Training Report

Internship Training Report

Submitted to

Lovely Professional University, Punjab

in partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:
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Registration Number: 11411397

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
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DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Dr. Saurabh Saxena (Internal supervisor), Lovely Professional University and Dr. MamtaKumari (MD), Chief Microbiologist (External supervisor), SRL Gurgaon. This work has not been submitted in part or in full in any other university for any degree or diploma.

Name Nitish Kumar Yadav

Date:

Place- Lovely Professional University

CERTIFICATE

This is to certify that **Mr./ Nitish Kumar Yadav** bearing **Registration Number** 11411397 has completed his/her Master of Science in Clinical microbiology internship under our guidance and supervision. This report is record of the candidate own work carried out by him/her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

Internal Supervisor
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Chief Microbiologist
Date: 3/5/16.
SRL, Gurgaon

SRL Diagnostics
Trusted by millions

Date - 29th April 2016

To Whomsoever It May Concern

This is to certify that **Mr. Nitish Kumar Yadav,** student **M.Sc (Clinical Microbiology) Lovely Professional University** has successfully completed his Project Training with our organization in the Lab Operations during the period from 1st January 2016 to 27th April 2016.

Project Statement: Isolation and Identification of Bacteria from Blood Sample and their Antibiotic susceptibility Test.

Project Guide: Dr. Mamta Kumari (Microbiologist)

During the period, the company found him performance to be good. He has demonstrated a positive attitude to learning.

We wish him the very best in all his future endeavors.

For **SRL Limited**

HR, Function

Rahul Singh
Senior Manager – Human Resources

ACKNOWLEDGEMENT

I consider it my bounded duty to thank the Almighty for his showers of blessings throughout my training and project work. You have given me the powers to believe in my passion and pursue my dreams.

This project is a fruit of the hard work done at SRL Diagnostic during my 4 months training tenure. Use of advanced automated systems here enhanced my interest several folds in clinical field by making me curious about their functioning.

At this moment of accomplishment, firstly I pay my homage to my guide, Dr. Saurabh Saxena for his constant guidance and encouragement.

I express my heartfelt gratification Dr. MamtaKumari and Dr. Yoginderpal Singh, who groomed me up and carved me with their experience and immense knowledge.

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ABSTRACT

Background: Bacterial blood stream infection constitutes a significant public health problem and it is an important cause of morbidity and mortality in patients worldwide. The aim of this study was to assess the prevalence of bacteria isolates from septicemia suspected patients and their antimicrobial susceptibility pattern.

Method: This laboratory based retrospective study of 1210 blood culture and susceptibility test was conducted in SRL diagnostic laboratory, Gurgaon. The sample were collected and processed following standard microbiological techniques as a part of the routine clinical management of the patient. Antibiotic susceptibility testing was done on pure culture isolates employing automated VITEK2 COMPACT system. The data was analyzed and result was summarized by using tables and graphs.

Result: Out of 1210 blood culture results, 155 (12.8%) were positive culture. The most prominent bacteria isolated from culture were *Salmonella typhi*followed by *Staphylococcus epidermidis* and *E.coli*. The Gram positive and Gram negative bacteria constituted 52.9% and 47.09% of culture respectively. The isolates show resistance to various antibiotics.

Conclusion: In the present study many pathogens isolated from blood culture showed high rates of resistance to commonly used antibiotics used to treat infection. Therefore, rational use of Antibiotics should be practiced.

Keywords- Bacteria, Blood stream infection, Antibiotics, Susceptibility, Blood culture

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LIST OF ABBREVIATION

Amp	Ampicillin
Tri	Trimethoprim
Cipi	Ciprofloxacin
Cfui	Cefuroxime
Ery	Erythromycin
Gen	Gentamycin
Dap	Daptomycin
Levi	Levofloxacin
MDR	Multi drug resistance
CONS	Coagulase negative Staphylococcus
SIRS	Systemic inflammation response syndrome
GNB	Gram negative bacilli
GPC	Gram positive cocci
AST	Antibiotic susceptibility test
BHI	Brain heart infusion

CHAPTER- I: INTRODUCTION

Blood is a versatile bodily fluid that performs a variety of functions in our body. It helps in transport of nutrients and oxygen, maintaining body temperature, killing of pathogen and removal of metabolic waste. Blood circulates throughout the body via veins, capillaries and reaches up to each and every vital organ of body such as brain, heart, liver etc. No flora in blood can be considered as normal flora. It is necessarily a sterile fluid. Blood acts as a transporting & communicating medium so, any infection in blood pose a serious threat to vital organs of body.

Although blood is itself equipped with various immunological cells such as phagocyte, macrophage, eosinophil etc. which are capable of killing the foreign pathogen but some microorganism are able to survive in the blood and they are not killed by these cells. Infections in blood may be caused by virus, bacteria and fungi. The infection of bacteria in blood is known as bacteremia. The cases of bacteremia is most frequently seen in neonates, old age persons and those suffering from immunodeficiency. Bacteremia is also common in people who are already been affected by or being treated for some other medical problem. In addition, medical treatment may bring a person in contact with new type of bacteria that is more invasive than those already residing in patient's body.

Wide range of pathogen including *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* has been isolated from blood stream infection and are leading cause of mortality and morbidity. Bacteremia can be fatal if not treated early. Antibiotics are recommended by doctors in case of bacteremia. As only antibiotics are able to kill blood stream infection pathogen but nowadays a new threat is emerging which is rise of resistance strain of bacteria. Earlier, bacterial infection were easily with antibiotics by now, resistant strain has the ability to overcome the effects of Antibiotics. Bacteria has developed the resistance through mutation and are capable of transferring the mutation in any species which is another major threat in itself. Antibiotic resistance is attained by bacteria due to overuse and abuse of antibiotics. Some strains of bacteria such as TB are reported to be TDR (Total drug resistance) i.e. no antibiotic will work against them. It is major problem in developing countries. Due to new advancements in diagnostic

technology, now it is much easier to detect organism in blood stream infection and obtain their antibiotic susceptibility pattern. Quicker diagnosis is of major importance in health care sector.

Approximately 200,000 cases of bacteremia and fungemeia occur worldwide with mortality rate ranging from 20%-50%. In recent cases there is striking increase noticed in incidents of enterobactericea and other Gram negative bacteria. MDR bacteria have been frequently reported as the cause of nosocomial outbreaks of infection in burn unit or as colonizer of wound of burn patient. The constant evolving antimicrobial resistance pattern has generated a need for constant antimicrobial sensitivity surveillance

CHAPTER- II.: AIMS AND OBJECTIVES

GENERAL OBJECTIVE

The aim of present study was isolationand identification of bacteria from blood sample and their antibiotic susceptibility test.

SPECIFIC OBJECTIVE

The objectives of the study are as follows:-

1. To isolate the organisms from blood samples.
2. To perform antibiotic susceptibility tests.
3. To study the distribution of organisms in different age groups and with relation to gender.

CHAPTER –III: LITERATURE REVIEW

3.1 STUDY OF MICROORGANISMS IN BLOOD INFECTION

Presently, more research works has been done compared to past years in India concerrning microbiology of blood infections. Likewise, more journals and literatures have been updated concerning wound infection in India. Identification of etiological agents and their antibiotics sensitivity patterns should be known for proper prognosis and treatment. Several studies have been conducted and have been put forward for prevention and control of different diseases in different parts of the world.

Kairavi*et al* (2010) was conducted study on 140 samples (46.20%) organisms were isolated. These included *Klebsiella* (66, 47.14%), *Staphylococcus aureus* (35, 25%), Coagulase negative *Staphylococci* (CONS) (5, 3.57%), *E.coli* (15, 10.71%), Proteus (5, 3.57%), *Acinetobacter* (3, 2.14%), *Pseudomonas* (6, 4.28%) and *Candida* (5, 3.57%). Majority of organisms isolated were resistant to commonly used antibiotics. Maximum sensitivity was seen by Cefoperazone/sulbactum (97%) & Piperacillin/tazobactum (98%) for Gram negative organisms & Vancomycin (100%) for Gram positive organisms. He was concluded that multi-drug resistance organisms were isolated from septicemia in neonates. Therefore great caution is required in selection of antibiotic therapy.

Garg *et al* (2007)was conducted study on Positive cultures were obtained in 493 (20.5%) cases. Among culture positive isolates, Gram-negative bacteria accounted for 67.5% cases; most common being Pseudomonas spp. (16%) followed by *s. typhi* and *S.paratyphi* A (14.2%). Of the pathogenic Gram-positive isolates, *Staphylococcus aureus* (8.3%) was the predominant isolate followed by *Enterococcus faecalis* (3.7%). Maximum Gram-negative isolates were sensitive to cefoperazonesulbactam combination (81%). Vancomycin sensitivity was reported in 100% *Staphylococcus aureus* and 83.3% *Enterococcus faecalis*. He was concluded that study provides information on antibiotic resistance of blood isolates.

It may be a useful guide for physicians initiating empiric therapy and will help in formulation of antibiotic therapy strategy in this part of the country. The predominant isolated pathogens from blood specimens were Gram positive bacteria in 301 (65.25%) patients, mainly 198 (65.78%) coagulase-negative *Staphylococci*. The Gram-negative bacteria were 140 (31.74%). About 66% of the Grampositive bacteria were coagulase-negative *Staphylococci*, while the gram-negative bacteria were mainly (*Escherichia coli*, *Enterobacter spp.*, *Klebsiella pneumoniae*, and *Pseudomonas spp.*). He was concluded that the majority of *S. aureus* were resistant to Oxacillin, Co-trimoxazole, Cephalotin, and Ampicillin. About two – third of the *E. coli* and *K. pneumonia* were resistant to Cephalotin.

Villegas *et al* (1994) conducted study on microdilution method was utilized for determining susceptibility to several antimicrobial agents in 142 bacterial blood culture isolates obtained during a one year period. Associated clinical features were also identified. Three cases of polymicrobialbacteriemia were found. Endocarditis was the most frequent source of bacteremia (28.5%) and the viridians streptococci were the most frequently isolated microorganism (53%). Surprisingly, half of the bacteremia episodes corresponded to a nosocomial infections, most of which were due to staphylococci (25%) and Enterobacter sp (22%). Viridians streptococci group were 61.5% resistant to penicillin (MIC > 0.12 micrograms/mL). These strains also showed a 31% resistance to ceftriaxone (MIC > 8 micrograms/mL). The staphylococcal strains showed a 19% resistance to oxacillin; this resistance occurred for coagulase negative *Staphylococcus* in 32% (6/19) and for *Staphylococcus aureus* in 9% (2/22). All Gram-positive microorganisms were susceptible to vancomycin. The enterobacteria groups were susceptible to most antimicrobial agents; nevertheless this group showed a 45% resistance to amikacin. In contrast, the non enterobacteria groups were resistant to most of the antimicrobial agents tested except to imipenem, ceftazidime and ciprofloxacin. When comparing susceptibility longitudinally, no significative changes were identified, but a significant increase was found in MIC5090 to amikacin and cephalothin when testing *Staphylococcus aureus*, and cefoperazone in the non enterobacteria group.

Obi *et al* (1996) was conducted study on results obtained revealed that only 303(37.1%) of the 817 total samples screened were positive for either monomicrobial or polymicrobial bacteremia. Two hundred and eighteen (71.9%) and 85 of positive cultures were Gram positive and Gram negative bacteria respectively. Coagulase negative staphylococci (CNS) strains were the predominant organisms isolated (42.9%). Other organisms isolated were *S. aureus* (11.6%), *E.coli* (6.9%), *Salmonella spp.* (8.3%), *Klebseilla spp.* (5.3%), whereas *Pseudomonas aeroginosa,* Enterobacter and Micrococcus species each accounted for less than 4%. Antibiogram patterns showed multiple resistance of *S.aureus*and CNS to Penicillin, Erythromycin and Methicillin. All isolates of *S. Pyogenes* (10), *S.pneumoniae*(18) And Micrococcus spp (10) were susceptible to penicillin. Ciprofloxacin, Clindamycin, Fusidic acid and Gentamycin were highly active against gram positive organisms except that Gentamycin was inactive against *S.pneumoniae*. Ceforoxime, Erythromycin and Ceftriazone also showed good activities against Gram positive organisms. All (10) isolates of *P. aeruginosa* were susceptible to Polymyxin B, Carbenicillin and Ciprofloxacin. Ciprofloxacin, Norfloxacin and Gentamycin were highly active against all Gram negative bacteria. He was conducted that for infections due to both Gram positive and Gram negative bacteria, Ciprofloxacin and Gentamycin would be appropriate for therapy whereas Fusidic acid and Clindamycin may, in addition, be recommended for Gram positive organisms. It is also concluded that a prevalence rate of 37.1% of bacteremic cases existed in the sampled population and that monomicrobial cases were more predominant.

In a study conducted by Ghadiri*et al* (2012), 129 blood stream positive samples were obtained from patients referring to Besat hospital over a two-year period (2009 & 2010). Antibiotic sensitivity was ascertained using the Kirby-Bauer disk diffusion technique according to CLSI guidelines. Patient's data such as gender and age were recorded. The ratio of gram-negative to gram-positive bacteria in BSIs was 1.6: 1. The most prevalent BSI pathogen was Coagulase-Negative Staphylococci (CoNS). The highest resistance rate of CoNS was against penicillin (91.1%) followed by ampicillin (75.6%), and the lowest rate was against vancomycin (4.4%). The present study showed that CoNS most common

causative agents of nosocomial BSIs and, and control of infection needs to be addressed in both antibiotic prescription and general hygiene.

In a previous study (Duran *et al,* 2012); methicillin resistance rate among 139 *S. aureus* isolates was found to be 16.5 and 25.9 % of *S. aureus* carried mecA gene. Of the 159 CoNSisolates, methicillin resistance rate was 18.9 and 29.6 % carried mecAgene. A total of 165 isolates were resistant to erythromycin, and contained at least one of the erythromycin resistance genes (ermA, ermB, ermC and msrA). Phenotypically, 106 staphylococcal isolates were resistant to tetracycline, 121 isolates carried either tetK or tetM or both resistance genes. The majority of staphylococci tested possessed the blaZ gene (89.9%). Eun-mi Koh*et al,* (2007) was conducted study on 536,916 blood specimens cultured, 24,877 (4.6%) from 13,102 patients were positive. Among the isolates, 93.1% were aerobic or facultative anaerobic bacteria, 3.3% anaerobes, and 3.6% fungi.*Escherichia coli*was isolated most frequently, followed by *Staphylococcus aureus*, αhemolytic*Streptococcus, Enterococcus* spp., and *Klebsiella pneumoniae*. The proportion of patients with*Enterococcus faecium* and *K. pneumoniae* gradually increased during this study. *Enterococcus, S. aureus*and α-hemolytic *Streptococcus* were frequently isolated from the age group of less than 2 yr. *E. coli, Enterococcus* spp., *K. pneumoniae* and *S. aureus* from the age group of over 50 yr. Oxacillin-resistant *S. aureus* decreased, whereas vancomycin-resistant *E .faecium* and imipenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* increased.

3.2 Blood culture

Blood culture refers to collection and inoculation of blood into culture medium with the aim of growing pathogenic bacteria for diagnostic purposes.

3.2.1 Bacteremia

The presence of viable bacteria in the bloodstream may be transient (e.g. following dental procedures), intermittent (e.g. undrained abscesses) or continuous (e.g. endovascular infection). Bacteremia refers to presence of pathogenic bacteria in blood. Bacteremia is not necessarily responsible for clinical sign, especially when it is present in transient sepsis, a term used less and less frequently. From now on sepsis is known as the general inflammatory reaction to infection. Sepsis usually combines a body temperature above 100°F, chills and sweats. In this case the culture of blood sample will enable the confirmation of bacteremia. Sepsis requires antibodies therapy immediately after drawing blood to prevent septic shock, the severe form of bacteremia. Depending on the culture result, antibiotics may be changed.

3.2.2 Systemic inflammatory response syndrome (SIRS)

A consequence of the host inflammatory response that can follow infection or other injury, defined as the clinical cluster of two or more of:

- Temperature >38°C or <36°C.
- Heart rate >90 beats/minute.
- Respiratory rate >20 breaths/minute or $paCO_2$<4.3 kPa (32 mmHg).

White cell count >12 000 cells/mm^3, <4 000 cells/mm^3, or >10% immature white blood cells (band forms)(Nobeka, Nusi 2010).

The concept of systemic inflammatory response syndrome is to describe the complex pathophysiologic response to an insult such as infection, trauma, burn, pancreatitis or a variety of injury. There was recognition that a diverse group of injuries produce a common inflammatory response in the host and provide attractive target for new anti inflammatory molecules designed to prevent further propagation and provide specific treatment.

SIRS AS A THERAPEUTIC TARGET - Late in his career, Roger bane proposed a new paradigm to explain pathogenesis of septic process taking into account the complexity and chaotic nature of sepsis response (Balk RA, 2000). He recognized that the process which was intended to benefit the host could potentially cause severe injury that could culminate to death. He suggested that there were series of 5 stages to sepsis cascade that could eventually result in multiorgan dysfunction/failure if not properly countered by anti inflammatory response.

Severe sepsis and SIRS remain important condition that consume resources, lead to complication and drastically change lines of affected patient

Undoubtedly, there will be scientific breakthroughs in our understanding of mechanism and pathophysiology that will lead to a more refined diagnosis, perhaps coupled with specific biomarker and/or PCR technology.

3.2.3 Severe sepsis

Sepsis is body's overwhelming and life threatening response to infection which can lead to tissue damage, organ failure and death. Sepsis can occur even after a minor infection. Sepsis is difficult to diagnose and treat. Sepsis is dangerous and can be fatal

Sepsis can occur as a result of both community acquired and health care associated infection. Pneumonia is most common cause accounting for almost half of all cases. Blood culture is positive only in one third of cases. *S.aureus*and *S.pneumoniae* are most common gram positive bacteria wherease*E.coli* ,*Klebseilla spp.* And *Pseudomonas aeroginosa* predominates among gram negative isolates.

Infection triggers a more complex, variable and prolonged host response in which both pro inflammatory and anti inflammatory mechanism can contribute to clearance of infection and tissue recovery on one hand and organ injury and secondary infection on other hand. The host response to sepsis is characterized by both pro inflammatory response and anti inflammatory immunosuppressive response(Y Zhang 2002). The direction, extent and

duration of these response are determined by host factor (genetic characteristics, age, and medication). Inflammatory responses are initiated by interaction between PAMP expressed by pathogen and PRR expressed by host cell at cell surface (TLR) and C type lectin receptor (CLR). The consequences of exaggerated inflammation iscolletral tissue damage and necrotic cell death, which result in release of Damage associated molecular pattern (DAMP). The immune system harbors humoral, cellular and neural mechanism that attenuate potentially harmful effects of pro inflammatory response. Phagocyte can switch to anti inflammatory phenotype that promote tissue repair and regulatory T cell and myeloid derived suppressor cell further reduce inflammation. Documented sepsis associated with organ dysfunction, hyper fusion or hypotension.

Perfusion abnormalities may manifest as, but are not limited to:

- Lactic acidosis.
- Oliguria.
- Acute alteration in mental state.
- Areas of mottled skin.
- Capillary refilling requiring ≥3 seconds.
- Disseminated intravascular coagulation.

Acute lung injury or acute respiratory disease can cause sepsis, including infection in skin, lungs, UTI, appendicitis or other part of body. An infection occur when germ enter a person's body and multiply causing illness, organ and tissue damage. There is no sign of symptoms of sepsis. It is rather a combination of symptoms, since sepsis is a result of an infection, symptoms can include infection sign (diarrhea, committing, sore throat etc). Pneumonic sepsis can be deadly. It kills more than 258,000 Americans each year and leaves thousands of survivor with life changing after effects. According to CDC, there are over one million of cases of sepsis each year and it is 9th leading cause of death related to disease.

Doctors diagnose sepsis by using a number of physical findings like fever, increased heart rate, increased breath rate. They also do lab test that check for sign of infection. Most of the symptoms of sepsis resembles to those in other conditions, making sepsis hard to diagnose in early stage.

People with sepsis are usually treated in hospitals. Doctors try to treat infection by keeping vital organs working and prevent drop in blood pressure. Antibiotic regimen is started as soon as possible.

3.2.4 Septic shock

The presence of severe sepsis plus one or both of the following:

- Systemic mean blood pressure <60 mmHg (or <80 mmHg if the patient has baseline hypertension) despite adequate fluid resuscitation.
- Maintaining systemic mean blood pressure >60 mmHg (or >80 mmHg if the patient has baseline hypertension) requires support with inotropic/vasoactive agents (Levy MM, 2003).

3.2.5 Multi-organ dysfunction/failure

Multi-organ failure is often a consequence of severe sepsis and septic shock, and refers to the presence of altered function affecting more than one organ in an acutely ill patient such that homeostasis cannot be maintained without intervention. Multiple organ dysfunction syndrome (MODS) is either primary (directly attributable to a well-defined insult) or secondary (as a consequence of the host response). MODS is characterized by, but not limited to, abnormalities in serum creatinine, platelet count, serum bilirubin, Glasgow coma score, pressure adjusted heart rate, and arterial oxygenation (Balk RA, 2000).

Multiple organ failure (MOF) has reached epidemic proportion in most intensive care units and is fast becoming the most common cause of death in surgical intensive care unit. Furthermore, inspire of development of successive generation of new and more powerful

antibiotics and increasing sophisticated technique of organ support, clearly new therapeutic strategies aims at preventing or limiting development of physiologic abnormalities that induce organ failure are needed to improve survival in critically ill patient.

MODS is comprised of activation and deregulation of multiple complex overlapping physiologic systems. It is overall humoral, cytokine and immunologic changes leading to system inflammation. Stress hormone such as catecolamines, cortisol, growth hormones, glucagons, insulin are elevated. Immune system activation take place, compliment activation, neutrophil and macrophage activation, free radicle liberation, toxic oxygen metabolites.

There is also an increase in metabolic demands such as oxygen consumption, gluconeogenesis and protein catabolism.

3.2.6 Sepsis and the significance of blood culture

Some 1.8 million cases of sepsis occur worldwide each year 6 however (Angus DC, 2001), owing to variable definitions and reporting, this is probably an underestimate. A more accurate estimate may be as high as 18 million, an incidence of 3/1000 population (Martin GS, 2003).

Sepsis is the most common cause of death in non-coronary intensive care units worldwide. The mortality rate from sepsis is generally between 30% and 70%, and is higher in persons with a pre-existing disease (Vincent JL, 2006). Elderly, critically ill and immunocompromised persons are particularly vulnerable (Dellinger RP, 2004). Population-based studies of incidence and prevalence of sepsis in South Africa have not been undertaken. There are several reviews of the pathophysiology of sepsis. The direct effects of invading micro-organisms, their toxic products, or both, may contribute to pathogenesis of sepsis. Important factors include endotoxin, bacterial cell wall components (peptidoglycan,

muramyl dipeptide, and lipoteichoic acid), and bacterial products such as staphylococcal enterotoxin B, toxic shock syndrome toxin-1, pseudomonas exotoxin A, and M protein of haemolytic group A streptococci. Cellular injury and organ dysfunction occur as a consequence of ischaemia, cytopathic injury and increased rate of apoptosis (Marshall JC, 1997). Panendothelial activation in sepsis also leads to widespread tissue edema, endothelial dysfunction, hypotension, up regulation of adhesion molecules, and impaired anticoagulant properties leading to MODS. Blood culture plays an integral role in the evaluation of sepsis.

Clinical feature of sepsis- Any infection that is present anywhere in the body can cause sepsis, including infection in skin, lungs (such as pneumoniae), UTI, appendicitis or other part of body. An infection occur when germ enter a person's body and multiply causing illness, organ and tissue damage. There is no sign or symptom of sepsis. It is rather a combination of symptoms, since sepsis is a result of an infection, symptom can include infection sign (diarrhea, vomiting, sore throat etc.). Sepsis alone accounts for death of approximately 158,000 Americans each year and leaves thousands of survivors with life changing after effects (Dellinger RP, 2004). According to CDC, there are over one million cases of sepsis reported each year in India and it is 9th leading cause of death related to disease.

Treatment- People with sepsis are usually treated in hospitals. Doctor try to treat infection by keeping vital organs working and preventing drop in blood pressure. Doctor prescribe antibiotics as soon as possible in case to sepsis (Harris RL, 1987).

3.2.7 Diagnosis of sepsis

Diagnosis- Doctor diagnose sepsis by using a number of physical findings like fever, increase heart rate, increased breath rate. They also do lab test that check for infection. Most of symptoms of sepsis resembles to those in other diseased conditions, making sepsis hard to diagnose in early stages. Sepsis is primarily a clinical diagnosis, supported by laboratory investigation and imaging. Culture of specimens from a sterile site is the gold standard

microbiological investigation and the key to successful diagnosis (Von Landenberg P, 2001). Occasionally, serology may aid diagnosis. Culture is a slow process compared with rapid molecular diagnostic tests that detect genetic material and can be used at the "point of care". The pneumonic for symptoms of sepsis goes like

S- Shivering fever, very cold

E-extreme pain or discomfort

P-pale or discolored skin

S- Sleepy, difficult to wake up

I-I feel like I might die

S- Shortness of breath

Although only 5 - 15% of blood cultures drawn in febrile patients are positive, approximately half of all patients with severe sepsis demonstrate bacteremia at the time of diagnosis. The presence or absence of a positive blood culture does not appear to influence outcome of sepsis, suggesting that prognosis is more related to severity of sepsis (Brun-Buisson C, 1996). Hospital-acquired infections (HAI) diagnosed from blood culture have a higher mortality than those acquired in the community (Lohen J, 1995).

3.3 Principles of sample collection for blood culture

The rate of isolation of microorganisms from blood is directly related to volume of blood collected

Quantity- It is recommended that a blood culture should consist of total 30 ml (for adults) and less that 10 ml for pediatrics collected from two venipuncture sites. Bottles making up the sets should be collected at the same time. It is critical that there is meticulous preparation of skin prior to venipuncture to prevent contamination of specimen.Blood cultures should be

drawn when there is a clinical suspicion of bloodstream infection. Blood should not be collected from indwelling arterial or venous lines unless an infected intravenous line is suspected (Norberg A, 2003). Should blood be drawn for culture from an indwelling line, a second specimen should be obtained from a peripheral site.

1. Two or more blood specimens should be collected using sterile technique at separate sites, before administering antibiotics (Washington JA II, 1986). Except in very unusual cases, no more than 3 sets of blood cultures should be collected in one 24-hour period. If all 4 sets are negative after 24 hours and sepsis is still suspected, more cultures may be collected. A larger number of cultures may have to be collected from persons already receiving antimicrobials although, if clinical condition allows, stopping antibiotics and re-culturing after 48 hours is preferred.

2. Ideally, a minimum of 10 ml of blood, depending on the blood culture system used, should be inoculated into a culture bottle when taking blood from adults (Connell TG, 2007). Adequate volumes of blood improve detection of pathogenic organisms and reduce time to detection.

3. Arterial blood culture provides no advantage over venous samples (Reller LB, 1982).

4. Changing needles between venipuncture and inoculation into culture bottles is controversial. Discontinuing this practice owing to lack of evidence that it decreases contamination and the increased risk of sharp injuries has been recommended. However, a meta-analysis challenges this recommendation, suggesting a slight overall benefit of switching needles (Spitalnic SJ, 1995). Until more definitive studies are available, we recommend that the risk of changing needles after venipuncture does not outweigh the benefit. More important ways to decrease contamination of blood culture bottles include the use of tincture of iodine as a disinfectant, avoidance of drawing blood through existing intravenous lines and disinfecting the membrane of the blood culture bottle (Eskira S, 2006).

5. It is a medico-legal requirement that the patient's notes must always document acquiring blood for culture, including indication, site, time of taking blood culture and a legible name (NtobekoNtusi, 2010).

6. There should be a balance between volume of blood collected and clinical condition of patient.

7. Vials that has been expired or show signs of contamination (such as turbidity, bulging septum) should not be used.

Site of collection- Initial collection should be peripheral venipuncture, if possible, second site may be central line if sepsis is suspected or peripheral access sites are unavailable. For pediatric patients, peripheral sites are preferred, the decision to obtain a sample from second site may be at the discretion of physician. Peripheral blood samples should be collected distally (toward the extremities) of any venous/arterial line in same limb.

Site preparation- vigorously cleanse the skin over the venipuncture site in a circle approximately 5 cm in diameter with 70% alcohol. Scrubbing should continue for at least 30 seconds. Venipuncture site should not be touched after preparation and prior to phlebotomy.

Before collection mark the appropriate volume on the vials. For adults mark off 10 ml above the level of vial.For pediatrics mark off the volume based on patient age and weight. The cap of bottles should be removed and disinfected with an alcohol swab and allowed to dry. Do not use iodine as it may damage the septum

Order of draw/collection- collect blood culture first starting with the aerobic vial followed by anaerobic lytic vial from the first site. After collection, mix the bottle thoroughly by gentle inversion. Butterfly needle procedure (preferred method except for neonatal patient)- Remove butterfly needle and tubing from the package

Perform vein puncture by inserting the needle with the rubber cover directly into the bactec bottle. The needle and vacuatiner holder must be held down to keep the needle from popping out of vial.

Remove the vial when blood flow has reached the mark that has been made on vial, indicating the appropriate fill levels. For alternative syringe draw-perform vein puncture with needle and syringe and draw the proper amount of blood. Inoculate the blood into appropriate blood culture vials. Needle should not be recapped. After collection, bottles should be mixed throughouly by gentle inversion. Vials should be labelled with following information-

Patients name, age lab ID number, day and time of collection and site of location.

3.4 Optimal number of blood cultures

The optimal number of blood cultures that should be obtained in a febrile patient varies according to the suspected diagnosis or clinical condition, the suspicion of underlying infection, and the urgency of the need for treatment (Lee A, 2007).

- One blood culture is rarely, if ever, sufficient or advisable. A positive result on a single culture is difficult to interpret, unless an unequivocal pathogen is isolated.
- Two blood cultures are usually adequate when continuous bacteremia is anticipated.
- Three blood cultures are reasonable when intermittent bacteremia is suspected (Washington JA II, 1975).

Furthermore, the probability of obtaining a false-positive result increases steadily as more blood cultures are performed

3.5 Timing of blood cultures

Few systematic studies have been conducted on the timing of blood cultures and the optimal interval between successive tests. The ideal is to collect blood at time intervals ranging from one to several hours. However, it is sufficient and appropriate to obtain blood from 2

separate sites within minutes of each other from patients who are acutely ill or those in whom the likelihood of continuous bacteremia is high. By contrast, in patients suspected to have intermittent bacteremia, it is advisable to obtain multiple blood cusltures 6 - 36 hours apart (Weinstein MP, 1996). Antibiotics should not be withheld in sick patients apart from a reasonable delay to collect more than 1 culture sample.

3.6 Volume of blood to be taken for culture

A minimum of 10 ml (and preferably 20 ml) of blood should be obtained from adults (Weinstein MP, 1996) and 1 - 5 ml (and preferably more) from infants and children (Brown DR, 1995). The yield from blood culture depends upon the volume of blood cultured, the yield in adults increasing approximately 3% per ml of blood cultured. Maintaining the correct ratio of blood to culture medium is important. Most blood culture bottles contain the anticoagulant sodium polyanetholsulphonate (SPS). SPS can inactivate lysozyme, some antibiotics, and parts of the complement cascade. However, SPS can also be toxic to certain fastidious organisms. Adding sufficient blood to the bottle helps negate this latter effect. A dilution of blood in broth by a ratio of 1:5 has been recommended (O‟Hara CM, 2003).

3.7 Antibiotic resistance

3.7.1 Characteristics of Antibiotics

Today, there are about 4 000 compounds with antibiotic properties. Antibiotics are used to treat and prevent infections, and to promote growth in animals.

Antibiotics are derived from three sources: moulds or fungi; bacteria; or synthetic or semi-synthetic compounds. They can be used either internally or topically, and their function is to either inhibit the growth of pathogens or to kill them. Antibiotics can thus be divided into Bacteriostatic drugs, which merely inhibit the growth of the pathogen, and Bactericidal drugs, which actually kill the bacteria. However, the distinction is not absolute, and depends on the drug concentration, the bacterial species, and the phase of growth (Y. Zhang 2007).

Antibiotics are more effective against actively growing bacteria, than against no growing persists or spores. When two antibiotics are used in combination, the effect could be additive, synergistic, or antagonistic.

Antibiotics can also be divided into broad-spectrum and narrow-spectrum antibiotics. Some antibiotics, such as Pyrazinamide, have an even narrower spectrum, and can be used merely against *Mycobacteriumtuberculosis*.

Antibiotics are of five major class-

1. Cell wall inhibitors, such as Penicillin and Vancomycin.
2. Inhibitors of nucleic acid synthesis, such as Fluoroquinolones, which inhibits DNA synthesis, and Rifampin, which inhibits RNA synthesis.
3. Protein synthesis inhibitors, such as Aminoglycoside.
4. Anti-metabolites, such as the sulfa drugs.
5. Antibiotics that can damage the membrane of the cell, such as Polymyxin B, Gramicidin and Daptomycin (Y. Zhang 2007).

Antibiotics are divided into various categories on the basis of their mode of action -

1. Beta lactam antibiotics- They are bactericidal drugs. They inhibits bacteria cell wall by interference with the synthesis of peptidoglycan. The bacterial enzyme that are affected by beta lactam are called penicillin binding protein (PBPs). The affect of beta lactam is mostly expressed against multiplying bacteria that are building their cell wall intensively. Beta lactam are not toxic and have minimal concentration dependent adverse effect. The extent of dosing is extremely high in case of penicillin. Most important undesirable effect are allergic reaction of various intensity. Phlebitis while intravenous administration(hyperosmolar solution), local pain and infiltrate with intramuscular administration and thrombocytopenia. Eg.-penicillin, cephalosporin.

2. Glycopeptides- They are bactericidal drug inhibiting bacterial cell wall synthesis in a step prior to beta lactam action. They may also injure bacterial protoplast or interfere with RNA synthesis because of large molecule that does not penetrate into periplasmic space of Gram negative bacteria, their antibacterial spectrum is narrow and involve only GP microbes. These drugs are not absorbed from gastrointestinal tract. Penetration across biological barrier is poor. The drug is excreted almost exclusively by glomerular filtrate.

3. Aminoglycosides- They have very strong and rapid bactericidal effect on bacteria. They act in several sites of bacterial cell (outer membrane, ribosomes). A very important feature of aminoglycosides- is synergism with wall affecting antibiotics (beta lactam, glycopeptides). This synergism is expressed against some Gram positive as well as some Gram negative bacteria. Aminoglycosides are not effective against anaerobes, spirochete, obligatory intracellular pathogen and capsulated pathogen.

4. Nitroimidazoles- They are bactericidal narrow spectrum antibiotics effective against anaerobes and some protozoa. The drug are well absorbed from gastrointestinal tract. They posses excellent penetration across biological barrier such as blood brain barrier and placental barrier. They are metabolized 40% in liver and excreted mainly by kidney.

5. Chloramphenicol- They posses both bactericidal and bacteriostatic properties against a variety of microbes including GP and GN bacteria, anaerobes, spirochete and obligatory intracellular pathogen. It inhibit protein synthesis on the ribosomal level. It has toxicity for bone marrow manifested by anemia, leucocytopenia and thrombocytopenia

6. Lincosamides- They are two static antibiotics reversibly inhibiting protein synthesis on ribosomal level. However, they have narrow spectrum effective against Gram positive bacteria and anaerobes. They are absorbed from Gastrointestinal tract but do not cross biological barrier.(Mahdinejind et.al. 2009)

3.7.2 Drug Resistance

The Scope of the Drug Resistance Problem

Drug resistant bacteria have been posing a major challenge to the effective control of bacterial infections for quite some time. In the case of *M. tuberculosis*, MDR-TB and XDR-TB are becoming an increasingly significant problem. Antibiotic resistant worldwide, cause challenge. Multiple resistant organism render therapy more precarious and costly. Individual may succumb to MDR infection because all available drug have failed especially in developing world.(Rani et.al. 2012))The resistance problem can be seen simplistically as an equation with two main component, the antibiotic or antimicrobial drug which inhibit susceptible organism and select resistance ones. Drug resistance is mobile. The gene for resistance can be transferred among bacteria of different taxonomic and ecological group by mean of mobile genetic element such as bacteriophage, plasmids, naked DNA or transposons.

Drug resistance refers to a situation in which the drugs that usually destroy the bacteria no longer do so. It implies that people can no longer be effectively treated against the bacteria (Y. Zhang 2007). Consequently, they are ill for longer periods of time; and they face a greater risk of dying. Furthermore, epidemics are prolonged, putting more people at a risk of becoming infected.

3.7.4 Causes for Drug Resistance

The main origin of resistance to antibiotic is their misuse. As underlined by European center for disease prevention and control, there are basically 3 types of misuse-

1. Unnecessary prescription of broad spectrum antibiotics in place of a better targeted antibiotic, through more precise diagnosis.

2. Inadequate use by patient, not respecting either dosage or duration of treatment.

Some bacteria are naturally resistant to certain antibiotics, others can acquire resistance through some kind of mutation in some of their gene, when they are exposed to an

antibiotic. This resistance, either acquired or natural, can spread to other bacterial species and since bacteria can easily exchange genetic material among each other, even if they are from different species (Prasad et.al. 1996). A recent WHO report made a clear case that resistance of common bacteria to antibiotics has reached alarming level in many parts of the world. There is an increase of resistance in common bacteria such as *E.coli* and also *s.aureus, klebsiella pneumoniae, pseudomonas aeroginosa.* To limit the increasing resistance to antibiotics, the ECDC(European centre for disease prevention and control) considers 3 strategic area of interventions-

1. Prudent use of available antibiotics and when possible infection prevention through appropriate vaccination.

2. Hygiene precaution for control of cross transmission of resistant strain between persons.

3. Research and development of antibiotic with a novel mechanism of action.. Antibiotic use in animal husbandry is also creating antibiotic resistant bacteria (Y. Zhang 2007).

3.7.5 Natural and Acquired Resistance

Antibiotic resistance can be divided into natural resistance and acquired resistance.

Natural resistance means that the bacteria are „intrinsically" resistant. For example, Streptomyces has some genes responsible for resistance to its own antibiotic. Other examples include organisms that lack a transport system or a target for the antibiotics. In other cases, the resistance can be due to increased efflux activity. Acquired resistance refers to bacteria that are usually sensitive to antibiotics, but are liable to develop resistance. Acquired resistance is often caused by mutations in chromosomal genes, or by the acquisition of mobile genetic elements, such as plasmids or transposons, which carry the antibiotic resistance genes.

3.7.6 Genetic and Phenotypic Resistance

Broadly speaking, antibiotic resistance could also be divided into genetic drug resistance, which is the one most commonly discussed, and phenotypic drug resistance, which is a more subtle type. Genetic resistance is due to chromosomal mutations or acquisition of antibiotic resistance genes on plasmids or transposons (Y. Zhang 2007). Phenotypic resistance is due to changes in the bacterial physiological state, such as the stationary phase, antibiotic persists, and the dormant state.

3.7.7 Genetic Drug Resistance Mechanisms

Until the 1950s, it was not clear how the bacteria acquire drug resistance. Then, Joshua LEDERBERG devised replica plating, and demonstrated that the antibiotic resistant mutants are pre-existing. Thus, the antibiotics merely selected these mutants. Then, in 1988, John CAIRNS showed that when the bacteria are not growing, they are nevertheless able to acquire new mutations, due to some genetic alteration process. Those mutations are called adaptive mutations. It was never formally proven that adaptive mutations cause antibiotic resistance; however, it is possible, particularly in non-growing forms of bacteria (Y. Zhang 2007). There are five major mechanisms of antibiotic drug resistance, which are due to chromosomal mutations:

1. Reduced permeability or uptake.
2. Enhanced efflux.
3. Enzymatic inactivation.
4. Alteration or over-expression of the drug target.
5. Loss of enzymes involved in drug activation. This mechanism is relatively new.

3.8 MDR Resistance

MDR has become a major problem in medical development. It is an emerging phenomenon in various organism throughout the evolutionary scale(Harvey et.al. critical care 2011).

When antibiotic like penicillin was discovered, they were treated as miracle drug of century. This scene has suddenly changed. Now population is confronted with new resistant type of bacteria. The cause of resistance is attributed to amplification of bacterial MDR genes. Most bacterial MDR comes under major facilitator families, which include arabinose/symporter of E.coli & glucose facilitators of eukaryotes. MFS have 12 trans membrane 12 alpha helical domains and use proton motive force as a source of energy. Qac A is one of first MDR protein identified in bacteria. Qac A is a membrane pump which efflux out several drug in a proton motive force dependent manner. enrA and enrB are the two gene coded by E.coli which confer resistance to uncoupleres (CCCP) and other antimicrobial agent. The function of these proteins are to form channel between inner and outer membrane (Prasad et.al. 1996))The Multi-drug Resistance (MDR) mechanism can be caused by different mechanisms in different organisms. For example, in 1959, the Japanese found Shigella species that were resistant to Sulfonamides, Streptomycin, Chloramphenicol, and Tetracycline. The resistance was due to plasmid, which carried different antibiotic resistance genes. The other MDR mechanism is due to sequential accumulation of chromosomal mutations in different drug resistant genes, as in the case of MDR-TB and XDR-TB (Y. Zhang 2007).

Multidrug resistance phenomenon is not restricted to mammalian or microbial cell. It has been identified in yeast cell as well. About 25 genetic determinant associated with multidrug resistance has been characterized in saccharomyces cerevisiae, Candida albicans. The gene product encoded by these yeast fall into 3 categories (class of proteins)- ABC, MFS and transcriptional regulators (Stuart bleny& Bonnie Marshall 2004). According to most accepted model, drugs are removed by the transporter directly from plasma membrane(lipid bilayer) thus, drugs are thrown out and are unable to reach cytoplasm. Conceptually, the multidrug transporter work as a hydrophobic vacuum cleaner which removes drug from the membrane. Drug transporter could be an enzyme 'flippase' which would bind the drug from inner leaflet and flip it to outer leaflet from where drug diffuse out to extracellular spaces.(Harvey et.al. 2011). Efforts are being made to produce more effective antibiotics by

modification of specific group of antibiotic molecules. A possible approach could be to increase the spontaneous influx of drug by making them sufficiently lipophilic so that efflux can be counter balanced by rapid influx.

3.9 Antibiotics susceptibility pattern

Antibiotic susceptibility is the term use to describe susceptibility of bacteria to antimicrobial. Antibiotic susceptibility test is usually carried out to determine which antibiotic will be most effective in treating bacterial infection in vivo. Testing for antibiotic susceptibility can be done by Kirby Bauer's diffusion technique. It is a manual technique in which the antibiotic discs are placed on to Meuller Hinton agar. A clear zone of inhibition indicates poor growth of bacteria, so the antibiotic is sensitive to bacteria. The susceptibility category implies that isolate are inhibited by the usually achievable concentration of antimicrobial agent when the recommended dosage is used for site of infection. Automated system like Vitek2 COMPACT can be used, not only for carrying out antibiotics susceptibility test but also for identification of isolated organism. The result of antimicrobial susceptibility testing should be combined with the clinical information and experience when selecting most appropriate antibiotic for the patient.

CHAPTER- 1V: MATERIAL AND METHOD

Following materials are used during the study period given below:-

4.1 Equipments

- Vitek 2 compact
- Bactec 9120

4.2 Gram staining

Gram staining method was named after Danish bacteriologist Hans Christian Gram. Gram staining method is used to classify bacteria into Gram negative and Gram positive category. It consist of four components

1. Primary stain (crystal violet)

2. Mordant (iodine)

3. Decolorizer (alcohol-acetone)

4. Counterstain (safranin)

Procedure

1. The smear on glass slide is covered with few drops of primary stain. Primary stain renders all bacteria violet uniformly.

2. After a minute of exposure, the slide is washed with the water and then the smear is treated with few drops of Gram iodine and allowed to act for a minute. This result in formation of dye-iodine complex. Gram iodine works as a mordant.

3. Then the slide is washed with water & decolorized with acetone-alcohol. This process of decolorization should not exceed 30 second for thin smear.

4. After the smear is decolorized, wash it with water and treat with few drops of counter stain.

Bacteria which holds up primary dye is called Gram positive and those which get decolorized and subsequently take counter stain is called Gram negative.

4.2.1 Composition and Preparation of Gram Stain Reagents

a) Crystal Violet Stain

Composition	gram/liters
Solution A	
Crystal violet	**2 gm**
Ethanol	20 ml
Solution B	
Ammonium oxalate	0.8 gm
Distilled water	80ml

Preparation

1. Mix solution A to solution B and store 24 hours to dissolve the stain completely.
2. Filter the solution through filter paper after 24 hours and then use.
3. Label the reagent bottle with preparation date and reagent name.

b)**Gram's Iodine Solution**

Composition	**gram/lit**er
Potassium iodide	2 gm
Iodine	1 gm
Distilled water	100 ml

Preparation

1. Dissolve the potassium iodide in distilled water and then add iodine.
2. Store in a tightly stopper bottle with preparation date and reagent name.
3. **c) Acetone-alcohol decoloriser**

Composition	**volume (ml)**
Acetone	250
Ethanol	250

Preparation

1. Mix the 250 ml of acetone into a 250ml ethanol.
2. Store in a tightly stopper bottle with preparation date and reagent name.

d)Counterstain Solution

Composition	**gram/liters**
Safranine	0.34 gm
Absolute alcohol	10 ml
Distilled water	90 ml

Preparation

1. Dissolve the safranine in 0.34 gm into 10 ml absolute alcohol and then add 90 ml distilled water.
2. Filter the solution through filter paper and then use.
3. Label the reagent bottle with preparation date and reagent name.

4.3 MEDIA

BD BACTEC™ Blood culture instrument and media are designed to meet the wide range of need required by laboratory. It is quite efficient in enhancing safety to health care workers. It will increases recovery significantly and faster time to detection as compared to other automated system. BACTEC media with patented resins have shown to effectively remove antibiotics (penicillin, cephalosporin and vancomycin). It has unique bottle design allow use of standard and readily available safety adapters for blood collection, reducing risk of needle stick injury. It's faster diagnosis help in reducing chances of septic shock and significantly increase survival rates. It provides all the components necessary for safe draw of blood.

4.3.1 BACTEC PLUS Aerobic/F Culture Vial

This general purpose media provide good recovery and time of detection.

Optimum blood volume for each vial is 8 to 10 ml; 3 to 10 ml of blood is acceptable.

a) Each vial contains:

- 25 ml Enriched Soybean-Casein Digest broth (TSB).
- 0.05% Sodium Polyanetholesulfonate (SPS).
- Cationic and Non-ionic Adsorbing Resins.
- Carbon dioxide (CO_2).
- Oxygen (O_2).
- Sensor for the detection of fluorescence.

b) Store at 2° to 25° C.

4.3.2 BACTEC™ PLUS Anaerobic/F Culture Vial

It incorporates lysing agent for detection of phagocytized organism

Optimum blood volume for each vial is 8 to 10 ml; 3 to 10 ml of blood is acceptable.

a) Each vial contains:

- 25 ml pre-reduced enriched Soybean-Casein Digest broth.
- 0.05% SPS.
- Resins.
- CO_2 and Nitrogen gas (N_2).
- Sensor for the detection of fluorescence.

b) Store at 2° to 25° C.

4.3.3 BACTEC PEDS PLUS/F Culture Vial

It is specifically designed for pediatrics and other low blood volume sample. It has low SPS concentration which improve recovery of specific pediatric pathogen (*Neisseria* etc.).

Optimum blood volume for each vial is 1 to 3 ml; 0.5 to 5 ml of blood is acceptable.

a) Each vial contains:

- 40 ml Enriched Soybean-Casein Digest broth.
- 0.02% SPS.
- Resins.
- CO_2.
- O_2.
- Sensor for the detection of fluorescence.

b) Store at 2° to 25° C

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4.3.4Blood Agar (Hi-Media)

Blood agar is enriched medium used to culture bacteria or microbe that do not grow easily. Such bacteria are called "Fastidious" as they demand a special, enriched nutritional environment. It is used to grow wide range of pathogen such as Hemophilusinfluenzae, streptococcus pneumoniae, Neisseria spp. It is also required to detect and differentiate haemolytic bacteria, especially streptococcus spp. It also is a differential medium in allowing the detection of hemolysis (destruction of RBC). Hemolysis can be alpha or beta hemolysis.

Composition	**gram/liters**
Peptic digest of casein	**500**
Tryptone	**10**
Sodium chloride	**5**

Agar	**1.5**
Final pH at 25º C	**7.3**

Preparation

As directed by the manufacturing company 40 gm of blood agar base was dissolved in 1000 ml distilled water. The medium was then sterilized by autoclaving at 15 lbs pressure (121 ºC) for 15 min. Then the prepared medium was cooled to about 40-50 ºC, to which 50 ml of sterile defibrinated blood was added aseptically. Then the medium was poured into petriplates.

4.3.5 MacConkey Agar (Hi-Media)

MacConkey Agar is a selective medium. It is also recommended for selective isolation and differentiation of lactose fermenting and non lactose fermenting bacteria.

Composition	**gram/lit**
Pancreatic digest of animal tissue	**17.0**
Peptone	**3.0**
Lactose	**10.0**
Sodium Chloride	**5.0**
Bile salt	**1**

Agar	**15**
Neutral red	**0.03**
Final pH	**6.9 to 7.3**

Preparation

As directed by the manufacturing company, 51.53 gm of the medium was dissolved in 1000 ml distilled water. It was sterilized by autoclaving at 15 lbs pressure (121 ºC) for 15 minutes.

4.3.6 BHI (Brain-Heart infusion) media

BHI is general purpose liquid medium used in cultivation of Fastidious and non-fastidious microorganisms including aerobic and anaerobic bacteria. It serve as a base of supplement media containing 0.1% agar.

Principle of procedure

In the formulation containing 6.5% NaCl, the salt act as differential and/or selective agent by interfering with membrane permeability. The addition of 0.1% agar aid in cultivation of anaerobic microorganism because its consistency yield condition to reduce oxygen tension.

Composition	**gm**
Calf brain, infusion form.	7.7
Beef heart, infusion form.	9.8
Peptone	2.0

Dextrose	2.0
Sodium chloride	5.0
Disodium phosphate.	2.5

Growth is indicated by presence of turbidity. The bottle of BHI is sub cultured after 24 hour and 48 hour on MacConkey and Blood Agar and then the Agar plates are incubated in incubator at 37°C and result is seen after 24 hours.

4.3.7 Biosafety cabinet class 2

The modern class II microbiological safety cabinet, now regarded as probably the most common in use safety cabinet employed around the world. Class II cabinet provide both kind of protection (of sample and of environment). Since make up air is also HEPA filtered. There are 4 types: Type A1, Type A2, Type B1 and Type B2. Each type's requirement are defined by NSF international standard 49. Cabinet air may be recirculated back into the laboratory or ducted out of building by means of a "thimble" connection whereby the balance of cabinet is not disturbed by fluctuation in the building exhaust system. The thimble must be designed to allow for proper certification of cabinet (i. e. provide access to permit scan testing of HEPA filters). It maintains average face velocity of 0.5 m/s. It have ducts and plenums under negative pressure. The principle of operation involves using a fan mounted in top of cabinet to draw a curtain of sterile air over the product that are being handled. The air is then drawn underneath the work surface and back up to the top of cabinet where it passes through the HEPA filter. The air being drawn acts as barrier to potentially contaminated air coming back out to the operation. The class II cabinets are offered with glass slide Windows and advantage to this is to offer more natural lighting, giving user a more comfortable working environment as well as allowing viewing of the work procedure for training etc. Pre- drilled holes allow for pass through of cables, vacuum lines etc. as well as allowing service fixture to be fitted easily. Class II cabinet employ design with

angled front window which give ergonomically better seating position leading to safer and comfortable operations.

4.4 METHODS

All positive blood cultures from adults and infants at Super Religare laboratories, diagnostic center, Haryana, from 1 Jan 2016 - 31 March 2016 was included in this study. Bactec 9120 blood culture system with aerobic resin blood culture media is being used in lab. The blood culture consist of 5 ml of blood obtained either by vein puncture or intravenous access device. The blood sample is inoculated in bactec bottle and they are loaded in Bactec 9120 blood culture system. The medical record of patients were reviewed by one of the investigators to determine the clinical significance of results.

4.4.1 AUTOMATED BLOOD CULTURE

PRINCIPLE

The BACTEC 9000 series of blood culture instruments are designed for the rapid detection of microorganisms in clinical specimens. The sample to be tested is inoculated into the vial which is entered into the BACTEC instrument for incubation and periodic reading. Each vial contains a sensor which responds to the concentration of CO2 produced by the metabolism of microorganisms or the consumption of oxygen needed for the growth of microorganisms. The sensor is monitored by the instrument every ten minutes for an increase in its fluorescence, which is proportional to the increasing amount of CO2 or the decreasing amount of O2 present in the vial. A positive reading indicates the presumptive presence of viable microorganisms in the vial.

4.4.1.1 INSTRUMENT

BACTEC 9120 (BACTEC Fluorescent Series)

Microorganisms, if present in the blood samples, metabolize nutrients in the BACTEC culture vial and release CO_2 into the medium or utilize the oxygen in the medium. The instrument

monitors the fluorescence of the vial sensor which increases as CO_2 is produced or oxygen is utilized. Analysis of the rate and amount of CO_2 produced or O_2 utilized enables the instrument to determine if the vial is positive; i.e., the presumptive presence of viable organisms.

Fig Bactec 9120

a) Computer and peripherals

The system computer stores all the system software, including the application software which controls instrument operations and the user interface, which enables the user to enter patient information, view results, print reports, identify errors, etc.

NOTE: The microprocessor associated with each rack is responsible for the actual testing of the vials and positivity analysis.

b) Barcode Scanner

The barcode scanner is located on the inside of the instrument to provide the ability to activate barcode commands and to scan vial labels for specimen identification. When the door is opened and an activity is initiated, the scanner is activated and is ready to read the vial barcode. The barcode scanner for the

BACTEC 9050 is located on the front of the instrument and is activated when the door is opened and the vial entry or vial removal soft keys are pushed.

Fig.- inserting area of Bactec bottle.

4.4.1.2SPECIMEN COLLECTION

a) SITE SELECTION

1.Select a different body site for each culture drawn.

2. Avoid drawing blood through indwelling intravascular catheters unless blood cannot be obtained by venipuncture. Blood collected from intravascular catheters should be done with the knowledge that contamination may be an issue.

b)SITE PREPARATION (PERSIST Povidone Iodine Prep)

1 .Open the PERSIST package by tearing completely through at the side notches and twisting.

2. Leave the package over the end of the swab stick to prevent gloves from becoming covered with solution.

3. Apply PERSIST by beginning at the intended venipuncture site, working in a circular motion with friction, covering an area of 2-3 inches in diameter. Do not return to the center of the site once swab has moved outward to the periphery. Persist™ should be applied with friction and the site prepped 30 seconds to 1 minute.

4. Allow PERSIST solution to air dry.

5. DO NOT touch or palpate the area after cleansing.

c)DISINFECTING BLOOD CULTURE VIALS

1. Remove the flip-off caps from BACTEC culture vials.

2. Wipe top of each vial with a separate 70% isopropyl alcohol pad and allow to dry.

3. Do not use iodine to disinfect tops of vials.

VENIPUNCTURE

1. Avoid touching the venipuncture site. If it is necessary to touch the site after it has been cleaned, wipe your fingers with povidone iodine before touching the site.

2. When using the Blood Collection Set ("butterfly") the phlebotomist MUST carefully monitor the volume collected by using the 5 mL graduation marks on the vial label. If the volume is not monitored, the stated maximum amount collected may be exceeded. This condition may adversely create a "false" positive result, due to high blood background.

3. If using a needle and syringe, typically a 20 mL syringe is used for adults. Draw 16 to 20 mL of blood for one blood culture set (aerobic and anaerobic). Aseptically inject 8 to 10 mL of specimen into each vial. Aseptically inject 3 to 5 mL into the MYCO/F LYTIC vial.

4. For pediatric patients, a 3 mL syringe is frequently used. Draw 1 to 3 mL of blood and transfer the entire amount into BACTEC™ PEDS PLUS/F vial.

5. After all specimens have been collected from the individual, use a sterile alcohol pad to remove the povidone-iodine solution from the venipuncture site.

6. Continue to care for the venipuncture site following guidelines recommended by your institution.

7. The inoculated BACTEC vials should be transported as quickly as possible to the laboratory.

4.4.1.3 VOLUME

The volume of blood cultured is critical because the number of organisms per mL of blood in most cases of bacteremia is low, especially if the patient is on antimicrobial therapy. In infants and children, the number of organisms per mL of blood during bacteremia is higher than adults, so less blood is required for culture.

1. **Children:** 1 to 5 mL of blood per venipuncture. Transfer the entire amount to a BACTEC™ PEDS PLUS/F vial.
2. **Adult:** 7-10 mL of blood per venipuncture. If it is impossible to draw the required amount, aliquot as follows:

Amount per Venipuncture	**Amount in BACTEC plus Aerobic Vial**	**Amount in BACTEC plus Anaerobic Vial**
7-10 mL	Split equally between aerobic and anaerobic vials	
10-12 mL	5 - 7 mL	5 mL
5-9 mL	entire blood amount	0

NOTE: Optimum recovery of isolates will be achieved by adding 8 to 10 mL of blood (BACTEC PEDS PLUS/F: 1 - 3 mL; BACTEC MYCO/F LYTIC - 3 to 5 mL). The use of lower or higher volumes may adversely affect recovery and/or detection times.

4.4.1.4 SPECIMEN LABELING

a) Each vial should be labeled with the appropriate patient information:

- Patient's name
- Hospital number (Patient ID)
- Date and time of collection

- Collector's initials
- Site of venipuncture
- Or other information as per facility

b) Each request slip should also have all the information above.

4.4.1.5 NUMBER AND TIMING

Most cases of bacteremia are detected using two to three sets of separately collected blood cultures. More than three sets of blood cultures yield little additional information. Conversely, a single blood culture may miss intermittently occurring bacteremia and make it difficult to interpret the clinical significance of certain isolated organisms.

4.4.1.6 PROCESSING NEW BLOOD CULTURES

a)ENTERING DATA AND LOADING INSTRUMENT

BACTEC 9120

i. Entering Data into the Computer

- From the Main menu, press [F3] Culture Complete the following fields:
  - Patient's ID
  - Patient's Name
  - Accession Number
  - Collection Date
  - Collection Time
  - Hospital Service

- Advance the cursor to the sequence number field. With the computer's barcode scanner, scan the vial barcode label. The sequence number, media type, station number, and the status field are then filled in automatically.
- Check the protocol field. (Default protocol is 5 days for blood cultures and 42 days for MYCO/F LYTIC).

NOTE: To change the length of the testing protocol, use the [↓] to advance the cursor to the protocol field and enter the desired value (1 to 42 days).

- Press [F10] to save the entry.
- Enter a different Accession number for each culture.

ii. Loading the Instrument:

- Take the new vials to the instrument and open the instrument doors.
- Scan the vial's barcode label. Listen for the beep indicating a successful scan and look for the station with the illuminated GREEN and RED LED.
- Insert the vial into that station.
- Repeat the above step for each of the new vials.

NOTE: If you haven't already entered the patient's data into the computer, be sure to write down the station number on the patient's requisition slip to use for logging patient's information into the computer at a later time.

b) POSITIVE CULTURES

i.BACTEC 9120: The system will notify the presence ofpresumptive positive vials in several ways:

- An audible alarm sounds (if configured) and the computer. Title and Cabinet windows flash.

- The Positive Indicator Lamp on the front of the instrument illuminates (yellow).
- On the computer's instrument status display, the station number and the total positives in the summary winders displayed in FLASHING GREEN, FLASHING RED.

To Remove the Positive Vials:

- Press [F2] to acknowledge the alarm.
- Open the instrument doors, and using the instrument barcode scanner scan the menu option REMOVE POSITIVES. Listen for a beep indicating that the item was scanned successfully.
- Find the station with the FLASHING GREEN and RED LEDs. Remove the vial and scan its barcode using the instrument barcode scanner. Listen for the beep and the LEDs will extinguish.
- If the instrument is configured to show related vials, the vial with the same patient ID and Accession number will be indicated by a solid GREEN LED. If you wish to remove this vial for subculture, scan its label with the instrument barcode scanner after you remove the vial from its station.

NOTE: A Gram stain and a subculture should be performed from each presumptive positive vial.

c) NEGATIVE CULTURES

i. Negative cultures may exist as ongoing negatives (in protocol) and out-of-protocol negatives.

ii. The BACTEC 9120 instrument status screen.

iii. Out-of-protocol negatives will be displayed in flashing green digits on the BACTEC 9120 computer's instrument status display. Also, the negative

count in the summary window of the computer will flash green. Out-of-protocol negatives in the BACTEC 9120are displayed as a solid circle with a minus sign (-) in the Main Status Display. The summary region reflects the number of negatives in the instrument. Also, the REMOVE NEGATIVES soft key appears when the door is opened.

iv. To Remove Out-of-Protocol Negatives from the BACTEC 9120 instrument systems:

- Open the instrument doors.
- Scan the menu option, REMOVE NEGATIVES using the instrument barcode scanner. Listen for a beep from the scanner indicating the item was scanned successfully.
- Find the station(s) with FLASHING GREEN LEDs and remove the vial.
- Scan the vial barcode label using the instrument barcode scanner. Listen for the beep indicating a successful scan and the GREEN LEDs will extinguish.
- Repeat the above two steps to remove additional negatives.

d) PROCESSING AN INSTRUMENT - POSITIVE VIAL

I Remove the vial from the instrument and place in a biological safety cabinet.

ii.Use a venting needle

iv. Remove aliquot from the vial for stain preparations (Gram).

v. Subculture vials according to the Gram stain results.

vi. Report preliminary results only after stain preparation.

Sub-culture of Positive Samples

The positive samples were sub-cultured for aerobic bacteria only and the study did not include anaerobic bacterial culture. The positive samples were streaked on to MacConkey agar and Blood Agar plates. The inoculated plates were incubated at 37^{o} C for 24 hours in CO_2 incubator.

4.4.2 ISOLATION AND IDENTIFICATION OF ORGANISMS

After overnight incubation, the culture plates incubated aerobically were examined for bacterial growth and identified using standard microbiological techniques which involve colony characteristic, staining reactions, microscopy and biochemical properties and also the antibiotic susceptibility pattern were noted.

4.4.2.1 IDENTIFICATION WITH VITEK2 COMPACT

Vitek2 COMPACT is an automated microbiology system utilizing growth based technologies. This system is available in 3 formats (vitek2 COMPACT, vitek2, vitek2 XL). The system accommodates the same colorimetric reagent cards that are incubated and interpret automatically. This format focuses on industrial microbiology testing environment which also is having application for low to middle volume clinical laboratories. Colorimetric reagent card (BCL) is used to identify the spore forming Gram positive bacilli (Bacillus and related genera). The other colorimetric reagent card (Gram negative, Gram positive, YST) also applies.

4.4.2.2 Reagent card

The reagent card have 64 well that can each contain an individual test substrate. Substrate measures various metabolic activities such as acidification, alkalization, enzyme hydrolysis and growth in presence of inhibitory substance. An optically clear film present on the both sides of card allows for appropriate level of oxygen transmission while maintaining sealed vessel that prevent contact with organism substrate mixture. Each card has a pre inserted transfer tube for inoculation. Cards are been bar coded that contain information on product type, lot number and expiration date.

Fig. VITEK card

There are currently 4 reagent cards available for identification of different organism classes.

1. Gram Positive card

2. Gram Negative card

3. YST (for yeast)

4. BCL- GP spore forming bacilli

4.4.2.3 SUSPENSION PREPARATION

A sterile swab or application stick is used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganisms in 3.0 ml of sterile saline (aq. 0.45% to 0.5% NaCl), pH- 4.5 to 7 in a 12×75 mm clear plastic or polystyrene test tube. The turbidity is adjusted accordingly and measured using a turbidity meter called Densicheck™.

4.4.2.4 INOCULATION

Identification cards are inoculated with microorganism suspension using an integrated vacuum apparatus. A test tube containing the microorganism suspension is placed into a special rack (cassette) and identification card is placed in neighboring slot while inserting the transfer tube

into the corresponding suspension tube. The filled cassette is placed manually into a vacuum chamber. The organism suspension is forced through the transfer tube into micro channel that fill all the test wells.

4.4.2.5 CARD SEALING AND INCUBATION

Inoculated card are passed by a mechanism, which cut off transfer tube and seal the card prior to incubate in carousel. The carousel incubator can accommodate up to 30 or up to 60 cards. All cards are incubated at 35.5°C±1.0°C. Each card is removed from carousel incubator once every 15 minutes and transported to optical system for reaction reading and then returned to the incubator until the next reading time.

Fig VITEK2 compact

4.4.2.6 OPTICAL SYSTEM

A transmittance optical system allows interpretation of test reaction using different wavelength in visible spectrum. During incubation, each test reaction is read every 15 minutes to measure

either turbidity or colored product of substrate metabolism. In addition, a special algorithm is present to eliminate false reading due to small bubbles that may be present.

4.4.2.7 GP (Gram positive) card

GP card is used for automated identification of 115 taxa of the most significant non sporing Gram positive bacteria (primarily cocci). When representative species or sub species of these collective designation are included, the total number of taxa claimed by Gram positive is over 120. The Gram positive identification card is based on establishment biochemical method and newly developed substrate. There are 43 biochemical test measuring carbon source utilization, enzymatic activity and resistance. Final result is obtained in approximately 8 hours or less.

4.4.2.8 GN (Gram negative) card

GN card is used for automated identification of 135 taxa of the most significant fermenting and non fermenting Gram negative bacilli. When representative taxa of these collective designations are included, total number of taxa claimed by GN is over 160. There are 47 biochemical test and one negative control well. In a recent multisite study, in which performance of VITEK2 GN card was evaluated, misidentification occurred at 3.0% and no identification occurred at 0.2%(David Pincus microbial identification, 2011).

4.4.2.9 PROCEDURE

The colonies are first isolated from Agar plate and they are diluted to make appropriate suspension.

This dilution is made by of 0.47-0.55 mcF with the help of normal saline.

Densicheck™ is used to check dilution. The colonies are transferred to polystyrene tube containing 3ml normal saline and then inserted in Densicheck™.

For each sample, two suspensions are made and placed in a special rack. One tube holds AST card and other tube hold ID card.

The rack consists of bar code which is scanned by machine after insertion in machine.

The result is provided by machine within 8 hours and can be obtained in printable form.

VITEK 2 compact is an automated microbial identification system that provides highly accurate and reproducible results as shown in multiple independent studies. It offers a state of the art technology platform for phenotypic Identification methods (http:// pda.org/bookstore).

CHAPTER- V: RESULTS

The study was conducted out in the SRL Diagnostic laboratory from 1 Jan - 31 March 2016. The whole training period cover 4 months. Blood samples were collected, processed and analyzed by standard microbiological techniques. In this study 1210 samples were collected with 155 positive and 1108 with no growth and 29 with contamination.

Table 1: Organisms isolated and their percentage

Organism	*No. of isolate*	*Percentage*
Staphylococcus hominis	*15*	*9.6%*
Staphylococcus epidermidis	20	12.9%

Staphylococcus haemolyticus	8	5.16%
Enterococcus faecalis	2	1.29%
Salmonella paratyphi	15	9.67%
Enterococcus spp	3	1.93%
Klebsiella pneumonia	8	5.16%
Pseudomonas aeruginosa	2	1.29%
E.coli	15	9.6%
Salmonella typhi	25	16.12%
Staphylococcus warneii	*1*	*0.64%*

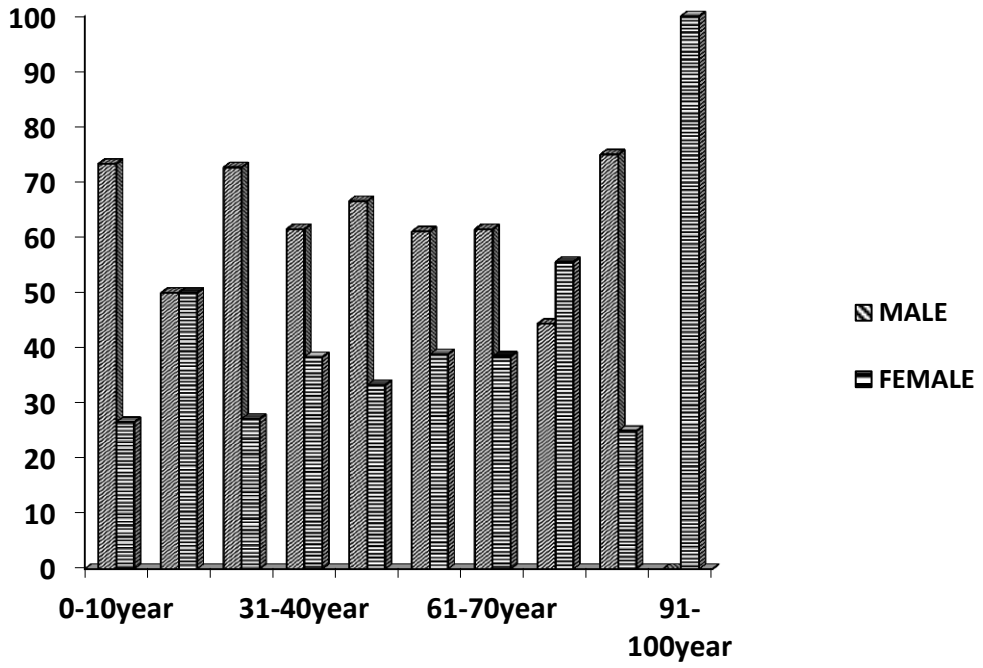
5.1 Distribution of organism isolated according to age and gender wise

Out of 1210 blood sample collected 53.3% were from male patient were positive, 46.69% from female patient were positive cases. Samples were collected from age group ranging from 1 day to 91 years. Age group under <10 years age was found to be most affected age group and age group under 20-30 years was found to be least affected age group.

Table 2: Age/gender wise distribution of positive cases

AGE	TOTAL SAMPLE	MALE		FEMALE	
		POSITIVE SAMPLE	%	POSITIVE SAMPLE	%

<10 years	30	22	73.33%	8	26.68%
11-20 years	18	9	50%	9	50%
21-30 years	22	16	72.72%	6	27.27%
31-40 years	13	8	61.53%	5	38.46%
41-50 years	9	6	66.66%	3	33.33%
51-60 years	18	11	61.11%	7	38.88%
61-70 years	13	8	61.53%	5	38.46%
71-80 years	9	4	44.44%	5	55.55%
81-90 years	4	3	75%	1	25%
91-100 years	1	0	100%	0	0%



Graph depicting positive cases in male and female population

5.2 Antibiotic susceptiblity test

Table 3: Percentage of antibiotic resistance on Gram negative bacteria

Drugs	Amp	Tri	Cipi	Cfui	Ctx	Casi	Imni	Meri	Ert
E.coli	66.6%	66.6%	80%	66.6%	73.3%	20%	9.09%	7.14%	10%
S. paratyphi	27.2%	0%	15.38%	100%	28.57%	12.5%	0%	0%	0%
P. aeruginosa	83.6%	50%	0%	0%	0%	50%	50%	50%	0%
K. pneumoniae	83.4%	66.6%	83.4%	83.4%	83.3%	83.3%	75%	75%	50%
S.typhi	4.76%	6.25%	33.33%	100%	10%	4%	0%	0%	0%

Table 4: Percentage of antibiotic resistance on Gram positive bacteria

Drugs	Ery	Trii	Gen	Cipi	dap	Levi
S. hominis	81.81%	83.3%	0%	27.27%	0%	42.3%
S.epidermidis	87.5%	60%	62.5%	62.5%	12.5%	62.5%

Coagulase negative staphylococcus	57.14%	12.5%	0%	0%	0%	40%
E. feacum	50%	75%	12.5%	50%	0%	50%
S.haemolytics	83.3%	75%	83.3%	100%	0%	83.3%

CHAPTER-VI: CONCLUSION

The current study was carried out in microbiology laboratory of SRL diagnostic, gurgaon with an aim to isolate and identify bacteria from blood sample and to study its antibiotic susceptibility pattern. In this study 1210 samples were collected in period from 1 Jan to 31 March, from different patients and different areas. The pathogenic microorganisms were identified by culture and biochemical test and their antibiotic susceptibility test were performed through VITEK2 COMPACT automated system.

Distribution of organism isolates of 1210 samples were collected, processed and analyzed through standard microbiological procedure. Out of which 155 sample were having bacterial infection and they showed growth. A total of 26 bacterial species were isolated with single growth.

Out of total 155 positive cases (82, 52.9%) were Gram positive bacteria and (73, 47.09%) were Gram negative bacteria. Among total 155 bacterial isolates, *Salmonella typhi (25 , 16.12%)* found to be most isolated organism followed by *Staphylococcus epidermidis (20, 12.9%) Staphylococcus hominis(15, 9.6) E.coli (15, 9.6%) Salmonella paratyphi (15, 9.6%), Coagulase negative Staphylococcus (14, 9.03%) Klebsiella pneumoniae (8, 5.16%) Staphylococcus haemolyticus (8, 5.16%), Enteroccus faecalis (2, 1.29%), Staphylococcus aureus (2, 1.29), Pseudomonas aeroginosa (2, 1.29), Staphylococcus cohinii (2, 1.29), Staphylococcus warneii (1, 0.64%) Pseudomonas stutzeri (1, 0.64%), Morgenella morgnii (1, 0.64%), Burkholderia capacia (1, 0.64%), Acinetobacter dentrificans (1, 0.64 %), Staphylococcus saprophyticus (1, 0.64%) , Serritia rubiddea (1, 0.64%0*

6.1 AGE AND GENDER WISE DISTRIBUTION OF PATIENT WITH POSITIVE CASES

Out of 1210 samples collected, single growth was found in 100% , no multiple growth were seen and No growth was observed in (1008) samples. 645 were male patient and 545 were female patient. The relative higher number of cases in males may be due to their greater participation in outdoor activities.

Samples were collected from patient's age ranging from 1 day to 91 years. The age group <10 years was the most affected age group whereas the age group 20-30 year was least affected age group.

6.2 ANTIBIOTIC SUSCEPTIBILITY TEST OF ORGANISM ISOLATED

Antibiotic susceptibility test of Gram negative organism

In vitro Antibiotic susceptibility test of Gram negative organism indicates that Imipeneum was found to be most sensitive antibiotic followed by Tetracycline and Ciprofloxacin was least affected antibiotic.

Antibiotic susceptibility test of Gram positive organism

In vitro Antibiotic susceptibility test of Gram positive organism shows that Daptomycin was most sensitive antibiotic and Erythromycin was least sensitive.

CHAPTER- VII:SUMMARY

The overall result of study can be summarized as follow:

1. Total 1210 samples were collected, processed and analyzed through standard microbiological techniques.

2. Out of 1210 sample (645, 53.3%) was of male patient and (545, 46.69%) was of female patient. The bacterial growth was found to be higher in female patient than in male patient.

3. Age group <10 was found to be most affected age group.

4. The samples have shown single growth and no multiple growth was seen.

5. A total of 26 bacteria was isolated out of which predominant in Gram positive bacteria was *Staphylococcus epidermidis* and in Gram negative *Salmonella typhi.*

6. Among Gram positive isolates Daptomycin was most sensitive while in Gram negative isolates Imipeneum was most sensitive antibiotic.

CHAPTER- VIII: BIBLIOGRAPHY

1. Asrat D, Amanuel Y. Prevalence and antibiotic susceptibility pattern of bacterial isolates from culture in Tikur Anbessa hospital, Addis Ababa, Ethiopia. Ethiop Med J. 2001;39(2):97–104.
2. Daniel RK, Scott AF, James MB, Sanjay S. Brief Report: Incidence, Etiology, Risk Factors, and Outcome of Hospital-acquired Fever. J Gen Intern Med. 2006;21:1184–1187.
3. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of sepsis in the US: Analysis of incidence,outcome, and associated costs of care. Crit Care Med 2001;29:1303-1310.
4. Yung A. Approach to undifferentiated fever in adults. In: Cameron P, Jelinek G, Kelly A, et al., editors. Textbook of adult emergency medicine. 1st ed. Edinburgh: Churchill Livingston; 2000. pp. 303–307.
5. JoAnn D. Antibiotic resistance: The ongoing challenge for effective drug therapy. JAAPA.2009;22(3):18–22

6. Agnihotri N Karlowsky JA, Jones ME, Draghi DC et al. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann Clin Microbiol Antimicrob. 2004; 3: 3-7.
7. 13. Gupta V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms-a fiveyear study. Burns 2004; 30: 241-243.
8. Mason Jr AD. McManus AT, Pruitt BA Jr: Association of Burn Mortality and Bacteremia: a 25-Year Review. Arch Surg 1986, 121: 1027-1031.
9. Wasserman D. Systemic complications of extended burns. Ann Chir Plast 2001; 46: 196–209.
10. Pruitt BA, McManus AT, Kim SH et al. Burn wound infections: current status. World J Surg 1998; 22: 135–145.
11. Ntobeko Ntusi, Lindsey Aubin, Stephen Oliver, Andrew Whitelaw, Marc Mendelson, 2010
12. Balk RA. Severe sepsis and septic shock. Definitions, epidemiology, and clinical manifestations. Crit Care Clin 2000;16:179-192.
13. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESCIM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 2003;31:1250-1256.
14. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003;348:1546-1554.
15. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006;34:344-353
16. Dellinger RP, Carlet JM, Masur H, et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. Crit Care Med 2004;32: 858-873.
17. Marshall JC, Watson RW. Apoptosis in the resolution of systemic inflammation. In: Vincent JL, ed. Yearbook of Intensive Care and Emergency Medicine. Berlin: Springer-Verlag, 1997:100.
18. Peduzzi P, Shatney C, Sheagren J, Sprung C. Predictors of bacteremia and gram-negative bacteremia in patients with sepsis. Veterans Affairs Systemic Sepsis Cooperative Study Group. Arch Intern Med 1992;152:529-535.
19. Harris RL, Musher DM, Bloom K, et al. Manifestations of sepsis. Arch Int Med 1987;147:1895-1906

20. von Landenberg P, Shoenfeld Y. New approaches in the diagnosis of sepsis. Isr Med Ass J 2001;3:439-442.
21. Cohen J. Microbiological requirements for studies of sepsis. In: Sibbald WJ, Vincent JL, eds. Clinical Trials for the Treatment of Sepsis. Berlin: Springer-Verlag, 1995:29
22. Brun-Buisson C, Doyon F, Carlet J, et al. Bacteremia and severe sepsis in adults: A multicenter prospective survey in ICUs and wards of 24 hospitals. French Bacteremia-Sepsis Study Group. Am J Resp Crit Care Med 1996;154:617-624.
23. Shorr AF, Tabak YP, Killian AD, et al. Healthcare-associated bloodstream infection: A distinct entity? Insights from a large U.S. database. Crit Care Med 2006;34:2588-2595.
24. Norberg A, Christopher NC, Ramundo ML, et al. Contamination rates of blood cultures obtained by dedicated phlebotomy vs. intravenous catheter. JAMA 2003;289:726-729.
25. Washington JA II, Ilstrup DM. Blood cultures. Issues and controversies. Rev Infect Dis 1986;8:792-802.
26. Connell TG, Rele M, Cowley D, et al. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. Pediatrics 2007;119:891.
27. Reller LB, Murray PR, MacLowry JD. Blood Cultures II. In: Washington JA II, Cumitech IA, eds. Washington DC: American Society for Microbiology,1982.
28. Spitalnic SJ, Woolard RH, Mermel LA. The significance of changing needles when inoculating blood cultures: A meta-analysis. Clin Infect Dis 1995;21:1103-1106.
29. Y. Zhang, Clin Pharmacol Ther. 2007, 82:595-600.
30. Eskira S, Gilad J, Schlaeffer P, et al. Reduction of blood culture contamination rate by an educational intervention. Clin Microbiol Infect 2006;12:818-821.
31. Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of blood stream infections in adults: how many blood cultures are needed? J Clin Microbiol 2007;45:3546-3548
32. Washington JA II. Blood cultures: principles and techniques. Mayo Clin Proc 1975;50:91-98.
33. Weinstein MP. Current blood culture methods and systems: Clinical concepts, technology and interpretation of results. Clin Infect Dis 1996;23:40-46.

34. Brown DR, Kutler D, Rai B, et al. Bacterial concentration and blood volume required for a positive blood culture. J Perinatol 1995;15:157-159.

35. O'Hara CM, Weinstein MP, Miller JM. Manual and automated systems for detection and identification of microorganisms. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. Manual of Clinical Microbiology, 8th ed. Washington DC: ASM Press, 2003.

36. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteraemia and fungaemia. Clin Microbiol Rev 1997:10:444-465.

37. Riley JA, Heiter BJ, Bourbeau PP. Comparison of recovery of blood culture isolates from two BacT/ALERT FAN aerobic blood culture bottles with recovery from one FAN aerobic bottle and one FAN anaerobic bottle. J Clin Microbiol 2003;41:213-217.

38. Horvath LL, George BJ, Hospenthal DR. Detecetion of fifteen species of Candida in a automated blood culture system. J Clin Microbiol 2007;45:3062-3064.

39. Mehdinejad M, Khosani AD, Morvaridi A Study of prevelance and antimicrobial susceptibility pattern of bacteria isolate from blood culture 2009:04

40. Vanita Ravi N, Kaunan Gopal, Venkat Narendra M, VRD nagesh, Venkat sune M A retrospective study on blood stream infection and antibiotic susceptibility pattern in hospital 2011

41. Rajendra Prasad, S. Krishna murthy, Ramasane Prasad, Vinita Gupta Multidrug resistance: An emerging threat 1996,vol. 71: current science

42. Thomas C Harvey, Robert A Fowler and Nick Daneman ; Duration of antibiotic therapy for bacteremia, a systemic overview and meta analysis 2011

43. Kreig NR, Holt JG Bergey's manual of systemic bacteriology vol. 1 William and Wilkins, Baltimore, Maryland 1984

44. Derek C August and Tom van der Poll , severe sepsis and septic shock 21,2013. 369:840-51.

45. Stuart B levy & Bonnie Marshall. Antibacterial resistance worldwide causes, challenges and responses: 2004 December vol II

REFRENCE OF BOOK

1. CKJ Paniker (2005), Text Book of Microbiology, 7th edition, University press (India) pvt ltd. Hyderabad, India