

Water use and reuse for agriculture – food safety challenges

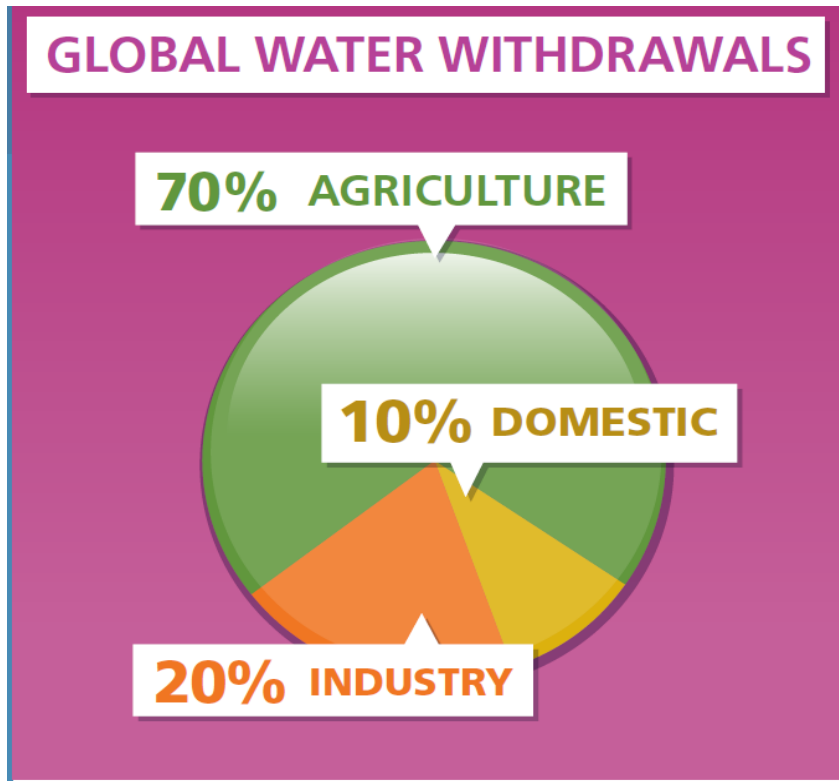
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- In industrialized nations, however, industries consume more than half of the water available for human use.
- Freshwater withdrawals have tripled over the last 50 years.

INCREASE IN WATER WITHDRAWALS BY 2025

50%

DEVELOPING
COUNTRIES

18%

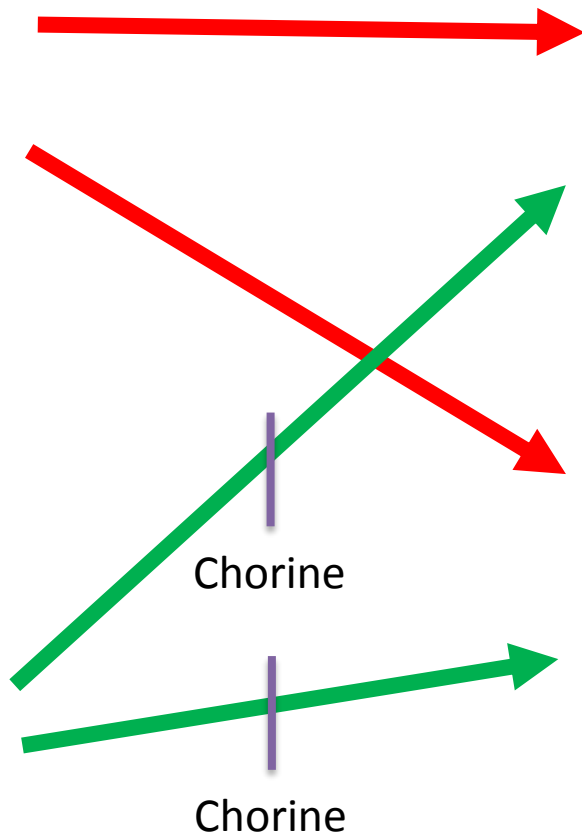
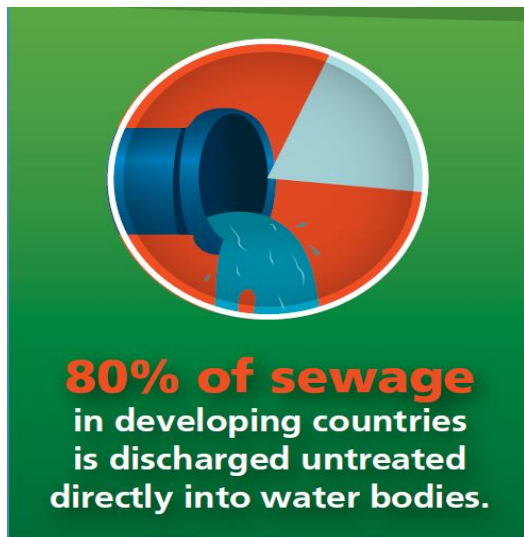
DEVELOPED
COUNTRIES

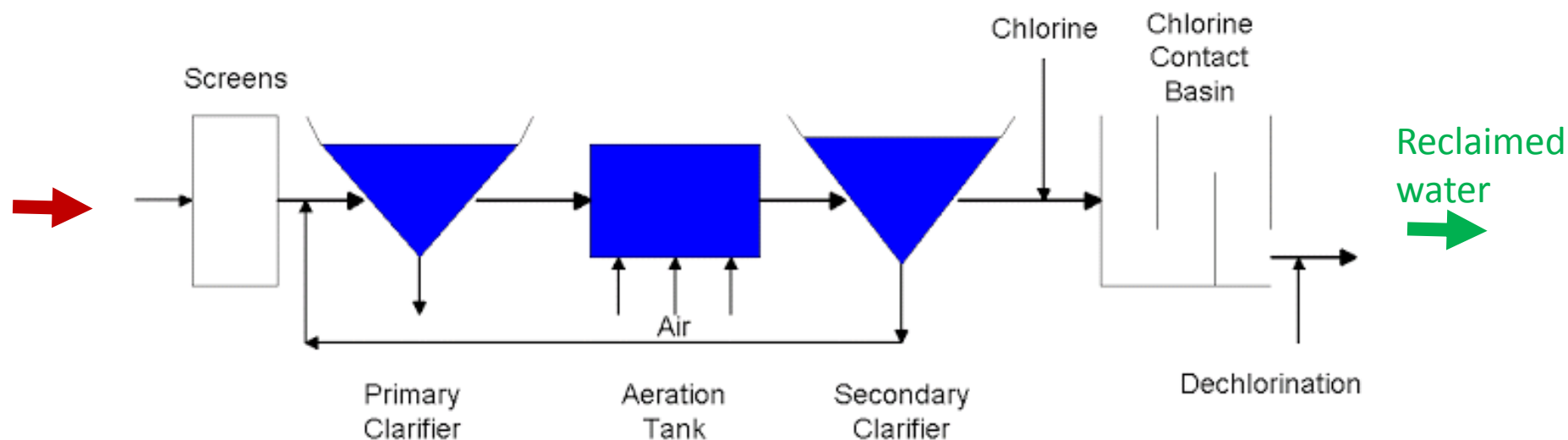
**By 2025,
1800 million
people will be living
in countries or
regions with absolute
water scarcity,
and two-thirds
of the world population
could be under stress
conditions**

- Changes in lifestyles and eating habits are requiring more water consumption per capita;
- The production of biofuels has also increased sharply in recent years, with significant impact on water demand;
- Almost 80% of diseases in low income countries are associated with water, causing some three million early deaths.

- Reclaimed water-treated wastewater
- The use of reclaimed water in agriculture is driven by:
 - water scarcity situations,
 - its nutrient value,
 - it is seen as a viable economic strategy, and
 - an environmental sustainable practice.







- Barriers to the use of treated wastewater for irrigation:
 - Social acceptance.
- Scientific issues approached:
 - Chemical aspects:
 - Presence of antibiotics.
 - Microbiological:
 - The legislation only focuses on the analysis of faecal coliforms and intestinal parasite eggs,
 - And:
 - Antibiotic resistance bacteria
 - Antibiotic resistance genes
 - Pathogenic viruses



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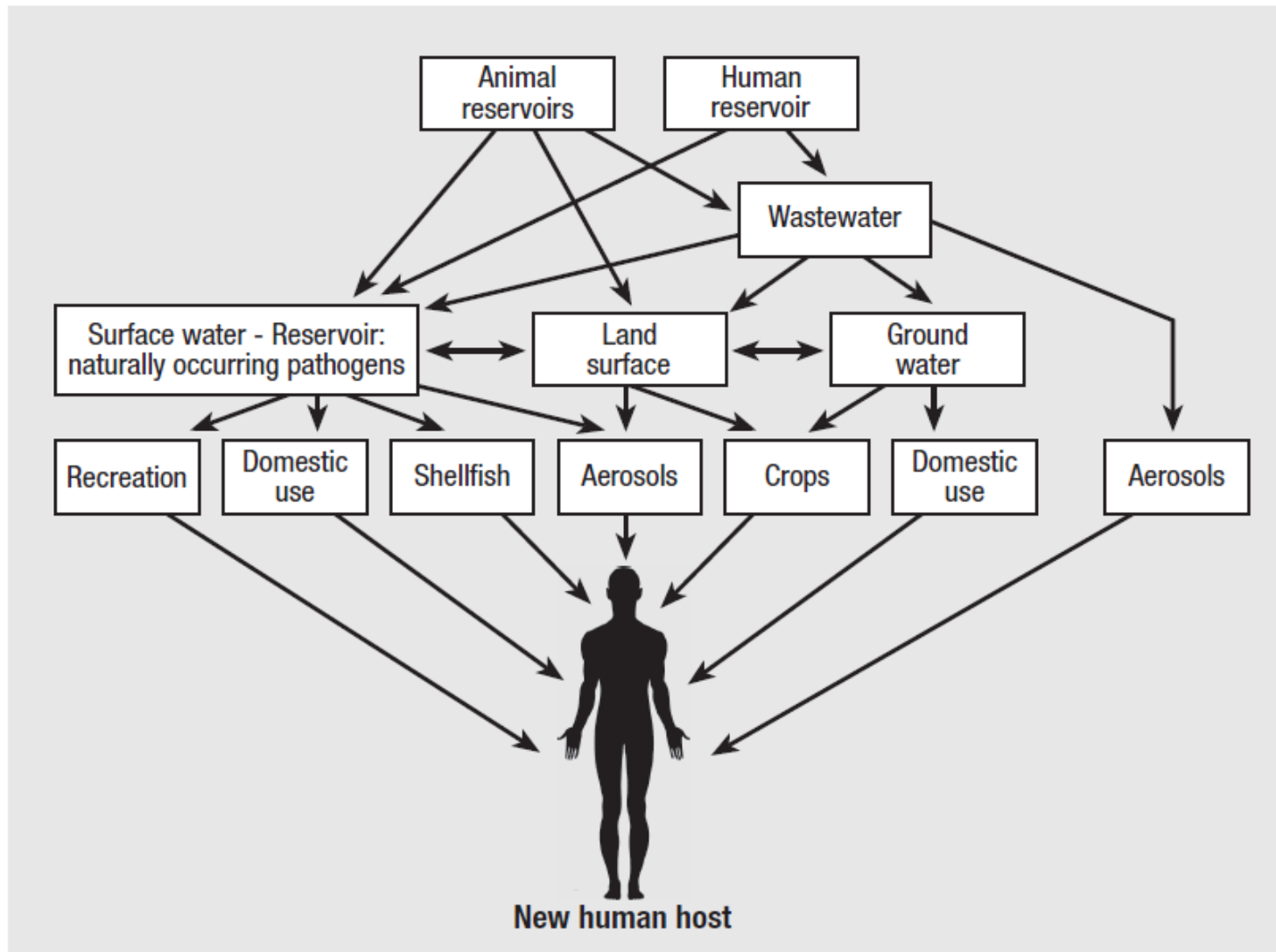


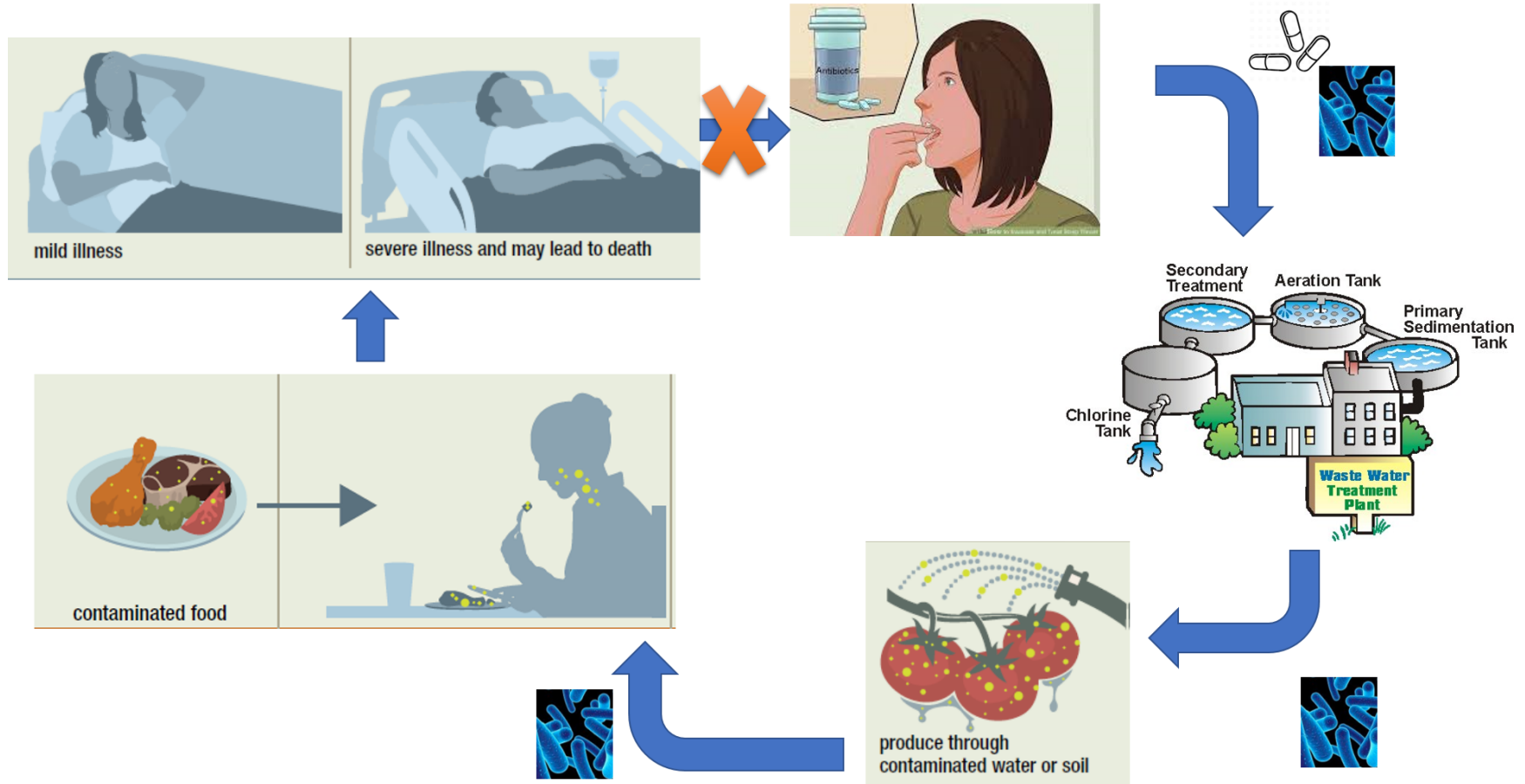
- Antibiotics commonly present in wastewater:
 - Tetracycline, sulphonamides, quinolones and macrolides.
- Attention should also be paid to:
 - Trimethoprim, cephalosporins, carbapenems and vancomycin.

How? By LC-Mass spectrometry

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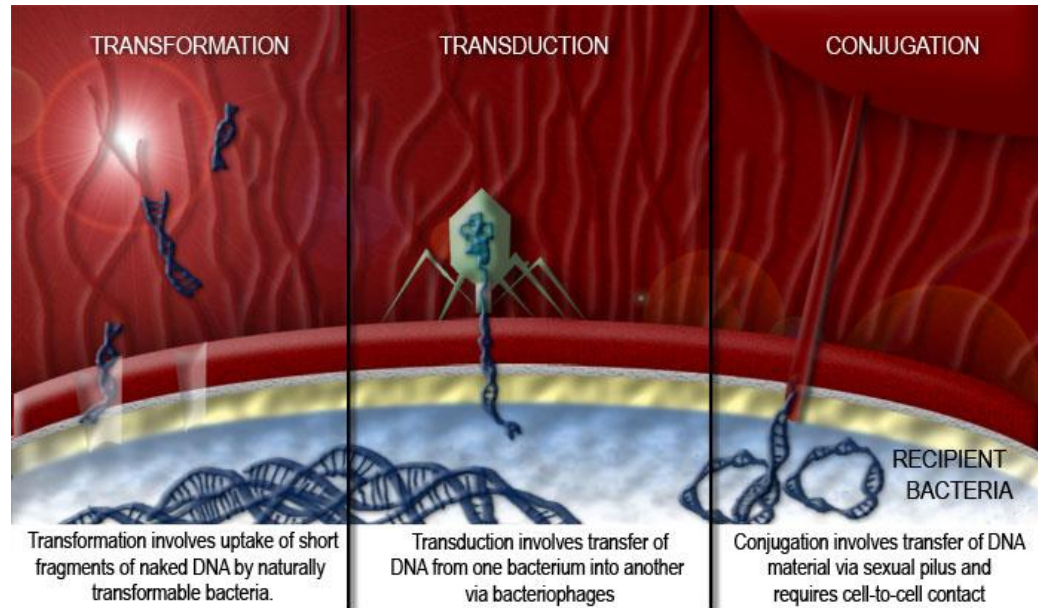
- What?
 - AB resistant bacteria, such as extended spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae*, *Aeromonas*, *Pseudomonas*, *Enterococci*, *Escherichia coli* and *Salmonella* are frequently found in wastewater.
 - These genera have strains known for their pathogenic potential, aggressive diseases outcomes and have been described as resistant to disinfection processes.
 - Some have even been detected in higher numbers in sewage effluent when compared to sewage influent.



WWTP are hotspots for AB resistance proliferation and dissemination

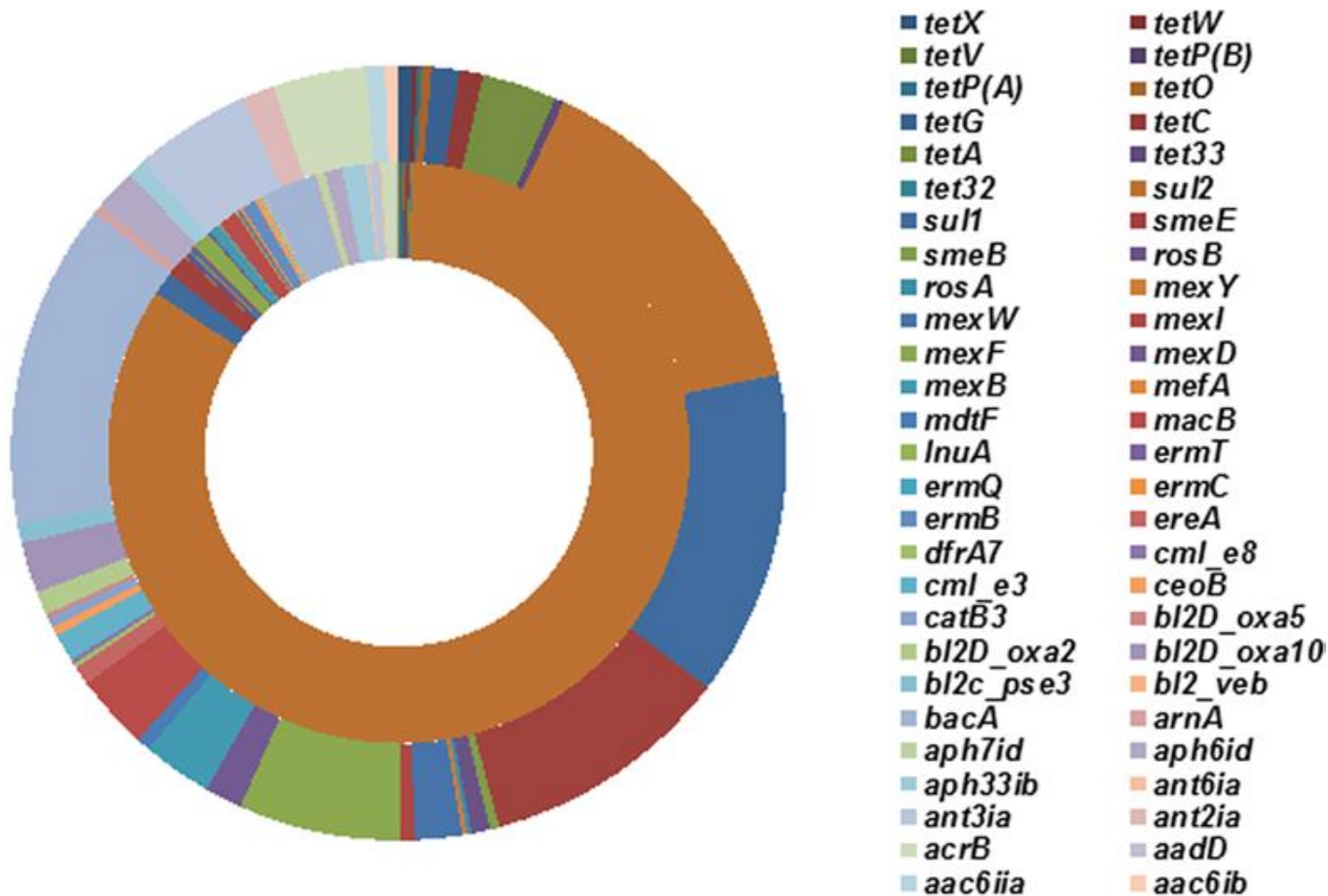
- How?
 - qPCR and NGS.

- Extracellular genomic DNA – eDNA:
 - DNA survival from microbial and spontaneous chemical degradation is due to binding to:
 - Biofilms,
 - Clay minerals,
 - Larger organic molecules,
 - Other charged particles:
 - which shield the adsorbed DNA from nuclease activity
 - Binding of nucleases also inhibits their ability to hydrolyse extracellular eDNA.
 - Humic acids, of which some are resistant to decay, also bind DNA molecules due to a negative surface charge.



- The eDNA can be released from the cells in which case it may bind to inorganic particles.
 - Protect the DNA from microbial and spontaneous chemical degradation
- Extracellular DNA may also be incorporated into the genomes of bacteria by:
 - Transformation
 - Transduction
 - Conjugation

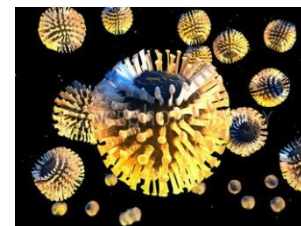
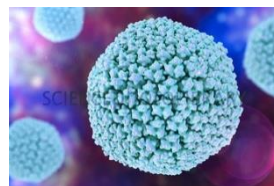
- What?
 - Antibiotic resistance genes (WWTP):
 - Tetracycline (tet)
 - Sulphonamide (sul)
 - Quinolone (qnr)
 - Macrolides (erm, msrA, mefA)
- How?
 - qPCR
 - NGS



Relative abundance of antibiotic resistance genes (ARGs) in sludge
Huang, K et al 2014 Int J Mol Sci 15: 10083-10100

The most commonly described viruses in wastewater are:

- Adenoviruses
- Noroviruses
- Enteroviruses
- Rotaviruses
- Hepatitis viruses
- Astroviruses



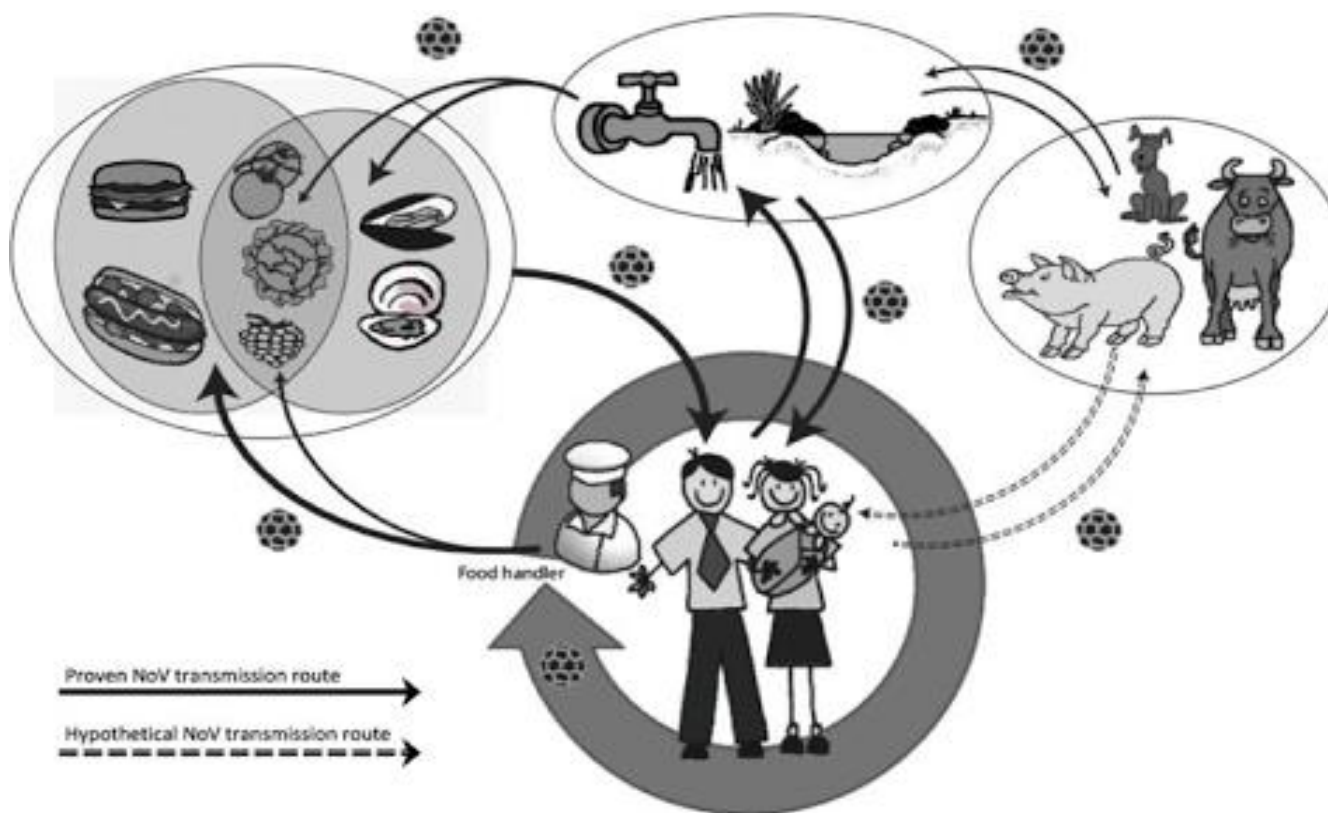
Waterborne viruses are stable due to the lack of a lipid envelope, which renders them a resistance to the environmental agents and disinfection processes

- 600 million, or almost 1 in 10 people in the world, fall ill after consuming contaminated food
 - 420 000 people die
 - 125 000 children under the age of 5 years die
- Diarrhoeal diseases are responsible for more than half of the global burden of foodborne diseases
 - 550 million people fall ill
 - 220 million children
 - 230 000 deaths every year
 - 96 000 children
- Other major contributors to the global burden of foodborne diseases

Norovirus

Hepatitis A

Virus transmission



- Infective doses are extremely low
 - only a few viral infectious particles (less than 100)
- Infected persons shed viral particles in stool and vomit
 - at peak level of 10^7 - 10^{10} viral copies per gram of faeces
- Illness lasts only a day or two
 - shedding of virus can continue for up to 60 days
- Viruses do not replicate in food under any temperature and/or water activity
 - they require living cells to replicate
- Viruses do not induce alterations of food ingredients
 - food smells, looks and tastes normal
- Viruses can persist for extended periods
 - in conditions which can otherwise inactivate common foodborne pathogenic bacteria.



Conventional detection:

- qPCR towards specific viruses types and genotypes more related to food outbreaks
- Cell culture infectivity assays (when viruses are culturable)

Novel approach:

- Viral metagenomics
 - Viruses identification by next generation sequencing



182085	460433	190674	152057	154	559	1042	304	29031	282675	87659	155178	18787	60114	18754	22756	Adenoviridae
2972	96	239	387	29850	7	494	57	1211	13	124	47	20338	27	335	219	Reoviridae
746	19	123	19	1676	24	153	8	1553	65	159	6	516	10	53	6	Picornaviridae
412	12	32	33	2040	20	54	26	237	4	6	8	785	13	31	8	Astroviridae
207	20	21	31	929	0	14	10	245	6	29	5	142	3	6	4	Picobirnaviridae
146	1	3	3	263	2	4	0	313	8	8	2	354	2	11	2	Caliciviridae
3	6	18	7	8	18	33	12	1	8	12	5	5	7	22	2	Parvoviridae
1	5	12	7	7	7	27	8	4	10	13	4	5	13	36	6	Circoviridae
11	0	0	2	13	1	14	1	29	3	36	0	2	2	37	3	Poxviridae
15	0	0	1	21	0	9	0	29	10	37	0	4	1	22	2	Herpesviridae
3	0	0	0	25	0	0	0	2	0	0	0	6	0	0	0	Hepeviridae
0	1	2	1	0	0	0	0	1	3	4	2	5	6	8	2	Polyomaviridae
0	2	2	1	0	0	0	0	0	1	1	0	1	1	3	0	Papillomaviridae
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	Anelloviridae
PEG_NUC	PEG_MIN	PEG_QIA	PEG_POW	SMF_NUC	SMF_MIN	SMF_QIA	SMF_POW	MAF_NUC	MAF_MIN	MAF_QIA	MAF_POW	GW_NUC	GW_MIN	GW_QIA	GW_POW	

Mathis Hjort Hjelmsø1 et al. PLOS One, 2107

- Further treatment
 - Chemical barriers?
 - Microbiological barrier / inactivation / death?
- Greenhouse cultivation of soft fruits
 - Watered with tap water
 - Watered with wastewater
 - Watered with treated wastewater



- Acceptance by stakeholders
- Improvement of reclaimed water treatments
- Knowledge based suggestions for food and water safety legislation

Heal the world

Heal The World
Make It A Better Place
For You And For Me
And The Entire Human Race
There Are People Dying
If You Care Enough
For The Living
Make A Better Place
For You And For Me

Michael Jackson Lyrics

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