SIMPYFYING PROTOZOA ASSESSMENT IN WATER TREATMENT STSTEMS

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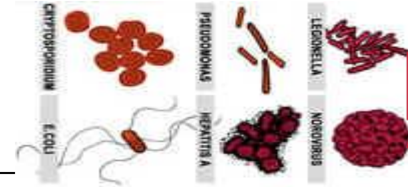
Water-Borne Illnesses…

- Causes more than **2.2 million deaths** per year
- About **1.4 millions of these deaths are children**
- Worldwide, an **economic loss of nearly 12 billion US dollars** per year is estimated

Waterborne Pathogens…



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Micro-organisms	Major diseases	Persistence in water
Bacteria		
Salmonella spp	Typhoid, paratyphoid, gatroenteritis	Moderate/may multiply
Vibrio cholerae	Gastroenteritis, cholera	Short to long
Pathogenic *E. coli*	Acute diarrhoea, bloody diarrhoea and gastroenteritis	Moderate
Virus		
Rotavirus	Gastroenteritis	Long
Adenovirus	Gastroenteritis	Long
Norovirus	Gastroenteritis	Long
Protozoa		
Giardia	Gastroenteritis	Moderate
Cryptosporidium	Gastroenteritis	Long

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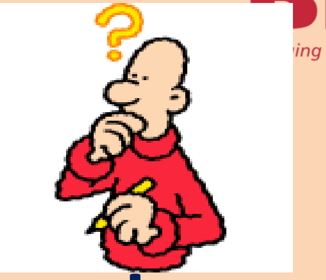
Need for Assessment



- Monitoring water quality- regulatory, voluntary
- Surveillance/monitoring
- Microbial risk assessment
- Outbreak Investigation
- Source tracking
- Track Emerging Waterborne Pathogens

Protozoa in Water

- Of 1428 waterborne disease outbreaks worldwide (1991 -2008), **49.6%-bacteria, 39.3%-viruses, 11.1%- parasites** (Yang et al, 2012).
- **Of the protozoa, *Cryptosporidium* spp are most resistant to chemical inactivation, therefore it is used as the reference culture for assessing technologies used for inactivation of protozoa.**
- **Most disinfection** based studies done with **chlorine, however, *Cryptosporidium* is resistant** to chlorine at concentrations typically applied for pathogen inactivation (Russel et al, 2003; WHO, 2003).

- **Inadequate data** on presence of protozoan pathogens in water and **lack of correlation** with bacterial indicators

Water purification methods

1. Exclusion based: filtrations including depth and membrane filters
2. Disinfection based: Chlorine, Chlorine Dioxide, Ozone, UV, etc

Issues of concern in evaluating water for total Microbial safety:

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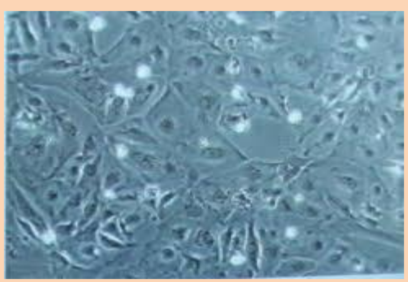
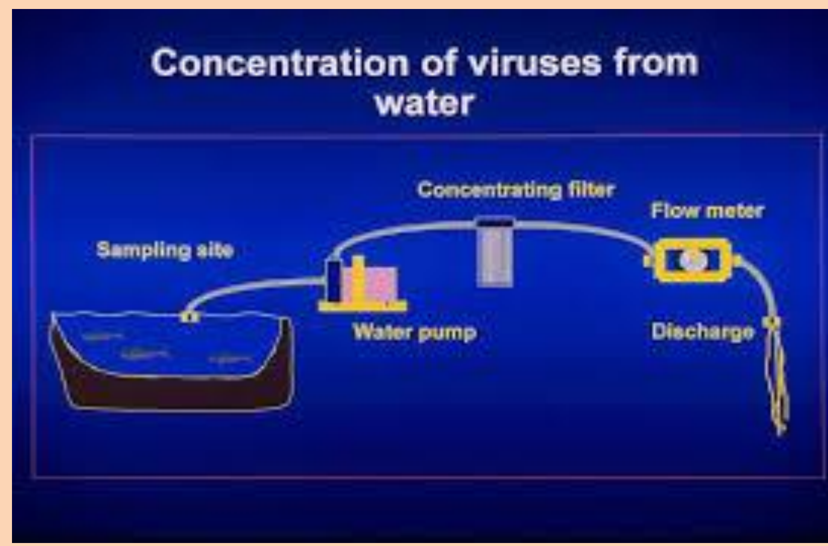
1. Bacteria: *E.coli* used as gold standard for monitoring water quality; indicates bacterial safety,

   Is *E.coli* the best indicator???

2. Non-bacterial Pathogens: Viruses and protozoa: their occurrence is low, thus require concentration from large volumes of water.

3. Detection systems are highly competency driven.





Methods to Evaluate efficiency of water treatment Systems to make water Protozoa Safe

1. **Exclusion**: Irradiated cysts of *Cryptosporidium, Giardia*
2. **Inactivation/Disinfection** based:
   a. **UV**: Bacteriophage MS2 most resistant to UV, so used are reference microorganism for all 3 groups
   b. **Chemical disinfectants:** Live protozoa like *Cryptosporidium* & *Giardia* recommended by WHO, EPA, NSF.

Use of Surrogates: WHO and other agencies recommended surrogates:

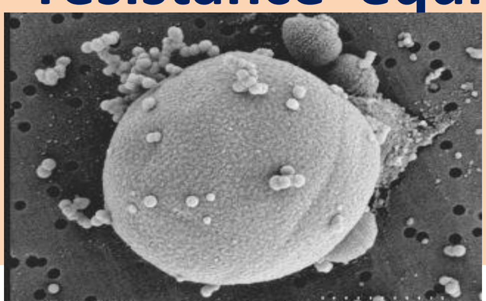
a. Microspheres for exclusion (well accepted)
b. Clostridium perfringens & Bacillus subtilis disinfectants??

Our Study Focus: Design effective Surrogate When ozone is the disinfectant

- **Chlorine**: limited effect on inactivating protozoa
- **Ozone**, **UV** more effective, specially *Cryptosporidium* (EPA, 1999; Russel et al, 2003)
- Presently recommended surrogates: **Conflicting reports on their suitability and most data are with Cl** (Hijnen et al,2002; Kaymak et al,2005).

Objective of the present study:

- **Designing a simple, low cost surrogate, with ozone resistance equivalent to that of real pathogen**



Methodology & Results

- **Bacterial spore formers** were isolated from environments exposed to **ozone** and their ozone resistance was determined through ozone disinfection experiments
- **Inactivation profile of *Cryptosporidium*** was determined in water in presence of ozone
- Based on resistance pattern obtained, **3 spore-forming bacteria** which gets reduced by 3 log cycles at ozone concentrations similar to that of *Cryptosporidium* (11-12 ppm, as reported in litrature)
- Recommended strain of *B.subtilis A*TCC 6633 gets reduced by 3 log cycles at concentration much lower ozone 6ppm

Conventional *VS* Surrogate

- Requires live protozoa
- Procurement of protozoa- extremely difficult
- Requires lab set-up for protozoa cultivation, detection using immunoflorescence, molecular methods
- Special training for protozoa handling, detection
- Extremely expensive (10-20X)
- Either not used, or being used only at terminal stage of product development
- Regulatory Monitoring ??

- Bacterial Spore adequate
- Not so for bacteria
- Basic microbiology lab set-up adequate
- Basic microbiology training adequate, minimum safety issue
- Economical
- Can be used for standardizing treatment systems at all stages of product development
- Quite Feasible

What Next..

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- Molecular identification of the selected isolates- to ensure non-pathogenic strain
- Standardization of sporulation and protocol development and validation for its usage by simple labs
- Submit standardization data to EPA & WHO for initialization of the isolate/s in standards
- Check the isolate/s for **resistance to other disinfectants, viz Chlorine, Bromine, Chorine dioxides**, etc and have **broader/more universal application**

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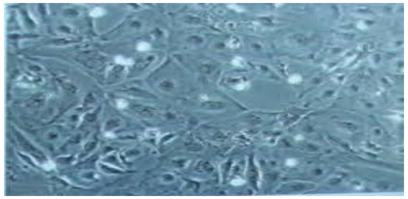
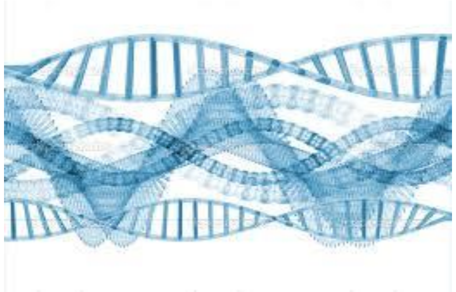
Bacterial & non- Bacterial: Detection Systems

- Cultivation:

a. Bacteria- Classical

b. Virus & Protozoa- Cell culture





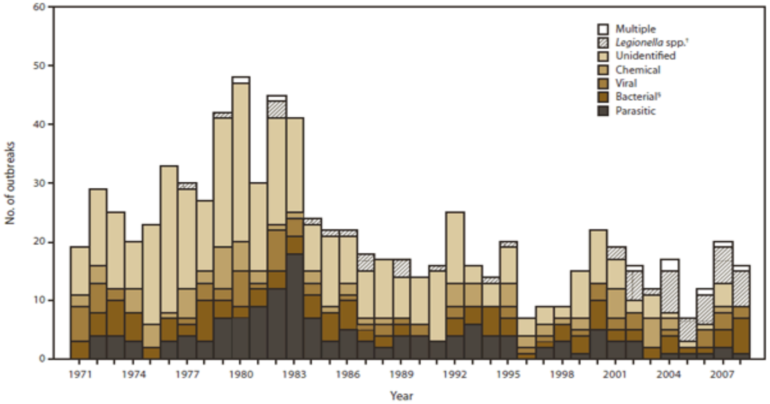
- Molecular

a. PCR

b. qPCR

c. Multiplex PCR

d. Metagenomics- unknown etiology



Area for Collaborations..

- **Comprehensive water quality surveillance to:**
  1. Identify the indigenous pathogens
  2. Formulate water treatment strategies
  3. Review existing water quality specifications
- **Develop cost-effective methodologies for detection of non-bacterial pathogens & include them in surveillance programs**
- **National capacity building for implementation of water safety strategies**

About Bhavan’s Research Center

- Unique Experimentation in the Andheri, Campus of Bharatiya Vidya Bhavan
- Partnership between Microbiology Department of Bhavan’s College and SPJIMR, the management school to set up a lab for Academia-Industry partnership with internal funding, on a self-sustaining basis
- The lab now, set in an area of 9000 sqft , is ISO 17025 (NABL) accredited and works actively with industries in the areas Food, Water and Hygiene
- R&D projects and analysis are handled by a dedicated pool of trained staff or students, under constant supervision of faculty
- Focus Area:

1. Antimicrobial studies for hygiene based products
2. Predictive Microbiology
3. QMRA
4. Pathogen detection and evaluation, including biosensor-based technology
5. Building capability in non-bacterial pathogens detection and evaluation in foods and water
6. Collaborating with national and international scientists in areas of Water & food safety, surrogate designing for protozoa, bacteriophage application for reducing pathogens in water contaminated with sewage

Prior work by the group in water

- Member of BIS technical committee for water purifier evaluation protocol development
- Lab validation of protocol for assessment of water purifiers for WQA- C**ompleted**
- Part of a group for rapid detection of coliform using mobile kit- **Phase 1 completed**
- Evaluation of the slime removal of the water purifier testing units to bring about bacterial reduction- **Completed**
- Standardization of Rapid Coliphage assay using β-galactosidase and applying it for testing performance of water purifiers- **Completed**
- Feasibility study of using bacteriophage for reduction of enteric pathogens in domestic sewage and wastewater effluent-Supported by **CPCB –on-going**
- Monitoring and controlling the water quality of Bhavan’s College lake for recreational purpose, using Cholrine dioxide- **on-going**
- Evaluation of Commercial available coliform detection/enumeration kits- **On-going**
- Designing bacterial surrogate to monitor inactivation of Cryptosporidium by ozonation

Ct Values (mgCmin/L) for *Cryptosporidium* Inactivation by Chlorine Dioxide[1]

	Log Credit	Water Temperature, ° C				
		10	15	20	25	30
Chlorine Dioxide	0.5	138	89	58	38	24
	1.0	277	179	116	75	49
	2.0	553	357	232	150	98
	3.0	830	536	347	226	147

Ct Values (mgCmin/L) for *Cryptosporidium* Inactivation by Ozone[2]

Ozone	0.5	4.9	3.1	2.0	1.2	0.78
	1.0	9.9	6.2	3.9	2.5	1.6
	2.0	20	7.8	7.8	4.9	3.1
	3.0	30	12	12	7.4	4.7

USEPA - Long Term 2 Surface Water Treatment Rule - 2005

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