A Molecular Shift in Microbial Targets: Redefining Water Quality and Public Health through Omics and Emerging Pathogens

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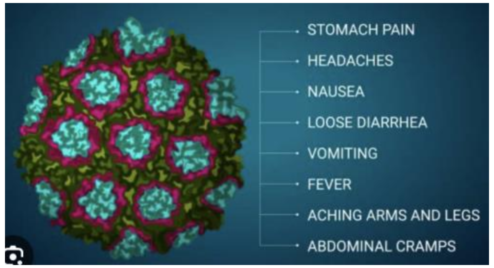
Why We Need Better Microbial Targets

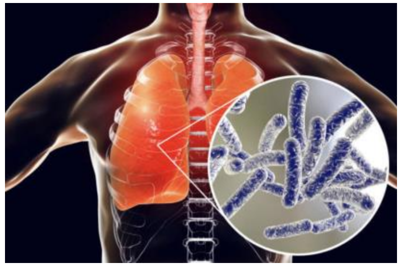
- Foundation of safe drinking water, reuse, irrigation and recreational waters: set regulations, validate treatment processes, protect public health, build trust & guide investment
- **A good target**
  1. Reflects real health risks (viruses, bacteria, protozoa) people are exposed to
  2. Detects pathogens early, at low levels, and in infectious forms
  3. Shows predictable response of pathogens to treatment (chlorine, UV, filtration)
  4. Practical: cost-effective, rapid, field-deployable, standardised
  5. Actionable: align with policy and guidelines

Two Major Groups of Waterborne Pathogens



Enteric pathogens (Faecal Origin)

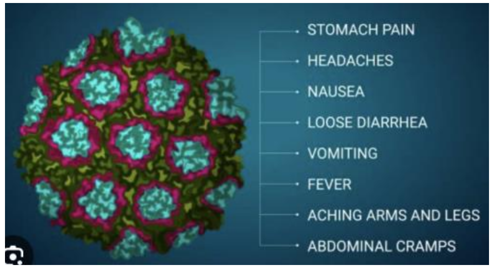
- Introduced through faecal contamination
- Traditional focus of water treatment: important targets when designing treatment processes and meeting log reduction goals in drinking water guidelines
- Do not generally amplify in pipes and environment in general



Opportunistic pathogens (Environmental Origin)

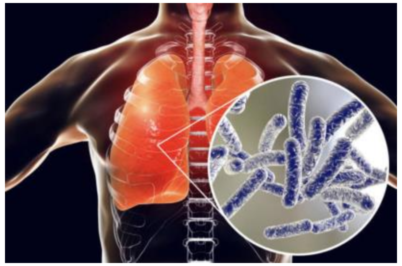
- Multiply throughout water systems (water filters and pipes)
- Most important to manage in premise plumbing systems and near points of human exposure (air conditioning systems, cooling towers)
- Can be present in high numbers in water systems

Traditional Microbial Targets



Enteric pathogens (Faecal Origin)

- **Traditional faecal Indicators target *E. coli* and enterococci**
- **Do not index the behaviour of human viruses & parasitic protozoa**
- Viruses (e.g., *Norovirus*) smallest pathogens, breakthrough filters and more disinfection resistant
- Parasitic protozoa (*Cryptosporidium*/*Giardia*) have oo/cysts that are very resistant to chlorination

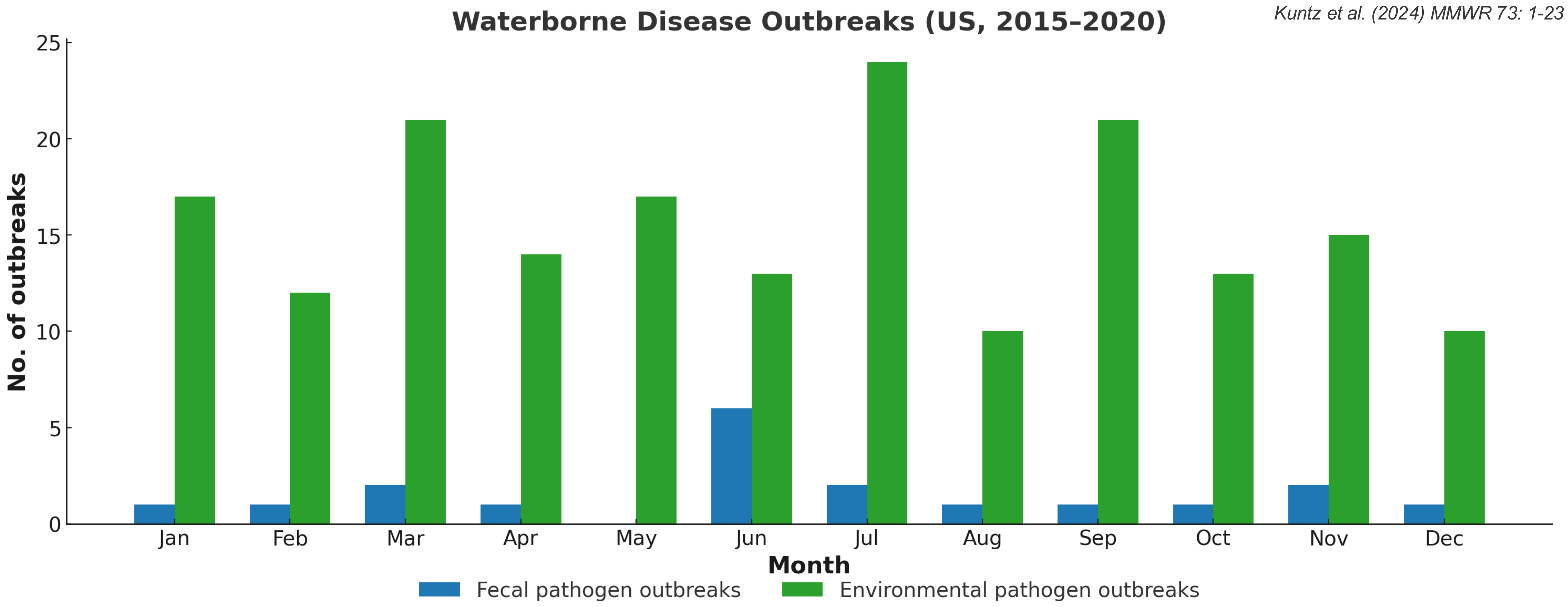


Opportunistic pathogens (Environmental Origin)

- **Miss environmental/opportunistic pathogens**
- *Legionella pneumophila*, *L. longbeachae, etc.*
- Non-tuberculous mycobacteria (NTM)
- *Pseudomonas aeruginosa*
- Mostly cause lung and skin infections, predominantly seen in healthcare settings
- Major blind spot in our current monitoring system

Traditional microbial indicators have significant limitations in comprehensively assessing water quality and the presence of diverse pathogens. A more comprehensive approach is needed to address these gaps and ensure the protection of public health.

Waterborne Health Burden:
Faecal Pathogens vs Environmental Pathogens



Most outbreaks were driven by environmental pathogens and biofilm-associated outbreaks.

Health & Economic Burden of Waterborne Pathogens

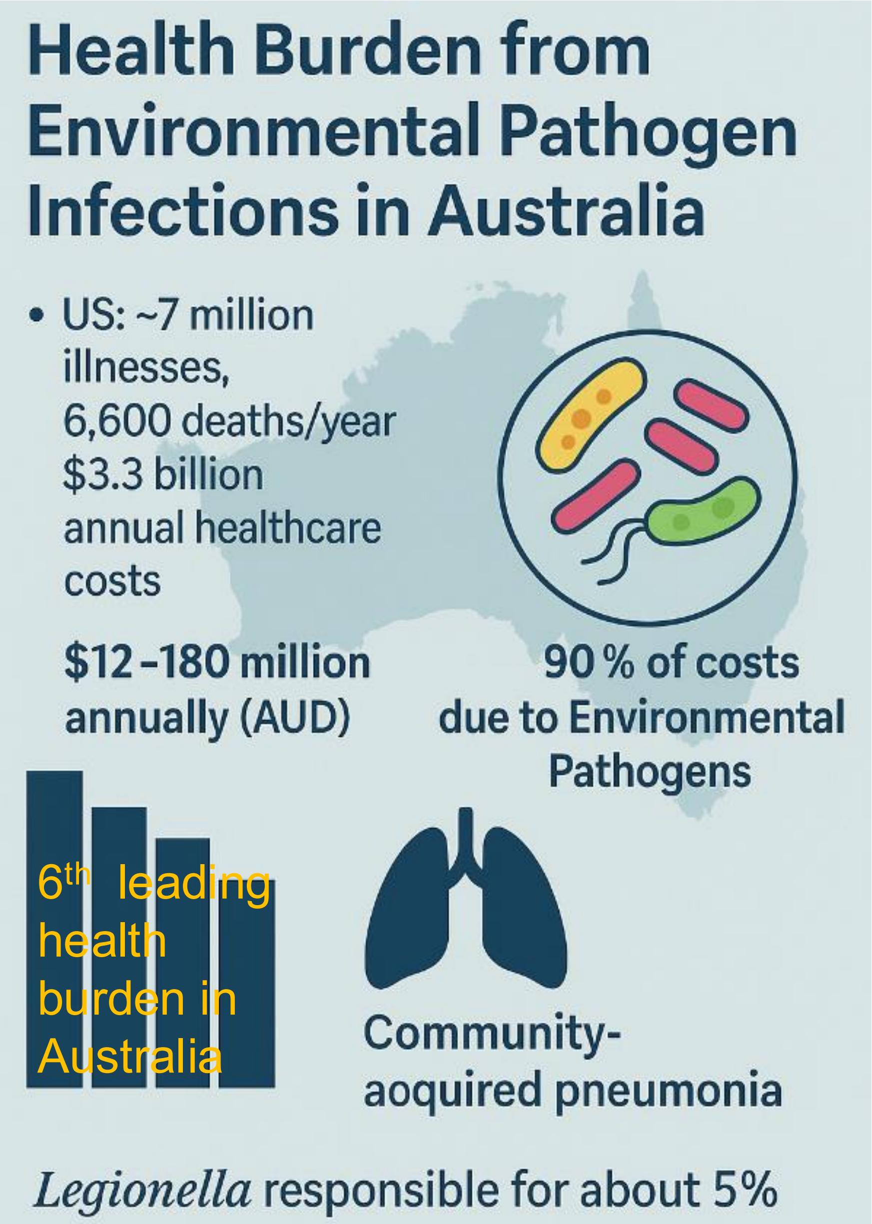
~90% of costs from **environmental pathogens** (*Legionella, NTM, P. aeruginosa*)

US

- In the US: ~7 million illnesses & 6,600 deaths each year
- Direct healthcare costs: **US$3.3 billion annually**

Australia

- Translated to Australia: ~**500,000 illnesses & 500 deaths annually**
- Estimated costs: **AU$ ~$370 million per year**
- Community-acquired pneumonia is ranked 6th for health burden in Australia, costing **AU$500 million a year** (*Legionella* responsible for ~5%)



[1] Graham *et al.* (2023) Emerg Infect Dis. 29:1173-1182

Faecal Source Pathogens – Current & Emerging Markers

Current and Emerging:

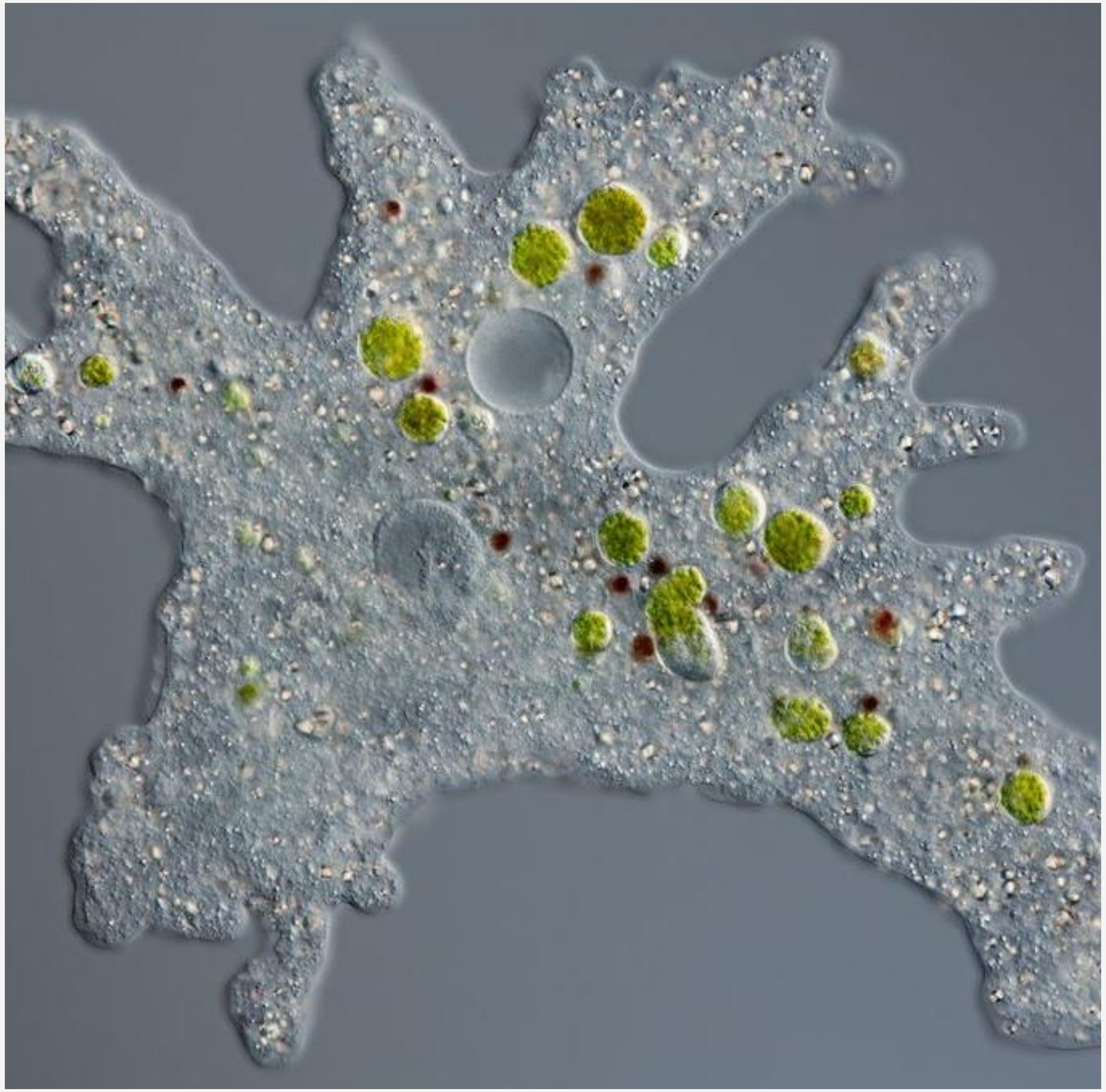
- ***Bacterial targets:***
  - *E. coli/*enterococci
- **Viral surrogates (qPCR):**
  - crAssphage
  - PPMoV (Pepper Mild Mottle Virus)
  - plasmid pBI143 (14 x crAssphages)
- **Parasitic protozoan surrogates:**
  - *Bacillus pumilus* spores (with viability testing)

Multi-Omics:

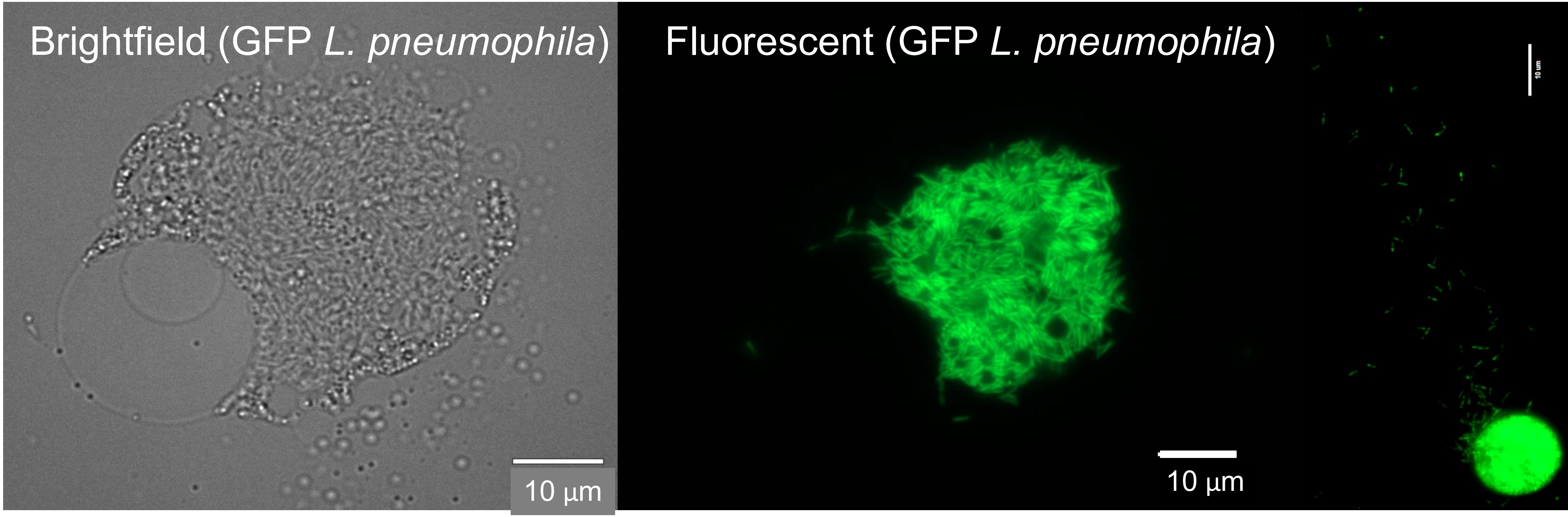
- Metagenomics to identify more numerous targets (so spiking not needed)
- Transcriptomics: assess viability & disinfection response
- Currently still expansive, but can help us to identify targets for which inexpensive detection platforms can be developed

Environmental Pathogens: Designing New Targets

Amoebae play a key role in the lifecycle of many environmental pathogens. Pathogens can multiply inside **amoebae** and be released through **extracellular vesicles**, gaining protection from disinfectants and UV.



Legionella pneumophila in biofilm – grow and multiply in Amoebae cells, then disperse via extracellular vesicles

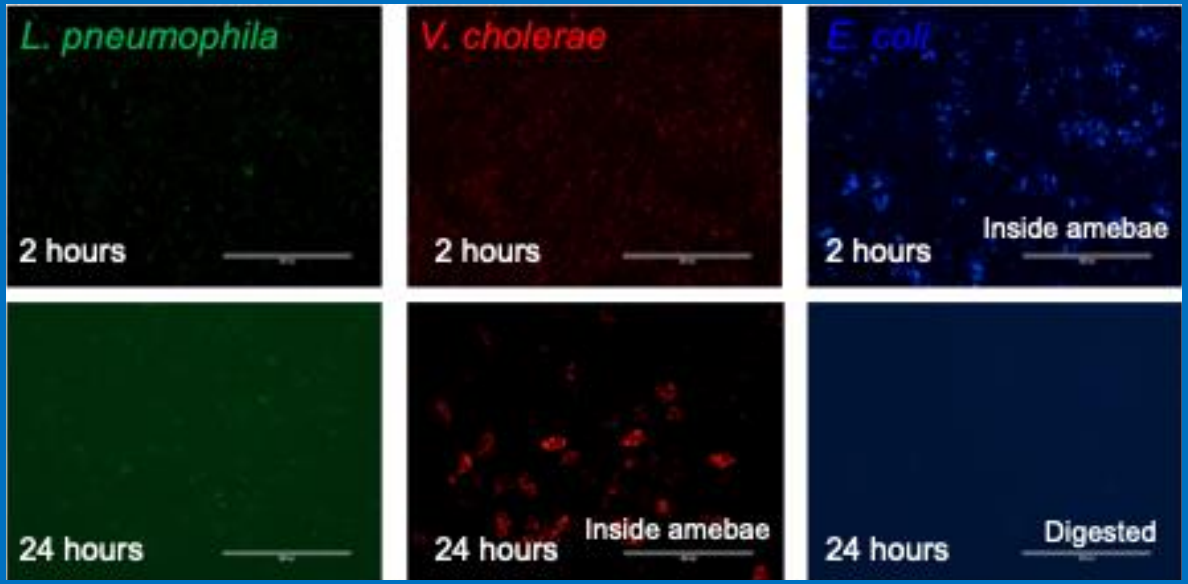


Shaheen & Ashbolt (2018) *Exposure and Health 10:* 201-209

Amoeba, vesicles and *Legionella pneumophila*

Ashbolt's team has pioneered studies on:

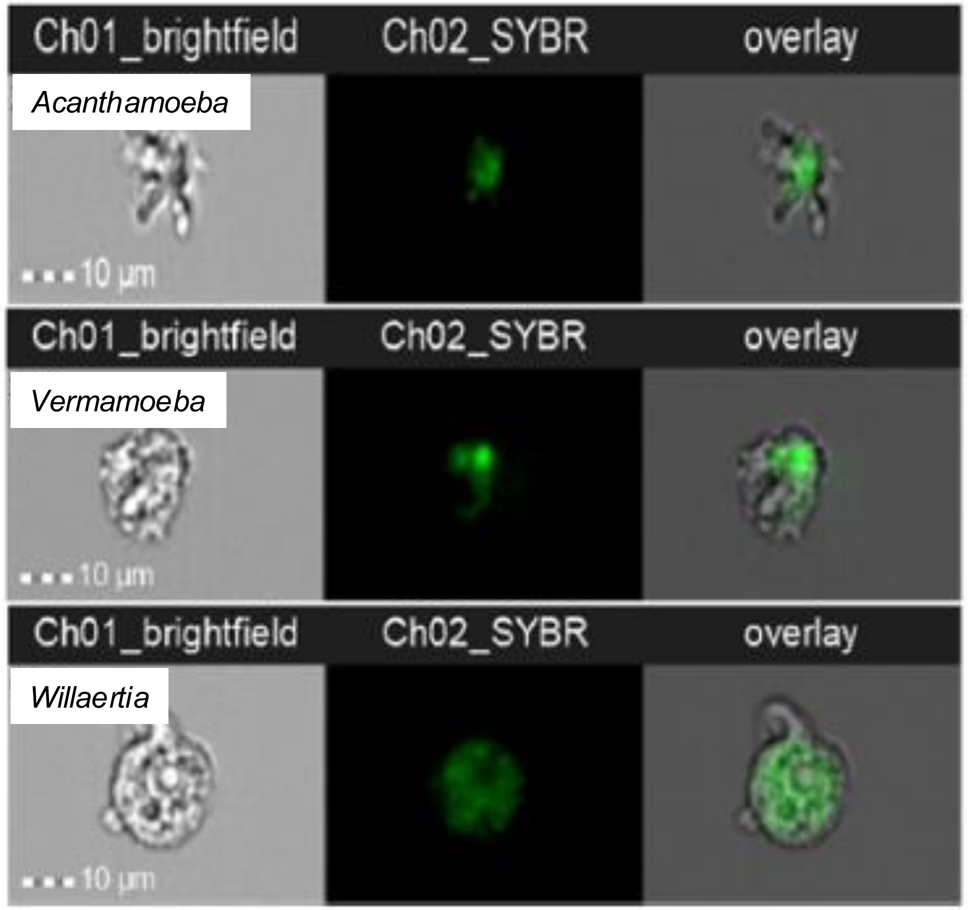
- **Amoeba** diversity and behaviour in water systems (influence of temperature, flow conditions, pipe materials, corrosion products, disinfectant etc.)
- **Amoeba-protection** from secondary disinfectant and heat treatments
- **Extracellular vesicle** sizes & numbers, microbial content, and roles in amoeba predation/ intracellular host pathogen amplification
- **QMRA-defined** control point concentrations near points of exposure

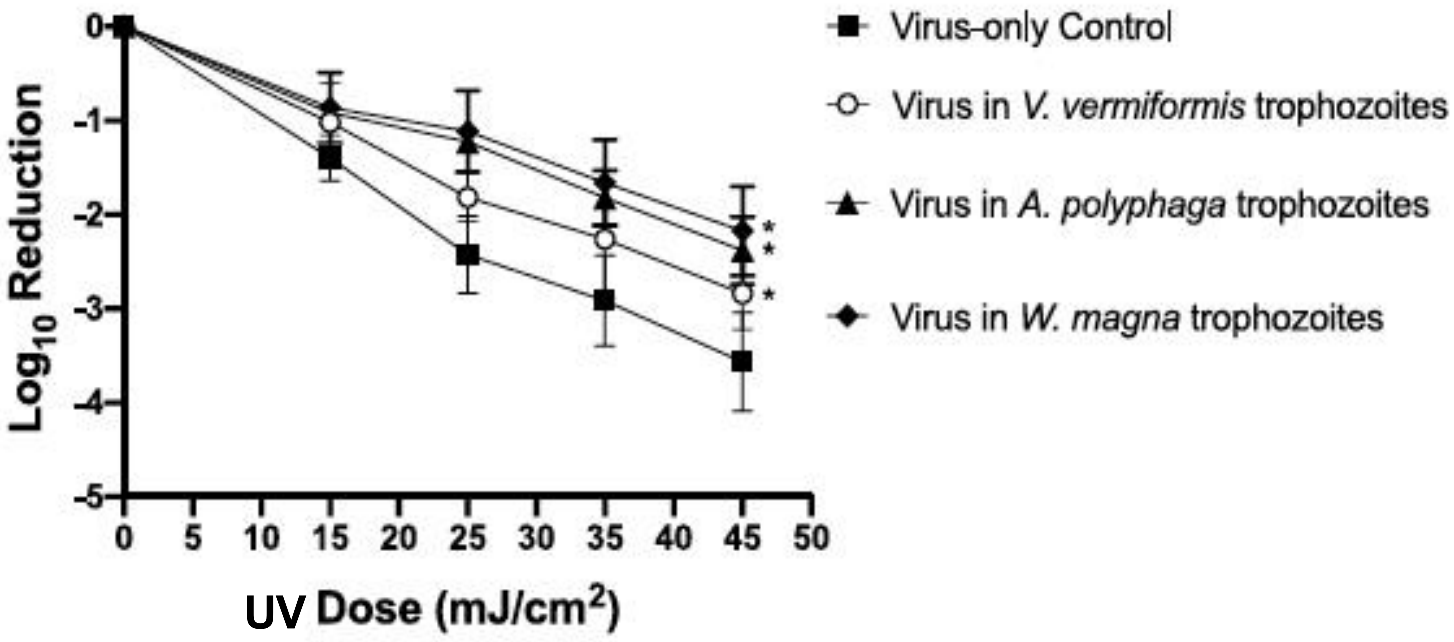


- Amoebae feed on non-virulent *E. coli* and *V. cholerae* before *L. pneumophila*.
- Once inside amoebae, *L. pneumophila* can switch off the digestive system of amoebae.
- Microbial and eucaryotic vesicles play various ecologic roles, such as mediating pathogenicity or acting as vectors for ***inter-cellular communication*** across bacteria, archaea, protists and metazoans.

Storey et al. (2004) The efficacy of heat and chlorine treatment against thermotolerant... Scan J Inf Dis 36: 656-62
Schoen & Ashbolt (2011) An in-premise model for Legionella exposure during showering events. Wat Res 45: 5826-36
Buse et al. (2014) Preferential colonization and release of Legionella… Int J Hyg Environ Health 217: 219-225;
Shaheen & Ashbolt (2018) Free-living amoebae supporting intracellular growth… Exposure Health 10: 201-9
Lu et al. (2025) Meta-transcriptomic response to copper corrosion in drinking water biofilms. Microorganisms 7: 1528

UV protection of pathogens within amoebae (faecal & environmental pathogens)





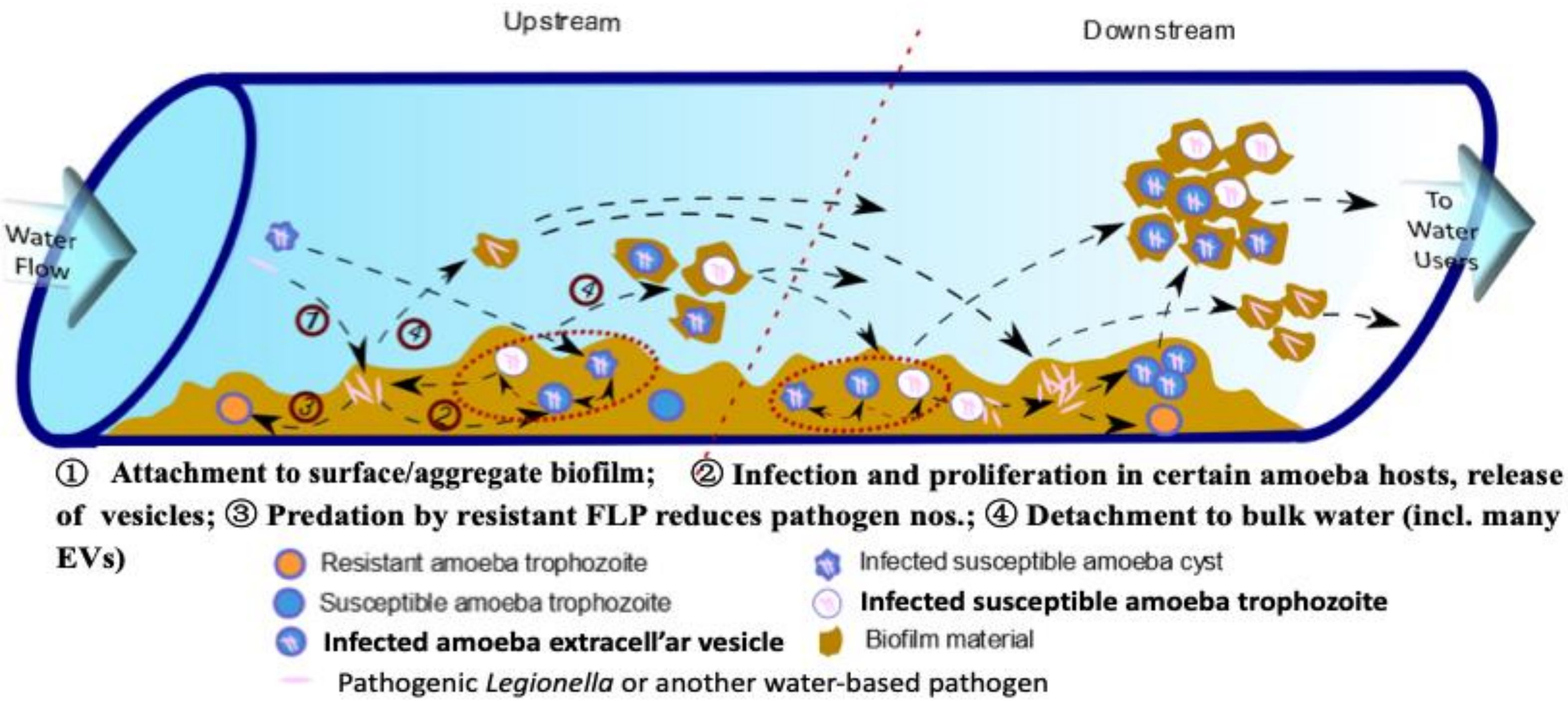
Amoebae provide 1-2 Log_{10} UV protection

Folkins et al. (2020) Environ Sci Tech 54: 10201-10206; Dey et al. (2021) Biofilms & Microbiomes 7: 25; Dey et al. (2022) J Wat Health 20: 83-91

Conceptual model to inform WSP management of *Legionella* in engineered water system biofilms

Water Safety Plan (WSP) management should include:

- Frequent chlorinated water filter media backwashes
- Removal of drinking water sediments from reservoirs/distribution
- Minimise stagnation periods and avoid 25-45 °C water (e.g., constant recirculation hot/cold premise plumbing, auto flushing of distal outlets, thermostatic mixers with minimal dead space)
- Replace copper and iron pipes with cross-linked polyethylene (PEX) (Cu-, Fe-oxides select for *Legionella* growth)



① Attachment to surface/aggregate biofilm; ② Infection and proliferation in certain amoeba hosts, release of vesicles; ③ Predation by resistant FLP reduces pathogen nos.; ④ Detachment to bulk water (incl. many EVs)

[1] *Ashbolt (2023) Front Cell Inf Microbiol 13: 1200478*

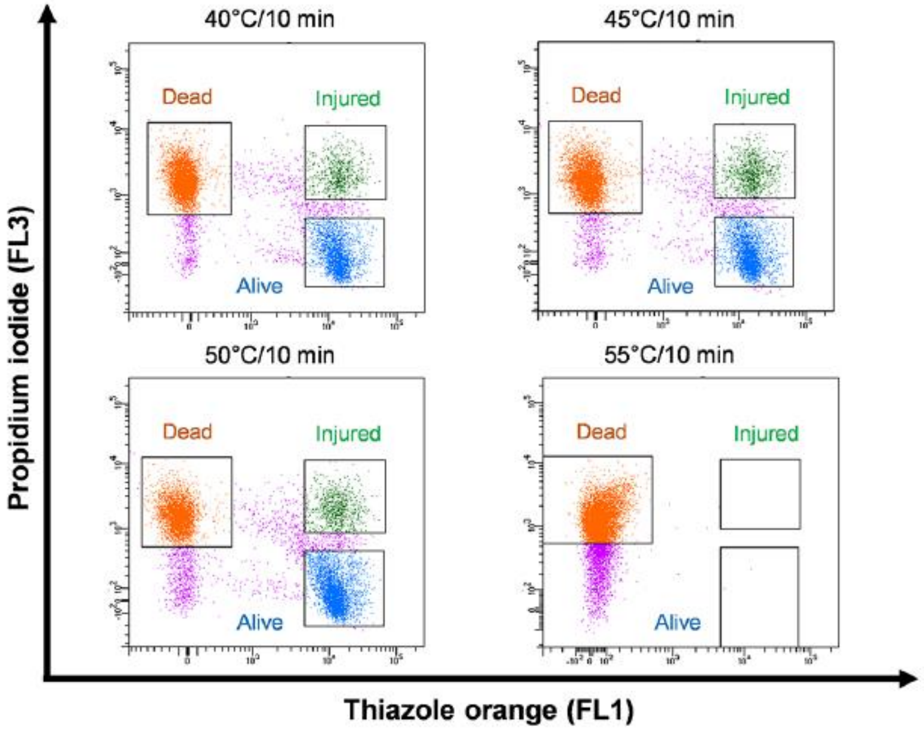
Environmental Source Pathogens – Method 1 Targeting Pathogens Directly

Targeting *Legionellae, Mycobacteria* etc

- Live/Dead differentiation can be done with PMA-qPCR
- Flow cytometry better live/dead resolution
- Isothermal amplification techniques (LAMP) for field testing

Traditional approach for DNA extraction does not extract all

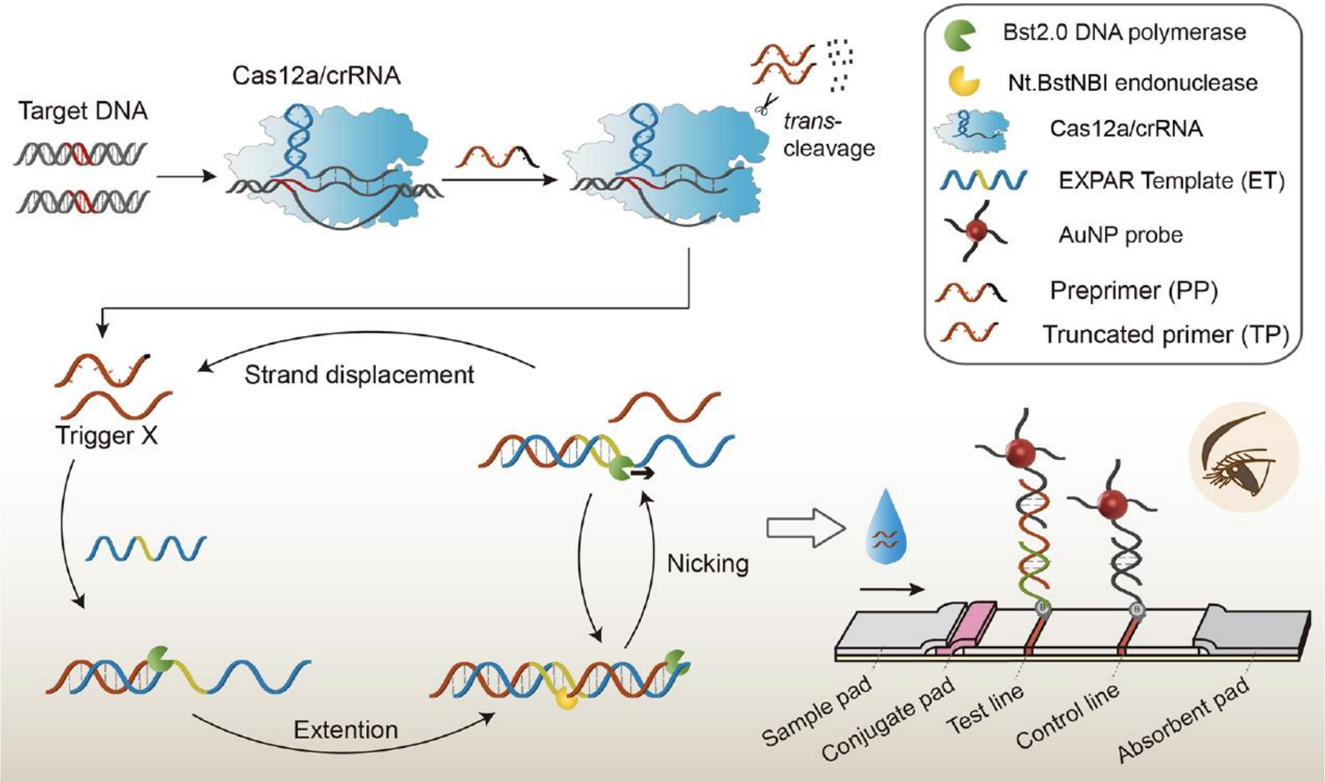
- Such as misses viable cells within amoebal cysts
- Needs to extract DNA from within amoebae
- Requires additional steps for cyst lysis/staining



Live, injured and dead *L. pneumophila* resolution by Flow Cytometry (Naire *et al.* 2023 Front Microbiol 14: 1094877)

Environmental Source Pathogens – Method 2 Targeting Amoeba Numbers

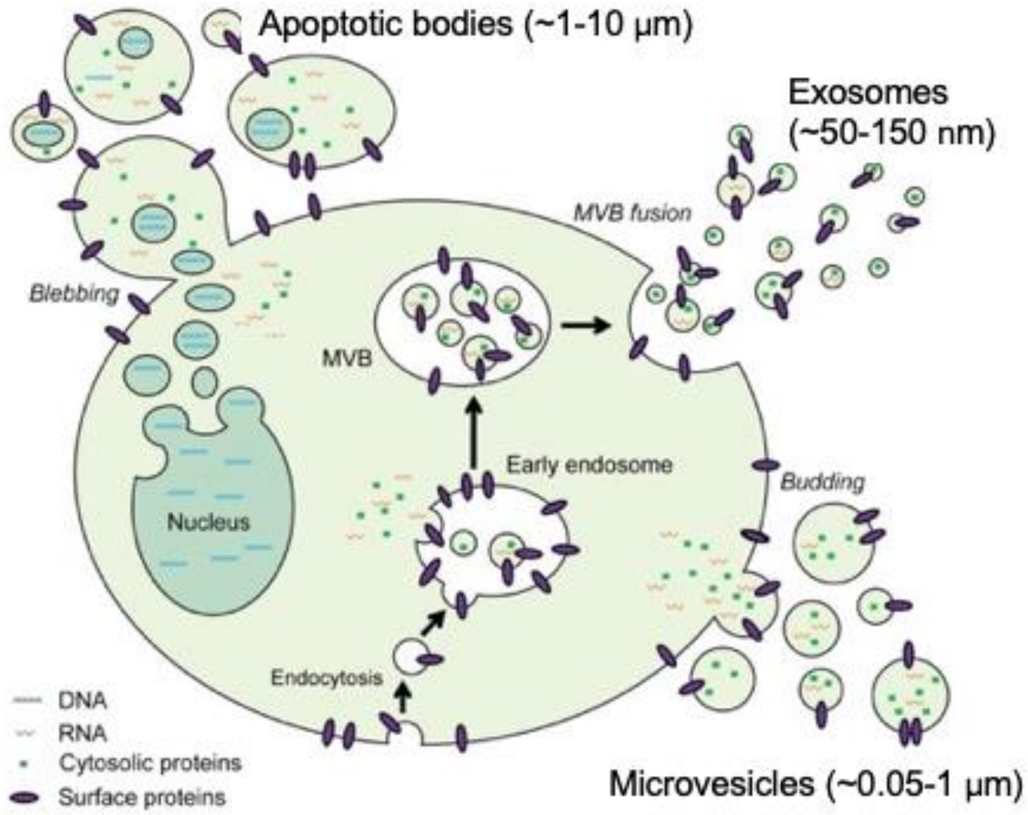
- Amoeba could act as an effective early warning system – when the conditions are right for environmental pathogens to develop and spread
- In-field lateral flow device (LFD) could offer easy and inexpensive approach
  - However, while used in medicine, not yet developed for water testing



Huang *et al.* 2025 ACS Sens 20250912

Environmental Source Pathogens – Method 3 Targeting Extracellular Vesicles

- Tiny packages released by amoebae
- Can carry pathogens → major pathway to contribute to both infection & contamination
- Three types; **apoptotic bodies** most promising (large, abundant, easy to detect)
- Approach for vesicles detection has been developed for medical applications – via surface-specific protein targets (epitopes) and multi-omics on their molecular cargoes



What is Missing for Pathogen Management – Integrating Approaches are Required

- Reliable, inexpensive, practical for the field/online covering both faecal and environmental pathogens
  - **Faecal indicators**: e.g., recreational or irrigation waters
    - Detection with faecal source marker (e.g., ***E. coli*/enterococci, pBI143**)
    - Water treatment verification, faecal and viability marker (e.g**., pBI143, *E. coli*/enterococci, *Bacillus* Spores**)
  - **Environmental pathogen indicators**: e.g., filter media or distribution systems
    - *Amoeba (e.g., Acanthamoeba/Vermamoeba* cysts vs trophozoites active) or extracellular Vesicles
- **Do not ignore emerging risks**, such as antimicrobial resistance (AMR)
  - AMR is a critical issue across faecal and environmental pathogens (High-priority to ID within resistomes)

Future Directions in Pathogen Management

Develop & validate microbial markers for Water System Application

- **Broad range markers** Viruses, protozoa, and environmental pathogens
- **Leverage omics technologies** Metagenomics, proteomics, metabolomics for discovery
- **Integrate AI & data analytics** To identify patterns and link markers to health risks

Build practical monitoring tools

- Rapid, field-ready, scalable methods for water systems
- Inexpensive, field/IoT deployment

Translate science into policy

- Ensure markers support regulatory targets and reuse schemes

By leveraging cutting-edge technologies from the medical field, we can unlock new possibilities for water pathogen detection and monitoring, paving the way for safer drinking water and water reuse.

Acknowledgements

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ARC Industry Laureate Fellowship

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- Water Research Australia

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- Queensland Health
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- US EPA