CHAPTER I

1. INTRODUCTION

Bacteria may enter the blood stream, giving rise to bacteraemia, from a focus of infection within the body, from a surface site with a normal flora, or by the introduction into the vascular system of contaminated material. These organisms are normally cleared from blood within a few minutes, so that bacteraemia is often entirely silent and transient. However, if the immune system is overwhelmed or evaded, organisms persists in the blood and the symptoms and signs of septicaemia may supervene, either briefly until the organisms are eliminated or as a more constant or recurrent event(Phillips and Eykyn, 1990). Bacteraemia may be defined as a condition of simple presence of bacteria in blood without any multiplication which is often silent and transient. Septicaemia is a condition in which bacteria circulate and either continuously poured in the blood stream from a focus in the body or may actively multiply in the blood (Chakraborty, 2003).

Bloodstream infections are potentially life-threatening and require rapid identification and antibiotic susceptibility testing of the causative pathogen in order to facilitate specific antimicrobial therapy (Cleven *et al.,* 2006). Both gram positive and gram negative bacteria causes bacteraemia and septicaemia. Gram negative septicaemia, also known as endotoxic shock, is more severe than gram positive septicaemia. Many septic episodes are nosocomial and may be due to microorganisms with increased antimicrobial resistance. *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative staphylococci (CoNS), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus* spp., *Streptococcus* spp., *Candida albicans*, and *Enterobacter cloacae* are the most frequent etiological agents of bactaerimia and fungaemia in Europe and the United States (Cleven *et al.*, 2006).

The presence of living microorganisms in the blood of a patient is usually indicative of a serious invasive infection requiring urgent antimicrobial therapy. The mortality associated with bloodstream infections may range from 20% to 50% and depends on several factors, including the pathogen and host (Reimer *et al.*, 1997**)**. It is estimated that 2 million patients per year in the United States acquire infections while in hospitals, approximately 350,000 (10-20%) of these infections involve the bloodstream, and 90,000 (4.5%) are fatal. In Nepal, febrile illness is one of the most common reasons for medical consultation. The etiology of

bloodstream infection in febrile patients is poorly characterized in Nepal, mainly due to limited laboratory resources, a poor recording system and an inadequate number of trained personnel.

***Salmonella* infection (enteric fever):**

Although advances in public health and hygiene have led to the virtual disappearance of **enteric fever** (more commonly termed **typhoid fever**) from much of the developed world, the disease remains endemic in many developing countries. Typhoid fever is caused by *Salmonella* ***enterica*** serovar Typhi (*S.* Typhi), a Gram negative bacterium. A similar but often less severe disease is caused by *S* Paratyphi A and, less commonly, by *S* Paratyphi B (Schotmulleri) and *S* Paratyphi C (Hirschfeldii) (Bhutta, 2006). These highly adapted, human-specific pathogen have evolved remarkable mechanisms for persistence in their host that help to ensure its survival and transmission (Parry *et al*., 2002). The fever is characterized by the classic prolonged fever, sustained bacteraemia without endothelial or endocardial involvement, and bacterial invasion of and multiplication within the mononuclear phagocytic cells of liver, spleen, lymph nodes, and peyer patches of the ileum (Sharma *et al.,* 2002*)*..

Typhoid fever was an important cause of illness and death in the overcrowded and unsanitary urban conditions of the United States and Europe in the 19th century (Osler, 1912). The provision of clean water and good sewage systems led to a dramatic decrease in the incidence of typhoid in these regions. Today most of the burden of the disease occurs in the developing world, where sanitary conditions remain poor. Reliable data from which to estimate the burden of disease in these areas are difficult to obtain, since many hospitals lack facilities for blood culture and up to 90 percent of patients with typhoid are treated as outpatients (Parry *et al*., 2002). Typhoid is usually caused by ingestion of food or water contaminated by fecal or urinary carriers excreting *Salmonella* Typhi *and* Paratyphi. It is a sporadic disease in developed countries that occurs mainly in returning travelers, with occasional point-source epidemics (Ackers *et al*., 2000) In endemic areas, identified risk factors for disease include eating food prepared outside the home, such as ice cream or flavored iced drinks from street vendors, (Black *et al*., 1985; Luby *et al*., 1998),drinking contaminated water (Mermin *et al*, 1999) having a close contact or relative with recent typhoid fever,(Black *et al*., 1985; Luxemburger *et al.,* 2001) poor housing with inadequate facilities for personal hygiene, (Gasem *et al.,* 2001) and recent use of antimicrobial drugs(Luby *et al*., 1998).

In Nepal typhoid fever is known as "bisham joauro" meaning fever with poison. The fever is prevalent in mountains, valleys and southern belts of Nepal as an endemic disease with its peak incidence in May to August. Typhoid fever is one of the leading diagnosed fevers in most of the hospitals in Nepal (Hale, 1999; Rauniar *et al*., 2000). Typhoid outbreak was responsible for the deaths of many Nepalese from the time it was known as one of the causes of the fever. However, typhoid fever was considered responsible for deaths of many patients admitted in the hospitals in Kathmandu, the capital of Nepal, in late 1960s when the National Public Health Laboratory (NPHL) came into being (Sharma *et al.*, 2002*).*

Investigators from the US Centers for Disease Control and Prevention estimate that there are 21.6 million typhoid cases annually, with the annual incidence varying from 100 to 1000 cases per 100 000 population. The global mortality estimates from typhoid have also been revised downwards from 600 000 to 200 000, largely on the basis of regional extrapolations (Bhutta, 2006). In endemic areas, children aged 1 to 5 years are at the highest risk because of waning passively acquired maternal antibody and a lack of acquired immunity. In more recent years, prospective studies have shown that even where the incidence in patients is highest in adolescents and young adults, the overall incidence of blood culture–confirmed disease generally is highest in children aged 3 to 9 years and declines significantly in late adolescence **(**Corales and Schmitt, 2005).

Thus, rapid and reliable detection of bloodstream infections, including characterization of the pathogen to the species level and determination of its antibiotic susceptibility pattern, is crucial for several reasons:
(i) Appropriate antimicrobial agents can be selected, and thus, unnecessary treatment with ineffective antibiotics can be avoided (ii)
The prognosis of the patients can be improved
(iii) The acquisition of resistance in pathogens may be decelerated
(iv) Expenditure on antimicrobials and overall hospital costs can be reduced. (Barenfanger *et al.*, 1999; Doern *et al.*, 1994**)**

This study not only helps in the treatment of the patient more efficiently but will also help in generating surveillance data which will be useful to formulate an antibiotic policy in a given hospital.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To determine the prevalence of different types of bacteraemia and septicaemia from blood specimen in children visiting Kanti Children Hospital, Maharajgunj, Kathmandu and to determine antibiotic susceptibility pattern of isolates

2.2 SPECIFIC OBJECTIVES

1) To isolate and identify the pathogenic bacteria from blood sample collected from patients visiting Kanti Children Hospital, Maharajgunj, Kathmandu.
2) Age wise and Gender wise prevalence of bacteraemia and septicaemia.
3) To determine the pattern of antibiotic susceptibility test of bacterial isolates
4) To determine prevalence of MDR isolates.
5) To find correlation of Ciprofloxacin susceptibility with respect to Nalidixic acid resistant *Salmonella* spp.

CHAPTER-III

3. LITERATURE REVIEW

3.1 BACTERAEMIA AND SEPTICAEMIA

Bacteraemia means the presence of bacteria in blood and consequently may cause the invasion of cardiovascular system. The consequences of this event can range from transient, self-limiting infection to the one that is life threatening. Bacterial infections of the vascular system can be rapidly fatal or they can smolder for months causing gradual decline in health. Usually the bacteria are carried into the bloodstream by the flow of lymph from an area of infection in the tissues. Infection of the cardiovascular system can have devastating effects because infectious agents become systemic, they can be carried to all parts of the body producing disease in one or more vital organs or causing the circulatory system itself to stop functioning. Bacteraemia means the presence of viable bacteria in the blood, as demonstrated by positive blood culture. Septicaemia is an imprecise term applied to a bacteraemia accompanied by symptoms suggesting that the bacteria are multiplying within the blood stream. (Mahon and Manuselis, 1995)

The first case of bacteraemia was reported by Brill (previously called as *Bacillus pyocyaneus*, now *Pseudomonas aeroginosa*) in 1899. Ten years later, fewer than 40 cases had been reported worldwide, with less than 30 additional cases in the 15 years following that. Between 1954 and 1974 the reported incidence of bacteraemia increased 20 fold. And bacteraemia is now the thirteenth leading cause of death in the united state. (Mahon and Manuselis, 1995)

3.2 BLOOD AND ITS DEFENCE MECHANISM

Blood is a highly specialized tissue and as a liquid, it has no shape and confirms to the boundaries of the vascular tree. The circulating blood can be considered sterile under normal circumstances. Nevertheless, in most people, a few bacteria circulate everyday. The bacteria that circulate are members of the normal flora that spills over mechanical barriers such as the skin or mucosae. They are usually non pathogenic and are quickly cleared without any ill-effect. On the other hand, pathogenic microorganisms are not always removed from blood, but may persist there. Bacteria may travel throughout the body and seed various tissues to cause secondary infections. An enormous variety of microbial species can cause such

infections (Schaechter *et al.*, 1989). The presence of microorganisms in it represents failure of the defence mechanism (Duerden *et al.*, 1987).

Blood contains powerful antimicrobial systems, including leucocytes, immunoglobulin and complements. The chemical system acts in the moving bloodstreams pathogens, for example antibodies and complement can attach to circulating bacteria. On the other hand cellular defences such as neutrophils act efficiently only after the blood has delivered them to a suitable site in another tissue (Schaechter *et al.*, 1989). Once parasites penetrate the sub-epithelial layer they become prey to host's second line of defence which includes cellular elements of the blood, the lymphatic system and other non-specific resistance factor (Boyd and Marr, 1980)

3.3 CLINICAL SIGNS AND SYMPTOMS

Only about one third of patients experience the classic sign and symptoms of bacteraemia, which may include abrupt onset of chills, fever, or hypothermia, and hypotension. Forty percent of patients experience prostration and diaphoresis. Tachypnea is an early sign of bacteraemia. Delirium, stupor or agitation, vomiting and nausea, oliguria, or anuria occurs in 50% of the patients with gram-negative bacillary bacteraemia. Ecthyma gangrenosum, a central necrotic area surrounded by an erythematous base, is strongly correlated with gram negative bacteraemia. The failure of the body to mount an elevated temperature is also associated with increased mortality among neonates and the elderly. Altered clinical laboratory values that may be indicative of bacteraemia include the following:

- thrombocytopenia (50- 60% of the patients with gram-negative bacilli)
- leukocytosis or leucopenia
- acidosis with lactic acid
- abnormal liver function tests (especially hyperbillirubinemia)
- coagulopathy (more than 60% of the time)
- disseminated intravascular coagulation (5-10% of the patients with gram-negative bacillary bacteraemia)
- elevations in C-reactive protein, haptoglobin, and fibrinogen (Mahon and Manuselis, 1995)

3.4 COMPLICATIONS

a. Shock is the gravest complication of septicaemia. In the septic shock, the presence of bacterial products and the host's responding defensive components act to shut down major host physiological systems. Manifestations include a drop in blood pressure, increase in heart rate, impairment of function of vital organs (brain, liver, kidney, and lungs), acid-base problems and bleeding problems. Endotoxin (or the core of the lipopolysaccharides, lipid A) has been shown to mediate numerous systemic reactions, including a febrile (producing fever) response, and the activation of complement and certain blood- clotting factors. Although most Gram-positive bacteria do not contain endotoxin, and the effects of their in blood stream may be equally devastating to the patients (Forbes *et al.*, 1998).

b. Disseminated Intravascular Coagulation (DIC) is a disastrous complication of sepsis. DIC is characterized by numerous small blood vessels becoming clogged with clotted blood and bleeding as a result of the depletion of coagulation factors. DIC can occur with septicaemia involving any circulating pathogen, including parasites, viruses, and fungi, although it is most often a consequences of gram-negative bacterial sepsis (Boyd and Marr, 1980)

3.5 CAUSATIVE ORGANISMS

The commonest organisms causing septicaemia were pneumococci, streptococci (predominantly *Streptococcus pyogens)* and *Staphylococcus aureus* in the preantibiotic era. The introduction and continued use of antibiotics has brought important changes: there has been a striking increase in the number of bacteraemias caused by the gram negative bacilli a corresponding decrease in the number of streptococcal and pneumococcal bacteraemias.

Anaerobic bacteraemia is uncommon and polymicrobial bacteraemia is found in a small number of episodes.

Bacteria most commonly isolated from the cases of bacteraemia and septicaemia are as follows: (Cheesebrough, 2000)

a. Gram-negative microorganisms (60-70%):
    i) *Salmonella* spp. *(S.* Typhi, *S* Paratyphi A, *S* Paratyphi B, *S* Paratyphi C*),*
    ii) *Brucella* spp.

iii) *Haemophilus influenzae*

iv) *Escherichia coli*

v) *Klebsiella pneumoniae,*

vi) *Proteus mirabilis and Proteus vulgaris,*

vii) *Enterobacter* spp.

viii) *Bacteroides* spp.

ix) *Yersinia pestis*

x) *Acinetobacter* spp.

b. Gram-positive microorganisms (20-40%):

i) *Staphylococcus aureus*

ii) *Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus faecalis,* Viridans streptococci, Anaerobic streptococci

iii) Coagulase negative *Staphylococcus* (CoNS)

iv) *Listeria monocytogens*

v) *Clostridium perfrigens, Bacillus spp., Pseudomonas spp.*

vi) *Bacillus anthracis*

c. Others:

i) *Nesseria meningitidis*

ii) *Mycobacterium tuberculosis* (HIV associated tuberculosis)

iii) *Leptospira* spp.

iv) *Borrelia* spp.

v) *Bartonella bacilliformis.*

3.6 PATHOGENESIS

Once the bacteraemia is established, the patient develops signs and symptoms of bacteraemia and septicaemia through several mechanisms (Baron *et al.,* 1990). Gram-negative and certain other microorganisms contain lipopolysaccharides (LPS), which may be released during the normal growth cycle of bacteria and often the destruction of bacteria by a drop in circulating granulocytes, and a febrile response occurs and patient develop several symptoms (Baron *et al.,* 1990*).* Gram positive organisms do not have lipopolysaccharide in their cell wall, but other cell wall components such as peptidoglycan can produce pathophysiological effects like to those of LPS, albeit less effectively on a weight-for weight basis (Phillips and Eykyn, 1990). Some gram-positive bacteria produce exotoxins and in any case the effects of their presence in the bloodstream may be equally devastating to the patient (Baron *et al.*, 1990).

3.7 SOURCE OF BACTERAEMIA

i) Intravascular sources: In this case, the sources are within the hosts own body. As sources are within the body, bacteria are present in blood stream in fairly constant rate. e.g. endocarditis or infections of blood vessel, mycotic aneurysm, suppurative thrombophlebitis and intravenous catheter-associated bacteraemia (Forbes *et al.*, 2002). These sources can seed the bloodstream directly (Reese and Douglas, 1983).

ii) Extra vascular sources: e.g. wound furuncles, invasive procedures and abscesses. In a classic report, Bennett and Beeson emphasized that extra-vascular focus of infection gain entrance into the bloodstream through the lymphatics. Although lymph nodes are efficient filters of bacteria and are effective in localizing extra vascular foci of infection, their efficacy may be decreased if (a) the number of bacteria reaching the node is excessive (b) there is increased perfusion pressure of the lymphatic (i.e. presence of oedema and/or manipulation of the areas) or (c) usually virulent organisms are involved (Reese and Douglas, 1983).

The complex relationship between the various types of source and consequences of bacteraemia and septicaemia (Blood stream invasion) by bacteria are shown in fig-1

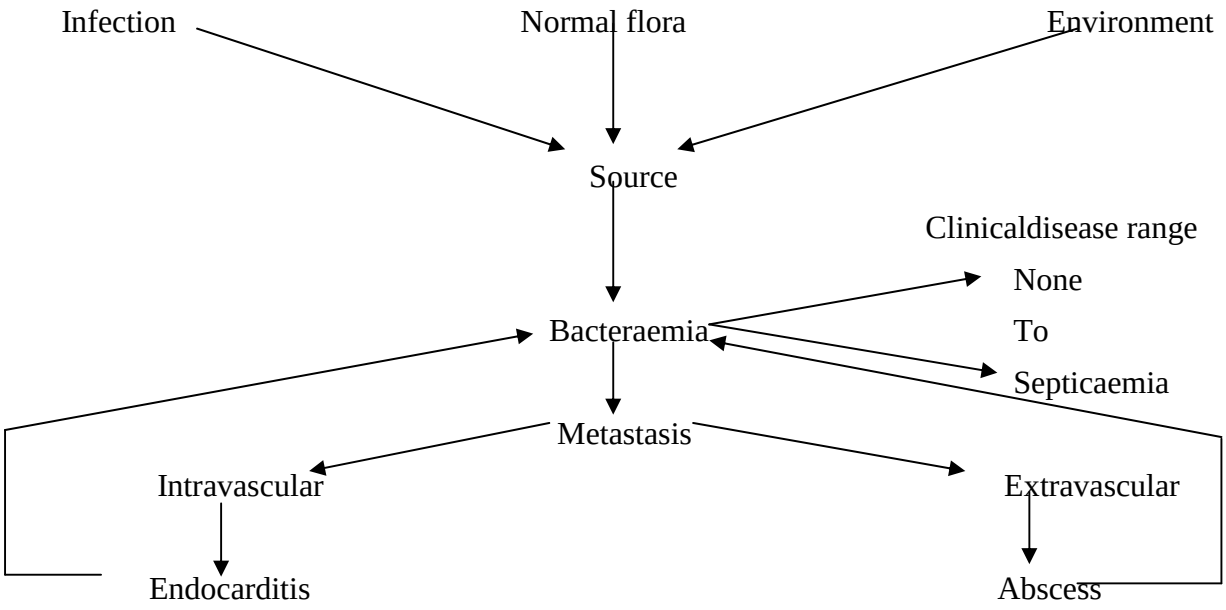


Figure1 The origin of bacteraemia and septicaemia (Phillips and Eykyn, 1990)

3.8 CLINICAL PATTERN OF BACTERAEMIA

The clinical pattern of bacteraemia was characterized as transient, intermittent or continuous by earlier workers (Reller *et al.*, 1982; Campos, 1989).

Transient bacteraemia: This is also known as incidental bacteraemia as bacteria are being entered in blood stream as a result of minor events such as brushing of teeth, chewing of food, manipulation of infected tissue, instrumentation of contaminated mucosal surface, surgery involving non-sterile site. These bacteria are effectively and rapidly removed by mononuclear phagocytic system in liver, spleen and circulating phagocytic cell (Tortora *et al.*, 2004). The precise duration of a transient bacteraemia is poorly defined in the literature. Studies looking at the duration of bacteraemia after dental extractions and endoscopy suggest that the great majority of these bacteraemia last 5 to 15 minutes only. Thus for a workable definition, it is reasonable to think of a transient bacteraemia as lasting for several minutes, not several hours or days (Reese and Douglas, 1983).

Sustained or continuous bacteraemia: Continuous bacteraemia are documented when several blood cultures are positive and spread out over time i.e. several hours to days. These bacteraemia are particularly suggestive of intravascular processes such as endocarditis, septic thrombonephritis or an infected artery. These infections in cardiovascular system are extremely serious and are considered life-threatening. Occasionally, an intra-abdominal abscess can cause a sustained gram-negative bacteraemia without an associated intravascular infection. A continuous bacteraemia from an extra vascular source is usually a poor prognostic sign for it implies that local and general host defence mechanisms have not been able either to contain or clear the infection (Reese and Douglas, 1983).

Intermittent Bacteraemia: These are defined as those in which the blood cultures are intermittently positive (without therapy) i.e. they are "recurrent transient" bacteraemia. The intermittent nature of the bacteraemia implies intermittent obstruction of an infected area (e.g. biliary or genitourinary) or intermittent manipulations of an infected area (Reese and Douglas, 1983).

3.9 CLASSIFICATION OF BACTERAEMIA:

Bacteraemia can be categorized into two classes:

1) Community Acquired Bacteraemia

2) Hospital Acquired Bacteraemia

3.9.1 Community Acquired Bacteraemia:

It often arises in previously healthy persons. It is more often severe than hospital acquired Bacteraemia. Many cases occur in association with demonstrable infection at local site from which organism entered the blood as in pneumococcal pneumonia (Phillips and Eykyn, 1990).

The organisms responsible for causing community acquired bacteraemia come predominantly from the patient's own commensal flora, for example, *Staphylococcus aureus*, *Escherichia coli*, pneumococci etc., though why they become invasive is not always clear. Pathogens may also be acquired from other persons, from animals or from environmental sources. Often the organisms are isolated from the blood culture will also be present at a local site of infection (Phillips and Eykyn, 1990).

The common cause of community acquired bacteraemia, as of bacteraemia in general, is *E. coli.* The majority of these incidents occur in association with urinary tract infections, 80% of the females, Bacteraemic urinary tract infections have a variety of presentations, ranging from the classical symptoms of acute pyelonephritis (usually in young women) to a rather non-specific illness in elderly or diabetic patients, again usually female. In elderly men they usually occur in association with retention of urine. The second most frequent focus of infection for community acquired *E. coli* bacteraemia is the biliary tract, either acute cholecystitis or less commonly acute cholangitis (Phillips and Eykyn, 1990).

Streptococcus pneumoniae accounted for 13% isolates in the Nottingham study, 16% in the Colorado study and for 25% in the St. Thomas' study. All series confirm that the majority of cases of pneumococcal bacteraemia (and indeed of all pneumococal infection) are community acquired: 86, 81 and 87% respectively. Most of these incidents occur in the association with respiratory tract infection, usually pneumonia (Phillips and Eykyn, 1990).

Community acquired bacteraemia due to *Staphylococcus aureus* occurs with moderate frequency. These are often severe with maximum systemic disturbances (infection of bone or joint). In most cases, there will be an obvious metastatic infection and infection of bone or joint are the commenest one. Endocarditis is the second focus of infection in community acquired staphylococcaemia (Phillips and Eykyn, 1990).

3.9.2 Hospital acquired bacteraemia:

According to the definitions proposed by the Centers for Disease Control and Prevention, hospital acquired bacteraemia (or nosocomial bacteraemia) is defined in a patient with a clinically important blood culture positive for a bactaerium that is obtained more than 49 hours after being admitted to the hospital (Garner *et al.*, 1988).

The ever increasing numbers of intensive procedures that have become part of modern high technology medicine have brought a concomitant increase in hospital acquired bacteraemia (Phillips and Eykyn, 1990).

The commenest organism isolated is *E. coli* but its frequency is at least a little lower in hospital acquired infections than in those acquired in the community. In ever one-half of the hospital acquired infection, the organisms come from the urinary tract and about a quarter of these are associated with urethral catheter or other urological instrumentation, about a quarter come from the gastrointestinal tract (Phillips and Eykyn, 1990).

S. aureus is second in frequency to E. coli as a source of hospital acquired bacteraemia. The main local site of infection in hospital acquired Staphylococcaemia is a vascular access site. A small but significant number of these patients subsequently developed endocarditis. The other main local site of infection is a surgical wound (Phillips and Eykyn, 1990).

The commenest of the Gram-negative bacilli are *Klebsiella* spp., *Pseudomonas aeruginosa* and *Proteus mirabilis*. Other most frequently encountered are *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Morganella morganii* and *Acinetobacter* spp. An increased frequency of coagulase-negative staphylococcus bacteraemia has been reported from USA and Europe as well as from the UK. These bacteraemia most commonly occur in patients in intensive therapy units and among premature neonates. Most of these bacteraemia are associated with infected intravascular catheters. *S. epidermidis* is the major isolate from hospital acquired bacteraemia (Phillips and Eykyn, 1990).

Nosocomial blood stream infections have been divided into two categories, primary and secondary.

A. Primary blood stream infection: Bloodstream Infection (BSI) occurs without any recognizable focus of infection with the same organism at another anatomic site at the time

of positive blood culture. Episodes of BSI secondary to intravenous or arterial lines are typically classified as primary bacteraemia (Apostolopoulous *et al.,* 2003).

B. Secondary blood stream infection: Secondary BSI is an infection that develops subsequent to a documented infection with the same microorganism at another body site. Note that if purulent thrombonephritis exists or if local infection (defined as redness, tenderness and pus) is present at the site of an intravenous line, the bacteraemia is considered as secondary to an intravenous line (Apostolopoulous *et. al.,* 2003).

3.10 TYPES OF SEPTICAEMIA

Septicaemia may be caused by a single organism or more than one microorganism. On the basis of the number of microorganisms involved, the septicaemia can be divided into monomicrobial and polymicrobial septicaemia.

Monomicrobial septicaemia: Septicaemia caused by a single type of microorganism is called monomicrobial septicaemia. This type of septicaemia is more commonly found in community acquired cases and usually recognized as part of a specific illness as when *Streptococcus pneumoniae* septicaemia complicates pneumonia with pyelonephritis (Weinstein *et al.,* 1986).

Polymicrobial septicaemia: Polymicrobial septicaemia refers to infections in which more than one microorganism has been recovered from the blood culture within 48 hour period. It may account for about 6-7% of all septicaemia and possesses a number of problems both in detection and in management. Polymicrobial septicaemia is more common in the hospital acquired than in community acquired septicaemia. Such septicaemia frequently occurs in surgical transplants, obstetrics and gynaecology and on medical service (Apostolopoulous *et al.,* 2003).

3.11 RISK FACTORS

The increased incidence of bacteraemia over the past 25 years appears to center around the following:

i) decreased immune competency of selected patient populations
ii) Increased use of invasive procedures
iii) age of patient

iv) administration of drug therapy

i) Gram-negative bacteraemia seem more increased in frequency in persons who have neoplasias, especially carcinomas, hematologic malignancies, and connective tissue diseases. Persons with other chronic underlying diseases, such as diabetes and cirrhosis, and those who are receiving immunosuppressive therapy are also at increased risk for bacteraemia.

ii) Increased use of life support system, respirators, and other invasive diagnostic procedure may play a role in gram-negative bacteraemia. Indwelling urethral catheters, suprapubic catheters, and intravenous pyelograms predisposes patients to catheter infections. These devices penetrate otherwise sterile areas and encourage colonization with bacteria from surrounding tissue. A greater potential of bacteraemia also occurs after surgery involving the gastrointestinal and biliary tracts.

iii) A bimodal distribution of bacteraemia has been observed. In the very young, increased bacteraemia is observed owing to a defect in humoral immunity, whereas in older popylations, bacteraemia is due to a general decrease in immune competency.

iv) The administration of broad spectrum antimicrobials reduces sensitive normal flora and favours colonization and invasion by gram negative bacteraemia. Immunisuopressive agents, especially steroids and anti cancer chemotherapeutics, place patients at increased risk far bacteraemia. (Mahon and Manuselis, 1995).

3.12 SALMONELLAE INFECTION

3.12.1 General review:

The bacterial genera *Salmonella* can cause a wide spectrum of clinical illness, of which there are four major syndromes, each with its own diagnostic and therapeutic problems which are the best considered separately. They include enteric fever, gastroenteritis, bacteraemia with or without meta-static infection and the asymptomatic carrier state (Lewis, 1995). Enteric fever is a generalized acute infectious disease characterized by cyclic course, definitive temperature curve, general intoxication, bacteraemia and affection of the lymphatic apparatus of the small intestine through which the infection implants itself in the organism upon entrance of the causative agent into gastrointestinal tract (Bunin, 1980). This septicaemic disease is caused by member of certain salmonella serotypes such as Typhi,

Paratyphi A, Paratyphi B, Sendai (Old, 1990). Certain other Salmonellae serotypes that cause septicaemia also frequently give rise to pyaemic lesion in the internal organ. The chief of these are member of serotypes Choleraesuis, Paratyphi C, Dublin and certain rare Enteritidis.

Table 1 Clinical diseases induced by Salmonellae

	Enteric Fever	**Septicaemias**	**Gastroenteritis**
Incubation period	7-20 days	Variable	8-48 hours
Onset	Insidious	Abrupt	Abrupt
Fever	Gradual, then high plateau with "typhoidal" state	Rapid rise, then spiking "septic " temperature	Usually low
Duration of disease	Several weeks	Variable	2-5 days
Gastrointestinal Symptoms	Often early constipation; bloody diarrhoea	Often none	Nausea, vomiting, diarrhoea at onset
Blood Cultures	Positive in 1^{st}-2^{nd} weeks of disease	Positive during high fever	Negative
Stool Cultures	Positive from 2^{nd} week on; negative earlier in disease	Infrequently positive	Positive soon after onset

Source: Brooks *et al.*, 2002.

Salmonellae are primarily intestinal parasites of man and animals, both domestic and wild they are also isolated from the blood and internal organs of vertebrates. They are frequently found in sewage, river and other waters and soil in which they do not multiply significantly. Provided the environmental conditions are suitable, they may survive for weeks in waters and for years in soil. Some serotypes are adapted to specific hosts; e.g. Abortusovis, Gallinarum, Typhi and Typhisuis are confined respectively to sheep, fowl, man and swine (or closely related species). The majority, however, do ubiquitous serotypes inhabit a wide range of hosts (Old, 1990).

Salmonella enterica serotype Typhi, *Salmonella enterica* serotype Paratyphi 'A' and 'B' and *Salmonella enterica* serotype Cholerasuis are primarily infective for humans and infections with these organisms implies acquisition from a human source (Brooks *et al.*, 2002). *S. Typhi* can be identified in the laboratory by several biochemical and serological tests. One of the most specific is that of polysaccharide capsule Vi, which is present in about 90% of all freshly isolated *S.* Typhi and has a protective effect against the bactericidal action of the serum of infected patients. This capsule provides the basis for one of the commercially available vaccines. Vi antigen is present in some other bacteria (*Citrobacter freundii*, *Salmonella* Paratyphi C and *Salmonella* Dublin) but not in exactly the same genetic context. The ratio of disease caused by *S.* Typhi to that caused by *S.* Paratyphi is about 10 to 1 in most of the countries where this matter has been studied (Bhutta, 2006)

***S.* Typhi:** It is the cause of typhoid fever in man and is very rarely isolated from other animals which it does not appear to infect under natural conditions (Old, 1990). It is serologically positive for LPS antigen O9 and O12, protein flagellar antigen Hd and polysaccharide capsular antigen V_i (Agarwal *et al.*, 2004).

***S.* Paratyphi A:** It was first isolated by Gwyn in 1898. *Salmonella* Paratyphi A is an important cause of enteric fever in Asia, the Middle East, Africa and South America. Host-adapted to man, it is infrequently isolated from other animals. It occurs naturally in H1 phase. Among subspecies-I salmonellae, it is unusual in being H_2S negative, xylose non-fermenting, lysine-decarboxylase negative and often anaerogenic (Ewing, 1969).

***S.* Paratyphi B:** *Salmonella* Paratyphi B has been isolated from cases of enteric fever in man. The synonym Schottmuelleri has precedence but is rarely used by clinical microbiologists. Some strains lack O antigen 5 i.e. is unable to O-acetylate abequose; it is difficult to understand why this property should be common in strains from carriers. Two broad categories of strain exist: (1) strains which cause an enteric-type fever, form a mucoid (slime) wall, do not ferment d-tartarate, are diphasic and are rarely isolated from animals, i.e. the group called '*Salmonella* Paratyphi B' by Kauffmann; (2) strains which cause an acute but mild enteritis, do not form a mucoid wall, are d-tartarate fermenting and are not uncommonly isolated from animals and food (Old, 1990).

***S.* Choleraseuis:** When first isolated by Salmon and Smith (1885), it was thought to be the agent of hog cholera, which is now known to be a virus disease. The natural reservoir of this

host-adapted serotype is pigs in which it commonly causes enteritis, though generalized infections also occurs. It is occasionally isolated from other animals, including man, for whom it is an important pathogen, not because it gives rise to many infections, but because it is highly invasive and its effects are severe. About 50% of recorded human infections are associated with prolonged pyrexia, usually of the septic type. Over one-third result in local pus formation and about 20% are fatal. The common pyaemic manifestations include pneumonia, septic arthritis, osteomyelits, meningitis and endocarditis (Old, 1990).

3.12.2 Pathogenesis :

During an acute infection, *S.* Typhi multiplies in mononuclear phagocytic cells before being released into the bloodstream. After ingestion in food or water, typhoid organisms pass through the pylorus and reach the small intestine. They rapidly penetrate the mucosal epithelium via either micro fold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophages (Mp) that ingest the bacilli but do not generally kill them. Some bacilli remain within macrophages of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where there is further multiplication and ingestion by Mp. It is believed that typhoid bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this silent primary bacteraemia the pathogen reaches an intracellular haven within 24 hours after ingestion throughout the organs of the reticuloendothelial system (spleen, liver, bone marrow, etc.), where it resides during the incubation period, usually of 8 to 14 days. The incubation period in a particular individual depends on the quantity of inoculum, i.e. it decreases as the quantity of inoculum increases, and on host factors. Incubation periods ranging from 3 days to more than 60 days have been reported. Clinical illness is accompanied by a fairly sustained but low level of secondary bacteraemia (approx. 1 to10 bacteria per ml of blood) (WHO, 2003).

3.12.3 Symptoms:

The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise, and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications. Many factors influence the severity and overall clinical outcome of the infection. They include the duration of illness before the initiation of appropriate therapy, the choice of antimicrobial treatment, age, the previous exposure or vaccination history, the virulence of the bacterial strain, the quantity of inoculum ingested, host factors (e.g. HLA

type, AIDS or other immunosuppression) and whether the individual was taking other medications such as H2 blockers or antacids to diminish gastric acid. Patients who are infected with HIV are at significantly increased risk of clinical infection with *S.* Typhi and *S.* Paratyphi (Gotuzzo *et al.*, 1991). Evidence of *Helicobacter pylori* infection also represents an increased risk of acquiring typhoid fever (WHO, 2003).

Acute non-complicated disease: Acute typhoid fever is characterized by prolonged fever, disturbances of bowel function (constipation in adults, diarrhoea in children), headache, malaise and anorexia. Bronchitic cough is common in the early stage of the illness. During the period of fever, up to 25% of patients show exanthem (rose spots) on the chest, abdomen and back (WHO, 2003).

Complicated disease: Acute typhoid fever may be severe. Depending on the clinical setting and the quality of available medical care, up to 10% of typhoid patients may develop serious complications. Since the gut-associated lymphoid tissue exhibits prominent pathology, the presence of occult blood is a common finding in the stool of 10-20% of patients, and up to 3% may have melena. Intestinal perforation has also been reported in up to 3% of hospitalized cases. Abdominal discomfort develops and increases. It is often restricted to the right lower quadrant but may be diffuse. The symptoms and signs of intestinal perforation and peritonitis sometimes follow, accompanied by a sudden rise in pulse rate, hypotension, marked abdominal tenderness, rebound tenderness and guarding, and subsequent abdominal rigidity. A rising white blood cell count with a left shift and free air on abdominal radiographs are usually seen. Altered mental status in typhoid patients has been associated with a high case-fatality rate. Such patients generally have delirium or obtundation, rarely with coma. Typhoid meningitis, encephalomyelitis, Guillain-Barré syndrome, cranial or peripheral neuritis, and psychotic symptoms, although rare, have been reported. Other serious complications documented with typhoid fever include haemorrhages (causing rapid death in some patients), hepatitis, myocarditis, pneumonia, disseminated intravascular coagulation, thrombocytopenia and haemolytic uraemic syndrome. In the pre-antibiotic era, which had a different clinical picture, if patients did not die with peritonitis or intestinal haemorrhage, 15% of typhoid fever cases died with prolonged persistent fever and diseases for no clear reason. Patients may also experience genitourinary tract manifestations or relapse, and/or a chronic carrier state may develop (WHO, 2003).

Carrier state: 1-5% of patients, depending on age, become chronic carriers harbouring *S.* Typhi in the gallbladder (WHO, 2003)

3.12.4 Case definition:

Confirmed case of typhoid fever: A patient with fever (38°C and above) that has lasted for at least three days, with a laboratory-confirmed positive culture (blood, bone marrow, bowel fluid) of *S.* Typhi (WHO, 2003).

Probable case of typhoid fever: A patient with fever (38°C and above) that has lasted for at least three days, with a positive serodiagnosis or antigen detection test but without *S.* Typhi isolation (WHO, 2003).

Chronic carrier: Excretion of *S.* Typhi in stools or urine (or repeated positive bile or duodenal string cultures) for longer than one year after the onset of acute typhoid fever. Short-term carriers also exist but their epidemiological role is not as important as that of chronic carriers. Some patients excreting *S.* Typhi have no history of typhoid fever (WHO, 2003).

3.13 SOME OTHER BACTERAEMIC ILLNESS

3.13.1 Infective endocarditis

Infective endocarditis, formerly known as bacterial endocarditis, is a grave disease characterized by a microbiological inflammation of the endocardial lining of the heart chambers, great vessels or valves (Weatherall *et al.*, 1987). In pre-antibiotic era, endocarditis was always fatal. The incidence of infective endocarditis is approximately 1.7-6.2 cases per 100 000 patient years (Mylonakis and Calderwood, 2001) although rates are higher in at risk cohorts such as intravenous drug users. Men are more often affected than women (in a ratio of 2:1), and the incidence progressively increases with age. Underlying degenerative aortic and mitral valve disease now predominate over rheumatic disease, although in one recent French study 47% of patients with infective endocarditis presented without previous knowledge of an underlying cardiac disorder. (Hoen *et al.*, 2002) The relation to dental surgery has been overemphasized in the past, and infective endocarditis is now more likely in the context of previous valve surgery or as a consequence of iatrogenic or nosocomial infection (Bouza *et al.*, 2001).

It could be classified in to acute, sub-acute and chronic by the length of the time from unset of to death (Cruickshank *et al.*, 1975). This classification is largely of historical interest because it is related to the unusual cause of untreated diseases. The classical presentation is with fever, heart murmurs, petechia, embolization, enlarged spleen and haematuria. Death results unless the patient is appropriately treated with bactericidal antibiotics.

The most common causative organisms of infective endocarditis are:

1. Viridians streptococci
2. *Enterococcus faecalis*
3. *Staphylococcus aureus*
4. *Haemophilus* spp.:
5. HACEK Bacteria (*Haemophilus aphrophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae*)

Other fastidious microorganisms such as *Campylobacter* spp., *Capnocytophaga* spp., *Rothia dentocariosa, Flavobacterium* spp., *Abiotrophia* spp. and *Chromobacterium* spp. may also be isolated from blood cultures (Forbes *et al.*, 2002). Apart from these groups of microbes CoNS, Brucellae, *Neisseria*, coli-form, *Pseudomonas* spp. (in drug abusers) rarely causes endocarditis.

3.13.2 Brucellocis

Brucellosis in man is alternatively known as undulant fever because clinical pattern often consists of periods of bacteraemia and fever alternatively with a febrile period. Fatigue, drenching sweats, malaise, headache, anorexia, weight loss, joints and muscle pain are the common features of clinical picture that is often difficult to recognize. In some patients, the illness is mild and in others, it is disabling and may persist for years if not recognized and correctly treated (Duerden *et al.*, 1987).

3.13.3 Staphylococcal infection

Staphylococcus aureus is one of the commonest causes of bacteraemia and such infection with it often takes the severe form known as staphylococcal septicaemia. Staphylococcal bacteraemia due to haemodialysis catheter have been reported from United Kingdom (Peacock *et al.*, 1999).

The staphylococci will have entered the bloodstream from a detectable local lesion but much more commonly there will be no evidence of local sepsis. Such bacteraemia are sometimes referred to as primary. Most cases of acute osteomyelitis occur in children but *Staphylococcus aureus* is also the commonest cause of vertebral osteomyelitis in adults (Phillips and Eykyn, 1990).

Coagulase negative staphylococci (CoNS) are frequently isolated from blood cultures, where they may be only a contaminant or the cause of bacteraemia. Determining whether an isolate of CoNS represents a true CoNS bacteraemia is difficult, and there is no single criterion with sufficient specificity.

Staphylococcus epidermidis and other coagulase negative staphylococci have been increasingly detected. The isolation of coagulase negative staphylococci with same biochemical and antibiotic susceptibility patterns from more than one blood culture from a patient should alert the clinician to the possibility of a clinically significant isolate. *Staphylococcus epidermidis* is the most common etiological agent of prosthetic valve endocarditis (Saint *et al.*, 2000).

3.13.4 Enterobacterial and Pseudomonads infections

Pseudomonas aeruginosa may be the cause of a great variety of bacteraemia, septicaemia, endocarditis, pyelonephritis and pneumonia. *Pseudomonas* bacteraemia occur after oral surgery.

Escherichia coli is the commonest isolate from blood cultures. Determination of *E. coli* septicaemia in non-perforated appendicitis has been reported from Ireland (Ruff *et al.*, 1994). These organisms, like those responsible for bacteraemic urinary-tract infections have a variety of presentations ranging from the classic symptoms of acute pyelonephritis (usually in young women) to a rather non-specific illness in elderly or diabetic patients (Phillips and Eykyn, 1990).

Klebsiella pneumoniae is also responsible for the bacteraemic illness. Nosocomial neonatal septicaemia due to *K. pneumoniae* has been reported from Mexico (Arredondo-Garcia *et. al*., 1992). Similarly Al-Rabea *et al.,* 1998 reported *K. pneumoniae* blood stream infection in neonates from Saudi Arabia and most of them are due to catheter-related infection and extrinsic contamination of infusates may have been occurred.

3.14 LABORATORY DIAGNOSIS

Because bacteraemia frequently portends life threatening condition, the prompt detection and recovery of microorganism from the blood is of paramount importance. Besides this, demonstration of endotoxin is increasingly considered useful because its presence in a patient infected with gram-negative microorganism is a good predictor of the eventual development of clinical syndrome of septicaemia (Phillips and Eykyn, 1990).

Blood culture system comprise (1) aseptic collection, (2) culture of this in a liquid medium to allow organisms present in small numbers to multiply, (3) a system or combination of systems to detect them and (4) a final phase of subcultures so that the bacteria can be identified and their antibiotic susceptibility determined (Phillips and Eykyn,1990).

3.14.1 Specimen collection:

Blood must be collected with sterilized syringe. The vein from which blood is to be collected must be chosen before skin is cleaned with 70% ethanol followed by tincture of iodine solution. This will minimize the risk of contamination with normal skin flora and hence major source of confusion in interpretation of result is avoided. If patient has an existing intravenous line, the blood should be drawn below the existing line because blood drawn above the line will be diluted with fluid being infused. It is less desirable to draw blood through a vascular shunt of catheter because these prosthetic devices are difficult to decontaminate completely (Forbes *et al.*, 2002).

i. Timing of sample: In order to attain a maximum yield, blood specimen must be collected at an appropriate time (Phillips and Eykyn, 1990). The number of bacteria found in the blood varies from time to time in the same patient and it is clearly desirable to take sample when many are present (Stokes *et al.,* 1993). If a regular periodicity of fever can be established, then the most appropriate time to draw blood culture will be just before raise in the temperature. In bacterial endocarditis and other endovascular infection, organisms are released into blood stream at fairly constant rate, so that timing of culture is not so

important. Blood cultures should always be collected before antibiotic treatment has begun (Reimer *et al.*, 1997).

ii. Number of blood samples: The number of blood specimen that should be drawn for culture and the period of time over which this is done depends in part upon the severity of the clinical illness. In hyper acute infection e.g. gram-negative sepsis with shock or staphylococcus sepsis, it is appropriate to culture two blood specimens obtaining from different anatomic sides over a period of 10 minutes. Current guidelines for infective endocarditis recommend that three sets of blood cultures are drawn one hour apart before the introduction of antibiotic treatment (Horstkotte *et al.*, 2004)—the first two sets are positive in more than 90% of cases. A total of three blood cultures yields the infecting bacteria in more than 95% of bacteraemic patients. If the initial three cultures are negative and occult abscess, fever or unknown origin or some other obscure infection is suspected, additional blood specimen should be cultured before antimicrobial therapy is started (Brooks *et al.*,2002). Similarly, a patient develops septicaemia from an acute infection of the urinary tract; a sample of urine and a single blood culture are generally significant for clinical purposes.

iii. Volume of blood: In case of adult, several studies show that less than 30 CFU/ml of bacteria was found with clinically significant bacteraemia. Therefore sufficient volume of blood is required for successful detection of bacteraemia (Forbes *et al.*, 2002). A number of studies have shown that increasing the volume up to 10-20 ml is associated with an increased frequency of positive cultures. In case of children, it is not safe to take large volume of blood for culture. Fortunately, the numbers of organisms present in the blood of infants with septicaemia is usually high and thus smaller volume is sufficient (NCCLS, 1998).

New data suggest that anaerobic blood bottles may not be necessary for obtaining accurate and clinically relevant results. Two aerobic bottles may be more valuable because of increase in the volume of blood culture. A total of 20 to 30 ml of blood may be optimal (Miller, 1996).

Types of broth media:

The most frequently used liquid media are either nutrient, glucose, Tryptic Soya or Brain Heart Infusion Broth but that some laboratories use a diphasic medium (solid and liquid

medium in the same bottle) or liver infusion for Brucellosis or bile salt broth for enteric fever (Phillips and Eykyn, 1990).

Blood dilution:

The blood sample should be diluted between 1 in 5 and 1 in 10 in the culture medium in order to reduce the concentration of natural antibacterial constituents such as serum complement, immunoglobulin, lysozyme, monocytes and to a sub-effective level. The dilution also reduces the concentration of any therapeutically administered antibiotic (Collee *et al.*, 1996). Clotting of blood is also prevented by dilution (Stokes *et al.*, 1993).

Anticoagulant used in broth media:

The anticoagulant in blood culture media must not harm the bacteria and must prevent clotting of the blood which would entrap the bacteria and prevent their detection. The most commonly used preparation in blood culture media, today is 0.025-0.03% solution of Sodium Polyanethol Sulphonate (SPS) (Liquoid). In addition to its anticoagulant properties, SPS is also anticomplementary, antiphagocytic and interferes with the activity of certain antimicrobial agents, notably the aminoglycosides (Forbes *et al.*, 2002). In the vast majority of cases, liquoid increases the isolation rate and also the growth rate (Shanson, 1989). Unfortunately, liquoid is inhibitory, at least in some media, for some organisms, including some strains of micro-aerophilic streptococci, *Neisseria* spp., *Gardenerella vaginalis* and *Peptostreptococcus anaerobius*. The addition of 1-2% gelatin has been shown to counteract this inhibitory action of SPS (Forbes *et al.*, 2002).

Ghimire., 1995 from Nepal showed that the anticoagulant liquoid was far better than sodium citrate for the recovery of gram-positive bacteria specially pneumococcus and other α and β haemolytic streptococci.

3.14.2 Subculture from blood cultures

The most important recent advances in blood culture technology have been in methods for the detection of growth in the liquid medium after it has been incubated at 37°C. Such growth may be visible as turbidity or as colonies and with haemolytic organisms, the blood may be haemolysed. However, the most sensitive means of detection is subculture of a small volume on appropriate solid medium (Phillips and Eykyn, 1990).

Initial subculture may include chocolate agar, 5% sheep blood agar, MacConkey agar, anaerobic supplemented blood agar and a selective medium for *Bacteroides* spp., such as bacteroides-bile aesculin agar. Bottles are then re-incubated for 5-7 days unless the patient's conditions require special consideration (Forbes *et al.*, 2002).

3.14.3 Identification

The isolated organisms are identified by Gram staining procedure, biochemical tests and agglutination tests (Chakraborty, 1998).

3.14.4 False positive results

It is necessary to determine the significance of the positive blood culture. Guidelines that can assist in distinguishing probable pathogen from contaminants are as follows:

i) Probable contaminants:

Growth of *Bacillus* spp.; *Corynebacterium* spp.; *Propionibacterium acnes* or coagulase negative staphylococci in only one of several cultures.

Growth of multiple organism from only one of several culture (polymicrobial growth is uncommon).

The clinical presentation and/or course is not consistent with sepsis (physician based, not laboratory based criteria).

The organism causing the infection at a primary site of infection is not the same as that isolated from the blood culture.

ii) Probable pathogen:

Growth of the same microorganism in repeated cultures obtained either at different times of from different times or from different anatomical sides.

Growth of certain organism in culture obtained from patients suspected of endocarditis; such as enterococci or gram-negative rods in patients with clinical gram-negative sepsis.

Growth of certain pathogen such as member of enterobacteriaceae, *Streptococcus pneumoniae,* gram-negative anaerobes and *Streptococcus pyogenes.*

Isolation of commensal microbial flora from blood culture obtained from patients suspected to be bacteraemic (e.g. Immunosuppressed patients or those having prosthetic devices).

3.14.5 Other methods

Conventional blood culture techniques are labour intensive and time consuming. During these times of cost constraints in health care and a corresponding requirement for clinically relevant care, the development of instrumentation for blood cultures has been accomplished. Instruments can rapidly and accurately detect organism in blood specimens. By using such instrumentation, laboratories that process many blood cultures can also provide results cost effectively (Forbes *et al.*, 2002). Some of them are:

BACTEC SYSTEMS: Many laboratories use the BACTEC system (Becton Dickinson Microbiology Systems, Md.), which measures the production of carbon dioxide (CO_2) by metabolizing organisms. Blood or sterile body fluid for routine culture is inoculated into bottles that contain the substrates.

Bac T/ALERT MICROBIAL DETECTION SYSTEM: Other laboratories use the Bac T/Alert System (Organon Teknika, Durham, N. C.) which measures CO_2 derived pH changes by a colorimetric sensor in the bottom of each bottle.

ESP SYSTEM: The ESP Culture System II (Trek Diagnostic Systems, Inc, Westlake, Ohio) differ the other previously discussed systems in that microbial growth is detected by the consumption and/or production of gases as organisms metabolize nutrients in the culture medium.

VITAL: Another continuous monitoring blood culture system is the Vital (bioMerieux, Inc., Hazelwood, Mo.,USA). A fluorescent molecule that decreases its fluorescent output in the presence of CO_2, changes in pH or modification of oxidation-reduction is incorporated in the broth solution and serves as an indicator, detecting any organism present in the culture (Forbes *et al.*, 2002).

3.14.5 Antimicrobial susceptibility of the isolates

Bacterial susceptibility to antimicrobial agents is done in vitro by Kirby-Bauer disc diffusion technique using fresh broth culture of isolates in Mueller-Hinton agar medium and

commercially available antibiotic discs. On the basis of zone-size compared with that of control strains, the result is interpreted (Greenwood *et al.*, 2000).

The antibiotic susceptibility of the causative organism will rarely be known early enough to provide definitive guidance for the initial treatment of a septicaemic patient though occasionally examination of the buffy coat from a blood-culture bottle may have provided some suggestive information. Initial selection of an antibiotic must depend on prediction of the likely pathogen and its usual susceptibility to antibiotics (Collee *et al.*, 1996).

Once a pathogen has been isolated and its antibiotic susceptibility is known, definitive therapy should be given (Phillips and Eykyn, 1990). Laboratory monitoring of therapy is necessary and for which the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the antimicrobial agent for the isolated organisms must be determined. The routine disc diffusion technique with a fixed concentration of drug is not adequate for guiding treatment of infective endocarditis. The measurement of MIC and MBC of antibiotic helps to determine adequate dose of the antibiotic to be used for ensuring the serum levels that can penetrate the valves and kill the organisms (Chakraborty, 1998).

If the focus of infection is known, the nature and antibiotic susceptibility of the possible cause may also be known. In a burnt patient, there is a reasonable probability of *Pseudomonas* and one should use ticarcillin with gentamicin, tobramycin or amikacin. The aminoglycosides choice would be influenced by knowledge of the antibiotic sensitivities of the local gram- negative rods. For other patients, not in shock, cefotaxime alone would probably be adequate. Persons with overwhelming sepsis should be given cefotaxime and an aminoglycoside because one may not have a second chance (Weatherall *et al.*, 1987).

3.14.6 The effect of antimicrobials and the problem of drug resistance

The overuse and misuse of the antimicrobials have led to the death of the sensitive strains leaving resistant strains to survive, multiply and infect new hosts. Bacteria most likely to cause hospital infection are usually highly resistant to antimicrobials. The fact that bacteria which are multiple drug resistant are uncommon outside the hospital means that they are not biologically dominant, without the aid of antibiotics to depress resident bacteria, they are unable to establish themselves (Stokes *et al.*, 1993).

There are four mechanisms which are responsible for the ability of micro organism to resist the action of antimicrobials (Boyd, 1995). They are:

a) Interference with transport across the cell-wall or cytoplasmic membrane.
b) Enzymatic modification of the drug.
c) Alteration of the antimicrobial target in the microorganism.
d) Synthesis of resistant metabolic pathways.

The most frequent antimicrobial resistance mechanism in bacteria is plasmid mediated enzymatic inactivation. Plasmids are extra-chromosomal DNA that represents a reasonable stable but dispensable gene pool in bacteria. They impart considerable additional genetic information to their bacterial host. The kind of antibiotic inactivation is unique to R-plasmids (Hugo and Russel, 1983). The result of a study carried out by Khor and Jegathesan (1983) in Malaysia demonstrated that transferable resistant plasmids play a major role in antibiotic resistance in Enterobacteriaceae.

3.15 GLOBAL SCENARIO OF BACTERAEMIA AND SEPTICAEMIA

A prospective study of bloodstream infection performed between February 1999 and July 2000, there were 193 C-BSI and 230 H-BSI. The large majority of C-BSI were caused by bacterial pathogens susceptible to narrow-spectrum antibiotics, particularly in children. H-BSI were infrequently due to enterococci (4%), Candida (3%) or methicillin-resistant Staphylococcus aureus (0.4%). No BSI was due to vancomycin-resistant enterococci or extended-spectrum beta-lactamase producing Enterobacteriaciae. (Diekema *et al.*, 2003)

A study conducted in the United States in 2002 revealed that coagulase-negative staphylococci (42.0%), *Staphylococcus aureus* (16.5%), *Enterococcus faecalis* (8.3%), *Escherichia coli* (7.2%), *Klebsiella pneumoniae* (3.6%), and *Enterococcus faecium* (3.5%) were the most frequently isolated bacteria from blood cultures and susceptibility to expanded-spectrum β-lactams such as ceftriaxone were high for coagulase-negative staphylococci (98.7%), *S. aureus* (99.8%), *E. coli* (97.3%), *K. pneumoniae* (93.3%), and *Streptococcus pneumoniae* (97.2%). Susceptibilities to fluoroquinolones were variable for *K. pneumoniae* (90.3-91.4%), *E. coli* (86.0-86.7%), *S. aureus* (84.0-89.4%), coagulase-negative staphylococci (72.7-82.7%), *E. faecalis* (52.1%), and *E. faecium* (11.3%). (Karlowsky *et al.*, 2004)

In the surveillance from ICDDRB, 2004 between May and July 2004, among, 999 children were enrolled; blood samples were collected from 886 children. 63% were male and 87% were less than 2 years including 19% neonates, 27% between two to five months, 30% between 6 to 11 months and 24% between 12-23 months. 85 bacterial isolates were obtained from blood of them *Klebsiella pneumoniae* and *Streptococcus pneumoniae* were the most frequently isolated.

In all 1,201 children with suspected septicaemia from Calabar, Nigeria 539 (44.9%) were females and 662 (55.1%) were males. The majority of the patients were newborns (533; 44.4%) and infants (252; 21.0%). Bacteria were isolated in 552 (45.9%) of the 1,201 patients studied. The rate of isolation was highest among newborns (271/533: 50.8%). The overall rate of isolation reduced with increasing age but the types of organisms cultured did not vary with age. The most frequent isolates were *Staphylococcus aureus* (48.7%) and *Coliforms* (23.4%). Unidentified gram-negative rods (8.0%), *Pseudomonas aeruginosa* spp. (5.8%), *Streptococcal* spp. (4.7%) and *Chromobacterium* spp. (4.5%) were also fairly frequently identified. *Staphylococcus aureus* had the highest susceptibility to Ceftriazone (100%), Cefuroxime (100%), Azithromycin (100%), Erythromycin (90.1%) and Gentamicin (86.6%). *Coliforms* were most susceptible to Ceftazidime (78.8%), Ceftriaxone (83.3%), Cefuroxime (76.5%) and Azithromycin (92.9%) (Meremikwu *et al*.**,** 2005).

A study was performed by Berkley *et al.,* 2005; blood was cultured from 19,339 inpatients. Of a total of 1783 infants who were under 60 days old, 228 had bacteraemia (12.8%) and also 866 of 14,787 children who were 60 or more days of age (5.9%) had bacteraemia. Among infants who were under 60 days old, *Escherichia coli* and group B streptococci predominated (14 %and 11 %, respectively). Among infants who were 60 or more days of age, *Streptococcus pneumoniae,* nontyphoidal salmonella species, *Haemophilus influenzae,* and *E. coli* accounted for more than 70 % of isolates. The minimal annual incidence of community-acquired bacteraemia was estimated at 1457 cases per 100,000 children among infants under a year old, 1080 among children under 2 years, and 505 among children under 5 years.

A retrospective review of all the 390 neonatal blood cultures carried out in Nigeria, 390 neonatal samples constituted 25% of all blood samples.22% were positive for bacterial growth, yielding gram-negative bacilli (GNB) and gram- positive cocci (GPC) in almost equal proportion, predominantly *Klebsiella pneumoniae* (86%) and Staphylococcus aureus

(81%). The sensitivity of the Staphylococcus aureus isolates to amoxicillin-clavulanic acid, cefuroxime, ciprofloxacin, chloramphenicol and erythromycin were 89%, 85%, 75%, 71% and 64% respectively (Iregbu *et al.,* 2006).

A study performed in Saudi Arabia, revealed that 220 pediatric patients had BSI, of whom 147 (67%) were males. 173 (78.6%) of the isolates were Gram positive bacteria and included the following: *Staphylococcus epidermidis* (55.4%), *Staphylococcus aureus* (9.5%), *Streptococcus pneumoniae* (4.5%), and *Enterococcus faecalis* (4%). Gram negative bacteria were 44 (20%) and included *Escherichia coli* and *Klebsiella pneumoniae* (3.6% each). None of the Gram positive isolates were vancomycin resistant. Three isolates of *Klebsiella pneumoniae* and one isolates of *Pseudomonas* spp. were multiresistant (Babay *et al.*, 2005).

Blood cultures were performed on 891 children (aged 1 month to 16 years) who presented at the Jos University Teaching Hospital. 139 (15.6%) were positive to blood culture. *Staph. aureus* was the commonest bacterial agent isolated and accounted for 36.0%, *Klebsiella* spp. and *Salmonella* spp. each accounted for 18.7% and 15.8% respectively. Other bacterial agents isolated included E. coli 7.9%, coliforms, 6.5%, *Pseudomonas* spp. 3.6%, *Proteus* spp. 2.2% and, miscellaneous organisms 9.3%. 40% of the *Staphylococcus aureus* isolated was resistant to cloxacillin. 1.7% of the *Staphylococcus aureus* isolated were resistant to all the antibiotics (Angyo *et al.*, 2001).

In a one year study of bacteraemia in children carried out in Myanmar, children aged one month to 12 years were included and bacteria were isolated from 65 cases (54.2%). The commonest organism isolated was *Salmonella Typhi* (43.1%). Others included *Escherichia coli* (12.3%), *Staphylococcus aureus* (7.7%), *Pseudomonas aeruginosa* (7.7%); *Streptococcus*, *Shigella*, Diplococcus, *Klebsiella* and *Acinetobacter* were also isolated. The *Salmonella Typhi* were resistant to conventional antibiotics; ampicillin, amoxicillin, chloramphenicol, and co-trimoxazole (Shwe *et al.*, 2002)

A total of 48 isolates of *K. pneumoniae* and 4 additional isolates of *Klebsiella* spp. were recovered from blood samples of 48 patients. A total of 36 *E. coli* isolates were recovered from 33 patients, and a total of 37 isolates of various *Salmonella enterica* serotypes (two *S. Typhi*, 15 *S. Typhi*murium, 19 *S.* Enteritidis, and 1 *S.* Newport) were isolated from 37 patients. Fifteen patients had polymicrobial infections, of which eight patients had mixed

infections with pathogenic bacteria other than *E. coli*, *Klebsiella* spp., and salmonellae (Blomberg *et al.***,** 2005).

A total of 120 sets of blood samples were cultured aerobically from 60 children with clinically diagnosed septicaemia at Kenyatta National Hospital, Nairobi. *Salmonella Typhi*murium was the most frequently isolated bacteria (63%), followed by *Staphylococcus aureus* (15.8%). Most of the patients were 13 months to 4 years old (45%). 31.7% of the patients had positive blood cultures. *Staphylococcus aureus* was the second most common bacteria (15.8%) among these patients (Odhiambo *et al.,* 1991).

Of the 920 patients at the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, 233 (25.3%) had blood samples positive by culture. 13 different bacterial species were identified from positive blood cultures of which six predominated: *Staphylococcus aureus* (30.5%), Coliforms (17.6%), *Klebsiella* spp. (14.2%), *Pseudomonas aeruginosa* (9.2%), *Escherichia coli* (7.9%) and *Salmonella Typhi* (5.9%). The in vitro antibiotic, disc sensitivity pattern of the isolates showed they were relatively resistant to ampicillin and penicillin G. Septicaemia cases were recorded in all age groups but incidence in females was slightly but not significantly higher than in males (Ako-Nai *et al.,* 1990).

Over the period of thirteen months 4,368 blood samples (for blood culture) were collected from children in the age group below 14 years, 1,001 cases (22.9%) were culture positive. Incidence of bacteraemia in neonates was 521(33.94%). Gram negative organisms were the most predominant isolates being (88.8%) where the commonest were *Klebsiella* spp. 471 (47.1%) followed by *Salmonella* spp. 162 (16.2%) and *Pseudomonas* spp. 80 (8%) whereas in gram positive, *Staphylococcus aureus* 76 (7.6%) was the most common. (Sharma *et al.* 2002)

Data on 2364 consecutive episodes of BSI at Birmingham Children's Hospital, a total of 1224 (51.8%) episodes were community-acquired. Intravascular devices (IVDs) were the most common source of infection, accounting for 48.9% of episodes. Gram-positive, gram-negative and anaerobic bacteria accounted for 66.2%, 31.3% and 0.4% of isolates, and 2.2% were yeasts. Coagulase-negative staphylococci, *Staphylococcus aureus* and enterococci accounted for over 50% of all isolates. Of these, only enterococci were predominantly hospital-acquired. *Neisseria meningitidis* was the most common cause of community-acquired BSI in previously healthy children. Antibiotic resistance, especially in

Enterobacteriaceae, *Staphylococcus aureus* and enterococci, was more common (Gray, 2004).

In a study conducted at GMC, Chandigarh,, on 5,704 blood samples that were collected from patients with fever/sepsis admitted to Government Medical College and Hospital, Chandigarh, India,among the 567 samples, *Pseudomonas aeruginosa* (19.75%), *Escherichia coli* (15.17%), *Klebsiella pneumoniae* (14.99%), *and Salmonella Typhi* (12.87%) were the most frequently isolated Gram-negative bacteria other than *Citrobacter, Acinetobacter, Proteus,* and *Enterobacter* spp. collectively accounting for 80.96% of the isolates. *Staphylococus aureus* (13.86%) and *Enterococcus feacalis* (2.35%) were most frequently isolated Gram-positive bacteria other than other *Streptococcus* and *Staphylococcus* spp. collectively accounting for 18% of the isolates. Among the antibiotics used for susceptibility testing of most of the Enterobacteriaceae weer sensitive to amikacin (76.61%) against Enterobacteriaceae and lactose non-fermenters were sensitive to ciprofloxacin(65.17%). All of the Gram-positive isolates were sensitive to vancomycin (100%), and 89.74% were sensitive to ciprofloxacin (Mehta *et al.,* 2005).

In a study in children in Southern Israel, 1561 isolates 793, (50%) gram-positive and 768, (49%) gram-negative organisms, respectively) and 13 fungi were recovered. Most frequent gram-positive organisms were *Streptococcus pneumoniae* (509 isolates, 32% of all isolates, 64% of all gram-positive), *Staphylococcus aureus* (137, 9%, 17%) and *Streptococcus pyogenes* (46, 3%, 6%). Enterobacteriaceae spp. were the most frequent gram-negative pathogens (279, 18%, 36%), followed by *Brucella* (205, 13%, 27%). *S. pneumoniae* was the most common pathogen in children less than12 months and 1 to 5 years age; *Brucella* was the most frequent pathogen in children more than 5 years of age (Gur *et al.,* 2006).

In a study of bacterial isolates from neonatal septicaemia over a period of 5 years (July 1998-June 2003) at the Government Medical College Hospital, Chandigarh, India, the study was carried to determine the bacterial profile, the antimicrobial susceptibility of the isolates, and the change in trends over the study period. A total of 3,064 blood samples for blood culture were obtained, out of which 588 (19.2%) were positive for bacterial isolates. Gram-negative bacilli (58.5%) predominated over gram-positive cocci (41.5%). *Staphylococcus aureus* was found to be most common isolate (35.0%).Amikacin was found to be the most effective drug against Gram-negative bacteria (Agnihotri *et al.,* 2004).

3.16 NATIONAL SCENARIO

Clinically suspected 77 cases of neonatal **septicaemia** admitted to the pediatric ward of BP Koirala Institute of Health Sciences, Dharan, Nepal, were evaluated by blood culture. The blood culture was positive for bacterial growth in 46 (59.7%) cases. Gram-negative bacteria were isolated in 33 (71.7%) cases, the most common being *Escherichia coli*, followed by *Klebsiella* spp., *Enterobactor* spp. *Staphylococcus aureus* was the most common Gram-positive bacteria. The bacterial isolates were most sensitive to gentamicin (74.5%) and ciprofloxacin (74.5%) followed by chloramphenicol (59.9%). They were most resistant (78.8%) to ampicillin.

In a study done by Niroj *et al.* (2003) at Kathmandu Model Hospital, incidence of bacteraemia of children below 10 years was found to be 10.14%.

A study conducted at Tribhuwan University teaching Hospital (TUTH), revealed that among 49 children of age less than 10 years, the incidence of bacteraemia was found to be 12.24% and the organism isolated were *Salmonella* Typhi, *Salmonella* Paratyphi A, *Staphlylococcus aureus* and *Pseudomonas aeruginosa* (Wagle *et al.*, 2002).

3.17 MAGNITUDE OF SALMONELLAE INFECTION

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are no bacteriology laboratories in most areas of developing countries. These factors are believed to result in many cases going undiagnosed. It has been estimated that approximately 17 million cases of typhoid fever and 600 000 associatd deaths occur annually (Ivanoff, 1994). However, the estimates have been biased because study populations have usually been in areas of high incidence. Preliminary results from recent studies conducted in Bangladesh by ICDDRB show an incidence of approximately 2000 per 100 000 per year (Punjabi, 1998).

It is important to note that reports from some provinces in China and Pakistan have indicated more cases of paratyphoid fever caused by *S.* Paratyphi A than by *S.* Typhi. In areas of endemicity and in large outbreaks, most cases occur in persons aged between 3 and 19 years. In 1997, for example, this age range was reported during an epidemic of the disease in

Tajikistan. Nevertheless, clinically apparent bacteraemic *S.* Typhi infection in children aged less than three years has been described in Bangladesh, India, Jordan, Nigeria, and elsewhere (Sinha *et al.,* 1999; Saha *et al.,* 2001).

In Indonesia there is a mean of 900 000 cases per year with over 20 000 deaths. In Indonesia, people aged 3 to19 years accounted for 91% of cases of typhoid fever and the attack rate of blood-culture-positive typhoid fever was 1026 per 100 000 per year. A similar situation was reported from Papua New Guinea. When typhoid fever was highly endemic in certain countries in South America the incidence of clinical typhoid fever in children aged less than 3 years was low. In Chile, however, single blood cultures for all children aged less than 24 months who presented at health centres with fever, regardless of other clinical symptoms, showed that 3.5% had unrecognized bacteraemic infections caused by *S.* Typhi or *S.* Paratyphi (Ferrecio *et al.,* 1988).

In South America the peak incidence occurred in school students aged 5 to19 years and in adults aged over 35 years. This kind of study has not been conducted in other areas of endemicity. Between 1% and 5% of patients with acute typhoid infection have been reported to become chronic carriers of the infection in the gall bladder, depending on age, sex and treatment regimen. The propensity to become a carrier follows the epidemiology of gall bladder disease, increasing with age and being greater in females than in males. The role of chronic carriers as a reservoir of infection was studied in Santiago, Chile, where a crude rate of 694 carriers per 100 000 inhabitants was found (Levine *et al.,* 1982).

CHAPTER –IV

4. MATERIALS AND METHODS

This study was conducted at Kanti Children's Hospital's Microbiology lab from 1st June, 2006 to 30th September, 2006. During this period blood samples from 1671 patients suspected of bacteraemia and septicaemia were processed in the microbiological laboratory. The samples were processed according to the standard protocol.

4.1 MATERIALS

Different materials required for present work were mentioned in Appendix IV.

4.2 METHODS

4.2.1 Sample collection

The blood samples were collected either by doctors or nurses or laboratory technicians using standard aseptic techniques. First of all each patient was provided with one culture bottle containing Brain Heart Infusion (BHI) broth. Three blood culture bottles were provided to each patient suspected of infective endocarditis, for the collection of 3 blood samples at three different times differing 1 hour. These culture bottles were provided according to the age of the patients. The blood collection procedure was as follows (Cheesbrough, 1984):

1. Using a pressure cuff, a suitable vein was located in the arm.

2. The skin over the vein was cleansed in a circle approximately 5 cm in diameter with 70 % alcohol, rubbing vigorously.

3. Starting in the centre of the circle, 2 % tincture iodine was applied in ever widening circles until the entire circle has been saturated with iodine. The iodine was allowed to remain on the skin for at least 1 minute. The timing was crucial; so a watch or timer should be used.

4. Using a sterile syringe and size 21 gauge needles, appropriate volume (5-10 ml) of blood was withdrawn by inserting the needle into the vein.

5. After the needle has been removed, the site was cleansed with 70 % alcohol again, as many patients are sensitive to iodine.

For Children: 2-3 ml of blood was drawn from the patient and dispensed in the culture bottle containing BHI broth in a ratio of 1:5.

For infective endocarditis suspected children:

For infective endocarditis, three consecutive blood samples were collected at an interval of one hour.

Inoculation of blood sample into the culture broth was done immediately after collection i.e. in laboratory, in wards and emergency rooms wherever the sample was collected.

4.2.2 Processing of the sample

Immediately after the blood culture bottles were received in the laboratory, they were provided with laboratory identification numbers and further processed.

The culture bottles were incubated at 37°C. Incubation was continued upto 7 days and subcultured at 24 hrs, 48 hrs, 72 hrs or unless the visible growth was obtained. The blood culture requested for infective endocarditis was hold for a maximum of 2 weeks. The day of collection of sample was defined as the first day in this study.

4.2.3 Macroscopic examination of blood culture bottle

It was the primary step of sample processing. The culture bottles were examined daily for any visible growth such as turbidity, haemolysis of red blood cells, formation of gas bubbles and formation of clot or formation of discrete colonies. This step helped in the presumptive diagnosis of positive broth cultures as shown in Table 2.

Table 2 Macroscopic examinations of blood culture bottle (Shakya , 2001).

S.N.	Observation	Presumptive Diagnosis
1.	Uniform turbidity with gas bubble formation	Enteric organism (Gram negative bacilli)

2.	Partial haemolysis of blood with greenish tinge	*Streptococcus* spp. (Gram positive cocci)
3.	Haemolysis with cotton ball colony	*Streptococcus* spp. (Gram positive cocci)
4.	Jelly like coagulum throughout the broth, discrete colonies on the surface of red cell layer and in the broth	*Staphylococcus* spp. (Gram positive cocci)

4.2.4 Microscopic examination

The microscopic examination for the macroscopically positive cultures was performed by Gram staining method. The microscopic examination of broth was used for the presumptive diagnosis of bacteria as shown in Table 3.

Table 3 Microscopic examination of broth (Shakya , 2001)

S. N.	Gram-staining	Presumptive Diagnosis
1.	Gram-positive cocci in cluster	*Staphylococcus* spp.
2.	Gram-positive cocci in chain	*Streptococcus* spp.
3.	Gram-positive bacilli	*Bacillus* spp.
4.	Gram-negative bacilli	Members of Enterobacteriaceae

The Gram staining procedure is mentioned in the Appendix II.

4.2.5 Subculture from broth culture

The broth cultures were sub-cultured on Blood agar and MacConkey agar pates. Repeated subcultures of the culture bottles were made at different times during their incubation from 24 hours to 96 hours.

The composition and preparation of Blood agar and MacConkey agar are mentioned in the Appendix III.

The MacConkey agar plate and blood agar plate were incubated at 37°C aerobically and carbon dioxide jar respectively in ordinary incubator.

The subculture plates were examined after 18 hrs incubation. Blood agar plates were observed for growth and haemolysis. MacConkey agar plates were observed for growth of lactose fermenter and non-lactose fermenter organisms.

4.2.6 Identification of isolates

Identification of significant isolate was done by using microbiological techniques as described in the Bergey's manual which involves morphological appearance of the colonies, staining reactions, biochemical properties and serotyping if required in specific cases. Standard protocol provided by Cheesbrough (1984) and Collee *et al.* (1996) was followed for identification of bacteria isolated from blood specimens.

Pure culture for identification: Each of the organisms was isolated in pure form before performing biochemical and other tests. Gram staining of an isolated colony was done from primary culture. For Gram negative organism, a speck of single isolated colony from MA and for Gram positive, the same from BA was transferred into the nutrient broth and incubated at 37ºC for 4 hours. It was then subcultured on dried nutrient agar plate and incubated at 37ºC for 24 hours. Thus obtained overnight incubated culture of organism on nutrient agar was used to perform catalase, oxidase, other biochemical and antibiotic susceptibility test. The Gram-staining procedure is mentioned in the Appendix-IV.

Biochemical Test: Appropriate biochemical tests were performed for the confident identification of the bacterial isolates. For that, the pure colonies on the media plates were inoculated onto different biochemical media.

- Gram positive organisms were identified primarily on the basis of their response to Gram’s staining, catalase, oxidase, coagulase, Optochion (5 µg) susceptibility tests and bile solubility test.
- The biochemical tests used for the identification of Gram negative bacterial isolates include Catalase test, Oxidase test, Indole test, Methyl red test, Voges Proskauer test,

Citrate utilization test, Triple Sugar Iron (TSI) test, Urease test, Motility test, Sulphide production test and Gas production test.

The composition of biochemical media and reagents and their preparation are described in the Appendix-III. The procedure for performing biochemical tests are mentioned in Appendix-V.

4.2.7 Antibiotic susceptibility testing

The antimicrobial susceptibility testing of the isolates towards various antimicrobial disks was done by modified Kirby-Bauer disk diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) using Mueller Hinton agar (MHA).

- Mueller Hinton Agar was prepared and sterilizd as instructed by the manufacturer.
- The pH of the medium 7.2-7.4 and the depth of the medium at 4 mm (about 25 ml per plate) were maintained in petri dish.
- Using a sterile wire loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into Mueller Hinton broth tube and was incubated at 37ºC for 2-4 hrs.
- After incubation, the turbidity of the suspension was matched with the turbidity standard of Mc Farland tube number 0.5.
- Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture technique. The plate was left for about 5 minutes to let the agar surface dry.
- Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) was placed, evenly distributed on the inoculated plates, not more than 6 discs were placed on a 90 mm diameter petri dish.
- After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and compared with standardized zone interpretative chart provided by the company.

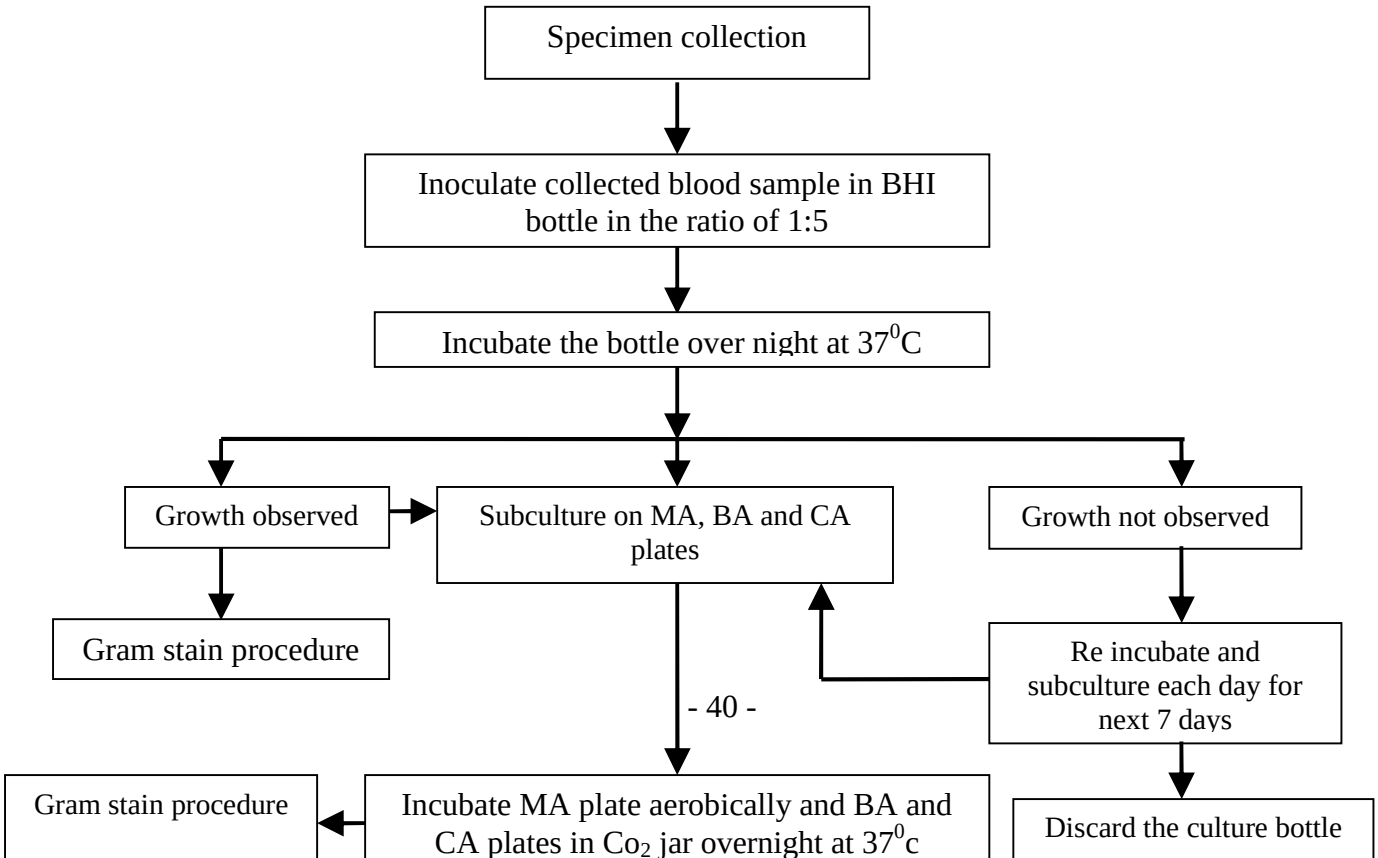
The preparation and composition of Mueller Hinton Agar medium is mentioned in the Appendix-III. The detailed about antibiotic discs used and its interpretative chart are mentioned in VIII respectively.

4.2.8 Quality control

To obtain reliable microbiological result, it is necessary to maintain quality control. Quality of each test was maintained by using standard procedures. The quality of each agar plates prepared was by incubating one plate of each lot on the incubator. Control strains of *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used for the standardization of the Kirby-Bauer test and also for correct interpretation of zone of diameter. Quality of sensitivity tests was maintained by maintaining the thickness of MHA at 4 mm and the pH at 7.2-7.4. Similarly antibiotics discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures.

4.2.11 Data analysis

Chi- square test was applied to find significance of the values. Test of present work are shown in appendix IX.



Flow chart of methodology

(Adapted from Cheesebrough, 2000)

CHAPTER-V

5. RESULTS

Study population:

A total of 1671 blood samples were collected from the children attending Kanti Children Hospital to study prevalence of bactaerimia and septicaemia**.**

Table 4 Age and gender wise distribution of patient

Age group	Male		Female		Total	
	Number	**%**	**Number**	**%**	**Number**	**%**
0-45 days	133	12.41	64	11.79	197	11.79
45days-1yr	249	23.22	127	22.5	376	22.5
1yr -5yrs	366	34.14	232	35.79	598	35.79
5yrs -10yrs	213	19.87	127	20.35	340	20.35
10yrs-14yrs	111	10.35	49	9.6	160	9.6
Total	**1072**	**64.15**	**599**	**35.84**	**1671**	

During the study period, 1072 (64.15%) were male and 599 (35.84%) were female. The age group of between 1 to 5years had the maximum requests of 598 (35.79%) blood culture. The age group 45 days to 1 year was second with 376(22.5%) requests. Age group 10-14 years requested the least with 160 (9.6%) requests.

Table 5 Result of culture positivity among indoor and outdoor patients

Types of patient	Total		Culture positive		Culture negative	
	Cases	%	Cases	%	Cases	%
Indoor	**119**	7.12	17	14.28	102	85.71
Outdoor	**1552**	92.88	162	10.43	1390	89.56
Total	**1671**	**100**	**179**	**10.71**	**1492**	**89.29**

Out of 119 (7.12%) samples collected from indoor patients only 17 (14.28%) cases showed culture positive. Similarly, 1552 (92.88%) blood samples were collected from outdoor patients and 162 (10.43%) showed positive culture result. The result is tabulated in Table 5.

Table 6 List of isolation pattern of bacterial isolates

S.N.	Types of bacteria	Frequency	Percentage (N=179)
1.	**Gram Negative Bacteria**	**118**	**65.93**
i.	*Salmonella* Typhi	70	39.11
ii.	*Salmonella* Paratyphi A	14	7.82
iii.	*Escherichia coli*	24	13.41
iv.	*Proteus* spp.	1	0.56
v.	*Klebsiella* spp.	5	2.79
vi.	*Acinetobacter* spp.	2	1.12
vii.	*Enterobacter* spp.	2	1.12
2.	**Gram positive bacteria**	**61**	**34.07**
i.	*Staphylococcus aureus*	52	29.05
ii.	*Streptococus pneumoniae*	4	2.23
iii.	*Streptococcus viridans*	5	2.79
	Total	**179**	**100**

As shown in Table 6, out of 179 bacterial isolates, 118 (65.93%) were Gram negative and 61 (34.07%) were Gram positive. *Salmonella* Typhi (39.11%) was found to be most predominant among all bacterial isolates which was followed by *Staphylococcus aureus* (29.05%), *Escherichia coli* (13.41%) and *Salmonella* Paratyphi A (7.82%). Only one isolate (0.56%) of *Proteus* spp. was obtained among the study population. Among Gram negatives,

Salmonella Typhi 70(59.32%) was found to be predominant and among Gram positive *Staphylococcus aureus* 52 (85.24%) was found to be predominant.

Table 7 Distribution pattern of the bacterial isolates in blood cultures of different age group

Types of organism isolated	**Age group**					**Total**
	0-45 days	**45days-1yr**	**1 yr- 5 yr**	**5 yr-10 yrs**	**10-14 yrs**	
Salmonella Typhi	0	9	22	24	15	70
Salmonella Paratyphi A	0	2	4	3	5	14
Staphylococcus aureus	31	11	10	0	0	52
Escherichia coli	16	6	0	1	1	24
Klebsiella spp.	4	0	0	1	0	5
Acinetobacter spp.	1	0	1	0	0	2
Streptococcus pneumoniae	0	0	3	1	0	4
Streptococcus viridans	1	3	1	0	0	5
Proteus spp.	1	0	0	0	0	1
Enterobacter spp.	2	0	0	0	0	2
Total (Percentage)	**56 (31.28%)**	**31 (17.32%)**	**41 (22.91%)**	**30 (16.76%)**	**21 (11.73%)**	**179 (100%)**

Out of 179 bacterial isolates, highest number of bacteria was isolated agegroup below 45 days (31.28%) followed by the age group 1yr- 5yrs (22.91%) and age group 10-14 yrs was found to be least affected (11.73%). Also age groups below 45 days were most affected by wide range of bacteria with 7 types of isolates and were mainly affected by *Staphylococcus aureus* followed by *Escherichia coli* and *Klebseilla pneumonaie* whereas age group 10-14 years ware affected by the narrow range of bacteria with only 3 types of isolates. *Salmonella* Typhi was found to be predominant among the age group of 5yrs-10yrs followed by age group 1yr-5yrs.

Table 8 Gender wise distribution of bacterial isolates

S.N.	Organisms isolated	Male		Female		Total	
		N	%	N	%	N	%
Gram negative bacteria							
1.	*Salmonella* Typhi	47	40.17	23	37.09	70	39.11
2.	*Salmonella* Paratyphi A	4	3.41	10	16.12	14	7.82
3.	*Escherichia coli*	16	13.67	8	12.9	24	13.41
4.	*Proteus* spp.	1	0.8	0	0	1	0.56
5.	*Klebsiella* spp.	4	3.41	1	1.6	5	2.79
6.	*Acinetobacter* spp.	2	1.7	0	0	2	1.12
7.	*Enterobacter* spp.	1	0.8	1	1.6	2	1.12
Gram positive bacteria							
1.	*Staphylococcus aureus*	28	23.93	24	38.70	52	29.05
2.	*Streptococcus pneumoniae*	4	3.41	0	0	4	2.23
3.	*Streptococcus viridans*	4	3.41	1	1.6	5	2.79

(N= Number of isolates)

A total of 179 bacteria belonging to 10 different species were isolated from blood samples, which are tabulated in table 5.7. Among the isolates, *Salmonella* Typhi (40.17%) was found to be the most predominant organism followed by *Staphylococcus aureus (23.93%), Escherichia coli (*13.67%) in case of male and similarly in female *Staphylococcus aureus(38.70%)* was most predominant and followed by *Salmonella* Typhi *(3*7.09%) *Salmonella* Paratyphi A *(16.12%)* and *Escherichia coli (12.9%).* All isolates of *Streptococcus pneumoniae* are isolated from male.

Table 9 Distribution of the isolates among the outdoor and indoor patients

S.N.	Organisms isolated	Indoor		Outdoor		Total	
		N*	%	N	%	N	%
Gram negative bacteria							
1.	*Salmonella* Typhi	1	0.56	69	38.55	70	39.11
2.	*Salmonella* Paratyphi	0	0	14	7.82	14	7.82

	A						
3.	*Escherichia coli*	9	5.03	15	8.38	24	13.41
4.	*Proteus* spp.	0	0	1	0.56	1	0.56
5.	*Klebsiella* spp.	1	0.56	4	2.23	5	2.79
6.	*Acinetobacter* spp.	0	0	2	1.12	2	1.12
7.	*Enterobacter* spp.	0	0	2	1.12	2	1.12
Gram positive bacteria							
1.	*Staphylococcus aureus*	6	3.35	46	25.70	52	29.05
2.	*Streptococcus pneumoniae*	0	0	4	2.23	4	2.23
3.	*Streptococcus viridans*	0	0	5	2.79	5	2.79
	Total	**17**	**9.50**	**162**	**90.50**	**179**	**100**

(* N= Number of isolates)

As shown in above Table, all the organisms isolated were found to be predominant among the outdoor patients (90.50%) than among indoor patients (9.50%). Only one *Salmonella* Typhi was isolated from an indoor patient. Among ten different types of bacterial isolate, *Escherichia coli* were found to be most predominant one among indoor patients.

5.2 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES OBTAINED FROM BLOOD CULTURE

Table 10 Antibiotic susceptibility pattern of *Escherichia coli* (N=24)

Antibiotics used	**Antibiotic Susceptibility Pattern**					
	Resistant		**Intermediate**		**Sensitive**	
	N	**%**	**N**	**%**	**N**	**%**
Cephalexin	20	83.33	0	0	4	16.66
Ofloxacin	4	16.66	3	12.5	17	70.83
Ciprofloxacin	9	37.5	10	41.66	5	20.83
Cephotaxime	17	70.83	0	0	7	29.16
Chloramphenicol	11	45.83	0	0	13	54.16
Cotrimoxazole	18	75.00	3	12.50	3	12.50

Amikacin	8	33.33	4	16.66	12	50.00

(N= Number of isolates)

E.coli was found to be most susceptible towards Ofloxacin (70.13%) followed by Chloramphenicol (54.16%) and Amikacin (50%). Most of the *E.coli* strains were found to be resistant to Cephalexin (83.33%) and Cotrimoxazole (75%).

Table 11 Antibiotic susceptibility pattern of *Staphylococcus aureus* (N=52)

Antibiotics used	Antibiotic Susceptibility Pattern					
	Resistant		Intermediate		Sensitive	
	N	%	N	%	N	%
Cloxacillin	15	28.84	0	0	37	71.15
Ofoxacin	7	13.46	0	0	45	86.53
Ciprofloxacin	10	19.23	4	7.69	38	73.07
Cephotaxime	20	38.46	1	1.92	31	59.61
Chloramphanicol	10	19.23	0	0	42	80.76
Cotrimoxazole	30	57.69	0	0	22	42.30
Amikacin	5	9.61	0	0	47	90.38

(N= Number of isolates)

Staphylococcus aureus was found to be highly susceptible towards Amikacin (90.38%) followed by Ofloxacin (86.53%) and Chloramphenicol (80.76%) where as susceptibility towards Cotrimoxazole (42.30%) was found comparatively low.

Table 12 Antibiotic susceptibility pattern of other gram negative isolates

S.N.	Isolates	antibiotics used	Antibiotic Susceptibility Pattern					
			Resistant		Intermediate		Sensitive	
			N	%	N	%	N	%
1	*Proteus* spp. (N=1)	Cephalexin	0	0	0	0	1	100
		Ofloxacin	0	0	0	0	1	100
		Ciprofloxacin	0	0	0	0	1	100
		Cephotaxime	1	100	0	0	0	0
		Chloramphenicol	0	0	0	0	1	100
		Amikacin	0	0	0	0	1	100
2	*Klebsiella* spp. (N=5)	Cephalexin	4	80.00	0	0	1	20.00
		Ofloxacin	0	0	0	0	5	100
		Ciprofloxacin	0	0	2	40.00	3	60.00
		Cephotaxime	1	20.00	0	0	4	80.00

		Chloramphenicol	2	40.00	0	0	3	60.00
		Cotrimoxazole	2	40.00	0	0	3	60.00
		Amikacin	2	40.00	1	20.00	2	60.00
3	*Acinetobacter* spp. (N=2)	Tobramycin	1	50.00	0	0	1	50.00
		Ofloxacin	0	0	0	0	2	100
		Ciprofloxacin	0	0	0	0	2	100
		Cotrimoxazole	2	100	0	0	0	0
		Cephotaxime	2	100	0	0	0	0
		Chloramphenicol	2	100	0	0	0	0
		Amikacin	0	0	0	0	2	100
4	*Enterobacter* spp. (N=2)	Cephalexin	0	0	0	0	2	100
		Ofloxacin	0	0	0	0	2	100
		Ciprofloxacin	0	0	0	0	2	100
		Cephotaxime	0	0	1	50.00	1	50.00
		Chloramphenicol	1	50.00	0	0	1	50.00
		Cotrimoxazole	1	50.00	0	0	1	50.00
		Amikacin	0	0	1	0	1	50.00

(N= Number of isolates)

Ofloxacin (100%) was found to be the most effective antibiotic for *Klebsiella* spp. followed by Cephotaxime (80%). Amikacin, Ciprofloxacin, Chloramphenicol and Cotrimoxazole (60%) were found to be equally susceptible towars *Klebsiella* spp. whereas most of the isolates were resistant to Cephalexin (80%)

Enterobacter spp. were found to be 100% sensitive towards Cephalexin, Ofloxacin and Ciprofloxacin whereas Cephotaxime, Chloramphenicol, Cotrimoxazole and Amikacin, all were 50% sensitive.

Ofloxacin, Ciprofloxacin and Chloramphenicol were shown to be 100% susceptible by *Acinetobacter* spp. whereas Cotrimoxazole, Cephotaxime and Chloramphenicol 100% resistant.

The single isolates of *Proteus* spp. was found to be sensitive to all the antibiotics used except for Cephtaxime which was found resistant.

Table 13 Antibiotic susceptibility pattern of other gram positive isolates

S.N.	Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
			Resistant		Moderate		Susceptible	
			N	%	N	%	N	%
	Streptococcus pneumoniae (N=4)	Penicillin	0	0	0	0	4	100
		Erythromycin	0	0	0	0	4	100
		Cotrimoxazole	1	25.00	0	0	3	75.00
		Ciprofloxacin	0	0	0	0	4	100
		Chloramphenicol	2	50.00	0	0	2	50.00
		Ampicillin	0	0	0	0	4	100
	Streptococcus viridans (N=5)	Penicillin	1	20.00	1	20.00	3	60.00
		Erythromycin	4	80.00	0	0	1	20.00
		Cotrimoxazole	3	60.00	0	0	2	40.00
		Ciprofloxacin	2	40.00	1	20.00	2	40.00
		Chloramphenicol-	3	60.00	0	0	2	40.00
		Ampicillin	1	20.00	0	0	4	80.00

(N= Number of isolates)

Streptococcus pneumoniae was found to be 100% susceptibible towards Penicillin, erythromycin and Ampicillin followed by Cotrimoxazole (75%). The least effective one is found to be Chloramphenicol (50%)

Ampicillin (80%) was found to be most effective against *Streptococcus viridans* followed by Penicillin (60%) and most of the isolates (80%) were found to be resistant towards Erythromycin.

Table 14 MDR of bacterial isolates

Organism	N	Resistant to			
		1 antibiotic	2 antibiotics	MDR strain	
				≥3 antibiotics	%
Acinetobacter spp.	2	0	0	2	100
Escherichia coli	24	1	4	18	75.00
Streptococcus viridans	5	0	1	3	60.00
Enterobacter spp.	2	0	0	1	50.00
Klebsiella spp.	5	2	1	2	40.00
Staphylococcus aureus	52	17	7	16	30.76
Salmonella Typhi	70	28	1	1	1.42
Salmonella Paratyphi A	14	10	0	0	0
Streptococcus pneumoniae	4	3	0	0	0
Proteus spp.	1	1	0	0	0
Total (%)	179	62(34.63%)	14(7.8%)	43	24.02%

Out of 179 isolates 43 strains (24.02%) were resistant to ≥3 antibiotics and were considered as multidrug resistant. It was found that 62(34.63%) isolates were resistant to 1 antibiotic and 14(7.8%) isolates were resistant to 2 antibiotics. Among the MDR strains, 75% (18 out of 24) of *E. coli* were found to be MDR. Similarly, 30.76% (16 out of 52) of *Staphylococcus aureus* were found to be MDR. All the isolates (100%) *Acinetobacter* spp. was found to be MDR.

5.3 PREVALENCE OF INFECTION BY SALMONELLAE IN CHILDREN

Table 15 Prevalence of Salmonellae infection

Total blood sample cultured	*Salmonella* positive		*Salmonella* negative	
	N	%	N	%
1671	84	5.02	1587	94.98

Out of 1671 patient, only 84 patients (5.02%) showed the positive results towards *Salmonella* spp**.**

Table 16 Age wise and gender wise distribution of *Salmonella* spp.

Age group	*Salmonella* Typhi			*Salmonella* Paratyphi A			Total (1671)	
	Male (1072)	Female (599)	Total (1671)	Male (1072)	Female (599)	Total (1671)		
	No.	No.	No.	No.	No.	No.	No.	%
0-45 days	0	0	0	0	0	0	0	0
45days-1yr	5	4	9	0	2	2	11	13.09
1yr - 5yrs	15	7	22	2	2	4	26	30.95
5-10yrs	16	8	24	0	3	3	27	32.14
10-4yrs	10	5	15	2	3	5	20	23.80
Total (%)	47 (4.3%)	23 (3.8%)	70 (4.18%)	4 (0.3%)	10 (1.6%)	14 (0.8%)	84 (5.02%)	

Occurrence of *Salmonella* spp. was found to be highest among the age group 5-10yrs (32.14%) followed by 1-5 yrs (30.95%) and 10-14yrs (23.80%) respectively. *Salmonella* spp. was least seen among the age groups 45-1 yr (23.80%) whereas there was no isolates from age group below 45 days.

Highest percentage of *Salmonella* Typhi was isolated from the male patient (4.3%) and *Salmonella* Paratyphi A from the female patient (1.6%).

Table 17 Antibiotic susceptibility pattern of *Salmonella* Typhi (N=70)

Antibiotics used	Antibiotic Susceptibility Pattern					
	Resistant		Intermediate		Susceptible	
	N	%	N	%	N	%
Cephotaxime	0	0	0	0	70	100
Ofloxacin	0	0	0	0	70	100

Ciprofloxacin	0	0	12	17.14	58	82.28
Amoxycillin	0	0	0	0	70	100
Chloramphenicol	1	1.42	0	0	69	98.57
Cotrimoxazole	2	2.85	0	0	68	97.14
Nalidixic acid	30	42.58	0	0	40	57.14

(N= Number of isolates)

The antibiotic susceptibility pattern of *Salmonella* Typhi showed that 100% of the isolates were sensitive to Cephotaxime, Ofloxacin, Amoxycillin and followed by Chloramphenicol (98.5%), Cotrimoxazole (97.18%) and Ciprofloxacin (82.28%). 42.58% of the isolates were found to be resistant towards Nalidixic acid.

Table 18 Antibiotic susceptibility pattern of *Salmonella* Paratyphi A (N=14)

Antibiotics used	Antibiotic Susceptibility Pattern					
	Resistant		Intermediate		Sensitive	
	N	%	N	%	N	%
Cephotaxime	0	0	0	0	14	100
Ofloxacin	0	0	3	21.42	11	78.85
Ciprofloxacin	0	0	5	35.71	9	64.28
Amoxicillin	0	0	0	0	14	100
Chloramphenicol	0	0	0	0	14	100
Cotrimoxazole	0	0	3	21.42	11	78.57
Nalidixic acid	9	64.28	0	0	5	35.71

(N= Number of isolates)

Out of 14 isolates of *Salmonella* Paratyphi A, all of them were found to be 100% susceptible towards Amoxicillin, chloramphenicol and Cephotaxime. Susceptibility of the organism towards Ofloxacin and Cotrimoxazole was found to be 78.85% each and were found to be 78.85% susceptible towards Chloramphenicol. 64.28% of the isolates were found to be resistant towards Nalidixic acid.

Table 19 Ciprofloxacin susceptibility pattern of Nalidixic acid resistant *Salmonella* spp.

Nalidixic acid resistant *Salmonella* spp.	**Sensitive**		**Intermediate**		**Resistance**		**Total**
	Number	**%**	**Number**	**%**	**Number**	**%**	
S. Typhi	20	66.66	10	33.33	0	0	30
S. Paratyphi A	5	55.55	4	44.44	0	0	9

All together, there were 30 nalidixic acid resistant *Salmonella* Typhi and 9 nalidixic acid resistant *Salmonella* Paratyphi A in which 66.66% S. Typhi were sensitive to ciprofloxacin and 33.33% were intermediate to ciprofloxacin but in case of *S.* Paratyphi A 44.44% were intermediate and other 55.55% were sensitive.

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

The study was carried out from June 2006 to July 2007 and all together 1671 blood cultures were performed from clinically suspected bacteraemic and septicaemic patients visiting Kanti Children's hospital, Kathmandu to determine the prevalence of bacteraemia and septicaemia in the children of below 14 years and also to determine the prevalence of enteric fever in children. The samples received are from clinically suspected patients of enteric fever, pneumonia, meningitis, infective endocarditis and other suspected bacteraemic illness.

The load of outdoor patient for blood culture were high in frequency i.e. 1552 (92.55%) in number whereas frequency of indoor patient was 119 (7.12%). The majority of the patients belong to age group 1 to 5 years and represented 35.79% of the total population studied and the frequency of male patient were higher than the female. In this study, male child patient were 1072 (64.15%) and female child patient were 599 (35.84%). A study done by Meremikwu *et al.,* 2005 found the higher percentage of male patient of 55.1% and Babby *et al.* (2005) also found the higher male percentage of 67%. Supporting to our study, a study conducted by ICCDR, B found the male percentage requesting for blood culture is 63%.

Out of 1671 blood samples processed 179 (10.71%) samples showed culture positive. Johnston and Sell (1964) found culture positive result of 7.1%. Sharma *et al.* (2002) reported 22.9% of culture positive result whereas Meremikwu *et al.* (2005) reported 45.9%. Osinupebi and Olajubu (1998) yielded 24.1% of positive blood culture. Also the study carried out in the children below 10 years by Wagle (2004) and Amatya (2005) gave positive percentage of 12.24% and 10.14% respectively.

In our study, coagulase-negative Staphylococci (CoNS) were considered as contaminants and were rejected thus our result showed lower growth positive result. Though CoNS was isolated most often, only 12.4% were clinically significant (Weinstein *et al.*, 1997). According to Reese and Douglas (1983), CoNS are commonly isolated representing 15-03% of positive blood culture isolates and approximately 90% of these are contaminants although they can be pathogenic. Since CoNS are common commensal organisms with little

pathogenicity, a positive blood culture for CoNS may represent either contamination or true bacteraemia, and for this reason, many studies require more than one positive blood culture and in our study single blood culture sample was taken in most of the case except in infective endocarditis. Other laboratory evidence of infection, such as an elevated CRP level, must also be performed to distinguish CoNS bacteraemia from contamination.

Apart from CoNS, present study also excluded *Bacillus* spp. and *Micrococcus* spp. as a contaminant but finding of this microorganism must be reported if patients had suspected endocarditis or in immunocompromized . Contamination of blood cultures is believed to occur mainly during venipuncture procedure which was very difficult in children and also due to later stages of laboratory handling and processing of the specimen.

The study encompassed all the samples requested for suspected bacteraemia. Only monomicrobial infection was found during the study period, not the polymicrobial infection. Polymicrobial bacteraemia commonly occur in the setting of intra-abdominal abscesses as well as in patients who have underlying malignancies or are immunosuppressed. The polymicrobial bacteraemia can also be seen in alcoholics with liver disease, patients with decubiti and occasionally in the cardiac surgical patients who develop endocarditis post operatively (Reese and Douglas, 1983).

Tibrewal (1999) reported monomicrobial bacteraemia as the most common prevalent (97.54%) than polymicrobial bacteraemia. Finegold, 1983 in his report stated that polymicrobial bacteraemia should be always considered as a possibility; it is found in 5 to 10% cases. This may be obvious if initial gram stained film shows a mixed morphology or subcultures are mixed.

The present study includes children of all age groups but children below 45 days reveals a rather high rate (31.28%) of isolation of bacterial pathogens from blood cultures. The isolation rate is comparable to rates reported in other studies of Nigerian neonates with suspected septicaemia in Calabar (50.6%) (Antia-Obong et al., 1992), Ilorin (30.8%) (Mokuolu and Adesiyun, 2002) and Ife (55%) (Ako-Nai *et al.*, 1999)

Outdoor patient showed maximum bacterial isolates than indoor patients. Out of total blood samples requested for culture, 119 (7.12%) samples were collected from indoor patients and only 17 (1.02%) out of 119 cases showed culture positive. Similarly, 1552 (92.88%) blood

samples were collected from outdoor patients and 162 (9.69%) showed positive culture result.

Out of total male patients, only 111 (10.35%) cases showed positive culture result and out f total female patients, 68 (11.35%) showed positive blood culture result. This result shows that prevalence rate of bacteraemia and septicaemia is slightly higher in female. There is no significant association of occurrence of bacteraemia and septicaemia in male and female patients ($p>0.05$). A study performed by Ako-Nai *et al.,* 1990 also supported our study that incidence in females was slightly but not significantly higher than in males.

In present study, among 179 bacterial isolates, 118 (65.93%) were Gram negative and 61 (34.07%) were Gram positive.Gram negative organisms were found to be predominant in our study which was similar as reported by Osinupebi and Olajubu (2003) and Sharma (2002). The predominance of Gram-negative organisms reported as etiological agents of bacteraemia and septicaemia was also seen in the study done by Mehta *et al.* (2005) where Gram-negative bacteria accounted for 80.96% of the cases whereas in the study by Babay *et al.*, 2005 revealed the predominance of gram positive bacteria(78.6%).

In all 10 different bacterial species were identified from the positive culture of which the predominant were *Salmonella* Typhi (39.11%) which was followed by *Staphylococcus aureus* (29.05%), *Escherichia coli* (13.41%) and *Salmonella* Paratyphi A (7.82%). Only one isolate (0.56%) of *Proteus* spp. was obtained among the study population. We did not isolate anaerobes because our laboratory techniques may not have been sensitive enough to detect obligate anaerobes. Similar bacterial isolates was obtained in a study done by Angyo *et al.* (2001*)* where 139 bacterial isolates were isolated and *Staphylococcus aureus* (36.0%) was predominant.

In this study, children of below 45 days were most affected by wide range of bacteria with 7 types of isolates namely; *Staphylococcus aureus, Escherichia coli, Klebsiella* spp., *Acinetobacter* spp., *Streptococcus viridans, Proteus* spp. and *Enterobacter* spp. This result is also supported by Iregbu *et al.*(2006) and Berkley *et al.* (2005).In a study by Agnihotri *et al.* (2004), observed predominancy of Gram negative bacilli in neonates and infants and found that *Staphylococcus aureus* was predominant organism isolated and the other organism isolated were *Klebsiella* spp., *E. coli, Acinetobacter* spp., *Pseudomonas aeroginosa, Enterobacter* spp., *Enterococcus faecalis , Proteus* spp., which support our study. Also data

from Pakistan reveal that *S. aureus*, *Klebsiella*, and *E. coli* are the common organisms isolated in neonatal units at Karachi and Peshawar, and most of these strains are multidrug resistant (Rahman *et al*., 2002; Anwer *et al*., 2002)

In a similar study performed in Nigeria, Bacteria was isolated in 552 (48.9%) of samples with highest rates among newborns (271: 50.8%). The most frequent isolates were *Staphylococcus aureus* (48.7%) and Coliforms (23.4%). Unidentified gram-negative rods (8.0%), *Pseudomonas aeruginosa* spp. (5.8%), Streptococcal spp. (4.7%) and *Chromobacterium* spp. (4.5%) (Meremikwu *et al.*, 2005).

According to our study, *Salmonella* Typhi was the most isolated pathogen from outdoor patient accounting 38.55% and *Escherichia coli* was isolated from the indoor patient accounting 5.03%. where as in outdoor patient it accounted 8.35%. Similarly *S.* Paratyphi A accounted 7.82% in outdoor patient where as none was isolated in indoor patient. The second most isolated among bacterial isolates is *Staphylococcus aureus* which is 29.05%. These results were supported by Schwartz *et al.*, 1990; Thapa (1991), Ghimire (1995), Shrestha (1996).

As depicted in table 5, the patients below the age group of 45 days showed the maximum culture positive and it covered 31.28% of the total culture positive isolates followed by age group of 1 to 5 years covering 22.91%. In our study *Staphylococcus aureus* was isolated in highest number, followed by *E. coi*, in age group below 45 days and *Salmonella* Typhi was in highest number in age group between 5 to 10 years. *Streptococcus pneumoniae* was mostly isolated from age group between 1 to 5 years in our study and similar result was

reported by Diekema *et al*., 2002.

In present study ofloxacin remains the principle antibiotic of choice based on its effectiveness on both gram positive and gram negative bacteria.

Among the isolates of *E. coli*, Ofloxacin (70.83%) was most effective followed by Chloramphenicol (54.16%) whereas Ciprofloxacin and Cotrimoxazole were least effective (20.83% and 12.50% respectively).In our study not a single isolate was 100% sensitive to the antibiotics used. Luzzaro *et al.,* 2002 from Italy showed that *E. coli* were 42.0% resistant towards ampicillin and ciprofloxacin resistance was seen in 12.4% cases. *E. coli* are increasing found to produce extended spectrum of beta- lactamases (ESBLs). These enzymes

render bacteria resistant to most beta lactam antibiotics, sparing only the carbapenams and cephamycins. Out breaks of *E. coli* producing CTX-M ESBLs have been found in number of regions in England where some of these infections have resulted in bacteraemias (CDR Weekly, 2004).

All isolates of *Klebsiella* spp. were sensitive to Ofloxacin (100%) but resistant to Cephalexin. All *Acinetobacter* spp. were sensitivite to Ofloxacin, Ciprofloxacin and Amikacin while resistant to Cotrimoxazole, Cephotaxime, and Chloramphenicol. Isolates of *Proteus* spp. was found to be sensitive towards all antibiotics used except Cephotaxime. *Enterobacter* spp. was sensitive to Cephalexin, Ofloxacin and ciprofloxacin.

In a study by Trenholme *et al.*, 1989 reported that 31 gram-negative bacteria were sensitive to Cephotaxime and the study conducted by Ghimire, 1995 showed that chloramphenicol was the most effective antibiotics followed by amoxycillin and cotrimoxazole against gram-negative rods.

But Tibrewal, 1999 found that ciprofloxacin was the most effective antibiotic (93.26%) followed by tetracycline (77.53%) whereas ampicillin was found to be 20.22% effective against all the 89 gram-negative isolates.

Shakya, 2001 showed that cotrimoxazole and gentamicin were effective antibiotics against gram-negative isolates with 88.42%. Wagley, 2004 showed that ceftazidime was the drug of choice for gram-negative isolates (96.35%). Chloramphenicol and ceftriaxone were the second choice with 94.16% effectiveness. Ciprofloxacin and ofloxacin were found to be 89.05% and 78.83% sensitive to gram negative bacteria respectively.

In our study, *Staphylococcus aureus* was found to be most susceptible to Amikacin (90.38%) followed by Ofloxacin (86.53%) and Chloramphenicol (80.76%). Cotrimoxazole is the least effective antibiotics (42.30%). Wagle, 2004 repoted Cephalexin, Ciprofloxacin and Cloxacillin as effective antibiotics (80%) toward *Staphylococcus aureus* and ampicillin the least effective .The study performed by Karki and Parija (1999) found that *Staphylococcus aureus* were most sensitive to gentamicin (74.5%) and ciprofloxacin (74.5%) followed by chloramphenicol (59.9%).

All the isolates of *Streptococcus pneumoniae* were sensitive toward Penicillin (100%), Erythromycin (100%) Ciprofloxacin (100%) and Ampicillin (100%) whereas to Cotrimoxazole and Chloramphenicol, were 75% and 50% sensitive respectively. A study published by ICDDRB, 2004 also reported, resistant to cotrimoxazole and susceptible to Ampicillin, Penicillin and Ciprofloxacin which supports our result. But in a study by Diekema *et al.*, 2002 reported Penicillin resistance among *S. pneumoniae* was highest in children younger than 5 years. In case of *Streptococcus viridans* the most sensitive antibiotic was Ampicillin followed by penicillin and the least sensitive was Erythromycin.

Multiple drug resistance (MDR) was defined as resistance to three or more of the antimicrobial agents evaluated in the study (Sunenshine *et al.*, 2007). Antimicrobial resistance is a global problem. It is now generally accepted as major public health issue and has significant implication on health and patient care. Resistance to antimicrobial drugs is associated with high morbidity and mortality, high health-care cost and prolonged hospitalization. The problem of the drug resistance to antimicrobial drug is more troublesome to developing countries. The World Health Organization (WHO) and the European Commission (EC) have recognized the importance of studying the emergence and determinants of resistance and the need for strategies for its control.

In our study out of 179 isolates 43 strains (24.02%) were resistant to 3 or more drugs and were considered as multidrug resistant. Among the MDR strains, 75 %(18 out of 24) *E. coli* were found to be MDR. Similarly, 30.76 %(16 out of 52) *Staphylococcus aureus* were found to be MDR. *Streptococcus viridans* accounted for 60.00 %(3 out of 5) isolated. All the isolates (100%) *Acinetobacter* spp. (2 out of 2) was found to be MDR.

Acinetobacter spp. is emerging as an important pathogen in traditional and nontraditional healthcare settings. Its ability to infect healthy hosts and its propensity to develop antimicrobial drug resistance has caused concern among the infectious diseases community (Sunenshine *et al.*, 2007).

The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used (Levy, 1991) Resistant strains are now reported against all available classes of antibiotics (Kunin, 1983 and Levy, 1991). The morbidity and mortality rates because of MDR strains among the very young, elderly population and among immunocompromised patients are very high (Kunin, 1983). The recent increase of MDR strains in hospital has

started to pose great difficult in selecting antimicrobial agents for the management of the infection they caused and obviously the cost in the management of infection caused by MDR strains will be definitely high because of need of acquiring new drug which is of course will be high in cost as well as the cost of prolong staying in the hospital. Some factors responsible for the emergence of resistant strains in hospital include the indiscriminate use of antibiotics, the prolonged hospitalization, the increase in uses of insertion devices etc. Moreover, results obtained from these surveillance systems must be used to implement preventive programmes and policy decisions to prevent the emergence and spread of antimicrobial resistance.

Salmonellae infection is a systemic illness with a significant morbidity and mortality in developing countries. Poor sanitation, over crowding, low standard of living, lack of medical facilities and indiscriminate use of antibiotic lead to endemicity of typhoid fever and multi-drug resistant strains of *Salmonella* Typhi in developing countries (Brown *et al.*, 1996; Therefall *et al.*, 1998).

In our study period, the prevalence and distribution of *Salmonella* spp. among the children were studied and patient with enteric fever complain fever, abdominal pain, headache and vomiting as the common presenting symptoms The duration of illness at the time of blood culture is less than 1 week for 71.25% and duration of illness for 1 to 2 week is 28.75%. There is no patient with positive blood culture complaining of illness more than 2 weeks.

All vertebrates including farm animals, birds and reptiles appear capable of harbouring Salmonellae within gut and is excreted in faeces. If people consume faecal contaminated water or food, they acquire infection. In a study carried out in Kathmandu valley, the source of water was not regarded as safe up to the guidelines recommended by WHO (DISVI, 1990). In our study it was found that 78.75% of the patient with enteric fever used unboiled water for drinking purpose and the source of water used is either from tap or well whereas only 21.25% drink boiled water.

The high prevalence of *Salmonella* spp in bloodstream isolates in febrile patients in our study corresponds to the findings of previous studies performed in Nepal (Biswas *et al.*, 2004; Murdoch *et al.*, 2004; Sharma *et al.*, 2006). Enteric fever is one of the causes of fever in most of the hospitals in this country (Hale *et al*, 1999).

In our study, most of the patients with enteric fever were within the age group of 1 to 5 years and 5 to 10 year (30.95% and 32.14% respectively). According to Corales and Schmitt, 2005, in endemic areas, children aged 1 to 5 years are at the highest risk because of waning passively acquired maternal antibody and a lack of acquired immunity. In more recent years, prospective studies have shown that even where the incidence in patients is highest in adolescents and young adults, the overall incidence of blood culture–confirmed disease generally is highest in children aged 3 to 9 years and declines significantly in late adolescence (Corales and Schmitt, 2005). Also according to Graham SM, (2002) disease due to Typhi is common in children younger than 5 years old in developing countries. According to Sekarwana *et al*., 1989, the greater numbers of typhoid fever were in more than 5 years old (64%). Gupta *et al.,* 1985 stated that while no age group seems to be immune, typhoid fever was most common (77.6%) in young adults.

Out of 1671 blood culture requested cases, total 84 (5.02%) cases of enteric fever were identified and among which 14 (16.66%) cases were isolated with *Salmonella* Paratyphi *A* and 70 (83.33%) cases were isolated with *Salmonella* Typhi. This indicates that the incidence of typhoid fever is higher than that of paratyphoid fever with ratio 5:1. No *Salmonella* Paratyphi B and C were isolated during the study period. The prevalence of infection of *Salmonella* Typhi in male is found to be 4.3% where as in female it is 3.8%. Also the prevalence of infection of *Salmonella* Paratyphi A in male is 0.3% where as in female it is 1.6% but according to Corales and Schmitt, 2005 there do not exist any predilection between male and female.

The isolates of *Salmonella* spp. showed 100% susceptibility towards cephotaxime and amoxycillin. Ciprofloxacin showed least effectiveness in both species of *Salmonella* spp. As shown in table 17 Cephotaxime, Ofloxacin and Amoxicillin was the first drug of choice to treat typhoid fever caused by *Salmonella* Typhi with 100% efficiency. In children with possible multidrug-resistant typhoid, a third-generation cephalosporin (eg, cefotaxime) is the drug of choice if quinolones are to be avoided (although ciprofloxacin is being used increasingly in children with typhoid fever) (Corales and Schmitt, 2005).

With reference to table 18, Cephotaxime, Amoxycillin or Chloramphenicol was the first drug of choice for *Salmonella* Paratyphi A as their susceptibility rate was found to be 100%. Present study showed that efficacy rate of ofloxacin, cotrimoxazole and ciprofloxacin towards *S.* Paratyphi A was 78.85%, 78.57% and 64.28% respectively.

Typhoid fever is the most common clinical diagnosis among febrile patients presenting to hospital in Kathmandu. *Salmonella enterica* serovar Typhi (*S. enterica* serovar Typhi) and *Salmonella enterica* serovar Paratyphi A (*S. enterica* serovar Paratyphi A) with decreased susceptibility to fluoroquinolones and resistance to nalidixic acid are common in recent years (Shirakawaa *et al*., 2005). Nalidixic acid resistance is a marker for predicting low-level resistance to ciprofloxacin among *S.* Typhi *a*nd also an indicator of treatment failure to ciprofloxacin. Hence, it is suggested that all *S.* Typhi isolates should be screened for nalidixic acid resistance along with ciprofloxacin (Kapil *et al.*, 2002). Any isolate that shows resistance to nalidixic acid should be reported as intermediately susceptible to ciprofloxacin. The clinician should be advised to change the antibiotic. Such strains have been found to be endemic in different parts of the world including India. All patients with nalidixic acid resistant strains should be treated with higher doses of ciprofloxacin or ofloxacin. There are reports that when the sensitivity pattern indicates resistance to Nalidixic Acid, which is marker for delayed clinical response to fluoroquinolones, it is necessary to increase the oral dose of Ciprofloxacin or treat the patient with third generation cephalosporin like Ceftriaxone (Rodrigues *et al.*, 1998).

In our study, 42.58% of isolated *Salmonella* Typhi and 64.28% of isolated *Salmonella* Paratyphi A were resistant to Nalidixic Acid and out of 39 isolated strain of Nalidixic acid resistant *Salmonella* isolates, 44.44% of *Salmonella* Paratyphi Aand 33.33% of *Salmonella* Typhi showed decrease in susceptibility to ciprofloxacin though there is not a single isolate which is resistant to that antibiotic. The association of resistivity pattern of Ciprofloxacin with Nalidixic acid of *Salmonella* spp. is statistically significant ($p>0.05$). Rodrigues *et al.*, (1998) reported 82 to 88% resistance to nalidixic acid among their isolates with an associated increase in MIC to ciprofloxacin from 0.125 to 1.5µgm/mL. A single point mutation in the Quinolone resistance-determining region of the topoisomerase gene gyrA in *Salmonella* usually leads simultaneously to resistance against Nalidixic Acid and decreased Ciprofloxacin susceptibility (Nair and Sudarsana, 2004).

It has been suggested that resistance to nalidixic acid may be an indicator of low-level resistance to ciprofloxacin (Launay *et al*., 1997; Murdoch *et al.*, 1998; Vasallo *et al*., 1998). Hence screening isolates of *Salmonella* spp. by the nalidixic acid susceptibility test is incorporated to alert the treating physicians of the possibility of the failure to ciprofloxacin therapy in patients with enteric fever. The current NCCLS breakpoints may have to be re-

evaluated for salmonellae and clinicians may have to revert back to the older options for enteric infections. This implies that quinolones may no longer be the drug of choice in treating enteric fever (Joshi *et al.,* 2004; Kadhiravan *et al***.,** 2005**).**

6.2 CONCLUSION

This study underlines the pattern of bacterial isolates in the children attending Kanti Children Hospital, Maharajgunj and their antibiotic susceptibility pattern. The study revealed that prevalence of Salmonellae bacteraemia in children was found to be most common among the children of age more than one year whereas *Escherichia coli* prevalence was found to be in neonates. The highest sensitivity was shown to Ofloxacin by gram-negative isolates and MDR *Escherichia coli* were isolated significantly.

The other finding of this study is that there is significant association of resistivity pattern of Ciprofloxacin with Nalidixic acid resistant *Salmonella* spp. which implies that it is important to identify nalidixic acid resistance in *Salmonella* spp. as a predictor for decreased fluoroquinolone susceptibility.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. Out of 1671 blood samples, 179 blood samples showed growth positiveand the figure represented 10.71% of the total number of the patients studied.

2. The highest number of the bacterial isolates was obtained from the age group of 0-45 days (neonates) and 1 to 5years with 31.28% and 22.91% respectively of positive culture result.

4. Among the 179 positive cases, 111 cases were from male patients and 58 cases were from female patients and there was no significant association of culture positive and culture negative cases between genders.

5. Among 21 bacterial isolates, 118(65.93%) were Gram negative organisms where as 61 (34.07%) were Gram positive organisms. Thus Gram negative organisms were found to be predominant in our study group

6. Altogether 10 different species of the bacterial isolates were isolated in our study. Among them, *Salmonella* Typhi (39.11%) and *Staphylococcus aureus* (29.05%) were found to be most predominant which is followed by *Escherichia coli (13.41%) Salmonella* Paratyphi A (7.82%), *Streptococcus viridans* (2.79%), *Klebsiella* spp. (2.79%) *Streptococcus pneumoniae* (2.23%), *Acinetobacter* spp. (1.12%), *Enterobacter* spp. (1.12%) and only one isolate *Proteus* spp. *was* obtained from culture.

7. *In vitro* antibiotic susceptibility test showed that ofloxacin remained sensitive to most of the gram negative isolates. Though cephalexein and cotrimoxazole were found to be most resistant but the least effective antibiotic which has lowest sensitivity is ciprofloxacin.

8. Forty three multi-drug resistant isolates including only one species of Salmonella were isolated and among 43 isolates 18 *E. coli* (75%) were MDR.

9. Out of 1671 blood culture referred cases, 84 isolate of *Salmonella* spp. were isolated and out of 84 isolates 70 were isolates of *Salmonella* Typhi and 14 were isolates of *Salmonalla* Paratyphi A.

10. Occurrence of enteric fever was highest among age-groups 5 to 10 years followed by 1 to 5years (32.14% and 30.95% respectively) and highest percentage of *Salmonella* Typhi (4.3%)was isolated among male whereas *Salmonella* Paratyphi A (1.6%) among female.

11. Out of 80 enteric fever positive patients studied, 75% used unboiled water for drinking purpose reflecting the poor microbiological quality of water in Kathmandu valley as enteric fever is considered as water borne disease.

12. Amoxicillin and cephotaxime were found to be most effective antibiotic for typhoid bacteria and the least effective was ciprofloxacin (79.76%).

13. All 84 isolates of salmonellae were tested against nalidixic acid in which 39 strains were resistant. 30 out of 70 isolates of *Salmonella* Typhi were resistant to nalidixic acid and out of 14 *Salmonella* Paratyphi A 9 were Nalidixic acid resistant.

14. Only 66.66% of nalidixic acid resistant *Salmonella Typhi* showed sensitive to Ciprofloxacin and in case of nalidixic acid resistant *Salmonella Paratyphi* A 55.55% were sensitive to ciprofloxacin.

7.2 RECOMMENDATIONS

1. As the study conducted in Kanti children Hospital does not represent whole scenario of country, the surveillance should be carried out through out the year covering wide geographical region in order to obtain information regarding variation of pathogen and their antibiotic sensitivity profile.

2. The background of patients visiting the hospital should be collected. On the basis of background and their co-relation to the condition of occurrence of bacteraemia and septicaemia in the country could be linked.

3. The early reporting of gram-stain results from blood cultures, combined with early liaison, results in more rational and cost effective treatment. So, further research should be conducted in assessing the impact of blood culture reporting and clinical liaison on the empiric treatment of bacteraemia.

4. Although the prevalence of MDR isolates from blood culture are low but prudent use of antibiotics should be encouraged in order to control the emergence of drug emergence.

5. Molecular studies of MDR strains should be carried out in order to ascertain the location of drug resistance genes and to characterize the mechanism of drug resistance.

6. The MIC of ciprofloxacin should be done for Nalidixic acid resistant Sallmonellae.

7. In endemic countries, like Nepal, the most cost-effective strategy for reducing the incidence of typhoid fever is the institution of public health measures to ensure safe drinking water and sanitary disposal of excreta. The effects of these measures are long-term and reduce the incidence of infections.

8. In the absence of such a strategy, mass immunization with typhoid vaccines at regular intervals also considerably reduces the incidence of infections

CHAPTER VIII

8. REFERENCES

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APPENDIX-I
Questionnaire

1. Patient's ID: Date:

2. Patient's name:

3. Age:

4. Sex:

5. Address:

6. Patient type: OPD/ Emergency/Ward

7. Clinical signs and symptoms:

Clinical Sign and symptoms	Yes	No
Fever		
Abdominal pain		
Headache		
Vomiting		
Cough		
Constipation		
Diarrhea		
Back pain		
Chills and rigor		
Red spots		

8. Duration of illness: less than1 week / 1 to 2 weeks / More than 2 weeks

9. Temperature record:

10. Prior consultant:

11. Prior treatment of antibiotics: Yes/No
If yes, name of antibiotic:

13. Type of drinking water: Boiled / Unboiled

APPENDIX-II

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

A. EQUIPMENTS

Hot air oven	Advantec (Japan)
Incubator	Yamato (Japan)
Autoclave	Ravi (India)
Refrigerator	Sanyo (Japan)
Microscope	Olympus (Japan)
Centrifuge	Remi (India)
Weighing Machine	Chyo MP (Japan)

B. MICROBIOLOGICAL MEDIA

Blood agar base	Mueller Hinton broth
Brain heart Infusion broth	Salmonella Shigella agar
MacConkey agar	Simmon's Citrate agar
MR-VP medium	Sulphur Indole Motility agar
Mueller Hinton agar	Triple Sugar Iron agar
Nutrient agar	Urea broth

C. CHEMICALS AND REAGENTS

3%Hydrogen peroxide	Barritt's reagent
Crystal violet	Kovac's reagent
Gram's iodine	Barium chloride
Absolute (95%) alcohol	Sulphuric acid
Safranine	Normal saline

D. ANTIBIOTIC DISCS

All the antibiotics discs used for the susceptibility tests were from Hi-Media Laboratories Pvt. Limited, Bombay, India. The antibiotics used were as follows

Ampicillin (10mcg)	Cloxacillin (1mcg)
Amikacin (30mcg)	Cotrimoxazole (1.25/23.75mcg)
Cefotaxime (30mcg)	Erythromycin (15mcg)
Ceftriazone(30mcg)	Ofloxacin (5mcg)
Chloramphenicol (30mcg)	Penicillin (10 mcg)
Ciprofloxacin (5mcg)	

E. IDENTIFICATION DISCS/OTHER DISCS

Bacitracin, Optochin/ 'X' factor, 'V' factor

F. MISCELLANEOUS

Conical flasks, Cotton, Distilled water, Droppers, Forceps, Glass slides and cover slips, Immersion oil, Inoculating loop, Inoculating wire, Lysol, Measuring cylinder, Petri dishes, Pipettes, Plastic containers, Spatula, Test tubes, Wooden applicator sticks

APPENDIX-III

A. COMPOSITION AND PREPARATION OF DIFFERENT CULTURE MEDIA

The culture media used were from two companies:

a. Hi-Media Laboratories Pvt. Limited, Bombay, India.

b. Oxoid Unipath Ltd. Basingstoke, Hampshire, England

(All compositions are given in grams per liter and at 25^0C temperature)

1. Blood agar base (Oxoid, England)

Blood agar base (infusion agar) + 5-10% sheep blood

Ingredients	gm/liter
Protease peptone	15.0
Liver extract	2.5
Yeast extract	5.0
Sodium Chloride	5.0
Agar	15.0
Final pH (at 25^0C)	7.4±0.2

Direction: 42.50 grams of the blood agar base medium was suspended in 1000 ml distilled water, dissolved by boiling and sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes. After cooling to 45-50^0C, 7% sterile defibrinated sheep blood was added aseptically, then mixed with gentle rotation and immediately poured in sterile petriplates.

2. MacConkey Agar (Hi Media Laboratories)

Ingredients	gm/liter
Peptic digest of animal tissue	1.5
Casein enzymic hydrolysate	1.5
Pancreatic digest of gelatin	17.0
Lactose	10.0
Bile salts	1.50
Sodium chloride	5.0
Crystal violet	0.001
Neutral Red	0.03
Agar	15.0
Final pH (at 25^0C)	7.1±0.2

Direction: 51.5 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes. The medium was poured into sterile petriplates.

3. Mueller Hinton Agar (Hi Media Laboratories)

Ingredients	gm/liter
Beef, Infusion form	300.0
Casein Hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH (at 25^0C)	7.4±0.2

Direction: 38 grams of the medium was suspended in 1000 ml distilled water and the medium was warmed to dissolve completely. It was sterilized by autoclaving at 121ºC (15 lbs pressure) for 15 minutes and poured into sterile petriplates.

4. Nutrient Agar (Hi Media Laboratories)

Ingredients	gm/litre
Peptic digest of animal tissue	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Agar	15.0
Final pH (at 25^0C)	7.4±0.2

Direction: 28 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

5. Mueller Hinton Broth (Hi Media Laboratories)

Ingredients	gm/liter
Beef, Infusion form	300.0
Casein Hydrolysate	17.5
Starch	1.5
Final pH (at 25^0C)	7.4±0.2

Direction: 21 grams of the medium was dissolved in 1000 ml of distilled water, boiled and dispensed into small containers. It was then sterilized by autoclaving at 121˚C for 15 minutes.

6. Nutrient Broth (Hi Media Laboratories)

Ingredients	gm/liter
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.50
Yeast extract	1.50
Final pH (at 25^0C)	7.4±0.2

Direction: 13 grams of the medium was dissolved in 1000 ml of distilled water, boiled and dispensed into small containers. It was then sterilized by autoclaving at 121˚C for 15 minutes.

7. Brain Heart Infusion Broth (Hi Media Laboratories)

Ingredients	gm/liter
Calf brain infusion from	200.0
Beef heart, infusion from	250.0
Proteose peptone	2.0
Dextrose	2.0
Sodium chloride	5.0
Disodium phosphate	2.0
Final pH (at 25^0C)	7.4±0.2

Direction: 37 grams of the medium was dissolved in 1000 ml of distilled water, boiled and dispensed into blood collecting bottles. It was then sterilized by autoclaving at 121˚C for 15 minutes.

8. Salmonella Shigella Agar (Hi Media Laboratories)

Ingredients	gm/liter
Peptic digest of animal tissue	5.0
Beef extract	5.0
Lactose	10.0
Bile salts mixture	8.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	15.0
Final pH (at 25^0C)	7.0±0.2

Direction: 63 grams of the medium was suspended in 1000 ml distilled water and the medium was warmed to dissolve completely. It was sterilized by autoclaving at 121ºC (15 lbs pressure) for 15 minutes and poured into sterile petriplates.

B. COMPOSITION AND PREPARATION OF DIFFERENT BIOCHEMICAL TESTS MEDIA

1. MR-VP Medium (Hi-Media laboratories)

Ingredients	gm/litre
Peptone	5.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final pH (at 25^0C)	6.9±0.2

Direction: 15 gm powder was dissolved in 1000 ml of distilled water & mixed well. 3 ml of medium was distributed in each test tube and autoclaved at 121^0C for 15 minutes.

2. Sulphide Indole Motility (SIM) medium (Hi Media Laboratories)

Ingredients	gm/litre
Beef extract	3.0
Peptone	30.0
Peptonized iron	0.2
Sodium Thiosulphate	0.25
Agar	3.0
Final pH (at 25^0C)	7.3±0.2

Direction: 36 grams of the medium was suspended in 1000 ml of distilled water and dissolved completely. Then it was distributed in tubes to a depth of about 3 inches and sterilized by autoclaving at 121ºC for 15 minutes.

3. Simmon's Citrate Agar (Hi Media Laboratories)

Ingredients	gm/litre
Magnesium sulfate	0.2
Mono-ammonium phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Agar	15.0
Bromothymol Blue	0.08
Final pH (at 25^0C)	6.8±0.2

Direction: 24.2 grams of the medium was dissolved in 1000ml of distilled water. 3ml medium was distributed in test tubes and sterilized by autoclaving at 121^0C for 15 minutes. After autoclaving, tubes containing medium were tilted to form slant.

4. Triple Sugar Iron Agar (TSI) (Hi Media Laboratories)

Ingredients	gm/litre
Peptone	10.0
Tryptone	10.0
Yeast extract	3.0
Beef extract	3.0
Dextrose	1.0
Lactose	10.0
Sucrose	10.0
Ferrous sulphate	0.2
Sodium chloride	5.0
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0
Final pH (at 25^0C)	7.4±0.2

Direction: 65 grams of the medium was dissolved in 1000ml of distilled water and sterilized by autoclaving at 15 lbs (121^0C) pressure for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of length.

5. Urea Base Agar (Hi Media Laboratories)

Ingredients	gm/litre
Peptone	1.0
Dextrose	1.0
Sodium chloride	5.0
Dipotassium phosphate	1.2
Monosodium phosphate	0.8
Phenol red	0.012
Agar	15.0
Final pH (at 25^0C)	7.4±0.2

Direction: 24 grams of the medium was suspended in 950 ml of distilled water and sterilized by autoclaving at 121^0C for 121 minutes. After cooling to about 45^0C, 50 ml of 40% urea solution was added aseptically, mixed well and distributed 5 ml amount in sterile test tubes.

C. COMPOSITIN AND PREPARATION OF DIFFERENT STAINING AND TESTS REAGENTS

1. For Gram's Stain

(a) Crystal Violet solution

Crystal Violet	20.0 g
Ammonium Oxalate	9.0 g
Ethanol or Methanol	95 ml
Distilled Water (D/W) to make	1 litre

Direction: In a clean piece of paper, 20 gm of crystal violet was weighed and transferred to a clean brown bottle. Then, 95 ml of ethanol was added and mixed until the dye was completely dissolved. To the mixture, 9 gm of ammonium oxalate dissolved in 200 ml of D/W was added. Final volume was made 1 litre by adding D/W.

(b) Lugol's Iodine

Potassium Iodide	20.0 g
Iodine	10.0 g
Distilled Water	1000 ml

Direction: To 250 ml of D/W, 20 gm of potassium iodide was dissolved. Then 10 gm of iodine was mixed to it until it was dissolved completely. Final volume was made 1 litre by adding D/W.

(c) Acetone-Alcohol Decoloriser

Acetone	500 ml
Ethanol (Absolute)	475 ml
Distilled Water	25 ml

Direction: To 25 ml D/W, 475 ml of absolute alcohol was added, mixed and transferred into a clean bottle. Then immediately, 500 ml acetone was added to the bottle and mixed well.

(d) Safranin (Counter Stain)

Safranin	10.0 g
Distilled Water	1000 ml

Direction: In a clean piece of paper, 10 gm of safranin was weighed and transferred to a clean bottle. Then 1 litre D/W was added to the bottle and mixed well until safranin dissolved completely.

2. Normal saline

Sodium Chloride	0.85 g
Distilled Water	100 ml

Direction: The sodium chloride was weighed and transferred to a leak-proof bottle premarked to hold 100 ml. Distilled water was added to the 100 ml mark, and mixed until the salt was fully dissolved. The bottle was labeled and stored at room temperature.

3. Biochemical Test Reagents

(a) Catalase Reagent (For Catalase test)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

Direction: To 97 ml of D/W, 3 ml of hydrogen peroxide was added and mixed well.

(b) Oxidase Reagent (impregnated in a Whatman's No. 1 filter paper) (For Oxidase Test)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD)	1 gm
Distilled Water	100 ml

Direction: This reagent solution was made by dissolving 1 gm of TPD in 100 ml D/W. To that solution strips of Whatman's No. 1 filter paper were soaked and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

(c) Kovac's Indole Reagent (For Indole Test)

Isoamyl alcohol	30 ml
p-dimethyl aminobenzaldehyde	2.0 g
Conc. Hydrochloric acid	10 ml

Direction: In 30 ml of isoamylalcohol, 2 g of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to that, 10 ml of conc. HCl was added and mixed well.

(d) Methyl Red Solution (For Methyl Red Test)

Methyl red	0.05 g
Ethyl alcohol (absolute)	28 ml
Distilled Water	22 ml

Direction: To 28 ml ethanol, 0.05 gm of methyl red was dissolved and transferred to a clean brown bottle. Then 22 ml D/W was added to that bottle and mixed well.

(e) Barritt's Reagent (For Voges-Proskauer Test)

Solution A	
α-naphthol	5.0 g
Ethyl alcohol (absolute)	100 ml

Direction: To 25 ml ethanol, 5 g of α-naphthol was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

Solution B	
Potassium hydroxide	40.0 g
Distilled Water	1000 ml

Direction: To 25 ml D/W, 40 gm of KOH was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

(f) Turk's Reagent

Glacial acetic acid	1.5ml
Distilled water	98.5ml

Direction: To 98.5 ml of distilled water, 1.5ml of Glacial acetic acid was added and mixed well. Then 2-3 drops of Crystal violet was added.

5. **McFarland tube (No. 0.5)**

0.5 ml of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2.H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% w/v) with constant stirring. The McFarland standard was thoroughly mixed to ensure that it is evenly suspended. Using matched cuvettes with a 1 cm light path and water as a blank standard, the absorbance was measured in a spectrophotometer at a wavelength of 625 nm. The acceptable range for the turbidity standard is 0.08-0.13. The standard was distributed into screw-cap tubes of the same size and volume as those used to prepare the test inoculum. The tubes were sealed tightly to prevent loss by evaporation and stored protected from light at room temperature. The turbidity standard was then vigorously agitated on a vortex mixer before use. Standards may be stored for up to 6 months, after which time they should be discarded.

APPENDIX-IV

A. GRAM-STAINING PROCEDURE

First devised by Hans Christian Gram during the late 19th century, the Gram-stain can be used effectively to divide all bacterial species into two large groups: those that take up the basic dye, crystal violet (Gram-positive) and those that allow the crystal dye to was out easily with the decolorizer alcohol or acetone (Gram-negative). The following steps are involved in Gram-stain:

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with crystal violet stain and allowed to remain without drying for 1 minute.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.
7. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer. Thicker smear requires more aggressive decolorizing.
8. The slide was flooded with counter-stain (safranine) for 1 minute and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 100X.

B. SLIDE AGGLUTINATION TEST FOR IDENTIFICATION OF *Salmonella* spp.

1. A drop of normal saline was kept on a clean glass slide.
2. A dense suspension of the organism is made. The organism was taken from nutrient agar.
3. A drop of respective antiserum was added to the organism suspension and mixed well with a wooden stick.
4. The slide was observed for the clumping of the suspension. Positive test was indicated by rapid complete agglutination of the test organism.

The suspension without adding antisera should be kept as a control to detect autoagglutination

APPENDIX-V

METHODOLOGY OF BIOCHEMICAL TESTS USED FOR IDENTIFICATION OF BACTERIA

A. Catalase test

This test is performed to demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide.During aerobic respiration, in the presence of oxygen, microorganisms produces hydrogen peroxide, which is lethal to the cell itself. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, the main exception being *Streptococcus* spp.

Procedure: A small amount of a culture from Nutrient Agar plate was taken in a clean glass slide and about 2-3 drops of 3% H_2O_2 was put on the surface of the slide. The positive test is indicated by the formation of active bubbling of the oxygen gas. A false positive reaction may be obtained if the culture medium contains catalase (e.g. Blood Agar) or if an iron wire loop is used.

B. Oxidase test

This test is performed for the detection of cytochrome oxidase in bacteria which catalyzes the transport of electrons between electron donors. In the presence of redox dye Tetramethyl-*p*-phenylene diamine dihydrochloride, the cytochrome oxidase oxidizes it into a deep purple colored end product Indophenol which is detected in the test. The test is used for screening species of *Neisseria, Alcaligenes, Aeromonas, Vibrio, Campylobacter* and *Pseudomonas* which give positive reactions and for excluding the Enterobacteriaceae, all species of which give negative reactions.

Procedure: A piece of filter paper was soaked with few drops of oxidase reagent (Whatman's No. 1 filter paper impregnated with 1% tetramethyl-p-phenylene diamine dihydrochloride). Then the colony of the test organism was smeared on the filter paper. The positive test is indicated by the appearance of blue-purple color within 10 seconds.

C. Indole Production test

This test detects the ability of the organism to produce an enzyme: 'tryptophanase' which oxidizes tryptophan to form indolic metabolites: indole, skatole (methyl indole) and indole acetic acid. The enzyme tryptophanase catalyses the deamination reaction attacking the

tryptophan molecule in its side chain and leaving the aromatic ring intact in the form of indole.

Procedure: A smooth bacterial colony was stabbed on SIM (Sulphide Indole Motility) medium by a sterile stab wire and the inoculated media was incubated at 37°C for 24 hours. After 24 hours incubation, 2-3 drops of Kovac's reagent was added. Appearance of red color on the top of media indicates indole positive. Indole if present combines with the aldehyde present in the reagent to give a red color in the alcohol layer. The color reaction is based on the presence of the pyrrole structure present in indole.

D. Methyl Red test

This test is performed to test the ability of an organism to produce and maintain stable acid end product from the fermentation of glucose to give a red color with the indicator methyl red and to overcome the buffering capacity of the system. Medium used in the study was Clark and Lubs medium (MR/VP broth, pH 6.9). Methyl red is an indicator which is already acid and will denote changes in degree of acidity by color reactions over a pH range of 4.4-6.0.

Procedure: A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of methyl red reagent was added and mixed well. The positive test was indicated by the development of bright red color, indicating acidity and negative with yellow color.

E. Voges-Proskauer (VP) test

The principle of this test is to determine the ability of some organisms to produce a acetyl methyl carbinol, a neutral end product (acetoin) or its reduction product 2, 3-butanidiol during fermentation of carbohydrates. An organism of the Enterobacteriaceae group is usually either methyl red positive and Voges- proskauer- negative or methyl red negative and Voges-Proskauer positive. The Voges proskauer test for acetoin is used primarily to separate *E. coli* from *Klebsiella* and *Enterobacter* species.

Procedure: A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of Barritt's reagent was added and shaken well for maximum aeration and kept for 15 minutes, positive test is indicated by the development of pink red color.

F. Citrate Utilization test

This test is performed to detect whether an organism utilizes citrate as a sole source of carbon for metabolism with resulting alkalinity. The medium used for citrate fermentation (Simmon's Citrate medium) also contains inorganic ammonium salts. Organisms capable of utilizing citrate as its sole carbon source also utilizes the ammonium salts present in the medium as its sole nitrogen source, the ammonium salts are broken down to ammonia with resulting alkalinity.

Procedure: A loopful of test organism was streaked on the slant area of Simmon's Citrate Agar medium and incubated at 37°C for 24 hours. A positive test was indicated by the growth of organism and change of media by green to blue, due to alkaline reaction. The pH indicator bromothymol blue has a pH range of 6.0-7.6, i.e. above pH 7.6; a blue color develops due to alkalinity of the medium.

G. Motility test

This test is done to determine if an organism was motile or non-motile. Bacteria are motile by means of flagella. Flagella occur primarily among the bacilli; however a few cocci forms are motile .Motile bacteria may contain a single flagella. The motility media used for motility test are semisolid, making motility interpretations macroscopic.

Procedure: Motility of organism was tested by hanging drop and cultural method. In cultural method, the test organism was stabbed in the SIM medium and incubated at 37ºC for 48 hours. Motile organisms migrate from the stabline and diffuse into the medium causing turbidity. Whereas non-motile bacteria show the growth along the stabline, and the surrounding media remains colorless and clear.

H. Triple Sugar Iron (TSI) Agar Test.

The TSI agar is used to determine the ability of an organism to utilize specific carbohydrate incorporated in the medium (glucose, sucrose and lactose in concentrations of 0.1%, 1.0% and 1.0% respectively), with or without the production of gas (indicated by cracks in the media as well as an air gap at the bottom of the tube) along with determination of possible hydrogen sulfide production (detected by production of black color in the medium). A pH indicator (phenol red) included in the medium can detect acid production from fermentation

of these carbohydrates and it gives yellow reaction at acidic pH, and red reaction to indicate an alkaline surrounding.

Procedure: The test organism was streaked and stabbed on the surface of TSI and incubated at 37°C for 24 hours .Acid production limited only to the butt region of the tube is indicative of glucose utilization, while acid production in slant and butt indicates sucrose or lactose fermentation. The results are interpreted as follows:

a. Yellow (Acid)/ Yellow (Acid), Gas, H_2S → Lactose/ Sucrose fermenter, H_2S producer.

b. Red (Alkaline) / Yellow (Acid), No Gas, No H_2S → Only Glucose, not lactose/ Sucrose fermenter, not aerogenic, No H_2S production.

c. Red (Alkaline) / No Change → Glucose, Lactose and Sucrose non-fermenter.

d. Yellow (Acid)/ No Change → Glucose- Oxidiser.

e. No Change / No Change →Non-fermenter.

I. Urea Hydrolysis test:

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia and carbon dioxide. Ammonia thus produced changes the color of indicator (phenol red) incorporated in the medium.

Procedure: The test organism was inoculated in a medium containing urea and the indicator phenol red. The inoculated medium was incubated at 37°C overnight. Positive organism shows pink red color due to the breakdown of urea to ammonia. With the release of ammonia the medium becomes alkaline as shown by a change in color of the indicator to pink.

J. Coagulase test

This test is used specifically to differentiate species within the genus *Staphylococcus*: *S. aureus* (usually positive) from *S. saprophyticus, S epidermidis* (negative). A positive coagulase test is usually the final diagnostic criterion for the identification of *S. aureus.* Free coagulase and bound coagulase are the two types of coagulase possessed by this organism; most strains possess both free and bound coagulase.

Slide Coagulase Test

Bound coagulase (Clumping Factor) is detected by slide test. The bound coagulase is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma.

Procedure: For slide coagulase test, a drop of physiological saline was placed on three places of a slide, and then a colony of the test organism was emulsified in two of the drops to make thick suspensions. Later a drop of plasma was added to one of the suspensions and mixed gently. Then a clumping was observed within 10 seconds for the positive coagulase test. No plasma was added in second suspension. This was used for the differentiation of any granular appearance of the organism from true coagulase clumping. The third drop of saline was used for a known strain of coagulase positive staphylococci.

Tube Coagulase Test

This test is carried out to detect production of free coagulase. Plasma contains coagulase reacting factor (CRF) which activates free coagulase. The activated coagulase acts upon prothrombin thus converting it to thrombin. Thrombin converts fibrinogen into fibrin which is detected as a firm gel (clot) in the tube test. Tube test is performed when negative or doubtful results are obtained in slide coagulase test.

Procedure: In the tube coagulase test, plasma was diluted 1:10 in physiological saline. Four small tubes were taken, one for test organism, one for positive control, one for negative control, and one to observe self clotting of plasma. Then 0.5 ml of the diluted plasma was pipetted into each tube and 0.5 ml of test organism, 0.5 ml of positive control (*S. aureus* culture), and 0.5 ml negative control (*S. epidermidis* culture) was added to three tubes, to the fourth tube, 0.5 ml sterile broth was added. After mixing gently, all tubes were incubated at 37^0C on a water bath for 6 hours and observed for gel formation in every 30 minutes. The clotting is observed by gently tilting the tube for positive coagulase test.

APPENDIX-VI

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM BLOOD SAMPLE

Bacteria	Morphological Characteristics	Cultural Characteristics
Salmonella Typhi	Gram negative rods of 1-3µm×0.5-8µm in size, motile with peritrichous flagella, non capsulated and non sporing.	On MA: Flat, circular colonies about 0.5-1mm in diameter, colorless and opaque.
Salmonella Paratyphi A	Gram negative rods of 1-3µm×0.5-8µm in size, motile with peritrichous flagella, non capsulated and non sporing.	On MA: Slightly raised, circular colonies about 1-2mm in diameter, colorless and translucent.
Streptococcus pneumoniae	Gram positive ovoid or lanceolate cocci arranged in pairs 1-3µm×0.4-0.7µm size, aerobic and anaerobic, nonsporing, motile, capsulated	On BA: Raised, circular about 1mm in diameter, grow well when supplemented with CO_2. The colonies are alpha (α) haemolytic. On MA: No growth
Escherichia coli	Gram negative rod of 1-3µm×0.4-0.7µm size, aerobic and anaerobic, nonsporing, motile, noncapsulated	On BA: Large 1-4 mm in diameter, grayish white, moist, smooth, convex and opaque. The colonies may appear mucoid and some strains are haemolytic. On MA: Bright pink colonies due to lactose fermentation, smooth, glossy and translucent.
Staphylococcus aureus	Gram positive, spherical cocci, 0.8-1 µm in diameter,	On BA: Large, 2-4 mm diameter. Circular, smooth with glistening surface, entire edge,

	non sporing, facultative anaerobe, non-motile, except for rare strains, non capsulated. They are arranged in characteristics grape like clusters or in small groups, pairs, singles and short chain(less than five cocci in line).	soft butyrous consistency and opaque. The pigmentation is golden yellow to cream coloured.Some strains are beta-haemolytic when grown aerobically. On MA: Small (pin head size), 0.1-0.5mm, pink or pink orange due to lactose fermentation. Some strains are non-lactose fermenting.
Klebsiella spp.	Gram negative, short and thick rods of 1-2µm × 0.8µm size, nonsporing, nonmotile and capsulated.	Large dome shaped moist and usually viscid or mucoid colonies when cultured on BA and MA. Most Klebsiella species are lactose fermenting.
Acinetobacter spp.	Gram negative, short, stout, non- motile rods that become almost coccoid, frequently capsulated, strict aerobes.	They grow well on ordinary media and form white of cream, glistening smooth and often rather viscid colonies about 1mm in diameter.
Streptococcus viridans	Gram positive cocci, non sporing and non-motile.	On BA: Alhpa (α) haemolytic colonies are obtained.
Proteus spp.	Gram negative rods of 1-3 µm × 0.4-0.6µm size, non capsulated, nonsporing motile rods.	On BA: when cultured aerobically, most strains are swarming type and have a characteristic fishy odour. On MA: *Proteus* species produce individual non-lactose fermenting colonies after overnight incubation at 35ºC to 37ºC. Swarming is prevented on MA because this media contains bile salts.
Enterobacter spp.	Gram negative rods, non sporing, noncapsulated.	About 2 to 3 mm in diameter, moist, yellowish coloured, LF, motile organism.

APPENDIX-VII

Table: Distinguishing reactions of the common and pathogenic Enterobacteriaceae

Species	Test/ substrate											
	lac	mot	gas	ind	VP	cit	PDA	ure	lys	H_2S	inos	ONPG
E. coli	+	+	+	+	-	-	-	-	+	-	-	+
Shigella groups A, B, C	-	-	-	±	-	-	-	-	-	-	-	-
Sh. sonnei	-	-	-	-	-	-	-	-	-	-	-	+
Salmonella (most serotypes)	-	+	+	-	-	+	-	-	+	+	±	-
S. typhi	-	+	-	-	-	-	-	-	+	+	-	-
S. paratyphi A	-	+	+	-	-	-	-	-	-	-	-	-
C. freundii	±	+	+	-	-	+	-	±	-	±	-	+
C. koseri	±	+	+	+	-	+	-	±	-	-	-	+
K. pneumoniae	+	-	++	-	+	+	-	+	+	-	+	+
K. oxytoca	+	-	++	+	+	+	-	+	+	-	+	+
E. aerogenes	+	+	++	-	+	+	-	-	+	-	+	+
E. cloacae	+	+	+	-	+	+	-	±	-	-	-	+
Hafnia alvei	-	+	+	-	+	-	-	-	+	-	-	+
Serratia marcescens[b]	-	+	±	-	+	+	-	-	+	-	±	+
P.mirabilis	-	+	+	-	±	±	+	++	-	+	-	-
P. vulgaris	-	+	+	+	-	-	+	++	-	+	-	-
M. morganii	-	+	+	+	-	-	+	++	-	±	-	-
Providencia rettgeri	-	+	-	+	-	+	+	++	-	-	+	-
P. stuartii	-	+	-	+	-	+	+	±	-	-	+	-
P. alcalifaciens	-	+	+	+	-	+	+	-	-	-	-	-
Yersinia enterocolitica[c]	-	-	-	±	-	-	-	±	-	-	±	+
Y. pestis	-	-	-	-	-	-	-	-	-	-	-	±
Y. pseudotuberculosis	-	-	-	-	-	-	-	+	-	-	-	±

[a] lac, inos, fermentation of lactose, inositol; mot, motility; gas, gas from glucose; ind, indole production; VP, Voges-Proskauer; cit, Citrate utilization (Simmons'); PDA, phenylalanine

deaminase; ure, urease; lys, lysine decarboxylase; H_2S, H_2S produced in TSI agar; ONPG, metabolism of *o*-nitrophenyl-β-D-galactopyranoside.

[b] Some strains of Serratia marcescens may produce a red pigment

[c] *Yersinia* are motile at 22°C.{Key: +, ≥85% of strains positive; -, ≥ 85% of strains negative; 16-84% of strains are positive after 24-48 hour at 36°C}

(Source: Collee *et al.* 1996)

APPENDIX-VIII

ZONE SIZE INTERPRETATIVE CHART

Antimicrobial Agents used	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Susceptible (mm or more)
Amikacin	Ak	30µg	14	15-16	17
Amoxycillin	Am	30µg	13	14-16	17
Amoxycillin/Clavulanic acid	Ac	20/10µg (30µg)	19	-	20
Ampicillin	A	10µg	18	19-21	22
Cefotaxime	Ce	30µg	14	15-22	23
Ceftriaxone	Ci	30 µg	13	14-20	21
Chloramphenicol	C	30µg	12	13-17	18
Ciprofloxacin	Cf	5 µg	15	16-20	21
Cloxacillin	Cx	5 µg	12	12-13	14
Cotrimoxazole (Trimethoprim/ Sulphonamide)	Co	1.25/23.75µg	10	11-15	16
Erythromycin	E	15 µg	13	14-22	23
Ofloxacin	Of	5 µg	12	13-15	16
Penicillin When testing with *S.pneumoniae*	P	10 mcg	-	-	20

(Source: Product Information Guide, Hi-Media Laboratories Pvt. Limited, Bombay, India)

APPENDIX-IX

DATA ANALYSIS (CHI-SQUARE TEST)

1. Association of occurrence of bacteraemia and septicaemia between genders

Gender	Culture positive	Culture negative	Total
Male	111	961	**1072**
Female	68	531	**599**
Total	**179**	**1492**	**1671**

Test statistic is χ^2

Ho: There is no significant association of occurrence of bacteraemia and septicaemia in male and female patients.

H1: There is significant association of occurrence of bacteraemia and septicaemia in male and female patients.

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=1.271$

Thus χ^2_{cal} (1.271) < χ^2_{tab} at $\Im=0.05$ and d.f. = 1 i.e.3.841

Hence, Ho is accepted i.e. there is no significant association of occurrence of bacteraemia and septicaemia in male and female patients.

2. Association of resistivity pattern of Ciprofloxacin with Nalidixic acid of *Salmonella* spp

	Ciprofloxacin susceptible	Ciprofloxacin intermediately sensitive	Total
Nalidixic acid sensitive	42	3	**45**
Nalidixic acid resistant	25	14	**39**
Total	**67**	**17**	**84**

Test statistic is $\chi 2$

Ho: There is no significant association of resistivity pattern of Ciprofloxacin with Nalidixic acid of *Salmonella* spp.

H1: There is significant association of resistivity pattern of Ciprofloxacin with Nalidixic acid of *Salmonella* spp.

From $\chi^2 = \Sigma (0-E)^2/E$ we find $\chi^2 = 11.05$

Thus χ^2_{cal} (11.05) > χ^2_{tab} at $\Im = 0.05$ and d.f. =1 i.e 3.841

Hence, Ho is rejected i.e. the association of resistivity pattern of Ciprofloxacin with Nalidixic acid of *Salmonella* spp is statistically significant.