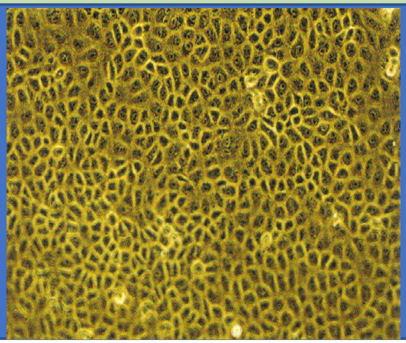
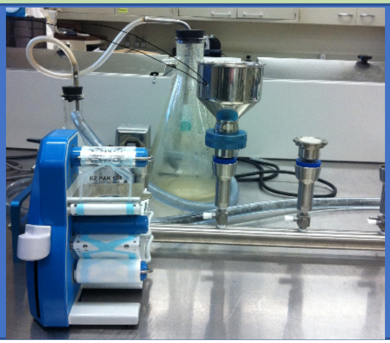
Office of Research and Development


Opportunistic Pathogens in Premise Plumbing

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Emerging Contaminants – Marquette University

October 22, 2018

Premise Plumbing

- Premise Plumbing - All materials that carry water within buildings

- Materials vary widely from Copper, Lead, PVC, Cast Iron

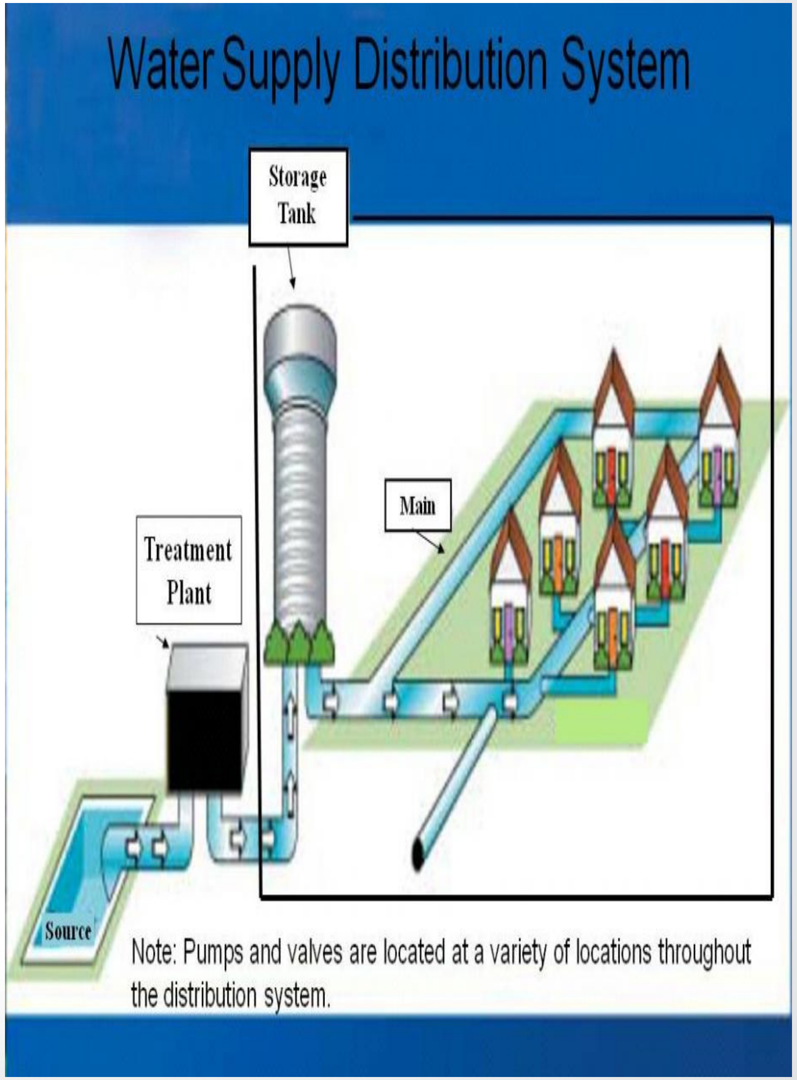
- Premise Plumbing- distribution system on a small scale

Premise Plumbing Similar to Distribution System

- Distribution Systems are complex systems in which water is treated, and then delivered to consumers.

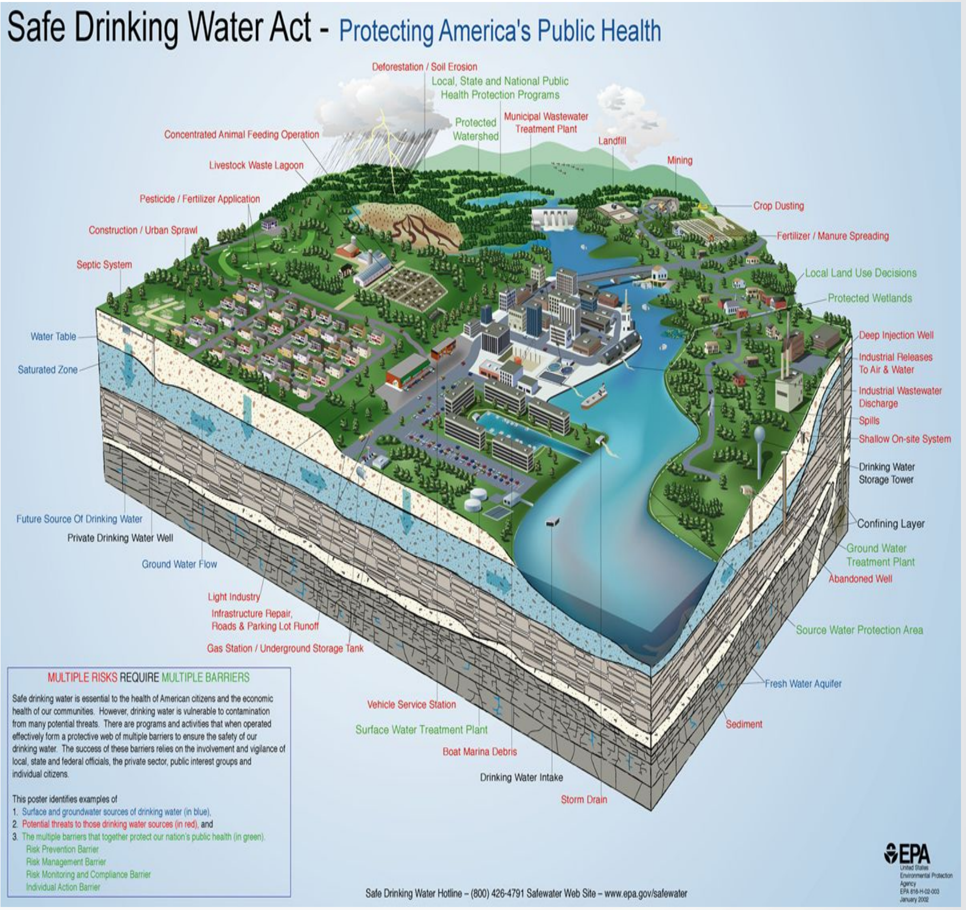
- Distribution Systems would include all the material needed to take source water to treatment as well as treated water to consumer.
  - Pipes
  - Pumps
  - Storage facilities
  - Fire protection needs

- Distribution systems also contain many different plumbing materials.



EPA Drinking Water Regulations

- EPA regulates water delivered to consumers through a number of regulations
  - Safe Drinking Water Act (SDWA, 1974)
  - Surface Water Treatment Rule (SWTR, 1989)
  - Long Term 2 Enhanced Surface Water Treatment Rule (LT2)
  - Ground Water Rule (GWR, 2006)
- EPA regulations typically stop at the water meter
  - Lead and copper rule exception – Sample collection is at tap



Opportunistic Premise Plumbing Pathogens (OPPPs)

- What is the problem with only regulating to the water meter?

- Premise plumbing systems can be very complex
  - Hot-water systems are often built in a recirculation loop
  - Water flows in many different directions depending on design of building
  - May be different interactions between cold-water systems and hot-water systems
  - Dead legs and long runs in the system
  - High surface to volume ratio

- Residuals required by regulations may be decreased or completely eliminated because of premise plumbing complexity.

Opportunistic Premise Plumbing Pathogens (OPPPs)

- Goal of water treatment is not to sterilize water but to inactivate pathogens that could be present.

- What microbes are present in finished drinking water?
  - Heterotrophic bacteria
  - Protozoans – amoeba, ciliates, slime molds
  - Opportunistic pathogens – ***Legionella, Pseudomonas, Mycobacterium****, Acinetobacter, Sphignomonas, Stenotrophomonas, Aeromonas, Methylobacterium*, and **Antibiotic resistant microbes (AMR)**
  - Other Pathogens found in drinking water that is not properly treated – Escherichia coli, Salmonella, Shigella, Campylobacter, Cryptosporidium, Giardia

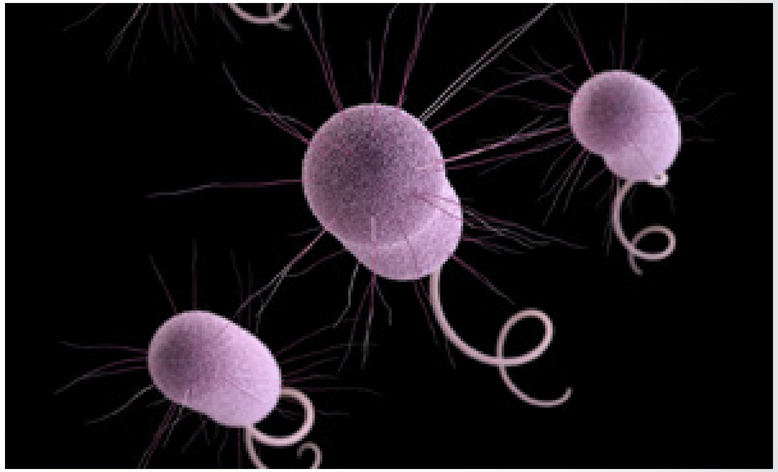
What makes premise plumbing a great niche for OPPPs?

- Premise Plumbing may have low disinfection residuals.
- OPPPs can survive in low nutrient, low oxygen environments.
- OPPPs interact with protozoans, such as amoeba. This may enable them to resist treatment and persist in these environments.
- OPPPs are known to form biofilms, in which microorganisms aggregate together to form complex structures attached to pipe walls. Cell-to-cell communication, called quorum sensing, is involved in cell attachment and detachment in biofilms.

Premise Plumbing Pathogens

- Focus on the most common premise plumbing pathogens
  - *Pseudomonas spp.*
  - *Mycobacterium avium* complex and other nontuberculosis mycobacteria (NTM)
  - *Legionella spp.*
  - Antimicrobial Resistant Microbes (AMR)

Pseudomonas Spp.



- Discovered in 1894 by Walter Migula
- Gram negative aerobic bacterium , measures 1.5-5µm in length
- Motile - single polar flagella
- Over 140 different species
  - *P aeruginosa*, *P fluorescens*, *P putida*, *P cepacia*, *P stutzeri*, *P maltophilia*, and *P putrefaciens*
- *Pseudomonas aeruginosa* and *Pseudomonas maltophila* are associated with 80% of human infections involving pesudomonads.

Pseudomonas aeruginosa

- 8-11% of all nosocomial infections in Europe and the US from 2001-2010 (Hirden et al., 2008)

- Produces several substances which allows it to outcompete other microbes in the same environment.
  - Studies demonstrate that it produces antimicrobial substances to other Gram positive bacteria (Haba et al., 2003, Kim et al. 2000, McClure et al., 1996)
  - Produces extracellular polymeric substances in biofilms which allow for increased resistance to disinfection (Xue et al., 2013)

- *P. aeruginosa* in premise plumbing is most often found in POU areas, such as faucets, drains, and showerheads, more than it is seen in the premise plumbing distribution system. (Mena and Gerba, 2009)

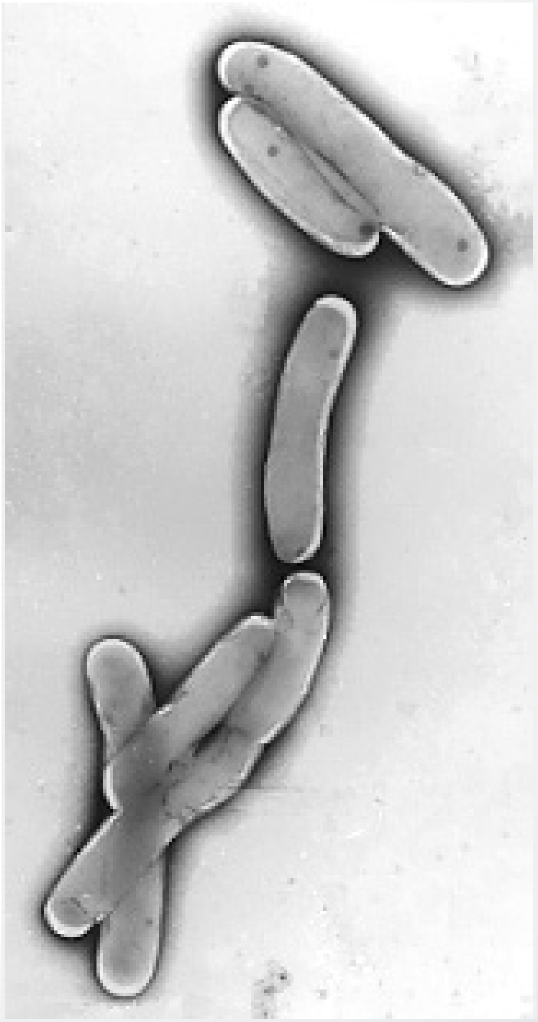
Mycobacterium Avium (NTM)

- Waterborne illness caused by nontuberculous mycobacteria (NTM) cost nearly $1.8 B for in-patient and out-patient treatment in 2010 ([a]Thomson et al, 2015).
- Pulmonary NTM infections account for almost half of all NTM hospitalizations in the US, and are typically caused by *Mycobacterium avium* (MA) and *M. intracellulare (MI)*
- In addition to pulmonary infections, they can cause skin, soft tissue, lymph node, systemic infections, among others
- Primary source of human exposure: **WATER**

CCL's 1 and 2: *Mycobacterium avium* Complex (MAC)

CCL's 3 and 4: *M. avium*

4 subspecies: *M. avium* subsp. *hominissuis*
M. avium subsp. *avium*
M. avium subsp. *silvaticum*
M. avium subsp. *paratuberculosis*



- [a]Thomson et al (2015) Ann Am Thorac Soc, Vol 12:1425–1427

Occurrence of M. *avium* and *M.* *intracellulare* in potable water in buildings and homes in the US

- 40 sites across the US (8 homes, 32 buildings) provided a variety of waters:
- groundwater + no treatment, chlorine, or chloramine
- surface water + chlorine or chloramine
- mixed ground and surface water + chlorine or chloramine = 2
- 68 total taps (1 to 2 taps/site)
- Samples collected 4 times during the course of a year

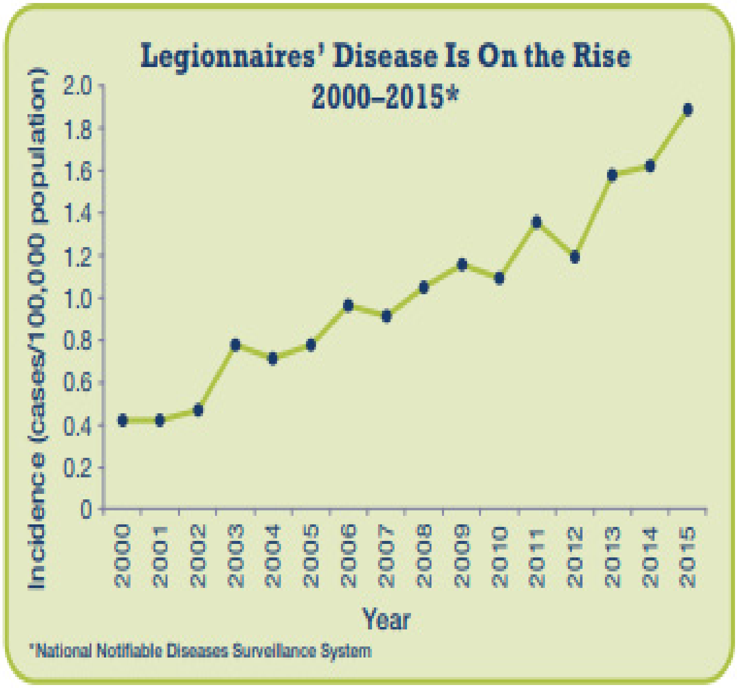
Occurrence of *M. avium* and *M. intracellulare* in tap water in the U.S.

- 83% (33/40) of sites were positive for MAC by at least one of two detection methods
- No geographical distribution patterns were observed
- Seven sites were consistently negative
- *M. intracellulare* was detected more frequently and at higher concentrations than *M. avium* by qPCR (28% and 18%)
- *M. avium* was detected more frequently than *M. intracellulare* by culture (13 and 6%)

Target	Mean of qPCR positives (target copies L^{-1})	Median of qPCR positives (target copies L^{-1})	No. isolates obtained by culture
M. avium	1.43×10^3	4.16×10^1	101
M. intracellulare	4.37×10^5	5.97×10^1	23

Legionella infections

- According to CDC estimates *Legionella* infections have risen steadily over the last 15 years
- Legionella infections are almost exclusively associated with premise plumbing
- Discovered in 1976 at American Legion Conference



In the United States, reported cases of Legionnaires' disease have increased by nearly four and a half times since 2000. More illness occurs in the summer and early fall but can happen any time of year.

Legionella spp.

- 66 species (serogroups)

L. adelaidensis
L. anisa
L. beliardensis
L. birminghamensis
L. bozemanii (2)
L. brunensis
L. busanensis
L. cardiaca
L. cherrii
L. cincinnatiensis
L. clemsonensis
L. donaldsonii
L. drancourtii
L. dresdenensis
L. drozanskii
L. dumoffii
L. erythra (2)

L. fairfieldensis
L. fallonii
L. feeleii (2)
L. geestiana
L. genomospecies
L. gormanii
L. gratiana
L. gresilensis
L. hackeliae (2)
L. impletisoli
L. israelensis
L. jamestowniensis
L. jeonii
L. jordanis
L. lansingensis
L. londiniensis
L. longbeachae (2)

L. lytica
L. maceachernii
L. massiliensis
L. micdadei
L. monrovica
L. moravica
L. nagasakiensis
L. nautarum
L. norrlandica
L. oakridgensis
L. parisiensis
L. pittsburghensis
L. pneumophila (15)
L. quateirensis
L. quinlivanii (2)
L. rowbothamii
L. rubrilucens

L. sainthelensi (2)
L. santicrucis
L. shakespearei
L. spiritensis
L. steelei
L. steigerwaltii
L. saoudiensis
L. taurinensis
L. thermalis
L. tucsonensis
L. tunisiensis
L. wadsworthii
L. waltersii
L. worsleiensis
L. yabuuchiae

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19 species associated with human disease

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19 species associated with human disease

Dresden panel of mAbs (17), groups Lp into sg 1 to 15

Lück, C. et al. 2013. Typing Methods for *Legionella*, p. 119-148. *In* C. Buchrieser and H. Hilbi (ed.), *Legionella.*

sg1, >80% clinical isolates

Protozoan Species that Support Intracellular *Legionella* spp.

Amoeba

Acanthamoeba castellani
A. culbertsoni
A. hatchetti
A. polyphaga
A. palestinensis
A. royreba
Comandonia operculata
Echinamoeba exudans
Filamoeba nolandi

Naegleri fowleri
N. gruberi
N. jadini
N. lovaniensis
Paratetramitus jugosis
Vahlkampfia spp
V. jugosa
V. ustiana

Vermamoeba spp.
V. cantabrigiensis
V. vermiformis

Ciliate

Tetrahymena pyriformis
T. thermophila

Slime Mold

Dictyostelium discoideum

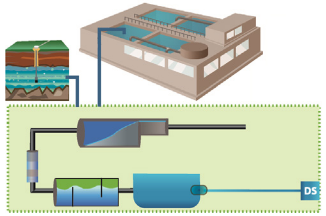
Nematode

Caenorhabditis spp.

Adapted from Lau & Ashbolt. 2009. J Appl Microbiol 107:368-378.
Brassinga et al. 2010. Cell Microbiol 12:343-361.

Legionella Environmental Distribution

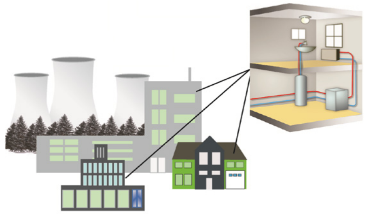
Treated water - reclaimed (i.e. sewage treatment plant), ground, and surface



- $3 \times 10^3 - 8 \times 10^4$ CFU L^{-1}
- $290 - 2.5 \times 10^3$ CE L^{-1}

L. anisa
L. adelaidensis
L. bozemanii
L. donaldsonii
L. dumoffi
L. fairfieldensis
L. fallonii
LLAP 2, 3, 4, 7, 8
L. londiniensis
L. micdadei
L. pneumophila
L. quateirensis
L. sterigerwaltii
L. wadsworthii
L. waltersii
L. worsleiensis

Distributed water - premise plumbing, e.g. residences, apartments, recreational facilities, hospitals, and cooling towers



- $43 - 6 \times 10^5$ CFU L^{-1}
- $< 2 \times 10^3 - 8 \times 10^5$ GU L^{-1}
- $1.6 \times 10^5 - 2 \times 10^6$ CE L^{-1}

L. anisa
L. bozemanii
L. cherrii
L. dumoffi
L. erythra
L. feelii
L. gormanii
L. gresilensis
L. hackeliae
L. longbeacheae
L. lytica
L. maceachernii
L. micdadei
L. pneumophila
L. rubrilucens
L. saintcrucis
L. sterigerwaltii

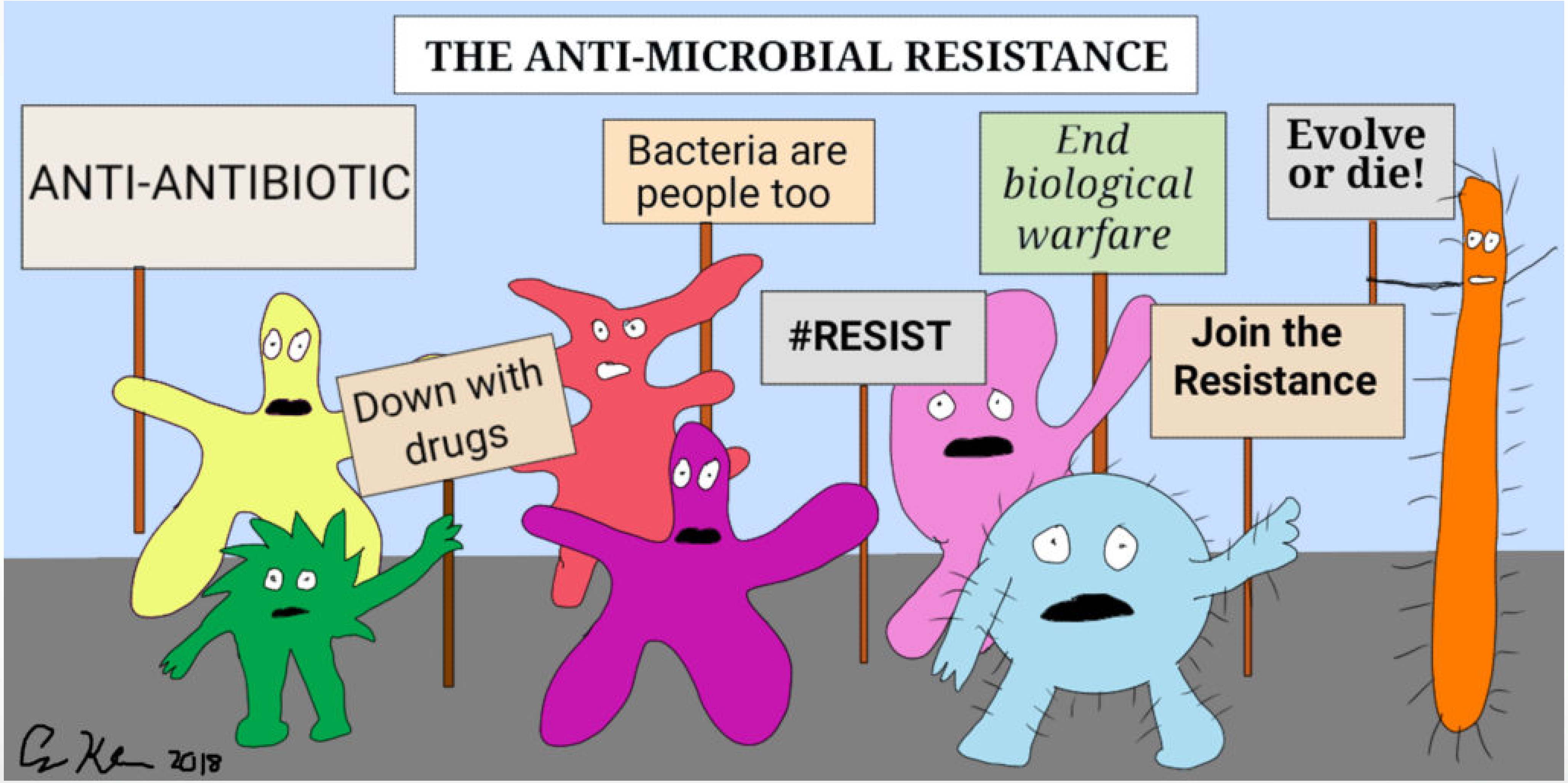
Soil – garden, compost, potting

- $10^2 - 10^8$ CFU g^{-1}
- $10^4 - 10^6$ GU g^{-1}

L. anisa
L. birminghamensis
L. bozemanii
L. cincinnatiensis
L. dumoffi
L. feelii
L. gormanii
L. gratiana
L. impletisoli
L. jamestowniensis
L. longbeacheae
L. micdadei
L. nautarum
L. oakridgensis
L. pneumophila
L. quinlivanii
L. saintcrucis
L sainthelensis
L. wadsworthii
L. yabuuchiae

Papadakis et al. 2018. Int J Environ Res Public Health
Schalk et al. 2014. Int J Infect Dis.
van Heijnsbergen et al. 2016. Appl Environ Microbiol.
van Heijnsbergen et al. 2014. J Appl Microbiol.

Antimicrobial Resistant Microbes (AMR)



AMR in the headlines

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Epidemiology and Surveillance

A large, refractory nosocomial outbreak of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Escherichia coli* demonstrates carbapenemase gene outbreaks involving sink sites require novel approaches to infection control

V Decraene, H. T. T. Phan, R George, D. H. Wyllie, O Akinremi, Z Aiken, P Cleary, A Dodgson, L Pankhurst, D. W. Crook, C Lenney, A. S. Walker, N Woodford, R Sebra, F Fath-Ordoubadi, A. J. Mathers, A. C. Seale, M Guiver, A McEwan, V Watts, W Welfare, N Stoesser, J Cawthorne, the TRACE Investigators' Group

APMIS Suppl. 2003;(116):1-47.

Pseudomonas aeruginosa chromosomal beta-lactamase in patients with cystic fibrosis and chronic lung infection. Mechanism of antibiotic resistance and target of the humoral immune response.

Ciofu O[1].

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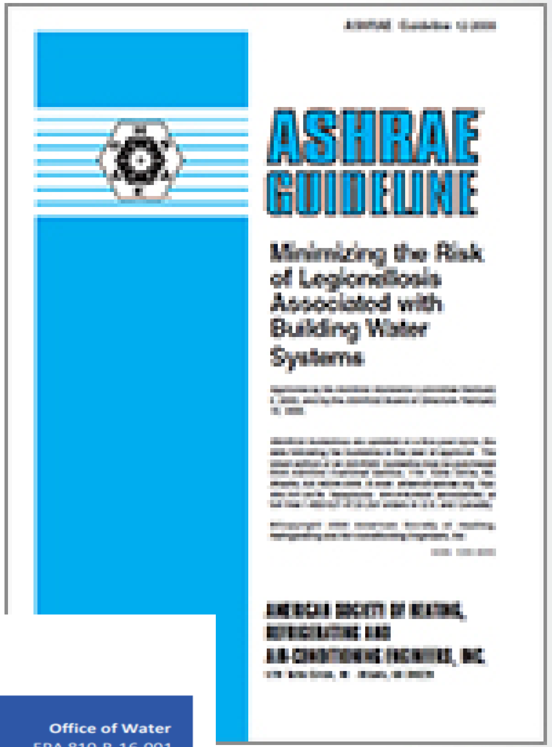
RESEARCH LETTER – Pathogens & Pathogenicity

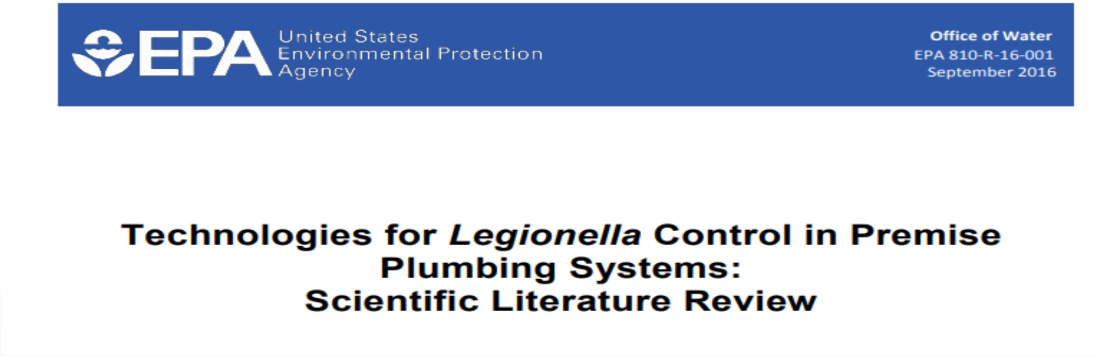
Comparative genomics of a drug-resistant *Pseudomonas aeruginosa* panel and the challenges of antimicrobial resistance prediction from genomes

J. Jeukens, I. Kukavica-Ibrulj, J. G. Emond-Rheault, L. Freschi and R. C. Levesque*

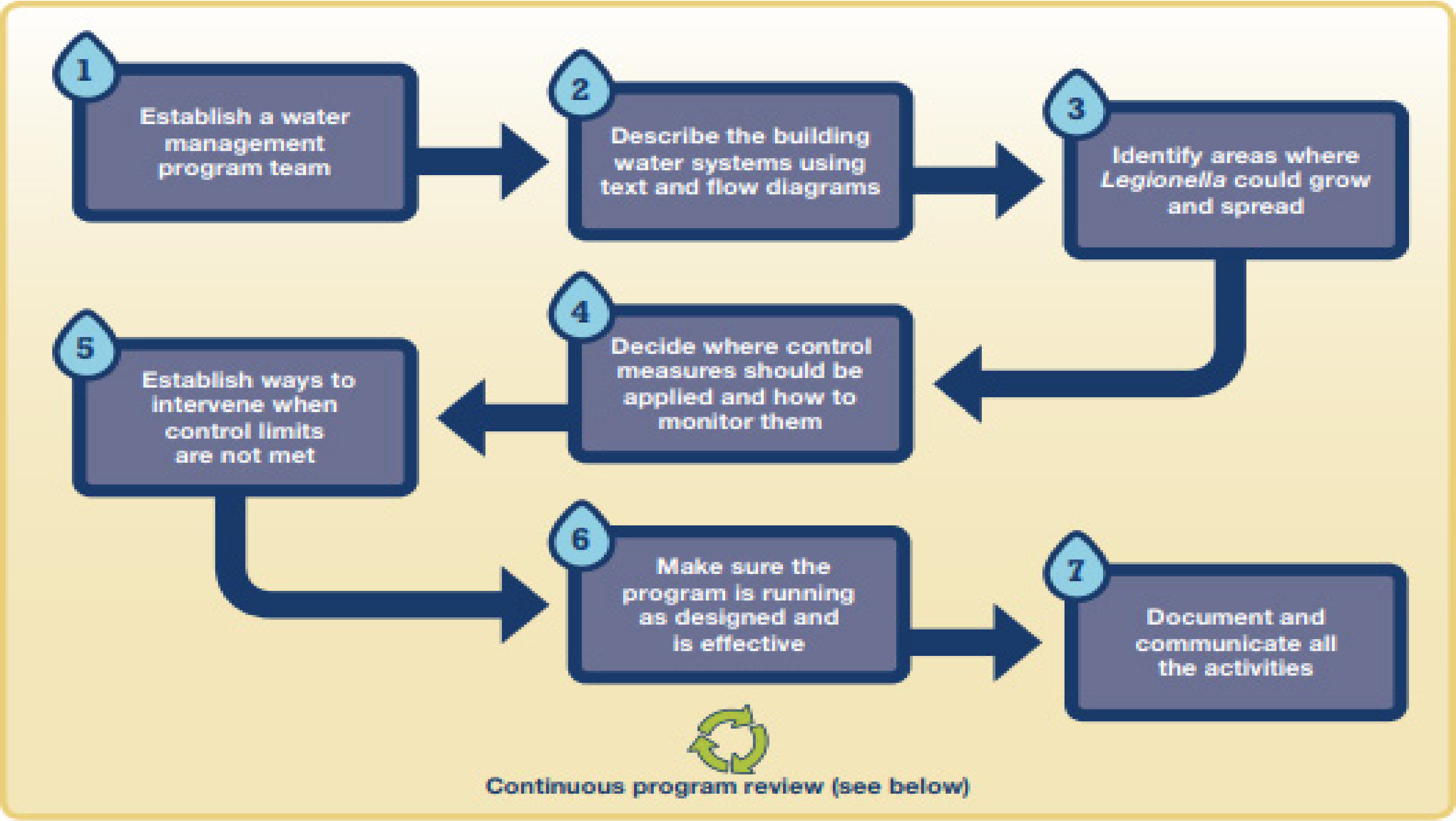
What Can Building Owners / Managers Do?

- Develop a water management plan
  - CDC toolkit
  - ASHRAE Guidance
  - EPA – Literature review





Building Water Management Plan



Questions?

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The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for external publication. Any opinions expressed in this paper are those of the author (s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.