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Bacteriological profile and antibiotic sensitivity pattern of isolates from blood culture in suspected septicemic patients attending tertiary care hospital

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ABSTRACT

Introduction: Septicemia is a leading cause of morbidity and mortality in India. Blood culture remains the gold standard for the diagnosis of septicemia. The antimicrobial sensitivity pattern differs in different studies. Knowledge of likely causative organisms of septicemia and their antimicrobial sensitivity pattern can help to start appropriate therapy in order to minimize morbidity and mortality.
Aim: To isolate the etiological organisms causing sepsis and study the antimicrobial susceptibility profile and its mechanism of resistance pattern.
Materials and Methods: The observational study of 206 positive blood culture was carried out in the Department of microbiology, tertiary Care Hospital during the period from December 2015 to November 2016 and processed by standard conventional method. Antibiotic susceptibility pattern of isolates was studied by Kirby Bauer Disc diffusion technique.
Observations & Results: Total 1281 samples were received during the study period of which 206 (16.08%) samples were found to be positive. Bacteria isolated include CONS (15.04%), S.aureus (14.08%), Klebsiella pneumonia (19.90%), Acinetobacter spp (10.19%), Escherichia coli (8.74%), Pseudomonas spp (7.77%), Salmonella typhi (1.46%) and Salmonella paratyphi A (0.49%). Majority of organisms Isolated were resistant to commonly used antibiotics. Imipenem showed 83% and colistin 94.69% sensitivity for gram negative organisms. Methicillin resistance was found in 2.91% *Staphylococcus aureus* Isolated. The Gram positive bacteria showed high resistance to Penicillin G (75%) but they were highly susceptible to Azithromycin (70%), Levofloxacin (80%) Linezolid (100%) and Vancomycin (98%)
Conclusions: *Klebsiella pneumoniae* was the most predominant etiological agent of septicemia. Every hospital should monitor its antibiotic sensitivity pattern against the common isolates that can serve as a basis for empirical therapy in emergency conditions. Considering the burden of mortality resulting from septicemia, better diagnostic facilities should be employed for the early detection of septicemia and rational use of narrow spectrum and antibiotics is recommended.

1. Introduction

Septicemia is defined as the presence of microorganisms or their toxins in the bloodstream.[1] Bacteremia indicates the presence of bacteria in the circulating blood; it may be transient, continuous or intermittent.[2] Septicemia is a leading cause of morbidity and mortality in India. Neonates are particularly vulnerable to infection (septicemia) because of their weak immune system.[3] Septicemia may present with nonspecific signs and symptoms – severe febrile episodes with fever, chills, malaise, tachycardia, mental confusion, hyperventilation, hypotension or shock.[4] Various conditions in which bacteria are present in the blood stream include manipulation of infected tissues, instrumentation of contaminated mucosal surfaces, bacterial endocarditis, typhoid fever, undrained abscesses, meningitis, pneumonia etc. causing significant septicemia.[5] Septicemia is caused either by a single type of organism or it may be caused

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by multiple species of bacteria. Recent literature suggests that the incidence of polymicrobial bacteremia is increasing. Blood culture remains the gold standard for the diagnosis of septicemia.[6,7]

The vascular compartment is sterile and usually intact. Microbes gain entry from breakages of blood vessels adjacent to skin or mucous surfaces or by phagocytic cells carrying organisms into capillaries or the lymphatic system. Gram-negative lipids (endotoxins) or Gram-positive toxins initiate a cascade of events involving cytokines, interleukin-2, vascular mediators and platelets leading to hypotension. This process becomes irreversible and produces failure of all major organs so sepsis is life threatening emergency that demands urgent diagnosis and treatment. High rate of antibiotic resistance against bacterial pathogen has worsened the situation. Detection of causative organisms and their antibiotic susceptibility is crucial for diagnosis of sepsis in order to initiate the appropriate antibiotic treatment therapy which reduces the adverse effects of antibiotic treatment on patient prognosis; hence we had done study to identify most common organisms and its sensitivity pattern in our hospital.

The information obtained from this study will help in de-escalating the antibiotics and to prevent indiscriminate and unnecessary use of antibiotics which contribute to the emergence of drug resistance strains in environment. In this study we had included all age group of suspected septicemic patient attending tertiary care hospital.

2. Materials and Methods

After approval from Institutional Ethical Committee, Out of 1281 total blood culture samples of suspected septicemic patients, 206 positive blood culture samples were included in present study. The observational study was carried out in the Department of microbiology, tertiary Care Hospital during the period from December 2015 to November 2016.

2.1. Sample collection

Careful skin preparation before collecting the blood sample is of paramount importance to reduce the risk of introducing contaminants into blood culture media. Choose the vein by touching the skin before it has been disinfected. Using 2% iodine soaked gauze cleanse the skin over the venepuncture site in a circle approximately 5 cm in diameter. Allow the iodine to dry on the skin for at least one minute. The timing is critical. Starting in the center of the circle apply 70% alcohol soaked gauze and clean the skin. Insert the needle into the vein and withdraw blood. Do not change needle before injecting the blood into the culture bottle. Standard precautions require that phlebotomists wear gloves for blood drawing. At least 10 ml of blood should be obtained from adult and 5 ml from children for each venepuncture. It is generally recommended that routine blood cultures be obtained from different venepuncture sites at least 1 hour apart. In cases of an acute febrile episode that may require immediate empiric antibiotic therapy, two separate venepunctures should be performed from opposite arms. It is generally accepted that two blood culture bottles should be inoculated from each venepuncture - an aerobic bottle and an anaerobic bottle. The venous blood injected into brain heart infusion in the ratio of one part of blood to five parts of the broth containing Sodium polyanetholsulfonate in concentration of 0.025% to 0.05% is the best anticoagulant available for blood. In addition to its anticoagulant properties SPS is also anticomplementary, antiphagocytic and interferes with the activity of some antimicrobial agents. Blood drawn for culture must not be allowed to clot.

In addition to the volume of blood cultured and type of medium chosen, the dilution factor for the blood in the medium must be considered. For this purpose a 1:5 or 1:10 ratio of blood to unmodified medium has been found to be adequate in conventional blood cultures. All commercial blood culture systems specify the appropriate dilution.

Culture medium consists of brain heart infusion broth (BHIB). Blood culture bottles were incubated at 37 °C aerobically in incubator overnight and After incubation blood culture bottles were carefully examined for macroscopic evidences of growth such Hemolysis of RBC, Gas in medium, Uniform or surface turbidity, surface pellicle and white grains on the surface or deep in the blood layer. Then after performing Gram Staining first do subculture was made after 18-24 hours onto Blood agar, MacConkey agar and Chocolate agar plate, if no any growth is indicated, they were further incubated for overnight and again sub cultured. This was repeated at 48 hours, 72 hours and on 7^{th} days before declaring culture negative. Next day growth of organism was noted. If there was growth, colony morphology was studied and Gram staining was done. Once organism was isolated, identification of bacteria and its spp. was done by standard bacteriological identifications methods like colony morphology, motility and different biochemical reactions. Organisms like *Salmonella*, its identity was confirmed by slide agglutination reaction.

Antibiotic susceptibility testing was done by Kirby Bauer method of disk diffusion method. The isolates were grown in peptone water by incubating it at 37°C for 2 hours and turbidity matched with 0.5 MacFarland standard tube. They were then lawn cultured onto Mueller Hinton agar (MHA) plate and commercial antibiotic discs were placed on the surface. The plates were incubated overnight at 37°C in the incubator and a report of sensitive, intermediate or resistant was interpreted also resistant pattern was identified as CLSI guideline. As For all the tests, positive and negative controls were kept.

3. Observation & Result

Culture positivity is 16.08 %, 206 positive blood culture out of 1282 total blood culture samples. Out of 96 Blood culture sample of male & 110 of female. A total of 206 patients were included in this study 55.34% cases were of pediatric age group and 44.66% were adults.

Table 1: Showing study of age distribution (No. of cases – 2O6)

Age group	No.of cases	Percentage (%)
<1 month	81	39.32
1 month to 1 year	14	6.80
2 year to 5 year	11	5.34
6 year to 12 year	6	2.91
13 year to 18 year	2	0.97
>19 year	92	44.66
Total	206	100

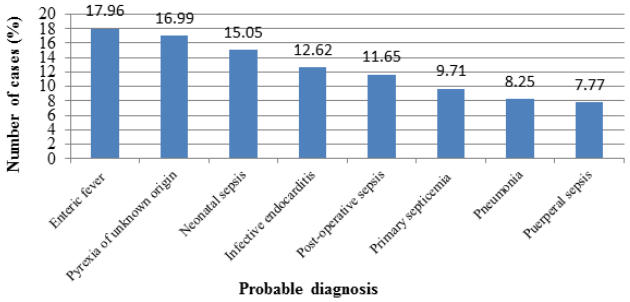

Fig. 1: Various probable diagnosis in patients of septicemia

Figure 1 shows the incidence of various clinical diagnosis in patients of septicemia: Enteric fever (17.96%) was the commonest disease followed by pyrexia of unknown origin (16.99%) and neonatal sepsis (15.05%). Other underlying disorders were infective endocarditis, post-operative sepsis, primary septicemia, pneumonia and puerperal sepsis.

Study shows positive culture in relation to appearance of growth of pathogens: Maximum isolates were noticed within 36 hours i.e. 92.23% and 2.43% after 36 to 48 hours in blood culture. Further subcultures was performed on next 5^{th} days, 5.34 % organisms were isolated from blood culture on 5^{th} day.

Gram negative organisms (54.85 %) were predominant followed by Gram positive organisms (45.14 %)

4. Discussion

Definitive diagnosis tests on a positive blood culture, to identify the pathogen and determine its antibiotic susceptibility pattern. With this background, the present study was conducted in the Tertiary Care Hospital during December 2015 to November 2016 to study the

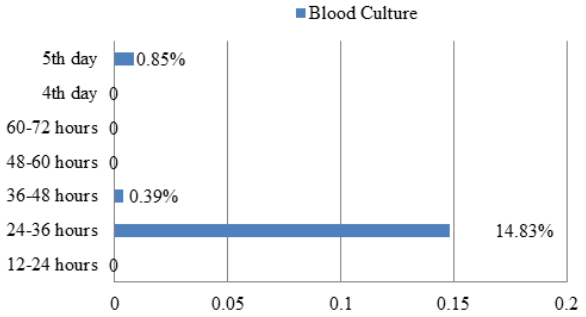

Fig. 2: Blood cultures in relation to appearance of growth of pathogens

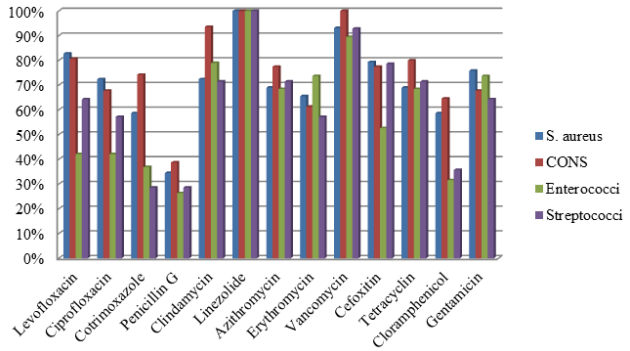

Fig. 3: Antimicrobial sensitivity of Gram positive isolates (% of sensitive

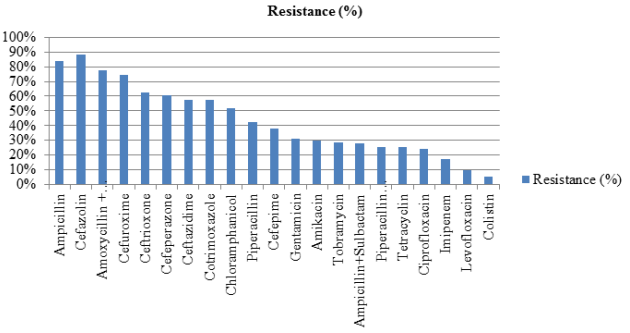

Fig. 4: Resistant pattern of various drugs for gram negative isolates

bacteriological profile of septicemia and their antibiotic susceptibility pattern. Most of the studies conducted in India and abroad are related with septicemia in neonates and infants.[8] Reports of bacteriological profile of septicemia in patients of all age groups are very few. With this background the present study was undertaken to know the profile of septicemia in patients from all age groups.

Septicemia is a leading cause of morbidity and mortality in India. Sepsis remains the most important cause of multi-organ dysfunction syndrome (MODS) all over the world. Septicemia remains a major and challenging clinical

Table 2: Showing isolation of bacteria in various clinical conditions: (n=206)

Bacteria isolated	Probable Diagnosis								
	Enteric fever	PUO	Neonatal Sepsis	Infective endocarditis	PostOperative Sepsis	Primary Septicemia	Pneumonia	Puerperal septicemia	Total
Klebsiella pneumonia	8	4	7	1	3	5	6	7	41
CONS	6	5	3	8	4	2	1	3	32
Staphylococcus aureus	9	2	3	2	4	3	4	2	29
Acinetobacter spp.	1	4	6	1	3	1	4	1	21
Enterococci spp	1	6	1	6	2	3	0	-	19
E.coli	-	6	4	2	2	2	-	2	18
Pseudomonas spp	5	-	4	-	3	2	1	1	16
Streptococci spp	4	2	-	6	1	-	1	-	14
Citrobacterspp	-	4	2	-	1	2	-	-	9
Salmonella species	3	1	-	-	-	-	-	-	4
Enterobacter spp.	-	1	1	-	1	-	-	-	3
Total	37	35	31	26	24	20	17	16	

Table 3: Showing various Gram positive organisms isolate from blood culture (No. of cases-206)

Organisms isolated		No. of isolates	Percentage (%)
Coagulase negative staphylococcus species		31	15.04
	MSSA	23	11.16
Staphylococcus aureus	MRSA	6	2.91
Enterococci spp.		19	9.22
Streptococcus spp.		14	6.80
Total		93	45.14

Table 4: Showing Gram negative organisms isolated from blood culture (No. of cases-206)

Organisms isolated	No. of isolates	Percentage (%)
Klebsiella pneumoniae	41	19.90
Acinetobacter spp	21	10.19
Escherichia coli	18	8.74
Pseudomonas spp	16	7.77
Citrobacter spp.	9	4.37
Salmonella typhi	3	1.46
Enterobacter spp.	4	1.94
Salmonella paratyphi A	1	0.49
Total	113	54.85

problem throughout the world.

There were 46.61% males and 53.39% females. Various studies carried out in India shows that septicemia is more commo n in males. Khatua *et al*[9] (1986) postulated that the factors regulating the synthesis of gamma globulin are probably situated on the X chromosome. Immunity in males is less than females. Testosterone can suppress the immunity whereas the estrogen has beneficial effect.[10] In our study septicemia in female patients was found to be more common. This is because of inclusion of puerperal sepsis cases in this study. Puerperal sepsis is a major cause of maternal mortality in a community.[11] Patients with positive blood culture were more likely to die during hospitalization than patients without positive blood cultures.

There are various organisms can cause septicemia. The causative organisms in sepsis vary from place to place and the frequency of the causative organisms is different in different hospitals and even in the same hospital at different time.

Enteric fever was the commonest clinical presentation (17.96%) followed by pyrexia of unknown origin (16.99%), Neonatal sepsis (15.05%) and infective endocarditis (12.62%). Amatya *et al*[12] (2007) found that enteric fever was the commonest clinical diagnosis. The result of our study is also consistent with the study of Amatya *et al*[12]

In the present study, Blood culture positivity was 16.08%. The result of our study is consistent with the study of Agnihotri et al[13] and Sudharshan et al.[14] Kumar, Qunibi, Neal et al[15] (2001) had reported that a period of 36 hours is

enough to rule out sepsis. In the present study, out of 206 isolates 190 (92.23%) isolates were detected within 24 to 36 hours.

In the present study out of total isolates 206, Gram negative organisms were predominant 113(54.85%) followed by Gram positive organisms 93 (45.14%).

Isolation of CONS as most common Gram Positive organisms can't be overlooked as commensals or contaminants because in all those patient signs of Septicemia were present and CONS grown as single isolate and many of risk factor were present.[16,17] Careful evaluation shoud be done before instituting therapy to avoid unnecessary use of antibiotics[18] S.aureus is also important blood stream infection which is rapidly increasing due to suboptimal adherence to infection control practices, wide use of multiple antibiotics and increasing prevalence of diabetes mellitus Therefore whenever instituting empiric antibiotic therapy for suspected septicemia, coverage of *Staphylococcus aureu*s must be emphasized.

Although the most common probable diagnosis was enteric fever, Salmonella isolates are in 1.94% cases only. Mathur *et al*[19] (1994) reported 2.4% and Kumar *et al*[20] (2004) reported 5.4% and Atul Garg[21] reported 14.2% *Salmonella typh*i septicemia by blood culture. The reason of low isolation rate may be due to the habit of antibiotic administration in patients of fever before the blood is collected for culture. The emphasis should be given on collection of blood for paired blood culture from all probable septicemic patients before administering any antibiotics.

Gram negative multidrug resistant organisms were the main cause of septicemia in all age groups. Therefore great caution is required in selection of antibiotic therapy.[22]

The antimicrobial sensitivity pattern differs in different studies as well as at different times in the same hospital. In the view of the above facts the strategy of antibiotics usage in patients should be reviewed regularly even in the same hospital.

Sensitivity pattern of Gram Positive Isolates:

In our study most Gram negative isolates were resistant to *Ampicillin* (84%); Mehdinejad *et al*[23] (2009)observed that the Gram negative bacilli showed highest resistance to ampicillin (98.5%). In the present study, *enterobacteriaceae* isolates were resistant to *ceftazidime* (87.5%) and *cefotaxime* (76.79%) respectively. As many as 67.15% *gram negative* isolates in the present study were sensitive to *amikacin*. *Gram negative*p (7.77 %) strains in our study. The 16 strain showed susceptibility to piperacillin/tazobactum (75%), imipenem (68.75%), ciprofloxacin (81.25%), amikacin (75%), gentamicin (43.75%), and ceftazidime (37.5%).

4.1. Sensitivity pat tern of Gram Positive Isolates

*Staphylococcus aureu*s and *Coagulase negative staphlylococcu*s species showed 100% sensitivity to linezolid, 94.62 % sensitivity to vancomycin and maximum resistance to penicillin G (62.37%). In the present study we isolated 19(9.22%) strains of *Enterococci*. The strain was found sensitive to linezolid (100 %) and vancomycin (89.47%). *Streptococcus species* was found to be 100% sensitive to linezolid, 92.86 % sensitive to vancomycin and 28.57% sensitive to penicillin G. The antimicrobial sensitivity pattern differs in different studies as well as at different times in the same hospital. In the view of the above facts the strategy of antibiotics usage in patients should be reviewed regularly even in the same hospital.

5. Conclusion

No age was exempted from septicemia. Klebsiella pneumoniae was the most predominant etiological agent of septicemia. Antibiotic sensitivity of different organisms was variable but Levofloxacin and Piperacillin + Tazobactam were found to be effective against the majority of organisms. Antibiotic resistance to commonly used cephalosporins is increased among Gram negative organisms. Colistin, Imipenem, piperacillin + tazobactum and Levofloxacin are the best alternative to which organism are highly sensitive. Methicillin resistant staphylococcus aureus (MRSA) and multidrug resistant organisms is a great risk for epidemic among admitted patients. Considering the burden of mortality resulting from septicemia, better diagnostic facilities should be employed for the early detection of septicemia and rational use of narrow spectrum antibiotics is recommended.

6. Source of Funding

None.

7. Conflict of Interest

None.

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