

JOURNAL OF HYGIENE SCIENCES

Committed to the advancement of Clinical & Industrial Disinfection & Microbiology

VOLUME - I

ISSUE - I

JAN-FEB 2008

Editorial

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There is a proverb which, says “Hygiene is two thirds of health”. If you are concerned about a healthy body then you should be aware of hygiene. Hygiene science is basically related with the degree of cleanliness of a person and his surrounding environment. For over a century back people were not aware of health and hygiene. With the changing era, science has played a pivotal role in encouraging human being for the betterment of life. And obviously, a recent developed thought about health and hygiene among public indicates an improvement in life style. But this awareness of hygiene science has to reach out to the masses of urban as well as rural areas. So with the advent of the New Year we have come up with **Journal of Hygiene Sciences** to pave the way for quality health care for the masses.

Journal of Hygiene Sciences is focused on the advancement of clinical as well as industrial disinfection and microbiology. It will track and highlight the trends in the latest technology in the field of disinfection and microbiology.

Talking about this issue, Mini Review highlights the emergent risk of listeriosis on public health and searches a solution to fight against it. You can go through all about hydrogen peroxide fumigation a recent trend of aerial fumigation. A lot more proper information is required about the regular laboratory practices for the microbiologists and clinicians, and we have decided to acknowledge some good practices in our Best Practices section. Our In Profile section will remind you the person who has kept his great contribution in the field of microbiology. You will find some interesting topics in our Did You Know section. And surely you have a curiosity to know about the tiny microorganisms, we will not dim your hope. Bug of the Month section is there to fulfill your curiosity about microorganism. Readers can cover a large range of basic but important knowledge about microbiology and disinfection in our Encyclopedia section. Check it out Relax Mood and In Focus pages.

Our intention is to walk along with you and form an integral bond with you. Share your opinions, queries or problems with us. We will try to solve it. An American author Henry Miller quoted “nine- tenths of our sickness can be prevented by right thinking plus right hygiene- - nine-tenths of it”. With this vision and mission Tulip Group has come up with **Journal of Hygiene Science**. We are looking forward to your active participation to make our effort successful and everlasting. **Journal of Hygiene Science** wishes our readers a peaceful and healthy 2008.

Listeriosis

- An emergent risk to Public Health

Listeriosis is an illness caused by the bacterium *Listeria monocytogenes* that is acquired by eating contaminated food. Certain groups of individuals are at great risk for listeriosis. These are pregnant women (and their unborn children) and immunocompromised persons.

Microbiology

Listeria monocytogenes is a small, Gram-positive bacillus, with a tendency to occur in chains. It exhibits slow tumbling motility at room temperature (25°C) but not at 37°C. This is because the bacillus, with a tendency to occur in chains, produces peritrichous flagella. It is aerobic or microaerophilic. *Listeria* survives in temperatures from below freezing (-7°C) to body temperature but it grows best at -18°C to 10°C.

Reservoirs

Listeria monocytogenes are widely distributed in nature. Reservoirs for *L. monocytogenes* are soil, water, mud, silage, mammals and fowl. As it is a bacterium found in soil and vegetation, it is easily contracted and transmitted by herd animals. It is also found in grazing areas, state water supplies and poorly prepared animal feed. It can live in the intestines of humans, animals and birds for long periods of time without causing infection. *Listeria* has also been isolated from fish, oysters, ticks and flies.

Epidemiology

Listeria is widely distributed in nature. Most areas of human listeriosis are believed to occur sporadically, but food borne and nosocomial outbreaks have been documented. Foods associated with infection include unpasteurized milk, soft cheeses, processed meats and contaminated vegetables. Unlike most other food borne pathogens, *Listeria* tends to multiply in refrigerated foods those are contaminated. *Listeria* has also been found in raw fish, shellfish and fish products, raw meat, poultry and their products. Cell mediated immunity defends human against intracellular pathogen like *Listeria monocytogenes*. So the individuals with suppressed cell mediated immunity are most susceptible to the devastating effects of listeriosis. Pregnant women naturally have a depressed cell mediated immune system because to reduce the chance of the rejection of fetus. Pregnant women are about twenty times more likely than other healthy adults to get listeriosis. Pregnancy presents a complex immunological problem for the mother's body. The fetus contains traits from the father that are antigenically foreign to the mother, and therefore her immune system should reject the fetus. This does not occur, however, because the mother's cell-mediated immunity is down regulated by increasing progesterone levels during pregnancy. Although this immunomodulation allows the fetus to survive, it also increases susceptibility to intracellular pathogens that are normally attacked by the cellular immune system. About one third of listeriosis cases happen during pregnancy. The incidence of

listeriosis in the newborn is 8.6 per 100,000 live births. The immune system of fetus and new borne are very immature and are extremely susceptible to this intracellular pathogen. Infants also respond inadequately to listerial infection. Examination of the in vitro immune response to *L. monocytogenes* by one-year-old infants who previously had a severe listerial infection at birth revealed that they produced neither antibodies nor a cell-mediated response to *L. monocytogenes*. In other words, their immune systems had no memory of encountering this pathogen previously. In contrast, the mothers of these infants did respond immunologically to a challenge with *L. monocytogenes*. A number of factors may affect the immune response and susceptibility to listerial infection in the elderly. Results from numerous studies comparing the immune response of people 65 years of age with that of young adults indicate that T cell functions (proliferative responses, interleukin-2 production, antibody production) tend to decline with age. In addition, there is a decline in the number of naive T cells that would be able to respond to new infections. The aging process decreases the efficiency of B cell and T cell mediated immunity. Major surgery, poor nutrition, and lack of exercise can also diminish cell-mediated immune responses. Surgery causes a transient decrease in T cell proliferation and cytokine production. Surveys have indicated that diets of as many as one third of the elderly are deficient in some vitamins and/or trace elements. In a clinical trial, persons receiving supplements of vitamins and minerals had a significantly higher immune status and levels of IL-2 and CD4+ T cells as compared to unsupplemented controls. Other adults, especially transplant recipients and lymphoma patients, are given necessary therapies with the specific intent of depressing immune T cells and these individuals become especially susceptible to *Listeria monocytogenes*. Persons with AIDS suffer from listeriosis 65-145 times more frequently than the general population. Persons who take glucocorticosteroid medications are also at increased risk. The elderly and certain debilitated patients (such as those on dialysis or alcoholics) are at minor increased risk for listeriosis. Persons with cancer, diabetes or kidney diseases are also at risk for listeriosis. Gastrointestinal function may also be altered in the elderly. Gastric acidity levels are decreased by atrophic gastritis, infection with *Helicobacter pylori*, and the use of antacids. This reduction in acidity lowers an important barrier to the establishment of intestinal food borne infections. Gastrointestinal motility may also decline, thereby reducing clearance of pathogens from the intestinal tract. Finally, use of antibiotics to treat illness destroys much of the competing microflora in the gastrointestinal tract, permitting the survival and penetration of *L. monocytogenes*. Although all elderly people probably have less efficient immune responses than healthy young adults, the extent of an individual's immune deficiency, and therefore susceptibility to infections such as

listeriosis, depends on gastrointestinal function as well as nutritional and lifestyle practices and overall health

Pathogenesis and Clinical manifestations of *Listeria*

Listeria monocytogenes is presumably ingested with raw, contaminated food. An invasion secreted by the pathogenic bacteria enables the listeriae to penetrate host cells epithelial lining. The bacteria are widely distributed so this event may occur frequently. Normally the immune system eliminates the infection before it spreads. Adults with no history of listeriosis have T lymphocytes primed specially by *Listeria* antigens. If the immune system is compromised, systemic disease may develop. *Listeria monocytogenes* multiplies not only extracellularly but also intracellularly within macrophages after phagocytosis or within parenchymal cells, which are entered by induced phagocytosis. *Listeria* can attach to and enter mammalian cells. The bacterium is thought to attach to epithelial cells of the Gastrointestinal tract by means of D-Galactose receptors on the host cells. The bacteria are then taken up by induced phagocytosis. An 80 kDa membrane protein called internalin probably mediates invasion. Internalin A was first identified as a listerial surface protein that is required for the penetration of *L. monocytogenes* into non phagocytic cells such as epithelial cells. A related protein internalin B, plays a role in invasion of hepatocytes in the liver. Internalin A on the surface of listerial cells binds to a surface protein, E-cadherin on the surface of host epithelial cells. This interaction apparently stimulates the phagocytosis of *L. monocytogenes* cells. In addition to the internalins, another surface protein on *L. monocytogenes*, p104, has recently been identified and shown to play a role in adhesion to intestinal cells. After engulfment, the bacterium may escape from the phagosome before phagolysosome fusion occurs mediated by a toxin, which also acts as a hemolysin known as listeriolysin O (LLO). Survival of the bacterium within the phagolysosome may be aided by its ability to produce catalase and superoxide dismutase, which neutralize the effects of the phagocytic oxidative burst. LLO can also act as an inflammatory stimulus by inducing endothelial cell activation and neutrophil activation. Some additional genetic determinants are necessary for further steps in the intracellular life cycle of *L. monocytogenes*. One particular gene product, Act A (encoded by *act A*) promotes the polymerization of actin, a component of the host cell cytoskeleton, on the bacterial surface. Within the host cell environment, surrounded by a sheet of actin filaments, the bacteria reside and multiply. The growing actin sheet functions as a propulsive force that drives the bacteria across the intracellular pathogens until they finally reach the surface. Then, the host cell is induced to form slim, long protrusions containing living *L. monocytogenes*. Adjacent cells, including parenchymal cells, engulf those cellular projections. By such a mechanism, direct cell-to-cell spread of *Listeria* in an infected tissue may occur without an extracellular stage. *L. monocytogenes* produces two other hemolysins besides LLO, phosphatidyl inositol specific phospholipase C (PI-PLC) and phosphatidyl choline specific phospholipase C

(PC-PLC). Unlike LLO, which lyses host cells by forming a pore in the cell membrane, these phospholipases disrupt membrane lipids such as phosphatidyl inositol and phosphatidyl choline (lecithin). PI-PLC is synthesized in an active form PC-PLC is produced as an inactive precursor. A bacterial zinc dependent metalloprotease and a host cell cysteine protease are required to cleave off part of the precursor and activate the phospholipase. An operon called *lma BA* encodes a 20 kDa protein located on the bacterial surface. This protein induces delayed type hypersensitivity and other cell mediated immune responses. In order to adapt to adverse environmental conditions (high or low pH, temperature, osmotic conditions), many bacteria have chaperone proteins that assist in the proper refolding of proteins or assembly of protein subunits and proteases which process proteins that cannot be altered conformationally. Some of the Clp (caseinolytic proteins) group of proteins, which act both as chaperones and as proteolytic enzymes, have been identified as having a role in pathogenesis of *L. monocytogenes*. ClpCATPase is a general stress protein that aids in disruption of the vacuolar membrane and the intracellular survival of listeriae. ClpC also modulates expression of the ActA protein and the internalins, apparently acting at the transcriptional level. Another ATPase, ClpE, also plays a role in listerial pathogenesis. ClpP serine protease is required for growth under stress conditions and has been shown to affect the activity of listeriolysin O and ClpP serine protease is required for growth under stress conditions and has been shown to affect the activity of listeriolysin O and escape from vacuoles. Protein p60 is murein hydrolase enzyme that catalyzes a reaction during the final stage of cell division of *L. monocytogenes*. It is normally present on the cell surface as well as being secreted into the surrounding medium. It is important for phagocytosis of *L. monocytogenes* by some cell types. All of these virulence factors participate in specific ways in the infection process, and in addition each may also affect host cell signal transduction in ways that enhance the spread of infection. Growth temperature, pH, and availability of iron have been shown to affect the expression of some virulence factors in *L. monocytogenes*. *Listeria* can survive and grow at low temperatures (4-25°C), but under these conditions listeriolysin O production is reduced or abolished. However, it takes only 2 hours at 37°C for LLO levels to return to normal. Therefore, it is likely that *Listeria* in refrigerated foods would recover their infectivity during passage through the intestinal tract of warm blooded animals. It has been reported that exposure to low pH (4.5-4.9) reduces production of listeriolysin O. Growth in an iron-rich medium enhanced the invasiveness of *L. monocytogenes*. Thus pathogenic *Listeria* enters the host primarily through the intestine and targets liver. In the liver, *Listeria* actively multiply until a cell controls the infection mediated immune responses. In normal individuals, the continual exposure to listerial antigens probably contributes to the maintenance of anti-listeria memory T cells. But in debilitated and immunocompromised patients, the unrestricted proliferation of

Listeria in the liver may result in prolonged low level bacteremia, leading to invasion of the preferred secondary target organs (like brain & gravid uterus) and to overt clinical disease. *Listeria monocytogenes* is a facultative intracellular parasite able to survive in macrophages and to invade a variety of normally non-phagocytic cells, such as epithelial cells, hepatocytes and endothelial cells. Central nervous system infections are one of the most serious manifestations of listeriosis. *L. monocytogenes* may cause meningitis, frequently accompanied by seizures, or rhomboencephalitis, an infection of the brain stem. In order to infect the central nervous system, listeriae must penetrate the bloodbrain barrier, which maintains biochemical homeostasis within the brain and spinal cord. This barrier consists of epithelial cells bound together with tight junctions, which prevent the passage of even small molecules like antibiotics. However, *L. monocytogenes* can invade these human brain microvascular endothelial cells (HBMEC) in culture. Once within these cells, bacteria replicate and go on to invade adjacent cells. Listeriae can also invade the microvascular endothelial cells directly from infected macrophages which may have been carried to the brain in the blood stream from the intestine. Macrophages containing *L. monocytogenes* circulate throughout the body. At the placenta, listeriae from macrophages can infect endothelial cells and then the fetus, thereby precipitating premature labor or death of the fetus. Infants can also be infected during passage through the birth canal and develop sepsis or meningitis several days later. Although the amniotic fluid surrounding the fetus kills or stops the growth of some bacteria, it appears to have no adverse effect on *L. monocytogenes*. Human defend themselves from bacterial attack using three strategies: (1) general, non-specific resistance factors, (2) cell-mediated immunity, and (3) antibody-mediated immunity. Antibody-mediated immunity does not appear to play a significant role in protection from listeriosis. Non-specific host defenses for food borne pathogens include stomach acidity, which is lethal to many bacteria in food, and the normal gut microflora which are usually so numerous that they occupy all available niches in the intestine and consume essential nutrients, thereby preventing incoming bacteria from getting established. *L. monocytogenes* is known to be a poor competitor; this probably explains why the majority of adults exposed to this bacterium do not become sick. Infection of epithelial and macrophage cells by *L. monocytogenes* strongly modulates the complex host cell signalling systems affecting the introduction and activity of a number of non-specific proteins and cells which counteract or support listerial infections. Cell-mediated immunity is probably the main host defense against listeriosis with CD4+ T helper lymphocytes activating macrophages. The effectiveness of immune reactions in different individuals is modified by genetic and nutritional factors. Pregnancy and aging also affect the effectiveness of the immune response. During early stages of listeriosis, neutrophils and macrophages migrate to the spleen and liver, destroying most of the listerial cells that have arrived there from the small intestine. Some listeriae escape by invading hepatocyte cells. Neutrophils are also recruited to the

utero-placental unit and the central nervous system to kill listeriae invading these organs. Although neutrophils and macrophages can attach to and lyse cells containing listeriae, some macrophages do not kill listeriae. Macrophages that allow survival of listeriae contain too much or too little intracellular iron and have interleukin-10 on their surface. This is known to suppress macrophage function. An early inflammatory response is important for limiting growth and spread of *L. monocytogenes* in the body, but it is not sufficient to destroy all listeriae. In all of these cell types, pathogenic *Listeria* go through an intracellular life cycle invading early escape from the phagocytic vacuole, rapid intracytoplasmic multiplication, bacterially induced actin based motility and direct spread to neighbouring cells, in which they reinitiate the cycle. In this way, *Listeria* disseminate in host tissues sheltered from the humoral arm of the immune system.

It is believed that ingestion of as few as 1000 cells of *Listeria* can result in illness. After ingestion of food contaminated with *Listeria*, incubation periods for infection are in the range of 3-70 days, usually 4-21 days. Five days to three weeks after ingestion, *Listeria* has access to all body areas and may involve the central nervous system, heart, eyes or other locations. There are four distinct clinical syndromes of listeriosis:

(a) Infection in pregnancy- *Listeria* can proliferate asymptotically in the vagina and uterus. If the mother becomes symptomatic, it is usually in the third trimester. Symptoms include fever, myalgias, arthralgias and headache. Maternal infection with *Listeria* can result in chorioamnionitis, premature labour, spontaneous abortion or stillbirth.

(b) Neonatal infection- There is two types of listeriosis in the new born baby: early onset disease and late onset disease. Early onset disease refers to a serious illness that is present at birth and usually causes the baby to be born prematurely. Babies infected during the pregnancy usually have a blood infection (sepsis) and may have a serious, whole body infection called granulomatosis infantisepticum. Early onset neonatal listeriosis is usually associated with sepsis or meningitis. When a full term baby becomes infected with *Listeria* during childbirth, that situation is called late onset disease. Commonly, symptoms of late onset listeriosis appear about two weeks after birth. Babies with late term disease typically have purulent meningitis.

(c) CNS infection- *Listeria* has a predilection for the brain parenchyma, especially the brain stem and the meninges. Mental status changes are common. Seizures occur in at least 25% of patients. Encephalitis, meningitis, meningoencephalitis and abscesses can all occur.

(d) Gastroenteritis- *L. monocytogenes* can produce food borne diarrheal disease which typically is non invasive. The median incubation period is 1-2 days with diarrhea lasting for 1-3 days. Meningitis occurs in about half of the cases of adult listeriosis. Other diseases, which have been caused by *Listeria monocytogenes*, include brain abscess, eye infection, hepatitis (liver disease), peritonitis (abdominal infection), lung infection, joint infection, arthritis, heart disease, bone infection and gall bladder infection.

Diagnosis and treatment

Listeriosis may be diagnosed and treated by infectious disease specialists and internal medicine specialists. The only way to diagnose listeriosis is to isolate *Listeria monocytogenes* from blood, cerebrospinal fluid or stool. The amniotic fluid may be tested in pregnant women to check the listerial infection. *Listeria* can be identified by performing following biochemical tests like Esculin hydrolysis, Voges Proskauer test, Nitrate reduction, Methyl red test, Catalase and carbohydrate utilization tests (Glucose, Xylose, Lactose, Mannitol, Rhamnose, Ribose etc.). Fraser broth is a medium recommended as a primary as well as secondary enrichment medium for the isolation and enumeration of *L. monocytogenes* from foods, environmental specimens and animal feeds. Using several antibiotics can treat listeriosis. Ampicillin in combination with an aminoglycoside such as gentamicin is the therapy of choice. *Listeria* is not susceptible to cephalosporins of any generation. Therefore, cephalosporins should not be used to treat *Listeria* infections. Successful treatment can also be done with trimethoprim and sulfamethoxazole. Because the bacteria are an intracellular pathogen so treatment may be difficult and the treatment periods may vary. Usually pregnant women are treated for two weeks; newborn, two to three weeks; adults with mild disease, two to four weeks; persons with brain abscesses, six weeks; and person with endocarditis, four to six weeks. Patients are often hospitalized for treatment and monitoring.

Control measures

Human cases of *Listeria* are sporadic and treatable. *Listeria* remains an important threat to public health, especially among those most susceptible to this disease. With the increase of the numbers of immunocompromised people, the risk multiplies. The fact that listeriosis is a disease that can easily be transmitted from mother to fetus through the placenta is worrisome to an expectant mother. The public is not the only group that should learn more about *Listeria*. Sometimes many doctors overlook the possibility of *Listeria* food poisoning. While current regulations allow alternative methods in controlling *L. monocytogenes* in post-lethal processing, caution must be exercised in selecting which method or methods to implement. Ionizing radiation, high pressure processing, pulsed electric fields, ultra violet light and several other novel technologies have been investigated for effectiveness in eliminating or reducing post-processing populations. These methods have shown success, but *L. monocytogenes* control generally seems to require more severe treatment parameters than other bacteria. Appropriate good manufacturing practices and proper sanitation procedures are the best defense. A Food-manufacturing unit should consider following practices:

- Raw and ready to eat food processing areas should be physically separated.
- Traffic patterns must be established and maintained to eliminate the potential for cross contamination between raw and ready to eat food processing areas and materials.
- Personnel, materials, equipment etc. used in raw product areas should be kept separate from those in ready to eat food

processed areas.

- Adequate hand washing and sanitizing facilities should be provided at each food-processing unit.
 - Employees must be trained in and must follow proper hand washing and sanitizing procedures each time they enter the room and their hands may become contaminated.
 - *L. monocytogenes* thrives in "niches" in plant structures, floor drains and difficult to clean areas of processing equipment, usually in biofilms, which are difficult to remove unless promptly identified and removed, these pockets of contamination can continuously shed *L. monocytogenes* cells into the product stream. So floor drains should be cleaned properly. But clean up procedures should be avoided during production.
 - Food processing areas should be kept, as dry as possible, standing water should be removed frequently. Drips and leaks should be repaired properly.
- Chlorine, quaternary ammonium compounds and peracetic acid based disinfectants are effective against *L. monocytogenes*. When niches of contamination are found or if biofilms are present, higher levels of the sanitizer in conjunction with mechanical action may be necessary to eliminate the hazard. After using the higher concentration, the equipment should be rinsed properly. People in high-risk groups for listeriosis should avoid the following high-risk foods:

- Ready to eat seafood such as smoked fish and smoked mussels, oysters or raw seafood.
- Prepared or stored salads including coleslaw and fresh fruit salad.
- Drinks made from fresh fruit or vegetables where washing procedures are unknown (excluding canned or pasteurized juices).
- Precooked meat products, which are eaten without further cooking or heating such as pate, salami and cooked diced chicken.
- Any unpasteurized milk or milk products.
- Soft serve ice creams.
- Soft cheeses, such as Brie, Camembert, Ricotta and Feta (these are safe if cooked and served hot).
- Ready to eat foods, including left over meats, which have been dipped.
- Raw vegetable garnishes.

During food handling and storage following precautions should be taken to avoid listerial infection in healthy individuals:

- Washing of hands is necessary before preparing food and between handling raw and ready to eat foods.
- All food should be covered during storage.
- All cooked food should be placed in the refrigerator within one hour of cooking.
- Raw poultry, fish and meat should be stored on the lowest shelves of the refrigerator to prevent them from dripping onto cooked and ready to eat foods.
- Cooked foods should not be handled with the same utensils used for raw foods, unless they have been thoroughly washed with disinfectants.
- All food from animal origin should be cooked thoroughly.
- Foods should be reheated until the internal temperature of the food

reaches at least 70°C.

- All raw vegetables, salads and fruits should be well washed before eating or juicing and consumed fresh.
- Food should defrost by placing it on the lower shelves of a refrigerator or should use a microwave oven. Foods are regularly tested for the presence of *L. monocytogenes*. If there is an outbreak of listerial food infection following measures has to be taken:
 - Food history should obtain from patient.
 - Contaminated foods can be recalled if necessary.
 - Epidemiological investigation of cases should be used to detect outbreaks and to determine source.
 - Molecular sub typing should be used to determine the association between isolate from cases and any foods positive for *L. monocytogenes*. Government agencies and the food industry of different countries have taken steps to reduce contamination of food by the *Listeria* bacterium. The Food and Drug Administration and U.S. Department of Agriculture monitor food regularly. When processed foods have found to be contaminated, monitoring and inspection are intensified and if necessary, the implicated food is recalled. The National Center for Infectious Diseases (NCID) is studying listeriosis in several states to help measure the impact of prevention activities and recognize trends in disease occurrence. Early detection and

reporting of outbreaks of listeriosis to local and state health departments can help identify sources of infection and prevent more cases disease.

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Encyclopedia

Biofilms are a collection of microorganisms surrounded by the slime they secrete, attached to either an inert or living surface. It is a complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix. Biofilms are also often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances.

Steps in biofilm development

1. Surface conditioning - In contact with water, organic molecules adhere to the surface. These organics neutralize the surfaces charge that may repel approaching bacteria.
2. Adhesion of "pioneer" bacteria - Planktonic (free-floating) bacteria first attach themselves by electrostatic attraction and physical forces. Some of these cells will permanently adhere to the surface with their extracellular polymeric substances, or sticky polymers.
3. Slime formation - The extracellular polymers consist of charged and neutral polysaccharide groups that not only cement the cell to the wall of the material, but also act as an ion exchange system for trapping and concentrating trace nutrients from the water. As nutrients accumulate, the pioneer cells reproduce. The daughter cells then produce their own exopolymers, greatly increasing the volume of ion exchange surface.
4. Secondary colonizers - Besides trapping nutrient molecules, the exopolymer web also snares other types of microbial cells through physical restraint and electrostatic interaction. These secondary colonizers metabolize wastes from the primary

colonizers as well as produce their own waste which other cells then use.

5. Fully functioning biofilm - The mature, fully functioning biofilm is like a living tissue on the surface. It is a complex, metabolically cooperative community made up of different species each living in a customized micro niche. An anaerobic layer may develop underneath the aerobic biofilm. As the film grows to a thickness that allows it to extend through the quiescent zone at the object wall into zones of more turbulent flow, some cells will be sloughed off. *Pseudomonas aeruginosa* is a common "pioneer" bacterium, which can adhere to stainless steel, even to electro polished surfaces, within 30 seconds of exposure.

Examples

Biofilms can be found on rocks and pebbles at the bottom of most streams or rivers and often form on the surface of stagnant pools of water. Biofilms are important components of foodchains in rivers and streams and are grazed by the aquatic invertebrates upon which many fish feed. In industrial environments, biofilms can develop on the interiors of pipes, which can lead to clogging and corrosion. Biofilms on floors and counters can make sanitation difficult in food preparation areas. Infectious processes in which biofilms have been implicated include common problems such as urinary tract infections, catheter infections, middle-ear infections, formation of dental plaque, gingivitis, coating contact lenses, and less common but more lethal processes such as endocarditis, infections in cystic fibrosis, and infections of permanent indwelling devices such as joint prostheses and heart valves.

Hydrogen Peroxide A better fumigant than Formaldehyde

Importance of fumigation

Vaporous decontamination involves the application of a decontaminant in the vapour phase for the decontamination of enclosed spaces. In vapour form these decontaminants have the potential to decontaminate hard to reach and complex surfaces including heating, ventilating and air conditioning systems, internal components of electronic equipment as well as cracks in floors, walls and other surfaces which may be contaminated by aerosolized biological agents or agent vapours. Microbiological safety cabinets, hospital wards, OTs and ICUs should always be fumigated to maintain the sterile environment. Microbiological safety cabinets, if they have been used for hazardous microorganisms, must be fumigated in the following circumstances:

- After a major spillage or a spillage where in accessible surfaces have been contaminated.
- Before any maintenance work on the cabinet where access to potentially contaminated parts is necessary (including filter and pre filter changes).
- Before carrying out filter penetration tests.
- When there are any changes in the nature of the work that result in significantly different risks.

To avoid microbiological contamination pharmaceutical industries should also carry fumigation procedure. Only a trained responsible person with adequate knowledge of the procedure and the precautions to be followed should always carry out fumigation. Fumigants have remarkable capacities for diffusion, a property essential to their function. Some readily penetrate rubber and neoprene personal protective gear, as well as human skin. They are rapidly absorbed across the pulmonary membrane, gut and skin. Special adsorbents are required in respirator canisters to protect exposed workers from airborne fumigant gases. Even these may not provide complete protection when air concentrations of fumigants are high. The packaging and formulation of fumigants is complex. Fumigants those are gases at room temperature (methyl bromide, ethylene oxide, sulfur oxide, hydrogen cyanide, sulfuryl fluoride) are provided in compressed gas cylinders. Liquids are marketed in cans or drums. A number of vaporous sterilants can be used to inactivate biological agents such as *Bacillus anthracis* and these include chlorine dioxide, ethylene oxide, propylene oxide, ozone, formaldehyde and hydrogen peroxide.

Formaldehyde as a fumigant

Formaldehyde vapour is most commonly used for fumigation purpose. Formaldehyde (formalin) is a commercially available 40% solution of formaldehyde vapour in water. When formalin is heated formaldehyde vapour is generated in quantity. It acts as an alkylating agent, inactivating microorganisms by reacting with carboxyl, amino, hydroxyl and sulphydryl groups of proteins as well as amino groups of nucleic acid bases. To act as a

disinfectant formaldehyde must dissolve at adequate concentration, in a film of moisture in the immediate vicinity of the organisms which are to be killed. Water vapour generated in the process of dispersing formaldehyde provides the essential optimum level of relative humidity and so it is important to ensure that water is added to the formalin prior to vapourization. Too much formaldehyde results in the deposition of sticky deposits of paraformaldehyde and cabinets may contribute to filter blockage. Fumigation is most effective above a temperature of 20°C and relative humidity of 65%. At temperatures below 18°C formaldehyde fumigation is less effective. Below 9°C, formaldehyde sublimates and is less easy to vapourize. The formaldehyde should be left to disperse within the cabinet for at least six hours. So formaldehyde fumigation has some advantages. It is cheap, not readily inactivated by organic materials and not harmful to fabrics, paints or metals.

But formaldehyde has following drawbacks:

(a) Toxic properties - It is very hazardous substance. Formaldehyde vapour is irritating to the eyes and to membranes of the upper respiratory tract. In some individuals it is a potent sensitizer, causing allergic dermatitis. OSHA and IARC recognize formaldehyde as a suspect carcinogen. In addition, it has been associated with asthma like symptoms. High concentrations of formaldehyde may cause laryngeal edema, asthma or tracheobronchitis. Aqueous solutions in contact with the skin cause hardening and roughness, due to superficial coagulation of the keratin layer. Ingested formaldehyde attacks the membrane lining of the stomach and intestine, causing necrosis and ulceration. Absorbed formaldehyde is rapidly converted to formic acid. The latter is partly responsible for the metabolic acidosis that is characteristic of formaldehyde poisoning. Circulatory collapse and renal failure may follow the devastating effects of ingested formaldehyde on the gut, leading to death.

(b) Physical properties - Formaldehyde is explosive at 7.75% in dry air. The vapour is irritant. Maximum occupational exposure limit is 2ppm. Formaldehyde vapour is most effective at a relative humidity >65% and a temperature above 24°C. A concentration of at least 0.05g of formaldehyde per m³ is required. Excessive quantity should not be used.

(c) Chemical interaction - Under certain conditions formaldehyde can react with hypochlorite and other chlorine containing chemicals to form bis (chloro methyl) ether which is a known lung carcinogen. Chlorine containing compounds must therefore be removed from rooms and cabinets before fumigation.

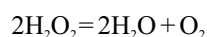
(d) It penetrates poorly and its effectiveness depends upon the temperature and humidity. The vapour can also combine with many common substances and under certain conditions will readily polymerize. Use of excessive amounts may cause deposition of polymers within the cabinet and may contribute to

filter blockage.

- (e) Formaldehyde does not have a residual effect and it is not effective against dried spores.
- (f) It cannot reach the surfaces, which are in intimate contact.
- (g) Contact time of formaldehyde fumigation is 6-48 hours.
- (h) Presence of organic matter reduces efficacy of formaldehyde.

Hydrogen peroxide: A new trend in fumigation

Formaldehyde may cause a considerable risk to health and there is an increasing impetus for finding a safer alternative. Hydrogen peroxide has been considered as an alternative method for fumigation. Aqueous hydrogen peroxide has a long history of use as a disinfectant. The disinfection mechanism of hydrogen peroxide is based on the release of free oxygen radicals:



Aqueous hydrogen peroxide is active against a wide range of organisms and has broad-spectrum activity. Hydrogen peroxide is being used in liquid as well as vapour form. These systems have been widely used for sterilization of pharmaceutical applications including production filling lines, sterility testing environments, production rooms, lyophilisers and more recently for the decontamination of laboratory animal, research and biosafety laboratory facilities. Hydrogen peroxide vapour system may be classified as "wet" or "dry" processes. Hydrogen peroxide vapour can be introduced into a given area up to a certain concentration, dependent on the isolator temperature and humidity, to a saturation level or dew point. If the concentration of the hydrogen peroxide increases above this level it will condense onto the surfaces of the isolator. In case where micro condensation is formed and maintained during the cycle, this is considered as a "wet" process. If the vapour concentration is maintained below the dew point during the cycle, this is considered as a "dry" process. In dry process, vapourous hydrogen peroxide may only be used in sealed enclosures to avoid uncontrolled leakage of hydrogen peroxide as well as the inflow of unconditioned air. The vapourous hydrogen peroxide concentration is maintained below the condensation point to prevent condensation of liquid peroxide on surfaces. The dry process cycle has four phases (i) dehumidification, (ii) conditioning, (iii) decontamination/ sterilization, (iv) aeration. The process parameters of the dehumidifying, conditioning, sterilization and aeration phases are determined on the basis of measurements of the air temperature and humidity in the enclosure. Commercially available hydrogen peroxide gas generators are designed to provide biological decontamination within enclosures and rooms, providing a layer of hydrogen peroxide micro condensation, deactivating microorganisms on all exposed surfaces. The decontamination cycle consists of three phases (i) preconditioning, (ii) gassing, (iii) aeration. During the gassing phase liquid hydrogen peroxide is pumped onto a hot plate in an air stream and is flash evaporated turning

all the liquid into hydrogen peroxide vapour. The hydrogen peroxide vapour is delivered to the chamber or enclosure at an elevated temperature. As the flash evaporation process continues the concentration of the vapour in the enclosure increases until the vapour dew point is reached. At this point the vapour will start to condense onto all cooler surfaces and it is the deposition of hydrogen peroxide condensate that facilitates the decontamination. When an appropriate contact between all surfaces of the enclosure and the vapour mixture has been reached, hydrogen peroxide is passed through a catalyst and decomposed to water and oxygen. A drier removes water vapour produced in this process. This aeration phase continues until all of the peroxide has been removed to a safe level. Hydrogen peroxide along with the silver ions has a good synergistic effect against microorganisms. Silver ion itself has a good microbicidal activity. Silver ions are rapidly attracted to the surfaces of microorganisms, which can lead to disruption of cell wall and membrane functions by affecting the structures and functions of proteins. Silver binds to sulfhydryl, amino and carboxyl groups on amino acids, which leads to protein denaturation. The interaction of silver with thiol groups in enzymes and proteins plays an important role in bacterial inactivation. Silver has been shown to especially inhibit cell wall metabolism, respiration and electron transport. It also has been shown to bind to DNA and to inhibit replication and transcription. Silver nitrate acts on the surfaces of bacteria, indicative of cell wall and membrane damage. As a fumigant hydrogen peroxide has some advantages.

- Hydrogen peroxide fumigation has good residual effect.
- It reaches the surfaces, which are in intimate contact.
- Contact time of hydrogen peroxide is only one hour.
- It is ecofriendly.

So hydrogen peroxide shows a good microbicidal activity with minute amount of silver salts. It is claimed that the mode of action of vapourized hydrogen peroxide is distinct from liquid hydrogen peroxide but regardless of the exact mode, there is increasing evidence that hydrogen peroxide is a broad spectrum, rapid antimicrobial and this broad spectrum efficacy has been shown against a wide range of microorganisms.

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Antony van Leeuwenhoek

Birth: October 24, 1632

Death: August 30, 1723

Nationality: Dutch

Known for: Inventor of Microscope

Science progresses in small steps and giant leaping bounds. Inventions come from true scientists and non scientists. One of the most important inventions in the scientific world was by one of those non scientist. With skill, diligence, patience and an endless curiosity Leeuwenhoek made some of the most important discoveries in the history of biology.

Leeuwenhoek was born in Delft, Holland on October 24, 1632. He was the son of Philips Thoniszoon, a basket maker and Margriet Jacobsdochter van den Berch, who came from a family of brewers. He did not pursue any university degrees or higher education.

At the age of sixteen he secured an apprenticeship with a Scottish cloth merchant in Amsterdam. He returned to Delft at the age of twenty and established himself as a linen draper and haberdasher. As a textile merchant he was not satisfied using available lens to examine his fabrics, so he learned to grind his own. This led him to construct simple microscopes.

It is believed that Leeuwenhoek had been inspired to take up microscopy by having seen a copy of Robert Hooke's illustrated book "*Micrographia*", which depicted Hooke's own observations with the microscope. He constructed more than 500 microscopes of which fewer than ten have survived to the present day. In reality all of Leeuwenhoek's instruments were simply powerful magnifying glasses, not the compound microscopes of the type used today. It is an extremely simple device, using only one lens mounted in a tiny hole in the brass plate. The specimen was mounted on a sharp point in front of the lens its position and focus adjusted by turning two screws. The entire instrument was only 3-4 inches long and had to be held up very close to the eye; it required good lighting and great patience to use. Leeuwenhoek's simple microscopes magnified objects to over 200 times actual size, with clear and brighter images. He grinded small lenses of short focal length and obtained a resolving power that was greater than that of the early compound microscopes had been invented nearly 40 years before Leeuwenhoek was born. But due to some technical difficulties in building them, early compound microscopes were not practical for magnifying objects more than about twenty or thirty times natural size. Lens technology also caused a number of viewing problems like spherical aberration, chromatic aberration etc.

Leeuwenhoek was also distinguished by an immense curiosity to observe just about anything that would fit under his lenses and he made careful, detailed observations, he succeeded in making some of the most important contributions in the history of biology. In 1674, he made the first observations of microbes. He

observed bacteria, protozoa from various sources, such as rainwater, pond and well water and the human mouth and intestine. Leeuwenhoek referred these tiny organisms as "animalcules". He also observed spermatozoa. In 1677 Leeuwenhoek described for the first time the spermatozoa from insects, dogs and man. Leeuwenhoek also made extensive investigation of reproduction in plants. He was particularly interested to the blood vessels and the blood cells. In 1682 he discovered the banded pattern of muscle fibers. Van Leeuwenhoek's discovery of microorganisms along with experiments done by Francesco Redi, Lazzaro Spallanzani and Louis Pasteur did overturn the traditional belief of spontaneous generation of life. He also calculated the size of microbes. He studied nerve fibers, the structure of the lens of the eye, the structure of bone, teeth and hair, the corn weevil, the grain moth, the flea, the spinning apparatus of a spider, the brain of a fly and many more.

Both his colleagues and the public recognized Leeuwenhoek's scientific achievements during his lifetime. Although Leeuwenhoek had no formal scientific training, the astounding and detailed nature of his discoveries resulted in his induction as a full member of the Royal Society in 1680, where he joined the ranks of many other scientific luminaries of his day. He started writing letters to the newly formed Royal Society of London, describing his observations under microscopes. His first letter contained some observations on the stings of bees. For the next fifty years he corresponded with the Royal Society. His letters written in Dutch, were translated into English or Latin and printed in the "*Philosophical Transactions of the Royal Society*". In 1699 he was appointed as a correspondent of the Paris Academic des sciences and in 1716 the Louvain College of Professors awarded him a silver medal.

Leeuwenhoek with enormous curiosity and enthusiasm achieved the height of success. In a letter he mentioned ".....my work, which I've done for a long time, was not pursued in order to gain the praise now I enjoy, but chiefly from a craving after knowledge, which I notice resides in me more than in most other men. And theoretical, whenever I found out paper, so that all ingenious people might be informed thereof." This great man continued his observations until the last days of his life. After his death on August 30, 1723, the pastor of the New Church at Delft wrote to the Royal Society : ".....Antony van Leeuwenhoek considered that what is true in natural philosophy can be most fruitfully investigated by the experimental method, supported by the evidence of the senses; for which reason, by diligence and tireless labour he made with his own hand certain most excellent lenses, with the aid of which he discovered many secrets of Nature now famous throughout the whole philosophical world."

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Enjoy the humour

▪ *Deep within a forest a little turtle began to climb a tree. After hours of effort he reached the top, jumped into the air waving his front legs and crashed to the ground. After recovering, he slowly climbed the tree again, jumped, and fell to the ground. The turtle tried again and again while a couple of birds sitting on a branch watched his sad efforts. Finally, the female bird turned to her mate. "Dear," she chirped, "I think it's time to tell him he's adopted."*

▪ *On opening his new store, a man received a bouquet of flowers. He became dismayed on reading the enclosed card, that it expressed "Deepest Sympathy". While puzzling over the message, his telephone rang. It was the florist, apologizing for having sent the wrong card. "Oh, it's alright," said the storekeeper. "I'm a businessman and I understand how these things can happen." "But," added the florist, "I accidentally sent your card to a funeral party." "Well, what did it say?" ask the storekeeper. "'Congratulations on your new location!'" was the reply.*

Track your brain

Down

1. Vague airborne principle believed to cause disease. 2. Country in which Reed performed his studies. 3. Koch's solid culture medium had a _____ like consistency. 4. Pasteur's country. 5. Fabricius suggested this fungus caused plant disease. 7. Disease agent isolated by Löffler. 9. Leeuwenhoek's microscope had a single _____ 11. His broth experiments supported spontaneous generation. 12. Type of culture obtained by Koch. 13. Related ticks to Rocky Mountain spotted fever. 16. Leeuwenhoek's letters number over _____ hundred. 17. Disease of silkworms studied by Pasteur. 18. Insect that can transmit Texas fever. 21. Environment in which Leeuwenhoek observed microorganisms. 22. Broth experiments opposed spontaneous generation. 24. Recognized three forms by which contagion passed. 25. Believed by Pasteur to be a source of disease microorganisms. 28. Developed the condenser for use in microscopy. 31. Animal infected by anthrax bacillus. 33. One of the founders of the science of microbiology. 34. Studied cholera during an outbreak in London. 35. Won the Nobel Prize for work on malaria and mosquitoes. 38. Proved that a specific microorganism causes a specific disease. 40. Source of aqueous humour used by Koch. 41. Used by Abbé to increase microscope magnification. 44. Article of clothing sold by Leeuwenhoek.



Thoughts to live by

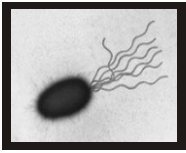
- The first step in acquisition of wisdom is silence, the second listening, the third memory, the fourth practice, the fifth teaching others. (Solomon Ibn Gabriol).
- The ultimate measure of a man is not where he stands in moments of comfort, but where he stands at times of challenge and controversy. (Martin Luther King, Jr).
- The future belongs to those who believe in the beauty of their dreams. (Eleanor Roosevelt).
- Love is an emotion experienced by the many but enjoyed by the few. (George Jean Nathan).
- Success is the good fortune that comes from aspiration, desperation, perspiration and inspiration. (Evan Esar).



Across

4. Insect that transmits plague. 6. Microorganisms poorly studied during the classical Golden age. 8. Leeuwenhoek's term for microorganisms. 10. Pasteur's flasks had the neck of a _____. 13. Related diphtheria to a toxin. 14. Mass of bacteria isolated by Koch. 15. Attempted to disprove spontaneous generation by covering meat. 19. Isolated the gas gangrene bacillus. 20. Used chlorine water for hand disinfection in hospital. 22. Related a specific insect to Texas fever. 23. Environment where anthrax spores is located. 26. Souring attracted attention of Pasteur. 27. Isolated the bacterium that causes dysentery. 29. Believed by Pasteur to be the cause of spoilage in wine. 30. Proved that tsetse flies transmit African sleeping sickness. 32. First described by Robert Hooke. 36. Tetanus bacilli grow where there is _____ oxygen. 37. Dutch investigator who reported the existence of microorganisms. 39. Needed by algae and cyanobacteria for photosynthesis. 42. In the classical Golden _____ microbiology experienced substantial growth. 43. Searched for a magic bullet to treat syphilis. 45. Produced by the bacterium in cases of diphtheria. 46. Studied the role of mosquitoes in yellow fever epidemics. 47. Causative agent studied by Kitasato.

Check your Answers on Page 16



Pseudomonas species

In 1882, Gessard first discovered *Pseudomonas* species, a strictly aerobic, Gram negative motile bacillus. They are ubiquitous and being found in soil, water, plants and animals. *Pseudomonas* is a clinically as well as industrially significant microorganisms. Some are opportunistic pathogen, often causing nosocomial infections and some species form biofilms, causing an emergent threat to the food industry. Some of the common species of *Pseudomonas* are *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas cepacia* (now known as *Burkholderia cepacia*), *Pseudomonas mallei* (now known as *Burkholderia mallei*) etc.

Cultural characteristics and Biochemical reactions

It is an obligate aerobe but can grow anaerobically in the presence of nitrate. It has very simple nutritional requirements. The organism is able to grow in a wide range of temperatures (6-42°C) but 37°C is the optimum temperature for growth. *Pseudomonas* isolates may produce three types colony. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples produce some different colony morphology. One type has a fried egg appearance, which is large, smooth, with flat edges, and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance. Many strains are hemolytic on blood agar. Mucoid appearance of the colony is due to the extracellular polysaccharide material produced by the organism. In absence of oxygen nitrates act as an ultimate electron acceptor. Indole, methyl red, Voges Proskauer and hydrogen sulfide tests are negative. Most *Pseudomonas* species are motile by means of one or more polar flagella. *Pseudomonas aeruginosa* produces a number of pigments. Pyocyanin and fluorescein are the major two among them. Pyocyanin is a bluish green phenazine pigment soluble in water but not in chloroform. Other pigments produced are pyorubin (red) and pyomelanin (brown). Cetrimide broth is used for isolation and cultivation of *Pseudomonas* species.

Pathogenesis

Pseudomonas aeruginosa is an opportunistic pathogen. The pathogenesis of pseudomonal infections is multi factorial and complex. It infects the pulmonary tract, urinary tract, burns, wounds and causes wide range of diseases, which include septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis and dermatitis. Most pseudomonal infections are both invasive and toxigenic. The three major stages of pseudomonal infection are (a) bacterial attachment and colonization, (b) local invasion and (c) blood stream dissemination and systemic disease. *Pseudomonas* species colonize to the epithelial cells of the upper respiratory tract with the help of fimbriae. These adhesions bind to specific galactose or mannose or sialic acid receptors of epithelial cells. Mucoid strains produce an exopolysacchride (alginate), which helps to attach to the tracheobronchial mucin. This alginate slime forms the matrix of the *Pseudomonas* biofilms, which protects the bacteria from the host defenses. *Pseudomonas* starts invading tissues by the production of extracellular enzymes and toxins that break down physical barriers and damage host cells. *Pseudomonas aeruginosa* produces some extracellular enzymes (elastase, alkaline protease) and extracellular protein toxins

(Exoenzyme S, Exotoxin A) that enhance the virulence of the organism. *P. cepacia* was first identified as a human pathogen that causes endocarditis. Subsequently, the organism has been found in numerous catheter-associated UTIs, wound infections, and IV catheter associated bacteremias. *P. mallei* causes glanders, a serious infectious disease of animals (primarily horses, although it also has been isolated in donkeys, mules, goats, dogs, and cats). Transmission is believed to occur through direct contact. Glanders transmission to humans is rare and presumably occurs through inoculation of broken skin or the nasal mucosa with contaminated discharges. Manifestation of the disease in humans varies, ranging from an acute localized suppurative infection, acute pulmonary infection, or acute septicemic infection to chronic suppurative infection. Fulminate disease with multiple organ system involvement occurs with septicemic infection.

Hazards associated with *Pseudomonas* in Clinical & Industrial Field

Pseudomonas species is a common human saprophyte; it rarely causes disease in healthy person. They cause infection mostly in compromised hosts include disrupted physical barriers to bacterial invasion (eg. burn injuries, urinary catheters, dialysis catheters, endothelial tubes) and dysfunctional immune mechanisms (eg. AIDS, cystic fibrosis). *Pseudomonas aeruginosa* can cause following infections in different parts of human body.

- i. Respiratory tract infections- *Pseudomonas* causes infections in lower respiratory tract of immunocompromised patients. Primary pneumonia occurs in patients with neutropenia following chemotherapy and in patients with AIDS.
- ii. Endocarditis - *Pseudomonas aeruginosa* infects heart valves of IV drug users and prosthetic heart valves. Non-specific symptoms include fever and malaise, with more specific symptoms depending on which cardiac valve is involved.
- iii. Central Nervous System infections- *Pseudomonas aeruginosa* causes meningitis and brain abscesses. Most infections extend from a contiguous structure such as the inner ear or paranasal sinus or are inoculated directly by means of head trauma, surgery or invasive diagnostic procedures or spreads from a distant site of infection such as the urinary tract.
- iv. Bacteremia and Septicemia - *Pseudomonas aeruginosa* causes bacteremia may be acquired via medical devices in hospitals and nursing homes.
- v. Ear infections - *Pseudomonas aeruginosa* is the predominant bacterial pathogen in external otitis (i.e. swimmer's ear). Malignant otitis externa and chronic otitis media is also caused by *Pseudomonas*.
- vi. Eye infections - *Pseudomonas* can colonize the ocular epithelium by means of a fimbrial attachment to sialic acid receptors and produces some extracellular enzymes that cause a rapid progressive and destructive lesion. It is also a common cause of bacterial keratitis, scleral abscess and endophthalmitis in adults and ophthalmia are trauma, contact lens use, predisposing ocular conditions, exposure to an ICU environment and AIDS.
- vii. Bone and joint infections - *Pseudomonas* infections of bones and joints result from direct inoculation of the bacteria or the hematogenous spread of the bacteria from other primary sites of

infection. It causes chronic contiguous osteomyelitis.

viii. Urinary tract infections - Most pseudomonal urinary tract infection are hospital acquired and iatrogenic due to catheterization, instrumentation and surgery.

ix. Skin infections- *Pseudomonas aeruginosa* can cause a variety of skin infections. Pseudomonal infections flourish at high moisture contents in skin like in the ear of swimmer, toe webs of athletes, in the perineal region and under diapers of infants, hot tub users. It also has emerged as an important source of burn wound sepsis.

x. Gastrointestinal infections.- *Pseudomonas aeruginosa* can produce disease in any part of the gastrointestinal tract from the oropharynx to the rectum. As in other forms of *Pseudomonas* disease, those involving the GI tract occur primarily in immunocompromised individuals. The organism has been implicated in perirectal infections, pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis. The GI tract is also an important portal of entry in *Pseudomonas* septicemia. Epidemics of pseudomonal diarrheal diseases occur in children.

Other than causing infections *Pseudomonas* also causes contamination in dental unit waterlines by means of biofilm formation. This opportunistic pathogen successfully colonizes on synthetic surfaces, increasing the concentration of the pathogens in water to potentially dangerous levels in dental unit. *Pseudomonas* has the capability to develop biofilm by adhering to the surfaces of equipments or gasket and surrounded themselves with a protective layer of polysaccharides and grow into a network of micro colonies and water channels. These channels serve to supply nutrients and remove bacterial waste products. When *Pseudomonas* grows in biofilm, they pose generally no safety threat, but they can be sloughed off during production, contaminate the food and accelerate spoilage.

Currently *Pseudomonas* species limits the shelf life of processed fluid milk at 4°C. In addition to the ability of *Pseudomonas* species to grow in high numbers during refrigerated storage, many of these strains produce heat stable extracellular lipases; proteases and lecithinases, which can further contribute to milk spoilage. Many of these enzymes remain active even after thermal processing. Degradation of milk components through various enzymatic activities can reduce the shelf life of processed milk. *Pseudomonas* is also associated with the following food spoilages. It causes souring and putrefaction of fresh meat. It also causes souring and greening of cured meat, discolouration of fish, green rots on eggs, odour and slime in poultry products. It forms green colouration in sugar products, honey and syrups. These biofilms of *Pseudomonas* may also be found on the surfaces of food and beverage processing and packaging equipment such as product lines, filters, hoppers and stuffers, on plastic cutting boards, stainless steel and plastic conveyor systems, mixers, grinders, slicers etc.

Control Measures

Pseudomonas species is a common inhabitant of soil, water and vegetation. It is found on the skin of some healthy persons. Within the hospital, *Pseudomonas* finds numerous reservoirs like respiratory equipment, foods, sinks, taps. In hospitals, *P. aeruginosa* can also be spread through fecal material. Also *Pseudomonas* has been isolated from many natural and manufactured foods, and therefore, foods have been implicated as sources of infection in hospitals. Visitors constantly reintroduce it into the hospital environment on fruits, plants, and

vegetables. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated reservoirs and by the ingestion of contaminated foods and water. *Pseudomonas* should be considered in the differential diagnoses in any probable gram-negative infections. Often, the effect of this organism causes concern, as it can cause severe hospital-acquired infection, especially in immunocompromised hosts. Furthermore, a concomitant antibiotic resistance is often present, which makes the choice of treatment difficult. Therefore, *Pseudomonas* organisms should always be treated with two antipseudomonal antibiotics, each with different mechanisms of action. Often, treatment is achieved with a combination of an aminoglycoside or quinolone with another antipseudomonal antibiotic. When infection is localized and external, treatment with 1% acetic acid irrigations or topical agents such as polymyxin B or colistin is effective. Necrotic tissue must be formed debris and abscesses must be drained. Other antibiotics used include amikacin, tobramycin and gentamicin. Several penicillins, including carbenicillin, ticarcillin, piperacillin, mezlocillin, and azlocillin, are active against *Pseudomonas*. *Pseudomonas* contamination causes significant economic losses for the food industry. Moreover, this biofilm results in equipment damage, product contamination and energy losses.

The spread of *Pseudomonas* species can best be controlled by observing proper isolation procedures, aseptic techniques and careful cleaning and monitoring of respirators, catheters and other instruments can best control the spread of *Pseudomonas* species. The consequences of Pseudomonal biofilm formation in food plants can be serious leading to the contamination of the product comprising shelf life and food safety. Because of these problems, it is extremely important to take the proper measures to prevent the formation of biofilms. Isolation and identification should be done by choosing proper selective medium for *Pseudomonas* species and thereby, treating the patients with effective anti microbial agents. At last but not the least proper cleaning and sanitizing practices with high level disinfectants can be highly effective to control *Pseudomonas* contamination in clinical as well as in industrial field.

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Quaternary Ammonium Compounds

Quaternary ammonium compound (QAC/ Quats) is the term used to describe a diverse group of chemicals. They are cationic, surface active (surfactant) chemicals used as disinfectants, detergents and sanitizers. Chemically the QACs are synthetic derivatives of ammonium chloride. They have a general structure in which a nitrogen atom is linked to four carbon atoms, the sum of which forms a positively charged cation. The cation is bonded to a negatively charged anion, usually chloride or bromide, thus forming a salt. The carbon atoms linked to the nitrogen atom are actually alkyl groups which may all be alike or all different or in various combinations of such.

Historical Aspects and Generations of Quaternary Ammonium Compounds

W.A. Jacobs and colleagues first recognized QACs in 1915/1916. But QACs were prominently synthesized in 1935 when a long aliphatic chain was attached to a quaternary nitrogen atom. These first borne molecules have lead up to many generations of quaternary ammonium compounds. Different generations of QACs, starting from simple QAC to modern QAC are explained as follows:

First generation QACs: The first generation QACs was the simple compounds than other generations. These QACs have been around for a long time. It has been found that biocidal activity peaks at a carbon chain length of C₁₄. The major raw materials of QACs, tertiary amine, use to be synthesized from only alcohol. And these could be the probable reason for the various alkyl chain lengths of QACs. They are affected by hard water. The positively charged Calcium and Magnesium ions compete with the QAC for the negatively charged bonding sites on the bacteria. This affect can be reduced with the addition of chelating agents, which bond themselves to the Calcium & Magnesium ions, and free reactive sites of the microorganism are now free to complex with the QAC.

Second generation QACs: To increase the efficacy and hard water tolerance of the first generation QACs were carried out by substituting an ethyl group for a hydrogen on the aromatic ring. The ultimate compound is an alkyl dimethyl ethyl benzyl ammonium chloride or EBC quat. Second generation QAC has higher biocidal activity.

Third generation QACs: Third generation QAC was developed in 1955. It was a mixture of first and second generation QAC, known as "Dual Quat". Compare to first and second generation QAC this blend has a higher biocidal activity, stronger detergency and less toxic to the user.

Fourth generation QACs: It was known as "Twin Quats". These products showed outstanding germicidal performance and improved hard water tolerance.

Fifth generation QACs: It is nothing but the blend of second and fourth generation Quats. They have a good germicidal activity and are active under more hostile conditions and are safer to use. Sixth and seventh generation of QACs include the polymeric type quats and blends of polymeric quats.

Mode of Action of QACs

The mode of action of QAC has not been understood completely. QACs inhibit enzyme activity. In high concentrations they can bind to nucleic acid also. The majority of applications for quaternary ammonium compounds are related to their very strong affinity for surfaces, which makes them powerful surfactants. The QACs adsorb on almost any surface resulting in the formation of a monolayer. The main target of QAC is bacterial cell surface. Negatively charged bacterial membrane are attacked by QACs and adsorbed onto the cell surface. Then

they start diffusing through the outer layer. QACs bind to the cytoplasmic membrane and disrupt the cytoplasmic membrane. Gram positive organisms are more sensitive to QACs than Gram negative organisms. In case of Gram negative bacteria outer membrane acts as a significant barrier. So QACs displace cations from outer membrane and promote own entry across the outer membrane.

The surfactant action of the QACs is often increased by coformulation with other surfactants insuring the removal as well as the destruction of microorganisms.

This dual mode of the both killing and removal reduces or completely removes the bacterial and fungal population from the treated area. In low concentrations QACs are mycobacteriostatic. They are sporistatic at low concentrations but not sporicidal even at very high concentrations. Activity of QACs depends on the nature of virus. Mostly enveloped viruses are sensitive towards QAC.

Benzalkonium Chloride: A widely used Quaternary Ammonium Compound

Benzalkonium chloride is a mixture of alkyl benzyl dimethyl ammonium chloride of various alkyl chain lengths. It has been considered one of the safest synthetic biocides known and has a long history of efficacious use. Products formulated with benzalkonium chloride need a great care. Certain organic compounds including soap, anionic surfactants can inactivate benzalkonium chloride. It acts same like other QACs on microorganisms. Benzalkonium chloride solutions are rapidly acting anti-infective agents with a moderately long duration of action. Applications are extremely wide ranging from disinfectant formulations to microbial corrosion inhibition in the oilfield sector.

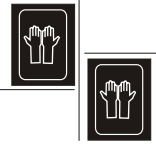
Applications and Implications in Health Care & Industry

Quaternary ammonium compounds are widely used for clinical as well industrial purpose. QACs has the capability to coat and penetrate soils, making them suitable for disinfecting environmental, non food contact areas such as floor drains, walls, external equipment surfaces and restroom facilities. They are also applied to control mildew and unpleasant odours in storage rooms, garbage containers, etc.

QACs are used to disinfect food contact surfaces like lines, tanks, equipment parts and cooking utensils. In addition, they are frequently used to sanitize drinking glasses, eating utensils and kitchen preparation areas. QACs has a residual effect. Residual films can also interfere with the growth and metabolism of cultured microorganisms used in the manufacture of foods such as cheese and beer. A residual film can also have advantage in that it provides anti microbial activity over an extended period of time. This is certainly a desirable property when used in environmental areas, cutting/chopping blocks, utensils, storage rooms and various equipment surfaces. QACs can be applied by any of the common techniques, including spraying, soaking, manual brushing/sponging. But quaternary ammonium compound in a concentrated mist or fog can be toxic. Use of QACs should be done in a proper way otherwise microorganisms may develop resistance against them.

References

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Hand Hygiene Techniques

Hand hygiene is a means of achieving a reduction in, or removal of visible soiling, transient or resident microbes and or other hazardous or toxic substances.

Historical perspective

For over a century, skin hygiene, particularly of the hands, has been accepted as a primary mechanism to control the spread of infectious agents. The concept of cleansing hands with an antiseptic agent probably emerged in the early 19th century. In 1846, Ignaz Semmelweiss realized the significance of hand transfer pathogenic bacteria during an outbreak of puerperal fever at obstetrics ward of General Hospital of Vienna. At the same time Dr. Oliver Wendell Holmes had concluded health care practitioners transmitted that puerperal fever. As a result of the seminal studies by Semmelweiss and Holmes, hand washing generally became accepted as one of the most important measures for preventing transmission of pathogens in health care facilities. But hand hygiene awareness started growing exponentially since the early 20th century. Then CDC recommended a guideline for hand washing with non antimicrobial soap between the majority of patient contacts and washing with antimicrobial soap during invasive procedures in 1975 & 1985. Recent studies and developments are going on to improve hand hygiene practices in health care facilities.

Physiology and microflora of the skin

In order to understand the principles of safe hand washing, one must understand the physiology and normal flora of the skin. The primary function of the skin is to reduce water loss, provide protection against abrasive action and microorganisms act as a permeability barrier to the environment. Skin basically composed of four layers, considering from outer layer to inner most layer, the superficial region, the viable epidermis, the dermis and hypodermis. The dermis and subcutaneous tissues are free from micro flora. Bacterial floras are on and within the epidermis and can become established in the hair follicles and in the sweat and sebaceous glands.

Normal human skin is colonized with microorganisms and the skin provides nutrients for selected colonizing microbes in the form of lipids and proteins (keratin). Micro flora of the skin can be classified as resident flora and transient flora.

Resident floras are considered as permanent inhabitants of the skin and are more resistant to removal. Coagulase negative staphylococci, members of the *Corynebacterium*, *Propionibacterium* and *Acinetobacter* and certain members of the enterobacteriaceae family reside to the resident micro flora. The presence of resident micro flora on the skin prevents to colonize other pathogenic microorganism. Transient micro flora are those, which are picked up during daily activities and may be shed on skin scales. This flora mainly colonize the superficial layers of the skin and can be effectively removed or substantially reduced to a low level by hand washing or by using

some antiseptic hand rub. Transient microorganisms can be of any type, from any source with which the body has had contact. This flora mainly includes *Escherichia coli*, *Salmonella*, *Shigella*, *Clostridium perfringens* and Hepatitis A virus. High level of transient microorganisms attaches to hand, fingertip and fingernail surfaces when hands are not properly washed. A resident flora is considered as permanent inhabitants of the skin and is more resistant to removal.

Significance of Hand Hygiene

Health care associated infections are an important cause of morbidity and mortality among hospitalized patients worldwide. Transmission of health care associated pathogens most often occurs via the contaminated hands of health care workers. Effective hand hygiene removes transient microorganisms, dirt and decreases the risk of cross contamination from patients, patient care equipment and the environment. Hand hygiene is the single most important strategy to reduce the risks of transmitting organisms from one person to another or from one site to another on the same patient. Cleaning hands promptly and thoroughly between patient contact and after contact with blood, body fluids, secretions, excretions, equipment and potentially contaminated surfaces is an important strategy for preventing health care associated infections.

Indications and Guidelines for Hand Hygiene Decision Making

Following indications and guidelines are given for proper hand hygiene decision-making. Hands must be decontaminated:

- Immediately before and after each and every episode of direct patient contact.
- After contact with body fluids or excretions, mucous membranes and wound dressings.
- On arrival to, and before leaving the work place.
- Before and after manipulating any invasive device.
- Before and after undertaking clinical procedures.
- Before donning sterile gloves and also after removing gloves.
- Before and after handling food.
- After using restroom.

The choice of using alcohol based hand rub, anti microbial soap or surgical hand preparation is based on:

- The degree of hand contamination.
- The degree of activity which requires less or reduced bio burden.
- Transmission and patient risk factors.
- Invasive or surgical procedure.

Alcohol based hand rub is used to destroy transient flora and part of resident flora on unsoiled hands. Anti microbial soap can be used to remove soil and transient flora. For surgical hand asepsis it is necessary to remove transient flora and reduce resident flora by using either anti microbial disinfectant or alcohol and chlorhexidine based preparation.

Techniques of Hand Hygiene

Different types of hand hygiene techniques are described below.

Procedure for hand washing:

- i. Hand washing takes only 1-1.5 minutes. Person should stand near sink, but should avoid touching it, as the sink itself may be a source of contamination.
- ii. In case of lever operated paper towel dispenser person should dispense a portion of towel before washing hands.
- iii. Washing hands with high level disinfectant is recommended.
- iv. Person should wet their hand with tepid water. Splashing should be avoided. Hands, sleeves and clothing should keep away from moisture. Use of hot water is avoided because repeated exposure to hot water may increase the risk of dermatitis.
- v. Person should apply the amount of product necessary to cover all hand surfaces.
 - Hands are rubbed palm to palm.
 - Right palm over left dorsum with interlaced fingers interlocked.
 - Palm to palm with fingers interlaced.
 - Backs of fingers to opposing palms with fingers interlocked.
 - Rotational rubbing of left thumb clasped in right palm and vice versa.
 - Hands are rinsed with water.
 - Hands are dried thoroughly with a single use/disposable towel.
 - Towel should be used to turn off the faucet for handle-operated faucets to prevent contaminating your hands.

Procedure for using alcohol based hand rub:

Onto hands that are not visibly soiled, the person should apply product to palm of one hand and rubs hand together, covering all surfaces of hands fingers until hands get dry. Manufacturer's recommendations should be followed for product volume and this procedure should take approximately 20 seconds.

Procedure for surgical hand antisepsis:

- i. All the jewellery and watches should be removed before beginning the surgical hand scrub.
- ii. Debris is removed from underneath fingernails using a disposable nail cleaner under running water.
- iii. Surgical hand antisepsis using an anti microbial soap or an alcohol based hand rub with persistent activity is recommended before donning sterile gloves when performing surgical procedures.
- iv. Contact time of scrubbing should be 2-6 minutes. Long scrub time is not necessary.
- v. The manufacturer's instructions are followed while using an alcohol based hand rub with persistent activity. Before applying the alcohol solution pre wash of hands and forearms should be done with a non-anti microbial soap and dried completely.
- vi. After application of the alcohol-based product as recommended hands and forearms should allow drying thoroughly before donning sterile gloves.

Selection of Hand Hygiene agents and facilities for its potential use

While selecting a hand hygiene agent following things should be considered:

- Personnel should provide with efficacious hand hygiene products that have low irritancy potential, particularly when these products are used multiple times per shift. This recommendation applies to products used for hand antisepsis

before and after patient care in clinical areas and to products used for surgical hand antisepsis by surgical personnel.

- To maximize the acceptance of hand hygiene by personnel the product is included with some special characteristics like fragrance, colours and consistency (i.e. feel). For soaps, ease of lathering also may affect user performance.
- The product should be provided with skin emollients to reduce the skin irritancy and dryness.

Following facilities should be provided to increase the potentiality of the hand hygiene products:

- Hand washbasins should be easily accessible.
- Warm water must be available for hand washing in all clinical areas, by means of mixer taps or temperature controlled water.
- Liquid dispensers should function properly and deliver an appropriate volume of product.
- Disposable hand towels should be used instead of using communal hand towels or hot air driers.

Some administrative measures should be taken to increase the potentiality of the procedure:

- Improved hand-hygiene adherence should be made as an institutional priority and appropriate administrative support and financial resources should be provided.
- A multidisciplinary program should be implemented to improve adherence of health personnel to recommended hand-hygiene practices.
- As part of a multidisciplinary program to improve hand-hygiene adherence, HCWs should be provided with a readily accessible alcohol-based hand-rub product.
- To improve hand-hygiene adherence among personnel who work in areas in which high workloads and high intensity of patient care are anticipated, an alcohol-based hand rub should be made available at the entrance to the patient's room or at the bedside, in other convenient locations, and in individual pocket-sized containers to be carried by HCWs.

The following performance indicators are recommended for measuring improvements in HCWs' hand-hygiene adherence:

- (a). Periodically monitor and record adherence should be done as the number of hand-hygiene episodes performed by personnel/number of hand-hygiene opportunities, by ward or by service. Feedback should be to personnel regarding their performance.
- (b) The volume of alcohol-based hand rub (or detergent used for handwashing or hand antisepsis) should be monitored used per 1,000 patient-days.
- (c). Monitor adherence to policies dealing with wearing of artificial nails.
- (d). When outbreaks of infection occur, assess the adequacy of health-care worker hand hygiene.

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Air, land and water are essential elements around which diverse life forms on our planet thrive and survive. Precious needs have to be protected against the challenge mounted by microbes in day-to-day life as well as professional settings in order to insulate mankind from a multitude of infectious agents. Build up of resistance and development resistant strains continues to challenge preventive healthcare and infection control professionals globally. To overcome these challenges and empower infection control professionals **BioShields** has researched, designed and developed potent, effective and safe disinfectant and antiseptic solutions for medical, industrial and general use. These products have been carefully designed to deliver effective and safe solutions in each product category such as,

- Hand care
- Antiseptics
- Skin preps
- Environment and Surface
- Instruments

Hitmax™ is liquid microbiocidal hand wash soap from BioShields Hand care range. It has a wide application in medical, industry and also in general. It has a broad-spectrum activity and acts as virucidal, bactericidal and fungicidal. It is mildly perfumed and effective cleanser for organic matter with skin emollients, which leaves soft feel.

The microbial population in environment is large and complex. To study the characteristics of one species, that species must be separated from all the other species, i.e. it must be isolated in pure culture. For the cultivation of microorganisms chemically defined media and certain complex raw materials (such as peptone, meat extract, yeast extract etc) are needed. Many special purpose media, bases, supplements, indicators & stains, test kits are needed to facilitate enumeration, isolation and identification of certain types of bacteria. **Microxpress** has come up with Accumix Dehydrated Culture Media, Bases and Supplements to address the needs of professional microbiologists across the full spectrum of Clinical, Analytical, Industrial and Research Laboratories, globally.

Microxpress has some Accumix dehydrated culture media, Ready to use kits and supplements for the isolation and identification of *Listeria monocytogenes*. Accumix *Listeria* Identification Agar Base (known as Polymixin Acriflavin lithium chloride Ceftazidime Esculin Mannitol, PALCAM) is used for the isolation of *Listeria monocytogenes* from food. By using Accumix Fraser Broth Base isolation and enumeration of *Listeria monocytogenes* can be done from foods, environmental specimens and animal feeds. Accumix *Listeria* Selective Supplement is recommended for the selective isolation and identification of *Listeria monocytogenes*. Ready to use *Listeria* identification kit, which comprises of twelve miniature biochemical tests for identification of *Listeria* species.

Highlights of the coming issue

