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Editorial

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We would like to thank all our readers for their precious inputs & encouragement in making this Journal a successful effort. Here's another issue of JHS with loads of valuable information, kindly flip a few pages to believe us…………

Mini Review: Clinical microbiology deals with the prevention, diagnosis and treatment of infectious diseases. The study of characteristics of pathogens, their modes of transmission, mechanisms of infection and growth a treatment can be devised. Microbiological culture is the primary method used for isolating infectious disease for study in the laboratory. Let us see the different Medias used for microbiological testing.

Current Trends: It may not be the most glamorous of winter tasks but cleaning out greenhouses, gutters and water butts is an important one. Cleaning greenhouses, whether glass or plastic, greatly improves the growing environment for plants. By removing the algae, moss and grime it lets in more light and helps control pests and diseases too.

In Profile: We learned so far that many animal and human diseases caused my microbes. But microbes can also cause plant diseases. One of the pioneer of studying the relationships between microbes and plant is Thomas J. Burril, who was probably the first to study the plant disease "Fire-blight".

Bug of the Month: ***Burkholderia pseudomallei*** (also known as ***Pseudomonas pseudomallei***) is a gram-negative, bipolar, aerobic, motile rod-shaped bacterium. It infects humans and animals and causes the disease melioidosis. It is also capable of infecting plants. *B. pseudomallei* is not fastidious and grows on a large variety of culture media (blood agar, MacConkey agar, EMB, etc.). Ashdown's medium (or *Burkholderia cepacia* medium) may be used for selective isolation.

Did You Know? The University of Nottingham has developed a technique to produce chemically functionalized spider silk that can be tailored to applications used in drug delivery, regenerative medicine and wound healing. The chosen molecules can be 'clicked' into place in soluble silk protein before it has been turned into fibres, or after the fibres have been formed. This means that the process can be easily controlled and more than one type of molecule can be used to 'decorate' individual silk strands.

Best Practices: We have seen in the earlier issue, Biomedical waste (BMW) has recently emerged as an issue of major concern not only to hospitals and nursing homes, but also to the environmental and law enforcing agencies, media, and the general public. BMW forms approximately 1%–2% of the total municipal solid waste stream. Health care waste is a heterogeneous mixture, which is very difficult to manage as such. This topic is continued

Don't forget to ease your mind with a light humour in our Relaxed Mood section…….

Clinically Important Culture Media's & their applications

Clinical microbiology deals with the prevention, diagnosis and treatment of infectiousdiseases. The study of characteristics of pathogens, their modes of transmission, mechanisms of infection and growth a treatment can be devised. Infections may be caused by bacteria, viruses, fungi, and parasites. The pathogen that causes the disease may be exogenous (acquired from an external source; environmental, animal or other people) or endogenous (from normal flora).

Microbiological culture is the primary method used for isolating infectious disease for study in the laboratory.Regularly used Medias for microbiological testing are as given below:

1. **Alkaline Peptone Water**

   Alkaline Peptone Water is recommended for enrichment of Vibrio species prior to plating onto a selective medium, such as TCBS Agar.It is useful for preliminarily enrichment of Vibrios from faeces or other contaminated materials.

2. **Blood Agar Base**

   It is recommended as a base to which blood is added for use in the isolation and cultivation of fastidious pathogenic microorganisms like Neisseria, Streptococci etc. Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. It also helps in visualizing the haemolytic reactions. (Alpha, Beta& Gamma). Beta Haemolysis: Complete breakdown of red blood cells. This haemolysis produces a clearing of the blood cells that surrounds the colony.- (S.aureus)

   Alpha Haemolysis: Partial breakdown of red blood cells. Haemolysis produces a greening of the blood cells that surround the colony. - (S.pneumoniae)

   Gamma Haemolysis: Do not haemolyze the red blood cell. - (S. epidermidis).

3. **Brain Heart Infusion Broth**

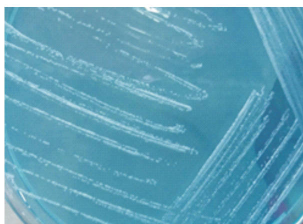
   Useful for cultivating pathogenic cocci and other fastidious organisms associated with blood culture. It is useful for cultivating a wide variety of microorganisms since it is a highly nutritive medium.

4. **CLED Agar with Andrade Indicator**

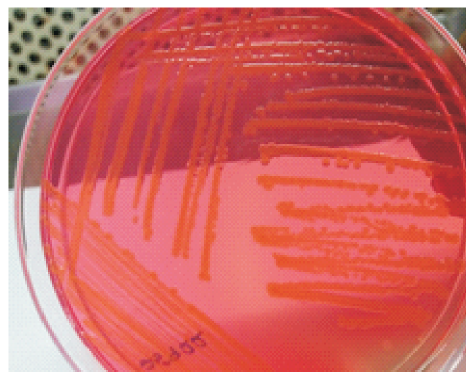
   Used for isolation, enumeration & identification of Urinary Tract Infection. It is a double indicator medium. It gives proper differentiation between lactose and non-lactose fermenter.

   Colour of the medium changes at different pH values which helps in easy identification.

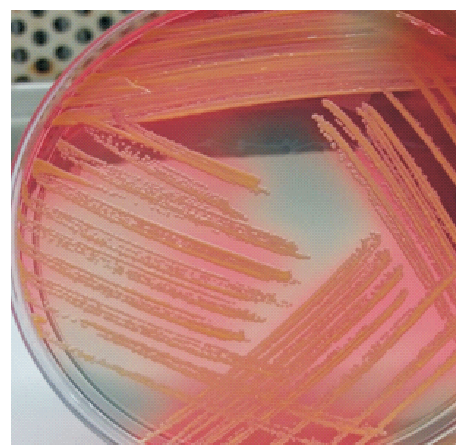
   This medium provides sharper differentiation between lactose-fermenters (LF) and lactose-non-fermenters (NLF). Addition of Andrade's indicator enhances the appearance of colony and aids in the identification of microorganisms.



Candida albicans on CLED Agar



Escherichia coli on CLED Agar



Staphylococcus aureus on CLED Agar

5. **Kligler Iron Agar**

   Used for differentiation, identification of gram negative bacteria on the basis of fermentation of dextrose, lactose & H_2S production. When lactose & dextrose are fermented, acid is produced which reacts with phenol red pH indicator hence changing colour. Acid production is indicated by change of colour from red to yellow. Combination of ferric ammonium citrate & sodium thiosulphate enables detection of hydrogen sulfide production which is indicated by black colour either throughout the butt or in a ring formation near top of the butt.

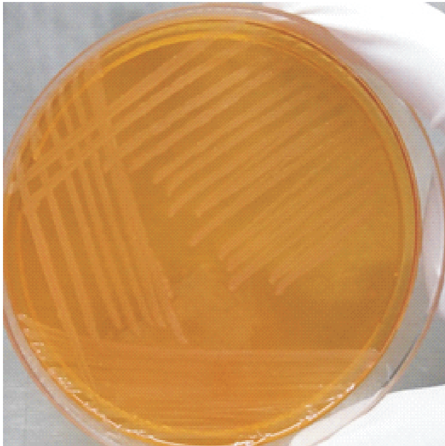
6. **Lowenstein Jensen Medium**

   This is a simple formulated medium which requires supplementation to support growth of Mycobacteria. Glycerol & egg mixture are added, which provides fatty acids & protein for growth of Mycobacterium. Malachite green acts as selective agent preventing the growth of majority of contaminants surviving decontamination of the specimen while encouraging the growth of Mycobacteria.

7. **MacConkey Agar with Crystal Violet, Sodium Chloride (NaCl) and 0.15% Bile Salts**

   It is recommended for the selective isolation and differentiation of coliform organisms and other enteric pathogens based on lactose fermentation. Lactose

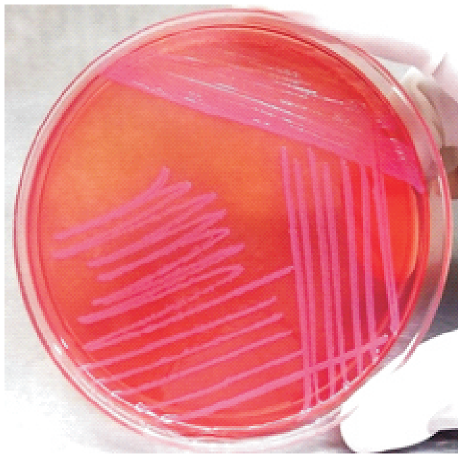
fermenting bacteria forms brick red colonies surrounding bile precipitate zone, this is due to local pH drop around the colony due to lactose fermentation whereas non-lactose fermenting bacteria cannot utilize lactose. They use peptone, which forms ammonia, this raise the pH of the agar so color of the colony appears colourless.



Salmonella Typhimurium on MacConkey Agar

8. MacConkey Agar without Crystal Violet, Sodium Chloride and with 0.5% Sodium Taurocholate

Used to differentiate between lactose and non-lactosecoliform organisms and other enteric pathogens, permitting growth of certain gram positive Enterococci & Staphylococci. Also restricting the swarming of Proteus species from specimen such as urine which may contain large number of Proteus species.



Escherichia coli on MacConkey Agar

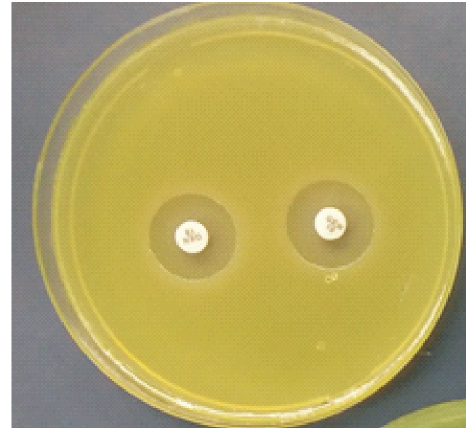
9. MR- VP Medium

This medium is used for differentiation of bacteria by means of methyl red and Voges Proskauer reaction. This medium is also referred as Buffer Peptone Glucose Broth and Glucose Phosphate Broth. Methyl Red positive organisms ferment dextrose and produce high level of acid due to which on addition of methyl red indicator (pH indicator) red colour is formed. In Voges Proskauer, organisms ferment dextrose in small amounts which is converted to acetoin. On addition of Voges Proskauer reagent (Baritt Reagent A&B and Creatine), acetoin gets oxidized and produces red colour. This is positive reaction for Voges Proskauer.

10. Mueller Hinton Agar

It is used for Antibiotic Sensitivity Testing (AST). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard. Mueller Hinton Agar has been selected by the CLSI for several reasons as given below.

I. It results in good batch-to-batch reproducibility.
II. It is low in sulfonamide, trimethoprim, and tetracycline inhibitors.
III. It results in satisfactory growth of most bacterial pathogens.
IV. Large amount of data has been collected concerning susceptibility tests performed with this medium.



Mueller Hinton Agar with antibiotic disc

11. Nutrient Agar

It is a general purpose medium, used for maintaining microorganisms, cultivating fastidious organisms by enriching with blood. It is non-selective media useful in routine cultivation of microorganisms. This medium has very simple formulation which provides nutrients necessary for growth of organisms.

12. Sabouraud Dextrose Agar

It is used for cultivation of pathogenic and non-pathogenic fungi, especially dermatophytes. Dermatophytes are fungi that cause skin, hair, and nail infections.
Low pH of the medium 5.6, helps the growth of fungi and slightly inhibitory to bacteria in clinical specimens.

13. Simmon's Citrate Agar

This media is used for the differentiation between Enterobacteriaceae and the members of aerogenes group on the basis of citrate utilization as sole carbon source.

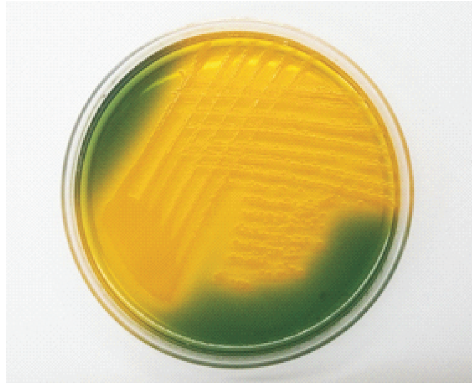
14. Salmonella Shigella Agar

Used as selective and differential media for isolation of enteric bacilli especially Salmonella Species. SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

15. TCBS Agar

It is used for isolation and cultivation of Vibrio's causing Cholera and food poisoning from clinical and non-clinical specimens. TCBS Agar is also recommended by APHA for the selective isolation of V. cholerae and

V.parahaemolyticus. Inhibition of gram-positive bacteria is achieved by the incorporation of oxgall.



Vibrio cholerae – on TCBS Agar

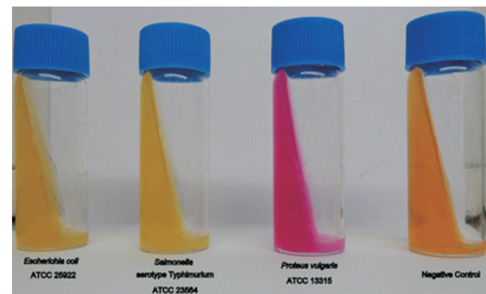
16. Triple Sugar Iron Agar

Used for differentiation of gram-negative enteric bacilli based on dextrose, lactose and sucrose fermentation and hydrogen sulphide production. Depending on variety of acids, the colour of the medium changes from red to yellow. Gas production (CO_2) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. Hydrogen Sulphide (H_2S) production is indicated by blackening in the butt of the tube.

Triple Sugar Iron Agar

17. Urea Agar Base, Christensen

Recommended for the detection of urease production, particularly by members of the genus Proteus.



Urea Agar Base Christensen

Disinfection in agricultural sector (contd.)

Cleaning and Disinfecting the Greenhouse

Clean greenhouse work table with non-porous surface

Hose nozzle hung in greenhouse

First Steps to a Clean Greenhouse:

If you have had re-occurring problems with diseases such as Pythium root rot or insects such as fungus gnats, perhaps your greenhouse and potting areas need a good cleaning. Over the course of growing a crop, infectious microbes accumulate and algae flourish on moist surfaces harboring fungus gnats and shore flies.

Attention to greenhouse sanitation and disinfecting are steps that growers can between crop cycles. Some growers wait until the week before opening a greenhouse before cleaning debris from the previous growing season. It is better to clean as early as possible to eliminate over-wintering sites for pests to reduce their populations prior to the spring growing season. Pests are much easier to prevent than to cure.

Although disinfecting should be done routinely, timing does not always permit this extra effort. Take the opportunity to thoroughly clean greenhouses between crop cycles when greenhouses are totally empty.

Benefits to Disinfecting the Greenhouse

Many pathogens can be managed to some degree, by the use of disinfectants. For example, dust particles from fallen growing medium or pots can contain bacteria or fungi such as Rhizoctonia or Pythium . Disinfectants will help control these pathogens. In addition to plant pathogens, some disinfectants are also labeled for managing algae which is a breeding ground for fungus gnats and shore flies.

Managing Algae

Algae are a diverse grouping of plants that occur in a wide range of environments. Algae growth on walks, water pipes, equipment, greenhouse coverings, on or under benches and in pots is an ongoing problem for growers. Algae form an impermeable layer on the media surface that prevents wetting of the media and can clog irrigation and misting lines, and emitters. It is a food source for insect pests like shore flies, and causes slippery walkways that can be a liability risk for workers and customers. Recent studies have shown that algae are brought into the greenhouse through water supplies and from peat in the growing media. Once in a warm, moist environment with fertilizer, the algae flourish.

Proper water management and fertilizing can help to slow algae growth. Avoid over-watering slow-growing plants and especially crops early in the production cycle. Allow the surface of the media to dry out between watering.

Avoid excessive fertilizer runoff and puddling water on floors, benches, and greenhouse surfaces. The greenhouse floor should be level and drain properly to prevent the pooling of water prior to installing a physical weed mat barrier.

Algae management involves an integrated approach involving sanitation, environmental modification and frequent use of disinfectants.

Irrigation water can also be a source for pathogens and algae. For information on water treatment technologies for control of algae see the Water Education Alliance for Horticulture: http://www.watereducationalliance.org/

Greenhouse Benches and Work Tables

If possible, use benches made of wire that can be easily disinfected. Wood benches can be a source for root rot diseases and insect infestations. Algae tend to grow on the surface of the wood creating an ideal environment for fungus gnats and shore flies, and plant pathogens can grow within the wood. Plants rooting through containers into the wood will develop root rot if conditions are favorable for pathogen activity. Disinfect benches between crop cycles with one of the labeled products listed below. Keep in mind that disinfectants are not protectants. They may eradicate certain pathogens, but will have little residual activity.

Bench tops and work tables should be made of a non-porous surface such as a laminate that can be easily disinfected. Avoid using bare wood for these tasks.

Cleaning Containers

Plant pathogens such as Pythium, Rhizoctonia and Thielaviopsis can survive in root debris or soil particles on greenhouse surfaces. If a crop had a disease problem, then avoid re-using containers. It is also a good idea to avoid planting crops that are prone to Thielaviopsis problems, such as pansies, in containers that have been previously used. Research has shown that Thielaviopsis spores are capable of surviving on recycled plug trays and infecting new crops.

Containers to be reused should be washed thoroughly to remove soil particles and plant debris before being treated with a disinfectant, even if there is no evidence of disease in the crop. Debris and organic matter can protect pathogens from coming in contact with the disinfectant solution.

Disinfectants for Greenhouses

There are several different types of disinfectants that are currently used in the greenhouse for plant pathogen and algae control. They are quaternary ammonium compounds, hydrogen dioxide, hydrogen peroxide &peroxyacetic acid, hydrogen peroxide, peroxyacetic acid and octanoic acid, odium carbonate

peroxyhydrate (GreenClean Pro Granular Algicide) and chlorine bleach. Alcohol, although not used as a general disinfectant is mentioned here because it is used by growers to disinfect propagation tools. All these products have different properties. If possible, disinfectants should be used on a routine basis both as part of a pre-crop clean-up program and during the cropping cycle.

Quaternary ammonium chloride salts. Q-salt products, commonly used by growers are quite stable and work well when used according to label instructions. Q-salts are labeled for fungal, bacterial and viral plant pathogens, and algae. They can be applied to floors, walls, benches, tools, pots and flats as disinfectants. Instructions recommend that surfaces be air-dried after treatment except for cutting tools. The label recommends soaking cutting tools for 10 minutes before use, then using the wet tool on plants. One way to do this is by having two cutting tools, one pair to use while the other is soaking. Some have higher organic tolerances and longer residual activity on hard surfaces.

Q-salts are not protectants. They may eradicate certain pathogens, but will have little residual activity. Contact with any type of organic matter will inactivate them. Therefore, pre-clean objects to dislodge organic matter prior to application. Because it is difficult to tell when they become inactive, prepare fresh solutions frequently (twice a day if in constant use). The products tend to foam a bit when they are active. When foaming stops, it is a sign they are no longer effective. No rinsing with water is needed.

Hydrogen Dioxide and Peroxyacetic Acid Hydrogen dioxide kills bacteria, fungus, algae and their spores immediately on contact. It is labeled as a disinfectant for use on greenhouse surfaces, equipment, benches, pots, trays and tools, and for use on plants. Label recommendations state that all surfaces should be wetted thoroughly before treatment. Several precautions are noted. Hydrogen dioxide has strong oxidizing action and should not be mixed with any other pesticides or fertilizers. When applied directly to plants, phytotoxicity may occur for some crops, especially if applied above labelled rates or if plants are under stress. Hydrogen dioxide can be applied through an irrigation system. As a concentrate it is corrosive and causes eye and skin damage or irritation. Product containing this molecule is a strong oxidizing agent used as an algaecide on greenhouse structures and floor and is labelled for use in chemigation.

Sodium Carbonate Peroxyhydrate is a granular and activated with water. Upon activation, sodium carbonate peroxyhydrate breaks down into sodium carbonate and hydrogen peroxide. Green Clean is labelled for managing algae in any non-food water or surfaces. Non-target plants suffer contact burn if undiluted granules are accidentally spilled on them.

Chlorine bleach. There are more stable products than bleach to use for disinfecting greenhouse surfaces. Chlorine bleach may be used for pots or flats, but is not recommended for application to walls, benches or flooring. When used properly, chlorine is an effective disinfectant and has been used for many years by growers. A solution of chlorine bleach and water is short-lived and the half-life (time required for 50 percent reduction in strength) of a chlorine solution is only two hours. After two hours, only one-half as much chlorine is present as was present at first. After four hours, only one-fourth is there, and so on. To ensure the effectiveness of chlorine solutions, it should be prepared fresh just before each use. The concentration normally used is one part of household bleach (5.25 percent sodium hypochlorite) to nine parts of water, giving a final strength of 0.5 percent. Chlorine is corrosive. Repeated use of chlorine solutions may be harmful to plastics or metals. Objects to be sanitized with chlorine require 30 minutes of soaking and then should be rinsed with water. Some would say that rinsing is not necessary. Bleach should be used in a well-ventilated area. It should also be noted that bleach is phytotoxic to some plants, such as poinsettias.

Alcohol (70 percent) is a very effective sanitizer that acts almost immediately upon contact. It is not practical as a soaking material because of its flammability. However, it can be used as a dip or swipe treatment on knives or cutting tools. No rinsing with water is needed.

Disinfectants should be used on a routine basis both as part of a pre-crop clean up program and during the cropping cycle.

Good Agricultural Practices (GAP) Farm Food Safety: Using Chlorine Sanitizers in Produce Wash Tanks

The use of diluted chlorine bleach solutions in fresh produce wash water is a common and inexpensive practice among small scale growers and packers. To use this sanitizer safely and effectively, it's important to understand why we should add a sanitizer to wash water, the different kinds of chlorine sanitizers available, and what factors influence their effectiveness.

Why add a sanitizer to produce wash water?

It's important to understand that if produce becomes contaminated with harmful microorganisms in the field, we can never assume that sanitizer wash treatments can make the product completely safe to eat. The few cells that survive may still be enough to make someone sick. This is why it is critically important to follow Good Agricultural Principles (GAP) that prevent contamination from occurring. Then why should we add a sanitizer to wash water? One reason is to make sure that the water source cannot be a source of contamination. Another reason is to prevent the transfer of harmful microbes present on a few fruits or vegetables to an entire produce lot, therefore magnifying a single isolated contamination incident into one that could affect many people. A continuous presence of a sanitizer also prevents small amounts of harmful microbes from growing in the wash water or on equipment surfaces and eventually getting on the product.

What do we mean by the term "chlorine sanitizer"?

When we discuss chlorine sanitizers we usually are referring to a class of antimicrobial chemicals known as "hypochlorites." There are two common forms of hypochlorite disinfectants.

Sodium hypochlorite. You may recognize this form as common liquid bleach used to whiten or disinfect clothes in the laundry. Bleach can be purchased with varying concentrations of this active ingredient written on the label, usually between 5 and 12% sodium hypochlorite. Concentrated sodium hypochlorite solutions are sold as a highly alkaline product so that it remains stable over time. Therefore, they are very caustic and should be handled with care to avoid splashing that can damage the skin and eyes.

Calcium hypochlorite. This form of hypochlorite is sold as a dry granular material. It is used most commonly in the home as a disinfectant for swimming pools. For water disinfection, it is available as pellets than slowly dissolve under a stream of water. Because calcium hypochlorite, it is in a non-liquid form, it is easier to measure out and therefore less hazardous to use. All sanitizers are regulated by the U.S. Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA). It is a violation of federal law to use ordinary liquid bleach or dry powders that are not specifically labeled for disinfection purposes. Make sure the label of the container indicates that it is approved for microbial disinfection.

References:

http://agriculture.vermont.gov/sites/ag/files/pdf/consumer_protection/Chlorine_Sanitization_Produce_Wash_Water.pdf
https://ag.umass.edu/greenhouse-floriculture/fact-sheets/cleaning-disinfecting-greenhouse

Thomas Burrill

We learned so far that many animal and human diseases caused my microbes. But microbes can also cause plant diseases. One of the pioneer of studying the relationships between microbes and plant is Thomas J. Burril, who was probably the first to study the plant disease "Fire-blight". He represents the American pioneer spirit of the time. His life story is impressive and much worth for us to learn.

Thomas was born on April 25, 1939, near Pittsfield, Massachusetts. His father was John Burrill and mother Mary Francis. John Burrill was a native of Penrith, England, came to the United States in 1818, settling first in Pawtucket, Rhode Islands, where he worked in Slater's cotton mill. The family was influnced by the tide of westward emigration at the time, and moved to Stephenson County in northern Illinois when Thomas was only nine years of age (1848). They settled on a farm. Thomas helped his father on the farm and attended country school a few months each winter. At the age of nineteen, he entered high school in Freeport. Because he was a painfully and self-consciously boy, he felt misery in school and quitted. Next year, he tried again in the Rockford high school, where he graduated and entered the Illinois State Normal University at the age of twenty-three (1862) and graduated in 1865.

Thomas was a hard learning student. At the Illinois State Normal University, he came in contact with good teachers. His teacher of botany was Dr. J. A. Sewall, Curator of the Museum of the State Natural History Society located in Normal. Thomas was inspired by him greatly. Because he attended the annual meetings of the Society of Natural History held in nearby in Bloomington, Thomas was further stimulated to his love for natural history.

His career was a rather lucky one. During 1865 to 1868, Thomas was appointed the position of Superintendent of the Urbana public schools. But he spent a lot of his spare time to study the flora of the Urbana area. In the summer of 1867, he was selected as a botanist to accompany Major John W. Powell to explore the Grand Canyon of the Colorado and surrounding regions. Next year in the spring of 1868, he was called to the Illinois Industrial University (Now the University of Illinois in Champaign/ Urbana) in Urbana to teach algebra. He was made Assistant Professor of Natural History and Botany later. Two years later, 1870, he was promoted to the professorship of Botany and Horticulture. Academic advancement at those days was much easier and quicker than now. He then became established for a life of active and influential service on the faculty of the University of Illinois. He was professor of Botany and Horticulture in 1870 to 1903, and professor of Botany in 1903-1912. He took charge of the university library as its first librarian and was secretary of the faculty in 1970 to 1883. He was dean of College of Science from 1878-1884.; dean of general faculty, 1894-1901; vice president of the university, 1879-1912; botanist of the agricultural experiment station, 1888-1912. Upon his retirement in 1912, he was made professor emeritus until his death in1916.

Thomas Burrill began his academic career by teaching. He taught most of the day in many subjects. He was a horticulturist to the experiment station. He planted with his own hands most of the trees on the campus of the University of Illinois. He had a extensive love for trees. He was frequently heard to say, "Were I a heathen, not knowing the true God, I would not worship the sun,----but would bow in adoration to the trees and herbs of the fields. " In an Oriental old saying " It takes tenths of years to plant trees; it takes hundredth years to nourish human talents." He indeed planted the trees and also nourished human talents.

He was a hard working man. His worked from sun-up to midnight or until things should be done at that day. He was very much interested with the natural history of the State of Illinois. In 1869, he organized a group including himself, collected a large number of natural history specimens from Chicago to Cairo in the State of Illinois. He brought back specimens including 582 plants for preservation and study. In 1875, he made a collection of the woods of the state for the Centennial Exposition. In addition to teaching, he wrote reports, collected specimen from everywhere in the State of Illinois, served on numerous committees, nourished young scientists and did experiments. His administrative skill also greatly contributed to the growth and development of the University of Illinois. With little remnant of his time, he was charged by the Board with the sales of mules. He lived a strenuous life indeed.

The most significant contribution of Burrill to science is in the field of Phytopathology, in which he is indeed a pioneer. Burrill became interested in the disease of pear-blight. He noticed that might be a bacterial disease. Although the bacterial disease in animals is common, bacterial disease in plant is never heard. Burrill presented his preliminary study on this malady in State Horticultural Society in 1878. He described the organism seen in the tissue. He transmitted the disease by transferring the exudation from the diseased plant to bark of healthy trees. Finally he conclusively proved that bacteria caused disease in plants and identified the organism as *Micrococcus amylovorus.* These results were published in the Reports of Illinois Industrial University in 1882 and also in the American Naturalists in 1883.

Although his findings were accepted generally by American botanists, but were doubted by his contemporary in Europe. Through much effort, his theory was proved to be totally correct.

He continued to study the bacterial diseases of sorghum and brown corn in 1886 and 1888. In 1885 to 1891, he became active in taxonomic work and planed to publish a volume on the

Cryptogenic flora of Illinois. Unfortunately he did not finish his publications, because he was appointed as acting president for the University; the administrative duty diverted much of his time. But instead, he published two monographs: one was on the rusts, the other on the mildews of Illinois. Both of them were valuable mycological literature. From, 1902, he carried on extensive investigation on the bitter-rot of apple, which caused a great damage in Southern Illinois. He also engaged in the study of ear-rots of corn, potato scab, rust of blackberry, and raspberry, and peach "yellow." All of this work, owned him as a pioneer in the field of phytopathology. Professor Burrill was devoted to agriculture for the benefit of better living in the state of Illinois. His contribution to the Illinois College of Agriculture is immensely.

Professor Burrill was granted the degree of A.M. by the Northwestern University in 1876. In 1881, the University of Chicago conferred the honorary degree of Ph.D. upon him. The University of Northwestern University also granted him the honorary degree of LL.D. degree in 1893. In 1912, just before his formal retirement, the University of Illinois conferred him the honorary degree of LL.D. There were many honors he received from the various scientific societies of which he was a member. He was elected President of the National Bacteriological Association in 1916. He was member of the American Association for the Advancement of Science and elected Vice-President in 1885. He was President of the American Microscopy Society, 1885-1903, and secretary, 1886-1889. He was also a member of Botanical Society of America, International Botanical Society, American Society of Naturalists, American Academy of Arts and Sciences, Illinois Academy of Science,and the University Club, Urbana, Illinios.

Professor Burrill's public service is also very impressive. In 1889, he was invited as one of three Commissioners by the U.S. Department of Agriculture to settle a controversy concerning a prevalent bacterial disease of pigs. In 1895, he was appointed by Governor John P. Altgeld of Illinois to investigate and report on the subject of tuberculosis in the state prisons.

After his retirement, he became engaged in active research on symbiosis between nodule bacteria and legiminous plants. He carried this research till the end of his life in 1916.

One more thing worthy of mention, Professor Burrill was among the first American botanists to introduce and use the compound microscope in the biological laboratory. He was also the first to organize a bacteriological laboratory in an American State University; and one of the two or three first American writers on plant diseases.

Professor Burrill was a generous and sincerely religious man. He was an ideal companion and a resourceful person, and always was capable of seeing the amusing side of a situation. And his life is full of vigorous activities. He enjoyed his study of plant life, horticulture and forestry. He also enjoyed amateur photography and camp life. He laid the foundation of phytopathogy, which extends the horizon of the contribution of microbiology. He published more than 100 research papers on scientific and educational subjects.

Burrill married Sarah Helen Alexander, daughter of Ephraim Alexander of Schenectadys, New York, on July 22, 1868 at Urbana, Illinois. Two children were born to them. He died at Urbana, Illinois, April 14, 1916.

WILLIAM SHAKESPHERE

Three sentences for getting SUCCESS:

a) know more than other
b) work more than other
c) expect less than other

BONNIE BLAIR

Winning doesn't always mean being first , winning means you're doing better than you've done before -

THOMAS EDISON

I will not say I failed 1000 times , I will say that I discovered there are 1000 ways that can cause failure - -

LEO TOLSTOY

Everyone thinks of changing the world, but no one thinks of changing himself...

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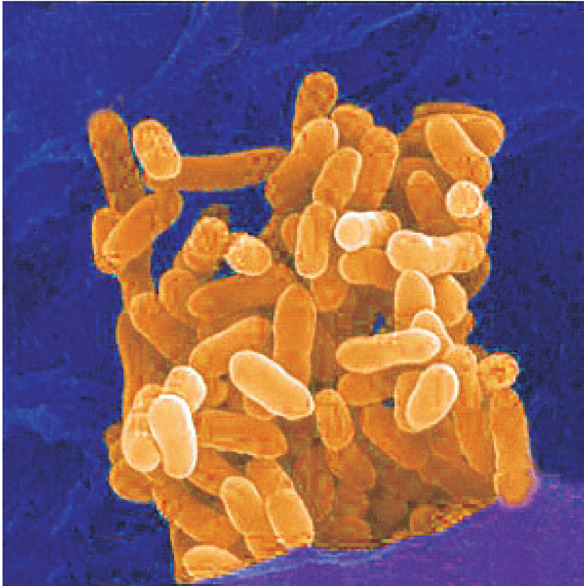
Burkholderia pseudomallei



Domain:	Bacteria
Phylum:	Proteobacteria
Class:	Betaproteobacteria
Order:	Burkholderiales
Family:	Burkholderiaceae
Genus:	*Burkholderia*
Species:	***B. mallei***

Burkholderia pseudomallei (also known as ***Pseudomonas pseudomallei***) is a gram-negative, bipolar, aerobic, motile rod-shaped bacterium. It is a soil-dwelling bacterium endemic in tropical and subtropical regions worldwide, particularly in Thailand and northern Australia. It infects humans and animals and causes the disease melioidosis. It is also capable of infecting plants.

B. pseudomallei measures 2–5 μm in length and 0.4–0.8 μm in diameter and is capable of self-propulsion using flagella. The bacteria can grow in a number of artificial nutrient environments, especially betaine- and arginine-containing ones.

In vitro, optimal proliferation temperature is reported around 40°C in neutral or slightly acidic environments (pH 6.8–7.0). The majority of strains are capable of fermentation of sugars without gas formation (most importantly, glucose and galactose; older cultures are reported to also metabolize maltose and starch). Bacteria produce both exo- and endotoxins. The role of the toxins identified in the process of melioidosis symptom development has not been fully elucidated.

Identification

B. pseudomallei is not fastidious and grows on a large variety of culture media (blood agar, MacConkey agar, EMB, etc.). Ashdown's medium (or *Burkholderia cepacia* medium) may be used for selective isolation. Cultures typically become positive in 24 to 48 hours (this rapid growth rate differentiates the organism from *B. mallei*, which typically takes a minimum of 72 hours to grow). Colonies are wrinkled, have a metallic appearance, and possess an earthy odour. On Gram staining, the organism is a Gram-negative rod with a characteristic "safety pin" appearance (bipolar staining). On sensitivity testing, the organism appears highly resistant (it is innately resistant to a large number of antibiotics including colistin and gentamicin) and that again differentiates it from *B. mallei*, which is in contrast, exquisitely sensitive to a large number of antibiotics. For environmental specimens only, differentiation from the nonpathogenic *B. thailandensis* using an arabinose test is necessary (*B. thailandensis* is never isolated from clinical specimens). The laboratory identification of *B. pseudomallei* has been described in the literature.

The classic textbook description of *B. pseudomallei* in clinical samples is of an intracellular, bipolar-staining, Gram-negative rod, but this is of little value in identifying the organism from clinical samples. Some suggest the Wayson stain is useful for this purpose, but this has been shown not to be the case.

Laboratory identification of *B. pseudomallei* can be difficult, especially in Western countries where it is rarely seen. The large, wrinkled colonies look like environmental contaminants, so are often discarded as being of no clinical significance. Colony morphology is very variable and a single strain may display multiple colony types, so inexperienced laboratory staff may mistakenly believe the growth is not pure. The organism grows more slowly than other bacteria that may be present in clinical specimens, and in specimens from nonsterile sites, is easily overgrown. Nonsterile specimens should, therefore, be cultured in selective media (e.g., Ashdown's or *B. cepacia* medium). For heavily contaminated samples, such as faeces, a modified version of Ashdown's that includes norfloxacin, amoxicillin, and polymyxin B has been proposed.

Even when the isolate is recognised to be significant, commonly used identification systems may misidentify the organism as *Chromobacterium violaceum* or other nonfermenting, Gram-negative bacilli such as *Burkholderia cepacia* or *Pseudomonas aeruginosa*. Again, because the disease is rarely seen in Western countries, identification of *B. pseudomallei* in cultures may not actually trigger alarms in physicians unfamiliar with the disease.

The pattern of resistance to antimicrobials is distinctive, and helps to differentiate the organism from *P. aeruginosa*. The majority of *B. pseudomallei* isolates are intrinsically resistant to all aminoglycosides (via an efflux pump mechanism), but sensitive to co-amoxiclav: this pattern of resistance almost never occurs in *P. aeruginosa* and is helpful in identification. Unfortunately, the majority of strains in Sarawak, Borneo, are susceptible to aminoglycosides and macrolides, which means the conventional recommendations for isolation and identification do not apply there.

Molecular methods (PCR) of diagnosis are possible, but not routinely available for clinical diagnosis. Fluorescence *in situ* hybridisation has also been described, but has not been clinically validated, and it is not commercially available.[In Thailand, a latex agglutination assay is widely used, while a rapid

immunofluorescence technique is also available in a small number of centres.

Disinfection

B. pseudomallei is susceptible to numerous disinfectants, including benzalkonium chloride, iodine, mercuric chloride, potassium permanganate, 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and to a lesser extent, phenolic preparations.[1] *B. pseudomallei* is effectively killed by the commercial disinfectants, Perasafe and Virkon. The microorganism can also be destroyed by heating to above 74 °C for 10 min or by ultraviolet irradiation. *B. pseudomallei* is not reliably disinfected by chlorine.

Medical importance

B. pseudomallei infection in humans is called melioidosis; its mortality is 20 to 50% even with treatment.

Antibiotic treatment and sensitivity testing

The antibiotic of choice is ceftazidime. While various antibiotics are active *in vitro* (e.g., chloramphenicol, doxycycline, co-trimoxazole), they have been proven to be inferior *in vivo* for the treatment of acute melioidosis. Disc diffusion tests are unreliable when looking for co-trimoxazole resistance in *B. pseudomallei* (they greatly overestimate resistance) and Etests or agar dilution tests should be used in preference. The actions of co-trimoxazole and doxycycline are antagonistic, which suggests these two drugs ought not to be used together.

The organism is intrinsically resistant to gentamicin and colistin, and this fact is helpful in the identification of the organism. Kanamycin is used to kill *B. pseudomallei* in the laboratory, but the concentrations used are much higher than those achievable in humans.

Pathogenicity mechanisms and virulence factors

B. pseudomallei is an opportunistic pathogen. An environmental organism, it has no requirement to pass through an animal host to replicate. From the point of view of the bacterium, human infection is a developmental "dead end". Strains which cause disease in humans differ from those causing disease in other animals, by possessing certain genomic islands. It may have the ability to cause disease in humans because of DNA it has acquired from other microorganisms. Its mutation rate is also high, and the organism continues to evolve even after infecting a host. *B. pseudomallei* is able to invade cells (it is an intracellular pathogen). It is able to polymerise actin, and to spread from cell to cell, causing cell fusion and the formation of multinucleated giant cells. It possesses a uniquely fusogenic type-6 secretion system that is required for cell-cell spread and virulence in mammalian hosts. The bacterium also expresses a toxin called lethal factor 1. *B. pseudomallei* is one of the first Proteobacteria to be identified as containing an active type-6 secretion system. it is also the only organism identified that contains up to six different type-6 secretion systems.

B. pseudomallei is intrinsically resistant to a large number of antimicrobial agents by virtue of its efflux pump mechanism. This mediates resistance to aminoglycosides (*AmrAB-OprA*), tetracyclines, fluoroquinolones, and macrolides (*BpeAB-OprB*).

Vaccine candidates

No vaccine is currently available, but a number of vaccine candidates have been suggested. Aspartate-β-semialdehyde dehydrogenase (*asd*) gene deletion mutants are auxotrophic for diaminopimelate (DAP) in rich media and auxotrophic for DAP, lysine, methionine, and threonine in minimal media. The Δ*asd* bacterium (bacterium with the *asd* gene removed) protects against inhalational melioidosis in mice.

Development of antibiotic synthetic spider silk

Development of antibiotic synthetic spider silk

After five years' work an interdisciplinary team of scientists at The University of Nottingham has developed a technique to produce chemically functionalized spider silk that can be tailored to applications used in drug delivery, regenerative medicine and wound healing.

The Nottingham research team has shown for the first time how 'click-chemistry' can be used to attach molecules, such as antibiotics or fluorescent dyes, to artificially produced spider silk synthesized by E.coli bacteria. The research, funded by the Biotechnology and Biological Sciences Research Council (BBSRC) is published today in the online journal *Advanced Materials*.

The chosen molecules can be 'clicked' into place in soluble silk protein before it has been turned into fibres, or after the fibres have been formed. This means that the process can be easily controlled and more than one type of molecule can be used to 'decorate' individual silk strands.

Nottingham breakthrough

In a laboratory in the Centre of Biomolecular Sciences, Professor Neil Thomas from the School of Chemistry in collaboration with Dr Sara Goodacre from the School of Life Sciences, has led a team of BBSRC DTP-funded PhD students starting with David Harvey who was then joined by Victor Tudorica, Leah Ashley and Tom Coekin. They have developed and diversified this new approach to functionalising 'recombinant' -- artificial -- spider silk with a wide range of small molecules.

They have shown that when these 'silk' fibres are 'decorated' with the antibiotic levofloxacin it is slowly released from the silk, retaining its anti-bacterial activity for at least five days.

Neil Thomas, a Professor of Medicinal and Biological Chemistry, said: "Our technique allows the rapid generation of biocompatible, mono or multi-functionalized silk structures for use in a wide range of applications. These will be particularly useful in the fields of tissue engineering and biomedicine."

Remarkable qualities of spider silk

Spider silk is strong, biocompatible and biodegradable. It is a protein-based material that does not appear to cause a strong immune, allergic or inflammatory reaction. With the recent development of recombinant spider silk, the race has been on to find ways of harnessing its remarkable qualities.

The Nottingham research team has shown that their technique can be used to create a biodegradable mesh which can do two jobs at once. It can replace the extra cellular matrix that our own cells generate, to accelerate growth of the new tissue. It can also be used for the slow release of antibiotics.

Professor Thomas said: "There is the possibility of using the silk in advanced dressings for the treatment of slow-healing wounds such as diabetic ulcers. Using our technique infection could be prevented over weeks or months by the controlled release of antibiotics. At the same time tissue regeneration is accelerated by silk fibres functioning as a temporary scaffold before being biodegraded."

The medicinal properties of spider silk recognized for centuries.

The medicinal properties of spider silk have been recognized for centuries but not clearly understood. The Greeks and Romans treated wounded soldiers with spider webs to stop bleeding. It is said that soldiers would use a combination of honey and vinegar to clean deep wounds and then cover the whole thing with balled-up spider webs.

The idea came together at a discipline bridging university 'sandpit' meeting five years ago. Dr Goodacre says her chance meeting at that event with Professor Thomas proved to be one of the most productive afternoons of her career.

Dr Goodacre, who heads up the SpiderLab in the School of Life Sciences, said: "I got up at that meeting and showed the audience a picture of some spider silk. I said 'I want to understand how this silk works, and then make some.'

"At the end of the session Neil came up to me and said 'I think my group could make that.' He also suggested that there might be more interesting 'tweaks' one could make so that the silk could be 'decorated' with different, useful, compounds either permanently or which could be released over time due to a change in the acidity of the environment."

The approach required the production of the silk proteins in a bacterium where an amino acid not normally found in proteins was included. This amino acid contained an azide group which is widely used in 'click' reactions that only occur at that position in the protein. It was an approach that no-one had used before with spider silk -- but the big question was -- would it work?

Dr Goodacre said: "It was the start of a fascinating adventure that saw a postdoc undertake a very preliminary study to construct the synthetic silks. He was a former SpiderLab PhD student who had previously worked with our tarantulas. Thanks to his ground work we showed we could produce the silk proteins in bacteria. We were then joined by David Harvey, a new PhD student, who not only made the silk fibres, incorporating the unusual amino acid, but also decorated it and demonstrated its antibiotic activity. He has since extended those first ideas far beyond what we had thought might be possible."

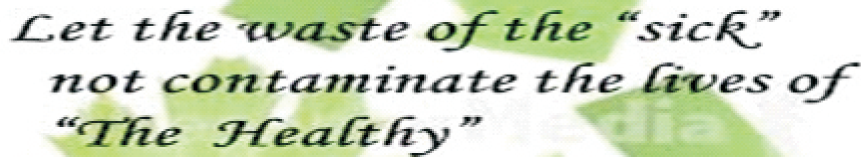
David Harvey's work is described in this paper but Professor Thomas and Dr Goodacre say this is just the start. There are other joint SpiderLab/Thomas lab students working on uses for this technology in the hope of developing it further.

David Harvey, the lead author on this their first paper, has just been awarded his PhD and is now a postdoctoral researcher on a BBSRC follow-on grant so is still at the heart of the research. His current work is focused on driving the functionalized spider silk technology towards commercial application in wound healing and tissue regeneration.

Where will we be in 5 years' time?

Dr Goodacre said: "It is likely that this paper is just the start of a very exciting range of studies using the new spider silk material. Some of the future work will also be supported by other, neat ideas from the world of spiders and their silk, which the SpiderLab is currently trying to unravel."

Biomedical Waste Management (part 2)



Disposal Procedures for Infectious Liquid Waste:

Sanitary Sewer Disposal Methods

The sanitary sewer system is designed for the disposal of certain liquid wastes. Use of the sanitary sewer reduces the chance for leaks or spills during transport and thereby reduces disposal costs. Chemical disinfection is done prior to sewer disposal with the aim to eliminate micro-organisms or to reduce the microbial load. Chemical treatment usually involves the use of 1% sodium hypochlorite solution with a minimum contact period of 30 min or other standard disinfectants like, 10-14 gm of bleaching powder in 1 litre water, 70% ethanol, 4% formaldehyde, 70% isopropyl alcohol, 2.5% povidone iodine, or 6% hydrogen peroxide.

Disinfection of culture media differs a little from the usual disinfection process, where due to the high microbial load and the rich protein content of the media plates, rigorous disinfection is required, where inactivation should be done by 5.23% sodium hypochlorite, in a 1:10 dilution and should be left for a minimum of 8 h covered and then finally disposed down the sanitary sewer, followed by flushing with a lot of cold water for a minimum period of 10 min.

Sodium hypochlorite solution, also known as bleach, is a broad-spectrum disinfectant that is effective for enveloped viruses (HIV, HBV, HSV), vegetative bacteria (Pseudomonas, Staphylococcus, and *Salmonella*), fungi (e.g., Candida), mycobacterium (M. tuberculosis and M. bovis), and non-enveloped viruses (Adenovirus and Parvovirus), should be stored between 50 and 70°F. Undiluted household bleach has a shelf life of 6 months to 1 year from the date of manufacture, after which it undergoes degradation at a rate of 20% per year until a total degradation to salt and water. Though a 1:10 concentration of bleach solution has a shelf life of 24 h only, some manufacturer prepared 1:10 bleach solutions, contain a stabilizer that increases the shelf life to approximately 18 months.

Hypochlorite (1%) is inefficient in decontaminating blood containing hypodermic needles.

Infectious biomedical waste and sharps have a potential hazard of transmission of pathogens. Among sharps, used needles form a major share and disinfection by 1 % hypochlorite is recommended in biomedical waste management rules of India. The aim of the present study was to evaluate the efficacy of hypochlorite for the decontamination of needles. Needles (16 g) filled with suspensions of standard strains and clinical isolates of gram positive and gram negative bacteria in plain normal saline and in human blood containing anticoagulant, were exposed to 1% hypochlorite and the surviving bacteria were subjected to viable counts. The observations indicated that 85 - 90 % of the needles filled with bacterial suspensions in saline are disinfected to a level of >5 log bacterial reduction (standard disinfection) on exposure to hypochlorite but only 15 to 30% needles contaminated with the challenge bacteria suspended in blood showed >5 log reduction in viable counts. Thus, hypochlorite treatment is inadequate for disinfecting needles contaminated with pathogenic bacteria in presence of blood and should not be recommended as an option for disinfection of the needles.

Medical waste generated in hospital can be a serious health hazard for the patients in the hospital, hospital staff and if reaches to the community, can become a community health hazard. In order to control this biohazard MOEF (Ministry of Environment and Forests) enacted the Biomedical waste rules (BMW) 1998. The sharps and specifically needles have been given special emphasis since needle stick injuries during handling or medical procedures have the potential to transmit blood borne infections. The diseases that can be transmitted include AIDS, hepatitis B, hepatitis C, malaria and many other bacterial and viral diseases. The sharps are to be disposed in special coloured puncture proof containers. The treatment options suggested by BMW rules are chemical disinfection with 1% hypochlorite or autoclaving or microwaving. The needles are to be mutilated after treatment. The rules also recommend monitoring of chemical disinfection from time to time. However, there is no data available on monitoring of chemical disinfection of needles.

Hypodermic needles range in length from 1 to 6 inches and thickness of 24 to 16 g are used for a number of clinical procedures in a hospital setup. In case of hypodermic needles which are attached to plastic or rubber tubing, the usual practice in Indian setups is to just cut the needle portion and immerse the needles in 1% sodium hypochlorite. The other needles after use are either directly added to hypochlorite containers or mutilated by cutting part of the needle mechanically in needle cutters or by burning the tip portions in electric needle destroyers before immersing in hypochlorite solutions. The studies with bacterial challenges to test the efficacy of chlorine releasing solutions are numerous but chlorine solutions used have been in the range of (0.0003 to 0.25%). The only studywith organic soiling used albumin (1%) and plasma (10 to 50%) which rendered 0.25%

hypochlorite totally ineffective. Further, in a study blood was used as organic material using *Staphylococcus aureus* as the challenge organism and quantitative suspension test was performed using 1% hypochlorite. To the best of our knowledge, studies on efficacy of hypochlorite have been undertaken for conditions simulating spillage but not for needle decontamination. The penetration of hypochlorite into the narrow lumen of needles, especially those filled with blood and body fluids, remains doubtful. Surprisingly scientific data to prove the efficacy of 1% hypochlorite for decontamination of hypodermic needles is not available. Hence, this study was designed to determine the efficacy of 1% sodium hypochlorite for disinfection of contaminated hypodermic needles with or without blood as organic soil.

Materials and methods

Sodium hypochlorite

Sodium hypochlorite having an initial Cl2 concentration of 10% (checked by chlorinometer, Qualigens, India) was used to prepare in-use solution of 1% sodium hypochlorite.

Bacterial cultures

The standard strains of *S.aureus* NCTC 6538 and *E.coli* NCTC 10418 which were procured from Haffkine Institute for Training, Testing and Research, Mumbai, were used for the experiments. Isolates of *Staphylococcus aureus*, coagulase negative staphylococci, *E.coli*, *Klebsiella* species and *Pseudomonas aeruginosa* were obtained from clinical samples of patients.

Bacterial inoculum

Two sets of inoculum were used for the study:

a) Plain inoculum-Bacterial growth from fresh overnight grown nutrient agar slants were harvested in sterile normal saline to match the turbidity of 0.5 McFarland standard (1 x 108 CFU/mL). Suspension was vortexed to prevent clumping of bacterial cells.
b) Blood inoculum-CPDA (Citrate Phosphate Dextrose and Adenine) anticoagulated blood from blood bank was used for preparing the inoculum. Equal volumes of plain inoculum and blood were mixed to prepare the inoculum.

New sterile hypodermic needles (16 g) from blood donor set were used in the study for the experimental ease of flushing the needles and sterile 1% sodium thiosulphate solution was used after exposure of needles to neutralize residual chlorine present.

Procedure

Sterile hypodermic needles were filled with inoculum (plain or in blood) by aspiration with syringe. The needles were separated from the syringe and immersed in 1% sodium hypochlorite solution (300 mL) for 30 minutes. The needles were picked up with sterile forceps and 1ml sodium thiosulphate (1%) was passed through the needles with a sterile syringe and collected in sterile tubes. Thereafter, dilutions were made in sterile normal saline. The undiluted and serial dilutions were further subjected to viable bacterial count by plating over nutrient agar in duplicate. The plates were incubated at 37^0C for 48 hours. Ten needles filled with plain inoculum of bacteria were exposed to hypochlorite solution in each experiment and a second set of ten needles filled with inoculum in blood were exposed similarly. As a control, three needles in each experimental set were filled up with plain and blood containing bacterial inoculum and immersed in normal saline and processed like exposed test needles. The experiments were repeated second time using identical number of needles for test and control.

Statistical analysis

Post- hypochlorite exposure reduction in CFU count for the bacterial suspension in plain normal saline versus bacterial suspension in blood were compared by Wilcoxon sign rank test.

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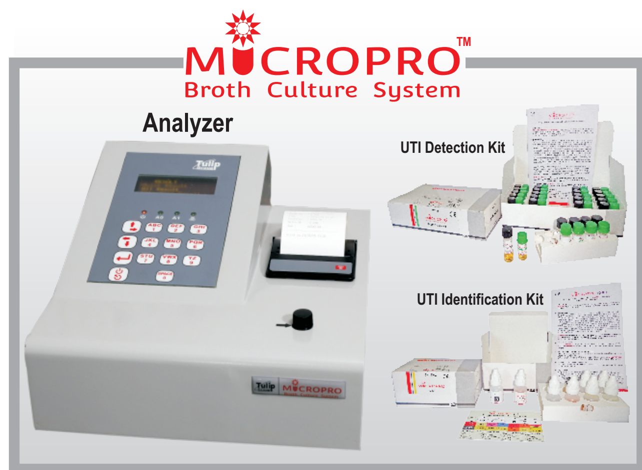

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USAGE DIRECTIONS :

● Pre & post-surgery skin cleaning & antisepsis : Use undiluted ● Surgical, post operative, non surgical dressing : Use undiluted, once day/alternate ● Antisepsis during minor incisions, scopy, catheterization, first aid, cuts, bites, stings etc : Use undiluted ● Chronic wound management (diabetic foot, pressure and arterial/ venous leg ulcers) : Use undiluted ● First aid : Use undiluted

**AMPs- Antimicrobial Peptides

***BI-Biocompatibility Index measures an antiseptic agent's antimicrobial activity in relation to its cytotoxicity

Not recommended for infants below 9 months except on medical advice.

Highlights of the coming issue

JOURNAL OF HYGIENE SCIENCES

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