CET

CENTER FOR ENVIRONMENTAL TRAINING

Bacteria: Evaluating Exposure and Health Risks

Will begin at 9:00 a.m. (PST)

Participants will be in listen only mode.

Download the PDF of this presentation (case sensitive):

http://www.emlab.com/m/media/Bacteria-webinar.pdf

Presented by:

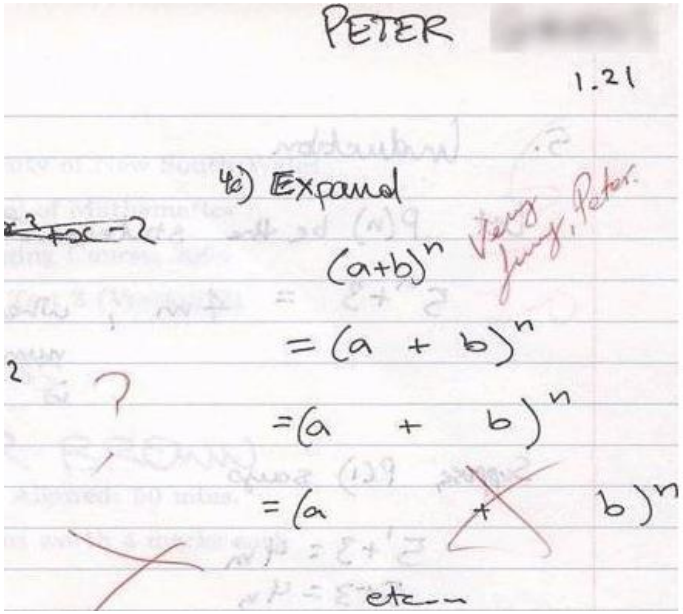
Harriet Burge, Ph.D.

EMLAB P&K

THE PROVEN LEADER®

A TestAmerica Company

Continuing Education Units (CEUs)



To receive a certificate of attendance, you must complete the survey after the webinar:

- Click on the survey link in the "Thank you" email (sent 1 hour after this webinar).
- Complete survey by this Friday, March 7, 2014.
- You will receive an email in 2-3 weeks when your certificate is ready.

Outline

- Introduction to bacteria and bacterial classification
- Distribution and role of the bacteria in the natural environment
- Overview of the health effects of bacteria
- Overview of sampling and analytical methods for bacteria
- *Legionella* and Legionnaires' disease
- Mycobacteria
- MRSA in health care settings
- Sewage: evaluating hazards

Introduction To Bacteria

- Naming bacteria
- Morphology
  - Macroscopic
  - Microscopic
- Classification
- Physiology

Bacterial Diversity

3 Distinct Domains, separated on the basis of biochemistry, genetics and cellular structure.

Eukarya

Archaea

Bacteria

Naming Bacteria

Binomials:

- Genus: *Escherichia*
- Species: *coli*

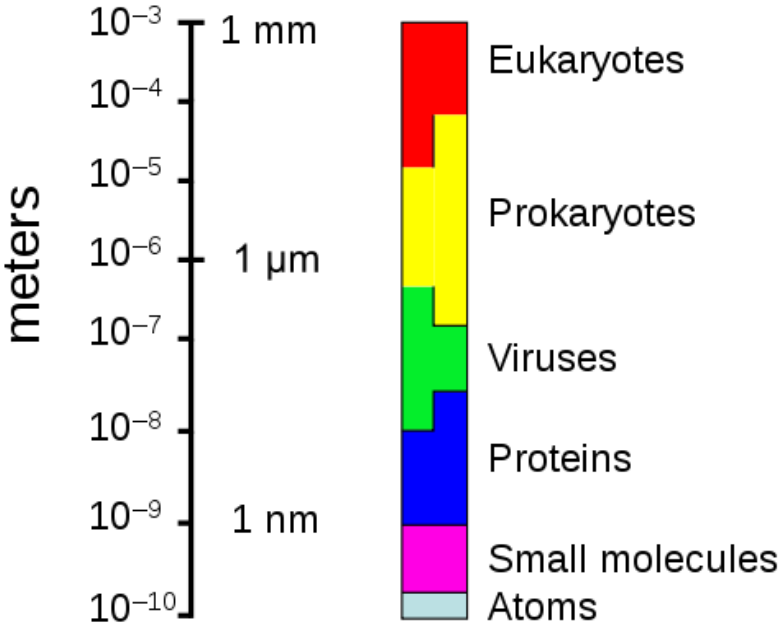
- Genus: *Staphylococcus*
- Species: *aureus*

The U.S. Food and Drug Administration is warning consumers in Puerto Rico that two hand sanitizers – "Bee-Shield Hand Sanitizer" with Aloe Vera (10 fl. oz. or 1 gallon bottles) and "MD Quality Hand Sanitizer" with Aloe Vera (10 fl oz. bottles) – contain high levels of a bacteria, *Burkholderia cepacia*, that can cause serious infections in humans. March 3, 2010

Morphology

Size

- 0.1 to about 600 µm over a single dimension
- *Escherichia coli:* 1.1 to 1.5 µm by 2.0 to 6.0 µm
- On surfaces, usually present as colonies with a few or millions of cells
- When airborne, usually on rafts (e.g., skin scales) (>10µm) or in droplet nuclei (one or more bacteria surrounded by dried mucous) (>2µm).

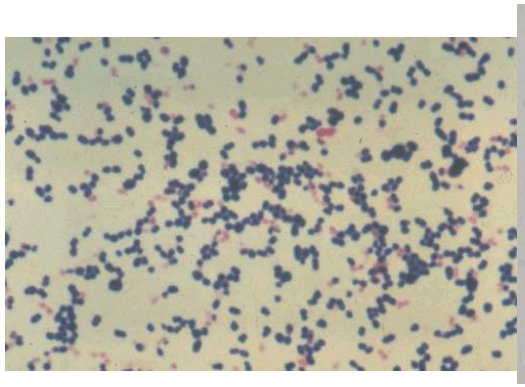


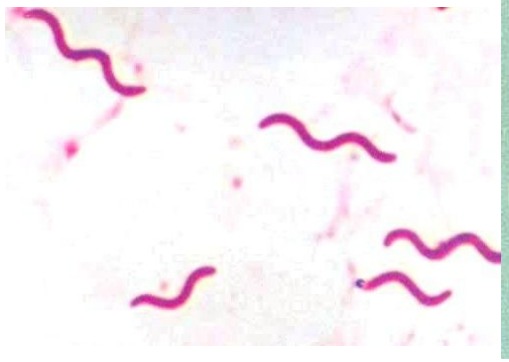
Source: http://en.wikipedia.org/wiki/File:Relative_scale.svg

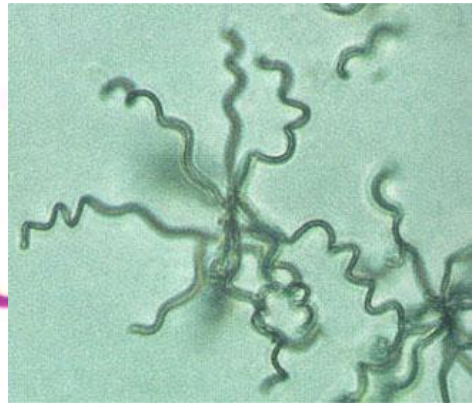
Morphology (cont'd)

Shape

- Cocci: Spherical
  - *Staphylococcus aureus*
- Bacilli: Rod shaped
  - *Escherichia coli*
  - *Pseudomonas aeruginosa*
- Spirilli: Spiral rods;
  - *Borrelia burgdorfii*
  - *Helicobacter pylori*
- Filamentous: long branching strands
  - *Thermoactinomyces vulgaris*







Source: http://en.wikipedia.org/wiki/File:Bacterial_morphology_diagram.svg

Morphology (cont'd)

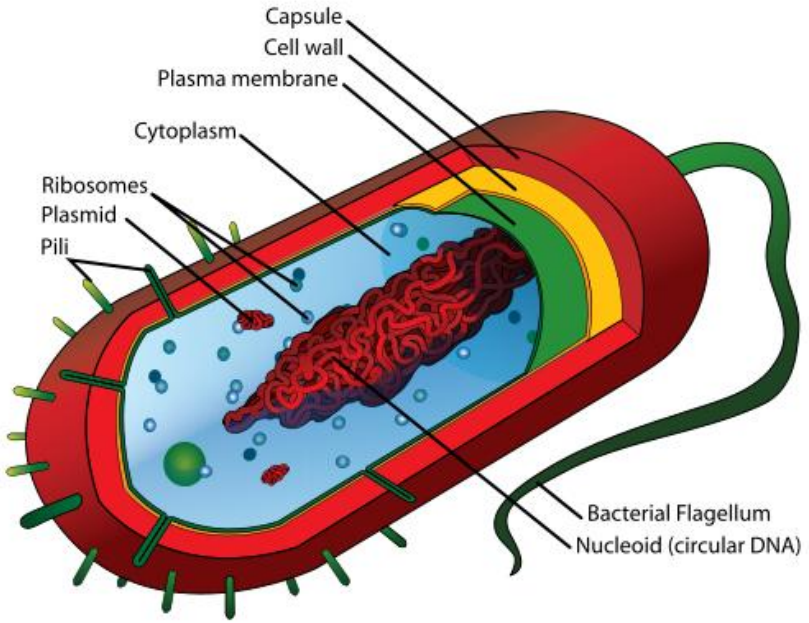
Appendages

- Flagellae
- Pili
  - Type IV
  - Reproductive
- Fimbriae

According to Live Science the bacteria found in yogurt can come to your teeth's rescue as the bacteria that has been used in the making of yogurt has been found to help prevent plaque from sticking to teeth and scientists are currently working on introducing it into toothpaste.

Bacterial Cell Structure

- DNA: loosely organized, no membrane bound nucleus
- RNA: in cytoplasm
- Ribosomes: make protein from amino acids under instruction from RNA
- Plasma membrane: lipid/protein layer with selective permeability
- Cell wall:
  - Peptidoglycan
  - Lipopolysaccharide (Gram negative)
- Capsule (polysaccharides)



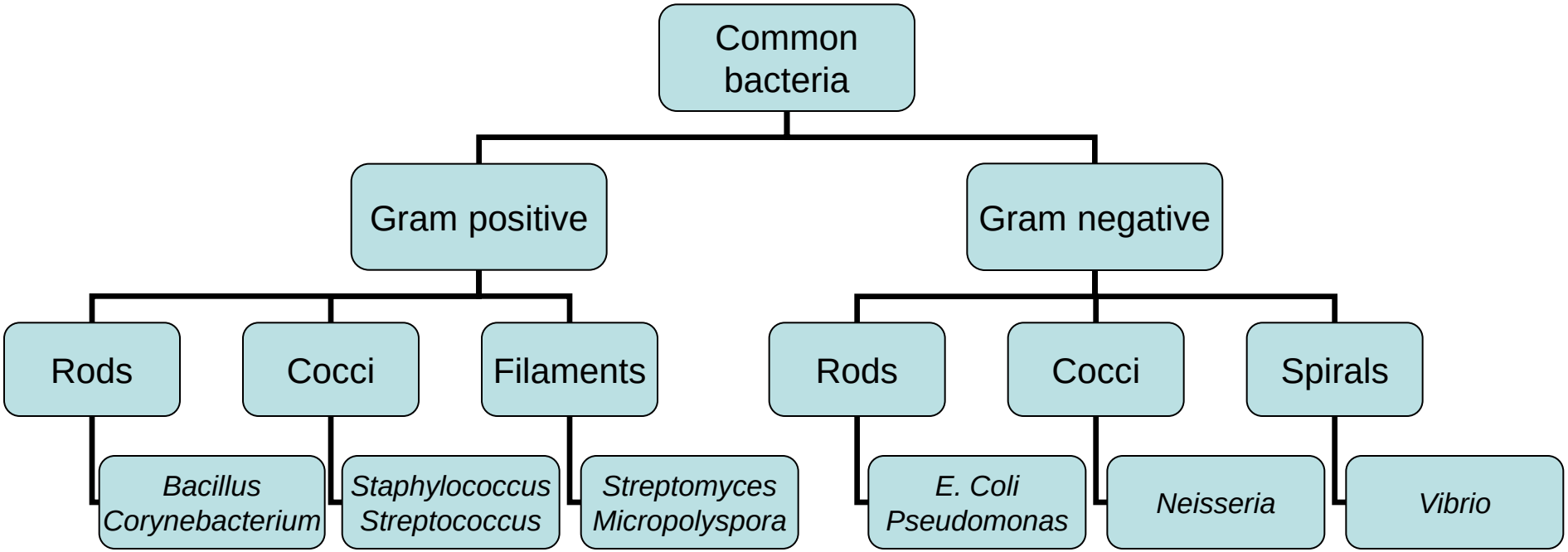
Source: http://en.wikipedia.org/wiki/File:Average_prokaryote_cell-_en.svg

Biofilms: A Larger Structure

- In nature, most bacteria are bound to surfaces in biofilms
- Few microns to half a meter (yes, meter) in depth.
- Complex arrangement of cells & extracellular components including networks of channels to diffuse nutrients.

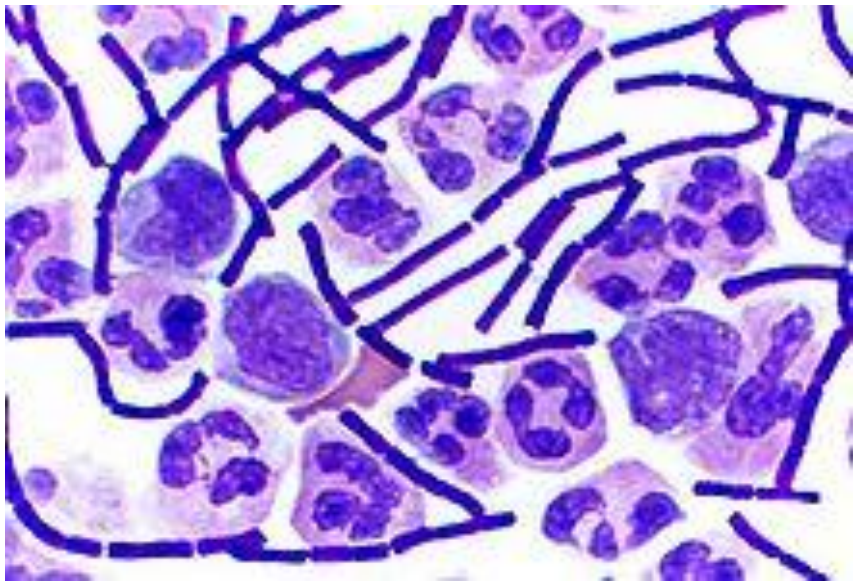
Source: wikipedia.org/wiki/Biofilm

Practical Classification



The Gram Stain

- Add **Crystal Violet;** wait 1 minute; rinse with water
- Add **Iodine;** wait 1 minute; rinse with water
- Add **Acetone Alcohol;** wait 10-15 sec**.;** rinse with water
- Flood slide with **Safranin;** wait 1 minute; rinse with water
- Gently blot the slide dry.
- View under oil immersion (1000x) with a bright-field compound microscope.



Source: http://en.wikipedia.org/wiki/Gram_staining

Gram Negative (Pink)

Cells with a thin lipopolysaccharide cell wall do not retain the violet dye

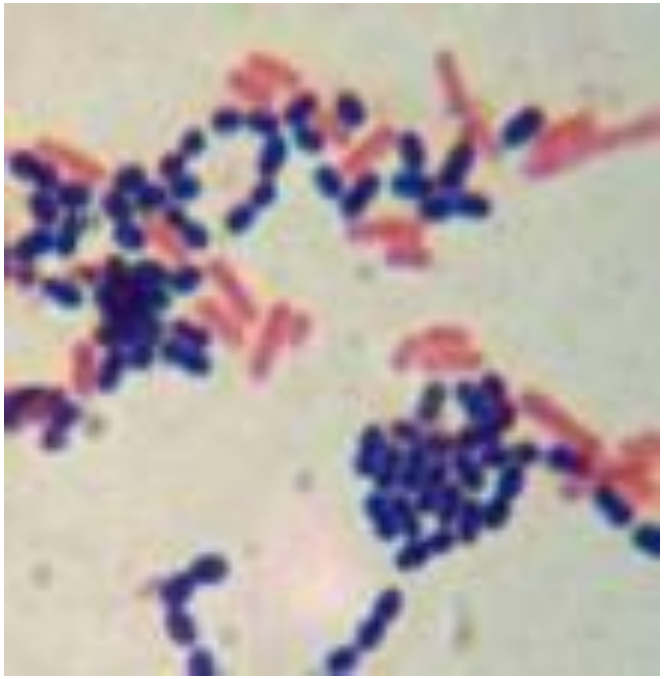
- *E. coli*
- *Legionella pneumophila*
- *Pseudomonas aeruginosa*
- *Neisseria gonorrhea*
- Etc.

Source: http://en.wikipedia.org/wiki/Gram_staining

Gram Positive

Cells with a thick peptidoglycan cell wall do retain the violet dye

- *Staphylococcus*
- *Bacillus*
- *Corynebacterium*
- *Actinomycetes*



Acid Fast

Cells with mycolic acid in outer wall: retain a dye when treated with acid. All acid fast bacteria are Gram positive.

- *Mycobacterium*
- *Nocardia*
- Some amoebal cysts (not a bacterium)

Physiology

- **Psychrophiles:** Grow best at cold temperatures. <10˚ C
- **Mesophiles:** Grow best at medium temperatures. All pathogenic bacteria are mesophiles. 10-40˚ C
- **Thermophiles:** Grow best at hot temperatures. 40-80˚ +C

Physiology (cont'd)

- **Obligate Anaerobes:** anaerobic fermentation; cannot survive in the presence of oxygen.
- **Obligate Aerobes:** strictly oxidative respiration and require oxygen for survival.
- **Facultative Anaerobes:** fermentation in the absence of O_2, or respiration in its presence.
- **Aerotolerant Anaerobes:** never undergo oxidative respiration but can tolerate the presence of oxygen.
- **Microaerophilic:** facultative anaerobes, but they prefer low O_2-concentration conditions.

Physiology (cont'd)

- Professor Alan Parsons and Dr. Richard Heal of QinetiQ Ltd, claim to have shown that physically separated colonies of bacteria can transmit signals conferring resistance to commonly used antibiotics
- (volatile organic compounds)?

Distribution and Role of the Bacteria in the Natural Environment

- Ubiquity:
  - "We live now in the 'Age of Bacteria.' Our planet has always been in the Age of Bacteria, ever since the first fossils—bacteria, of course—were entombed in rocks more than 3 billion years ago. Bacteria are—and always have been—the dominant forms of life on Earth" *Steven J Gould*
  - All surfaces on earth and all water contain bacteria
  - Approximately five nonillin ($5x10^{30}$) bacteria on Earth, forming much of the world's biomass.

Environmental Bacteria

How many bacteria are acceptable/normal?

Mean Concentrations of Total Airborne Culturable Bacteria (Sum of Mesophilic and Thermophilic Bacteria) (CFU/m^3), by Location (Indoors/Outdoors) and Season.

	Indoor		Outdoor	
	Summer	**Winter**	**Summer**	**Winter**
Total Gram+ rods	10.6	11.4	33.6	43.6
(Actinomycetes)	(2)	(1.2)	(6.4)	(3.4)
(Bacillus species)	(6.9)	(6.6)	(19.9)	(23.4)
(Other Gram+ rods)	(1.7)	(3.5)	(7.3)	(16.9)
Gram+ cocci	48.3	28.7	26.2	21.8
Gram– rods	3.5	2.6	14.9	11
Gram– cocci	1.6	1.3	1.1	3.3
Unknown	51.8	42.6	89.1	114.7
Total bacteria (All groups)	116	86.7	165	194.5

Tsai, F.C.; Macher, J.M. 2005. Concentrations of airborne culturable bacteria in 100 large U.S. office buildings from the BASE study. Indoor Air 15(Suppl 9):71-81.

Environmental Bacteria: A Few Standards

Drinking water (EPA):

- HPC (no more than 500 bacterial colonies per ml)
- Coliform baceria (no more than 5.0% samples total coliform-positive in a month)
- *Legionella* (no limit, but EPA believes that if *Giardia* and viruses are removed/inactivated, *Legionella* will also be controlled)

Water

- A million ($10^{6)}$ bacterial cells in a milliliter of fresh water
- $5x10^{8}$ in an 8 ounce glass of water
  - *Pseudomonas, Flavobacterium* and *Acinetobacter*
- Old, dirty water filters seem to make water taste better. Bacteria that thrive on dirty water filters can reduce the distasteful earthy tinge in tap water.

E. coli

- *E. coli* is a gut organism, but is common in soil and water where animal feces are present.
- Approximately 0.1% of the total bacteria within an adult's intestines (on a Western diet) is represented by *E. coli*.
- *E. coli* and other bacteria in our intestines are essential and provide Vitamin K and B-complex vitamins.
- *E. coli* O157:H7 is a specialized rare strain that causes serious infections. It is native to cows, not people.

Soil

- A teaspoon of productive soil generally contains between 100 million and 1 billion bacteria. That is as much mass as two cows per acre. (Elaine R. Ingham)
- Decomposers: can break down pesticides and pollutants in soil
- Mutualists form partnerships with plants. The most well-known of these are the nitrogen-fixing bacteria (ex. *Rhizobium*)
- Plant pathogens: *Xymomonas* and *Erwinia* species, and species of *Agrobacterium* that cause gall formation in plants

Plant Surfaces

- Bacteria multiply on the surface of plants and are aerosolized by wind and rain action.
- In the air, water clumps around bacteria forming condensation nuclei, leading to rainfall.
- Precipitation returns bacteria to the ground. Even if one bacterium lands on a plant, it can multiply and form groups, thus causing the cycle to repeat.
  - *Pseudomonas syringae*
- Epiphytic bacteria can increase water permeability of leaf cuticles, which increases the availability of water and dissolved compounds.

Air

- Bacteria are globally distributed in the atmosphere and are believed to play a large role in formation of snow and rain.
- Over 2,000 different kinds of bacteria may be present in the air on any given day (*Gary Anderson).*
- Air bubbles breaking at the Air-water interface remove bacteria that concentrate at the interface. Bubbles eject the bacteria into the atmosphere. Bacterial concentrations in the drops may range from 10 to 1,000 times that of the water.
- Bacteria may reproduce within airborne droplets
- Diarrhea-causing *Arcobacter* and ulcer-inducing *Heliobacter* have been recovered from air.

EMLab P&K's IAQ Pocket Guide

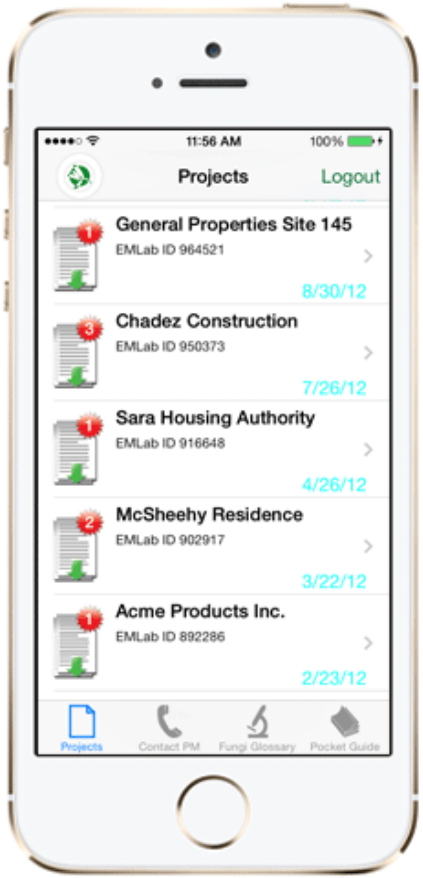
- Sampling and regulatory guidelines for: Fungi, Asbestos, Bacteria, Allergens, and Industrial Hygiene
- Updated MoldRange™ data from over 350,000 spore trap samples
- Data interpretation guidelines

To order a complimentary Pocket Guide, complete the survey after the webinar.

Limit 1 per person per calendar year. United States and Canada only. The "Thank you" email (sent an hour after the webinar) will include a link to the survey to order your Pocket Guide.

EMLab P&K Mobile App

Access your lab results: Easier. Faster. Anywhere you are.



- Instant alerts when your reports are available
- Review your projects, reports and invoices
- Browse EMLab P&K's Pocket Guide and fungal library
- Contact your Project Manager with the tap of the screen

http://www.emlab.com/mobile

Available on the App Store

ANDROID APP ON Google play

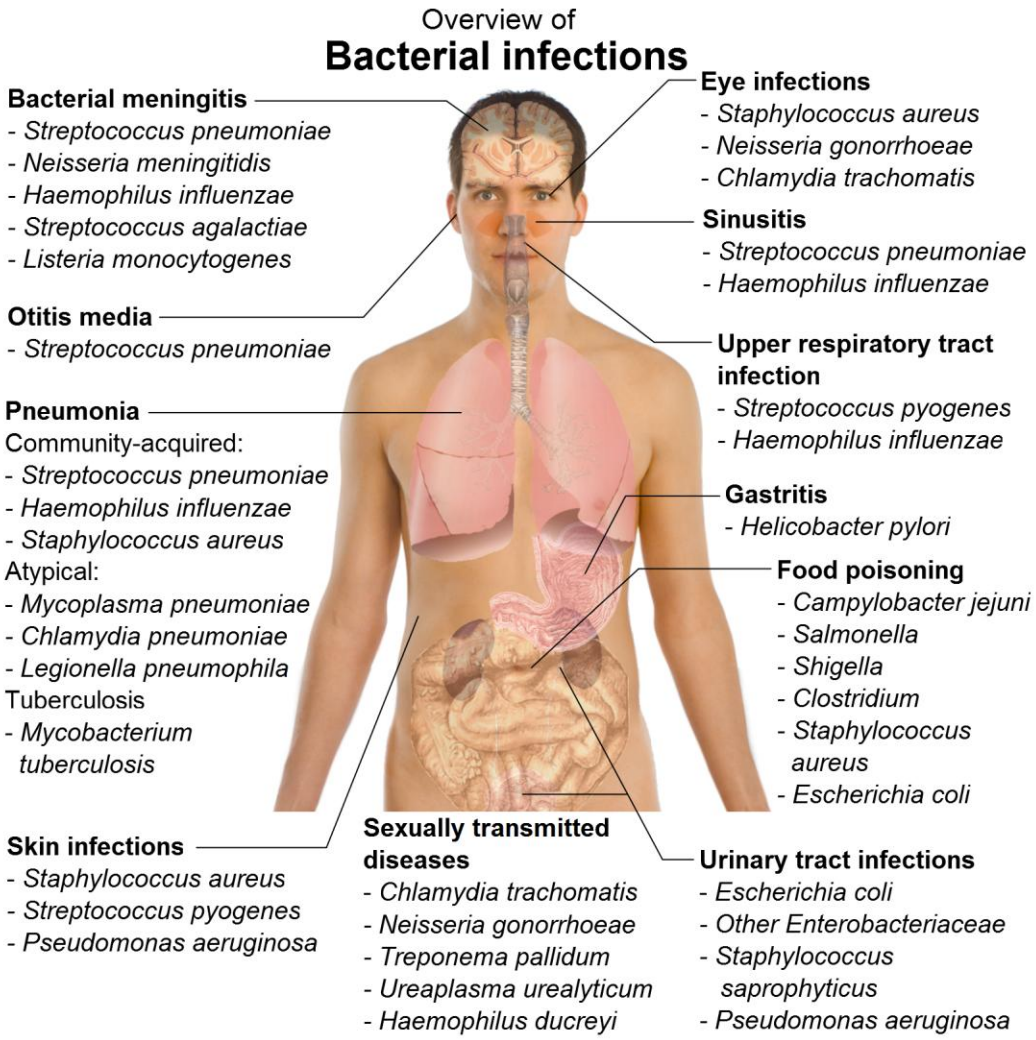
Overview of Health Effects of Bacteria

- Infection
- Allergy
- Toxicosis
- Symbiosis

Infection

Invasion by, and multiplication of, pathogenic microorganisms in a bodily part or tissue, which may produce subsequent tissue injury and progress to overt disease through a variety of cellular or toxic mechanisms.

Overview of Bacterial Infections



http://en.wikipedia.org/wiki/File:Bacterial_infections_and_involved_species.png

Environmental Bacteria – Pathogens

Pathogens

- Obligate pathogens
  - must cause disease in order to be transmitted from one host to another (e.g. *Mycobacterium tuberculosis*)
- Opportunistic pathogens
  - can be transmitted from one host or substrate to another without having to cause disease
  - In a host whose immune system is not functioning properly, the bacteria can cause an infection that leads to a disease (e.g. *Pseudomonas aeruginosa)*

Opportunistic Pathogens

Opportunistic pathogens are often hospital acquired.

- Bacteria
  - *Legionella*
  - *Staphylococcus aureus*
  - *Serratia marcescens*
  - *Pseudomonas aeruginosa*
- Fungi (Eukarya, not bacteria)
  - *Candida albicans*
  - *Aspergillus* species (Aspergillosis)
  - *Mucor/Rhizopus/Absidia* (Mucormycosis)
  - *Cryptococcus neoformans*

Virulence

- **Virulent infection:** infection by an organism that can infect anyone without specific antibodies.
- **Virulence**: a measure of how readily an organism can infect a host.
  - Highly virulent agents may cause infection following exposure to a single organism. (e.g., *Mycobacterium tuberculosis*)
  - Less virulent organisms may require higher doses before infection occurs (e.g., *Bacillus anthracis*)
  - Avirulent organisms may only infect those without a functioning immune system (e.g, *Pseudomonas aeruginosa*)

The Infection Process

- Invasion or reactivation and growth of the organism
- Overwhelming communities of bacteria
- Immune reactions leading to disease
- Release of bacterial toxins that cause the symptoms of disease

Internal Risk Factors For Infection

Poorly Developed or Impaired Immunity

- **Age.** Neonates and the elderly are at increased risk of bacterial infections.
- **Nutritional status.** Malnutrition results in a depressed immune system
- **Genetic predisposition.** The Human Genome Project increased our ability to locate specific genes related to infectious disease susceptibility (Bentley, DR, 2000).
- **Immunosuppression** via disease or medications
- **Lack of induced specific antibodies**

Routes of Exposure

- Exogenous: those that originate outside the body
  - Food, water, air, surfaces, other people
  - Ingestion, inhalation, other entry points
- Endogenous: caused by bacteria within the body that cause disease when the body's resistance is lowered

Pathways To Infection

- **Airborne infection:** contracted by inhalation of microorganisms or spores suspended in air, on water droplets, or dust particles, or in *droplet nuclei (dried droplets)*.
- **Droplet infection:** contracted by inhalation of respiratory pathogens suspended for a brief time on liquid particles exhaled by someone already infected.
- **Direct contact:** infection contracted by touching an infected person or contaminated surface.
- **Endogenous infection:** due to reactivation of organisms present in a dormant focus, as occurs in tuberculosis, etc.
- **Tunnel infection:** subcutaneous infection of an artificial passage into the body that has been kept open.

Respiratory Infections

- Upper respiratory tract infections (URI)
  - Leading cause of time lost from work and school
  - Bacteria account for up to 25 percent of URI (the rest are viral)
  - *Streptococcus* and *Haemophilus influenzae*
- Otitis media
  - Middle ear infection: most common bacterial infection in U.S. children
  - *Streptococcus pneumoniae*

Respiratory Infections (cont'd)

- Lower respiratory tract infections (LRI)
  - Acute, chronic pneumonia and bronchitis
  - LRI occur in both healthy and immunocompromised individuals
  - *Streptococcus pneumoniae*
- Tuberculosis (TB)
  - Affects 15 million people in the U.S. Fewer develop disease which depends on nutritional status, age, HIV. incarceration
  - *Mycobacterium tuberculosis*

Gastrointestinal Infection

- Infectious diarrhea is a leading cause of morbidity and mortality worldwide.
- In the US, 100 million people are affected every year.
- Most diarrhea is viral but bacteria also important.
  - 50% restrict activities
  - 10% consult physicians
  - 250,000 require hospitalization
  - approximately 3000 die
  - *Campylobacter, Salmonella, Shigella,* and *E. coli O157:H7*

Campylobacter jejuni

- Most common cause of bacterial diarrhea in the US.
- Over 1 million Americans are affected yearly.
- Antibiotics in poultry- and cattle-feed linked to the increasing incidence of drug-resistant *C. jejuni*
- Transmission via contaminated food (especially chicken) and water, or contact with infected animals (especially cats and puppies)

Salmonella

- Second most frequent cause of bacterial disease in U.S.
- In 2002, more than 44,000 cases were reported to the CDC. Incidence may be 30 or more times greater than reported.
- Diarrhea, fever, and abdominal cramps.
- The elderly, infants, and people with impaired immune systems are at greater risk of severe disease.
- Transmission is via exposure to contaminated food (especially eggs) or water, or contact with infected animals (reptiles).

Escherichia coli O157:H7

- Severe diarrheal disease called hemolytic uremic syndrome.
- An estimated 73,000 cases are reported in the United States annually.
- Transmission is through contaminated hamburger meat, apple cider, and fruits and vegetables.

Shigella

- Most common symptoms are diarrhea, vomiting, stomach cramps, fever, flatulence, nausea, and constipation.
- An estimated 448,240 cases occur in the U.S. yearly.
- Groups at highest risk in the U.S. are children in child care centers, individuals in custodial institutions, and international travelers.

Helicobacter pylori

- Most common chronic infection in humans causing chronic gastritis, peptic ulcer disease, and some types of stomach cancer.
- Half of the world's population is infected.
- Drinking coffee or alcohol and smoking increase your risk for an ulcer from *H. pylori*.
- Impairs absorption of nutrients, altering the balance of iron, vitamin B12, folic acid, alpha-tocopherol, vitamin C, and beta-carotene.
- Acute infection causes abdominal pain, weight loss, nausea, and vomiting.
- Has been found in river, creek, and lake water in central Pennsylvania.

Skin Infection

- Skin infections include:
  - Impetigo, boils, carbuncles, cellulitis, and complications from burns,
  - *Staphylococcus aureus,*
  - *group A streptococci,*
  - *Pseudomonas aeruginosa*,
  - Impetigo, a skin infection caused mostly by group A streptococci, can cause severe kidney inflammation, sometimes resulting in kidney failure.

Contagious Bacterial Infection

- Exogenous sources
- Highly virulent
- Risks depend on type of organism
  - Direct contact
  - Droplet contact
  - Airborne

Allergy

- IgE allergy
  - Most patients with nasal polyposis and/or chronic sinusitis possess bacteria-specific IgE, while subjects with only allergic rhinitis do not;
  - Multiple bacterial species isolated from chronically infected sinuses are capable of inducing IgE-mediated sensitization. (*Calenoff et al 1993)*
- IgG/cell mediated allergy
  - Machining coolant aerosols
  - Thermophilic actinomycetes

Endotoxins

General definitions

- **Endotoxins**: Toxic compounds found inside bacteria and other pathogens, lipopolysaccharides, cell-associated.
- **Exotoxins**: Secreted in soluble form, extracellular, diffusible.

Endotoxins (cont'd)

Endotoxins (in particular)

- Component of cell walls from gram-negative bacteria (*E. coli*, *Salmonella* etc.)
- Lipopolysaccharide (LPS) – Potent stimulator of the immune system

Detection with Limulus Amebocyte Lysate

Endotoxins (cont'd)

- Cell wall of Gram negative bacteria
- Induces fever, irritant, immune stimulant
- Geometric mean endotoxin concentrations: (in EU/mg):
  - Bedroom floors, 35.3 (5th–95th percentile, 5.0–260);
  - Bedding, 18.7 (2.0–142);
  - Family room floors, 63.9 (11.5–331);
  - Sofas, 44.8 (6.4–240);
  - Kitchen floors, 80.5 (9.8–512).
- Significant relationships between increasing endotoxin levels and diagnosed asthma, asthma symptoms in the past year, current use of asthma medications, and wheezing among adult residents.

Endotoxins (cont'd)

- Airborne endotoxin concentration: 0.49 ± 3.49 EU/m^3
- Doubling of the air endotoxin concentration was associated with an increase of 0.32 illness episodes per year ($p = 0.0003$).
- Short-term exposure in the air at levels > 45 EU/m^3 linked with decreases in lung function over the course of a single day. Longer-term exposures to endotoxin levels as low as 10-28 EU/m^3 may be linked with chronic decreases in lung function.

Endotoxins (cont'd)

- The ACGIH recommends that exposures more than ten times background levels be considered a concern if there are complaints of respiratory symptoms, and that exposures of 100 times background be avoided at all times.
- The Dutch Expert Committee on Occupational Standards of the National Health Council proposed a health-based recommended limit value for workers of 50 EU/m^3 over an eight-hour exposure period.

Endotoxins – Exposure Limits

- Recommendation of ~100 EU/m^3 as maximum exposure limit. Background levels of >10 EU/m^3 are of concern and >30 EU/m^3 should be avoided.
- Normal concentrations indoors: <1 EU/m^3
- Higher concentrations may indicate water damage.
- Concentrations increase up to 100-fold in rooms of smokers.

Endotoxins – Exposure Limits (cont'd)

When and where are endotoxins a problem?

- Occupational environment:
  - Waste collectors
  - Organic household composting facilities
  - Cotton mills
  - Power plants with biomass as biofuel
  - Biotech Industry
  - Metal grinding (metal working fluids)

Endotoxins – Asthma

- Relationship between endotoxin and asthma is still unclear: Some studies indicate that exposure to endotoxins may protect against allergic asthma but is a risk factor for non-allergic asthma.
- Smoking, presence of furred pets and cleaning regime influence endotoxin levels.
- Health effects are significant both in short and long term but depend on dose

Endotoxins – Sampling

- Preferred sample type is air (endotoxin-free filter cassettes)
- 250 – 1000 Liter sample volume
- Dust can also be used as sample type

Endotoxins – Control and Prevention

- Controlling water reduces possibility of Gram-negative bacteria (and endotoxin)
- Removal of contaminated materials and HEPA vacuuming can reduce endotoxin levels
- Do not smoke

Lethality of Bacterial Protein Toxins

Toxin	Toxic dose (mg)	Host	Compared to Strychnine
Botulinum toxin	0.8×10^{-8}	Mouse	3×10^{6}
Tetanus toxin	4×10^{-8}	Mouse	1×10^{6}
Shiga toxin	2.3×10^{-6}	Rabbit	1×10^{6}
Diphtheria toxin	6×10^{-5}	Guinea pig	2×10^{3}

Symbiosis

- *Lactobacillus acidophilus* is a harmless bacterium that resides in your intestines.
- *Lactobacillus acidophilus* helps you digest food, destroys some disease-causing organisms, and provides nutrients to your body.
- May also help prevent asthma.
- *E. coli* provides vitamins.

Sampling and Analysis

- Type of sample collection and choice of analytical methods depends on:
  - Hypothesis or goals (monitoring)
  - Expected concentrations
  - Agent(s) of concern
  - Standards/guidelines

Types of Samples and Relevant Hypotheses or Goals

- Water
  - This water contains sewage organisms
  - This water contains *Legionella pneumophila*
- Surface
  - Potential pathogens are falling into wounds during surgery
  - Residual contamination is present on these surfaces
- Bulk
  - This slime contains *Legionella pneumophila*
  - This humidifier water contains Thermophilic actinomycetes
- Air
  - Exposure is occurring to this agent
  - This activity produces aerosols containing this agent

Analytical Methods: Bacteria

- Culture
- Microscopy
- Stains
- DNA methods
- Bioassays
- Immunoassays
- HPLC
- GCMS

Culture

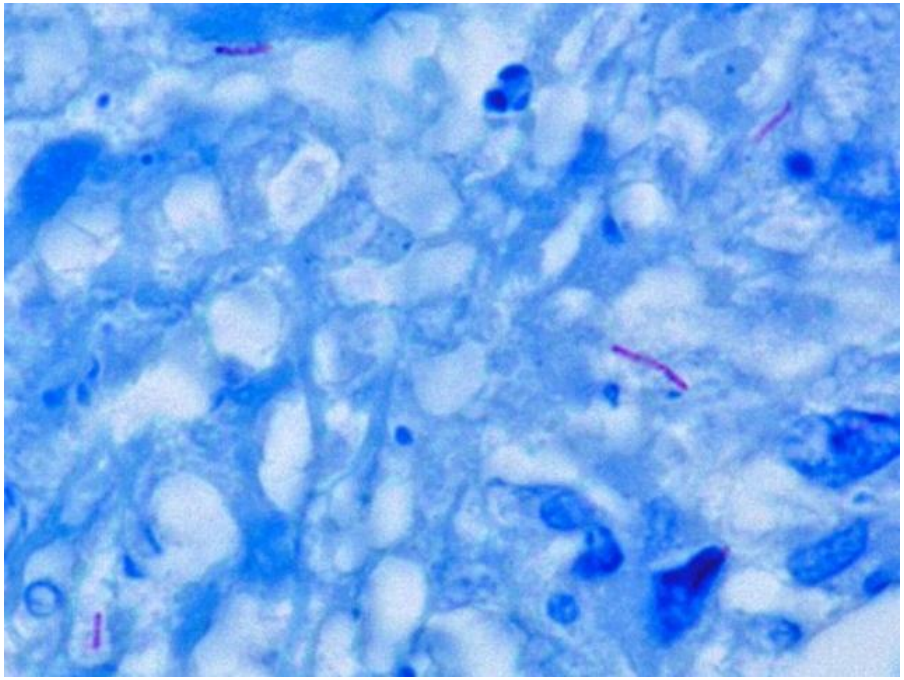
- Requires organism to be alive
- Recovers only organisms that can reproduce under the provided conditions
  - Best to use a broad spectrum medium which allows damaged bacteria to recover
- Always underestimates concentrations and diversity
- Hypotheses:
  - Potential pathogens are falling into wounds during surgery
  - *Legionella pneumophila* is growing in this humidifier

Microscopy

- Vital Staining
  - Use of stain that differentiates living from dead bacteria
  - All cells can be counted and % viable calculated
- Fluorescence staining
  - Allows microscopic or flow cytometric counting of cells
- Fluorescent antibody staining
  - Allows counting of specific organisms
- Can be used on all types of samples
- Hypotheses:
  - The bacterial aerosol has xx living and xx non-living organisms
  - This treatment kills bacteria

Other Kinds of Staining

- All require 1000x oil immersion microscopy
- Gram stain
- Acid fast staining
  - Mycobacterium cells are present in this sample
- Acridine orange (a fluorescent stain)
  - Total concentration of bacterial cells in this sample is xx



Mycobacterium tuberculosis (stained red) in tissue (blue).
Source: http://en.wikipedia.org/wiki/acid-fast

DNA Methods

- For identification
- For monitoring populations
  - This specific strain of *Legionella pneumophila* is present in this cooling tower
  - Track composition of bacterial populations are present in this aerosol
  - The bacterial population in this biofilm is the same as or different than the one in a different biofilm.

Bioassays

- Limulus assay for endotoxin
  - Depends on the horseshoe crab
  - Quantitative only within each batch of lysate
  - Internal controls essential for every assay
    - This sample contains more endotoxin than the outdoor air

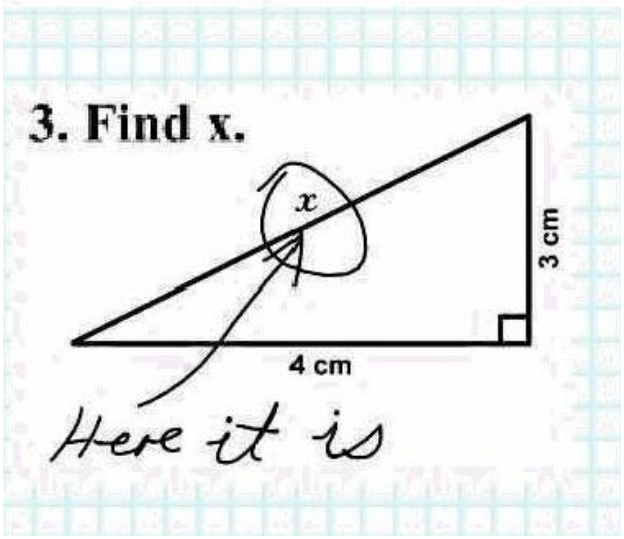
Immunoassays For Specific Bacteria

- There are immunoassays for surrogates of bacteria considered possible biowarfare agents (e.g., *Bacillus globigii* as a surrogate for *Bacillus anthracis*).
- Obviously it is possible since bacteria can stimulate an antibody (immune system) response. Such methods have not been widely used.

HPLC, GCMS

- Chemical methods used for measurement of bacterial and other biological chemicals.
- HPLC: high pressure liquid chromatography
- GCMS: gas chromatography mass spectroscopy
- Fatty acid analysis used for identification of bacteria in bulk samples

Continuing Education Units (CEUs)

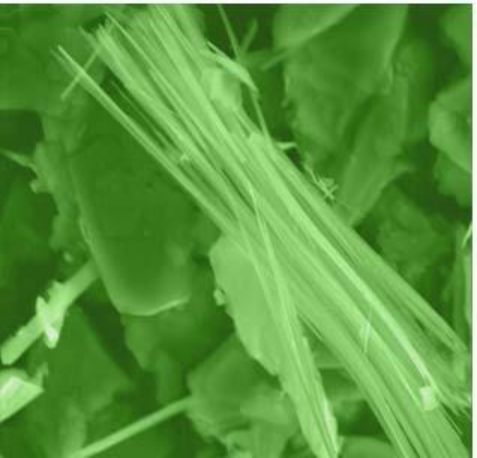


To receive a certificate of attendance, you must complete the survey after the webinar:

- Click on the survey link in the "Thank you" email (sent 1 hour after this webinar).
- Complete survey by this Friday, March 7, 2014.
- You will receive an email in 2-3 weeks when your certificate is ready.

CET

CENTER FOR ENVIRONMENTAL TRAINING



Five Minute Break

Presented by:

EMLAB P&K

THE PROVEN LEADER®

A TestAmerica Company

Legionella and Legionnaires' Disease

- Nature of *Legionella*
- Natural reservoirs
- Human exposure
- Sampling strategies
  - Hypotheses
  - Sampling plans
  - Data interpretation

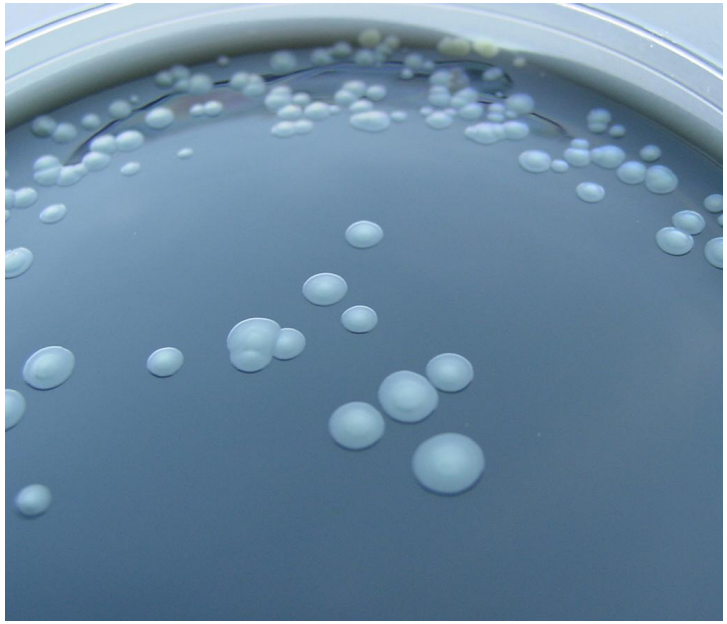
Nature and Ecology

- Gram negative rod-shaped bacterium
- Widely distributed natural inhabitants of waters.
- Significant multiplication restricted to 20° C to 45° C
- Growth promoted by other micro organisms: amoebae amplify *Legionellae* multiplication
- Other bacteria and algae provide nutrients
- Low concentrations of metals such as iron, zinc and potassium enhance proliferation
- The constituents of certain types of rubbers used in rubber fittings in water and cooling systems can also support the multiplication of *L. pneumophila.*

Legionella

Legionella

- Gram negative bacteria common in many environments
- Approx. 50 species and 70 serogroups have been described
- *Legionella* is the causative agent of Legionellosis (Legionnaires' disease and Pontiac fever)



Legionellosis

Legionnaires' disease

- The first recognized outbreak of the disease occurred 1976 in Philadelphia
- As many as 221 people were treated and 34 deaths occurred.
- The source was identified as the *Legionella* bacterium and found in the cooling tower of a hotel's air conditioning system.
- Over 90% of legionelloses are caused by *Legionella pneumophila*

Skyline Philadelphia

Legionellosis (cont'd)

Legionellosis takes two distinct forms:

1. **Pontiac fever:** respiratory illness without pneumonia, symptoms resemble acute influenza
2. **Legionnaires' disease:** symptoms include fever, chills, cough, muscle aches, headache, tiredness, loss of appetite, loss of coordination (ataxia), and occasionally diarrhea and vomiting.
   - *L. pneumophila* infections may be subclinical.
   - Antibodies present in up to 25% of adults tested.

Legionellosis (cont'd)

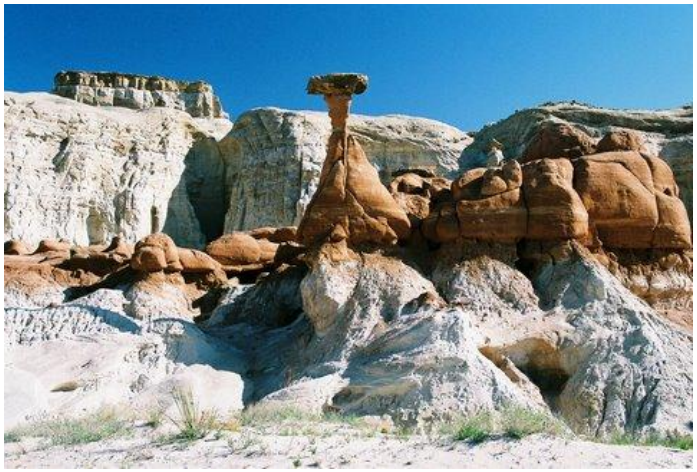
- 2-10 day incubation
- One of the top three causes of community-acquired pneumonia
- 8,000 to 18,000 people get legionellosis in U.S. each year
- Many cases go undiagnosed
- Transmission is not person to person
- Worldwide distribution, although outbreaks of Legionnaires' Disease are more common in the northeast U.S., England, Australia, the Netherlands
- Treatable with antibiotics if diagnosed early
- Diagnosed with chest x-rays and laboratory confirmation

Legionellosis (cont'd)

Community and Hospital Acquired

Risk factors:

- Age
  - Highest risk in elderly >65
  - Not common in people <50
  - Very rare in people <20
- Smoking
- Pre-existing chronic obstructive pulmonary disease (COPD), diabetes
- Compromised immune system



Epidemiology

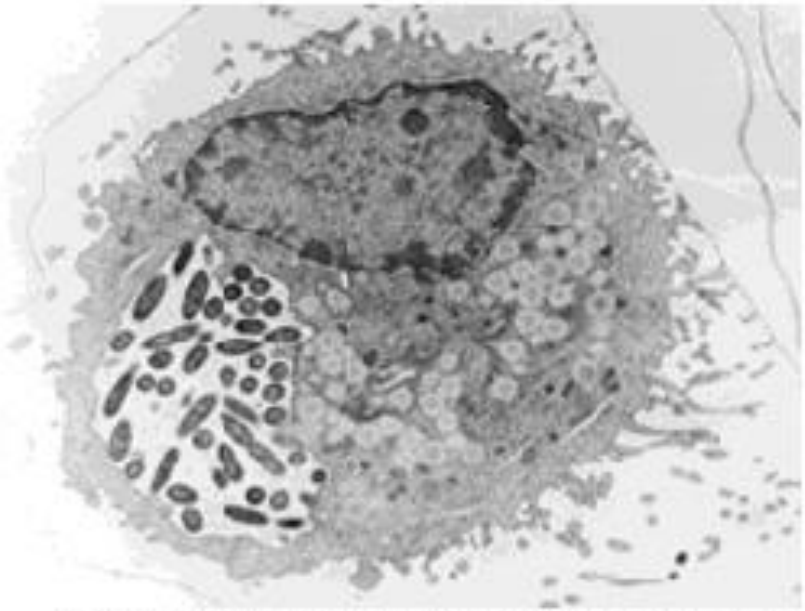
Infection and Transmission

- *Legionellosis* infection occurs after inhaling water droplets that originated from a water source contaminated with *Legionella*.
- Typical manmade water sources include cooling towers, evaporative coolers, hot water systems, showers, whirlpool spas, architectural fountains, room-air humidifiers, ice-making machines, misting equipment.
- Environmental sources for *Legionella* are freshwater ponds, rivers and creeks.
- *Legionella* survives in the environment as intracellular parasites of freshwater protozoa.

Epidemiology (cont'd)

Temperature requirements for growth:

- *Legionella* bacteria will grow in water at temperatures 20°C to 50°C (68°F to 122°F).
- Ideal growth conditions are in stagnant water (95°F to 115°F)



Legionella pneumophila multiplying inside a human monocyte
M. Horwitz

Sampling – Swabs and Air

Wear Respiratory Protection:

- Wear appropriate respiratory protection during the examination of water systems if a significant potential exists for exposure to high concentrations of contaminated aerosols (e.g. operating spray humidifier).
- **Swabs:** Sampling of biofilm (slime) or on water outlets (e.g. inside of shower heads). Use sterile swab and keep moist.
- **Air:** Air samples collected on special culture plates with an Andersen-type sampler rarely demonstrate the presence of *Legionella* in the air. Not recommended.

Sampling – CDC Method

Water samples:

Non-potable water source

- Examples: cooling towers, chillers, condensate pans, surface water in reservoirs, sprinklers.
- Collect 250 mL water from the bottom or side of the vessel or reservoir.

Potable water source

- Use 1 liter bottles containing thiosulfate to neutralize chlorine.
- Collect a 250-mL to 1-Liter “pre-flush” sample of the first water drawn from bottom drains and outlet valves of storage tanks, sumps, and water heaters as will as faucets and showerheads.
- Run water until temperature stabilizes and collect a second “post-flush” sample when water temperature is constant (after ~60 sec.).

Sampling – Shipping

Shipping

- Samples should be protected from temperature extremes such as sunlight or other external heat or cold sources during transport and storage, for example, temperatures below 3°C (37°F) and above 30°C (86°F).
- Use non-leaking sealed containers and overnight shipping.
- Label sample clearly and include Chain of Custody.

Source: http://en.wikipedia.org/wiki/File:Fedexgroundtruck.jpg

Sampling – Analysis

- Impinger or a six-stage microbial impactor for detecting legionellae in air around a cooling tower contaminated with *L. pneumophila* ($1.2 \pm 0.3 \times 10^5$ CFU/100 ml).
- *L. pneumophila* SG 6 detected in the air around the cooling tower by the impinger (0.09 CFU/l. air).
- No legionellae were detected by the impactor with *Legionella-selective* agar plates (WYOa) because the plates were overgrown with fungi.
- PCR (rep-PCR, AP-PCR) were used to assess relationships among *Legionella* isolates from the air and the cooling tower water. *L. pneumophila* SG 6 isolated from the aerosols produced rep-PCR and AP-PCR fingerprints identical to those of *L. pneumophila* SG 6 strains from the cooling tower water.

Water Treatment

Water treatment options to eradicate *Legionella*

- Thermal Eradication
- Copper-Silver Ionization (ionization unit)-best long term treatment
- Chlorination
- Ozonation
- Chlorine Dioxide
- Ultraviolet Irradiation (point of delivery treatment)

Heat treatment:

70-80° C (158-176° F): Disinfection range

At 66° C (151° F): Legionellae die within 2 minutes

At 60° C (140° F): Legionellae die within 32 minutes

At 55° C (131° F): Legionellae die within 5 to 6 hours

Legionella – Analysis

***Legionella* testing:**

- CDC and ISO method are commonly used.
- Culture analysis: 10 – 14 days. Culture analysis is considered the “gold standard.”
- Detection of several species and serotypes of *Legionella* can be done by culture on selective media followed by species- or type-specific staining.
- PCR test for *L. pneumophila* can be performed in 1-2 days and is helpful in outbreak situations.

Legionella – Thresholds

- No concrete threshold and action limits for *Legionella*.
- The European Working Group for *Legionella* Infections (EWGLI) published the following guidelines and action limits for cooling towers.

CFU of *Legionella* per Liter	**Action**
< 1000	System under control
1000 – 10,000	Review program operation. Conduct re-sampling. Review of control measures and risk assessment should be carried out to identify any remedial actions.
> 10,000	Implement corrective action. The system should immediately be re-sampled.

Legionella Action Levels (cfu/ml)

The OSHA Technical Manual offers the following guidelines for interpreting *Legionella* analysis.

Action	Cooling Tower	Domestic Water	Humidifier
1	100	10	1
2	1,000	100	10

- Action 1: Prompt cleaning and/or biocide treatment of the system.
- Action 2: Immediate cleaning and/or biocide treatment. Take prompt steps to prevent employee exposure.

Legionella – Thresholds

- Threshold limits for potable water especially in hospitals and nursing homes should be considerably lower.
- Goal for “sensitive locations” is a zero count for *Legionella* (detection limits are typically around 100 cfu/liter.

Legionella – More Information

More information and literature can be found at:

- http://en.wikipedia.org/wiki/Legionnaire%27s_Disease
- http://www.cdc.gov/legionella/index.htm
- http://www.ewgli.org/

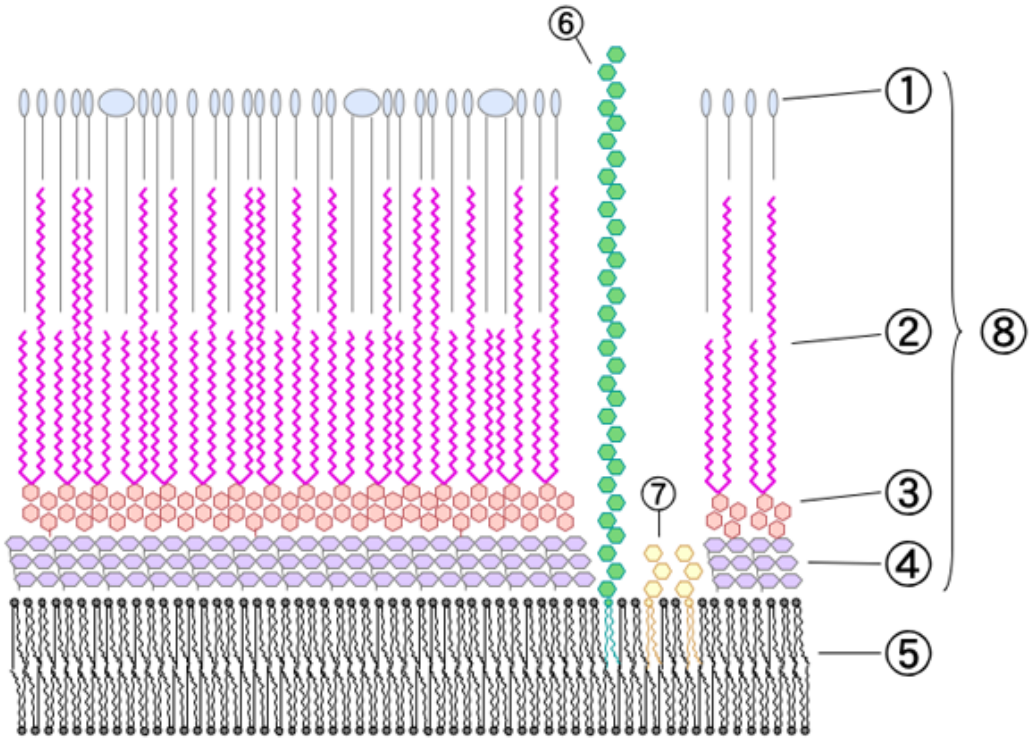
Mycobacteria

- Aerobic non-motile bacteria (curved rods)
- Acid-alcohol fast
- Gram-positive (taxonomically, not in the actual staining procedure)
- Unusually thick cell wall containing mycolic acids
- Slow growing

TEM Micrograph or *Mycobacterium tuberculosis*

Source: Wikipedia, the free encyclopedia (online)

Mycobacteria – Cell Wall



Source: Wikipedia, the free encyclopedia (online)

Mycobacteria cell wall

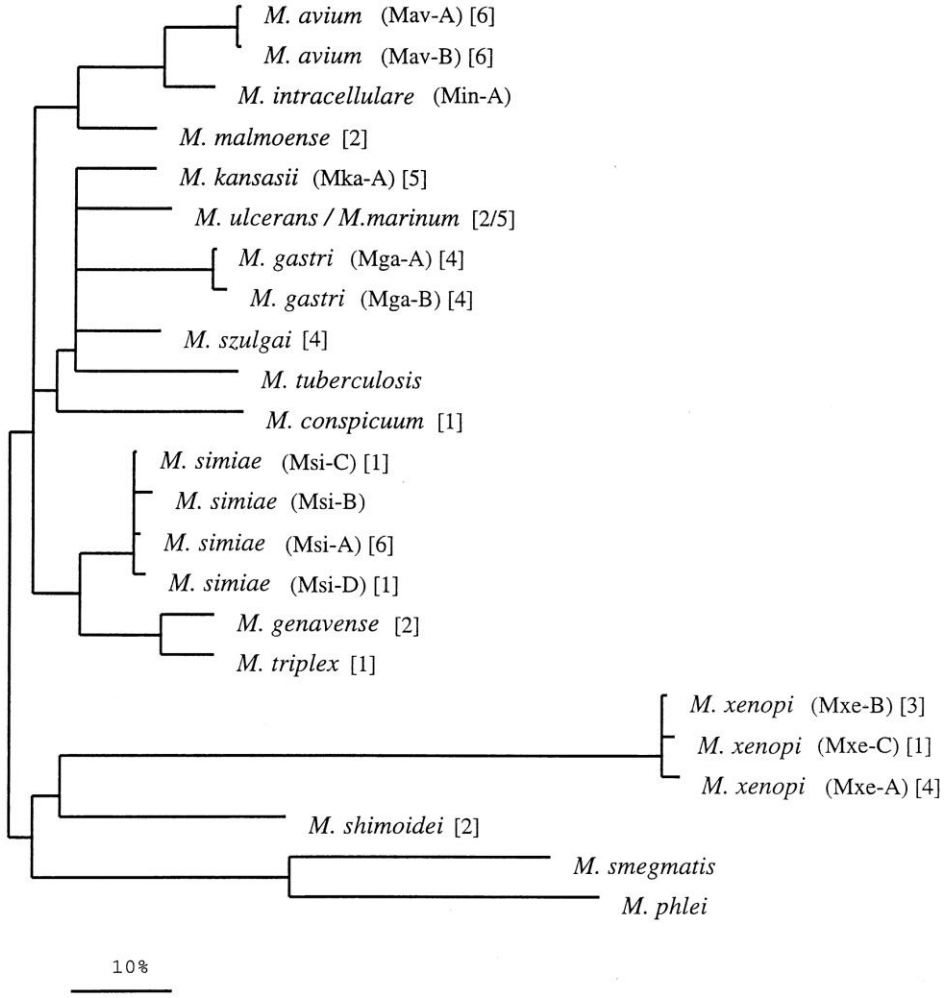
1. outer lipids
2. mycolic acid
3. polysaccharides (arabinogalactan)
4. peptidoglycan
5. plasma membrane
6. lipoarabinomannan (LAM)
7. phosphatidylinositol mannoside
8. cell wall skeleton

Mycobacteria – Classification

- **Runyon classification**: Characterization of environmental mycobacteria based on the rate of growth, production of pigment
- Runyon I: **Photochromogens** (slow growing, produce a yellow-orange pigment when exposed to light)
- Runyon II: **Scotochromogens** (slow growing, produce a yellow-orange pigment in light or in the dark)
- Runyon III: **Nonchromogenic** (slow growing, and do not produce pigment)
- Runyon IV: **Rapid Growers** (colonies visible in 5 days)

Mycobacteria – Classification (cont'd)

- Classification by DNA sequence analysis
- Other characterization/ classification systems use a variety of chemical reactions and properties



Distance matrix tree showing the divergence of ITS sequences of the investigated mycobacteria.

Source: Roth et al. 1998.

Two Distinct Groups of Mycobacteria

Virulent Pathogens

- *Mycobacteria tuberculosis*
- *M. leprae*

Infect all those exposed who do not have specific immunity.

Opportunistic Pathogens

- *M. avium complex*
- *M. chelonea*
- *M. fortuitum*

and many more

True inhabitants of the environment (saprophytes, commensals & symbionts). Over 90 species of NTM (NonTuberuberculosis Mycobacteria) identified.

Environmental Mycobacteria

Nomenclature:

- Environmental mycobacteria = nontuberculous mycobacteria (NTM) or mycobacteria other than tuberculosis mycobacteria (MOTT)

Definition:

- Common saprophytes in all natural ecosystems, including water, soil, food, dust, and aerosols. Some species are also pathogenic for humans or animals, causing pulmonary and cutaneous disease, lymphadenitis, and disseminated infections.

Environmental Mycobacteria Are…

- True inhabitants of a wide variety of environmental reservoirs, including natural and municipal water, soil, aerosols, protozoans, animals, and humans.
- Resistant to high chlorine and biocide levels
- Able to form biofilms
- Slow growing and difficult to detect

Diseases Caused by Environmental Mycobacteria

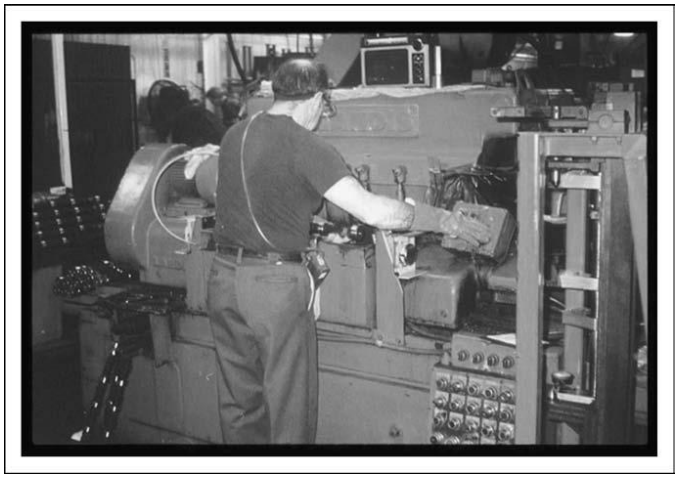
- Pulmonary disease (hot-tub lung) (a hypersensitivity disease
- Lymphadentitis (inflammation of lymph nodes)
- Infections of soft tissue/skin
- Disseminated disease (e.g. AIDS patients)
- Associated with Crohn’s disease (chronic bowel disease)

Transmission and Infection

- Transmission of environmental mycobacteria is not from human-to human or animal-to-human. Disease results from environmental exposure
- Aerosol associated infections (hypersensitivity pneumonitis)
  - Occupational: metal working fluids, life guards (swimming pools)
  - Home: aerated hot tubs, spas, humidifiers, water damaged building material

Mycobacteria in Metalworking Fluids

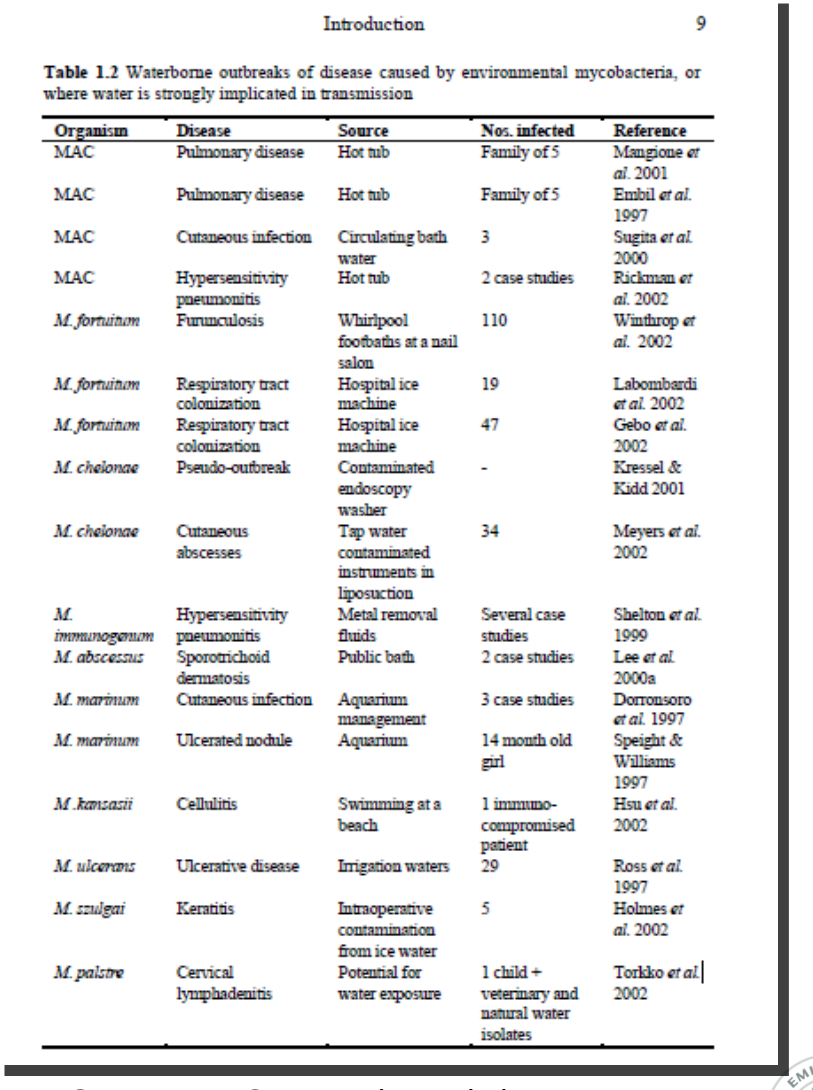
- Hypersensitivity pneumonitis (HP) has been associated with exposure of workers to metalworking fluids
- Recent work has focused on the presumed relationship between the microbiological contamination of MWF with *M. immunogenum* as the cause of hypersensitivity pneumonitis



Source: Current Opinion in Allergy and Clinical Immunology 2009, 9:97–102

Mycobacteria in Water

- Mycobacteria are commonly found in municipal water.
- A number of mycobacteria infections were reported and documented since the 1970s.
- Mycobacteria infections are often misdiagnosed and likely underreported.



Introduction 9

Table 1.2 Waterborne outbreaks of disease caused by environmental mycobacteria, or where water is strongly implicated in transmission

Organism	Disease	Source	Nos. infected	Reference
MAC	Pulmonary disease	Hot tub	Family of 5	Mangione *et al.* 2001
MAC	Pulmonary disease	Hot tub	Family of 5	Embil *et al.* 1997
MAC	Cutaneous infection	Circulating bath water	3	Sugita *et al.* 2000
MAC	Hypersensitivity pneumonitis	Hot tub	2 case studies	Rickman *et al.* 2002
M. fortuitum	Furunculosis	Whirlpool footbaths at a nail salon	110	Winthrop *et al.* 2002
M. fortuitum	Respiratory tract colonization	Hospital ice machine	19	Labombardi *et al.* 2002
M. fortuitum	Respiratory tract colonization	Hospital ice machine	47	Gebo *et al.* 2002
M. chelonae	Pseudo-outbreak	Contaminated endoscopy washer	-	Kressel & Kidd 2001
M. chelonae	Cutaneous abscesses	Tap water contaminated instruments in liposuction	34	Meyers *et al.* 2002
M. immunogenum	Hypersensitivity pneumonitis	Metal removal fluids	Several case studies	Shelton *et al.* 1999
M. abscessus	Sporotrichoid dermatosis	Public bath	2 case studies	Lee *et al.* 2000a
M. marinum	Cutaneous infection	Aquarium management	3 case studies	Dorronsoro *et al.* 1997
M. marinum	Ulcerated nodule	Aquarium	14 month old girl	Speight & Williams 1997
M. kansasii	Cellulitis	Swimming at a beach	1 immuno-compromised patient	Hsu *et al.* 2002
M. ulcerans	Ulcerative disease	Irrigation waters	29	Ross *et al.* 1997
M. szulgai	Keratitis	Intraoperative contamination from ice water	5	Holmes *et al.* 2002
M. palstre	Cervical lymphadenitis	Potential for water exposure	1 child + veterinary and natural water isolates	Torkko *et al.* 2002

Source: WHO – Mycobacteria in Water

Epidemiology

- 1.8 cases of nontuberculous diseases per 100,000 persons in the U.S. were estimated in the early 1980s. No current data for the U.S. available.
- Recent analysis from Ontario (Canada) found average annual increase of 8.4% for the isolation prevalence of NTM.
- Detection of NTM has improved significantly.

Epidemiology (cont'd)

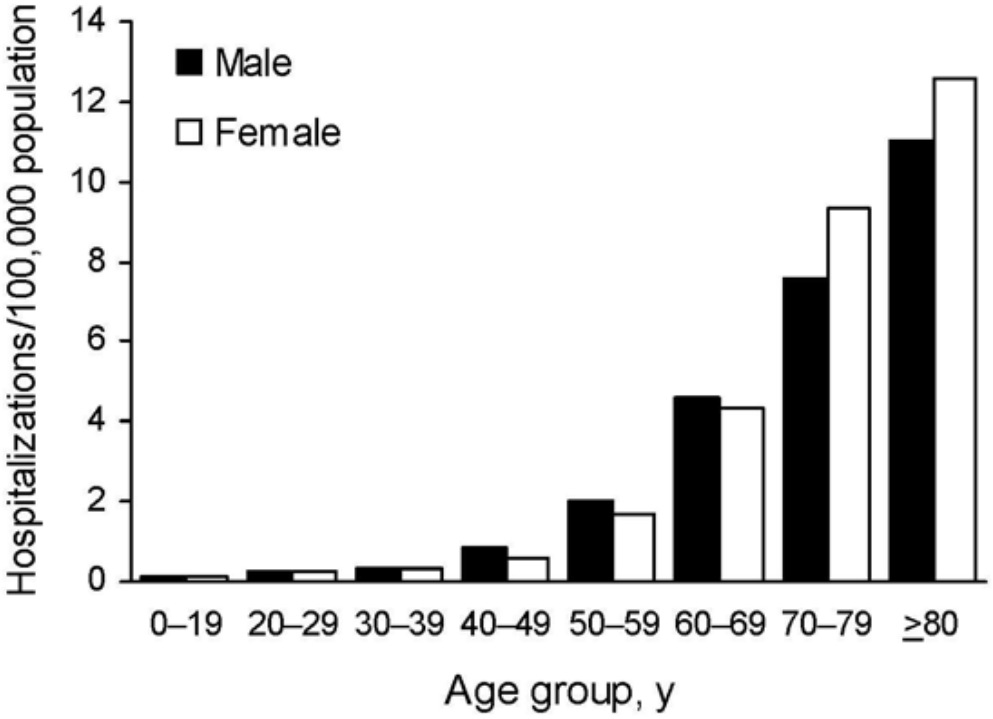
Primary diagnoses, non-AIDS pulmonary NTM-associated hospitalizations, HCUP-SID, USA, 1998–2005*		
ICD-9 code	**Primary diagnosis**	**No. (%)**
310	Pulmonary NTM	5,148 (31.25)
482	Pneumonia	1,156 (7.01)
49121	Obstructive chronic bronchitis with acute exacerbation	821 (4.98)
51881	Acute respiratory failure	392 (2.38)
4280	Congestive heart failure, unspecified	225 (1.37)
4941	Bronchiectasis with acute exacerbation	216 (1.31)
2765	Volume depletion	196 (1.19)
515	Postinflammatory pulmonary fibrosis	186 (1.13)
5070	Aspiration pneumonia caused by inhalation of food/vomitus	176 (1.07)
	Other primary diagnosis <1% of population	7,959 (48.3)

*NTM, nontuberculous mycobacteria; HCUP, Healthcare Cost and Utilization Project; SID, state inpatient databases; ICD-9, International Statistical Classification of Diseases, Revision 9.

Source: http://www.cdc.gov/eid/content/15/10/1562.htm

Epidemiology (cont'd)

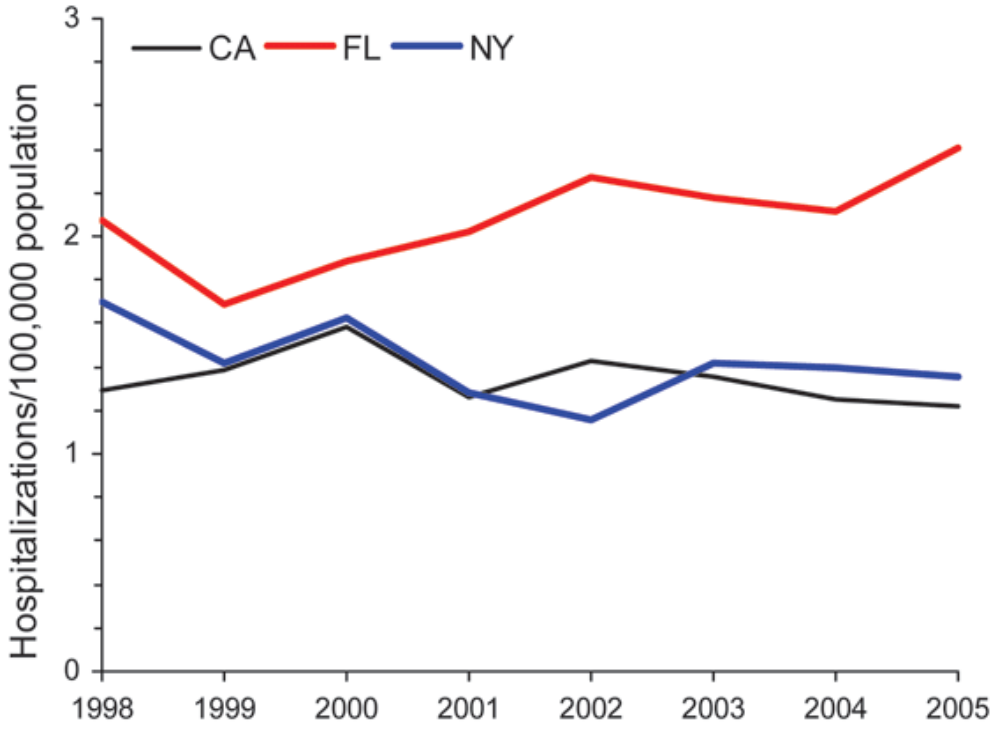
Average annual prevalence of non-AIDS pulmonary nontuberculous mycobacteria-associated hospitalizations by age group and sex, Healthcare Cost and Utilization Project state inpatient databases, USA, 1998–2005.



Source: http://www.cdc.gov/eid/content/15/10/1562.htm

Epidemiology (cont'd)

Age-adjusted prevalence of non-AIDS pulmonary nontuberculous mycobacteria-associated hospitalizations among men, California (CA), Florida (FL), and New York (NY), USA, Healthcare Cost and Utilization Project state inpatient databases, 1998–2005.



Source: http://www.cdc.gov/eid/content/15/10/1562.htm

Mycobacteria Summary

- Non-tuberculosis mycobacteria (NTM) is a significant and increasing cause of pulmonary illnesses in the United States.
- Prevalence of pulmonary NTM-associated hospitalizations is increasing in selected geographic areas.
- It has been estimated that in the U.S., 25% to 50% of individuals with AIDS will develop NTM diseases, primarily attributable to MAC.
- Waterborne NTM have been associated with hospital (nosocomial) infections worldwide.

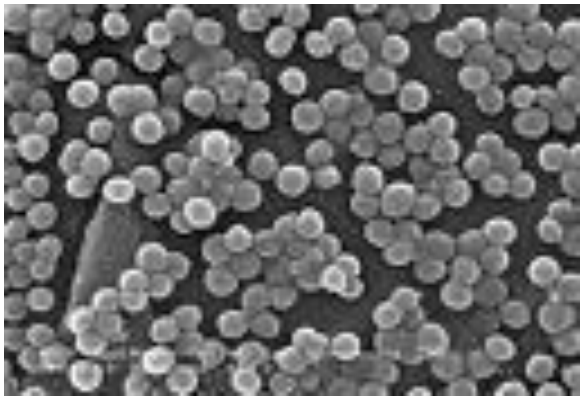
Mycobacteria Summary (cont'd)

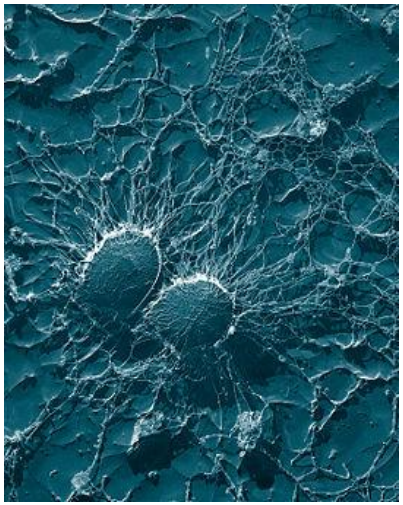
- In particular metalworking fluids and water in hospital transplant units should be monitored for NTM.
- Environmental mycobacteria (nontuberculous mycobacteria) are distinctly different from the obligate pathogens of the *M. tuberculosis* complex.
- NTM have been identified in numerous environmental sources, including water.
- NTM are not thought to be transmitted by the human to human route, but are instead thought to be transmitted from environmental sources.

MRSA and *Staphylococci* – Overview

Staphylococcus aureus

- Gram-positive bacteria
- 0.5 – 1 μm diameter
- Commonly found on the skin and in the nose of humans





MRSA and *Staphylococci* – Overview

- Approximately one third of the world's population has *S. aureus* bacteria on their body at any given time.
- About 1% of people carrying *S. aureus* have MRSA (CDC estimated).
- Spread from one person to another through casual contact or contaminated objects.
- Estimated 94,000 cases of MRSA infections in the U.S. per year and nearly 19,000 deaths.

Staphylococci – Symptoms

Skin infections

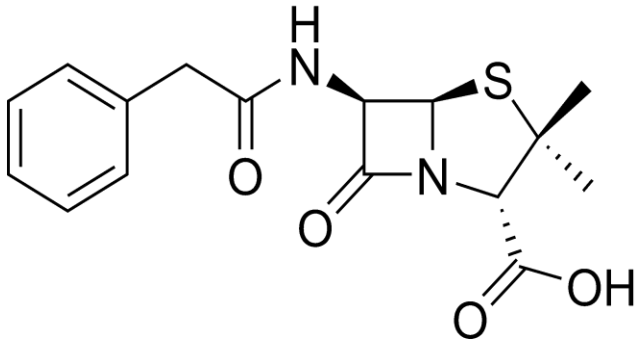
- Folliculitis (hair roots)
- Impetigo (blisters)
- Skin abscesses
- Cellulitis
- Necrolysis

S. aureus – Symptoms

- *Staphylococci* tend to infect skin but can travel through the bloodstream and involve almost any site in the body, particularly the heart and the bones.
- May infect the respiratory tract, mainly in people with chronic lung disease or influenza and lead to staphylococcal pneumonia.
- Other severe and life-threatening infections with *staphylococci* include victims of severe burns and heart infections.

MRSA – Resistance

- MRSA is a resistant variation of the common bacterium *Staphylococcus aureus*. It has evolved an ability to survive treatment with beta-lactam antibiotics, including penicillin, methicillin, and cephalosporins
  - Hospital acquired (HA)
  - Community acquired (CA)



Hospital Acquired MRSA

- Most infections with MRSA occur in hospitals and healthcare facilities (HA-MRSA) including:
  - Surgical would infections
  - Urinary tract infections
  - Blood stream infections and pneumonia

Hospital Acquired MRSA (cont'd)

Risk factors for HA-MRSA:

- Current or recent hospitalization
- Residing in a long-term care facility
- Invasive devices
- Recent antibiotic use

Prevention:

- Ask hospital staff to wash their hands
- Wash your own hands frequently
- Make sure invasive devices are kept sterile

Community Acquired MRSA

Community acquired MRSA recently gained much attention in the news media.

Risk factors:

- Young age (children)
- Participating in contact sports
- Sharing towels and athletic equipment
- Weakened immune system
- Living in crowded and/or unsanitary conditions
- Association with health care workers

Community Acquired MRSA (cont'd)

Prevention

- Washing hands frequently
- Keep personal items personal
- Keep wounds covered
- Shower after athletic games or practices
- Do not participate in athletic games or practices if you have infected wounds
- Sanitize items
- Get tested if you have a skin infection
- Use antibiotics appropriately

Diagnosis and Environmental Testing

Clinical testing

- Sample from an infected site or a nasal swab
- Clinical laboratory performs the testing

Environmental testing

- Collect swab samples from items that are frequently touched such as door knobs, keyboards, athletic equipment etc.
- Environmental laboratory can perform the testing for MRSA

Sewage – Evaluating Hazards

- Nature of Sewage
- Microorganisms
- Human exposure
  - Direct contact
  - Ingestion
  - Aerosols
- Sampling Strategies
  - Hypotheses
  - Sampling plans
  - Data Interpretation

Nature of Sewage

- Sewage is the water found in sewers.
- “Used” water from homes, workplaces, surface runoff.
- Contains waste products of human, animal, vegetable and mineral origin.
  - Suspended solids
  - Solutes
  - Bacteria and other sewage microorganisms
- On average each of us generates between 135 and 180 liters of sewage each day.
- This sewage is over 99.9% liquid, with less than 0.1% being solid.

Who Is At Risk?

- Employees involved in sewer inspection and maintenance work
- Construction workers who repair or replace live sewers
- Water company employees who work with sewage treatment plants
- Agricultural and forestry workers who may be exposed to sewage sludge
- Sludge tanker drivers/operators and associated maintenance staff
- Plumbers
- Employees who clean and maintain the underside of railway carriages and empty aircraft sewage compartments and other types of portable lavatories

Aerosol Exposure

Wastewater Treatment

- Average concentrations of 17000 cfu/ml of mesophilic, 2100 cfu/ml of TSA-SB bacteria (bacteria associated with certain waterborne virulence factors) in the water.
- In the aerosol of the fixed-film reactor 3000 cfu/m^3 mesophilic and 730 cfu/m^3 TSA-SB bacteria.

What Are The Health Risks?

- Gastroenteritis: cramping stomach pains, diarrhea and vomiting
- Weil's disease: a flu-like illness with persistent and severe headache; damage to liver, kidneys and blood may occur and the condition can be fatal
- Hepatitis: inflammation of the liver, and jaundice
- Occupational asthma: attacks of breathlessness, chest tightness and wheezing; produced by the inhalation of living or dead organisms
- Infection of skin or eyes
- Rarely, allergic alveolitis: (inflammation of lung) with fever, breathlessness, dry cough, and aching muscles and joints

How Do Sewage Micro-organisms Enter The Body?

- Hand-to-mouth contact during eating, drinking and smoking,
- Wiping the face with contaminated hands or gloves, or by licking splashes from the skin.
- Skin contact, through cuts, scratches, or penetrating wounds, i.e. from discarded hypodermic needles.
- Aerosols landing on surfaces of the eyes, nose and mouth.
- By breathing them in, as either dust, aerosol or mist.

Coliforms

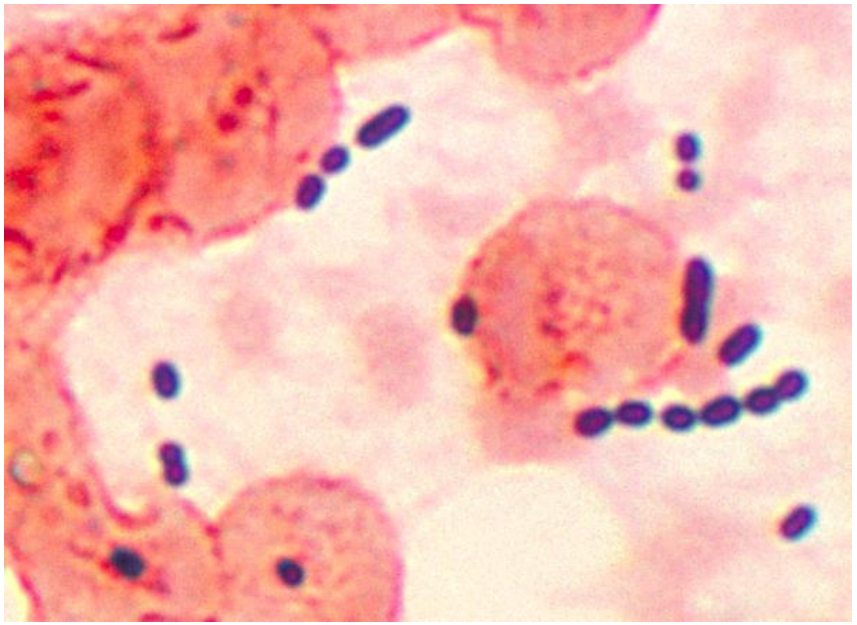
- Rod-shaped Gram-negative non-spore forming organisms that ferment lactose with the production of acid and gas when incubated at 35-37° C.
- Coliforms are abundant in the feces of warm-blooded animals, but can also be found in the aquatic environment, in soil and on vegetation.
- TAXA
  - *Citrobacter*
  - *Enterobacter*
  - *Escherichia*
  - *Hafnia*
  - *Klebsiella*
  - *Serratia*
  - *Yersinia*

Water Quality Coliform Guidelines

- Culture based
  - 200 colonies of fecal coliform bacteria /100ml for primary contact recreation
  - 1000 colonies of fecal coliform bacteria /100ml for secondary contact recreation
- Swimming beaches:
  - geometric mean of **126 *E.coli*** bacteria per 100 ml of water (fresh water)

Enterococcus

- May provide a higher correlation than fecal coliforms with many human pathogens.
- In 2004, *Enterococcus spp.* took the place of fecal coliforms as the new federal standard for water quality at public beaches.



Enterococcus infection in pulmonary tissue.
Source: wikipedia.org

- The acceptable level of contamination is very low,
  - Hawaii: 7 colony forming units per 100 ml of water
  - Geometric mean of **35** / 100 ml of water for five samples over 30 days and an instantaneous (single sample) standard of 104 / 100 ml of water (salt water).

Sampling Equipment and Supplies

Air pumps & samplers

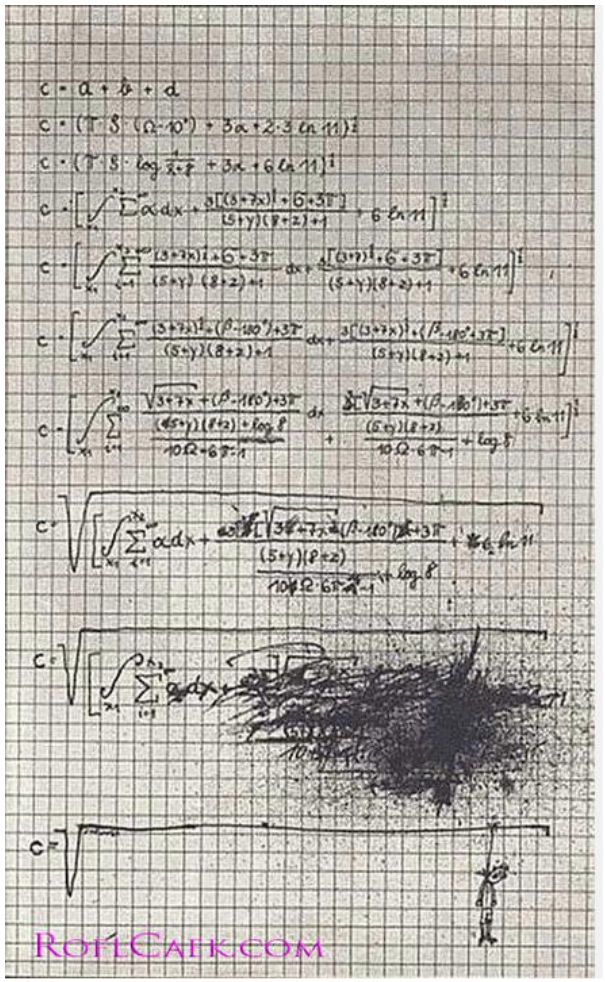
Swabs

Water bottles

Media plates

Shop online at www.emlabpk.com/store

Continuing Education Units (CEUs)



To receive a certificate of attendance, you must complete the survey after the webinar:

- Click on the survey link in the "Thank you" email (sent 1 hour after this webinar).
- Complete survey by this Friday, March 7, 2014.
- You will receive an email in 2-3 weeks when your certificate is ready.

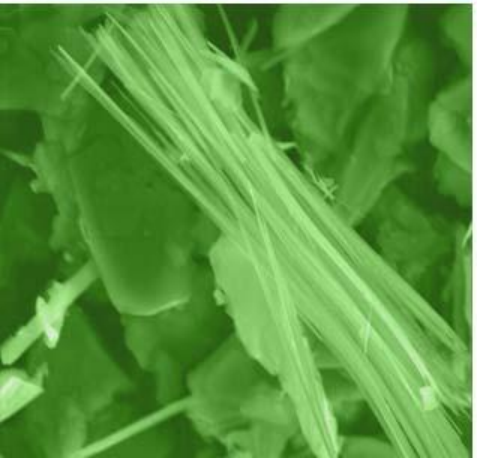
Thank you for your time!

Questions about Bacteria:
DGallup@emlabpk.com

All other questions:
webinars@emlabpk.com

CET

CENTER FOR ENVIRONMENTAL TRAINING



Appendix

Presented by:

EMLab P&K

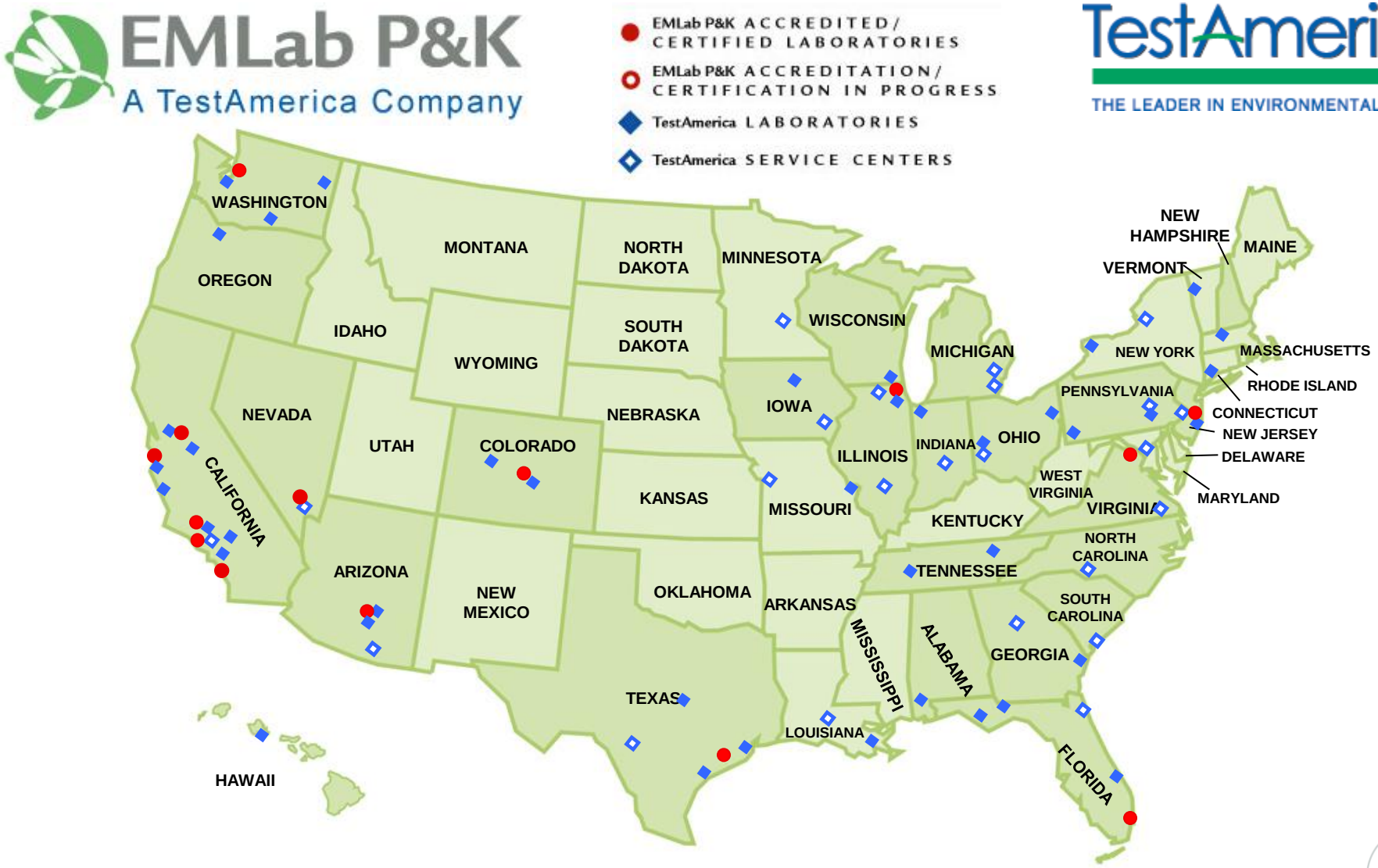
EMLAB P&K

THE PROVEN LEADER®

A TestAmerica Company

Locations Nationwide to Serve You

(Addresses on following slides)



EMLab P&K Locations Near You

For the most current list of locations, please visit us at www.emlabpk.com
Contact individual laboratories for service capabilities and scopes of accreditation.

EMLab P&K
A TestAmerica Company
LABORATORIES

Arizona - Phoenix
1501 West Knudsen Drive
Phoenix, AZ 85027
phone: 800.651.4802
fax: 623.780.7695
AIHA EMLAP# 102297

California - San Bruno
1150 Bayhill Drive, Suite 100
San Bruno CA 94066
phone: 866.888.6653
fax: 650.829.5852
AIHA EMLAP# 102856

New Jersey - Marlton
3000 Lincoln Drive East, Suite A
Marlton, NJ 08053
phone: 866.871.1984
fax: 856.489.4085
AIHA EMLAP #103005

EMLab P&K
A TestAmerica Company
MICROLABS

MicroLabs in bold are AIHA Accredited as documented by the Scope of Accreditation Certificate.

California - Glendale
1010 N. Central Ave.
Suite 420
Glendale, CA 91202
phone: 866.465.6653

California - Irvine
17461 Derian Ave
Suite 100
Irvine, CA 92614
Phone: 866.465.6653

California - Sacramento
880 Riverside Parkway
West Sacramento, CA 95605
phone: 866.888.6653

California - San Diego
9089 Clairemont Mesa Blvd.
Suite 106
San Diego, CA 92123
Phone: 866.465.6653

Colorado - Denver
4955 Yarrow Street
Arvada, CO 80002
phone: 800.651.4802

Florida - Ft. Lauderdale
6301 NW 5th Way
Suite 2850
Ft. Lauderdale, FL 33309
phone: 877.711.8400

Illinois - Chicago
655 W. Grand Ave.
Suite 205
Elmhurst, IL 60126
phone: 866.871.1984

Nevada - Las Vegas
6000 S. Eastern Ave.
Suite 5E
Las Vegas, NV 89119
phone: 866.888.6653

Texas - Houston
6310 Rothway St.
Houston, Texas 77040
phone: 800.651.4802

Virginia - Fairfax
3929 Old Lee Highway
Unit 91C
Fairfax, Virginia 22030
phone: 866.871.1984

Washington - Seattle
19515 North Creek
Parkway N, Suite 100
Bothell, WA 98011
phone: 866.888.6653

TestAmerica Laboratories

For your convenience, you can drop off samples for EMLab P&K at these locations.
For the most current list of locations, please visit us at www.emlabpk.com

TestAmerica
THE LEADER IN ENVIRONMENTAL TESTING

ALABAMA - Mobile
900 Lakeside Drive
Mobile, AL 36693
phone: (251) 666-6633

ALASKA - Anchorage
2000 W. International Airport Rd., Suite A10
Anchorage, AK 99502
phone: (907) 563-9200

ARIZONA - Phoenix
4625 E. Cotton Center Blvd., Suite 189
Phoenix, AZ 85040
phone: (602) 437-3340

CALIFORNIA - Irvine
17461 Derian Ave., Suite 100
Irvine, CA 92614
phone: (949) 261-1022

CALIFORNIA - Los Angeles
3585 Cadillac Ave, Suite A
Costa Mesa, CA 92626
phone: (714) 258-8610

CALIFORNIA - San Francisco
1220 Quarry Ln.
Pleasanton, CA 94566
phone: (925) 484-1919

CALIFORNIA – W. Sacramento
880 Riverside Pkwy
West Sacramento, CA 95605
phone: (916) 373-5600

COLORADO - Denver
4955 Yarrow St.
Arvada, CO 80002
phone: (303) 736-0100

CONNECTICUT
128 Long Hill Cross Rd.
Shelton, CT 06484
phone: (203) 929-8140

FLORIDA - Pensacola
3355 McLemore Dr.
Pensacola, FL 32514
phone: (850) 474-1001

FLORIDA - Tallahassee
2846 Industrial Plaza Dr.
Tallahassee, FL 32301
phone: (850) 878-3994

FLORIDA - Tampa
6712 Benjamin Rd., Suite 100
Tampa, FL 33634
phone: (813) 885-7427

GEORGIA - Savannah
5102 LaRoche Avenue
Savannah, GA 31404
phone: (912) 354-7858

HAWAII - Honolulu
99-193 Aiea Heights Dr.
Suite 121
Aiea, HI 96701
phone: (808) 486-5227

ILLINOIS - Chicago
2417 Bond Street
University Park, IL 60484
phone: (708) 534-5200

INDIANA - Valparaiso
2400 Cumberland Drive
Valparaiso, IN 46383
phone: (219) 464-2389

IOWA - Cedar Falls
704 Enterprise Drive
Cedar Falls, IA 50613
phone: (319) 277-2401

MASSACHUSETTS - Westfield
53 Southampton Road
Westfield, MA 01085
phone: (413) 572-4000

MISSOURI - St. Louis
13715 Rider Trail North
Earth City, MO 63045
phone: (314) 298-8566

NEW JERSEY - Edison
777 New Durham Road
Edison, NJ 08817
phone: (732) 549-3900

NEW YORK - Buffalo
10 Hazelwood Drive, Ste. 106
Amherst, NY 14228
phone: (716) 691-2600

OHIO - Dayton
4738 Gateway Circle
Kettering, OH 45440
phone: (937) 294-6856

OHIO - North Canton
4101 Shuffel Street NW
North Canton, OH 44720
phone: (330) 497-9396

OREGON - Portland
9405 SW Nimbus Avenue
Beaverton, OR 97008
phone: (503) 906-9200

PENNSYLVANIA - King of Prussia
1008 W. Ninth Ave.
King of Prussia, PA 19406
phone: (610) 337-9992

PENNSYLVANIA - Pittsburgh
301 Alpha Drive
Pittsburgh, PA 15238
phone: (412) 963-7058

TENNESSEE - Knoxville
5815 Middlebrook Pike
Knoxville, TN 37921
phone: (865) 291-3000

TENNESSEE - Nashville
2960 Foster Creighton Dr.
Nashville, TN 37204
phone: (615) 726-0177

TEXAS - Austin
14050 Summit Dr., Ste. A100
Austin, TX 78728
phone: (512) 244-0855

TEXAS - Corpus Christi
1733 N. Padre Island Drive
Corpus Christi, TX 78408
phone: (361) 289-2673

TEXAS - Houston
6310 Rothway Street
Houston, TX 77040
phone: (713) 690-4444

VERMONT - Burlington
30 Community Drive, Suite 11
South Burlington, VT 05403
phone: (802) 660-1990

WASHINGTON - Richland
2800 George Washington Way
Richland, WA 99354
phone: (509) 375-3131

WASHINGTON - Seattle
5755 8th Street East
Tacoma, WA 98424
phone: (253) 922-2310

WASHINGTON - Spokane
11922 E. 1st Ave.
Spokane, WA 99206
phone: (509) 924-9200

WISCONSIN - Watertown
602 Commerce Dr.
Watertown, WI 53094
phone: (920) 261-1660

TestAmerica Service Centers

For your convenience, you can drop off samples for EMLab P&K at these locations.
For the most current list of locations, please visit us at www.emlabpk.com

TestAmerica
THE LEADER IN ENVIRONMENTAL TESTING

ALABAMA - Birmingham
Bessemer, AL 35022
phone: (205) 641-0019

ARIZONA - Tucson
1870 W. Prince Road, Suite 59
Tucson, AZ 85705
phone: (520) 807-3801

CALIFORNIA - Anaheim
1016 East Katella Ave.
Anaheim, CA 92805
phone: (714) 328-7971

CALIFORNIA - Ontario
1014 East Cooley Dr., Suite A
Colton, CA 92324
phone: (909) 370-4667

FLORIDA - Jacksonville
8933 Western Way, Suite 1
Jacksonville, FL 32256
phone: (904) 519-9551

FLORIDA - Orlando
8010 Sunport Drive, Suite 116
Orlando, FL 32809
phone: (407) 851-2560

GEORGIA - Atlanta
6500 McDonough Drive, Suite C-10
Norcross, GA 30093
phone: (678) 966-9991

ILLINOIS - Chicago
655 W. Grand Ave., Suite 205
Elmhurst, IL 60126
phone: (630) 758-0262

ILLINOIS - Decatur
920 W. Pershing Road
Decatur, IL 62526
phone: (217) 872-0340

IOWA - Davenport
736 Federal St., Suite 2202
Davenport, IA 52803
phone: (563) 323-7944

LOUISIANA - Baton Rouge
6113 Benefit Dr.
Baton Rouge, LA 70809
phone: (225) 755-8200

MARYLAND - Baltimore
5710 Executive Drive, Suite 106
Baltimore, MD 21228
phone: (410) 869-0085

MASSACHUSETTS - Boston
240 Bear Hill Rd., Suite 104
Waltham, MA 02451
phone: (781) 466-6900

MICHIGAN - Ann Arbor
6095 Jackson Road
Ann Arbor, MI 48103
phone: (734) 205-2530

MICHIGAN - Pontiac
339 W. Walton Blvd.
Pontiac, MI 48340
phone: (800) 526-4951

MINNESOTA - Minneapolis
7204 West 27th Street, Suite 114
St. Louis Park, MN 55426
phone: (800) 593-8519

MISSOURI - Kansas City
601 NW 39th Street
Blue Springs, MO 64015
phone: (800) 276-1286

MISSOURI - St. Louis
13715 Rider Trail North
Earth City, MO 63045
phone: (314) 302-8354

NEVADA - Las Vegas
6000 S. Eastern Ave., Suite 5E
Las Vegas, NV 89119
phone: (702) 429-1264

NEW JERSEY - South Jersey
520 Fellowship Rd., Suite A-106
Mt. Laurel, NJ 08054
phone: (856) 222-1990

NEW YORK - Albany
25 Kraft Ave.
Albany, NY 12205
phone: (518) 438-8140

NEW YORK - New York City
231 W. 29th St., Suite 904
New York, NY 10001
phone: (212) 643-2367

NEW YORK - Syracuse
118 Boss Rd.
Syracuse, NY 13211
phone: (315) 431-0171

NORTH CAROLINA - Charlotte
I-85 South Bldg.
2858 Queen City Dr., Suite B
Charlotte, NC 28208
phone: (704) 392-1164

OHIO - Cincinnati
11416 Reading Road
Cincinnati, OH 45241
phone: (513) 733-5700

SOUTH CAROLINA - Charleston
1436-A North Point Lane
Mt. Pleasant, SC 29464
phone: (843) 849-6550

TEXAS - Beaumont
3012 Spurlock Road
Nederland, TX 77627
phone: (409) 540-5302

TEXAS - Dallas/Ft. Worth
2100 N. Highway 360
Suite 1401
Grand Prairie, TX 75050
phone: (972) 988-0500

TEXAS - San Antonio
404 E. Ramsey, Suite 208
San Antonio, TX 78216
phone: (210) 344-9751

VIRGINIA - Virginia Beach
5135 Cleveland Street
Virginia Beach, VA 23462
phone: (757) 671-1291

WASHINGTON - Seattle
19515 North Creek Parkway N
Suite 100
Bothell, WA 98011
phone: (425) 420-9293

EMLab P&K Products

Buy equipment and supplies for sampling allergens, asbestos, bacteria, mold, fungi, and more

Shop online at www.emlabpk.com/store

Authorized Distributor for:

CENTER FOR ENVIRONMENTAL TRAINING

When quality and accuracy are critical.

Analytical Services: Allergens, Asbestos, Bacteria, Fungi, PCR, Radon, & USP <797>

Toll-Free: 866.888.6653 • **www.emlabpk.com**