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Editorial

Contents

As the year advances and moves into the month of March, here's another issue of the Journal packed with information that is relevant and useful in different aspects of health and hygiene.

Mini Review opens to Skin Infections and Related Disorders. Skin provides an excellent barrier against bacterial infection and is comprised of 3 principal layers; namely the epidermis, dermis and subcutaneous layers, separated from the underlying muscle by the fascia. Although many bacteria come in contact with or even reside on the skin, they are normally unable to establish an infection and are considered as normal flora. However bacterial skin infections and disorders do occur, as a result of any break in the skin which predisposes the tissue to infection. Therefore proper care and treatment must be administered immediately.

Current Trends focuses on Antimicrobial Peptides (AMP) and PHMB, which is a heterodisperse mixture of polymers and is a synthetic compound which is structurally similar to naturally occurring antimicrobial peptides (AMPs). The structural similarities between AMPs and PHMB mean that the latter can enter bacterial cell membranes and kill bacteria in a similar way to AMPs. The primary targets appear to be the outer and cytoplasmic membranes. PHMB is thought to adhere to and disrupt target cell membranes, causing them to leak potassium ions and other cytosolic components which results in bacterial cell death.

Our In Profile scientist Susumu Tonegawa is a Japanese who discovered the genetic mechanism that produces antibody diversity, for which he was awarded the Nobel Prize in Physiology or Medicine, Stockholm, Sweden in1987.

Bug of the Month is the etiological agent of Lyme disease, a spirochete *Borrelia burgdorferi* which was first recognized in the United States in 1975 by Dr. Allen Steere, following a mysterious outbreak of juvenile rheumatoid arthritis near the community of Old Lyme, Connecticut. The spirochete responsible is transmitted by the bite of an infected tick.

Did You Know emphasizes that fruits and vegetables can act as vehicles of pathogen transmission and therefore it is essential that raw fruits and vegetables have to be thoroughly washed and cleaned before being consumed or used in cooking.

Antimicrobial Susceptibility Testing is the need of the hour, and the same is the core of Best Practices for this issue. With rapid increase in drug resistance among microbes it is a must to analyze antimicrobials before being used as therapeutic measures and the importance of this testing in medicine and thus, better patient management in the future.

As you look forward to reading these interesting topics, we thank you for your contributions towards the Journal and look forward to your feedback and suggestions.

Skin Infections and Related Disorders

The skin provides an excellent barrier against bacterial infections. Although many bacteria come in contact with or even reside on the skin, they are normally unable to establish an infection and are considered as normal flora, which are kept under continuous check by the skin, via surface secretions. However bacterial skin infections do occur, which can range in size from a tiny spot to the entire body surface and can differ in seriousness as well, from harmless to life threatening condition.

Many types of bacteria can infect the skin. The most common are Staphylococcus and Streptococcus. Skin infections caused by less common bacteria may develop in people while hospitalized or living in a nursing home, while gardening, or while swimming in a pond, lake, or ocean.

Some people are at particular risk of contracting skin infections. For example, people with diabetes are likely to have poor blood flow, especially to the hands and feet, and the high levels of sugar in their blood diminishes the ability of white blood cells to fight infections. People with human immunodeficiency virus (HIV) or AIDS or other immune disorders and those undergoing chemotherapy are at higher risk as well, because they have a weakened immune system. Skin that is inflamed or damaged by sunburn, scratching, or other trauma is more likely to be infected. In fact, any break in the skin predisposes the tissue to infection.

Skin is comprised of 3 principal layers; namely the epidermis, dermis and subcutaneous layers, separated from the underlying muscle by the fascia. Each layer has different characteristics and a different spectrum of infections. The keratinized layer can be infected by lice, papilloma virus and some fungi. Of the bacterial diseases that infect the skin, impetigo is the most superficial, followed (as one goes deeper into the skin) by erysipelas, cellulitis and fasciitis. A kind of fasciitis called necrotizing fasciitis is often called a flesh-eating infection due to the ability of the causative bacteria to quickly move along the plane of the fascia and cause widespread necrosis. Folliculitis, an infection of the hair follicle, can be quite superficial or form a deeper abscess. Any of the infections except the very superficial can, by toxigenic mechanisms or direct spreading, progress to shock and even death. By far the commonest bacteria causing infections of the skin are *Staphylococcus aureus* and *Streptococcus pyogenes*. These can lead to the very serious conditions; toxic shock and toxic shock-like syndrome, but even in the absence of these complications, can be very painful and cause widespread damage. *Haemophilus influenzae* may cause erysipelas or cellulitis, particularly in children, whereas burn infections are most frequently caused by either Staphylococcus or Pseudomonas. The enteric bacteria, which have the ability to cause Gram negative sepsis, can also be a problem any time there is large scale damage to the skin. The spore-forming anaerobes, the Clostridia, also can cause very dangerous infections including myonecrosis or gas gangrene as well as tetanus that begin by skin penetration. Traumatic infections caused by animal bites or scratches have a unique set of causative agents with rabies being the most feared.

Skin infections and disorders can be caused by a vast variety of microbes, including bacteria, fungi, viruses and parasites, however the present article will focus on bacterial infections.

Cellulitis is defined as a spreading bacterial infection of the skin and the tissues immediately beneath the skin.

Many different bacteria may be responsible for Cellulitis. The most common are those of the *Streptococcus* species. Streptococci spread rapidly in the skin because they produce enzymes that hinder the ability of the tissue to confine the infection. Staphylococcus bacteria can also cause cellulitis, as can many other bacteria, especially after bites by humans or animals or after injuries in water or dirt.

Small breaks in the skin resulting from scrapes, punctures, burns and skin disorders serve as a portal of entry for the infecting bacteria.

Bacteria usually enter through small breaks in the epidermis that result from scrapes, punctures, burns, and skin disorders. Areas of the skin that become swollen with fluid (edema) are especially vulnerable. Individuals with poor blood circulation (chronic venous insufficiency) are more prone to cellulitis. However, cellulitis can also occur in skin that is not obviously injured.

Symptoms

Albeit cellulitis can occur in different parts of the body, it commonly develops on the legs. The first symptoms are redness, pain, and tenderness over an area of skin. These symptoms are caused both by the bacteria themselves and by the body's attempts to fight the infection. The infected skin becomes hot and swollen as a result of inflammation and may look slightly pitted, like an orange peel. Fluid-filled blisters, which may be small (vesicles) or large (bullae), sometimes appear on the infected skin. The borders of the affected area are not distinct, except in a form of cellulitis called erysipelas.

Systemic symptoms may vary, commonly people with cellulitis feel only mildly ill, but some may have a fever, chills, rapid heart rate, headache, low blood pressure, and confusion.

As the infection spreads, nearby lymph nodes may become enlarged and tender (lymphadenitis), and the lymphatic vessels may become inflamed. Sometimes, bacteria spread through the blood (bacteremia), which can cause more serious illness.

When cellulitis affects the same site repeatedly, especially the leg, lymphatic vessels may be damaged, causing permanent swelling of the affected tissue.

Diagnosis and Treatment

The physician usually diagnoses cellulitis based on its appearance and symptoms. Laboratory identification of the bacteria from blood, pus, or tissue specimens usually is not necessary unless a person is seriously ill or the infection is not responding to drug therapy. Sometimes, doctors need to perform tests to differentiate cellulitis from a blood clot in the deep veins of the leg (deep vein thrombosis, DVT), because the symptoms of these disorders are similar.

Prompt treatment with antibiotics can prevent the infection from spreading rapidly and reaching the blood and internal organs. Antibiotics that are effective against both streptococci and staphylococci (such as dicloxacillin or cephalexin) are used. People with mild cellulitis may take antibiotics by mouth. Those with rapidly spreading cellulitis, high fever, or other evidence of serious infection often receive intravenous antibiotics (such as oxacillin or nafcillin). Also, the affected part of the body, when possible, is kept immobile and elevated to help reduce swelling. Cool, wet dressings applied to the infected area may relieve discomfort.

Symptoms of cellulitis usually disappear after a few days of antibiotic therapy. However, symptoms often get worse before they get better probably because, with the death of the bacteria, substances that cause tissue damage are released. When this occurs, the body continues to react even though the bacteria are dead. Antibiotics are continued for 10 days or longer even though the symptoms may disappear earlier.

Erysipelas is a superficial form of cellulitis typically caused by streptococci.

Erysipelas results in a painful, red, raised patch on the skin. The edges have a distinct border and do not blend into the nearby normal skin. The patch feels warm and firm to the touch. It occurs most frequently on the legs and face. People often have a high fever, chills, and a general feeling of illness (malaise). Doctors usually base the diagnosis on the characteristic appearance of the rash.

Antibiotics given by mouth, such as penicillin, can cure the infection. For a severe infection, intravenous penicillin is needed. Cold packs and drugs for pain may relieve discomfort. Fungal foot infections may be an entry site for infection and may require treatment with antifungal drugs to prevent recurrence.

Erythrasma is infection of the top layers of the skin caused by the bacterium *Corynebacterium minutissimum.*

Erythrasma affects mostly adults, especially those with diabetes and those living in the tropics. Erythrasma often appears in areas where skin touches skin, such as the webs of the toes, and genital area—especially in men, where the thighs touch the scrotum. The armpits, skin folds under the breasts or on the abdomen, and the area between the vaginal opening and the anus (perineum) are prone to this infection, particularly among those with diabetes and among obese middle-aged women. The infection can produce irregularly shaped pink or brown patches that may later turn into fine scales. In some people, the infection may even spread to the torso and anal area.

Although erythrasma may sometimes be confused with a fungal infection, doctors can easily diagnose erythrasma because skin infected with Corynebacterium glows coral-red under an ultraviolet light.

An antibiotic given by mouth, such as erythromycin or tetracycline, can eliminate the infection. Antibacterial soaps, such as chlorhexidine, may also help. Topical drugs such as erythromycin and clindamycin are also effective. Antifungal creams such as miconazole may be helpful if yeast or fungus is present in the affected areas as well. Erythrasma may recur, necessitating a second treatment.

Folliculitis and **skin abscesses** are pus-filled pockets in the skin resulting from bacterial infection. They may be either superficial or deep, affecting just hair follicles or deeper structures within the skin.

Folliculitis is a type of skin abscess that involves the hair follicle. Abscesses are commonly caused by *Staphylococcus aureus* bacteria and appear to be pus-filled pockets on the skin surface. Sometimes the bacteria enter the skin through a single hair follicle, small scrape, or puncture, although often there is no obvious point of entry. People who have poor hygiene or chronic skin diseases or whose nasal passages contain *Staphylococcus* are more likely to have episodes of folliculitis or skin abscesses. A weakened immune system, obesity, old age, and possibly diabetes are also common risk factors. However some people may have recurring episodes of infection for unknown reasons.

Doctors may try to eliminate *Staphylococcus* from people prone to recurring infections by instructing them to wash the entire body with antibacterial soap, apply antibiotic ointment inside the nose, and take antibiotics by mouth.

Folliculitis: Folliculitis is an infection of a hair follicle. It appears like a tiny white pimple at the base of a hair. There may be only one infected follicle or many. Each infected follicle is slightly painful, but the person otherwise does not feel sick.

Some people develop folliculitis after exposure to a poorly chlorinated hot tub or whirlpool. This condition, sometimes called "hot-tub folliculitis" or "hot-tub dermatitis," is caused by the bacterium *Pseudomonas aeruginosa*. It begins anytime from 6 hours to 5 days after the exposure. Areas of skin covered by a bathing suit, such as the torso and buttocks, are the most common sites.

Sometimes stiff hairs in the beard area curl and reenter the skin (ingrown hair) after shaving, causing irritation without substantial infection. This type of folliculitis is called pseudofolliculitis barbae.

Folliculitis is treated with antibacterial cleansers or topical antibiotics. Large areas of folliculitis may require antibiotics taken by mouth. Hot-tub folliculitis goes away in a week without any treatment. However, adequate chlorination of the hot tub is necessary to prevent recurrences and to protect others from infection. Folliculitis caused by ingrown hairs is treated by a number of methods with varying success. For severe, recurring problems, doctors may take a bacterial culture (a sample of pus is sent to a laboratory and placed in a culture medium that allows microorganisms to grow). The results of the culture are used to guide choice of antibiotic. The person may also need to temporarily stop shaving.

Skin Abscesses: Skin abscesses, also called boils, are warm, painful, pus-filled pockets of infection below the skin surface that may occur on any body surface. Abscesses may be one to several inches in diameter. Furuncles are smaller, more superficial abscesses that by definition involve a hair follicle and the surrounding tissue. Carbuncles are multiple furuncles that are connected to one another below the skin surface. If not treated, abscesses often come to a head and rupture, discharging a creamy white or pink fluid. Bacteria may spread from the abscess to infect the surrounding tissue and lymph nodes. The person may have a fever and feel generally sick.

A skin abscess may go away with application of warm compresses. Otherwise, a doctor treats an abscess by cutting it open and draining the pus. After draining the abscess, a doctor makes sure all of the pus has been removed by washing out the pocket with a sterile salt solution. Sometimes the drained abscess is packed with gauze, which is removed 24 to 48 hours later. If the abscess is completely drained, antibiotics usually are not needed. However, if the abscess is on the middle or upper part of the face, antibiotics that kill staphylococci, such as dicloxacillin and cephalexin, may be used because of the risk that the infection will spread to the brain. Antibiotics also are needed if the infection has spread or if the person has a weakened immune system.

People who have recurrent skin abscesses can wash their skin with liquid soap that contains special antiseptics, or they can take antibiotics for 1 to 2 months.

Hidradenitis suppurativa is inflammation of the apocrine sweat glands, resulting in painful accumulations of pus under the skin.

Hidradenitis suppurativa develops in some people after puberty because the apocrine sweat glands (the specialized sweat glands under the arms, in the genital area, around the anus, and under the breasts) are chronically blocked. Doctors do not know why the blockage occurs, however it is not evident that it is not related to

the use of deodorants or powders or to underarm shaving. The blockage causes the glands to swell and rupture, frequently leading to infection by various bacteria. The abscesses (pus-filled pockets) that result are painful and foul smelling and tend to recur. After several recurrences, the skin in the area becomes thick and scarred.

Hidradenitis suppurativa resembles common skin abscesses. A doctor makes the diagnosis based on the location of the abscesses and on the fact that they recur often.

For people with mild cases, a doctor injects corticosteroids into the area and prescribes antibiotics, such as tetracycline or erythromycin, to be taken by mouth. Clindamycin applied topically is also effective. In some cases, a doctor cuts open the abscesses to drain the pus. In severe cases, an anti-inflammatory drug, may be given by mouth. Laser treatment has also been used. In severe cases, cutting out the involved area followed by skin grafting may be necessary.

Impetigo is a skin infection, caused by *Staphylococcus aureus*, *Streptococcus pyogenes*, or both, that leads to the formation of scabby, yellow-crusted sores and, sometimes, small blisters filled with yellow fluid.

Impetigo is common. It affects mostly children. Impetigo can occur anywhere on the body but most commonly occurs on the face, arms, and legs. The blisters that may form (bullous impetigo) can vary from pea-sized to large rings and can last for days to weeks. Impetigo often affects normal skin but may follow an injury or a condition that causes a break in the skin, such as a fungal infection, sunburn, or an insect bite. Poor hygiene and a moist environment are also risk factors. Some people have Staphylococcus bacteria living in their nose without causing disease (they are considered nasal carriers). These nasal bacteria may cause repeat infection in the person and sometimes in others.

Impetigo is itchy and slightly painful. The itching often leads to extensive scratching, particularly in children, which serves to spread the infection. Impetigo is very contagious—both to other areas of the person's own skin and to other people. Impetigo typically causes clusters of sores to rupture and develop a honey-colored crust over the sores. Bullous impetigo is similar except that the sores typically enlarge rapidly to form blisters. The blisters burst and expose larger bases, which become covered with honey-colored varnish or crust.

Diagnosis is based on the appearance of the rash. If people have repeated infections, a swab of the nose is taken and sent to the laboratory to determine if they are a nasal carrier of staphylococci.

The infected area should be washed gently with soap and water several times a day to remove any crusts. Small areas are treated with topical antibiotics. If large areas are involved, an antibiotic taken by mouth may be needed. People who are nasal carriers are treated with topical antibiotics applied to the nasal passages.

Lymphadenitis is an inflammation of one or more lymph nodes, which usually become swollen and tender.

Lymphadenitis almost always results from an infection, which may be caused by bacteria, viruses, protozoa, rickettsiae, or fungi. Typically, the infection spreads to a lymph node from a skin, ear, nose, or eye infection or from such infections as infectious mononucleosis, cytomegalovirus infection, streptococcal infection, tuberculosis, or syphilis. The infection may affect many lymph nodes or only those in one area of the body.

Symptoms and Diagnosis

Infected lymph nodes enlarge and are usually tender and painful. Sometimes, the skin over the infected nodes looks red and feels warm. The person may have a fever. Occasionally, pockets of pus (abscesses) develop. Enlarged lymph nodes that do not cause pain, tenderness, or redness may indicate a serious different disorder, such as lymphoma, tuberculosis, or Hodgkin lymphoma. Such lymph nodes require a doctor's attention.

Usually, lymphadenitis can be diagnosed on the basis of symptoms, and its cause is an obvious nearby infection. When the cause cannot be identified easily, a biopsy (removal and examination of a tissue sample under a microscope) and a culture (a sample is sent to a laboratory and placed in a culture medium that allows microorganisms to grow) may be needed to confirm the diagnosis and to identify the organism causing the infection.

Treatment

Treatment depends on the organism causing the infection. For a bacterial infection, an antibiotic is usually given intravenously or by mouth. Warm compresses may help relieve the pain in inflamed lymph nodes. Usually, once the infection has been treated, the lymph nodes slowly shrink, and the pain subsides. Sometimes the enlarged nodes remain firm but no longer feel tender. Abscesses must be drained surgically.

Lymphangitis is inflammation of one or more lymphatic vessels, usually caused by a streptococcal infection.

Streptococci bacteria usually enter the lymphatic vessels from a scrape or wound in an arm or a leg. Often, a streptococcal infection in the skin and the tissues just beneath the skin spreads to the lymph vessels. Occasionally, staphylococci or other bacteria are the cause.

Lymphangitis is characterized by red, irregular, warm, tender streaks which develop on the skin in the affected arm or leg. The streaks usually stretch from the infected area toward a group of lymph nodes, such as those in the groin or armpit. The lymph nodes become enlarged and feel tender.

Common symptoms include a fever, chills, a rapid heart rate, and a headache. Sometimes these symptoms occur before the red streaks appear. The spread of the infection from the lymph system into the bloodstream can cause infection throughout the body, often with startling speed. The skin or tissues over the infected lymph vessel becomes inflamed. Rarely, skin ulcers develop. Sometimes, bacteria enter the bloodstream (bacteremia).

The diagnosis of lymphangitis is based on its typical appearance. A blood test usually shows that the number of white blood cells has increased to fight the infection. Doctors have difficulty identifying the organisms causing the infection unless the organisms have spread through the bloodstream or pus can be taken from a wound in the affected area.

Most people recover quickly with antibiotics that kill staphylococci and streptococci, such as dicloxacillin, nafcillin, or oxacillin.

Necrotizing skin infections, including necrotizing cellulitis and necrotizing fasciitis, are severe forms of cellulitis characterized by death of infected tissue (necrosis).

Most skin infections do not result in the death of skin and nearby tissues. Sometimes, however, bacterial infection can cause small blood vessels in the infected area to clot. This clotting causes the tissue fed by these vessels to die from lack of blood. Because the body's immune defenses that travel through the bloodstream (such as white blood cells and antibodies) can no longer reach this area, the infection spreads rapidly and may be difficult to control. Death can occur, even with appropriate treatment.

Some necrotizing skin infections spread deep in the skin along the surface of the muscle (fascia) and are termed necrotizing fasciitis.

Other necrotizing skin infections spread on the outer layers of skin and are termed necrotizing cellulitis. Several different bacteria, such as Streptococcus and Clostridia, may cause necrotizing skin infections, although in many people the infection is caused by a combination of bacteria. This streptococcal infection in particular has been termed "flesh-eating disease" by the lay press, although it differs little from the others.

Some necrotizing skin infections begin at puncture wounds or lacerations, particularly wounds contaminated with dirt and debris. Other infections begin in surgical incisions or even healthy skin. Sometimes people with diverticulitis, intestinal perforation, or tumors of the intestine develop necrotizing infections of the abdominal wall, genital area, or thighs. These infections occur when certain bacteria escape from the intestine and spread to the skin. The bacteria may initially create an abscess in the abdominal cavity and spread directly outward to the skin, or they may spread through the bloodstream to the skin and other organs.

Symptoms and Diagnosis

The onset of cellulitis may be the commencement of necrotizing disease. The skin may look pale at first, but quickly becomes red or bronze and warm to the touch, and sometimes becomes swollen. Later, the skin turns violet, often with the development of large fluid-filled blisters (bullae). The fluid from these blisters is brown, watery, and sometimes foul smelling. Areas of dead skin turn black (gangrene). Some types of infection, including those caused by Clostridia and mixed bacteria, produce gas. The gas creates bubbles under the skin and sometimes in the blisters themselves, causing the skin to feel crackly when pressed. Initially the infected area is painful, but as the skin dies, the nerves stop working and the area loses sensation.

The person usually feels very ill and has a high fever, a rapid heart rate, and mental deterioration ranging from confusion to unconsciousness. Blood pressure may fall because of toxins secreted by the bacteria and the body's response to the infection.

A doctor makes a diagnosis of necrotizing skin infection based on its appearance, particularly the presence of gas bubbles under the skin. X-rays may show gas under the skin as well. The specific bacteria involved are identified by laboratory analysis of infected fluid and tissue samples. However, treatment must begin before a doctor can be certain which bacteria are causing the infection.

Prognosis and Treatment

The overall death rate is about 30%. Older people, those who have other medical disorders, and those in whom the disease has reached an advanced stage have a poorer outcome. A delay in diagnosis and treatment and insufficient surgical removal of dead tissue, worsen the prognosis.

The treatment for necrotizing fasciitis is surgical removal of the dead tissue plus intravenous antibiotic therapy. Large amounts of skin, tissue, and muscle must often be removed, and in some cases, an affected arm or leg may have to be amputated. Some doctors recommend treatment in a high-pressure (hyperbaric) oxygen chamber, but it is not clear how much this helps.

Staphylococcal scalded skin syndrome is a reaction to a staphylococcal skin infection in which the skin blisters and peels off as though burned.

Certain types of staphylococci bacteria secrete toxic substances that cause the top layer of the epidermis to split from the rest of the skin. Because the toxin spreads throughout the body, staphylococcal infection of a small area of skin may result in peeling over the entire body. Staphylococcal scalded skin syndrome occurs almost exclusively in infants and young children under the age of 6. It rarely occurs in older people except for those with kidney failure or a weakened immune system. Like other staphylococcal infections, staphylococcal scalded skin syndrome is also contagious.

Symptoms

Symptoms begin with an isolated, crusted infection that may look like impetigo. In newborns, the infection may appear in the diaper area or around the stump of the umbilical cord. In older children, the face is the typical site of infection. In adults, the infection may begin anywhere. In all people with this disorder, scarlet-colored areas appear around the crusted area within a day of the beginning of infection. These areas may be painful. The skin may be extremely tender and have a wrinkled tissue paper–like consistency. Then, other large areas of skin distant from the initial infection redden and develop blisters that break easily.

The top layer of the skin then begins peeling off, often in large sheets, with even slight touching or gentle pushing. The peeled areas look scalded. Within another 1 to 2 days, the entire skin surface may be involved, and the person becomes very ill with a fever, chills, and weakness. With the loss of the protective skin barrier, other bacteria and infective organisms can easily penetrate the body, causing what doctors call superinfections. Also, critical amounts of fluid can be lost because of oozing and evaporation, resulting in dehydration, leading to further deterioration in the patient's condition.

Diagnosis and Treatment

A diagnosis is made by the appearance of skin peeling after an apparent staphylococcal infection. If no signs of staphylococcal infection are observed, doctors often perform a biopsy, in which a small piece of skin is removed and sent to the laboratory to be tested. Swabs taken from the nose, the thin mucous membrane that covers the eyes (conjunctiva), the throat, and the nasal passages and upper throat (nasopharynx) are sent to the laboratory to be cultured for bacteria.

Treatment is with antibiotics for at least a week. Local wound care with topical emollients reduces the itching and protects the skin from drying out.

Treatment

Topical drugs (drugs applied directly to the skin) are a mainstay of treating skin disorders. Systemic drugs are taken by mouth or given by injection and are distributed throughout the body. Rarely, when a high concentration of a drug is needed at the affected area, a doctor injects the drug just under the skin (intradermal injection).

Topical Preparations

In a topical preparation, the active ingredient, or drug, is mixed with an inactive ingredient (vehicle). The vehicle determines the consistency of the product (for example, thick and greasy or light and watery) and whether the active ingredient remains on the surface or penetrates the skin. Depending on the vehicle used, the same drug can be placed in an ointment, cream, lotion, solution, gel, oil, foam, or powder. In addition, many preparations are available in different strengths (concentrations).

Ointments (such as petroleum jelly) are oily and contain very little water. They are messy, greasy, and difficult to wash off. Ointments are most appropriate when the skin needs lubrication or moisture. Ointments are usually better than creams at delivering active ingredients into the skin. A given concentration of a drug is more potent in an ointment than in a cream. Ointments are less irritating than creams and much less irritating than gels, lotions, and

solutions for open wounds such as erosions or ulcers.

Creams , the most commonly used preparations, are emulsions of oil in water, meaning they are primarily water with an oil component. (An ointment is the opposite, some water mixed mostly with oil.) Creams are easy to apply and appear to vanish when rubbed into the skin. They are relatively non-irritating.

Lotions are pharmaceutical compositions similar to creams but contain more water. They are actually suspensions of finely dispersed, powdered material in a base of water or oil and water. They are less effective than ointments, creams, and gels at delivering drugs and are considered of lower potency for a given drug concentration. Lotions have a number of beneficial effects. They are easy to apply to hairy skin, and they are particularly useful for cooling or drying inflamed or oozing lesions, such as those caused by contact dermatitis, athlete's foot (tinea pedis), and jock itch (tinea cruris).

Baths and soaks are used when treatment must be applied to large areas of the body. This technique is most often used in the form of sitz baths for over-the-counter (OTC) treatments of mild skin problems such as hemorrhoids. Baths are not often used to apply potent prescription drugs because of difficulties controlling the amount of drug delivered.

Solutions are liquids in which a drug is dissolved. The most commonly used liquids are alcohol, propylene glycol, polyethylene glycol and plain water. Solutions are convenient to apply but tend to dry rather than moisturize the skin. However, this drying effect is useful for wet, oozing (weeping) skin disorders. Depending on the vehicle used, solutions can be irritating to the skin, particularly when those containing alcohol and propylene glycol are applied to open wounds.

Powders are dried forms of substances that are used to protect areas where skin rubs against skin—for instance, between the toes or buttocks, in the armpits or groin, or under the breasts. Powders are used on skin that has been softened and damaged by moisture (macerated). They may be mixed with active drugs such as antifungals.

Gels are water- or alcohol-based substances thickened without oil or fat. The skin does not absorb gels as well as it absorbs preparations containing oil or fat. Gels may be quite irritating on open wounds and diseased skin.

Damaged skin may be an entry point for pathogens and microbes into the system in general, however immediate action in the form of a good first aid and immediate medical assistance is often the first and the best option in preventing diseases and further complications associated with wounds.

Reference: Merck Manual.

Encyclopedia

Renal lithiasis can be defined as the consequence of an alteration of the normal crystallization conditions of urine in the urinary tract. In a healthy individual, during the residence time of urine in the urinary tract, crystals either do not form or are so small they are eliminated uneventfully (asymptomatic crystalluria). When normal urine crystallization conditions become altered, however, the rate of crystal nucleation and growth may become such that the crystals cannot be easily eliminated due to their size. In some cases, altered urinary conditions affecting crystallization are related to specific underlying disorders such as hyperparathyroidism, which is associated with hypercalcularia; tubular acidosis, which is associated with hypercalcularia and hypocitraturia; and some genetic alterations, which are associated with hyperoxaluria, hypercystinuria and hypercalciuria. However, in many cases it is not possible to clearly identify the underlying disorder. Indeed, in nearly all renal calculi cases, crystal formation is attributable to a combination of diverse factors that may or may not be associated with an underlying disorder. These factors can be classified into two main: urine composition factors and renal morphoanatomy factors.

Urine composition factors are important in crystal formation as urine is a metastable liquid containing several coexisting substances that can crystallize to generate renal calculi. These substances are present at supersaturated levels (the system contains higher amounts of solute than that corresponding to the solubility), meaning the urine is in an unstable state, and a stable urine state will eventuate through crystallization of the excess solute. The ease of crystallization depends on the degree of supersaturation, the presence of preformed particles (so-called heterogeneous nucleants that act as promoter substances) and the level if crystallization inhibitors. These latter substance inhibit crystal nucleation and / or growth.

There are two main renal morphoanatomy factors that can affect crystal formation. The first of these is the presence of cavities (formed by renal calices) with low urodynamic efficacy that retain urine for long periods. The second is an altered epithelium covering the renal papillae, which can arise from events such as damage to the anti-adherent glycosaminoglycan layer that covers the uroepithelium, necrosis, or the presence of subepithelial calcifications.

The development of renal calculi usually involves both urine composition and renal morphoanatomy factors. Thus, not all people with hypercalciuria, hypocitraturia or hyperuricuria, for instance, will develop renal calculi. The effect of a factor on renal lithiasis depends on the nature and magnitude of other factors when a particular renal stone is generated. For example, while some alterations to the uroepithelium alone may not be serious enough to cause stone formation, when combined with other factors stone formation might occur. Thus, precise knowledge of the factors involved in the development of a given renal calculus is vital, and adequate study of the calculus structure and composition allows identification of an important number of possible etiologic factors related to its formation. Many such factors can be adequately modified through diet, as this has a significant effect on urine composition. Indeed, altering inappropriate habitual dietary patterns should be the main measure for preventing kidney stones.

There are various types of renal calculi namely; Calcium oxalate monohydrate papillary calculi, Calcium oxalate monohydrate unattached calculi, Calcium oxalate dihydrate calculi, Calcium oxalate dihydrate/hydroxyapatite mixed calculi, Hydroxyapatite calculi, Struvite infectious calculi, Brushite calculi, Uric acid calculi, Calcium oxalate/uric acid mixed calculi, Cystine calculi

Conclusion: Preventive measure for avoiding renal calculus formation involve specific dietary considerations. While there are specific dietary factors to be considered for different calculi, there is also a general list of dietary measures that can be recommended in order to avoid any renal calculus formation: ● Daily intake of a suitable liquid volume (minimum 2 L water/day) ● Avoid strictly vegetarian diets ● Avoid excessive animal protein diets ● Avoid excessive salt (NaCl) consumption ● Avoid excessive vitamin C and/or vitamin D consumption ● Consume phytate-rich products (natural dietary bran, legumes and beans, whole cereals) ● Avoid exposition to cytotoxic substances (i.e., analgesics abuse, residual pesticides, organic solvents and cytotoxic drugs).

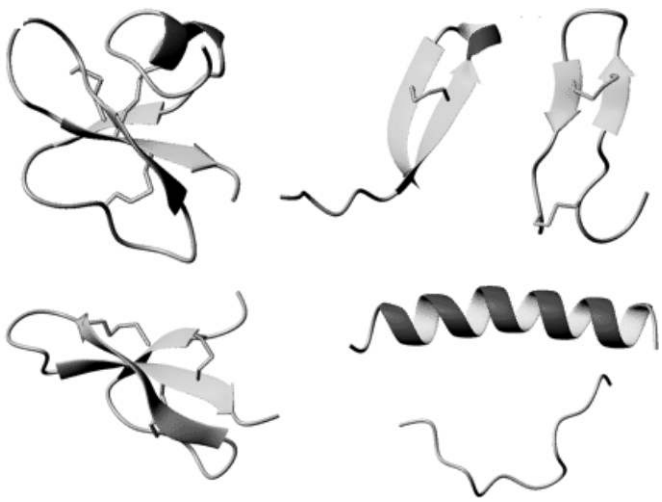
Reference: Review: Renal lithiasis and nutrition by Felix Grases et al; 2006

Antimicrobial Peptides (AMP) and PHMB

Due to an alarming rise in the occurrence of antibiotic resistant bacterial strains, identification of new antimicrobial compounds has become one of the frontier areas in biomedical research. The emergence of antibiotic-resistant strains is linked with the overuse and misuse of antibiotics and is long-established, widely-studied problem. The focus is now being shifted to the use of antimicrobial ingredients, other than antibiotics, present in disinfectants, antiseptics and sanitizers, for the occurrence of resistant strains. It is important to understand that, by reducing the number of infection outbreaks through effective hygiene, the number of antibiotic courses prescribed can be lowered, which can in turn reduce the impact of antibiotic resistance.

As a promising approach for the reduction of bacterial pathogens in wounds, the innate immune system has aroused new scientific interest. It is well known that the innate immunity is triggered immediately after microbial infection to produce antimicrobial compounds. Based on this fact recent studies show that several metabolites of unusual structure and exhibiting biological activities are expressed in many vertebrate, invertebrate and bacterial species. Some of these bioactive metabolites have biomedical potential. **A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from animals and plants during the past two decades. Among them, cationic peptides are the most widespread. These** peptides have been found to play a major role as cutaneous antimicrobial and immunomodulating agents, known as antimicrobial peptides (AMPs) or host defence peptides (HDPs). AMPs form part of the ancient, nonspecific innate immune system, which is the principal defense system for the majority of living organisms. In many cases, their primary role is in the killing of invading. pathogenic organisms. The value of antimicrobial peptides in innate immunity lies in their ability to function without either high specificity or memory, and their small size makes them easy to synthesize. **In spite of the astonishing diversity in structure and chemical nature displayed by these molecules, all of them present antimicrobial activity, a condition that has led researchers to consider them as "natural antibiotics" and as such a new and innovative alternative to chemical antibiotics with a promising future as biotechnological tools. A resulting new generation of anti microbial peptides (AMPs) with higher specific activity and wider microbe-range of action could be constructed, and hopefully endogenously expressed in genetically-modified organisms.** The knowledge acquired in the past two decades and the discovery of antimicrobial peptides (AMPs) make them the basic element of a novel generation of drugs for the treatment of bacterial and fungal infections. AMPs show remarkable specificity for prokaryotes with low toxicity for eukaryotic cells. This is a characteristic that has favored their investigation and exploitation as potential new natural drugs.

Antimicrobial peptides are a unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure. Antimicrobial peptides generally have between 12 and 100 amino acids. They are defined as molecules less than 10 kDa in mass which show antimicrobial properties. These evolutionarily conserved peptides are usually positively charged and are amphiphilic (have both a hydrophobic and hydrophilic side) in nature, that enables the molecule to be soluble in aqueous environments yet also enter lipid-rich membranes. The major classes of antimicrobial peptides include (i) α-helices, (ii) β-sheet and small proteins, (iii) peptides with thio-ether rings, (iv) peptides with an over representation of one or two amino acids, (v) lipopeptides, and (vi) macrocyclic cystine knot peptides. The most prominent structures are amphiphilic peptides with two to four-strands, amphipathic-helices, loop structures, and extended structures.

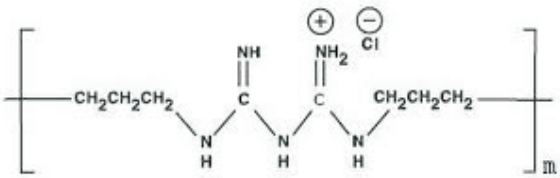


Various Antimicrobial peptides

Unlike the majority of conventional antibiotics it appears as though antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators. In addition to important antimicrobial properties, growing evidence indicates that AMPs alter the host immune response through receptor-dependent interactions. Cathelicidins and defensins are major groups of epidermal AMPs. AMPs have been demonstrated to kill Gram positive and Gram negative bacteria (including strains that are resistant to conventional antibiotics), mycobacteria, enveloped viruses, fungi and even transformed or cancerous cells. Examples of antimicrobal peptides include magainins, alamethicin, pexiganan or MSI-78, human antimicrobial peptide, LL-37, defensin, protegrins etc. In addition to the wide spectrum of antimicrobial activities reported for these molecules, a number of naturally occurring peptides and their derivatives have been developed as novel anti-infective therapies for conditions as diverse as oral mucositis, lung infections associated with cystic fibrosis (CF), cancer, viral and topical skin infections. Pexiganan has been shown to be useful to treat infection related diabetic foot ulcer. AMPs have been shown to be important in such diverse functions as angiogenesis, wound healing, and chemotaxis.

On the other hand, the antiseptic/antimicrobial compound polyhexamethylene biguanide or PHMB belongs to the biguanide group. Biguanides are important class of cationic surface active antimicrobial agents, which are used as antiseptics & disinfectants. PHMB is a polymeric biguanide. It is a polycationic linear polymer comprising of a hydrophobic backbone with multi-cationic grouping separated by

hexamethylene chain. The basic molecular chain of PHMB can be repeated from 2 to 30 times, with increasing polymer chain length correlating with increasing antiseptic/antimicrobial efficacy. Polyhexamethylene biguanides (PHMB) are mixtures of polymeric biguanides with an average polymer length (*n*) of 5, but containing high ($n > 15$, mol. wt 3300) and low molecular weight material ($n = 2$, mol. wt 400). Studies involving discrete molecular weight fractions of PHMB have shown that antimicrobial activity of PHMB increases with increasing polymer length.



PHMB

PHMB is a heterodisperse mixture of polymers and is asynthetic compound which is structurally similar to naturally occurring antimicrobial peptides (AMPs). The structural similarities between AMPs and PHMB mean that the latter can enter bacterial cell membranes and kill bacteria in a similar way to AMPs (Moore and Gray, 2007). The primary targets appear to be the outer and cytoplasmic membranes. PHMB is thought to adhere to and disrupt target cell membranes, causing them to leak potassium ions and other cytosolic components which results in bacterial cell death. There is also evidence that following penetration into target cells, PHMB binds to DNA and other nucleic damaging or inactivating bacterial DNA.

PHMB is chemically stable and non volatile compound. It has very low surface activity, having a surface tension essentially identical to water. PHMB consequently can be readily water rinsed from surfaces & do not have residual streaks or tackiness. It is odorless, non foaming, clear & colorless. PHMB is effective & stable over a wide pH range (4-10). It is easy to handle & apply. PHMB is clear and colourless, soluble in water, glycols & alcohol but insoluble in non polar solvents like petroleum, ethers or toluene. In medicine PHMB was introduced by Willenegger in 1994 as an antiseptic in abdominal surgery. 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. PHMB containing wound rinsing solutions show superior wound healing due to its antimicrobial efficacy as well as its ability to maintain the moist environment. *In-vitro* and *in-vivo* studies into the effectiveness of PHMB in wound care have demonstrated that the product may also have other 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 buildup of necrotic tissue and so promoted connective tissue regeneration. Wiegand *et al.,* (2008) demonstrated that PHMB can have a positive effect on tissue proliferation. Use of PHMB, 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.

PHMB has been successfully tested for wound irrigation, wound care dressings (Surgical & non surgical), diabetic foot ulcer management, pre-operative antiseptic for surgery, sanitizer, and preservative in topical ophthalmic preparations, inter-operative irrigation, peri-operative cleansing products, treatment of fungal infections, contact lens cleaning solutions and swimming pool cleaners.

Similarities between AMPs and PHMB

1. Both are structurally similar
2. AMPs generally contain 12 to 100 amino acids while the basic molecular chain of PHMB can be repeated 2 to 30 times
3. Both are cationic in nature
4. Both are low molecular weight compounds (molecular weight of AMPs is not more than 10 KD while molecular weight of PHMB varies from 400 to 3300 depending on the number of polymers it contains)
5. Both have the novel non specific mode of action against pathogens. They target the outer and cytoplasmic membranes of pathogens and kill the pathogen by disrupting membranes, interfering with metabolism and targeting the cytoplasmic components
6. Both require a short contact time to induce killing of pathogens
7. Both have broad spectrum of activity against pathogens
8. In contrast to conventional antibiotics both PHMB and AMPs do not appear to induce resistance in pathogens
9. Both are safe and non cytotoxic to human cells
10. Both play an important role in wound repair and healing
11. Both are excellent candidates for development as novel therapeutic agents

Both AMPs and PHMB are antiseptics/antimicrobials that are gaining importance as alternatives to conventional antibiotics. AMPs and PHMB are exciting candidates as new antiseptic/antimicrobial agents due to their broad antimicrobial spectra, highly selective toxicities, and the difficulty for bacteria to develop resistance to these peptides. These compounds bind to bacterial cell membrane and induce cell lysis by destroying membrane integrity, in a similar way that penicillin and cephalosporin antibiotics do. Antiseptics/antimicrobial compounds have been in use for much longer than antibiotics yet resistance to antiseptics/antimicrobial compounds present much less of a problem. Antiseptics such as PHMB are an alternative to antibiotic prophylaxis and are less likely to generate resistance.

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Susumu Tonegawa

Birth: September 6, 1939

Nationality: Japanese

Known for: Discovery of the genetic mechanism that produces antibody diversity

Susumu Tonegawa was born in Nagoya, Japan on September 6th, 1939, and was the second of the family's four children. His father was an engineer working for a textile company that had several factories scattered in rural towns in the southern part of Japan. His parents firmly believed that education is the best asset that parents can give to their children. As his elder brother and he reached adolescence, his parents decided to send them to Tokyo so that they could receive a better education.

He commuted to the prestigious Hibiya high school from his Uncle's home in Tokyo. During his high school years he developed an interest in chemistry, so upon graduation, he chose to take an entrance examination for the Department of Chemistry of the University of Kyoto, the old capital of Japan. After having failed once, he was admitted to this University in 1959.

A certain political event/experience, which had Susumu as a passive participant may have been a major factor in making him give up his original goal of becoming a chemical engineer to pursue the academic life.

Tonegawa then became fascinated by the then blossoming science of molecular biology when in his senior year he happened to read the papers by Francois Jacob and Jacques Monod on the operon theory. He decided to pursue graduate study in molecular biology and was accepted by Professor Itaru Watanabe's laboratory at the Institute for Virus Research at the University of Kyoto, one of a few laboratories in Japan where U.S.-trained molecular biologists were actively engaged in research. However, only two months after he started his work in the laboratory, Professor Watanabe suggested that he should carry out his graduate study in the United States. Since the labs in Japan including his own were inadequate for graduate training programs in molecular biology laboratories and offered to help Susumu in applying to some major universities in the United States.

With the additional help of Dr. Takashi Yura, then an assistant professor in Watanabe's laboratory, he was admitted to the graduate school of the Department of Biology of the University of California at San Diego that had recently been established by Professor David Bonner in La Jolla.

At the University of California, San Diego he studied in the laboratory of Professor Masaki Hayashi, carrying out a thesis project on the transcriptional control of phage lambda and received his Ph.D. in molecular biology in 1968. He remained in Professor Hayashi's laboratory as a postdoctoral fellow working on the morphogenesis of a phage, ØX174, until early 1969. Then he moved, also as a postdoctoral fellow, across the street to the laboratory of Dr. Renato Dulbecco at the Salk Institute.

Like many others, he believed that the golden age of prokaryotic molecular biology was coming to an end and that the great excitement would be in higher organisms. However, the complexity of high organisms was baffling and the necessary tools seemed hopelessly insufficient. Small tumor viruses like polyoma and simian virus 40, the biological material primarily dealt with in Dulbecco's laboratory, seemed to offer a bridge for the gap between prokaryotes and eukaryotes.

His project was to define the transcripts of SV40 during lytic infection and in transformed cells. Since this was the pre-restriction enzyme and pre-recombinant DNA age, the information he could obtain was very limited. However, being a member of the best laboratory in the field he glimpsed the excitement of the cutting edge of scientific research. Unfortunately, as an awardee of a Fulbright travel grant, his U.S. visa was to expire by the end of 1970 and he had to leave the country for at least two years before he was eligible for another U.S. visa.

Renato mentioned the newly established Basel Institute for Immunology in Basel, Switzerland, and suggested that the time might be ripe for a molecular biologist to tackle immunological problems, though Susumu had very little knowledge of immunology, he decided to take Dr. Dulbecco's advice and sent an application letter to the Director of the Institute, Professor Niels Kaj Jerne, who offered him a two-year contract.

By the winter of 1971, Susumu found himself surrounded by immunologists in this small town located in the middle of Europe. The first year in the Institute was not easy for him, since he held an interest in work on SV40, but was also keenly aware that he would not be able to take much advantage of the circumstances if he isolated himself by pursuing that subject. He therefore decided to study immunology in the hope of finding an interesting project.

An immunologist, Dr. Ita Askonas, and a geneticist, Charles Steinberg, were very helpful, on his entering the new field. By the end of 1971, he was introduced to the great debate on the genetic origins of antibody diversity. He felt he could contribute to resolving this question by applying the recently invented techniques of molecular biology, namely, restriction enzymes and recombinant DNA. Initially he worked only with skillful technicians, Monica Shöld and Rita Schuller, but was soon joined by Drs. Nobumichi Hozumi, Minoru Hirama, and Christine Brack. Later, as his research group expanded, he worked with many capable postdoctoral fellows and devoted technical assistants. In addition, Charles Steinberg was a very important collaborator and consultant, particularly in the initial phase of the research.

The research progressed with amazing speed from 1974 to 1981, the year he left Basel. The whole team worked hard. And finally the work resolved the long held debate on the genetic origin of antibody diversity. It turned out that this diversity is generated by somatic recombination of the inherited gene segments and by somatic mutation. Director Niels Jerne was quick to understand the importance of this approach and became a staunch supporter of the research in its early phase.

The research projects on which Tonegawa decided to take up, concerned two major problems, namely to investigate the role of somatic rearrangement in the activation of the rearranged antibody gene, and the second was to extend the research in Basel to "the other half" of the immune system, namely, to the antigen receptor of T cells. Susumu and his team could contribute to the understanding of both problems by discovering a tissue-specific transcriptional enhancer in the immunoglobulin heavy chain gene and by identifying, cloning, and sequencing genes coding for the polypeptide subunits of the T cell receptor.

Tonegawa has been a recipient of many professional honors which include the NOBEL PRIZE in Physiology or Medicine, Stockholm, Sweden (1987) among many others.

Reference: http://nobelprize.org/nobel_prizes/medicine/laureates/1987/tonegawa-autobio.html

Enjoy the humour

Signboard outside a Pathology Clinic:
"For You , it may be your piss and shit,! But for us, its our bread and butter!!"

A baby mosquito came back after its first fight.
Dad asked: How did you feel?
It replied: Dad it was wonderful everyone was clapping for me.
Moral: Take everything in your stride.

2 friends were attending a boring lecture.
1st friend: "Even my ass has fallen asleep..!"
2nd friend: "Yeah man I know, I heard it snoring 3 times..!"

Thoughts to live by

- In order to be irreplaceable one must always be different. (Coco Chanel)
- Do what you feel in your heart to be right- for you'll be criticized anyway. You'll be damned if you do, and damned if you don't. (Eleanor Roosevelt)
- Absence sharpens love, presence strengthens it. (Benjamin Franklin)
- There are people who have money and people who are rich. (Coco Chanel)
- There are two things a person should never be angry at, what they can help, and what they cannot. (Plato)

Track your brain

1. Skin is separated from the underlying muscle by the _____.
2. _____ is an infection of the hair follicle.
3. _____ is one of the most feared infections caused by animal bites.
4. Large fluid filled blisters are referred to as _____.
5. _____ results is painful, red, raised patch, on the skin.
6. _____ leads to the formation of scabby, yellow crusted sores.
7. AMPs have been shown to be important in such diverse functions as _____, wound healing and chemotaxis.
8. _____ has been shown to be useful to treat infections related to diabetic foot ulcers.
9. Susumu Tonegawa discovered the _____ mechanism that produces diversity for which he received the Nobel prize in 1987.
10. *Borrelia burgdorferi* is a _____ which causes Lyme disease.
11. _____ are most effective at pH 2 – 5 and at concentrations ranging from 6 – 13 ppm.
12. Washing fruits and vegetables with _____ prior to consumption is a preferred practice.

Check your Answers on Page 16

Borrelia burgdorferi

Lyme disease was first recognized in the United States in 1975 by Dr. Allen Steere, following a mysterious outbreak of juvenile rheumatoid arthritis near the community of Old Lyme, Connecticut. Parents were perplexed by the high incidence of arthritis among their children and called for an investigation; this revealed that the children were suffering from a "new" disease. Factors such as rural location of Lyme outbreak and the onset of illness during summer and early fall suggested that the transmission of the disease was by an arthropod vector.

Subsequently In 1982, the etiological agent of Lyme disease was discovered by Willy Burgdorfer, who isolated spirochetes belonging to the genus Borrelia from the mid-guts of *Ixodes* ticks. He showed that these spirochetes, reacted with immune sera from patients that had been diagnosed with Lyme disease. Thus, the etiologic agent was given the name *Borrelia burgdorferi*.

Biology of Spirochetes

Borrelia burgdorferi, is a spirochete. Spirochetes are a group of phylogenetically-distinct bacteria that have a unique mode of motility by means of axial filaments (endoflagella). Spirochetes are widespread in viscous environments and they are found in the intestinal tracts of animals and the oral cavity of humans. The spirochetes have a unique cell surface which accompanies their unique type of motility. The endoflagella are contained within the periplasmic space between a semi rigid peptidoglycan helix and a multi-layer, flexible outer membrane sheath. When the filaments rotate within this space, the spirochetes move in cork-screw fashion.

Spirochetes are usually much longer than they are wide, and often their width is below the resolving power of the light microscope. Dark-field microscopy must be used to view spirochetes. Dark field microscopy utilizes a special condenser which directs light towards an object at an angle, rather than from the bottom. As a result, particles or cells are seen as light objects against a dark background.

The spirochetes are not classified as either Gram-positive or Gram-negative. When *Borrelia burgdorferi* is Gram-stained, the cells stain a weak Gram-negative by default, as safranin is the last dye used. Borrelia, like most spirochetes, does have an outer membrane that contains an LPS-like substance, an inner membrane, and a periplasmiic space which contains a layer of peptidoglycan. Therefore, it has a Gram-negative bacterial type cell wall, despite its staining characteristics.

Cultivation

Borrelia burgdorferi can be cultivated in vitro. However, the bacterium is fastidous and requires a very complex growth medium. The medium used to grow *Borrelia burgdorferi* is called Barbour-Stoenner-Kelly (BSK) medium. It contains over thirteen ingredients in a rabbit serum base. *Borrelia burdorferi* has an optimal temperature for growth of 32^{o}C, in a microaerobic environment. Even under optimal conditions, the generation time is slow, about 12 – 24 hours.

Borreliae from ticks and from the blood, skin, and cerebrospinal fluid of Lyme disease patients have been successfully cultivated in BSK medium. BSK solidified with 1.3% agarose allows the production of colonies from single organisms.

Other Surface proteins

The outer membrane of *Borrelia burgdorferi* is composed of various unique outer space proteins (Osp) that have been characterized (Osp A through Osp F) and are presumed to play a role in virulence. Osp A and Osp B are by far the most abundant outer surface proteins.

Pathogenicity

Borrelia burgdorferi invades the blood and tissues of various infected mammals and birds. The natural reservoir for it is thought to be the white-footed mouse. Ticks transfer the spirochetes to the white-tailed deer, humans, and other warm-blooded animals after a blood meal on an infected animal. In humans, dogs, and many other animals, infection with *Borrelia burgdorferi* results in the pathology of Lyme Disease.

Transmission of Lyme Disease

Lyme disease is spread by the bite of ticks of the genus Ixodes that are infected with *Borrelia burgdorferi*. Ixodes, commonly known as the deer tick (or bear tick), normally feeds on the white-footed mouse, the white-tailed deer, and certain other mammals. It is responsible for transmitting the spirochetes to humans in the northeast and north-central US. On the pacific Coast, the bacteria are transmitted to humans by the western black-legged tick, and in the southeastern states by the related black-legged tick. Spirochete prevalence in adult Ixodes ticks is highly variable depending on geographic location.

Epidemiology and disease description

Lyme disease is the most common tick-transmitted disease in the world. There are roughly 20,000 new cases of Lyme disease in the United States each year. Although sporadic cases are reported in many states, Lyme disease primarily occurs in three distinct regions: in the Northeast from Massachusetts to Maryland, in the Midwest in Wisconsin and Minnesota, and in the West in northern California. Lyme disease is quite seasonal due to the life cycle of the tick. In the Northeast and Midwest, disease generally begins to appear between May 1 and October 30, with the majority of cases occurring in June and July. In California, the disease occurs throughout the year, with a slight increase in the summer months.

The two species of ticks known to transmit the disease are the deer tick *Ixodes scapularis* (also called *Ixodes dammini*) in the northeastern and midwestern United States, and *Ixodes pacificus* in the West. Ticks live in wooded and grassy areas and survive by attaching to animal hosts and sucking their blood. They attach to deer, field mice, other wild animals and humans, as well. *Ixodes pacificus* also feeds on birds and cold blooded animals, such as lizards. Other ticks, such as the common dog tick, do not transmit Lyme disease.

Symptoms of Lyme disease

Human Lyme borreliosis generally occurs in stages, with remissions and exacerbations and different symptoms at each stage. The first stage of Lyme disease is a rash known as erythema migrans (EM), which usually occurs several days to a month after the tick bite, and consists of a small red lesion that later expands to form a ring-shaped rash - "bull's eye" - a bright red ring encircling the bite and a clear area at the center. This rash varies in size - it can be as small as a dime or it can cover a person's entire back; it usually marks the site of the bite or it can appear with several others throughout the body.

Flu-like symptoms, such as headaches, stiff neck, muscle aches

and fatigue may also be present. About half of all infected people never develop the rash, making it more difficult to diagnose the illness.

If left untreated, Lyme disease can progress to a second stage of the disease, which occurs within the next several weeks. This disseminated infection involves joint pain and may bring about complications in the nervous system or the heart. Neurologic complications, such as inflammation of the brain and its covering membranes and inflammation of the nerve roots and facial paralysis, occur in about 15 percent of all patients. Symptoms may last several months, but usually disappear completely.

Heart disease: Symptoms occur in about 8 percent of the patients, and include dizziness, shortness of breath and an irregular heart rhythm. Generally, they disappear completely within weeks.

Arthritis: May progress in stage 3 of the disease (persistent infection). Joint pain may appear weeks or even years after the rash, and it affects more than 50 percent of the patients. The large joints - knees, shoulders, elbows, ankles and wrists - are usually involved. They become swollen and painful. The first attack usually lasts about a week, but recurrent attacks are quite common. At this stage, a small number of people may also develop neurological abnormalities, such as somnolence (drowsiness), loss of memory, mood swings and inability to concentrate.

Diagnosis of Lyme disease

Due to the variety of symptoms, a diagnosis cannot always be made quickly and accurately. If the patient knows about a recent tick bite, and demonstrates a classic rash described above, a straightforward diagnosis can be made and the most difficult part is over.

Unfortunately, because of the small size of the nymphal *I. scapularis* tick, the inciting bite often goes undetected. If Lyme disease is suspected, the physician can order a blood test to check for infection. The blood test, however, may be negative during the early phases of the disease, and false positives have been known to occur. Specialized tests to identify the genetic material of *Borrelia burgdorferi* within arthritic joints may aid in the diagnosis of Lyme arthritis.

Difficulty in diagnosing Lyme Disease is compounded because in addition to failure in spotting a tick bite and the absence of a rash, other ailments - such as systemic lupus erythematosus or rhematoid arthritis - are sometimes wrongly diagnosed as Lyme disease. Moreover, leptospirosis and syphilis, also caused by spirochetes, can misleadingly give lab results similar to those of Lyme disease.

Treatment of Lyme Disease

The various manifestations of Lyme disease are generally treatable with oral antibiotic therapy, except for objective neurological abnormalities, which require intravenous therapy. Lyme arthritis can be treated successfully in most patients by a number of alternative antibiotics including tetracycline, doxycycline, erythromycin or amoxicillin. Response, however, is often delayed until many weeks after completion of the course, which suggests that noninfectious inflammatory mechanisms (perhaps involving uncleared antigenic material) can contribute to the perpetuation of arthritis in many patients. Another 10 percent or 15 percent respond to a second course of a different oral antibiotic or to a course of parenteral antibiotic therapy. The earlier the disease is treated, the shorter and less severe the symptoms will be.

Arthritis in advanced Lyme disease requires doxycycline, 100 mg orally twice a day for 30-60 days; amoxicillin, 500 mg four times a day for 30-60 days; or ceftriaxone, 2 g/d IV, in one or two doses, for 14 to 28 days. The addition of corticosteroids to the regimen is not recommended. If an arthritic patient fails to respond to oral medication, IV antibiotic therapy should be considered.

The diagnosis and treatment of Lyme disease presents numerous obstacles. People can be bitten by a tick without knowing it, and even when the bite is noticed, all ticks are not infected. The red circular rash around the bite, the telltale sign of an infected tick, does not always appear. Furthermore, Lyme disease symptoms mimic other diseases, such as fibromyalgia and multiple sclerosis, and no tests can reliably identify the presence of *Borrelia burgdorferi*.

Patients younger than 9 years or age, or pregnant or lactating women, are treated with amoxicillin or penicillin because doxycycline can stain the permanent teeth developing in young children or unborn babies. Patients allergic to penicillin are given erythromycin.

Lyme disease patients with neurological symptoms are usually treated with the antibiotic ceftriaxone, which is given intravenously once a day for a month or less. Most patients experience full recovery.

Lyme disease patients experiencing heart symptoms are treated with antibiotics, such as ceftriaxone or penicillin, given intravenously for about two weeks. If these symptoms persist or are severe enough, patients may also be treated with corticosteriods or given a temporary internal cardiac pacemaker. People with Lyme disease rarely experience long-term heart damage.

Following treatment for Lyme disease, some people continue to have persistent fatigue and achiness. This general malaise can take months to subside, although it generally does so spontaneously without requiring antibiotic therapy.

Prevention of Lyme disease

No FDA approved vaccine to prevent Lyme disease is presently available.

The best way to prevent Lyme disease is to avoid areas that are known to contain ticks, especially during the summer months. If you are going into wooded or grassy areas, wear light-colored clothing that fits tightly around the ankles and wrists, and tuck the pants into boots or socks. Spray your clothing with tick repellent. When you return, examine your body and clothing for ticks - they are very small, so look carefully.

How to remove an attached tick

Ticks must removed as soon as possible, because the risk of Lyme disease transmission increases significantly after 24 hours of attachment. Brush off any ticks that are not attached and use tweezers to remove those that are. To remove them, grasp the tick's mouth-parts as close to the skin as possible, and use a slow steady pressure while pulling straight out. Don't attempt to jerk the tick out. Try not to squeeze the tick's body or tear the skin. If the tick's mouth parts remain in the skin, however, don't worry - the bacteria that causes Lyme disease is located in the tick's belly. After removing the tick, always wash the area immediately with soap and water, alcohol or antiseptic.

Pets should be checked as they come into the house.

Other popular tick-removal tactics - such as butter, fingernail polish, gasoline, kerosene, petroleum jelly, rubbing alcohol, and lit matches or cigarettes - are usually ineffective and can even be hazardous.

Many ticks are disease free, so a tick bite does not mean you will automatically develop Lyme disease. If any possible symptoms develop, report them promptly to the physician.

Fruits and Vegetables as Vehicles of Pathogen Transmission

It has become a well known and documented fact for over a century that raw fruits and vegetables have served as vehicles of transmission of human pathogens. Contamination of fruits and vegetables and thus the spread of pathogens and diseases is more common in developing countries as compared to developed countries due to various underlying factors, including the continued use of untreated wastewater and manure as fertilizers for the production of fruits and vegetables which is a major contributing factor to contamination that causes many foodborne disease outbreaks.

Bacteria such as *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes*, all capable of causing infections, are normal inhabitants of many soils, whereas Salmonella, Shigella, *Escherichia coli* and Campylobacter reside in the intestinal tracts of animals, including humans and are more likely to contaminate raw fruits and vegetables through direct or indirect contact with feces, sewage, untreated irrigation water or surface water. Post harvest handling may also be responsible for contamination with bacteria, viruses and parasites at the site of transport and vending.

Other factors, that may also contribute to the increase in diseases associated with fruits and vegetables include application of improperly composted manures to soils in which fruits and vegetables are grown, changes in packaging technology viz; the use of modified or controlled atmosphere and vacuum packaging, extended time between harvesting and consumption, and changing food consumption patterns. Increased global trade in raw fruits and vegetables, as well as increased travel in general, could also increase the risk of produce-associated diseases. Finally, the susceptibility of the public to food borne diseases, at least in more developed countries, is changing due to increased number of people who are elderly, immunocompromised or have chronic diseases. This change in social demographics is likely to lead to increased risk of illness associated with the consumption of raw produce that otherwise may contain levels of pathogens innocuous to healthy individuals.

Microbes commonly present on the surface of raw fruits and vegetables may comprise of chance contaminant from soil or dust, or bacteria or fungi that have grown and colonized by utilizing nutrients exuded from plant tissues. Bacterial groups commonly found on plant vegetation are those that test positive as for coliforms or fecal coliforms.

Fruits which have to be peeled off before consumption like mangoes, oranges and bananas seem lesser significant in the transmission of microbes as compared to fruits which are consumed whole. However care must be taken to ensure that pathogens present on the rind/skin of these fruits are not transferred to the edible part, and thus consumed. Its a preferred practice to wash the fruit prior to peeling, to mitigate the numbers of microbes. Alternatively microorganisms that may get trapped on the inner leaves of certain vegetables can be particularly difficult to remove by routine cleansing practices.

In these cases, in order to minimize the potential risk to acquire infection or an intoxication associated with raw fruits and vegetables, key sources of contamination from the environment to the table must be identified and in turn specific measures and interventions to prevent and / or minimize the risk of contamination must be considered and appropriately implemented. In cases where the possibility of contamination cannot be excluded, the application of the most effective decontamination processes should be considered. Application of good hygienic practice during production, transport and processing, combined with Hazard Analysis Critical Control Point (HACCP) system, will definitely reduce the contamination of fruits and vegetables and reduce the risk of illness associated with these foods.

A Simple domestic practice of washing raw fruits and vegetables in hot water or water containing detergent or permanganate salts aids to remove a portion of pathogens and spoilage microbes that may be present. Washing fruits and vegetables in potable water or rinsing in potable water would aide in removing microorganisms. Additional 10-fold or 100-fold reductions can sometimes be achieved by treatment with disinfectants. It is noteworthy to mention that viruses and protozoan cysts on fruits and vegetables usually exhibit higher resistance to the effect of disinfectants than do bacteria or fungi.

A wide variety of treatments are known to be partially effective in removing disease causing microbes from the surface of whole and cut raw fruits and vegetables or from contact surfaces during handling. These treatments should be considered as methods of disinfection, causing reductions in populations of microorganisms but not always yielding fruits and vegetables completely free of pathogens.

Each type of disinfectant has its own efficacy in killing or eliminating microbial cells. Effectiveness depends on the nature of the cells as well as the characteristics of fruits and vegetable tissues and juices. Some disinfectants are best suited for use in disinfectant direct contact washes, while others may be apt for equipments or containers used to process, store or transport fruits and vegetables.

Common disinfecting treatments include the use of:

Chlorine: Liquid chlorine and hypochlorites are moderately effective disinfectants for surfaces that may come in contact with fruits and vegetables during harvest, processing, and for whole and cut fruits and vegetables. To disinfect produce, chlorine used at concentrations of 50 – 200 ppm with a contact time of 1 – 2 minutes is sufficient.

Chlorine dioxide (ClO_2): The oxidizing power of ClO_2 is about 2.5 times that of chlorine, and its activity is not affected by pH. This compound must be generated on site since it can be explosive when concentrated. Its mechanism of action involves disruption of cell protein synthesis and membrane permeability. Its used in varied concentrations depending on the purpose and the material for which the equipment is being disinfected.

Bromine: Bromine has had limited use either alone or in combination with chlorine compounds in water treatment programmes. Bromine is selective in its activity against microbes and is effective against a few pathogens at a concentration of 200 ppm with a contact time of 15 minutes.

Iodine: Iodine compounds are widely used for sanitizing food processing equipment and surfaces. Iodophores are less corrosive than chlorine at low temperatures. Iodophores are most effective at pH 2 – 5 and at concentrations ranging from 6-13 ppm.

Other disinfectants include:

Trisodium phosphate (TSP): Is known to be effective in removing Salmonella from poultry and red meats.

Quaternary ammonium compounds: Are cationic surfactants, used largely to sanitize floors, walls, drains, and equipment and other food-contact surfaces in fruit and vegetable processing plants. This group of disinfectant has potential for application to surfaces of uncut fruits and vegetables which subsequently would have their peel, rind or skin removed prior to consumption.

Acids: Organic acids naturally found in or applied to fruits and vegetables behave primarily as fungistats, bacteriostats. Also washes and sprays containing organic acids, particularly lactic acid, have been successfully used to disinfect beef, lamb, pork and poultry carcasses.

While every effort must be made to prevent contamination of fruits and vegetables during the phases of production, transport, processing and handling, much improvement is still required in many parts of the globe if hygienic production of fruits and vegetables is to be ensured.

Antimicrobial Susceptibility Testing

Introduction

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple task, and is beyond doubt that emergent antibiotic resistance is a serious global problem.

The widespread use of antibiotics provide a selective pressure in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs as well. Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

Principle

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal ,Walker and others at the turn of the century, the discovery of antibiotics made these tests (or their modification) too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field .The Oxford group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. It is imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

Factors Influencing Antimicrobial Susceptibility Testing

i) pH

The pH of each batch of Mueller-Hinton agar should be checked when the medium is prepared. The agar medium should have a pH between 7.2 to 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:

* Macerate a sufficient amount of agar to submerge the tip of a pH electrode. * Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup. * Use a properly calibrated surface electrode.

ii) Moisture

If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35^{o}C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

iii) Effects of Thymidine or Thymine

Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Mueller-Hinton agar that is as low in thymidine content as possible should be used. Satisfactory media will provide essentially clear, distinct zones of inhibition, 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

iv) Effects of Variation in Divalent Cations

Variation in divalent cations, principally magnesium and calcium, will affect results of aminoglycoside and tetracycline tests with *P. aeruginosa* strains. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of Mueller-Hinton agar must conform to the control limits.

v) Testing strains that fail to grow satisfactorily

Only aerobic or facultative bacteria that grow well on unsupplemented Müeller-Hinton agar should be tested on that medium. Certain fastidious bacteria such as *Haemophilus* spp., do not grow sufficiently on unsupplemented Mueller-Hinton agar. These organisms require supplements or different media to grow, and they should be tested on the media prescribed for them.

Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	Dilution	Diffusion&Dilution
Stokes method	Minimum Inhibitory Concentration	E-Test method (AB BIODISK)
Kirby-Bauer method	i) Broth dilution ii)Agar Dilution	

In this article we will emphasize on disk diffusion method

Reagents for the Disk Diffusion Test

1. **Müeller-Hinton Agar Medium**

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

* It shows acceptable batch-to-batch reproducibility for susceptibility testing. * It is low in sulphonamide, trimethoprim, and tetracycline inhibitors. * It gives satisfactory growth of most nonfastidious pathogens. * A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

2. **Preparation of antibiotic stock solutions**

Antibiotics may be received as powders or tablets. It is recommended to obtain pure antibiotics from commercial sources, and not use injectable solutions. Powders must be accurately weighed and dissolved in the appropriate diluents to yield the required concentration, using sterile glassware. Standard strains of stock cultures should be used to evaluate the antibiotic stock solution. If satisfactory, the stock can be aliquoted in 5 ml volumes and frozen at -20ºC or -60ºC.

Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven.

The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers 0.005 ml of antibiotics to each disc.

Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.
2. The plates are inverted and placed in an incubator set to at appropriate conditions.

Reading Plates and Interpreting Results

1. After appropriate incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light then the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed.
2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.
3. Based on the sizes of the zones of inhibition, the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested.

Quality Control in Antibiotic Susceptibility Testing

QC is performed to check the quality of medium, the potency of the antibiotic, to check manual errors. Quality control strains should be included daily with the test. Not more than 1 in 20 results should be outside accuracy limits. No zone should be more than 4 standard deviations away from midpoint between the stated limits.

If, for reasons of expense or manpower constraints, it is not possible to include all strains on a daily basis, then the following guidelines should be followed:

The frequency can be decreased to once weekly if proficiency has been demonstrated by

1. Performing QC daily for 30 days with less than 10% inaccuracy for each drug
2. Proficiency testing is repeated for each new drug included in the testing
3. All documentation is maintained indefinitely
4. Proficiency testing is repeated for each new batch of media or reagents

All tests must be within accuracy limits if QC is done once weekly.

Reference strains for quality control

Escherichia coli ATCC 25922 (beta-lactamase negative)
Escherichia coli ATCC 35218 (beta-lactamase positive)
Staphylococccus aureus ATCC 25923 (beta-lactmase negative, oxacillin susceptible)
Staphylococccus aureus ATCC 38591 (beta-lactmase positive)
Pseudomonas aeruginosa ATCC 27853 (for aminoglycosides)
Enterococcus faecalis ATCC 29212 (for checking of thymidine or thymine level of MHA)
Haemophilus influenzae ATCC 49766 (for cephalosporins)
Haemophilus influenzae ATCC 10211 (for medium control)
Neissseria gonorrheae ATCC 49226

Stock cultures should be kept at -70^{O}C in Brucella broth with 10% glycerol for up to 3 years. Before use as a QC strain, the strain should be subcultured at least twice and retested for characteristic features. Working cultures are maintained on TSA slants at 2-8^{O}C for up to 2 weeks.

Reference: Manual on Antimicrobial Susceptibility Testing; Dr. M. K. Lalitha

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.

Track your brain

1. Fascia
2. Folliculitis
3. Rabies
4. Bullae
5. Erysipelas
6. Impetigo
7. Angiogenesis
8. Pexiganan
9. Genetic
10. Spirochete
11. Iodophores
12. Permanganate

BioShields Presents Nusept

Composition - 1% w/v Poly (hexamethylene biguanide) hydrochloride, Perfume, Fast green FCF as color.

Description: NUSEPT™ is a new generation, powerful, non stinging, safe, highly effective and resistance-free microbicidal antiseptic solution. NUSEPT™ is an ideal antiseptic for use in medical settings. The main active ingredient of NUSEPT™ is poly (hexamethylenebiguanide) hydrochloride (PHMB). PHMB is a polymeric biguanide. There is no evidence that PHMB susceptibility is affected by the induction or hyper expression of multi-drug efflux pumps, neither there have been any reports of acquired resistance towards this agent.

ACTIVITY : Broad spectrum: Bactericidal, Fungicidal & Virucidal.

CONTACT TIME :1 min (undiluted & 10% v/v solution), 5 min (5% v/v solution), 10 min (2.5% v/v solution).

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

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