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PREVALENCE OF MICROBIAL ISOLATES IN BLOOD CULTURES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PROFILES

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ABSTRACT
The aim of our study was to determine the epidemiological profile and the antibiotic susceptibility of bacteria identified in blood culture to improve empirical antibiotherapy. The study was retrospective in nature and carried out at the Microbiology Laboratory of Shalamar Medical and Dental College, Lahore; data generated from blood culture specimens over 2 year period (Aug 2009 – Aug 2011) was compiled, relevant information such as age, sex, organism recovered and antibiotic susceptibility patterns were obtained from patients records. During the 2years period 450 blood cultures were received from pediatrics / neonatology wards / OPD and from adult patients. In a total of 450 blood cultures, 75 yielded monomicrobial growth. An incidence of positive blood culture was 16.6% (75/450) that includes Gram – negative bacilli 60% and Gram – positive cocci 40%. The most frequently identified species were Staphylococcus epidermidis (16%), Staphylococcus saprophyticus (10.7%), Staphylococcus aureus (13.3%) and Enterobacteriaceae accounted for 60% included Escherichia coli 16%, Klebsiella pneumonae 13.3% and pseudomonas aeroginosa10. 10.7% the most common bacterial isolates, and the other bacteria isolated were Citrobacter / Enterobacter the least 5.4%, Proteus spp. and Salmonella typhi / paratyphi; were 8% acinotobacter and pasturella were 6.6%. Antimicrobial susceptibility to 16 antimicrobial agents were determined by minimum inhibitory concentration (MIC) using standard Kirby Bauer's method. None of the antibiotics tested was 100% active against isolates. Staphylococcus aureus and coagulase negative Staphylococci showed no resistance to glycopeptides. Most Gram positive isolates were (100%) sensitive to vancomycin, and 50 – 75% of the Gram – negative isolates were sensitive to ciprofloxacin and amikacin. It is concluded that E.coli, Klebsiella and Staphylococcus aureus remain the principal organisms responsible for blood infection in a tertiary care setting.

INTRODUCTION

Bacteraemia, is the presence of bacteria in the blood, an episode of the presence of bacterial colonization of 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.[1-3] Septicaemia on the other hand is obviously a medical emergency. Bloodstream Infections (BSIs) are a major cause of morbidity and mortality worldwide.[4] Usually, the bloodstream is sterile. 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.[3,5] 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.[6-7] Bacteriological culture to isolate the offending pathogen and knowledge about sensitivity pattern of the isolates remains the main stay of definitive diagnosis and management of BSI. Microorganisms present in blood stream continuously or intermittently are threat to every organ in the body. Blood stream infection (BSI) is a serious problem that needs immediate attention and treatment. It is a cause of high mortality especially if caused by multidrug resistant bacteria.

The results of bacteriological cultures and antibiotic susceptibility tests take 3 – 4 days. Thus it is a common practice to institute early empirical therapy with broad – spectrum antibiotics in patients presenting with clinical features suggestive of bacteraemia. The present retrospective analysis was carried out to determine the pattern of bacterial agents responsible for blood stream infection (BSI) in a tertiary care hospital of Lahore 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

Present study was based on retrospective analysis of data about blood culture results, of specimens sub-

mitted for culture to Microbiology Laboratory of Shalamar Teaching Hospital, Lahore.

Four hundred fifty blood culture bottles (both paediatric and adult) containing appropriate amount of blood in Tryptic Soy Broth with SPS were received from the patients visited or admitted to Shalamar Hospital, Lahore; during the period Aug. 2009 – Aug 2011. These were incubated overnight at 35°C. After 24 hours incubation these samples were sub cultured on Blood and MacConkey's agar plates and incubated at 35°C overnight. Identification of growth was based on colony morphology, Gram staining and appropriate biochemical test performing.[6] Susceptibility to different antibiotics based on the type of growth was performed on Muller Hinton agar by standard Kirby Bauer's method.[7] Antibiotic susceptibility of bacterial isolates from blood determined for Gram positive includes Augmentin, ciprofloxacin, erythromycin, gentamycin and vancomycin. Gram negative bacilli specially enterobacteriaceae family tested against amikacin, augmentin, ciprofloxacin, ceftriaxone, cefotaxime, cotrimoxazole and gentamycin. For non-fermenter the plus carbinicillin and pipercillin sensitivity was analysed. Finally two combinations of antibiotics like sulbactum + cefoperazone and ceftazidime + clavulinic acid only for Gram negative isolates were tested.

Table 1: *Incidence of micro-organisms in 75 positive blood cultures*

Micro-organisms	*Number (%)*
Staphylococcus aureus	10 (13.3%)
Coagulase Negative Staphylococci	20 (25.1%)
Proteus species	01 (1.33%)
Klebsiella pneumonae	10 (13.3%)
Escherichia coli	12 (16%)
Salmonella typhi / paratyphi	05 (6.7%)
Citrobacter / Enterobacter	04 (5.3%)
Acinetobacter	04 (5.3%)
Pasturella	01 (1.33%)
Pseudomonas spp	08 (10.7%)

Table 2: *Antimicrobial susceptibility pattern of bacteria isolated from blood.*

Antibiotics	Disc contents ug/ml	Entero-bacteriaceae	Non-fermenter	Gram – positive cocc
Amikacin	30	73.8	57.3	---------
Augmentin	30	46.2	26.8	70
Ciprofloxacin	5	50.0	52.6	80
Ceftriaxone	30	53.8	52.6	---
Cefotaxime	30	54.0	57.8	---
Gentamicin	10	50.0	47.3	66.6
Imipenem	100	---	57.0	----
Erythromycin	30	---	---	63.3
Vancomycin	20	---	---	100
Cotrimoxazole	25	46.2	63.2	50
Cefoperazone+ sulbactum	30 + 75	84.2	84.2	---
Ceftazidime + clavlinic acid	30 + 10	80.5	80.5	

RESULT

The present retrospective analysis revealed that, in a total of 450 blood cultures, 75 yielded monomicrobial growths. An incidence of positive blood culture was 16.6% (75/450) which includes Gram – negative bacilli 60% (45/75) and Gram – positive cocci 40% (30/75). The most frequently identified species were (13.3%), Staphylococcus aureus. Staphylococcus epidermidis (16%), Staphylococcus saprophyticus (10.7%) and Enterobacteriaceae accounted for 60%, Escherichia coli 16% Klebsiella spp 13.3% and pseudomonas 10.7% were the most common bacterial isolates, Proteus spp. and Salmonella typhi / paratyphi; were 8% acinotobacter and pasturella were 6.6% and Citrobacter / Enterobacter spp the least 5.4%, (Table 1). Amongst the antibiotic used for susceptibility testing Amikacin showed the highest activity (73.8%) against the enterobacteriaceae and ciprofloxacin (78.9%) against non-fermenters. However cefoperazone + sulbactum showed maximum activity (84.2%) for Gram negative isolates. For Gram positive bacteria vancomycin and ciprofloxacin showed the highest activity (Table 2).

DISCUSSION

In the present retrospective study 75 (16.9%) blood culture were positive for growth. The rate (16.9%) of bacterial isolation in the blood culture in this study was relatively low compared to some previous studies done in Nigeria, namely; (44.9%),[8] (Pakistan, Islamabad) (42%),[10] (44%),[3] (68.4%).[11] While in other studies the incidence of microbial recovery is relatively low, in Nigeria, (4.1%),[11] (6.1%).[13] In the concurrent analysis the incidence of bacterial isolation was (16.9%). It is in accordance with the study of other workers (18.2%),[14] (20.2%),[15] (22%)[16] and (24.5%)[17] in previous studies carried out on blood culture samples collected from patients of suspected BSI from different hospitals.

In the present analysis gram positive bacterial agents account for (30%) while (60%) isolates were gram negative. It is in accordance with the study of other workers (49%) and (51%) (Elouenassann et al; 2008) (69.3%) and (30.7%),[14] (52.7%) and (47.3%).[15] The most frequent Gram negative organism isolated were E.coli (16.0%), klebsiela pneumonia (13.3%), pseudomonas aerogenosa (10.7%) being the commonest microbes. Staphylococcus aureus (13.3%) followed by coagulase negative staphylococci (25.1%) were the major gram positive isolates. There was a total of 60.0% of gram negative bacteria isolation, with predominance of E. coli (16.0.3%), Klebsiella (13.3%). Similarly, prevalence of E.coli / Klebsiella pneumonae gram – negative bacterial etiology of septicemia in children has been recorded by several other Nigeria authors[16] reporting from eastern Nigeria and central Nigeria, respectively. In India,[14,15,19,20] reported E. coli and klebsiella the most prevalent isolate. In another study (Elouenassann et al; 2008) recovered acinetobacter followed by staphylococcus the most frequently identified microbe. On the other hand, some Nigerian authors had recorded preponderance of S. aureus as bacterial cause of septicemia in neonates[21] reporting from northern Nigeria, (Tambeker et al; 2007) claimed staphylococcus the most common cause of bacteremia. Pseudomonas aeroginosa, one of the important non-fermenter was isolated in (10.7%) of cases where as it has been reported to be (7.63%)[15] and (9.8%).[23] It might be that most of the patient in study were indoor cases. Acinetobacter was isolated in (5.3%) culture which has been reported (13.6%) by[18] (32%).[20] Salmonella species and Enterobacter were reported (6.7%) and (2.7%) respectively in present study, Regarding Enterobacter sepsis was expressed in a report from Islamabad (80.9%).[10] Salmonela infection was reported by (Karuiki S et al; 2006) (3.5%).[25] Thus, predominance of either the gram – positive or gram – negative bacterial isolates is influenced by geographical location. In some studies candida isolates were (4.73%)[15] (1.4%)[25] (karunakaran R et al; 2007) and (5.9%).[23] While candida isolates were not recovered in the current study.

In this retrospective analysis antimicrobial used for susceptibility testing in Gram negative bacilli, Amikacin, showed (73.05%) sensitivity against enterobactriaceae family, similar results were documented (76.6%)[19,13] highly active drug against gram negative (fermenter) bacteria while for non-lactose fermenters, including, pseudomonas, acinetobacter and proteus, Ciprofloaxine showed the highest antimicrobial activity (78.9%), followed by amikacin (57.8%). In other studies Ciprofloxacin the most effective drug for gram positive and gram negative bacteria (87%).[14,20] All the antimicrobials used for Gram positive isolates, Vancomycine, showed the highest (100%) activity. This can be compared to the results (87%).[17] The present study showed that Ceftriaxone and Cefotaxime were highly effective in vitro against gram negative, especially E. coli. This is consistent with the study of (Elouenassann et al; 2008)[19,27] while, (93.7%)[25,10] (83%)[11] recovered that high resistance seen amongst Enterobacteriaceae against 3rd generation cephalosporins. However, cefoperazone + sulbactum (a combination of drug put up for Gram negative isolates showed the highest activity (82.6%) among all antimicrobial agents. Combination of anti-microbial agents is often prescribed as empiric therapy for suspected BSI. In the present study, a significance percentage of gram negative isolates showed in vitro susceptibility to combination therapy consisting of Sulbactum + cefoperazone and Ceftazidime + Clauvilinic acid. It is important for clinician to be updated with current data concerning the efficacy of commonly prescribed agents, and selection of anti-microbial to be used for empiric therapy based on rate of susceptibility and site of infection.

In ***Conclusion*** a regular epidemiological study of blood culture isolates and determination of susceptibility to antibiotics are necessary to improve empirical therapy.

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