

Editorial

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A quick glance at this issue of **The Journal of Hygiene Sciences** shows our continued commitment to publish a journal of high standard which is devoted exclusively to the topics of Microbiology & Disinfection.

Mini Review Section - Enteric bacteria are bacteria of the intestines can be represented as the *Gut flora*, which are always present and usually harmless; conversely the other group is the *Pathogenic bacteria* of bacterial gastroenteritis (infectious diarrhea). Many members of this family are a normal part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. *Escherichia coli* is one of the most important model organisms, and its genetics and biochemistry have been closely studied.

Current Trends Section – It has been known for many years that alcohol is an effective disinfectant; studies have shown that it is a better disinfectant when it is neither too weak nor too concentrated. Disinfectant efficacy is optimal at concentrations between 50% and 80%. Many institutions use 70% alcohol blends as standard.

Alcohol is suitable as a combined cleaning and disinfection agent provided no proteins are present, as alcohol will fix these to a surface by a process of protein denaturing. In this instance a separate cleaning agent should be used.

In Profile – Jayaraman Gowrishankar (born 1956) is an Indian medical microbiologist. He was awarded in 1991, the Shanti Swarup Bhatnagar Prize for Science and Technology, the highest science award in India, in the Biological sciences category. He was awarded in 2013, the Padma Shri, India's fourth highest civilian honour, for his contribution towards the field of science.

Bug of the Month - Burkholderia cepacia complex is a group of Gram-negative, non-spore forming bacilli composed of approximately 17 closely-related species which are grouped into nine genomovars. This is often referred to as the *Burkholderia cepacia* complex (BCC). It was originally recognized by W. H. Burkholder in the 1950s for the distinct stench it caused in onion bulbs, known as "sour skin" disease for its vinegar-like odor.

Did You Know? – More than 415 million people worldwide are living with diabetes, and frequently need to inject themselves with insulin to manage their blood sugars. Human cells can be genetically engineered into living factories that efficiently manufacture and deliver hormones and signaling molecules, but most synthetic biological circuits don't offer the same degree of sensitivity and precision as digital sensors.

Best Practices - Although endoscopic equipment has been implicated in transmitting infection, it appears as if virtually all transmissions have been due to errors in the process of cleaning and disinfecting the equipment or in breakdown of general infection control practices with the exception of newer duodenoscopes. This topic review will discuss infectious agents that can potentially be transmitted during gastrointestinal endoscopy and outline the recommendations from various societies for the cleaning and disinfection of gastrointestinal endoscopes.

So let's go on & explore the information.....

Identification and Isolation of Enteric Pathogens

Identification and description

Enteric bacteria are bacteria of the intestine scan be represented as the *Gut flora*, which are always present and usually harmless; conversely the other group is the *Pathogenic bacteria* of bacterial gastroenteritis (infectious diarrhea). Many members of this family are a normal part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. *Escherichia coli* is one of the most important model organisms, and its genetics and biochemistry have been closely studied.

The taxonomic family Enterobacteriaceae

The Enterobacteriaceae are a large family of Gram-negative bacteria that includes, along with many harmless symbionts, many of the more familiar pathogens, such as *Escherichia coli*, *Salmonella*, *Klebsiella*, *Yersinia pestis*, and *Shigella*. Other disease-causing bacteria in this family include *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter*. This family is the only representative in the order Enterobacteriales of the class Gammaproteobacteria in the phylum Proteobacteria. Members of the Enterobacteriaceae can be trivially referred to as enterobacteria or "enteric bacteria", as several members live in the intestines of



Figure. *Citrobacterfreundii*, one member of the family

animals. In fact, the etymology of the family is enterobacterium with the suffix to designate a family (aceae)—not after the genus *Enterobacter* (which would be "Enterobacteraceae")—and the type genus is *Escherichia*.

Characteristics:

Members of the Enterobacteriaceae are large, rod-shaped, and are typically 1–5 μm in length. They appear as small grey colonies on blood agar. Like other proteobacteria, enterobacteria have Gram-negative stains, and they are facultative anaerobes, grow rapidly in aerobic as well as anerobic conditions, fermenting sugars to produce lactic acid and various other end products. Most also reduce nitrate to nitrite, although exceptions exist (e.g. *Photorhabdus*). Unlike most similar bacteria, enterobacteria generally lack cytochrome C oxidase, although there are exceptions (e.g. *Plesiomonasshigelloides*). Most have many flagella used to move about, but a few genera are non-motile. They are not spore-forming. Catalase reactions vary among Enterobacteriaceae.

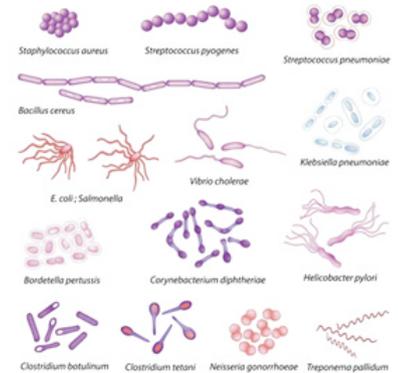
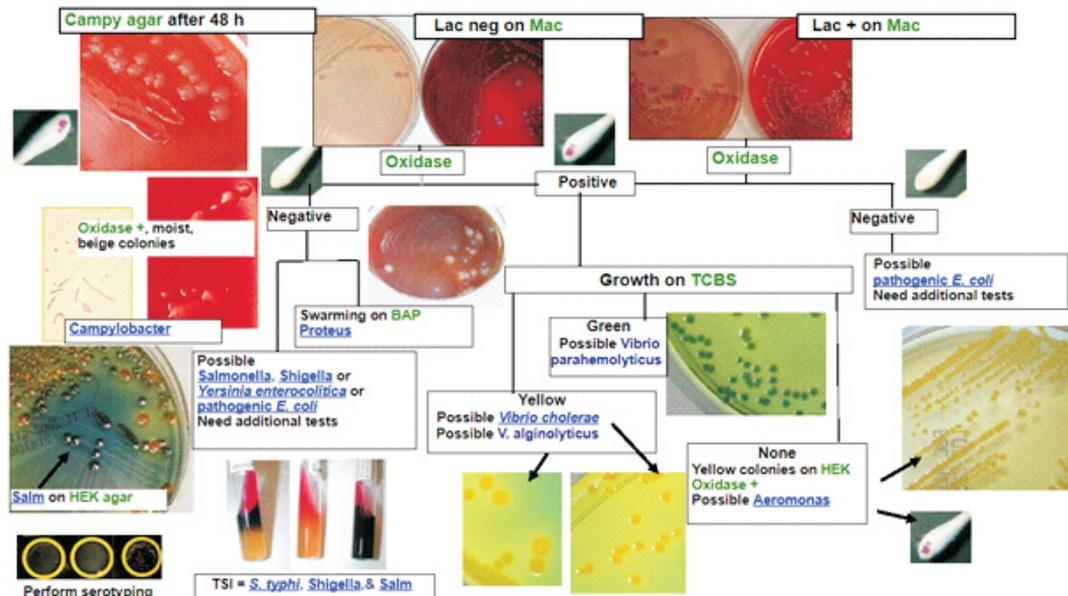


Figure: Illustration of the shape and appearance of different Enteric bacteria

Flow chart for identification of enteric fecal pathogens



Most members of Enterobacteriaceae have peritrichous, type I fimbriae involved in the adhesion of the bacterial cells to their hosts. Some enterobacteria produce endotoxins. Endotoxins reside in the cell wall and are released when the cell dies and the cell wall disintegrates. Some members of the Enterobacteriaceae produce endotoxins that, when released into the bloodstream following cell lysis, cause a systemic inflammatory and

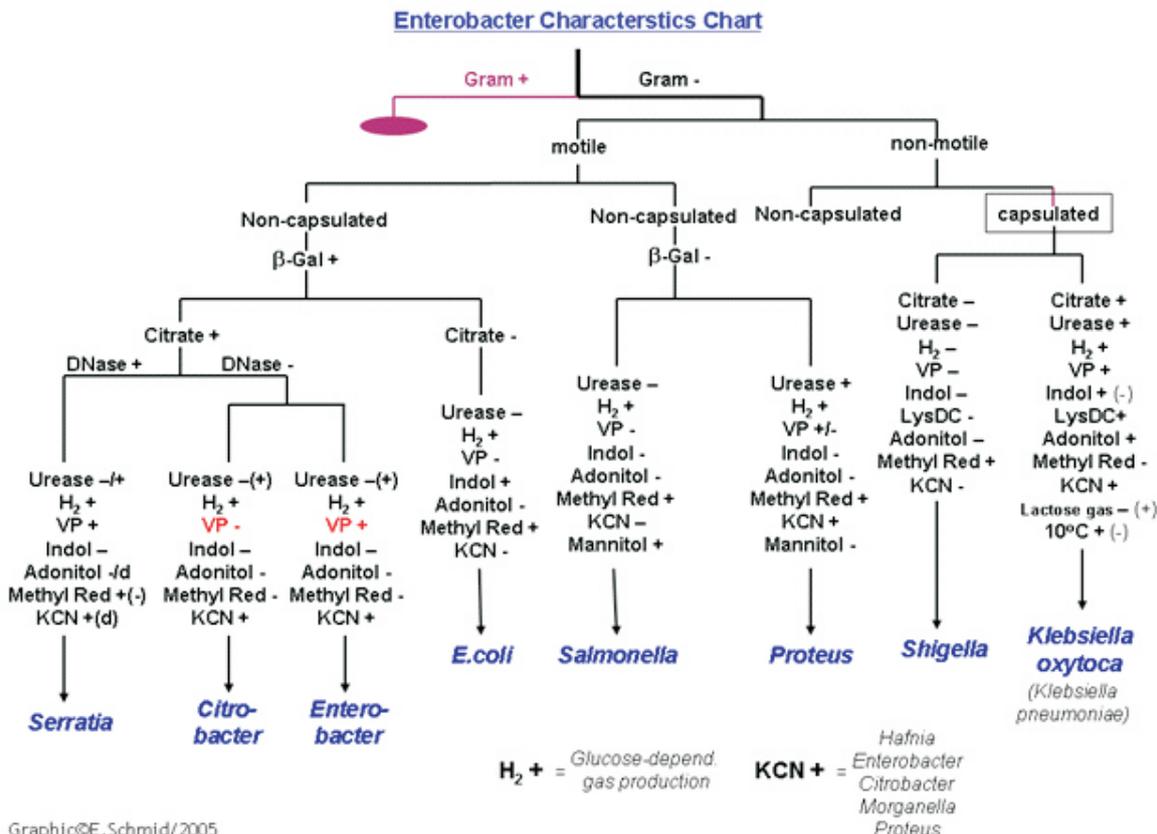
vasodilatory response. The most severe form of this is known as endotoxic shock, which can be rapidly fatal.

Identification: To identify different genera of Enterobacteriaceae, a series of tests are carried out in the laboratory which include:

- i. Phenol red test
- ii. Tryptone broth test

- iii. *Phenylalanine agar* for detection of production of deaminase, which converts phenylalanine to phenylpyruvic acid
- iv. *Methyl red* or *Voges-Proskauer* tests depend on the digestion of glucose. The methyl red tests for acid end-products. The *Voges Proskauer* tests for the production of acetylmethylcarbinol.

- v. *Catalase* test on the nutrient agar, tests the production of catalase enzyme, which splits hydrogen peroxide and releases oxygen gas.
- vi. *Oxidase* test on the nutrient agar, tests for the production of the enzyme oxidase, which reacts with an aromatic amine to produce a purple color.
- vii. *Nutrientgelatin* tests to detect activity of the enzyme gelatinase.



Graphic©E.Schmid/2005

In a clinical setting, three species make up 80 to 95% of all isolates identified. These are *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.

Antibiotic resistance: Several Enterobacteriaceae strains have been isolated which are resistant to antibiotics including carbapenems, which are often claimed as "the last line of antibiotic defense" against resistant organisms. For instance, some *Klebsiella pneumoniae* strains are carbapenem resistant.

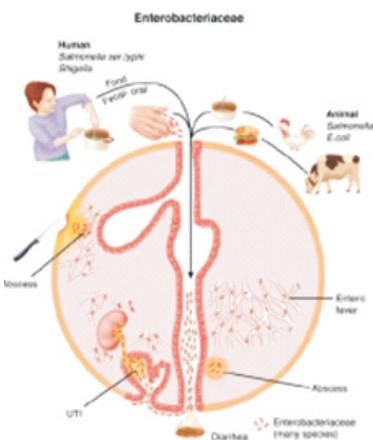
What type of disease do Enterobacteriaceae produce?

Two categories based on the type of diseases caused:

A. **Opportunistic Infection** in normally sterile environments such as Urinary tract infection, wounds, soft tissue infection, pneumonia and meningitis

B. **Diarrhea**

Diarrhea defined as unusual frequency of bowel action, with the passage of loose, unformed faeces. It may be associated with symptoms such as abdominal cramps, nausea and malaise,



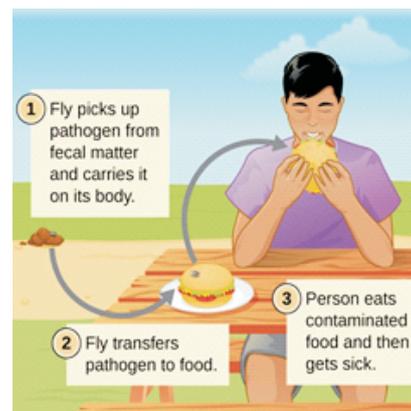
and with vomiting, fever and consequent dehydration. Patients with visible blood and mucus in the faeces, suggesting inflammation of the bowel, accompanied by symptoms such as abdominal cramps and constitutional disturbance, may be said to be suffering from dysentery. Diarrhea maybe of 3 types:

1. **Dysentery:**

Inflammatory diarrhea with WBCs, and/or blood in stool that is caused by invasive and cytotoxic strains.

2. **Watery Diarrhea:** Dominated by fluid loss, this is caused by enterotoxins.

3. **Enteric Fever:** Febrile, the GI tract is simply the port of entry for a systemic disease.



Outbreaks

Epidemics may occur as a result of person to person spread of infection, through ingestion of infected food and water and from

direct contact with animals. Food borne outbreaks are defined as 2 or more cases of a similar illness resulting from the ingestion of a common food and currently are estimated to cause 3 million deaths worldwide per year. A water borne outbreak is defined as 2 or more cases of a similar illness resulting from the drinking of water from a common source or contact with water used for recreational purposes from a collective source. Water borne outbreaks are estimated to cause the death of 2 million children annually as a consequence of diarrheal disease.

Isolation of Enteric Pathogens

National Standard Method (NSM) outlines the bacteria responsible for enteric infection and methods for their isolation. It recommends routine screening of faeces for *Campylobacter*, *Salmonella*, *Shigella* and *Escherichia coli* O1572 on all diarrheal (semi-formed or liquid) faeces obtained from the clinically susceptible patients. However, consideration should be given to the "three day rule" for collection of faeces samples from hospitalized patients (see section below "Collection of faeces samples"). In addition faeces may be screened for individual organisms as indicated by clinical details.

Faecal parasites are a common cause of diarrhea in immunosuppressed or immunocompromised individuals. Infection with the common gastroenteritis viruses may be prolonged in patients who are immunocompromised; this can have important infection control implications. The possibility of herpesvirus infections should also be considered in certain groups. Cytomegalovirus (CMV) colitis may be a cause of diarrhea in some transplant patients and CMV may cause exacerbations of symptoms in chronic inflammatory bowel conditions such as Crohn's disease and ulcerative colitis.

Mycobacterium tuberculosis and *Mycobacterium avium-intracellulare* (MAI) have been isolated from faeces in immune-compromised patients. The isolation procedure is unreliable, has a low success rate. Both these organisms may be isolated from blood cultures in disseminated infection.

Collection of faeces samples

Some clinicians advocate the use of a "3 day rule". This rule is derived from the low numbers of faecal pathogens isolated from patients who have been hospitalized for longer than 3 days.

It suggests that faecal samples from these patients should not be cultured except under the following circumstances:

- those in-patients suffering diarrhea within three days of admission
- adults with nosocomial diarrhea only if one of the following is applicable: (a) aged 65 or more with pre-existing disease causing permanently altered organ function. (b) patients who are HIV positive. (c) patients with neutropenia. (d) suspected nosocomial outbreak.
- those with suspected non-diarrheal manifestations of enteric infections

Conformity to this "three day rule" relies on appropriate clinical information accompanying the specimen.

Bacteria commonly associated with gastrointestinal infections

Campylobacter, *Helicobacter* and *Arcobacter* species, *Salmonella*, *Shigella*, *Escherichia coli*, *Vibrio* species

TECHNICAL INFORMATION/LIMITATIONS

A study showed no significant differences in the isolation rates of *Salmonella* species or *Campylobacter* species when faecal samples were plated directly or when diluted prior to inoculation to culture media: dilution significantly reduces the amount of competing flora without compromising low numbers of

pathogens⁷⁶. It was also shown that there were fewer subcultures for *Campylobacter* species when using a dilute inoculum, thus reducing labor costs.

A study in 2002 comparing xylose lysine desoxycholate (XLD), desoxycholate (DCA), α - β chromogenic medium (ABC) and mannitol lysine crystal violet brilliant green agar (MLCB) found that XLD plus MLCB is the optimal combination when employing direct plating. MLCB was shown to be the best, single direct plating medium for non-*typhisalmonellae*, whereas XLD remains the most effective for routine diagnostic work.

The rate of isolation of *Campylobacter* species is higher and the growth of competing flora is less when an incubation temperature of 42°C is used in preference to 37°C. However, recovery of organisms such as *Arcobacter* species and *H. cinaedi* may be compromised.

There are various technical problems associated with recovery of this diverse group of bacteria from samples of faeces:

- Organisms may be sensitive to selective agents incorporated into *campylobacter* agars (e.g. *C. upsaliensis*, *C. hyointestinalis* and *H. fennelliae* are sensitive to cephalothin)
- *Arcobacter* species, and *H. cinaedi* may not grow at 42°C
- *C. hyointestinalis* may require a hydrogen tension greater than that regularly supplied by commercially-available micro-aerobic atmosphere generating kits.

Overall, the contribution to human disease in the UK provoked by this grouping of bacteria is believed to be small. For this reason the incubation temperature, choice of selective agars etc. recommended in standard methods are primarily aimed at detecting *C. jejuni*, *C. coli* and *C. lari*.

The results of a study of the performance of lactose and mannitol selenite broths as enrichment media when plated on XLD Agar and DCA (Deoxycholate Citrate Agar) for the isolation of *Salmonella* species has led to the proposal that routine diagnostic laboratories subculture mannitol selenite broths to XLD.

Toxin detection is considered to be adequate for investigation of sporadic cases of *C. difficile*. However, in outbreaks when epidemiological studies are required, toxin detection plus isolation of *C. difficile* is the optimal approach. If the workload is too heavy for the laboratory to perform both tests, then the toxin tests should be performed and a faeces specimen should be stored at 4°C or -20°C for later culturing and typing of isolates.

References:

- (1) List of genera included in families - Enterobacteriaceae". List of Prokaryotic Names with Standing in Nomenclature. Retrieved 26 June 2016.
- (2) Don J. Brenner; Noel R. Krieg; James T. Staley (July 26, 2005) [1984 (Williams & Wilkins)]. George M. Garrity, ed. The Gammaproteobacteria. Bergey's Manual of Systematic Bacteriology. 2B (2nd ed.). New York: Springer. p. 1108. ISBN 978-0-387-24144-9. British Library no. GBA561951.
- (3) http://inst.bact.wisc.edu/inst/index.php?module=book&type=user&func=displayarticle&art_id=268.
- (4) Dorlands Medical Dictionary: Enterobacteriaceae.
- (5) MacFaddin, Jean F. Biochemical Tests for Identification of Medical Bacteria. Williams & Wilkins, 1980, p 441.
- (6) Centers for Disease Control and Prevention.
- (7) Snyderman DR. Food poisoning. In: Gorbach SL, Bartlett JG, Blacklow NR, editors. Infectious Diseases. 2nd ed. Philadelphia: WB Saunders Company; 1998. p. 768-81.
- (8) Swaminathan B, Beebe J, Besser J. Investigation of foodborne and waterborne disease outbreaks. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH, editors. Manual of Clinical Microbiology. 7th ed. Washington D.C.: American Society for Microbiology; 1999. p. 174-90.

Alcohols in Disinfection



They are capable of dissolving lipids, which makes them effective against lipid-wrapped viral cells such as HIV and hepatitis A. They are inexpensive and relatively easy to handle, although their vapors are flammable.

ISOPROPYL ALCOHOL

Isopropyl alcohol, often called IPA or Isopropanol, is similar in function and structure to ethanol. It evaporates at a similar rate and destroys bacterial and viral cells by the same mechanism. However, it is not as effective at dehydrating living tissue and so is a better solution for disinfecting skin than ethanol.



Ethanol dehydrates cells more readily.

Ethanol may be used in purely surface-cleaning applications, but isopropyl alcohol can also double as antiseptic and is often used in hospitals.

Alcohol such as ethanol and isopropanol is a prevalent component of these substances which are widely used particularly for disinfecting hands and other parts of the skin, as well as surfaces and surgical tools. The great advantage of an alcohol as major disinfecting substance is to be seen in its instantaneous activity against microorganisms so that surfaces treated with an alcohol as disinfectant can be further used after a only a short period of time.

Another advantage of alcohol based disinfectants is the residue-free evaporation of the active ingredient which minimizes any subsequent contact with potentially harmful remainders on the treated surface and also renders superfluous any subsequent rinsing with water or the like.

A mixture comprising at least 70 wt % of ethanol or methanol and 1-10 wt % of glycerol. The mixture is said to be effective against naked viruses. Many institutions use 70% alcohol blends as standard.

Alcohol has the tendency of drying-out human skin which in particular after applying an alcohol based disinfectant for a

number of times in a certain time period tends to develop cracks, which may provide shelter for the microorganisms to be decimated, and in the worst case even provide a gateway for microorganisms into the body, resulting in an infection.

Alcohol is suitable as a combined cleaning and disinfection agent provided no proteins are present, as alcohol will fix these to a surface by a process of protein denaturing. In this instance a separate cleaning agent should be used.

The alcohol used can be either IPA or denatured ethanol. There is very little difference in efficacy between the two alcohols – the results are almost identical. The choice is reduced to other factors. IPA dries more readily but has a strong acrid smell and a lower occupational exposure limit than denatured ethanol. However, denatured ethanol has a sweeter smell that some users may find unpleasant.

Mode of Action:

Protein denaturation also is consistent with observations that alcohol destroys the dehydrogenases of *Escherichia coli*, and that ethyl alcohol increases the lag phase of *Enterobacter aerogenes* and that the lag phase effect could be reversed by adding certain amino acids. The bacteriostatic action was believed caused by inhibition of the production of metabolites essential for rapid cell division.

Microbicidal Activity:

Methyl alcohol (methanol) has the weakest bactericidal action of the alcohols and thus seldom is used in healthcare. The bactericidal activity of various concentrations of ethyl alcohol (ethanol) was examined against a variety of microorganisms in exposure periods ranging from 10 seconds to 1 hour. *Pseudomonas aeruginosa* was killed in 10 seconds by all concentrations of ethanol from 30% to 100% (v/v), and *Serratiamarcescens*, *E. coli* and *Salmonella typhosa* were killed in 10 seconds by all concentrations of ethanol from 40% to 100%. The gram-positive organisms *Staphylococcus aureus* and *Streptococcus pyogenes* were slightly more resistant, being killed in 10 seconds by ethyl alcohol concentrations of 60%–95%. Isopropyl alcohol (isopropanol) was slightly more bactericidal than ethyl alcohol for *E. coli* and *S. aureus*.

Ethyl alcohol, at concentrations of 60%–80%, is a potent virucidal agent inactivating all of the lipophilic viruses (e.g., herpes, vaccinia, and influenza virus) and many hydrophilic viruses (e.g., adenovirus, enterovirus, rhinovirus, and rotaviruses but not hepatitis A virus (HAV) or poliovirus). Isopropyl alcohol is not active against the non-lipid enteroviruses but is fully active against the lipid viruses. Studies also have demonstrated the ability of ethyl and isopropyl alcohol to inactivate the hepatitis B virus (HBV) and the herpes virus, and ethyl alcohol to inactivate human immunodeficiency virus (HIV), rotavirus, echovirus, and astrovirus.

In tests of the effect of ethyl alcohol against *M. tuberculosis*, 95% ethanol killed the tubercle bacilli in sputum or water suspension within 15 seconds. In 1964, Spaulding stated that alcohols were the germicide of choice for tuberculocidal activity, and they should be the standard by which all other tuberculocides are compared. For example, he compared the tuberculocidal activity of iodophor (450 ppm), a substituted phenol (3%), and isopropanol (70% volume) using the mucin-loop test

(10^6 *M. tuberculosis* per loop) and determined the contact times needed for complete destruction were 120–180 minutes, 45–60 minutes, and 5 minutes, respectively. The mucin-loop test is a severe test developed to produce long survival times. Thus, these figures should not be extrapolated to the exposure times needed when these germicides are used on medical or surgical material.

Ethyl alcohol (70%) was the most effective concentration for killing the tissue phase of *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum* and the culture phases of the latter three organisms aerosolized onto various surfaces. The culture phase was more resistant to the action of ethyl alcohol and required about 20 minutes to disinfect the contaminated surface, compared with <1 minute for the tissue phase.

Isopropyl alcohol (20%) is effective in killing the cysts of *Acanthamoeba culbertsoni* (560) as are chlorhexidine, hydrogen peroxide, and thimerosal.

Uses:

Alcohols are not recommended for sterilizing medical and surgical materials principally because they lack sporicidal action and they cannot penetrate protein-rich materials. Fatal postoperative wound infections with *Clostridium* have occurred when alcohols were used to sterilize surgical instruments contaminated with bacterial spores. Alcohols have been used effectively to disinfect oral and rectal thermometers, hospital pagers, scissors, and stethoscopes. Alcohols have been used to disinfect fiber optic endoscopes but failure of this disinfectant have lead to infection. Alcohol towelettes have been used for years to disinfect small surfaces such as rubber stoppers of multiple-dose medication vials or vaccine bottles. Furthermore, alcohol occasionally is used to disinfect external surfaces of equipment (e.g., stethoscopes, ventilators, manual ventilation bags), CPR manikins, ultrasound instruments or medication preparation areas. Two studies demonstrated the effectiveness of 70% isopropyl alcohol to disinfect reusable transducer heads in a controlled environment. In contrast, three bloodstream infection outbreaks have been described when alcohol was used to disinfect transducer heads in an intensive-care setting.

The documented shortcomings of alcohols on equipment are that they damage the shellac mountings of lensed instruments, tend to swell and harden rubber and certain plastic tubing after prolonged and repeated use, bleach rubber and plastic tiles and damage tonometer tips (by deterioration of the glue) after the equivalent of 1 working year of routine use. Tonometer biprisms soaked in alcohol for 4 days developed rough front surfaces that potentially could cause corneal damage; this appeared to be caused by weakening of the cementing substances used to fabricate the biprisms. Corneal opacification has been reported when tonometer tips were swabbed with alcohol immediately before measurement of intraocular pressure. Alcohols are flammable and consequently must be stored in a cool, well-ventilated area. They also evaporate rapidly, making extended exposure time difficult to achieve unless the items are immersed.

Cetyl alcohol is used in the cosmetic industry as an opacifier in

shampoos, or as an emollient, emulsifier or thickening agent in the manufacture of skin creams and lotions. It is also employed as a lubricant for nuts and bolts, and is the active ingredient in some "liquid pool covers" (forming a surface layer to reduce evaporation and retain heat).



People who suffer from eczema can be sensitive to cetyl alcohol, though this may be due to impurities rather than cetyl alcohol itself. However cetyl alcohol *is* sometimes included in medications used for the treatment of eczema.

Antiviral activity of alcohol for surface disinfection

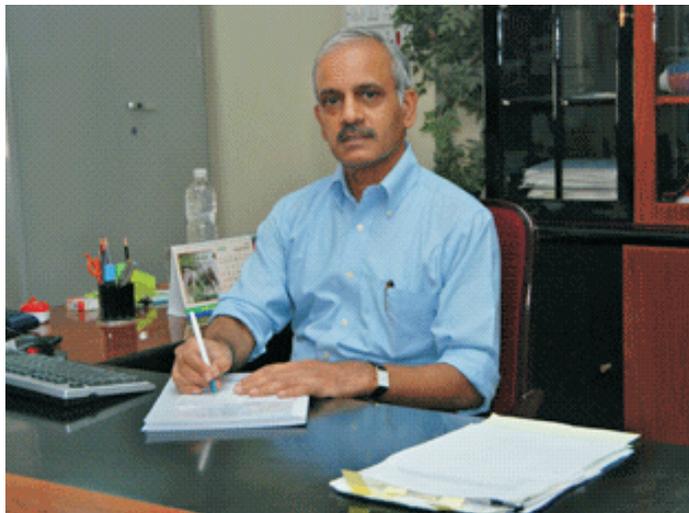
Abstract: Bacteria and viruses from the patient's mouth travel with dental splatter and spills. A surface disinfectant should possess antiviral activity as well as antibacterial action. Because of frequent and 'open' application in the dental, such a disinfectant should be non-toxic, non-allergenic and safe for the hygienist. It now appears that high-concentration alcohol mixtures (i.e. 80% ethanol, 5% isopropanol) are not only excellent antibacterials, but quickly inactivate HIV as well as hepatitis B and hepatitis C viruses. Compared to alternative surface disinfectants, use of high-concentration alcohol for the spray-wipe-spray method of surface disinfection in dentistry appears safe. However, dried matter should be wiped and hydrated.

During patient treatment, droplets and smears containing saliva and blood contaminate the very surroundings. Diluted by spray and cooling water from the dental unit, these so called 'dental spills and splatter' are not only found on gloves, garments and instruments, but do reach virtually any surface, utensil, handle, switch and button around. Bacteria and viruses from the patient travel along with these dental spills. While protective gloves, masks and garments are changed and instruments sterilised, bacteria and viruses remain on those surfaces, utensils, etc. that cannot or are not changed or removed for sterilisation or disinfection. Therefore, an in situ disinfection between patient remains a very important cross-infection control measure. A disinfectant for this purpose should not only possess antibacterial, but, very importantly, should also possess antiviral activity. It should be effective, but most importantly, safe for frequent application by the dental team and dental hygienist.

References:

- <http://www.nzhealth.co.nz/knowledge/alcohol-as-disinfectants>
- <https://www.google.com/patents/US20150201621>
- <https://www.cemag.us/article/2008/06/when-use-alcohol-disinfectant>
- http://onlinelibrary.wiley.com/doi/10.1034/j.1601-5037.2003.00032.x/epdf?r3_referer=wol&tracking_action=preview_click&show_checkout=1&purchase_referrer=www.readcube.com&purchase_site_license=LICENSE_DENIED
- http://www.osap.org/?FAQ_Instrum_Disinf1
- <https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html>

Jayaraman Gowrishankar



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Washington, DC--June 6, 2012 The 2012 Moselio Schaechter Distinguished Service Award will be presented to Jayaraman Gowrishankar, Ph.D., Director, Centre for DNA Fingerprinting and Diagnostics (CDFD), and leader of the Laboratory of Bacterial Genetics, CDFD, Hyderabad, India. This award, named in honor of Professor Moselio Schaechter, former ASM President, honors an ASM member who has shown exemplary leadership and commitment towards the substantial furthering of the profession of microbiology in research, education or technology in the developing world. Gowrishankar is honored for "his dual responsibilities as the Director of the DNA diagnostics facility as well as the leader of a team of scientists, and also for his direct contributions to basic microbiology," states his nominator, Laszlo Csonka, Purdue University.

Gowrishankar received his undergraduate degree in medicine at the Christian Medical College in Vellore, South India. In 1979, he took a step back from a future career as a physician and followed a love of basic research. He pursued graduate studies in bacterial genetics under Jim Pittard at the University of Melbourne, Australia. He received his Ph.D in 1983, and returned to India to begin his independent research career as a Scientist and Group Leader at the Centre for Cellular and Molecular Biology in Hyderabad. In 2000, Gowrishankar moved to the CDFD, where he has been Director for the last six years and head of the Laboratory of Bacterial Genetics.

Research in Gowrishankar's laboratory has covered different aspects of the physiology and genetics of *Escherichia coli*, including osmoregulation, the occurrence of spontaneous mutations in non-dividing cells, arginine export, replication fork

progression, and factor-dependent transcription termination. With about fifty papers published in the peer-reviewed journals (most with no more than two co-authors), he has been one of the most successful *E. coli* labs outside of the developed world. In addition, several of his students have gone on subsequently to establish their own independent research programs in microbiology. His group was also the first in India to patent and earn royalties from a genetically modified organism, in the form of an engineered *E. coli* strain for salt-induced overproduction of recombinant proteins.

Gowrishankar is also known for his many contributions outside of the laboratory. "While continuing his research at CDFD, he also plays a key role as Institute Director to set up the diagnostic systems for DNA tests of heritable genetic disorders, and moreover to provide young scientists with the chance to train in bacterial molecular genetics," explains Akira Ishihama, Hosei University, Tokyo, Japan. Csonka elaborates, "CDFD houses approximately twenty scientists conducting basic research in diverse areas ranging from bacterial physiology to bioinformatics and structural biology, whose research is overseen and guided by Gowrishankar."

Over the years, Gowrishankar has been engaged in science-related public activities in India and beyond, including participating in training programs on intellectual property protection and exploitation, contributing to the Indo-Japan Collaborative Program on Modern Biology, and publishing a variety of articles on matters of science policy. In a letter in ASM News in 1994, he spoke of the inequity inherent in the imposition of page charges for papers published by groups from the developing world, and appealed for their waiver. He is an elected Fellow of the major Science Academies in India as also of the International Molecular Biology Network for Asia and the Pacific Rim, and has received several major national awards and honors in recognition of his achievements in science.

Max Gottesman, Columbia University Medical Center, summarizes: "I have been struck by his quick intelligence and his encyclopedic knowledge of scientific literature. For me, he has been a source of many useful suggestions. He is open and interactive--I have the utmost admiration for Gowrishankar, and support him without hesitation.



Great Thoughts by Great People

If we cannot love the person whom we see, how can we love God,
Whom we cannot see?

—Mother Teresa

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

—Bonnie Blair

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

—Leo Tolstoy

If someone feels that they had never made a mistake in their life, then it
means they had never tried a new thing in their life.....

—Einstein

No one can make you feel inferior without your consent.

—Eleanor Roosevelt

Without a sense of urgency, desire loses its value.

—Jim Rohn

There are two ways of meeting difficulties: you alter the difficulties, or
you alter yourself to meet them.

—Phyllis Bottome

A man can succeed at almost anything for which he has unlimited
enthusiasm.

—Charles Schwab

In the middle of difficulty lies opportunity.

—Albert Einstein

When you know what you want, and you want it badly enough, you'll
find a way to get it.

—Jim Rohn

If you are not big enough to lose, you are not big enough to win.

—Walter Reuther

Challenges are what make life interesting; overcoming them is what
makes life meaningful.

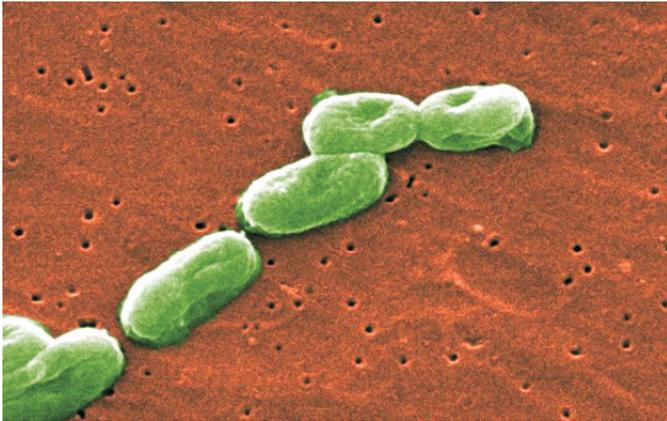
—Joshua J. Marine



Funny Quotes

1. Before I got married I had six theories about bringing up children; now I have six children and no theories.
—John Wilmot
2. Always forgive your enemies; nothing annoys them so much.
—Oscar Wilde
3. Laughing at our mistakes can lengthen our own life. Laughing at someone else's can shorten it.
—Cullen Hightower
4. We learn something every day, and lots of times it's that what we learned the day before was wrong.
—Bill Vaughan
5. Don't ever wrestle with a pig. You'll both get dirty, but the pig will enjoy it.
—Cale Yarborough
6. An inventor is simply a fellow who doesn't take his education too seriously.
—Charles F. Kettering
7. Some people like my advice so much that they frame it upon the wall instead of using it.
—Gordon R. Dickson

Burkholderia cepacia



Taxonomy

Domain = Bacteria

Phylum = Proteobacteria

Class = Betaproteobacteria

Order = Burkholderiales

Family = Burkholderiaceae

Genus = *Burkholderia*

Species = *B. cepacia*, *B. multivorans*, *B. cenocepacia*, *B. vietnamiensis*, *B. stabilis*, *B. ambifaria*, *B. dolosa*, *B. anthina*, *B. pyrrocinia*

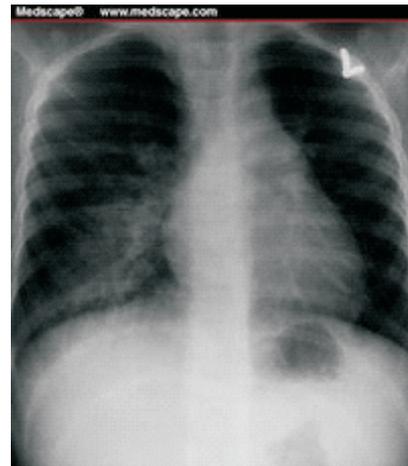
Description

Burkholderia cepacia complex is a group of Gram-negative, non-spore forming bacilli composed of approximately 17 closely-related species which are grouped into nine genomovars. This is often referred to as the *Burkholderia cepacia* complex (BCC). It was originally recognized by W. H. Burkholder in the 1950s for the distinct stench it caused in onion bulbs, known as "sour skin" disease for its vinegar-like odor. *B. cepacia* has emerged as a human respiratory opportunistic pathogen in individuals with weakened immune systems or chronic lung disease, especially cystic fibrosis (CF) patients, within the past 30 years and has therefore received increased attention from the scientific community. BCC potentially causes abscesses and bacteremia, but this condition is highly uncommon in patients without CF. Pulmonary colonization of *B. cepacia* can cause accelerated decline in lung functions and cause "cepacia syndrome," which is a progressive pneumonic illness that is fatal and essentially untreatable.

The organisms possess a remarkably large genetic make up with a total DNA content of more than 7 megabases and up to 3 megabase-sized replicons. BCC was formerly classified as a *Pseudomonas*, but was transferred to the *Burkholderia* genus in 1992 on the basis of phenotypic characteristics, 16S rRNA sequences, DNA-DNA homology values, and cellular lipid and fatty acid composition. It poses little medical risk to healthy individuals, but weakened immune systems or chronic lung diseases, such as cystic fibrosis, may have increased susceptibility. BCC often causes infections in hospitalized patients as well. Various traits distinguish BCC from other CF pathogens, such as its inherent resistance to many antibiotics, high transmissibility, and association with greater virulence. Because of these factors, management of *B. cepacia* complex patients is difficult.

Ecological Role

BCC bacteria exist throughout the environment. These bacteria can act as a powerful pesticide, capable of eliminating many soil-borne plant pathogens. In contrast, there is potential for the *B. cepacia* complex to act as a biofertiliser for rice that is cultivated in low fertile, low acidic soils. Though initially discovered as a plant pathogen and capable of acting as a pesticide, these bacteria generally interact with plants in an ecologically beneficial manner. Additionally, their metabolic capacity and substantial genetic diversity allow them to degrade significant pollutants such as trichloroethylene. Scientists are utilizing the diverse metabolic properties of BCC to create an assistive agent for bioremediation of contaminated environmental sites. Currently, it is not possible to determine strains of *B. cepacia* that can be safely used in agriculture without potentially harming humans, which causes discourse between the scientific and agricultural communities.



Infection of cystic fibrosis patients

From: medscape.com Cystic fibrosis patient with *B. cepacia* infection

Cystic fibrosis is a genetic, life-threatening disease that primarily affects the digestive system and the lungs. It is the most common potentially lethal autosomal recessive disease in North America, and it affects approximately 1 in 2,000 live births among Caucasians worldwide. The major cause of morbidity and mortality in CF patients is chronic microbial colonization of major airways, which lead to exacerbations of pulmonary infection. Common CF pathogens are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *haemophilus influenzae*. Reports of *B. cepacia* in CF patients appeared in the late 1970s and early 1980s. In 1984 the first thorough description of the clinical significance of its colonization and infection was published. Strains of BCC are frequently associated with aggressive pneumonia that is accompanied by rapidly fatal bacteremia, while *P. aeruginosa* infections are much less invasive in nature.

Transmission

Susceptible persons can acquire *B. cepacia* organisms through person-to-person contact, contact with contaminated surfaces, and exposure to it in the environment (i.e. soil and water).

Transmission from contaminated medicines, nasal spray, mouthwash, and sublingual probes have been recorded also. *B. cepacia* complex is highly transmissible between cystic fibrosis patients and can be transferred from one CF patient to another in hospitals, health clinics, and social environments.

Epidemiology

B. cepacia may colonize and cause respiratory tract infections in cystic fibrosis patients. It also may cause catheter-related infections in patients on hemodialysis or cancer patients through formation of biofilms. In patients treated with ceftazidime antibiotics and fluoroquinolones, there have been reports of *B. cepacia* nosocomial pneumonia. Soft-tissue, skin, surgical-wound, and genitourinary tract infections with *B. cepacia* have been reported. In cystic fibrosis patients who contract *cepacia* syndrome, the pneumonic illness is fatal.

Virulence Factors

B. cepacia bacteria are able to produce a diverse collection of potential virulence factors, though not all of them have demonstrated a role in pathogenesis of human disease thus far. A substantial amount of research has been undertaken to define the virulence factors expressed by *B. cepacia* bacteria because of its effects of cystic fibrosis patients.

Lipopolysaccharide (LPS), composed of O-antigen, core oligosaccharide, and lipid A, is a common virulence factor in Gram-negative bacteria. However, the LPS of BCC is distinct because the core oligosaccharide contains less phosphate and 3-deoxy-D-manno-oct-2-ulosonic acid than most other Gram-negatives. Additionally, 4-amino-4-deoxyarabinose moieties are attached to the phosphate residues in the lipid A backbone, which have been implicated in resistance to some antibiotic effects. Compared to the LPS of the other major CF pathogen, *P. aeruginosa*, the LPS of BCC is 4-5 times more endotoxic and induces heightened activity of neutrophil burst and induction of interleukin-8 (IL-8) from epithelial cells.

Lipase has been shown to effect the invasion of lung epithelial cells in BCC bacteria, but little is specifically known as to how. Metalloproteases, specifically ZmpB, play a crucial role in the virulence of BCC bacteria in lung tissue. ZmpB is proteolytically active against a great number of proteins in the lung's extracellular matrix, including type IV collagen and fibronectin. It has also shown its ability to destroy members of the immune system.

BCC bacteria express one of two flagellin types, which are distinguished by RFLP patterns of the *fliC* gene and subunit size. These flagella have been shown to contribute to lung epithelial cell invasion through in vitro experimentation.

Because of *P. aeruginosa's* ability to grow in biofilms, it is thought that other CF pathogens may also be able to form biofilms. BCC has grown biofilms in vitro which show increased resistance to ciprofloxacin and ceftazidime when compared with their planktonic counterparts. Biofilms may be highly advantageous for BCC bacteria, especially in the environment of a CF lung, because the bacteria may be better protected from antibiotics and host-defence mediators.

Clinical Features

Burkholderia cepacia complex infections are difficult to recognize because of their similarity to all other lung infections. These symptoms are often already occurring in cystic fibrosis patients, which causes even more difficulty in detecting the infection. Common symptoms include fever, cough, shortness of breath, congestion, and wheezing.

Diagnosis

Currently, the sole method of diagnosis for *B. cepacia* complex infection is the culturing of the patient's sputum. A culture will indicate which strains of *Burkholderia*, if any, are present within the patient.

Treatment

Clinical indicators of BCC infection vary. *Cepacia* syndrome is almost universally fatal, but aggressive treatment has yielded some success in specific cases. BCC is often resistant to many common antibiotics, and treatment decisions are typically made on a case-by-case basis. Because it is resistant to many antibiotics, effective therapies and treatments are not straightforward. Therefore, management efforts are primarily aimed at prevention of infection.

Prevention

Hand hygiene and other infection control procedures can reduce the risk of transmission. CF patients should not share hospital rooms with each other and should limit contact in outpatient clinics, such as CF summer camps.

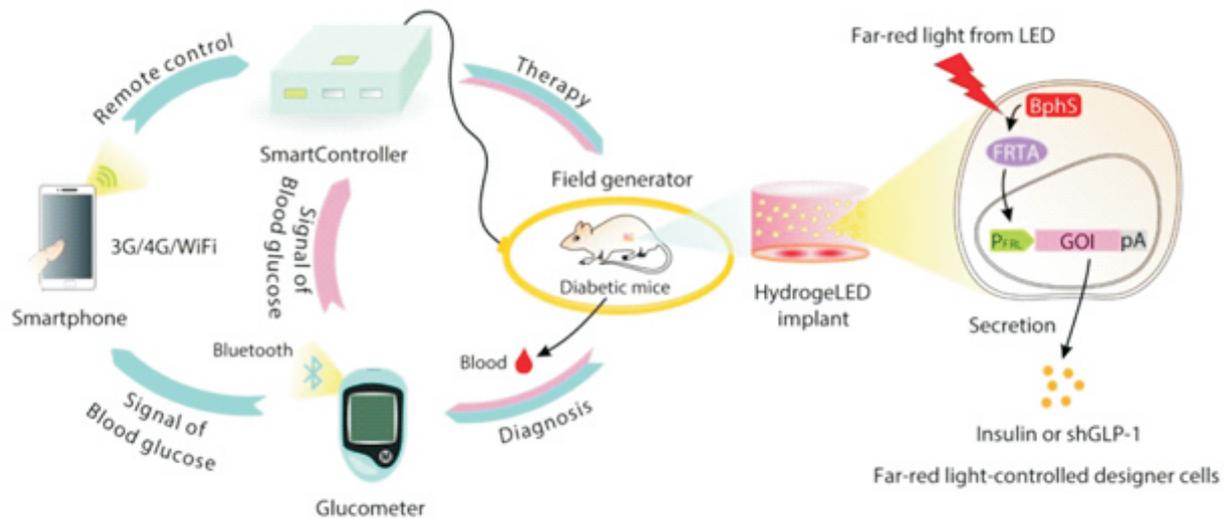
Host Immune Response

Little is known about the specific immune responses of those infected with BCC pathogens, but it is evident that persons with weakened immune systems, especially chronic lung issues, are especially susceptible to the pathogens. Its high transmissibility creates even more vulnerability for patients with weakened immune systems and reinforces that cystic fibrosis patients should be kept separate from one another to prevent further infection.

References:

1. Miller, S.C.M., LiPuma, J.J., Parke, J.L. "Culture-Based and Non-Growth-Dependent Detection of the *Burkholderia cepacia* Complex in Soil Environments." Applied and Environmental Microbiology.
2. Mahenthiralingam, E., Urban, T.A., Goldberg, J.B. "The multifarious, multireplicon *Burkholderia cepacia* complex." Nature.
3. Hua, C.N.C., Tokeshi, J. "Emergence of *Burkholderia cepacia* in Honolulu: A Case of Nursing Home-acquired *B. cepacia* sepsis. Hawai'i Journal of Medicine and Public Health.
4. Jones, A.M., Dodd, M.E., Webb, A.K. "*Burkholderia cepacia*: current clinical issues, environmental controversies and ethical dilemmas." European Respiratory Journal.
5. Chu, K.K., Davidson, D.J., Halsey, K., Chung, J.W., Speert, D.P. "Differential Persistence among Genomovars of the *Burkholderia cepacia* Complex in a Murine Model of Pulmonary Infection." American Society for Microbiology.

Smartphone-controlled cells help keep diabetes in check



Cells engineered to produce insulin under the command of a smartphone helped keep blood sugar levels within normal limits in diabetic mice.

More than 415 million people worldwide are living with diabetes, and frequently need to inject themselves with insulin to manage their blood sugars. Human cells can be genetically engineered into living factories that efficiently manufacture and deliver hormones and signaling molecules, but most synthetic biological circuits don't offer the same degree of sensitivity and precision as digital sensors.

Combining living tissues and technology, Jiawei Shao et al. created custom cells that produced insulin when illuminated by far-red light (the same wavelengths emitted by therapy bulbs and infrared saunas).

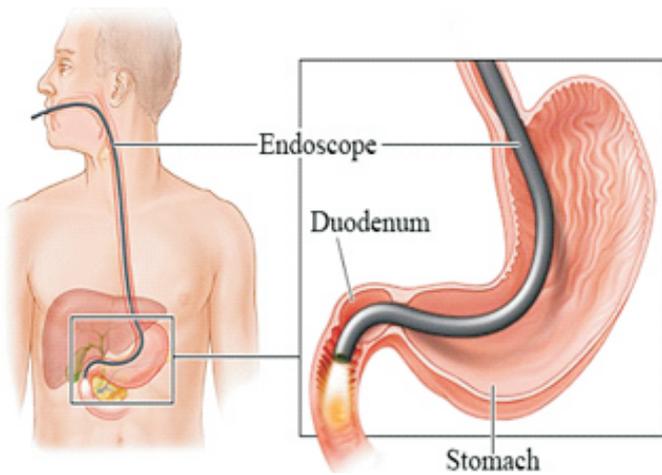
The researchers added the cells to a soft bio-compatible sheath that also contained wirelessly-powered red LED lights to create HydrogeLEDs that could be turned on and off by an external electromagnetic field.

Implanting the HydrogeLEDs into the skin of diabetic mice allowed Shao and colleagues to administer insulin doses remotely through a smartphone application. They not only custom-coded the smartphone control algorithms, but designed the engineered cells to produce insulin without any "cross-talk" between normal cellular signaling processes.

The scientists went on to pair the system with a Bluetooth-enabled blood glucose meter, creating instant feedback between the therapeutic cells and the diagnostic device that helped diabetic animals rapidly achieve and maintain stable blood glucose levels in a small pilot experiment over a period of several weeks.

The authors say that successfully linking digital signals with engineered cells represents an important step toward translating similar cell-based therapies into the clinic. A related Focus by Mark Gomelsky highlights the findings further.

Endoscope Disinfection - Part II



Inventory

Conduct an endoscope inventory to identify all endoscopes and method of reprocessing in use by the facility. Information reviewed for each endoscope should include but is not limited to the:

- Endoscope manufacturer and model
 - Location of use
 - Number of procedures performed
 - Location of the endoscope manufacturer's IFUs
 - Location for reprocessing
 - Equipment used for HLD and/or sterilization
 - Status of the endoscope (i.e., retired, out for repair, in use)
- Ensure that each endoscope has a unique identifier to facilitate tracking. Tracking should include the ability to determine when specific endoscopes were used for specific patients, loaned to other units or facilities, reprocessed, or repaired. Tracking is also essential for responding to device or product recalls.

Disinfection/Sterilization Breach or Failure

- Breaches in adherence to essential disinfection and sterilization steps can be a result of malfunctioning of equipment and/or human error. Each breach is a result of unique circumstances and should be evaluated to determine the risk of disease transmission. A multi-disciplinary team that includes infection prevention, risk management, and endoscopy personnel should review each event carefully to determine the necessary corrective steps and the need for patient notification.
- There are several resources available to assist in a breach evaluation. The multi-disciplinary team should use one or more of these documents to guide their investigation.
- When a breach involves a suspicion of patient exposure to an improperly reprocessed endoscope, the decision to notify patients of their potential exposure should be made in consultation with an infection preventionist and state and local health departments.
- If a healthcare provider suspects persistent bacterial contamination of an endoscope following reprocessing, either because of an increase in infections after endoscopic procedures or because of the results of microbiological culturing of endoscopes, the healthcare provider should file a

voluntary report through MedWatch, the FDA Safety Information and Adverse Event Reporting program.

Discard enzymatic detergents after each use, because these products are not microbicidal and will not retard microbial growth.

Lubricating Agents

Use of lubricating agents is an effective way of extending the use life of some medical devices. Lubricants may reduce the friction commonly associated with metal-on-metal movements and thereby reduce device wear and corrosion.

If applicable, the reprocessing instructions should recommend lubricating agents, or a class of lubricating agents (e.g., water soluble lubricants) that are compatible with the medical device, its intended use, and with any subsequent processing steps such as sterilization. Also, labeling for the reusable device should refer to the lubricating agent labeling for preparation and use instructions of those agents. If your reprocessing instructions specify the use of lubricating agents, you should validate the device reprocessing methods using the lubricating agents under the conditions of use of the device.

Caution should be exercised when using oil-based and silicone based lubricants, as they may coat and protect surface microorganisms and reduce the effectiveness of certain sterilization methods, including steam and EO. They may even provide nutrients for microbial growth.



Figure 3. Chemical Damage from Reprocessing

Susceptibility of Resistant Organisms:

Organisms of concern in gastroenterology settings—such as *Clostridium difficile*, *Helicobacter pylori*, *Escherichia coli*, Human immunodeficiency virus (HIV), Hepatitis C virus, Hepatitis B virus, multidrug-resistant *M. tuberculosis*, Vancomycin-resistant enterococcus (VRE), and Methicillin-resistant *Staphylococcus aureus* (MRSA)—are susceptible to high-level disinfectants and sterilants (Rutala et al., 2008; ASGE Standards of Practice Committee et al., 2008).

Outbreaks of infection have been traced to lack of adherence to reprocessing guidelines, endoscopes which are damaged or difficult to clean, and AER design problems or failures such as breakdowns in AER water filtration systems (Rutala et al., 2008). Reports of carbapenem resistant (CRE) transmission have led to challenges in achieving effective HLD, requiring added reprocessing steps in all phases.

The complex design of duodenoscopes has prompted manufacturers to implement changes in reprocessing (FDA, 2015).

Prions and other transmissible spongiform encephalopathies (TSE), including Creutzfeldt-Jakob disease (CJD) and variant Creutzfeldt-Jakob disease (v-CJD) are resistant to conventional

disinfectants and sterilants. In order for an endoscope or medical/surgical device to act as a vehicle of prion transmission, it must come in contact with infective tissue (Rutala & Weber, 2013). TSEs and CJD are confined to the central nervous system and are transmitted by exposure to infectious brain, pituitary, or eye tissue. Since endoscopes do not come in contact with brain, pituitary, or eye tissue, transmission is highly unlikely (ASGE Standards of Practice Committee et al., 2008; Nelson & Muscarella, 2006; Rutala & Weber, 2013).

Determining Minimum Effective Concentration (MEC)

The high-level disinfectants/sterilants must be monitored to ensure they maintain their effectiveness.

The following factors result in a gradual reduction of the effectiveness of reusable high-level disinfectants/sterilants (Rutala et al., 2008; ASGE Standards of Practice Committee et al., 2008)

1. Decreased concentration because of challenging loads of microbes and organic matter
2. Dilution by rinse water from endoscopes or items not sufficiently dried
3. Aging of the chemical solution

Each solution's minimum effective concentration (MEC) and reuse life are established by the manufacturer. Monitor minimum effective concentration according to the disinfectant/sterilant manufacturer's instructions and maintain a log of test results.

Reusable high-level disinfectant/sterilants must be disposed and replaced whenever the MEC fails or the reuse life expires, whichever comes first. Chemical HLD/sterilants that are single use and prepared onsite also need to be tested. It is important to use the product-specific test strip or chemical monitoring device (AAMI, 2015). Because chemical test strips deteriorate with time, the bottle should be labeled with the manufacturer's expiration date and date when opened, and the strips should be used (or discarded) within the period of time specified by manufacturer. Follow the manufacturer's recommendations regarding the use of quality control procedures to ensure the strips perform properly (Rutala et al., 2008). Document quality control results. If additional chemical solution is added to an AER or basin (if manually disinfected), the reuse life should be determined by the first use/activation of the original solution. The practice of "topping off" of the chemical does not extend the reuse life (Petersen et al., 2011)

References

- <https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM253010.pdf>
https://www.sgna.org/Portals/0/HLD_FINAL.pdf?ver=2017-01-06-085346-147
https://www.sgna.org/Portals/0/MS_guideline_reprocessing_GI_endoscopes.pdf?ver=2017-01-09-151750-253
<https://www.cdc.gov/hicpac/recommendations/flexible-endoscope-reprocessing.html>



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CONTACT TIME : ● 1 minute (undiluted & 10% v/v solution)

● 5 minutes (5% v/v solution) ● 10 minutes (2.5% v/v solution)

ACTIVITY : Broad spectrum: Bactericidal, Fungicidal and Virucidal



Structurally similar to AMPs**	Enhance the immune response by functioning as immunomodulators
Maintain hydrobalance	Facilitate wound healing
Anti-biofilm effect	Effective in chronic & diabetic wounds
BI***>1	● Non cytotoxic ● Helps in re-epithelization
No known resistance	Effect against wide range of microbes
● Non-stinging ● Non-staining	Good patient compliance

APPLICATIONS :

● Pre & post surgery skin and mucous membrane antisepsis ● Surgical and non-surgical wound dressings ● Chronic wound (Diabetic foot ulcers, pressure ulcers, arterial/venous leg ulcers) management ● Routine antisepsis during minor incisions, catheterisation, scopy etc ● First aid

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

