Piperacillin – Tazobactam and Ceftriaxone. Least resistance was seen with Amikacin, Gentamycin and Cefaclor. Esch.coli isolates showed no resistance to Amoxyclav , Ofloxacin and Piperacillin – Tazobactam. Least resistance was observed with Amoxycillin,Imipenem and Gentamicin (16.7%). In Acinetobacter isolates , no resistance was seen with Amoxyclav, Amikacin,,Ciprofloxacin, Ofloxacin, Ceftazidime, Cefaperazone , Imipenem , Piperacillin, Ceftriaxone and Gentamicin. Least resistance was seen with Amoxycillin, Cefotaxime and Cefaclor (33.3 %). Proteus isolates were not resistant to Amoxycillin,Amikacin, Ceftazidime and Cefaclor.And least resistance to Ciprofloxacin, Ofloxacin, Cefaperazone, Cefataxime, Cotrimoxazole, Imipenem and Gentamicin. Isolates of Pseudomonas showed no resistance to Amikacin,Ciprofloxacin, Ofloxacin, Ceftriaxone and Gentamicin. Least resistance was seen to Amoxycillin,Amoxyclav, Ceftazidime, Cefaperazone, Cefotaxime, Cotrimoxazole, Imipenem, Piperacillin-Tazobactam and Ceftazidime. 32.6% of our Klebsiella and Esch.coli isolates were ESBL producers.

Overall, the most sensitive drugs for Grampositive isolates were Amikacin , Erythromycin and Ofloxacin and the most sensitive drugs for Gramnegative isolates were Amoxyclav and Piperacillin- Tazobactam.

Discussion: In the present study , blood culture positivity was seen in 120 (22.9%) cases which is consistent with the studies of Kamga et al 28.3% [7] and Atul Garg et al 20.5% [6] whereas Kavitha et al and Roy et al have reported 44% and 47.5% positivity in blood cultures respectively . [8,9] This variation might be due to most patients given antibiotics before they come to the hospital . [10] In the concurrent study , the incidence of gram positive organisms was 75 (61.5%) while 48(39.3%) were gram negative isolates. It is in accordance with the studies of K amga et al and Karlowsky et al who reported incidence of gram positive isolates to be 56.2% and 78.1% respectively and gram negative bacilli as 43.8% and 21.9% respectively. [7, 11] But in most of the studies gram negative organisms have taken over gram positive organisms in hospital setings.[4,12,13,14] This suggests that infections by gram positive organisms constitute a significant threat to septicaemia in our locale and other developing country settings and the spectrum of organisms is subject to geographical alterations.[8,9]

In our study Staphylococcus was isolated in 29.2% of cases and CONS in 23.5% of cases which is consistent with the study of Arora U et al where the reported isolation of these organisms was 27.3% and 20.16% respectively. [10] However, Roy et al and Karlowsky et al have reported 14 % and 16.5% isolation of Staphylococcus and 16.5% and 42% isolation of CONS respectively.[9,10] Given that CONS isolated from blood are often skin contaminants which are clinically insignificant [15] and Staphylococcus being the predominant isolate in our hospital blood culture isolates , we suggest that initial therapy of septicaemia in hospitals in our area be aimed at treating Staphylococcus aureus infection .

The isolation of Streptococci in our study was 5.8% which is in accordance with the study of

Karlowsky et.al., and in contrast with the study of Kamga et al, who reported percentage of isolation of Streptococci as 4.5% and 9.5% respectively. [10,7] Enterococcal isolation in our study was 2.5% which is in acccordance with the studies of Atul garg et al and Manjula et al showing an isolation of 3.7% and 2.4% respectively. [6,11]

In our study ,organisms belonging to Family Enterobacteriaceae (87.3%) were the predominant gram negative isolates which is consistent with the studies of Karlowsky et al 82.2%) and Kamga et al (79.6%) [10,7] whereas Enterobacteriaceae were reported as 40.75% by Atul garg et al.This shows that Enterobacteriaceae still remain important group of pathogens in Septicaemias

Staphylococcus aureus isolates in our study exhibited Oxacillin resistance of 37.1 % which is consistent with the studies of Kavitha et al and Karlowsky et al who reported a percentage of 49.5 % and 29% [10,8] . This is in contrast with the studies of Atul garg et al who reported a percentage of 75.6%. [6] Vancomycin resistance in our Staphylococcal isolates was 25.7 %. Kamga etal recorded an isolation of 32 % which was in accordance with our study.In contrast the studies of Sharma Metal and Roy etal reported no resistance to Vancomycin . [9,12] Keeping in view the Oxacillin resistance of 37.1% and vancomycin resistance of 25.7% in Staphylococcal isolates in our area , drugs like Quinopristin/Dalfopristin , Cotrimoxazole or Linizolid could be considered more useful in the treatment of MRSA than Vancomycin .[16] . CONS isolates in our study showed no resistance to multiple drugs indicating that CONS isolates from blood are mostly contaminants. [15] The incidence of vancomycin resistant enterococci in our study is 33.3% which is accordance with the study of Diaz Granados et al who reported percentage of VRE as 42.3% . [17] The other studies on VRE which were in contrast to our study were those of Mathur etal and Kapoor et al who reported 1.8 % and 8% VRE respectively. [18,19] The increasing glycopeptide resistance in hospitals could be due to increased use of Vancomycin in hospital settings which emphasizes the importance of strict antibiotic policy to prevent emergence and spread of antibiotic resistance in bacteria.[17]

Among the gram negative isolates , the Enterobacteriaceae isolates in our study showed no resistance to combination drugs like Amoxyclav and Piperacillin – Tazobactam . This is in accordance with the studies of Atul garg et al , Kavitha et al and Karlowsky et al,[6,8,10] . In our study, the Pseudomonas isolate was not susceptible to combination drugs.This is in contrast to the studies of Rahbar Metal and Vargese GK et al in which the susceptibility to Piperacillin- Tazobactam was 68% and 58% respectively. [18,19] The efficacy of Piperacillin – Tazobactam can be expected only in clinical centres in which Pseudomonas aeruginosa resistance to Piperacillin is low.[9] ESBL producers detected in our study were 32.6% which is in accordance with the study of Kavitha et al and Arora et al who reported prevalence of ESBL producers as 32% and 34.4% respectively. [8,10]

Conclusion: Staphylococcus aureus and organisms belomging to Family Enterobacteriaceae are the leading causes of septicaemia., a pattern similar to that of other low income countries . The most sensitive drugs for gram positive isolates were Erythromycin , Ofloxacin ,Ceftriaxone , Imipenem and Piperacillin- Tazobactam and the most sensitive drugs for gram negative bacteria were Amoxyclav and Piperacillin- tazobactam .Increasing incidence of drug resistant organisms like Vancomycin resistant Staphylococcus aureus,Vancomycin resistant Enterococci and ESBL producing Klebsiella and Esch.coli calls for increased efforts to ensure more rational use of these drugs .Clinicians should exercise caution in their use of Vancomycin in order to preserve this useful antibiotic and prolong its therapeutic usefulness

and replace its use by drugs like Cotrimoxazole and Linizolid.

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