JOURNAL OF
HYGIENE SCIENCES
Committed to the advancement of Clinical & Industrial Disinfection & Microbiology
VOLUME - IV ISSUE - I JAN-FEB 2011

Contents

Editorial

As 2010 steps out and ushers in 2011, the Journal continues to provide its readers with the best topics that are not only important but are also beneficial to individuals from the health sector.

This year's first issue opens to, Wound Management as the topic for the 'Mini Review' Wound management is a challenging job. One of the first steps in wound healing and management is to determine the underlying etiology followed by local wound care. All wounds are treated differently depending on how they occurred and their severity.

Vaccines have continuously evolved from its nascent composition to the more sophisticated and effective prophylactic measures available today. Current Trends focuses on Vaccines and Its Impact. The article describes the evolution of the wide array of vaccines. Vaccines serve not only to prevent diseases but have also served in the eradication of fatal infections such as small pox. Therefore these have evidently decreased morbidity and drastically reduced mortality.

In Profile comprises of excerpts on the life and achievements of Hermann Joseph Muller, who is best known for his remarkable discovery of the physiological and genetic effects of radiation, which earned him the Nobel Prize in 1946.

Relax Your Mood is provided with humor to cheer you, thoughts to live by and a quick quiz that will make you recall your current issue readings.

Bug of the Month, *Vibrio cholerae* is the etiological agent of cholera. Cholera is characterized by rice-water like stools and is responsible for excessive loss of body fluids. The corrective as well as therapeutic remedy for the same is Oral Rehydration Solution as recommended by the W.H.O. Cholera can be fatal if not treated immediately.

Did You Know emphasizes on the different Antimicrobial Properties of Tea. The use of tea is clearly still a long way from clinical application, but there are promising leads in the dental context. The concept of being able to exploit an antimicrobial agent which is a new chemical entity found in an abundantly available and renewable source is indeed a beguiling one.

Best Practices have to be implemented especially when the item/s in question are food. Food, Hygiene and Microbiology are crucial aspects that must be critically assessed and maintained in order to prevent instances of food poisoning and casualties.

We are immensely thankful to all our customers, well wishers and readers for their co-operation and support and look forward to exploring new horizons with you, in the year ahead. We wish you a Happy New Year, with Fresh Beginnings, Pretty Rainbows, Lovely Smiles, Blooming Flowers, Soft Fragrances, Warm Hugs, and a lot of happiness and prosperity to make it a cheerful experience.

Wound Management

Skin is the largest organ in the human body. The primary function of normal intact skin is that it can control microbial populations living on skin surface from entering underlying layers or organs and thus protects the body from pathogens. Skin also plays a crucial role in sustaining life through regulation of water and electrolyte balance, thermoregulation and resistance against mechanical, thermal and chemical aggression. The skin is composed of three main layers – epidermis, dermis and hypodermis. The epidermis or outer layer is made of mostly dead cells with a protein called keratin. This makes the layer waterproof and is responsible for protection against the environment. The dermis or middle layer is primarily responsible for structure and support. The subcutaneous fat layer also known as hypodermis is primarily responsible for insulation and shock absorbency. Cells on the surface of the skin are constantly being replaced by regeneration from below with the top layers sloughing off. The repair of an epithelial **wound** is merely a scaling up of this normal process.

Exposure of subcutaneous tissue with a wound provides a moist and warm environment for microbial organisms. However factors such as wound type, depth, location, quality, level of tissue perfusion and anti-microbial efficacy or resistance is important for examining microbial effects on wounds.

When this skin barrier is disrupted due to any cause - ulcers, burns, neoplasm or trauma it cannot function adequately. A wound is defined as a break in the epithelial integrity of the skin. However, the disruption could be deeper, extending to the dermis, subcutaneous fat, fascia, muscle or even the bone.

Wounds are generally classified as, wounds without tissue loss (eg. in surgery), and wounds with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments eg. venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and dermabrasions. Wounds are also classified by the layers involved, superficial wounds involve only the epidermis, partial thickness wounds involve only epidermis and dermis, and full thickness wounds involve the subcutaneous fat or deeper tissue. Wounds are divided into acute (post traumatic wounds, burns, skin tears etc) and chronic wounds (leg ulcers, pressure ulcers, diabetic foot ulcers).

Acute wounds: are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries. Acute wounds are mainly divided into surgical and traumatic wounds.

Surgical wounds: are skin incisions, made with the objective of eliminating a skin lesion or creating a path for reaching deep organs. Surgical incisions cause minimal tissue damage. They are made with precision in an environment where aseptic and antiseptic techniques reduce the risk of infection, with the best of instruments and the facility to control hemostasis. Penetrating trauma may involve minimal damage to skin and connective tissue, though deeper damage to vessels, nerves, and internal organs may occur.

Traumatic wounds: are contused wounds characterized by - torn and irregular edges, presence of devitalized tissue fragments, presence of foreign matter (gravel, etc.).

Types of Surgical and Traumatic Wounds

Type of Wound	Cause	Result
Incision	Surgical (rarely trauma)	Penetrating
Laceration	Usually trauma	Torn tissue
Confusion	Usually trauma, skin may be intact	Extensive tissue damage
Abrasion	Usually trauma	Superficial epithelial
Combination	Usually severe trauma	Life threatening

A **chronic wound** is the loss of cutaneous substance resulting from a pathological process which progresses over an indeterminate time period. The normal process of healing is disrupted at one or more points in the phases of hemostasis, inflammation, proliferation and remodelling. Chronic wounds result from various causes, including venous (chronic venous leg ulcers or CVLUS), arterial, neuropathic, pressure, vasculitis and burns. Although chronic ulceration can affect any anatomical region, the most common site is the lower limb. Ulcers secondary to venous hypertension and venous insufficiency accounts for nearly 70 per cent of all leg ulcers, with diabetes and arterial disease contributing towards a significant proportion of the rest. As all these conditions are more prevalent in older people they are more susceptible to leg ulcers. In addition, the increased occurrence and longevity of these ulcers are further compounded by the detrimental effects aging has on the skin and the wound healing process.

Restoration of tissue continuity after injury is a natural phenomenon, infection, quality of healing, speed of healing, fluid loss and other complications that enhance the healing time represents a major clinical challenge. Majority of wounds heal without any complication. However, chronic non-healing wounds involving progressively more tissue loss gives rise to the biggest challenge to wound-care product researchers. Unlike surgical incisions where there is very little tissue loss and easy to heal, chronic wounds disrupts normal process of healing and is often not sufficient in itself to effect repair. Delayed healing is generally a result of compromised wound physiology and typically occurs with venous stasis, diabetes, or prolonged local pressure. Second major challenge is the prevention of scarring, keloid formation or contractures and a cosmetically acceptable healing.

Normal wound healing involves a complex and dynamic but superbly orchestrated series of events leading to the repair of injured tissues. A completely healed wound, usually seen after simple injury, is defined as one that has returned to its normal anatomical structure, function and appearance within a reasonable period of time. It is also defined as one that has attained complete skin closure without drainage or dressing requirements. In contrast to these some wounds fail to heal in a timely and orderly manner, resulting in chronic, non-healing wounds.

Wound healing process may be broadly divided into five continuous phases, namely hemostasis, inflammation, proliferation ,maturation or remodeling and epithelialisation. The platelets present in the exposed blood aggregates and a temporary plug is formed reducing bleeding (hemostasis). The phagocytes act to clear debris and destroy the ingested material (inflammation). New vessels are formed and carry oxygenated blood to the wound bed. The fibroblast cells lay down a network of collagen fibers surrounding the neovasculature of the wound (proliferation). Next the process of remodeling of the collagen fibers laid down in the proliferation phase occurs. Finally the process of laying down new skin or epeithelial cells takes place. Skin forms a barrier between the outer environment and the healed wound.

Acute wounds are expected to heal within a predictable and specified time frame and with minimal intervention although in severe cases such as gunshot wounds, anti-microbial therapy or surgical intervention may be necessary. In contrast, chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue. Pathophysiological abnormalities that may predispose to the formation of chronic wounds such as leg ulcers, foot ulcers, and pressure sores include compromised tissue perfusion as a consequence of impaired arterial supply (peripheral vascular disease) or impaired venous drainage (venous hypertension) and metabolic diseases such as diabetes mellitus.

Wound management is a challenging job. One of the first steps in wound healing and management is to determine the underlying etiology followed by local wound care. All wounds are treated differently depending on how they occurred and their severity. There are signs and symptoms to look in wound infection management. Superficial wounds like scraps are obvious to the eye. They may have more of a slow bleeding and are usually caused by abrasive surfaces. Lacerations go through all of the skin layers and the bleeding may be much greater. Puncture wounds are often caused by sharp pointed objects such as knives or glass. Puncture wounds can sometimes be caused by humans or animal bites as well.

An important part of wound management is realizing the potential dangers of wound infection. Surgery itself carries a 1 to 5% risk of wound infection and if proper care is not taken, there is a 27% chance of endogenous contamination. Wound infection and presence of pathogens in the skin and body are primarily responsible for delayed wound healing although host immune response and local environmental factors such as tissue necrosis, hypoxia and ischemia impair immune cell activity. Antiseptics, antibiotics, antimicrobial therapy, vacuum assisted wound closure, enzymatic and surgical debridement, pressure reduction in wounds and complementary and alternative therapies are the common techniques of wound management.

Tissue viability is considered as a growing specialty that primarily addresses all aspects of skin and soft tissue wounds including acute surgical wounds, pressure ulcers, and leg wounds and ulceration. Tissue viability is not just restricted to wound management and covers professional aspects of wound care, nursing and also a wide range of organizational, political and socioeconomic issues. Wound management and tissue viability are intricately related. Healing process in acute wounds has been extensively studied and the knowledge obtained from these studies have been used for the care of chronic wounds with the assumption that non healing chronic wounds suggest an aberration of the normal tissue repair process. However the healing process associated with chronic wounds is quite different from that of acute wounds. Usually in chronic wounds, the sequence of events which lead to repair in acute cases becomes stuck or disrupted at different stages of the healing process and before the normal healing process could be resumed, the barrier to the healing process has to be recognized and correct techniques have to be applied. Thus for appropriate understanding of the healing process and the interventions necessary to speed up healing and to repair chronic wounds, it is necessary to understand the underlying molecular events.

Wound bed preparation is the management of wound that accelerates endogenous healing and facilitates the effectiveness of therapeutic measures and is an important concept in wound management. Wound bed preparation is an educational tool in wound management and several key issues form part of wound management and tissue viability. These include status of wound bed preparation, analysis of acute and chronic wound environment, wound bed preparation in the clinic, cellular components of the wound bed preparation concept, and analysis of the components of wound bed preparation. The most significant advancement in wound care came with Winter's study in 60's, which showed that occluded wounds healed much faster than dry wounds and moist wound healing environment optimized the healing rates. He demonstrated that when wounds on pigs are kept moist, epithelialisation is twice as rapid as on wounds allowed to dry by exposure to air. Later Hinman and Maibach confirmed Winter's work on human beings in 1963. An open wound, which is directly exposed to air, will dehydrate and a scab or scar is formed. This forms a mechanical barrier to migrating epidermal cells and are then forced to move in a deeper level of tissue, which prolongs the healing process. Moist healing prevents the formation of scab as the dressing absorbs wound exudate secreted from the ulcer.

Current wound management techniques include

1. Hydro balanced dressings containing PHMB (Polyhexamethylene Biguanide)

- Absorbs exudate
- Prevents tissue dehydration & cell death (HydroBalance)
- Reduces pain
- Promotes wound closure
- Minimizes microbial burden levels
- Reduced chances of pathogenic organisms developing resistance, due to nonspecific mechanisms of action of **PHMB**

2. Hydrocolloid dressings & Hydrogels

- As they are occlusive, hydrocolloid dressings do not allow water, oxygen, or bacteria into the wound
- Hydrocolloids cannot be used if the wound or surrounding skin is infected
- Hydrogels contain up to 95% water
- Maintain the moist wound environment
- Can not absorb much exudate and should be reserved for dry wounds or wounds with minimal to moderate drainage

3. Alginates & Absorptive dressings

- Absorb moderate to high amounts of wound drainage
- Are not recommended for wounds with light exudate

- Should not be used on dry wounds or wounds with minimal drainage as it could dehydrate the wound, delay healing
- In dry wounds this dressing does not form gel and may adhere to granulation tissue causing trauma

4. Dressings impregnated with collagen, silver, zinc, or other factors

- Stimulates wound healing
- Silver compounds cause argyria (discoloration)
- Silver ions have been linked to cell toxicity
- Dressings containing silver can absorb large amounts of fluid but do not hydrate and are not appropriate for dry wounds

5. Composite dressings

- Composite dressings have multiple layers
- Appropriate for wounds with minimal to heavy exudate, healthy granulation tissue, necrotic tissue (slough or moist eschar), or a mixture of granulation and necrotic tissue
- Composite dressings need to be used cautiously if the patient is dehydrated or has fragile skin

6. Foam dressings containing PHMB

- Uniform dispersion of **PHMB** within the matrix
- As exudate is absorbed into the dressing & structural changes occur, **PHMB** attacks bacteria in the wound fluid & minimizes microbial burden levels
- **PHMB** prevents tissue dehydration & cell death (HydroBalance)
- **PHMB** reduces pain
- **PHMB** promotes wound closure
- Reduced chances of pathogenic organisms developing resistance, due to nonspecific mechanisms of action of **PHMB**

7. Antibiotics

Antibiotics effective against Gram-positive, Gram-negative organisms and also both aerobic and anaerobic organisms are needed to treat severe or limb-threatening infections. Patients with such wounds need to be hospitalized and treated with intravenous antibiotics. Mild to moderate infections with localized cellulitis are generally treated on an outpatient basis with oral antibiotics.

Although antibiotics are, in general, considered safe and well-tolerated, they have been associated with a wide range of adverse effects. The effects can be very serious depending on the antibiotics used and the microbial organisms targeted. The safety profiles of newer medications may not be as well established as those that have been in use for many years. Adverse effects can range from

- Fever and nausea
- Major allergic reactions including photodermatitis and anaphylaxis.
- Diarrhea caused by the anaerobic bacterium *Clostridium difficile*, which results from the antibiotic's disruption of the normal balance of the intestinal flora.
- An antibiotic-induced disruption of the population of the bacteria normally present as constituents of the normal vaginal flora may also occur, and may lead to overgrowth of yeast species of the genus Candida in the vulvo-vaginal area.
- Side-effects can result from interaction with other drugs, such as elevated risk of tendon damage from administration of a quinolone antibiotic with a systemic corticosteroid.
- Certain antibiotics administered (e.g.aminoglycosides, vancomycin) can cause significant permanent hearing loss.

The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have an enhanced ability to survive doses of antibiotics that would have previously been lethal.

- Antibiotics like Penicillin and Erythromycin, which used to be one-time miracle cures are now less effective because bacteria have become more resistant.
- Antibiotics themselves act as a selective pressure that allows the growth of resistant bacteria within a population and inhibits susceptible bacteria. Antibiotic selection of pre-existing antibiotic resistant mutants within bacterial populations was demonstrated in 1943 by the Luria–Delbrück experiment.
- Survival of bacteria often results from an inheritable resistance. The underlying molecular mechanisms leading to antibiotic resistance can vary. Intrinsic resistance may naturally occur as a result of the bacteria's genetic makeup.
- The bacterial chromosome may fail to encode a protein that the antibiotic targets. Acquired resistance results from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. Antibiotic-producing bacteria have evolved resistance mechanisms that have been shown to be similar to, and may have been transferred to, antibiotic-resistant strains.
- The spread of antibiotic resistance mechanisms occurs through vertical transmission of inherited mutations from previous generations and genetic recombination of DNA by horizontal genetic exchange. Antibiotic resistance is exchanged between different bacteria by plasmids that carry genes that encode antibiotic resistance that may result in co-resistance to multiple antibiotics. These plasmids can carry different genes with diverse resistance mechanisms to unrelated antibiotics but because they are located on the same plasmid multiple antibiotic resistance to more than one antibiotic is transferred. On the other hand, cross-resistance to other antibiotics within the bacteria results when the same resistance mechanism is responsible for resistance to more than one antibiotic is selected for.

Use of antibiotics should be reserved for systemic infections. Inappropriate use of antibiotics increases chances of development of resistant strains. This has led to a renewed interest in the use of antiseptics (for topical use only) for wound management. Antiseptics can provide a useful alternative to antibiotics.

8. Antiseptics

Antiseptics destroy or inhibit the growth and development of microorganisms in or on living tissue. Unlike antibiotics that act selectively on a specific target, antiseptics have multiple targets and a broader spectrum of activity, which include bacteria, fungi, viruses, protozoa, and even prions. Antiseptics are also considered superior to topical antibiotics when their rates of causing contact sensitization are compared.

Antiseptics have been commonly used on wounds to prevent or treat infection. However, due to the cytotoxicity, delay in wound healing and safety issues of several antiseptics (povidone iodine, iodine, silver), their use is **generally** avoided on open wounds. Use of safe and non cytotoxic antiseptics (such as **PHMB**) on open wounds helps in prevention and treatment of infection

which increases the rate of the healing process. The main rationale for using antiseptics on open wounds is prevention and treatment of infection and, therefore, increased rate of the healing process. It is established that infections may delay healing, cause failure of healing, and even cause wound deterioration. Microbial pathogens delay wound healing through several different mechanisms, such as persistent production of inflammatory mediators, metabolic wastes, toxins and maintenance of the activated state of neutrophils, which produce cytolytic enzymes and free oxygen radicals. Prolonged inflammatory response contributes to host injury and delays healing. Moreover, bacteria compete with host cells for nutrients and oxygen necessary for wound healing. Wound infection can also lead to tissue hypoxia, render the granulation tissue hemorrhagic and fragile, reduce fibroblast number and collagen production, and damage the process of reepithelization.

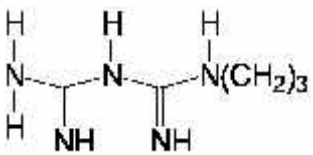
All chronic wounds are colonized by bacterial population, bacterial number above a critical concentration can decrease the wound healing rate and may have deleterious effects on the wound healing process. Increased bacterial numbers in pressure ulcers have been implicated as significant participants of chronic ulceration. Several studies have demonstrated that bacterial number above 105 or 106 organisms per gram can cause local disease to skin or can delay wound healing.

Antibiotic resistance of skin wound flora has emerged as a significant problem, and measures to prevent it should be taken. Generally, antiseptics aim at eliminating all pathogenic bacteria of the wound, while antibiotics are effective only to certain bacteria that are sensitive to them. Although resistance towards antiseptics has been reported, it is to a significantly lesser degree than reported with antibiotic usage. Payne, *et al.,* state that the sensible use of antiseptics could help decrease the usage of antibiotics, preserving their advantage for clinically critical situations. Antiseptics are also considered superior to topical antibiotics when their rates of causing contact sensitization are compared.

It is evident that wound healing is a complex and dynamic process of restoring cellular structures and tissue layers and requires a sophisticated local wound management. **Thorough and gentle wound cleaning,** keeping the wound moist and necrosis and detritus-free, are **crucial for wound healing. Successful wound management** involves -

- **Adequate antisepsis:** The antiseptic used should be able to perform adequate antisepsis & disinfection. Traditional antiseptics score low in this point due to resistance development, low bioburden tolerance and cytotoxicity.
- **Reduced inflammation**
- **Epithelialisation:** This step involves regeneration of epithelial cells of the skin. Epithelialisation is a critical factor in wound-healing process in all wounds, particularly in chronic wounds like diabetic foot ulcers & burn wounds. Therefore, the antiseptic/topical solution or cream used should be such that the wound ecosystem remains conducive to epithelialisation. Unfortunately, the traditional antiseptics fail miserably in this area too. PVI solution, the most common antiseptic is cytotoxic and actually delays the wound-healing process.
- **Hydrobalance:** This is another critical factor for the wound healing process. Adequate moisture should be present in the wound ecosystem to facilitate rapid epithelialisation & healing.
- **Not harming the cells involved in the repair process**
- **Patient-compliance: The antiseptic solution used should be non-irritating and non-staining. It should also not be malodorous.**

PHMB is a polymeric biguanide. Biguanides are an important class of cationic surface active antimicrobial agents, which are used as **antiseptics & disinfectants**. **PHMB** is a heterodisperse mixture of polymers. The basic molecular chain of **PHMB** can be repeated from two to 30 times, with increasing polymer chain length correlating with increasing antiseptic/antimicrobial efficacy. Unlike iodophores, biguanides are active even in presence of interfering substances such as blood & serum which makes them ideal for use in wound management.



PHMB is active against broad spectrum of microorganisms including antibiotic resistant bacteria such as MRSA & VRE, viruses (HIV, HBV), pathogenic food borne organism such as E. coli 0157 & *Campylobacter* sp. The fact that biguanide groups are separated by a C6 aliphatic hydrocarbon chain gives **PHMB** a particularly high efficacy power on a wide range of micro-organism.

PHMB is a synthetic compound structurally similar to naturally occurring **antimicrobial peptides (AMPs). AMPs** are important in innate immune response, are produced by the majority of living organisms, have a broad spectrum antimicrobial activity and have been suggested as therapeutic alternatives to antibiotics.

Management of microbial burden and moisture from exudate in chronic wounds are two of the most important aspects of wound bed preparation. It is difficult to create a moist environment conducive to wound healing without promoting excessive bacterial growth that may interfere with normal healing. Wound infection may delay healing and even cause wound deterioration by prolonging the inflammatory stage, competing for nutrients and oxygen, and leading to tissue hypoxia with subsequent increased fragility of granulation tissue, reduction of fibroblasts and collagen, can lead to the damage of re-epithelialisation.

Typically, neutral physiological solutions are used to create a moist environment. **Latest research shows that, use** of safe and non cytotoxic antiseptics on open wounds helps in prevention and treatment of infection which increases the rate of the healing process. Antiseptics that provide better moisture and manage the bacterial load may not only improve the wound environment but also might increase patient comfort, improve quality of life and lower the cost of nursing care.

PHMB containing wound rinsing solutions are superior to salt solutions (saline or Ringer solution) as wounds treated with **PHMB** containing wound rinsing solution show superior wound healing due to its antimicrobial efficacy as well as its ability to maintain the moist environment. In wound care specifically,

PHMB has previously been demonstrated to block *Pseudomonas aeruginosa*-induced infection (Cazzaniga *et al.,* 2000) and prevent degradation of wound fluid and skin proteins *in vitro* (Werthen *et al.,* 2004). It can also kill a diverse range of bacteria and fungi (Lee *et al.*, 2004).

Wound healing also requires great care to ensure no damage or harm to vital and especially to naturally functionally important structures. Keeping this objective in mind, the application of **PHMB** containing wound rinsing solutions seems to be a highly appropriate concept in supporting these effects and to promote wound healing. **PHMB** has good tissue compatibility based on its activity against the acid lipids contained within the bacterial cell membranes and minor effect on the neutral lipids of human cell membranes. This helps to prevent damage to the surrounding healthy tissue**.**

In vitro and *in vivo* studies into the effectiveness of **PHMB** in wound care have demonstrated that the product has many benefits in wound management. Daeschlein *et al.,* (2007) reported that the product may reduce pain and malodour, while Mueller and Krebsbach (2008) found that its use reduced fibrin slough and prevented the build-up of necrotic tissue and so promoted connective tissue regeneration. Wiegand *et al.,* (2008) demonstrated that **PHMB** can have a positive effect on tissue proliferation.

Advantages of PHMB in wound management

- ✓ Uniform dispersion within the matrix
- ✓ Does not damage or harm the surrounding healthy tissue
- ✓ Promotes epithelisation (regeneration of epithelial cells of the skin) & healing
- ✓ Maintains hydrobalance in the wound
- ✓ Non-irritating/non-staining & non-malodourous
- ✓ Not cytotoxic to human cells
- ✓ No skin sensitization/irritation
- ✓ Can be used for thyroid patients
- ✓ Very high safety profile (LD_{50} >2000 mg/kg body weight)

Use of **PHMB** based antiseptics, in the form of gels, wound irrigants and dressings, is ideal in care and management of wounds including chronic wounds, burns and diabetic foot ulcers. Over the past years end-use (ready-to-use) wound care products containing **PHMB** have been successfully launched including wound rinsing solutions, wound gels and dressings. Special features qualify **PHMB** for growing and effective application in wound care management.

Encyclopedia

Bacteremia is the presence of bacteria in the blood. Blood is normally a sterile fluid, so the detection of bacteria in the blood is always abnormal. There are various routes which serve as portals of entry of the bacteria into the bloodstream like pneumonia or meningitis, during a surgery, or the introduction of catheters and other foreign bodies including intravenous drug abuse.

Bacteremia can have many consequences. The immune response to the bacteria can cause sepsis and septic shock, which has a relatively high mortality rate. Bacteria can also be transported by blood to various parts of the body (referred to as hematogenous spread), thus causing infection away from the original site of infection.

Causes: Several types of bacteria live on the surface of the skin or colonize the moist linings of the urinary tract, and other internal surfaces. These bacteria are normally harmless as long as they are kept in check by the body's natural barriers and the immune system. People in good health with strong immune systems rarely develop bacteremia. However, when bacteria are introduced directly into the circulatory system, especially in a person who is ill or undergoing aggressive medical treatment, the immune system may not be able to cope with the invasion, and symptoms of bacteremia may develop. Therefore bacteremia is most common in individuals who are already affected by or being treated for some other medical problem. In addition, medical treatment may bring a person in contact with new types of bacteria that are more invasive than those already residing in that person's body, further increasing the likelihood of bacterial infection.

Conditions which increase the chances of developing bacteremia include: ● Immune suppression, either due to HIV infection or drug therapy. ● Antibiotic therapy which changes the balance of bacterial types in the body. ● Prolonged or severe illness. ● Alcoholism and other drug abuse. ● Malnutrition. ● Disease or drug therapy that causes ulcers in the intestines.

Common immediate causes of bacteremia include: ● Drainage of an abscess, including an abscessed tooth. ● Urinary Tract Infections, especially in the presence of a bladder catheter. ● Decubitus ulcers (pressure sores). ● Intravenous procedures using unsterilized needles, including IV drug use. ● Prolonged IV needle placement. ● Use of ostomy tubes, including gastronomy (surgically making a new opening into the stomach), jejunostomy (surgically making an opening from the abdominal wall into the jejunum), and colostomy (surgically creating an artificial opening into the colon).

The bacteria most likely to cause bacteremia include members of the Staphylococcus, Streptococcus, Pseudomonas, Haemophilus, and Escherichia genera.

Symptoms: Fever over 101 ^{o}F (38.3 ^{o}C), Chills, Malaise, Abdominal pain, Nausea, Vomiting, Diarrhea, Anxiety, Shortness of breath, Confusion.

Diagnosis: Bacteremia is diagnosed by culturing the blood for bacteria. Samples may need to be tested several times over several hours. Blood analysis may also reveal an elevated number of white blood cells. Blood pressure is monitored closely; a decline in blood pressure may indicate the onset of septic shock.

Treatment: Bacteremia may cause no symptoms, but may be discovered through a blood test for another condition. In this situation, it may not need to be treated, except in patients especially at risk of infection, such as those with heart valve defects or whose immune systems are suppressed.

Prognosis: Prompt antibiotic therapy usually succeeds in clearing bacteria from the bloodstream. Recurrence may indicate an undiscovered site of infection. Untreated bacteria in the blood may spread, causing infection of the heart (endocarditis or pericarditis) or infection of the covering of the central nervous system (meningitis).

Prevention: Bacteremia can be prevented by preventing the infections which often precede it. Good personal hygiene, especially during viral illness, may reduce the risk of developing bacterial infection. Treating bacterial infections quickly and thoroughly can minimize the risk of spreading infection. During medical procedures, the burden falls on medical professionals to minimize the number and duration of invasive procedures, to reduce patients' exposure to sources of bacteria when being treated, and to use scrupulous technique.

Vaccines and their Impact

Vaccine development, which began with Edward Jenner's observations in the late 18th century, has entered its 4th century. From its beginnings, with the use of whole organisms that had been weakened or inactivated, to the modern-day use of genetic engineering, it has taken advantage of the tools discovered in other branches of microbiology.

Like the consumption of clean water has dramatically reduced mortality, so has the advent and the use of vaccines. It is however significant to mention that there are many diseases for which there are no vaccines and nor candidate preparations for the elimination or prevention of certain communicable diseases. The lack or the difficulty in providing vaccines arises from the fact that either the preparations may not be safe or the presentation of the antigens is improper to elicit a desirable immune response.
Most of the current vaccines have been created by adaptation of living organisms to growth in conditions that attenuate their virulence, by preparation of suspensions of killed microbes or through the concentration and purification of proteins or polysaccharides from pathogens. Molecular biology has come forth like a new lease to vaccine sciences since it has provided multiple new tools with which vaccines can be developed. These tools may enable making use of more cellular immune responses in addition to the antibody responses that are key to the success of almost all of the vaccines now in use.

The era of attenuated vaccines started with the development of poxvirus vaccine related to smallpox used by Jenner, the physical attenuation of organisms by Louis Pasteur, and the use of passage in vitro or in vivo by workers early in the 20th century. The discovery in mid century of the technique of cell culture for viruses enabled the development of many live vaccines. Whereas for inactivated vaccines, the story began in the late 19th century with killed whole bacteria or viruses, a technique which continues to yield important biologicals. Later development depended on taking apart of the microbes and using extracts, purified proteins, purified polysaccharides, or detoxified extracellular products (toxoids). The chemical linkage of proteins to polysaccharides dramatically increased the immunogenicity of vaccines based on bacterial capsules.

New Strategies for Live Vaccines
Live attenuated vaccines have been among the most powerful for the purpose of disease control and even eradication, owing to the strong antibody and cellular responses elicited by them. However, they have also been associated with genetic instability and residual virulence. A number of strategies are now available for dealing with those issues, namely: reassortment, reverse genetics, recombination, deletion mutants, codon deoptimization, and control of replication fidelity.

Reassortment: is not really a new strategy but has been used frequently for the creation of influenza vaccine strains that bear new hemagglutinins (HAs) and also grow well in vitro and, more recently, for the development of a vaccine against the human rotaviruses that cause infantile gastroenteritis

Reverse genetics: involves the alteration of DNA complementary to viral negative RNA strands and reconstitution of the viruses through co-infection of cells with plasmids containing those cDNAs. The phenotypic characteristics of the resulting viruses can then be correlated with the genetic changes made to the cDNAs. In this way, new properties can be induced in many different negative-stranded RNA viruses, including that of attenuation.

Recombination: allows the insertion of desirable genes of one microbe into the genome of another. One example is the parainfluenza 3/respiratory syncytial virus (RSV) recombinant developed at the NIH. A parainfluenza virus was attenuated by reverse genetics and recombination with a naturally attenuated bovine parainfluenza 3 virus.

Numerous molecular strategies have been put forth to effect attenuation more rapidly than by passage in unnatural conditions in tissue culture or in animals that are not normal hosts. The simplest strategy employs deletion mutants, such as those used to attenuate dengue viruses or polioviruses through excision of portions of 5' noncoding regions. Other strategies include codon deoptimization, which attenuates by changing the original nucleotide triplets preferred by the virus to code for amino acids to triplets less often used to code for the same amino acids; enhancement of replication fidelity of error-prone RNA polymerases through mutation, thus reducing the likelihood of reversion from attenuation to virulence; and insertion of RNA into vaccine strains that are blocked by complementary micro-RNA in some host cells but not in others. These strategies can create new attenuated viruses for use in live vaccines.

DNA Plasmids
The serendipitous discovery that injection of DNA plasmids containing gene inserts of interest is followed by transcription and translation to proteins that are carried to antigen-presenting cells in the bone marrow was followed by optimism concerning the value of this strategy in vaccinology. That optimism has not been justified heretofore by the results of immunization of humans, chiefly because the induction of antibodies has been poor. However, recent results have indicated that this defect appears to be ripe for correction through the use of either of two modalities: electroporation or adjuvantation.
Electroporation uses an externally applied electrical field to increase the permeability of the cell plasma membrane, making it easier in molecular biology to introduce a new substance to the cell. This may be a new drug, a molecular probe, or a snippet of DNA. Through a variety of processes, electroporation causes a number of very small pores to open in the plasma membrane. With careful control of the strength of the electrical field and the duration of the cell's exposure to it, the pores will reseal themselves after a short period of time. Adjuvants that also enhance immunogenicity of vaccines including DNA plasmids consisting of poloxamers and cationic lipids. Through the use of these two modalities, protective levels of antibody against pathogens such as avian influenza virus and measles virus are induced.
Thus, the problems involving induction of B-cell-based immunity conferred by DNA plasmids may be solved, but in any case, DNA plasmids have the virtue of priming T-cell immunity and T-cell memory responses, even in the presence of passively

transmitted maternal antibodies. The latter property makes DNA potentially interesting for immunization of newborns. Of more interest, DNA is now clearly one of the best priming agents in prime-boost regimens.

Reverse Vaccinology

Reverse vaccinology is a term coined by Rino Rappuoli for the mining of microbial genomes, usually bacterial, to find proteins of vaccine interest. Biochemical, serological and microbiological methods have been used to dissect pathogens and identify the components useful for vaccine development. Although successful in many cases, this approach is time-consuming and fails when the pathogens cannot be cultivated in vitro, or when the most abundant antigens are variable in sequence. Now genomic approaches allow prediction of all antigens, independent of their abundance and immunogenicity during infection, without the need to grow the pathogen in vitro. This allows vaccine development using non-conventional antigens and exploiting non-conventional arms of the immune system. Since the process of vaccine discovery starts in silico using the genetic information rather than the pathogen itself, this novel process can be named reverse vaccinology. The process begins with the sequencing of a genome followed by computer analysis of DNA open reading frames (ORFs) to predict which will produce surface or secreted antigens. The candidate ORFs are then inserted into *E. coli* bacteria for expression of the corresponding proteins. The expressed proteins are used to immunize the mice from which serum samples are collected to test for bactericidal activity and surface localization. If the serum samples show that the protein is conserved in multiple bacterial strains, it can then be included in vaccine development.

Newer variations on reverse vaccinology include pan-genome reverse vaccinology and comparative genome analysis. The former consists of genomic sequencing of numerous strains of a particular organism in order to identify antigens present in common. These then become components of a vaccine that should cover all or almost all strains. This approach has been fruitful for group B streptococci. In comparative genome analysis, the objective is to identify antigens produced only in pathogenic strains, which is important for organisms that exist in both pathogenic and nonpathogenic forms.

Prime – Boost

These heterologous prime-boost immunizations elicit immune responses of greater height and breadth than can be achieved by priming and boosting with the same vector. The first immunogen initiates memory cells; the second immunogen expands the memory response, leading to an augmented immune response. Outside of the immune response to the common vaccine insert, which undergoes a tremendous boost, the two agents do not raise responses against each other and thus do not interfere with each other's activity. In AIDS vaccine development, DNA plasmids coding for HIV proteins have often been used as the prime, whereas poxviruses containing the genes for the same proteins have often been used as the boost.

Other miscellaneous strategies are also being analyzed.

Adjuvants

Adjuvants are added to many vaccines to increase their immunogenicity and efficacy. Aluminum salts (alum) have been widely used as adjuvants and are generally considered safe. The incorporation of antigens and adjuvants into nanoparticles and nanoemulsions is another way of increasing immune responses, as they can be taken up by antigen-presenting cells, even on a mucosal surface.

Aluminum salts however have their limitations in terms of adjuvant effect, and a wide range of novel adjuvants are now being evaluated for use in new or improved vaccines. These adjuvants include immunostimulators, microparticulate carriers and emulsions as well as various combinations of these.

Vaccine Manufacture

Over the past two centuries, immunization programs have led to the elimination and/or control of several different infectious diseases including smallpox, polio, measles, mumps and rubella. More recently, the past 30 years have seen significant changes both in the number of businesses involved in vaccine manufacture and the production systems used. These include fewer companies, higher costs, and a shift from egg-based to cell-based processes, to reduce allergenicity, optimize quality and increase output.

While more vaccines are now available than ever before, they have long presented special issues for producers, particularly regarding scale-up, affordability, and change of pace. Enduring challenges include ensuring good laboratory and manufacturing process, maintenance of product stability, and preparing a 100% safe and effective product. With time-to-market and flexibility increasingly important to improve cost-effectiveness, producers are becoming increasingly cognizant of the latest technologies to simplify development and manufacturing.

While the market remains dominated by a select group of key players, technology transfer is increasing between small biotech companies and big pharma. More significantly, with emerging markets set to dominate vaccine manufacturing by 2023, globalisation is being taken seriously as the quickest way to develop a product and increase market access.

Conclusion

While eradication of some historic diseases has led to a lower need for prophylaxis, increasing need for seasonal vaccines for influenza, emerging diseases such as West Nile and SARS, the need for vaccines for widespread diseases such as Malaria, the development and approval of vaccines for conditions such as HPV, and the resurgent threat of bioterrorism has given impetus to finding new and rapid methods for producing vaccines. Disease resistance of some strains of organisms, such as tuberculosis, difficulties in providing vaccines suitable for immunosuppressed patients, as well as the need to provide vaccines to greater numbers of people at lower cost, are all challenges facing manufacturers and governments alike.

In the 21st century, a number of problems stand out as the biggest in vaccinology viz.: immaturity of responses in the newborn owing to poor antigen processing; postmaturity of responses in the elderly owing to deficiencies of naïve T cells; maintenance of both effector and central memory cells after the antigen is no longer present; adjuvants capable of selectively stimulating distinct cell types such as B, Th1, Th2, Th17, Treg, $CD4^+$, $CD8^+$, or dendritic cells; and mucosal immunization with nonreplicating antigens. These are large problems, but as stated by Maurice Hilleman, "Vaccinology is a field in which dreams may be turned into realities. It is an activity which is heavily overshadowed by uncertainties, but can be conquered by persistent rational pursuits and by selective choices needed to surmount the hills and mountains in the quest"

Reference: Vaccines: The Fourth Century by Stanley A. Plotkin

Hermann Joseph Muller

Birth: December 21, 1890

Death: April 5, 1967

Nationality: American

Known for: Physiological and genetic effects of radiation (X-ray mutagenesis)

Hermann Joseph Muller was born in New York City on December 21, 1890. His father, born in New York, had continued the grandfather's art metal works (the first in the U.S.A.), but was not by inclination a business man, and, although he died in 1900, he early awoke in the boy a lively interest in the nature of the universe and in the process of evolution, as well as in the welfare of men in general. The boy's mother, Frances Lyons Muller, had also been born in New York City. She, as well as the father, encouraged in the boy a broad sympathy, an interest in living things, and a love of nature.

He was brought up in Harlem, first attending public school there and later Morris High School (also public) in the Bronx. There he and his classmates Lester Thompson and Edgar Altenburg founded what was perhaps the first high-school science club.

At Columbia College he was before the end of his first year fascinated by the subject of biology. Reading by himself in the summer of 1908 R.H. Lock's (1906) book on genetics, his interests became centered in that field. Courses soon afterwards taken under E. B. Wilson influenced him profoundly, as did also his reading, independently of courses, of works by Jacques Loeb and by other writers on experimental biology and physiology. In I909 he founded a students' biology club, which was participated in, among others, by Altenburg, and by two students, Bridges and Sturtevant, who had entered Columbia a year later.

For his first two years of graduate work, since there was no opening offered to him in zoology, he managed to obtain a scholarship (1910-1911) and then a teaching fellowship (1911-1912) in physiology, the latter at Cornell Medical College, while keeping up with genetics on the side and doing various extra jobs, such as teaching English to foreigners in night school. Finally, however, he obtained a teaching assistantship in zoology at Columbia (1912-1915). The first summer (1911) of graduate work was spent in studies at Woods Hole, the rest in laboratory teaching at Columbia. During these five years he was seriously overworked. In all this period he was chiefly interested in the Drosophila work which Morgan had opened up, and from 1910 on he closely followed this research and was an intimate member of the group, although he did not have an opportunity for much experimental work of his own on this material until 1912. Then he was able to begin his investigation of the simultaneous inter-relationships of many linked genes, which supported the theory of crossing-over and constituted his thesis. At the same time he undertook his analysis of variable, multiple-factor, characters by means of the device of 'marker genes'. This extended the validity both of chromosomal inheritance and of gene stability, and led later (1916) to his theory of balanced lethals.

Called to the Rice Institute, Houston, as Instructor, by Julian Huxley, he taught varied biological courses (1915-1918), and began studies on mutation. During this time and the two years following, when he was again at Columbia (1918-1920), now as instructor, elaborated methods for quantitative mutation study. Altenburg, who had meanwhile moved to the Rice Institute, and he, partly in collaboration, obtained the first results in this field (1918-1919), including evidence that made probable an effect of temperature. He then (1920) returned to Texas, this time to the University, at Austin, as Associate Professor, and from 1925 on as Professor, teaching mainly genetics and evolution, and doing research mainly on mutation.

In late 1926 he obtained critical evidence of the abundant production of gene mutations and chromosome changes by X-rays (published 1927). This opened the door to numerous researches, many of them carried on with the aid of students and co-workers, both at his own and other institutions, in the twenty years that followed. These had been briefly outlined in his Nobel Lecture, since they, together with the first discovery of the effect, constitute the work for which the Nobel Award was granted. They include studies on the mechanisms of the gene mutation effects and of the structural changes, on the roles which each kind of changes, when spontaneously occurring, play in evolution, and on the properties of genes and of chromosome parts (e.g. eu- versus hetero-chromatin), as disclosed by studies in which the chromosomes were broken and rearranged.

This later work was carried on at a succession of places. In 1932 he was awarded a Guggenheim Fellowship and for a year worked at Oscar Vogt's institute in Berlin, in Timoféeff's department of genetics. At the request of N. I. Vavilov, he then spent 3 1/2 years as Senior Geneticist at the Institute of Genetics of the Academy of Sciences of the U.S.S.R., first in Leningrad later (1934-1937) in Moscow, with a considerable staff of co-workers. With the rise of the Lysenko anti-genetics movement, he moved to the Institute of Animal Genetics, University of Edinburgh (1937-1940); here numerous graduate students, largely from India, took part. Then, from 1940 to 1945, he did both teaching and research at Amherst College, being professor ad I interim there from 1942 to 1945. At Amherst he completed a large-scale experiment showing the relationship of aging to spontaneous mutations. Finally, in 1945, he accepted a professorship in the Zoology Department at Indiana University, Bloomington, Indiana. Here again devoted his time chiefly to work on radiation-induced mutations, using them on one hand for purposes of genetic analysis and on the other hand in the study of how radiation produces its biological effects.

Muller has contributed over 300 articles on biological subjects to the scientific publications of learned societies. His principal books are The Mechanism of Mendelian Heredity with T. H. Morgan and others, 1915 and 1922, Out of the Night - a Biologist's View of the Future, 1935, 1936, and 1938, and Genetics, Medicine and Man with C. C. Little and L. H. Snyder, 1947.

In 1946, Muller was awarded the 1946 Nobel Prize in Physiology or Medicine, "for the discovery that mutations can be induced by X-rays".

He was President of the 8th International Congress of Genetics in 1948 and of the American Humanist Association during 1956-1958. He has received Doctor of Science degrees from the Universities of Edinburgh (1940), Columbia (1949) and Chicago (1959), the honorary Doctor of Medicine from Jefferson Medical College (1963), the Annual Award of the American Association for Advancement of Science (1927), the Kimber Genetics Award (1955) and the Darwin-Wallace Commemoration Medal (1958). He was Pilgrim Trust Lecturer (Royal Society) and Messenger Lecturer (Cornell University) in 1945, and was designated Humanist of the Year by the American Humanist Association in 1963.

Muller married his first wife, formerly Jessie M. Jacobs, in 1923 - they had one son, David Eugene. In 1939 he married Dorothea Kantorowicz - they have one daughter, Helen Juliette.

Enjoy the humour

If titanic was made in India.....

1. There would be 10 times as many people on the ship.
2. Hero and heroine would float in cold water for days and still survive, but the villain would die in the first dip.
3. The iceberg would be sent by the heroine's father to teach the hero a lesson.
4. None of the women would float due to heavy designer saris.
5. And, lastly half of the rescue boats would be reserved for SC/ST/OBC!!!

A dress shop received this note:
"Dear Sir: you have not yet delivered that maternity dress I ordered. Please cancel the order. My delivery was faster than yours."

As the speaker of the evening sat down, he coughed. His upper denture fell to the floor and broke. A guest at his side realized the man's plight, dug into his pocket and came up with a set. The speaker-to-be tried them, but they were too big. The helpful guest supplied another set. They were too small. The third set fit.
The speaker got along perfectly with the borrowed teeth, and as he sat down, returned them with thanks.
"By the way," he said, "are you a dentist?"
"No. An undertaker."

Thoughts to live by

- Have faith in God. God has faith in you. (Edwin Louis Cole)
- To be alive at all involves some risk. (Harold MacMillan)
- You will find as you look back upon your life that the moments when you have truly lived are the moments when you have done things in the spirit of love. (Henry Drummond)
- A true friend never gets in your way unless you happen to be going down. (Arnold H. Glasow)
- Love is not love which alters when it alternation finds. (William Shakespeare)

Track your brain

1. Leg ulcers are an example of _____ wounds.
2. Healing of the wound commences with _____.
3. Local environmental factors such as tissue necrosis, hypoxia and _____ impair immune cell activity.
4. The process by which phagocytes act to clean debris and destroy the ingested material is referred to as _____.
5. _____ dressings do not allow water, oxygen and bacteria into the wound.
6. _____ also means discoloration of the skin caused by silver containing compounds.
7. _____ allows the insertion of desirable genes of one microbe into the genome of another.
8. Detoxified extracellular products used to immunize susceptible individuals are referred to as _____.
9. Hermann Joseph Muller is known for X – ray _____.
10. Oral _____ serves as the mainstay of treatment for cholera.
11. The complex oxidized polyphenol in tea is often called _____.
12. Vitamin 'P' effect refers to the strengthening of _____.
13. Maize, potatoes and rice are rich sources of _____.
14. Salmonella releases _____ which are primarily responsible for food poisoning.

Check your Answers on Page 16

Vibrio cholerae

Vibrio cholerae, a member of the family Vibrionaceae, is a facultatively anaerobic, Gram-negative, non-spore-forming curved rod, about 1.4–2.6 mm long, capable of respiratory and fermentative metabolism; it is well defined on the basis of biochemical tests and DNA homology studies (Baumann, Furniss & Lee, 1984). The bacterium is oxidase-positive, reduces nitrate, and is motile by means of a single sheathed polar flagellum. Growth of *V. cholerae* is stimulated by addition of 1% sodium chloride (NaCl). However, an important distinction from other *Vibrio* spp is the ability of *V. cholerae* to grow in nutrient broth without added NaCl.

Differences in the sugar composition of the heat-stable surface somatic "O" antigen are the basis of the serological classification of *V. cholerae* first described by Gardner & Venkatraman (1935); currently the organism is classified into 206 "O" serogroups. Until recently, epidemic cholera was exclusively associated with *V. cholerae* strains of the O1 serogroup. All strains that were identified as *V. cholerae* on the basis of biochemical tests but that did not agglutinate with "O" antiserum were collectively referred to as non-O1 *V. cholerae*. The non-O1 strains are occasionally isolated from cases of diarrhea and from a variety of extra-intestinal infections, from wounds, and from the ear, sputum, urine, and cerebrospinal fluid.

Pathogenicity for humans and virulence factors

The major features of the pathogenesis of cholera are well established. Infection due to *V. cholerae* begins with the ingestion of contaminated water or food. After passage through the acid barrier of the stomach, the organism colonizes the epithelium of the small intestine by means of the toxin-co-regulated pili and possibly other colonization factors such as the different haemagglutinins, accessory colonization factor, and core-encoded pilus, all of which are thought to play a role. Cholera enterotoxin produced by the adherent vibrios is secreted across the bacterial outer membrane into the extracellular environment and disrupts ion transport by intestinal epithelial cells. The subsequent loss of water and electrolytes leads to the severe rice-water like diarrhea characteristic of cholera.

The existence of cholera enterotoxin (CT) was first suggested by Robert Koch in 1884 and demonstrated 75 years later by De (1959) and Dutta, Pause & Kulkarni (1959) working independently. Subsequent purification and structural analysis of the toxin showed it to consist of an A subunit and 5 smaller identical B subunits. The A subunit possesses a specific enzymatic function and acts intracellularly, raising the cellular level of cAMP and thereby changing the net absorptive tendency of the small intestine to one of net secretion. The B subunit serves to bind the toxin to the eukaryotic cell receptor, ganglioside GM1. The binding of CT to epithelial cells is enhanced by neuraminidase.

Monitoring and Assessment

Sampling and sample preparation

For the investigation of surface waters, water samples should be collected in sterilized bottles following standard procedures. Plants should be collected in sterile polyethylene bags, and phytoplankton and zooplankton should be collected using plankton nets and kept in sterile glass bottles. Sediment should be collected by a core sampler and kept in sterile polyethylene bags. All field samples should be transported to the laboratory inside a cooled container (at about 4–10 °C) and processed within 6 hours (Donovan & Van Netten, 1995).

Analytical methods: Culture methods, immunological and molecular methods, methods performance

A qualitative enrichment procedure is normally performed for the detection of *V. cholerae* from food or environmental samples. Quantitative procedures, either direct plating or most probable number (MPN), are required only occasionally. Culture media that were developed for the isolation of *V. cholerae* from feces in clinical laboratories have also generally been used for the isolation of *V. cholerae* from foods or the environment. Alkaline peptone water (APW) is the standard medium for enrichment of *V. cholerae*, although several nutrient-rich modifications of APW, such as blood–APW and egg–APW are also used. Thiosulfate–citrate–bile-salts–sucrose agar (TCBS) is a highly selective differential medium that is most commonly used for the isolation of *V. cholerae* ; its selective ingredients suppress the growth of most of the interfering organisms such as coliforms, pseudomonads, aeromonads, and other Gram-positive bacteria. The advantage of TCBS is its sucrose–bromothymol blue diagnostic system, which distinguishes the yellow sucrose positive colonies of *V. cholerae* from other colonies.

For isolation of *V. cholerae* from the environment the following procedures are recommended:

1) 10 g of plant material are homogenized with 100 ml of normal saline in a blender.
2) 10 ml of plankton sample should be homogenized in a PTFE-tipped tissue grinder using a stirrer.
3) 1 ml of plant homogenate, 10 ml of plankton homogenate, 50 ml of water, and 1.0 g of sediment are enriched in either APW or bile–peptone broth overnight at 37 °C.
4) All samples are then plated on TCBS agar or taurocholate–tellurite–gelatin agar and incubated at 37 °C for 18–24 hours.

Suspected *V. cholerae* strains transferred from primary isolation media can be identified by means of a standard series of biochemical media used for identification of members of the Enterobacteriaceae and Vibrionaceae families. Both conventional tube tests and commercially available enteric identification tests are suitable for identifying *V. cholerae*. A crucial test for differentiation of *V. cholerae* from Enterobacteriaceae is the positive oxidase test. Other key traits for distinguishing *V. cholerae* from other species include fermentation of D-glucose with acid production (without gas), maltose, D-mannitol, sucrose, and trehalose. Most strains are also motile at 37 °C, metabolize lysine and ornithine, and show a positive string test (a mucoid "string" is formed when a large loop of growth from a noninhibitory agar medium is suspended in a drop of 0.5% aqueous selection deoxycholate and then drawn). The absence of arginine metabolism is also frequently used for differentiation. However, the most important test for identification of *V. cholerae* O1 or O139 is agglutination in antisera raised against O1 or O139.

Various simpler schemes for identification of *V. cholerae* are available for use in developing countries. One involves the inoculation of suspected *V. cholerae* colonies from the isolation plate into a single tube, multitest medium which is based on the principles of triple sugar iron (TSI) and Kligler iron agar (KIA) medium (Kaper, 1979). Cultures yielding an alkaline slant (K) over acid (A) butt, with no gas or H_2S, are then tested for oxidase and reactivity with O1 or O139 antisera, using growth taken from the multitest medium. Extensive evaluation has revealed that 97.9% of the oxidase-positive strains that yield a K/A reaction in the multitest medium have biochemical reactions consistent with those of *V. cholerae*. Strains of *V. cholerae* that do not agglutinate in either O1 or O139 antisera should be labeled as non-O1 non-O139.

Specific probes for the A and B subunit genes of CT have been used to detect the location of these genes in the *V. cholerae* genome and in differentiating between clones of *V. cholerae*. Molecular diagnostic tests, such as PCR, are now being developed for both clinical and environmental monitoring of *V. cholerae* O1 and O139.

Control

Emission, Transport, and Survival in the Environment

Most Vibrio species are ubiquitous in estuarine and marine environments and are also found in fresh water provided that there is a certain minimal level of sodium ions. The cholera vibrio, however, was long considered to be an exception, in that it was believed not to be an environmental organism but associated with water only as a result of sewage contamination.

Thus, until the late 1970s, *V. cholerae* was considered by most workers in the field to be an organism whose normal habitat was the human gut and to be incapable of surviving for more than a few days outside the gut. The reason for this belief was a general failure to isolate the organism from the water unless there were cholera cases in the immediate vicinity. During epidemics, toxigenic *V. cholerae* O1 or O139 can be isolated from the local fresh water as well as from patients but disappears from the environment after the epidemic subsides.

While there is no doubt that the fecal–oral route of cholera transmission is of primary concern because of its importance in the development of secondary cases and in the subsequent spread of the disease, it does not fully explain seasonal recurrence of the disease in some areas or outbreaks that occur where fecal–oral transmission is unlikely. Traditional culture techniques for isolating *V. cholerae* from water are frequently unsuccessful. More advanced techniques, however, using direct immunofluorescence microscopy, DNA hybridization, PCR, and improved culture methods, have frequently isolated both O1 and non- O1 strains, even in the absence of traditional fecal indicator bacteria such as *Escherichia coli* and fecal streptococci. This suggests either that *V. cholerae* can survive longer in the environment than other fecal organisms or that *V. cholerae* is an environmental organism in its own right.

Effects of drinking water treatment

Ensuring safe drinking-water implies both securing a safe source and maintaining safety up to the point of consumption. This is equally true of sophisticated piped distribution systems, of water collected by householders from sources such as wells, and of water provided to the consumer by any other means.

Groundwater sources such as wells and springs are often believed to be of good quality with regard to bacterial pathogens transmitted by the fecal–oral route. However, such sources are readily contaminated by fecal material, especially where there are potential sources of contamination nearby or where contaminants may be carried by surface waters. Protection measures need to be properly applied.

Surface-water sources should generally be considered to be susceptible to fecal contamination and, therefore, to contamination by *V. cholerae*. However, the organism can be easily eliminated from drinking-water by appropriate treatment.

It has long been accepted that slow sand filtration is effective for removal of *V. cholerae* during drinking-water treatment. The biological processes that are responsible for water purification occur more slowly at low temperatures, and ice formation on filter surfaces has been associated with unacceptable deterioration in effluent water quality. The use of open filters should therefore be avoided in regions where temperatures can drop below 0 °C.

Other common treatment methods such as coagulation, flocculation, sedimentation, and rapid filtration will significantly reduce numbers of *V. cholerae*, but should be seen as preparatory treatments to be followed by disinfection.

Most chemical disinfectants effectively eliminate *V. cholerae* under normal operating conditions (principally concentration and time) provided that water is clear (i.e. free of particulates). The same is true of physical disinfection methods, such as the use of ultraviolet light.

Recontamination of "safe" water is a significant concern. When water has to be collected, there are several opportunities for recontamination, by recipients, and during handling or extraction from storage for use. In piped supply systems recontamination is also a significant risk, especially where the supply is discontinuous or of low pressure and where there is appreciable leakage. To minimize the health risks associated with recontamination, use of a residual disinfectant is recommended.

Boiling is generally advised but for poor populations this is not affordable. Use of potassium aluminium sulfate, 500 mg/litre, has been claimed to kill *V. cholerae* but the taste of water is unacceptable to many. Chlorine-releasing agents (such as calcium hypochlorite or bleaching powder) are very effective and less expensive. For domestic chlorination 1% stock solution is prepared by adding enough water to 4 teaspoons (16 g) of hypochlorite or 10 teaspoons (40 g) of bleaching powder to make 1 litre. Three drops of stock solution should be added per litre of water, which should be allowed to stand for 20–30 minutes before use (Clark, 1956). Various disinfecting solutions, containing about 1% chlorine, are available commercially, as are water purification tablets and liquid preparations containing chlorine.

Treatment

Cholera is now an easily treatable disease. The prompt administration of oral rehydration salts to replace lost fluids nearly always results in cure. In especially severe cases, intravenous administration of fluids may be required to save the patient's life. However, if left untreated cholera can be fatal.

Antimicrobial Properties of Tea

Tea, a beverage is an infusion of variously processed leaves of one of the varieties of an evergreen shrub, *Camellia sinensis* L. It is the most widely drunk beverage in the world. Green tea, popular in the Far East, differs from the black tea familiar in the West in that an oxidation step (called "fermentation") occurs in the processing of the latter compound but not the former compound. Although it has little nutritional value per se, tea is refreshing, mildly stimulating, and produces a feeling of well being. The latter two properties have been assumed to be due to caffeine, about 50 mg of which is present in a cup of tea; caffeine is known to have "stimulant and anti-soporific actions, that elevate mood, decrease fatigue and increase capacity for work". However, other components of a cup of tea, notable, the polyphenols, may also contribute to the effects of tea, in view of their known pharmacological properties.

The complex of oxidized polyphenols in tea is often called "tannin". It should be stressed, however, that unlike some compounds from other plants also given this generic name, tea tannins are not harmful. Contrary to widespread belief, tea does not contain tannic acid.

Biological Effects of Tea

Non microbiological effects: Tea has exhibited a wide range of beneficial effects ranging from physiological to pharmacological effects. These include slowing the catabolism of catecholamines, strengthening capillaries ("vitamin P effect"), exerting an anti-inflammatory effect by enhancing the effectiveness of ascorbic acid, acting as an antioxidant, inhibiting angiotensin-converting enzyme, having a hypocholesterolemic action, and inhibiting the growth of implanted malignant cells.

Microbiological effects: In a certain early report describing the microbiological effects of tea, an army surgeon recommended the use of tea in soldier's water bottles as a prophylactic measure against typhoid. Until recently, good evidence for a useful antimicrobial activity of tea was missing. Although there had been several reports of the antibacterial effects of tea in vitro and in vivo, mainly against intestinal pathogens, these were somewhat superficial and fragmentary.

Within the past few years, the situation has changed. A series of well-conducted, systematic studies, mainly from Japan, now suggests that tea extracts show several useful antimicrobial effects. Toda et. at. found that extracts of tea inhibited and killed *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella flexneri*, *Shigella dysenteriae*, and *Vibrio* spp., including *Vibrio cholerae*. Toda et al. later reported that tea at concentrations identical to those found in the beverage (a "cup" of tea contains ca. 3 mg of solids per ml) inhibited methicillin-resistant *S. aureus*. A similar finding was made with respect to *Bordetella pertussis*. Other workers showed that aqueous extracts of green tea inhibited cariogenic streptococci, including *Streptococcus mutans*; activity against other harmful mouth flora has been reported in a certain patent literature. Tea extracts have been found to be active against *Clostridium* spp. and phytopathogens such as *Erwinia* spp. and *Pseudomonas* spp.

Tea extracts prevented rotavirus and enterovirus from infecting monkey kidney cells in tissue culture; this was ascribed to interference with viral adsorption rather than a direct antiviral effect. Preventive and curative effects of tea on influenza virus have been claimed in a patent.

Killing of pathogenic protozoa by tea extracts has been reported in the Russian-language literature, but it is difficult to assess the significance of this.

At a subcellular level, these observations have been extended by the demonstration that extracts of black and green tea inhibited the hemolytic activities of staphylococcal alpha toxin and the thermostable direct hemolysin of *Vibrio parahemolyticus* against rabbit erythrocytes. A potentially valuable anticariogenic effect is suggested by the inhibition of the synthesis of insoluble glucans by *S. mutans*.

Biological Activities of Tea Components

Nonmicrobiological activities: It has been reported that the polyphenol moiety found in the tea extracts is primarily responsible for many of the physiological activities attributed to it. Some of the more interesting of these facts include activation of leukocytes in various ways, antioxidant and antimutagenic activities, lowering of plasma cholesterol levels), and protection from the effects of radiation.

Microbiological activities in vitro: The polyphenol fractions of tea have been closely examined for their antimicrobial properties. Several studies have shown that purified catechin fractions from green and black tea, and ECG and EGCG in particular, inhibit the growth of many bacterial species and possess anticariogenic properties. Specifically, a commercially available preparation of tea polyphenols, Sunphenon, prevented the attachment of a cariogenic *S. mutans* strain to hydroxyapatite and also inhibited its glucosyltransferase activity. Hattori et al.and Fukai et al. reported that the activities of the the aflavins were similar to those of the simple catechins, thus casting doubt on the importance of the gallate moiety in the antimicrobial activity of black tea extracts. These compounds display activity at cup-of-tea concentrations, unlike caffeine. ECG and EGCG, but not EC or EGC, have been reported to be powerful antagonists of human immunodeficiency virus reverse transcriptase, causing 50% inhibition at concentrations of 10 to 20 ng/ml. Ikigai et al. showed that EC was much less active than EGCG. *S. aureus* was more susceptible than *Escherichia coli*, consistent with a much greater binding of EGCG to staphylococci.

The flavonols quercitin, kaempferol, and myricetin showed activity against Gram-positive bacteria and phytopathogenic fungi in a screening test. Volatile flavor components make up a very small fraction of flush and tea leaf (10 to 20 ppm) but play an important part in providing taste.

Microbiological activities of tea in vivo: In microbiological terms, much of the postulated benefit to be derived from tea drinking is anecdotal. Toda et al. found that a mixture of tea catechins protected rabbits from an experimental infection caused by *V. cholerae* and suggests that patients with cholera could benefit if tea extracts were added to oral rehydration solutions.

It is evident from the above that "the cup that cheers but does not inebriate" contains a veritable witches' brew of biologically active ingredients. The use of tea as a prophylactic measure is clearly still a long way from clinical application, but there are promising leads in the dental context. The concept of being able to exploit an antimicrobial agent which is a new chemical entity found in an abundantly available and renewable source is truly an enticing one.

Reference:

Antimicrobial Properties of Tea by J. M. T. Hamilton Miller

Food, Hygiene, and Microbiology

Food microbiology is an important field in sciences which requires a lot of attention, since what one consumes should not only be healthy and nutritious but also hygienic and free from pathogens.

Food Hygiene

Food contamination projects a major health risk to communities and is a leading cause of disease outbreaks and transmission. Food that is kept too long can go bad and often contains toxic chemicals or pathogens, and food-stuffs that are eaten raw, such as fruits or vegetables, can become contaminated by dirty hands, unclean water or flies and other such vectors. Improperly prepared food can also cause chemical poisoning. Half cooked or over cooked food for example hard boiled eggs and over cooked meat are bad for health.

To promote good health, therefore, food should be properly prepared and stored.

Food Preparation at Home

Families must understand the principles of basic hygiene and know how to prepare food safely:

- Before preparing food, hands should be washed with soap or ash.
- Raw fruit and vegetables should not be eaten unless they are first peeled or washed with clean water.
- It is also important to cook food properly, particularly meat. Both cattle and pigs host tapeworms that can be transferred to humans through improperly cooked meat; for this reason, raw meat should never be eaten.
- Eggs, too, must be cooked properly before eating, since they may contain salmonella, a virulent pathogen.
- The kitchen itself should be kept clean and waste food disposed off carefully to avoid attracting vermin, such as rats and mice that may transmit diseases.
- Keeping food preparation surfaces clean is critical, because harmful organisms can grow on these surfaces and contaminate food.
- Fresh meat should be cooked and eaten on the same day, unless it can be stored in a refrigerator; if not, it should be thrown away immediately.
- Cooked food should be eaten while it is still hot and should not be left to stand at room temperature for long periods of time, since this provides a good environment for pathogens to grow.
- Food that is ready to eat should be covered to keep off flies and should be thrown away if not eaten within 12 – 16 hours.
- If food must be stored after cooking, it should be kept covered and in a cool place, such as a refrigerator and if a refrigerator is not available, food can be stored on ice blocks or in a preservative such as pickling vinegar or salt.
- Food that is already prepared, or food that is to be eaten raw, must not come into contact with raw meat as this may contain pathogens that can contaminate the other foods (particularly if slaughtering was not carried out hygienically).

Hotels and Restaurant

In many urban centers food is bought and consumed at eating-houses (cafes, restaurants or canteens). If basic health and safety rules for storing, preparing and handling food are not followed in the eating-houses; these places will represent a health hazard for the customers and may cause serious disease outbreaks. The most important aspects of food hygiene in these establishments relate to sanitation, water supply and personal cleanliness:

- Eating-houses should have clean water for washing and drinking, and separate sanitation facilities, away from the kitchen area, for customers, cooks and food-handlers.
- The staff should have clean uniforms each day and have regular medical check-ups.
- The cooks, chefs and waiters should be the most particular in their own personal hygiene so as to prevent any contamination of food handled by them.
- Food to be served should be prepared fresh always and any that is spilled or not used should be disposed off.
- The kitchens and eating areas must be kept clean and free of vermin and insects.
- Eating-houses should also be well-ventilated, with adequate lighting.

Street Food Vendors

Street food-vendors are common in urban and peri-urban areas, but they also operate in rural areas, particularly if there is a market or community fair. Although people enjoy food from these vendors, in many cases the food is of poor quality and it represents a serious health risk. In part, this is because the street vendors have little or no access to safe water supplies or sanitation facilities, and they commonly cook and handle food with dirty hands. Raw foodstuffs, too, cannot be kept in safe storage places and are easily contaminated by vermin and insects. Moreover, the street vendors often keep cooked food at ambient (environmental) temperatures for prolonged periods of time and may heat the food only slightly before serving. All these factors may make the food from street vendors dangerous.

What causes food poisoning?

Food poisoning is usually caused by micro-organisms (germs), including bacteria, viruses and molds. The spread of these germs can be prevented by practicing good food hygiene.

The most serious types of food poisoning are caused by bacteria. Bacteria multiply best in a moist environment between 5°C and 63°C. Just a single bacterium on an item of food, left out of the fridge overnight, could generate many millions of bacteria by the morning, enough to make you ill if eaten. Storing food below 5°C prevents bacteria from multiplying, and cooking food at temperatures over 70°C will kill off any existing bacteria.

Food poisoning and other food borne hazards

The term 'food poisoning' is commonly used to cover a wide variety of illnesses or clinical conditions affecting the gastrointestinal tract. The very large majority of such illnesses found in developed countries result from the consumption of contaminated food or drink, and because they are caused by infection with or the presence of bacteria, these organisms will receive the greatest attention here. However, it is necessary to consider, albeit more briefly, other forms of food poisoning and food-borne hazards since these may sometimes be of concern and pose serious health hazards in other parts of the world.

Bacterial food poisoning

It may be helpful to distinguish between bacterial food poisoning and food-borne bacterial infections. In the former the causative organism multiplies in the food and by its heavy growth induces illness by one mess or another after ingestion of the contaminated food. In food-borne infections the food merely acts as a carrier for the causative organism which does not require to multiply in the food.

Food poisoning is characterized by an acute gastroenteritis (inflammation of the lining of the alimentary canal) following

ingestion of food in which multiplication of bacteria has taken place, the ingested viable bacteria continuing to grow within the host's body to produce the typical symptoms. Salmonellas are principally responsible for this type of food poisoning in which toxins are released as the bacterial cells disintegrate. This toxin type (often termed an intoxication) is a genuine toxin having been produced by the bacteria which have grown in the food prior to consumption. The toxin also causes an acute gastroenteritis but ingestion of viable bacteria is usually not a prerequisite of the induction of the disease. Bacteria causing toxin food poisoning include *Clostridium perfringens* and *Staphylococcus aureus*.

Improving Hygiene

- Maintaining high levels of personal and kitchen hygiene are important and effective ways to stop germs from spreading.
- Wash your hands and nails with hot, soapy water before handling food, between handling cooked and uncooked foods, and after going to the toilet.
- Rinse your hands well and dry them on a clean hand towel, a disposable paper towel, or under a hand dryer. Wet hands transfer germs more effectively than dry hands.
- Use different cloths for different jobs (eg washing up and cleaning surfaces). Wash them regularly on the hot cycle or soak in a dilute solution of bleach.
- Wipe down and disinfect surfaces and utensils regularly, using a detergent or dilute solution of bleach - always read the safety instructions first.
- Wash up using hot, soapy water - use rubber gloves if necessary.
- Don't handle food if you have stomach problems such as diarrhea and vomiting, or if you're sneezing or coughing frequently.
- Cover up cuts and sores with waterproof plasters.
- If possible, remove rings, watches and bracelets before handling food. Germs can hide under these.

Bacteria can spread from raw food, in particular meat, to food that has already been cooked or is eaten raw, such as salads.

- Use separate chopping boards for preparing raw meat, poultry and seafood and for fresh produce such as salads, fruits and vegetables.
- Never use a marinade that has already been used on raw meat for cooked food, unless it has been boiled thoroughly.
- Always use a clean plate to serve food.
- After using a knife or other utensil on raw meat, clean it thoroughly before using it on other foods.

Storing food correctly

- It's very important that food is stored in the right place (eg fridge or freezer) and at the correct temperature.
- Always check labels for guidance on where and how long to store food, in particular, fresh or frozen food.
- Store fresh or frozen food in the fridge or freezer within two hours of purchase - sooner if the weather is hot.
- Allow meal leftovers to cool to room temperature before storing them in the fridge, ideally within two hours of preparation. If necessary, divide leftovers into smaller portions to help food cool more quickly.
- Use up leftovers within two days. Cooked rice should only be kept for one day.
- Store raw food such as meat in airtight containers at the bottom of the fridge to prevent juices or blood from dripping onto other food.
- Defrost frozen foods in the fridge. Place them on a plate or in a container as they defrost so they don't drip on or contaminate other foods.
- Don't overfill the fridge - food may not cool properly.
- Keep the fridge at less than 5°C and the freezer at less than -18°C - consider getting a thermometer.
- Don't store opened tins of food in the fridge - transfer the contents to a suitable airtight container instead.

Cooking Food Safely

If food isn't cooked at a high enough temperature, bacteria can still survive. The following advice will help you to cook safely.

- Follow the recipe or packet instructions for cooking time and temperature, ensuring the oven is pre-heated properly.
- Food should be piping hot (steaming) before serving.
- Take special care that pork, sausages, burgers and poultry are cooked thoroughly and aren't pink in the middle. Using a clean skewer, pierce the meat. When cooked properly, the juices run clear. Lamb and beef joints and steaks can be cooked rare, but must be thoroughly sealed (browned) on the outside.
- When microwaving, stir food well from time to time to ensure even cooking.
- Only reheat food once and serve piping hot.
- Eggs contain harmful bacteria which can be dangerous to pregnant women, older people and babies. Don't serve eggs with runny yolks, or egg-containing foods that won't be cooked, for example homemade mayonnaise.

Eating Out

When eating out, it's also important to consider food hygiene. You can't usually inspect the kitchens in restaurants, cafés or pubs, but there are certain warning signs of poor hygiene standards that you can look out for:

- Unhygienic personnel, dirty dining areas, toilets, cutlery or crockery
- Rubbish and overflowing bins outside - these could attract vermin
- Hair or insects in food
- Raw food and ready to eat food displayed together
- Hot food that isn't cooked properly and cold food that is served lukewarm

Promoting nutrition

A healthy and well-balanced diet is essential for good health. Undernourishment and malnourishment can lower resistance and make individuals/kids more likely to suffer from infectious diseases. It is important to make children's food less spicy than adult food, because their stomachs are small, children can eat only small portions and need to be fed more frequently than healthy adults. It is also important that children are fed not just foods high in starch or carbohydrate (for instance rice). Although these foods can quickly make a child feel full, he or she may become malnourished if other key foodstuffs are not eaten.

A well-balanced diet usually has a mixture of food:

- Proteins (for example beans, peas, meat, fish or eggs).
- Carbohydrates (such as maize, potatoes, cassava, rice and many other staple foods).
- Vitamins (such as vegetables, fish, fruits or milk).
- Fats or oils (such as cooking oil).

Sometimes not all these foods are available and it is important that community members ask health workers how to make best use of available foods for a balanced diet. In many situations, nutrition can be improved by changing agricultural or gardening practices. Often, even small plots of land can provide nutritious food provided that the right crops are grown. Health workers or agricultural extension workers can be asked for advice about which crops to grow to provide community members with well-balanced diets.

Reference: Food Hygiene Microbiology and HACCP by S. J. Forsythe, P. R. Hayes.

Microxpress introduces **Biogram antimicrobial susceptibility discs** (As per US-FDA / W.H.O. recommendations and CLSI standard design).
Since there is a rapid rise in resistance in microbes worldwide; it is imperative to screen out the antibiotics to which the microbial strain is susceptible in order to use only these drugs for effective therapy.
The method of choice for clinical microbiologists for in-vitro antimicrobial susceptibility testing is the Disc Diffusion Method. Acceptance of the in-vitro disc susceptibility method has been aided by its simplicity and rapidity. The Kirby-Bauer technique for disc susceptibility testing has been recommended by the CLSI (Clinical Laboratory Standards Institute), and is approved by the US-FDA and is also recommended by W.H.O.

Biogram antimicrobial susceptibility discs

W.H.O. Parameters (As per the Technical report series 610, 1977)	Feature	**Biogram antimicrobial susceptibility discs**
Quality of antibiotics	Antibiotics used for impregnating on the disc should be as per international pharmacopoeia	✓
Paper	Paper used for disc diffusion should not have any inhibitory action on the antibiotics	✓
Solvent	Solvent used for impregnating antibiotics should not have any inhibitory activity on the antibiotics.	✓
Drying	Drying process should be such that only the solvents are dried without acting on the antibiotics impregnated on the discs.	✓
Packaging	Packaging used should be free from moisture to prevent deterioration of antibiotics. Also it should have a desiccant which indicates presence of moisture with colour change.	✓
Size of antimicrobial disc	Size of antimicrobial disc should be 5-7 mm	✓

Biogram Pack Sizes
The Biogram antimicrobial discs are available in pack size of 5 and 10 cartridges.
Each pack of 5 / 10 cartridges pack contains: ● 5 cartridges / 10 cartridges ● Each aluminium foil pouch contains one single cartridge which accommodates 50 discs and desiccant. ● Single disc dispenser ● Package insert.

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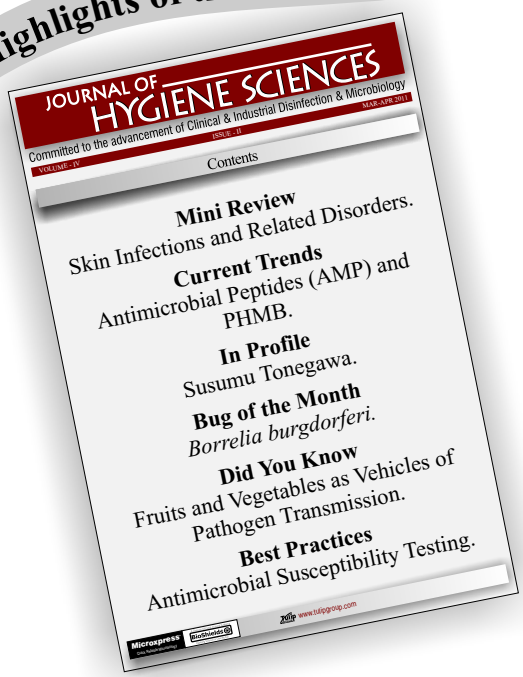
APPLICATIONS :
Medical: In Hospitals, Nursing homes, Medical colleges, Pathological laboratories for Inter-operative irrigation. Pre & post surgery skin and mucous membrane disinfection. Post-operative dressings. Surgical & non-surgical wound dressings. Surgical Bath/Sitz bath. Routine antisepsis during minor incisions, catheterization, scopy etc. First aid. Surface disinfection.
Industrial: In Pharmaceutical industry, Food & beverage industry, Hotel industry etc. General surface disinfection. Eliminating biofilms.

USAGE DIRECTIONS :

Surgical, postoperative, non surgical dressings	–	Use undiluted
Pre & post surgery, skin cleaning & disinfection	–	Use undiluted
Surgical/Sitz bath	–	Add 50 ml of NUSEPT™ in 1L of water & use
Antisepsis during minor incisions, catheterization,	–	Use undiluted scopy, first aid, bites, cuts stings etc
Midwifery, nursery & sickroom	–	Use undiluted
General surface disinfection	–	Add 100 ml of NUSEPT™ in 1L of water and gently mop the floor or surfaces

Highlights of the coming issue



Printed and published by D.G. Tripathi, Edited by Gracinda Mesquita for and on behalf of Tulip Diagnostics (P) Ltd., Tulip House, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex, Post Office Goa - 403 202, India. Fax: 0832 2458544, **Website: www.tulipgroup.com.**