

“Automated Blood Culture for the Detection of Septicemia”

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Abstract

Blood culture is a microbiological culture of blood. It is employed to detect infections that are spreading through the bloodstream (such as bacteremia, septicemia amongst others). The blood culture represents a critical tool for the health care professional as a means of detecting the dangerous presence of living organism in the blood stream. A positive blood culture can suggest a definitive diagnosis, enable the targeting of therapy against the specific organisms and provide prognostic value (Bryan C.S 1989). In blood culture, false positive arises due to contamination which occurs when organisms that are not actually present in a blood sample are grown in culture.

Faced with a positive blood culture result, clinicians must determine whether the organism represents a clinically significant infection associated with great risk of morbidity and mortality. Further complicating the issue in recent years is the increasing use of central venous catheters (CVC) and other indwelling vascular access devices (Anonymous 1986, Data summary from October 1986- April 1996, issued in May 1996)(National Nosocomial Infections surveillance system 2004, Data summary from January 1992 through June 2004, issued October 2004)

Interpretation of culture result for patients with these devices in place is particularly challenging because while these individuals are at increased risk for bacteremia, such results may also indicate culture contamination or colonization of the line. It has been demonstrated in a number of studies of both adult and pediatric patients (Bates, D.W; Goldman and T.H. Lee 1991)(Segal, G.S; and J.M Chamberlain 2000)(Souvenir, D;D.E. Anderson, Jr; S Polpant, H Mroch, S.Askin, J.Anderson, J.Claridge, J. Eiland, C. Malone, M.W Garrison, P.Watson, and D.M Campbell 1998) .

The college of American pathologists (CAP) Q Probes quality improvement study involved the prospective examination of 497,134 blood culture specimen from 640 U.S health care institutes (Schifman, R.B and A. Pindur 1993). While for other, it has more than 5% of their blood culture were contaminated. The financial impact of blood culture contamination has been described in a number of studies (Bates, D.W; L. Goldman and T.H Lee 1991) (Dunagan, W.C; R.S Woodward, G. Medoff, J.L Gray III, E. Casabar, M.D Smith, C.A Lawrenz and E. Spitznagel. 1989)(Waltzman, M.L; and M. Harper 2001)

Bates et al. found that contaminant results, compared with true negative result, were independently associated with increased subsequent charges and intravenous antibiotic charges (Bates D.W; L.Goldman and T.H Lee 1991). In a subsequent study focused on blood culture contamination caused by coagulase negative staphylococci, Souvenir et.al reported that almost half of the patients with a false positive result were treated with antibiotics, often with Vancomycin (Souvenir D; D.E Anderson, Jr; S Palpant, H Mroch, S. Askin, J. Anderson, J. Claridge, J. Eiland, C. Malone, M.W. Garrison, P.Watson and D.M Campbell 1998).

In a retrospective study of 9,959 blood cultures performed in children aged 1 month to 18 years, Thuleret. Al found that 26% of children with false positive culture who were initially evaluated as outpatients were subsequently admitted to the hospital on the basis of initial culture results (Thuler, L.C; M. Jenicek, J.P Turgeon, M.Rivard, P. Lebel and M.H Lebel 1997). The presence of living microorganisms in blood has substantial clinical importance. From the diagnostic standpoint a positive blood culture yielding a clinically important microorganism represents either failure of host defences to contain an infection at its primary focus. A positive blood culture however is not always clinically significant, since contamination may occur or the positive result may represent the presence of microorganisms in the blood (Everett E.D, Hirschmann IV 1997).

A Comprehensive analysis of positive blood culture in 1990s, authored by two of the current investigators (Weinstein M.P, Reller L.B, Murphy J.R, Lichtenstein K.A, 1983). The primary goal is to determine the current microbiology, epidemiology and importance of positive blood culture, with special references to similarities and differences (Gold, Polsky 1986).

Identification of organisms:

Bates et. al. found that the identification of organisms was the most important predictor for differentiate the contaminated blood culture results from indicating bacteremia or septicemia (Bates D.W; and T.H Lee 1991)

Weinstein et.al study positive blood culture in adult inpatients from 3 hospitals around the country suggested that certain organisms should almost always be thought to represent true septicemia or fungemia when isolated from a blood culture (Weinstein, M.P; M.L Towns, S.M Quartey, S.Mirrett, L.G Reimer, G. Parmigiani and L.B Reller 1997). These organisms included Staphylococcus aureus, S.pneumoniae, E.coli and other Enterobacteriaceae, Pseudomonas aeruginosa and candida albicans. Weinstein's personal observation is that the following organisms always represent a true infection when isolated from blood culture, Streptococcus pyogenes, S.agalactiae, Haemophilus influenza, members of all bacteriology fragilis group, all candida species and Cryptococcus neoformans (Weinstein, M.P 2003)

Along the same vein, certain organisms have been found to represent contamination in a significant proportion of cases. These organisms include coagulase negative staphylococci, Bacillus species, Micrococcus species, Streptococci, Enterococci and clostridium perfringens (Weinstein M.P; M.L Towns, S.M Quartey, S. Mirrett, L.G Reimer, G.Parmigini and L.B Reller 1997) .However, it is crucial to recognize that each of these organisms can also represent true bacteremia or septicemias. Of these organisms, the ones that are thought to represent true bacteremia or septicemia are corynebacterium species, Bacillus species and other B. Anthracis (Weinstein M.P 2003).

Coagulase negative staphylococci were usually believed to represent contamination when isolated from blood cultures. Hence it is the most common blood culture contaminants which represents 70% to 80% of all contaminated blood cultures (Calfee, D.P; and B.M Farr 2002)(Norberg, A.N.C.Christopher, M.L Ramunodo, J.R Bower and S.A Berman 2003)(Rubin L.G; P.J Sanchez, J.Siegel, G. Levine, L.Saimann 2002)

Weinstein et al found that even though only 12.4% of coagulase negative staphylococcal isolates were clinically significant, they ranked as the third most common cause of septicemia. Similarly, other organisms can be difficult to interpret when isolated from blood cultures. One study found that enterococci were pathogens 70% of the time, whereas viridians group streptococci were pathogens 38% of the time (Weinstein, M.P; M.L Towns, S.M Quarty, S.Mirrett, L.G Reimer, G.Parmigiani and L.B Reller 1997). Clostridium perfringens was contaminants 77% of the time, whereas other clostridium species were true pathogens 80% of the time. Bloodstream infections involve only a single organism, prompting clinicians to sometime conclude that a blood culture bottle that grows multiple organisms is contaminated. However, studies have found that 6% to 21% of all true bacteremia are polymicrobial, usually in patients in high risk group (Sharma, M.K, Riederer, L.B Johnson and R. Khatib 2001).

Multiple coagulase negative species have been found to cause polyclonal coagulase negative staphylococci infections (Galdbart, J.O; A.Morvan, (N.Desplace and N.elSolh 1999) (Van Eldere, J; W.E Peetermans, S.VanLierde and J. Van Eldere, 1997).

Number of positive blood culture bottles within a blood culture sets:

Another method that has been used by health care workers to differentiate contaminated blood cultures from culture that represent septicemia in the number of blood culture bottles that exhibit growth within a given blood culture set. Theoretically, if one bottle exhibits growth within a given set. The likelihood of contamination is greater. However, there is at least one study that suggested that these criteria should not be used for this purpose (Mirrett S; L.B Reller, C.A Petti, C.W Woods, M.L Wilson, M.P Weinstein 2001).

In the study, Mirrett et al. found that the number of bottles positive for coagulase negative staphylococci within blood culture sets comprising two, three or four bottles was not correlated with the likelihood of infections using clinical parameters(Mirrett S; L.B Reller, C.A Petti, C.W Woods, M.L Wilson, M.P Weinsten 2001)

Usually, a set of blood cultures involve one aerobic bottle and one anaerobic bottle in an attempt to optimize the isolation of both aerobic and anaerobic organisms. It makes sense intuitively that if growth of a given organisms is more likely in aerobic conditions than anaerobic conditions, the number of positive culture bottles within a set that consist of one aerobic conditions and one anaerobic bottle should not be used to differentiate contaminated from clinically significant cultures. Recent studies have found that the presence of growth for a given organism varies between the aerobic and anaerobic bottle. One study of 644 positive blood culture sets found that 413(59.8%) were recovered from both bottle, 206(29.8%) were recovered only from aerobic bottle and 2(10.4%) were recovered only from anaerobic bottle (Saito, T; K Senda, S. Takaura, N. Fujihara, T. Kudo, Y.Linuma, M.Tanimoto and S.I schiyama 2003).

In that study, the aerobic bottle was significantly superior to the anaerobic bottle for both recovery and detection time for overall organisms and there was no significant difference in detection time for facultative anaerobic bacteria between the two bottles. Another study of coagulase negative staphylococcal isolates found that the majority (59.7%) of isolates grew in the aerobic bottle only, whereas 27.7% grew in both bottles and 12.5% grew only in the anaerobic bottle (Khatib, R; K.M Riederer, J.A Clark, S. Khatib, L.E Briski and F.M Wilson 1995). Another factor in determining contamination that has been explored by several investigators is the amount of time required for the organism to grow in the culture medium. It is thought that perhaps the blood from septicemic patients will have a much higher inoculum of bacteria than a contaminated culture. Theoretically, it follows that a larger inoculum will grow faster than a small inoculum, a theory that seems to have verified in prior studies of catheter related bloodstream infections (Catton; J.A; B.M Dubbins, P. Kite, J.M Wood, K.Eastwood, S. Sugden, J.A Sandae, D. Burke, M.J Mc Mahon and M.H Wilcox 2005)(Frankin J.A; A.H Gaur, J.L Shenep, X.J Hu and P.M Flynn 2004)(Innes, G.K Roland, E. Grafstein and J.M Christenson 2000)(Rogers, M.S; and B.A Oppenheim 1998).

In support of this theory, several studies have shown that cultures that become positive more than 3 to 5 days after incubation have been more likely to represent contamination (Evans, M.R; A.L Truant, J. Kostmann and L. Locke 1991)(Hans. X.Y; and A.L Truant 1999)(Herwaldt, L.A; B.B Hulbert and P.C Migneault 1992)(Huang. A.H; J.J Yan and J.J Wee 1998)(Kurlat I; B.J Stoll and J.E Mac Gowan Jr. 1989)(Mozes B; L.B Reller; C.A Petti; C.W Woods, B. Vaiziran, R. Sivadas and M.P Weinstein 2003). Because it can be difficult to obtain more than one set of cultures in the pediatric populations.

Haimi- Cohen et al used clinical parameters to differentiate true coagulase negative infections from contaminants and found that a time to positivity of <15 hr had a positive predictive value of 84% for true infection in children (Haimi- Cohen, Y;S Shaffnoori, V. Tucci and L.G Rubin 2003)

Bates et al found that the time to growth was a useful variable in a multivariable algorithm for predicting true septicemia from a positive culture results, although it did not perform as well as either the identification of the organism or the presence of multiple positive cultures (Bates, D.W; and T.H Lee 1995). Souvenier et al study which differentiated coagulase negative staphylococcus contaminated cultures from septicemia or bacteremia in the time for detection of a positive culture.

Khatib et al's molecular study of 47 episodes of multiple positive culture for coagulase negative staphylococci found that time to growth does not help differentiate cultures that grew identical strains from culture sets that differed by strain. The difference in time to detect the contaminants versus true positive coagulase negative staphylococci was statically significant. The time to growth and sensitivity for detecting growth can be expected to change, making the use of this technology in this regard questionable.

Population studies:

In the pediatric population, investigators have studied the risk of occult bacteremia, given the widespread adoption of vaccinations to prevent infection caused by Haemophilus Influenza and streptococcus pneumonia (Alpern, E.R; E.A Alessandrini, L.M Bell, K.N Shaw and K.L; McGowan 2000)(Bandyopadhyay, S; J. Bergholte, C.D Blackwell, J.R Friedlander and H. Hennes 2002). In a study of well appearing, highly

febrile children aged 2 to 36 months, Stoll and Rubin found that the incidence of occult bacteremia was 0.91% and concluded that universal blood culture testing in this population may be unnecessary.

Herz et al conducted a retrospective review of blood cultures obtained from children aged 3 to 36 months during a five year period from 1998 to 2003. Bacteremia rates dropped from 1.62% to 0.71% from the first year to the last year of the study period, while the contaminant to pathogen ratio increased from 1:2:1 to 2:3:1, leading the authors to agree with Stoll and Rubin about the decreased role for blood culture testing in highly febrile children in this age group. Innes et al found that during a 6 month period at a hospital, blood culture result from patients seen in the emergency department were rarely helpful. With only 2.1% of 767 cultures yielding potentially helpful, with only 2.1% of retrospective study of 1,350 patients who had blood cultures taken in the emergency department, only 0.52% had results that potentially affected their management.

Contaminated blood cultures are a particular challenge for infants and children for several reasons. Evidence suggests that contamination occurs more frequently in this population, particularly in young infants. In addition, concerns about the risk of occult bacteremia it have led to use blood culture and empirical therapy, particularly in children less than 3 year of age (Baraff, L. J; J.W Bass. G.R Fleisher, J.O Klein, G.H McGacken, Jr; K.R Powell, D.L; Schriger, et al 1993). The current era of influenza and pneumococcal vaccination, the risk of occult bacteremia has significantly lowered. As a result, the use of blood culture testing on this patient's population is associated with a lower positive predictive value. Moreover, analysis of current practice patterns reveals that in most cases, only single blood cultures are collected. In an effort to reduce unnecessary discomfort, pediatricians often use existing intravenous catheters for obtaining cultures instead of peripheral venipuncture.

Ramsook et al found that similar results in a 6 month study of 2,431 pediatric blood cultures with contamination rates of 3.4% for specimen collected via intravenous catheters versus 2.0% for those obtained by separate venipuncture. Ramsook single blood cultures are particularly common in pediatric patients combined with the increased utilization of catheter based culture contamination challenging, particularly when coagulase negative staphylococci are grown in culture (Ammann, R.A; A. Hirt, A.R Luthy and C. Aebi 2003) .

Neonatal septicemia is an important cause of morbidity and mortality among neonates in India. With an estimated incidence of approximately 4% in intramural livebirths (Indian Pediatric 1997). Delayed treatment until clinical recognition of signs and symptoms of sepsis entails risk of preventable mortality, presumptive antibiotic therapy may result in overtreatment. A wide variety of bacteria both aerobic and anaerobic can cause neonatal septicemia. Blood culture was done for all neonates suspected to have septicemia.

All blood culture was collected from a peripheral vein with proper aseptic precautions before starting any antibiotic therapy. Approximately 3 ml of blood was inoculated into brain heart infusion broth and incubated at 37 c. subcultures were made on both blood and Mac conkey agar after 24 and 48 hrs. Growth if any, was identified by the standard bacteriological techniques (Cruickshank k, Duguid J P, Marmion BP, Swain RHA, Livingstone 1975), including gram staining, colony characteristics, biochemical properties. Antibiotic sensitivity was performed by Stroke's Disc Diffusion method (Strokes EJ, Arnold 1975).

Antimicrobial susceptibility Test:

The result of direct antimicrobial susceptibility testing of positive blood cultures have been shown to correlate well with those of standardized tests. In most of the foregoing studies, blood culture samples were subculture to broth to allow the adjustment of the inocula before susceptibility testing (Johnson, J.E; and J.A Washington III 1976) (Kiehn, T.E; C. Capitolo and D. Armstrong 1982) (Mirrett, S; and L. BarathReller 1979) (Wegner, D.L; C.R Mathis and T.R Neblett 1979).

Reller et al recommend that the inoculum density should be adjusted to match a 0.5 Mcfarland turbidity standard for direct susceptibility tests for blood cultures (Reller. L.B; P.R Murray and J.D Maclowry 1982). It is common practice in many laboratories to report presumptive

susceptibilities after 4 to 6 hr incubation of direct disk diffusion tests from unadjusted blood cultures. This study was undertaken to test the accuracy of 4 to 6 hr readings of disk diffusion tests that were inoculated directly from positive blood cultures.

Arbo et al found that blood cultures have a limited effect on antibiotic choices as they are observed that there was underutilization of the culture and sensitivity results. The most common used antibiotic was Quinolone particularly Ciprofloxacin (39%) followed by Chloramphenicol (22%) and Co- amoxiclav (16%). Monotherapy was also started in 50% cases of secondary bacteremia, the combinations of Clindamycin+ an aminoglycoside and Ciprofloxacin + Aminoglycoside and Ciprofloxacin + Aminoglycoside were the preferred antibiotic. Evaluation of the antibiotic regimen given showed only 7 out of 29 or 24.1% were used. This is comparable to the study of Edwards Id, et al 1973, Arbo MDJ 1994, Kunin CM, Tupasi TE, Craig WA 1973 and Castle M et al 1977.

In the study of Arbo, modification of antibiotic therapy consisted of addition of antibiotics. This is also true in our study, wherein 48% of the antibiotic therapy is superfluous. Both the studies of Kunin and Castle showed less than 50% of approximately Antibiotic use. In neonates, Vancomycin is still the drug of choice of S.aureus but resistance to this drug has also been reported (Jick S. 1997). A combination of Ciprofloxacin and Amikacin appears to be the best choice for infections due to Klebsiella. These findings are in tandem with the National Neonatal Perinatal Database.

Treatment with Ciprofloxacin is also indicated in multidrug resistant S.aureus in the pediatrics age group, but its use in neonates is still experimental due to lack of safety data (Karthikeyan G, Premkumar K 2001, Jick S 1997).

Automated blood culture system:

The advent of automated blood culture detection systems allows significantly earlier detection of most aerobic bloodstream pathogens than manual systems. (Rohner P et al 1995)

Introduction

Septicemia is the presence of bacteria in the blood (bacteremia) and is often associated with severe disease. Its alternative name is blood poisoning (bacteremia with sepsis). Septicemia is a serious, life-threatening infection that gets worse very quickly. It can arise from infections throughout the body, including infections in the lungs, abdomen, and urinary tract. It may come before or at the same time as infections of the bone (osteomyelitis), central nervous system (meningitis), or other tissues. Sepsis is a

severe illness caused by overwhelming infection of the bloodstream by toxin-producing bacteria. Its alternative name Systemic inflammatory response syndrome (SIRS) .Sepsis is caused by bacterial infection that can originate anywhere in the body. Blood cultures should be obtained (PRIOR to initiation of antimicrobial therapy) for any patient, in whom there is suspicion of bacteremia, including hospitalized patients with fever and leukocytosis or leukopenia. Circumstances in which blood cultures are especially important include sepsis, meningitis,

osteomyelitis, arthritis, endocarditis, pneumonia, and fever of unknown origin.

The infection is often confirmed by a positive blood culture, though blood cultures may be negative in individuals who have been receiving antibiotics. In sepsis, blood pressure drops, resulting in shock. Major organs and systems, including the kidneys, liver, lungs, and central nervous system, stop functioning normally. Systemic inflammatory response syndrome or SIRS is evidence of the body's ongoing inflammatory response. When SIRS is suspected or known to be caused by an infection, this is sepsis. Severe sepsis occurs when sepsis leads to organ dysfunction, such as trouble breathing, coagulation or other blood abnormalities, decreased urine production, or altered mental status. If the organ dysfunction of severe sepsis is low blood pressure (hypotension), or insufficient blood flow (hypoperfusion) to one or more organs (causing, for example, lactic acidosis), this is septic shock. Even though bacteremia and sepsis are closely

related, they are two separate conditions. The simple presence of bacteria in the blood is known as bacteremia. It may be transient, where small quantities of bacteria are in the

blood for a limited period of time, or it can be sustained, where the bacteria persist and multiply in the bloodstream. The sustained form of bacteremia is usually what leads to sepsis, which is the body's immune response to the presence of the bacteria. This potentially fatal condition, sometimes referred to as blood poisoning, involves an inflammatory response by the entire body and is characterized by increased body temperature, heart rate, and respiratory rate, and in its severe form can lead to organ failure, extreme low blood pressure, or septic shock.

Sepsis can lead to multiple organ dysfunction syndrome (MODS) (formerly known as multiple organ failure), and death. Organ dysfunction results from local changes in blood flow, from sepsis-induced hypotension (< 90 mmHg or a reduction of ≥ 40 mmHg from baseline) and from diffuse intravascular coagulation, among other things. Sepsis can be defined as the body's response to an infection. An infection is caused by microorganisms or bacteria invading the body and can be limited to a particular body region or can be widespread in the bloodstream. Sepsis is acquired quickest with infections developed in surgery and physical contact with someone

with sepsis. Just over one-third of sepsis patients have positive blood cultures, mainly due to inadequate sampling volumes (50% of adults have < 1.0 CFU/mL blood) and the prior use of antibiotics. However, 20-30% of sepsis patients are given inappropriate empirical antibiotics. The Clinical and Laboratory Standards Institute guidelines recommend paired culture sets to help discriminate between contaminant organisms and true pathogens; four 10-mL bottles (2 sets) should be used for the initial evaluation to detect about 90-95% of bacteremias and six 10-mL bottles (3 sets) should be used to detect about 95-99% of bacteremias. It has also been shown that the positivity rate increased by 15-35% with resin-based media in patients on antibiotics. For diagnosing catheter-related bloodstream infections, differential time-to-positivity is one method recommended to help determine whether the catheter is truly the source of infection. The proper training of personnel with regard to drawing an appropriate blood volume and the importance of clear labeling of culture bottles is also of critical importance. Furthermore, if the contamination rate is relatively high, hiring dedicated staffs that are well-trained in order to get a lower blood culture contamination rate may be cost-effective. It is because high

false-positive blood culture rates due to contamination are associated with significantly increased hospital and laboratory charges.

Bacteremia is the presence of viable bacteria in the bloodstream. Likewise, the terms viremia and fungemia simply refer to viruses and fungi in the bloodstream. These terms say nothing about the consequences this has on the body. For example, bacteria can be introduced into the bloodstream during tooth brushing (Lockhart, P. B.; Brennan, M. T.; Sasser, H. C.; Fox, P. C.; Paster, B. J.; Bahrani-Mougeot, F. K. (2008) This form of bacteremia almost never causes problems in normal individuals. However, bacteremia associated with certain dental procedures can cause bacterial infection of the heart valves (known as endocarditis) in high-risk patients (Wilson, W.; Taubert, K. A.; Gewitz, M.; Lockhart, P. B.; Baddour, L. M.; Levison, M.; Bolger, A.; Cabell, C. H. et al (2007). Conversely, a systemic inflammatory response syndrome can occur in patients without the presence of infection, for example in those with burns, polytrauma, or the initial state in pancreatitis and chemical pneumonitis (Bone, R.; Balk, R.; Cerra, F.; Dellinger, R.; Fein, A.; Knaus, W.; Schein, R.; Sibbald, W. (1992).

Blood cultures are used to detect the presence of bacteria or yeasts in the blood, to identify the microorganism(s) present, and to guide treatment. Two or more blood cultures are typically ordered and collected as consecutive samples. Often, a complete blood count (CBC) is ordered along with or prior to the blood culture to determine whether the person has an increased number of white blood cells, indicating a potential infection. Sometimes other testing is also performed, such as a chemistry panel to evaluate the health status of a person's organs, or a urine, sputum, or cerebrospinal fluid (CSF) culture to help identify the source of the original infection. This is especially true when a person has symptoms associated with a urinary tract infection, pneumonia, or meningitis.

It is important to eliminate all of the bacteria that are causing the problem. For some infections, several weeks of treatment are necessary. This is especially important if you have endocarditis, which requires weeks of antibiotic therapy to cure. The bacteria or yeast must grow in the nutrient media before they can be detected and identified. Usually this happens within a couple of days, but in some cases and with some microorganisms it can take longer. Furthermore, some microorganisms are

present in the blood in very small numbers. They must have a sufficient time to reproduce. Additional blood cultures may be drawn to determine if bacteria present in the culture are persistent in the blood stream (true pathogens). If they are not present in follow-up cultures, then bacteria from the skin may have contaminated the initial cultures. Additional blood cultures may also be drawn if you continue to have signs of sepsis but no microorganism is recovered from the first cultures collected and grow to quantities that can be detected. In general, patients with bacteremia are likely to have low quantities of bacteria in the blood, even in the setting of severe clinical symptoms. For this reason, multiple blood cultures, each containing large volumes of blood, are required to detect bacteremia. Prior to initiation of antimicrobial therapy, at least two sets of blood cultures taken from separate venipuncture sites should be obtained. The technique, number of cultures, and volume of blood are more important factors for detection of bacteremia than timing of culture collection; these are discussed further in the following sections.

Technique — Careful technique is important to avoid contamination of the blood culture media by normal skin flora during the

process of collection. This is important because normal bacterial skin flora can cause systemic disease such as infective endocarditis, and in some circumstances blood culture contamination can make it difficult to distinguish between false positive results and true infection. Important measures to reduce contamination include effective disinfection of the venipuncture site and avoiding blood culture collection through existing intravenous lines.

The tourniquet should be applied and the vein palpated before disinfection of the venipuncture site. Thereafter the venipuncture site should be cleansed with 70 percent alcohol followed by 1 to 2 percent tincture of iodine or chlorohexidine. The disinfectant should be allowed to dry for one to two minutes before blood is aspirated. If further palpation of the vein is necessary after skin preparation, a sterile glove should be worn. Alcohol should be used to disinfect the septum of culture bottles after removal of their flip caps. Blood should be collected directly into culture bottles during the venipuncture procedure, rather than into transport tubes sent to the laboratory for subsequent transfer of blood into the culture bottle.

Definitions for this study for the most part were identical to those of Weinstein et al. (WeinsteinMP, MurphyJR, RellerLB, LichtensteinKA 1983)

Only a few will be detailed here. New or modified definitions also will be detailed.

True septicemia vs. contamination. Each positive blood culture was assessed critically by one of the investigators, each an infectious disease physician. All isolates were categorized as true-positives, contaminants, or of unknown clinical significance. The categorical decision was made after the following factors were taken into account: the patient's clinical history, physical findings, body temperature at the time of the blood culture, leukocyte count and differential cell counts, number of positive blood cultures out of the total number performed, results of cultures of specimens from other sites, imaging results, histopathologic findings, and clinical course and response to therapy. If the clinical significance of the positive culture was not clear on the basis of the available information, the isolate was categorized as being of unknown significance.

Episode. An episode of bacteremia, fungemia, or mycobacteremia was defined by the first positive blood culture in a series

or by any new positive blood culture result that occurred >48 hours after the previous positive result, unless it was clear to the investigator that the new positive culture was part of the same episode. For example, a positive blood culture for *Staphylococcus aureus* on the fifth day of antibiotic therapy for *S. aureus* endocarditis was considered part of the original bacteremic episode.

Community- vs. hospital-acquired septicemia. Each episode was classified as either community- or hospital-acquired septicemia, according to guidelines of the Centers for Disease Control and Prevention (Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM 1988) Septicemia in nursing home patients with positive blood cultures was always classified as hospital-acquired, whereas that in patients transferred from other outside facilities was classified according to their total period of hospitalization at both institutions.

The effect of age on outcome of bacteremia and fungemia was assessed according to the decade of life of the infected patients. As shown in figure 1, septicemia-associated mortality gradually increased with age and was especially notable after the age of 70 years. The infection-associated mortality for

septicemic patients over 70 years of age was 24.2%, compared with 15.2% for patients 70 years of age or less. In comparison with other patients, the relative risks of death from bacteremia and fungemia for patients between 71 and 80 years of age and >80 years of age were 2.58 and 3.4, respectively. There were no significant differences in infection-associated mortality between septicemic men and women.

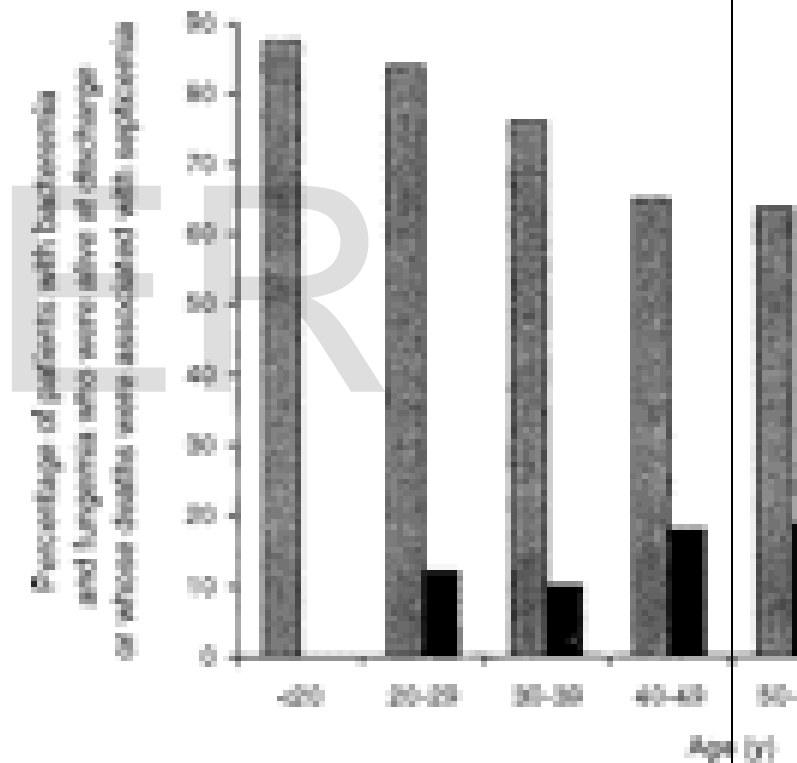


Figure 1. Outcome of bacteremia or fungemia, according to age group of patients (▨ = alive at discharge; ■ = death, associated with septicemia).

Aims and Objectives

Aim: Automated blood culture for detection of Septicemia

Objectives:

- 1) To study the profile of bacterial isolates from blood culture
- 2) To study antimicrobial resistance pattern of the isolates
- 3) To assess the advantages of Automated blood culture
- 4) To study the frequency of blood culture positivity in clinically suspected cases of septicemias.

Review of Literature

Blood culture is a microbiological culture of blood. It is employed to detect infections that are spreading through the bloodstream (such as bacteremia, septicemia amongst others). The blood culture represents a critical tool for the health care professional as a means of detecting the dangerous presence of living organism in the blood stream. A positive blood culture can suggest a definitive diagnosis, enable the targeting of therapy against the specific organisms and provide prognostic value (Bryan C.S 1989). In blood culture, false positive arises due to contamination which occurs when organisms that are not actually present in a blood sample are grown in culture.

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summary from January 1992 through June 2004, issued October 2004)

Interpretation of culture result for patients with these devices in place is particularly challenging because while these individuals are at increased risk for bacteremia, such results may also indicate culture contamination or colonization of the line. It has been demonstrated in a number of studies of both adult and pediatric patients (Bates, D.W; Goldman and T.H. Lee 1991)(Segal, G.S; and J.M Chamberlain 2000)(Souvenir, D;D.E. Anderson, Jr; S Polpant, H Mroch, S.Askin, J.Anderson, J.Claridge, J. Eiland, C. Malone, M.W Garrison, P.Watson, and D.M Campbell1998) .

The college of American pathologists (CAP) Q Probes quality improvement study involved the prospective examination of 497,134 blood culture specimen from 640 U.S health care institutes (Schifman, R.B and A. Pindur 1993). While for other, it has more than 5% of their blood culture were contaminated. The financial impact of blood culture contamination has been described in a number of studies (Bates, D.W; L. Goldman and T.H Lee 1991) (Dunagan, W.C; R.S Woodward, G. Medoff, J.L Gray III, E. Casabar, M.D Smith, C.A Lawrenz

and E. Spitznagel L. 1989)(Waltzman, M.L; and M. Harper 2001)

Bates et al. found that contaminant results, compared with true negative result, were independently associated with increased subsequent charges and intravenous antibiotic charges (Bates D.W; L.Goldman and T.H Lee 1991). In a subsequent study focused on blood culture contamination caused by coagulase negative staphylococci, Souvenir et.al reported that almost half of the patients with a false positive result were treated with antibiotics, often with Vancomycin (Souvenir D; D.E Anderson, Jr; S Palpant, H Mroch, S. Askin, J. Anderson, J. Claridge, J. Eiland, C. Malone, M.W. Garrison, P.Watson and D.M Campbell 1998).

In a retrospective study of 9,959 blood cultures performed in children aged 1 month to 18 years, Thuler et. Al found that 26% of children with false positive culture who were initially evaluated as outpatients were subsequently admitted to the hospital on the basis of initial culture results (Thuler, L.C; M. Jenicek, J.P Turgeon, M.Rivard, P. Lebel and M.H Lebel 1997). The presence of living microorganisms in blood has substantial clinical importance. From the diagnostic standpoint a positive blood

culture yielding a clinically important microorganism represents either failure of host defences to contain an infection at its primary focus. A positive blood culture however is not always clinically significant, since contamination may occur or the positive result may represent the presence of microorganisms in the blood (Everett E.D, Hirschmann IV 1997).

A Comprehensive analysis of positive blood culture in 1990s, authored by two of the current investigators (Weinstein M.P, Reller L.B, Murphy J.R, Lichtenstein K.A, 1983). The primary goal is to determine the current microbiology, epidemiology and importance of positive blood culture, with special references to similarities and differences (Gold, Polsky 1986).

Identification of organisms:

Bates et. al. found that the identification of organisms was the most important predictor for differentiate the contaminated blood culture results from indicating bacteremia or septicemia (Bates D.W; and T.H Lee 1991)

Weinstein et.al study positive blood culture in adult inpatients from 3 hospitals around the country suggested that certain organisms should almost always be thought to represent true septicemia or fungemia

when isolated from a blood culture (Weinstein, M.P; M.L Towns, S.M Quartey, S.Mirrett, L.G Reimer, G. Parmigiani and L.B Reller 1997). These organisms included Staphylococcus aureus, S.pneumoniae, E.coli and other Enterobacteriaceae, Pseudomonas aeruginosa and candida albicans. Weinstein's personal observation is that the following organisms always represent a true infection when isolated from blood culture, Streptococcus pyogenes, S.agalactiae, Haemophilus influenza, members of all bacteriology fragilis group, all candida species and Cryptococcus neoformans (Weinstein, M.P 2003)

Along the same vein, certain organisms have been found to represent contamination in a significant proportion of cases. These organisms include coagulase negative staphylococci, Bacillus species, Micrococcus species, Streptococci. Enterococci and clostridium perfringens (Weinstein M.P; M.L Towns, S.M Quartey, S. Mirrett, L.G Reimer, G.Parmigini and L.B Reller 1997) .However, it is crucial to recognize that each of these organisms can also represent true bacteremia or septicemias. Of these organisms, the ones that are thought to represent true bacteremia or septicemia are corynebacterium species,

Bacillus species and other B. Anthracis (Weinstein M.P 2003).

Coagulase negative staphylococci were usually believed to represent contamination when isolated from blood cultures. Hence it is the most common blood culture contaminants which represents 70% to 80% of all contaminated blood cultures (Calfee, D.P; and B.M Farr 2002)(Norberg, A.N.C.Christopher, M.L Ramunodo, J.R Bower and S.A Berman 2003)(Rubin L.G; P.J Sanchez, J.Siegel, G. Levine, L.Saimann 2002)

Weinstein et al found that even though only 12.4% of coagulase negative staphylococcal isolates were clinically significant, they ranked as the third most common cause of septicemia. Similarly, other organisms can be difficult to interpret when isolated from blood cultures. One study found that enterococci were pathogens 70% of the time, whereas viridians group streptococci were pathogens 38% of the time (Weinstein, M.P; M.L Towns, S.M Quarty, S.Mirrett, L.G Reimer, G.Parmigiani and L.B Reller 1997). Clostridium perfringens was contaminants 77% of the time, whereas other clostridium species were true pathogens 80% of the time. Bloodstream infections involve only a single organism,

prompting clinicians to sometime conclude that a blood culture bottle that grows multiple organisms is contaminated. However, studies have found that 6% to 21% of all true bacteremia are polymicrobial, usually in patients in high risk group (Sharma, M.K, Riederer, L.B Johnson and R. Khatib 2001).

Multiple coagulase negative species have been found to cause polyclonal coagulase negative staphylococci infections (Galdbart, J.O; A.Morvan, (N.Desplace and N.elSolh 1999) (Van Eldere, J; W.E Peetermans, S.VanLierde and J. Van Eldere, 1997).

Number of positive blood culture bottles within a blood culture sets:

Another method that has been used by health care workers to differentiate contaminated blood cultures from culture that represent septicemia in the number of blood culture bottles that exhibit growth within a given blood culture set. Theoretically, if one bottle exhibits growth within a given set. The likelihood of contamination is greater. However, there is at least one study that suggested that these criteria should not be used for this purpose (Mirrett S; L.B Reller, C.A Petti, C.W Woods, M.L Wilson, M.P Weinstein 2001).

In the study, Mirrett et al. found that the number of bottles positive for coagulase negative staphylococci within blood culture sets comprising two, three or four bottles was not correlated with the likelihood of infections using clinical parameters(Mirrett S; L.B Reller, C.A Petti, C.W Woods, M.L Wilson, M.P Weinsten 2001)

Usually, a set of blood cultures involve one aerobic bottle and one anaerobic bottle in an attempt to optimize the isolation of both aerobic and anaerobic organisms. It makes sense intuitively that if growth of a given organisms is more likely in aerobic conditions than anaerobic conditions, the number of positive culture bottles within a set that consist of one aerobic conditions and one anaerobic bottle should not be used to differentiate contaminated from clinically significant cultures. Recent studies have found that the presence of growth for a given organism varies between the aerobic and anaerobic bottle. One study of 644 positive blood culture sets found that 413(59.8%) were recovered from both bottle, 206(29.8%) were recovered only from aerobic bottle and 2(10.4%) were recovered only from anaerobic bottle (Saito, T; K Senda, S. Takaura, N. Fujihara, T. Kudo, Y.Linuma, M.Tanimoto and S.I schiyama 2003).

In that study, the aerobic bottle was significantly superior to the anaerobic bottle for both recovery and detection time for overall organisms and there was no significant difference in detection time for facultative anaerobic bacteria between the two bottles. Another study of coagulase negative staphylococcal isolates found that the majority (59.7%) of isolates grew in the aerobic bottle only, whereas 27.7% grew in both bottles and 12.5% grew only in the anaerobic bottle (Khatib, R; K.M Riederer, J.A Clark, S. Khatib, L.E Briski and F.M Wilson 1995). Another factor in determining contamination that has been explored by several investigators is the amount of time required for the organism to grow in the culture medium. It is thought that perhaps the blood from septicemic patients will have a much higher inoculum of bacteria than a contaminated culture. Theoretically, it follows that a larger inoculum will grow faster than a small inoculum, a theory that seems to have verified in prior studies of catheter related bloodstream infections (Catton; J.A; B.M Dubbins, P. Kite, J.M Wood, K.Eastwood, S. Sugden, J.A Sandae, D. Burke, M.J Mc Mahon and M.H Wilcox 2005)(Frankin J.A; A.H Gaur, J.L Shenep, X.J Hu and P.M Flynn 2004)(Innes, G.K Roland, E. Grafstein and J.M Christenson

2000)(Rogers, M.S; and B.A Oppenheim 1998).

In support of this theory, several studies have shown that cultures that become positive more than 3 to 5 days after incubation have been more likely to represent contamination (Evans, M.R; A.L Truant, J. Kostmann and L. Locke 1991)(Hans. X.Y; and A.L Truant 1999)(Herwaldt, L.A; B.B Hulbert and P.C Migneault 1992)(Huang. A.H; J.J Yan and J.J Wee 1998)(Kurlat I; B.J Stoll and J.E Mac Gowan Jr. 1989)(Mozes B; L.B Reller; C.A Petti; C.W Woods, B. Vaiziran, R. Sivadas and M.P Weinstein 2003). Because it can be difficult to obtain more than one set of cultures in the pediatric populations.

Haimi- Cohen et al used clinical parameters to differentiate true coagulase negative infections from contaminants and found that a time to positivity of <15 hr had a positive predictive value of 84% for true infection in children (Haimi- Cohen, Y;S Shaffnoori, V. Tucci and L.G Rubin 2003)

Bates et al found that the time to growth was a useful variable in a multivariable algorithm for predicting true septicemia from a positive culture results, although it did not perform as well as either the identification of the organism or the

presence of multiple positive cultures (Bates, D.W; and T.H Lee 1995). Souvenier et al study which differentiated coagulase negative staphylococcus contaminated cultures from septicemia or bacteremia in the time for detection of a positive culture.

Khatib et al's molecular study of 47 episodes of multiple positive culture for coagulase negative staphylococci found that time to growth does not help differentiate cultures that grew identical strains from culture sets that differed by strain. The difference in time to detect the contaminants versus true positive coagulase negative staphylococci was statically significant. The time to growth and sensitivity for detecting growth can be expected to change, making the use of this technology in this regard questionable.

Population studies:

In the pediatric population, investigators have studied the risk of occult bacteremia, given the widespread adoption of vaccinations to prevent infection caused by Haemophilus Influenza and streptococcus pneumonia (Alpern, E.R; E.A Alessandrini, L.M Bell, K.N Shaw and K.L; McGowan 2000)(Bandyopadhyay, S; J. Bergholte, C.D Blackwell, J.R Friedlander and H. Hennes 2002). In a study of well appearing, highly

febrile children aged 2 to 36 months, Stoll and Rubin found that the incidence of occult bacteremia was 0.91% and concluded that universal blood culture testing in this population may be unnecessary.

Herz et al conducted a retrospective review of blood cultures obtained from children aged 3 to 36 months during a five year period from 1998 to 2003. Bacteremia rates dropped from 1.62% to 0.71% from the first year to the last year of the study period, while the contaminant to pathogen ratio increased from 1:2:1 to 2:3:1, leading the authors to agree with Stoll and Rubin about the decreased role for blood culture testing in highly febrile children in this age group. Innes et al found that during a 6 month period at a hospital, blood culture result from patients seen in the emergency department were rarely helpful. With only 2.1% of 767 cultures yielding potentially helpful, with only 2.1% of retrospective study of 1,350 patients who had blood cultures taken in the emergency department, only 0.52% had results that potentially affected their management.

Contaminated blood cultures are a particular challenge for infants and children for several reasons. Evidence suggests that contamination occurs more frequently in this

population, particularly in young infants. In addition, concerns about the risk of occult bacteremia it have led to use blood culture and empirical therapy, particularly in children less than 3 year of age (Baraff, L. J; J.W Bass. G.R Fleisher, J.O Klein, G.H McGacken, Jr; K.R Powell, D.L; Schriger, et al 1993). The current era of influenza and pneumococcal vaccination, the risk of occult bacteremia has significantly lowered. As a result, the use of blood culture testing on this patient's population is associated with a lower positive predictive value. Moreover, analysis of current practice patterns reveals that in most cases, only single blood cultures are collected. In an effort to reduce unnecessary discomfort, pediatricians often use existing intravenous catheters for obtaining cultures instead of peripheral venipuncture.

Ramsook et al found that similar results in a 6 month study of 2,431 pediatric blood cultures with contamination rates of 3.4% for specimen collected via intravenous catheters versus 2.0% for those obtained by separate venipuncture. Ramsook single blood cultures are particularly common in pediatric patients combined with the increased utilization of catheter based culture contamination challenging, particularly when coagulase negative

staphylococci are grown in culture (Ammann, R.A; A. Hirt, A.R Luthy and C. Aebi 2003) .

Neonatal septicemia is an important cause of morbidity and mortality among neonates in India. With an estimated incidence of approximately 4% in intramural livebirths (Indian Pediatric 1997). Delayed treatment until clinical recognition of signs and symptoms of sepsis entails risk of preventable mortality, presumptive antibiotic therapy may result in overtreatment. A wide variety of bacteria both aerobic and anaerobic can cause neonatal septicemia. Blood culture was done for all neonates suspected to have septicemia.

All blood culture was collected from a peripheral vein with proper aseptic precautions before starting any antibiotic therapy. Approximately 3 ml of blood was inoculated into brain heart infusion broth and incubated at 37 c. subcultures were made on both blood and Mac conkey agar after 24 and 48 hrs. Growth if any, was identified by the standard bacteriological techniques (Cruickshank k, Duguid J P, Marmion BP, Swain RHA, Livingstone 1975), including gram staining, colony characteristics, biochemical properties.

Antibiotic sensitivity was performed by Stroke's Disc Diffusion method (Strokes EJ, Arnold 1975).

Antimicrobial susceptibility Test:

The result of direct antimicrobial susceptibility testing of positive blood cultures have been shown to correlate well with those of standardized tests. In most of the foregoing studies, blood culture samples were subculture to broth to allow the adjustment of the inocula before susceptibility testing (Johnson, J.E; and J.A Washington III 1976) (Kiehn, T.E; C. Capitolo and D. Armstrong 1982) (Mirrett, S; and L. BarathReller 1979) (Wegner, D.L; C.R Mathis and T.R Neblett 1979).

Reller et al recommend that the inoculum density should be adjusted to match a 0.5 Mcfarland turbidity standard for direct susceptibility tests for blood cultures (Reller. L.B; P.R Murray and J.D MacLowry 1982). It is common practice in many laboratories to report presumptive susceptibilities after 4 to 6 hr incubation of direct disk diffusion tests from unadjusted blood cultures. This study was undertaken to test the accuracy of 4 to 6 hr readings of disk diffusion tests that were inoculated directly from positive blood cultures.

Arbo et al found that blood cultures have a limited effect on antibiotic choices as they are observed that there was underutilization of the culture and sensitivity results. The most common used antibiotic was Quinolone particularly Ciprofloxacin (39%) followed by Chloramphenicol (22%) and Co- amoxiclav (16%). Monotherapy was also started in 50% cases of secondary bacteremia, the combinations of Clindamycin+ an aminoglycoside and Ciprofloxacin + Aminoglycoside and Ciprofloxacin + Aminoglycoside were the preferred antibiotic. Evaluation of the antibiotic regimen given showed only 7 out of 29 or 24.1% were used. This is comparable to the study of Edwards Id, el al 1973, Arbo MDJ 1994, Kunin CM, Tupasi TE, Craig WA 1973 and Castle M et al 1977.

In the study of Arbo, modification of antibiotic therapy consisted of addition of antibiotics. This is also true in our study, wherein 48% of the antibiotic therapy is superfluous. Both the studies of Kunin and Castle showed less than 50% of approximately Antibiotic use. In neonates, Vancomycin is still the drug of choice of S.aureus but resistance to this drug has also been reported (Jick S. 1997). A combination of Ciprofloxacin and Amikacin appears to

be the best choice for infections due to Klebsiella. These findings are in tandem with the National Neonatal Perinatal Database.

Treatment with Ciprofloxacin is also indicated in multidrug resistant S.aureus in the pediatrics age group, but its use in neonates is still experimental due to lack of safety data (Karthikeyan G, Premkumar K 2001, Jick S 1997).

Automated blood culture system:

The advent of automated blood culture detection systems allows significantly earlier detection of most aerobic bloodstream pathogens than manual systems. **(Rohner P et al 1995)**

MATERIALS AND METHOD

Place of study: - Microbiology Lab of Care hospital and Vishakha clinical microbiology laboratory.

Period of study: - From 2nd of January 2012 to 15th April 2012

Subject of study:-.Automated Blood culture for the detection of Septicemia.

Equipments:

- Beaker

- Conical flask
- Test tube
- Petri plate
- Inoculating loop
- Forcep
- Measuring cylinder
- Blood culture bottle
- Incubator
- Bac T Alert 3D system
- Cabinet (Hood)
- Burner
- Slide
- Autoclave
- Digital balance
- Refrigerator

Chemicals:

- Alcohol
- Methylene blue
- Crystal violet
- Saffranine
- Gram iodide
- Distilled water
- Muller Hinton agar
- Mac Conkey agar
- Blood agar

Sample: Blood sample from patient.

Methods:

1) Specimen collection for blood culture:

The skin of veinpuncture site was disinfected by applying 70% alcohol in water with 1% iodine for atleast 1 min and allow it to dry. 5-10 ml of blood was collected.

The collected blood was then inoculated directly in Bac T/ Alert blood bottles.

2) Blood culture:

Blood culture were carried out as per standard protocol (Krishna el at 2001, forbes B A et al 2002 & vandepitte T et al 2003)

Glucose broth was inoculated for 24 hr at 34- 35°c . A sterile culture usually showed a layer of sedimented red blood covered by a pale yellow transparent broth.

Growth was observed by-

- ✓ A floccular deposit on top of the blood layer
- ✓ Turbidity
- ✓ Coagulation of the broth
- ✓ Production of gas

Culture was done on blood agar and mac conkey agar after 24 hrs, 48 hrs, 72 hrs, and 5th day. If after 5th day, no growth was obtained, the sample was considered as negative. If it is

positive, colony morphology and standard biochemical tests were performed. (Refer Appendix 1,2)

3) *Bac T/Alert 3D System:*

Automated blood culture system designed to do blood culture in both aerobic and anaerobic cultures. The capacity is 120 blood culture bottles.

It is quick for the identification of bacteria grown in the bottle. It helps in quality control incorporated and also in data management. 24 hrs continuous scanning of bottles were done for growth.

The Power of Colorimetric Technology:

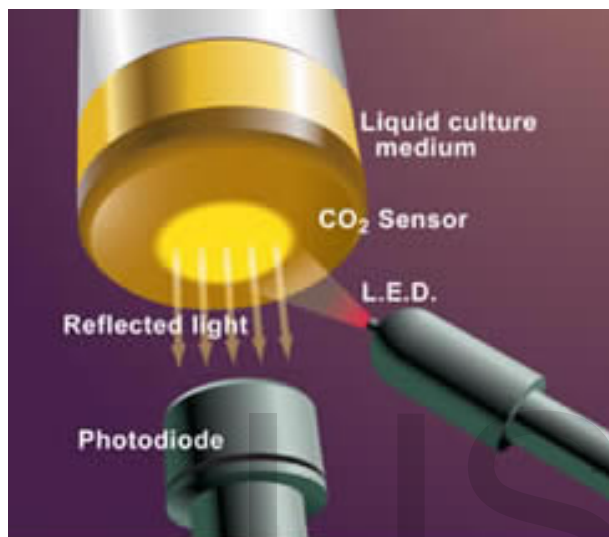
Results are the most important aspect of any microbial detection system. Built on bioMérieux's patented colorimetric technology, the BacT/ALERT 3D demonstrates excellent recovery of a wide range of organisms with >95% recovery within 24 hours and >98% within 72 hours. BacT/ALERT Culture Media offer the ability to detect aerobic microorganisms and high-acid-producing organisms, such as lactobacillus, yeasts and molds. BacT/ALERT 3D's patented colorimetric sensor-and-detection technology detects microorganism growth by tracking CO₂

production. Notification of positives is immediate and results are dependable.

Working

1. Microorganisms multiply in the media, generating CO₂. As CO₂ increases, the sensor in the bottle turns yellow.
2. Measuring reflected light, the BacT/ALERT 3D monitors and detects color changes in the sensor.
3. Algorithms analyze the data to determine positivity, and the laboratory is notified immediately with visual and audible alarms.
4. Changes in the sensor are permanent and visible to the unaided eye, unlike any other method.





BacT/ ALERT ® 3D MEDIA:

Bottle Type- BacT/ALERT PF (Pediatric FAN)

Media formulation- 20 ml peptone-enriched tryptic soy broth, supplemented with brain heart infusion solids and activated charcoal.

Characterization of isolates:

i) Colony morphology: colony morphology of isolated culture was studied

such as shape, size, colony and nature of

ii) Microscopic Appearance

Gram staining: The isolated cultures were stained by gram staining procedure (Cappuccino and Sherman, 1996). Bacterial smear were treated in following order, crystal violet, Alcohol, saffranine. Wash with water after each steps, smear were blot dried and observed microscopically. (Refer appendix 2)

5) Antimicrobial susceptibility test:

Antimicrobial susceptibility testing was performed by Kirby – Bauer disc diffusion susceptibility method performed in accordance to NCCLS guidelines for gram positive and gram negative isolates.

Muller Hinton agar plate is used for susceptibility. (Refer appendix 3)

At least four morphologically similar colonies from an agar medium were touched with a wire loop and transferred to a tube containing 5 ml of nutrient broth. It is then incubated for 3-4 hrs. at 37°C to produce a suspension i.e. turbidity. The density of suspension was matched with 0.5 MC Farland standard.

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Population

Growth

No

Total

growth

Adult	14	58	72
Children	20	92	112
Newborn	15	51	66
Total	49	201	250

After that a sterile cotton swab was dipped into the suspension and extra fluid was removed by rotating the swab against the side of the tube. It is then streaked over the entire surface of the plate in four directions.

The antibiotic discs were applied to the agar surface with a sterile forceps and gently pressed down to ensure contact. Commercially available discs (Hi Media, Mumbai) with known potency were used. (Refer Appendix 4).

Observation and results:

Table 1:

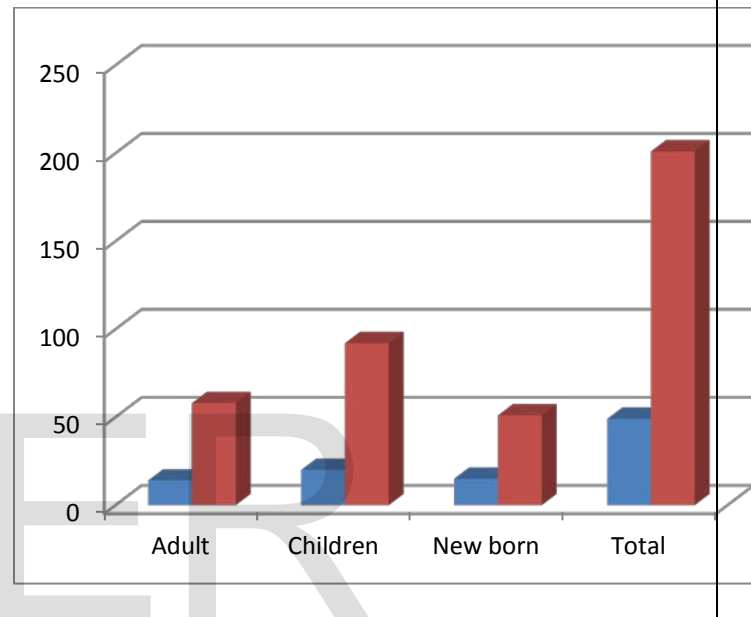


Table no 1 show that there are 250 blood culture samples from which 49 were showing positive blood culture sample in adults while 58 samples were showing negative culture in adult, while in children positive blood culture sample were found to 20 out of 112, and 92 were found to be negative in case of children. While new born shows positive culture 15 out of 66. And 51 were negative cultures in new born. From this table we can say that, the positivity arises more in children than in adult and new born patients.

Table 2:

Organisms	Number	%(n=49)
Pseudomonas	11	22.4
Staphylococcus	20	40.8
Klebsiella	6	12.2
Acinetobacter	1	2.04
E.coli	8	16.3
Proteus	1	2.04
Candida	1	2.04
Salmonella typhi	1	2.04

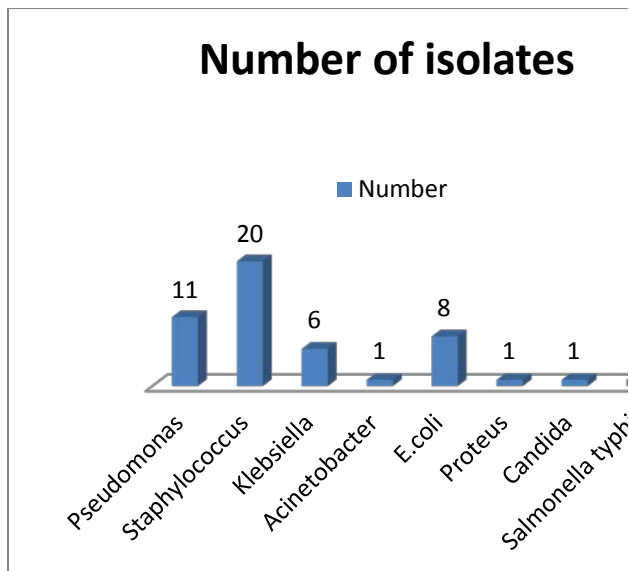


Table 2 shows that, out of 49 positive blood culture samples *Staphylococcus aerusinos* were found to be 40.8% which is maximum among all. While *pseudomonas* shows 22.4%, *klesiella* 12.2% and *Acinetobacter*, *Proteus mirabilis*, *candida*, and *salmonella typhi* shows 2.04%, while *E.coli* shows 16.3% positivity.

Table 3:

Organism	Adult	Children	New bor
Staphylococcus	3	10	7
Pseudomonas	5	5	1
E.coli	3	2	3
Klebsiella	2	1	3
Acinetobacter	0	1	0
Proteus	0	0	1
Mirabilis			
Salmonella	0	1	0
Typhi			
Candida	1	0	0
Total	14	20	15

Table 3 shows that, In children the number of *Staphylococcus* were found to be maximum while it adult *pseudomonas* were

found to be more. In new born Staphylococcus were found to be maximum.

Klebsiella

E.coli

Proteus mirabilis

Acinetobacter

Salmonella typhi

Table 4:

Sample	Total No. of sample	Total No. of positive sample
Central line	8	1
Peripheral line	234	48
Both	8	0
Total	250	49

Table 5 shows that, candida shows positive blood culture in central line while in peripheral line organisms like Pseudomonas, Klebsiella, E.coli, Proteus mirabilis, Acinetobacter, Salmonella typhi in appropriate number.

The blood samples were taken from central line or peripheral line or both. Table 4 shows that central line blood culture shows less positivity than peripheral line. While central line+ peripheral line shows no positivity. Peripheral line shows 48 samples positive out of 234.

Table 6:

Antibiotic sensitivity for pseudomonas

Antibiotic	No(n=11)	%
I	10	90.9
MR	8	72.7
PT	5	45.5
AK	9	81.8
G	10	90.9
LE	11	100
OH1	8	72.7
CF	7	63.6
Total	20	

Table 5:

Sample	Positive sample	Organisms	Total
Central line	1	Candida	1
Peripheral line	48	Pseudomonas	48
		Staphylococcus	20

CPT	0	0
CA	0	0
CI	4	36.4
CS	2	18.2
CL	4	36.4
PB	8	72.7
TOB	0	0
TC	1	9.1

Table 7:

Antibiotic sensitivity for Staphylococcus

Antibiotic	No(n=20)	%
VA	18	90
TE	17	85
LZ	17	85
AK	18	90
G	18	90
LE	19	95
OF	2	10
CF	6	30
CD	6	30
E	4	20
CA	10	50
CI	3	15
CU	5	25
CP	1	5
AC	12	60
A	1	5

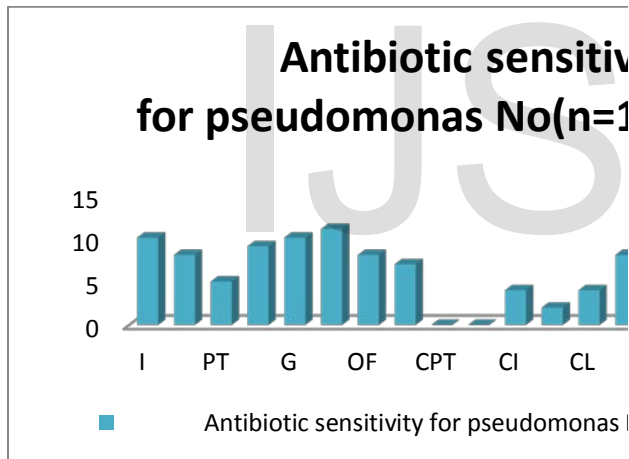


Table 6 shows the antibiotic sensitivity pattern for pseudomonas which shows 100% sensitivity for levofloxacin, while it is less sensitive to Ticarcillin+calvulanic acid (TC).

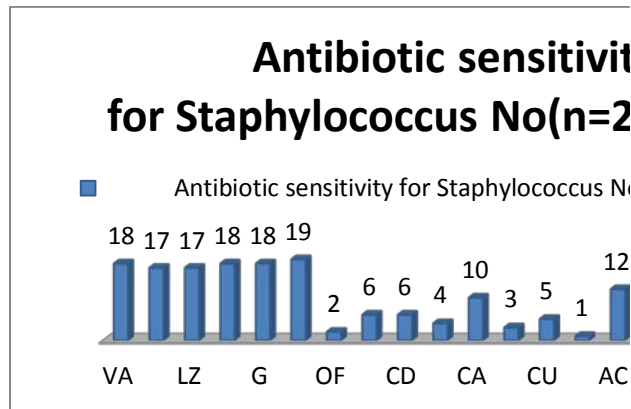


Table 7 shows sensitivity pattern of *Staphylococcus aeruginosa* which shows higher sensitivity for levofloxacin while it is less sensitive to amikacin and Cefoperazone.

Table 8:

Antibiotic sensitivity for GNB(E.coli, Klebsiella, proteus, samonella)

Antibiotic	No(n=15)	%
I	16	93.8
MR	14	93.3
PT	13	86.7
AK	13	86.7
G	14	93.3
LE	16	93.8
OF	9	60

CF	5	33.3
CPT	3	20
CA	3	20
CI	5	33.3
CU	2	13.3
CFX	6	40
AC	8	53.3
A	5	33.3
CS	10	66.7

Table 8 shows the sensitivity pattern of Gram negative bacteria which include *E.coli*, *Klebsiella*, *proteus* and *salmonella*.

Discussion

Out of 250 patients, 49 patients blood culture sample were found positive which includes *Pseudomonas* (22.4%), *Staphylococcus* (40.8%), *Klebsiella* (12.2%), *Acinetobacter* (2.04%), *E.coli* (16.3%), *Proteus mirabilis* (2.04%), *Candida* (2.04%), *Salmonella typhi* (2.04%) and 201 blood culture sample were negative. In adult, the total positive samples were 72 in which 14 were found to be positive (19.5%). In children, the total samples were 112 in which 20 were found to be positive (17.9%). While in new born individuals there are 66 sample in which 15 were found to be positive (22.7%).

As compared to all these individuals, the populations of children with positive blood culture sample were found to be more i.e 20 as compared to adult (14), and new born (15). The principal hypothesis examined in this study was that the rate of contamination of blood cultures taken at central line would be lower than that of blood cultures taken from peripheral line. Out of the 250 patients, 12.5% were taken during central line insertion, 20.5% were taken from peripheral veinpuncture.

The process of obtaining blood cultures can be divided broadly into three parts- preparing the phlebotomy site, drawing the blood and inoculating the blood into the culture bottles. All three parts can be sources of blood culture contaminations. However, in the process of drawing blood between central and peripheral blood culture. Central line insertion involves the use of a larger needle which involves considerable manipulations of the skin and subcutaneous tissues during catheter insertion and takes longer than veinpuncture. Approximately, 20% of skin bacteria live within the deeper layers of the dermis and subcutaneous tissue into which topical antiseptics cannot penetrate (Brown, E; R.P. Wenzel & J.O Hendley 1989, Selwyn, S; & H. Ellis 1972) and these bacteria could be contaminating centrally drawn cultures. Similarly, discarding the initial blood volume at phlebotomy for blood cultures decreased blood culture contamination (Patton, R.G; & T. Schmitt 2010). The longer time required for central line insertion also increase exposure to environmental contaminants.

Cultures obtained by peripheral veinpuncture are often drawn for investigations of fever or leukocytosis,

possibly associated with a lower pretest probability of bacteremia.

Staphylococcus aureus was the most predominant isolates (65%) followed by *E.coli* (29.3%). This observation is in accordance with reports from two other developing countries (Weber M W, Carlin JB, Gatchalian S, Lehman D, Muhe L, Mulholland EK, WHO young infants study group 2003). Similarly in a study conducted by Martin M Meremikwv et al in Nigeria found that *Staphylococcus aureus* was isolated in 48.7% and *E.coli* in 23.4% as the most frequent isolates (Martin M Meremikwv, Chukwuemoka E Nwachukwv Anne E Asuque, Joseph U Okebe 1 and Simon J Utsala 2005). In our study, *Staphylococcus aureus* was found to be 40.8% followed by *pseudomonas* 22.4%, while *Acinetobacter*, *Proteus mirabilis*, *Candida* and *Salmonella typhi* were found to be less i.e 2.04%

Aziz et al reported that *staphylococcus aureus* (25%) and *E.coli* (12.1%) as the most pathogenic bacteria recovered from blood sample. While in our study, *Staphylococcus aureus*(40.8%) and *pseudomonas* (22.4%) was the most pathogenic bacteria. *E.coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* were

found to be highly prevalent among new born individuals (Martin M Meremikwv, Chukwuemoka E Nwachukwv Anne E Asuque, Joseph U Okebe 1 and Simon J Utsala 2005). *Staphylococcus aureus* was found to be more sensitive to Chloramphenicol (87.5%), Ofloxacin (76.5%) and Ciprofloxacin (72%).

While in our study it is found to be more sensitive to Vancomycin (90%), Linezolid (85%), Amikacin (90%), Gentamicin (90%) followed by Levofloxacin (95%) while in gram negative bacteria Imipenem, Meropenem was found to be more sensitive. Ghanshyam D Khumhar (V G Ramachandran and Piyush Gupta 2002) in his study done at India also found that *E.coli* and *Klebsiella* is sensitive to Amikacin but *Staphylococcus aureus* was more sensitive to Vancomycin. While in our study, *Pseudomonas* shows 100% sensitivity against Levofloxacin.

Automated blood culture is designed to do blood culture for both aerobic and anaerobic cultures. Automated blood cultures comprises of Bac T Alert 3D 120 systems which has a capacity of 60 blood culture bottles. It helps in quick identification of bacterial growth in the bottles. 24 hours continuous scanning is

done which helps to identify the growth of organisms. It's easy to use and it is time saving. In manual blood culture, blood culture is done manually i.e 1 ml of sample is taken from blood culture bottle with the help of syringe and subculture it on Mac conkey and blood agar plate. Growth is observed after 24 hours. It is then again subculture for 5 days and final results are noted down. It is time consuming and proper handling should be taken while performing.

Figure 1: Bac T Alert 3D



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Figure 3: Positive blood culture shows change in colour of the media



Figure 2: Blood culture bottles: Green bottles is for adult patients, Yellow bottle if for pediatric patients and orange bottle is for anaerobic culture for adult and pediatric patients.

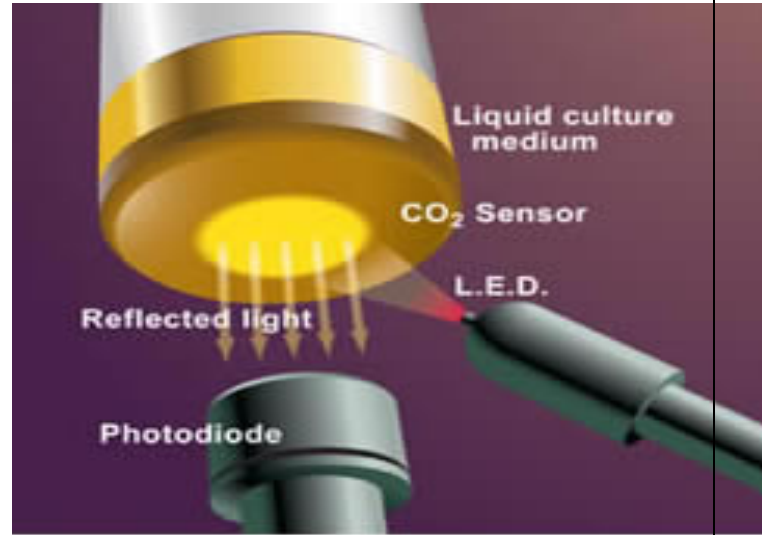


Figure 4: Reflected light helps in to identify the positive blood culture bottle



Figure 5 : Mac conkey agar showing Lactose and non-lactose fermenting bacteria.

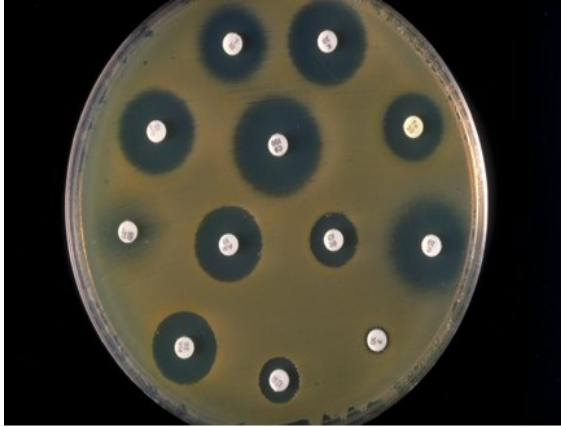


Figure 6: Antibiotic sensitivity pattern

Conclusion

Septicemia is the presence of bacteria in the blood (bacteremia) and is often associated with severe disease. Its alternative name is blood poisoning (bacteremia with sepsis). Out of 250 blood culture samples, 49 patients' samples were found to be positive which include adult, children and new born patients. Children population with positive blood culture were found to be more as compared to adult and new born. Organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida*, *Klebsiella*, *Proteus mirabilis*, *Acinetobacter*, and *Salmonella typhi* were isolated from these blood culture samples. Each isolated were then treated with antibiotic to observe the sensitivity pattern of these organisms. Each isolates were treated with different Antibiotic to see the effect or sensitivity

Pattern against those organisms. Out of these isolates *Staphylococcus* were found to be the pathogenic bacteria. It shows higher sensitivity against Vancomycin, and shows low sensitivity against Amikacin and ciprofloxacin. Subculturing of the positive blood culture is done manually or by using automated blood culture system. Automated blood culture shows accurate result and it shows a quick identifications of organisms present in the sample, while in manually it is time consuming and can sometime lead to false result. Hence, person showing the symptoms of fever may show the sign of septicemia which can be treated or decreased down by using antibiotic against that pathogen.

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