CSE 2017

Surveillance of Antibiotic Resistance in the environment

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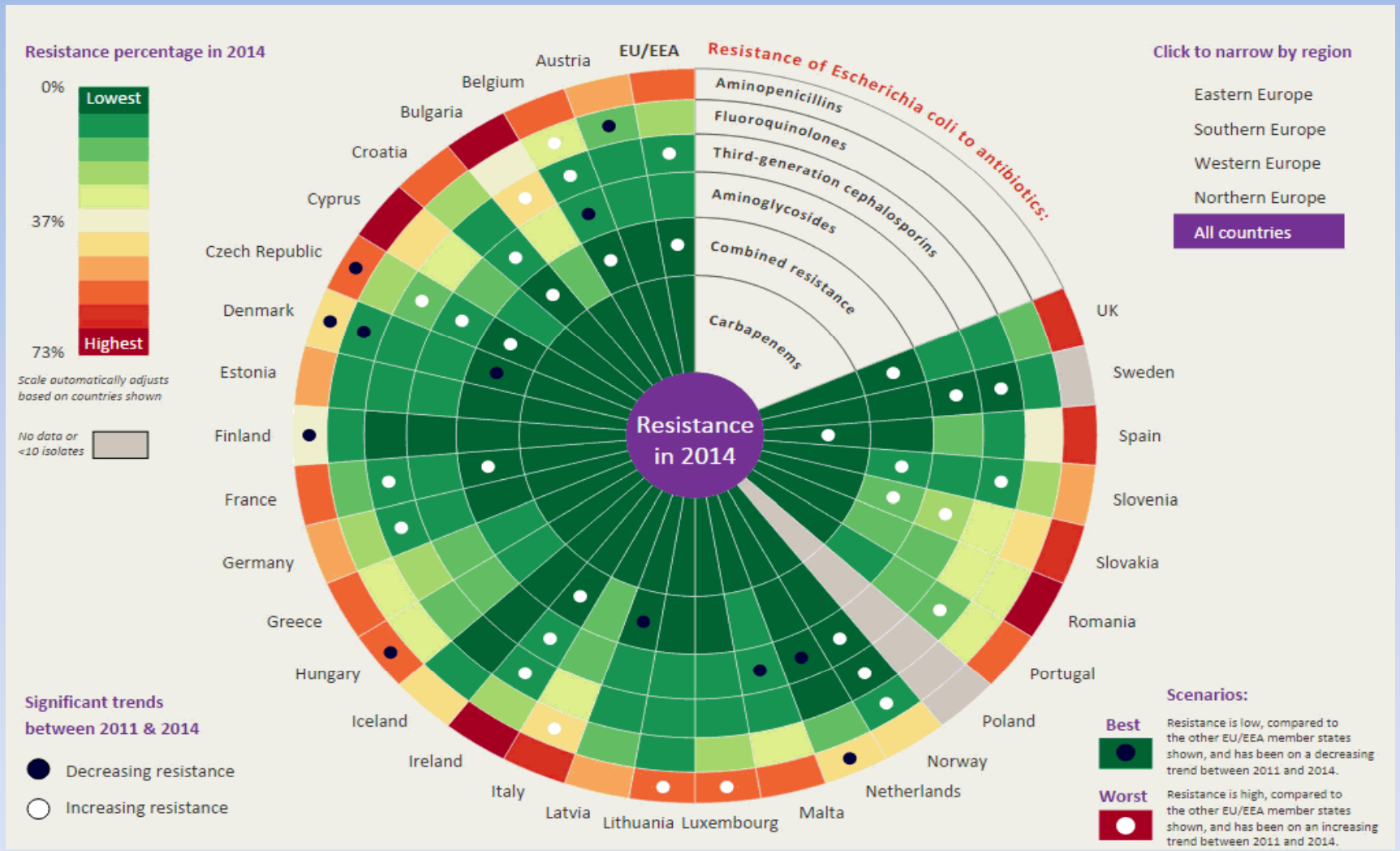
Global Spread of AMR

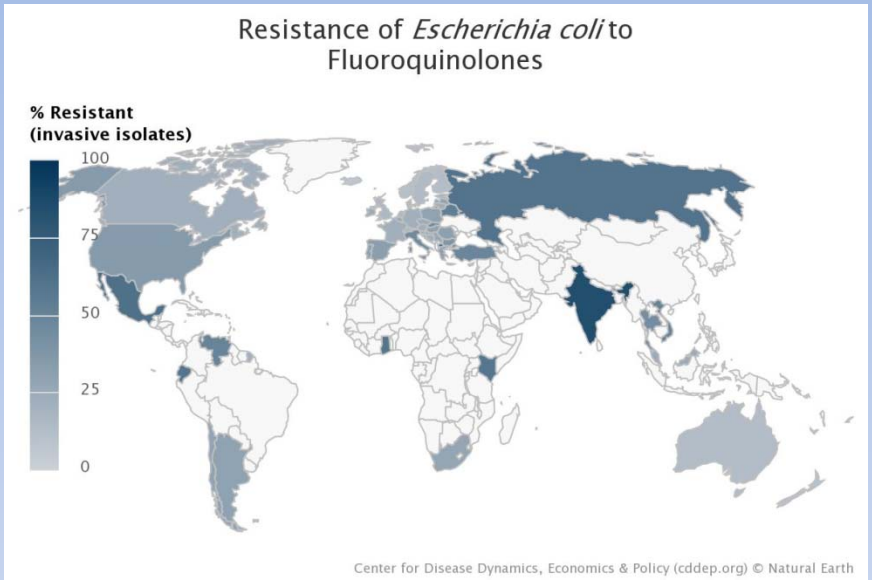


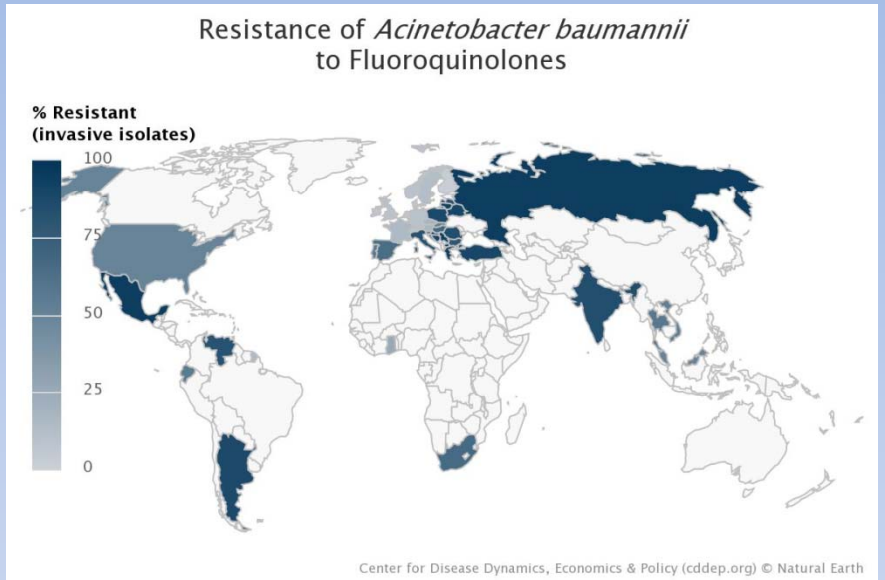
Data from European Center for Disease Prevention and Control
Image Courtesy – BIVDA DIAGNOSTICS (https://www.bivda.co.uk/)

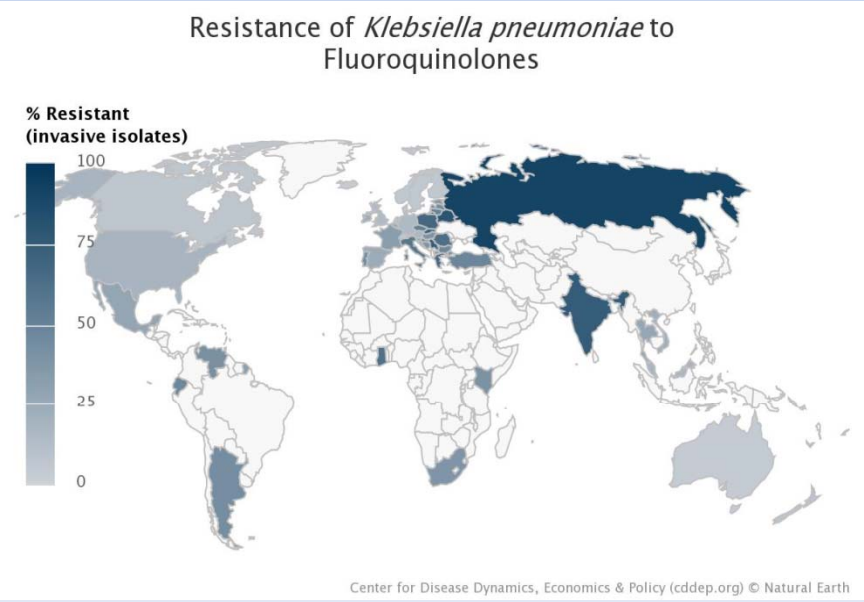
How big is this problem?

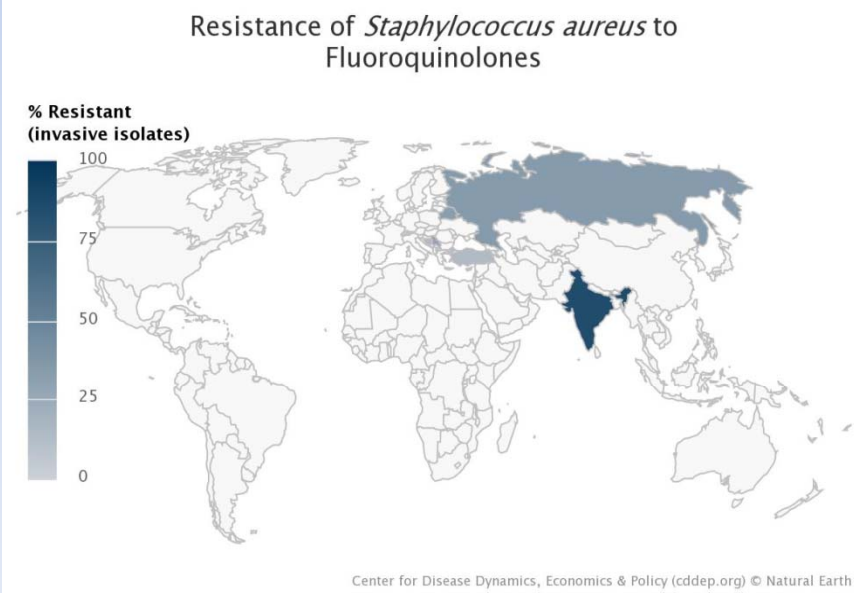
- On an average, Indians consume around 11 antibiotic tablets every year
- India is now the world's largest consumer of antibiotics with 62% increase in consumption reported over the last decade
- 95% of antibiotics in general are known to be excreted without being altered
- A research study published in the British Journal "Lancet" in 2010 by lead author Karthikeyan Kumarasamy about NDM1 superbug created a storm in the heathcare sector of India
- After this episode, recently in 2016, Indian researchers have identified *mcr*-1, which has resistance to the last mile antibiotic colistin human has access it

Where are we?


Resistance of *Escherichia coli* to Fluoroquinolones
% Resistant (invasive isolates)
100
75
50
25
0
Center for Disease Dynamics, Economics & Policy (cddep.org) © Natural Earth


Resistance of *Acinetobacter baumannii* to Fluoroquinolones
% Resistant (invasive isolates)
100
75
50
25
0
Center for Disease Dynamics, Economics & Policy (cddep.org) © Natural Earth


Resistance of *Klebsiella pneumoniae* to Fluoroquinolones
% Resistant (invasive isolates)
100
75
50
25
0
Center for Disease Dynamics, Economics & Policy (cddep.org) © Natural Earth


Resistance of *Staphylococcus aureus* to Fluoroquinolones
% Resistant (invasive isolates)
100
75
50
25
0
Center for Disease Dynamics, Economics & Policy (cddep.org) © Natural Earth

MICROBIAL RESISTANCE IN INDIA

Data collected at various hospitals during 2007-11 through clinical samples and microbiology analysis shows a high rate of resistance to most of the standard antibiotics.

Year	Location	Samples	Organism	Resistance rate to drugs
2007	Delhi	Stool samples	V. Cholera 01	**96%** to furazolidone, cotrimoxazole, nalidixic acid
2007	Kolkata	284 clinical samples	Metalo beta lecatmase	**43.3%** to standard antibiotics
2007	Lucknow	2,995 blood samples	Klebsiellaspp	**98.28%** to ampicillin, ticarcillin, piperacillin
2008	Puducherry	261 clinical samples	Staphylococcus	**72.34%** to oxacillin
2009	Nagpur	1,300 nasopharyngeal swabs	MRSA	**4.16%** to standard antibiotics
2010	Vellore	176 clinical samples	P. aeruginosa	**42.6%** to carbapenem
2010	Puducherry and other parts	31 clinical samples	K. pneumoniae	**93.55%** multi-drug resistance
2010	Mangalore	83 CA-MRSA clinical samples	Staphylococcus aureus	**92.8%** to penicillin, **31.32%** to erythromycin
2010	New Delhi	83 OPD payoderma cases	CA-MRSA	**9.6%** to standard antibiotics
2010	Mangalore	180 clinical samples	Enterococcus strains	**16.67%** to **42.86%** to aminoglycosides
2011	Sikkim	291 clinical samples	MRSA	**38.14%** to standard antibiotics
2011	New Delhi	3,984 clinical samples	Gram negative, Pseudomonas Acinetobacter, etc.	**50%** to carbapenems, **66%** to aminoglycosides, **76%** to fluroquinilones, **88%** to third-generation cephalosporins, **66%** to betelactam combinations, **58%** to methacillin

MRSA: Methicillin-resistant Staphylococcus aureus; CA-MRSA: Community-associated MRSA; OPD: Out patient department

Source: Journal of Natural Science, Biology and Science

Image source - The perils of irrational antibiotic use – Livemint –Aug 2017

Antibiotics and its Persistence

- Emerging chemicals of environmental concern
- Widely used in human and veterinary medicine
- Most frequently detected in various compartments of aquatic environment

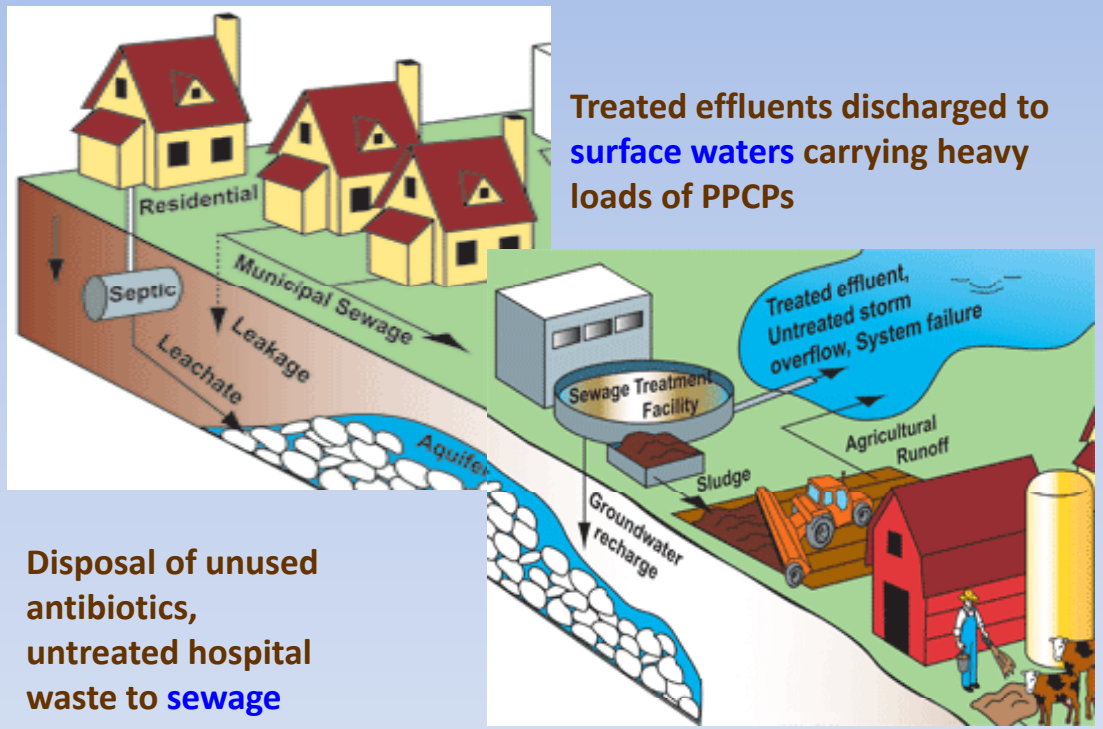
- Persistent in solid environmental matrices for long time.
- Persistence depends on their
  - photostability
  - binding
  - adsorption capacity
  - degradation rate
  - leaching

- Incomplete removal in WWTP - breeding grounds for antibiotic resistant bacteria

- Strongly sorbing - tend to accumulate in soils or sediment

- Highly mobile pharmaceuticals have a potential to resist degradation and tend to leach into the groundwater and to be transported with the drainage water and surface water run-off.

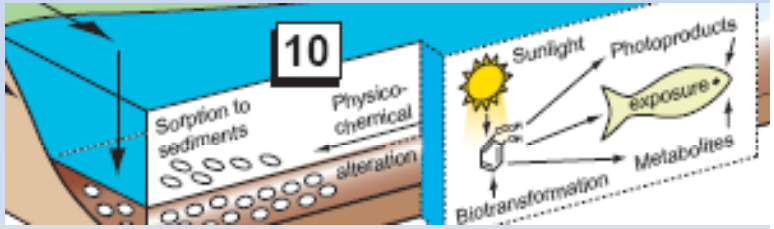
SOURCE AND FATE OF ANTIBIOTICS



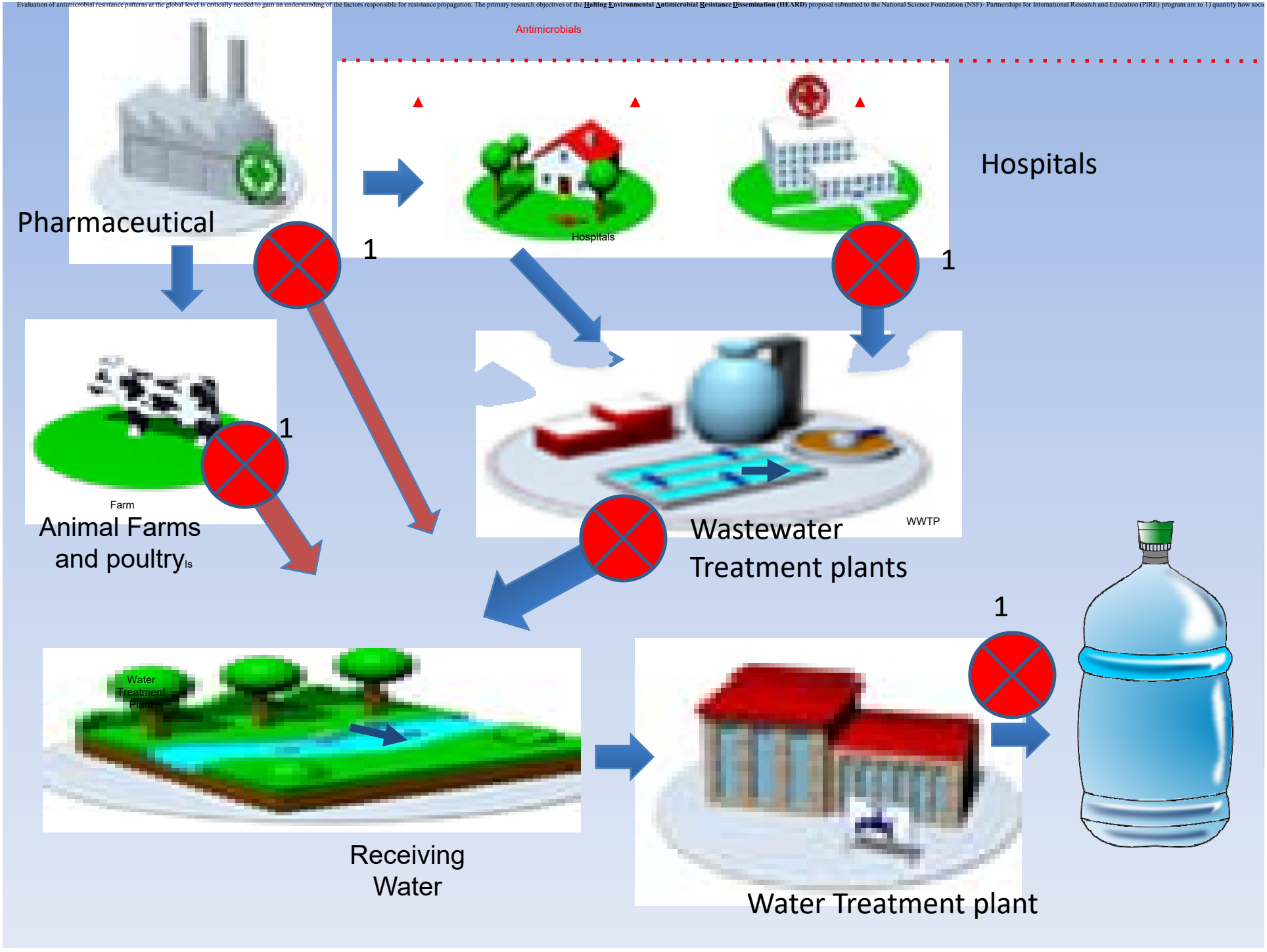
Treated effluents discharged to surface waters carrying heavy loads of PPCPs

Disposal of unused antibiotics, untreated hospital waste to sewage

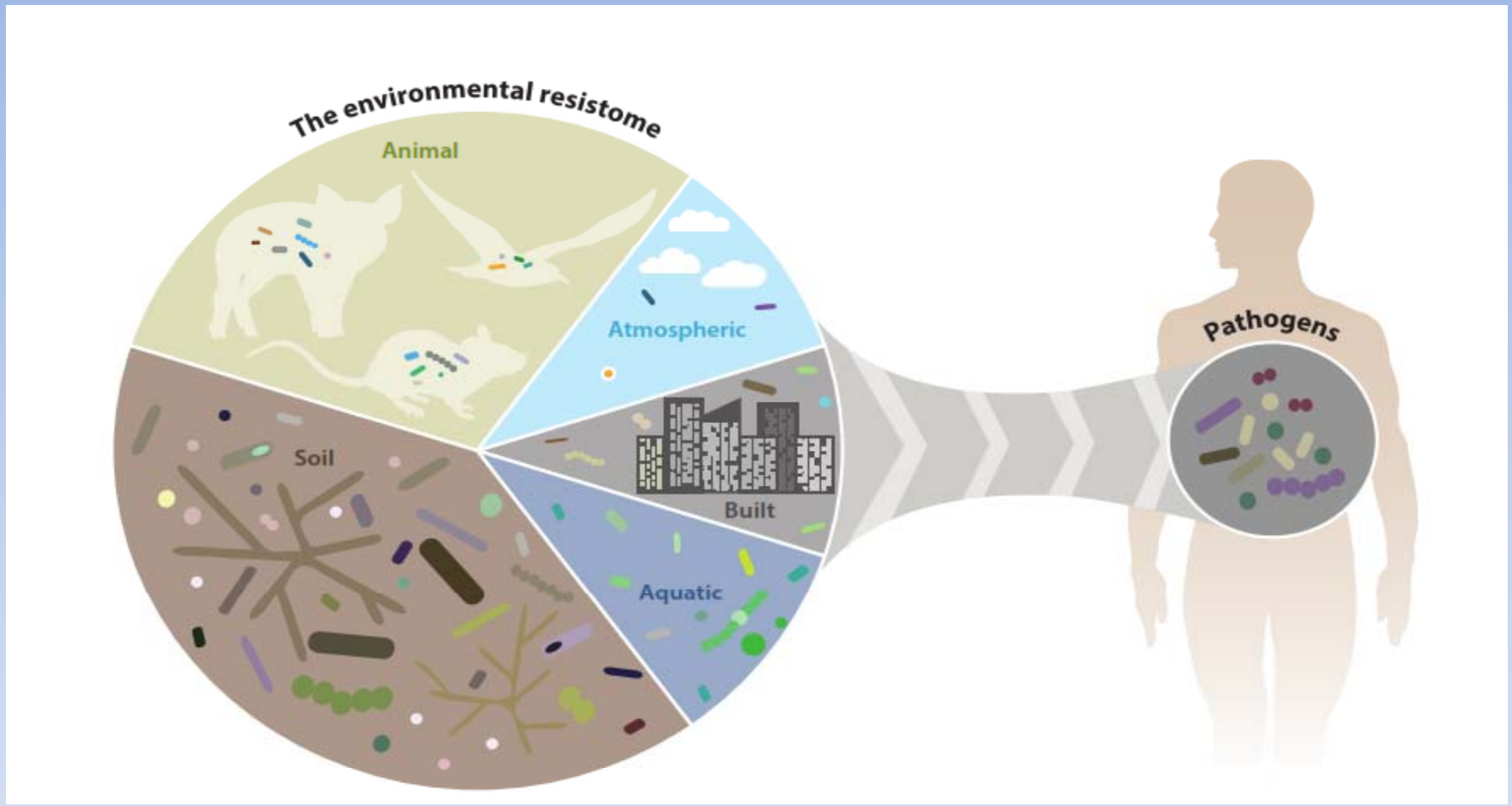
Fate of antibiotics - Phototransformation, Physicochemical alteration, biotransformation, volatilization



Evaluation of antimicrobial resistance patterns at the global level is critically needed to gain an understanding of the factors responsible for resistance propagation. The primary research objectives of the **Halting Environmental Antimicrobial Resistance Dissemination (HEARD)** proposal submitted to the National Science Foundation (NSF)- Partnerships for International Research and Education (PIRE) program are to 1) quantify how soci



Environment - The single largest source and reservoir of resistance



Surette, M., & Wright, G. D. (2017). Lessons from Environmental Antibiotic Resistome. *Annual Review of Microbiology*, *71*(1).

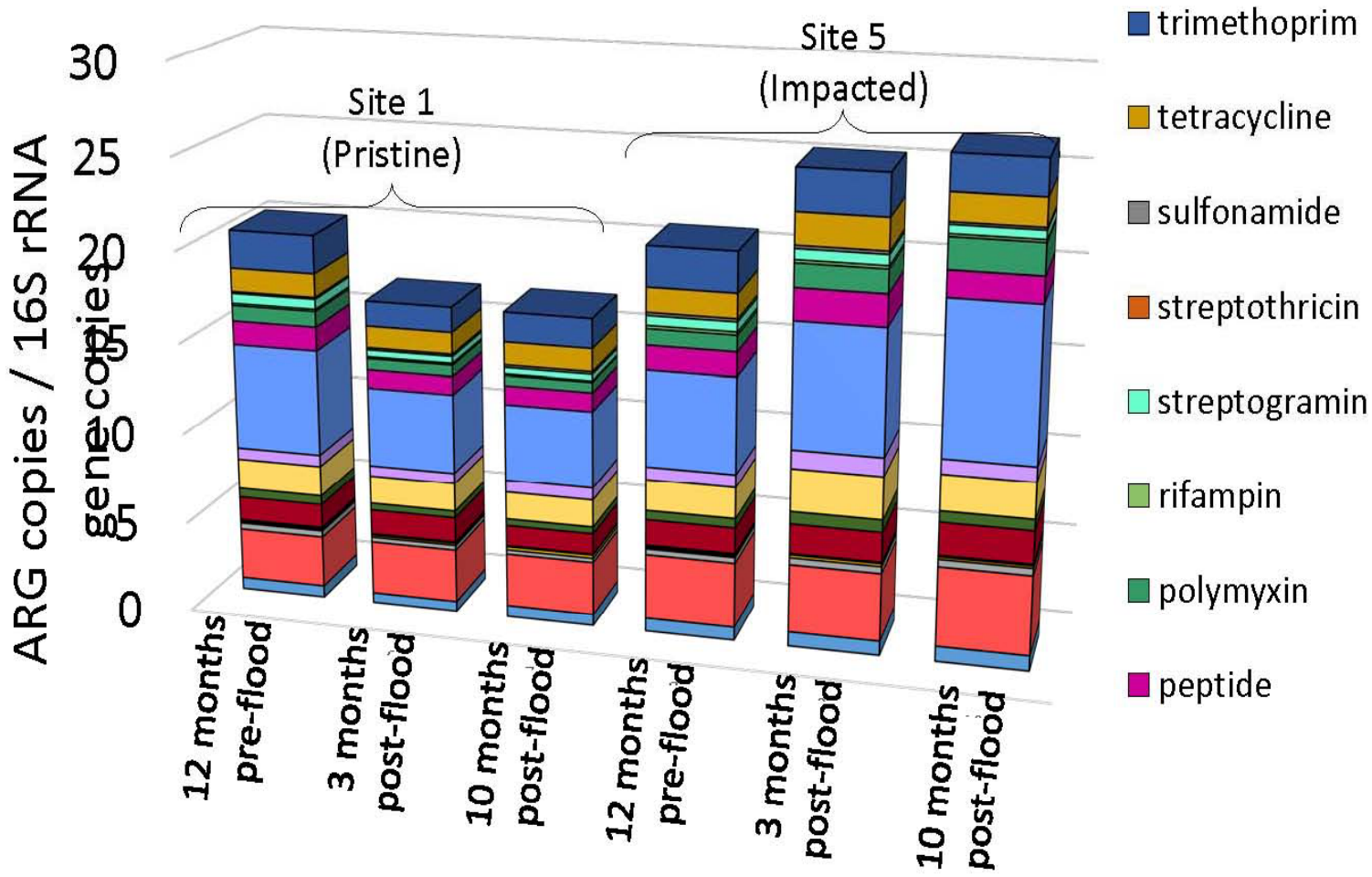
Status of antibiotics monitoring in India

S.No	NAME OF THE COMPOUND	LOCATION	REPORTED RANGE (ng/L)	REFERENCE
1.	Ciprofloxacin	Patancheru Enviro	28,000–31,000	Larsson *et al.*,
	Losartan	Tech Ltd.; PETL	2,400–2,500	2007
	Cetrizine	Hyderabad	1,300–1,400	
	Metoprolol		800–950	
	Enrofloxacin		780–900	
	Citalopram		770–840	
	Norfloxacin		390–420	
	Lomefloxacin		150–300	
	Enoxacin		150–300	
	Ofloxacin		150–160	
	Ranitidine		90–160	
			•Rnnges in µg/L	
2.	Amoxicillin	Wastewater from Vasanthgunj STP, Delhi	53-58, ND-62.5, 22.9-23.3, ND, 23-52.1	Mutiyar and Mittal, 2014

Impact of Human Inputs on AMR in Environment

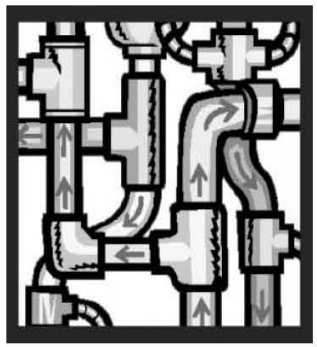
- Phenomenon is now well-documented:
  - ARGs elevated in Duluth harbor receiving WWTP effluent (LaPara et al. 2011)
  - River in Cuba (Knapp et al. 2012)
  - Swiss Lake (Czekalski et al. 2014)
  - Viable MRSA and vancomycin-resistant *Enterococci* have been detected in reclaimed water intended for reuse (Sapkota and colleagues 2012, 2013)

Metagenomic Profiling of 1000 year storm impact on Poudre River ARGs



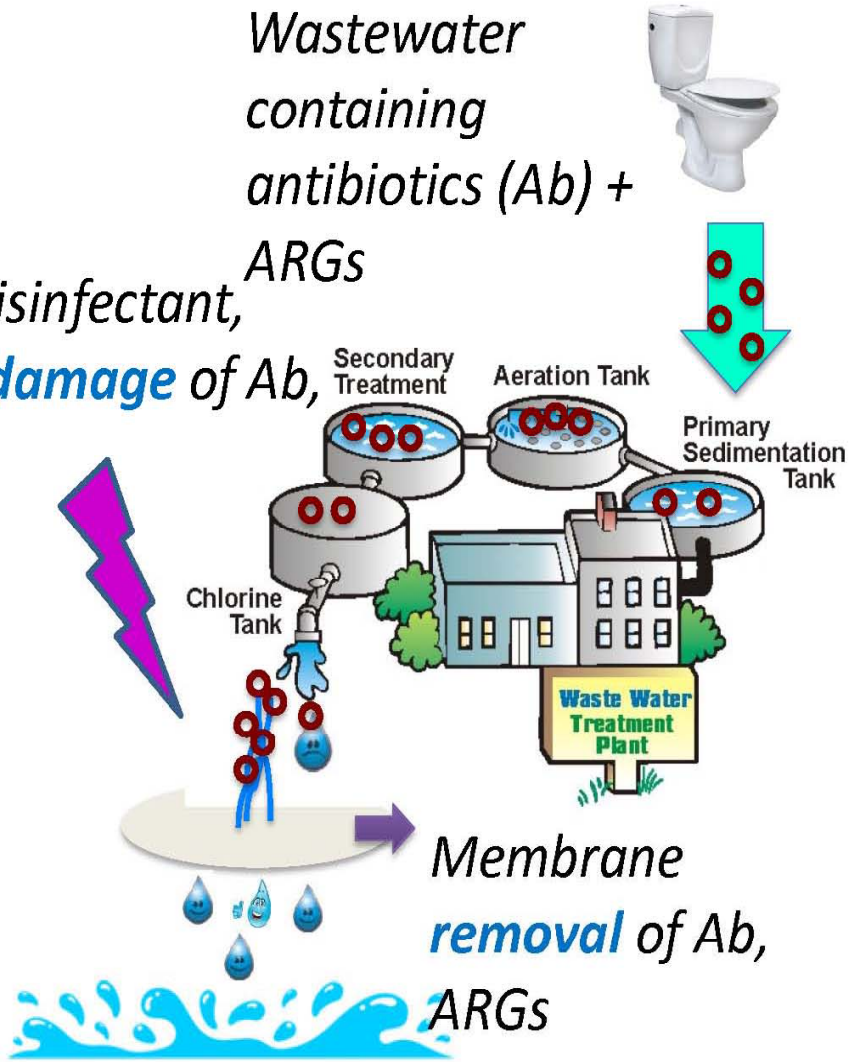
Management of Antibiotic Resistance Risk in Sustainable Water Systems

- DNA/Ab **Removal**
- DNA/Ab **Damage**
- Management of **Distribution System** and Other Infrastructure



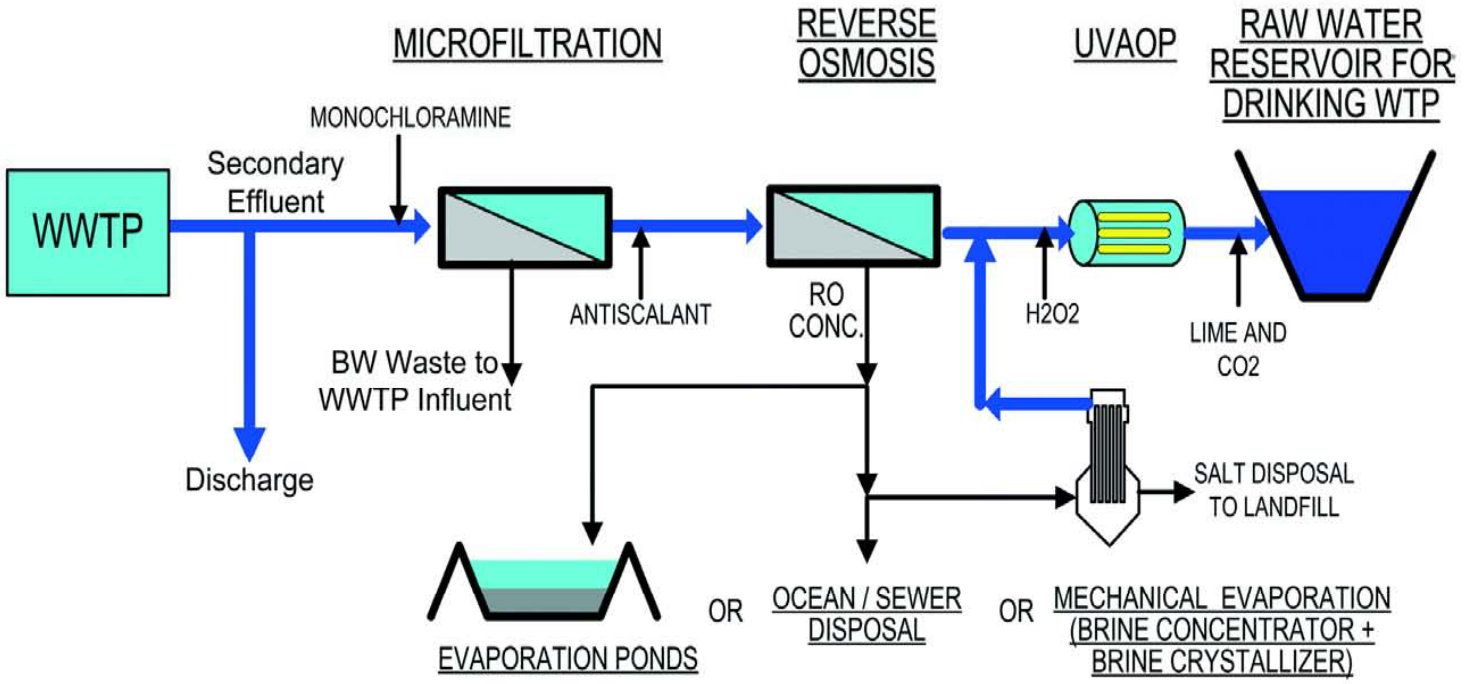
Wastewater containing antibiotics (Ab) + ARGs

*UV, disinfectant, AOP **damage** of Ab, ARGs*



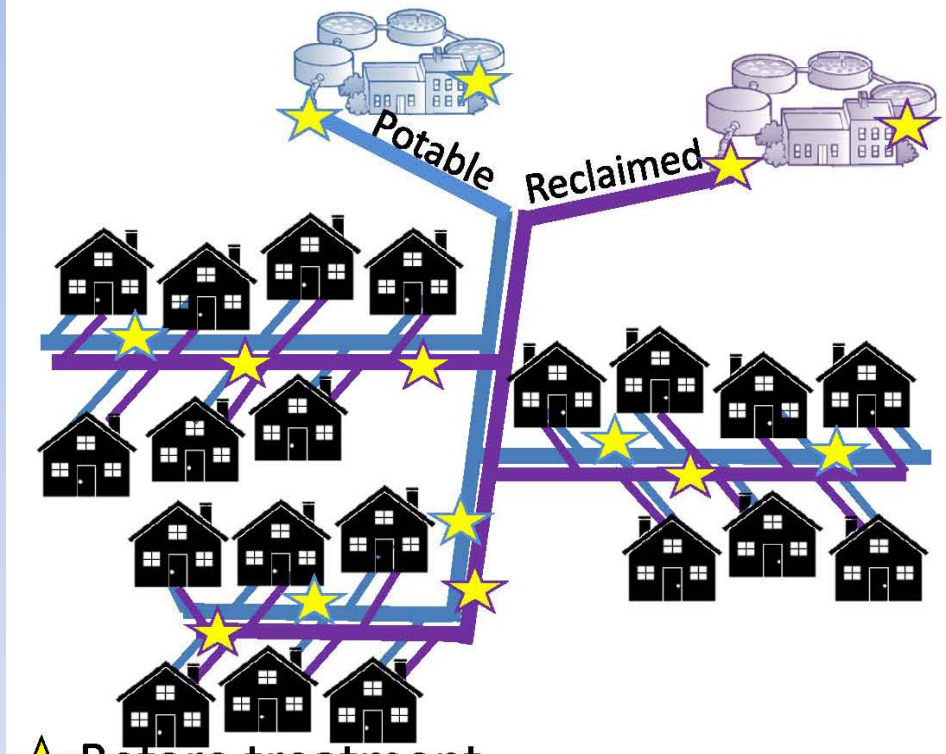
*Membrane **removal** of Ab, ARGs*

Direct Potable Reuse



Schimmoller et al. *ES:WR&T* 2015 CH2MHill

Recycled Water Distribution Systems



★ Before treatment

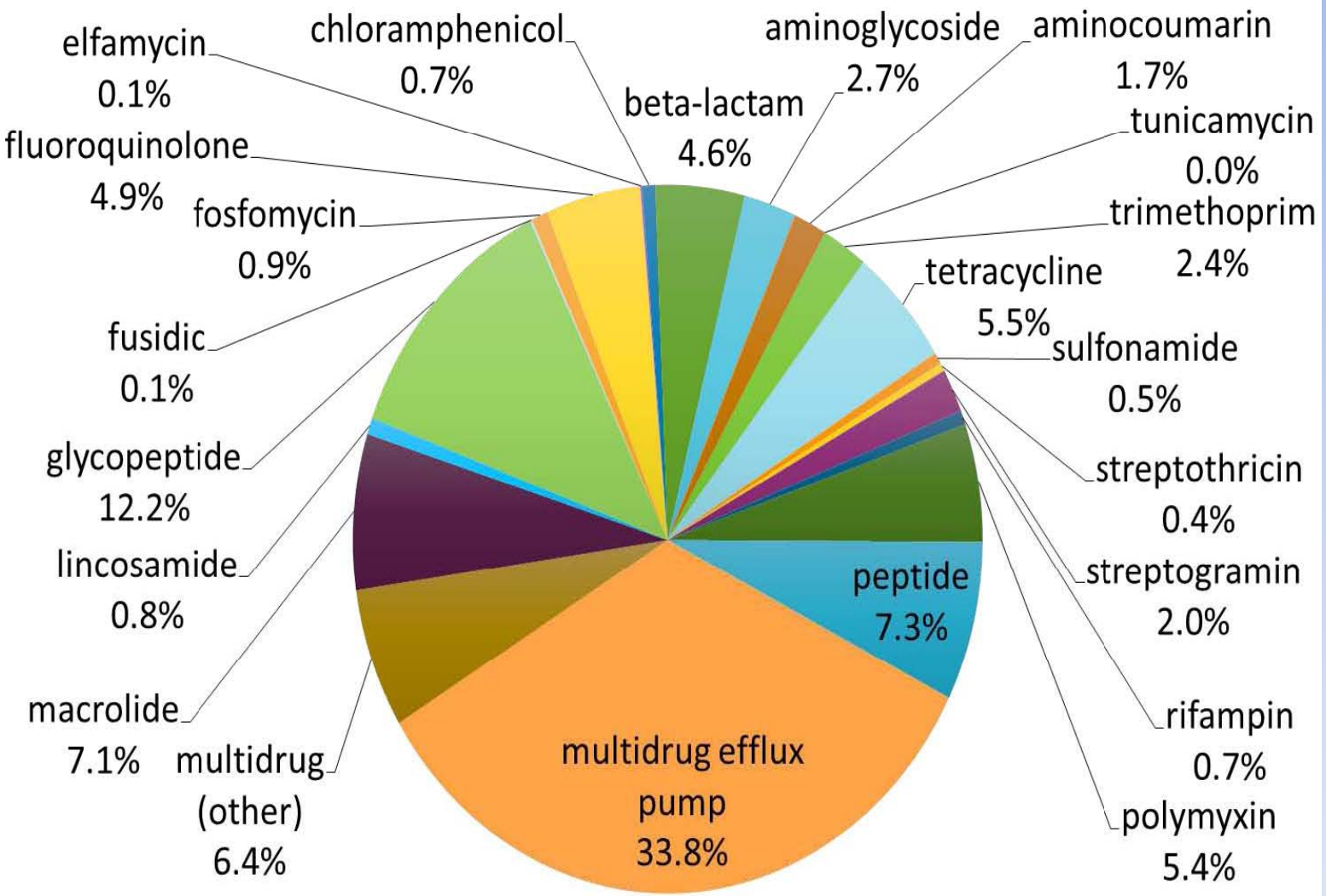
★ Point of Entry

★ 5 Dist. System (Point of Use)

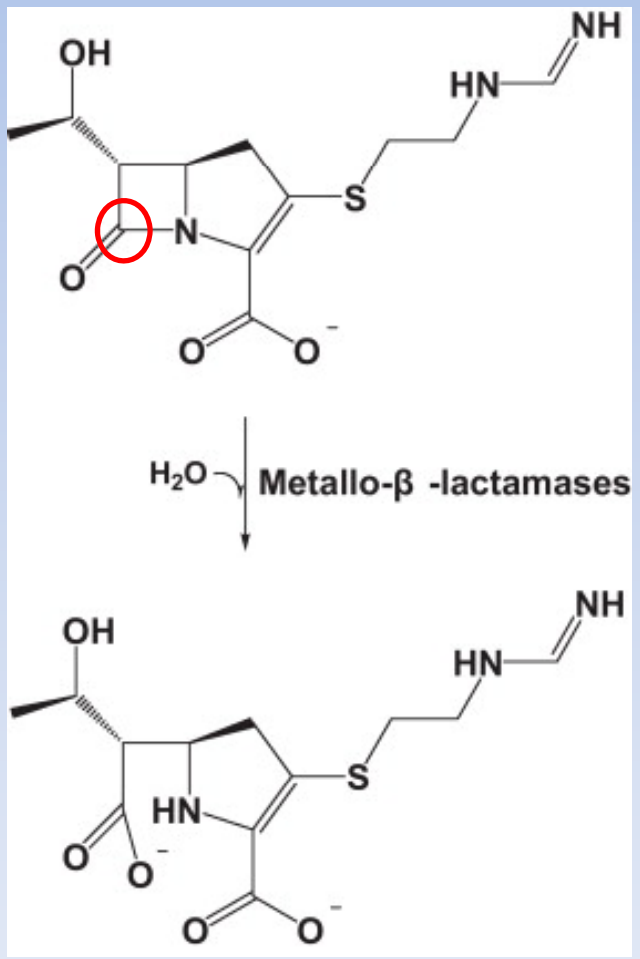
Water Chemistry

– pH, temperature, disinfectant residual, DO, turbidity, metals, anions

Metagenomics: Average ARG Composition in Reclaimed Water



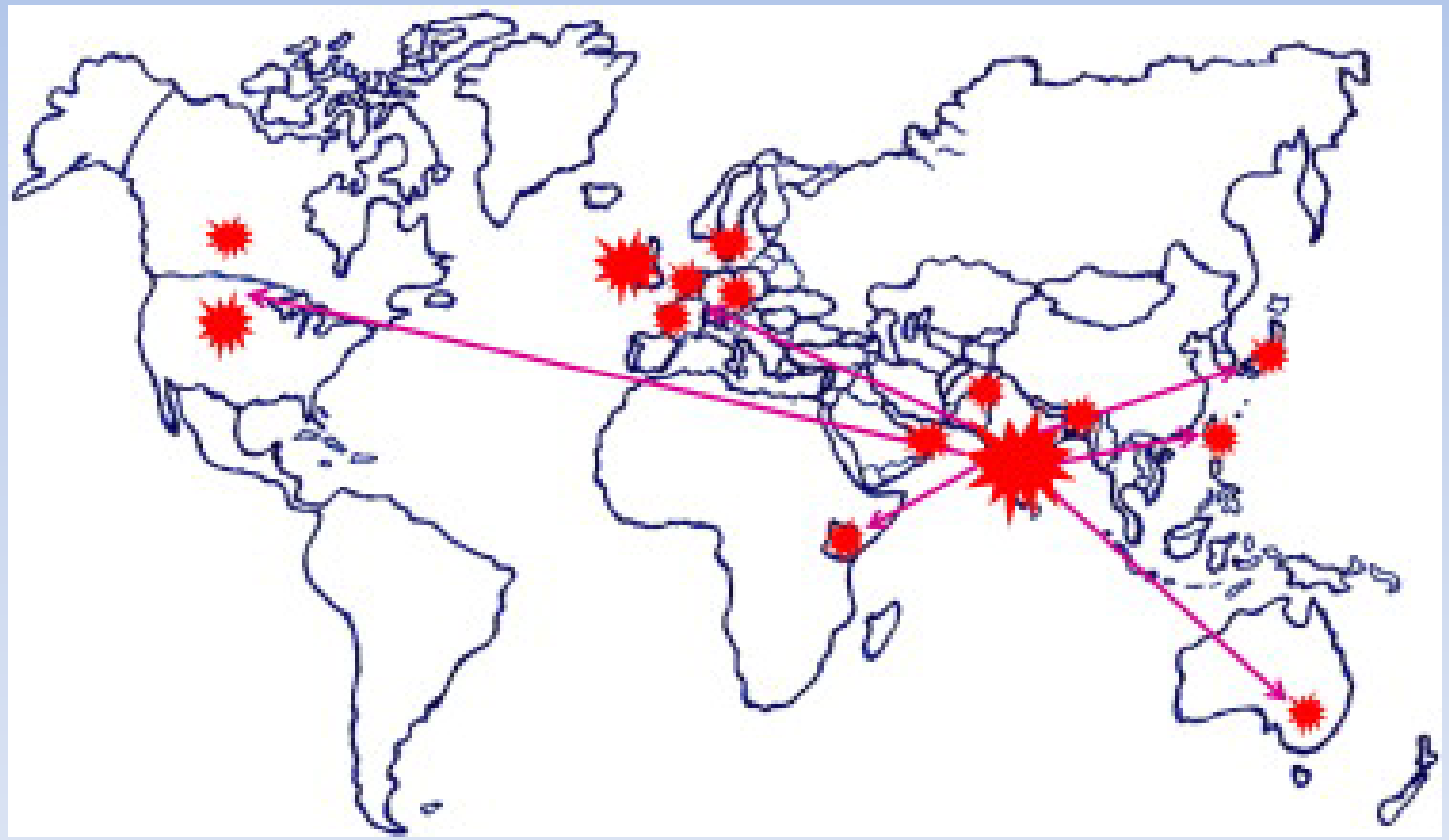
Superbugs include beta-lactam resistant bacteria that carry the New Delhi metallo beta lactamase (NDM-1) gene



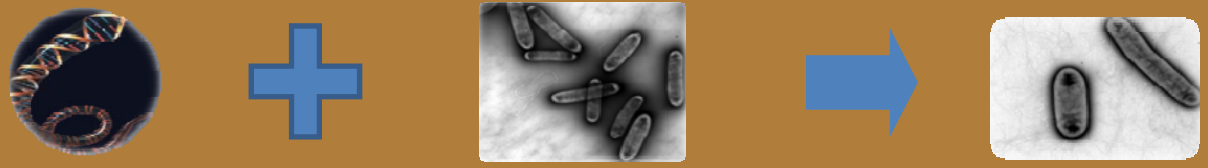
NDM-1

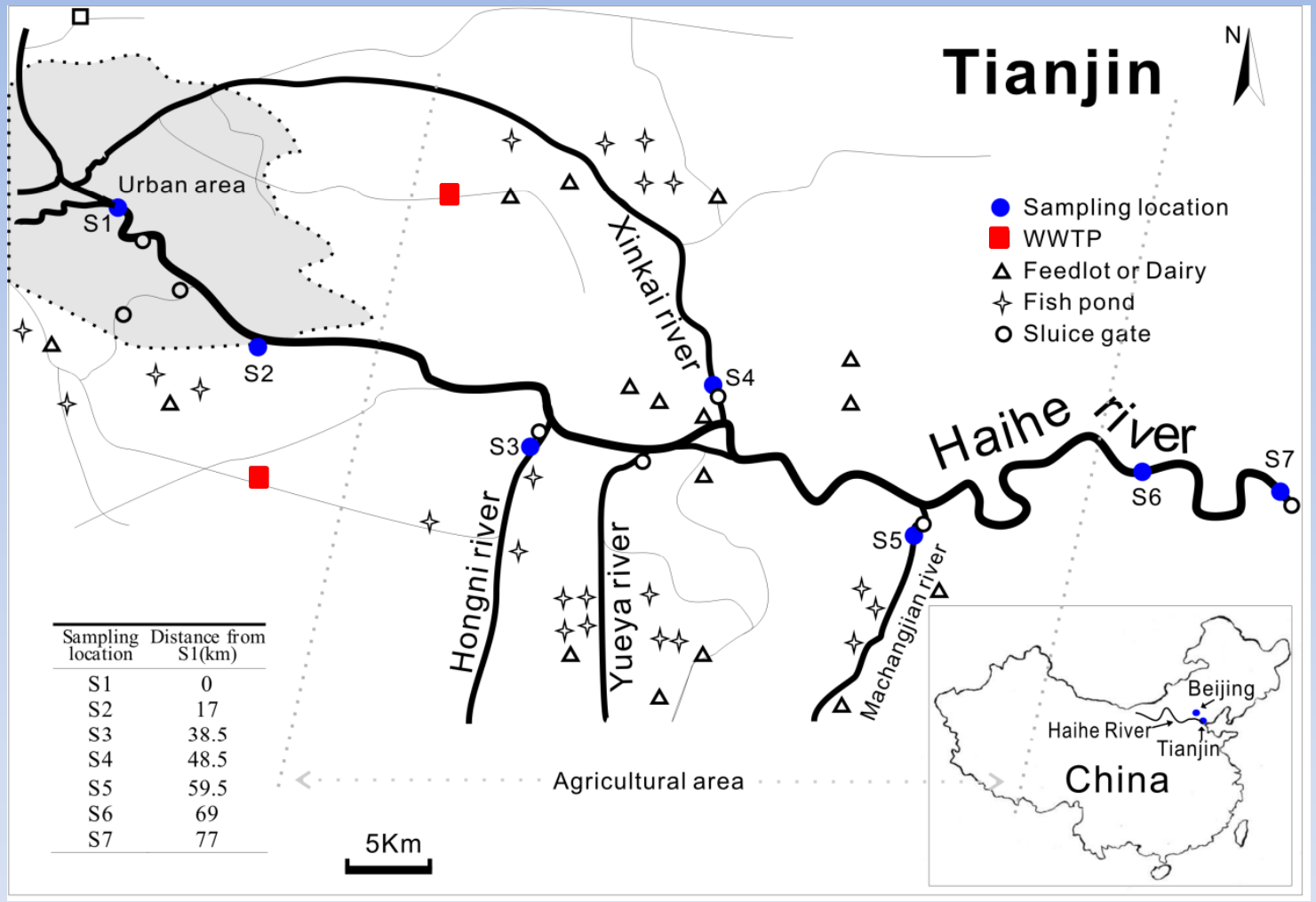
- Produces an enzyme that counteracts effect of beta-lactam type antibiotics;
- Many of the initial patients had visited India/Pakistan for cosmetic surgery;
- *Alarm over gene's ability to replicate across bacterial species.*

Spread of NDM-1 in 2010



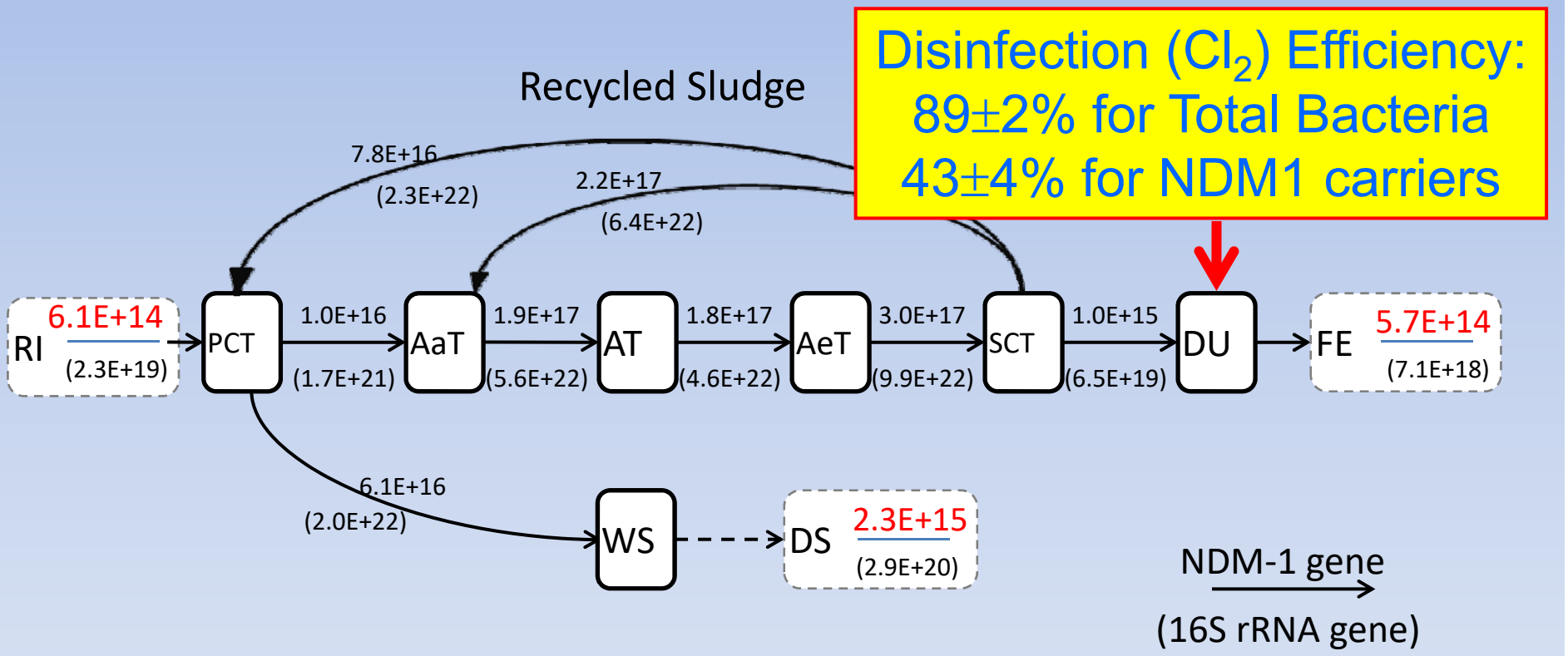
Are superbugs reaching sewage treatment plants, and if so, how do they behave though the activated sludge process?





Sampling location	Distance from S1(km)
S1	0
S2	17
S3	38.5
S4	48.5
S5	59.5
S6	69
S7	77

Proliferation of Multi-drug Resistant "Superbugs" (NDM-1 positive) from Sewage Treatment Plants

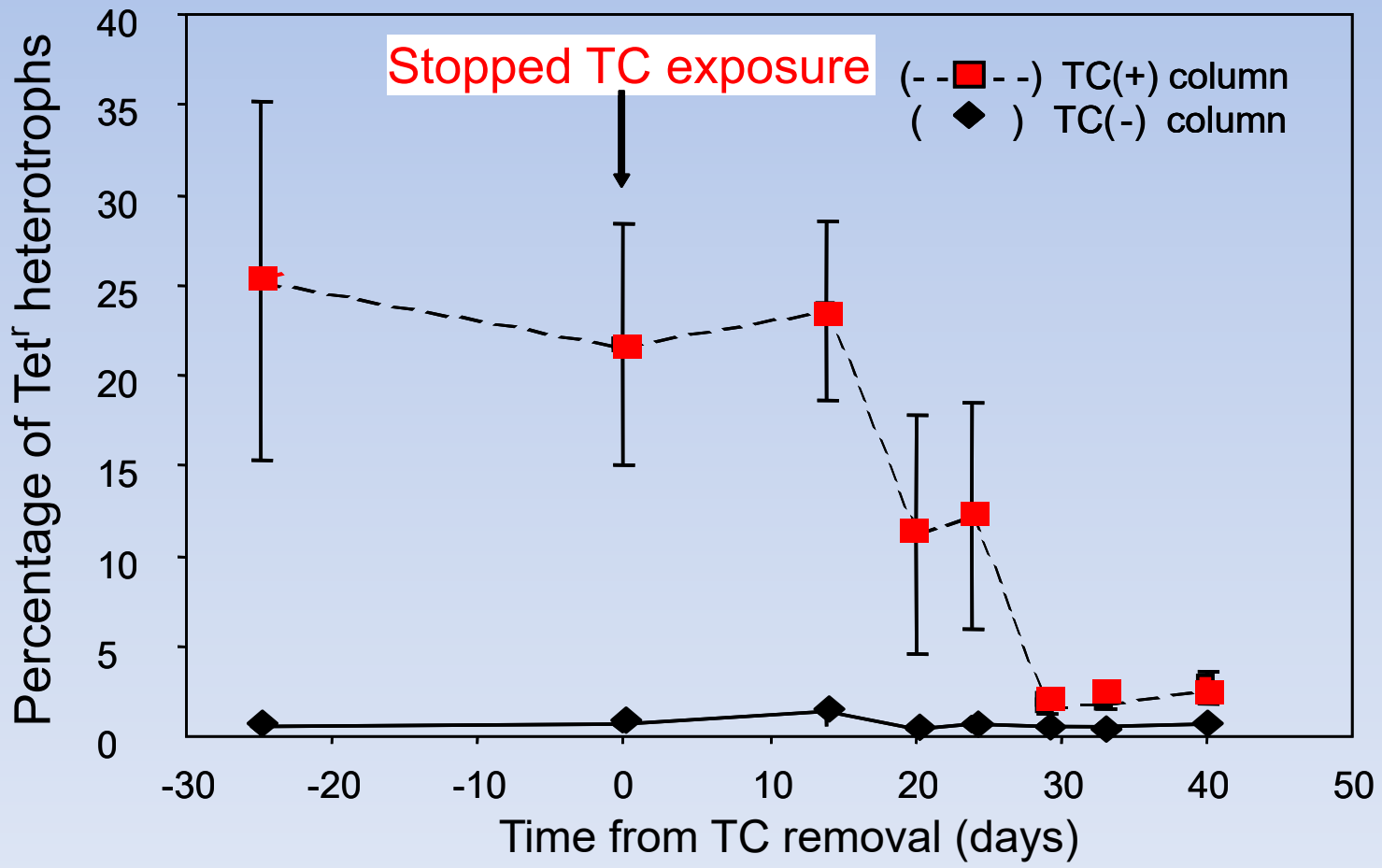


NDM-1 flow output (genes/day) was
4.6-fold greater than influent values

Yi L., F. Yang, J. Mathieu, D. Mao, W. Qing, and P.J.J Alvarez (2013). ES&T Letters.

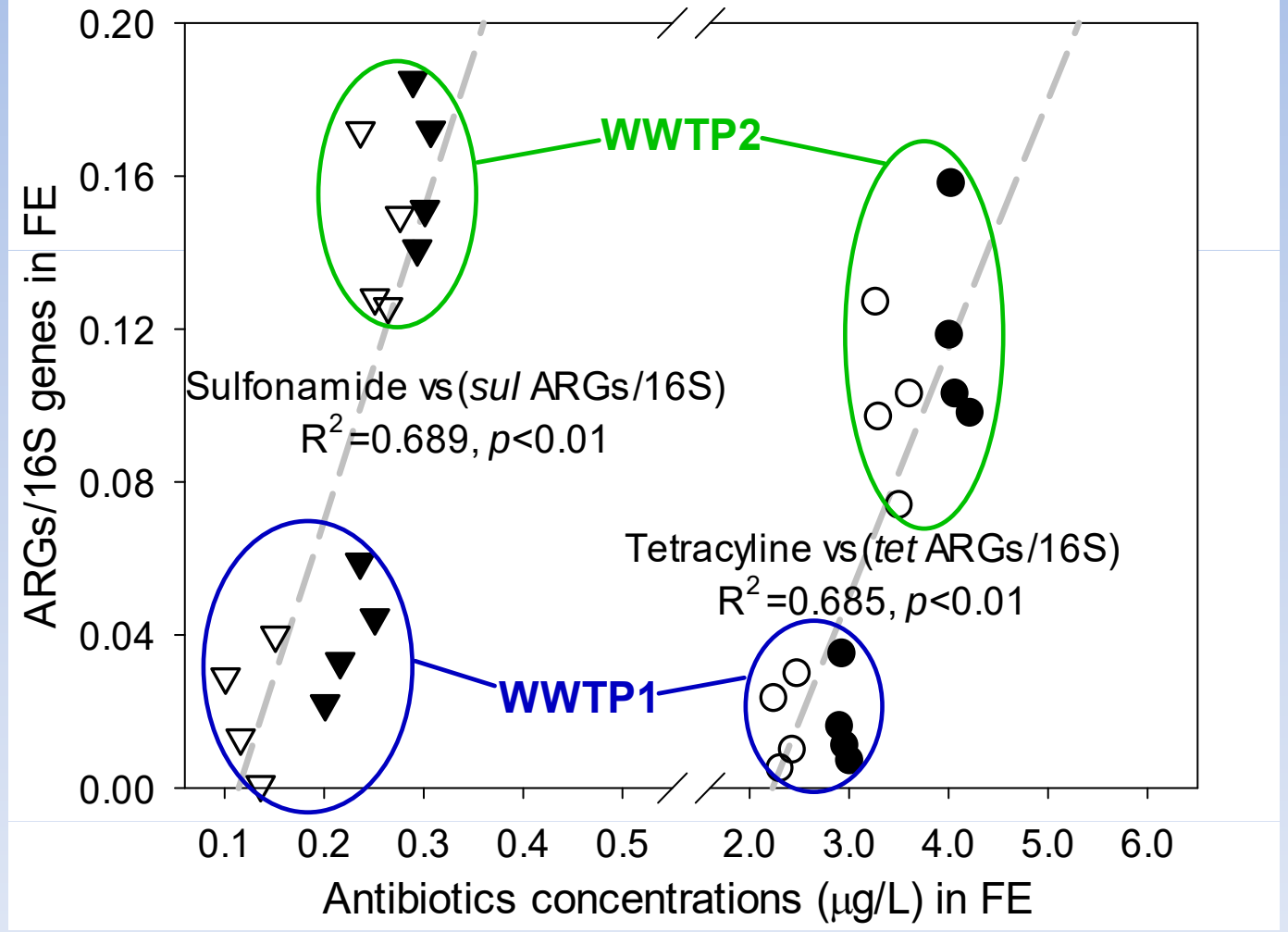
What can WWTPs do to mitigate the proliferation of NDM-1 and other antibiotic-resistant genes (ARGs)?

Antibiotics exert selective pressure for ARG maintenance



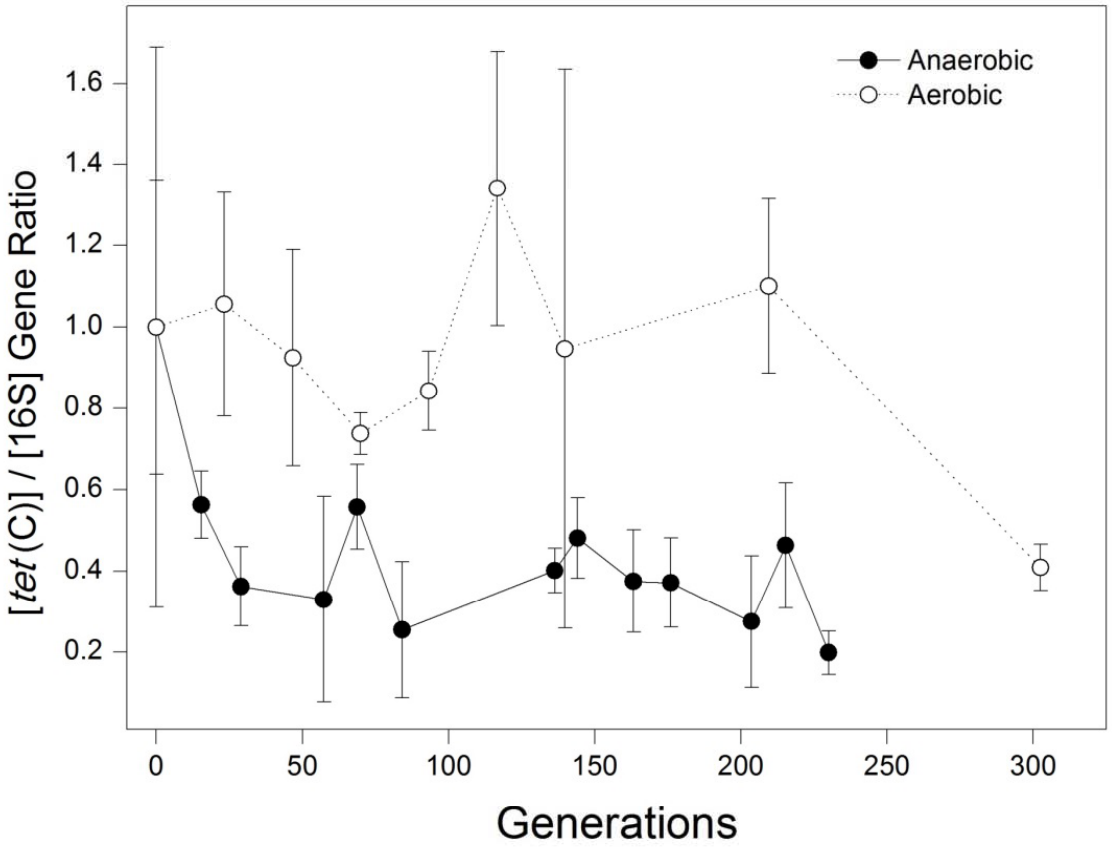
Rysz M., and P.J.J. Alvarez (2004). Water Research

Positive correlation between antibiotic concentrations and the relative abundance of the corresponding ARGs (ARG/16S rRNA genes) in the final effluent (FE)



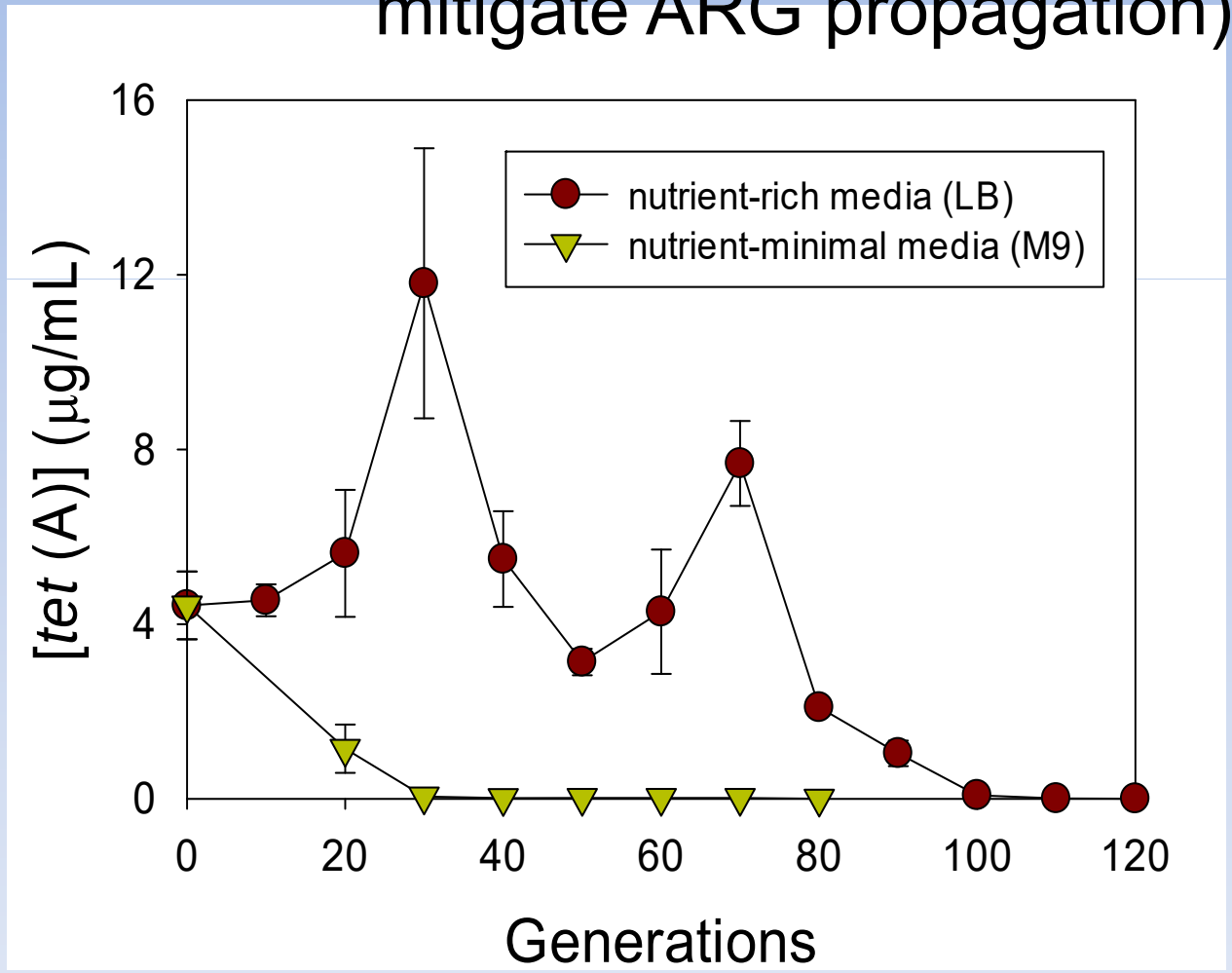
Luo Y., S. Yu, M. Rysz, D. Mao, F. Yang, J. Hou and P.J.J Alvarez (2015). Water Research

Anaerobic conditions yield less metabolic energy needed for plasmid maintenance, and bacteria loose Tet^R faster



Rysz M., W.R. Mansfield, J.D. Fortner, and P.J.J. Alvarez (2013). Environ. Sci. Technol.

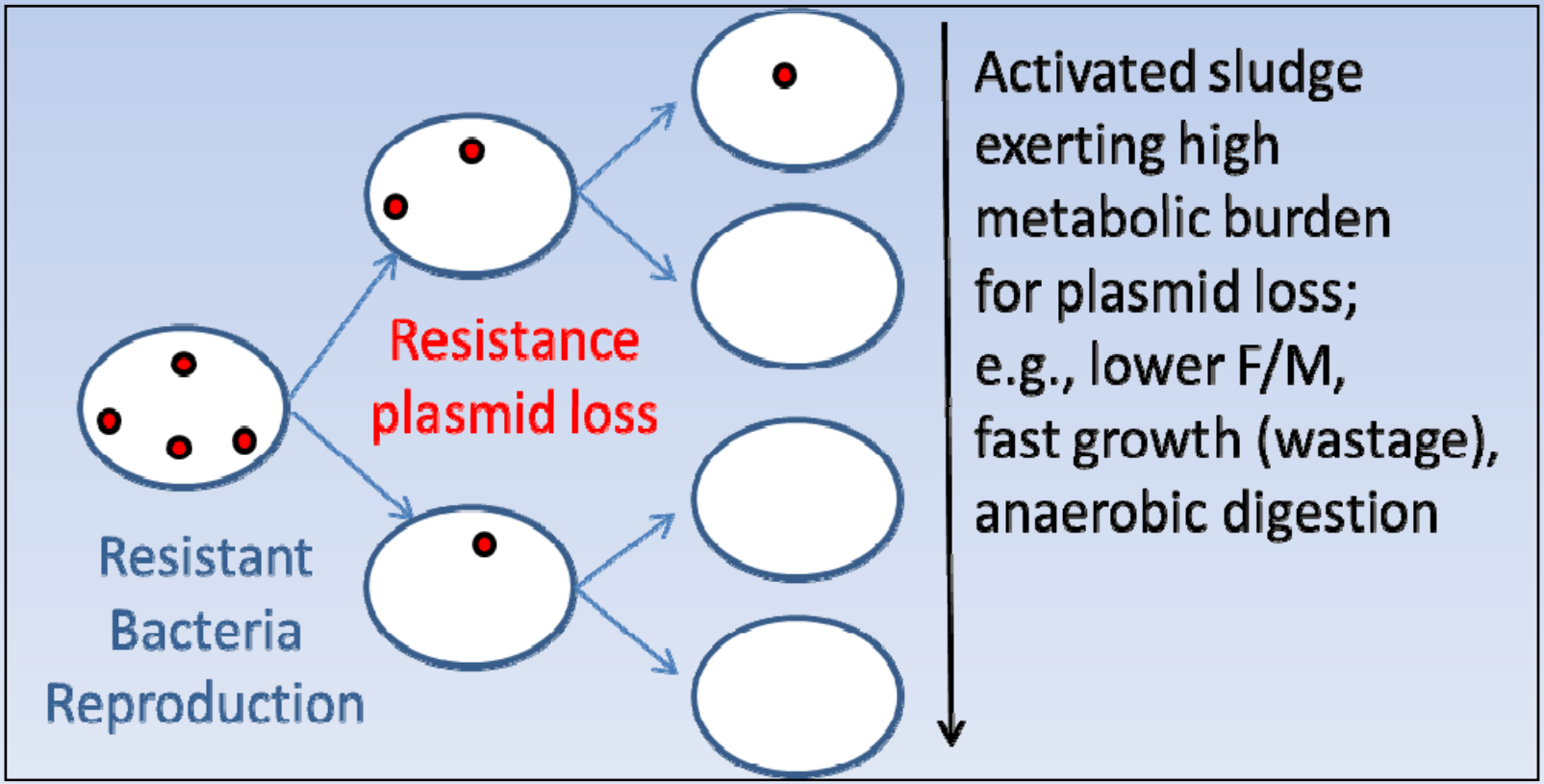
Growth on minimal medium results in faster ARG plasmid loss (suggesting that low F/M ratio may mitigate ARG propagation)



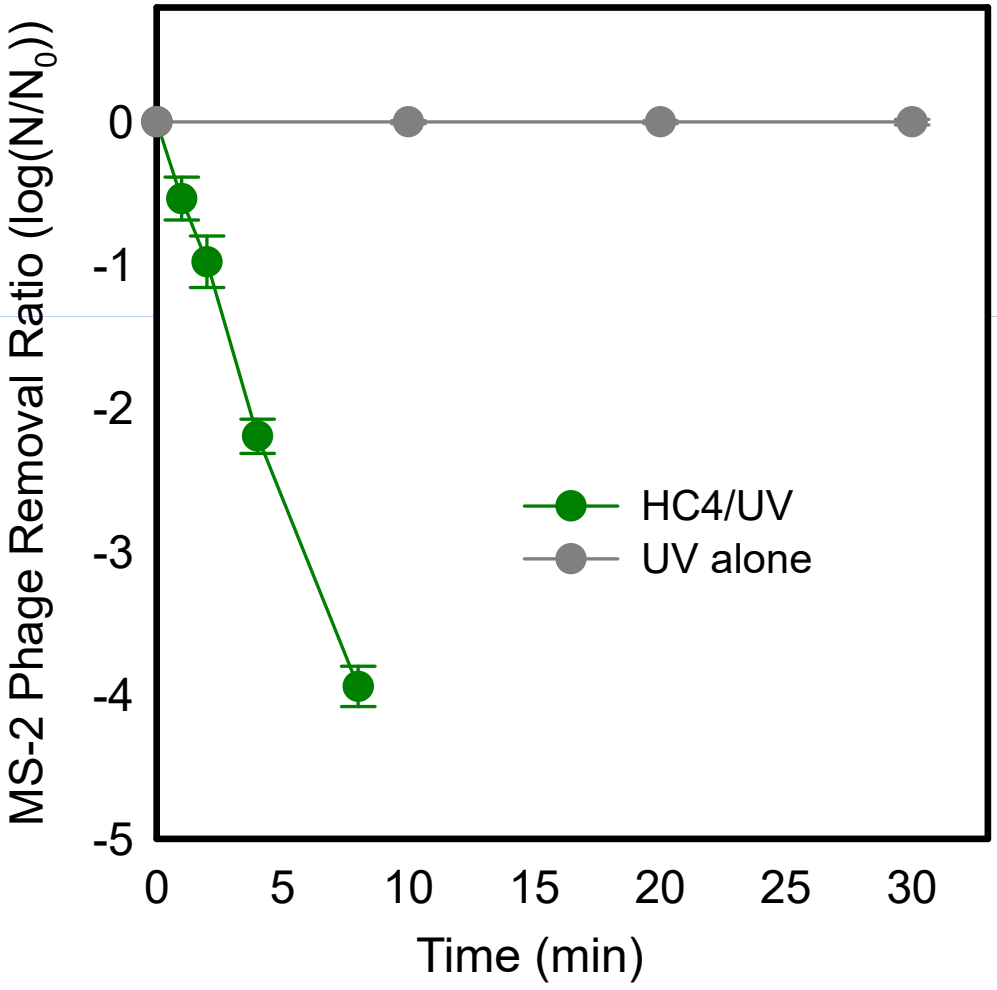
Rysz M., W.R. Mansfield, J.D. Fortner, and P.J.J. Alvarez (2013). Environ. Sci. Technol.

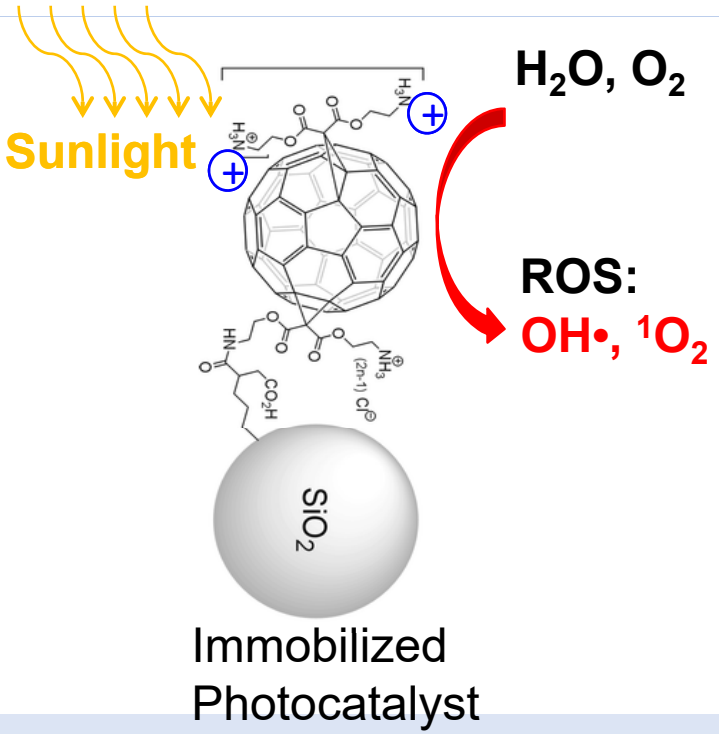
1) Manipulate WWTPs operation variables

Increase metabolic burden of plasmid maintenance (e.g., low F/M and longer contact time in anaerobic digesters) to promote loss of resistance.



2) Improve disinfection of WWTP effluent (nano-photocatalysis?)





Lee, Mackeyev, Cho, Wilson, Kim and Alvarez (2010). *Environ. Sci. Technol.*44: 9488–9495.

Conclusions

- AMR is everywhere in the environment and rapidly propagating
- WWTPs could serve as breeding grounds and point sources for multidrug-resistant bacteria
- Disinfection by chlorination may not reliably mitigate the discharge of “superbugs”
- Need improved effluent disinfection.
- Manipulation of operation variables to increase the burden of ARG maintenance and replication should also be considered.

Surveillance strategies

Methodology

Sample Collection from dairy farm/wastewater/ aquaculture farm

↓

Filtration thro 0.45 micron glass fiber filters

↓

pH was adjusted to 2 with 3.5 M Hcl

↓

Solid Phase Extraction (Oasis HLB)

↓

LC-MS Analysis

↓

Antibiotic concentration quantification

↓

Risk Assessment

Surveillance steps

- Sampling
- Sample processing
- Chromatographic separation
- Detection and Data analysis

Sampling

- Selection of small fraction of matrix enough in volume to still accurately represent the environment
- Representativeness and integrity
- Care should be taken in following aspects:
  - choose proper sampling method
  - Location and frequency of sampling
  - Number of samples to be collected
  - Storage and handling of samples

Sampling

- Sampling frequency – indicator of representativeness
  - Low sampling freq – underestimate the conc
  - 24 hr composite sampling – better for environmental analysis
- Sample decomposition by
  - Temp
  - UV irradiation
  - Microbes
  - Chemical reactivity

Sampling

- Sample collected in brown amber glass bottles. Plastic bottle – unsuitable
- Storage at low temperature (4 °C) in dark
- Tetracycline - easily degraded
- Storage at high temp – enhance bacterial growth – loss of analyte
- Addition of preservatives – to avoid decomposition
  - Acidification to pH 2 with Hcl for macrolides
  - Sulphuric acid for erythromycin, sulfamethoxazole and trimethoprim

Sampling

- Filtration
  - Performed immediately after arriving lab
  - 0.45 or 0.2 micron glass fiber filters

- necessary to remove particles from the water samples which can plug up the SPE cartridges

Stability

- Antibiotics were stable at ambient temperature in containers and in SPE cartridges for 2 weeks
- Stability increased if stored at low temp
- Stock standard solutions – methanol – stored at 4 degree for 1 month

Sample Processing

- Main goal is to :
  - concentrate analytes in sample
  - to remove interferences from matrix and
  - to prepare analyte in suitable form for subsequent chromatographic analysis

- Highly influenced by physico-chemical properties of the sample and matrix.

Sample Processing

- Sample processing involves:
  - Adjustment of pH solution
    - Knowledge of p*K*a of analyte is very essential
- Most antibiotics are acidic substances;
- Acidification 2 units under pKa values of target analytes in water samples in order to obtain their neutral or acidic forms is required and allows the retention of these substances in the most commonly used SPE sorbent polymeric Oasis HLB column

Chelators

- Addition of chelators
- Environmental matrices - contain compounds with divalent or polyvalent cations. The antibiotics from the group of tetracyclines, Fluoroquinolones and Macrolides form complexes with those ions.
- These antibiotics - sorb to the residual metals on SPE cartridges and glassware resulting in irreversible binding to the cartridge and lowering recovery.
- Chelating agents -EDTA, oxalic acid and citric acid are usually applied to decrease the tendency for antibiotics to bind to cations in the matrix, to improve peak shape and to prevent interferences during the extraction of antibiotics

Solid Phase Extraction

- SPE - most preferred technique which replaced classical liquid-liquid extraction (LLE) and become the most common sample preparation technique in the environmental area.
- Best technique for extraction of analytes from aqueous matrix.

- Advantages
  - Improved selectivity, specificity and reproducibility, lower organic solvent consumption, shorter sample preparation time

SPE

- Sample volume – usually 500 or 1000 mL surface water or groundwater
- Wastewater sample – lower volume – complex matrix – organic matter high – matrix effect.
- In evaluation of efficiency of WWTPs 100 ml influent and 250 ml effluent can be used.
- Matrix effect - Matrix constituents in sample may form complexes with the target compounds, preventing their interaction with the sorbent

SPE

- Multiresidue analysis – choice of SPE sorbent critical
- Selection of the most suitable SPE sorbent has to be done with the respect to
  - Polarity of analytes
  - Sample matrix
- Oasis Hydrophilic lipophilic Balance (HLB) catridges – most robust recovery - suitable for polar and non-polar solvents
- Chemical composition containing the lipophilic divinylbenzene units and the hydrophilic N-vinylpyrrolidone units allowing working in wide range of pH

Sampling Musi river, Hyderabad for Antibiotics with Large Volume Solid Phase Extraction apparatus (LVSPE)

•Hyderabad city is built across the Musi river.

• City is well known as hub for pharmaceutical manufacturing industry.

•Patancheru ETP wastewater has been discharging into Musi river for years.

• More than 40 anbiotic manufacturing factories located in this area

LVSPE

- Novel solid phase extraction device developed in Germany for chemical and effect based analysis.
- Device can sample 50 L of water at a time.
- Combination of SPE with a prefiltration cartridge to separate suspended particulate matter (SPM) from the water phase
- Implementation of a pressurized system to force the water through the extraction columns

LVSPE working components

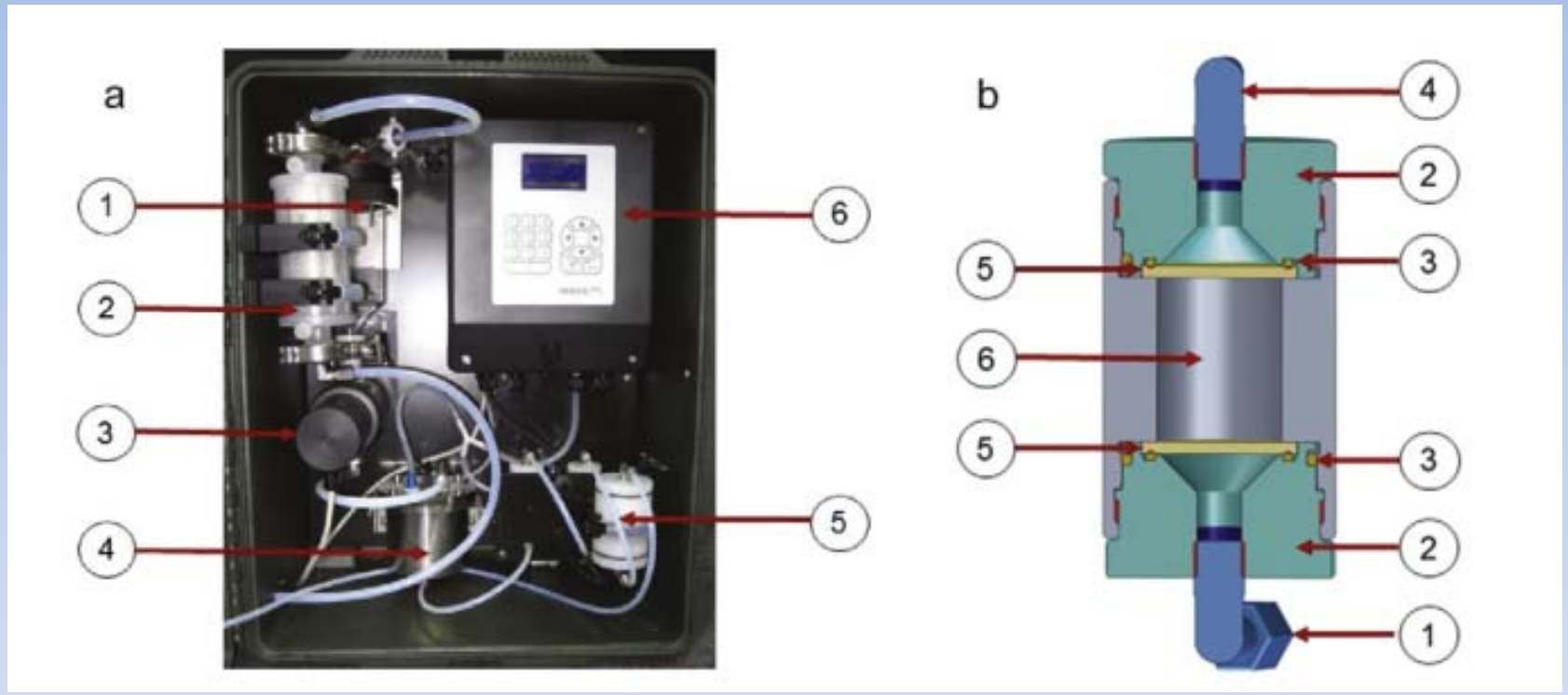


Fig. 1. (a) Picture of the LVSPE50 device; (1): Dosing system (500 mL), (2): pre-filter (3): ball valve, (4): pressure chamber (550 mL), (5): extraction cartridge, (6): controller (Photo by MAXX GmbH); (b) Scheme of the LVSPE50 cartridge, body and screw caps, made of polyvinylidene fluoride; (1): inlet fitting, (2) lower and upper screw caps with mortises to take in the (3) silicone tights (O-rings), (4) outlet fitting, (5) glass filter disc, (6) body containing the sorbent.

Schulze, T.(2017) *Science of the Total Environment*, *581*, 350-358.

Sampling Musi with LVSPE

Halting the propagation of AMR

- From the pharma industries,hospitals, poultry, livestock and fisheries
- At WWTPs in each of the above sources
- Eliminating Antibiotics and AMR genetic material
- Tracking and survelliance of soil, surface water bodies and groundwater
- Halting the AMR propagation drinking water and food sources

Conclusions

- WWTPs could serve as breeding grounds and point sources for multidrug-resistant bacteria
- Disinfection by chlorination may not reliably mitigate the discharge of “superbugs”
- Need improved effluent disinfection.
- Manipulation of operation variables to increase the burden of ARG maintenance and replication should also be considered.

Novel Methods of Treatment

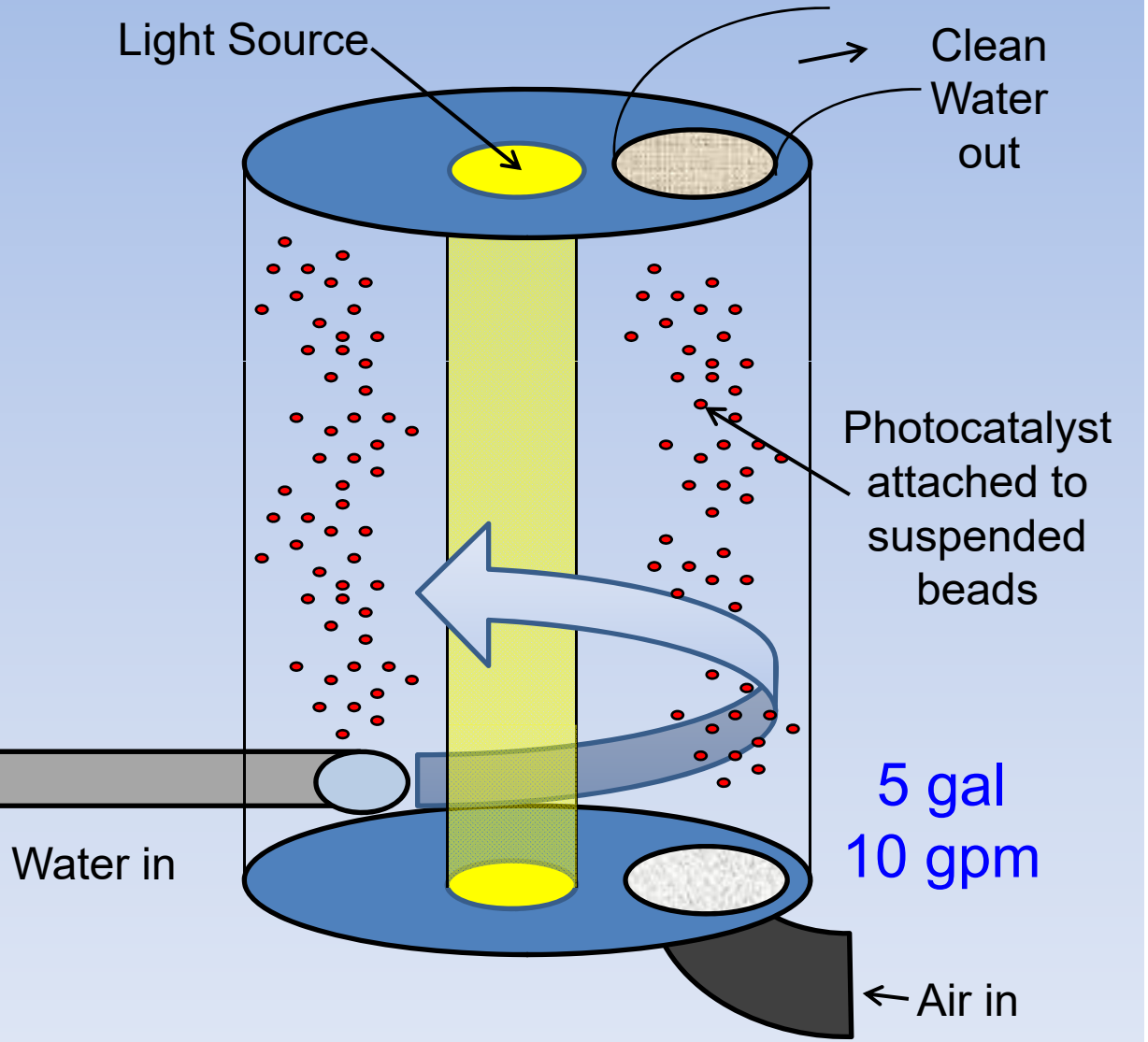
- Removal of antibiotics
  - – Water
  - - Waste water
- Disinfection
  - – Water and Wastewater

  Changing the wastewater treatment

  ❖Tricking the Resistant bacteria

  ❖Changing the process parameters

Fluidized Bed Photocatalytic Reactor



Environmental Engineer and Scientist: Applied Research and Practice

RESEARCH OUTCOMES

- An understanding into which antimicrobial drugs primarily from pharmaceutical effluents, hospitals, livestock, poultry and aquaculture are of concern to public health
- Is there a link between the antibiotics in the environment and the increased prevalence of AMR associated with drug resistant diseases
- How effective is the biological treatment systems in degrading antibiotics and How AMR propagate in different treatment plants during different treatment practices.
- Development of the targeted treatment technology for antimicrobial drugs which can be adopted in effluent treatment plants and water treatment plants.
- Alternate disinfections strategies to halt the resistant genetic material propagation

National Collaborators

- **IITR, Roorkee:** Dr. Gargi Singh
- **NIE (ICMR), Chennai**: Dr. P. Manickam, Dr.CP Girishkumar, Dr.Tarun Bhatnagar
- **RDGMC, Ujjain**: Prof. Vishal Diwan, Prof. Manju Purohit
- **VIT, Vellore**
- **Need more labs to network**

International Collaborators

- **PIRE Group (Partnerships for International research and education) for HEARD (Halting Environmental Antibiotic Resistance Dissemination) –** Dr.Peter Vikesland (Virginia tech, Blacksburg, VA24061); Dr. Amy Pruden (Virginia tech, Blacksburg, VA24061),

- **Indo-German Center for Sustainability – Sustainable water management research -** Dr. Henner Hollert, RWTH Aachen University

- **Helmholtz Center for Environmental Research** – UFZ, Leipzig, Germany – Dr.Werner Brack

- **Division of Infectious Diseases and Tropical Medicine, Leipzig University Hospital,** Germany - Dr. Christoph Lubbert

Acknowledgements