Ordinarily, man lives comfortably in an environment that teems with microorganisms. Not many persons are concerned about this, nor they need to be, since most microbes are harmless commensals, and some are actually beneficial. People therefore ordinarily enjoy a state of 'Peaceful-coexistence' that does not require any special attention or effort on their part. Moreover, a sanitary civilization has so reduced exposure to dangerous pathogens that relatively simple personal hygiene suffices to gain for all the added protection they ordinarily need from pathogens.

However, when person enter a hospital as patient the situation is remarkably different. There, they come into close association with a multitude of different people and with many more and different microorganisms than they do normally. Many of the organisms that they encounter are pathogens. Furthermore greater exposure to more and different microorganisms than people are normally accustomed engenders the like hood of developing an infection during hospitalization.

Infection is as old as mankind itself. The Arabs learned several years ago that cauterization of a wound with hot metal prevented infection. This was common procedure
despite the fact that the patient would be scarred for life. Even though cauterization was traumatic, it gave the victims, more fighting chance to overcome the effects of disease agents, [Wistreich and Lachtman : 1973]

The French Surgeon Pare in 1537 treated gun shot wounds with bandages soaked in egg yolk, turpentine and other materials. The turpentine served as chemical cautery and egg material contained antibacterial enzymes, lysozyme. [Wistreich and Lechtman ; 1973]

The concept of antisepsis was introduced largely by Semmelweiss and Lester, [Wistreich and Lechtman ; 1973]. Before the relationship of microorganisms to infection was established. Infection rates in hospitals were so high that these infections were often referred to as 'pest houses'. It was only a little more than a century ago that Ignatz Semmelweiss, a Hungarian Physician working in Vienna observed that incidence of child bed fever, known as puerperal fever, was very much higher in Obstetrical Ward in Vienna in General hospitals operated by physicians, than a similar ward run by midwives. Semmelweiss observed that the midwives washed their hands frequently whereas as physicians and medical students, after performing autopsies treated the patients without changing their blood splattered cloths or washing hands. In order to lower the occurrence of infection
in his maternity ward he required the attendent to wash their hands with chlorinated lime \([\text{CaCl}_2]\). The infection rate dropped from 12% to 1%.

Unfortunately Semmelweiss's efforts to persuade the other physicians, the necessity of cleanliness and disinfection ended in failure and he was dismissed from hospital staff, ridiculed, he eventually left Vienna in 1854 and fate led him to insanity. [Wistriech and Lechtman ; 1973].

In early 1800s microorganisms were considered biological non entities. Infections were believed to be caused by some magic power in air or by imbalance of body fluids. It took incredible tenacity of Pasteur to show the world that microorganisms could not only ferment fruit juice to wine but also cause spoilage of wine. The evolution of germs theory of infection by Louis Pasteur gave the dreaded complication of wounds after injuries a new significance, [Altemeier ; 1980].

Sir Joseph Lister in England undertook the challenge of preventing surgical infections. Lister reasoned that the very basis of surgical infection, sepsis, might be microbial in nature. Subsequently to prevent access of microorganisms to wounds he designed a system which came to be known as antiseptic surgery. He included heat sterilization of
instruments and application of Carbolic acid to wounds by means of dressing, [Wistreich and Lechtman ; 1973].

Robert Koch, the German bacteriologist published a monograph "The cause of infection in wounds" which showed for the first time the specificity of different types of bacteria for causing distinctive types of clinical pictures. These pointed to some limitations of antisepsis and set the stage for next advance, [Altemeier ; 1980]

Neuber applied mercury chloride as surgical antiseptic as well as for disinfection of operating rooms. He also insisted on wearing clean gowns on patients and clean apparens by the surgeons in operation rooms, [Wisterich and Lechtman ; 1973]

Near the begining of twentieth century Van Bergmann developed the principle and practice of aseptic surgery introducing the aseptic era which offered the hope of surgical intervention completely free from infection. This compounded the significance of Lister's discoveries and a new day dawned in practice of surgery, [Altemeier;1980]

Another new era, the golden era of antibiotic therapy was introduced in 1940s when penicillin became available and about a decade later broad spectrum antibiotics were
introduced, [Agarwal ; 1972]

The germ theory of infectious disease was accepted, and asepsis was widely believed in and practiced, until antibacterial chemotherapeutic agents such as sulfonamides and antibiotics such as penicillin came into use. These agents were so effective at the outset that emphasis on asepsis was decreased and reliance was placed upon the two new agents. However, it was soon learnt that a selection process was going on where by the susceptible organisms were eliminated and resistant organisms were retained in the hospital environment. Unfortunately, the resistant organisms appeared to retain their pathogenicity, and much to the consternation of all concerned, the incidence of hospital-acquired infections rebounded, [American - Hospital Association ; 1979]

INFECTIONS OF NEWBORN :

The normal foetus is sterile until shortly before birth, as long as amniotic membrane remains intact. After birth the newborn infant who has essentially no microbial flora, is immediately exposed to the microbial world and inoculated with millions of bacteria and other microbes.

After birth, the infant is at the mercy of his environment.
He may acquire infection from the hands of his attendants, from apparatus used in his resuscitation or general care, particularly from humidifying units of such equipments, from the air or from his feeds, [Davies ; P.A.;1971].

Bacterial colonization is not synonymous with infection; but higher the colonization rate with potential pathogens, greater is the risk of frank clinical infection. This is because the host immunity is yet to develope and largely dependent on the maternal immunoglobulins. Which are passively transferred across placenta. At this stage some commensals may become opportunistic pathogens particularly in compromised neonates who must remain in hospital for the treatment of congenital abnormalities. [Rotimi and Duerden; 1981].

The most important neonatal factor predisposing to infection is prematurity or low-birth weight. There is 3 to 10 fold higher incidence of sepsis, meningitis or urinary tract infections in these infants than in full term, normal birth weight infants, [Glasgow and Overall ; 1983].

The premature infants defences against invasion by microorganisms are limited in several respects. In term of humoral immune systems, the preterm neonate is compromised at birth by having lower concentrations of IgG than his
Neonatal IgG being largely of maternal origin and transferred mostly as the foetus nears term. The premature infants, because of early birth are deficient in IgG antibodies. Newborn infants also lacks IgM antibodies at birth. Because of their large molecular size IgM cannot permeate the placenta. It may be also deficient in endogenous immunoglobulin production. These factors may combine to produce a state of hypogammaglobulinemia with an associated increased susceptibility to infection.

Multiple factors are involved in the spread of the pathogens in hospital e.g. their presence in infected patients.

The common bacteria that involved in bacterial infections of the newborn are gram positive cocci like Staphylococci, Streptococci, Pneumococci and gram negative bacilli like E. coli, Klebsiella species, Proteus Species and Pseudomonas aeruginosa, since these bacteria are known to colonise remains a potential source for infection, especially, premature infants have immature defences to meet this unique challenge.
INFECTION OF BURNS:

Burns are among the oldest injuries that afflict mankind. There is evidence that certain herbs for burn treatment were utilized by Neanderthal man living in the fertile crescent as early as 60,000 BC. There is general agreement that the earliest account of the treatment of burn is recorded in Papyrus Ebers, which was written about 1600 BC.

During the Seventh and Sixth centuries BC, there arose in Greece a series of nature philosophers who sought to explain how the world as they saw it come to be and how the process of change occurred. These ideas set the stage for the development of theories of medicine. The elaboration of medical theory was built upon a base of elements, qualities and humors. The elements of earth, air, fire and water were perceived as the basic components of the universe. From the medical perspective, this category included all living things. Elements were then characterized according to their qualities, wet, dry, hot and cold reflecting sensory interpretation. The humors were described as blood, phlegm, black bile and yellow bile. The task of Greek physician was first to determine the imbalance of elements, qualities and humors, to add a prognosis, and then to prescribe drugs that would promote the reestablishment of an appropriate balance.
Hippocrates of Cos was an outstanding physician in Greece during the later part of the fifth century BC. He exerted tremendous influence upon medical practice of his day, and a collection of Greek medical papers summarizing the period were given his name Hippocratic Corpus which emphasizes the role in practice of diagnostic evaluations, drug effectiveness, surgery, and anatomical considerations. The earliest mention of the treatment of burns by Greek physicians is in Hippocratic Corpus. In these papers, special significance was emphasis on avoidance of suppuration by wound cleansing. The wounds were irrigated by use of clean water or wine, and wound dryness was sought. Greek physicians of the fourth and third centuries BC prescribed medication of burn wounds. "Melt old pig’s fat mixed with pine resin and bitumen, smear this on a piece of linen cloth, warm it before the fire and apply as a bandage." The Greek physicians were expert in medical practice and in pharmacology. They inherit a strong tradition of herbal medicine; some of which may be traced back to the Ebers Papyrus, 11 Centuries before their time.

During the early days of the Roman Empire, Cornelius Celsus wrote a remarkable book on medicine "De Medicina (AD 14-37)". His section on the treatment of burn is similar to that of Greek physicians. During Celsus’s evolution new herbs were described for burn wounds.
During the Middle Ages, local treatment of burns became stabilized. Rhazes (850-923) formulated white ointment composed of white lead and oil of roses in wax. Rhazes emphasized the use of rose water cooled by snow, and Avicenna, a prominent Arabian physician (980-1037) extolled the virtues of ice water in the care of burn injury. Brunchwig in 1494 and Veigo in 1514, wrote accounts of the treatment of gunshot wounds and regarded them as poisoned burns.

The concept that the use of heat would diminish the pain in burn was an old one and had been practiced by Avicenna in the tenth century and subsequently through the Middle Ages.

The idea of burning out the burn is referred to in Shakespeare's "King John" (1602) (Act III, Scene I); where in Cordinal Pandulph says to king Philip, "And falsehood cures falsehood as fires cool fire within scorched veins of one new burned .......". Although the concept of treating fire with fire is popularly associated with Ambrose Pare, in fact, Pare, who decided that such treatment was contraindicated. Working as army surgeon he noted that gunshot wounds heated better without being treated with boiling oil, and would heal best by local cleansing and care.
In 1799, Sir James Earle treated his patients with severe burns with ice as antitode. The treatment of burns by baths in such common practice today is of relatively recent origin in history. In fact baths for burns were promoted by Von Hebra (1816-1880), a Viennese dermatologist. He designed and constructed a wooden tub in which the patient was immersed, often for weeks, in continuously warm water.

The history of fluid replacement-therapy for burns begins with Sushruta, who first described clinical symptoms of a burn patient and emphasized fever and enormous thirst as characteristic.

In 1607, Fabry recommended that the blisters should be cut to avoid infection, in deep burns he made incisions to let moisture escape, having observed that otherwise gangrene and infection would occur. These may be considered the first recorded escharotomies. The Indian Surgeon Sushruta, whose collected works (800 BC) are called the Samhita, who recommended to debride severe burns with loose skin and flesh.

Several new approaches to the local treatment of the burn wound had unfolded at the turn of the 20th century. Copeland in Alabama in 1887 first described the open or exposure method for treating burns. Exposure therapy was
also recommended by Sneve in 1905 and John in 1910, but the use of this method lost favor later because of unavoidable infection. Exposure therapy was repopularized by Wallance in 1947 and is still in use widely but selectively today. Of the local treatments used during the early 20th century, the most important was that of Davidson, who originated the use of tannic acid Spray in 1925. The use of this technique produced a dry eschar which was thought to decrease fluid loss and infection and relieved pain. This method was used widely for 20 years until in 1944, McClure pointed out that tannic acid was toxic to liver. Aldrich 1933, recommended the use of gentian Violet as a topical escharotic because of its effectiveness in the control of gram positive infections, Staphylococcus and hemolytic Streptococci. Later because the gentian violet failed to control gram negative infections, he added other dyes such as acriviolet and brilliant green. The method however, fell into disrepute because of the failure of the drug to control the surface infection and the fact that beneath the eschar created by the dyes, severe infection often occurred, (Aldrich; 1933, 1937)

The use of petrolatum gauze dressing with pressure and strict immobilization was popularized by Allen and Koch in 1942.
The use of Allen-type dressing plus exposure therapy was continued during the 1950s, but it became clear that the primary cause of patients death was septicaemia due to Staphylococci and the source of these organisms was in the burn wound. As the semisynthetic penicillins became available to control resistant staphylococcal infection, Pseudomonas and other gram negative infections were in the ascendancy. In 1965 Mayer introduced the use of ½ % Silver nitrate soaks for the control of surface wound infection, and about the same time Moncrief introduced the topical use of Sulfamylon. These two agents ushered in a new topical therapeutic era for control of gram positive and gram negative infection. In 1969, Fox introduced Silver sulfadiazine for the topical treatment of burn wound infection. This drug to-date has been quite successful in treating both gram positive and gram negative burn wound infection. However, resistant Pseudomonas organism and others have appeared, and it is apparent that the final chapter in burn wound sepsis control has not yet been written.

In addition to the use of topical and parenteral agents of an antibacterial nature to assist in the control of burn wound infection, hosts of other approaches have been used, chief among which are the use of various types of human and animal skin as a means of providing temporary wound coverage.
and assisting in control of local infection.

Nosocomial infections have been increasing for the past two decades. Five to fifteen percent of the patients admitted in the hospital acquire these infections and this is mainly due to presence of pathogenic and potentially pathogenic microorganisms in high concentration in hospital environment, instrumental procedures on the patients and lowered resistance in some cases. These infections are frequently due to multiple drug resistant organisms which have emerged as a result of ever indulgence of antibacterial agents in treating the patients.

A large proportion of patients who attend a hospital, because they need treatment for some ailment caused by the invasion of their tissue by microbes. The bacterial population of the hospital is likely to be more dangerous than that of similar large institutions attended by a comparable number of healthy people.

Infections that are acquired by patients during hospitalization, with diagnosis confirmed by clinical or laboratory evidence are called nosocomial infections. The infecting organisms may originate either from endogenous sources, as indigenous commensal flora carried by the patient, as many of them have infected discharging wounds or
harbour virulent bacteria in their respiratory passages, on their skin and elsewhere, or from exogenous sources, as recent acquisitions from animate or inanimate objects within the hospital.

Many factors may influence the development of nosocomial infection.

MICROBIAL AGENTS:

There are many kinds of pathogens to which a patient may be exposed during hospitalization. The infections resulting from such exposure depends in part on the species of pathogens, its resistance to antimicrobial agents being administered to the patients, its virulence, and the number introduced to the patient.

The modern hospital environment favours bacteria with special properties. For success they need to be able to withstand and grow in the presence of as many antibiotics as possible. Especially those commonly employed such as penicillins and tetracycline. In addition the microbs may withstand or actually multiply in dilute antiseptics commonly applied to wounds or skin.

Species which inhabit sites from which they are easily shed
will constantly maintain their numbers in the hospital dust and air. In addition, they are virulent in the sense that small numbers only are needed to establish colonization or wound invasion for their success as "hospital pathogens".

The widespread use of antibiotics has virtually abolished streptococcal wound infection although many strains, even of this organism, have within the last ten years become resistant to tetracycline and may in future acquire resistance to sufficient drugs to stage a comeback.

Streptococcus pyogenes was a common cause of septicaemia. These were commonly isolated in post mortem.

Streptococci were commonly isolated from throat of the patients and nursing staff. At the time of outbreaks streptococci of single serotype was isolated, indicating the role of cross infection and hospital environment, [Lowbury; 1954].

A major change has taken place in the etiology of infectious diseases in the past two decades. Formerly specific infections were the greater problem. These diseases have not disappeared, but opportunistic infections attributed to the organisms formerly believed to be "harmless" have proved to be a serious problem. These opportunistic pathogens have
became a continuing and growing problem in hospital population. Bringing a large number of sick people under a single roof has many advantages, but it also has one serious drawback of nosocomial infection.

Among the various organisms causing hospital infections Staphylococci and gram negative bacilli are posing a special problem because of their resistance to many antimicrobial drugs. Since the introduction of penicillin in 1941, the Staphylococci had persistently displayed a potential to develop resistance to virtually every new antistaphylococcal drug discovered. In last few years this problem of hospital Staphylococcal cross infection seems to have become more manageable and due credit must be given to semisynthetic penicillins tolerant to penicillinase, newer antimicrobial drugs and internationally accepted methods of epidemiological studies.

Staphylococci isolated from patients during hospital stay by Lowbury were predominantly of a single strain of phage group III (type 80). Eighty to hundred percent of Staphylococci were penicillin resistant.

The incidence of infection caused by relatively less virulent gram negative micro organisms such as Pseudomonas aeruginosa have begun to increase appreciably. Though
Pseudomonas aeruginosa inhabit gastrointestinal tract or colonize on skin, they seldom cause illness in humans who have competent immunity. In contrast infections with Pseudomonas aeruginosa occur in patients on antibiotic and immunosuppressive therapy. Indiscriminate use of antibiotics promotes the growth of relatively resistant. Pseudomonas aeruginosa without competition. Immunosuppressive drugs diminish host defense and "open the door" to Pseudomonas aeruginosa invasion. Because of its tendency to grow or at least survive in moist environment, its relative resistance to antimicrobial drug, insensitivity to common disinfectants and moreover its ability to grow in disinfectants like cetavelon, Pseudomonsa aeruginosa infection is a special hazard to the patients who are hospitalized. It causes hospital infections of burns, wounds, skin, urinary tract, respiratory tract, ear, eye and meninges. It has a tendency to invade the blood stream, leading to septicaemia. Pseudomonas aeruginosa is also isolated from equipment which retain moisture, especially respirator and resuscitator. Patients with severe burns, patients undergoing major surgery, patients with cystic fibrosis and malignancy, those who are receiving radiotherapy and immunosuppressive therapy are "high risk" patients for Pseudomonas infection and infection in these patients is severe and may terminate in death.
Coagulase negative staphylococci has long been recognised as opportunistic pathogen. In the last two decades, serious infections have been increasingly seen in immunocompromised patients. - [Harris (1985)].

Because of their prevalence on the skin and frequent implantation of foreign devices in patients during hospitalization, coagulase negative staphylococci are ideally situated to cause serious infections in such individuals. - [Parisi (1985)].

Coagulase negative staphylococci constitute 4% of all bacterial isolates from nosocomial infection of genitourinary tract and surgical wounds, - [Jay (1983)]. It is the nosocomial pathogen of prosthetic valves, C.S.F. shunts, joint prostheses, vascular prostheses post operative wounds, septicaemia, abscess and osteomyelitis - [Archer et al (1985)].

Bacterial endocarditis is a serious problem occurring due to coagulase negative staphylococci - [Culliford et al 1976]. They are the major pathogens isolated from patients undergoing bone marrow transplant and cardiac surgery [Archer et al (1985), Parisi (1985).] They are common blood culture isolates in the neonatal ICU, - [Freeman et al (1990)].
In the last ten years, the major increasing problem associated with coagulase negative staphylococci is, their resistance to various antimicrobial drugs. Since the introduction and wide spread use of antibiotics, the acquirement of gradually increased antibiotic resistance by CONs was reported by many workers. Progressive emergence of these organisms, resistance to various antibiotics was evident from the study of Barber et al. It was observed by Coarse and Williams that S. epidermidis was the most resistant of gram positive cocci showing increased resistant to all antibiotics. They found that about half of the strains from the lesions of hospital patients were resistant to penicillin; streptomycin, tetracycline and 10% of strains were resistant to Cloxacillin. In general they concluded that frequency of antibiotic resistance and the 'width' of resistance spectrum were greater in strains of hospital sources than in those from outside hospitals - [Corse et al (1968)].

Medical management of coagulase negative staphylococci infection is complicated by high incidence of resistance to methicillin reported for this organism. To prevent major therapeutic problem in future due to immense multiresistant strains, use of those life saving antimicrobial weapons should be rationalised and one should be elieant in identifying the resistant strains by screening every
isolates routinely by antibiotic testing, - [Sunderam et al (1982)].

Following the improvement in the control of infections by Pseudomonas aeruginosa other gram negative organisms including Klebsiella species have come into greater prominence as agents of hospital infection in patients with diminished resistance, - [Weil et al 1966, Steinhauer et al 1966].

In a study of bacteriological patterns of hospital infections McNamara et al (1967) found that gram negative bacilli accounted for almost two third (64.5 percent) of hospital acquired diseases. The organism most prevalent were E. Coli, Pseudomonas and Klebsiella pneumoniae in almost equal proportions. - [Weil et al 1966, Steinhauer et al 1966], Klebsiella species in particular have been responsible and the apparent ease with which these organisms can spread, especially to debilitated patients is a matter of concern. - [Price and Sleigh 1970, Hill et al, 1974].

Klebsiella species is an organism of low pathogenicity for healthy subjects but is an important pathogen in patients with poor resistance.
SOURCES OF INFECTION:

The source of infecting organism may be exogenous, from another patient or a member of the hospital staff, or from the inanimate environment in the hospital; or it may be endogenous, from the patient's own flora. Which at the time of infection may include organisms brought into hospital at admission and others acquired. The characteristic of hospital flora are increased pathogenicity, multiple-drug resistance and transmission by endogenous source and cross-infection.

Patients own flora forms a source of gram negative organisms; secondly by the environmental and the hospital personnel, who are carriers. Broadly speaking staphylococcal or streptococcal infections are primarily cross infections where as gram negative bacilli, self infections or environmental infections, - [Laboratory manuel on Hospital infections, 1980].

Chief source of staphylococcal infection is the nasal carriers of staphylococci. Nasal carriage is high in medical and paramedical staff.

Air borne infection and air-borne pathogens in operation theatre and ward is an important source of wound infection.
Blankets, infected beddings, pillows, mattresses are sources of infection.

Hospitals admit infectious patients for treatment and also generate further source of infection. In recent years opportunities for the spread of infection in hospital have been increased by the progressive replacement of multi-purpose wards by special units designed for the treatment of patients with similar complaints, for example, burn wards, urological units and intensive care unit, in which similar procedures are often applied serially to a number of patients by the same staff team, providing numerous opportunities for contact spread of infection.

The immediate source of infective micro organisms in the hospital are patients, persons, fomites, food and arthropods.

Persons who may transmit pathogenic microorganisms within the hospital include all hospital personnel, visitors and patients, whether they have a clinical disease or are asymptomatic carriers.

Environmental Sources:

The current importance of moist objects and fluids as sources of certain gram negative aerobic bacilli is mainly
attributed to

1. The increase use of invasive techniques for diagnosis and treatment.

2. The introduction of complicated medical devices that are difficult or even impossible to clean and decontaminate.

3. The central production of fluid medicaments and weak solutions of 'disinfectants' and their storage under conditions that permit subsequent bacterial multiplication.

Widespread use of antimicrobial agents:

When in hospital, the patients is likely to encounter highly antibiotic resistant strains of a number of bacterial pathogens, including staphylococci, various gram negative aerobic bacilli, coliform bacteria, Pseudomonads and other non-fermentative organisms. About one in four of all patients who enter a hospital receive courses of antibiotics during their stay, but in some wards or departments this proportion is much greater. Profound changes take place in the flora of antibiotic treated patients. Sensitive organisms are often eliminated from the bowel, skin and oropharynx and replaced by resistant organisms that are
usually acquired by cross-infection from other persons in the hospital. Strains of a number of potentially pathogenic organisms, particularly of *Staphylococcus aureus* and various gram negative aerobic bacilli that are resistant to widely used antimicrobial agents tend to accumulate in hospitals.

**PATHOGENS IN THE INANIMATE ENVIRONMENT:**

**Dry Environment Sites:**

Bacteria mainly gram positive that have a considerable resistance to desiccation are often found at dry environmental sites, and in the first hour, *staphylococcus aureus* may halve in numbers while *E. Coil* decreases by 90 % to 99 %. In subsequent hours and days the number of both decrease more slowly. Under normal circumstances airborne bacteria of human origin are almost entirely gram positive, but when very large number of gram negative bacilli are liberated into air, for example by patients with infected burns, sufficient will survive to be detected by air sampling.

The bacterial flora of surfaces in hospital rooms is constantly being added to by organisms from various sources, these die out at different rates. At most times an equilibrium is reached, with gram positive cocci and aerobic
spore bearers predominating. Washing or disinfecting of floors temporarily reduced their numbers, but these return to the original level within few hours [Ayliffe et al, (1966, 1967)].

There is a strong clinical impression that gram positive cocci that cause septic infections have been derived from an animate source.

Clostridium perfringens and clostridium tetani can be found in the dust in most hospital rooms, but they rarely appear to be responsible for infection.

Wet environmental sites:

A great variety of bacterial pathogens both gram positive and gram negative, can persist for several days at wet environmental sites, contaminated from a human source, from there they may be transferred by contact to other patients.

Organisms that multiply at moist situations may have come from a non-human source or from a patient.

Before the bacteriological era Ignat3 Semmelweis thought that puerperal sepsis was probably acquired by patient from sources of contamination in the hospital and transferred
through the hands of the doctors and nurses. After introduction of routine hand rinsing with chlorinated lime before examination of the patients in the Vienna Maternity Hospital, he found that the death from periperal sepsis fell from 12 percent to about 1 percent.

There is a good anecdote quoted by Wangensteen (1976), "When the Letvian surgeons Ernst Von Bergmann came to Vienna, he was visited by his former colleague from Riga. He inquired of the professor, "What is new in surgery?" Bergmann replied "Today we wash our hands before we operate rather than afterwards!"

This explains in brief the story of infections in the earlier part of the 19th century.

Theories of infections and sources date back to Frocostoro, a contemporary of Vesalius. He wrote many articles on medical subjects. But his greater work concerned the nature of contagion. He recognised that contagion could be transmitted through the air, hands, clothing and other objects communicating infection, - [Wangensteen, 1976].

The occurrence of nosocomial infections depends basically on the following factors: microorganisms, the host, the hospital staff, visitors, fellow patients and the
Bourdillon and Colebrook (1946) showed that agitation of contaminated textiles, for example removal of dressing caused a build up and sustained high level of air borne bacteria including wound pathogens, which could be prevented by planum ventilation of the room. This provided comparatively germ free air for the patients. In controlled trials of planum ventilated rooms it was clearly shown that the added infection with staphylococcus pyogenes and Pseudomonas aeruginosa was considerably lower than in the same room with out use of planum ventilation, - [Lowbury, 1954].

In a study on serological types of Pseudomonas aeruginosa - [Lowbury and Fox, 1954], it was found that each ward had, at a time its own predominant serotype of Pseudomonas aeruginosa. Though the dressing room was a common for all wards, the serotype each ward had, were different showing cross infection. Air, floor dust, nurses hands, the bedding and bandages of patients were shown to be demonstrable reservoirs and vectors of these organisms. Streptococci were commonly isolated from the throat of the patients and the nursing staff. At the time of outbreaks Streptococci of Single serotype were isolated indicating the role of cross-infection and hospital environment - [Lowbury, 1954].
Although Staphylococcus produces various types of infections, the nosocomial infections of Staphylococci are more common and so the investigation for the sources of Staphylococci in hospitals are necessary. For surgical wound infections, the potential sources are numerous and the routes more complex, - [Wilson and Miles, 1975]. Opinions differs as the relative importance of the operating room and the ward as the place of infection. Indeed this probably varies from hospital to hospital and at different times in the same hospital, - [Shooter et al, (1958); Bassett et al, (1963)].

Healthy carriage of staphylococci is harmful and it is a potent source of infection [William, (1963)]. Miles et al, (1944) and Barber et al, (1949) showed that the carriage rate amongst hospital staff was much higher than in the general population. There are several other studies showing that the staff working in hospital have a higher carriage rate, - [Ghose et al, (1962); Seth et al, (1973); Talib et al, (1973)].

The nasal carriage rate of pathogenic Staphylococci in the hospital staff in this country has been reported between 20% to 95 % by various workers, - [Chitale, (1956); Sayad et al, (1959); Hardas et al, (1964); Varma et al, (1965)].
Earlier workers have suggested the anterior nares as the essential reservoir, but it has been found that staphylococci get colonized on various parts of the skin, - [Dyke, (1960)]. In an early survey the back of the wrist was found to be the most common site, - [Williams, (1946)].

Organisms under nails and on the hairs are untouched by systemic antibiotics and can infect patients as well as carrier himself, - [Hughes and Davies, (1973)].

The highest rates of carriage are seen in young infants, - [Williams et al, (1966)].

Hurst (1960) is of the opinion that infants due to their heavy staphylococcal load are more often responsible for the contamination air as compared to adults.

Septic skin lesions, dressings, plasters etc. also serve as source of Staphylococcus aureus. The work of Bordillan and his colleagues, demonstrated how in certain circumstances the removal of pus soaked dressings or plaster casts from wounds could disperse large number of bacteria in air, - [Bourdillan et al, (1948); Girdlestone and Bourdillan (1951)].

Skin lesions have been found to be a source of secondary
cases of infection in medical wards, - [Hare and Cook, (1961); Alder and Gillespie, (1964)].

Staphylococci are also introduced into the ward by patients visitors who happen to be suffering from sepsis.

Sources of staphylococci in the environment are the beds, blankets of patients, dust from floors, ceilings, walls, clothing etc. Also the contamination of gowns and uniforms worn in burn unit and the transfer of patient’s Staphylococci by means of nurses uniforms was reported, - [Hamberaeus, (1973)].

The importance of contamination of floor dust and blankets with staphylococcus aureus cannot be ignored. Experimental work has shown that the organisms do not loose their virulence fourteen days after being shed, - [Williams et al, (1966)].

Further Caplan, (1962) revealed an appropriate reduction in the degree of environmental contamination by disinfecting blankets.

Staphylococci in dust have greater powers of survival than have gram negative bacilli, - [Lowbury and Fox, (1953); McDude and Hall, (1963, 1964)]. Contaminated dust may be
suspended in the air by activities such as bed making and dry sweeping of floors, - [Ayliffe and Barber, (1963)].

Gram negative bacilli were different in many ways than the alimentary flora and were clearly acquired from cross infection, - [Lowbury, (1960)]. Pseudomonas aeruginosa was a common pathogen, but it was isolated from only 3 percent cases of normal stool, - [Lowbury and Fox, (1954)]. In some reports the incidence of Pseudomonas aeruginosa has been found between 6-12 percent, - [Shooter et al, (1966); Stoodley and Thom, (1970)]. In patients of diarrhoea a higher percentage (21 percent) has been observed by Stoodley and Thom (1970). After admission in hospital Shooter et al. (1966) found an incidence of Pseudomonas aeruginosa carriage up to 20 percent. In India the incidence of intestinal carriage has been found to be rather low, - [Shrivastava et al, (1969)].

Hambraeus (1973) made studies regarding the dispersal of Staphylococcus aureus from burns patients, relation between nasal carriage by the staff and exposure to air born Staphylococcus aureus and the transfer of the carrying particles within the ward. Nasal carriage rate by the staff correlated with the air count. The transfer of staphylococcus within the ward was 6 to 20 times more than that would have been expected by air movements only.
Wormald (1970) observed that the rate of infection did not change with change of place. Streptococcal infections increased due to higher communicability, Pseudomonas aeruginosa was found rather late. About one third of cases could be traced to one source, that was one patient. Basin out flow in new premises showed Pseudomonas aeruginosa serotypes, not found on patients in the unit.

Kominos et al, (1972) found cross infection among patients and showed several serotypes at one time. Cultures from hands of Nurses yielded same types of Pseudomonas aeruginosa. Direct handling of the patients by nurses was found to be the principle mode of transmission.

Hambraeus (1977) found that staff Nurses were carriers of staphylococcus aureus of phage group III, which was found in most of the cases. Some of the cases also had same phage types weeks before actual infection indicating direct transfer and aerial transfer. Bed room air settle plate count was 1800 colonies per square meter. But corridor count was low at that time.

Smith et al, (1973) isolated hospital strains of staphylococcus aureus from patients skin and nares after admission. But immediately after admission the hospital strains were not isolated from the same patients.
Alexander (1971) stresses the importance of endogenous sources of infection, patients own gastro intestinal tract, respiratory tract and genito urinary tract as the source of infection. Hummel (1970) tried reverse isolation methods and found patients own flora to be an important source of infection, along with air borne infection. Munster (1971), Pruitt (1970), Skornic (1970) could locate the source of infection from lungs and stressed the role of lungs in causing endogenous infection. Bull (1971) reviewed the causes of mortality due to burns and found that the experience in 1946 to 1964 hardly changed. The rate of infection and sources depended mostly on depth, percentage of burns and hospital environment.

Sengupta et al (1975) formed post operative wound sepsis to be 68 percent a rather high incidence, but attributed this to local causes. Previous records ranged from 0.8 to 40.9 percent [Dineen and Pierce, 1958; Jaffrey and Skloroff, 1958 Srivastava et al 1969]. Usha Udagaonkar et al (1985) found post operative wound sepsis to be 30.43 percent; and suggested that in staphylococcal infections, operation theater was the possible source of infection; and nasal carriage rate of staphylococcus aureus in hospital personnel was 43.47 percent.

There was a steady rise of gram negative infections
especially due to Pseudomonas aeruginosa when the wounds were followed from first week to third week by Sengupta et al (1975) a. Now Pseudomonas aeruginosa has become the most important organisms especially in burns. Pseudomonas aeruginosa appears rather late in hospital stay, if not controlled it invades blood stream and poses great threat [Pierson and Feller, 1970; Sengupta, et al 1972].

Pseudomonas aeruginosa infection is also seen in dialysis units (Wagnild et al 1977), urological wards (Foreman 1966). Phillips (1966) found other sources of infection in the form of contaminated lignocaine jelly used for tracheal catheters. Established inanimate sources include physiological saline, soaps, antiseptics solutions, eye drops, creams and jellies [Editorial B.M.J. 1967].

Steroid cream was found to be contaminated by Noble and Savin (1960). MacLeod (1958), found that commonly used articles in the hospital that contain standing water or retain moisture such as urine bottles and bed pans, were frequently contaminated with Pseudomonas aeruginosa.

Surgical instruments have been found to transmit the organisms if inadequately sterilized [Moore and Foreman, 1966]. Sputum and skin carriers may cause either auto infection [Kamegi et al 1973, Shooter et al, 1966, Sutter et
al 1966]. Or cross infection in wards [Subramanian et al 1974].

Depending on the method of transmission and host factors any organism can be a pathogen.

ROUTES OF TRANSMISSION:

Microorganisms can be transmitted within the hospital environment by any of four routes, contact, air, a common vehicle or a vector. More than one route may be operational in the transmission of a pathogen during a single episode, and the same pathogen can be transmitted by different routes on different occasions.

Aerial transfer:

Organisms of human origin reach the air either as droplets form respiratory tract or as dry particles from the body surface. The mouth is the chief source of respiratory droplets. The size of the droplet particles determines the range of their dispersion and the depth of their penetration into the respiratory tract.

Dry infective particles generated from the body surface of size 15-25 μ sphere, stay in the air sufficiently long for
infection to be transmitted from one end of a hospital ward to other.

The constant inhalation of Staphylococcus aureus in ward air is an important determinant of nasal colonization by antibiotic resistant strains.

Organisms from non-human sources are most often disseminated aerially when they are liberated as liquid droplets generated mechanically from fluids. The organisms responsible are generally gram negative bacilli that have multiplied in the fluid. Respiratory apparatus that delivers humidified air is responsible for many of these infections.

Contact Transfer:

Contact transmission can occur by the direct, indirect, or droplet route. Direct contact transmission refers to spread from the source to a recipient directly without an intermediate, for example, the faecal-oral spread of hepatitis via contaminated hands. Staphylococci can also be transmitted by the direct contact route.

Indirect spread indicates that the organisms are transferred from the source to a recipient via an intermediate object.
Patient to patient and health personnel to patients transfer of infection is usually indirect, for example, the well known spread of gram negative organisms on the hands of hospital personnel. Transmission usually takes place within a few hours, not only gram positive cocci but also gram negative aerobic bacilli will be transferred, particularly from heavily contaminated objects.

The outer coating of staff members may become contaminated externally by contact with patients or their bedding. Contact transfer to other patients is facilitated when parts of the garment become wet. The contact infections from 'Wet' inanimate sources are usually caused by nutritionally unexacting gram negative bacilli, occurring frequently in the modern hospital.

Droplet spread diseases are those whose infectious agents are transmitted from the source in the form of droplets to a recipient who is within several feet of the source, for example, streptococcal disease and in influenza. Droplets never become independently airborne.

Common vehicle means that multiple cases of a disease are related to the same source of infection. Some etiologic agents can be transmitted by more than one route. For example, staphylococci can be transmitted by contact, air,
or a common vehicle, though the most common route is direct contact.

INFECTION FROM THE HANDS:

The resident flora of the skin of the hand is primary source and its transient flora an intermediate reservoir of infection. The relevant microorganisms can readily be demonstrated on the hands of staff and may easily be transferred to the skin of others by brief contact, - [Marples and Towers, 1979]. The importance of such contact in colonization and infection has been demonstrated with staphylococcal disease in a nursery for new born babies, - [Mortimer et al, 1962].

The most important microorganisms spread by hand contact are Staphylococcus aureus, Streptococcus pyogenes and gram negative bacilli such as Klebsiella and Serratia Spp. and many other hospital pathogens including Candida albicans, - [Burnie, 1986]. Casewell and Desai (1983) demonstrated that strains of gram negative bacilli particularly Klebsiella and Serratia that has caused outbreaks in which infection was spread by staff from patient to patient.
HOST SUSCEPTIBILITY:

A considerable proportion of patients are already prone to infection when they enter hospital, and others are rendered most susceptible to it by procedures or medication that they undergo after admission.

Significant factors in the host include age, immune status, type of underlying disease and effects of diagnostic and therapeutic procedures. For example, the extremes of life infancy and old age are associated with a decreased resistance to infection. Patients with chronic disease, such as certain types of cancer, leukemia, diabetes mellitus, lymphoma or nephrosis may be more susceptible to nosocomial infection than other patients. Additional host factors are nutritional status, alcoholism, lowered local resistance and hypogammaglobulinemia.

Local:

Pre-existing wounds or other surface lesions may render patients susceptible to invasion by microorganisms. Surgical operations and variety of other traumatic procedures used for diagnosis or treatment provide further opportunities for organisms to enter the tissues.
General:

The components of the body's general defences against microorganisms, include the activities of phagocytes, humoral immunity and cell mediated immunity. One or more of these defence mechanisms are impaired in a variety of diseases, as also by treatment with certain drugs and by irradiation, systemic disease in immunodeficient patient may be a consequence of invasion by organism.

Susceptibility in newborn babies:

Newborn babies are somewhat immunodeficient, in prematurely born, however, the deficiency may be profound, and persists until around the date of normal birth. It manifests itself mainly in increased susceptibility to infection by various gram negative aerobic bacilli especially E. Coli and Pseudomonas aeruginosa and to Staphylococcus aureus and Group-B-streptococci.

Several abnormalities in the immunity mechanisms of newborn and particularly of premature infants have been described which include - 1) low serum levels of various components of complement 2) defective function of granulocytes, notably decreased powers of migration, chemotaxis and intracellular killing. 3) Absence of IgM, which does not cross the
placenta and is said to be important in preventing invasion by E. coli and similar organisms.

Nosocomial infections have been increasing for the past two decades. Five to fifteen percent of the patient admitted in the hospital acquire these infections and this is mainly due to presence of pathogenic and potentially pathogenic microorganisms in high concentration in the hospital environment. These infections are especially due to multiple drug-resistant organisms which have emerged as a result of over indulgence of antibacterial agents in treating the patients.

Range of bacteria causing nosocomial infection has been changing over the years. During Second World War Streptococci were the main organisms. Afterwards Staphylococci took over till around 1960, but in the last two decades gram negative rods have replaced gram positive cocci. This changing pattern is mainly due to, use of broad spectrum antibiotics, immunosuppressive drugs and prolonged surgical procedures.

INCIDENCE OF HOSPITAL INFECTION:

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in the hospital acquire these infections and this is mainly due to presence of pathogenic and potentially pathogenic microorganisms in high concentration in the hospital environment, instrumental procedures on the patients and lowered resistance in some cases. These infections are especially due to multiple drug resistant organisms which have emerged as a result of over indulgence of antibacterial agents. in treating the patients.

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The incidence of hospital infection of all kinds in India varies from 8 per cent to 33 percent, as against 3 percent to 15 percent in U.S.A., and that is mostly due to staphylococci and Pseudomonas aeruginosa. The incidence of Pseudomonas aeruginosa infection is about 5 percent to 30 percent in India as against 8 to 10 per cent in U.S.A. of the total infection reports published, - [Wahba 1977; Shriniwas 1977 b]
Septic infection due to Staphylococcus aureus is world wide in distribution and is of particular importance among hospital patients. Lack of knowledge about the virulence of staphylococcus aureus for man under natural conditions greatly impede understanding of the role of the organisms as a cause of septic disease. Thirty to fifty percent of healthy individuals carry the organism on the body surface but clinical disease is relatively infrequent [Sengupta et al; 1982] and serious illness is rare in normal person. Yet epidemics of Staphylococcal sepsis is relatively frequent complication of surgical operations.

There is a general impression that post operative wound sepsis is becoming more common. This belief is probably based on reports of outbreaks of exceptional severity, with sepsis rates between 10 \% to 37 \%, [Public Health Laboratory Service Report (1960)]. Review of Indian literature shows that there is awareness of this problem but few reports are available, Chawala et al (1964) at All India Institute of Medical Sciences, New Delhi, found the sepsis rate to be 32\%, Wasek et al (1965), at S.S.K.M. Hosp. Calcutta have reported 23.4 \% rate, Talwar et al (1969) at S.M.S. Medical College Hospital, Jaipur found 20 \% infection rate, while Srivastava et al (1969) at S. N. Medical College, Agra reported an overall rate of surgical infection to be 20.19\%, Agarwal (1972) at S. N. Medical College Hospital, Agra have
recorded the rate of 20 %, Subramanian et al (1973) at A.I.I.M.S. Delhi, reported an overall surgical rate to be 24.76 %, Sengupta et al (1977) at Aurangabad Medical College Hospital, recorded a high rate of 68 %, At Jhansi Vinod Kumar et al (1979) reported a rate of 29 %. Agarwal et al (1980) at Lucknow found the infection rate to be 44%, Usha Udgaonkar and Bhavthankar (1985) found the infection rate 30.43 %.

Infection rate shown in Western literature has always been much less than shown at home.

In Britain, Clarke (1957) reported a sepsis rate of 13.6 %. Jeffery and Sklaroff (1958) found that sepsis developed in 26.1 % of clean operation and this was serious in 9.8 %, Burnett et al (1958) recorded a sepsis rate of 7.6 %, Dineen and Pearce (1958) have reported the incidence of wound infection to be 0.8 % to 1.4 % in a ten years study. Public Health Laboratory Service Report (1960), in a pilot survey of twelve hospitals on 3276 operations in England and Wales recorded it to be 2.5 % to 27 % with a mean of 9.7 %. Forbes (1961) recorded it to be 6.7 % at Baltimore, Thoburn et al (1968) reported the infection rate to be 2.3 %, Cruse and Poord (1980) recorded it to be 1.5 % at Foothills hospital, Canada.
Thus it is seen that in Indian series also the infection rate various from 5.1 % to 68 %. It is suggested that incidence of infection rate various depending upon the case material, hospital environment. Therefore comparison of infection rate in different hospitals in different parts of the world is not very useful, instead a periodic survey in the same hospital is more informative [Barret et al (1968)].

Besides this, wound sepsis is not equally common in all branches of surgery. Subramanian et al (1973) showed highest infection rate in bone operations and abdomino-perineal surgery and lowest infection rate was observed in head and neck surgery. This they pointed out could be due to rich blood supply and early mobilization of body defences.

The incidence of serious infections in the burned patient varies with the size of the burn. With current methods of topical chemotherapy, serious infections are not expected in otherwise healthy patients with burn involving less than 30 percent of the total body surface area. A progressive increase in the incidence of serious infection, however, is seen as the size of the burn increases.

The important relationship of the age of the patients and the size of the burn to the risk of infection has been emphasized by many authors and was especially well
documented by Thomsen. Suffice it to say that the incidence of infection increases with age (dramatically so in patients over the age of 60) and with size of burn (infection affects nearly all patients with more than 40 percent burn). - [Bennett and Brachman, (1979)].

The greater incidence of infection in recent years is giving rise to growing concern. The fact that these infections still occur and that they are on the increase can be attributed to the emergence of resistant strains of various organisms. This has been essentially due to injudicious use of antibiotics and greater reliance on antibiotics rather than the use of time honoured methods of a sepsis. This therefore, creating a worse problem than those in pre-antibiotic era - [Bhargawa et al, (1966)]. The predominance of antibiotic resistant Staphylococcus aureus a cause of open wound and post operative infection has been reported by many workers, - [Lowbury, (1955); Shooter et al, (1957); Shooter et al, (1958)]. Such infections have actually increased with increased use of antibiotics perhaps through selections of more virulent and more communicable strains through widened scope of surgery and through deficiencies in aseptic technique.

Advent of powerful antibiotics in 1950’s have lulled the surgeons into complacency and the procedure of practice of
asepsis inside operation theatre called as operation theatre ritual is an absolute procedure. Quick emergence of resistant bacteria against most powerful looking antibiotics have given these persons a shock and it is an established fact that we have a hoard of pathogenic bacteria at present in parallal with extensive use of chemotheraputic agents, resistant evey known antibiotic. Thus, dependence of antibiotic for destroying the bacteria in wound is fast becoming a false hope and we are again turning back from antisepsis to asepsis, - [Agarwal, (1972)].

The increasing prevalence of antibiotic resistant organisms in hospitals and a rising infection rate despite the antibiotic prophylaxis suggested that the two problems might be related, and the antibiotics by supression of the sensitive strains, help in selective survival and multiplication of resistant strains, - [Wasek et al, (1965)].

Of late contagious transferable drug resistance of bacteria to multiple drugs has assumed importance. There is evidence that contagious resistance is common and wide spread, is receiving the attention of medical and public health investigators.

The readily transmissible R plasmids are a source of
anxiety. Since they are selected by antibiotics, they are higher in developing countries. Most of the organisms are resistant because of plasmids they carry. These are often unstable and may be lost particularly if there is no antibiotic selective pressure. Instead of concentrating on modifying antibiotics it may be advantageous in long term to look for the agents that will eliminate plasmids from bacterial cell and prevent their transmission from organism to organism, [Ayliffe, (1979)]. The problem has a direct bearing in hospital infection.

Urinary tract infections are of major importance not only because they are common but also because they may be the source of invasion of bacteria or their products into the bloodstream. This may lead to renal damage, disseminated infection, the syndrome of gram negative sepsis, and considerable morbidity. The urinary tract is the most common site of infection that originates during hospitalization; and urinary tract infection constitute the largest single cause of gram negative bacterial sepsis in hospitals, - [Calvin Kunin, (1979)].

Urinary tract infections in hospitalized patients arise most commonly from instrumentation of the tract and to a lesser extent from bloodborne infection from a distant site. In addition, they may occur at times as a complication of
surgical procedures or from lesions in adjacent structures that produce fistulas from the vagina, rectum, or other structures adjacent to the bladder. The most common cause of these infections, however, is the urinary catheter, particularly when the catheter remains in place for prolonged periods.

The frequency of nosocomial urinary tract infection depends almost entirely on the frequency of conditions in patients that lead to instrumentation of the urinary tract and the policies and procedures of the medical care system in the hospital. Composite data from the National Nosocomial infection study indicate that about 40 percent of the nosocomial infections reported in 1971 through 1974 involved urinary tract and that the overall rate of such infections was 1.4 per 100 hospitals discharges. It can be estimated that at least two-thirds of these infections occurred in patients with catheterization or instrumentation of the urinary tract. The specific rate for catheter associated urinary tract infections was about 1 per 100 discharges and these accounted for about 30 percent of all nosocomial infection. Thus, the urinary tract is the site most commonly involved in nosocomial infections, - [Calvin Kunin, (1979)].

Most urinary tract infection are due to the gram negative
aerobic bacilli found in the gut. The most common organisms found in uncomplicated infections are Enterobacteriaceae. Of these, Escherichia Coli is the most common and account for roughly 80 percent of infections; followed by Klebsiella, proteus and Enterobacter. Pseudomonas, Staphylococci and group-D streptococci account for about 5 to 12 % of the remaining organisms in uncomplicated infection. In contrast, patients with so called complicated or surgical urinary tract infection who have been treated with multiple courses of anti-microbial agents tend to be infected less often with E. coli and more often with the other organisms. Infections with Pseudomonas indole positive proteus species and entrobacter are generally the most difficult to treat because of the refractoriness of these organisms to the commonly used antimicrobial agents and because such infections frequently are found in association with abnormal voiding mechanisms that interfere with host defences to infection, - [Calvin Kunin, (1979)].

Organisms such as Serratia marcescens, Acinetobacter and Candida albicans may produce disease in patients subjected to instrumentation, particularly with indwelling urinary catheters. - [Calvin Kunin, (1979)].

The distribution of bacteria that colonize catheterized patients will vary depending on the selective pressure
exerted by the use of antimicrobials and on the duration of catheterization. In general, E. coli remains the most common organism, but it will be replaced by more resistant bacteria and by yeasts when antibiotics are heavily used, - [Calvin Kunin, (1979)].

Striking changes have occurred in the medical and surgical care provided for neonates during the last decade and half. While standards have continued to improve for infants cared for in term newborn nurseries, the quality and sophistication of care provided for the premature infant and the sick newborn in neonatal intensive care units have improved many fold. These advances however, have been accompanied by significant risks of nosocomial infection. The center for Disease control National Nosocomial Infections Study (NNIS) have reported a combined nosocomial rate of 1.7 percent for all infants discharged from the newborn nursery service. Approximately 70 percent of the infants are discharged from community or community-teaching hospitals, with a reported neonatal nosocomial infection rate of 0.9 to 1.1 percent. In contrast, the NICUs of two university medical centers have recently reported nosocomial infection rates of about 24 percent, with over 15 percent of the infants in one NICU developing one or more infections. - [James Allen and Thomas Oliver, 1979].
About 35 percent of the infections reported by the NNIS hospitals were caused by Staphylococcus aureus, most of which were superficial cutaneous or eye infections. Escherichia Coli caused over 12 percent of the reported infections, including most of the urinary tract infections and gastrointestinal infections. E. Coli or group B Streptococcus was isolated from almost 32 percent of neonatal meningitis cases. [James Allen and Thomas Oliver 1979]. Pseudomonas species, Klebsisllla species and Staphylococcus aureus caused most of the pneumonia and other lower respiratory tract infections in this series. Group-B streptococcus caused only 2.5 percent of the nursery associated infections. Staphylococcal infections are still common in neonates, most infections during the 1970s were less serious than those occurring during the pandemic years of the mid 1950s through the early 1960s, when phage group-I Staphylococcus aureus, particularly phage type 80/81, was prevalent. Many Staphylococcal infections currently seen in neonates are caused by phage group-II Staphylococci.

Incidence of septicaemia is higher among the neonates due to deficiency in their inherent protective mechanism humoral and cellular immunity. It extends between 1 in 500 to 1 in 1600 live births with significant mortality rate ranging from 24-58 percent [Sharma et al 1987]. MacCracken and Shinfield (1966) studied the neonatal Septicaemia over a
decade and reported that 1) There was great increase of gram negative Septicaemia over last five years, 2) Commonest gram Positive organisms was staphylococcus aureus and Gram negative organisms was E. Coli.

Bhakoo et al (1968) studied prematures with septicaemia and concluded that gram negative bacetria i.e. E. Coli, Proteus mirabilis. Streptococcus faecalis dominated infection in first two months of life. Major factor in high mortality rate in gram negative Septicaemia is probably the emergence of drug resistant strains of bacteria.

Somu et al (1976) reported that out of 725, 554 blood cultures were positive. Gram positive, coliform, gram negative organisms were present in 35.7, 36.9 and 64.3 percent respectively with ratio of gram positive to Gram negative septicaemia as 1:2. Staphylococcus pyogenes was the commonest in gram positive group, E. Coli was commonest in Gram negative group, followed by Pseudomonas and Proteus. Enterococci were also an important cause of septicaemia. Extensive investigation carried out in England and Wales in recent years (1979-80) shows overall incidence 9.2 % (1671 patients amongst 18193 pts) with 61 % of infections were certain 25 % were probable and 14 % as doubtful - [Meers P. D., Ayliffe GAJ et al 1981, Journal of hospital infection 2 (suppli) : 1].
Another large study in U.S.A. showed total incidence of 5.7% amongst 169526 admissions in which urinary tract infections accounted for 42 %, surgical wounds 23.8 %, pneumonia 10.5% and bacterimia 4.8 %. - [Haley et al(1985)].

Similar surveys in the U.S.A and Europe and recent World Health Organisation Surveys observed prevalence of about 10 % have been fairly constant in recent years.
NOSOCOMIAL INFECTIONS IN NEWBORN

Striking changes have occurred in the medical and surgical care provided for neonates during the last decade and a half. While standards have continued to improve for infants cared for in term newborn nurseries, the quality and sophistication of care provided for the premature infant and the sick newborn in neonatal intensive care unit (NICU) have improved manyfold. These advances, however, have been accompanied by significant risks of nosocomial infection.

Neonates are particularly susceptible to infection for several reasons.

The normal foetus is sterile until shortly before birth, as long as amniotic membrane remains intact. After birth the newborn infant who has essentially no microbial flora is immediately exposed to the microbial world and inoculated with millions of bacteria and other microbes. [Bhatia et al. 1988; Rotimi and Duerden 1981].

After birth, the infant is at the mercy of his environment. He may acquire infection from the hands of his attendants, from apparatus used in his resuscitation or general care, particularly from the humidifying units of such equipment,
from the air or from his feeds, [Davis 1971].

Bacterial colonization is not synonymous with infection but the higher the colonization rate with potential pathogens, the greater is the risk of frank clinical infection; [Barr D.G.D; 1974].

When infants are colonized heavily at sites other than rectum, they are more likely to have bacterial infections than if lightly or insignificantly colonized, [Davis 1971].

The skin and the mucus membranes of the body are directly accessible to the external environment, soon after birth an infant is born these surfaces become populated by a characteristic flora. The skin becomes contaminated during passage through the birth canal; the mucus membranes may be sterile at birth but becomes contaminated within hours of the many organisms, that reach these surfaces. Only those that are particularly suited to grow in such environments become established. These constitute the "normal flora", which remains remarkably constant. The bacteria of the normal flora do not cause disease unless accidently involved into normally protected regions of the human body or as a result of physiological changes within the host. For example, a radical change in diet or infection with a virus may alter the conditions within the body in such a fashion
that a member of the normal flora can become pathogenic, [Stainer et. al. 1977].

MACHANISMS OF COLONISATION

Bacterial colonisation of body surfaces requires first that the organisms become firmly attached and second proliferate under existing conditions. [Gibbons; 1977].

Normal microbial associates and pathogenic microorganisms alike must overcome various obstacles to colonise animal epithelial surfaces. These obstacles are -

- unidirectional flow of fluids over epithelial surfaces.
- mucociliary clearance systems.
- Epithelial cell turnover.
- local immuno systems.
- receptor analogues.
- non-specific host antimicrobial agents.
- Variations in PH or Oxidation - reduction potential.
- Microbial competitors.

The continous undirectional flow of material through most visceral channels whether from peristaltic activity, gravitational forces or mucocilliary clearance systems dictates that organisms within channels such as the
gastrointestinal tract will be swept away with other intraluminal materials, if they are not attached to underlying epithelial surfaces. Adherence to epithelial cells not only prevents expulsion of the microorganisms but may also stimulate their growth, [Marshall and Britton; 1980].

The adhesion of microorganisms to host mucosal surfaces is necessary prerequisite for successful microbial colonisation and infection, [Gibbons, 1977].

Bacterial adherence is remarkably cell specific. In the human oropharynx, bacteria with the strongest affinity for particular epithelial cell types in vitro are the same microorganisms colonising the cell types in vivo. Nevertheless, microorganisms with a limited ability to colonise particular host surfaces directly may do so in vivo, through a process of interbacterial aggregation. [Wittenberger et. al.; (1977)], have shown that glycosyltransferase produced by streptococcus salivarius greatly facilitates the adhesion of an otherwise poorly adherent bacterium to smooth surfaces.

Gibbons, (1977), pointed out that the apparent preference of particular bacteria for certain tissues over others (tissuetropism). For example, streptococcus pyogenes is
virtually limited to the nasopharynx and skin of humans; whereas E. Coli organisms are the most common cause of genitourinary tract rarely colonised the nasopharyngeal cavity, [Beachey 1981].

There are exceptions, of course, as pointed out by Johanson et. al. (1979), hospitalized patients who have underlying diseases often become colonised in their upper respiratory tracts by E. Coli and other Gram negative bacteria.

The idea of the specificity of the interaction between bacteria and host tissue is further supported by the species specificity of certain bacetrial infections. For example, gonococcal infections are limited to humans, group A streptococcal infections to humans.

The striking cell-specificity of bacteria appears to be mediated by the attraction of species of specific microbial adhesins (also called lectins) to complementary host cell-specific receptors (Beachey 1981). Adhesins are microbial surface antigens that frequently exist in the form of filamentous projections designated as pilli or fimbriae. [Duguid and Old, 1980].

Albumin like proteins or glycoproteins present in the membranes of epithelial cells appear to be the receptors for
same gram positive cocci, [Beachey; 1981].

A primary function of the antibody forming system of mucosal surfaces is to discourage adherence of unwelcome microorganisms through the production of specific secretory IgA, [Williams and Gibbons 1972]. Finally, the resident microorganisms themselves pose a barrier to colonisation, [Mackowick; 1982].

BACTERIAL COLONISATION IN NEWBORN

As a foetus develops within the uterus it is effectively protected from most environmental influences, including infectious agents, by placenta and the amniotic fluid, [Finegold, S.M. and Baron E. J.; 1986].

After birth, the neonates rapidly colonised by acquisition of initial flora and environmental flora; [Davis, 1971].

Initial colonization is fortuitous, depending on the first suitable organism to arrive at a particular site as well as on such factors as the route of delivery; the amount of vernix caseosa present at birth [Noble; 1976].

The acquisition of certain bacteria after birth by the upper respiratory and gastrointestinal physiology. Such
colonisation is usually beneficial but, in neonatal period the dividing line between normal and invasive flora may be narrow.

Witkowski (1935), found that the infants mouth becomes colonised shortly after birth by Staphylococci, Streptococci, lactobacilli and coliform bacilli. Within a few days the flora was largely of adults type, except for Streptococci which appear only after dentition. He found that the maternal vagina and upper respiratory tract was the source of the colonising organisms.

Cunliffe (1949) observed a rise in the incidence of staphylococcus aureus in the nose of infants from 8.6 % on the first day to 76 % on the forth day and about 100 % at the end of first week. He also found that the staphylococci of the same phage type (47B) isolated from infant’s noses, wrists of nurses, were responsible for outbreak of neonatal impetigo. From his study he concluded that the infants in hospital are not usually infected by their mothers but more commonly indirectly from other babies, with the hands or clothing of attendants and the air as probable vehicle of infection.

Sarkany and Gaylarde (1967), found coagulase negative Staphylococci and diptheroids on the skin of newborn
immediately after birth. The same organisms were cultured by them from the maternal vagina just before delivery. They found that the skin of infants born by caesarean section was sterile. So they concluded that the birth canal was the source of colonizing organisms.

Whether the delivery of mother is made by the vaginal or abdominal route however, cultures of the infant’s nose, throat, umbilicus and rectum made immediately after birth are sterile in the majority, over the first days of life, there is steady increase in colonisation at these sites, and it occurs most rapidly in the rectum. Several workers are agreed that the umbilicus becomes colonised more quickly than nose and certain areas of the skin such as perineum and axillae may become colonised more quickly than others, [Hurst, 1960; Smith and Bloomfield, 1950; Torrey and Reese, 1945].

Mathur et. al. (1967) studied bacterial flora in throats of neonates in early neonatal life and found Streptococcus viridans, staphylococcus albus, staphylococcus aureus, N. Catarrhalis, Kleb. aerogenes, Micrococcus tetragenes, Pseudomonas Pyocyanea, Pneumococcus, Proteus and Diphtheriods. They suggested that in view of various microbes present in throats of newborns it may safely be called as one of the reservoir of infections. They also
found in majority of cases same bacteria present in the throat of the neonates, were found in the throat of mothers.

Bhatia et. al. (1988), studied bacterial flora of newborns at birth and after 72 hours of age. They found, at birth, most of newborns were sterile and 72 hours of age none of infants were found to be sterile. The Gram positive organisms especially Staph. epidermidis were predominant at birth. Whereas, after 72 hours they were replaced by Gram negative rods. The umbilicus and rectum were found to be more heavily colonised, compared to nose, ear and throat. The Gram-negative pathogens like E. Coli and klebsiella were also frequently isolated from nose and throat at 72 hours. They also found that Gram-negative growths were more common in special care nursery babies compared to rooming in babies.

Bhatia et. al. (1989), studied bacterial flora in newborns and their mothers. They found that the organisms colonising at various anatomic sites in newborns were causative agents for septicaemia. The same organisms were also found in their mothers.

The acquisition of aerobic Gram-negative bacilli after birth is rapid. Innoculation of infants delivered by vaginal route is significantly more than those delivered by
abdominal route. The gestational age, type of delivery, type of feeding are associated with significantly different colonisation patterns of anaerobic and aerobic bacteria in the first week after birth.

ACQUISITION OF ENVIRONMENTAL FLORA:

The problem of staphylococcal illness, which many hospitals faced in the 1950's and which may be no means confined to their maternity units, has gradually resulted in a better understanding of the acquisition and dispersal of this organism by patients and staff [Davies 1971].

Newborn infants and adults are heavy dispersers of staphylococci from their skin into the air; [Echenwald et. al. (1960), Hare and Thomas, (1956)]. The perineum has been shown to be a particularly heavily contaminated site.

According to Davis and Noble (1962), the bacteria are carried on epithelial scales which become detached by movement, friction of clothing, or bedding, and dispersed into environment.

The organisms carried on shed skin scales survive for considerable periods in dust, but airborne contamination is only partly responsible for floor deposition, and other
sources such as shoes and trolley wheels are thought to be equally important, [Ayliffe et. al. 1967].

It seems almost certain that Staphylococci are transferred to the newborn infant in the first place by the hands of his attendants, [Hurst, 1960; Cunliffe, 1949; Wolinsky et. al; 1960].

Gram negative bacilli are found in dust much frequently than Gram positive organisms, but a whole host of them flourish in water and form a particular hazards for the humidifying units of apparatus from where they may be transmitted to infants, [Sever, J. L. 1959].

The 'Waterbugs' have been the cause of many nursery epidemics. Perhaps the most notorious opportunist of them all, and the most frequently reported is Pseudomonas aeruginosa. It has isolated from incubators, [Barrie D. (1965)]; suction and resuscitation apparatus, [Bassett et. al. 1965], eye drops, hand lotions and other disinfectant fluids, [Morse and Schonbeck, 1968], and Sinks, Washbasins and their taps [Fierer et. al. 1967]. From the latter trio it is almost impossible to eradicate, [Kohn, J. 1967].

Though the hands may play a secondary role where the 'water bugs' are concerned, they no doubt transmit the
enterobacteriae back to the infants; [Balassanian and Wolinsky, 1968], as seems likely in adult patients; so that the acquisition of E. coli for instance may be the same as the Staphylococci, though their environmental habitats differ, [Davies P. A. 1971]. Colonization of the throat with Gram negative organisms is significantly less likely in healthy mature infants nursed with their mothers, than in ill and low birth weight infants in a special nursery, [Farmer K. 1968; Davies et. al. 1970]; and sick infants in such nurseries are more likely to be colonised with Gram negative bacteria than well infants, whether mature and normal weight, or of low birth weight, [Davies et. al. 1970].

*Klebsiella aerogenes* is becoming increasing importance in hospital infections and outbreaks of infection in neonates associated with increased colonisation have been reported; [Adler et. al. 1970; Hill et. al. 1974].

Hospital gowns have been routinely used in the nursery and post partum areas as a method of preventing colonization and infection in newborn, [Colney and Donowitz 1986;].

According to Barr (1974), bacterial colonization is not synonymous with infection but the higher the colonization rate with potential pathogens, the greater is the risk of
frank clinical infection. According to Davies (1971), when infants are heavily colonised at sites other than the rectum, they are more likely to have bacterial infection than lightly or insignificantly colonised. This hold true whether such colonization is Staphylococcal or Gram negative, - [Gillespie et. al. 1958; Davies et. al. 1970].

Bacterial infections are more likely to occur in those moderately or heavily colonised with Gram-negative bacilli, than Gram-positive or mixed flora, [Davies et. al. 1970].

INFECTIONS OF THE NEWBORN

General Consideration :

Infections are a frequent and important cause of morbidity and mortality in the neonatal period. As many as 2 % of fetuses are infected in utero and upto 10 % of infants are infected during delivery or the 1st month of life.

Several general factors contribute to the frequency and severity of neonatal infections and emphasize the importance of early and accurate diagnosis and appropriate therapy. First, a variety of organisms, including bacteria, viruses, fungi, protozoa, chlamydia and mycoplasma, are etiologic agents. Second, the presentng clinical features, in the
neonates with infection may subtle and may mimic the feature of other common diseases during this period. As a result, the diagnosis of infection is often missed; or delayed till the process has become widespread.

Third, some routine laboratory tests available to aid in the diagnosis of infection appears to be imprecise or do not provide the rapid results needed. Fourth, the host resistance mechanisms present in the newborn infant, particularly the sick premature infant, may be immature and easily overcome by invading microorganisms. Infections therefore, may become fulminant and cause death within a few hours or days; despite appropriate and intensive antimicrobial therapy. Fifth, many bacterial infections are caused by organisms relatively resistant to antibiotics particularly the Gram-negative enteric bacilli. These infections are difficult to treat, and the dose of antibiotics that can safely be used is limited by toxic side effects.

Frequency and Specific Predisposing Factors:

A variety of maternal and neonatal factors are associated with increased frequency or severity of infections. Mothers susceptible to certain pathogens (e.g. rubella or cytomegalovirus) may acquire an acute primary infection and
transmit the microorganism transplacentally to the foetus. On the other hand, mothers who are immune (e.g. to measles or a particular strain of group B streptococcus) have antibody in their serum that can pass transplacentally and provide passive protection for the neonate against infection after birth. During epidemic periods, the incidence of maternal and congenital disease may be several fold higher. The use of vaccines against maternal infections, such as rubella, has reduced the frequency of congenital infections. Much higher rates of vaginal colonization with group B streptococcus and genital infection with herpes simplex virus occur in women with multiple sexual partners; the rates of infection in neonates born to such women are correspondingly higher.

An important variable in the increased risk of neonatal sepsis in infants born of mothers with prolonged rupture of membranes is the development of ascending infection of the amniotic fluid, which then leads to congenital aspiration pneumonia, in the foetus and subsequently production of neonatal sepsis. However, amniotic and foetal infection can occur with rupture of membrane for less than 24 hr, and membrane may be ruptured for more than 24 hr without infection developing. Maternal urinary tract infections are also associated with an increased incidence of disease in the neonate. The maternal genital tract may be colonized
with a wide variety of organisms that do not necessarily cause disease in the mother, but may result in a heavy inoculum for the neonate at the time of birth and cause significant illness during the newborn period. These organisms include group B Streptococcus, E. Coli, Gonococcus, Listeria, Chlamydia, Candida, Herpes simplex virus and Cytomegalovirus. Intrauterine asphyxia may cause aspiraton of infected amniotic fluid and result in congenital pneumonia. Difficult or traumatic delivery is associated with an increased frequency of infections during the neonatal period.

The most important neonatal factor predisposing to infection is prematurity, there is a 3-10 fold higher incidence of sepsis, meningitis, or urinary tract infection in premature infants than in full term newborns. Males have an approximately 2 fold higher incidence of sepsis, meningitis and urinary tract infections than females, suggesting the possibility of a sex-linked factor in host susceptibility. Resuscitation at birth, particularly if it involves endotracheal intubation, insertion of an umbilical vessel catheter, or both, is associated with increased risk of bacterial infection. The presence of underlying disease, such as hyaline membrane disease; or congenital defects, such as meningomyelocele, predisposes to infection by acting as a portal of entry for organisms or by compromising host
resistance. The majority of infants cared for in a neonatal intensive care unit are exposed to a variety of diagnostic and therapeutic procedures that may also compromise host defences and provide a portal of entry for organisms e. g. Umbilical vessel catheters, endotracheal tubes, EKG monitor leads, foetal scalp electrodes, intravenous catheters and so on. In addition these infants may be exposed to antibiotic resistant organisms carried on the hands of personnel or contaminated equipment.

After birth, the neonates rapidly acquire commensal bacteria that colonise the skin and mucous membranes. The host defence mechanisms in the form of inflammatory response, immunoglobulins, complement, lysozyme, C-reactive proteins and phagocytosis are not well developed at this stage and some commonsals may became opportunist pathogens, [Rotimi and Duerden, 1981].

Basically two factors contribute to a relatively high incidence and increased severity of infections among newborn, is poor host resistance and greater exposure to infection, [Singh, 1978].

Most babies are sterile at birth but get rapidly colonized subsequently. Bacterial infections in utero and during delivery may occur in the following situations, [Singh and

1 - Prolonged rupture of membranes (>7-24 hours)
2 - Unclean or multiple vaginal examinations.
3 - Infected birth passage e. g. Gonococci, Candida, Streptococcus haemolyticus, Listeria monocytogenese and E. coli.
4 - Difficult and prolonged delivery.
5 - Meconium stained or foul smelling liquor.
6 - Birth asphyxia with difficult resuscitation.

1 - Poor host resistance:

The most important neonatal factor predisposing to infection is prematurity or low birth-weight. There is 3 to 10 fold higher incidence of sepsis, meningitis or urinary tract infections in these infants than in full-term, normal birth weight infants, - [Glasgow and Overall, 1983].

The full-term infant is equipped with defence mechanisms against infection and can bring them into action when necessary. If prematurely born, the baby is even less able to withstand infection and more liable to suffer serious consequences, - [Vulliamy and Johnston, 1987].

The premature infant's defences against invasion by microorganisms are limited in several respects. In term of
humoral immune system, the preterm neonate is compromised at birth by having lower concentration of IgG than his counterpart, - [Conway et al, (1985)].

Neonatal IgG being largely of maternal origin and transferred mostly as the foetus nears term. The premature infants, because of early birth are therefore deficient in IgG antibodies.

The concentration of immunoglobulin in the serum increase during foetal development, at birth the concentration is lowest in individual with lowest birth-weight. The infant is unable to synthesize appreciable amounts of immunoglobulin during the first few weeks of life and the level in the plasma falls, reaching about one third of the birth value at one month of age.

The levels remains essentially unchanged between one and three months of age, when the rate of synthesis of immunoglobulin equals that of catabolism.

Haworth et al. (1965) estimated levels of immunoglobulins in premature infants. They stated that there was no significant correlation between the concentration of immunoglobulins in the plasma of premature infants and the incidence of infection in them.
Fulginity et al. (1966) measured serum immunoglobulins in normal and in immunodeficiency states. They confirmed that IgG levels in cord blood were comparable with those of mothers. IgM exists in cord blood in small amounts and was fetal in origin. Very small amount of IgA was present in cord blood and therefore it was not quantifiable.

West et al. (1962) stated that IgG is selectively transferred from mother to the newborn across placenta but cord blood does contain small amount of IgM which is of fetal origin. About IgA he noted that it does not cross placenta and is absent from plasma of newborn infants. Smith and Eitzman (1964) studied development of immune response of human infant and found out that premature infants are by no means immunologically incompitant and are able to produce IgG and IgM antibodies after birth. Alford et al. (1967) suggested that demonstration of augmented levels of serum immunoglobulins resulting from intrauterine infection could be utilized in selection of neonates at high risk for having intrauterine infection and thus may prove useful in establishing an early diagnosis of such infants. Further in 1969, they confirm their earlier finding of raised IgM in cord blood in intrauterine infection; but here they stated that not all intrauterine infections could be detected on the basis of cord IgM elevations.
Contrary to this observation Miller et. al. (1969) raised a query for the presumption of intrauterine infection if the cord IgM values are elevated. On the same line of thinking studies were carried out to see if neonatal infections could be diagnosed early by measuring IgM levels in neonates because clinical signs of infections in neonates are often not definite.

Blankenship et. al. (1969) and Korines et. al. (1969) stated that IgM elevations occurs several days after the onset of physical signs of infection in neonates and therefore its elevation in blood could be used as test for early detection of neonatal infection.

Soward and Monif (1972) studied the IgM levels between 22 and 37 week of gestation and they concluded even IgM levels were influenced by gestational age and that in evaluating the significance of a given IgM level in premature and low-birth weight infant this factor must be taken into consideration.

In Indian literature also reports are available on immunoglobulin concentration in newborn.

Chandra et. al. (1970) reported data of concentration of serum albumin and IgA, IgM and IgG in first 24 hours of life in Indian newborns. They found that IgG levels in Indian
children were relatively lower during first year of life and that of IgA and IgM were higher during the same period compared to similar data from other advanced countries. From this comparison they found the higher incidence of malnutrition and infection in our country.

Prasad et. al. (1971) studied IgG; IgM and IgA levels in normal neonates, in premature, in neonates with acute infections and in neonates with congenital malformations. They found that IgA fraction was absent in cord blood but present in detectable amounts within the first week. Compared to normal neonates, premature infants appeared immunologically inferior but not immunologically incompetent. They also found there was generally appreciable rise in IgA and IgM fractions in response to infection but no change was found in cases with congenital malformations.

Kelkar and Kirthy (1979) studied that there was no significant difference in the pattern of synthesis of immunoglobulin in Indian children compared to that described in the developed countries.

Gupta et. al. (1980) and Malik et. al. (1980) studied the levels of IgG, IgM and IgA in cord blood of newborns and have reported that the levels of IgA and IgM in cord blood
were higher than those reported by Western Workers and they stated that this could be explained due to the high endemicity of parasitic and bacterial infections in our country.

Tandon et. al. (1984) studied maternal and cord serum IgG levels in relation to gestation and intrauterine growth. They found that the IgG levels in cord blood serum of preterm babies were significantly lower than the cord serum IgG levels in full-term babies. They stated that there was no correlation between maternal and cord serum IgG levels.

Both humoral and cell mediated immune response is deficient in the newborn especially amongst those born early and infants with sever intrauterine growth retardation; [Manerikar et. al. 1975]. The materno-foetal transfer of IgG antibodies occurs mostly during the third trimester of pregnancy. Preterm infants because of early birth, are therefore deficient in IgG antibodies. The normal infant lacks IgM antibodies at birth. Because of their large molecular size they cannot pass the placenta. The newborn baby fails to localize infection due to poor phagocytic ability of the leucocytes and deficient inflammatory response, [Meharban Singh 1978].
Greater exposure to infections:

There are greater opportunities, especially for low birth-weight babies to become exposed to infection because they demand greater handling and come in contact with a variety of gadgets viz. resuscitators, incubators, ventilators, catheters, infusion sets, tracheal tubes, masks etc. Unless great care is excercised it is difficult to render the equipment sterile. Warmth and excessive humidity in the nursery is also conducive to bacterial proliferation. [Meharban Singh; 1978].

ROUTES AND TYPES OF BACTERIAL INFECTION:

Bacterial infections in the newborn may be acquired in utero (intrauterine), at the time of birth (perinatal) or after birth and during neonatal period (postnatal).

As many as 2% of foetuses are infected in uterus and upto 10% of infants are infected during delivery or the first month of life, [Glassgow and Overall, 1983].

INTRAUTERINE:

The pathway by which bacterial infection may reach the developing foetus have been clearly summerised by Benirschke
A transplacental passage from the maternal blood stream appears to be the most important.

Transplacental passage of organisms from maternal blood stream, from peritoneal cavity, via fallopian tubes, from infected uterine walls, ascending infection from vagina either via ruptured membrane and uterine walls and across decidua are the possible pathways for intrauterine infection of foetus.

Morison (1963), has pointed out that large sparse blood vessels and absence of capillaries in the sub-amniotic tissues of the chorion make the entry of bacteria or bacterial toxins into the foetal blood stream. Listeria monocytogenes, vibricfetus, and Staphylococcus aureus were reported as common organisms for causing intrauterine infection, [Davies ; 1971].

PERINATAL :

Infection may spread to the baby from infected liquor amnii before delivery or by contamination during passage through the birth canal, - [Vulliamy and Johnston; 1987]. The majority of infants probably do not encounter bacteria or their toxins until they reach the vagina [Davies 1971].
According to Weinstein (1938), there appears to be little variation in vaginal flora between pregnant and non-pregnant individuals, despite the higher hydrogen ion concentration of the vaginal secretions during pregnancy. Staphylococci, Streptococci, Diptheroids and anaerobes are the common inhabitants, E. Coli being less often found, antibiotics may have a modifying influence, not all aspects of which are desirable. Following ampicillin treatment to febril women in labour, all types of streptococci, disappeared. E. Coli and Proteus mirabilis species lessened, Klebsiella species and other Gram-negative bacilli, largely resistant to antibiotics, increased, [Felton, and Williams, 1967].

Pathogens such as Listeria monocytogenes or Neisseria gonorrhoeae harboured in chronic cervical lesions, may be acquired by the newborns in their passage through the birth canal and cause serious infections. Maternal carriers of bowel pathogens, often symptomless, may also infect their infants during delivery and outbreaks of Salmonellosis, Shigellosis as well as infections with enteropathogenic coliform organisms have started in this way in newborn nurseries, [Barr, D.G.D., 1974, Rowe et. al. 1969].

According to Bhatia et. al. (1989) neonatal bacterial infections, which prove fatal after the first week of life are by and large horizontally transmitted and nosocomial in origin.
This is in contrast to infections transmitted by mother which causes most of the sepsis related to early neonatal death. The latter originate in maternal genitourinary tract and are mostly acquired during parturation.

POSTNATAL:

Infections acquired after birth are the result of environmental exposure either in the hospital or the community. After birth, umbilical cord, and mucus membrane, become important routes for entry of organisms in newborn, by apparatus, attendants and by air. Colonization of skin and mucus membrane occurs after birth by bacteria which are commensals. But demarcating line between the pathogenic organisms is faint. A particular organism may give rise to variety of infections depending upon time, route of entry and host factors.

In the first few days of life the baby becomes colonized by bacteria which are usually harmless but which occasionally cause disease and the pattern of pathogenicity changes over the years. [Vulliamy and Johnston 1987].

According to Cunliffe (1949) infants in hospital are not usually infected by their mothers but more commonly directly from other babies, with the hands or clothing of attendants.
and the air as probable vehicle of infection.

Barber and Burston (1955) also confirmed that mothers do not infect their babies. According to Duncan and Walker (1942) mother's breasts are infected from the noses of their babies. While Anderson, et al. (1961) and Wolinsky, et al. (1962) thought that the mothers or their visitors can sometimes be the source of infection. Shaffer, et al. (1957) suggested that the nursing staff was infected from the babies.

Cedergren et al., (1962) believed that a carrier among the staff could be the original source of infection. However, Gezon (1960) found no difference between the infection rate of babies looked after by non-carriers. However, the staphylococci may eventually be spread, the original source of infection is probably an adult who may be a nasal carrier, a non-nasal carrier, - [Hare and Ridley, (1958)]. Or a clinically infected case, [Davies, 1971]. Selbie, (1953) found that staphylococci from clinically infected cases were on the whole more active than those from nasal carriers but there were strains of high and low activity in both groups. Probably an open lesion is a more dangerous source of infection than a carrier but the potential danger of carriers can not be ignored, [Crosse, (1966)].
ETIOLOGY:

While coliform infections have for long been regarded as a particular hazard of the neonatal period, the bacterial colonisation of the newborn has shown changing patterns.

Earlier reports of neonatal infection commonly incriminated the beta-haemolytic Streptococci an organism responsible for puerperal fever, a cause of much maternal morbidity and mortality, [Boissard and Eton, (1956)].

During the 1940’s and 1950’s, the haemolytic Streptococcus was gradually ousted by Staphylococcus aureus and reports began to appear from many parts of the world describing the widespread colonisation of the newborn with this organism, [Baldwin et al, (1957); Standrup et al, (1959); Forfar et al, (1953)].

However, in 1961 to 1963 beta-haemolytic Streptococci were the most frequent single cause of neonatal sepsis at the Boston city Hospital, accounting for 25 % of such infections, - [Eickhoff et al, (1964)]; and were causing outbreaks of infection elsewhere; - [Nash et al, (1965)]. A determined onslaught against the staphylococcus included such majors as 'rooming in' for mothers and babies, - [Mortimer et al, (1966)], nasal creams, - [Jennison and
The use of triple dye or occlusive dressings for the umbilicus, - [Huntingford et al, (1961)] but the application of hexachlorophene to the skin of infants, and to the hands of attendants appeared to be the most effective resulting in a significant fall of nasal carriage of staphylococci by hospital newborns, - [Simon et al, (1961); Plueckhahn, (1961)].

Healthy carriage of staphylococci is harmful and it is a potent source of infection, - [William, (1963)].

The nasal carriage rate of pathogenic Staphylococci in the hospital staff in this country has been reported between 20-95 percent by various workers, - [Chitale, (1956); Sayed et al, (1959); Hardas et al, (1964); Verma et al, (1965)]. Doctors and nurses are a special danger to their patients as there are several studies showing that the staff working in hospital have a higher carriage rate, - [Ghosh-Ray and Walia, (1962); Seth et al, (1973); Talib et al, (1973)].

The highest rates of carriage are seen in young infants - [Williams et al, (1966)]. Hurst (1960) is of the opinion that infants due to the heavy Staphylococcal load more often responsible for the contamination of the air as compared to adults.
Sources of Staphylococci in the environment are beds, blankets of patients, dust from floors, ceiling, walls, clothing etc. Also the contamination of gowns and uniforms worn in wards and the transfer of patient’s Staphylococci by means of nurses uniforms was reported, - [Hamberaeus, (1973)].

Most of the coagulase positive Staphylococci are responsible for hospital infection. Coagulase may contribute to pathogenicity by inactivity a bacteriocidal substance in normal serum or by protecting the cocci with a fibrin barrier against phagocytosis, - [Cruickshank, (1976)].

The coagulase negative Staphylococci have considered ubiquitous commensals that are most commonly non pathogenic culture contaminants. Recently, however, coagulase negative Staphylococci have emerged as significant nosocomial pathogens in many neonatal nurseries, - [Battisti et al, (1981); Baumgart et al, (1983); Fleer et al, (1983); Munson et al, (1983)].

Neonatal sepsis and meningitis with coagulase negative Staphylococci are associated with significant morbidity and mortality rate as high as 10 percent, - [Baumgart et al, (1983), Noel and Edelson, (1984)]. Staphylococcus epidermidis is more likely than other species of coagulase
negative staphylococci to be presenting in blood cultures from neonates with significant disease, - [Hall et al, (1987); Dunne et al, (1987)]. Strains of coagulase negative Staphylococci that are resistant to semisynthetic penicillins, gentamycin or numerous antibiotics are also more likely to be pathogenic, - [Dunne et al, (1987)].

Klebsiella aerogenes is becoming of increasing importance in hospital infection and outbreaks infection in neonates associated with intestinal colonization, have been reported; - [Adler et al, (1970); Hill et al, (1974)]. Nosocomial outbreaks of Klebsiella infection resistant to late generation cephalosporins have been observed by, Kenneth et al, (1993).

Pseudomonas aeruginosa poses a special problem in hospital infection, since it is an ubiquitous organism present in soil, water and in many moist environments. Also, it is unique among pathogens because it can infect any living being, ranging from plants through invertibrates to human beings, - [Shriniwas, (1977)].

Pseudomonas aeruginosa has a special ability to survive in water and moist objects. Pseudomonas aeruginosa has always been an important human pathogen, and many workers believe that it causes more infections in hospital now than
formerly, - [Finland et al, (1959); Barber, (1961)].

Sengupta et al, (1976) reported Ps. aeruginosa as the leading cause of hospital infection.

Equipment that contains standing water or retain moisture is a common source of contamination with Pseudomonas aeruginosa and this was reported by many workers in different equipments such as air-cooling apparatus, [Anderson, (1959)], resuscitation equipment for premature infants, - [Bassette et al, (1965)], respirators, - [Phillips and Spencer, (1965)], Urine bottles and bed pans, - [Mcleod, (1958)] and brushes, - [Aylliffe et al, (1965)].

Warner and Moser, (1970) reported hospital infection in nursery caused by Pseudomonas aeruginosa and enterobacteria when the children were washed after birth with feacally contaminated water.

Whithy and Rampling, (1972) found that certain objects such as sink traps, floor cloths, mops were frequently contaminated, on the other hand when similar areas were examined in domestic homes, Ps. aeruginosa was rarely isolated. Pseudomonas may grow to concentration of $10^6$ organisms/ml in distilled water which appears perfectly clear, growth of Pseudomonas in distilled water,
disinfectants and medication is the factor sited most commonly in single source outbreaks of Pseudomonas infection in hospital. In newborn nurseries infection generally has been transmitted to the infants by the hands of personnel, from wash basin surfaces, and from solutions used to rinse suction catheters; [Nelson; (1992)].

BACTERIAL INFECTIONS IN NEWBORN :

Bacterial sepsis and meningitis continue to be major causes of morbidity and mortality in the newborn infants. This is despite of improvements in antimicrobial therapy advances in neonatal life support measures and the prompt recognition of perinatal risk factors for infection.

Sepsis Neonatorum :

Sepsis neonatorum is frequently devastating, with majority of survivors having significant neurologic sequelae as a consequences of central nervous system involvement, septic shock or hypoxemia secondary to severe parenchymal lung disease or persistent pulmonary hypertension.

The overall incidence of neonatal sepsis varies between 1 and 8 cases per 1000 live births. Approximately one third of septic newborn develop meningitis. Multiple risk factors
for perinatal infections have been identified. These factors can generally be divided between maternal and neonatal observations.

1. Maternal risk factors:

Obstetrical factors include premature onset of labor, premature rupture of membranes (PROM), and maternal peripartum infection. In studies in pregnant women with cervical colonization with Group-B-beta-hemolytic Streptococci, (GBS); the attack rate for perinatally acquired sepsis in newborn infants, born to GBS-colonized women is 1 to 2 percent, but this rate increases to 15.2 percent with premature onset of labor (< 37 weeks), 10.7 percent for chorioamnionitis or PROM > 24 hours, and 9.7 percent for maternal postpartum bacteremia.

Overt chorioamnionitis and maternal sepsis are relatively uncommon, so the only maternal indicator of intrauterine infection, may be intrapartum fever.

Neonatal risk factors:

The single most important risk factor is low birth weight. In the study of Boyer et al, (1983); the attack rate for sepsis was 26 times greater in infants weighing less than
1000 g. compared with those weighing more than 2500 g. In other studies, rate of sepsis was 8 times greater in infants weighing 1000 to 1500 g compared with those weighing 2000 to 2500 g; [Buetow et al, 1965]; and meningitis occurred from 3 to 17 times more often in those weighing less than 2500 g compared with infants weighing more than 2500 g. Considering low birth weight and maternal risk factors, an attack rate of 7.6 per 1000 and a mortality rate of 33 percent were observed for the combined risk factors of birth weight less than 2500 g; rupture of membranes (ROM) more than 18 h, and intrapartum maternal temperature greater than 37.5°C. By comparison, infants without these risk factors had an attack rate of 0.6 per 1000 and a mortality rate of 6 percent.

In neonatal period the infant may suffer from a general blood-stream bacterial infection which has been acquired antenatally or postnatally. In the newborn, especially in premature infants, the primary site of infection is usually not obvious and the symptoms are generalized and varied, [Silverman, (1961)].

Yippo (1919) pointed out that septicaemia is an important and relatively frequent cause of morbidity and mortality of newborn infants, He reported in 10 out of 14 infants dying between the fourth and fifth day, bacteria were demonstrable in the blood stream. Since that time improvement in
obstetric and pediatric techniques has undoubtedly reduced the incidence of septicaemia, [Silverman (1961)].

Sex and Septicemia:

In the series of Buetow et al, (1965) septicemia was more frequent in male infants, the male incidence rate being 6.5 % as compared with 4.5 % for females. It was also noted that the predominance of septicemia in the male infant was significantly increased among prematurely born children with a birth weight in excess of 1,500 gm. In the 1001-1,500 gm. weight group, more female had septicemia, perhaps as a consequence of a higher neonatal mortality in males and the fact that 60 % of all fatalities in this weight group occurred during the initial 48 hours of life, before the usual onset of septicemia, thus leaving more females in the population susceptible to bacterial invasion. Gram negative bacilli septicemia were noted with greater frequency in the male.

Among the previous reviews of neonatal septicaemia male infants were involved in 70 % of cases; [Nyhan and Fousek, (1958); Dunham, (1933)]. Several evaluations of Staphylococcal disease and colonization rates among newborn have shown an increased male susceptibility with male; female ratios varying from 1.3 - 2.1; [Plueskhahn, (1964)];
Thompson et al, (1963). Both eye infections and omphalitis due to Gram-negative organisms have been observed to occur about 1.5 times more frequently in male babies [Plueckhahn and Banks, (1964)].

Beutow et al, (1965) observed that newborn male have a greater susceptibility to bacterial invasion.

Neonatal septicaemia was brought to attention of paediatric physician by Dunham in 1933. It was considered as one of the important mile stone in early clinical paediatrics. Septicaemia caused by coliform bacilli and other intestinal organisms has been recognised since the the report of Jacob in 1909 and Fetly and Keefer in 1924. However bacteriaemic shock as clinical entity was first described in 1951 by Waisbren. Over subsequent ten years many experimental studies were conducted using cell wall of Gram negative bacteria i. e. endotoxin, in dogs; [MacLean, 1977].

Periodic reviews of this topic present evidence that Septicaemia still occurs and that it remains a very difficult clinical problem; - [Christo et al, (1990); Hall et al, (1987); Silverman, (1949)]. There is general agreement that the incidence of neonatal septicaemia is disproportionally high in premature infants and is higher in males than in females, - [Silverman, 1961].
Nyhan and Fousek (1958) noted that the distribution of causal agents has changed. Prior to 1944 beta-haemolytic Streptococci were the most frequently occurring organism. Since that time infections with coliform organisms have been more common. Septicaemia due to Pseudomonas aeruginosa in premature infant is of particular interest, because this organism usually does not invade human beings except in the presence of debility or prolonged treatment with antibiotics. Among newborn premature infants it may be associated with a fulminating infection. Nyhan and Fousek (1958) reported that among 13 newborn infants with septicaemia due to Pseudomonas aeruginosa 10 were premature. Besides Pseudomonas aeruginosa other Gram-negative bacilli like E. coli, Klebsicilla species are also predominant.

In premature newborns coagulase negative Staphylococci are frequent blood culture isolates. Staphylococcus epidermidis is more likely than other species of coagulase negative Staphylococci to be present in blood cultures from neonates with significant disease, - [Hall, et al, (1987), Mathur et al, (1967)].

In Indian neonates 55 to 80 % of neonatal sepsis is caused by Gram-negative organisms, especially Citrobacter species are known to cause sepsis in newborns, - [Choudhary et al, (1975); Chetan et al, (1987); Christo et al, (1990)].
MENINGITIS:

Most neonatal meningitis is due to infection by either E. coli or the Group-B streptococci. Much less often Listeria monocytogenes, Pseudomonas aeruginosa or Proteus are responsible.

Citrobacter diversus has been increasingly recognised as a cause a life-threatening neonatal meningitis with frequent abscess formation. This condition is associated with high mortality and extremely poor prognosis; [Giacoia and West, (1989)].

In United States, the case fatality rate has been estimated to be 34% and 91% of survivors have mental retardation; while Christo, et al, (1990) noted case fatality in Indian neonates was 61%.

OPHTHALMIA NEONATORUM:

This condition refers to inflammation of the conjunctiva within the first month of life. Causative agents include topical antimicrobial agents (chemical conjunctivitis), bacteria and herpes virus. Bacterial conjunctivitis is caused by Neisseria gonorrhoeae, Chlamydia, Staphylococci, Pneumococci, Streptococci, E. coli and other Gram-negative bacteria.
1. Chemical conjunctivitis: This is predominantly seen with silver nitrate 1% used as prophylaxis against bacterial conjunctivitis.

2. Neisseria gonorrhoeae:
Gonococcal opthalmia neonatorum usually presents as conjunctivitis with chemosis, purulent exudate and lid oedema starting 1 to 4 days after birth. Clouding or perforation of the cornea or panophthalmitis also may be present.

A prenatal clinic in North Carolina reported infection rate of 7.5 percent with recurrence rate of 30 percent by delivery. There is an increased risk of Gonococcal opthalmia neonatorum with premature rupture of membranes in infected women. Bacterial conjunctivitis, Staphylococcus is the most common cause of bacterial conjunctivitis; Gram negative organisms, Pseudomonas aeruginosa may be involved.

OSTEOMYELITIS:

This is an uncommon infection in the neonatal period. When present, it may result from sepsis, direct inoculation in association with heel sticks and scalp electrodes, extension from soft tissue infections.

The most common organisms are staphylococcus aureus,
Group-B-streptococcus, gram negative bacteria, Neisseria gonorrhoeae and candida. There is a good correlation between the most common causes of bacteremia in the newborn and the frequency of bone infections caused by a particular organism. Any bone can be involved, but most frequently, infection occurs in the femur, humerus, tibia, radius and maxilla. Osteomyelitis of the skull has been associated with use of scalp electrodes.

URINARY TRACT INFECTION: (UTI)

This varies with birth weight increasing from 1 percent in infants weighing more than 2500 g to 3 percent for infants weighing less than 2500 g. Bacteriuria may signal generalised sepsis with haematogenous spread to the kidney. Alternatively, a primary UTI may result in blood-stream infection. There is a greater incidence in male infants than in female infants.

The most common organism is E. coli, but other Gram negative bacteria, especially Klebsiella pneumoniae, and entero cocci are also causative agents.
INFECTION OF SKIN:

The newborn infant may develop a variety of rashes associated with bacterial disease. Some of these are related to systemic infection, while others are the direct result of primary cutaneous disease.

The most frequently encountered clinical manifestations of localized skin infections are pustules, vesicles, cellulitis and abscesses.

The common bacteria colonizing the skin of the newborn infant include coagulase negative Staphylococci, Staphylococcus aureus, Streptococci, Gram negative enterics (including E. coli) and diphtheroids.

The colonizing organisms will vary with the vaginal flora present at the time of delivery and the organisms present in the environment of the nursery.

1. Pustules:

Pustules in the newborn infants are most commonly caused by Staphylococcus aureus, but they must be distinguished from similar appearing lesions of erythema toxicum.
2. Cellulitis:

The causative agents are usually Streptococci and Staphylococcus aureus, gram-negative enteric bacteria or anaerobes also may be present with cellulitis associated with disruption of the skin (abrasions, scalp electrodes).

Omphalitis:

This is characterized by erythema or induration with purulent discharge from the umbilical stump. Both gram negative and gram positive organisms may be involved and in the setting of poor maternal immunity and poor aseptic technique.

Fungal Infections:

A. Mucocutaneous Candidiasis:

Fungal infections in the well, immuno competent term infant are genrally limited to immunocutaneous disease. The offeding organism is candida albicans. Oral candidiasis (thrush) is usually responsive to nystatin oral suspension. Although mucocutaneous candidiasis most commonly occurs postnatally, rare intrauterine infections have been reported. Intrauterine infection occurs by means of
ascending route and can result in mucocutaneous or disseminated disease.

B. Disseminated Candidiasis:

Systemic candidiasis has emerged as a serious nosocomial infection occurring in very low birth weight infants. As many as 3 percent of very low birth weight infants develop systemic candidiasis.

Risk factors may include prolonged use of antibiotics, parenteral hyperalimentation and intravenous fat emulsions, assisted ventilation and the use of contaminated monitoring equipment, Candida albicans remains the most common pathogen, but other candida species should be considered. Candida sepsis may result in meningitis, arthritis, endophthalmitis, endocarditis.

The incidence of nosocomial candida infection, including, outbreaks has been increasing. The mechanisms for many outbreaks have not been identified. The candida outbreaks with identified mechanisms of transmission including one NICU outbreak were caused by extrinsic contamination of intravenously administered medications or vascular-access apparatus; - [Robert Sherertz et al, (1992)].
In a Neonatal Intensive Care Unit, Robert Sherertz et al, (1992) found that five infants apparently acquired candida bacterimia as a result of contaminated retrograde medication syringes. In two infants they observed same strains of C. albicans both from the retrograde syringes and from the bloodstream.

Diarrhoea:

Infectious diarrhoea may follow invasion of the intestinal mucosa by staphylococcus aureus, E. coli, Shigella and transmission occurs by the faeco-oral route and the neonate usually infected at the time of birth by organisms present in maternal stool or after birth by spread of organism from other infected infants on the hands of personnel.
Although fire has been a geologic and ecologic feature of this planet since its inception, the history of its generation and control by man is lost in antiquity. Man's use of fire to warm himself, cook his food and heat his water offers major advantages, but the accidental production of burn injury is inevitable.

It is reasonable to assume that burns are among the oldest injuries that afflict mankind. The ancient healers had recognised the importance of burn wound care. Jackson states that the "PAPYRUS EBERS" written C 1500 B. C. recommends the rubbing of a warmed oiled frog on burned area as an escharifying agent. The escharifying agent produce dry, rough tissue which is inimical to bacterial growth, - [Jackson, (1970)].

Haider Sneve, (1905) wrote, "In burns involving large areas of skin the patient is exposed to death, first from shock, second from toxaemia due to absorption of poisons from the injured surfaces, third from loss of function of the absent skin covering and fourth from exhaustion due to long continued fight for recovery".

In appreciation of the experienced of Sneve the physicians
in his times started using escharifying agents to produce dry, rough tissue inimical to bacterial growth, - [Jackson, (1970)].

First clinical accounts of infection of burns can be recognised as early as in 1607 A.D. Fabricious (1607) speaks of an "ulcer profundum et putridium" after the separation of the slough. Dupuytren (1833) observed abundant suppuration, fever, wasting and death in patients of deep burn. Lister was also aware of the burn infection. But unlike his contemporaries, - [Pirrie, (1867); Maylard, (1892)]; he did not extend his antiseptic discipline to the treatment of burns; probably he considered it as impossible objectives, - [Lister, (1909); Dunbar, (1934)].

Local and general injury by bacteria colonizing a burn wound was recognised by different authors, - [Lustgarten, (1891); Stockis, (1903)]. But detail study was not undertaken until many years later. In 1926, Pack reported that practically every burn wound gets contaminated within a few hours, commonest organism being Streptococci, Staphylococci and Pseudomonas aeruginosa. These findings were extended by Aldrich, (1933) and Cruick Shank, (1935) who attributed the toxaemia to the invading organisms. These authors and Wilson et al, (1938) emphasized the importance of Streptococci. Marsch, (1935), Heggie and Heggie, (1942)
regarded the staphylococcus aureus as an important pathogen of burn wound. In United States Langohr et al, 1947 reporting a detailed bacteriological study of the burned surface considered haemolytic Streptococci and Staphylococci particularly damaging to the deep burns and later so especially when present with Proteus group of bacteria.

It is clearly evident that the burns patients are easily susceptible to infection. Burnt areas are directly exposed to the atmosphere. The resultant necrotic tissue forms a good culture medium for the growth of organism. If the burns are of the second degree or third degree then the deeply involved tissue favours the growth of the organisms. In the deeper tissue microabscesses and pockets are formed which are not easily accessible to the superficial dressings and may lead to dissemination of the infection in the generalised form, - [Lowbury, (1972)].

Infection has been described as, the cardinal problem in the treatment of burns ------------ causing, conversion of second degree burns to third degree burns, - [Artz et al, (1969)] and creating greatest threat to survival [Altmeir and MacMillan, (1968)]. Fatal septicaemia resulting from local infection can best be avoided by preventing development of infection, - [Moncrief, (1970); Linkner et al, (1972)].
Despite numerous advances in medical care and, specifically, care of burn injuries, infection continues to be the leading cause of death following hospitalization for thermal injury. Before the days of effective tropical treatment septicemia and/or burn wound sepsis accounted for 80% of the deaths occurring after first five days of hospitalization in patients, at U. S. Army Institute for Surgical Research between 1953 and 1962.

Before penicillin, a 30% burn injury could be expected to have a 50% mortality; and Streptococcal infections were the most common cause of death. With the control of Streptococcal infection by penicillin, Staphylococcus aureus emerged as the dominant pathogen. When effective antistaphylococcal antibiotics were introduced, the gram negative organisms emerged, the most prominent of them being Pseudomonas aeruginosa. Finally, fungi and viruses became prominent organisms causing death in severely burned patients in 1970s. Control of infection by tropical therapy, improvement in nutrition and aggressive wound care have markedly improved the outlook for patients with major burn. Despite these remarkable accomplishment in burn care infection remains a constant threat.

Because of the nature of the injury, burn wounds are invariably contaminated with microbes. The incidence of
serious infection in the burn patient clearly varies with the size of the burn, - [John A. Boswick, (1987)]. Age also affects the incidence of infection. Infection increases dramatically in patients over 60 years of age. The incidence and severity of infection vary greatly with the overall medical and nutritional state of the patient and the type of infecting organism.

Because of the presence of organisms in the necrotic tissue of the burn wound and because of the elaboration of endogenous pyrogens, fever is frequent in patients in large burn injuries. The white blood cell count (WBC) is quite variable following burn injury and the level of white cell does not indicate that infection is or is not present. However, a shift in the differential to more immature forms of neutrophil series strongly suggest the development of serious infection.

Infections of the burn wound can be classified as either non invasive or invasive.

Non invasive burn wound infection:

Burn wounds are never sterile despite claims made to the contrary, which reflect inadequate microbiological techniques. The eschar is a dead and denaturized biological
material that readily support growth of a wide variety of microorganisms. Although initially the burn wound has only a few organisms, this usually progresses to heavy colonization during the first few days following injury. Colonization of burn wound with a single type of organisms may occur, but more often there is a mixture. During the second and third postburn weeks, development of a granulation layer at the interface between viable and nonviable tissue is associated with increased resistance to invasion and separation of the eschar. The number of bacteria in the exudate or eschar may vary from as few as 10 up to 10 billion per gram of material. Lysis of the eschar may occur at a more rapid rate partly because of bacterial enzymes.

Noninvasive infection may be generally considered to be that limited primarily to the burn eschar or exudate. It is characterized by rapid separation of eschar and increased or heavy exudation of purulent material from the wound.

Invasive Burn Wound Infection:

Infections of the burn wound may invade the underlying viable tissue. Invaded granulation tissue becomes edematous and pale and does not bleed briskly when debrided with a spong or knife. Culture of the invaded normal tissue characteristically show more than 1,000,000 bacteria per gram.
of tissue. The onset may be sudden, but more often invasion is superimposed in a patient who already has purulent drainage; leukocytosis and fever. Early in process there may be increased number of nonsegmented neutrophils in blood and further elevation in white cell count. As the process advances, the patient may become hypothermic and the white count may be depressed although there is still a shift to the left, which often becomes more marked. The patient may become non responsive and the process progress to death with toxemia, some times without septicemia. Invasive burn wound sepsis requires early detection and extremely vigorous therapy.

Septicaemia:

Bacteremia usually occurs with invasive burn wound sepsis, but burn wound sepsis may occur without detectable bacteremia. Transient bacteremia without detectable evidence is not found frequently. Therefore, bacteremia cannot be equated with either septicemia or burn wound sepsis. It is not unusual to recover more than one organism from blood cultures, indicating that such patients have a generalized lack of resistance to infection that is associated with abnormalities of host defence.

Pseudomonas aeruginosa infection is much more commonly seen
in severe and deep burn injuries than the superficial and trivial injuries. Infection originating in devitalized burn tissues extends as cellulitis, lymphangitis and finally invade blood-stream, - [Sengupta et al, (1972)].

Blood stream invasion is being frequently reported nowadays which is mostly fatal and indicated the failure of antibiotics to control the infection, - [Altemeir et al, (1962); Rabin et al, (1961)].

In the study conducted by Thomson et al, (1971) in the period between 1945-1965 a total of 3195 children admitted in hospital with burns of all causes were studied. Eleven children out of 3195 died due to septicaemia and the causative organism was Pseudomonas aeruginosa in most of the cases.

In 10 years experience of MacMillan (1975) at Cincinnati Unit of Shriners Burns Institute 671 children were treated for burns, in most of them mortality was due to septicaemia.

Smith et al, (1975) noted growing incidence of septicaemia. Seventeen out of twenty patients who died due to burns had septicaemia. Invading organisms in maximum number of cases was Pseudomonas aeruginosa which was closely followed by Staphylococcus and Enterobacteria. Pseudomonas aeruginosa
had caused 8 deaths while others including fungi had caused remaining deaths.

The number of organisms in the burn wound site has proven to be very useful in assessing the state of Septicaemia in most of the cases. From the biopsies bacterial quantitation has been shown to be a good indicator. If bacterial count increases to more than $10^4$ organisms per gram of tissue, then the bloodstream invasion was observed invariably. This factor has been used in bacteriological monitoring of the patients, - [Loeble et. al; (1974)].

Cason et al, (1968) reported 16 deaths due to septicaemia out of 46 cases, the organisms included Pseudomonas aeruginosa, Proteus, Klebsiells and other coliform bacilli.

SEPTIC PHLEBITIS:

Burn patients often require prolonged intravenous therapy; and patients with larger burns who most need IV therapy have decreased numbers of available site for venipuncture. Hence one is often reluctant to remove and replace a well functioning venous line.

However, when intravenous lines are left in place for more than 48 hours, the incidence of infection is remarkably and
progressively higher, especially when plastic cannulas or catheters are used instead of stainless steel needles. When the signs and symptoms of generalized infection are present in a burn patient, one must suspect that septic phlebitis has occurred. A careful examination of previous intravenous sites is warranted. When the complication occurs, it is necessary to surgically remove the entire affected vein. - [Prutt et. al; (1970)].

PNEUMONIA:

Pneumonias occur frequently following burn injury, especially in patients with smoke inhalation injury or burns of the upper respiratory tract, in those who require prolonged intubation for any reason, and in those who require tracheostomy. Several years ago, it was generally recommended that tracheostomy be performed in any patient with burns about the head and neck. However, experience has shown that this is necessary only in extreme circumstances and that the incidence of serious complications; including pneumonia, is higher following tracheostomy. When pneumonia does occur, every attempt should be made to obtain an adequate sample for culture and sensitivity, preferably by transtracheal aspiration or by nosotracheal suctioning.

Pneumonias following burn injury are most commonly caused by
gram-negative bacilli and frequently have broad resistance patterns to the commonly used antibiotics.

URINARY TRACT INFECTIONS:

Urinary tract infections are usually associated with prolonged, often unnecessary catheterization. It is seldom essential to leave a catheter in place for more than a few days, and this device is often misused in the burn patient. Routine monitoring of urine from indwelling catheters should be done by needle aspiration on a regular basis two to three times a week. Candiduria is often but may reflect active infection or septicemia, especially when mycelia can be demonstrated.

Infections by Specific Organisms:

Group A Streptococcus pyogenes is a highly transmissible pathogen that can cause abrupt deterioration in the wound with rapid progression to death. The infection is associated with an increase in wound pain, redness, induration and swelling. Since it invades normal tissues, the most significant sign of Streptococcal infection is redness extending from the margin of the burn wound. Affected patient may have spiking fever, flushing of the face, and rapid tachycardia. Shock occurs late in the
course. Most Streptococcal infections are seen within the first week following burn injury.

Donor sites and freshly grafted wounds may also become infected with the beta-haemolytic Streptococcus but the clinical course is less abrupt, and the loss of grafts or conversion of a donor site to a full thickness injury is usually a more major consideration than invasive sepsis.

Invasive infections of the burn wound with Staphylococcus aureus or S. epidermidis have a more insidious course, often with two to five days elapsing from the onset of symptoms to a full-blown infection. Early dissolution of the granulation tissue is characteristic in burn wounds. Patients become disoriented, are usually hyperpyretic with a leukocytosis, and frequently develop a gastrointestinal ileus. Shock may occur and is sometimes accompanied by renal failure.

Infections caused by S. epidermidis, once uncommon, are being seen with alarming frequency in many burn units. These bacteria are often quite resistant to antibiotic therapy.

Superinfection not infrequently follows systemic antibiotic therapy for Staphylococcal infections, therefore, therapy
should be restricted to a relatively short period of time.

It is well to remember that Staphylococcus aureus usually cannot be eradicated from the wound until it is covered by graft or replaced by another pathogen.

Pseudomonas aeruginosa, rarely causes infections in immunologically normal individuals, but it may invade and become highly virulent in an immunodepressed burn patient. It grows well in moist environments, including the burn wound. Invasion may occur either abruptly or slowly. Typically, the burn wound develops a green, foul smelling discharge over a two to three day period; but in more rapidly advancing and invasive infections, the eschar may become dry with a shaggy green exudate, often progressing to patchy areas of necrosis within hours. Sometimes the pseudomonas infections do not have a greenish or bluish exudate. Ecthyma gangrenosum is a necrotic lesion occurring in nonburned tissues associated with metastatic involvement of blood vessels. It is a late sign often seen in patients with septicaemia.

During the past several years increasing numbers of infections have occurred with Escherichia, Klebsiella, Proteus, Enterobacter, and Providencia. These organisms colonize the burn wound from self contamination and from the
environment. They become pathogenic primarily when competing organisms are eliminated from the burn wound by antibiotic therapy.

Candida albicans and other Candida species are frequently cultured from burns wounds, but invasive infection is quite uncommon. When it occurs, the granulating wound may become dry and flat; with a yellow or orange colour. Systemic candidiasis is much more common and is most often associated with invasive therapeutic measures, including central and even peripheral venous lines left in place for a prolonged period of time.

Increasing numbers of mycotic infections of the burn wound associated with improved control of burn wound sepsis caused by gram-negative and gram positive bacteria have been reported. Aspergillus, Mucor and Rhizopus have been the most common pathogens. The clinical manifestations include ulceration, induration, oedema, early separation of the eschar, muscle necrosis, and conversion of a wound to a deeper injury.

PREDISPOSING FACTORS:

Despite numerous advances in medical care and, specifically care of burn injuries, infection continues to be the leading
cause of death following hospitalization for thermal injury.
Before the days of effective topical treatment, septicemia
and/or burn wound sepsis accounted for 80% of the deaths
occurring after the first five days of hospitalization in
patients at the U. S. Army Institute for Surgical Research
between 1953 and 1962. Even in the last ten years,
approximately 73% of deaths after the initial five days
result directly or indirectly from septic processes.

Infections occur in the burn patients because:
1. Environmental and therapeutic factors
   fail to control the bacterial load.

2. Altered host resistance.

**Environmental and Treatment Factor:**

Because of the nature of the disease being treated burn
wards have become important reservoirs for antibiotic
resistant pathogens that cause nosocomial infections.

Open wounds, frequent dressing changes and debridement, high
bacterial densities on the burn wound, and intensive nursing
care all contribute to perpetuation of the problem. Patient
treatment areas become especially contaminated and moistened
environment such as sinks, water taps and floor drains may
develop and maintain high bacterial densities. Visitors, fomites and food may provide external source of bacteria and should be controlled.

The knowledge of these details helps to explain the peculiar features of the epidemiology of burn wound infections.

Bourdillon and Colebrook (1946) showed that agitation of contaminated textiles for example removal of dressing caused a build up and sustained high level of air borne bacteria including wound pathogens which could be prevented by planum ventilation of the room. This provided comparatively germ free air for the patients.

In a study on serological types of Pseudomonas aeruginosa [Lowbury and Fox, (1954)], it was found that each ward had at a time its own predominant serotype of Pseudomonas aeruginosa. Though the dressing room was common for all wards the serotype each ward had, were different showing cross infection. Air, floor dust, nurse’s hands, the bedding and bandages of the patients were shown to be demonstrable reservoirs and vectors of these organisms.

Hambraeus (1973) made studies regarding the dispersal of Staphylococcus aureus from burns patients, relation between nasal carriage by the staff and exposure to air borne
Staphylococcus aureus and the transfer of the carrying particles within the ward. Nasal carriage rate by the staff correlated with the air count. The transfer of staphylococcus within the ward was 6 to 20 times more than that would have been expected by air movement only.

Kominos et al, (1972) found cross-infection among patients and showed several serotypes at one time. Cultures from hands of nurses yielded same types of Pseudomonas aeruginosa. Direct handling of the patients by nurses was found to be the principle mode of transmission. Sepetjian et al, (1974) found catheters and I. V. fluid cannulae mostly responsible for septicaemia.

Pseudomonas aeruginosa infection is also seen in dialysis units [Wagnild et al, (1977)]. Urological ward [Moore and Foreman, (1966)]. Phillips (1966) found other sources of infection in the form of contaminated lignocaine jelly used for tracheal catheters. Established inanimate sources include physiological saline, soaps, antiseptics solutions, eye drops, creams and jellies, - [Editorial B. M. J., (1967)].

Steroid cream was found to be contaminated by Noble and Savin (1960). MacLeod (1958) found that commonly used articles in the hospital that contain standing water or
retain moisture such as urine bottles and bed pans, were frequently contaminated with Pseudomonas aeruginosa.

ALTERED HOST RESISTANCE

The most obvious alteration of the resistance to bacterial invasion occurs when the normal skin is destroyed. The skin functions as an aesthetic container for the human body providing protection for internal organs, regulation of temperature, and a barrier to prevent protein and water loss. The skin is also the most important defense mechanism against microbial invasion. Any injury that compromises skin integrity threatens the ability to coexist with surrounding microorganisms. Devitalized tissues serve as a pabulum for microbial growth and with decreased blood flow to the burn wound as a contributing factor, invasion into adjacent viable tissue is a natural and expected consequence. Less apparent but equally important are alterations in host resistances to infection.

The immune system of defence against microbial pathogens is quite complex. The immune system can be conveniently divided into two broad categories:

the first is adaptive or acquired immunity and
the second is non adaptive or innate immunity.
Adaptive immunity is mediated by lymphocyte function; on the other hand non specific immunity involves an interplay of phagocytic cell (neutrophils and mononuclear phagocytes), opsonic proteins (antibody and certain complement component), and the vascular response.

**Lymphocyte Function:**

Lymphocytes are divided into three main categories: T cell, which develop under influence of the thymus. B cells, which develop under the influence of gut associated primary lymphoid organs, and cells having neither B-cell nor T cell characteristic.

T lymphocytes participate in cell-mediated immune responses. T-cells, sensitized to a given antigen may participate in delayed hypersensitivity reaction, cytolytic reaction of target cells and the released of a large number of mediators known as lymphokines. Persons known to have abnormal T-cell function have increased susceptibility to infections by most fungi, protozoa such as Pneumocystis carinii and a variety of viral agents including the herpes group of viruses, and cytomegalovirus but not hepatitis B virus.

In severe burn, T-cell function is usually abnormal the ability to express delayed type sensitivity reactions to
skin test antigens has been shown to be associated with an increased susceptibility to bacterial infection. A major cause of T-lymphocyte abnormalities after burn injury seems to be related to elevated levels of prostaglandins in the blood (especially PGE$_2$). It is probable that fungal infections, especially from candida species, are related to defects in T-cell function in burn patients, but the evidence remains fragmentary.

B-lymphocytes function primarily to synthesized immunoglobulins and antibodies to specific antigens. The antibodies of greatest importance in resistance to infection belongs to the IgG and IgM classes of immunoglobulin, IgG being more important. In very large burns, especially when sepsis has already occurred, the antibody response may be depressed.

Phagocytic cells:

Phagocytic cells may be classified as granulocytic or mononuclear. Both play important roles in resistance to infection, but neutrophils are responsible for primary defense against most bacterial infection.

Acquired and hereditary defects in the function of neutrophils have been associated with infections caused by
common pathogens, namely staphylococcus aureus, Aspergillus and gram-negative enteric bacilli. Both number and functional integrity of neutrophils are necessary for resistance to infection and when they number less than 500 neutrophils per cubic millimeter bacterial infection frequently occur, Neutrophil function is often abnormal in patients with severe burn injury and the severity of the abnormality has been related directly to the incidence and severity of systemic invasion by bacteria.

Mononuclear phagocytes play a less well defined role in resistance to infection in man. Recent work has shown that mononuclear phagocytic cells have critical regulatory roles for both lymphocytes and granulocytes. They may have both facilitatory and suppressor functions and they secrete numerous regulatory products including interleukin 1 (IL 1), complement components and prostaglandins.

Opsonins:

Opsonins are substances in the blood that promote ingestion and intracellular killing by phagocytic cells. The process of opsonization is initiated by a reaction between specific antibody and antigenic determinants of the cell walls of microbes. This in turn may activate the classical or alternative pathway of complement. When activated these
serum proteins mediate inflammation and tissue damage, and facilitate ingestion of microorganisms by phagocytic cells. The complement system plays a role in bacterial activity, neutralization of virus, opsonization leading to phagocytosis of microorganisms. Fresh human serum has bacterial activity that is dependent on an intact complement system. Complement activation results in bacteriolysis, however, the functional properties of the Clavage products of complement activation may be more important in host defence against infection; than are the actual bacteriolytic reactions themselves.

A decrease in the complement components C$_3$A and C$_5$A occurs in the circulation following thermal injury suggesting that thermal injury nonspecifically activates the complement system. Activation of complement in the injured tissue results in an inflammatory response due to the release of histamine and serotonin by C$_3$A and C$_5$A. Thus, edema formation in the injured tissue may be complement dependent. In addition, a consumptive opsoninopathy of C$_3$, proper in, and Factor B occurs following thermal injury. Thus, if complement activity is suppressed, the response of chemotactic cells and subsequent phagocytosis is also depressed.

Within a few hours following burn, injury, the exudation of
serum proteins into the injured tissue results in pronounced
call in serum levels of many opsonic proteins, especially
IgG. In addition, complement is activated via the
alternative pathway. However, these components
characteristically rise to normal or above during the second
post burn week because of an increased rate of synthesis.
Severe active infection may result in a fall in opsonic
proteins because of consumption. Perhaps the most important
cause of abnormally low serum immunoprotein levels following
burn injury; however, is malnutrition.
DEVELOPMENT OF THE CONCEPT OF SEPSIS:

Normal skin offers an effective barrier to the penetration of surface bacteria primarily because of its anatomic structure and antibacterial activity of its secretions. Injury due to burns destroys this barrier and provides a portal of entry. Alexander (1971), Kefalides et al (1960), Lowbury (1960).

The "Universal Challenge" posed by the thermally injured person has stimulated research in burns by workers from various disciplines and many valuable and unforgettable lessons have been learnt in sepsis, wound care and reconstructive surgery over the years, - [Dressler; (1971)].

The first mention of the problem of management of the burned patient are their in the works of Aulus Cornelius celus (100 A. D.). However, the first worker to comment on infection in burns was fabricus (1607). Who speaks of an "ulcus profundum et putridum" after separation of the burns slough.

In the early part of the nineteenth century sepsis present in burns and other surgical wards was believed to have been caused by absorption of putrid substances from wounds into blood, - [Alteimer, (1975); Dupuytren in (1833); (quoted in Brit. Med. Jour. 1 : 994 (1960) found abundant suppuration,
fever, wasting and death in patients with deep burns even when they were not extensive.

Bird, 1855 (Quoted in Brit. Med. Jour. 1:994 1960) said of the fever and inflammation in burned patients, that they were not essentially different from such symptoms after other kinds of injuries.

Many workers isolated microorganisms from pus without understanding their full implications. Notable amongst these were [Dupuytren (1855), Von Recklinghusen (1871), Klebs, (1872) - (Quoted in Post. Grad. Med. Jour. 48 : 338, (1972)].

However, the idea that microorganisms caused sepsis was not evolved until Schwann (1837) and Pasteur, 1863 - (Quoted in Davis Christoper Textbook of Surgery) asserted that putridity was a biological process due to growth of microorganisms in the wound.

Listerian Period: This was ushered in by Lister (1865) when he developed his technique of antiseptic surgery on the basis of microbial origin of sepsis. Yet Lister failed to extend his antiseptic discipline to the treatment of burns, probably because he felt that the defence of burns against contamination was impossible. However, some of his
contemporaries did attempt to extent antisepsis to the management of the burned patient.

The final crystallisation of the microbial concept of sepsis was due to the work of Koch, (1876) and Ogston, (1880) (Quoted in Text book of Surgery) who actually demonstrated cocci in chain and Cluster in pus. The detailed study of bacterial flora was not under taken until many years later.

PATHOGENESIS OF BURN WOUND SEPSIS:

Local and general injury caused by bacterial colonization of burn wound was recognised by various workers, Lutgarten (1891) and Stockes, (1903) (Quoted in Brit. Med. Jour. 1:994, (1960)).

Now it is an accepted fact that a sterile burn wound scarcely ever exist and carefully taken cultures invariably show a variety of microorganisms, - [Alexander, (1971)]. The organisms gains access to the burn wound either from the environment or from the patients own gastro intestinal tract. The high infection rate in the burns patient points to hospital infection as a more probable source of bacteria, - [Alexander, (1971); Lowbury, (1972)].

The burn eschar is a dead devascularized tissue which
provides a favourable milieu for the growth of microorganisms. Thus, the viable bacteria in burn wound increases with passage of time, - [Alexander, (1968); Arts and Moncrief, (1969), Bretano et al, (1967)]. Bacterial count depends on the thickness of eschar which itself depends on the temperature at which burn was made. Three hours after the burn injury has occurred, the bacterial count is similar to normal skin. It reaches a maximum three days after burns in a partial thickness burn and seven days after burn in a full thickness burn, - [Alexander, (1971); Fisher, (1969); Porkova and Konickova (1973) and Alexander, (1971)]. Types and incidence of organisms isolated from partial and full thickness burn are similar, although the number varies considerably. The average bacterial count in an infected partial thickness burn is $3.5 \times 10^7$, while that in a full thickness infected burn is $4.9 \times 10^8$, - [Baxter et al, (1971); Bretano et al, (1967); Clarkson et al, (1967); Georgiade et al, (1969); Lawrence et al, (1972); Loebl et al, (1974)].

Pruitt et al, (1968) felt that more than $10^6$ organisms/gm of tissue was an indication of severe infection, thus, making the burn wound a portal of generalized sepsis and also for toxicity from bacterial toxins.

Intra-eschar colonization is followed by bacterial invasion
of the underlying tissue and blood vessels with or without distant visceral lesions. Where there was no evidence of bacterial invasion of burn wound beyond the necrotic tissue, full thickness burns were seen to heal within 28 days, - [Grover and Lawrence, (1971)].

Local infection of the burn wound can convert a second degree burn into a full thickness burn by thrombosis of dermal vessels or bacterial destruction of the remaining epithelial tissues and thus, causes delayed healing. [Alexander, (1971); Fisher, (1969); Porkova and Konickova, (1973)]. Purulent discharge, offensive odour, oedematous granulation, toxicity and increase in bacterial count are all signs of infection, - [Alexander, (1971)].

Defining difference between colonization and invasive infection of the burn wound is no easier than defining the difference between bacteremia and septicaemia. Invasive burn wound sepsis can best be equated to penetration of bacterial growth into viable tissue beneath the eschar. Deep colonization of bacteria and constant invasion of bacteria into neighbouring healthy tissue, seems to be a major factor which leads to progressive deterioration of patient in cases of burns. Such colonization may result from multiplication of bacteria preexisting in the deeper parts of the dermis or from invasion of deeper tissue by
bacteria on the surface, - [Mon Crief et al, (1964); Pellar et al, (1973)]. The number of bacteria present in the burn eschar or in the biopsies of burn wound is one of the best objective signs of the presence of sepsis. The number and types of bacteria in the burn wound are of importance in the development of sepsis.

A slough which separates by the third week forms a hospitable medium for many bacteria. Here, the bacteria can multiply undisturbed by natural defences and are protected against the action of antibiotics. After the slough has separated, the surface of the burn consists of granulation tissue on which, Streptococcus pyogenes or Staphylococcus aureus flourish. Gram negative bacteria however, grow less well in serum and appear to be at a disadvantage, - [Colebrook et al, (1960)]. Larger the area of burn and longer is the time of healing the greater is the likelihood of colonizaion by pathogenic organisms such as Pseudomonas aeruginosa and relatively transient saphrophytes such as Clostridium Welchii, - [Lowbury, (1960), Sutter et al, (1966)]. Development of defence against sepsis in burns varies with age, extent of burn and interval following injury and hospitalisation.

Acute burn injury causes a generalized vascular response involving both burnt and non-burnt areas resulting in
accumulation of water and plasma proteins in extra vascular spaces. The host is unable to localize inflammatory cells in the burnt tissue. After the first few days, a layer of granulation tissue develops beneath the burn wound. A healthy base of granulation tissue can resist infection by $10^6 - 10^7$ bacteria per gram of burnt tissue. Resistance is related to the ability of the capillary network to sequester a large number of phagocytic cells. This capability is decreased as granulation tissue is replaced by scar tissue partially explaining the late susceptibility of the burned patient to infection, - [Alexander, (1971)].

During the first two to four days there is a fall in gammaglobulins, plasma proteins and complement, which return to normal by second week. Failure to attain normal levels is usually associated with increased catabolic states and is commonly seen in sepsis. The patient usually die, - [Auturson et al, (1969); Liljedehl et al, (1963)].

Abnormalities of cell mediated immunity and antibacteiral function of the neutrophils have been shown to be major factors in the development of sepsis, - [Alexander, (1971); Munster, (1970)].

Thus, compared with specific fevers and with other infections which arise in healthy tissue. Infection of
burns presents a confused picture. This is due to various factors. Most important is difficulty in determining which pathogenic changes are caused by infection and which by the trauma caused by the burns. Mixed rather than pure cultures are isolated from burns and many burns heal well even when heavily colonised.

MICROBIAL FLORA OF BURN WOUNDS

Various stressful forces, such as extensive use of antibiotic therapy have influenced the pattern of microorganisms causing sepsis in the thermally injured patients.

Prior to the development of Sulfonamides and discovery of Penicillin the most common causative agents of burn wound sepsis were B-haemolytic Streptococci, Pneumococci and Staphylococcus aureus, - [Alteimer et al, (1962); Pruitt et al, (1971) Boswick, (1973)].

Pack (1926), [Quoted in Brit. Med. Jour. 1:994, (1960),] demonstrated Streptococci and Staphylococci in pus from burn patients. Aldrich (1933), Cruikshank et al, (1935) - [Quoted in New Eng. J. Med. 208-299, (1933)] also demonstrated Streptococci from the burned patient and occasionally Streptococci were demonstrated from Cardiac blood of fatally infected patients.

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In the early nineteen forties infection caused by haemolytic Streptococci was controlled by penicillin and thus, penicillin resistant Staphylococci became the predominant organism in the infected burn wound. Marsh (1935) and Heggie and Heggie (1945), and Longohr et al, (1947) - [Quoted in Brit. Med. Jour. 1:994, (1960)] were all able to demonstrate Staphylococcus aureus as an important pathogen in burn wound sepsis. There was a time however, after the discovery of penicillins when Staphylococci infections were well controlled. However, as certain strains began to produce penicillinase, they soon developed penicillin resistance and became the predominant organism found in burn wounds, till the early nineteen hundred and fifties. - Thomsen (1973).

During this period Pseudomonas organisms were ubiquitous to the burn wound and of little significance.

With the development of newer broad spectrum antibiotics and synthetic penicillins, gram positive infections were controlled and gram negative infections now began to predominate in burn wounds, - [Lowbury, (1972); Thomsen, (1973)]. Amongst the gram negative infections, Pseudomonas aeruginosa was the worst offender. - [Alteimer et al, (1975); Pavkova and Konickova, (1973); Pruitt et al, (1971)].
Clinical picture of gram negative burn infection was studied by Moncrief et al, (1964), who discussed the difficulties of isolating the bacteria. Stone (1966) and Pruitt et al (1968), also demonstrated the increasing incidence of gram negative septicaemia in burned patient. Development of these gram negative infections occurred in most cases while the patient was on antibiotic therapy. - Alteimer, (1973). Continued use of broad spectrum antibiotic led to the development of resistant strains. - [Kefalides et al, (1960)].

Staphylococci again made an appearance in the burns ward by the nineteen sixties. Rivera et al, (1956) and Clarkson and Greenwell (1958) - [Quoted in Ann. Surg. 178:436 (1973)] were the first to demonstrate multiresistant strains of Staphylococci in burned patients. Haynes et al, (1960) demonstrated that the isolation of Staphylococcus aureus had increased from 30 % in 1951 to 89 % by 1959, with increase in the multiresistant strains. Macmillan (1964) and Thomsen (1973) demonstrated multiresistant strains of Staphylococcus in the burns ward. These strains were usually isolated after antibiotic therapy, but quite often even without the use of that particular antibiotic to which resistance had been developed. Thomsen (1973), from burns unit, Copenhagen, isolated Staphylococcus aureus from 80 % of his patients. Out of the Staphylococci isolated, 94 %
were multiresistant. This character of multiresistance was often characteristic of a phage type, which was hospital acquired. The antibiotics to which multiresistance was demonstrated, was often related to the antibiotics used in that hospital, - Decoul et al, (1965) Quoted in Acta. Path. Microbial., Scand. B, 79/3:320.

With the institution of tropical therapy of the burn wound, sepsis caused by pseudomonas and other bacterial infections was controlled but mycotic infections increased. Most common fungal agent was candida albicans, - [Albano, Schmitt (1973), Alteimer et al, (1973)]. There was an initial stage of fungal colonization followed by invasion into the burn wound and later septicaemia and systemic mycosis.

Increased incidence of candida in burn wound was noted and reported by Mancrief et al, (1968) and Nash et al, (1971).

To summarise the present day flora, infected burns show the following features:

1 - An increase in gram negative infections.
2 - Secondary infection following antibiotic therapy.
3 - Increasing infections by gram negative bacteria formerly recognised as having little or no virulence.
4 - Infection by fungi and viruses.
Infection in burns has been studied from various angles by different research workers. In a continuing study, over a period of twenty years from 1942 to 1962, comprising of 1828 cutaneous burns patients, treated in the department of Surgery at the University of Cincinnati and the Cincinnati General Hospital, Altemier et al, (1962) made observations regarding the predominant and most important infecting bacteria. In their series the predominant and most important bacteria were Staphylococcus aureus, Streptococcus pyogenes, Proteus and Pseudomonas aeruginosa. These organisms accounted for fifty percent of all bacteria recovered from the infected burn wound and represented ninety percent of the bacteria causing death by infection complications.

In the period between 1942-1944 there was a high incidence of haemolytic Streptococcal and pyogenic Staphylococcal contamination. In early burn wound in their series they recorded a significant increase in the incidence of strains of Staphylococcus pyogens from twenty percent to fifty percent. Over the years, the incidence of Streptococcus pyogens gradually decreased. This reflects the general
pattern of change of organisms in the post-penicillin era, - [Altemeir et al, (1962)].

The infection pattern showed a gradual change in subsequent years. In the series of Altemeir et al, (1962), commonest organisms were Gram negative organisms especially Proteus and Pseudomonas aeruginosa. In their continuing study they observed that there was no relative decreased in the incidence of gram negative bacteria particularly Proteus and Pseudomonas aeruginosa. They noted a continuing high incidence of these two groups of bacteria after institution of antibiotics and chemoprophylaxis. This was seen progressively over the three week post burn period. This was more significant when it was recalled that these organisms were frequently found as contaminants in the initial wound cultures.

In the study of Lowbury, (1960) the examination of wound was done from the time of admission. He observed, initially up to a few hours the skin to be sterile due to burns. After 24 hours the bacteria were sparsely present as contaminants growing in superficial areas of skin. Different types of organisms were seen in the initial stages. Lowbury observed that during first two to three weeks period gram negative bacilli were predominant especially Pseudomonas aeruginosa and Proteus. These organisms were accompanied by
Staphylococcus aureus, Micrococcus, Streptococcus faecalis and Diphtheroid bacilli. Only occasionally Streptococcus pyogenes was seen, which was predominant in unhealed wounds. These organisms were conspicuous by appearing late in unhealed wounds. Penicillin resistant group II (Type 80) Staphylococci were also common. Lowbury (1960) observed an interesting finding that Proteus, Klebsiella and E. coli grow well in the initial phases of slough formation and when slough separated and fresh serum exudes out gram positive cocci grow better.

In series of Sengupta et al, (1972) incidence of Staphylococcus pyogenes infection was more than 50 percent. They feel that this may be due to the spread from hospital infection.

In subsequent years all the reports showed decline of Staphylococcal infection with significant rise in gram negative bacterial infections. Pseudomonas aeruginosa was the predominant of all.

Increasing incidence of Pseudomonas aeruginosa was also noted by several other research workers. Fatal Pseudomonas aeruginosa infection in severely burned patients initially was reported in England by Jackson and his associates, (1951). In a study of 275 burnt patients in Peru, Markley
et al, (1957) reported that Pseudomonas aeruginosa infection was the major cause of death after the initial two day post burn period. The experience at the Surgical research unit indicates a growing importance of these organisms in severely burnt patients.

Robin et al, (1961) in an autopsy and bacteriological study of 38 patients demonstrated Pseudomonas aeruginosa infection as a contributory factor in the death in 14 cases.

Infection originating in devitalized burn tissues extends as cellulitis, lymphangitis and finally invades blood stream, - [Sengupta et al, (1972)].

Pseudomonas aeruginosa poses a special problem in hospital infection, since it is an ubiquitous organisms present in soil, water and in many moist environments. Also, it is unique among pathogens because it can infect any living being, ranging from plants through invertebrates to human beings, - [Shrinivas, (1977)].

Pseudomonas aeruginosa has a special ability to survive in water and moist objects. Infection in healthy persons, except newborn, are nearly always trivial and superficial and are usually associated with warm, moist environment.
Ps. aeruginosa has always been an important human pathogen, and many workers believe that it causes more infections in hospital now than formerly, - [Forkner et al, (1958); Finland et al, (1959); Barber, (1961)].

Sengupta et al, (1976) reported Ps. aeruginosa as the leading cause of hospital infection. By the end of the 19th century Ps. aeruginosa was recognised as the cause of highly fatal septicaemic disease in man, - [Williams and Camercon, (1876); Brill and Libman, (1899)].

The interest of Ps. aeruginosa was centered mainly on its tendency to cause septicaemia and death in extensively burnt patients, - [Jackson et al, (1951); Markley et al, (1957); Tumbusch et al, (1961)] and in those whose defences were weakened by leukemia and other malignant disease as well as by the immunosuppressive treatment, - [Forkner et al, (1958); Margaretten et al, (1961)].

It was observed that Ps. aeruginosa appears rather late in hospital infection and if not controlled invades blood stream posing a great threat to prognosis of the patient, [Pierson and Fellar, (1970); Sengupta et al, (1972)].

The resistance of Ps. aeruginosa to almost all the antibiotics used in hospital is an important factor by which
the organism gains a selective advantage over others and the factor undoubtedly contributed to the relative and sometimes absolute increase in Ps. aeruginosa infection in the past years, - [Yow, (1952); Asay and Kock, (1960)].

Ps. aeruginosa are present in the bowel, nose or skin, but more so in the environment. Liljedahl et al, (1972) reported that in several cases Ps. aeruginosa was present for a long period of time in the ward environment without colonising patients being treated for open, extensive and recent burns.

The chief source of infection with Ps. aeruginosa is the open wounds of infected patients and burn cases in the ward (Barber, 1961). The bacteriology of burns differs from that of general surgical wounds.

Sevitt (1955) reported the conditions for the invasion of Ps. aeruginosa to be present in the more extensively burnt patient. Epidemic infections occured where burn provided both a continuing source of cross infection and a target for fresh infections, - [Lowbury and Fox, (1954)].

Infection of burn wounds is an integral part of burn accidents and a great deal of research has been carried out with it, - [Tumbush et al, (1961); Markley et al, (1957)].
The pigment pyocyanin produced no toxic effects in the animal tests but concentrations of it much lower than those found in the infected burns were reported toxic to tissue cultures of human skin, - [Cruickshank and Lowbury, (1953)] and according to Jackson et al, (1951) this may contribute in the failure of skin grafts of burn wounds.

Equipments that contain standing water or retain moisture is a common source of contamination with Ps. aeruginosa and this was reported by many workers in different equipments such as air-cooling apparatus (Anderson, 1959), resuscitation equipment for premature infants (Bassette et al, 1965), respirators (Phillips and Spencer, 1965). Urine bottles and bed pans (McLeod, 1958) and brushes (Ayliffe et al, 1965). Whitby and Rampling (1972) found that certain objects such as sink, taps, floor cloths, mops were frequently contaminated, on the other hand when similar areas were examined in domestic homes, Ps. aeruginosa was rarely isolated.

The organism can produced infection through vegetables also, Kominoz et al, (1972) were able to isolate Ps. aeruginosa on the hands of kitchen personnel as well as cutting boards and knives they used. Which suggested acquisition of the organism through contact with the vegetables.
MICROBIAL AGENTS RESPONSIBLE FOR NOSOCOMIAL INFECTIONS OF NEWBORN AND BURNS

It is common knowledge that a major change has taken place in the etiology of infectious diseases in the past two decades. Formerly specific infections were the greater problem. These diseases have not disappeared but opportunistic infections attributed to the organisms formerly believed to be "harmless" have proved to be a serious problem. These opportunistic pathogens have become a continuing and growing problem in hospital population.

Among the various organisms causing hospital infection Staphylococci and Gram negative bacilli are posing a special problem because of their resistance to many antimicrobial drugs.

The incidence of infections caused by relatively less virulent Gram negative micro-organisms such as Pseudomonas aeruginosa have began to increase appreciably. Though Pseudomonas aeruginosa inhabit the gastrointestinal tract or colonize on the skin, they seldom cause illness in humans who have competent immunity. In contrast, infections with Pseudomonas aeruginosa occur in patients with antibiotics and immunosuppressive therapy. Indiscriminate use of
antibiotics promotes the growth of relatively resistant Pseudomonas aeruginosa without competition.

Immunosuppressive drugs diminish host defence and "open the door" to Pseudomonas aeruginosa invasion. Because of its tendency to grow or at least survive in moist environment, its relative resistance to antimicrobial drugs, insensitivity to common disinfectants and more over its ability to grow in disinfectants like Cetavelon, Pseudomonas aeruginosa infection is a special hazard to the patients who are hospitalized.

It causes hospital infections of burns, wounds, urinary tract, respiratory tract. It has a tendency to invade blood stream, leading to septicaemia. Pseudomonas aeruginosa is also isolated from equipment which retains moisture, especially respirator and resuscitator. Patient with severe burns, patients undergoing major surgery, those who are receiving radio therapy and immunosuppressive therapy are "high risk" patients for Pseudomonas infection and infection in these patients is severe and may terminate in death.

A rational approach to the nosocomial infection by Pseudomonas aeruginosa is to study ecology and epidemiology of the infection. Several epidemiological typing methods have been used to place markers, or epidemiological
fingerprints, and to trace the source of infection. The methods currently used for typing of Pseudomonas aeruginosa are serological typing, bacteriophage susceptibility and bacteriocine production and sensitivity. The purpose of all these methods is to establish the relationship between the patient and environmental strains, and if this can be done the epidemiology of Pseudomonas aeruginosa infection can be defined.
DETERMINANTS OF INFECTION

**FIG. I** - In normal state, all the determinants (bacteria, environments and host defence mechanism) intersect at a point indicating zero probability of sepsis.

**FIG. II** - Increased dose of bacteria leads to sepsis.

**FIG. III** - Altered host defence mechanism leads to sepsis.

Figures modified from Meakins et al. (1980) 167
Pseudomonas aeruginosa

Pseudomonas aeruginosa was first named by Schroeter in 1872. Gessard first recognised the organism Pseudomonas aeruginosa in 1882 that it can be pathogenic to man and named it Bacillus pyocyaneous, Bacillus aeruginosa was the name given by Trevisan (1885), Pseudomonas pyocyanea by Lehmann and Neumann (1896) and Pseudomonas aeruginosa by Migula (1900), - [Bergey, 1974].

According to Bergey's manuual (1974) the Genus-Pseudomonas has been defined as pseudo-monas, or pseudo-monas Gr. pseudes false, Gr. monas a unit, monad; pseudomonas a false monad, ae. ru. gi. nosa means full of copper rust or verdigris, hence green.

Daudoroff et. al. (1968) have studied extensively the toxonomy of this genus. Pickett M. J. and Pederson M. M., (1970 b&c) again revised the toxonomy to be applicable to strains of Pseudomonas maltophilia, all others are oxidase positive and with rare exception are motile by means of polar flagella. Flagellation is rarely useful in identification of this genus. Several species are pigmented, but non pigmented strains of these species are frequently encountered. - [Pickett and Manclark (1970)].

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By carrying out analysis by Gower's coefficient Sheath et al. (1981) showed five major grouping in Genus Pseudomonas

(a) The fluorescent pseudomonads.

(b) Biochemically active species (p. cepacia, p. pseudomallei and allies).

(c) Moderately active free living species (p. acidovorans, p. alcaligens and allies).

(d) p. solamacecearum and allies.

(e) p. mallei, p. diminuta does not appear to be clearly distinct from vesicularis nor does p. alcaligens there may however be some difference between p. multivorans and p. cepacia.

The current classification of bacterial species belongs to genus Pseudomonas has been revived by Gilardi (1985). After several years of exhaustively observing various phenotypic characteristic has been divided the Pseudomonas species in six major groups:

1 - Fluorescent

2 - Stutzeri
Parallel to his work has been that of Pallezoni and others (1984), who have proposed a taxonomy based on RNA/DNA homology studies. The Palleroni classification has been presented in Burgey’s Manuel, 1984 which places the pseudomonads into one of five RNA homology groups, which in turn include several smaller DNA homology groups. There is sufficient parallel between the genotypic and phenotypic characteristic of Pseudomonas species for Gilardi to provide a combined classification.

Classification:
RNA group:
Fluorescent group.
p. aeruginosa
p. fluorescens
p. putida
Acidovorans group-
Stutizeri group-
p. stutzeri
p. mendocina
pseudomonas sepcies group-I

**RNA GROUP II**
pseudomallei group
p. pseudomallei
p. cepacia
p. sladioti
p. picketti

**RNA GROUP III**
p. acidovorans
p. testosterzoni
p. delavieldii

**RNA GROUP IV**
Diminuta Group
p. diminuta
p. vesicularis

**RNA GROUP V**
p. (Xanthomonas) maltophilia

The international committee on the Nomenclature of Bacteria, Judicial commission (1970) has accepted the name Pseudomonas
aeruginosa in place of pseudomonas pyocyanea, - [Shriniwas, (1974)].

ANTIGENIC STRUCTURE :

Bovin and Mesrobeanu (1937) extracted from P. aeruginosa with trichloracetic acid a Lipopolysaccharide hapten. The polysaccharide has a common core, and the side chain are composed predominantly of amino sugars that determine O antigenic specificity, - [Koneman et al, (1988); and Jawetz, (1989)].

O antigen can be recognised by precipitation reaction with acid or formamide extracts (Christie, 1948) or by tube or slide agglutination reactions with boiled or live bacterial suspension.

Habs (1957) described 12 'O' types, Lanyi and Bergan (1978) propose the later, and recognise 13 serologically unrelated 'O' group in P. aeruginosa : 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15, nine of which can be subdivided giving a total of 27 serologically distinguishable 'O' type.

The 'H' antigen of P. aeruginosa are difficult to distinguish from other heat labile antigen except by demonstrating specific inhibition of motility by the
corresponding antibody, - [Lanyi, (1970); Jawetz, (1989)].

CHARACTERIZATION:

Medically Important Pseudomonads:

Fluorescent Group:

Production of water soluble pyoverdin pigments that fluoresce under short wavelength (254 nm) ultraviolet light, - [KonemanEW, (1988)].

i) Pseudomonas aeruginosa:

Pseudomonas aeruginosa is the pseudomonad most frequently recovered from clinical specimens.

Pseudomonas aeruginosa is a gram negative bacillus 0.5 - 0.8 by 1.5 - 3.0 µm, actively motile with single polar flagellum, grows well on ordinary laboratory media. Pseudomonas aeruginosa can grow on 0.3 % cetrimide agar, - [Lowbury and Collins, (1955); Pandya and Bhatt, (1975)] have used 1 percent Dettol agar as selective medium.

The characteristic colony of Pseudomonas aeruginosa is irregularly round, diffuse, 2-3 mm in diameter with matt surface, - [Wahba and Darrel, (1965)]. There may be
different types which include rough (R), gelatinous (G),
Smooth rough (SR), Mucoid (M) and dwarf (D), - [Phillips, (1969)].

Buhlmann et al, (1961) described ability of pseudomonas aeruginosa to grow at 42°C is an important characteristic of the organism. No strains of pseudomonas aeruginosa grows at 4°C, - [Phillips, (1969)].

Most strains of Pseudomonas aeruginosa give characteristic fruity odour. (Habs and Mann, 1967). Attack many carbohydrates oxidatively (Hugh and Leifson, 1953). Many strain liquify gelatin (Kohn, 1953) and reduce nitrates to nitrite within 24 hours, - [Brown and Lowbury, (1965)].

The strains of Pseudomonas are oxidase positive (Kovacs, 1956) and arginine dihydralase positive (Thornley, 1960). Phillips (1969) reported that Pseudomonas aeruginosa reduces tetrazolium salts and this character is not exhibited by other pseudomonas. All strains utilizes citrate as a source of carbohydrate.

Most of the strains of Pseudomonas aeruginosa produce water and chloroform soluble pigment pyocyanin and fluorescent pigment insoluble in chloroform, fluorescin (King et al, 1954) some strain also produce pyorubin a dark red pigment;
10 to 15 percent strains are non pigment producers; though they produce a pigment when grown on special media, - [Cruickshank, 1975).

Pseudomonas aeruginosa is known to be a frequent contaminant of respiratory apparatus, humidifiers, drainage bottles and other hospital equipment and is highly resistant to many disinfectants and antibiotics, - [Dube and Shriniwas, (1965); Routree and Beard, (1968)].

Pseudomonas aeruginosa is present in intestinal tract in a variable proportion of a normal human beings. Ringen and Drake (1952) reported intestinal carrier rate of 11 percent, Lowbury and Fox (1954) of 3 percent; Darrell and Wahba (1964) from rectal swab 8.2 percent and shooter et al, (1966) 12 percent in general population.

The carrier rate of Pseudomonas aeruginosa in hospital patients is higher. It raise from 24 percent on second day of admission to 36 percent after stay in hospital for 3 days or more. The stools examined from students, nurses and Lab staff gave only 4 percent carrier rate (Shooter et al, (1966)]. Sutter and Hurst (1966) reported carrier rate in stools 3-10 percent.

Srivastava et al, (1975) observed that carriage rate of
Pseudomonas aeruginosa from hospital staff was very low and does not play role in transmission of hospital infections.

Hospital infections due to contaminated lotions, creams, instrumentation, resuscitation equipment is well documented, - [Ayliffe et al, (1966); Ridley, (1958); Noble and Savin, (1966); Bassett et al, (1965); Phillips and Spencer, (1965) and Phillips, (1966)]. Urine bottles and bed pans may be other sources of infection of endemic nature, - [McLeod, (1958)].

Broadly speaking Staphylococcal and Streptococcal infections are caused primarily by cross infection, where as infections due to gram negative bacilli are by environmental or self infection. The environment plays a very important role in Pseudomonas aeruginosa infections, - [Shriniwas, (1978)].

Pseudomonas aeruginosa has been known to be an opportunistic which invades the host tissue and establishes infections most readily when the host is already weakened by other causes such as burns, malignancy or diabetes mellitus. The factor which determine the virulence of the organism is however, very little understood, - [Pinghui Liv and Charles B Mercer, (1963)].

Pseudomonas is an organism of low pathogenicity but has
acquired great importance in wound sepsis, specially in burn wound infection. The general resistance of burn patient is very much lowered due to many known and unknown factors. There is a large raw area for the bacterial growth. Wide spread and extensive use of antibiotics, has decreased the population of sensitive organisms, giving an opportunity to other resistant organism; like Pseudomonas to proliferate. The incidence of Pseudomonas aeruginosa infection in burns reported by Sengupta et al, (1972) and Altemeir et al, (1962) was 24 percent and 50-55 percent respectively.

Pseudomonas aeruginosa infection is now adays hospital acquired by the wide use of broad spectrum antibiotics, corticosteroids, antimetabolites etc. (Williams Williams and Hyans, 1960). The incidence of hospital infection of all kinds in India varies from 8 percent to 33 percent as against 3 percent to 15 percent in USA and that due to Pseudomonas aeruginosa from about 5 percent to 30 percent in USA of the total infections according to different published reports, - [Wahba, (1977); Shrinivas, (1977 a)].

Epidemiological Study of Ps. aeruginosa :

Pseudomonas aeruginosa can causes a wide variety of infection especially in the compromised host. The incidence of hospital infection due to this organisms has increased in
the recent years. Therefore, it is important to determine the sources and routes of infection by this organism, so that measures may be taken to control and prevent the infection. Several methods such as phage typing, serotyping and bacteriocine typing have been used to characterize the strains. However, there is no unanimity of opinion regarding the selection of typing systems and it remains more a matter of opinion (Madhubala et al, 1977).

Aeruginocine (pyocine) typing:

Most of the strains of Pseudomonas aeruginosa produce a substance known as pyocine which inhibit the growth of the other strains of the same species. Pyocine first described by Jacob (1954) and studied in detail by Hamon (1956) belongs to the class of substances known as bacteriocines.

The bacteriocines are bacteriocidal substances apparently protein in nature, which are synthesized by some strains of bacteria and are active against some other strains of the same or closely related species (Nomura, 1967). Narrow specificity of aetron and being protein in nature, bacteriocines are distinguished from most of the classical antibiotics, - [Nomura, (1967)].
Homma and Suzuki (1961) showed that pyocine and the protein moiety of Pseudomonas aeruginosa endotoxin were chemically and immunologically related if not identical. Pyocine may be present naturally or can be induced by treatment of culture with ultraviolet light, hydrogen peroxide, mustard gas, and mitomycin. Pyocine was found to be stable at room temperature and 4°C as well as 60°C for 10 minutes.

Pseudomonas aeruginosa produce a variety of pyocines. These pyocines have been designated as R, S or P based on their physiological nature and ultrastructure.

The 'R' pyocines are high molecular weight substances non-diffusible and trypsin sensitive (Bradley, D. R. 1967). The 'R' pyocines have been studied in detail and are shown to be
rod like structures resembling the contractile tails of T-even phages.

The 'S' pyocines have not been studied in detail and the available evidence indicate that they are protein molecules with molecular weight of about 75,000, - [Kageyana, M and Kgami, (1962)].

Available studies suggest a strain of Pseudomonas aeruginosa may produce both 'S' and 'R' pyocines. Both the pyocines seem to be induced by the action of mitomycin (C). Pseudomonas aeruginosa strains belonging to similar pyocine types have been shown to produced pyocines of different biological characters. The differentiation of S and R pyocine activity may be of an epidemiological significance and it may assist in the discrimination of isolates clustered in particular aeruginocine type, - [N. Venkateswara Sarma, Shriniwas and R. V. Sriniwas, (1977)].

A common feature of bacteriophage and bacteriocine is that they adsorb to the specific receptors on the cell surface. Pyocine stops the synthesis of DNA, RNA and protein in a sensitive cell. One particle adsorbed to the cell wall is sufficient to kill the bacterial cell, - [Jacob, (1954)].

A. H. Wahba (1963) noted in cross streaking experiments that
an area of growth of an indicator strain often occurred in the centre of the zone of inhibition suggesting that Pseudomonas aeruginosa strains produce pyocines as well as other substances which antagonise their action.

According to Hamon (1956) pyocine are also protein in nature. Wahba (1963) described that pyocines were inactivated similarly by strains of Pseudomonas aeruginosa, strains of Proteus vulgaris and the proteolytic enzymes trypsin which provided some evidence that the inactivating substances are proteinases.

Holland (1962) found that the presence of bacterial slime protected otherwise sensitive organism against the lethal activities of megacine, the bacteriocine produced by Bacillus megaterium. Indicator strains which may in fact be sensitive to the bacteriocine may with abundant slime production appear to be resistant.

Hamon (1956) studied in detail of pyocines and found that pyocine can inhibit the growth of other strains of same species and it is active against quite closely related organisms only.

Holloway (1960) suggested that there is a considerable variation in pyocine activity of different strains within
the species and it could be used as a basis for a typing scheme. He described a method of grouping Pseudomonas aeruginosa by using lysogenicity and isolated 18 groups from 214 strains. This formed additional basis for the differentiation of the strains.

Wahba (1963) found that production of pyocines depends on the medium used, time of incubation and temperature of incubation. For investigation of the production of pyocines by various strains of Pseudomonas aeruginosa, Wahba, (1963) devised a nutrient agar by incorporating $10^{-5}$ molar iodoacetic acid, 0.1 percent sodium citrate and 0.1 percent dipotassium hydrogen phosphate for suppression of the pyocine inhibiting substances. The pyocine production is stable characteristic which is not lost on repeated subcultures or prolonged storage and might form the basis of typing system of Pseudomonas aeruginosa, - [Wahba, (1963)].

Darrell and Wahba (1964) developed that first pyocine typing system for Pseudomonas aeruginosa using a set of 12 indicator strains, tryptone soya agar containing iodoacetic acid, sodium citrate and dipotassium hydrogen phosphate as the typing medium and on incubation temperature 37°C. By this technique the authors recognised pyocine types namely A, B, C, D, F, G, K, L, O, P and a group which was non typable. The set of indicator strains used by authors were
M8, B10, S17, B26, B39, A52, 8/39, 10/55, H1/180, M283, H323 and E826. Later Wahba replaced strains H1/180, H323 and E826 by strains 584, 577 and 593 (Matsumato et al, (1968)].

The authors typed the Pseudomonas aeruginosa isolated from the patients and ward surroundings. 94 percent of dust of the burn wards and 86 percent of surgical ward isolates were of type B. Nontypable strains were found to be large in stool specimens (19 percent). There was preponderance of type B in the urinary tract infection. Only 7.6 percent of the strains were untypable by this method.

Based on Darrell and Wahba’s technique Gillies and Govan (1966) proposed another typing system using tryptosoya blood agar as typing medium, 8 different indicator strains and an incubation temperature of 32°C. For pyocine production and 37°C for rest of the procedures.

By this technique the authors recognised 37 pyocin types (1-37) and were able to type 88.4 percent of their strains. Govan and Gillies (1969) in addition to usual 8 indicator strains used five more indicator strains (A-E of Pseudomonas pyocyanea) and thus, were able to differentiate 8 subtypes in the most common isolate type-1.

Sjoberg and Lindberg (1967) using Wahbas’ indicator strains
could type 320 strains into 40 patterns out of a total 356 strains they tested. Kohn (1966) reported a simplification of pyocine typing using a cellulose acetate.

Kumari et al, (1974) found Wahba and Darrell’s modified sierra medium to be the best medium for pyocinin production.

Bergan (1968) found it necessary that all the bacterial material was removed before the indicator strains’ inoculation.

Farmer et al, (1969) devised a method to trace cross infection by Pseudomonas aeruginosa. Unknown strains were induced to produce pyocine by mitomycin C or lysis by phage. The lysates were then tested against 27 selected indicator strains and the zones of clearing were differentiated as to killing pyocine or lysis by phage.

Osman (1965) worked on the inhibitory effect of different pyocines to differentiate Pseudomonas isolates. He used the name pyocine to indicate the filtrate free of bacteria by filtering through the sintered glass filter and the filtrate having inhibitory activity against the Pseudomonas. He found that typing on the basis of pyocine sensitivity is both stable and reproducible Murakami (1970) did the typing of 236 strains by Gillies and Govan’s method using human and
ox blood and found that typability rate increased by 10% when ox blood was used. Zabranski and Day (1969) typed Pseudomonas aeruginosa by their ability to produce pyocine. The strains were isolated from urine, blood, sputum and stools. 80 percent of their strains were typable and were grouped into three major groups A, B, and D. There was no significant difference in the distribution of the types except that the urine specimens yielded the highest percentage of one type. By this procedure they were able to type 93 percent of their isolates.

Jacob et al, (1973) titrated the activity of 21 standard pyocines tested on 27 standard indicator strains and determine minimum effective concentrations (RTD) for all pyocines tested on certain indicator strains.

Shriniwas et al, (1971) using Wahba's indicator strains typed 300 strains of Pseudomonas aeruginosa isolated from clinical material. 85 percent of the strains could be typed by them and they found that maximum number of non typable strains were from stools and ear swabs. The authors reported that the method is fairly reliable and reproducible. Heckman et al, (1972) typed 639 strains by modified Gilles and Govan (1966) technique by replacing wire loop application, by streaking of indicator strains by 8 sterile glass rods which saved much time. By this method
they were able to type 88.4 percent of the strains. In their series 52.1 percent strains belonged to pyocine type-1, 11.6 percent to pyocine type 10 and 7.4 percent to pyocine type-3.

The principle and method of pyocine typing is simple but is faced with problem like variation in inhibition of test strains, indicator strains, auto and incomplete inhibition. The pattern of inhibition is reported to be changing, - [Tripathy and Chadwick, (1971)].

Govan and Gilles (1969) suggested a rapid control of the temperature and incubation time to overcome this difficulty. Wahba (1965) could differentiate a large number of pyocine pattern in a single serotype. He could find correlation between serotyping and pyocine typing, though the relationship was not very consistent. Matsumato (1968) did serotyping and pyocine typing of 266 strains of Pseudomonas aeruginosa and concluded that serotype 0-2, 0-3 (Verder and Evans, 1961) were the prevalent types. They also observed that specific sero and pyocine types persisted for a long time in the same focus.

Shriniwas (1974) has suggested that the use of term aeruginocine typing in place of pyocine typing because at the International Committee on the Nomenclature of Bacteria
Judicial Commission (1970) has accepted the name *Pseudomonas aeruginosa* in place of *Pseudomonas pyocyanea*. The author studied 1500 strains of *Pseudomonas aeruginosa* obtained from clinical material from six hospitals of different parts of India and found that only eight indicator strains namely, M8, B10, S17, B26, B39, A52, 8/39 and 10/55 were enough to classify the strains into aeruginocine types. A, B, C, D, F, G, K, L, O and P of Wahba (1964). Rest of the strains with varying inhibition pattern were further classified into 20 types designated as 1 to 20 on the basis of inhibition patterns found in more than one strain. There were still some strains left with uncommon inhibition patterns and were designated as unclassifiable. His isolates were type 1 (15.4 percent) and type A (8.1 percent) of Wahba.

Shriniwas (1975) did aeruginocine typing of 1000 strains of *Pseudomonas aeruginosa* isolated from heterogeneous clinical material and concluded that infection due to *Pseudomonas aeruginosa* in hospitals was caused by large number of different aeruginocine types. The most frequently isolated aeruginosa type was type 1 followed by type F and A.

Shriniwas (1976) on further typing of *Pseudomonas aeruginosa* strains not typable by aeruginosa typing method, observed whatever be the method of typing employed a certain proportion of non-typable strains were always encountered.
and their numbers varied depending on various factors like the source of the strains and method of typing employed.

Shriniwas (1974, 1975, 1976) by testing several bacteriocine typing systems found aeruginosa typing systems based on Darrell and Wahba’s system using 8 indicator strains which were subsequently increased to 10 indicator strains (Shriniwas and Menon, 1977). Tryptone glucose extract agar as typing medium and an incubation temperature of 32°C throughout the typing procedure was more suitable for typing isolates from this region. The use of additional indicator strains (AIIMS 785/76 and AIIMS 790/76) to existing set of indicator strains of Wahba gives better discrimination than the phage or serotyping methods and reduces clustering of aeruginocine types and nontypability of strains [Shriniwas and Menon, (1977)].

The authors observed that the nontypability was reduced from 21.1 percent (using 8 indicator strains) to 15.8 percent (using 10 indicator strains). Similarly the clustering in type F was reduced from 20.3 percent to 15.3 percent. The other common types 1, A, 3 and 21 were represented by a maximum of 7.7, 1.0, 1.5 and 3.8 percent strains respectively.

Chug et al, (1977) typed 426 human and 50 animal isolates of
pseudomonas aeruginosa by using 8 Wahba's indicator strains. The percentage of typable strains was high being 82.9 percent. Aeruginocine types 11, 9 and G were common both in human and animal material. The typing system was stable and reproducible on storage for several weeks. Naidu (1977) in her studies on aeruginocine typing of pseudomonas aeruginosa reported 77.5 percent strains typable with incidence of type-1 (23.2 percent) type-P (16.2 percent) and type-21 (11.8 percent). Her 17.4 percent strains were unclassifiable. No association was observed between aeruginocine type and the sites of infection.

Chug and Sabharwal (1978) typed 835 clinical isolates of Pseudomonas aeruginosa by using 8 indicator strains of Wahba. The typability was 78 percent, 23.7 percent belonged to pyocine type of Wahba, 32.9 percent to additional aeruginocine types (W.H.O., 1976) and 24.4 percent were unclassifiable. The in vitro reproducibility of typing was perfect in 100 strains on storage at 4°C for 16 weeks. The in vivo stability of typing was poor as only in 10 percent of cases, same aeruginocine type was seen in 3 replicate specimens collected from the same patient and the same site.

Indrani et al, (1978) carried pyocine typing of strains of Pseudomonas aeruginosa isolated from clinical specimens and hospital environment as per the technique out lined by
shriniwas (1974) with a single modification in the typing medium by incorporating Wahba's trial (Iodoacetic acid, Sodium citrate and dipotassium Hydrogen phosphate) which are meant to suppress the pyocine inactivating substances produced by the organism (Wahba, 1963). Out of the total 200 strains tested 166 strains were typable and 34 strains were non-typable. Among the 166 typable 101 strains belonged to definite pyocine types of Shriniwas with pyocine type F in the lead (32 percent) followed by type-1 (22 percent) type - 21 (17 percent) type 11 (7 percent), type - 2 (6 percent), type-p (4 percent), type-8 (2 percent) and D, L, 9, 10, 12, 14 and 19 (1 percent) each. 65 percent of strains fell in the category of unclassified type.

Jain et al, (1980) typed 203 strains of Pseudomonas aeruginosa by using 10 indicator strains (1 to 8 of Wahba and No. 9 and 10 of AIIMS New Delhi), and observed that a total of 56 inhibition patterns were produce by 124 typable strains. (61.1 percent), using 8 indicator strains a total of 44 inhibition patterns were produce by 124 typable strains. The common aeruginocine types encountered were type-A (7.8 percent) and type-1 (4.9 percent). The use of two additional indicator strains, viz AIIMS 785/76 and AIIMS 790/76 did not increase the typability but increased the discrimination.
Baveja et al, (1979) did aeruginocine typing of 647 strains of Pseudomonas aeruginosa according to Shriniwas method and reported 51.9 % strains unclassifiable, as well as lack of correlation between source of isolates and the aeruginocine type.

Shriniwas (1977) studied 517 strains of Pseudomonas aeruginosa typed by aeruginocine typing using ten indicator strains, phage typing by techniques of Blair and Williams using 21 phages and serotyping using 18 antisera. Out of total 517 strains 431 strains (83.3 percent) were typable by aeruginocine typing and produced 105 inhibition patterns with a maximum of 62 strains in F1. Typability by phage typing method was only 400 strains (77.3 percent) which were differentiated into 198 patterns. Four hundred and fifty strains (87.0 percent) were typable by serotyping.

Several typing methods based on serology, phage susceptibility as well as production of/or sensitivity to bacteriocines have been proposed for epidemiological typing of Pseudomonas aeruginosa either alone or in combination. However, there is no unanimity of opinion regarding the selection of typing system and it remains a matter of dispute (Madhubala et al, 1977). The authors in their study typed 200 strains of Pseudomonas aeruginosa by serological typing and aeruginocine typing and found that combination of
two methods increased the discrimination and typability considerably.

By observing such variability in results of classification, some workers suggested mnemonic method to increase the classifiability.

Farmer (1970) reported a mnemonic method of classification which greatly simplified the reporting of typing results. Paramsivan et al, (1978) did the protiocine typing applying mnemonic classification and this method of classification is useful for aeruginocine typing if 12 indicator strains are used.

Thus, the mnemonic method of classification can increased the classification to 100 %.

Aeruginocine typing method has drawbacks of high percentage of unclassified strains (Chug et al, 1977) high degree of clustering into a few common types. - (Gillies and Govan, 1966), Lack of in vivo stability (Kumari et al, 1974) and the time required to complete the test (Darrell and Wahba, 1964).

Out of numerous methods some methods used for recognising epidemic strains suffered from disadvantage of poor
discrimination, phage typing and pyocine typing gave better discrimination but less than complete reproducibility, - [Sutter and Hurst, (1966); Bergan, (1968)].

Discrimination was improved by the use of combined typing methods such as serology plus pyocine typing (Wahba, 1965) or pyocine production plus pyocine sensitivity, - [Farmer and Herman 1969, Tagg and Mushin, 1973].

However, this typing method has a high in vitro stability (Mushin and Ziv, 1973) and it is technically simple. So that it can be easily adapted by a clinical bacteriological laboratory, - [Shriniwas, (1974)].

Farmer and Herman (1974) found the value of pyocine typing to be well established in the study of nosocomial infections, however, in some situations misleading results occured.

Thus, aeruginocine typing has been recommended as an useful epidemiological tool in tracing the sources and rates of hospital cross infections with Pseudomonas aeruginosa.
II SEROLOGICAL TYPING:

Serological typing was found useful in the studies of hospital infection [Lowbury and Fox, (1954); Gould and McLeod, (1960); Wahba, (1965); Bassette et. al. (1965)].

According to Loiseau Marolleau (1973), the serotype provided evidence of mainly endogenous origin of the infection in hospital.

The typing of Pseudomonas aeruginosa on the basis of antigenic characters is one of oldest methods for typing these organisms, and a number of serotypes have been recognized by various investigators since 1926 [Fisher et. al. (1969)].

A satisfactory scheme of classifying Pseudomonas aeruginosa on the basis of heat stable 'o' agglutinogens was first put forward by Habs (1957) and he differentiated Pseudomonas aeruginosa into 12 serotypes.

These typing methods are mainly based on the somatic antigens; some workers have attempted to increase the discriminatory power by using flaggler antigens in addition to 'o' antigens, in an hierarchial manner [Shriniwas, (1977 a)]. Earlier attempts to prepare typing sera were hampered
by the use of antigens which did not stimulate good production of antibodies. [Lowbury and Fox, (1954)].

In 1957, Habs differentiated Pseudomonas aeruginosa into 12 serotype by agglutinating antiser a prepared in animals immunized with boiled suspensions of Pseudomonas aeruginosa and showed that the serological typing of Pseudomonas aeruginosa was practicable if suitable methods were used for preparation of sera. Verder and Evans (1961) developed a similar typing scheme. From these and other studies wider range of typing sera has been developed.

There are now more than 6 'national' serotyping systems used by the different regions of the world: Habs's system in Europe; Verder and Evan's system in North America; Lanyi's system in Hungary, Hommas system in Japan; Meitert's system in Romania and so on and there is considerable overlapping between these systems [Shriniwas, (1977 a)].

Fisher et. al. (1969) carried out the study by slide agglulitation using 7 Parke-Davis lipopolysachharide factor antiserum, and they could group 342 strains into 7 immunotypes. A similar type of study was carried on by Zierdt (1975). Al Dujaili and Harris (1974) used commercially available antisera. Wahaba (1965) used different antigens of various workers namely Habs's,
Verder's, Sandvik's and his own strains. In their study, four types (1, 2, 5 and 6) predominated, accounting for more than 60 percent of cultures tested. Agarwal and Talwar (1976) attempted to classify hospital strains by serological typing methods using unabsorbed 'o' sera.

Agarwal and Talwar (1976) found that 19.7 percent strains are non-typable, whereas Baveja et. al. (1977) could not type 16.7 percent of strains. The highest typability was observed by Madhubala et. al. (1977), who found 95 percent of the strains typable.

Some hospital strains showed cross reactions with Hab's 0:2 and 0:5 antisera and were placed in 0:2/5 group. Such overlappings were also observed by Hab's (1957) and Mikkelson (1970).

Wahba (1965) and Bergan (1972) did not observe the cross-relations as they used adsorbed sera.

Sero group 0:13 was usually encountered from bovine sources [Sandvik (1960)] and rarely from human sources [Mikkelson, (1970)] and [Bergan (1972)].

Diaz et. al. (1970) reported that when serological typing was possible, mucoid and non mucoid isolates from the same
specimen belonged to the same serotype.

Classification of Ps. aeruginosa on the basis of six 'H' antigenic factors [Parker et. al. (1976)] using inhibition of migration through nitrates agar [Pitt and Bardley, (1975) appears promising but needs further evaluation.

Recently a 16 antiserum slide agglutination has been developed from Habs system by Psudomonadaceae sub-committee of the American Society for Microbiology, chaired by P. V. Liu [Zierdt, (1975)]. This typing set is proposed as a reference set for general distribution. Typing sera using these antigens are commercially available from Pasteur Institute, Paris.

Though for from perfection, serotyping is fairly reproducible and easy to perform. However the test suffers from a poor discriminatory power, in addition to non availability of sera commercially in India, and expenses, labour and time involved in raising the sera. [Shriniwas, (1977 a)].

Agarwal and Talwar (1976) concluded that distribution of large numbers of strains into a few serogroups, limits the suitability of the test as a single method, but it may be useful in combination with phage typing.
III PHAGE TYPING:

The method of phage typing uses as its index the phages carried in latent or Lysogenic forms by strains to be typed [Holloway, (1960)]. We recognise a phage as lysis if it produce even one plaque upto confluent lysis on the unknown strains [Zierdt, (1975)]. Most strains carry many phages. Shionoya and co-authors (1972) found 10 phages by one Pseudomonas aeruginosa strain. Pyocines are very well differentiated from phages [Holloway, (1960)].

Pseudomonas aeruginosa phages are quite mutable. However, this can be overcome by keeping propagation minimal through large batch preparation and lyophilization and maintaining same host range [Zierdt, (1975)].

Phage typing of Pseudomonas aeruginosa was carried out by many workers in the last two decades. Different workers used different sets of phages and there are more than 10 sets of phages used so far, for typing [Shriniwas, (1977 a)].

Phage typing of Pseudomonas aeruginosa was studied by Gould and McLeod (1960), Graber et. al. (1962) and Shooter et. al. (1966). Gould and McLeod (1960) could show clear correspondence between typing by agglutination and typing by
phages. Garber et. al. (1962) found that certain groups of Pseudomonas phage types were most commonly isolated in all burn infections.

Shooter et. al. (1966) studied faecal carriage in hospital patients and tried to correlate the possible spread from patients to patient by using phage typing method.

Phage typing method was successfully used in epidemiological studies by many workers [Ayliffe et. al., (1965) and (1966); Sutter and Hurst, (1966); Phillips, (1966)]. Phage typing gives numerous pattern of reactions which are usually epidemiologically significant.

Postic and Finland (1961) using a set of 13 temperate phages at RTD could type 88.8 % of 161 hospital strains of Pseudomonas aeruginosa.

Garber et. al. (1962) used 21 phages and typed 92 % of 443 human isolates. Lindberg et. al. (1964) selected a set of phages and could classify 92 % of 1100 strain received from various countries, while Sjoberg and Lindberg (1968) using 18 phages originally isolated by Lindberg, could type 91.6 % of 667 strains of Pseudomonas aeruginosa.

Bergan (1972 evaluated various phage sets available and
analysed the lytic patterns obtained by each.

Two sets that are commonly employed now-a-days are Lindberg set, comprising of 20 phages which are primarily obtained from lysogenic cultures and Bergan's set of 22 phages [Shriniwas, (1977 a)].

Typing of *P. aeruginosa* was also attempted by Lysogeny [Holloway, (1960), Feary, et. al. (1963), Paterson, (1965)] as 70 % to 100 % were found to be lysogenic.

Ito and Kageyama (1970) isolated a bacteriophage related to R type of pyocines from a lysogenic strains of *Pseudomonas aeruginosa* and named ps₃.

Bergan (1972) reported on a new *Pseudomonas* phage typing set based on numerical allocating procedures of the lytic spectra of phages selected from previous typing sets.

Bergan and Listad (1972) indicated that difference in one and occasionally two reactions were consistant with a common origin of strains.

Wretlind et. al. (1973) did not find any difference in the characters studied between strains isolated from blood and strains isolated from other parts of body.
Bacteriophage typing of Pseudomonas aeruginosa was reported to be a useful tool in the sub classification of strains isolated from patients with burn wound infections [Lindberg and Latta, (1974)].

Agarwal (1976) evaluated the role of phage typing in grouping hospital strains of Ps. aeruginosa by using a set of phages received from Colinadale and observed that for each phage there were some other phages with which it was commonly associated and some with which it was rarely associated.

Standardization of phage typing is essential if results obtained from different laboratories are to be compared. An internationally accepted set of phages with their propagating strains is a must for reliability of the typing system [Shriniwas, (1977 a)]. Although the discrimination is better with phage typing, it has a greater degree of variability. Further it is too cumbersome to perform. Therefore it is less suited for epidemiological studies [Shriniwas, (1977 c)].

RIBOTYPING OF P. AERUGINOSA:

Accurate investigation of the nosocomial epidemiology of P. aeruginosa have not been possible, mainly because the only
typing methods available until recently (serotyping, phage typing and bacteriocine typing) have poor discriminatory power and use phenotypic markers which are relatively unstable.

With advent of molecular typing methods, studies have been carried out using genetic markers. When applied this to Ps. aeruginosa, these methods have shown that these markers were stable and that typability and reproducibility were very good.

Restriction fragment length of polymorphism of ribosomal DNA regions (ribotyping) of Ps. aeruginosa was evaluated as a tool for epidemiological purpose. Using four selected restriction enzymes Bam HI, Cla I, ECo RI and pst I, the typability and reproducibility of the method reached 100 %.

Dominique et. al. (1993) evaluated RNA gene restriction fragment analysis (ribotyping) for the typing of Ps. aeruginosa. They found the ribotyping method is very good since both the typability and reproducibility reached 100 % and another important feature is stability of a marker.

In their study they used, ribotyping method with four RES. Which proved to be valuable for the epidemiological investigation of Ps. aeruginosa. It confirmed that the
clinical isolates of the outbreak in the burn unit all belong to the same strain. The water system of bath was initially suspected on the basis of epidemiological data, serotype and pyocine type. However ribotyping suggested that this was probably not the source of the epidemic. Since environmental and clinical isolate belongs to different ribotype. This illustrate the value of ribotyping in providing precise data in Ps. aeruginosa outbreaks.

SEROLOGICAL AND IMMUNOLOGICAL DIAGNOSIS:

Shen, Brackett, et. al. (1981) showed specific Pseudomonas Immunoglobulin E antibodies in sera of patients with cystic fibrosis. Fernandes and his associates (1981) also showed, antibodies to cell envelope proteins of Pseudomonas aeruginosa. These serum antibiodies did not protect the cystic fibrosis patients against infection with Ps. aerugionsa.

Ashdown, (1981 a) stated the relationship and significance of specific immunoglobulin ‘M’ antibody response in clinical and subclinical melioidosis. Study indicates that the IgM immunofluorescent test may be useful in the treatment of the infection, since the result of the test were generally negative 3 to 6 months after administration of chemotherapy appropriate for melioidosis.
Ashdown (1981 b) in his another study demonstrated human antibodies to Ps. pseudomallie by indirect fluorescent antibody staining. He further stated that demonstration of specific IgM may be value in differentiating active from inactive infection.

There is evidence for autoantibody production associated with polyclonal B-cell activation by Pseudomonas aeruginosa [Garzelli, Campa et. al. (1982)] Wanger et. al. (1986) showed antibody by counter immunoelectrophoresis in the diagnosis and management of Pseudomonas aeruginosa bone and joint infection.

The coagulation test is a useful procedure to screen patients suspected for pulmonary infection caused by Ps. aeruginosa, providing a presumptive diagnosis when the result is positive. This procedure detect a soluble Pseudomonas aeruginosa antigen 5 in bronchial sceretion by a coagulation test. [Sofianon and Doumboyas, (1989)].

Pressler et. al. (1990) showed IgG subclass antibodies to Pseudomonas aeruginosa in sera from patients with chornic Ps. aeruginosa infection investigated by ELISA. They stated that, in the early stage of chornic Ps. aeruginosa infection, antibody titres in all four classes i.e. IgG, IgG₂, IgG₃ and IgG₄ were significantly higher. Elevated
levels of IgG$_2$ and IgG$_3$ antibodies to Ps. aeruginosa are a sign of poor prognosis of cystic fibrosis.

In patients with wound infections the amount of avidity of IgG antibodies to exotoxin A (ExA) and 7 Fisher's immunotypes of lipopoly saccharides were measured by ELISA. Significant increase in the amount of avidity of the antibodies to ExA in a majority of sera and an increase in amount of antibodies to LPS immunotypes 4 in sera of patients with moderate infection. [Trafny; Girzy bowski; Patzer et. al. (1991)].

Pfaller, Barrett, Koontz et. al. (1989) evaluated a direct fluorescent monoclonal antibody test (DFA; Genetic systems corporation, Seattle, Washington) for the detection of Ps. aeruginosa in blood culture broths obtained from patients. The DFA method can be performed in 50 minutes and appears promisingly as a rapid method for identification of Ps. aeruginosa bacteraemia.

ANTIBIOTIC SENSITIVITY

Although bacteria were observed almost three centuries ago, their significance in the production of diseases was not established. About 100 years ago, the work of Louis Pasteur (1822-1895) established the definite relationship between
the disease and the bacteria. Lord Lister (1827-1912) the professor of surgery at Glasgow University, considered that the microorganism so prevalent in the air, might be responsible for the frequent occurrences of inflammation and suppuration after surgery. In 1867 he revolutionised surgery introducing antiseptic technique. Wounds were sprayed and washed with carbolic acid during operations and subsequently they were protected from the air by dressings. The results which followed by this procedure were remarkably successful. Later Antiseptic surgery was replaced by "Aseptic surgery".

The 'Golden age' of antimicrobial therapy began with the mass production of penicillin in 1941.

Following the discovery of streptomycin from streptomyces collected from all over the world were tested and precious antibiotics like chloramphenicol, chlortetracycline and tetracycline hydrochloride etc. were discovered.

Pseudomonas aeruginosa is resistant to commonly used antibiotics in the hospital [Dube and Shriniwas, (1965); Tinne et. al. (1967)] and this is an important factor by which the organisms gain a selective advantage over others and is enabled to flourish where it is most likely to cause clinical infection. [Fekety and Murphy, (1972)].
Susceptibility of Pseudomonas aeruginosa to antimicrobial drug is low. Frank et. al. (1950) carried out antibiotic sensitivity of Pseudomonas aeruginosa and found that the penicillin and bacitracin were ineffective, chlortetracycline and chloramphenicol were active against most of the strains, polymyxin B and D were effective against all strains while a significant number was streptomycin resistant.

Until recently the only antibiotics with some therapeutic value in the treatment of Pseudomonas aeruginosa infections have been polymyxines [Lowbury, (1968); Kumari et. al. (1974)]. But polymyxine activity in vitro has only limited correlation with their in vivo efficacy [Lowbury (1968)].

Jackson et. al. (1951) found polymyxin 0.1 percent local spray or cream was found most effective in preventing the colonization of Pseudomonas aeruginosa.

Samuel, S Wright et. al. (1954) studied the cultural and biochemical characteristic of 110 strains of Pseudomonas aeruginosa recently isolated from the patients at the Bostan city Hospital and each was tested for susceptibility to antibiotics. The authors reported polymyxin B was the most active agent, oxytetracycline ranked next, it was somewhat more active than chlortetracycline against most of the strains.
A large proportion of strains were moderately sensitive to streptomycin and neomycin. Typical strains of Pseudomonas were moderately or highly resistant to erythromycin and chloramphenicol. When they compared these with the strains isolated in 1949; the recent ones showed a significant increase in the percentage of strains resistant to tetracycline, streptomycin and neomycin. The increasing resistance to tetracycline and neomycin was believed to be result of cross resistance with chlortetraycline and streptomycin respectively.

Farror (1954) successfully treated cases of Pseudomonas aeruginosa infections of external ear with polymyxin. Trapnell (1954) reported that isolates of Pseudomonas aeruginosa from meningitis were sensitive to chloramphenicol, polymyxin B and E and oxytetracycline.

Asay and Richard (1960) observed that 92 percent isolates were sensitive to polymyxin, 42 percent to streptomycin and only 5 percent to chloramphenicol. Postic and Finland (1961) reported that polymyxin B and colistin were equally effective against the organism; chloramphenicol was effective against 40 percent of strains.

Fekety et. al. (1962) reported that 93 percent of Pseudomonas aeruginosa strains tested against polymyxin and
colistin revealed comparable antimicrobial effect, tetracycline had bacteriostatic effect against 19 percent isolates and chloromycetin against none. Streptomycin was effective against 28 percent and kanamycin was effective in high concentration against 9-15 percent of Psudomonas aeruginosa strains. By disc diffusion technique (2-10 mcg/disc) 32 percent of Pseudomonas aeruginosa strains were sensitive to colistin whereas by tube method (5 mcg/ml) 95 % strains were sensitive. This variation in effectiveness of drug may be due to poor diffusion of antibiotics in agar plates.

Until the introduction of gentamycin and carbenicillin in late 1960's the antipseudomonas therapy was ineffective. Since then numorous reports have documented the value of carbenicillin and gentamycin [Jackson, (1967); Sharma et al, (1969); Alder and Finland (1971); Sengupta et. al. (1975); Bansal et. al. (1976)]; the use of these durgs for the therapy of such infection has increased - [Sengupta et. al. (1975)].

However occasional studies have reported the emergence of strains highly resistant to gentamycin as well as carbenicillin, or the both [Chattopadhyay, (1975); Lowbury et. al. (1969)].
In vitro synergism has been observed for the combination of gentamycin and carbenicillin against a majority of isolates [Andriole (1971) and (1974); Brumfitt et. al. (1967)]. The observations of in vitro synergism of carbenicillin and gentamycin has led to clinical trials with apparent success in the same patients [Smith et. al. (1970); Schimpff et. al. (1971); Riff et. al. (1971)].

Bhaskaran and Ram Mohan Rao (1963) in their study of the sensitivity pattern of the gram negative bacilli isolated from the urinary tract infection reported that the strains of Pseudomonas aeruginosa were sensitive to streptomycin 14.3 percent, chloramphenicol 14.3 percent and resistant to other antibiotics used.

Stone et. al. (1965) reported successful clinical trial with Gentamycin in treatment of infected burn wounds with gram negative bacilli including Pseudomonas.

Dube et. al. (1965) reported that 88 percent of the strains of Pseudomonas aeruginosa were resistant to all the commonly used antibiotics and this was an important factor by which the organism gain a selective advantages over others and is enabled to flourish where it is most likely to cause clinical infection [Finland et. al. (1959); Fekety and Murphy (1972)].
Banerjee and Arya (1968) reported ineffectiveness of streptomycin, tetracycline, chloramphenicol, nalidixic acid and nitrofurantoin against all strains of Pseudomonas aeruginosa.

Sharma et. al. (1969) in their study on Garamycin found that at a strength of 30 microgram per disc, 313 strains (72.7 percent) out of 432 strains were sensitive to that drug. The authors recommended plate dilution technique for gentamycin and showed that out of 242 strains 70.7 percent had MIC of 6.25 mcg/ml, 18.6 percent had MIC of 12.5 mcg/ml whereas balance of 9.7 percent strains were resistant to gentamycin.

Bhujwala (1969) reported on antibiotic sensitivity of strains of Pseudomonas aeruginosa isolated from urinary tract infection as 88.6 percent to mandalmine, 37.9 percent to streptomycin, 24.1 percent to pyroldinomethyl tetracycline, 37.4 percent to tetracycline hydrochloride and 10.3 percent to chloramphenicol.

Lowbury et. al. (1969) on study of 1452 strains of Pseudomonas aeruginosa found that strains were more commonly sensitive to polymyxin B and gentamycin.

Gohain et. al. (1969) reported that all strains of
Pseudomonas aeruginosa isolated from urinary tract infection were sensitive to streptomycin, gentamycin and polymyxin B but were resistant to chloramphenicol. Chitkara (1969) reported that 100 percent his isolates were sensitive to polymyxin B and sensitivity to streptomycin, ampicillin, chloramphenicol and nitrofurantoin was 18.2 percent, 4.5 percent and zero percent respectively. Kumari et. al. (1973) tested 100 strains of Pseudomonas aeruginosa for antibiotic sensitivity and reported that polymyxin B was the most effective drug (100 percent) followed by Kanamycin (44 percent), Sulphanamides (40 percent), chloramphenicol (33 percent), streptomycin (28 percent) and neomycin (15 percent).

Antibiotic sensitivity of 300 strains of Pseudomonas aeruginosa was studied by Shriniwas et. al. (1971). The authors reported that 100 percent sensitivity of organisms to polymyxin B. Response to other drugs was streptomycin 44.6 percent, chloramphenicol 12.6 percent and tetracycline 9 percent.

Shriniwas (1976) in his study on antibiotic sensitivity of Pseudomonas aeruginosa, found 2 to 3 percent of his strains were sensitive to all antibiotics, 10 percent strains to three antibiotics and 39.3 percent to two antibiotics. The author also found that urinary strains were resistant to
chloramphenicol and tetracycline and less sensitive to streptomycin.

Shriniwas and Srinivas (1977) reported that 99.6 percent - 100 percent strains were sensitive to polymyxin B and 95.7 - 98 percent to gentamicin. The authors also found that 98 - 100 percent of their strains were resistant to chloramphenicol, 96.9 - 99.8 percent strains resistant to tetracycline, 61.4 - 68.4 percent of strains resistant to septran. Resistant pattern to streptomycin and carbenicillin varied from 17.5 percent to 23.1 percent and 3.1 percent to 14.3 percent respectively.

Bhatia (1977) found sensitivity to antibiotics as to polymyxin B 100 percent, streptomycin 44.6 percent, and chloramphenicol 12.6 percent. Resistance to gentamycin and cepholoridine was 15.4 percent each.

Prabhakar (1980) in the study on antibiotic sensitivity of 400 strains of Pseudomonas aeruginosa found 97 percent were sensitive to gentamycin, 26 percent to kanamycin, 11 percent to ampicillin, 38 percent to streptomycin, 23 percent to chloramphenicol and 15 percent to tetracycline. No correlation was observed between aeruginocine types and antibiotic sensitivity.
Usha Udgaonkar et. al. (1985) in the study on post operative wound infections found that Pseudomonas aeruginosa was the most resistant organism amongst gram negative bacilli. The authors reported that 50 percent sensitivity of organisms to gentamycin. Response to other drugs was streptomycin 12.1 percent, kanamycin 9 percent and ampicillin 4.5 percent.

For some years the only useful antibiotics against P. aeruginosa were colistin and gentamycin, both of which are more less toxic. Carbenicillin is most toxic, is valuable but highly resistant strains have appeared.

Carbenicillin is used in life threatening situations in combination with gentamycin. Recently introduced tobramycin and amikacin are highly useful against gentamycin resistant strain; both are relatively toxic. Dudley et. al. (1991) compared effect of simultaneous versus staggered administration of ciprofloxacin with azolocilin. The simultaneous regimen constantly provided the greatest extent of killing all strains, particularly those, which are resistant to ciprofloxacin. The simultaneous dosing of an anti-Pseudomonal B lactum with ciproflox proved to be potentially useful against pseudomonas aeruginosa.

Kikuchi et. al. (1992) evaluated the clinical efficacy of a combination therapy of ceftazidine (CAZ) and tobramycin
(TOB) for intractable pulmonary infections mainly caused by Pseudomonas aeruginosa. In pneumonia, the overall efficacy rate was 60 %, in chronic respiratory tract infection caused by Pseudomonas aeruginosa, the efficacy rate was 82.6 %.

Clinically significant infection with Pseudomonas aeruginosa should not be treated with single drug therapy as the success rate is slow with such therapy and also because the bacteria can rapidly develop resistance when single drugs are employed [Jawetz et. al. (1989)].

The penicillins most useful against Ps. aeruginosa are ticarcillin, mezlocillin and piperacillin. These antibiotics should be used in combination with an aminoglycoside usually gentamycin, tobramycin or amikacin. Other drugs active against Pseudomonas aeruginosa includes aztereonam imopenem. The newer quinolones including ciprofloxacin and the newer cephalosporin including cflaperone and ceftriaxone. The susceptibility pattern of Pseudomonas aeruginosa vary geographically and susceptibility test should be done as an adjunct to selection of antimicrobial therapy. [Jawetz et. al. (1989)].

Very recently a vaccine has been prepared against Pseudomonas aeruginosa and was useful in same cases.
Recently, treatment of Pseudomonas aeruginosa infection with pyocines has been attempted experimentally on mice [Morrikin and Terry (1972); Haas et. al. (1974)]; but results obtained are not encouraging [Williams, (1976)].

Uppal, Prakash and Sharma (1982) studied 100 strains of Pseudomonas aeruginosa isolated from different clinical samples for their antibiotic resistant pattern. It showed a high incidence of multiresistance with Ampicillin, Chloramphenicol, Carbenicillin streptomycin, tetracycline. 50 strains (25 each from pus and urine) subjected to R-plasmid study showed the presence of R-plasmids in 33 (66 %) strains : 28 conjugative and 38 % non conjugative. A greater diversity of plasmids was seen among isolates from pus than urine.
Among the various organisms causing hospital infection Staphylococci and gram negative bacilli are posing a special problem because of their resistance to various antimicrobial drugs. Since the introduction of Penicillin in 1941, the Staphylococci had persistently displayed a potential to develop resistance to virtually every new anti staphylococcal antimicrobial drug discovered.

Septic infection due to Staphylococcus aureus is world wide in distribution and is of particular importance among hospital patients.

Staphylococcus aureus has been considered by some workers as the most important single organism responsible for hospital infection. [Clarke, (1957) and Browne et. al; (1959)]. Although Staphylococcus aureus was also one of the commonest organism as regards wound infection in the pre-antibiotic era, (Herschfield, 1941), Since the advent of antibiotics Staphylococcus aureus has become the most difficult organism in the wound infection, [Wasek et. al; (1965)].

Even though in recent years the gram negative organisms are being more frequently isolated from cases of hospital infection, staphylococcal nosocomial infection still poses a
Outbreaks of wound sepsis in epidemic form in some countries due to Staphylococci have been reported by various workers. [Shooter et. al. (1956); Mcdonald and Timbury, (1957)]. From time to time maternity units suffer periods when the prevalence of infection is sufficiently raised to justify the term epidemic [Williams (1959)].

Staphylococcus aureus infection in burns are common but surprisingly delay in healing less than they do in incised wounds, [Williams et. al; (1966)]. Invasive infections of the burn with Staphylococcus aureus or S. epidermidis have a more insidious course, often with two to five days elapsing from the onset of symptoms to a full-blown infection. Early dissolution of the granulation tissue is characteristic in burn wounds. Patients become disoriented, are usually hyperpyretic with a leukocytosis and frequently develop a gastro-intestinal ileus. Shock may occur and is sometimes accompanied by renal failure. It is well to remember that Staphylococcus aureus usually cannot be eradicated from the wound until it is covered by graft or replaced by another pathogen, - [J. Wesley Alexander, 1987].

Infections caused by S. epidermidis once uncommon, are being
seen with alarming frequency in many burn units. These bacteria are often quite resistant to antibiotic therapy.

Many other Staphylococcal diseases that occur in hospital patients are not associated with a specific surgical operation. Some may follow diagnostic or therapeutic procedures such as sepsis following injection transfusion, venus cut-down and in small proportion after cathatherisation, - [W.H.O. Technical Report, (1968)].

Staphylococcal pneumonia of infants tends to affect the premature or sickly but epidemics may occur among groups of healthy newborn infants in hospital, [Guthic and Montogomery, (1947); Browning, (1955); Disney et. al; (1956)].

Purulent blepharitis in infants is also commonly due to Staphylococcal infections, - [Williams et. al; (1966)].

General infection may take the form of a septicaemic with evidence of non suppurative damage to the liver, kidneys and lungs, [Powel (1961 a)]. Staphylococcal septicaemia or pyaemia often occurs in debilitated hospital patients, - [Smith and Vickers, (1960); Powel, (1961 b)].

Although Staphylococcus produces various types of infections
the nosocomial infections of Staphylococci are more common and so the investigations for the sources of Staphylococci in hospitals are necessary.

Staphylococci isolated from patients during hospital stay by Lowbury were predominantly of a single strain of phage group III (type 80). Eighty to hundred percent of Staphylococci were penicillin resistant.

In burn unit of Copenhagen, Thomson (1971) tried to find out the correlation between Staphylococcus aureus and transfer of infection. He found that the strains found in 1967 were different than those found in 1962. But any conclusion was not possible.

Hambraeus (1973) made studies regarding the dispersal of Staphylococcus aureus from burns patients, relation between nasal carriage by the staff and exposure to air borne Staphylococcus aureus and the transfer of the carrying particles within the ward. Nasal carriage rate by the staff correlated with the air count. The transfer of Staphylococci within ward was 6 to 20 times more than that would have been expected by air movement only.

Healthy carriage of Staphylococci is harmful and it is potent source of infection, [Williams; (1966)]. Miles et.
al. (1944) and Barber et. al. (1949) showed that the carriage rate amongst hospital staff was much higher than in general population.

The nasal carriage rate of pathogenic Staphylococci in the hospital staff in this country has been reported between 20-95 percent by various workers [Chitale (1956); Sayed et. al. (1959); Hardas et. al. (1964); Varma et. al. (1965)]. Doctors and nurses are a special danger to their patients as there are several studies showing that the staff working in hospital have a higher carriage rate, [Ghosh Ray and Walia, (1962); Seth et. al. (1973); Talib et. al. (1973)].

The highest carriage rates are seen in young infants [Williams et. al. (1966)]. Hurst (1960) reported that infants due to their heavy Staphylococcal load are more responsible for contamination of the air as compared to adults.

Hambraeus (1971) found that staff nurses were carriers of Staphylococcus aureus of phage group III, which was found in most of the cases. Some of the cases also had same phage types weeks before actual infection indicating direct transfer and aerial transfer. Bed room air settle plate count was 1800 colonies per square meter. But corridor was low at that time.
Sources of Staphylococci in the environment are the beds, blankets of patients, dust from floors, ceiling, walls clothing etc. Also the contamination of gowns and uniforms worn in burn unit and the transfer of patients Staphylococci by means of nurses uniform was reported, [Hamberaeus, (1973)].

Smith et. al. (1973) isolated hospital strains of Staphylococcus aureus from patient's skin and nares after admission. But immediately after admission the hospital strains were not isolated from the same patients.

Earlier worker have suggested the anterior nares as the essential reservoir, but it has been found that Staphylococci get colonised on various parts of the skin, [Dyke, (1960)]. In an early survey the back of the wrist was found to be the most common site, - [Williams (1946)].

Septic skin lesions, dressings, plasters etc. also serve as sources of Staphylococcus aureus. The importance of contamination of floor dust and blankets with Staphylococcus aureus cannot be ignored. Experimental work has shown that the organisms do not lose their virulence fourteen days after being shed, [Williams et. al. (1966)].

Staphylococci in dust have greater powers of survival than
have gram negative bacilli, [Lowbury and Fox (1953); Mc Dade and Hall, (1963, 1964)]. Contaminated dust may be suspended in the air by activities such as bed making and sweeping of floors, [Aylifee and Barber, (1963)].

The problem of distinguishing pathogenic from non pathogenic Staphylococci is continuously being discussed and several criteria like source, haemolysis, chromogenesis and coagulase production have been put forward. Of these properties the value of coagulase test was well established and it has been universally accepted as a criterion for the identification of pathogenic Staphylococci, - [Jaykumar and Bhaskaran (1969)].

Most of the coagulase positive Staphylococci are responsible for hospital infections. Coagulase may contribute to pathogenicity by inactivating a bactericidal substance in normal serum or by protecting the cocci with fibrin barrier against phagocytosis, - [Cruickshank, (1976)].

The other characters which are absent in Staphylococcus albus strains and thus useful for identification of Staphylococcus aureus are pigment production, DNase activity, phosphatase, hemolysis, leucocidins, anaerobic fermentation of mannitol and the susceptibility to phages of the Staphylococcus phage typing set.
The presence of golden yellow colonies helps to make a presumptive diagnosis of Staphylococcus aureus in mixed cultures. The DNase production is seen in about 25% of coagulase negative strains and helps to rule out coagulase negative organisms.

The phosphatase test is again used as a screening test in mixed cultures. A few strains of coagulase negative Staphylococci may give a positive test. Almost all strains of Staphylococcus aureus for some type of hemolytic exotoxins. Most coagulase positive human strains from Alpha and Delta toxins while Staphylococcus albus strains do not form Alpha, Beta or Delta toxins.

In the reference laboratories factors such as nutritional requirements and cell wall structure are used as differentiating determinants. The Staphylococcus aureus strains do not require biotin for growth whereas the epidermidis strains utilize them. The Staphylococcus aureus strains have a substance protein A on their cell wall which is absent in other strains.

The production of a nuclease which was able to survive boiling was first demonstrated by Chesbro and Auborn (1967) to differentiate Staphylococcus aureus from other species which produce nucleases which are not heat resistant.
The differentiation of other Gram positive cocci from Staphylococci is often difficult especially as some Micrococci have a lot of similarities with Staphylococci. A few tests for differentiating them have been put forward. The presence in Staphylococci of Ribitol in the cell wall, and presence of peptide cross bridges help to separate the two. The DNA (Guanidine and Cytosine) (G+C) content of Staphylococci is about 30-40% whereas Micrococci have about 66-70%.

The glycine containing penta and hexapeptide of the cell wall peptidoglycan is a useful way to recognize Staphylococci in the laboratory. Thomas (1964) has shown a good correlation between lysis by the enzyme lysostaphin and anaerobic growth and fermentation of Gram positive, catalase positive cluster forming cocci. The lysostaphin endopeptidases attacks the glycyl glycine linkages in the cross bridges of the Staphylococcal peptidoglycans. The teichoic acid on the cell wall of Staphylococci is also used to differentiate it from Micrococci.

Evans and Kloos (1972) found a medium for distinguishing Staphylococci and Micrococci. It is of interest as most M. saprophyticus and M. Lactis strains with similar (G+C) in their DNA like Staphylococci, behave like Staphylococci in this medium.
Differences between Staphylococci and Micrococci are listed below:

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<th>Staphylococci</th>
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<td>1. Morphology from cubical packets</td>
<td>-</td>
<td>Variable</td>
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<tr>
<td>2. Growth anaerobically</td>
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<td>3. Acid from Glucose anaerobically</td>
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<td>4. Susceptibility to lysostaphin</td>
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<td>5. Erythromycin</td>
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<td>6. Furoxan</td>
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<td>7. NaCl 15 %</td>
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<td>8. Acid from glycerol anaerobically</td>
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Penicillin resistance among Staphylococci is of two kinds. That induced by laboratory procedures is usually accompanied by morphological changes and is associated with alternations in glutamic acid metabolism [Burrows (1963)]. Penicillin fast staphylococci isolated from infections usually show no morphological changes and produce penicillinase. This type of resistance is the only one of serious clinical importance, [Spink, (1951); Finland, (1955)].

The majority of typical hospital Staphylococci are multiple drug resistant and actively produce penicillinase [Ayliffe and Barber, (1963)]. Richmond and his colleagues (1964) drew attention to the fact that strains resistant to two or more antibiotics usually produce more penicillinase than
Monson et al. (1954) detected penicillinase production and modified by estimating destruction of penicillin - G by means of Hydroxamine method by Boxer and Everett (1949) and by a cellulose acetate membranes by Knox and Smith (1961). The significance of penicillinase production that is resistance by virtue of decomposing the antimicrobial agents is underscored by the sensitivities of penicillin resistant strains of Staphylococci to 2, 6 dimethoxy phenyle penicillin which is not degraded by penicillinase to penicilloic acid [Fairbrother and Taylor, (1961); Thomson et al. (1960)].

Staphylococcal penicillinase is inducible and is excreted by the organism into the medium. The amount excreted however varies considerably in different strains. By analysis of mutants obtained in the laboratory it has been possible to identify two genes controlling penicillinase production 'P' gene which determines the structure of the enzymes and gene 'i', which controls its inducibility.

Three antigenic varieties of penecillinase have been distinguished; A, B and C. Type B occurs only in phage Gr. II strains and type A and C in strains of Groups I and III. The type B enzyme is much less active and has a lower
substrate affinity than types A and C. This may explain the lower frequency as causes of human infections of phage Gr. II than strains of groups I and III [Wilson and Miles, (1975)].

Since the discovery of penicillinase production a number of methods have been described to detect and quantitate this enzyme. Abraham and Chain (1940) detected this enzyme from bacteria, which could destroy penicillin. Since then a number of modifications and techniques have been made.

Many methods have been described for the detection of B-lactamase production by bacteria. Initially these methods were devised for screening B-lactamase producing Staphylococci but more recently have been used for detecting B-lactamase production by gram negative organisms also. The methods are based on chemical changes in a sensitive substrate which takes place following hydrolysis on the B-lactum bond in penicillins and cephalosporins.

**IODOMETRIC TEST:**

Rosenblatt Jon and Newmann (1978) have developed a rapid, simple and reliable slide test (modification of the iodometric test) for demonstrating penicillinase production. the test is performed on an ordinary glass microscopic
slide, using colonies from agar plate media and is completed within five minutes. Results with this method compared well with minimum inhibitory concentration of antibiotics as determined by an agar dilution method. [Rosenblatt and Neumann, (1978)].

The iodometric assay for penicillinase production depends upon the reaction between chemical iodine and starch which produce a purple lavender colour. Iodine will also combine with penicilloic acid which is produced by the action of penicillinase on penicillin. When penicilloic acid is present in the test solution, iodine combines with it and being unavailable for reaction with starch, no purple colour develops when starch is added to the solution.

The slide test adaptation of this method utilizes solutions described by Workman and Farmer (1970), in their study of the agar plate modification of Perez’s iodometric assay, using culture of staph. aureus.

PAPER STRIP METHOD - [Wheldon and Mary, 1977].

The principles of this method are as follows:

Strains of Staphylococcus aureus that are able to produce penicillinase are induced to do so by growth in a
subinhibitory concentration of methicillin. The induced beta lactamase hydrolyses penicillin in the media to form penicilloic acid resulting in a fall of PH. A suitable indicator is used to demonstrate this PH change.

This acidimetric principle has been used previously in an agar overley method [Wong and Soo-Hoo, (1976)]. In the paper strip method however benzyl penicillin and an indicator are incorporated into paper strips. When dessicated and under optimum conditions and test strips and retain their sensitivity for many months.

A simple test for penicillinase production was also suggested by Orstavik and Odegard (1971). For this test the plates with a diameter of 14 cms. containing Mueller-Hinton medium were seeded by flooding with 3 ml of suspension of penicillin sensitive strain of Staphylococcus aureus diluted so as to give a dense but not confluent growth of colonies. Surplus inoculum was removed with a pipette and the plates were allowed to dry for one hour at room temperature. A solid streak of the strain to be tested was then made on the surface of the plate and a filter paper disc containing of 10 mcg penicillin G was placed in the middle of the streak. Following half an hour of prediffusion at R.T. the plates were incubated at 36-37°C and read 18-20 hours later.
Strains without penicillinase production had no effect on the inhibition zones around the penicillin G. disc. All zones below streaks of bacteria presumably producing penicillinase however were of reduced size and not circular.

Various other methods for detection of Staphylococcal penicillinase have been described. [O'Collaghan et. al; (1972); Wong and Soo-Hoo, (1976)] but they are unsuitable for routine use because of their complexity.

EPIDEMIOLOGICAL MARKERS OF STAPHYLOCOCCI :

When it is desired to trace the spread of infection from one patient to another or from hospital personnel to patient, a method of difference is needed that will distinguish each separate strain.

Growth on glycerol monocetate and resistance to low concentration of mercuric chloride, [Moore (1960)] can be used as markers of epidemic strains of Staphylococci, however, other epidemiological markers such as serological typing, phagetypeing and antibiotyping are more selective for epidemiological study to investigate the source of infection.

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The study of bacteriophages or bacterial viruses has passed through several phases since their discovery by Twort (1915). Early attempts to use them into treatment of bacterial infections were successful but their values in epidemiology of bacterial strains was appreciated largely owing to the work of Cragie, [Cragie and Yen (1938); and Felix (1955)].

Wilson and Alkinson (1945), adopting essentially the same method as that used for the Vi phage typing of typhoid bacilli, prepared and standardised phage filtrates and adopted some of the phages to new propagating strains.

The method of Wilson and Alkinson forms the basis of current phage typing procedures, but several refinements have been added [Anderson and Williams, (1956)]. Additional phages have been added to those in routine use and some of the original ones have been discarded.

A basic set of phages was established by international agreement in 1963 and had since undergone several modifications [Reports, 1959; 1963; 1967; 1971]. With 22 phages many hundreds of different patterns of susceptibility are possible, but certain combinations occur much more
frequently than other. Thus it is possible to divide Staphylococci into corresponding phage groups, - [Williams et. al; (1966)].

Until late in 1975 the usefulness of phage typing of Staphylococcus aureus as an epidemiological tool was hampered by the increasing number of isolates non-typable with the basic set of phages [Shayegani et. al; 1976]. At that time the international subcommittee on phage typing of Staphylococci (Report, 1970-1974) recommended a revised set including three new phages (94, 95, 96) which have been added to the miscellaneous group of the international basic set Gr, IV is eliminated i.e. phage 42 D and 187 are dropped from the basic set.

Pether (1968) reported reproducibility of the lytic patterns better with the basic set at R.T.D. x 1000 by a block of 20 extra phages.

A heat shock treatment developed by Ma and Mandle (1961) was reported effectively for previously non typable Staph. aureus strains. Use of revised international basic set of phages and selective use of the heat shock treatment would enable typing of about 90 percent of Staphylococcus aureus isolates [Shayegani et. al; (1978)].
Correlation between the virulence of Staph. aureus and their phage types is well documented [Bargoon and Goldstein (1961) and Krynki et. al. (1970)]. However, another group of investigators believe that such relationship if any is not significant [Hurst (1960); Singh (1972)]. Burgoon and Goldstein (1961) have suggested that phage type 80/81 is more virulent than other Staphylococci Cohen et. al; (1964) observed that phage type 54 may be less capable than type 80/81 to cause infection in normal person.

It has become obvious that epidemics of cross-infection in hospital are commonly due to one or other of relatively small number of types [Williams, (1959); Williams and Jevons, (1961)]. The classic example of one such is type 80/81 first recognised in Australia in 1953 [Rountree and Freeman, (1955)]. Since then this strain has been isolated from a number of severe epidemics of Staphylococcal infection [Gillespie and Alder (1957)]. Chatterjee and Aikat (1964) in their study isolated 53/75/77 as the predominant phage type which was responsible for an epidemic in the nursery as well as causing a serious outbreak of infection in the surgical unit.

The predominance of group-I and III is reported by various investigators [Rosendal and Bulow, (1967); Parker (1974)]. Several others [Ghosh-Ray and Paul, (1961); Chatterjee and
Aikat, (1964); Chatterjee et. al; (1968)] have stated that hospital strains of Staph. aureus predominantly belong to phage group III, others like Blair and Carr (1960), have reported predominance of strains belonging to Gr-I. Agarwal et. al; (1963) have reported strains belonging to mixed group to be the commonest isolates from clinical sources. Rajvanshi et. al; (1967) reported the hospital strains predominantly belonged to phage Gr-III followed by mixed group pattern.

 Besides the phage group, the predominant phage pattern amongst the individual groups are found to be different in several studies. The predominant types isolated previously among Gr I strains belonged to the 80/81 or 52/52A complex. Bhujawala and Mahapata (1972) reported type 29 as the commonest in Gr-I and 3A, 3A/3C in Gr II.

 Though phage typing is widely used in many countries the phage typing pattern is of little value in predicting the pathogenicity or the probability of spread of a particular strain of Staph. aureus.

 SEROLOGICAL TYPING :

 On the basis of agglutination with antisera of coagulase positive Staphylococci a number of strains can be
distinguished into specific protein types. Cowan, (1939) using slide agglutination technique recognised three serotypes, I, II, III and a number of minor types, but since then number of types were added. Hobbs (1948) recognised thirteen types.

Serotyping has been used in epidemiological studies of Staphylococcus aureus, but the information obtained has been less satisfactory than that obtained by phage typing. The antigenic structure of Staphylococcus aureus is complex, the demonstration of a particular antigen may be difficult and the production of antisera is complicated [Pereira, (1961); White et. al; (1962)]. Pereira, (1961) stated that continued cultivation in the laboratory leads to antigenic changes complicating the serological picture. He observed that two major agglutinating antigens (13 and 17) of freshly isolated strains tended to be lost and replaced by others on continued subcultures in laboratory.

Despite the complexities of serotyping there are some obvious advantage in the use of serotyping than phage typing and other marker systems, [Cohen J. O. (1974)]. The most promising is the ability to type most human strains of Staphylococcus aureus including those that are phage non typable. A new method for serological classification "Tachikowa Method" which satisfies the purpose of practical
use in the laboratory has been described, - [Saito et. al; (1974)].

**ANTIBIOTYPING**

Staphylococcus aureus has tremendous potentialities to acquire resistance to any new antibiotic. Hospitals are reservoirs of resistant strains, - [Barber and Rozwadowska Dowzenks (1948); Howe (1954); Breaven and Burry (1956); Wise et. al; (1959)].

Cogent evidence has also been presented by some workers that many of these resistant strains of Staphylococcus aureus have very special biological characteristics. These include high communicability from infected persons or carriers to contacts, a tendency to produce nasal carriers, a tendency to produce lesions of the integument septic complications of wounds and invasive tendency of the sites of reduced resistance [Rountree and Freeman, (1955); Mudd, (1958)].

Thus these strains keep up the vicious cycle of cross infection within the hospital environment and it is logical to accept that increasing number of resistant Staphylococcus will have great impact on the incidence of wound infection, - [Wasek et. al; (1965)].
Strains of Staphylococcus aureus isolated from different patients and carrier differ in their degree of sensitivity to particular antibiotics. So from time to time antibiotic sensitivity has been useful as epidemiological marker.

Most of the strains infecting patients and carriers outside the hospital are sensitive to various antibiotics but most of those (75 %) infecting in hospitals are resistant to penicillin and many other antistaphylococcal antibiotics [Finland, (1955)]. Chatterjee and Aikat, (1964) and Sahai et. al; (1967) also reported a higher incidence of resistance among the hospital strains as compared to community strains. In India, the rapid emergence and high incidence of antibiotic resistant strains of Staphylococci have been reported by a number of workers [Gupta and Chakrovarty, (1954); Trivedi and Sarkar, (1954); Myers and Acharya, (1956)].

Chatterjee and Aikat, (1964) reported incidence of 80.2 percent penicillin resistant Staphylococci. Gupta et. al. (1965) reported an increase in resistance to penicillin from 71.43 to 83.63 percent within five years. In series of Sengupta et. al; (1969), resistance to penicillin was found in 94 percent of the strains.

The antibiotic resistant strains frequently isolated from
hospitals reported to belong predominantly to phage Gr. III [Ghosh-Ray and Paul, (1961); Chatterjee and Aikat (1964); Chatterjee et. al; (1968)]. Phage Gr. I and II included a large majority of strains which were reported either sensitive to all antibiotics or resistant to penicillin alone [Alder et. al; (1956); Agarwal et. al. (1963); and Sahai et. al. (1967)]. Phage group III have an unenviable reputation of acquiring resistance to antibiotics other than penicillin more easily than most other Staphylococci, - [Williams et. al. (1966)].
COAGULASE NEGATIVE STAPHYLOCOCCI

Ability of Coagulase negative Staphylococci (CoNS), member of the family Micrococcaceae to become an opportunistic pathogen has long been recognised (Holt, 1969). In the past two decades, Serious infections have been increasingly seen in immunocompromised patients (Harris, 1985). Because of their prevalence on the skin and frequent implantation of foreign devices in patients during hospitalization, coagulase negative Staphylococci are ideally situated to cause serious infections in such individuals, - [Parisi, (1985)].

Production of Coagulase as a criterion of pathogenicity of Staphylococci has been universally accepted. Formerly; coagulase negative Staphylococci were regarded as non-pathogenic, nontoxic and biochemically inactive and ignored as accidental contaminants in clinical medicine, [Vijayalakshmi, 1980]. Much debate has developed whether CoNS represent contaminants or pathogens; (Martin et al, 1989). But work in last twenty years has shown that coagulase negative Staphylococci are pathogenic and should not be considered as harmless commensals.

Coagulase negative Staphylococci constitute 4 % of all bacterial isolates from nosocomial infection of
genitourinary tract and Surgical Wounds, (Jay, 1983). It is the nosocomial pathogen of prosthetic valves, CSF Shunts, joint prostheses, Vascular prostheses, post operative wounds, Septicemia, abscesses and osteomyelitis, (Archer et al, 1985). They are the common blood culture isolates in the neonatal ICU, - [Freeman et al, (1990)].

In last ten years, the major increasing problem associated with coagulase negative Staphylococci is, their resistance to various antimicrobial drugs. This is seen significantly in hospital environment, (Jesson et al, 1969). All hospital isolated strains are resistant to almost all antibiotics.

Because Coagulase negative staphylococcal infections are indolent and often clinically silent, diagnosis and therapy is difficult. It is clear from the present evidences that CoNS have changed status from 'non-pathogens' to that of 'Opportunistic pathogen' in the text book, - [Williams Reo, 1973].

Considering the fact that they are often considered as harmless commensals or contaminants, CoNS must now been individually evaluated as potentially true pathogens.

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INTRODUCTION:

The genera Staphylococcus, Micrococcus and Planococcus are members of family Micrococcaceae. The majority of Micrococci, Staphylococci and Planococci are free living saprophytes but the main habitat of Staphylococci is the surface of primates and other mammals, - [Finegold and Baran, (1986)].

HISTORICAL ASPECTS:

In 1883, Sir Alexandar Ogston detected cluster forming cocci in pus from the patients. However, the credit for the generic name for Staphylococci went to Rasenbach. Unfortunately, many microbiologists were not ready to accept the name Staphylococcus and so the organisms were included in the genus Micrococcus. In 1900, Staphylococci were divided into two subgenera (1) Aurococcus Aureus (2) Albococcus epidermis. Further classification was made by studying their ability to produce pigments by Andrews and Gorden, (1905 - 1906). But the classification had certain drawbacks e. g. certain anomalous strains of Staphylococci could not be differentiated accurately so this method fell into dispute.

Loeb, (1903) described coagulase test for the first time.
He demonstrated ability of Staphylococci to coagulase goose plasma. Similarly, Much, (1908) reported coagulase test with rabbit and horse plasma. However, its clinical significance was uncertain and the test was neglected for two decades. In 1922, a close relationship between mannitol fermentation and coagulase reaction and its role in pathogenesis of Staphylococcal infection was convincingly shown by, Dudgeon - Hine et al, 1968. Based on coagulase test, Staphylococci were divided into coagulase positive and coagulase negative strains.

Christiae and Wilson, 1941 found that with human strains of Staphylococcus, fibrinolytic activity generally runs parallel with coagulase production and pathogenicity.

In 1944, Smith and Hale, Showed that for coagglutination a third substance in addition to coagulase and fibrinogen must be present. This third substance which they called as coagulase reacting factor (CRF). Its roll was in production of thrombin like substance which converts fibrinogen into fibrin. This was the basis of coagulase test. Coagulase is thermostable substance, i. e. considerable activity is retained even after heating at 100°C for thirty minutes or after autoclaving. It is found abundantly in infusion or digest broth during logarithmic phase of growth and its release from the cell in evidence by the presence of
Borchardt and Pierce (1964) suggested that coagulase was not the factor responsible for staphylococcal survival within leucocytes. They suggested that some unknown factor, either, heat stable protected antigen, phagocytosis, inhibiting substances or an unknown factor other than coagulase may be contributing to Staphylococcal survival within or outside the phagocytic cell. - [Borchardt et al, (1964)].

CHARACTERS OF CONS:

A) MORPHOLOGICAL: (Schleifer, 1986)

CONS are defined as gram positive clustering cocci that produces catalase but not coagulase. CONS are of 0.5 to 1.5 mcm in diameter and are present in pairs or tetrads. They are non-sporing, nonmotile, some produce capsule. Cell wall of CoNS contains peptidoglycan and techoic acid. The aminoacid present in the peptidoglycan is L-lysine. Some strains produce slime. They are facultative anaerobes. They grow in presence of 10 % NaCl and below 18-40°C. Their G+C content is 30-39 mol %.
B) CULTURAL CHARACTERS:

Medium for primary culture is blood agar containing 5% sterile defibrinated sheep, rabbit, or bovine blood. Plates are incubated at 34-37°C for 18-24 hours under aerobic conditions. The colonies grown are usually circular, smooth, raised, butyrous, opaque, and 1-3 mm in diameter. Colonies should be allowed to grow at least three days at 34-37°C for further differentiation into genus and species. Specimens from heavily contaminated sources (e.g. faeces) should be inoculated on selective media such as mannitol salt agar, phenylethyl alcohol agar, colombia CNA agar or trypticase soya agar. A liquid medium such as thioglycolate broth can be used.

C) BIOCHEMICAL CHARACTERS:

As and when the CoNS were being discovered from various clinical infections, people tried to study various other biochemical properties of these organisms to find out possible correlation between biochemical activities and the pathogenicity; [Jaykar and Bhaskaran, (1970)]. Activities like pigment production, haemolysin production, mannitol fermentation and production of enzymes such as phosphatase, DNase hyaluronidase were studied.
A.C. Baird Parker (1963) in his classification of the Staphylococci says that variants of S. aureus may be found which have lost the ability to produce free or bound coagulase or which are unable to utilize mannitol anaerobically. It would appear unlikely that under normal circumstances a strain of S. aureus would lose all characteristics though possible loss of two characters could occur. However, mutants lacking in coagulase ability to utilize mannitol and other properties have been reported. Hence when a bound and free coagulase negative Staphylococci are isolated which ferment mannitol, there is every possibility that strains are coagulase negative variants of S. aureus; [Heltberg and Brunn, (1984)]. A positive result in either a coagulase test or a clumping factor test is essential in routine identification of S. aureus. To identify coagulase negative S. aureus in clinical specimens, a simple test is desirable to separate them from coagulase negative Staphylococci species proper, such as S. saprophyticus and S. epidermidis. Ordinary coagulase positive Staphylococcus strains are characterised by small inhibition zones around polymyxin discs as opposed to the CONS. By this test, correct identification of number of s. aureus variants, not detected by routinely performed coagulase test was possible, - [Heltberg and Brunn, (1984); Korman, (1963)].
In 1957, Weckman and Catlin studied the association between coagulase production by Staphylococci and their DNase activity using a Viscometric technique. They found that coagulase positive Staphylococci were more active DNase producers than CONS strains. They suggested that high DNase activity might be useful determinative characteristics supplementary to coagulase and phosphatase test reactions in identifying pathogenic Staphylococci; - [Parisi, (1985)].

Jones et al, (1964) studied S. epidermidis and found following characters:

1. Ability to grow anaerobically in standardised complex medium containing Glucose.

2. Inability to ferment mannitol and to produce coagulase.

3. Reduction of nitrate to nitrite.

Choudhary and Aikat (1968) studied some biochemical properties of CoNS and found that Carbohydrates such as lactose, glucose, maltose, mannitol, glycerol, sucrose, erythritol, fructose and mannose were utilised by coagulase negative Staphylococci with the production of acid.

In 1968, another property of S. epidermidis was studied by
Kleck and Donahue. They demonstrated that typical hemolysin produced by cultures of S. epidermidis was d lysin. Strains producing d lysin were isolated from noses of healthy carriers or from the blood of patients with endocarditis. All nasal strains were capable of elaborating hemolysin but only 83% of clinically significant blood culture isolates. They observed that cultures of CONS in aerated brain heart infusion broth can produce significant yields of hemolysins. The hemolysin was extremely thermostable and was inhibited by normal rabbit serum. Its inactivation by trypsin indicated that it was a protein but its antigenecity was not established. The marked ability of cultures of S. epidermidis to produce d lysin detracts from its significance as an important virulence factor for Staphylococci. Its molecular weight is 100,000. It was responsible for damage and cytopathic effect seen when various strains of CoNS were grown on monolayer of mouse skin fibroblast.

Epsilon toxin is similar to d toxin of S. aureus. d lysin is a non-antigen product and dectrophoretically heterogenous with strong detergent like activity.

In 1986, one more characteristic of S. epidermidis was shown by Janada (1986). They showed that elastase, an inducible enzyme was elaborated by s. epidermidis only and not by
other CONS. Its synthesis was medium dependent. Its importance has been postulated as it promotes systemic invasion from localised body site and helps in persistance.

CLASSIFICATION:

CONS form a heterogeneous group of organism. They can be classified into many biotypes as suggested by Baird-Parkar or Kloos-Schleifer method. Kloos-Schleifer method employs an extensive battery of tests which is not possible in ordinary laboratories. In such cases Baird-Parker scheme may be used which employs parameters like, glucose, maltose, mannitol lactose utilization, acetoin production phosphatase production and coagulase test; [Schleifer (1986)].

Baird-Parker (1963) divided Staphylococci into two genera. They suggested that two genera from family Micrococcaceae, Staphylococci and Micrococcii could be separated by the ability of former to ferment glucose anaerobically. They performed Hugh-Leifson for their classification, he divided Staphylococci which ferment glucose anaerobically into six biotypes S I to S VI and micrococcii which did not ferment glucose anaerobically into eight biotypes M I to M VIII. His S I was S. aureus. He combined S II and S V and formed single group so he was left with four biotypes of CoNS which he renamed S. epidermidis. For classification, various
biochemical tests performed were acetoin production, phosphatase test and acid from mannitol maltose and lactose. He biotype s. epidermidis S II - S V constitute s. epidermidis biotypes I, S III forms biotypes 2, S. IV biotypes 3 and S VI biotypes 4; - (Baird - Parker, 1963). After that, in 1974, Baird - Parker found that the first four biotypes of his genus micrococcus resembled Staphylococci in G + C content of the DNA and in cell wall composition so he named them as S. Saprophyticus. Therefore, M I to M IV are same as S. Saprophyticus biotype 1 to 4. S. epidermidis and S. Saprophyticus were differentiated by sensitivity to Novobiocin; - [McTaggart and Elliott, (1989)]. A modified oxidase test and susceptibility to furazolidon and lysostaphin were evaluated in conjunction with staph-ident strip to accurately differentiate between Staphylococci and micrococci.

Differentiation of S. epidermidis by phage typing had been accompanied with limited success, problems still exist with descrimination, reproducibility and typability of strains, standardisation of phage typing methods under development of an international set of phages are still under investigation; - (Stephenson and Tabagchali, 1986). Some of the more commonly used phage systems are those of Verboef et al, (1971). S. epidermidis found to carry two distinct groups of phages, of which one is active against S.
epidermidis strains and other against S. aureus strains. In 1969, Staphylococci were isolated from skin, nose, blood and colonised valves were examined using set of phages active in CONS by; - [Holt, (1969)].

In 1975, Kloos and Schleifer redefined S. epidermidis and S. saprophyticus describing four biotypes of each.

Nineteen species are recognised in the genus Staphylococcus and several others are recently under investigation. The genus Staphylococcus can be subdivided into atleast four species groups on the basis of DNA/DNA relationship and phenotypic characterisation; - [Schleifer, (1986)].


Pal et al, (1989) found S. epidermidis biotype 1 as a frequent isolate (51.1 %) of total 75 CoNS.

Newer technique for identification of CoNS : 

Identification of CONS by restriction enzyme analysis of plasmid DNA was found to be reproducible in vivo and vitro. It was very effective in determining the similarities and
differences among blood isolates, - [Lauerdiere et al, (1978)].

It has been proved that as an additional marker for epidermiological studies of infections caused by S. epidermidis, showing variation in bacteriological characters. With the increase in prevalence of CoNS as nosocomial pathogens, a new method of epidemiological typing was by immunoblot finger printing, - (Burnie et al, 1988). Commercial kits are available for rapid identification are many and have been compared with the Standard Coagulase Test; - (Gerson, et al, 1988). Latex particles coated with human plasma can be very useful for simultaneous and rapid detection of clumping factor and protein A. Rapid particle agglutination assays have also been developed for the detection of fibrinectin, fibrinogen and collagen receptors; - [Naidu et al, (1988)].

Machanism of pathogenicity and virulence :

The microbiology of pathogenic bacteria includes many examples of low frequency events that are important to the virulence of the organism. In addition to phase variation in adhesion expression other examples include conjugation, appearance of capsules, appearance of antimicrobial resistance, appearance of Serum resistance and antigenic
variation.

In all these examples, when the bacterial cell confronts a new environment, the changed characteristic leads to the selective survival of changed cell. The progeny of the variant in turn become the predominant phenotype. Slime production may function as a virulence factor for CONS by promoting bacterial colonization of indwelling devices; - [Christensen et al, (1990)].

The fact that certain 'virulence factors' are often found associated with pathogenic strains of CONS and the very properties of these factors in vitro, encourage the belief that it must be one or probably a combination of these factors. As virulence is not determined by any one factor but by several factors that bestows pathogenicity in vivo. But in spite of many toxins and enzymes identified, it is possible that virulence for Staphylococci for man is mediated by more sophisticated and as yet unknown factors.

Factors determining virulence of CoNS are:

1. Epsilon toxin
2. Elastase enzyme
3. Slime production
4. Urease enzyme
5. B-Lactamase
6. Production of enterotoxins.
Diseases caused by CoNS

Urinary tract infection:

In 1924, Hellestram actually demonstrated the presence of Staphylococcus albus in renal stones composed of calcium carbonate and suggested that these bacteria could participate in formation of phosphate stones. Inspite of this early proposal, Staphylococcal infection of urinary tract was overlooked for all these years. Lately, there has been renewed interest in the subject. In 1974, the urinary tract infection due to CoNS was reported by Hermansson et al; The significance of infection in children aged 1 month to 15 years was unknown. The fact that renal function was temporarily affected, suggested that the renal parenchyma was involved and that these infection should be examined treated and checked throughly as other urinary tract infections.

CONS which are commensals of urethera are often found as contaminants in urine but may cause infection in a diseased or damaged urinary tract.

It was shown in 1990 that establishment of urinary tract infection depends upon the interaction of bacterial virulence factor with the host. Similarly maintenance of urinary tract infection is influenced by interactions
between bacterial attachment to urothelial cells. It has been assumed that urethelium remains intact and offers consistently uniform surface for attachment and catheter associated urinary tract infection in patients have demonstrated that during urinary tract infections the urothelium is disrupted and disorganised with exfoliation of superficial urothelial cells exposing the underlying immature cells. Preferential colonization of these immature cells has also been described. Bacterial multiplication within urinary tract is limited by 'Washout' effect of urine and antibacterial activity of the bladder; - (MaCTaggart et al, 1990). It was suggested that antibacterial effect was due to macrophages associated with the urothelium.

Bacteraemia/Septicaemia:

Septicaemia was defined as bacteraemia with clinical and laboratory evidence suggestive of infection.

According to recent national survey, S. epidermidis caused 8-9% of primary nosocomial bacteraemia. The first demonstration of bacteraemia due to CONS was by Cunleiff in 1943. He demonstrated that blood stream was sometime invaded by Staphylococci that were indistinguishable from the saprophytic cocci by generally accepted laboratory tests. He further said that the cultivation of such
organisms from blood though rightly casted doubt upon the technique of blood culture, it could not be too readily dismissed as a contaminant. He suggested that repeated isolation from the blood of the same organism in a poured plate may be taken as evidence of actual bacteraemia; - [Cunleiff and Gillam, (1943)]. Forse and Coworkers reported as epidemic of nosocomial S. epidermidis bacteraemia in patients in their surgical intensive care unit during 1977 and 1978. They concluded that CONS were the leading organism causing hospital acquired bacteraemia and were associated with mortality in excess of that due to the underlying disease alone. Moreover, they significantly prolong the hospital stay; - [Cunleiff et al, (1943); Martin et al, (1989)].

The effect of nosocomial CONS bacteraemia on antibiotic administration prolongation of hospital stay and mortality had been investigated by Freeman et al, (1990) in neonatal ICU. Previous studies have demonstrated that the risk of bacteraemia is strongly associated with both low birth weight and duration of exposure to neonatal ICU. In their case, two bacteraemia within first 24 hours of life was not considered nosocomial in origin.
Skin and wound infections:

In 1985, Harris reported nosocomial intraabdominal abscess caused by CONS. The pathogenesis of CONS causing intraabdominal abscess was uncertain. The gastrointestinal tract harbours these organisms as part of its normal flora. CoNS could have been deposited intraabdominally during disruption of gastrointestinal tract that transpired in three of his four reported cases.

Although once regarded as an innocuous member of the normal skin flora, S. epidermidis is now recognised as an important opportunistic pathogen. It is an organism routinely found on the skin and hospital environment. The prevalence on the skin surface being 85 to 100%, in nose, mouth and nosopharynx 90%, in vagina and uterine cervix 35 to 80%. Noble (1969) believed that S. epidermidis was found excessively on damaged skin than the skin of normal persons.

Infections associated with foreign devices:

Staphylococcus epidermidis is known to cause infections in patients with compromised resistance to microbial diseases such as prosthetic heart valves; CNS shunts, vascular catheters, ventricular shunts etc., - [Wade et al, (1983)].
S. epidermidis caused mild infections following operations or instrumental manipulations of the lower urinary tract.

In 1984, Staphylococci as a predominant organisms constituting 59 % prosthetic joint infections were reported by Inman et al. They found s. epidermidis as predominant species in both early and late infections.

Meningitis :

Staphylococcal meningitis may be due to secondary invasion of nervous system as the result of traumatic wounds or nearby infection. It is prone to occur with cavernous sinus, thrombosis or epidural abscess.

S. albus infections have been a frequent indolent meningitic complication of ventriculo-atrial and ventriculo-peritoneal shunts. In some cases more than one Staphylococcal sub group may colonise the shunt at a time, each having a different antibiotic spectrum. Sequential shunt infections may be due to the same or different subgroups, - [Weil, ].

Meningitis caused due to CONS was reported in eleven patients by Pal and Ayyagari, (1989). They studied resistance pattern in them and concluded that CoNS isolated from meningitis were resistant to methicillin. They were
belonging to S. epidermidis biotype 1, 2 and 3 and s. cohni.

Meningitis below one year of age, was reported by Vijayalaxmi et al, (1980) in two children.

Antibiotic Sensitivity patterns of Coagulase negative Staphylococci:

Along with the adherence of CoNS to various foreign devices, antibiotic resistance is also an increasing problem. Since the introduction and widespread use of antibiotics, the acquisition of gradually increased antibiotic resistance by CoNS was reported by many workers. Progressive emergence of these organisms, resistance to various antibiotics was evident from the study of Barber et al, (1964). It was observed that CoNS strains isolated from hospital environment were more resistant to antibiotics than isolated from healthy carriers; - [Powell and Sandarson; (1987)].

Bacterial resistance to an antimicrobial agent is of three types.
1) Enzymatic - Which is mediated by extra chromosomal factor such as plasmids. (e. g. penicillinase production).
2) Innate - Which is probably chromosomally mediated (e. g. methicillin resistance).
3) Tolerance - Which may represent a defect in bacterial
It was observed by Coarse and William that S. epidermidis was the most resistant of gram positive cocci showing increased resistance to all antibiotics. They found that about half of the strains from the lesions of hospital patients were resistant to Penicillin, Streptomycin, Tetracyclin and 10% of strains were resistant to cloxacillin. In general they concluded that frequency of antibiotic resistance and the 'width' of the resistance spectrum were greater in strains from hospitals and they were also greater from Staphylococci than those as Micrococci, [Corse and Willims, (1968)].

Medical management of CONS infection is complicated by high incidence of resistance to methicillin reported for this organism. The majority of CoNS had been shown sensitive to cephalosporins, [Lauerdie et al, (1978)]. In 1978, Archer et al, showed that rifampicin is effective to cure infections of s. epidermidis which were due to insertion of indwelling devices especially CSF shunts, prosthetic heart valve etc. Griebal et al in 1981 reported that emergence of methicillin, gentamycin and tobramycin resistance among hospital Staphylococci represent a serious and persistant problem. It was also shown that resistance to Erythromycin had increased from 32 - 50 %, to chloramphenicol from 13-21%
to gentamycin from 2-24 %, to kanamycin from 29-51 % and methicillin from 22-33 %. Nearly three times more number of CONS strains had shown resistance to erythromycin than s. aureus reported by Sunderam in 1982. He told that use of penicillin as the first antibiotic to come into general therapeutic use is under shade, since the emergence of penicillinase producing Staphylococci. In their study CoNS resistance to penicillin was 41.7 %. Resistance to penicillin may relate to production of enzyme β-Lactamase or penicillinase. The first of β-lactam resistant strain developed in Europe; - [Jesson et al, (1969)].

In 1982, Richardson and Marples discussed the changing antibiotic resistance. They found that infection caused by s. epidermidis does not occur often but clinically significant strains are resistant to various antibiotics and among all, resistance to penicillin G was Common.

S. epidermidis shows broader and more variable range of resistance to various antibiotics when compared with s. aureus. The resistance is usually plasmid mediated. Intra and inter species transfer is known. Organisms become penicillin resistant. Methicillin resistance is chromosomal in origin and not plasmid mediated, - [Choudhary and Basu Malik, (1970)].
Archer et al, (1985) found that some strains of gentamycin resistant S. epidermidis recovered in culture surveys were found to be resistant by virtue of aminoglycoside modifying enzymes, the gene for which were encoded on plasmids. The plasmids were also found to be self transmissible by cell to cell contact (conjugative) and able to mobilise smaller plasmids that contained genes specifying resistance to additional antibiotics. In their study, they showed gentamycin resistance has become common among CONS that colonize patients in some hospitals. Gentamycin resistant CONS recovered from 74 % of patients and staff in a Cardiac Surgery Unit. In that encoded gentamycin resistance on plasmids that were transferred between Staphylococci by cell to cell contact. Identical plasmids were found in isolates colonizing patients and surgical staff and infecting inserted valves. The presence of these plasmids in such a large number of CoNS has important implications -

1) Gentamycin is an important antibiotic for treating infections caused by methicillin resistant CONS. Since the majority of CoNS (nosocomial) isolates are methicillin resistant, gentamycin resistant further limits therapeutic options.

2) Conjugative transfer can readily spread plasmid DNA among bacteria. The use of antibiotics for therapy or prophylaxis may select antibiotic resistance transconjugation in vivo.
Thus, since these conjugative plasmids can mobilise transfer of additional smaller non-conjugative plasmids that encode resistance to other antibiotics.

Pal and Ayyagari in 1989, isolated methicillin resistant strains from various infections, such as Urinary tract infections, endocarditis, meningitis. All these strains were sensitive to Vancomycin. In case of neonatal infections Miller, et al, (1990) observed that antibiotic resistant strains were spread from neonate to neonate on the hands of medical and nursing staff leading to colonization of premature neonates in ICU with in first week of life.

Pushpa Agarwal (1991) is of opinion that many times multidrug resistant endogenous infections by S. epidermidis could be due to acquisition of drug resistance from other genera of endogenous organisms, co-existing in the body.

To prevent major therapeutic problem in future, due to immense multi resistant strains, use of those life saving antimicrobial weapons should be rationalized and one should be eligent in identifying the resistant strains by screening every isolates routinely by antibiotic testing.

Thus, CONS have taken a crucial pathogenic role are have become an important opportunsitic pathogen with exhibition
of multiple drug resistance, penicillinase production and possession of different biological properties e.g. enzymes production, slime production, etc. which promote them to invade body site, adhere to foreign devices and help them in persistance of infection. The organisms and of low virulence but acquire capability of producing disease when the host defences are compromised.
KLEBSIELLA SPECIES

With the changing pattern of nosocomial infections focus of attention has been shifted from Gram positive cocci to gram negative bacilli and anaerobic organisms. The change in the character of hospital cross-infection is mainly due to the advent of the antibiotic era. Recognition of the serious problems caused by antibiotic resistant Staphylococci was followed by increasing awareness of the progressively more prominent role played by endogenous gram-negative bacilli, fungi and other opportunistic pathogens. The continually evolving patterns of nosocomial infections have been well documented in a series of reports from several medical centres, - [Finland et. al. (1959); Ayliffe et. al. (1965); Watt and Okubadejo (1967)]. In a study of bacteriological patterns of hospital infections McNamara et. al. (1967) found that Gram-negative bacilli accounted for almost two-thirds (64.5 percent) of hospital acquired infection. The organisms most prevalent were E.coli, Pseudomonas and Klebsiella pneumoniae in almost equal proportions [Weil et. al. (1964), Steinhauer et. al. (1966)]. Klebsiella species in particular have been responsible and the apparent ease with which these organism can spread, especially to debilitated patient is a matter of concern [Price and Sleigh (1970); Hill et. al. (1974)].
The organisms of Klebsiella-aerobacter-serratia group (KAS group) are constitutes of normal enteric flora of man. [Weil et. al. (1964)]. Like the other enterobacteriaceae they are also opportunistic pathogens and are thus frequently found in urinary tract infections, peritonitis, infected wounds and less commonly in pneumonia, empyema, meningitis and septicemic infections; [Finland (1960); Weil et. al. (1964)]. Finland et. al. (1959) pointed out that whether this increase in the incidence of this kind may not be related to the resistance to the commonly used antibiotics that is often found in the members of the KAS group. In the Bronx-Lebanon Hospital centre a survey covering the years between 1953-1964 showed that about 30 percent of infections with enterobacteriaceae involved organisms of the KAS group [Weil et. al. (1964)]. Of these about 80 percent were Klebsiellae. A similar prevalence of members of the KAS group was reported from Boston, [Kislak (1964)].

The reasons behind this changing pattern are variable. The use of the broad spectrum antibiotics has contributed to the suppression of susceptible bacteria, including those of normal flora and the complexities of modern medicine with the use of immunosuppressive and cytotoxic drugs, corticosteriods, prolonged surgical procedures, mechanical instrumentation and an aging population have all played roles. Klebsiella spp. is an organism of low pathogenicity.
for healthy subjects but is an important pathogen in patients or tissues with poor resistance.

Most reports on nosocomial Klebsiella infection are retrospective studies of outbreaks caused by multi drug-resistant strains and prospective studies in the periods following such outbreaks. As most hospitals have no continuous surveillance for the detection of nosocomial infection, attention is usually drawn to the problem by an outbreak of infection or by the clustering of multi drug-resistant infections in the ward.

The organisms are ubiquitous in nature. They have been isolated from soil, water and vegetation. They are commensals of man and animals occurring in intestine. However being opportunistic pathogens, they are capable of producing human infections, mainly of urinary and respiratory tract, and less frequently infections like meningitis, bacteraemia, gastroenteritis and others. The organism is known to be the responsible agent of 6-9 percent of all nosocomial infections. Various studies reported from India on hospital infection have shown varying rates of hospital infection. In the series of Shrivastava et. al. (1969) hospital infection rate was 20.19 percent of which Klebsiella organisms were 7.4 percent. Vinodkumar et. al. (1979) got 6.9 percent Klebsiella in 29 percent hospital
infection rate whereas in the series of Agarwal et. al. (1980) 31.8 percent Klebsiellae were reported in 44 percent hospital infection rate. In a similar study at Civil Hospital Sangli, Udgaonkar and Bhavthankar (1985) have reported 14.17 percent Klebsiella infection in a 30.43 percent hospital infection rate.

VARIOUS FACETS OF KLEBSIELLA

The genus Klebsiella has been placed in tribe Klebsielleae of the family Entrobacteriaceae in the Bergey's manual (1975). It was named so, after Edwin klebs who was a German bacteriologist.

MORPHOLOGY :

These organisms are non-motile, capsulated, Gram negative bacilli, 0.3-1.5 micron by 0.6-6.0 micron, arranged singly, in pairs or short chains. For demonstration of capsule the India ink method is to be preferred to the ordinary capsule stains since distortions due to drying are avoided and the capsular material present as "loose slime" can be seen as well as that ground individual organism [Duguid (1951)]. Capsular material is produced in greater amount in media containing a relative excess of carbohydrate [Duguid and Wilkinson (1953). Most Klebsiella strains are fimbriate but
some of the respiratory strains form an exception [Duguid, (1968)]. Two types of fimbriae are recognised, the thick type which adheres to red cells of the guinea pig and other animal except the ox, the reaction is inhibited by D-mannose (MS = mannose-sensitive). The thin type adheres to cell treated with tannic acid (ox cells most suitable) and is not influenced by mannose (MR = mannose-resistant). The ozaena and rhinoscleroma bacilli are invariably non-fimbriate, as are certain biochemically aberrant respiratory strains belonging to type 1 and 2.

Cultural Characteristic:

When much capsular material produced, the growth on agar is luxuriant, greyish-white, mucoid and almost diffluent. This is no doubt due to high proportion of water 92 percent in the capsular material [Toenniessen (1921)]. The cultural appearances of capsulated strains are subject to considerable variation. Non-mucoid variations appears on serial sub-culture on solid medium, particularly when the plates are incubated for several days [Toenniessen (1913)] either as translucent peripheral out-growths or as secondary colonies in the substance or on the surface of original colonies. Some times the whole colony may dry up and wither away, leaving an effuse translucent layer looking like ground glass called as "Suicide colonies" by Collins (1924).
Resistance and Metabolism:

The organisms are killed by moist heat at 55°C in half an hour. They may survive drying for months [Loewenberg, (1894)]. When kept at room temperature, culture remain viable as a rule for weeks or months. They are aerobic optimum temperature for growth is 37°C, the limits are 12°C and 43°C. They are resistant to a wide range of antibiotics than are most E. Coli strains. They are nearly always Ampicillin resistant, though they are usually sensitive to cephalosporins, in this they differ from enterobacter strains which are almost invariably cephalosporin resistant [Benner et. al. (1965)].

Their resistance to streptomycin, chloramphenicol and Tetracycline is variable, but they are usually sensitive to Gentamycin and to the polymixins. Strains that are endemic in hospitals are frequently kanamycin resistant. Many of the respiratory strains, on the other hand are more sensitive to antibiotics. A few of them are sensitive to as little as 2 micro gm. per ml. of Benzyl-penicillin and most of them are sensitive to Ampicillin, streptomycin, chloramphenicol and Tetracycline, - [Wilson and Miles, (1975)].
Biochemical Reactions

Members of this group ferment a wide range of sugars but their behaviour in this and other respects is far from uniform. Klebsiella aerogenes is the most active fermenter of the group producing acid and gas from all or nearly all of the sugars usually employed, with the possible exception of dulcitol. The respiratory Klebsiella are somewhat less active biochemically. Among them the scleroma bacillus is a fairly well defined variety. It is anaerogenic, does not acidify lactose, gives a positive M.R. reaction and a negative V.P. reaction, does not grow in Koser’s citrate medium, does not form lysin decarboxylate, and is urease and gluconate negative. The biochemical tests in current use clearly form an unsatisfactory basis for the definition of species in the Klebsiella group [Dubay (1968)].
Biochemical reactions of different members are as follows

[Wilson and Miles (1975)].

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<th>VP</th>
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<td>Koser’s citrate</td>
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<td>Urease</td>
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Over the past two decades the incidence of hospital-acquired infections caused by Klebsiella has greatly increased [Steinhauer et. al. (1966); Jans et. al. (1970)]. Multiple antibiotic resistant strains of k. aerogenes have caused serious outbreaks of infection which have often been extremely difficult to control [Price and Sleigh (1970); Habble et. al. (1972); Hill et. al. (1974) Curie et. al. (1978)]. Since asepsis was introduced some 70 years ago to control infection within hospitals, no measure of similar effectiveness has been added to prevent hospital infection. The use of antibiotics for prophylaxis and treatment of infection has not significantly diminished the incidence of nosocomial infection and may even have increased the prevalence of plasmids in the hospital bacterial pool [Finland and Mc Gowan, (1976)].

TYPING METHODS

With the increase in the incidence of gram negative bacillary infections, the surveillance of hospital infections has been hindered by the lack of an effective method of epidemiologic typing that could be adopted to most general laboratories. The following procedures for Klebsiella typing have been employed by different workers for the epidemiological study. Each system has its own merits and demerits and to achieve best results a
combination of two or more systems is recommended.

1. Colonial typing
2. Biotyping
3. Antibiogram typing
4. Serotyping
5. Phage typing
6. Bacteriocine typing

1. COLONIAL TYPING:

This is based on presence or absence of capsular (k), somatic (o) and slime (M) antigens. The strains of Klebsiella have been divided into 4 smooth and 4 rough forms as shown in the following table.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Smooth forms</th>
<th>Rough forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>K.O.</td>
<td>K.R. Non Mucoid, capsulated</td>
</tr>
<tr>
<td>3.</td>
<td>M.O.</td>
<td>M.R. Mucoid capsulated</td>
</tr>
<tr>
<td>4.</td>
<td>O.</td>
<td>R. Non Mucoid, non capsulated</td>
</tr>
</tbody>
</table>

This method is not at all practical, as it has two main
disadvantages:

1 - Spontaneous conversion of smooth forms to rough is known to occur.

2 - Detection of rough forms on culture plates is not easy as M.K.R., K.R. and M.R. forms may appear smooth on agar plates and only R. forms appear rough.

Non-mucoid variations appear on serial sub-cultures on solid medium, particularly when the plates are incubated for several days, either as translucent peripheral outgrowth or as secondary colonies in the substance or on the surface of original colonies, - [Toenniessen (1913)].

BIOTYPING:

Amongst the several biotyping procedures recommended by various workers, classification by Edwards and Ewing (1972) and Cowan and Steel (1974) are the most acceptable for implementation in diagnostic laboratories. Based on 17 tests, Edwards and Ewing (1972) divided Klebsiella genus into 3 species Cowen and Steel (1974) have divided them into 6 species.
Biochemical classification of Klebsiella species (Cowen and Steel 1974).

<table>
<thead>
<tr>
<th>Test or substrate</th>
<th>K.aero</th>
<th>K.Oxy</th>
<th>K.pneu</th>
<th>K.edw</th>
<th>K.edw</th>
<th>K.ozae</th>
<th>K.rhin</th>
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<tbody>
<tr>
<td>genes</td>
<td>+</td>
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<td>dsii</td>
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</tr>
</tbody>
</table>

| Growth in KCN     | +      | +     | -      | +      | +      | +      | +      |
| Citrate as        |        |       |        |        |        |        |        |
| C. source         | +      | +     | +      | +      | d      | d      | -      |
| Gluconates        | +      | +     | d      | d      | +      | -      | -      |
| Malonate          | +      | -     | +      | -      | d      | -      | +      |

| Carbohydrates     |        |       |        |        |        |        |        |
| Gas (Glu)         | +      | +     | +      | +      | -      | d      | -      |
| Acid (glu.)       |        |       |        |        |        |        |        |
| Duicitol          | d      | +     | +      | -      | -      | d      | -      |
| Inositol          | +      | +     | +      | +      | -      | +      | -      |
| Lactose           | +      | +     | +      | (+)    | (+)    | (+)    | d      |
| Maltose           | +      | +     | +      | +      | +      | +      | +      |
| Mannitol          | +      | +     | +      | +      | +      | +      | +      |
| Sucrose           | +      | +     | +      | +      | +      | +      | +      |
| MR. test          | -      | -     | +      | +      | d      | +      | +      |

278
<table>
<thead>
<tr>
<th>VP. test</th>
<th>+</th>
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<th>-</th>
<th>d</th>
<th>+</th>
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<td>Indole</td>
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<td>Gelatinhydrolysis d</td>
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<tr>
<td>Urease</td>
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<td>Lysine</td>
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<tr>
<td>de-carboxylase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>-</td>
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</tr>
</tbody>
</table>

+ 85-100 percent positive (all, most, many, usually).
d 16-84 percent positive (many, some).
- 0-15 percent positive (no, none, few, some).

() delayed reaction/delayed growth.

• Not known.

(d) Different reactions by different strains; positive are delayed.
As the number of tests employed by the two workers are almost the same, Cowen and Steel's classification has a definite advantage over Edward and Ewing's classification as it offers more discrimination.

SEROTYPING:

Upto date, serotyping has been found to be the best and most reliable method for typing Klebsiella. It is very reproducible and gives adequate discrimination. Its one disadvantage is that it is expensive and is time consuming [Shriniwas (1980)]. So far 80 serotypes of Klebsiella have been recognised. Various methods like agglutination, indirect immunofluorescence, complement fixation and capsular swelling have been employed for serotyping. Of all these capsular typing is the method most widely practiced. The procedure adopted is as follows, - [Wilson and Miles, (1975)].

The organism is grown on Bacto-worfel ferguson agar. A suspension of the organism is prepared in 0.85 percent saline, which matches the density of 1:100 dilution of No 3 MacFarland tube. On a clean slide a loopful each of specific antisera and normal rabbit sera are placed at two ends. To each, a drop of the suspension and a drop of
Toeffler’s methylene blue are added, mixed and covered with coverslip. An apparent increase in size of capsule in test mixture after 5 minutes, indicates a positive test. According to Edwards and Ewing, majority of K. pneumoniae belong to serotypes 1 and 2, K. ozanae to serotype 4 and K. rhinoscleromatis to serotyp 3.

Recently Riser et. al. (1976) have developed a new serotyping method for Klebsiella. They have compared indirect fluorescent typing method with an established method of capsular swelling. The fluorescent antibody (FA) technique was tested for standards and unknowns and the results were checked by capsular swelling, fluorescent typing gives correlation with the established capsular swelling technique but has greater sensitivity, allows more economical use of expensive antisera, possesses greater objectivity as it requires less operator skill in the reading of results. It resolves most of the cross-reactions observed with capsular swelling and has a higher percent success rate in identification.

**BACTERIOCINE TYPING (klebocine typing)**

Antibiotic like substances which are produced by many gram negative bacilli and are able to inhibit the growth of related strains were first described by Gratia (1925) and
were later called "colicines". [Gratia and Frederica (1946)]. The name bacteriocine was introduced by Jacob et al. (1953) as a general term to define a class of antibiotic substances produced by bacteria. They are distinguished from other antibiotics by their limited range of action and their chemical nature. Bacteriocines are usually active only against species of bacteria closely related to the strains producing them and they require specific receptor on the sensitive strain for their adsorption and killing action. They thus have the specificity of phages but cannot reproduce themselves. In contrast to other antibiotics, they are proteins. Sometimes complexed with lipopolysaccharides.

The interest in colicine production typing by using multiple indicators has been increasing over recent years and has been applied to the Shigella sonnei and Flexeneri [Gillies, (1964)], to the E. coli [McGeachie (1965), Heittiaratchy et al. (1973)], to the proteus species [Al jumali, (1975)], to the pseudomonas [Darrel and Whaba (1964); Whaba (1965); Gillies and Govan (1966); Chugh and Sabharwal (1978)].

The method for typing Klebsiella strains is still in its infancy and so far no universally acceptable set of klebocine producer strains is available. Different workers have used different sets of bacteriocines and their
typability has ranged 28.6 percent to 93 percent and the number of patterns produced have ranged between 10 to 50.

Hall (1971) tested eight hundred strains of Klebsiella isolated mainly from urine, sputum, faeces, wound swabs, high vaginal swabs, burns and other clinical specimens and also from environmental sources e.g. nail brushes, hand creams, toilet seats and from infant feeds in a hospital milk-kitchen using 10 indicator strains and found that 77 percent were typable and she got fifty distinct patterns. She found that this bacteriocine typing scheme was useful in investigation of hospital infection. During hospital survey, Klebsiella spp. were typed and found in jars of hand cream on a number of wards. These were typed and organisms of same type 3/ 4/ 7/ 23/ 24/ 27/ were ultimately isolated from the main supply jar in the hospital dispensary. Strains of the same bacteriocine type isolated also from opened and unopened bottles of a commercially produced hand washing preparation in use at number of different hospitals, showing that this contamination was probably introduced with supplies of the hand cream.

Another situation in which the scheme has been usefully applied was in investigations into contamination of infant feeds in hospital milk-kitchen. Strains of Klebsiella of bacteriocine type 4/7 and 3/4 /7/27 were shown to be present
in the infant feeds and in the faeces of the babies receiving the feeds. [Ayliffe et. al. (1970)]. Follows up studies on the faeces of neonates from other hospitals indicated that an epidemic strains of Klebsiella was present in the nurseries of their hospitals and each strain was of a different bacteriocine type.

This klebocine typing method is simpler to perform than serotyping and its discriminatory power reproducibility suggests that it will be of value, particularly in laboratories where capsular serotyping is not-available, and will, it is hoped, help more workers to study Klebsiella epidemiology. In laboratories where capsular antisera are available, the typing method is probably best used as an adjunct to serotyping with the two methods in combination providing greater discrimination between Klebsiella isolates, allowing more detailed epidemiological.

**ANTIBIOGRAM TYPING:**

Many workers have used the antibiotic sensitivity patterns alone or in conjunction with other method such as phage typing or serotyping for the epidemiological studies. Hall (1971) studied 106 strains of Klebsiella for their ability to produce bactericine and also studied the antibiogram of those isolates. She used Ampicillin, chloramphenicol,
colistin, kanamycin, Nalidixic acid, Nitrofurantoin, Streptomycin, Tetracycline, cephaloridine and Carbenicillin for testing the sensitivity. A high percentage of strains from all sources were resistant to Ampicillin and many were also resistant to chloramphénicol, streptomycin, tetracycline and cephaloridine. The percentage of strains tested which were resistant to kanamycin, colistin, Nalidixic acid and Nitrofurantoin were low, with the exception of the hospital epidemic strains, 84 percent of which showed resistance to kanamycin.

In the series of Bhargava et al. (1966) and in the series of Vinodkumar et al. (1979) sensitivity of Klebsiella to penicillin was found to be 0 percent. Streptomycin sensitivity was 46 percent in the series of Bhargava et al. (1966) and it was 33.33 percent in the series of Vinodkumar et al. (1979). Tetracycline sensitivity of Klebsiella in the series of Vinodkumar et al. (1979) was found to be 33 percent and kanamycin sensitivity was recorded to be 83 percent. In the series of Bhargava et al. (1966) sensitivity to chloramphenicol was 69 percent and it was 50 percent in the series of Vinodkumar et al. (1979). Sensitivity to Gentamycin was found to be 100 percent in the series of Vinodkumar et al. (1979).

A change in the susceptibility pattern is sometimes useful
in determining the source of outbreak with multiple drug resistant strains, whereas Casewell (1977) has reported nosocomial infection with Gentamycin resistant strains of Klebsiella. It is obvious that antibiogram typing is of limited value for epidemiological survey.
The organism now designated, Escherichia Coli was first isolated from faeces by Theodor Escherich, 1885 and since that time has been given a variety of names.

Adam (1927) called the pathogenic strains "Dyspepsie coli" or "Dyspepsie Strains". Bray (1948) isolated one particular strain from the faeces and labelled as 'B. coli neopolitanum'. Another serological distinct strain was also discovered [Giles and Sangster, (1948)], and was named 'B. coli neopolitanum Var Beta', - [Smith (1949)].

It is normal inhabitant in the intestinal canal of man and animal.

According to the recent classification, which is based on size, shape, staining reactions, spore-forming ability, different biochemical and nutritional requirements [Bergey’s Manual, 8th Ed], Esch. coli falls into 8 gram negative facultative, anaerobic rods.

Esch. coli are straight rods with peritrichate flagella and fimbriae of type one are found in Escherichia. When grown on medium with high osmolarity, most Escherichia cultures
produce extracellular slime. This is a colonic acid [Goebal, (1963)] which appears to be indentical with kauffman’s M substance.

On agar plates, growth is seen readily in 24 hrs. at 37°C. According to Smith and Conant (11th Ed.) on Endo’s medium and on Eosinmethylene blue medium, colonies of Esch. coli have a peculiar metallic sheen which is seen best when they are examined with reflected light.

Esch. coli is usually described as urease negative but certain groups of strains, e. g. members of O groups 26 from cases of infantile gastroenteritis [Le Minor et. al. (1954)], and strains from oedema disease of piglets [Quinchohn et. al. (1959)] are shown to contain a high proportion which give an allcaline reaction in Christensen’s urea medium. Many strains belonging to 0 group 138 and 149 were able to decompose urea, was reported by Soderland (1971).

H₂S producing variants of Esch. coli with multiple drug resistance is reported by Darland and Davis (1974).

Early attempts to study the antigenic structure of Esch. coli means of agglutionation revealed a great heterogenicity of antigenic factors [Mackie, (1913)]. Lovell, (1973),
using a precipitin test, was able to recognize two antigens
a specific soluble carbohydrate substance associated with
capsule and somatic antigen.

Kauffmann (1947) published a diagnostic scheme based on the
distribution of H, O and K antigens based entirely on the
reactions obtained in agglutination tests. Loss of K
antigen was described in serial isolates from patients in
the same way as loss of O antigens [Bettleheim and Taylor,
(1969)]. It was found, in a number of different serotypes
of Esch. coli that the genetic determinant can be passed
from one strain to another by epidemic transfer [Stirm et.
al. (1967)].

The large numbers of O, K and H antigens and the total
number of serotypes of Esch. coli is very high [Bergey’s
Manual, 8th Ed.] Pathogenicity apparently depends upon the
presence of certain types of O and K antigens.

TYPING METHODS:

First colicine to be described was the "principle V"
produced by one strain Esch. coli strain V, which inhibited
the growth of another strain of the species, strain. These
agents, occurring in the enterobacteriaceae were named
"colicines". [Gratia and Fredericq (1946)]. The methods
which have been commonly used for detecting colicin production are those of Abbott and Shannon (1958) and McGreachie (1966). Universal indicators used were Esch. coli Row or Esch. coli phi.

Colicine sensitivity and colicinogenic properties have been used as a means of identifying bacterial strains in epidemiological studies of Shigella Sonnei and pathogenic Esch. coli [Fredericq (1948 a) Halbert and Shannon (1958)].

**SEROLOGICAL TYPING :**

It was Goldschmidt (1933), who applied the serological methods to the identification of enteropathogenic coliform bacilli and made the first serious epidemiological study of gastro enterities due to Esch. coli.

After a complete antigenic scheme of Esch. coli was made available by Kauffman (1951), many serotypes were reported subsequently in the literature. Important amongst them were the 026:B6 by Taylor and Charter (1952), 0119:B14 by Smith (1953), 0127:B8 by Ewing et. al. (1955); 0142:B86 by Orskov et. al. (1960).

Clement et. al; (1953) and Trebedi and Sarkar (1955) reported 0111, 055, 026 and 026, 055, 03, 027, and 038 resp.
as causative organisms of infantile diarrhoea. Later, relationship between the common serotypes and the pathogenicity was observed by many workers [Sengupta and Sharma, (1967), Shriwastava et. al. (1968); Lawande (1972); Nath et. al. (1973); Nadkarni et. al. (1975); Deb et. al. (1977) etc].

Four new strains 0148, 0151, 0152 and 0153 were isolated from enteric diseases in man. [Orskov et. al. (1972)] 0149 : K91 was isolated from two piglets after autopsy and identified by the International center of Esch. coli in Copenhagen [Yalcin, (1971)].

Later, 0154; 0155 and 0156 Esch. coli strains representing strains from faeces and foods were established as antigenic test strains [Orskov et. al. (1973)]. Also, Esch. coli 0159 was found in out-break of enteritis amongst babies in a nursery unit [Gross et. al. (1976)].

RESISTOGRAM TYPING

Many chemical compounds exert selective toxicity within a species of micro-organism. This fact can be used to define a profile of a strain based on its resistance to selected compounds : the profile of the strain is referred to as its 'resistogram' [Elek and Higney, (1970)].

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Observations of the resistance of microorganisms to dyes for the purpose of typing was first employed Huddleson (1931) in distinguishing the different biological types of Brucella. The techniques of accurately testing sensitivity to antibiotics were not applied to antiseptics.

Elek and Higney (1970) reported resistogram typing as a new epidemiological tool and applied the principle to Esch. coli in urinary tract infection. Their results suggest that the method is comparable in efficiency with somatic serotyping of urinary strains of Esch. coli.

Esch. Coli infection

Esch. coli is a normal inhabitant of man and other animals. Jensen (1913) showed that some strain of Esch. coli were responsible for gastro-enteritis. Association of certain strains of Esch. coli with infantile diarrhoea was suspected shortly after the first world war, - [Adam, (1927)].

In man, the most common and important septic infection due to Esch. coli are of urinary tract about 80 % and which are not complicated by instrumentation or anatomical abnormality, - [Mond et. al. (1965)].
It is frequent cause of diarrhoea in pediatric wards and nurseries [Taylor et al. (1949); Taylor and Charter (1952); Adam (1956)].

Mortality and morbidity due to diarrhoea is high in infancy and in those parts of the world where malnutrition and unhygienic conditions are common.

Esch. coli are responsible for 30-40% of sporadic diarrhoea all over the world, [Gorbach et al. (1972); Rudoy and Nelson, (1975); Evans et al. (1977)]. Esch. coli was not recognised as pathogen as long as it was confined to the gastrointestinal tract. The first work as E. coli as a pathogen in babies was done by Adams in 1923. He described a strain of E. coli, which he could identify by biochemical tests and which was isolated from babies with diarrhoea. Goldschmidt in 1933 identified her strains from babies with diarrhoea by agglutination tests. During 1920s and 1930s several workers tried to identify specific types of Esch. coli as aetiological agents but no significant progress was made until a definite serotyping scheme was introduced by Kauffmann in the 1940s; [Kauffmann (1947)]. Epidemiological investigation of serious outbreaks of infantile enteritis in London and Aberdeen in the late forties clearly showed that certain serological types were responsible, - [Giles (1948)].
In 1949, E. coli serotypes such as 0111 : B4 and 055 : B5 from nursery out-breaks were reported by Taylor et. al. 1952, and Giles et. al. 1949 respectively. Later, confirmation and extension of these findings by other workers, showed that these two specific types had been isolated from different parts of the world, - [Kirby et. al. (1950); Magnusson et. al. (1950); Modica et. al. (1952)]. In addition to this epidemiological evidence for pathogenecity, volunteer experiments in both adults, [Ferguson and June (1952), June et. al. (1956)] and in children, [Neter and Shummwau, (1950)] with these two setorypes, confirmed that ingestion of large number of organisms of these two serotypes regularly resulted in diarrhoea.

Additional serotypes were identified in subsequent years by epidemiological investigation of outbreaks; by 1961 some 17 'O' serogroups had been recognised as causes of epidemic infantile enterities in many countries. The predominant 'O' serogroups reported so far are 18, 20, 25, 26, 28, 44, 55, 86, 111, 112, 114, 119, 125, 126, 127, 128 and 142. Six of these 'O' subgroups, namely 26, 55, 111, 119, 127 and 128 are found to be perticularly common.

The diarrhoeagenic E. coli at present can be therefore subdivided into -
i) Enteropathogenic E. coli (EPEC):
The pathogenic mechanisms of these organisms is not still understood.

ii) Entero- toxicogenic E. coli (ETEC):
These organisms produce exotoxins that induce secretory response by intestinal epithelium analogous to cholera toxin.

iii) Enteroinvasive E. coli

iv) Entero haemorrhagic E. coli: [Anantha Narayan and Panikar (1990)].

Sherwood et. al. (1972) reported toxin producing Esch. coli from cases of infantile diarrhoea causing fluid accumulation and gross distention of rabbit intestine.

When placed in the small intestinal lumen, the toxin caused electrolyte secretion apparently by stimulating mucosal adenyl cyclase thus increasing intracellular level of cyclic AMP; that is 3'5' cyclic adenosine monophosphate, - [Kimberg et. al. (1971); Evans et. al. (1972)].

Both the cholera and Esch. coli toxins as reported by Al-
Awquati et. al. (1972), caused an inhibition of net absorption and a reversal of net chloride movement in isolated ileal mucosa.

Esch. coli may cause sepsis in operation wounds and local abscesses in a variety of organs, [Wilson and Miles, (1975)].

Neonatal meningitis due to Esch. coli is usually attributed to infections from the mother at the time of birth. Esch. coli meningitis are known in adults and more commonly in infants, [Franklin et. al. (1968)].

It was reported that a limited number of well defined serotypes is closely associated with certain infections diseases in human infants and the young of the animals, - [Kauffmann and Orskov, (1956); Ewing et. al. (1963)].

ANTIBIOTIC RESISTANCE :

Esch. coli is ordinarily sensitive to many antibiotics, although moderately resistant to benzyl-penicillin, ampicillin, streptomycin, tetracycin and sulphonamide because they carry transferrable resistance determinants, - [Wilson and Miles, (1975)].
In Houston, during the winter of 1960 and 1961 colistin was used but very soon colistin resistant strains began to appear.

Organisms resistant to gentamycin was also found in 1971 (Riley, 1971). But it was reported as a useful drug for the treatment of selected cases of urinary tract infection, [Sengupta et. al. (1975)].

Strains with transferable resistance to one or more drugs can be isolated from the faeces of most members of the general population. [Levis, (1968); Datta, (1969)]. Cook (1971) reported the antibiotic sensitivity of Esch. coli from a variety of sources. They found that antibiotic resistant organisms were a little more common in the bowel of hospital patients than in normal population and related this to contamination of the hospital food with antibiotic resistant coliforms.

Esch. coli is widely distributed in the bowel of man and animals and its distribution in nature is related to this, it only being found in areas which are contaminated by faeces.

Very little evidence is known about spread from the environment. Esch. coli serotypes can survive in dust for a
considerable period [Rogers (1954)]. The incidence of Esch. coli in soil as in water is probably related very closely to the level of faecal contamination.

Studies on the epidemiology of Esch. coli [Turck et. al. (1969)] suggested that, as in the case with staphylococci the ecology and epidemiology of Esch. coli infections are changing constantly. This is not surprising because the coliform flora of the stools is constant flux, and it might be expected that this will be reflected in the epidemiologic behaviour of these organisms.
Of all the gram negative bacilli causing nosocomial infections Pseudomonas is the most important with Proteus following very closely. Like Pseudomonas, Proteus also shows multiple drug resistances.

Proteus belongs to family - Enterobacteriaceae  
Tribe - Proteae.

Proteus is pleomorphic and is so named after a Greek God of Ocean who took many shapes. Proteus has four species, namely pr. vulgaris, pr. mirabilis, pr. morganii, pr. rettgeri. Now a days Pr. Morganii is known as Morganella morganii and Pr. rettgeri as Rettgerella rettgeri.

Definition:

gram negative rods, usually motile by peritrichate flagella. Many strains have a characteristic growth (i.e. swarming); on agar media usually produce gas from glucose (except one species) but in small amount; do not acidify dulcitol and rarely acidify lactose; decompose urea and convert phenylalanine into phenyl - pyruvic acid; resistant to potassium cyanide, do not attack malonate, do not form lysine decarboxylase and arginine dihydrolase. Moles per cent G + C 36 - 53 (pr. morganii 51-53 other species 36-42).
Type Species:

Proteus Vulgaris

Proteus was found by Houser in 1885, who isolated three species from putrifying material such as meat and these were proteus mirabilis, proteus vulgaris and proteus zenkeri. The last one has been transferred to a separate genus kurthia.

Proteus Vulgaris (=common) and Proteus mirabilis (=wonderful) were originally classified on the basis of gelatin liquefaction. They were redefined by Wenner and Rettger in 1919 on the basis of maltose and sucrose fermentation. Proteus Vulgaris ferments maltose and sucrose promptly while proteus mirabilis is a maltose non-fermenter and late sucrose fermenter.

Proteus rettgeri was originally found by Rettger in 1904 but it was described by Hadley et al, in 1918 who called it as Bacterium rettgeri. It was transferred to genus Proteus by Rustigian and Stuart in 1943.

Proteus morganii was found by Morgan in 1906 and it was called as Morgan’s Bacillus No. 1.

As the Proteus organisms were first found in putrifying...
material, they were not considered to be pathogenic even though they were isolated in pure culture from various infected lesions.

Taylor in 1928 isolated Proteus from various infected lesions such as urinary tract infections, wound infections, septicaemias etc. and divided those strains into pathogenic, non-pathogenic and doubtfully pathogenic. He called them pathogenic only when they were isolated in pure culture.

From this time onwards Proteus has been regarded as one of the pathogenic organisms and has been reported as a cause of many epidemics especially of urinary tract infection in the hospital.

**BACTERIOLOGY**:

**Morphology**: These are Gram negative bacilli measuring about 1 - 3 mc long by 0.4 - 0.6 mc broad. Short, fat, coccobacillary forms are also seen. Young cultures on solid media in which swarming is seen, contain many organisms that are long, curved and filamentous, reaching 10, 20 or even 30 mc in length. These forms contain many nuclei without intervening septa. All members are actively motile by peritrichate flagella except for the non flagellated O
variants. All Proteus strains are fimbriate. Proteus is non-sporing and non-capsulated.

**CULTURAL CHARACTERISTICS:**

1) **Nutrient Agar** - Growth occurs all over the surface especially if it is moist, due to swarming.

2) **Blood Agar** - Swarming growth occurs on the medium, haemolysis is seen on rabbit blood agar but not on horse blood agar.

Swarming is readily seen in case of Proteus vulgaris and Proteus mirabilis at 37°C on ordinary agar (2 %), but in case of R. rettgeri and M. morganii it occurs on 1% agar plate incubated at temperature 20°C to 28°C.

Swarming is defined as "progressive surface spreading by the microbes from the edge of the parent colony." Its most characteristic form is, discontinuous swarming which begins after 4 hours of incubation, when a thin layer of growth appears around the site of inoculation and progresses outwards for 2 hours. The progress then ceases but layer of the growth thickens; at about 8th hour swarming starts again and a fresh ring of growth appears. Alternation of swarming and rest occurs at about every 4 hours until the whole plate
is covered. This gives countoured or rippled appearance to the surface of the growth. When the Swarming begins, edge of the growth consists of long slender forms which are in constant motion. When swarming ceases long forms are replaced by short forms. Few variants of Proteus vulgaris and Proteus mirabilis show uniform sheet of growth covering the whole surface or a compact ring of growth to a limited extent. This is called as "continuous swarming." In this type of Swarming long forms are not seen.

Swarming was first described by Cantu in 1911.

Cause of swarming has been a point for much speculation. Ruse and Munzer attributed swarming to the depletion of nutrients (1935). Lominsky and Lendrum (1947) suggested a role of negative chemotactic effects of metabolites which are formed at the site of stationary growth. When they reached a particular concentration they stimulate swarming.

Several methods have been found out to inhibit swarming, mainly to facilitate isolation of Gram positive cocci and organisms of enteric group.

These are as follows:

a) Narcotic drugs: Such as morphine, sodium phenobarbitone and chloral hydrate, the last one is used as 1 : 500 to 1 : 1000 final concentration in the medium.
b) Polyvalent rabbit antiserum against Proteus.
c) Alcohol 5 % to 6 %.
d) Six percent agar.
e) Boric acid 0.1 % (w/v) in heated blood agar.
f) Sodium azide - final concentration of 1 : 5000 to 1 : 10,000 in the medium.
g) Saltless agar

3) MacConkey's Agar: Colonies are colourless i.e. non-lactose fermenting, may be smooth but more often have a slightly rough surface with an irregularly crenated radially striated edge.
### BIOCHEMICAL REACTIONS

*(Edward and Ewing)*

<table>
<thead>
<tr>
<th>Test or substrate</th>
<th>Proteus vulgaris</th>
<th>Proteus mirabilis</th>
<th>Proteus morganii</th>
<th>Proteus rettgarri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl red</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>-</td>
<td>- or +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H₂S production</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Simon’s citrate</td>
<td>d</td>
<td>+ or (+)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>KCN</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatinase 22°C</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenyl-alanine deaminase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>+ or -</td>
<td>+</td>
</tr>
<tr>
<td>Oxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fermentation</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

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Lactose  -  -  -  -  -
Sucrose   +  d  -  d
Mannitol  -  -  -  + or -
Maltose   +  -  -  -

+ = positive  - = negative

d = different strains give different reactions +, (+), -
F = Fermentation  - or + = More strains negative
+ or - = More strains positive  (+) = Delayed positive.
HUMAN INFECTIONS:

All species of Proteus are pathogenic to man. They cause septic lesions in a variety of systems. These include wound infections, septicaemias, meningitis mastoid abscesses, urinary tract infections etc. They have been reported as causative organisms in epidemics of food poisoning also. They have been isolated in pure cultures from all of these infections from time to time. They are also found along with other organisms in wounds and burns and their presence is believed to favour infections due to pathogenic anaerobes.

Septicaemia due to Proteus have fetal outcome in majority of the cases. It may follow urinary tract infections or an infected bed sore or mechanical obstruction of an organ infected with Proteus such as biliary tract or uterus. In new borns meningitis accompanied with septicaemia may follow omphalitis.

Proteus is one of the important organisms causing urinary tract infections, either primary or acquired in hospitals. These are seen mainly in patients who have urinary tract obstruction. In such cases infection usually follows instrumentation of the urinary tract.
Serotyping of Proteus vulgaris and mirabilis [J. deLauvois, (1969)].

Serotyping is one of the earliest methods used to type the organisms. The above mentioned author used 20 "O" and 4 "H" antisera which were as follows:

C Antisera - 3, 5, 6, 7, 9, 10, 11, 13, 14, 16, 17, 18, 19, 20, 23, 24, 26, 28, 29, 30.

H Antisera - 1, 2, 3 and 4.

These were prepared by injecting the respective antigens in rabbits prepared as follows:

O antigens were prepared by heating 24 hours broth culture in saline. H antigens were prepared by making a saline suspension of the swarming growth on blood agar plates and subsequently formalising it. Sera were appropriately absorbed to make them specific.

Serotyping of 'O' antigens:

Diluted suspension of the organisms was taken and it was
mixed with an equal quantity of antiserum. It was incubated at 50°C for 18 hours. It was allowed to stand for 1 hour at room temperature and looked for agglutination.

When heat killed O antigen preparation of an organism was found autoagglutinating, then alcoholic extract from deoxycholate citrate agar was used, if this also was found autoagglutinating then the strain was investigated further.

Serotyping of H antigens:

A drop of bacterial suspension from swarming growth was mixed with a drop of antiserum and looked for agglutination after half a minute.

RESISTOTYPING: [Kashbur I.M. et al. (1974)].

Resistotyping is a method in which organisms are typed according to their resistance to various substances which are non-antibiotics but are still antibacterial. First report regarding such a typing is about E. coli by Elek in 1970 and second one is about Sh. sonnei by Elek et al. (1973).

The author used following substances and assign a reference letter to each of it as shown below:

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<table>
<thead>
<tr>
<th>Reference letter</th>
<th>Antibacterial Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sodium hydrogen selenite</td>
</tr>
<tr>
<td>B</td>
<td>Cetramide</td>
</tr>
<tr>
<td>C</td>
<td>Malachite green</td>
</tr>
<tr>
<td>D</td>
<td>Acriflavin</td>
</tr>
<tr>
<td>E</td>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td>F</td>
<td>Orcinol</td>
</tr>
<tr>
<td>G</td>
<td>Sodium periodate</td>
</tr>
<tr>
<td>H</td>
<td>Irgassan (H 3565 - Geigy)</td>
</tr>
<tr>
<td>I</td>
<td>Potassium tellurite</td>
</tr>
<tr>
<td>J</td>
<td>Chlorheximide diacetate</td>
</tr>
</tbody>
</table>

To each, Triphenyl tetrazolium chloride (TTC) was added and the mixture in 5 different concentrations was added to tryptose agar. Only in the case of sodium periodate TTC was added to agar directly to avoid precipitation, in the premix. Plates were dried and inoculated with overnight broth culture. Control plates without chemicals were also inoculated. The plates were then incubated at 37°C for 18 hours.

Strains showing less than 15 colonies or complete inhibition were labelled as sensitive for that chemical and were not recorded. Strains showing confluent growth were labelled as resistant to that chemical and assigned appropriate letter
e.g. A, B etc. Strains showing non confluent growth with more than 15 colonies with a particular chemical were recorded in parenthesis e.g. (A), (E) etc. Accordingly the strains were included in a particular group.

The plates containing the concentration of a chemical showing the typical pattern of inhibition of control strains were chosen for recording the pattern of unknown strains.

Strains were tested for reproducibility four times. Each time labelling of the strain was varied so that the worker was unaware of the identity of the strain.

Resistance to antibiotics (discs) was also recorded concentrations of antibiotics per disc were as follows:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>10 mcg</td>
</tr>
<tr>
<td>Cephaloridin</td>
<td>100 mcg</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25 mcg</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10 mcg</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 mcg</td>
</tr>
<tr>
<td>Sulphonamide</td>
<td>200 mcg</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>25 mcg</td>
</tr>
</tbody>
</table>
PROTEOCINE TYPING:

Al-Jumailis Method (1975):

This is a method of proteocine typing in which organisms is tested for its sensitivity to the proteocine produced by producer strains namely B.J. - 1 to 12.

Media used:

(A) Peptone water - PP₃ (broth)
Proteose peptone No. 3 (Difco) - 20 gm/liter
Sodium chloride - 5 gm/liter
PH - 7.4

(B) Bile salt Brain Heart infusion agar (BBHIA)
Brain heart infusion powder - 30 gms
Bile salts - 1.5 gms
Agar No. 3 - 10 gms
Distilled water - 1 liter
PH - 7.4.

Production of Proteocine:

Producer strains are inoculated into 5 ml. amounts of PP₃ broth and incubated at 25°C. After 18 hours this culture is
added to 50 ml of preheated (25°C) PP₃ broth and incubated at 25°C. After one hour mitomycin - c is added to each culture to give a final concentration of 1 μg/ml. and incubation is continued for further 24 hours. Then broth cultures are centrifuged at 3000 rpm for 20 minutes. The supernatant fluid is transferred to fresh sterile containers. Chloroform 0.5 ml is added to each flask the contents of which are agitated thoroughly for 5 minutes. The cultures are then centrifuged again at 3000 rpm. for 20 minutes and chloroform free sterilized proteocine preparation forms the supernatent which is finally removed to sterile screw capped bottles and stored at 4°C.

**Sensitivity Testing :**

Isolates to be screened for proteocine sensitivity are grown in 5 ml. PP₃ broth, incubated in water bath at 37°C for 7 hours, 2 ml of suspension of the culture containing 10⁷ organisms/ml. are flooded over the surface of BBHIA plates to produce uniform lawn of bacteria, excess broth being removed with sterile pasteur pipette. After drying standered drops of proteocine preparations are transferred to the surface of seeded agar plates using phage applicator. The plates were then dried and incubated at 37°C for 18 hours. Proteocine activity is indicated by punched out
zones of inhibition within the confluent growth of bacterial lawn, or by inhibition with discrete colonies within zones (partial inhibition).

Reading the results:
Inhibition, partial or complete is indicated by positive (+) sign and confluent growth by negative (-) sign.

Coding System:

Producer strains are divided into 4 groups as follows:
(1) 1, 2, 3, (2) 4, 5, 6, (3) 7, 8, 9, (4) 10, 11, 12.

As mentioned above signs are placed under each strain; (+) sign carries 4 marks when it is under the first member of the group, 2 marks when under the second member of the group, one mark when under the third member of the group. (-) sign carries 0 marks. All marks in a group are summed up; the number thus formed is a four figure number and it is the code for that particular strain.

\[
\begin{align*}
\text{e.g. } 1 & \quad 2 \quad 3 \quad 4 \quad 5 \quad 6 \quad 7 \quad 8 \quad 9 \quad 10 \quad 11 \quad 12 \\
+ \quad + \quad - \quad - \quad - \quad + \quad - \quad - \quad - \quad + \quad + \\
4 \quad + \quad 2 \quad + \quad 0 \quad 0 \quad + \quad 0 \quad + \quad 0 \quad 4 \quad + \quad 0 \quad + \quad 0 \quad 0 \quad + \quad 2 \quad + \quad 1 \\
6 \quad 0 \quad 4 \quad 3
\end{align*}
\]

Code No. 6043
STREPTOCOCCI

Streptococcus haemolyticus though is a relatively unimportant cause of hospital infection, must have the pride of place as it was the work on them that indicated so many potential paths of infection and our septic rituals were devised with streptococcal infections in mind. They had been a notorious cause of hospital infection, as a cause of puerperal fever and wounds sepsis in Nineteenth century, — [Williams et. al, (1966)].

Phyllis Rountree (1955) reported the incidence of B. haemolytic streptococci 11% in 1950 and 28% and 32% in 1953 and 1954. Which they attributed to increased throat carriers amongst the staff, Dineen (1964) found the incidence of streptococcus haemolyticus infection in only 3% of post operative cases.

In the united state Langohr et al, (1947) reporting a detailed bacteriological study of the burned surface considered haemolytic Streptococci and Staphylococci particularly damaging to the deep burns and latter so especially when present with Proteus group of bacteria. Altemeir et al, (1962) made observations regarding the predominant and the most important infecting bacteria. In their series the predominant and the most important bacteria
were Staphylococcus aureus, Streptococcus pyogenes, Proteus and Pseudomonas aeruginosa. These organisms accounted for fifty percent of all bacteria recovered from the infected burn wound and represented ninety percent of bacteria causing death by infectious complications.

In the period between 1942-1944 there was a high incidence of haemolytic Streptococcal and pyogenic Staphylococcal contamination. In the early burn wound in their series they recorded a significant increase in the incidence of strains of Staphylococcus pyogenes from twenty percent to fifty percent. Over the years, the incidence of Streptococcus pyogenes gradually decreased. This reflects the general pattern of change of organisms in the post penicillin era, - [Altemeir et al, (1962)].

Group A Streptococcus pyogenes is a highly transmissible pathogen that can cause abrupt deterioration in the burn wound with rapid progression to death. The infection is associated with an increase in wound pain, redness, induration and swelling. Since it invades normal tissue, the most significant sign of Streptococcal infection is redness extending from the margin of the burn wound. Donor sites and freshly grafted wounds may also become infected with the beta haemolytic Streptococci but the clinical coarse is usually less abrupt, and the loss of grafts or
conversion of a donor site to a full thickness injury is usually a more major consideration than invasive sepsis. In newborns and children it is responsible for skin rashes, sore throats etc. Williams et al, (1966) feel that now except in burns Streptococcus haemolyticus is of trivial importance, a change no doubt brought about by antibiotics.

FUNGUS:

Increasing numbers of mycotic infections of the burn wound associated with improved control of burn wound sepsis caused by gram negative and gram positive bacteria have been reported. Aspargillus, Mucor and Rhizopus have been the more common pathogens.

Candida infection has gained importance nowadays. Several authors have documented the rising incidence of fungi in burn wounds. MacMillan et al, (1972) reported 14 out of 65 deaths due to candidial systemic invasion from the burn wound.

are other who have reported a rising incidence of fungi in burn wound infections.

In newborn neonates it is responsible for thrush and diarrhoea.