It is quite obvious that patients are better treated in hospitals than anywhere else. Historically, hospitals have a notorious reputation for infection. However, congregating large number of sick under a single roof could easily facilitate the transmission of infectious disease from patient to patient. Before the mid-19th century, surgical patients commonly developed postoperative “irritative fever,” followed by purulent drainage from their incisions, overwhelming sepsis, and often death. It was not until Lister introduced the principles of antisepsis. Lister’s work changed surgery from an activity associated with infection and death to a discipline that could eliminate suffering and prolong life. The hazards of puerperal sepsis and the horrors of septic infection in the Pre-Listerian era have been well documented; admission to hospital in the mid-19th century was associated with the fear of gangrene and death. (37, 133)

Since that time, surgical and medical techniques have developed dramatically, basic Standards of building and hygiene have greatly improved and the identification and treatment of infecting micro-organisms have become possible in most cases. (37)

Despite such significant changes, infection acquired in hospitals, still remains one of the main causes of morbidity and mortality, leading directly or indirectly to an Enormous increase in the cost of hospital care and to the emergence of new health Hazards for the community. (37)

Nosocomial infection is a major public health problem throughout the world. WHO has described it one of the major infectious diseases having huge economic impact. It is estimated that at any point of time more than 1.4 million people are suffering from nosocomial infections in the world. There is no doubt that this figure is only the tip of the iceberg as the record keeping system in most of the developing/underdeveloped nations are quite poorly evolved and managed. More than 50% of these infections can be prevented if sufficient data about their risk factors are available and relevant preventive measures are adopted. In recent days, as more and more invasive diagnostic and therapeutic procedures as well as indiscriminate use of antibiotics are being plasticized, the problem of nosocomial infection is posing more complex challenges. (174)
A nosocomial infection (from nosocomiein, meaning hospital) — also called "hospital acquired infection" can be defined as: An infection acquired in hospital by a patient who was admitted for a reason other than that infection. An infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. This also includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility. \(^{(174)}\)

Patient care is provided in facilities which range from highly equipped clinics and technologically advanced university hospitals to front-line units with only basic facilities. Despite progress in public health and hospital care, infections continue to develop in hospitalized patients, and may also affect hospital staff. Many factors promote infection among hospitalized patients: decreased immunity among patients; the increasing variety of medical procedures and invasive techniques creating potential routes of infection; and the transmission of drug-resistant bacteria among crowded hospital populations, where poor infection control practices may facilitate transmission. \(^{(67)}\)

Nosocomial infections occur worldwide and affect both developed and resource-poor countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients. They are a significant burden both for the patient and for public health. A prevalence survey conducted under the auspices of WHO in 55 hospitals of 14 countries representing 4 WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed an average of 8.7% of hospital patients had nosocomial infections. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital. The highest frequencies of nosocomial infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8 and 10.0% respectively), with a prevalence of 7.7 and 9.0% respectively in the European and Western Pacific Regions. \(^{(67)}\)

The most frequent nosocomial infections are infections of surgical wounds, urinary tract infections and lower respiratory tract infections. The WHO
Introduction & Review of Literature

study, and others, has also shown that the highest prevalence of nosocomial infections occurs in intensive care units and in acute surgical and orthopaedic wards. Infection rates are higher among patients with increased susceptibility because of old age, underlying disease, or chemotherapy. (67)

IMPACT OF NOSOCOMIAL INFECTIONS
Hospital-acquired infections aid to functional disability and emotional stress of the patient and may, in some cases, lead to disabling conditions that reduce the quality of life. Nosocomial infections are also one of the leading causes of death. The economic costs are considerable. The increased length of stay for infected patients is the greatest contributor to cost. One study showed that the overall increase in the duration of hospitalization for patients with surgical wound infections was 8.2 days, ranging from 3 days for gynaecology to 9.9 for general surgery and 19.8 for orthopaedic surgery. Prolonged stay not only increases direct costs to patients or payers but also indirect costs due to lost work. The increased use of drugs, the need for isolation, and the use of additional laboratory and other diagnostic studies also contribute to costs. Hospital-acquired infections add to the imbalance between resource allocation for primary and secondary health care by diverting scarce funds to the management of potentially preventable conditions. (67)

THE MICROBIAL AGENT
The patient is exposed to a variety of microorganisms during hospitalization. Contact between the patient and a microorganism does not by itself necessarily result in the development of clinical disease — other factors influence the nature and frequency of nosocomial infections. The likelihood of exposure leading to infection depends partly on the characteristics of the microorganisms, including resistance to antimicrobial agents, intrinsic virulence, and amount (inoculum) of infective material. (67)

Many different bacteria, viruses, fungi and parasites may cause nosocomial infections. Infections may be caused by a microorganism acquired from another person in the hospital (cross-infection) or may be caused by the patient’s own flora (endogenous infection). Some organisms may be acquired
from an inanimate object or substances recently contaminated from another human source (environmental infection). \(^{(67)}\)

**THE ENVIRONMENTAL FACTORS**

Health care settings are an environment where both infected persons and persons at increased risk of infection congregate. Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for patients and staff. Patients who become infected in the hospital are a further source of infection. Crowded conditions within the hospital, frequent transfers of patients from one unit to another, and concentration of patients highly susceptible to infection in one area (e.g. newborn infants, burn patients and intensive care) all contribute to the development of nosocomial infections. \(^{(67)}\)

**BACTERIAL RESISTANCE**

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistance elements, antibiotics promote the emergence of multi drug resistant strains of bacteria; microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital. The widespread use of antimicrobials for therapy or prophylaxis (including topical) is the major determinant of resistance. Antimicrobial agents are, in some cases, becoming less effective because of resistance. As an antimicrobial agent becomes widely used, bacteria resistant to this drug eventually emerge and may spread in the health care setting. Many strains of *Pneumococci*, *Staphylococci*, *Enterococci*, and *M. tuberculosis* are currently resistant to most or all antimicrobials which were once effective. Multi drug resistant *Klebsiella species* and *Pseudomonas aeruginosa* are prevalent in many hospitals. This problem is particularly critical in developing countries where more expensive second-line antibiotics may not be available or affordable. \(^{(67)}\)

In determining the extent of hospital infection, the following categories should be considered. \(^{(37)}\)

1) Infections contracted and developing outside hospitals which require admission of the patient (e.g. pneumonia).
2) Infections contracted outside hospital which becomes clinically apparent when the patient is in hospital (e.g. measles).

3) **Infections contracted and developing within hospital (e.g. Post Operative Wound Infection).**

4) Infections contracted in hospital but not becoming clinically apparent until After the patient has been discharged (e.g. breast abscess).

5) Infections contracted by hospital staff as a consequence of their work, whether or not this involves direct contact with patients (e.g. Hepatitis B).

On an average, 5 – 10 % of all hospital patients will develop an infection as a result of their stay in hospital. Urinary tract infection, respiratory tract infections and **post operative wound infections** are the most common.\(^{(37)}\)

**Figure:-1: Frequency of Different Types of Nosocomial Infections** \(^{(108)}\)
Micro-organisms Causing Hospital infection:
The most important micro-organisms responsible for hospital infection are listed in Table-1.1 (37)

**Table-1.1 : Commonly Occurring Micro-organisms in hospital infection**

<table>
<thead>
<tr>
<th>Category</th>
<th>Micro-organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory infections</td>
<td><em>Haemophilus influenzae</em>, <em>Streptococcus pneumoniae</em>, <em>Staphylococcus aureus</em>,&lt;br&gt;<em>Enterobacteriaceae</em>,&lt;br&gt;<em>Respiratory viruses.</em></td>
</tr>
<tr>
<td>Wounds and skin sepsis</td>
<td><em>Staphylococcus aureus</em>,&lt;br&gt;<em>Escherichia coli</em>, <em>Klebsiella</em> spp.,&lt;br&gt;<em>Anaerobes</em>, <em>Faecal Streptococci</em>,&lt;br&gt;<em>Coagulase Negative Staphylococci</em> spp.</td>
</tr>
</tbody>
</table>

Presently, about 60% of hospital acquired infections are caused by aerobic Gram Negative Bacilli and about 30% by Gram Positive Cocci. Many Gram Negative bacilli such as *Pseudomonas aeruginosa* are opportunists Capable of causing infection in compromised patients (37).

**POST OPERATIVE WOUND INFECTIONS**
Modern surgery can be said to have its roots in the 19th century, when reconstructive, tissue-preserving operations began to replace amputations. Before the mid-19th century, surgical patients commonly developed postoperative “irritative fever,” followed by purulent drainage from their incisions, overwhelming sepsis, and often death.
It was not until the late 1860s, after Joseph Lister introduced the principles of antisepsis, which postoperative infectious morbidity decreased substantially. With the Lister's aseptic treatment of wounds, and when it was recognized that successful surgery was predicted on the technical skill of the surgical team, the limitation of the number of microorganisms introduced into the surgical wound, and the presence of systemic and local factors that enhance the ability of the patient to limit microbial replication and invasion. Lister's work radically changed surgery from an activity associated with infection and death to a discipline that could eliminate suffering and prolong life. (9)

A wound is the result of physical disruption of the skin, one of the major obstacles to the establishment of infections by bacterial pathogens in internal tissues. When bacteria breach this barrier, infection can result. The most common underlying event for all wounds is trauma. Trauma may be accidental or intentionally induced. The latter category includes hospital-acquired wounds, which can be grouped according to how they are acquired, such as surgically and by use of intravenous medical devices. (5)

The incidence of infection varies from surgeon to surgeon, from hospital to hospital, from one surgical procedure to another, and--most importantly--from one patient to another. During the mid 1970s, the average hospital stay doubled, and the cost of hospitalization was correspondingly increased when postoperative infection developed after six common operations. These costs and the length of hospital stay are undoubtedly lower today for most surgical procedures that are done on an outpatient basis, such as laparoscopic (minimally invasive) operations or those that require only a short postoperative stay (189).

In 1992, the US Centers for Disease Control (CDC) revised its definition of 'wound infection', creating the definition 'surgical site infection' (SSI) to prevent confusion between the infection of a surgical incision and the infection of a traumatic wound. Most SSIs are superficial, but even so they contribute greatly to the morbidity and mortality associated with surgery. Estimating the cost of SSIs has proved to be difficult but many studies agree that additional bed occupancy is the most significant factor. (189)
Surgical wound infections present a serious hazard to patients. Local complications include tissue destruction, wound dehiscence, incisional and deep hernias, septic thrombophlebitis, recurrent pain, and disfiguring and disabling scars. Systemic complications include toxemia, bacteremia, shock, metastatic infection, failure of vital organs remote from the infection, and death. The severity of each complication depends in large part on the infecting pathogen and on the site of infection. They are the third most frequent nosocomial infection in most hospitals and are an important cause of morbidity, mortality, and excess hospital costs. (3)

In general, a wound can be considered infected if purulent material drains from it, even without the confirmation of a positive culture. Infected wounds may not yield pathogens by culture because some pathogens are fastidious, culture techniques are inadequate, or the patient has received antimicrobial therapy. (3)

Wound site infections are a major source of postoperative illness, accounting for approximately a quarter of all nosocomial infections. National studies have defined the patients at highest risk for infection in general and in many specific operative procedures. Advances in risk assessment comparison may involve use of the standardized infection ratio, procedure-specific risk factor collection, and logistic regression models. (189)

Based on NNIS system reports, post operative wound infections are the third most frequently reported nosocomial infections among hospitalized patients. (133) Among surgical patients, post operative wound infections were the most common nosocomial infection, accounting for 38% of all such infections.

Why some patients develop postoperative surgical wound infection and others do not remains a mystery. There are many risk factors for infection, and mathematical scoring systems are often good predictors of infection; yet, some patients with a plethora of risk factors fail to develop surgical site infections. Even patients with established abdominal infection do not automatically develop wound infection.

Early experimental work, now confirmed in the clinical setting, dictates that bacteria must be in the wound to cause infection; the minimal infecting dose
will depend on the environmental conditions in the wound. The presence of foreign bodies, trauma, hematoma, etc., will enhance the effect of the inoculum; therefore, surgical debridement and careful surgery are necessary to reinforce the host defences. Some bacteria, e.g., *Staphylococcus aureus* and *Streptococcus pyogenes*, have a greater propensity to cause infection, so extensive infection-control practices are necessary to prevent or contain these pathogens. To minimize the risk of surgical site infection, individual patient risk factors must be identified and modified whenever possible. The patient should be prepared for the operation and appropriate skin antiseptics should be used on the operative site. The patient should be considered for preoperative antibiotic prophylaxis and, if appropriate, bowel preparation should be carried out.

Care and attention to the operation theatre environment is important, especially for cases in which airborne transmission of bacteria should be controlled, e.g., ultraclean air systems for implant surgery. In elective surgery, the source of bacteria that cause infection is either endogenous - the patient's normal flora (e.g. skin or bowel) or exogenous - the surgical staff or environment. Surgical expertise and theatre discipline are essential components in the fight against surgical sepsis.\(^{(61)}\)

Although the total elimination of wound infection is not possible, a reduction in the infection rate to a minimal level could have significant benefits in terms of both patient comfort and medical resources used.\(^{(102)}\)

The increased proportion of SSIs caused by resistant pathogens and *Candida* species may reflect increasing numbers of severely ill and immunocompromised surgical patients and the impact of widespread use of broad-spectrum antimicrobial agents.\(^{(133)}\)

Advances in infection control practices include improved operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial prophylaxis. Despite these activities, SSIs remain a substantial cause of morbidity and mortality among hospitalized patients. This may be partially explained by the emergence of antimicrobial-resistant pathogens and the increased numbers of surgical patients who are elderly.
and/or have a wide variety of chronic, debilitating, or immune-compromising underlying diseases. There also are increased numbers of prosthetic implant and organ transplant operations performed. Thus, to reduce the risk of SSI, a systematic but realistic approach must be applied with the awareness that this risk is influenced by characteristics of the patient, operation, personnel, and hospital. (133)
KEY POINTS (39, 81,189)

- About 06% of patients acquire an infection in hospital, and the incidence of hospital-acquired infections may be increasing.
- Common hospital-acquired infections are respiratory and urinary tract infections, surgical wound infections and infections associated with intravascular cannulas.
- The common hospital pathogens are Methicillin-resistant *Staphylococcus aureus* (MRSA), antibiotic-resistant Gram Negative Bacilli – ESBL, MBL, KPC producers and more recently, Vancomycin - resistant *Enterococci* (VRE).
- Surveillance is the cornerstone of effective infection control and prevention of hospital-acquired infections. Strategies to prevent both development of antibiotic resistance and spread of resistant organisms are necessary.
- Preventive strategies include prudent antimicrobial use, timely hand washing, aseptic technique, short hospital stays, minimal use and early removal of invasive devices, adequate staffing and an active infection control program.
- Post Operative Wound Infections (POWIs) are a real risk associated with any surgical procedure and represent a significant burden in terms of patient morbidity and mortality, and cost to health services around the world.
- A multitude of risk factors influence the development of POWIs and awareness of these will help to promote effective preventive strategies.

What do POWIs (SSI) give rise to?
- SSI’s increase hospitals stay by 7-10 days.
- SSI’s increase hospital costs.
- “Deep SSI’s” longer hospital stay and cost.
- SSI’s increase readmission rates.
- 40-60% SSI’s preventable with appropriate use of antibiotics.
A hospital is a place where sick and injured people go for medical and surgical treatment. Highly skilled health care professionals use the latest technology to make hospital visits as short and painless as possible. Medicine is a term used to describe the pills and syrups prescribed by doctors when people do not feel well. It also is the art and science of maintaining health, and includes the prevention and treatment of diseases and injuries. What we know about medicine today is the result of many discoveries made by men and women over thousands of years.

Before there were doctors and nurses, people tried to heal the sick. Evidence exists in prehistoric human skulls that were discovered with holes in them. Historians believe that medicine men cut holes into the heads of sick people to release evil spirits that they thought caused illnesses. Imagine being awake during brain surgery performed with crudely made tools and nothing to numb the pain. Ouch! Use the timeline to learn about progressively less painful advancements in the history of medicine.

ANCIENT TIMES

Many cultures in ancient times treated illnesses with magic and herbal remedies. People believed that the supernatural powers of a shaman (shaman), also known as a medicine man or witch doctor, healed the sick. Ancient Egyptians thought that their gods healed them. They also treated illnesses with herbal medicines and performed surgeries with metal instruments.

The ancient Greeks pursued medicine as a science. Hippocrates, the most famous physician of the time (c. 400 B.C.), believed that diseases had natural causes, not supernatural ones. He wrote that a proper diet and exercise affected the human body. Today, doctors still follow his advice to observe patients and use the facts to treat them.

Hippocrates:

... *He who desires to practice surgery must go to war,*

On the Surgery
DARK AND MIDDLE AGES

As the Roman Empire ended, Europe fell into the Dark Ages. Superstition crept back into beliefs about medicine, and people were taught that diseases were punishment from God. Much of what was learned from the Greeks and Romans was transferred to the new Islamic regions of Northern Africa, the Middle East and Spain. Medical schools and hospitals were built to support the work of Arabic doctors like Rhazes (900 A.D.) who further explored medicine as a science. He was the first doctor to identify the difference between the measles and smallpox 106.

THE RENAISSANCE

The Renaissance marked a new period of interest in art and science throughout Europe. People were curious about how the human body worked so they dissected dead bodies. This new study of the human anatomy contradicted earlier theories and brought about a more scientific approach to medicine. For hundreds of years, people thought that the heart made blood from food and drink, and the body absorbed it. An English doctor named William Harvey (1578-1657) showed that the heart recycles blood and acts as a pump to circulate it throughout the body 106.

A Brief History of Microbiology

Microbiology is the study of microorganisms, which are unicellular or cell-cluster microscopic organisms. Although much is now known in the field of microbiology, advances are being made regularly. In actual fact, the most common estimates suggest that we have studied only about 1% of all of the microbes in any given environment. Thus, despite the fact that over four hundred years have passed since the discovery of microbes, the field of microbiology is clearly in its infancy relative to other biological disciplines such as zoology, botany or even entomology 249.

Bacteria were first observed by Anton van Leeuwenhoek in 1676 using a single-lens microscope of his own design. The name "bacterium" was introduced much later, by Ehrenberg in 1828, derived from the Greek word
βακτηριον meaning "small stick". While Antony van Leeuwenhoek is often cited as the first microbiologist, the first recorded microbiological observation, that of the fruiting bodies of molds, was made earlier in 1665 by Robert Hooke\textsuperscript{249}.

Louis Pasteur (1822-1895) and Robert Koch (1843-1910) were contemporaries of Cohn’s and are often considered to be the founders of medical microbiology. Pasteur is most famous for his series of experiments designed to disprove the then widely held theory of spontaneous generation, thereby solidifying microbiology’s identity as a biological science.

**ANCIENT SURGERY INDIA**\textsuperscript{99,254}  
Indian physician Sushruta (600 BC) is an important figure in the history of surgery. He lived, taught and practiced his art of surgery on the banks of the Ganges in the area that corresponds to the present day city of Benares in Northern India. Because of his seminal and numerous contributions to the science and art of surgery he is also known by the title "Father of Surgery". Much of what is known about this inventive surgeon is contained in a series of volumes he authored, which are collectively known as the *Sushruta Samhita*. It is the oldest known surgical text and it describes in exquisite detail the examination, diagnosis, treatment, and prognosis of numerous ailments, as well as procedures on performing plastic surgery.

Varanasi on the banks of the Ganges is one of the holiest places in India. It is both the city of Buddha and a destination of pilgrimage for millions of Hindus who come to bathe in the holy river. It is also the home of Ayurveda, one of the oldest medical disciplines. Ayurveda means ‘science of life’, and its approach to the body is philosophical and holistic. Among the greatest of its ancient writings is the *Sushruta Samhita*, which describes the tradition of surgery in Indian medicine. Its author is believed to have been the scholar Sushruta, who lived over 3,000 years ago. Sushruta is said to have been given his knowledge by an incarnation of the god Vishnu. However, it is also suspected that he was simply reporting medical wisdom that had been passed down by word of mouth for centuries.
Sushruta’s general advice to physicians would certainly apply to doctors anywhere and in any age:

A physician who has set out on this path should have witnessed operations. He must be licensed by the king. He should be clean and keep his nails and hair short. He should be cheerful, well-spoken and honest.

The Victorian Revolution in Surgery

The 1944 Hollywood movie The Great Moment tells of the discovery of ether anaesthesia in Boston in the 1840s. This discovery was one of a trio of clinical innovations between the 1840s and the 1890s that collectively made up the Victorian revolution in surgery: anaesthesia, antisepsis, and x-rays. But did these “moments” really represent a revolution in surgery alone, or did they set in motion an even larger revolution in medicine? Viewed historically, these “discoveries” help us understand how medical innovations relate to science and technology. They also reveal how a new medical marketplace came to be and how market forces shaped modern medicine.

Twenty years later, the Glasgow-based surgeon, Joseph Lister, put forward his system of antiseptic surgery. Lister was correct in his view that surgical wound infection was the result of bacteria. By the 1880s, antiseptic surgery (or “Listerism”) had transformed into aseptic surgery as knowledge about pathogenic bacteria accumulated.

Development of modern surgery

Before the advent of anesthesia, surgery was a traumatically painful procedure and surgeons were encouraged to be as swift as possible to minimize patient suffering. This also meant that operations were largely restricted to amputations and external growth removals. In addition, the need for strict hygiene during procedures was little understood, which often resulted in life threatening post-operative infections in patients.

Beginning in the 1840s, surgery began to change dramatically in character with the discovery of effective and practical anesthetic chemicals such as ether and chloroform.
HISTORY OF ASEPSIS AND ANTI SEPTIC:

**Asepsis** is the practice to reduce or eliminate contaminants (such as bacteria, viruses, fungi, and parasites) from entering the operative field in surgery or medicine to prevent infection. Ideally, a field is "sterile" — free of contaminants — a situation that is difficult to attain. However, the goal is elimination of infection, not sterility.\(^{245}\)

**Antiseptics** is a term that is used sometimes as a synonym, but also applies to the uses of antiseptics. Antiseptics are agents that reduce or kill germs chemically and are applied to skin and wound surfaces. In contrast, disinfectants are chemicals applied to inert surfaces and are usually too harsh to be used on biological surfaces. Antibiotics kill specifically bacteria and work biochemically; they can be used externally or internally.\(^{245}\)

The first step in asepsis is cleanliness, a concept already espoused by Hippocrates. The modern concept of asepsis evolved in the 19th century.\(^{245}\)

An **antiseptic** (Greek αντι, against, and σηπτικος, putrefactive) is a substance that prevents the growth and reproduction of various microorganisms (such as bacteria, fungi, protozoa, and viruses) on the external surfaces of the body. Some are true germicides, capable of destroying the bacteria, whilst others merely prevent or inhibit their growth. The objective of antiseptics is to reduce the possibility of sepsis, infection, or putrefaction by germs. **Antibacterial** have the same objective but only act against bacteria. Antibiotics perform a similar function, preventing the growth or reproduction of bacteria within the body. Disinfectants operate on nonliving objects such as medical instruments.\(^{244}\)

The widespread introduction of antiseptic surgical methods followed the publishing of the paper *Antiseptic Principle of the Practice of Surgery* in 1867 by Joseph Lister, inspired by Louis Pasteur’s germ theory of putrefaction.

**Aseptic technique** refers to a procedure that is performed under sterile conditions. This includes medical techniques and laboratory techniques, such as with microbiological cultures.\(^{246}\)

Ayliffe et al. (2000) suggests that there are two types of asepsis: medical and surgical asepsis. Medical or clean asepsis reduces the number of organisms...
and prevents their spread; surgical or sterile asepsis includes procedures to eliminate micro-organisms from an area and is practiced by nurses in operating theatres and treatment areas.\textsuperscript{246}

\begin{itemize}
  \item 1847 - Semmelweis identifies surgeon’s hands as route of spread of puerperal infection.
  \item 1865 - Lister introduces hand and wound asepsis with the use of carbolic acid.
  \item 1800 - Von Bergmann invents the autoclave.\textsuperscript{205}
\end{itemize}

**Discovery of the importance of hygiene** \textsuperscript{247}

It was at the Vienna General Hospital that Semmelweis began investigating the causes of puerperal fever, against the resistance of his superiors who believed it to be non-preventable.

He instituted a policy of using a solution of chlorinated lime for washing hands between autopsy work and the examination of patients and the mortality rate dropped from its then-current level of 12.24% to 2.38%, comparable to the Second Clinic’s.

**Ignaz Semmelweis** (1818 – 1865) \textsuperscript{21,139,247}

"It is a disagreeable declaration for me to mention, that I myself was the means of carrying the infection to a great number of women”.

**Joseph Lister & Antiseptic Surgery (1827 – 1912)** \textsuperscript{22, 91, 234, 248}

"The notion that extensive experience is required for the administration of chloroform is quite erroneous, and does harm by weakening the confidence of the profession in this invaluable agent”

**Joseph Lister**

Before Lister’s day, a wound was so liable to cause serious trouble that the surgeon hesitated to inflict it upon any patient. He successfully introduced carbolic acid to sterilize surgical instruments and to clean wounds.\textsuperscript{164}

Lister also pioneered the use of catgut and rubber tubing for wound drainage.
A critical study of Lister's work on antiseptic surgery

The work of Pasteur on alcoholic and lactic acid fermentation demonstrated that minute organisms (germs) caused these fermentative changes. Lister applied these basic findings in the introduction of his antiseptic system. Its principles were based on the destruction of germs by antiseptics (carbolic acid) to prevent their entering the wound or spreading after surgery. Lister's work on antisepsis was therefore based on the germ theory of disease.

Germ Connection with wound sepsis

When, in 1865, Louis Pasteur suggested that decay was caused by living organisms in the air, which on entering matter caused it to ferment, Lister made the connection with wound sepsis.

He considered that microbes in the air were likely causing the putrefaction and had to be destroyed before they entered the wound.

Lister began to clean wounds and dress them using a solution of carbolic acid. He was able to announce at a British Medical Association meeting, in 1867, that his wards at the Glasgow Royal Infirmary had remained clear of sepsis for nine months.

HISTORICAL BACKGROUND OF WOUND

Wound infection is not a modern phenomenon. As early as 14-37AD there is documentary evidence that Cornelius Celsus (a Roman physician) described the four principal signs of inflammation and used 'antiseptic' solutions. Another Roman physician, Claudius Galen (130-200 AD) had such an influence on the management of wounds that he is still thought of by many today as the 'father of surgery'. It should also be remembered that he and some of his followers instigated the 'laudable pus' theory, which incorrectly considered the development of pus in a wound as a positive part of the healing process. Further historical references are listed below.
Table 1.2: Historical background:

| Historical background (1510-1994) |  
|-----------------------------------|------------------------------------------|
| Ambrose Pare (1510-1590)          | Encouraged wounds to suppurate           |
| Semmelweis (1818-1865), Pasteur (1822-1895) and Lister (1827-1912) | Accepted germ theory and introduced antiseptics |
| Florence Nightingale (1894)       | 'Not in bacteriology but looking into drains (for smells) is the thing needed'. Held a firm belief in the benefit of hand-washing and strict hygiene |
| Mary Ayton (1985)                 | Defined terminology in current use for wound infection |
| Vincent Falanga (1994)            | Identified the concept of 'critical colonisation' with fresh insights into chronic wound healing and non-healing wounds |

The ancient Egyptians were the first civilization to have trained physicians to treat physical ailments. Hippocrates (Greek physician and surgeon, 460-377 BC), known as the father of medicine, used vinegar to irrigate open wounds and wrapped dressings around wounds to prevent further injury.

The scale of wound infections was most evident in times of war. During the American Civil War, erysipelas (necrotizing infection of soft tissue) and tetanus accounted for over 17,000 deaths (anonymous, 1883). Because compound fractures at the time almost invariably were associated with infection, amputation was the only option despite a 25-90% risk of amputation stump infection.

As late as the nineteenth century, aseptic surgery was not routine practice. Sterilization of instruments began in the 1880s as did the wearing of gowns, masks, and gloves. Halsted (Professor of Surgery, Johns Hopkins University, United States, 1852-1922) introduced rubber gloves to his scrub nurse (and future wife) because she was developing skin irritation from the chemicals
used to disinfect instruments. The routine use of gloves was introduced by Halsted's student J. Bloodgood. Penicillin first was used clinically in 1940 by Howard Florey. With the use of antibiotics, a new era in the management of wound infections commenced. Unfortunately, eradication of the infective plague affecting surgical wounds has not ended because of the insurgence of antibiotic-resistant bacterial strains and the nature of more adventurous surgical intervention in immunocompromised patients and in implant surgery.

**WHEN ANTIBIOTIC ERA HAS ARRIVED?**

Lister studied the microbiology of air in his attempts to control wound infection associated with surgery. He used urine as a culture medium and noted that bacteria in cultures that were mixed with particular fungi appeared dead when viewed microscopically. Paul Ehrlich proposed that microbes could be destroyed by "magic bullets". He developed arsenical compounds to treat experimental trypanosmiasis and syphilis. These included atoxyl and salvarsan. They proved less dangerous than the former treatment for syphilis: mercury.

The introduction of penicillin, which heralded the antibiotic era, banished from hospitals the terrible cases of chronic sepsis, mainly caused by Staphylococcus aureus. Interest shifted in the 1950s, 1960s and 1970s to gram-negative bacilli; antibiotic-resistant *Enterobacteria*, such as *Escherichia coli*, *Klebsiella spp.* and later on to *Serratia spp.* which caused large outbreaks. Infection by *Pseudomonas aeruginosa* came into prominence with the increasing number of patients being rendered susceptible either by illness itself or by treatment.

More recently, of late, the extensive use of indwelling medical devices and possibly as a result of the introduction of new antibiotics coupled with their indiscriminate use, the Gram-Positive Cocci have once again emerged as the predominant causes of infection. Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococcus spp.* (VRE) and MRSA with reduced susceptibility to Vancomycin have posed serious problems.
The treatment appeared successful until the stock of drug became exhausted. The staphylococcal sepsis then re-established itself, and the patient subsequently died. Spurred by the success of this trial, other less severely ill patients were treated successfully...

...The antibiotic era had arrived.

HISTORICAL MILESTONES OF NOSOCOMIAL INFECTION AND SURGICAL INFECTIONS

One of the earliest records of hospital infections are perhaps those found in an Egyptian papyrus written around 3000 B.C. Needless to say, mere absence of documentation of bacterial infection does not exclude its prevalence prior to this time. Nearer home, in the Indian context a similar account of hospital infection is available in the ancient Ayurveda literature (ca. 600 B.C.) Again the famous Hindu physician Charaka and surgeon Sushruta (Ca. 400 B.C.) have also emphasized the need for prevention of infection in clinical practice. Elsewhere in the world too there is ample evidence that hospital infection were prevalent and documented in ancient times viz: the records of Herodotus on the conditions that prevailed in Greek and Roman hospitals in the period 1000 to 600 B.C., and the Hippocrates treatise (ca 400 BC) testifying the existence of infection.

In about 1861, Florence Nightingale in a much quoted remark in her book Notes on Hospitals.

“It may seem a strange principle to enunciate as the very requirement in a Hospital that it should do the sick no harm. The actual mortality in hospitals, especially in those of large crowded cities, is very much higher that any calculation founded on the mortality of the same class of diseases among patients treated out of hospital...”

In 1869 Simpson provided further evidence by the survey of the sequelae of amputation, which established that sepsis, gangrene and pyaemia were very much common in large urban hospitals than in rural practice.

At about this time Lister introduced his antiseptic theory, following the extensive use of carbolic acid to pack wounds, especially of compound
fractures, sterilize instruments and sutures, decontaminate his hands and as an air spray.

In 1883 Gustao Neubar introduced the use of masks and gowns in surgery, and Halsted in 1890 introduced the use of rubber gloves in surgery. Steam sterilization was discovered by von Bergman in 1896 and all these measures further increased the safety of surgery and contributed greatly in bringing down rates of infection by use of aseptic and antiseptic techniques.

During the period, when many fundamental discoveries in bacteriology were being made, other principles of hospital infection control were also simultaneously established. Flugge (1897, 1899) showed the importance of droplet and aerial spread in tuberculosis. By 1894, Hutinel and others had established basic isolation systems for diphtheria and other infectious diseases in children and fever hospitals.
HISTORICAL IMAGES:

IMAGE-1, MEDICINE IN ANCIENT EGYPT

IMAGE-2, SUSRUTA – SURGEON OF OLD INDIA
IMAGE-3, AMPUTATION: SURGERY BEGAN WITH THE TREATMENT OF EXTERNAL DISORDERS

IMAGE-4, AMPUTATION WITHOUT ANESTHESIA

IMAGE-5, THE INTERIOR OF THE OLD OPERATING THEATRE
IMAGE-6, ACCIDENT WARD. GUY’S HOSPITAL 1887

IMAGE-7, SEMMELWEIS: DEFENDER OF MOTHERHOOD

IMAGE-8, OPERATION USING LISTER’S CARBOLIC SPRAY INVENTED IN 1869
IMAGE - 9, JOSEPH LISTER (top left) WATCHES IN 1846, THE 1st OPERATION IN ENGLAND, UNDER ANESTHESIA. THE SURGEON IS ROBERT LISTON 87

IMAGE-10, ASEPTIC PRECAUTIONS BECAME UNIVERSAL BY 1900. SURGEONS AND NURSES WORE WHITE CAPS AND GOWNS. 194

IMAGE-11, EARLY DISINFECTION PHENOL BEING SPRAYED OVER AN OPERATION WOUND BY 19TH CENTURY SURGEONS 95
KEY TERMS USED IN THE STUDY

OPERATING SUITE
A physically separate area that comprises operating rooms and their interconnecting hallways and ancillary work areas such as scrub sink rooms. 09

OPERATING ROOM
A room in an operating suite where operations are performed. 09

Surgical Personnel
Any healthcare worker who provides care to surgical patients during the pre-, intra-, or postoperative periods. 09

Surgical Team Member
Any healthcare worker in an operating room during the operation who has a surgical care role. Members of the surgical team may be “scrubbed” or not; scrubbed members have direct contact with the sterile operating field or sterile instruments or supplies used in the field. 09

DEFINITION & CLASSIFICATION OF SURGICAL SITE INFECTIONS

DESCRIPTION OF SURGICAL SITE INFECTIONS
The Centers for Disease Control and Prevention (CDC) term for infections associated with surgical procedures was changed from surgical wound infection to surgical site infection in 1992. These infections are classified into incisional, organ, or other organs and spaces manipulated during an operation; incisional infections are further divided into superficial (skin and subcutaneous tissue) and deep (deep soft tissue-muscle and fascia). Detailed criteria for these definitions have been described. These definitions should be followed universally for surveillance, prevention, and control of surgical site infections. 189

CLASSIFICATION OF SURGICAL SITE INFECTIONS
In 1992, the CDC proposed standard definitions for postoperative infections involving the operative site to ensure that there was a common parlance for tracking incidence rates. The term surgical site infection refers to an infection
in the postoperative period involving the incision or deep space or organ accessed at the time of surgery. Rather than focusing solely on wound infections, these definitions extend to involve the broader spectrum of local postoperative infections. Thus, a pelvic abscess following colorectal surgery would be captured as an organ/space SSI, while a simple wound infection would be classified as a superficial SSI. If a SSI involves superficial and deep incisional sites, it is classified as a deep incisional SSI. Very occasionally a space infection drains through an incision. These infections rarely require reoperation and are considered a complication of the incision. As such, these are classified as deep incisional SSI.

DEFINITION OF SURGICAL SITE INFECTION

Wound infections have traditionally been thought of as infections in a surgical wound occurring between the skin and the deep soft tissues—a view that fails to consider the operative site as a whole. As prevention of these wound infections has become more effective, it has become apparent that definitions of operation-related infection must take the entire operative field into account; obvious examples include sternal and mediastinal infections, vascular graft infections, and infections associated with implants (if occurring within 1 year of the procedure and apparently related to it). Accordingly, the Centers for Disease Control and Prevention currently prefer to use the term surgical site infection (SSI). SSIs can be classified into three categories: superficial incisional SSIs (involving only skin and subcutaneous tissue), deep incisional SSIs (involving deep soft tissue), and organ/space SSIs (involving anatomic areas other than the incision itself that are opened or manipulated in the course of the procedure) (Figure -2).
FIGURE-2: CATEGORY OF SURGICAL SITE INFECTIONS. SURGICAL SITE INFECTIONS ARE CLASSIFIED INTO THREE CATEGORIES, DEPENDING ON WHICH ANATOMIC AREAS ARE AFFECTED. 127

FIGURE -3 CROSS-SECTION OF ABDOMINAL WALL SHOWING CDC CLASSIFICATIONS OF SURGICAL SITE INFECTION 127
SUPERFICIAL INCISIONAL SSI

Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following:

A. Purulent drainage, with or without laboratory confirmation, from the superficial incision.

B. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.

C. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat and superficial incision are deliberately opened by surgeon, unless incision is culture-negative.

D. Diagnosis of superficial incisional SSI by the surgeon or attending physician.

Do not report the following conditions as SSI:

A. Stitch abscess (minimal inflammation and discharge confined to the points of suture penetration).

B. Infection of an episiotomy or newborn circumcision site.

C. Infected burn wound.

D. Incisional SSI that extends into the fascial and muscle layers (see deep incisional SSI).

Note: Specific criteria are used for identifying infected episiotomy and circumcision sites and burn wounds.

Deep Incisional SSI

Infection occurs within 30 days after the operation if no implant † is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves deep soft tissues (e.g., fascial and muscle layers) of the incision and at least one of the following:

A. Purulent drainage from the deep incision but not from the organ/space component of the surgical site.
B. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38ºC), localized pain, or tenderness, unless site is culture-negative.

C. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathological or radiological examinations.

D. Diagnosis of a deep incisional SSI by a surgeon or attending physician.

Notes:

1) Report infection that involves both superficial and deep incision sites as deep incisional SSI.

2) Report an organ/space SSI that drains through the incision as a deep incisional SSI.

ORGAN/SPACE SSI

Infection occurs within 30 days after the operation if no implant† is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves any part of the anatomy (e.g., organs or spaces), other than the incision, which was opened or manipulated during an operation and at least one of the following:

A. Purulent drainage from a drain that is placed through a stab wound‡ into the organ/space.

B. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space.

C. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathological or radiologic examination.

D. Diagnosis of an organ/space SSI by a surgeon or attending physician.

* Horan TC et al. 113
† National Nosocomial Infection Surveillance definition: a nonhuman-derived implantable foreign body (e.g., prosthetic heart valve, nonhuman vascular graft, mechanical heart, or hip prosthesis) that is permanently placed in a patient during surgery.
‡ If the area around a stab wound becomes infected, it is not an SSI. It is considered a skin or soft tissue infection, depending on its depth.

SURGICAL WOUND CLASSIFICATION AND RISK OF SSI

Different surgical sites may contribute to the risk of developing clinical infection. Elective procedures have lower SSI rates than do emergency procedures. Stratification of various operations into groups that have similar risks for infection is important so that preventive strategies can be appropriately evaluated among similar patients, and so that quality monitors can be implemented to identify when infection rates are at variance from accepted trends and norms within an institution. An assessment of gross SSI rates without stratification is of only limited value, since overall rates are likely to be a reflection of patient risk rather than quality of performance.

The traditional wound infection classification system was developed in the wake of the ultraviolet light study of 1964. This classification system was primarily designed to provide a clinical estimate of the inoculum of bacteria likely to be encountered during the procedure and does not address the other determinants of infection defined above. Four separate classes of procedures were identified, each with a unique infection rate.

The wound classification scheme proposed by the National Research Council continues to be useful. The wound class has been shown to be independently predictive of wound infection in several large studies using multivariate analysis. In 1980, the Foothills Hospital study of 62,939 wounds generated a set of wound infection rates for the four wound classes: clean, 1.5%; clean contaminated, 7.7%; contaminated, 15.2%; and dirty 40%. Culver et al5 modified the SENIC risk index in 1991, but wound classification was the only risk factor that was unchanged from the original index. Garibaldi et al also
found surgical wound class (by stepwise logistic regression analysis) to be predictive of wound infection.

SURGICAL WOUND CLASSIFICATION:

**Class I / Clean:**
An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow non penetrating (blunt) trauma should be included in this category if they meet the criteria.

*Infection Risk (%):* <2

**Class II / Clean-Contaminated:**
An operative wound in which the respiratory, alimentary, genital or urinary tracts are entered under controlled conditions and without unusual contamination. Specifically, operations involving the biliary tract, appendix, vagina, and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered.

*Infection Risk (%):* <10

**Class III / Contaminated:**
Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (e.g., open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute, nonpurulent inflammation is encountered are included in this category.

*Infection Risk (%):* 15-20

**Class IV / Dirty-Infected:**
Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation.

*Infection Risk (%):* 40
ILLUSTRATIONS OF SURGICAL WOUND INFECTIONS

Figure 4:

1) NORMAL POST-SURGICAL WOUND HEALING - MEDICAL ILLUSTRATION

This medical exhibit depicts the progression of a post-operative septic surgical wound following abdominal surgery. The first illustration pictures the gross appearance of the wound on the female abdomen, with reactive, brown and murky infection drainage. The second illustration displays an enlarged cut-away view of the skin with the appearance of the sutured wound, progressing
to infection and dehiscence, or splitting open of the sutures. It includes labels for sutures, skin, fat, fat fascia, muscle, peritoneum and the abdominal cavity. The third illustration pictures the infected material in the subcutaneous space, extending through the disrupted fascia into the abdominal cavity.

3) SEPTIC SURGICAL WOUND MANAGEMENT - MEDICAL ILLUSTRATION

This medical exhibit depicts two (2) enlarged cut-away sections of the skin depicting septic surgical wound management for post-operative skin infections. The first illustration illustrates a septic wound beginning in the fatty subcutaneous space between the skin and fascia, draining through a skin incision. It includes labels for sutures, skin, fat, fat fascia, muscle, peritoneum
and the abdominal cavity. The second illustration pictures the usual management of the septic wound including removing the sutures superficial to the fascia, then opening the skin and fat, permitting drainage of the infected wound.

**PATHOGENESIS & DETERMINATIONS OF SURGICAL SITE INFECTIONS:**

**PATHOGENESIS OF SSI**

All surgical wounds are contaminated by bacteria, but only a minority actually demonstrates clinical infection. In most patients, infection does not develop because innate host defenses are quite efficient in the elimination of contaminants at the surgical site. 44

**THE HUMAN INFLAMMATORY RESPONSE**

With the creation of the surgical incision through the skin and into subcutaneous tissues, 5 critical initiators of the human inflammatory response are activated (Figure-5). Coagulation proteins and platelets are initially activated as part of the human haemostatic mechanism, but they also herald the onset of inflammation. Mast cells and complement proteins are activated, and bradykinin is produced from its ubiquitous protein precursors. The net effect of these 5 factors is vasodilation and increased local blood flow at the site of the surgical incision. While bulk flow is increased, flow velocity is reduced in preparation for margination of phagocytes. The simultaneous occurrence of increased vascular permeability and local vasodilation facilitates the formation of edema fluid, resulting in increased space between endothelial cells. The increased vascular permeability provides phagocytic access to the injured soft tissue, while edema provides aqueous conduits for the navigation of these phagocytes through the normally condensed extracellular tissues. Activation products from the 5 initiator events described above result in the production of nonspecific chemoattractant signals, while mast cells produce specific chemokine signals that “draw” specific neutrophil, monocyte and other leukocyte populations into the area of the surgical site. The important point of this discussion about inflammation is that tissue injury from the incision initiates the mobilization of phagocytes into the wound before bacterial
The abundant release of chemoattractant signals, products of tissue injury, orchestrates the movement of phagocytes into the wound. Chemoattractant signaling proteins bind to local vascular endothelial cells and upregulate selectin proteins on the endothelial surface of these cells, which results in neutrophil "rolling" on the endothelial surface within the post-capillary venule. Further interaction between neutrophil and endothelial cell adhesion proteins...
anchor the neutrophil to the surface of the endothelial cell, and the chemoattractant gradient then acts as a biological "beacon" to direct neutrophil movement toward the site of injury. Neutrophil presence at the surgical site allows systematic ingestion and digestion of any microbial contaminants from the operation. 44

By about 24 hours after creation of the surgical wound, monocytes enter the surgical site and initiate 1 of 2 different scenarios. When microbial contamination has been minimal and the early arriving neutrophils have been able to adequately control the bacteria that are present, then monocytes produce local chemical signals to regulate the wound-healing process. Myofibrocytes migrate into the fibrin matrix of the wound, and collagen deposition displaces its fibrin latticework. However, if microbial contamination and proliferation overwhelm the initial neutrophil infiltration, the monocyte assumes the role of a proinflammatory cell with the release of potent cytokines. Tumor necrosis factor (TNF)-alpha is produced and released by the monocytes and serves numerous functions; notably, it becomes a potent paracrine signal to upregulate vigorous neutrophil activity within the wound. TNF-alpha-stimulated neutrophils consume microbes, and lysosomal vacuoles may release reactive oxygen intermediates and acid hydrolases into the extracellular space from its lysosomal vacuoles. The extracellular release of reactive oxygen intermediates and the acid hydrolases results in lipid peroxidation of the local environment, with further tissue injury and further activation of the initiator signals. In this way, the entire inflammatory response is further intensified. Interleukin (IL)-1, IL-6, and other proinflammatory signals are released by the activated monocyte and serve as endocrine signals responsible for fever, stimulation of acute-phase reactants, and other responses.44

The net effect of vigorous neutrophilic stimulation, tissue autolysis, and sustained stimulation of inflammatory initiation is the creation of a wound space that is a host-pathogen battlefield. Ultimately, the wound space is filled with necrotic tissue, neutrophils, bacteria, and proteinaceous fluid that together constitute pus. The viable tissues around the infected wound typically exhibit the classic signs of inflammation. Wound rubor reflects local
vasodilation. *Calor* is the warmth of the vasodilated tissues resulting in increased heat conduction. *Tumor* reflects the presence of edema fluid about the wound. *Dolor* occurs from stimulation of nerve nociceptors by the numerous products of the inflammatory cascade and tissue injury. The discharge of pus from the wound interface via the incision completes the natural history of SSI. 44

**DETERMINANTS OF INFECTION**

Despite the fact that every surgical site is contaminated with bacteria by the end of the procedure, few become clinically infected. The interplay of 4 important determinants lead to either uneventful wound healing or SSI:

1. Inoculum of bacteria.
2. Virulence of bacteria.
3. Adjuvant effects of microenvironment.
4. Innate and acquired host defenses. 44

**INOCULUM OF BACTERIA**

The variable that has received the greatest amount of attention is the inoculum of bacteria lodged into the wound during the course of the operation. 2 Bacterial contaminants may enter the wound from the air in the operation room, or from the instruments or surgeon(s) that come into contact with the wound. The distal small intestine and the colon have very large concentrations of bacteria with $10^3 - 10^4$ bacteria/ml of distal small bowel content, $10^5 - 10^6$ bacteria/ml in the right colon, and $10^{10} - 10^{12}$ bacteria/g of stool in the rectosigmoid colon. Substantial numbers of bacteria are also present in the stomach of older patients who have hypo- or achlorhydria. 44

**VIRULENCE OF THE BACTERIAL CONTAMINANT**

A second determinant contributing to SSI is the virulence of the bacterial contaminant. The more virulent the bacterial contaminant, the greater the probability of infection. Coagulase-positive *Staphylococci* require a smaller inoculum than the Coagulase-negative species. Uncommon but virulent strains of *Clostridium perfringens* or Group A *Streptococci* require only a small inoculum to cause an especially severe necrotizing infection at the surgical
site. *Escherichia coli* have endotoxin in its outer cell membrane that gives it a particular virulence. *Bacteroides fragilis* and other *Bacteroides* species are ordinarily organisms of minimal virulence as solitary pathogens, but when combined with other oxygen-consuming organisms, they will result in microbial synergism and cause very significant infection following operations of the colon or female genital tract. While the virulence of the microbe is an important consideration in SSI, it represents the one variable that is intrinsic to the procedural site and the types of bacteria that already colonize the patient and cannot easily be controlled by preventive strategies.

**THE MICROENVIRONMENT OF THE WOUND**

A third variable that determines infection at the surgical site is the microenvironment of the wound. Adjuvant factors that are products or consequences of the surgical procedure may result in clinical infection by otherwise subinfectious inocula of bacteria. Hemoglobin at the surgical site is a well-known adjuvant substance. It is generally thought that the release of ferric iron during the degradation of red blood cells stimulates microbial proliferation. Necrotic tissue can act as a haven for contaminants to avoid phagocytic defenses of the host. Foreign bodies, particularly braided silk and other permanent braided suture materials, similarly harbor microbes and increase the probability of infection. Dead space within the surgical site also provides a local environment that fosters infection.

**INTEGRITY OF HOST DEFENSES**

The fourth determinant of SSI is the integrity of host defenses. Impaired host defenses can be viewed as innate or acquired. Innate impairment refers to the observation that intrinsic responses in some patients are less effective than in others. Variability is regularly found among all patients in various components of neutrophil function and macrophage mediator production. While innate differences may render some patients vulnerable to SSI and others very resistant, quantitating these differences remains elusive and their potential role in the management of clinical infection is speculative.

By contrast, acquired impairment of host responses is clearly related to increased rates of SSI. Shock and hypoxemia are positively associated with
SSI, especially in trauma patients. Transfusion appears to be immunosuppressive.\textsuperscript{7,8} Similarly, chronic illnesses, hypoalbuminemia, and malnutrition are significant factors. Hypothermia and hyperglycemia are also recognized as variables that impair the host response, while corticosteroids and other medications may also adversely affect the host and increase SSI rates.\textsuperscript{44}

**THE AGGREGATE EFFECT**

When all 4 determinants are evaluated in the aggregate, it becomes apparent that SSI is a very complex biological process and that determination of the causes of an infection in a specific situation can be problematic. The complexity of these individual variables also underscores the variety of issues that must be considered in the development of preventive strategies.\textsuperscript{44}

**INTEGRATION OF DETERMINANTS**

As operative infection rates slowly fall, despite increasingly complex operations in patients at greater risk, surgeons are approaching the control of infection with a broader view than simply that of asepsis and antisepsis. This new, broader view must take into account many variables, of which some have no relation to bacteria but all play a role in SSI.\textsuperscript{44}

To estimate risk, one must integrate the various determinants of infection in such a way that they can be applied to patient care. Much of this exercise is vague. In reality, the day-to-day practice of surgery includes a risk assessment that is essentially a form of logistic regression, though not recognized as such. Each surgeon's assessment of the probability of whether an SSI will occur takes into account the determining variables.\textsuperscript{127}

Probability of SSI = \( x + a \) (bacteria) + \( b \) (environment: local factors) + \( c \) (host defence mechanisms: systemic factors)
### Table-1.3:
DETERMINANTS OF INFECTION AND FACTORS THAT INFLUENCE WOUND INFECTION RATES.\(^{127}\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Determinant of Infection</th>
<th>Bacteria</th>
<th>Wound Environment (Local Factors)</th>
<th>Host Defence Mechanisms (Systematic Factors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial numbers in wound</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential contamination</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative shave</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of 3 or more diagnoses</td>
<td></td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>B</td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Duration of operation</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Abdominal operation</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>ASA class III, IV, or V</td>
<td></td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>O(_2) tension</td>
<td></td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose control</td>
<td></td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Normothermia</td>
<td></td>
<td>B</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Shock</td>
<td></td>
<td>B</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td>B</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

**Figure-6:** PROBABILITY OF SEPSIS IN NORMAL STATE. IN A HOMEOSTATIC, NORMAL STATE, THE DETERMINANTS OF ANY INFECTION PROCESS -- BACTERIA, THE SURGICAL SITE, AND HOST
DEFENSE MECHANISMS (REPRESENTED BY THREE CIRCLES) -- INTERSECT AT A POINT INDICATING ZERO PROBABILITY OF SEPSIS.¹²⁷

Pathogenesis of SSI

- Relationship equation

\[ \text{Dose of bacterial contamination} \times \text{Virulence} \]
\[ \text{Resistance of host} \]

\[ \text{SSI Risk} \]
MICROBIOLOGY OF SURGICAL WOUND INFECTIONS

BACKGROUND:
The medical definition of infection is 'invasion and multiplication of microorganisms in body tissues, which may be clinically inapparent or result in local cellular injury because of competitive metabolism, toxins, intracellular replication, or antigen-antibody response'. This series of events leads to progressive tissue destruction and eventual host demise if left unchecked.  

The infectious process begins with a disruption of the host mechanical barriers to microorganisms, the availability of microorganisms, and colonization. Louis Pasteur pointed out: 'The germ is nothing. It is the terrain in which it is found that is everything'. The field of hospital infection prevention started to get more attention by the end of 1960’s.

BACTERIA:
Clearly, without an infecting agent, no infection will result. Accordingly, most of what is known about bacteria is put to use in major efforts directed at reducing their numbers by means of asepsis and antisepsis. The principal concept is based on the size of the bacterial inoculum.

Wounds are traditionally classified according to whether the wound inoculum of bacteria is likely to be large enough to overwhelm local and systemic host defense mechanisms and produce an infection.

Figure-7: Wound infection rates.
The wound infection rate is shown here as a function of bacterial inoculum in three different situations: a dry wound with an adequate concentration of antibiotic (cephaloridine > 10 µg/ml), a dry wound with no antibiotic (placebo), and a wet wound with no antibiotic (placebo, wound fluid hematocrit > 8%).
DISTRIBUTION OF PATHOGENS IN SURGICAL WOUND INFECTION:
(A) USUAL PATHOGENS:

According to data from the NNIS system, the distribution of pathogens isolated from SSIs has not changed markedly during the last decade. *Staphylococcus aureus*, Coagulase-negative *Staphylococci*, *Enterococcus* spp., and *Escherichia coli* remain the most frequently isolated pathogens. An increasing proportion of SSIs are caused by antimicrobial-resistant pathogens, such as Methicillin - Resistant *Staphylococcus aureus* (MRSA), or by *Candida albicans*. 

Figure-8: Distribution of Pathogens Isolated from Surgical wound infection.
(B) UNUSUAL PATHOGENS:
Outbreaks or clusters of SSIs have also been caused by unusual pathogens, such as *Rhizopus oryzae*, *Clostridium perfringens*, *Rhodococcus bronchialis*, *Nocardia farcinica*, *Legionella pneumophila* and *Legionella dumoffii*, and *Pseudomonas multivorans*. These rare outbreaks have been traced to contaminated adhesive dressings, elastic bandages, colonized surgical personnel, tap water, or contaminated disinfectant solutions. When a cluster of SSIs involves an unusual organism, a formal epidemiologic investigation should be conducted.

Unusual Pathogens in Surgical Wound.
- *Rhizopus oryzae*: Elastoplast adhesive bandage.
- *Clostridium perfringens*: elastic bandage.
- *Rhodococcus bronchialis*: colonized heath care personal.
- *Legionella dumoffii* & *Pneumophila*: tap water.
- *Pseudomonas multivorans* – disinfectant solutions.

BACTERIAL PROPERTIES
Not only is the size of the bacterial inoculum important; the bacterial properties of virulence and pathogenicity are also significant. The most obvious pathogenic bacteria in surgical patients are Gram-Positive Cocci (e.g., *Staphylococcus aureus* and *Streptococci*).

The preoperative hospital stay has been found frequently to be an important contributing factor to wound infection rates. Bacteria with multiple antibiotic resistances (e.g., Methicillin-resistant *S. aureus* [MRSA], *S. epidermidis*, and Vancomycin-resistant *Enterococci* [VRE]) can be associated with significant SSI problems. In particular, *Staphylococci*, with their natural virulence, present an important hazard if inappropriate prophylaxis is used. SSIs caused by antibiotic-resistant organisms or unusual pathogens call for specific prophylaxis, perhaps other infection control efforts, and, if the problem persists, a search for a possible carrier or a common source.
The mechanisms by which microorganisms infect tissue and produce disease are complex and incompletely understood. For example, some pathogens may contain or produce toxins and other substances that increase their ability to invade a patient’s tissue, produce damage or survive in the tissue.

Table 1.4: PATHOGENIC EFFECTS OF VIRULENT MICROORGANISMS

<table>
<thead>
<tr>
<th>Toxin Production</th>
<th>Vigorous Stimulation of Immune Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super antigen release within the bloodstream that initiates an uncontrolled proliferation of T cells</td>
<td>Stimulation of T (thymus maturing) cell subsets allowing the release of cytokines that initiate cell and tissue damage</td>
</tr>
<tr>
<td>Super antigen production</td>
<td>Some species of micro-organisms such as the exotoxins of <em>Staphylococcus</em> and <em>Streptococcus</em> produce super antigens</td>
</tr>
<tr>
<td>Presence of biofilms</td>
<td>A microbial colony encased in an adhesive polysaccharide matrix that is usually attached to a wound surface. Biofilms present in the form of a transparent sticky film covering the wound surface. Cells in biofilms exhibit a decreased sensitivity to host immunological defence mechanisms, decreased susceptibility to antimicrobial agents and increased virulence. They have also been implicated in persistent infections</td>
</tr>
</tbody>
</table>

SOURCE OF PATHOGENS:  

A. Endogenous flora of the patient  
B. Operating theatre environment  
C. Hospital personnel  
D. Seeding of the operative site from distant focus of infection (prosthetic device, implants)
ENDOGENOUS FLORA:
For most SSIs, the source of pathogens is the endogenous flora of the patient's skin, mucous membranes, or hollow viscera. When mucous membranes or skin is incised, the exposed tissues are at risk for contamination with endogenous flora. These organisms are usually aerobic gram-positive cocci (e.g., *Staphylococci*), but may include faecal flora (e.g., anaerobic bacteria and gram negative aerobes) when incisions are made near the perineum or groin. When a gastrointestinal organ is opened during an operation and is the source of pathogens, gram negative bacilli (e.g., *E. coli*), gram-positive organisms (e.g., *Enterococci*), and sometimes anaerobes (e.g., *Bacillus fragilis*) are the typical SSI isolates. Seeding of the operative site from a distant focus of infection can be another source of SSI pathogens, particularly in patients who have prosthesis or other implant placed during the operation. Such devices provide a nidus for attachment of the organism. 09

EXOGENOUS FLORA:
Exogenous sources of SSI pathogens include surgical personnel (especially members of the surgical team), the operating room environment (including air), and all tools, instruments, and materials brought to the sterile field during an operation. Exogenous floras are primarily aerobes, especially gram-positive organisms (e.g., *Staphylococci* and *Streptococci*). Fungi from endogenous and exogenous sources rarely cause SSIs, and their pathogenesis is not well understood. 09

Exogenous sources of SSI pathogens are occasionally responsible. These include: 122

- Organisms from members of the surgical team (e.g., hands, nose or other body parts);
- Contaminated surfaces in the operating room, even the air; and
- Contaminated instruments, surgical gloves or other items used in the surgery.

RESISTANT PATTERN AND OUT BREAK:
According to data from the National Nosocomial Infections Surveillance System (NNIS), there has been little change in the incidence and distribution
of the pathogens isolated from infections during the last decade. However, more of these pathogens show antimicrobial-drug resistance, especially Methicillin-resistant *S. aureus*. Postoperative infections, including surgical site infections, were caused by multiple organisms in a multicenter outbreak due to contamination of an intravenous anesthetic, propofol. In this outbreak, CDC identified 62 patients at seven hospitals who had postoperative infections, primarily of the bloodstream or surgical site, after exposure to propofol. Only exposure to this anesthetic was substantially associated with these postoperative infections. In six of the seven hospitals, the same pathogen was isolated from several infected patients. The infections were due to extrinsic contamination of the protocol by the anesthesia personnel, who frequently carried the pathogens in lesions on their hands or scalp or in their nares. Lapses in aseptic technique and reuse of single-use vials for several patients were important factors in these outbreaks. This report stresses the importance of conducting a formal epidemiologic investigation when a cluster of infections involves an unusual organism such as *Moraxella osloensis* or *Serratia marcescens*.189

The consistency of the infecting organisms by surgical site underlies the rationale and success of prophylactic antimicrobial strategies. However, over the past decade there has been an increasing proportion of SSI caused by resistant pathogens and Candida species, a phenomenon related to surgical procedures on an increasing number of severely ill or immunocompromised patients and prevalent use of broad-spectrum antimicrobial agents. Outbreaks of SSI caused by unusual organisms should prompt formal epidemiologic investigation. Contaminated dressings, disinfectant solutions, and inadequate sterilization of surgical instruments have all been implicated. 16
### Table -1.5: Surgical site infections: common pathogens and preferred prophylactic antimicrobials

<table>
<thead>
<tr>
<th>Operative Site</th>
<th>Common Pathogens</th>
<th>Recommended Prophylactic Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac (sternotomy and cardiopulmonary bypass)</td>
<td><em>Staphylococcus aureus</em>, Coagulate-negative <em>Staphylococci</em>, Enteric Gram-Negative Bacilli</td>
<td>Cefazolin 1-2 g preinduction then q 8 h for 48 h</td>
</tr>
<tr>
<td>Non cardiac vascular: aortic resection and prosthetic bypass</td>
<td><em>S. aureus</em>, Coagulate-negative <em>Staphylococci</em>, Gram-Negative Bacilli</td>
<td>Cefazolin 1 g preinduction</td>
</tr>
<tr>
<td>Neurosurgery</td>
<td><em>S. aureus</em>, Coagulate-negative <em>Staphylococci</em></td>
<td>Cefazolin 1 g preinduction</td>
</tr>
<tr>
<td>Breast, herniorrphy</td>
<td><em>S. aureus</em>, Coagulate-negative <em>Staphylococci</em></td>
<td>Cefazolin 1 g preinduction</td>
</tr>
<tr>
<td>Head and neck (major procedures with breach of mucosa)</td>
<td><em>S. aureus</em>, <em>Streptococci</em>, oropharyngeal anaerobes <em>(e.g. Peptostreptococci)</em></td>
<td>Cefazolin 2 g preinduction</td>
</tr>
<tr>
<td>Orthopaedic</td>
<td><em>S. aureus</em>, Coagulate-negative <em>Staphylococci</em>, Gram-Negative Bacilli</td>
<td>Cefazolin 1-2 g preinduction</td>
</tr>
<tr>
<td>Thoracic: pulmonary or esophageal</td>
<td>Oral anaerobes, <em>S. aureus</em>, Coagulate-negative <em>Staphylococci</em> <em>Streptococcus pneumoniae</em>, Gram-Negative Bacilli</td>
<td>Cefazolin 1-2 g preinduction</td>
</tr>
<tr>
<td>Gastroduodenal</td>
<td>Gram-Negative Bacilli, <em>Streptococci</em>, oropharyngeal</td>
<td>Cefazolin 1-2 g preinduction</td>
</tr>
<tr>
<td>Section</td>
<td>Microorganisms</td>
<td>Antibiotics</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Biliary</td>
<td>anaerobes (e.g. <em>Peptostreptococci</em>)</td>
<td>Cefazolin (may be altered based on cultures) 1-2 g preinduction</td>
</tr>
<tr>
<td>Appendix, biliary tract,</td>
<td>Gram-Negative Bacilli, <em>S. aureus</em>, <em>Enterococci</em>, <em>Clostridia</em>, <em>Pseudomonas</em></td>
<td>Cefoxitin, cefotetan, or cefmetazole 1 g preinduction</td>
</tr>
<tr>
<td>colon, and rectum</td>
<td>anaerobes</td>
<td></td>
</tr>
<tr>
<td>Abdominal trauma</td>
<td>Gram-Negative Bacilli, anaerobes</td>
<td>Cefoxitin 2 g preinduction</td>
</tr>
<tr>
<td>Obstetric and gynecologic</td>
<td>Gram-Negative Bacilli, <em>Enterococci</em>, <em>Group B Streptococci</em>, anaerobes</td>
<td>Cesarean section: cefazolin 1 g after umbilical cord is clamped; other: cefotetan 12 g preinduction</td>
</tr>
<tr>
<td>Urologic</td>
<td>Gram-Negative Bacilli</td>
<td>Ciprofloxacin 500 mg orally/400 mg intravenously if preoperative catheterization or positive urine culture</td>
</tr>
</tbody>
</table>
**Klebsiella species:**

**Morphology & Staining:-**
Gram Negative, non-sporing, non-motile bacilli which tend to be short and thick, e.g. $1-2 \times 0.8 \ \mu m$. Virtually all freshly isolated strains form a well defined polysaccharide capsule which is readily visible in wet India ink films or by its ‘swelling’ reaction in films with homologous antiserum. The capsule is largest in cultures on sugar containing media, especially those with a high ratio of sugar to other nutrients. Some of what appears to be the same extracellular polysaccharide is secreted from the bacteria as a loose, soluble slime, and it is the accumulation of the loose slime that gives colonies their large ‘mucoid’ form.\(^{140}\)

Fimbriae of one or more of three types, 1, 3 and 6, are present in a majority of strains. Most strains produce both type 1 fimbriae and type 3 fimbriae, though in different phages of their growth they may form only the one type or the other or neither. A few strains form only type 1 fimbriae and a few form only type 3. *K. pneumoniae* forms only type 1 fimbriae.\(^{140}\)

**Images of morphology and Gram stain of Klebsiella**

<table>
<thead>
<tr>
<th>Phase contrast microscopy</th>
<th>Gram Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Phase contrast microscopy" /></td>
<td><img src="image2.png" alt="Gram Staining" /></td>
</tr>
</tbody>
</table>
ELECTRON MICROGRAPHS OF *Klebsiella*

Slime encapsulated bacilli bacteria with protruding hairs that give it a grip on its host.  

An advancing colony of Klebsiella bacteria is halted when it encounters an antibiotic.

**CULTURE CHARACTERISTIC**

Grow well on ordinary nutrient media and on glucose-ammonium salts agar unsupplemented with growth factors. Temperature range for growth is 12-43°C, optimum 37°C.

Colonies are large, raised, moist and viscid, i.e. mucoid. The degree of mucoidness depends on the amount of loose slime produced and this depends on the amount of carbohydrate in the culture medium as well as varying from strain to strain. The colonies of non-capsulated non-slime forming mutants resemble those of other non-capsulate coliform bacteria. Most strains ferment lactose and their colonies on MacConkey medium are pink, though this colour may not be clearly apparent in very mucoid colonies.

*Klebsiella pink mucoid colonies on MacConkey Agar Plate*
**TABLE-1.6**
Species classification of the genus *Klebsiella* by different taxonomic systems.\(^\text{183}\)

Classification by:

<table>
<thead>
<tr>
<th>Cowan</th>
<th>Bascomb</th>
<th>Ørskov</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. aerogenes</em></td>
<td><em>K. aerogenes/oxytoca/edwardsii</em></td>
<td><em>K. pneumoniae</em></td>
</tr>
<tr>
<td><em>K. edwardsii</em></td>
<td></td>
<td><em>subsp. pneumoniae</em></td>
</tr>
<tr>
<td>subsp. <em>edwardsii</em></td>
<td><em>K. pneumoniae</em></td>
<td>subsp. <em>ozaenae</em></td>
</tr>
<tr>
<td>subsp. <em>atlantae</em></td>
<td>sensu stricto</td>
<td>subsp. <em>rhinoscleromatis</em></td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>sensu lato</td>
<td><em>K. oxytoca</em></td>
</tr>
<tr>
<td><em>K. ozaenae</em></td>
<td><em>K. ozaenae</em></td>
<td><em>K. terrigena</em></td>
</tr>
<tr>
<td><em>K. rhinoscleromatis</em></td>
<td><em>K. rhinoscleromatis</em></td>
<td><em>K. planticola</em> (syn. K. trevisanii)</td>
</tr>
<tr>
<td><em>K. &quot;unnamed group&quot;</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td></td>
<td><em>K. ornithinolytica</em></td>
</tr>
</tbody>
</table>
Typing of *Klebsiella* Isolates

**Bio typing**

Bio typing based on an extended panel of biochemical and culture tests is certainly the most practicable method of typing for smaller laboratories that are epidemiologically not optimally equipped.

**Sero typing**

Sero typing is currently the most widely used technique for typing *Klebsiella* spp. It is based mainly on a division according to the capsule antigens.

**Phage Typing**

Phage typing of *Klebsiella* was first developed in the 1960s.

**Bacteriocin Typing**

Although capsule typing is the preferred method for *Klebsiella*, it has been advised to include an additional feature independent of the capsule type to enable more precise epidemiological analysis.

**Molecular Typing Methods**

Molecular typing methods, as applied to the genus *Klebsiella*, are still in their infancy.

Pathogenicity Factors of *Klebsiella*  

The current research into the pathogenicity of *Klebsiella* focuses on the group of five factors shown in figure-9.

**Figure-9: Schematic representations** of *Klebsiella* pathogenicity factors.
Diseases Caused By *Klebsiella*<sup>153</sup>

<table>
<thead>
<tr>
<th><strong>Pneumonia</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Chills</td>
<td>Chest pain</td>
</tr>
<tr>
<td>Cough</td>
<td>Dyspnea</td>
<td>Tachypnea</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>Rales</td>
<td>Lung consolidation</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>Mucoid sputum</td>
<td>Current jelly sputum</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Septicemia</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Chills</td>
<td>Anorexia</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Lethargy</td>
<td>Nausea</td>
</tr>
<tr>
<td>Vomiting</td>
<td>Tachycardia</td>
<td>Bacteremia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Wound Infection</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>Suppuration</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Erythema</td>
<td>Pain</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Burn Infection</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>Suppuration</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Erythema</td>
<td>Pain</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Urinary Tract Infection (cystitis)</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain on urination</td>
<td>Dysuria</td>
<td>Frequent urination</td>
</tr>
<tr>
<td>Urgent urination</td>
<td>Suprapubic pain</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Ankylosing spondylitis (autoimmune disease)</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Anorexia</td>
<td>Weight loss</td>
</tr>
<tr>
<td>Night sweats</td>
<td>Fatigue</td>
<td>Back pain</td>
</tr>
<tr>
<td>Joint pain</td>
<td>Spinal fusion</td>
<td></td>
</tr>
</tbody>
</table>
**Klebsiella pneumoniae Structural Genomics**

Structural genomics is a new and rapidly developing field in life sciences. The goal of this field is to discover and analyze the structures as well as functions of all proteins in nature in order to provide a foundation for a fundamental understanding of biology.

**Figure-10: Klebsiella pneumoniae Structural Genomics**

---

**LABORATORY INDICATIONS:**

**Diagnostic Factors**

<table>
<thead>
<tr>
<th></th>
<th>Pink colonies on MacConkey agar</th>
<th>Gas produced in TSI</th>
<th>Hydrogen sulphide negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>culture on MacConkey's agar or EMB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>growth on McConkey's and EMB agars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole negative</td>
<td>Lactose positive</td>
<td>Urease positive (weak)</td>
<td></td>
</tr>
<tr>
<td>No gas - TSI</td>
<td>Non motile</td>
<td>Non capsulated</td>
<td></td>
</tr>
</tbody>
</table>
A SIMPLIFIED BRANCHING FLOW DIAGRAM FOR GRAM-NEGATIVE RODS

Klebsiella species as a nosocomial pathogen:

The principal pathogenic reservoirs for transmission of Klebsiella are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks. Hospital outbreaks of multidrug-resistant Klebsiella spp., especially those in neonatal wards, are often caused by new types of strains, the so-called extended-spectrum-β-lactamase (ESBL) producers. The incidence of ESBL-producing strains among clinical Klebsiella isolates has been steadily increasing over the past years. ¹⁸³

Klebsiella is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics and showing characteristic radiographic abnormalities due to a severe pyogenic infection which has a high fatality rate if untreated. ¹⁸³

The vast majority of Klebsiella infections, however, are associated with hospitalization. As opportunistic pathogens, Klebsiella spp. primarily attack
immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction.  

TABLE –1.7: Hospital-acquired bacterial infections caused by *Klebsiella* spp.  

<table>
<thead>
<tr>
<th>Infection</th>
<th>% of infections caused by <em>Klebsiella</em></th>
<th>Rank&lt;sup&gt;a&lt;/sup&gt;</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTI</td>
<td>6-17</td>
<td>5-7</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>7-14</td>
<td>2-4</td>
<td></td>
</tr>
<tr>
<td>Septicemia</td>
<td>4-15</td>
<td>3-8</td>
<td></td>
</tr>
<tr>
<td>Wound infections</td>
<td>2-4</td>
<td>6-11</td>
<td></td>
</tr>
<tr>
<td>Nosocomial infections in intensive care unit patients</td>
<td>4-17</td>
<td>4-9</td>
<td></td>
</tr>
<tr>
<td>Neonatal septicemia</td>
<td>3-20</td>
<td>2-8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Ranking of *Klebsiella* compared to all other bacterial pathogens.

Table lists the most frequent nosocomial infections caused by *Klebsiella*. The urinary tract is the most common site of infection. *Klebsiella* accounts for 6 to 17% of all nosocomial urinary tract infections (UTI) and shows an even higher incidence in specific groups of patients at risk, e.g., patients with neuropathic bladders or with diabetes mellitus. As a cause of nosocomial gram-negative bacteremia, *Klebsiella* is second only to *Escherichia coli*.  

In pediatric wards, nosocomial *Klebsiella* infections are especially troublesome—particularly in premature infants and intensive care units. *Klebsiella* species are often the pathogens involved in neonatal sepsis, in both early-manifestation and late-manifestation infections.
Due to the extensive spread of antibiotic-resistant strains, especially of extended-spectrum β-lactamase (ESBL)-producing strains, there has been renewed interest in *Klebsiella* infections. ¹⁸³

**PSEUDOMONAS AERUGINOSA**

**INTRODUCTION:**

The biological identity of the genus *Pseudomonas* has changed dramatically in recent years during the transition between artificial classification based on phenotypic properties (e.g. Bergey's Manual of Systematic Bacteriology, 1st ed., 1986) and revisionist classification based on genotypic (phylogenetic) properties (e.g. Bergey's Manual of Systematic Bacteriology, 2nd ed., 2001). However, in either scheme, the genus comprises a relatively large and important group of Gram-negative bacteria. Members of the genus are found abundantly as free-living organisms in soils, fresh water and marine environments, and in many other natural habitats. They may also be found in associations with plants and animals as normal flora or as agents of disease.

**The Genus Pseudomonas**

The bacteriological criteria that distinguish the members of the genus *Pseudomonas* are given below in Table 1.8. ¹²³

**Table 1.8: General characteristics of the genus *Pseudomonas***

- Gram-negative
- Rod-shaped, 0.5-0.8 um x 1-3 um
- Strictly aerobic; the only anaerobic activities may be denitrification and arginine degradation to ornithine
- Motile by polar flagella; some strains also produce lateral flagella
- Oxidative, chemo organotrophic metabolism
- Catalase-positive
- Usually oxidase-positive
- No organic growth factors are required
- Diffusible and/or insoluble pigments may be produced
- GC content of the DNA: 58-68 mol%
Pseudomonas aeruginosa

Among Pseudomonas genus, Ps. aeruginosa has attracted the most attention from general and clinical microbiologists, geneticists, and biochemists. The list of materials from which this species can be isolated is almost endless; so that from a practical point of view, one can assume that the bacterium is present everywhere. Most strains of the species can be easily identified by a number of phenotypic characteristics never found in the same combination in other species. Most important among these are production of pigments, including pyocyanin, the ability to denitrify, and the ability to grow at 41°C. \(^{153}\)

Pseudomonas aeruginosa is the epitome of an opportunistic pathogen of humans. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner. \(^{153}\)

Pseudomonas aeruginosa is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed.

Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is 50 percent. \(^{153}\)

Pseudomonas aeruginosa is primarily a nosocomial pathogen. According to the CDC, the overall incidence of P. aeruginosa infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections. \(^{153}\)
CHARACTERISTICS

Gram stain of Pseudomonas aeruginosa cells

Electron micrographs of *Pseudomonas aeruginosa*
The rod-shaped *Pseudomonas aeruginosa* on cultured epithelial cells from the human respiratory tract.

CULTURE CHARACTERISTICS

The colonies of *Pseudomonas aeruginosa* are flat, grayish, with irregular edges, and with time they tend to spread on the surface of the agar. Mucoid colonies frequently appear among isolates from the respiratory tract of patients with cystic fibrosis. The mucoid extracellular substance is alginate. *Pseudomonas aeruginosa* isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large, smooth, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.
**Pseudomonas aeruginosa colonies on Nutrient Agar & Mac. Agar.**

**PIGMENTATION:**

*P. aeruginosa* is capable of producing several pigments, of which the most characteristic is **pyocyanin**. As far as is known, this blue pigment is an absolute diagnostic character, since no other species has been found to produce it. **Pyoverdin** is the fluorescent pigment most often produced, but the bacteria may be able to produce several additional pigments, including a reddish pigment, **pyorubrin**, and a brown pigment, **pyomelanin**. This pigment, in common with other melanins, is produced from aromatic amino acids such as tyrosine or phenylalanine, while pyorubrin production is enhanced by the addition of glutamate to the medium. Besides pyoverdin, which acts as a siderophore, the function of these pigments is obscure.\(^{123}\)
VIRULENCE DETERMINANTS:

Adhesins
- Fimbriae (N-methyl-phenylalanine pili)
- Polysaccharide capsule (glycocalyx)
- Alginate slime (biofilm)

Invasins
- Elastase, Cytotoxin (leukocidin), Alkaline protease
- Hemolysins (phospholipase and lecithinase)
- Siderophores and siderophore uptake systems
- Pyocyanin diffusible pigment

Motility / Chemotaxis
- Flagella

Toxins
- Exoenzyme-S, Exotoxin-A
- Lipopolysaccharides

Anti phagocytic surface properties
- Capsules, slime layers
- Lipopolysaccharides

Defence against serum bactericidal reaction
- Slime layers, capsules
- Lipopolysaccharides
- Protease enzymes

Defence against immune responses
- Capsules, slime layers
- Protease enzymes

Genetic attributes
- Genetic exchange by transduction and conjugation
- Inherent (natural) drug resistance
- R factors and drug resistance plasmids

Ecologic criteria
- Adaptability to minimal nutritional requirements
- Metabolic diversity
- Widespread occurrence in a variety of habitats
LABORATORY DIAGNOSIS

Diagnosis of *Pseudomonas* infection depends upon isolation and laboratory identification of the bacterium. It grows well on most laboratory media and commonly is isolated on blood agar or eosin-methylthionine blue agar. It is identified on the basis of its Gram morphology, inability to ferment lactose, a positive oxidase reaction, its fruity odor, and its ability to grow at 42°C. Fluorescence under ultraviolet light is helpful in early identification of *P. aeruginosa* colonies. Fluorescence is also used to suggest the presence of *P. aeruginosa* in wounds. ¹⁵³

OXIDASE TEST 2²⁴

Cytochrome oxidase is an enzyme found in some bacteria that transfers electrons to oxygen, the final electron acceptor in some electron transport chains. Thus, the enzyme oxidizes reduced cytochrome c to make this transfer of energy. Presence of cytochrome oxidase can be detected through the use of an Oxidase Disk which acts as an electron donator to cytochrome oxidase. If the bacteria oxidize the disk (remove electrons) the disk will turn purple, indicating a positive test. No color change indicates a negative test.

LABORATORY INDICATIONS: ²²⁵

- Oxidase Positive
- Beta-haemolytic
- Characteristic odour and color
- Motile

LABORATORY IDENTIFICATION OF *PSEUDOMONAS AERUGINOSA* ²¹⁷

Temperature and Media

- Hemolytic on blood agar plates (BAP); has three colony types.
- Produces extra-cellular pigments that diffuse into agar.
- Species are distinguished by temperature tolerance and biochemical tests, but many *Pseudomonas* are difficult to identify with conventional laboratory methods.
- Can be grown using many different Medias. Even grows in weak antiseptic, saline, and soap solutions. It has very simple metabolic needs.

**Important Chemical Tests**
- Oxidase positive

**Oxidation of Sugars**
- Can oxidize glucose and other sugars but does not turn the medium acid

**Pseudomonas IN POST OPERATIVE WOUND INFECTION**

The virulence and invasive capability of the organisms have been reported to influence the risk of infection, but the physiological state of the tissue in the wound and immunological integrity of the host seem to be of equal importance in determining whether infection occurs. Although not invariable, microbial densities of 105 or more organisms commonly indicate infection with less count reflecting contamination. Bacteriological studies have shown that post-operative wound infection is universal and that the bacteria types present vary with geographical location, bacteria resident on the skin, clothing at the site of wound, time between wound and examination. Within recent years, there has been a growing prevalence of Gram-negative organisms as causes of serious infections seen in many hospitals.

These organisms have almost replaced *Staphylococcus aureus* in nosocomial infection. Of the Gram-negative bacilli, *Pseudomonas aeruginosa* has been of particular interest. The incidence of *Pseudomonas aeruginosa* in post-operative wound infection is becoming more serious in developing countries because of relaxation in general hygienic measures, mass production of low quality antiseptic and medicinal solutions for treatment, difficulties in proper
definition of the responsibility among the hospital staff. The hospital-acquired nature of infections with *Pseudomonas aeruginosa* has been noted and while some patients suffer endogenous infections, the vast majorities are acquired from exogenous sources. It has also been observed that healthy carriers of *Pseudomonas aeruginosa* in the hospital environment account for about 28% while less in the open community. The objective of this study was to determine the occurrence of *Pseudomonas aeruginosa* in post-operative infection and its susceptibility to commonly used antibiotics.

**ENTEROBACTERIACEAE FAMILY – DEFINITION**

The Enterobacteriaceae are Gram Negative bacilli that are either motile with peritrichous flagella or non-motile, grow both aerobically and anaerobically on simple culture media and on MacConkey’s bile salt lactose agar, are oxidase negative and, with few exceptions, Catalase positive, and reduce nitrates to nitrites. They ferment glucose in peptone water with the production of either acid or acid and gas, and they break down glucose and other carbohydrates both fermentatively under anaerobic conditions and oxidatively under aerobic conditions.

The taxonomy of the genera, and species within the genera, has been the subject of repeated changes partly because the organisms in this heterogeneous group are relatively inert in the common biochemical tests.

**Genera of Enterobacteriaceae**

![Diagram of Enterobacteriaceae Genera]
**Escherichia coli**

**INTRODUCTION:**

The GI tract of most warm-blooded animals is colonized by *E. coli* within hours or a few days after birth. The bacterium is ingested in foods or water or obtained directly from other individuals handling the infant. The human bowel is usually colonized within 40 hours of birth. *E. coli* can adhere to the mucus overlying the large intestine.  

- *Escherichia coli* is a moderately-sized gram-negative bacillus.  
- Possess a peritrichous arrangement of flagella.  
- Facultative anaerobe.

The genus is named after Escherich, Theodor Escherich German paediatrician and bacteriologist, born November 29, 1857, Ansbach, Mittelfranken; died February 15, 1911, Vienna, who was the first to describe the colon bacillus under the name Bacterium coli commune (1885).

> "Once we understand the biology of *Escherichia coli*, we will understand the biology of an elephant".  
> Jacques Monod

**MORPHOLOGY AND STAINING**

Gram Negative, non-sporing bacilli measuring 1-3 um x 0.4-0.7 um. Most strains (about 80%) are motile, though motility is often feeble on primary isolation, and most strains (about 80%) are fimbriate. They are non capsulated straight rods arranged singly or in pairs.

**Gram Stain of *Escherichia coli***
CULTURE CHARACTERISTICS

It is an aerobe and facultative anaerobe. The temperature range is 10-46$^\circ$C (optimum 37$^\circ$C). They grow well on non-selective media, forming smooth, colorless colonies 2-3 mm in diameter in 18 hrs. on Nutrient agar. Since most strains ferment lactose rapidly, the colonies on MacConkey's agar medium are smooth, glossy and translucent pink-rose in color. On blood agar the colonies of some strains are surrounded by zones of haemolysis. On XLD (Xylose Lysine Deoxycholate) agar they produce yellow colonies due to acid from fermentation. On EMB (Eosin Methylene Blue) agar *E. coli* form bluish black colored colonies with metallic seen.

*Escherichia coli* Pink Lactose fermentor colonies on MacConkey’s Agar
Eosin Methylene Blue (EMB) Agar
Contains eosin & methylene blue to inhibit Gram positive bacteria. Eosin also acts as an indicator for fermentation. Lactose & sucrose are the sugars

*E. coli* Green metallic sheen (121)  
*Escherichia coli* on XLD Agar

Acid from fermentation lowers the pH and turns the phenol red from red (alkaline) to yellow (acid). No hydrogen sulfide is produced. ²³⁹

**Colonies of *Escherichia coli* on Blood Agar plate** ²¹⁹

Medium-sized grey colonies. The colonies have a characteristic odour.  
The same Blood Agar plate examined with transmitted light. There is a zone of beta-haemolysis
THE VIRULENCE DETERMINANTS OF PATHOGENIC *E. coli*

**Adhesins**
- CFAI/CFAII
- Type 1 fimbriae, P fimbriae, S fimbriae
- Intimin (non-fimbrial adhesin)

**Invasins**
- Hemolysin
- Siderophores and siderophore uptake systems
- *Shigella*-like "invasins" for intracellular invasion and spread

**Motility / chemotaxis**
- Flagella

**Toxins**
- LT toxin, ST toxin
- Endotoxin (Lipopolysaccharides)
- Shiga-like toxin, Cytotoxins

**Anti Phagocytic Surface Properties**
- Capsules
- K antigens
- Lipopolysaccharides

**Defence against serum bactericidal reactions**
- Lipopolysaccharides
- K antigens

**Defence against immune responses**
- Capsules
- K antigens
- Lipopolysaccharides
- Antigenic variation

**Genetic attributes**
- Genetic exchange by transduction and conjugation
- Transmissible plasmids
- R factors and drug resistance plasmids
- Toxin and other virulence plasmids
PROFILE OF *Escherichia coli* CHROMOSOME

The Profiling of *Escherichia coli* chromosome (PEC) database has been constructed to compile any relevant information that could help to characterize the *E. coli* genome, especially with respect to discovering the function of each gene.

![Escherichia coli chromosome diagram]

BIOCHEMICAL REACTIONS

Glucose, lactose, mannitol, maltose and many other sugars are fermented with the production of acid and gas. Typical strains do not ferment sucrose. The four biochemical tests widely employed in the classification of *Enterobacteria* are the indole, methyl red, Voges Proskauer and citrate utilization tests, generally referred to by the mnemonic IMViC.

*Escherichia coli* is indole and methyl red positive and VP and citrate negative (IMViC + + - -). Gelatine is liquefied. H2S is not formed, urea is not split and growth does not occur in KCN medium.

LABORATORY DIAGNOSIS

In order to distinguish *E. coli* from related species likely to be found naturally in the environment, a battery of tests called the IMViC reactions was developed in order to differentiate fecal coliforms from nonfecal coliforms. IMViC is an acronym in which the capital letters stand for Indole, Methyl red, Voges-Proskauer, and Citrate.) The IMViC set of tests examines: the ability of an organism to (1) produce Indole; (2) produce sufficient acid to change the color of Methyl red indicator; (3) produce acetoin, an intermediate in the
butanediol fermentation pathway (a positive result of the Voges-Proskauer test); and (4) the ability to grow on Citrate as the sole source of carbon. *Escherichia coli* is positive in the first two tests and negative in the second two; nonfecal coliforms are the opposite - negative in the first two tests and positive for the second two.

**RAPID METHODS FOR DETECTING *E. coli***

A fluorogenic detection method has been developed based on the cleavage of methylumbelliferyl-D-glucuronide (MUG) to the free methylumbelliferyl moiety, which fluoresces a blue color after irradiation with long-wave ultraviolet radiation. Although strains of *E. coli* are generally positive in this test, some strains of *Salmonella*, *Shigella*, and *Yersinia* are also capable of splitting MUG; the latter two genera are usually not present in food. A disadvantage is that enterohemorrhagic *E. coli* (EHEC) strains are generally negative in this test. MUG can be added to various selective media, so there is a great potential in its use for detecting *E. coli*.

Automated or semi-automated systems are also being used for the detection of *E. coli* as part of the detection methods for *Enterobacteriaceae*. Techniques involving impedance measurements have shown promise. Other techniques such as immunoassays and nucleic acid hybridization studies can also be used to enumerate *E. coli*, and DNA probes directed at a number of genes have also been developed.

**Escherichia coli** **AS NOSOCOMIAL AND SURGICAL WOUND PATHOGEN**

Aerobic Gram-negative species (like *Escherichia coli* and *Pseudomonas aeruginosa*) and anaerobic bacteria (*Bacteroides* spp. and Anaerobic *Streptococci*) can be important in surgical wounds related in particular to abdominal surgery. Diseases produced by the coliforms and *Proteus* can be grouped into three general categories:

1. Nosocomial or hospital-acquired infections: Forty percent of all nosocomial infections involve coliforms or *Proteus*. The primary sites for
infection include the urinary tract (E. coli), surgical wound (E. coli), lower respiratory tract (Klebsiella), and primary bacteremia (E. coli).

2. Infections in compromised patients: E. coli is responsible for 40% of neonatal bacterial meningitis infections.

3. Community acquired infections: E. coli accounts for 85% of urethrocystitis cases, 80% of chronic bacterial prostatitis cases and 90% of acute pyelonephritis cases. Proteus, Klebsiella and Enterobacter may produce urinary tract infections. Proteus may also be responsible for some renal infection stones, due to the production of the enzyme urease and subsequent alkalinisation and super saturation of urine. In addition, K. pneumonia is responsible for approximately 3% of bacterial pneumonia cases and is more severe than that produced by S. pneumoniae. E. coli can also produce several different types of diarrheal disease.
**Staphylococcus Species**\(^{154, 210, 211}\)

*Staphylococci* are Gram Positive Cocci that occur in grape-like clusters. They are ubiquitous and form the commonest cause of localized suppurative lesions in man. Their ability to develop resistance to penicillin and other antibiotics enhances their important as a human pathogen especially in the hospital environment. *Staphylococcus aureus* shows exceptional ability to appear in multiply drug resistant form.

*Staphylococci* were first observed in human pyogenic lesions by von Recklinghausen in 1871. Pasteur isolated the organism in 1880 from pus and produced abscesses in rabbit by inoculation of the organism. Sir Alexander Ogston, a Scottish surgeon, first showed in 1880 that the organism caused a number of pyogenic diseases in man. He introduced the name “Staphylococcus” which derived from the Greek staphyle (bunch of grapes) and kokkos (grain of berry).

**Characteristics of the genus Staphylococcus**\(^{154}\)

*Gram Positive cocci, Catalase Positive, Divide in more than one plan to form irregular, grape like clusters, capable of aerobic and anaerobic metabolism, occurs widely on the surfaces of man and other vertebrate animals.*

Although more than 20 species of staphylococcus are described in Bergey’s Manual (2001), only very few are medically important. They include: \(^{132, 211}\)

2. *Staphylococcus epidermidis* (*S. albus*): A normal Skin commensal
3. *Staphylococcus saprophyticus*: Found mainly on genitourinary mucous membrane and skin, similar to *Staphylococcus epidermidis* but resistant to novobiocin.

The association between virulence and pigment production was not found to be constant.

As distinction between pathogenic and non-pathogenic staphylococci is important in diagnostic practice, several attempts have made, but none was found reliable.
Now there is a general agreement between virulence and production of the enzyme coagulase and to a less extent, fermentation of mannitol.  

Staphylococci are therefore classified into two groups, on the basis of their ability to clot blood plasma by action of the enzyme coagulase. 

1. **Coagulase Positive Staphylococci**
2. **Coagulase Negative Staphylococci**

**Staphylococcus aureus**

**MORPHOLOGY & STAINING**

They are gram positive, spherical cocci, about 1 um in diameter, arranged characteristically in grape like clusters. They are non-sporing, non-motile, aerobic and normally facultative anaerobic cocci. Cluster formation is due to cells dividing sequentially in three perpendicular planes and the daughter cells getting located by the action of separation enzymes to yield typical irregular clusters.

**Mechanism of cluster formation by cells of Staphylococcus aureus**

*Staphylococci* divide perpendicular to last plane of division.

Gram Stain of *Staphylococcus aureus* in pustular exudates
CULTURE CHARACTERISTICS:

They grow readily on ordinary culture media under aerobic or anaerobic conditions within a temperature range of 10-42’ C, optimal temperature being 37’ C with pH of 7.4-7.6.

NUTRIENT AGAR:

After aerobic incubation for 24 h at 37’C, colonies are 1-3 mm in diameter and have a smooth glistening surface, an entire edge, a soft butyrous consistency and an opaque, pigmented appearance. In most strains the pigmentation is golden, with orange, yellow and cream-buff varieties, but in a few it is white. The pigment does not diffuse into the medium. Pigment production occurs optimally at 22’C and only in aerobic cultures. \(^{131, 210}\)
Colonies of *Staphylococcus aureus* on Nutrient agar & Blood agar

Golden yellow Colonies on N-agar & β-haemolysis on blood agar.

**MANNITOL SALT AGAR** 142, 163

This medium, which contains 1% mannitol, 7.5 % NaCl and 0.0025% phenol red in nutrient agar, is both a selective and an indicator medium. Most strains of *S. aureus* ferment mannitol and so form colonies surrounded by yellow zones due to acid production, whilst most other staphylococci fail to ferment mannitol and form colonies with red or purple zones.

**Mannitol Salt Agar**

Selective for *Staphylococcus* spp. Differentiates between *S. aureus* and *S. epidermidis*. *S. aureus* is able to ferment Mannitol.

<table>
<thead>
<tr>
<th>Mannitol Negative</th>
<th>Mannitol Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-fermentor</td>
<td>Fermentor</td>
</tr>
</tbody>
</table>

* S. epidermidis
* S. aureus
VIRULENCE FACTORS

- Antigens
  - Capsule
  - Adhesins
- Enzymes
  - Coagulase
  - Lipase
  - Hyaluronidase
  - Staphylokinase
  - Nuclease
- Toxins
  - $\alpha$-toxin
  - $\beta$-toxin
  - P-V Leukocidin
  - Enterotoxin
  - Exfoliative toxin
  - Toxic shock syndrome toxin

LABORATORY DIAGNOSIS:

The presence of *Staphylococci* in a lesion might first be suspected after examination of a direct Gram stain. However, small numbers of bacteria in blood preclude microscopic examination and require culturing first. The organism is isolated by streaking material from the clinical specimen (or from a blood culture) onto solid media such as blood agar, tryptic soy agar or heart infusion agar. Specimens likely to be contaminated with other microorganisms can be plated on mannitol salt agar containing 7.5% sodium chloride, which allows the halo-tolerant *Staphylococci* to grow. Ideally a Gram stain of the colony should be performed and tests made for catalase and coagulase production, allowing the coagulase-positive *S aureus* to be identified quickly. Another very useful test for *S aureus* is the production of thermo stable deoxyribonuclease. *S aureus* can be confirmed by testing colonies for agglutination with latex particles coated with immunoglobulin G and fibrinogen which bind protein A and the clumping factor, respectively, on the bacterial
Introduction & Review of Literature

cell surface. These are available from commercial suppliers (e.g., Staphaurex). The most recent latex test (Pastaurex) incorporates monoclonal antibodies to serotype 5 and 8 capsular polysaccharide in order to reduce the number of false negatives. (Some recent clinical isolates of *S. aureus* lack production of coagulase and/or clumping factor, which can make identification difficult). 214

Hospital strains of *S. aureus* are usually resistant to a variety of different antibiotics. A few strains are resistant to all clinically useful antibiotics except vancomycin, and vancomycin-resistant strains are increasingly-reported. The term MRSA refers to Methicillin resistant *Staphylococcus aureus*. Methicillin resistance is widespread and most Methicillin-resistant strains are also multiply resistant. A plasmid associated with vancomycin resistance has been detected in *Enterococcus faecalis* which can be transferred to *S. aureus* in the laboratory, and it is speculated that this transfer may occur naturally (e.g. in the gastrointestinal tract). In addition, *S. aureus* exhibits resistance to antiseptics and disinfectants, such as quaternary ammonium compounds, which may aid its survival in the hospital environment.

**IDENTIFICATION CHART OF GRAM POSITIVE COCCI:**
Staphylococcal disease has been a perennial problem in the hospital environment since the beginning of the antibiotic era. During the 1950's and early 1960's, staphylococcal infection was synonymous with nosocomial infection. Gram-negative bacilli (e.g. E. coli and Pseudomonas aeruginosa) have replaced the staphylococci as the most frequent causes of nosocomial infections, although the staphylococci have remained a problem, especially in surgical wounds. S. aureus responded to the introduction of antibiotics by the usual bacterial means to develop drug resistance: (1) mutation in chromosomal genes followed by selection of resistant strains and (2) acquisition of resistance genes as extra chromosomal plasmids, transducing particles, transposons, or other types of DNA inserts. S. aureus expresses its resistance to drugs and antibiotics through a variety of mechanisms.

Beginning with the use of the penicillin in the 1940's, drug resistance has developed in the staphylococci within a very short time after introduction of an antibiotic into clinical use. Some strains are now resistant to most conventional antibiotics, and there is concern that new antibiotics have not been forthcoming. New strategies in the pharmaceutical industry to find antimicrobial drugs involve identifying potential molecular targets in cells (such the active sites of enzymes involved in cell division), then developing inhibitors of the specific target molecule. Hopefully, this approach will turn up new antimicrobial agents for the battle against staphylococcal infections. In fact, in the past two years alternatives to vancomycin have been approved with the increase in VRSA (Vancomycin resistant S. aureus) & VISA (Vancomycin intermediate S. aureus) isolates.

**Coagulase-negative Staphylococci species:**

Coagulase-negative staphylococci (CONS), which historically have been viewed as contaminants when recovered in culture media, are now recognized as opportunistic pathogens of increasing importance in hospital-acquired infections. They are frequently found colonizing prosthetic devices and intravenous lines. CONS are capable of producing a variety of infections including deep-seated infections such as endocarditis and meningitis. Staphylococcus epidermidis is the most commonly isolated CONS and it appears to be the most resistant to antibiotics, making antimicrobial therapy
challenging. Treatment of the infection will very often require removal of a prosthetic device, if present. An adequate infection control program is imperative in prophylaxis against this infection.  

**CLINICAL SIGNIFICANCE:**
The clinical significance of Coagulase-Negative *Staphylococcus* species (CONS) continues to increase as strategies in medical practice lead to more invasive procedures. Hospitalized patients that are immunocompromised and/or suffering from chronic diseases are the most vulnerable to infection. Since CONS are widespread on the human body and are capable of producing very large populations, distinguishing the etiologic agent(s) from contaminating flora is a serious challenge. For this reason, culture identification should proceed to the species and strain levels.

Many of the CONS species are commonly resistant to antibiotics that are being indicated for Staphylococcal infections, with the exception of vancomycin. The widespread use of antibiotics in hospitals has provided a reservoir of antibiotic-resistant genes. Slime can reduce the immune response and opsonophagocytosis, thereby interfering with host defence mechanisms. As we become more aware of the various strategies used by CONS, we will be in a better position to compromise their defence mechanisms and improve treatment.

**MOST FREQUENT COAGULASE-NEGATIVE STAPHYLOCOCCI**

![Pie chart showing the most frequent coagulase-negative staphylococci.](chart.png)
Coagulase-negative staphylococci isolated from human specimens

<table>
<thead>
<tr>
<th>Novobiocin-susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. epidermidis</em></td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
</tr>
<tr>
<td><em>S. auricularis</em></td>
</tr>
<tr>
<td><em>S. capitis subsp. capitidis</em></td>
</tr>
<tr>
<td><em>S. capitis subsp. urealyticus</em></td>
</tr>
<tr>
<td><em>S. caprae</em></td>
</tr>
<tr>
<td><em>S. hominis</em></td>
</tr>
<tr>
<td><em>S. lugdunensis</em></td>
</tr>
<tr>
<td><em>S. pasteuri</em></td>
</tr>
<tr>
<td><em>S. saccharolyticus</em></td>
</tr>
<tr>
<td><em>S. schleiferi subsp. schleiferi</em></td>
</tr>
<tr>
<td><em>S. simulans</em></td>
</tr>
<tr>
<td><em>S. warneri</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Novobiocin-resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. saprophyticus subsp. saprophyticus</em></td>
</tr>
<tr>
<td><em>S. cohnii subsp. cohnii</em></td>
</tr>
<tr>
<td><em>S. Sciuri</em></td>
</tr>
</tbody>
</table>

Electron Micrographs of *Staph. epidermidis* Bacteria. SEM X9600
METHICILLIN RESISTANT *Staphylococcus aureus* 35, 56, 145

The treatment of infections due to *Staphylococcus aureus* was revolutionised in the 1940s by the introduction of the antibiotic penicillin.

Unfortunately, most strains of *Staphylococcus aureus* are now resistant to penicillin. This is because *Staphylococcus aureus* has 'learnt' to make a substance called β-lactamase that degrades penicillin destroying its antibacterial activity.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes. The evolutionary origins of MRSA are poorly understood, no rational nomenclature exists, and there is no consensus on the number of major MRSA clones or the relatedness of clones described from different countries.

Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus*. In 1961 there were reports from the United Kingdom of *S. aureus* isolates that had acquired resistance to Methicillin (Methicillin-resistant *S. aureus*, MRSA), and MRSA isolates were soon recovered from other European countries, and later from Japan, Australia, and the United States. MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community. The Methicillin resistance gene (*mecA*) encodes a Methicillin-resistant penicillin-binding protein (PBP) that is not present in susceptible strains and is believed to have been acquired from a distantly related species. *mecA* is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCCmec), of which four forms have been described that differ in size and genetic composition. Many MRSA isolates are multiply resistant and are susceptible only to glycopeptide antibiotics such as vancomycin and investigational drugs. MRSA isolates that have decreased susceptibility to glycopeptides (glycopeptide intermediately susceptible *S. aureus*, GISA), reported in recent years, are a cause of great public health concern.

Many studies have characterized MRSA isolates from individual hospitals or countries and have identified strains that appear to be well adapted to the
hospital environment, are established in several hospitals within a country, or have spread internationally (epidemic MRSA, EMRSA).

**Staphylococcus & Surgical Wound Infections**

The origins of the major MRSA clones are still poorly understood. Kreiswirth et. al. proposed that all MRSAs were descended from a single ancestral *S. aureus* strain that acquired mecA, but more recent studies show that some MRSAs are very divergent, implying that mecA has been transferred between *S. aureus* lineages. The data from MLST can be used to probe the evolutionary and population biology of bacterial pathogens and to predict ancestral genotypes and patterns of evolutionary descent within groups of related genotypes.

**Staphylococcal Surgical Wound Sepsis:**

Most of the patients who develop septic wounds due to *Staphylococcus aureus* are not infected by an epidemic hospital strain. The origin of the infecting strain of *Staphylococcus aureus* in sporadic cases is not always clear but in many cases is probably derived from the patient’s own skin flora. In the latter situation, the organism may sometimes enter the wound during the operation. *Staphylococcus aureus* is always present in large numbers in hospitals since it is carried in warm moist sites on the skin or in the noses of many patients and hospital staff, and it survives well in the dry environment. The carriage rate is higher in hospital than in the community.

**OPERATING THEATRE ASSOCIATED Staphylococcal SEPSIS:**

The surgical team is the most frequent source of outbreaks of operating theatre acquired sepsis. Any member of the surgical team who has skin sepsis, or who has skin lesions, including minor eczema which may be colonized by *Staphylococcus aureus*, is particularly likely to be a source of an outbreak. ‘Gentle’ handling of the tissues and good haemostasis by surgeons is important factors associated with low sepsis rates in theatre. Other measures which reduce -the chance of surgical wound infection involve the use of scrupulous aseptic technique.

**WARD-ASSOCIATED STAPHYLOCOCCUS WOUND INFECTIONS**

Outbreaks of staphylococcal sepsis generally originate in the ward rather than
the operating theatre and the usual source is an infected patient. The organism is spread from the infected patient usually by hands of staff; most often nursing staff, to one or more of the other patients in the ward. This may occur at the time of changing the dressings. Measures that reduce the chances of ward acquired *Staphylococcus aureus* wound infection include:

1. Isolation of patients in single rooms and suitable isolation nursing of patients who otherwise would discharge staphylococcal pus from the wound into the surgical ward. This isolation is particularly necessary when there is a lot of pus or the patient has a multiple antibiotic-resistant strain of *Staphylococcus*.

2. No-touch dressing techniques performed carefully—good education of nursing and medical staff is necessary. Medical staff should not touch the skin near the wound when 'peeping' at the wound. Care is also necessary when removing the sutures; adequate washing of hands between patients as well as no-touch techniques should be observed.

**CONTROL OF Staphylococcal OUTBREAKS:**
When the results of culture of the patient's swabs and swabs of the nose, hands, wrists, perineum and hairline of theatre staff, in theatre-suspected outbreaks, or ward staff, in ward-suspected outbreaks, are known the consultant microbiologist in charge of the investigations can advise appropriate measures to further control an outbreak. He or she will already have arranged for the isolation of patients who have been shown to be infected or significantly colonized by the epidemic strain. When a carrier of the epidemic strain is identified amongst hospital staff, the staff member may have to temporarily cease work with surgical patients until cleared of the organism.
Factors suggesting a theatre or ward source of outbreak of Staphylococcal wound sepsis:

<table>
<thead>
<tr>
<th>No.</th>
<th>Factor</th>
<th>Theatre Source</th>
<th>Ward Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Time of first recognition of the septic wound</td>
<td>a. Sepsis at the time of removing the first dressing b. Sepsis occurring between 1 and 8 days post operatively</td>
<td>a. Sepsis only after the first dressing was removed b. Sepsis developing after the third post operative day</td>
</tr>
<tr>
<td>2</td>
<td>Depth of Sepsis</td>
<td>Deep or superficial</td>
<td>Superficial</td>
</tr>
<tr>
<td>3</td>
<td>Hospital areas affected</td>
<td>Patients in different wards or different hospitals</td>
<td>Patients in the same ward</td>
</tr>
<tr>
<td>4</td>
<td>Surgical team and operating theatre details</td>
<td>All the patients: a. Operated by the same person in the surgical team or b. Same operating list or c. Same operating theatre</td>
<td>a. Different surgical team or b. Different surgical list or c. Different surgical theatres</td>
</tr>
<tr>
<td>5</td>
<td>Further microbiological investigations</td>
<td>Staph aureus strains of similar type isolated from a surgical team member or other theatre source and from patient’s wounds</td>
<td>Staph aureus strains of similar type isolated from patients in the ward and possibly from ward staff</td>
</tr>
</tbody>
</table>
Proteus, Providencia & Morganella species

INTRODUCTION

The taxonomy of *Proteus*, *Providencia*, and *Morganella* is a fascinating story that is enmeshed throughout the early history of the evolving science of microbiology. Species within these genera are not considered frank pathogens, unlike some of the other members of the *Enterobacteriaceae*, and are commonly isolated in clinical laboratories. As with other opportunistic pathogens, they may also cause morbidity and mortality. It is clear that while the more pathogenic members of the *Enterobacteriaceae*, such as *Salmonella*, *Shigella*, and *Escherichia coli*, may exact more urgent attention, the presence of any one of these less pathogenic genera in body fluids and in some deep or superficial lesions would lead one to suspect their potential etiologic nature.

Differentiation among the genera *Proteus*, *Providencia*, and *Morganella*

<table>
<thead>
<tr>
<th>Biochemical test or property</th>
<th><em>Proteus</em>&lt;sup&gt;&lt;small&gt;b&lt;/small&gt;&lt;/sup&gt;</th>
<th><em>Providencia</em>&lt;sup&gt;&lt;small&gt;b&lt;/small&gt;&lt;/sup&gt;</th>
<th><em>Morganella</em>&lt;sup&gt;&lt;small&gt;b&lt;/small&gt;&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate utilization</td>
<td>v</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Mannose fermentation</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin liquefaction (22°C)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>H₂S production (TSI)&lt;sup&gt;&lt;small&gt;c&lt;/small&gt;&lt;/sup&gt;</td>
<td>+</td>
<td>–</td>
<td>v</td>
</tr>
<tr>
<td>myo-Inositol fermentation</td>
<td>–</td>
<td>v</td>
<td>–</td>
</tr>
<tr>
<td>Lipase (corn oil)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>v</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td>Swarming</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Urea hydrolysis</td>
<td>+</td>
<td>v</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from reference 167

<sup>b</sup> Symbols (all data are reactions at 48 h unless otherwise specified): +, 90 to 100% positive; (+), 75 to 89.9% positive; v, 25.1 to 74.9% positive; (−), 10.1 to 25% positive; −, 0 to 10% positive.

<sup>c</sup> TSI, triple sugar iron.
THE GENUS PROTEUS

Current Classification

The genus *Proteus* currently consists of five named species (*P. mirabilis*, *P. penneri*, *P. vulgaris*, *P. myxofaciens*, and *P. hauseri*) and three unnamed genomospecies (*Proteus* genomospecies 4, 5, and 6).

Swarming growth of *Proteus vulgaris*

![Swarming growth of Proteus vulgaris](image)

PHENOTYPIC IDENTIFICATION OF THE SPECIES

Conventional methods

Biochemical test results for the four species of *Proteus*. Pompei et al. reported a methyl green-phenolphthalein phosphatase test which would accurately and simply separate members of the tribe *Proteeae*.

Commercial methods

With the ever-increasing cost of commercial identification methods, spot testing is both rapid and cost-effective and can be helpful in many instances. If the colonies on a sheep blood agar plate swarm and are oxidase negative and if a spot indole test using *para*-dimethylaminocinnemaldehyde reagent is negative, the probability of the culture being either *P. mirabilis* or *P. penneri* is very high. To separate these two species, a positive test for ornithine decarboxylase will be obtained with *P. mirabilis*. If the spot indole test is positive, the culture is most likely to be *P. vulgaris*. *P. penneri* is often described as indole-negative *P. vulgaris*. In most instances, identifications by rapid spot tests need not be confirmed by conventional or commercial methods.
Clinical Significance

*Protease* is widespread in the environment and make up part of the normal flora of the human gastrointestinal tract. Although *Escherichia coli* accounts for the largest percentage of cases of uncomplicated cystitis, pyelonephritis, and prostatitis.

*P. mirabilis* has been implicated in bacteremia, neonatal meningoencephalitis empyema, and osteomyelitis. *P. penneri* has been implicated in a case of bacteremia and concomitant subcutaneous thigh abscess in a neutropenic patient with acute lymphocytic leukemia and in nosocomial urosepsis in a diabetic patient from whom the organism was also subsequently isolated from bronchoalveolar lavage fluid and a pulmonary artery catheter tip.

The urease enzyme of *P. penneri* is also believed to be a leading cause of kidney stone formation; indeed, the organism has been isolated from the centre of a stone removed from a patient with persistent *P. penneri* bacteriuria. *P. penneri* has also been isolated from stool and infected conjunctiva.

Nosocomial transmission, while uncommon, has been reported. In 1983, Williams et al. reported on five patients in a cardiac surgery unit with septicemia caused by either *P. mirabilis*, *Morganella morganii*, or both organisms. No environmental source was identified, although O serotyping confirmed cross-infection of patients by both species.

In the last 10 years, there has been a report in the literature to suggest that *P. mirabilis* may play an etiopathogenic role in rheumatoid arthritis. This study showed that patients with rheumatoid arthritis have higher levels of urinary *Proteus* than do comparable healthy controls of either sex or women with non-rheumatoid-arthritis arthritic conditions, findings which are disputed by another group, perhaps due to a difference in the methods used in their studies.

Antimicrobial Susceptibility

The indole-negative *P. mirabilis* strains are generally more susceptible to antimicrobials than are *P. vulgaris*, *P. penneri*, and *P. hauseri*. *P. mirabilis* has intrinsic resistance to nitrofurantoin and tetracycline but is generally susceptible to the amino- and ureido-penicillins (ampicillin, amoxicillin, and piperacillin), cephalosporins (cefazolin, cefoxitin, cefuroxime, cefotaxime,
ceftazidime, ceftriaxone, ceftizoxime, and cefepime), aminoglycosides (amikacin, gentamicin, and tobramycin), imipenem, ciprofloxacin, and trimethoprim-sulfamethoxazole. However, high levels of ciprofloxacin resistance have been reported for *P. mirabilis* and *Providencia* spp. in hospitals where use of this agent is unrestricted. The intrinsic resistance to tetracycline can be used as an identification marker for this organism.

*Proteus penneri* is generally more resistant to penicillin than is *P. vulgaris*, and its susceptibility pattern more closely reflects that of *M. morganii* than that of *P. vulgaris*. These *Proteaeae* are generally susceptible to cefoxitin, broad-spectrum cephalosporins (cefotaxime, ceftriaxone, ceftizoxime, and ceftazidime), cefepime, aztreonam, aminoglycosides, ciprofloxacin, tazobactam, imipenem and may be resistant to cefazolin, cefprozil, cefuroxime, cefamandol, cefdinir, cefoperazone, loracarbef, ampicillin, and the ureidopenicillins.

**THE GENUS PROVIDENCIA**

**Current Classification**

The genus *Providencia* consists of five species: *P. alcalifaciens*, *P. heimbachae*, *P. rettgeri*, *P. rustigianii*, and *P. stuartii*.

**Clinical Significance**

Human isolates of *Providencia* species have been recovered from urine, throat, perineum, axilla, stool, blood, and wound specimens. *P. heimbachae* and *P. rustigianii* have also been isolated from penguins.

When Gomes first described *Eberthella* (now *Providencia*) *alcalifaciens* in 1944, the strain with which he worked was isolated from an 11-month-old child with dysentery. Haynes and Hawkey found a higher incidence of *P. alcalifaciens* in patients with diarrhoea than in healthy patients and suggested that this organism may be a cause of diarrhoea, particularly in children. Albert et al. have shown that *P. alcalifaciens* is capable of invading HEp-2 monolayers in rabbits, but the relevance to human disease is not clear.

*P. stuartii* has long been recognized as a pathogen for nursing home patients with chronic indwelling urinary catheters. A total of 21 to 61% of urinary tract
specimens in this population contain either P. mirabilis or P. stuartii, and the organisms may even result in a fatal bacteremia.

There have been rare incidents of P. rettgeri causing nosocomial infections. Traub et al. reported an outbreak of urinary tract infections caused by a highly resistant lactose-fermenting strain of P. rettgeri. While the organism was easily traceable because of this unusual biochemical characteristic, no common source of the outbreak was discovered. In a similar report, 10 patients had urinary tract infections caused by a highly resistant strain of P. rettgeri, and one death was believed to have been caused by these infections. The spread of infection was probably by contact with hospital personnel; the outbreak ended after the use of disposable gloves and contact isolation procedures were implemented. Other similar problems have been reported in the literature.

THE GENUS MORGANELLA

Current Classification
The genus Morganella currently consists of one species, Morganella morganii, with two subspecies, morganii and sibonii.

Clinical Significance
M. morganii is an opportunistic secondary invader that was originally thought to be the cause of summer diarrhoea. In 1986, Müller isolated M. morganii significantly more often from patients with gastrointestinal disease than from healthy controls. Case reports implicating this organism as a cause of disease, although rare, are scattered throughout the literature. For example, M. morganii has caused neonatal sepsis in an 11-day-old boy, a brain abscess in a neonate, and a tubo-ovarian abscess (originally mistaken for vasculitis attributed to Henoch-Schonlein purpura) in a 15-year-old girl. Reports involving M. morganii infections in immunocompromised individuals include chorioamnionitis and neonatal seizures in a pregnant woman, a postoperative foot infection in a diabetic, and pyomyositis and meningitis in AIDS patients. Schonwetter and Orson also described a case of atypical pyoarthritis due to M. morganii in an elderly patient. This case was atypical in that it had a very benign clinical presentation with minimal inflammatory
response over a prolonged period. Sica et al. reported a patient with acute lymphoblastic leukemia who underwent a resolving splenectomy for immune pancytopenia following an allogeneic bone marrow transplant. The patient developed pericarditis, from which *M. morganii* was isolated. The authors conclude that splenectomy could have been a predisposing factor for the development of this unusual complication.

Additional human sources from which the organism has been isolated include urine, gallbladder, stool, sputum and other respiratory samples, and assorted wound sites.

**Antimicrobial Susceptibility**

*M. morganii* is susceptible to many of the currently used antimicrobial agents, including ceftazidime, cefepime, aztreonam, imipenem, tazobactam, ciprofloxacin, tobramycin, and gentamicin. Strains are often resistant to the newer cephalosporins, including cefprozil, cefuroxime, loracarbef, cefdinir, and cefetamet. They can also be resistant to cefazolin, cefixime, cefpodoxime, and ampicillin.

As with strains of *Providencia* spp., *Morganella* spp. are capable of producing β-lactamases. When automated susceptibility testing is performed on these organisms, a 3- to 6-h time frame may not be adequate for expression of all of the bacterial resistance mechanisms and could result in a report of false susceptibility. False resistance also may occur in testing with aztreonam because elongation of cells just before lyses can be interpreted by the instrument as growth.

Laboratories must monitor susceptibility results involving these antimicrobials when using commercial systems.
Enterobacter species 43, 62, 93, 207

INTRODUCTION:

*Enterobacter* is a gram-negative bacillus belonging to the Enterobacteriaceae family. Other members of this family include *Klebsiella, Escherichia, Citrobacter, Serratia, Salmonella, Shigella* species, and many others. Enterobacteriaceae are the most frequent bacterial isolates recovered from clinical specimens. These bacteria have an outer membrane that contains, among other things, lipopolysaccharides from which lipid-A plays a major role in sepsis. Lipid-A, also known as endotoxin, is the major stimulus for the release of cytokines, which are the mediators of systemic inflammation and its complications.

In the microbiology laboratory, colonies of Enterobacteriaceae appear large, dull-gray, and dry or mucoid on sheep blood agar. All Enterobacteriaceae are glucose fermenters and consequently, are able to grow in aerobic and anaerobic atmospheres.

Many different species comprise the genus *Enterobacter*. For some, no evidence exists to date that proves they can cause human infections. The most frequently isolated species are *E. cloacae* and *E. aerogenes*, followed by *E. sakazakii*, which produces a characteristic yellow pigment. Other species rarely encountered in the clinic include *Enterobacter asburiae, Enterobacter gergoviae, Enterobacter taylorae, Enterobacter hormaechei*, and *Enterobacter cancerogenus*. *Enterobacter agglomerans* has been removed from the genus *Enterobacter* and renamed *Pantoea agglomerans*.

Background: *Enterobacter* species, particularly *Enterobacter cloacae* and *Enterobacter aerogenes*, are important nosocomial pathogens responsible for various infections, including bacteremia, lower respiratory tract infections, skin and soft tissue infections, urinary tract infections (UTIs), endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections. Risk factors for nosocomial *Enterobacter* species infections include hospitalization of greater than 2 weeks, invasive procedures in the past 72 hours, treatment with antibiotics in the past 30 days, and the presence of a
central venous catheter. Specific risk factors for infection with nosocomial multidrug-resistant strains of *Enterobacter* species include the recent use of broad-spectrum cephalosporins or aminoglycosides and ICU care.

These "ICU bugs" cause significant morbidity and mortality, and infection management is complicated by multiple antibiotic resistances. *Enterobacter* species possess inducible beta-lactamases, which are undetectable in vitro but are also responsible for resistance during treatment. Physicians treating patients infected with these bacteria are advised to avoid certain antibiotics, particularly third-generation cephalosporins, because resistant mutants can quickly appear. The crucial first step is appropriate identification of the bacteria. Antiobiotogram must be interpreted with respect to the different resistance mechanisms and their respective frequency, as is reported for bacteria belonging to this genus, even if the resistance mechanisms have not been detected by routine in vitro antibiotic susceptibility testing.

**PATHOPHYSIOLOGY:**

*Enterobacter* species rarely cause disease in a healthy individual. This opportunistic pathogen, similar to other members of the Enterobacteriaceae family, possesses an endotoxin known to play a major role in the pathophysiology of sepsis and its complications.

Although community-acquired infections are occasionally observed, nosocomial infections are, by far, the most frequent. Patients most susceptible to acquiring *Enterobacter* infections are those who stay in the hospital, especially the ICU, for prolonged periods. Other major risk factors include the prior use of antimicrobial agents, concomitant malignancy (especially haemopoietic and solid organ malignancies) hepatobiliary disease, ulcers of the upper gastrointestinal tract, use of foreign devices such as intravenous catheters, and serious underlying conditions such as burns, mechanical ventilation, and immunosuppression.

*Enterobacter* species contain a subpopulation of organisms that produce a beta-lactamase at low-levels. Once exposed to broad-spectrum cephalosporins, the subpopulation of beta-lactamase–producing organisms predominates. Thus, an infection that appears sensitive to cephalosporins at
the time of diagnosis may quickly develop into a resistant infection during therapy. Imipenem and cefepime have a more stable beta-lactam ring against the lactamase produced by resistant strains of Enterobacter.

**SKIN AND SOFT TISSUE INFECTIONS**

- In most cases, infections are hospital-acquired and include cellulitis, fasciitis, myositis, abscesses, and wound infections.
- *Enterobacter* can cause surgical wound infections in any body site, and these infections are clinically indistinguishable from infections caused by other bacteria.
- An outbreak of postsurgical *Enterobacter* mediastinitis has been reviewed by Palmer et al. Cases varied in severity (i.e., from fulminant bacteremic infections to less severe wound infections). The source was unknown, and a case-control analysis suggested that surgical complications and prophylaxis with cephalosporins were associated with the infection. The level of skin and wound colonization was high among cardiac surgery patients during this outbreak period. Implementing barrier isolation, restricting contacts, and reducing the duration of cephalosporin prophylaxis terminated this outbreak.
- Other *Enterobacter* wound infections are reported in the literature. Infected body sites include a posterior spinal wound, burn wounds (many reports), and different types of injuries involving multiple traumatized patients. In some cases, the infections were polymicrobial. Some authors have noted a trend of traditional wound bacteria (e.g., *S. aureus*) being replaced by *Enterobacter* and other nosocomial pathogens. Among traumatized patients, some wound infections are acquired before hospital admission. This was the case with agricultural mutilating wounds caused by corn harvesting machines. Gram-negative rods were predominant (81%), the most common being *Enterobacter* and *Stenotrophomonas maltophilia*.
- Anecdotal reports also demonstrate that *Enterobacter* occasionally cause community-acquired soft tissues infections in healthy individuals.
VARIOUS RESISTANCE MECHANISMS OF BACTERIA:
During the past 15 years, emergence and dissemination of β-lactam resistance in nosocomial Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, became a serious problem worldwide. Especially the increasing resistance to 3rd and 4th generation cephalosporins and carbapenems is of particular concern. Gram-negative bacteria pursue various molecular strategies for development of resistance to these antibiotics: (a) generation of extended-spectrum β-lactamases (ESBL) according to the original definition due to extension of the spectrum of already widely disseminated plasmid-encoded β-lactamases by amino acid substitution; (b) acquisition of genes encoding ESBL from environmental bacteria as, for instance the CTX-M-type β-lactamases from *Kluyvera* spp.; (c) high-level expression of chromosome-encoded β-lactamase (bla) genes as bla*OXA* or bla*ampC* genes due to modifications in regulatory genes, mutations of the β-lactamase promoter sequence as well as integration of insertion sequences containing an efficient promoter for intrinsic bla genes; (d) mobilization of bla genes by incorporation in integrons and horizontal transfer into other Gram-negative species such as the transfer of the ampC gene from *Citrobacter freundii* to *Klebsiella* species; (e) dissemination of plasmid-mediated carbapenemases as KPC and metallo-β-lactamases, e.g. VIM and IMP; (f) non-expression of porin genes and/or efflux pump-based antibiotic resistance. This mini-review summarizes the historical emergence of β-lactam resistance and β-lactamases as major resistance mechanism in enteric bacteria, and also highlights recent developments such as multidrug- and carbapenem resistance.  

EXTENDED SPECTRUM β-LACTAMASE (ESBL):
Besides fluorquinolones, β-lactam antibiotics are most frequently applied in treatment of bacterial infections. The large number of natural, semi synthetic and synthetic β-lactam antibiotics can be subdivided into 6 different structural subtypes:

- Penams (e.g. benzylpenicillin, ampicillin);
• Cephems which include classical cephalosporins, 2nd generation cephalosporins (e.g. cefotiam, cefuroxime), and also representatives of 3rd generation cephalosporins (e.g. cefotaxime, ceftazidime);
• Cephamycins as 7-α-methoxy cephalosporins (e.g. cefoxitin);
• Monobactams as monocyclic molecules (e.g. aztreonam);
• Penems with a 2,3-double bond in the fused thiazoline ring (e.g. faropenem); and
• Carbapenems (e.g. imipenem) with an unsaturated fused 5 membered ring differing from penem structure by possession of a carbon atom at position 1.

In the early 1950s, enteric bacteria that mediated resistance to the first penicillins attracted attention. In general, resistance of bacteria against β-lactam antibiotics relies on 3 basic principles:

(i) Possession of an altered or acquired penicillin binding protein (PBP) with low affinity for β-lactams (e.g. PBP2a in Methicillin resistant Staphylococcus aureus);
(ii) Efflux pumps that additionally use β-lactams as substrates (e.g. the mex system in Pseudomonas aeruginosa);
(iii) β-lactamases which cleave the amide bond of the β-lactam ring, thus inactivating the antibiotic agent.

The introduction of 3rd generation cephalosporins, which started with cefotaxime 30 years ago, was a milestone in antimicrobial chemotherapy. Undoubtedly as a consequence of selective pressure exerted by these new cephalosporins, resistance in Enterobacterial species emerged a few years later. At that time, 2 main causes were specified:

• expansion of the substrate spectrum of broad-spectrum TEM type and SHV-type β-lactamases which were already widely disseminated due to plasmid location of these genes (Jarlier et al., 1988; Sirot et al., 1988),
• Constitutive high-level expression of the intrinsic ampC gene, coding for a cephamycinase (cefoxitin as phenotypical indicator substrate) in species with an efficient ampC promoter such as
METHODS OF ESBL DETECTIONS: 64

1. DOUBLE DISK SYNERGY TEST:
   In this test discs of 3rd generation cephalosporins and augmentin are kept 30 mm apart from centre to enter on inoculated Mueller-Hinton (MH) agar. A clear extension of the edge of the inhibition zone of cephalosporin towards augmentin disc in interpreted as positive for ESBL production.

2. THREE DIMENSIONAL TEST:
   This test provides the advantage of simultaneous determination of antibiotic susceptibility and β-lactamase substrate profile. Inoculum produced in this method contains between 10⁹ and 10¹⁰ CFU/ml of cells that actively produce β-lactamase. Two types of inocula are prepared one disc diffusion test inoculum (O.D-0.5 McFarland standard) and a three dimensional inoculum. Plate is inoculated by disc diffusion procedure. A circular slit is cut on the agar 4 mm inside the position at which the antibiotic discs are placed. Conventional disc diffusion susceptibility test results are measured according to the CLSI recommendations. Distortion or discontinuity in the circular inhibiting zone is interpreted as positive for ESBL production.

3. INHIBITOR POTENTIATED DISC DIFFUSION TEST
   Cephalosporin disc is placed on clavulanate containing and without clavulanate containing MHA plates. More than 10 mm increase in the zone of inhibition on the clavulanate containing MHA plate indicates ESBL production.

4. DISK APPROXIMATION TEST:
   Cefoxitin (inducer) disc is placed at a distance of 2.5 cm from cephalosporin disc. Production of inducible β-lactamase is indicated by flattening of the zone of inhibition of the cephalosporin disc towards inducer disc by ≥1 mm.

5. MIC REDUCTION TEST:
An 8 fold reduction in the MIC of cephalosporin in the presence of clavulanic acid indicates production of ESBL.

6. **Vitek ESBL TEST:**

Four wells containing cards are inoculated. A predetermined reduction in growth of cephalosporin well containing clavulanic acid: when compared with the level of growth in well with cephalosporin alone indicates presence of ESBL.

7. **E TEST:**

The E test ESBL strip carries two gradients, on the one end, ceftazidime and on the opposite end ceftazidime plus clavulanic acid. MIC is interpreted as the point of intersection of the inhibition ellipse with the E test strip edge. Ration of ceftazidime MIC and ceftazidime clavulanic acid MIC equal to or greater than 8 indicates the presence of ESBL.

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**METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA):**

Antimicrobial resistance in *S. aureus* emerged soon after penicillin came into common use in the 1940s. During the next 2 decades, resistance of this
pathogen to penicillin became widespread, followed by increasing resistance to the new semi synthetic penicillinase-resistant antimicrobial drugs (e.g., Methicillin, oxacillin, nafcillin) (3). In the last 20 years, Methicillin-resistant S. aureus (MRSA) has spread throughout the world in healthcare settings, leading to an increased reliance on vancomycin for empiric treatment (4). Recently, S. aureus resistance to vancomycin, the last commonly used antimicrobial drug to which this organism was considered uniformly susceptible, has emerged (5). In addition, serious MRSA infection has been increasingly reported in persons without identified predisposing risk, including recent healthcare exposure (6).

Historically, resistance to the penicillinase-stable penicillins has been referred to as “Methicillin resistance” or “oxacillin resistance.” MRSAs are those strains of S. aureus that express mecA or another mechanism of methicillin resistance, such as changes in affinity of penicillin-binding proteins (PBP) for oxacillin (modified S. aureus [MOD-SA] strains).

**Detection of oxacillin resistance:**

Tests for mecA or for the protein expressed by mecA, the penicillin-binding protein 2a (PBP 2a, also called PBP2’), are the most accurate methods for prediction of resistance to oxacillin and can be used to confirm results for isolates of Staphylococci from serious infections. Isolates of staphylococci that carry the mecA gene, or that produce PBP2a (the mecA gene product), should be reported as oxacillin resistant. Isolates that do not carry mecA or do not produce PBP 2a should be reported as oxacillin susceptible. Because of the rare occurrence of resistance mechanisms other than mecA, if MIC tests are performed in addition to disk diffusion, isolates for which oxacillin MICs are ≥4µg/mL and are mecA negative or PBP 2a negative should be reported as oxacillin resistant. These isolates may test as susceptible to cefoxitin by disk diffusion.
RISK FACTORS FOR SURGICAL SITE INFECTION:

The term risk factor has a particular meaning in epidemiology and, in the context of SSI pathophysiology and prevention, strictly refers to a variable that has a significant, independent association with the development of SSI after a specific operation. Risk factors are identified by multivariate analyses in epidemiologic studies. Unfortunately, the term risk factor often is used in the surgical literature in a broad sense to include patient or operation features which, although associated with SSI development in univariate analysis, are not necessarily independent predictors. 80 The literature cited in the sections that follow includes risk factors identified by both univariate and multivariate analyses.

Table-XIII lists patient and operation characteristics that may influence the risk of SSI development. These characteristics are useful in two ways:

1. They allow stratification of operations, making surveillance data more comprehensible; and,
2. Knowledge of risk factors before certain operations may allow for targeted prevention measures. For example, if it is known that a patient has a remote site infection, the surgical team may reduce SSI risk by scheduling an operation after the infection has resolved.

An SSI prevention measure can be defined as an action or set of actions intentionally taken to reduce the risk of an SSI. Many such techniques are directed at reducing opportunities for microbial contamination of the patient’s tissues or sterile surgical instruments; others are adjunctive, such as using antimicrobial prophylaxis or avoiding unnecessary traumatic tissue dissection. Optimum application of SSI prevention measures requires that a variety of patient and operation characteristics be carefully considered.
### TABLE 1.9:

**PATIENT AND OPERATION CHARACTERISTICS THAT MAY INFLUENCE THE RISK OF SURGICAL SITE INFECTION DEVELOPMENT**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Duration of surgical scrub</td>
</tr>
<tr>
<td>Nutritional status</td>
<td>Skin antisepsis</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Preoperative shaving</td>
</tr>
<tr>
<td>Smoking</td>
<td>Preoperative skin prep</td>
</tr>
<tr>
<td>Obesity</td>
<td>Duration of operation</td>
</tr>
<tr>
<td>Coexistent infections at a remote body site</td>
<td>Antimicrobial prophylaxis</td>
</tr>
<tr>
<td>Colonization with microorganisms</td>
<td>Operating room ventilation</td>
</tr>
<tr>
<td>Altered immune response</td>
<td>Inadequate sterilization of instruments</td>
</tr>
<tr>
<td>Length of preoperative stay</td>
<td>Foreign material in the surgical site</td>
</tr>
</tbody>
</table>

(1) **PATIENT CHARACTERISTICS:**

In certain kinds of operations, patient characteristics possibly associated with an increased risk of an SSI include coincident remote site infections or Colonization, diabetes, cigarette smoking, systemic steroid use, obesity...
(>20% ideal body weight), extremes of age, poor nutritional status, and perioperative transfusion of certain blood products.

(a) **DIABETES**

The contribution of diabetes to SSI risk is controversial, because the independent contribution of diabetes to SSI risk has not typically been assessed after controlling for potential confounding factors. Recent preliminary findings from a study of patients who underwent coronary artery bypass graft showed a significant relationship between increasing levels of HbA1c and SSI rates. Also, increased glucose levels (>200 mg/dL) in the immediate postoperative period (<48 hours) were associated with increased SSI risk. More studies are needed to assess the efficacy of perioperative blood glucose control as a prevention measure.

(b) **NICOTINE USE**

Nicotine use delays primary wound healing and may increase the risk of SSI. In a large prospective study, current cigarette smoking was an independent risk factor for sternal and/or mediastinal SSI following cardiac surgery. Other studies have corroborated cigarette smoking as an important SSI risk factor.

(c) **STEROID USE**

Patients who are receiving steroids or other immuno suppressive drugs preoperatively may be predisposed to developing SSI, but the data supporting this relationship are contradictory. In a study of long-term steroid use in patients with Crohn’s disease, SSI developed significantly more often in patients receiving preoperative steroids (12.5%) than in patients without steroid use (6.7%). In contrast, other investigations have not found a relationship between steroid use and SSI risk.

(d) **MALNUTRITION**

For some types of operations, severe protein-calorie malnutrition is crudely associated with postoperative nosocomial infections, impaired wound healing dynamics, or death. The National Academy of Sciences/National Research Council (NAS/NRC), Study on the Efficacy of Infection Control (SENIC), and NNIS1 schemes for SSI risk stratification do not explicitly incorporate nutritional status as a predictor variable, although it may be represented
indirectly in the latter two. In a widely quoted 1987 study of 404 high-risk general surgery operations, theoretical arguments can be made for a belief that severe preoperative malnutrition should increase the risk of both incisional and organ/space SSI. However, an epidemiologic association between incisional SSI and malnutrition is difficult to demonstrate consistently for all surgical subspecialties. Multivariate logistic regression modelling has shown that preoperative protein calorie malnutrition is not an independent predictor of mediastinitis after cardiac bypass operations. In the modern era, total parenteral nutrition (TPN) and total enteral alimentation (TEA) have enthusiastic acceptance by surgeons and critical care specialists. However, the benefits of preoperative nutritional repletion of malnourished patients in reducing SSI risk are unproven. In two randomized clinical trials, preoperative “nutritional therapy” did not reduce incisional and organ/space SSI risk.

(e) PROLONGED PREOPERATIVE HOSPITAL STAY
Prolonged preoperative hospital stay is frequently suggested as a patient characteristic associated with increased SSI risk. However, length of preoperative stay is likely a surrogate for severity of illness and co-morbid conditions requiring inpatient work-up and/or therapy before the operation.

(f) PREOPERATIVE NARES COLONIZATION WITH Staphylococcus aureus
Staphylococcus aureus is a frequent SSI isolate. This pathogen is carried in the nares of 20% to 30% of healthy humans. It has been known for years that the development of SSI involving S. aureus is definitely associated with preoperative nares carriage of the organism in surgical patients. A recent multivariate analysis demonstrated that such carriage was the most powerful independent risk factor for SSI following cardiothoracic operations.

(g) PERIOPERATIVE TRANSFUSION
It has been reported that perioperative transfusion of leukocyte-containing allogeneic blood components is an apparent risk factor for the development of postoperative bacterial infections, including SSI. In three of five randomized trials conducted in patients undergoing elective colon resection for cancer, the risk of SSI was at least doubled in patients receiving blood transfusions. However, on the basis of detailed epidemiologic reconsiderations, as many as
12 confounding variables may have influenced the reported association, and any effect of transfusion on SSI risk may be either small or nonexistent. Because of methodological problems, including the timing of transfusion, and use of nonstandardized SSI definitions, interpretation of the available data is limited. A meta-analysis of published trials will probably be required for resolution of the controversy. There is currently no scientific basis for withholding necessary blood products from surgical patients as a means of either incisional or organ/space SSI risk reduction.

(2) OPERATIVE CHARACTERISTICS:

PRE-OPERATIVE ISSUES

(a) PREOPERATIVE ANTISEPTIC SHOWERING

A preoperative antiseptic shower or bath decreases skin microbial colony counts. In a study of >700 patients who received two preoperative antiseptic showers, chlorhexidine reduced bacterial colony counts nine fold (2.83102 to 0.3), while povidone-iodine or triclocarban medicated soap reduced colony counts by 1.3- and 1.9-fold, respectively. Other studies corroborate these findings. Chlorhexidine gluconate-containing products require several applications to attain maximum antimicrobial benefit, so repeated antiseptic showers are usually indicated. Even though preoperative showers reduce the skin’s microbial colony counts, they have not definitively been shown to reduce SSI rates.

(B) PREOPERATIVE HAIR REMOVAL

Preoperative shaving of the surgical site the night before an operation is associated with a significantly higher SSI risk than either the use of depilatory agents or no hair removal. In one study, SSI rates were 5.6% in patients who had hair removed by razor shave compared to a 0.6% rate among those who had hair removed by depilatory or who had no hair removed. The increased SSI risk associated with shaving has been attributed to microscopic cuts in the skin that later serve as foci for bacterial multiplication. Shaving immediately before the operation compared to shaving within 24 hours preoperatively was associated with decreased SSI rates (3.1% vs. 7.1%); if shaving was performed >24 hours prior to operation, the SSI rate exceeded 20%. Clipping
hair immediately before an operation also has been associated with a lower risk of SSI than shaving or clipping the night before an operation (SSI rates immediately before = 1.8% vs. night before = 4.0%). Although the use of depilatories has been associated with a lower SSI risk than shaving or clipping, depilatories sometimes produce hypersensitivity reactions. Other studies showed that preoperative hair removal by any means was associated with increased SSI rates and suggested that no hair be removed.

(C) PATIENT SKIN PREPARATION IN THE OPERATING ROOM

Several antiseptic agents are available for preoperative preparation of skin at the incision site (Table-XIV). The iodophors (e.g., povidone-iodine), alcohol-containing products, and chlorhexidine gluconate are the most commonly used agents. No studies have adequately assessed the comparative effects of these preoperative skin antiseptics on SSI risk in well-controlled, operation-specific studies. Alcohol is defined by the FDA as having one of the following active ingredients: ethyl alcohol, 60% to 95% by volume in an aqueous solution, or isopropyl alcohol, 50% to 91.3% by volume in an aqueous solution. Alcohol is readily available, inexpensive, and remains the most effective and rapid-acting skin antiseptic. Aqueous 70% to 92% alcohol solutions have germicidal activity against bacteria, fungi, and viruses, but spores can be resistant. One potential disadvantage of the use of alcohol in the operating room is its flammability. Both chlorhexidine gluconate and iodophors have broad spectra of antimicrobial activity. In some comparisons of the two antiseptics when used as preoperative hand scrubs, chlorhexidine gluconate achieved greater reductions in skin microflora than did povidone-iodine and also had greater residual activity after a single application. Further, chlorhexidine gluconate is not inactivated by blood or serum proteins. Iodophors may be inactivated by blood or serum proteins but exert a bacteriostatic effect as long as they are present on the skin.

There are reports of modifications to the procedure for preoperative skin preparation which include:

i. Removing or wiping off the skin preparation antiseptic agent after Application.

ii. Using an antiseptic-impregnated adhesive drape.
iii. Merely painting the skin with an antiseptic in lieu of the skin preparation procedure described above, or

iv. Using a “clean” versus a “sterile” surgical skin preparation kit.

However, none of these modifications has been shown to represent an advantage.

**(D) PREOPERATIVE HAND/FOREARM ANTISEPSIS**

Members of the surgical team who have direct contact with the sterile operating field or sterile instruments or supplies used in the field wash their hands and forearms by performing a traditional procedure known as scrubbing (or the surgical scrub) immediately before donning sterile gowns and gloves. Ideally, the optimum antiseptic used for the scrub should have a broad spectrum of activity, be fast acting, and have a persistent effect. Alcohol is considered the gold standard for surgical hand preparation in several European countries. When 7.5% povidone-iodine or 4% chlorhexidine gluconate was compared to alcoholic chlorhexidine (60% isopropanol and 0.5% chlorhexidine gluconate in 70% isopropanol), alcoholic chlorhexidine was found to have greater residual antimicrobial activity. No agent is ideal for every situation, and a major factor, aside from the efficacy of any product, is its acceptability by operating room personnel after repeated use.

**(e) MANAGEMENT OF INFECTED OR COLONIZED SURGICAL PERSONNEL**

Surgical personnel who have active infections or are colonized with certain microorganisms have been linked to outbreaks or clusters of SSIs. Thus, it is important that healthcare organizations implement policies to prevent transmission of microorganisms from personnel to patients.

**(f) ANTIMICROBIAL PROPHYLAXIS:**

Surgical antimicrobial prophylaxis (AMP) refers to a very brief course of an antimicrobial agent initiated just before an operation begins. AMP is not an attempt to sterilize tissues, but a critically timed adjunct used to reduce the microbial burden of intraoperative contamination to a level that cannot overwhelm host defences. AMP does not pertain to prevention of SSI caused...
by postoperative contamination. Intravenous infusion is the mode of AMP delivery used most often in modern surgical practice. Essentially all confirmed AMP indications pertain to elective operations in which skin incisions are closed in the operating room.

**Four Principles must be followed to maximize the benefits of AMP:**

1. **Use an AMP agent for all operations or classes of operations in which its use has been shown to reduce SSI rates based on evidence from clinical trials or for those represent a catastrophe.**

2. **Use an AMP agent that is safe, inexpensive, and bactericidal with an in vitro spectrum that covers the most probable intraoperative contaminants for the operation.**

3. **Time the infusion of the initial dose of antimicrobial agent so that a bactericidal concentration of the drug is established in serum and tissues by the time the skin is incised.**

4. **Maintain therapeutic levels of the antimicrobial agent in both serum and tissues throughout the operation and until, at most, a few hours after the incision is closed in the operating room.**

   Because clotted blood is present in all surgical wounds, therapeutic serum levels of AMP agents are logically important in addition to therapeutic tissue levels. Fibrin-enmeshed bacteria may be resistant to phagocytosis or to contact with antimicrobial agents that diffuse from the wound space.

A simple way to organize AMP indications is based on using the surgical wound classification scheme, which employs descriptive case features to postoperatively grade the degree of intraoperative microbial contamination. A surgeon makes the decision to use AMP by anticipating preoperatively the surgical wound class for a given operation.

AMP is indicated for all operations that entail entry into a hollow viscous under controlled conditions. Certain clean-contaminated operations, such as elective colon resection, low anterior resection of the rectum, and abdominoperineal resection of the rectum, also require an additional preoperative protective manoeuvre called “preparation of the colon,” to empty the bowel of its
contents and to reduce the levels of live microorganisms. This manoeuvre includes the administration of enemas and cathartic agents followed by the oral administration of non absorbable antimicrobial agents in divided doses the day before the operation.

AMP is sometimes indicated for operations that entail incisions through normal tissue and in which no viscous is entered and no inflammation or infection is encountered. Two well-recognized AMP indications for such clean operations are: (1) when any intravascular prosthetic material or a prosthetic joint will be inserted, and (2) for any operation in which an incisional or organ/space SSI would pose catastrophic risk. Examples are all cardiac operations, including cardiac pacemaker placement, vascular operations involving prosthetic arterial graft placement at any site or the revascularization of the lower extremity, and most neurosurgical operations. Some have advocated use of AMP during all operations on the breast.

By definition, AMP is not indicated for an operation classified as contaminated or dirty. In such operations, patients are frequently receiving therapeutic antimicrobial agents perioperatively for established infections. Cephalosporins are the most thoroughly studied AMP agents. These drugs are effective against many gram-positive and gram-negative microorganisms. They also share the features of demonstrated safety, acceptable pharmacokinetics, and a reasonable cost per dose. In particular, cefazolin is widely used and generally viewed as the AMP agent of first choice for clean operations. If a patient is unable to receive a cephalosporin because of penicillin allergy, an alternative for gram-positive bacterial coverage is either clindamycin or Vancomycin. Cefazolin provides adequate coverage for many clean-contaminated operations, but AMP for operations on the distal intestinal tract mandates use of an agent such as Cefoxitin (or some other second-generation cephalosporin) that provides anaerobic coverage. If a patient cannot safely receive a cephalosporin because of allergy, a reasonable alternative for gram-negative coverage is aztreonam. However, an agent such as clindamycin or metronidazole should also be included to ensure anaerobic coverage. The aminoglycosides are seldom recommended as first choices for AMP, either as single drugs or as components of combination regimens. The
routine use of Vancomycin in AMP is not recommended for any kind of operation. However, Vancomycin may be the AMP agent of choice in certain clinical circumstances, such as when a cluster of MRSA mediastinitis or incisional SSI due to Methicillin-resistant Coagulase-negative Staphylococci has been detected. A threshold has not been scientifically defined that can support the decision to use Vancomycin in AMP. The decision should involve consideration of local frequencies of MRSA isolates, SSI rates for particular operations, review of infection prevention practices for compliance, and consultation between surgeons and infectious disease experts. An effective SSI surveillance program must be operational, with careful and timely culturing of SSI isolates to determine species and AMP agent susceptibilities. Agents most commonly used for AMP (i.e. Cephalosporins) exhibit time-dependent bactericidal action. The therapeutic effects of such agents are probably maximized when their levels continuously exceed a threshold value best approximated by the minimal bactericidal concentration value observed for the target pathogens in vitro. When the duration of an operation is expected to exceed the time in which therapeutic levels of the AMP agent can be maintained, additional AMP agent should be infused. That time point for cefazolin is estimated as 3 to 4 hours. In general, the timing of a second (or third, etc.) dose of any AMP drug is estimated from three parameters: tissue levels achieved in normal patients by a standard therapeutic dose, the approximate serum half-life of the drug, and awareness of approximate MIC values for anticipated SSI pathogens. Basic “rules of thumb” guide decisions about AMP dose sizes and timing. For example, it is believed that a full therapeutic dose of cefazolin (1-2 g) should be given to adult patients no more than 30 minutes before the skin is incised. There are a few exceptions to this basic guide. With respect to dosing, it has been demonstrated that larger doses of AMP agents are necessary to achieve optimum effect in morbidly obese patients. With respect to timing, an exception occurs for patients undergoing caesarean section in whom AMP is indicated: the initial dose is administered immediately after the umbilical cord is clamped. If Vancomycin is used, an infusion period of approximately 1 hour is required for a typical dose. Clearly, the concept of “on-call” infusion of AMP is flawed simply because
delays in transport or schedule changes can mean that suboptimal tissue and serum levels may be present when the operation starts. Simple protocols of AMP timing and oversight responsibility should be locally designed to be practical and effective.

(3) OPERATIVE CHARACTERISTICS:

INTRAOPERATIVE ISSUES:

(A) OPERATING ROOM ENVIRONMENT

(1) Ventilation

Operating room air may contain microbial-laden dust, lint, skin squames, or respiratory droplets. The microbial level in operating room air is directly proportional to the number of people moving about in the room. Therefore, efforts should be made to minimize personnel traffic during operations. Outbreaks of SSIs caused by group A beta-haemolytic Streptococci have been traced to airborne transmission of the organism from colonized operating room personnel to patients. In these outbreaks, the strain causing the outbreak was recovered from the air in the operating room. It has been demonstrated that exercising and changing of clothing can lead to airborne dissemination of Group A Streptococci from vaginal or rectal carriage. Operating rooms should be maintained at positive pressure with respect to corridors and adjacent areas. Positive pressure prevents airflow from less clean areas into more clean areas. All ventilation or air conditioning systems in hospitals, including those in operating rooms, should have two filter beds in series, with the efficiency of the first filter bed being >30% and that of the second filter bed being >90%. Conventional operating room ventilation systems produce a minimum of about 15 air changes of filtered air per hour, three (20%) of which must be fresh air. Air should be introduced at the ceiling and exhausted near the floor. Detailed ventilation parameters for operating rooms have been published by the American Institute of Architects in collaboration with the U.S. Department of Health and Human Services (Table-XV). Laminar airflow and use of UV radiation have been suggested as additional measures to reduce SSI risk for certain operations.
Laminar airflow is designed to move particle-free air (called “ultraclean air”) over the aseptic operating field at a uniform velocity (0.3 to 0.5 µm/sec), sweeping away particles in its path. Laminar airflow can be directed vertically or horizontally, and recirculated air is usually passed through a high efficiency particulate air (HEPA) filter. HEPA filters remove particles >0.3µm in diameter with an efficiency of 99.97%. Most of the studies examining the efficacy of ultraclean air involve only orthopaedic operations. Charnley and Eftakkan studied vertical laminar airflow systems and exhaust-ventilated clothing and found that their use decreased the SSI rate from 9% to 1%. However, other variables (i.e., surgeon experience and surgical technique) changed at the same time as the type of ventilation, which may have confounded the associations. In a multicenter study examining 8,000 total hip and knee replacements, Lidwell et al. compared the effects of ultraclean air alone, antimicrobial prophylaxis alone, and ultraclean air in combination with antimicrobial prophylaxis on the rate of deep SSIs. The SSI rate following operations in which ultraclean air alone was used decreased from 3.4% to 1.6%, whereas the rate for those who received only antimicrobial prophylaxis decreased from 3.4% to 0.8%. When both interventions were used in combination, the SSI rate decreased from 3.4% to 0.7%. These findings suggest that both ultraclean air and antimicrobial prophylaxis can reduce the incidence of SSI following orthopaedic implant operations, but antimicrobial prophylaxis is more beneficial than ultraclean air. Intraoperative UV radiation has not been shown to decrease overall SSI risk.

**TABLE-1.10: PARAMETERS FOR OPERATING ROOM VENTILATION**

<table>
<thead>
<tr>
<th>Parameters for Operating Room Ventilation*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature</strong>: 68°-73°F, depending on normal ambient temp</td>
</tr>
<tr>
<td><strong>Relative humidity</strong>: 30%-60%</td>
</tr>
<tr>
<td><strong>Air movement</strong>: from “clean to less clean” areas</td>
</tr>
<tr>
<td><strong>Air changes</strong>: ≥15 total per hour ≥3 outdoor air per hour</td>
</tr>
</tbody>
</table>

*(American Institute of Architects, 1996)
(2) ENVIRONMENTAL SURFACES

Environmental surfaces in U.S. operating rooms (e.g., tables, floors, walls, ceilings, lights) are rarely implicated as the sources of pathogens important in the development of SSIs. Nevertheless, it is important to perform routine cleaning of these surfaces to re-establish a clean environment after each operation. There are no data to support routine disinfecting of environmental surfaces or equipment between operations in the absence of contamination or visible soiling.

When visible soiling of surfaces or equipment occurs during an operation, an Environmental Protection Agency (EPA)-approved hospital disinfectant should be used to decontaminate the affected areas before the next operation. This is in keeping with the Occupational Safety and Health Administration (OSHA) requirement that all equipment and environmental surfaces be cleaned and decontaminated after contact with blood or other potentially infectious materials. Wet-vacuuming of the floor with an EPA approved hospital disinfectant is performed routinely after the last operation of the day or night. Care should be taken to ensure that medical equipment left in the operating room be covered so that solutions used during cleaning and disinfecting do not contact sterile devices or equipment. There are no data to support special cleaning procedures or closing of an operating room after a contaminated or dirty operation has been performed. Tacky mats placed outside the entrance to an operating room/suite have not been shown to reduce the number of organisms on shoes or stretcher wheels, nor do they reduce the risk of SSI.

(3) MICROBIOLOGIC SAMPLING

Because there are no standardized parameters by which to compare microbial levels obtained from cultures of ambient air or environmental surfaces in the operating room, routine microbiologic sampling cannot be justified. Such environmental sampling should only be performed as part of an epidemiologic investigation.

(4) CONVENTIONAL STERILIZATION OF SURGICAL INSTRUMENTS

Inadequate sterilization of surgical instruments has resulted in SSI outbreaks. Surgical instruments can be sterilized by steam under pressure, dry heat,
ethylene oxide, or other approved methods. The importance of routinely monitoring the quality of sterilization procedures has been established. Microbial monitoring of steam autoclave performance is necessary and can be accomplished by use of a biological indicator. Detailed recommendations for sterilization of surgical instruments have been published.

(5) FLASH STERILIZATION OF SURGICAL INSTRUMENTS
The Association for the Advancement of Medical Instrumentation defines flash sterilization as “the process designated for the steam sterilization of patient care items for immediate use.” During any operation, the need for emergency sterilization of equipment may arise (e.g., to reprocess an inadvertently dropped instrument). However, flash sterilization is not intended to be used for either reasons of convenience or as an alternative to purchasing additional instrument sets or to save time. Also, flash sterilization is not recommended for implantable devices because of the potential for serious infections. Flash sterilization is not recommended as a routine sterilization method because of the lack of timely biologic indicators to monitor performance, absence of protective packaging following sterilization, possibility for contamination of processed items during transportation to operating rooms, and use of minimal sterilization cycle parameters (i.e., time, temperature, pressure).

To address some of these concerns, many hospitals have placed equipment for flash sterilization in close proximity to operating rooms and new biologic indicators that provide results in 1 to 3 hours are now available for flash-sterilized items. Nevertheless, flash sterilization should be restricted to its intended purpose until studies are performed that can demonstrate comparability with conventional sterilization methods regarding risk of SSI. Sterilization cycle parameters for flash sterilization are shown in Table-1.11.

**TABLE: 1.11: PARAMETERS FOR FLASH STERILIZATION CYCLES, ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION.**

<table>
<thead>
<tr>
<th>Gravity-Displacement</th>
<th>Minimum Exposure Time and Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonporous items</td>
<td>3 min at 132ºC (270ºF)</td>
</tr>
<tr>
<td>Nonporous and porous items</td>
<td>10 min at 132ºC 270ºF</td>
</tr>
</tbody>
</table>
### Pre-vacuum Table

<table>
<thead>
<tr>
<th>Pre vacuum</th>
<th>Minimum Exposure Time and Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonporous items</td>
<td>3 min at 132ºC (270ºF)</td>
</tr>
<tr>
<td>Nonporous and porous items</td>
<td>4 min at 132ºC (270ºF)</td>
</tr>
</tbody>
</table>

Association for the Advancement of Medical Instrumentation.

### (b) SURGICAL ATTIRE AND DRAPES

In this section the term *surgical attire* refers to scrub suits, caps/hoods, shoe covers, masks, gloves, and gowns. Although experimental data show that live microorganisms are shed from hair, exposed skin, and mucous membranes of operating room personnel, few controlled clinical studies have evaluated the relationship between the use of surgical attire and SSI risk. Nevertheless, the use of barriers seems prudent to minimize a patient’s exposure to the skin, mucous membranes, or hair of surgical team members, as well as to protect surgical team members from exposure to blood and blood borne pathogens (e.g., human immunodeficiency virus and hepatitis viruses).

#### (1) SCRUB SUITS

Surgical team members often wear a uniform called a “scrub suit” that consists of pants and a shirt. Policies for laundering, wearing, covering, and changing scrub suits vary greatly. Some policies restrict the laundering of scrub suits to the facility, while other facilities have policies that allow laundering by employees. There are no well-controlled studies evaluating scrub suit laundering as an SSI risk factor. Some facilities have policies that restrict the wearing of scrub suits to the operating suite, while other facilities allow the wearing of cover gowns over scrub suits when personnel leave the suite. The Association of Operating Room Nurses recommends that scrub suits be changed after they become visibly soiled and that they be laundered only in an approved and monitored laundry facility. Additionally, OSHA regulations require that “if a garment(s) is penetrated by blood or other potentially infectious materials, the garment(s) shall be removed immediately or as soon as feasible.”

#### (2) MASKS

The wearing of surgical masks during operations to prevent potential microbial contamination of incisions is a longstanding surgical tradition. However, some
studies have raised questions about the efficacy and cost-benefit of surgical masks in reducing SSI risk. Nevertheless, wearing a mask can be beneficial since it protects the wearer’s nose and mouth from inadvertent exposures (i.e., splashes) to blood and other body fluids. OSHA regulations require that masks in combination with protective eyewear, such as goggles or glasses with solid shields, or chin length face shields be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious material may be generated and eye, nose, or mouth contamination can be reasonably anticipated. In addition, a respirator certified by the National Institute for Occupational Safety and Health with protection factor N95 or higher is required when the patient has or is suspected of having infectious tuberculosis.

(3) **SURGICAL CAPS/HOODS AND SHOE COVERS**

Surgical caps/hoods are inexpensive and reduce contamination of the surgical field by organisms shed from the hair and scalp. SSI outbreaks have occasionally been traced to organisms isolated from the hair or scalp (*Staphylococcus aureus* and group A *Streptococcus*), even when caps were worn by personnel during the operation and in the operating suites. The use of shoe covers has never been shown to decrease SSI risk or to decrease bacteria counts on the operating room floor. Shoe covers may, however, protect surgical team members from exposure to blood and other body fluids during an operation. OSHA regulations require that surgical caps or hoods and shoe covers or boots be worn in situations when gross contamination can reasonably be anticipated (e.g., orthopaedic operations, penetrating trauma cases).

(4) **STERILE GLOVES**

Sterile gloves are put on after donning sterile gowns. A strong theoretical rationale supports the wearing of sterile gloves by all scrubbed members of the surgical team. Sterile gloves are worn to minimize transmission of microorganisms from the hands of team members to patients and to prevent contamination of team members’ hands with patients’ blood and body fluids. If the integrity of a glove is compromised (e.g., punctured), it should be changed as promptly as safety permits. Wearing two pairs of gloves (double-gloving)
has been shown to reduce hand contact with patients’ blood and body fluids when compared to wearing only a single pair.

(5) GOWNS AND DRAPES
Sterile surgical gowns and drapes are used to create a barrier between the surgical field and potential sources of bacteria. Gowns are worn by all scrubbed surgical team members and drapes are placed over the patient. There are limited data that can be used to understand the relationship of gown or drape characteristics with SSI risk. The wide variation in the products and study designs make interpretation of the literature difficult. Gowns and drapes are classified as disposable (single use) or reusable (multiple use). Regardless of the material used to manufacture gowns and drapes, these items should be impermeable to liquids and viruses. In general, only gowns reinforced with films, coatings, or membranes appear to meet standards developed by the American Society for Testing and Materials. However, such “liquid-proof” gowns may be uncomfortable because they also inhibit heat loss and the evaporation of sweat from the wearer's body. These factors should be considered when selecting gowns. A discussion of the role of gowns and drapes in preventing the transmission of blood borne pathogens is beyond the scope of this document.

(c) ASEPSIS AND SURGICAL TECHNIQUE
(1) ASEPSIS
Rigorous adherence to the principles of asepsis by all scrubbed personnel is the foundation of surgical site infection prevention. Others who work in close proximity to the sterile surgical field, such as anaesthesia personnel who are separated from the field only by a drape barrier, also must abide by these principles. SSIs have occurred in which anaesthesia personnel were implicated as the source of the pathogen. Anaesthesiologists and nurse anaesthetists perform a variety of invasive procedures such as placement of intravascular devices and endotracheal tubes, and administration of intravenous drugs and solutions. Lack of adherence to the principles of asepsis during such procedures, including use of common syringes and contaminated infusion pumps and the assembly of equipment and solutions in
advancement of procedures have been associated with outbreaks of postoperative infections, including SSI. Recommendations for infection control practices in anaesthesiology have been published.

(2) SURGICAL TECHNIQUE
Excellent surgical technique is widely believed to reduce the risk of SSI. Such techniques include maintaining effective hemostasis while preserving adequate blood supply, preventing hypothermia, gently handling tissues, avoiding inadvertent entries into a hollow viscus, removing devitalized (e.g., necrotic or charred) tissues, using drains and suture material appropriately, eradicating dead space, and appropriately managing the postoperative incision. Any foreign body, including suture material, a prosthesis, or drain, may promote inflammation at the surgical site and may increase the probability of SSI after otherwise benign levels of tissue contamination. Extensive research compares different types of suture material and their presumed relationships to SSI risk. In general, monofilament sutures appear to have the lowest infection promoting effects. A discussion of appropriate surgical drain use and details of drain placement exceed the scope of this document, but general points should be briefly noted. Drains placed through an operative incision increase incisional SSI risk. Many authorities suggest placing drains through a separate incision distant from the operative incision. It appears that SSI risk also decreases when closed suction drains are used rather than open drains. Closed suction drains can effectively evacuate postoperative hematomas or seromas, but timing of drain removal is important. Bacterial colonization of initially sterile drain tracts increases with the duration of time the drain is left in place.

Hypothermia in surgical patients, defined as a core body temperature below 36°C, may result from general anaesthesia, exposure to cold, or intentional cooling such as is done to protect the myocardium and central nervous system during cardiac operations. In one study of patients undergoing colorectal operations, hypothermia was associated with an increased SSI risk. Mild hypothermia appears to increase incisional SSI risk by causing vasoconstriction, decreased delivery of oxygen to the wound space, and subsequent impairment of function of phagocytic leukocytes (i.e., neutrophils).
In animal models, supplemental oxygen administration has been shown to reverse the dysfunction of phagocytes in fresh incisions. In recent human experiments, controlled local heating of incisions with an electrically powered bandage has been shown to improve tissue oxygenation. Randomized clinical trials are needed to establish that measures which improve wound space oxygenation can reduce SSI risk.

(4) OPERATIVE CHARACTERISTICS:
THE POSTOPERATIVE ISSUES

(a) Incision care
The type of postoperative incision care is determined by whether the incision is closed primarily (i.e., the skin edges are re-approximated at the end of the operation), left open to be closed later, or left open to heal by second intention. When a surgical incision is closed primarily, as most are, the incision is usually covered with a sterile dressing for 24 to 48 hours. Beyond 48 hours, it is unclear whether an incision must be covered by a dressing or whether showering or bathing is detrimental to healing. When a surgical incision is left open at the skin level for a few days before it is closed (delayed primary closure), a surgeon has determined that it is likely to be contaminated or that the patient’s condition prevents primary closure (e.g., edema at the site). When such is the case, the incision is packed with a sterile dressing. When a surgical incision is left open to heal by second intention, it is also packed with sterile moist gauze and covered with a sterile dressing. The American College of Surgeons, CDC, and others have recommended using sterile gloves and equipment (sterile technique) when changing dressings on any type of surgical incision.

(b) Discharge planning
In current practice, many patients are discharged very soon after their operation, before surgical incisions have fully healed. The lack of optimum protocols for home incision care dictates that much of what is done at home by the patient, family, or home care agency practitioners must be individualized. The intent of discharge planning is to maintain integrity of the healing incision, educate the patient about the signs and symptoms of
infection, and advise the patient about whom to contact to report any problems.

**SSI RISK STRATIFICATION:**  

*(a) Concepts*

Three categories of variables have proven to be reliable predictors of SSI risk:

- Those that estimate the intrinsic degree of microbial contamination of the surgical site,
- Those that measure the duration of an operation, and
- Those that serve as markers for host susceptibility.

A widely accepted scheme for classifying the degree of intrinsic microbial contamination of a surgical site was developed by the 1964 NAS/NRC Cooperative Research Study and modified in 1982 by CDC for use in SSI surveillance. In this scheme, a member of the surgical team classifies the patient’s wound at the completion of the operation. Because of its ease of use and wide availability, the surgical wound classification has been used to predict SSI risk. Some researchers have suggested that surgeons compare clean wound SSI rates with those of other surgeons. However, two CDC efforts—the SENIC Project and the NNIS system—incorporated other predictor variables into SSI risk indices. These showed that even within the category of clean wounds, the SSI risk varied by risk category from 1.1% to 15.8% (SENIC) and from 1.0% to 5.4% (NNIS). In addition, sometimes an incision is incorrectly classified by a surgical team member or not classified at all, calling into question the reliability of the classification. Therefore, reporting SSI rates stratified by wound class alone is not recommended.

Data on 10 variables collected in the SENIC Project were analyzed by using logistic regression modeling to develop a simple additive SSI risk index. Four of these were found to be independently associated with SSI risk: (1) an abdominal operation, (2) an operation lasting >2 hours, (3) a surgical site with a wound classification of either contaminated or dirty/infected, and (4) an operation performed on a patient having >3 discharge diagnoses. Each of these equally weighted factors contributes a point when present, such that the risk index values range from 0 to 4. By using these factors, the SENIC index
predicted SSI risk twice as well as the traditional wound classification scheme alone.

The NNIS risk index is operation-specific and applied to prospectively collected surveillance data. The index values range from 0 to 3 points and are defined by three independent and equally weighted variables. One point is scored for each of the following when present: (1) American Society of Anaesthesiologists (ASA) Physical Status Classification of >2 (Table-XVII), (2) either contaminated or dirty/infected wound classification, and (3) length of operation >T hours, where T is the approximate 75th percentile of the duration of the specific operation being performed.

The ASA class replaced discharge diagnoses of the SENIC risk index as a surrogate for the patient’s underlying severity of illness (host susceptibility) and has the advantage of being readily available in the chart during the patient’s hospital stay. Unlike SENIC’s constant 2-hour cut-point for duration of operation, the operation-specific cut-points used in the NNIS risk index increase its discriminatory power compared to the SENIC index.

(b) Issues
Adjustment for variables known to confound rate estimates is critical if valid comparisons of SSI rates are to be made between surgeons or hospitals. Risk stratification, as described above, has proven useful for this purpose, but relies on the ability of surveillance personnel to find and record data consistently and correctly. For the three variables used in the NNIS risk index, only one study has focused on how accurately any of them are recorded. Cardo et al. found that surgical team members’ accuracy in assessing wound classification for general and trauma surgery was 88% (95% CI: 82%-94%). However, there are sufficient ambiguities in the wound class definitions themselves to warrant concern about the reproducibility of Cardo’s results. The accuracy of recording the duration of operation (i.e., time from skin incision to skin closure) and the ASA class has not been studied. In an unpublished report from the NNIS system, there was evidence that over reporting of high ASA class existed in some hospitals. Further validation of the reliability of the recorded risk index variables is needed. Additionally, the NNIS
risk index does not adequately discriminate the SSI risk for all types of operations. It seems likely that a combination of risk factors specific to patients undergoing an operation will be more predictive. A few studies have been performed to develop procedure specific risk indices and research in this area continues within CDC’s NNIS system.

**TABLE: 1.12**: PHYSICAL STATUS CLASSIFICATION, AMERICAN SOCIETY OF ANESTHESIOLOGISTS:

<table>
<thead>
<tr>
<th>Code</th>
<th>Patient’s Preoperative Physical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normally healthy patient.</td>
</tr>
<tr>
<td>2</td>
<td>Patient with mild systemic disease.</td>
</tr>
<tr>
<td>3</td>
<td>Patient with severe systemic disease that is not incapacitating.</td>
</tr>
<tr>
<td>4</td>
<td>Patient with an incapacitating systemic disease that is a constant threat to life.</td>
</tr>
<tr>
<td>5</td>
<td>Moribund patient who is not expected to survive for 24 hours with or without operation.</td>
</tr>
</tbody>
</table>

Note: The above is the version of the ASA Physical Status Classification System that was current at the time of development of, and still is used in, the NNIS Risk Index. Meanwhile, the American Society of Anaesthesiologists has revised their classification system; the most recent version is available at [http://www.asahq.org/profinfo/physicalstatus.html](http://www.asahq.org/profinfo/physicalstatus.html).

**REVISED CLASSIFICATION OF AMERICAN SOCIETY OF ANESTHESIOLOGISTS**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>A normal healthy patient</td>
</tr>
<tr>
<td>P2</td>
<td>A patient with mild systemic disease</td>
</tr>
<tr>
<td>P3</td>
<td>A patient with severe systemic disease</td>
</tr>
<tr>
<td>P4</td>
<td>A patient with severe systemic disease that is a constant threat to life</td>
</tr>
<tr>
<td>P5</td>
<td>A moribund patient who is not expected to survive without the operation</td>
</tr>
<tr>
<td>P6</td>
<td>A declared brain-dead patient whose organs are being removed for donor purposes</td>
</tr>
</tbody>
</table>
SSI SURVEILLANCE METHODS

(a) Inpatient SSI surveillance

Two methods, alone or together, have been used to identify in-patients with SSIs:

1. Direct observation of the surgical site by the surgeon, trained nurse surveyor, or infection control personnel and
2. Indirect detection by infection control personnel through review of laboratory reports, patient records, and discussions with primary care providers.

The surgical literature suggests that direct observation of surgical sites is the most accurate method to detect SSIs, although sensitivity data are lacking. Much of the SSI data reported in the infection control literature has been generated by indirect case-finding methods, but some studies of direct methods also have been conducted. Some studies use both methods of detection. A study that focused solely on the sensitivity and specificity of SSIs detected by indirect methods found a sensitivity of 83.8% (95% CI: 75.7%-91.9%) and a specificity of 99.8% (95% CI: 99%-100%). Another study showed that chart review triggered by a computer-generated report of antibiotic orders for post caesarean section patients had a sensitivity of 89% for detecting endometritis. Indirect SSI detection can readily be performed by infection control personnel during surveillance rounds. The work includes gathering demographic, infection, surgical, and laboratory data on patients who have undergone operations of interest. These data can be obtained from patients' medical records, including microbiology, histopathology, laboratory, and pharmacy data; radiology reports; and records from the operating room. Additionally, inpatient admissions, emergency room, and clinic visit records are sources of data for those post discharge surgical patients who are readmitted or seek follow-up care.

To calculate meaningful SSI rates, data must be collected on all patients undergoing the operations of interest (i.e., the population at risk). Because one of its purposes is to develop strategies for risk stratification, the NNIS system collects the following data on all surgical patients surveyed: operation
date; NNIS operative procedure category; surgeon identifier; patient identifier; age and sex; duration of operation; wound class; use of general anesthesia; ASA class; emergency; trauma; multiple procedures; endoscopic approach; and discharge date. With the exception of discharge date, these data can be obtained manually from operating room logs or be electronically downloaded into surveillance software, thereby substantially reducing manual transcription and data entry errors. Depending on the needs for risk-stratified SSI rates by personnel in infection control, surgery, and quality assurance, not all data elements may be pertinent for every type of operation. At minimum, however, variables found to be predictive of increased SSI risk should be collected.

(b) Post discharge SSI surveillance
Between 12% and 84% of SSIs are detected after patients are discharged from the hospital. At least two studies have shown that most SSIs become evident within 21 days after operation. Since the length of postoperative hospitalization continues to decrease, many SSIs may not be detected for several weeks after discharge and may not require readmission to the operating hospital. Dependence solely on inpatient case-finding will result in underestimates of SSI rates for some operations (e.g., coronary artery bypass graft) (CDC/NNIS system, unpublished data, 1998). Any comparison of SSI rates must take into account whether case-finding included SSIs detected after discharge. For comparisons to be valid, even in the same institution over time, the post discharge surveillance methods must be the same. Post discharge surveillance methods have been used with varying degrees of success for different procedures and among hospitals and include (1) direct examination of patients' wounds during follow-up visits to either surgery clinics or physicians' offices, (2) review of medical records of surgery clinic patients, (3) patient surveys by mail or telephone, or (4) surgeon surveys by mail or telephone. One study found that patients have difficulty assessing their own wounds for infection (52% specificity, 26% positive predictive value), suggesting that data obtained by patient questionnaire may inaccurately represent actual SSI rates.

Recently, Sands et al. performed a computerized search of three databases to determine which best identified SSIs: ambulatory encounter records for
diagnostic, testing, and treatment codes; pharmacy records for specific antimicrobial prescriptions; and administrative records for re-hospitalizations and emergency room visits. This study found that pharmacy records indicating a patient had received antimicrobial agents commonly used to treat soft tissue infections had the highest sensitivity (50%) and positive predictive value (19%), although even this approach alone was not very effective.

(c) Outpatient SSI surveillance

Both direct and indirect methods have been used to detect SSIs that complicate outpatient operations. One 8 year study of operations for hernia and varicose veins used home visits by district health nurses combined with a survey completed by the surgeon at the patient’s 2-week postoperative clinic visit to identify SSIs. While ascertainment was essentially 100%, this method is impractical for widespread implementation. High response rates have been obtained from questionnaires mailed to surgeons (72%–90%). Response rates from telephone questionnaires administered to patients were more variable (38%, 44%, 81%, 457 and 85%455), and response rates from questionnaires mailed to patients were quite low (15%455 and 33%446). At this time, no single detection method can be recommended. Available resources and data needs determine which method(s) should be used and which operations should be monitored. Regardless of which detection method is used, it is recommended that the CDC NNIS definitions of SSI be used without modification in the outpatient setting.

RECOMMENDATIONS FOR PREVENTION OF SURGICAL SITE INFECTIONS 71

(A) RATIONALE

The Guideline for Prevention of Surgical Site Infection, 1999, provides recommendations concerning reduction of surgical site infection risk. Each recommendation is categorized on the basis of existing scientific data, theoretical rationale, and applicability. However, the previous CDC system for categorizing recommendations has been modified slightly.
Category I recommendations, including IA and IB, are those recommendations that are viewed as effective by HICPAC and experts in the fields of surgery, infectious diseases, and infection control. Both Category IA and IB recommendations are applicable for, and should be adopted by, all healthcare facilities; IA and IB recommendations differ only in the strength of the supporting scientific evidence. Category II recommendations are supported by less scientific data than Category I recommendations; such recommendations may be appropriate for addressing specific nosocomial problems or specific patient populations. No recommendation is offered for some practices, either because there is a lack of consensus regarding their efficacy or because the available scientific evidence is insufficient to support their adoption. For such unresolved issues, practitioners should use judgement to determine a policy regarding these practices within their organization.

(B) RANKINGS

Category IA. Strongly recommended for implementation and supported by well-designed experimental, clinical, or epidemiological studies.

Category IB. Strongly recommended for implementation and supported by some experimental, clinical, or epidemiological studies and strong theoretical rationale.

Category II. Suggested for implementation and supported by suggestive clinical or epidemiological studies or theoretical rationale.

No recommendation; unresolved issue.

Practices for which insufficient evidence or no consensus regarding efficacy exists.

(C) RECOMMENDATIONS

(1) PREOPERATIVE RECOMMENDATIONS:

(a) Preparation of the patient

1. Whenever possible, identify and treat all infections remote to the surgical site before elective operation and postpone elective operations on patients with remote site infections until the infection has resolved.

Category IA
2. Do not remove hair preoperatively unless the hair at or around the incision site will interfere with the operation. *Category IA*

3. If hair is removed, remove immediately before the operation, preferably with electric clippers. *Category IA*

4. Adequately control serum blood glucose levels in all diabetic patients and particularly avoid hyperglycemia perioperatively. *Category IB*

5. Encourage tobacco cessation. At minimum, instruct patients to abstain for at least 30 days before elective operation from smoking cigarettes, cigars, pipes, or any other form of tobacco consumption (e.g., chewing/dipping). *Category IB.*

6. Do not withhold necessary blood products from surgical patients as a means to prevent SSI. *Category IB*

7. Require patients to shower or bathe with an antiseptic agent on at least the night before the operative day. *Category IB*

8. Thoroughly wash and clean at and around the incision site to remove gross contamination before performing antiseptic skin preparation. *Category IB.*

9. Use an appropriate antiseptic agent for skin preparation. *Category IB*

10. Apply preoperative antiseptic skin preparation in concentric circles moving toward the periphery. The prepared area must be large enough to extend the incision or create new incisions or drain sites, if necessary. *Category II*

11. Keep preoperative hospital stay as short as possible while allowing for adequate preoperative preparation of the patient. *Category II*

12. No recommendation to taper or discontinue systemic steroid use (when medically permissible) before elective operation. *Unresolved issue*

13. No recommendation to enhance nutritional support for surgical patients solely as a means to prevent SSI. *Unresolved issue*

14. No recommendation to preoperatively apply mupirocin to nares to prevent SSI. *Unresolved issue*

15. No recommendation to provide measures that enhance wound space oxygenation to prevent SSI. *Unresolved issue*
(b) **Hand/forearm antisepsis for surgical team members**

1. Keep nails short and do not wear artificial nails. *Category IB*
2. Perform a preoperative surgical scrub for at least 2 to 5 minutes using an appropriate antiseptic. Scrub the hands and forearms up to the elbows. *Category IB*
3. After performing the surgical scrub, keep hands up and away from the body (elbows in flexed position) so that water runs from the tips of the fingers toward the elbows. Dry hands with a sterile towel and don a sterile gown and gloves. *Category IB*
4. Clean underneath each fingernail prior to performing the first surgical scrub of the day. *Category II*
5. Do not wear hand or arm jewellery. *Category II*
6. No recommendation on wearing nail polish. *Unresolved Issue*

(c) **Management of infected or colonized surgical personnel**

1) Educate and encourage surgical personnel who have signs and symptoms of a transmissible infectious illness to report conditions promptly to their supervisory and occupational health service personnel. *Category IB*
2) Develop well-defined policies concerning patient care responsibilities when personnel have potentially transmissible infectious conditions. These policies should govern (a) personnel responsibility in using the health service and reporting illness, (b) work restrictions, and (c) clearance to resume work after an illness that required work restriction. The policies also should identify persons who have the authority to remove personnel from duty. *Category IB*
3) Obtain appropriate cultures from, and exclude from duty, surgical personnel who have draining skin lesions until infection has been ruled out or personnel have received adequate therapy and infection has resolved. *Category IB*
4) Do not routinely exclude surgical personnel who are colonized with organisms such as *S. aureus* (nose, hands, or other body site) or group A *Streptococcus*, unless such personnel have been linked
epidemiologically to dissemination of the organism in the healthcare setting. *Category IB*

**(d) Antimicrobial prophylaxis**

1. Administer a prophylactic antimicrobial agent only when indicated, and select it based on its efficacy against the most common pathogens causing SSI for a specific operation and published recommendations. *Category IA*

2. Administer by the intravenous route the initial dose of prophylactic antimicrobial agent, timed such that a bactericidal concentration of the drug is established in serum and tissues when the incision is made. Maintain therapeutic levels of the agent in serum and tissues throughout the operation and until, at most, a few hours after the incision is closed in the operating room. *Category IA*

3. Before elective colorectal operations in addition to d2 above, mechanically prepare the colon by use of enemas and cathartic agents. Administer non-absorbable oral antimicrobial agents in divided doses on the day before the operation. *Category IA*

4. For high-risk caesarean section, administer the prophylactic antimicrobial agent immediately after the umbilical cord is clamped. *Category IA*

5. Do not routinely use vancomycin for antimicrobial prophylaxis. *Category IB*

**(2) INTRAOPERATIVE RECOMMENDATIONS:**

**(a) Ventilation**

1) Maintain positive-pressure ventilation in the operating room with respect to the corridors and adjacent areas. *Category IB*

2) Maintain a minimum of 15 air changes per hour, of which at least 3 should be fresh air. *Category IB*

3) Filter all air, recirculated and fresh, through the appropriate filters per the American Institute of Architects’ recommendations.299 *Category IB*

4) Introduce all air at the ceiling, and exhaust near the floor. *Category IB*
5) Do not use UV radiation in the operating room to prevent SSI. *Category IB*

6) Keep operating room doors closed except as needed for passage of equipment, personnel, and the patient. *Category IB*

7) Consider performing orthopaedic implant operations in operating rooms supplied with ultraclean air. *Category II*

8) Limit the number of personnel entering the operating room to necessary personnel. *Category II*

**(b) Cleaning and disinfection of environmental surfaces**

1) When visible soiling or contamination with blood or other body fluids of surfaces or equipment occurs during an operation, use an EPA-approved hospital disinfectant to clean the affected areas before the next operation. *Category IB*

2) Do not perform special cleaning or closing of operating rooms after contaminated or dirty operations. *Category IB*

3) Do not use tacky mats at the entrance to the operating room suite or individual operating rooms for infection control. *Category IB*

4) Wet vacuum the operating room floor after the last operation of the day or night with an EPA-approved hospital disinfectant. *Category II*

5) No recommendation on disinfecting environmental surfaces or equipment used in operating rooms between operations in the absence of visible soiling. *Unresolved issue*

**(c) Microbiologic sampling**

1) Do not perform routine environmental sampling of the operating room. Perform microbiologic sampling of operating room environmental surfaces or air only as part of an epidemiologic investigation. *Category IB*

**(d) Sterilization of surgical instruments**

1) Sterilize all surgical instruments according to published guidelines. *Category IB*

2) Perform flash sterilization only for patient care items that will be used immediately (e.g., to reprocess an inadvertently dropped instrument). Do
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not use flash sterilization for reasons of convenience, as an alternative to purchasing additional instrument sets, or to save time. *Category IB*

(e) **Surgical attire and drapes**

1) Wear a surgical mask that fully covers the mouth and nose when entering the operating room if an operation is about to begin or already under way, or if sterile instruments are exposed. Wear the mask throughout the operation. *Category IB*

2) Wear a cap or hood to fully cover hair on the head and face when entering the operating room. *Category IB*

3) Do not wear shoe covers for the prevention of SSI. *Category IB*

4) Wear sterile gloves if a scrubbed surgical team member. Put on gloves after donning a sterile gown. *Category IB*

5) Use surgical gowns and drapes that are effective barriers when wet (i.e., materials that resist liquid penetration). *Category IB*

6) Change scrub suits that are visibly soiled, contaminated, and/or penetrated by blood or other potentially infectious materials. *Category IB*

7) No recommendations on how or where to launder scrub suits, on restricting use of scrub suits to the operating suite, or for covering scrub suits when out of the operating suite. *Unresolved issue*

(f) **Asepsis and surgical technique**

1) Adhere to principles of asepsis when placing intravascular devices (e.g., central venous catheters), spinal or epidural anesthesia catheters, or when dispensing and administering intravenous drugs. *Category IA*

2) Assemble sterile equipment and solutions immediately prior to use. *Category II*

3) Handle tissue gently, maintain effective hemostasis, minimize devitalized tissue and foreign bodies (i.e., sutures, charred tissues, necrotic debris), and eradicate dead space at the surgical site. *Category IB*

4) Use delayed primary skin closure or leave an incision open to heal by second intention if the surgeon considers the surgical site to be heavily contaminated (e.g., Class III and Class IV). *Category IB*
5) If drainage is necessary, use a closed suction drain. Place a drain through a separate incision distant from the operative incision. Remove the drain as soon as possible. *Category IB*

(3) **POSTOPERATIVE INCISION CARE**

1) Protect with a sterile dressing for 24 to 48 hours postoperatively an incision that has been closed primarily. *Category IB*

2) Wash hands before and after dressing changes and any contact with the surgical site. *Category IB*

3) When an incision dressing must be changed, use sterile technique. *Category II*

4) Educate the patient and family regarding proper incision care, symptoms of SSI, and the need to report such symptoms. *Category II*

5) No recommendation to cover an incision closed primarily beyond 48 hours, nor on the appropriate time to shower or bathe with an uncovered incision. *Unresolved issue*

(4) **SURVEILLANCE**

1) Use CDC definitions of SSI without modification for identifying SSI among surgical inpatients and outpatients. *Category IB* b. For inpatient case-finding (including readmissions), use direct prospective observation, indirect prospective detection, or a combination of both direct and indirect methods for the duration of the patient’s hospitalization. *Category IB*

2) When post discharge surveillance is performed for detecting SSI following certain operations (e.g., coronary artery bypass graft), use a method that accommodates available resources and data needs. *Category II*

3) For outpatient case-finding, use a method that accommodates available resources and data needs. *Category IB*

4) Assign the surgical wound classification upon completion of an operation. A surgical team member should make the assignment. *Category II*
5) For each patient undergoing an operation chosen for surveillance, record those variables shown to be associated with increased SSI risk (e.g., surgical wound class, ASA class, and duration of operation). *Category IB*

6) Periodically calculate operation-specific SSI rates stratified by variables shown to be associated with increased SSI risk (e.g., NNIS risk index). *Category IB*

7) Report appropriately stratified operation-specific SSI rates to surgical team members. The optimum frequency and format for such rate computations will be determined by stratified case-load sizes (denominators) and the objectives of local, continuous quality improvement initiatives. *Category IB*

8) No recommendation to make available to the infection control committee coded surgeon-specific data. *Unresolved issue.*