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Editorial

Contents

Nosocomial infections are defined as infections that are not present or incubating in a patient at the time of admission to a hospital or health care facility. The Centers for Disease Control and Prevention (CDC) estimates that nosocomial infections occur in 5% to 10% of the patients annually. These types of infections also contribute to deaths and increase the cost of the health care system per year. Nosocomial infections continue to be a major concern for the health care industry. National Nosocomial Infections Surveillance (NNIS) is a voluntary, hospital-based reporting system established to monitor hospital-acquired infections and to guide the prevention efforts of infection control practioners. In Mini Review we have included a study of Nosocomial Infection and the control program.

Here we have again come up with an interesting topic in our Current Trends section. Earlier povidone iodine was widely used as antiseptics but it has some drawbacks. Now we have explained why Chlorhexidine gluconate is a preferred antiseptic over povidone iodine. Pasteurization is a process, which is frequently used in beverage and dairy industry and we have enlightened the biography of the discoverer of the process, Louis Pasteur in our In Profile section. Bug of the Month is focused on *Salmonella* species, which is causative agent of typhoid fever. It also causes food borne infections. Antigenic structure of *Salmonella* is an important criterion, discussed in this issue. As you are aware that antibiotic sensitivity testing is one of the most important tests performed by microbiologist in clinical segment. McFarland Standard No. 0.5 (turbidity standard) is mainly used in Antibiotic sensitivity testing for standardization of bacterial suspension and you can know lot more about it in our Did You Know section.

Autoclave is most widely used for sterilization of culture media and surgical supplies. It is the most reliable method among all the moist heat sterilization methods. Autoclave is a pressurized device designed to heat aqueous solutions above their boiling point to achieve sterilization. We have described all the details of operation, validation, and safety instructions about autoclave in our Best Practices segment. Some more interesting matters are there for you in Encyclopedia, Relax Mood and In Focus pages.

We would like to thank all our valued readers for appreciating and encouraging the previous two efforts. We want more support from you to make **Journal of Hygiene Science** more successful. You can communicate your views to us through fax or e-mail.

A Study of Nosocomial Infection and Control Program

Nosocomial Infection

The term nosocomial infection or hospital-acquired infection is applied to any clinical infection that was neither present nor was in its incubation period when the patient entered the hospital. Nosocomial infections may also make their appearance after discharge from the hospital, if the patient was in the incubation period at the time of discharge. It is also known as Healthcare associated infections. Within hours after admission, a patient's flora begins to acquire characteristics of the surrounding bacterial pool. Most infections those become clinically evident after 48 hours of hospitalization are considered hospital-acquired. Infections that occur after the patient's discharge from the hospital can be considered to have a nosocomial origin if the organisms were acquired during the hospital stay.

Historical Perspective

The modern era of infection control began in the early 1950s; the recognition and the awareness that the provision of medical and nursing care in an institutional setting (e.g., a hospital) could result in an increased risk for the acquisition of infection occurred more than 100 years ago. In the 1840s, Dr. Ignaz Philip Semmelweiss was concerned about the incidence of puerperal fever in obstetrics ward and its related mortality. He postulated that the puerperal fever that affected so many parturient women was caused by the transmission through the hands of medical personnel. Then he represented the first evidence indicating that cleansing heavily contaminated hands with an antiseptic between patient contacts may reduce the healthcare associated transmission.

Causes Of Nosocomial Infections

All hospitalized patients are at risk of acquiring an infection from their treatment or surgery. Some patients are at greater risk than others, especially young children, the elderly, and persons with compromised immune systems. The National Nosocomial Infection Surveillance System database compiled by the CDC shows that the overall infection rate among children in intensive care is 6.1%, with the primary causes being venous catheters and ventilator-associated pneumonia. The risk factors for hospital-acquired infections in children include parenteral nutrition (tube or intravenous feeding), the use of antibiotics for more than 10 days, use of invasive devices, poor postoperative status, and immune system dysfunction. Other risk factors that increase the opportunity for hospitalized adults and children to acquire infections are:

A prolonged hospital stay

Severity of underlying illness

Compromised nutritional or immune status

Use of indwelling catheters

Failure of health care workers to wash their hands between patients or before procedures

Prevalence of antibiotic-resistant bacteria from the overuse of antibiotics

The sources of the infecting organism are mainly from another patient or a member of hospital staff, or from the inanimate environment in the hospital and sometimes from patient's own flora. Any type of invasive (enters the body) procedure can expose a patient to the possibility of infection. Some common procedures that increase the risk of hospital-acquired infections include:

Urinary bladder catheterization

Respiratory procedures such as intubations or mechanical ventilation

Surgery and the dressing or drainage of surgical wounds

Gastric drainage tubes into the stomach through the nose or mouth

Intravenous (IV) procedures for delivery of medication, transfusion, or nutrition

Mode Of Transmission

Microorganisms are transmitted in hospitals by several routes, and the same microorganism may be transmitted by more than one route. There are five main routes of transmission -- contact, droplet, airborne, common vehicle, and vectorborne.

(a) Contact Transmission- It is the most important and frequent mode of transmission of nosocomial infections and divided into two subgroups: direct contact transmission and indirect contact transmission.

Direct Contact Transmission - This involves direct physical transfer between a susceptible host and an infected or colonized person, such as occurs between patient and hospital personnel when personnel are turning patients, giving baths, changing dressings, or performing other procedures requiring direct personal contact. Taking care of patients generally involves some direct contact. Direct contact transmission also can occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host.

Indirect Contact Transmission - This involves personal contact of the susceptible host with a contaminated intermediate object, usually inanimate, such as instruments, dressings, or other infective material. If proper care is not taken, personnel can contaminate objects when assembling or

handling critical equipment (such as respiratory therapy equipment, pressure-monitoring devices, cardiac bypass pumps) or during other procedures that involve inanimate objects.

(b) Droplet Transmission - Infectious agents may come in contact with the conjunctivae, nose, or mouth of a susceptible person as a result of coughing, sneezing, or talking by an infected person. These droplets are propelled through the air for a short distance (approx. 3 feet). Generally influenza virus is transmitted by droplet transmission.

(c) Air Borne Transmission - It occurs by dissemination of either droplet nuclei (residue of evaporated droplets that may remain suspended in the air for long periods of time) or dust particles in the air containing the infectious agent. Organisms carried in this manner are then inhaled by or deposited on the susceptible host. Microorganisms transmitted by airborne transmission include *Mycobacterium tuberculosis* and varicella viruses.

(d) Common Vehicle Transmission - It applies microorganisms transmitted to the host by contaminated items such as food, water, medications, devices, and equipment. Food borne illnesses are frequently caused by common vehicle transmission and occur when a number of people ingest the same contaminated food (e.g. staphylococcal food poisoning) or contaminated water.

(e) Vector Borne Transmission - It is of greater concern in developing countries for example, mosquito-transmitted malaria. Since agent and host factors are more difficult to control, interruption of the chain of infection in the hospital is directed primarily at transmission.

Types Of Hospital Acquired Infections

The most common types of nosocomial infections that could occur in the hospital set up are:

Surgical wound and other soft tissue infections
Urinary tract infections
Respiratory infections
Gastroenteritis
Meningitis

Surgical site infection - Invasive surgical procedures increase a patient's risk of getting an infection by giving bacteria a route into normally sterile areas of the body. An infection can be acquired from contaminated surgical equipment or from the hands of healthcare workers. Following surgery, the surgical wound can become infected from contaminated dressings or the hands of healthcare workers who change the dressing. Other wounds can also become easily infected, such as those caused by trauma, burns, or pressure sores that result from prolonged bed rest or wheel chair use. Factors most consistently associated with an increased incidence of postoperative infections are:

Over 60 years, of age
Preoperative stay in hospital
Long duration of the surgical procedure
Pre-existing infection at the site of the wound

Urinary Tract Infection - Urinary tract infection (UTI) is the most common type of hospital-acquired infection and has been shown to occur after urinary catheterization. Catheterization is the placement of a catheter through the urethra into the urinary bladder to empty urine from the bladder; or to deliver medication, relieve pressure, or measure urine in the bladder; or for other medical reasons. Normally, a healthy urinary bladder is sterile, with no harmful bacteria or other microorganisms present. Although bacteria may be in or around the urethra, they normally cannot enter the bladder. A catheter, however, can pick up bacteria from the urethra and give them an easy route into the bladder, causing infection. Bacteria from the intestinal tract are the most common type to cause UTIs. Patients with poorly functioning immune systems or who are taking antibiotics are also at increased risk for UTI caused by a fungus called *Candida*. The prolonged use of antibiotics, which may reduce the effectiveness of the patient's own immune system, has been shown to create favorable conditions for the growth of this fungal organism.

Respiratory Tract Infection - Pneumonia is another type of hospital-acquired infection. Bacteria and other microorganisms are easily introduced into the throat by treatment procedures performed to treat respiratory illnesses. Patients with chronic obstructive lung disease are especially susceptible to infection because of frequent and prolonged antibiotic therapy and long-term mechanical ventilation used in their treatment. The infecting microorganisms can come from contaminated equipment or the hands of health care workers as procedures are conducted such as respiratory intubation, suctioning of material from the throat and mouth, and mechanical ventilation. Once introduced through the nose and mouth, microorganisms quickly colonize the throat area. This means that they grow and form a colony, but have not yet caused an infection. Once the throat is colonized, it is easy for a patient to aspirate the microorganisms into the lungs, where infection develops that leads to pneumonia.

Epidemiology and Transmission of Infections among Hospital Personnel and Patient

Bacteria, viruses, fungi, or parasites can cause hospital-acquired infections. These microorganisms may already be present in the patient's body or may come from the environment, contaminated hospital equipment, health care workers, or other patients. Depending on the causal agents involved, an infection may start in any part of the body. Almost any transmissible infection may occur in the community at large or within the hospital and can affect both personnel and patients. Transmission can occur from personnel to patient as well as from patient to personnel.

Some of the following diseases are transmitted mostly from

health care personnel to patient and vice versa.

Acute Diarrhea - Various agents may cause diarrhea in patients and hospital personnel. *Salmonella*, *Shigella*, and *Campylobacter* species are among the common bacterial enteric pathogens. Rotavirus and other viral agents can cause gastroenteritis. *Giardia lamblia* and other protozoa are also frequent causes of diarrhea. Any of these agents may be nosocomially transmitted via the hands of personnel who are infected.

Hepatitis - Viral hepatitis has long been recognized as a nosocomial hazard. The agents that most commonly cause viral hepatitis are hepatitis A virus (HAV) and hepatitis B virus (HBV). Primarily the fecal-oral route transmits Hepatitis A. Fecal excretion of HAV is greatest during the incubation period of disease before the onset of jaundice. Once disease is clinically obvious, the risk of transmitting infection is decreased. Some patients admitted to the hospital with hepatitis A may still be shedding virus and are potentially infective. Personnel can help protect themselves and others from infection with HAV by always maintaining good personal hygiene, practicing thorough hand washing at all times.

Most nosocomial cases of hepatitis B unrelated to the transfusion of blood or blood products occur in hospital personnel rather than patients. Transmission occurs by parenteral or mucosal exposure to HBsAg-positive blood from persons who are carriers or have acute HBV infection. Often carriers of HBsAg and persons with acute infections are unrecognized and are therefore not known to be infective. Since droplets from the patient's mouth reach the face of the dentist during certain procedures, dentists might consider protecting their eyes, nose, and mouth from such exposure by using masks and protective eyewear. They can prevent direct contact with infective material in the mouth by routinely wearing gloves during dental procedures.

Herpes simplex viruses (HSV) can be transmitted among personnel and patients through either primary or recurrent lesions or through secretions. Many sites can become infected, exposed areas of skin are most likely to be involved, particularly when minor cuts, abrasions, or other skin lesions are present. Direct contact with lesions or infected secretions is the principal mode of spread. So it is a distinct hazard for nurses, anesthesiologists, dentists, respiratory care personnel, and other personnel who may have direct (usually hand) contact with either oral lesions or respiratory secretions from patients. Personnel can protect themselves from such infections by 1) avoiding direct contact with lesions, 2) wearing gloves on both hands or using "no-touch" technique for all contact with oral or vaginal secretions, and 3) thorough hand washing after patient contact.

Staphylococcus infection frequently occurs in human. In nosocomial transmission, there are two sources: a person with a lesion or an asymptomatic carrier. Persons with skin lesions due to *S. aureus* are most likely to disseminate these organisms. Direct contact is the major route of transmission.

In the hospital, infection is most likely to occur when a patient has unsuspected pulmonary or laryngeal TB, has bacilli-laden sputum or respiratory secretions, and is coughing or sneezing into air that remains in circulation. The best ways to protect others from a patient with TB are to maintain a high index of suspicion for TB and to institute appropriate precautions.

Nosocomial transmission of varicella- zoster infection among personnel and patients is well recognized. Appropriate isolation of hospitalized patients with known or suspected varicella or zoster can reduce the risk of transmission to personnel. Varicella is transmitted primarily via airborne spread by small particle aerosols (droplet nuclei) and by large particles (droplets).

Viral respiratory infections are common problems for infection control programs. The three chief mechanisms of transmission of respiratory viruses are 1) small-particle aerosols (droplet nuclei), 2) large particles (droplets), and 3) inoculation of viruses after direct contact with infective areas or materials. Different respiratory viruses may vary in the way in which they are transmitted. Small-particle aerosols are produced by talking, sneezing, or coughing and may transmit infection over a considerable distance. Large particles (droplets) are produced by sneezing and coughing and require close person-to-person contact for transmission. Person-to-person transmission can also occur by contaminating the hands by direct contact with infective areas or materials, then transferral of infective virus to mucous membranes of a susceptible person. Self-inoculation can also occur in this way. The nose and eyes, rather than the mouth, appear to be important portals of entry.

Pediatric patients appear to be at particular risk for complications from nosocomial respiratory tract infections. Infection in the elderly, patients with chronic underlying illness, and immunocompromised patients may also be associated with significant morbidity. Thus, it may be prudent to exclude personnel with viral respiratory infections from the care of these high-risk patients. Because large numbers of personnel may have viral respiratory illnesses during the winter, it may not be possible to restrict all such personnel from taking care of patients not in high-risk groups. Careful hand washing before patient contact is essential in preventing transmission. Masks might be beneficial in preventing transmission by large droplets from personnel to patients upon close contact. However, masks probably will not completely protect personnel from patients with respiratory illnesses because large particles and aerosols may still reach the eyes, and self-inoculation from contaminated hands can still occur by touching the eyes.

Personnel may be exposed to patients with cytomegalovirus (CMV) infection, but the risk of acquiring CMV infection from patients appears to be small. There are two principal

reservoirs of CMV in the hospital: 1) infants infected with CMV and 2) immunocompromised patients, such as oncology patients and those undergoing kidney or bone marrow transplant. Infection appears to be acquired only through intimate, direct contact with an excreter of CMV or contact with contaminated secretions. Virus can be shed in the urine, saliva, respiratory secretions, tears, feces, breast milk, semen and cervical secretions. A practical approach to reduce the risk of infection with CMV is to stress careful hand washing after all patient contacts and avoiding contact with areas or materials that are potentially infective.

Nosocomial transmission of *Neisseria meningitidis* to hospital personnel taking care of patients with meningococcemia, meningococcal meningitis, or lower respiratory infections is uncommon. In rare instances transmission to personnel from patients with meningococcemia or meningococcal meningitis has occurred through intensive direct contact with the infected person and direct contact with respiratory secretions without use of proper precautions. The most likely mode of spread from a person with infections at these sites is by large droplet secretions.

Pertussis, caused by *Bordetella pertussis*, is highly communicable. Nosocomial transmission of pertussis has been reported infrequently. Although infection occurs less commonly in adults and may be limited to mild respiratory illness, personnel with pediatric patient contact may be involved in transmission of pertussis to patients.

Scabies is a disease caused by infestation with the mite *Sarcoptes scabiei*. It is transmitted in hospitals primarily through intimate direct contact with an infested person, even when high levels of personal hygiene are maintained. Transmission to personnel has occurred during activities such as sponge-bathing patients or applying body lotions.

Reasons of Emerging Nosocomial Infections

Three major forces are involved in nosocomial infections. The first is antimicrobial use in hospitals and long-term care facilities. The increased concern about gram-negative bacilli infections in the 1970s to 1980s led to increased use of cephalosporin antibiotics. As gram-negative bacilli became resistant to earlier generations of cephalosporin antibiotics, newer generations were developed. Widespread use of cephalosporin antibiotics is often cited as a cause of the emergence of enterococci as nosocomial pathogens. About the same time, MRSA, perhaps also in response to extensive use of cephalosporin antibiotics, became a major nosocomial threat. Widespread empiric use of vancomycin, as a response to concerns about MRSA and for treatment of vascular catheter associated infection by resistant coagulase-negative staphylococci, is the major initial selective pressure for VRE. Use of antimicrobial drugs in long-term care facilities and transfer of patients between these facilities and hospitals have created a large reservoir of resistant strains in nursing homes. Second, many hospital personnel fail to follow basic infection control, such as hand washing between patient contacts. In ICUs, asepsis is often overlooked in the rush of crisis care. Third, patients in hospitals are increasingly immunocompromised. The shift of surgical care to outpatient centers leaves the sickest patients in hospitals, which are becoming more like large ICUs. This shift has led to the greater prevalence of vascular access associated bloodstream infections and ventilator-associated pneumonias.

Control Measures

The CDC (1985) on the efficacy of nosocomial infection control (SENIC) showed beyond doubt that increase in surveillance activities is able to directly bring down the rates of nosocomial infections. It is only too well known that nosocomial infections are most prevalent in certain high risk areas such as the intensive care renal dialysis and organ transplant units, burns ward, cancer ward, operation theatres, post-operation theatres, postoperative ward nursery and the geriatric ward. Hospitals take a variety of steps to prevent nosocomial infections, including:

Sterilization should be done of all reusable equipment such as ventilators, humidifiers, and any devices that come in contact with the respiratory tract.

Handwashing with high level disinfectants frequently is called the single most important measure to reduce the risks of transmitting microorganisms from one person to another or from one site to another on the same patient. Washing hands as promptly and thoroughly as possible between patient contacts and after contact with blood, body fluids, secretions, excretions and equipment or articles contaminated by them is an important component of infection control and isolation precautions. Although handwashing may seem like a simple measure, it is often not used or hand washing is performed incorrectly. Healthcare settings must continually remind practitioners and visitors to wash their hands thoroughly.

Strict attention should be given to aseptic (sterile) technique in the performance of procedures, including use of sterile gowns, gloves, masks, and barriers. In addition to handwashing, gloves play an important role in reducing the risks of transmission of microorganisms. Gloves are worn for three important reasons in hospitals. First, gloves are worn to provide a protective barrier and to prevent gross contamination of the hands when touching blood, body fluids, secretions, excretions, mucous membranes, and nonintact skin; the wearing of gloves in specified circumstances to reduce the risk of exposures to blood borne pathogens. Second, gloves are worn to reduce the likelihood that microorganisms present on the hands of personnel will be transmitted to patients during invasive or other patient-care procedures that involve touching a patient's mucous membranes and nonintact skin. Third, gloves are worn to reduce the likelihood that hands of personnel contaminated with microorganisms from a patient or a fomite can transmit these microorganisms to another patient. Wearing gloves does

not replace the need for handwashing, because gloves may have small, non-apparent defects or may be torn during use, and hands can become contaminated during removal of gloves.

Use of an antibacterial-coated venous catheter can destroy bacteria before they get into the blood stream. Use of silver alloy-coated urinary catheters can destroy bacteria before they migrate up into the bladder.

Frequent changing of dressings for wounds and use of antibacterial ointments under dressings can prevent the spread of infection.

Isolation precautions are designed to prevent transmission of microorganisms by common routes in hospitals. Because agent and host factors are more difficult to control, interruption of transfer of microorganisms is directed primarily at transmission.

Sterilization of medical instruments and equipment should be done to prevent contamination.

An infection control practitioner should be employed for every 200 beds.

The general use of antibiotics should be reduced to encourage better immune response in patients and reduce the cultivation of resistant bacteria.

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Encyclopedia

Pasteurization is the process of heating liquids (milk and milk products, beverages) for the purpose of destroying viruses and harmful organisms such as bacteria, protozoa, molds and yeasts. The process was named after its inventor, French scientist Louis Pasteur. Unlike sterilization, pasteurization is not intended to kill all pathogenic microorganisms in the food. Instead, pasteurization aims to achieve a logarithimic reduction in the number of viable organisms, reducing their number so they are unlikely to cause disease. Commercial-scale sterilization of food is not common, because it adversely affects the taste and quality of the product. Pasteurization typically uses temperatures below boiling since at temperatures above the boiling point for milk, casein micelles aggregate irreversibly.

There are two main types of pasteurization used presently: high temperature/short time(HTST) and ultra-high temperature (UHT). In the HTST process, milk is heated to 71.7 ºC (161ºF) for 15-20 seconds. UHT processing hold the milk at a temperature 138ºC (280ºF) for 2 seconds. In vat pasteurization the milk is heated 63ºC (145ºF) for 30 minutes. Pasteurization methods are usually standardized and controlled by national food safety agencies such as USDA. There are different standards for different dairy products, depending on the fat content and the intended usage. For example, the pasteurisation standards for cream differ from the standards for fluid milk, and the standards for pasteurizing cheese are designed to preserve the phosphatase enzyme, which aids in cutting. The HTST pasteurization standard was designed to achieve a 5-log reduction (0.00001 times the original) in the number of viable microorganisms in milk. This is considered adequate for destroying almost all yeasts, mold, and common spoilage bacteria and also to ensure adequate destruction of common pathogenic heat-resistant organisms (including particularly *Mycobacterium tuberculosis* and *Coxiella burnetii*). A newer method called flash pasteurization involves shorter exposure to higher temperatures, and is claimed to be better for preserving color and taste in some products.

Methods of Pasteurization

There are two basic methods of pasteurization, batch or continuous.

Batch method

The batch method uses a vat pasteurizer, which consists of a jacketed vat, surrounded by either circulating water, steam or heating coils of water or steam. In the vat the milk is heated and held throughout the holding period while being agitated. The milk may be cooled in the vat or removed hot after the holding time is completed for every particle.

Continuous Method

Continuous process method has several advantages over the vat method, the most important being time and energy saving. For most continuous processing, a high temperature short time (HTST) pasteurizer is used. The heat treatment is accomplished using a plate heat exchanger.

Significance

There are two distinct purposes for the process of milk pasteurization:

- Public Health Aspect- To make milk and milk products safe for human consumption by destroying all bacteria that may be harmful to health (pathogens).
- Keeping Quality Aspect- To improve the keeping quality of milk and milk products. Pasteurization can destroy some undesirable enzymes and many spoilage bacteria. Shelf life can be 7, 10, 14 or up to 16 days.

Chlorhexidine Gluconate

A Superior Antiseptic than Povidone Iodine

An antiseptic is a substance that prevents or arrests the growth or action of microorganisms either by inhibiting their activity or by destroying them. The term is used especially for preparations applied topically to living tissue. A skin antiseptic is a safe, nonirritating, antimicrobial-containing preparation that prevents overt skin infection.

John Pringle apparently first used the term "antiseptic" (Greek for "against putrefaction") in 1750 to describe substances that prevent putrefaction. As so often in the history of medicine a change of practice depended on the persistence of one man. For antiseptics, this man was John Lister. He chose phenol and applied it vigorously in surgery. The effect of Lister's practices was revolutionary and the antiseptic technique opened the way to great surgical advances.

Salient Features Of Antiseptics

An antiseptic has following features:

1) Antiseptics bind readily to bacteria; the amount absorbed increasing with an increasing concentration solution. The most important site of absorption is the cytoplasmic membrane.
2) The extent of killing of the bacteria is governed by three principal factors: (a) concentration of the antiseptic, (b) bacterial cell density, and (c) time of contact. The absorption of a given amount of the compound per cell leads to the killing of a definite fraction of the bacterial population in a chosen time interval.
3) The necessary characteristic of antiseptics is their bactericidal action, but there is often a low and rather narrow concentration range in which their effect is bacteriostatic. At these low concentrations, certain biochemical functions associated with the bacterial membrane may be inhibited.
4) In the presence of higher concentrations of antiseptic and after prolonged treatment, the compound usually penetrates the cell and brings about extensive ill-defined disruption of normal cellular functions.

Povidone Iodine - An earlier used Antiseptic

The first use of iodine in medical practice was as a remedy for bronchocele. The first specific reference to the use of iodine in wounds was made in 1839. Iodine was officially recognized by the Pharmacopeia of the United States in 1830. By the late nineteenth century iodine tinctures came into wide use as microbicides. Later povidone iodine is used as a topical antiseptic agent. Povidone iodine is water-soluble complex of iodine with polyvinylpyrrolidone. It is applied as an antiseptic in the form of solutions or ointments. It is used in cleansing and disinfecting the skin. It is widely used in preoperative and postoperative skin treatment. Despite the wide use of povidone iodine it also possess properties unsuitable for practical application. Povidone iodine has the following drawbacks:

- It is deactivated in the presence of organic materials (such as pus, blood and exudates) and it is cytotoxic to the fibroblasts in stronger concentrations.
- Intermediate antimicrobial action is slow.
- Absorption of iodine can occur in young children, in people with thyroid problems, and people with poor renal function.
- Povidone iodine is not recommended for long term wound cleansing.
- It has no residual effect.
- It can cause pain in open wounds and also cause allergic reactions to some hypersensitive patients.
- Its effects wear off after a relatively short time and it does discolour the skin.
- Bacterial resistance may occur mainly with *Staphylococcus aureus*.
- Povidone iodine disinfection is tedious and has to follow multi step procedure.

Chlorhexidine - The 'preferred' skin antiseptic

Chlorhexidine was first synthesized in 1950 in the laboratories of ICI Ltd. (England). It was found to possess a high level of antibacterial activity, low mammalian toxicity and a strong affinity for binding to skin and mucous membrane. These properties led to the development of chlorhexidine principally as a topical antiseptic for application to such areas as skin, wounds and mucous membranes and for dental use.

Chlorhexidine is 1,6-di (4-chlorphenyl-diguanido) hexane, a cationic bisbiguanide compound. Bisbiguanides are the primary second generation antiplaque agents. It is a strong base, at physiologic pH, is a large dicationionic molecule.

Chlorhexidine has a broad spectrum of antimicrobial activity including a wide range of Gram positive and Gram negative bacteria, yeasts, dermatophytes and some lipophylic viruses including HBV and HIV. At relatively low concentrations, the action of chlorhexidine is bacteriostatic and at higher concentrations, it is rapidly bactericidal, with the actual levels varied from species to species. The lethal process consists of a series of related cytologic and physiologic changes, some of which are reversible, that culminate in the death of the cell. The sequence is thought to be as follows:

- The rapid attraction toward the bacterial cell.
- Specific and strong adsorption to certain phosphate containing compounds on the bacterial surface.

- Overcoming the bacterial cell wall exclusion mechanisms.
- Attraction toward the cytoplasmic membrane.
- Leakage of low molecular weight cytoplasmic components, such as potassium ions and inhibition of certain membrane bound enzymes, such as adenosyl triphosphatase.
- Precipitation of the cytoplasm by the formation of complexes with phosphated entities, such as adenosine triophosphated entities, such as adenosine triphosphate and nucleic acids.

A bacterial cell is negatively charged, the nature of the ionogenic groups varying with bacterial species. It has been shown that, given sufficient chlorhexidine concentration and reaches a stable equilibrium within 5 minutes. The rapid electrostatic attraction of the cationic chlorhexidine molecules and the negatively charged bacterial cell undoubtedly contributes to the rapid rate of killing associated with chlorhexidine, although surface charge reversal is secondary to cell death. Chlorhexidine molecules are thought to compete for the negative sites on the peptidoglycan, thereby displacing metallic cations. The bacterial cytoplasmic membrane appears to be the important site of action. Several changes indicative of damage to the cytoplasmic membrane have been observed in bacterial populations treated with bacteriostaitic and bactericidal levels of chlorhexidine. Leakage of cytoplasmic contents is a classic indication of damage to the cytoplasmic membrane, starting with low molecular molecules typified by potassium ions. Sub lethally treated cells show a shrinkage or plasmolysis of the protoplast.

Chlorhexidine also has depathogenizing effect. Chlorhexidine inhibits the growth of the vegetative cells of spore forming bacteria at relatively low concentrations and also inhibits the spore germination. Chlorhexidine has good activity against viruses with a lipid component in their coats or with an outer envelope. These include many respiratory viruses, herpes, and cytomegalovirus.

Applications and Advantages of Chlorhexidine over Povidone Iodine

Nowadays chlorhexidine is used in general medicine for skin disinfection, surgical hand disinfection, preoperative whole body disinfection, hygienic hand disinfection, urology, obstetrics and gynecology, nasal cavity and throat, wounds and burns.

Skin disinfection

Chlorhexidine formulated in a detergent base is used extensively for disinfection of the hands of surgeons and nurses and also for whole body skin disinfection of patients undergoing surgery. Surgeons and nurses for hand disinfection also use alcohol-based chlorhexidine solutions with emollients. Alcohol based chlorhexidine solutions are particularly suitable for final stage skin preparation of the operation site; the area should be kept wet for at least 2 minutes to achieve the maximal effect.

Surgical Hand Disinfection

The objective of surgical hand disinfection is to render the skin free of bacteria, thus preventing the escape of organisms into the operation wound through the puncture in surgical gloves, which occur frequently during operation. The procedure must eliminate the transient organisms that are likely to be present on the skin and reduce the resident flora to as low level as possible. The agent should remain persistent on the skin to maintain the numbers of survivors at this low level through out the course of the operation.

The use of 2-4% chlorhexidine as handwash is found to be effective antiseptic in reducing the numbers of bacteria on the hands of the surgical personnel and maintaining these low numbers for several hours under gloves. A povidone iodine handwash is less effective initially and allowed the numbers of survivors on the hands to increase dramatically during the course of an operation. Chlorhexidine handwash significantly reduce a greater number of resident floras than povidone iodine handwash.

Preoperative Whole Body Disinfection

A significant proportion of postoperative wound infections are caused by microorganisms from the patient's own skin, which may be derived from sites remote from that of the operation. Chlorhexidine skin cleanser is found to reduce the skin floras and maintain this reduction for up to 1 week. Preoperative showering of patient with chlorhexidine is effective to control the infection rate.

Hygienic Hand Disinfection

The major source of infectious organisms within the hospital is the infected or heavily colonized patient, and the principal route of transmission is via the hands of hospital personnel. Washing hands with high level is therefore considered to be the single most important measure to prevent nosocomial infection.

Other than these Chlorhexidine gluconate can also reduce the risk for catheter-related blood stream infection by approximately 50% in hospitalized patients who require short-term catheterization. Povidone iodine is not persistent and allows a significant increase in bacterial numbers with time. So CDC has recommended chlorhexidine gluconate solution to prevent infections related to invasive devices. Chlorhexidine gluconate solution can also be used in pharmaceutical industry as well as in general purpose. Alcoholic chlorhexidine gluconate solution is much more effctive antiseptic than povidone iodine. So it is time for health practioners to shift from povidone iodine to more effective chlorhexidine gluconate to control the infection rate.

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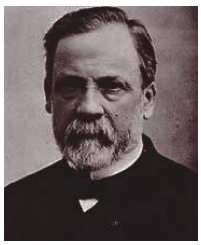

Louis Pasteur

Birth: December 27, 1822
Death: September 28, 1895
Nationality: French
Known For: Germ theory of disease, Pateurization Technique

Louis Pasteur, world-renowned French chemist and biologist, who founded the science of microbiology, proved the germ theory of disease, invented the process of pasteurization and developed vaccines for several diseases, including rabies.

Pasteur was born in Dôle on December 27, 1822, the son of a tanner, and grew up in the small town of Arbois. In 1847 he earned a doctorate at the École Normale in Paris, with a focus on both physics and chemistry. Becoming an assistant to one of his teachers, he began research that led to a significant discovery. He found that a beam of polarized light was rotated to either the right or the left as it passed through a pure solution of naturally produced organic nutrients, whereas when polarized light was passed through a solution of artificially synthesized organic nutrients, no rotation took place. If, however, bacteria or other microorganisms were placed in the latter solution, after a while it would also rotate light to the right or left.

After spending several years of research and teaching at Dijon and Strasbourg, Pasteur moved in 1854 to the University of Lille, where he was named professor of chemistry and dean of the faculty of sciences. This faculty had been set up partly to serve as a means of applying science to the practical problems of the industries of the region, especially the manufacture of alcoholic beverages. Pasteur immediately devoted himself to research on the process of fermentation. He studied lactic and alcoholic fermentation and showed that all fermentation is due to the presence of microorganisms.

The souring of wine and beer had been a major economic problem in France; Pasteur contributed to solving the problem by showing that heating the starting sugar solutions to a high temperature could eliminate bacteria. Pasteur achieved success by slightly modifying the process used with the broth. Boiling the wine would alter its flavour. Therefore, Pasteur heated the wine enough to kill most of the microbes present without altering the flavour. This process is later known as pasteurization.

Louis Pasteur demonstrated that the fermentation process is caused by the growth of microorganisms, and that the growth of microorganisms in nutrient broths is not due to spontaneous generation. He exposed boiled broths to air in vessels that contained a filter to prevent all particles from passing through to the growth medium, and even in vessels with no filter at all, with air being admitted via a long tortuous tube that would not allow dust particles to pass. Nothing grew in the broths; therefore, the living organisms that grew in such broths came from outside, as spores on dust, rather than spontaneously generated within the broth. This was one of the last and most important experments disproving the theory of spontaneous generation. While Pasteur was not the first to propose germ therapy, he developed it and conducted experiments that clearly indicated its correctness and managed to convince most of Europe it was true.

The country's enormous production of silk had suddenly been curtailed because of an epidemic illness among the silkworms. Despite having no prior experience with animal biology, Pasteur determined the silkworms were dying from two parasitic infections. Pebrine, in which black spots and corpuscles are generally, but not always, present on the worm. In such cases the worms die within the cocoons. In the second type of disease, flacherie, the worms exhibit no corpuscles or spots but fail to spin cocoons. Suspecting that certain microscopic objects found in the diseased silkworms (and in the moths and their eggs) were disease-producing organisms, Pasteur experimented with the separation of uninfected from infected silkworms, the cleanliness of silkworm colonies, and controlled breeding. He concluded that only in diseased and living eggs was the cause of the disease maintained; therefore, selection of disease-free eggs was the solution.

Pasteur also determined the natural history of anthrax, a fatal disease of cattle. He proved that anthrax is caused by a particular bacillus and suggested that animals could be given anthrax in a mild form by vaccinating them with attenuated bacilli, thus providing immunity from potentially fatal attacks.

Pasteur spent the rest of his life working on the causes of various diseases - including septicemia, cholera, diphtheria, fowl cholera, tuberculosis, smallpox and their prevention by means of vaccination. He is best known for his investigations concerning the prevention of rabies, otherwise known in humans as hydrophobia. After experimenting with the saliva of animals suffering from this disease, Pasteur concluded that the disease rests in the nerve centers of the body; when an extract from the spinal column of a rabid dog was injected into the bodies of healthy animals, symptoms of rabies were produced. By studying the tissues of infected animals, particularly rabbits, Pasteur was able to develop an attenuated form of the virus that could be used for inoculation. In 1885, a young boy and his mother arrived at Pasteur's laboratory; the boy had been bitten badly by a rabid dog, and Pasteur was urged to treat him with his new method. At the end of the treatment, which lasted ten days, the boy was being inoculated with the most potent rabies virus known; he recovered and remained healthy. Since that time, thousands of people have been saved from rabies by this treatment.

The French government honoured Pasteur with its highest award, the Legion of Honour. Louis Pasteur died at Saint Cloud on 28 September 1895 and was given a state funeral at the Cathedral of Notre Dame and his body placed in a permanent crypt at the Pasteur Institute. His work served as the springboard for branches of science and medicine such as stereochemistry, microbiology, bacteriology, virology, immunology, and molecular biology. Moreover, his work has protected millions of people from disease through vaccination and pasteurization.

References

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Enjoy the humour

- *Mary was discussing the various aspects and possible outcome of the insurance policy with the clerk at the Insurance Agency. During the discussion, she asked. "Suppose I take the life insurance for my husband today for a million dollars, and tomorrow he dies? What will I get?"*

The clerk eyed her suspiciously and replied, "Probably a life sentence."

- *A physician, a civil engineer, and a consultant were arguing about what was the oldest profession in the world.*

The physician remarked, "Well, in the Bible, it says that God created Eve from a rib taken out of Adam. This clearly required surgery, and so I can rightly claim that mine is the oldest profession in the world."

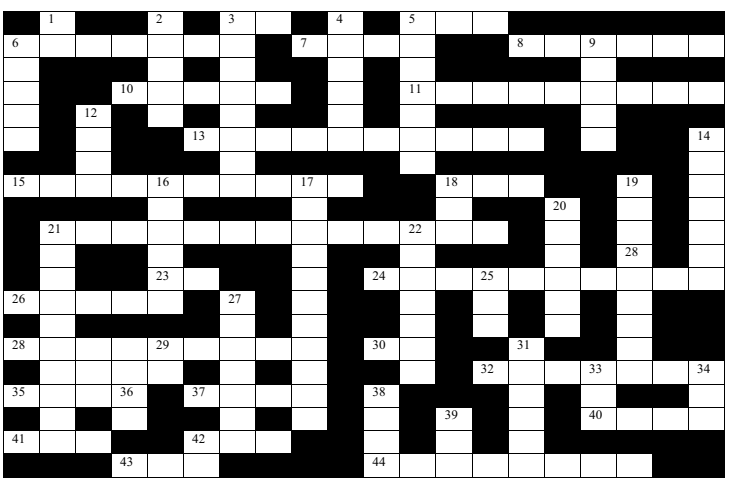
The civil engineer interrupted, and said, "But even earlier in the book of Genesis, it states that God created the order of the heavens and the earth from out of the chaos. This was the first and certainly the most spectacular application of civil engineering. Therefore, fair doctor, you are wrong: mine is the oldest profession in the world."

The consultant leaned back in her chair, smiled, and then said confidently, "Ah, but whom do you think created the chaos?"

Thoughts to live by

- The grand essentials of happiness are: something to do, something to love and something to hope for. (Allan K. Chalmers)
- Hold fast to dreams, for if dreams die, life is a broken winged bird that cannot fly. (Lanston Hughes)
- The secret of success in life is for a man to be ready for his opportunity when it comes. (Earl of Beaconsfield)
- Lucky is the man who is the first love of a woman, but luckier is the woman who is the last love of a man.
- Time is a companion that goes with us on a journey. It reminds us to cherish each moment, because it will never come again. What we leave behind is not as important as how we have lived. (Captain Jean Luc Picard).

Track your brain



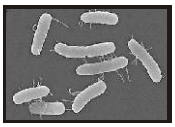
Across

3. Glycocalyx- producing bacterial species that causes anthrax(initials). 5. The genetic material of a bacterium. 6. A cluster of eight cocci. 7. Staining allows one to determine the shape,_____, and arrangement of bacterial cells. 8. Contains the enzyme lysozyme for bacterial destruction. 10. The mosaic model describes the structure of the cell membrane. 11. The cell membrane is the structure for nutrient _____ into the cell cytoplasm. 13. It includes capsules and slime layers. 15. Closed loop of DNA having the bacterial inheritance characteristics. 18. Nucleic acid found in plasmids. 21. The important chemical constituent of the bacterial cell wall. 23. Unit of measurement for viruses(abbr). 24. Spiral bacteria with a flexible cell wall. 26. A short hair-like projection for attachment and genetic transfer. 28. Bodies of RNA and protein that function in protein systhesis. 30. Bacterium that occurs as a _____ sarcina (initials). 32. Closed loop of DNA apart from the chromosome in the cytoplasm. 35. The slime of the capsule of *A. viscolactis* is_____ positive bacteria. 37. Teichoic acid is present in the cell walls of_____ positive bacteria. 40. Flagella permit a bacterium to_____. 41. A monotrichous bacterium has_____. 42. One of the disaccharides in the bacterial cell wall(abbr). 43. The cell membrane has_____ important organic constituents. 44. The name for the chromosomal region of a bacterium.

Down

1. Bacterium (initials) that appears as a grape like cluster. 2. A pair of bacterial cocci is a_____ coccus. 3. A bacterial rod. 4. A curved rod that resembles a comma under the microscope. 5. A polysaccharide existing as tangled fibers in the glycocalyx. 6. A thin, loosely bound glycocalyx is called the _____ layer. 9. About 40 percent of the cell membrane consists of_____. 12. The side chains of peptidoglycan consist of_____ amino acids. 14. The presence of a capsule contributes to the ability of a pathogen to cause_____ . 16. A capsule containing cause of tooth decay is *Streptococcus*_____ 17. A cytoplasmic body that helps a bacterium orient itself. 18. Nucleic acid forming chromosomes. 19. Many times the length of a bacterium but extremely thin; used for motility. 20. A bacterial sphere. 21. Antibiotic that interrupts construction of bacterial cell wall. 22. Serves as a buffer between bacterium and external environment. 25. Form displayed by typhoid, anthrax and diphtheria bacilli. 27. Alternative name of pilus. 29.Bacterium well known for its capsule(initials). 31. Layer of bacteria and other materials on tooth surface. 33. Microscope (abbr) used to visualize cell surfaces. 34. Used to stain cells for bright field microscopy. 36. Unlike_____ -karyotic cells, bacteria have no nuclei. 38. Common site of infection by staphylococci. 39. Type of cell(abbr) that cannot easily engulf encapsulated bacteria.

Check your Answers on Page 16



Salmonella species

The genus *Salmonella* consists of bacilli that parasitise the intestines of a large number vertebrate species and infects human beings, leading to enteric fever, gastroenteritis and septicemia with or without focal suppuration and the carrier state. The most important member of the genus is *Salmonella typhi*, the causative agent of typhoid fever. In 1880s, the typhoid bacillus was first observed by Eberth in spleen sections and mesenteric lymph nodes from a patient who died from typhoid. Robert Koch confirmed a related finding by Gaffky and succeeded in cultivating the bacterium in 1881. But due to the lack of differential characters, separation of the typhoid bacillus from other enteric bacteria was uncertain. In 1896, it was demonstrated that the serum from an animal immunized with the typhoid bacillus agglutinated (clumped) the typhoid bacterial cells, and it was shown that the serum of patients afflicted with typhoid likewise agglutinated the typhoid bacillus. Serodiagnosis of typhoid was thus made possible by 1896.

Morphology & Cultural Characteristics

Salmonellae are Gram negative rods and motile with peritrichate flagella. They are aerobic and facultatively anaerobic, growing readily on simple media over a range of pH 6-8 and temperature 15-41°C. On MacConkey and deoxycholate citrate media, colonies are colourless due to the absence of lactose fermentation. On Wilson and Blair bismuth sulphite medium, jet-black colonies with a metallic sheen are formed due to production of H_2S. Selenite F broth and tetrathionate broth are commonly employed as enrichment media. Salmonellae ferment glucose, mannitol and maltose, forming acid and gas. They give Methyl red positive but Voges Proskauer negative and Citrate negative.

Habitats

Salmonellae are disseminated in the natural environment (water, soil, sometimes plants used as food) through human or animal excretion. Humans and animals (either wild or domesticated) can excrete *Salmonella* either when clinically diseased or after having had salmonellosis, if they remain carriers. *Salmonella* organisms do not seem to multiply significantly in the natural environment (out of digestive tracts), but they can survive several weeks in water and several years in soil if conditions of temperature, humidity, and pH are favorable. The principal habitat of the salmonellae is the intestinal tract of humans and animals.

Food can become contaminated with *Salmonella* during the slaughter and processing of an animal, when food is handled by a person infected with *Salmonella* or from cross-contamination because of unsanitary food handling practices. The following listed below have been responsible for foodborne illnesses: raw and undercooked meat (especially poultry), raw fruits and vegetables (especially sprouts and cantaloupes) and their juices, raw and undercooked eggs, unpasteurized dairy products, like raw milk and raw milk cheeses.

Mode of Transmission

The mode of transmission for *S. typhi* is water, food borne or vertical transmission. It is primarily transmitted through fecal oral route. Humans normally acquire the disease from eating contaminated animal products or raw vegetables that have been contaminated. Other modes of zoonotic transmission include direct contact with livestock, wildlife, or pets, especially cats and turtles. Animal-animal transmission happens at the farm through contaminated food and water sources, pastureland, or contact with newly acquired animals. On the way to the slaughterhouse, stress and overcrowded conditions associated with transport increase the number of bacteria shed. Environmental conditions at the slaughterhouse can easily cause indirect transmission through contaminated equipment or people. Since one infected animal can infect an entire slaughterhouse, and therefore, expose many people to harmful animal products, safety precautions must be taken at three different levels, which must cooperate with each other - the farm, the slaughterhouse, and the preparation of food, by the consumer/handler.

Antigenic Structure

Salmonellae possess the following antigens based on which they are classified and identified: (a) Flagellar antigen, H; (b) Somatic antigen, O; (c) Surface antigen, Vi. H antigen present on the flagella is a heat labile protein. The H antigen is strongly immunogenic and induces antibody formation rapidly and in high titres following infection or immunization. The somatic O antigen is a phospholipids-polysaccharide complex. It is less immunogenic than H antigen and the titre of the O antibody induced after infection or immunization is lower. The Vi antigen is poorly immunogenic and only low titre antibody is produced after infection.

Pathogenicity & Clinical Manifestation

Salmonella infections in humans vary with the serovar, the strain, the infectious dose, the nature of the contaminated food and the host status. In the pathogenesis of typhoid the bacteria enter the human digestive tract, penetrate the intestinal mucosa (causing no lesion), and are stopped in the mesenteric lymph nodes. Bacterial multiplication occurs there, and part of the bacterial population lyses. From the mesenteric lymph nodes, viable bacteria and endotoxin may be released into the bloodstream resulting in septicemia. *Salmonella* excretion by human patients may continue long after clinical cure. Asymptomatic carriers are potentially dangerous when unnoticed. About 5% of patients clinically cured from typhoid remain carriers for months or even years. Antibiotics are usually ineffective on *Salmonella* carriage because the site of carriage may not allow penetration by the antibiotic.

The term enteric fever includes typhoid fever caused by *S. typhi* and paratyphoid fever caused by *S. paratyphi*. Typhoid is strictly a human disease. The incidence of human disease decreases when the level of development of a country increases (i.e., controlled water sewage systems, pasteurization of milk and dairy products). Where these hygienic conditions are missing, the probability of fecal contamination of water and food remains high and so is the incidence of typhoid. The incubation period is usually 7-14 days but may range from 3-56 days. The onset is usually gradual, with headache, malaise, anorexia, a coated tongue and abdominal

discomfort with either constipation or diarrhea. A soft palpable spleen is a constant finding. Hepatomegaly is also common. The most important complications are intestinal perforation, hemorrhage and circulatory collapse. Relapses may be seen during the convalescence, particularly when antibiotics treat the patients. They are due to a re-invasion of the bloodstream from the tissues in which typhoid bacilli are still proliferating at the time when the bacteraemic phase of the primary attack is brought to a close.

Salmonella can also cause food borne intoxication. About 12-24 hours following ingestion of contaminated food (containing a sufficient number of *Salmonella*), symptoms appear (diarrhea, vomiting, fever) and last 2-5 days. Spontaneous cure usually occurs. Contamination of meat (cattle, pigs, goats, chicken, etc.) may originate from animal salmonellosis, but most often it results from contamination of muscles with the intestinal contents during evisceration of animals, washing, and transportation of carcasses. When contaminated meat is ground, multiplication of *Salmonella* may occur within the ground meat and if cooking is superficial, ingestion of this highly contaminated food may produce a *Salmonella* infection. The incidence of foodborne *Salmonella* infection/toxication remains reletavely high in developed countries because of commercially prepared food or ingredients for food. Any contamination of commercially prepared food will result in a large-scale infection.

Egg-associated salmonellosis is an important public health problem in the United States and several European countries. *Salmonella enteritidis*, can be inside perfectly normal-appearing eggs, and if the eggs are eaten raw or undercooked, the bacterium can cause illness. A person infected with the *Salmonella enteritidis* usually has fever, abdominal cramps and diarrhea beginning 12 to 72 hours after consuming a contaminated food or beverage. The illness usually lasts 4 to 7 days, and most persons recover without antibiotic treatment.

Diagnosis

Laboratory diagnosis of *Salmonella* infections depends mainly on the isolation and identification of the causal *Salmonella* from a specimen of the patient's blood, feces, urine or vomit. Testing the patient's serum for salmonellae antibodies is useful only in the diagnosis of enteric fever (Widal reaction). For the diagnosis of pyrexial illnesses blood culture should be done. *Salmonella* epidemics may occur among infants in pediatric wards. The frequency and gravity of these epidemics are affected by hygienic conditions, malnutrition and the excessive use of antibiotics that select for multiresistant strains. Bacteremia occurs early in the disease and blood cultures are positive in approximately 90% of cases in the first week of fever. When blood samples from a patient with suspected enteric fever have been submitted for the Widal test, it is useful, as a routine to cultivate the clot after the serum has been removed. Salmonellae are shed in the feces throughout the course of the disease and even in convalescence, with varying frequency. Fecal cultures are almost as valuable as blood cultures in diagnosis. Salmonellae are shed in the urine irregularly and infrequently. A variety of serological tests are available for the diagnosis of enteric fever like widal agglutination test, counter immuno electrophoresis, latex agglutination test, etc. Widal reaction is a test for the measurement of H and O agglutinins for typhoid and paratyphoid bacilli in the patient's sera. The antigens used in the test are H and O antigens of *S. typhi* and the H antigens of *S. paratyphi* A and B. The detection of carriers is important for epidemiological and public health purposes. Laboratory tests are also useful in screening food handlers and cooks to detect carrier state. The identification of fecal carriers is by isolation of the bacillus from feces or from bile. For the detection of urinary carriers, repeated urine cultures should be carried out.

Treatment

Specific antibacterial therapy for enteric fever became available only in 1948 with the introduction of chloramphenicol. Though *S. typhi* is susceptible in vitro to many antibiotics such as streptomycin and tetracycline, it is ineffective in vivo. Ampicillin, amoxycillin, furazolidone and cotrimoxazole were the other drugs that had been found useful in the treatment of typhoid fever. Some of the strains of *Salmonella* have become resistant to chloramphenicol. At present, the drugs useful in treatment of such multiresistant typhoid cases are the later fluoroquinolones(e.g. ciprofloxacin, pefloxacin, ofloxacin) and the third generation cephalosporins (e.g. ceftazidime, ceftrioxone). A combination of antibacterial therapy along with the vaccine has been tried in the eradication of carrier state. This combination has also been used to prevent relapses.

Control Measures

Salmonellae infection can be effectively controlled by general measures, such as improvements in sanitation and provision of protected water supply. Many developed countries have been able to eliminate the risk by these measures. The following measures should be followed to defeat salmonellae infection:

- Handwashing is one of the best ways to prevent the spread of salmonellae related illness. Hands should be washed with high-level disinfectants after going to the toilet and before preparing food. Hand washing should be done before switching from preparing one type of food to another.
- Kitchen utensils must be properly washed before use with another type of food. Again, this stops bacteria being exchanged.
- Different cutting boards and knives should be used for preparing different foods.
- Foods like meat and egg should be cooked thoroughly.
- Bacteria can grow in the danger zone between 4°C and 60°C. Food should be kept in refrigerator at or below 4°C. Refrigeration at or below 4°C slows down most bacterial growth. Freezing at or below -18°C can stop it completely.
- Poultry must always be thoroughly cooked or boiled.
- Minced meat must always be thoroughly cooked or boiled.
- In most eggs, the salmonella bacteria exist only on the shell. Eggs should be scalded in boiling water for five seconds before use.

References

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McFarland Standards

Resistance to a variety of antimicrobial agents is emerging in bacterial pathogens throughout the world. Since the accuracy of the antimicrobial susceptibility data is associated with the performance standard of the test, strict adherence to the standard procedures is essential. The Kirby-Bauer disc diffusion susceptibility test, performed in accordance to NCCLS (National Committee on Clinical Laboratory Standards) method gives reliable results and hence predicts clinical efficacy of the antibiotic tested.

The emergence of antimicrobial resistant strains of pathogenic bacteria has become a great threat to the public health. Early detection of emerging trends in antimicrobial resistance may facilitate implementation of effective control measures. The laboratory testing of antibiotic susceptibility contributes directly to patient care and the expertise of the microbiology laboratory can have powerful influence on antibiotic usage and hence on the pressures that facilitate the emergence of antimicrobial drug resistance. Standardization of the inoculum in antimicrobial susceptibility testing by comparison of a broth culture with a barium sulfate standard has been recommended for routine performance of both the disc diffusion and the agar dilution test.

Principle

Light-scattering techniques have been utilized for many years to estimate the number of cells in a microbial suspension. The light scattered by particulate matter during its passage through the suspension causes the turbidity of a suspension. Accurate measurements of turbidity and hence bacterial growth can be obtained in two ways:

- By measuring the amount of scattered light directly, a procedure known as nephelometry. This is rarely used in practice.
- By measuring the light lost from the beam by scattering, a procedure known as turbidimetry. This can be measured easily by using Lambert Beer's law.

Latter method of turbidity estimation is the easiest and quickest way of calibrating a bacterial population. Standard suspension of insoluble barium sulphate precipitate described by McFarland (1907) and Brown (1919-20), which is a series of standards of different barium sulfate concentrations correspond to suspensions of different numbers of bacteria/ml.

Original McFarland standards were made by mixing specified amounts of barium chloride and sulfuric acid together. Mixing the compounds forms a barium sulfate precipitate, which causes turbidity in the solution. Other than barium sulfate various ionic compounds such as calcium carbonate and silver chloride have been used for many years for turbidity standards. Clays have been used widely. Representative of these are kaolinite, bentonite, illite and fuller's earth. These are generally unsatisfactory because suspensions must be shaken frequently, particle size changes with time, or turbidity is affected by light.

Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A $BaSO_4$ 0.5 McFarland standard may be prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard.
3. The Barium Sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.
4. These tubes should be tightly sealed and stored in the dark at room temperature.
5. The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appear, the standard should be replaced. Latex particle suspensions should be mixed by inverting gently, not on a vortex mixer
6. The barium sulfate standards should be replaced or their densities verified monthly.

Now there are McFarland standards prepared from suspensions of latex particles, which lengthens the shelf life and stability of the suspensions. The latex standards are prepared by suspending latex particles in a buffer solution. Sodium azide is added as a preservative. The standards are adjusted to an acceptable absorbance range with a one-centimeter light path set at 625nm. Adjusting bacterial suspension turbidity to a McFarland Standard produces bacterial counts in an expected range. As with the barium sulfate standards, a 0.5 McFarland Standard is comparable to a bacterial suspension of 1.5 X 10^8 CFU/ml. McFarland standard 1, 2, 3, 4 & 5are also comparable to 300, 600, 900, 1200 & 1500 millions CFU/ ml respectively.

Application and Interpretation

- In a tube of the same internal diameter as the standard a uniform suspension is prepared in saline of bacteria under test to a density greater than that required.
- A standard opacity tube of the required density is selected and shaken it vigorously until all the deposit is raised into uniform suspension.
- Bacterial suspension is compared with standard and viewed with oblique illumination against a dark background.
- The bacterial suspension is adjusted by dilution with saline until it matches the standard.

The approximate number of bacteria per milliliter can be estimated according to the opacity standard. But the numbers vary with the size of the bacteria.

Precautions

McFarland standard is used for *in vitro* purpose. It should be stored at 2-30ºC. It should be stored in original tube and should not be transferred from original tube.

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Moist Heat Sterilization By Using Autoclave

Sterilization is the freeing of an article from all living organisms, including bacteria and their spores.

Sterilization of culture media, containers and instruments is essential in microbiological work for isolation and maintenance of microbes. Sterilization can be done in a variety of ways, which can be conveniently categorized as follows:

(a) Physical Method- Sterilization can be done by heat, radiation and filtration. Again heat sterilization can be categorized into two:
- Dry Heat
- Moist Heat

(b) Chemical Methods

Moist Heat Sterilization

Moist-heat sterilization is achieved when water vapour at a definite temperature is introduced or generated at the level of the microorganisms to be inactivated and is maintained in such conditions for a definite time. Moist heat kills the organisms by coagulating and denaturing their enzymes and structural protein. Steam contacts a cooler surface, condenses, causing a huge decrease in volume and setting up a negative pressure that draws more steam. Condensation occurs as long as there is a temperature differential. Action of steam ensures: Surface heating, penetration, and protein coagulation. Sterilization by moist heat of the most resistant spores generally requires 121 °C for 15-30 minutes. Moist heat is used for the sterilization of culture media and all other materials through which steam can penetrate. Moist heat is more effective than dry heat. Sterilization can be done at lower temperatures in a given time at a shorter duration at the same temperature. Moist heat can be employed at temperatures below 100ºC, temperatures at 100ºC and temperature above 100ºC. Autoclaving is the most reliable method to do moist heat sterilization above 100ºC.

Autoclave

Autoclave is the method most widely used for sterilization of culture media and surgical supplies. It is a pressurized device designed to heat aqueous solutions above their boiling point to achieve sterilization. Charles Chamberland invented it in 1879. Under ordinary circumstances (at standard pressure), liquid cannot be heated above 100 °C in an open vessel. However, when water is heated in a sealed vessel such as an autoclave, it is possible to heat liquid water to a much higher temperature. As the container is heated the pressure rises due to the constant volume of the container. The boiling point of the water is raised because the amount of energy needed to form steam against the higher pressure is increased. The standard temperature and pressure for sterilization in autoclave is 121ºC at 15 lb/inch^2. This works well on solid objects; when autoclaving hollow objects, it is important to ensure that all of the trapped air inside the hollow compartments is removed. Autoclaves may achieve air removal by downward displacement, super atmospheric, transatmospheric or sub-atmospheric pulses.

Factors Influencing Sterilization by Heat

There are some factors, which influence the sterilization:
- Temperature and time- They are inversely related, shorter time is sufficient at high temperatures.
- Sterilization hold time- This is the time for which the entire load requires to be exposed to pure, dry, saturated steam at the effective temperature in order to ensure sterilization.
- Heat penetration time- Before the sterilization hold time can start, the load needs to be brought up to temperature. This is the heat penetration time. It varies with the different type of load.
- Number of microorganisms and spores-The number of survivors diminished exponentially with the duration of heating. It also depends on the species, strains and spore forming ability of the microbes.
- Depends on the nature of material- A high content of organic substances generally tends to protect spores and vegetative organisms against heat.

Operation Of Autoclave

Autoclave is used for sterilization as well as decontamination. Basic operating procedures for autoclave are arranging the load, setting of operating control, sterilization and unloading. Packaging is an important step for autoclaving. Some of the following points should be remembered while operating an autoclave:
- Volatile chemicals, radioactive compounds and sharps should not be kept for autoclave.
- Containers and autoclave bags should be utilized for autoclaving.
- Containers should not be overfilled.
- Packaging should be prepared in such a way that steam can penetrate easily.
- Incase of decontamination of biohazardous materials it must be capped at least with aluminum foils.
- The material to be autoclaved should not allow touching the sides or top of the chamber.

Various types of autoclaves are there like: a) Gravity displacement, b) Vacuum assisted, c) Simple transportable, d) Porous load. Gravity refers to the way steam enters from the top of the chamber and forces air out the drain at the bottom. The objective is to get all the air out of the chamber and replace it with steam. But in vacuum assisted model use a vacuum to remove air from the chamber before the steam is allowed to enter. In simple transportable autoclave the air is removed by turbulent displacement. In porous load autoclave the air may be extracted by an oil-seal high vacuum pump or be diluted out by a succession of steam pulses alternating with the drawing of a partial vacuum. Packaged and wrapped goods, linen and instruments with lumens

where there may be difficulty in removing the air or allowing sufficient steam penetration, can be effectively processed only in a porous load or multipurpose autoclave.

Safety Instructions

Some of the safety instructions should follow while handling an autoclave:

- Personal protective equipments (PPE) like laboratory coat, globes, eye protecting shield; closed toed shoes should be used during the unloading of autoclave.
- The door (lid) should be closed tightly during the time of loading.
- Chamber pressure should be checked before opening the autoclave.
- The door (lid) should be opened slowly to remove the steam.

Validation Approaches

The validation of moist heat sterilization processes may be performed using any of the three strategies outlined below. The approach selected should be appropriate and adequately supported.

a) Prospective Validation- This approach applies to new or modified processes and new equipment. The studies are conducted, evaluated and the process & equipment system certified prior to initiating routine production.

b) Concurrent Validation- This approach applies to existing processes and equipment. Concurrent validation studies are conducted during regular production and should only be considered for processes, which have a manufacturing and testing history indicating consistent quality production.

c) Retrospective Validation- This approach can only be applied to existing products, processes and equipment and is based solely on historical information. Normal processing records generally lack sufficient detail to permit retrospective validation.

Each stage of the evaluation of the effectiveness and reproducibility of a sterilization process should be based on a pre-established and approved detailed written protocol. A written change control procedure should be established to prevent unauthorized change to the protocol or process and restrict change during any phase of the studies until all relevant data are evaluated. The protocol should specify the following in detail:

- The process objectives in terms of product type, batch size, container/closure system, and probability of survival desired from the process;
- Pre-established specifications for the process, which include the cycle time, temperature, pressures and loading pattern;
- A description of all of the equipment and support systems in terms of type, model, capacity and operating range;
- The performance characteristics of each system, sub-system or piece of equipment in performance characteristics including pressure gauge sensitivity and response, valve operation, alarm systems functions, timer response and accuracy, steam flow rates and/or pressures, cooling water flow rates, cycle controller functions, door closure gasketing, and air break systems and filters;
- For new equipment: installation requirements and installation check points for each system and sub-system;
- For existing equipment, the necessary upgrading requirements or any compensatory procedures; justification for alternate procedures should be available;
- A description of the following studies like bioburden determination studies, empty chamber heat distribution studies, container mapping studies, loaded chamber heat penetration studies, microbiological challenge studies.

Indicating devices used in the validation studies or used, as part of post-validation monitoring or requalification must be calibrated. Physical and chemical indicators should be tested to demonstrate adequate predetermined response to both time and temperature. Detailed written test procedures and records of test results should be available. The indicators should be used before a written expiry date and stored to protect their quality. Biological indicators should be tested according to detailed written procedures for viability and quantitation of the challenge organism and for the time/temperature exposure response. For commercial indicators, a certificate of testing for each lot indicating the "D" value of the lot should be available. The biological indicator should be used before expiry and adequately stored. Following steps should be followed for validation testing:

- All autoclaves are to be run with cycle log recorders, which ensure that temperature parameters are achieved. Paper copies of the results are to be maintained for a period of 1 year. Any deviations are to be addressed.
- All loads (sterilization and decontamination) are to include items labeled with temperature sensitive tape. Any load where the tape has not changed color will be re-autoclaved with fresh tape.
- All autoclaves will have biological indicator testing performed to confirm efficacy of decontamination cycles. *Bacillus stearothermophilus* is used as biological indicator. Typical biological indicators for combined biological indicator/bioburden based steam sterilization models include: *B. stearothermophilus, C. sporogenes, B. coagulans* and *B. subtilis.*

Applications

Autoclaves are found in many medical settings and other places that need to ensure sterility of an object. Autoclaving is often used to sterilize medical waste prior to disposal in the standard municipal solid waste stream.

References

Practical Medical Microbiology, Mackie & McCartney, 13th Edition 1989, Edited by J.G. Collee, J.P. Daguid.

In this issue Bug Of the Month article focused on *Salmonella* species and **Microxpress** offers following Kit and dehydrated culture media for the isolation and identification of *Salmonella*:

- ***Salmonella* Identification Kit**
  A biochemical identification kit contains 12-miniature test panel.
- **SS Agar**
  A differential and selective medium for isolation of Salmonella and some Shigella species from clinical and non-clinical specimens.
- **Tetrathionate Brilliant Green Bile Broth**
  A medium for isolation and identification of *Salmonella*.
- **Tetrathionate Bile Brilliant Green Broth IP**
  A medium for isolation and identification of *Salmonella* in compliance with IP.
- **Tetrathionate Bile Brilliant Green Broth (Broth Medium I) EP**
  A medium for isolation and identification of *Salmonella* in compliance with EP.
- **Tetrathionate Bile Brilliant Green Broth (Broth Medium I) BP**
  A medium for isolation and identification of *Salmonella* in compliance with BP.
- **Tetrathionate Broth Base Medium USP**
  A medium for selective enrichment of *Salmonella* in compliance with USP.
- **Tetrathionate Broth Base, Hajna**
  A medium for selective enrichment of *Salmonella*, particularly in food and dairy products prior to isolation.

According to the Did You Know article related to McFarland Standard Microxpress recommends the following kit:

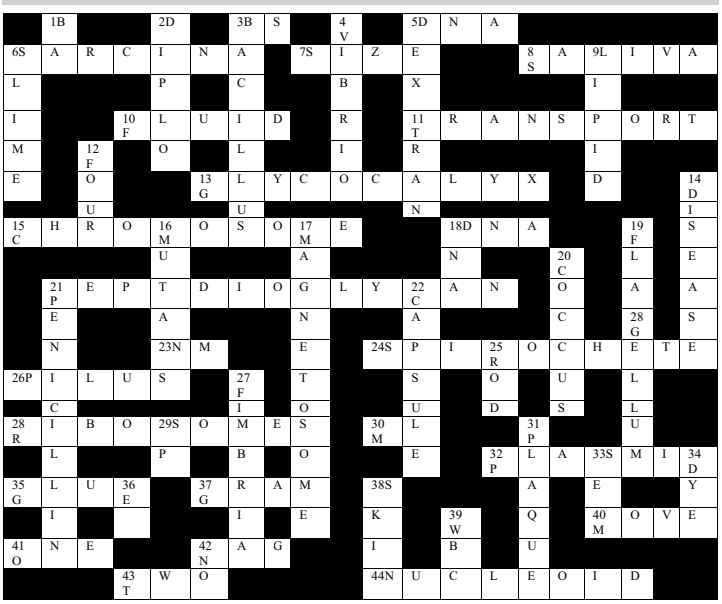
- **McFarland Standard No. 0.5**
  Turbidity standard, used in antimcrobial susceptibility testing.

In Mini Review we have discussed about nosocomial infection, and surgical site infections (SSI) were most common nosocomial infection. An important SSI prevention measure would include techniques directed at reducing microbial flora by localized skin prepping at the surgical site.

Our Current Trends topic is related to chlorhexidine gluconate: A superior antiseptic than povidone iodine. According to our articles **BioShields** recommends following antiseptic solutions for preparation and post closure of skin to prevent the nosocomial infection.

Skin Preps

- **Surgiprep- CHX™**
  It is an antiseptic solution for preparation and post closure of skin. Surgiprep-CHX™ can be used in major & minor surgical procedures. It can also be used in dermatological applications. It contains 2.5% v/v chlorhexidine gluconate solution IP, 63% v/v isopropyl alcohol IP and brilliant green as colour. Suriprep- CHX™ has following features:
  - Rapid Disinfection
  - Degreasing Effect
  - High Residual Effect
  - Quick Drying
  - Effective, Fugitive Skin Marking
  - Few Steps, Good Results
- **Surgiprep™**
  Surgiprep™ contains 2.5% w/v benzalkonium chloride solution IP, 63% v/v isopropyl alcohol IP and sunset yellow FCF as colour. It has the same features like Surgiprep-CHX™.



Highlights of the coming issue

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