

Contaminants of Emerging Concern in the Irish Aquatic Environment



Environmental Change Institute
National University of Ireland, Galway, Ireland



NUI Galway
OÉ Gaillimh

Martin Cormican

National University of Ireland, Galway



Emerging Concern

The Need for Perspective

Hazard Identification

Hazard Characterisation

Exposure Assessment

Risk Assessment

Contaminants / Hazards Of Emerging Concern

Pharmaceuticals

(Antimicrobial Agents/Endocrine Disrupting Compounds)

Personal Care Products

Chemical Disinfectants

Antibiotic Resistant Bacteria

Genetically Modified Organisms

Nanoparticles

Introduction

- Municipal effluent main pollution source in Ireland
- Environmental contaminants
- Novel pollutants, pharmaceuticals, endocrine disrupting compounds, nanoparticles...
- Aquatic environment particularly at risk
- Potential for Low level intermittent or continuous exposure in environment
- ? Chronic rather than acute effects

Emerging Monitoring Technology

Emerging Technologies for Monitoring

Microbial Source Tracking (MST)

Biomarkers for Environmental Contaminants

Novel Water Treatments

ANTIMICROBIAL RESISTANCE

INTRINSIC (NATURAL)

ACQUIRED RESISTANCE

Acquired Antimicrobial Resistance

Survival of the Fittest- Selection Pressure

In The Presence of Antibiotic

A Bacterial Cell with a Genetic Change that makes it tolerant of/resistant to the antibiotic has advantage

The Resistant Cell Will multiply – The Others will die₇

Selection Pressure

**Any Environment with Antimicrobial Agents &
Microbial Populations**

Gut (Human & Animal)

**Resistant Bacteria Will End Up In
Sewage/Septic Tank/Slurry**

Selection Pressure

**Any Environment with Antimicrobial Agents &
Microbial Populations**

Inanimate Environments

Contaminated by Antimicrobial Compounds

Obvious Environmental Antimicrobial Contaminants

Less Obvious Sources of Antimicrobial Therapeutic Antibiotics

(Animal & Human)

Swallowed / Injected

Distributed Throughout The Body Including GIT

Significant Proportion Excreted in Biologically
Active Form

In Urine

In Faeces

An Example - Ciprofloxacin

Synthetic antimicrobial agent

Chemical family- quinolones

Widely Used for Treatment of Infection

In Community

In Hospital

95% Of Dose Is Excreted in Urine/Faeces

Where Do Antibiotics And AMR Faecal Bacteria Go ?

GLOBAL ANTIMICROBIAL CONSUMPTION

100 000 TO 200 000 TONS

Humans

Animals

Plants

Looking for Antimicrobial-Resistant Bacteria in the Environment

Indicator bacteria with attitude

Well Established Systems for Looking for
E. coli and *Enterococci*

Limitations of Testing for Indicator Bacteria

Failure to detect indicator bacteria

Does not ensure absence of
Virus

Protozoan parasites

Does not ensure absence of other AMR
Bacteria

Acinetobacter/Pseudomonas spp.

Our Way of Detecting Antimicrobial Resistant Bacteria



From Left to Right: Effluent without added antimicrobial, Effluent with 4 mg/l of Ciprofloxacin, Effluent with 2 mg/L of Cefotaxime

Where Do You Look ?

Where Do You Look ?

Hospitals and Environments Directly Impacted by
Hospital Effluent

Ciprofloxacin (10 to 20Kg/Year)

95% Of Dose Is Excreted in Urine/Faeces
(Its Not Just Antimicrobial Agents)

WHAT WE FOUND

WASTEWATER FROM HOSPITAL

E. coli

OLDER AGENTS

AMPICILLIN RESISTANT 49%

STREPTOMYCIN RESISTANT 21%

SULPHONAMIDE RESISTANT 25%

TETRACYCLINE RESISTANT 20%

NEWER AGENTS

CEFOTAXIME RESISTANT 1%

CEFOXITIN RESISTANT 9%

CIPROFLOXACIN RESISTANT 7%

AVERAGES OF A NUMBER OF OBSERVATIONS

WHAT WE FOUND

E. coli

ANTIMICROBIAL RESISTANCE ALSO FROM
OTHER ENVIRONMENTAL WATER SOURCES
(WHERE-EVER WE LOOKED)

1. % RESISTANCE GENERALLY LOWER
2. LESS COMMONLY RESISTANT TO NEWER
ANTIMICROBIAL AGENTS

WHAT WE FOUND

EFFLUENT FROM HOSPITAL

Enterococcus species

VANCOMYCIN RESISTANCE 5.3%

WHAT WE FOUND

ACQUIRED ANTIBIOTIC RESISTANCE IN INDICATOR
BACTERIA

(*E. coli* and Enterococci)

IS COMMON IN MANY WATER SOURCES

WHAT WE FOUND

Extended Spectrum Beta-Lactamase Producing *E. coli*

Detected on Multiple Occasions

Hospital Effluent

Downstream From Hospital Inflow to Municipal
Effluent

AND ANTIBIOTICS TOO

EFFLUENT FROM HOSPITAL ANTIMICROBIAL SUBSTANCES

*Quinolones = $1\mu\text{g/ml}$

Macrolides = $0.02\mu\text{g/ml}$

Tetracyclines = $0.02\mu\text{g/ml}$

Aminoglycosides = $0.15\mu\text{g/ml}$

Penicillins = $0.05\mu\text{g/ml}$

A HIGH PROPORTION OF MANY ANTIBIOTICS GIVEN TO
HUMANS AND ANIMALS ARE EXCRETED UNCHANGED IN
URINE AND FAECES

*Biological Assay

AND THE ANTIBIOTICS TOO

EFFLUENT FROM HOSPITAL ANTIMICROBIAL SUBSTANCES

Chemical Confirmation
Solid Phase Extraction and MS

Unable to Confirm Ciprofloxacin

Did Detect Trimethoprim

(beta-blockers and caffeine)

Waste Water Treatment

Secondary Treatment

Marked Reduction in Total Numbers of *E. coli*

Antimicrobial Resistant *E. coli* Proportions Not Reduced

ESBL-*E. coli* in Outflow From WWTP

Predominant bla genes in Effluent and WWTP Outflow
Comparable to Predominant bla genes in Human Cases

Wider Acquatic Environment

Suggest (tenuous)

AMR E. coli

Rivers > Lakes

Areas of Intensive Agriculture > Low Intensive Land Use

ITS NOT JUST US

Kummerer K J Antimicrob Chemother 2003;52:5-7

Kummerer K Clin Microbiol Infect 2003;9:1203-1214

Espiagres E Int J of Hyg & Env Health 2006;209:103-107

da Silva MF FEMS Microbiol Ecol 2007;60:166-176

Blanch AR J Appl Microbiol 2003;94:994-1002

Reinthal FF Water Research 2003;37:1685-1690

Sahlstrom S Letters in Appl Microbiol 2006;43:46-52

Kummerer K J Antimicrob Chemother 2004;54:311-320

Chitnis V Water Research 2004; 38:441-447

Isidori M Science of the Total Environment 2005;346:87-89

Emmanuel E Environment International 2005;31:399-406

The Wider Community

**Pharmaceuticals & Personal Care Products
Extensively Used in General Community**

Prescription and Non-Prescription Medicines

The Wider Community

Personal Skin Care Products

Antiseptic

Triclosan

Dettol

Savlon

Sun Screen

Nanotechnology

Silver Nanoparticles

Carbon Nanoparticles

Potentially Persistent

Potential to Accumulate

(Stockholm Convention)

Genetically Modified Organisms

Deliberate Release

Accidental Release

(AMR-Determinants used in Cloning)

Genetic Determinants of Resistance

DNA Extracted from *E. coli* and *Enterococcus* free Water

Amplification and Sequencing of Specific Resistance Determinants

Resistance Genes Were Detected (Preliminary)

Municipal Effluent and Sludge

**Specific Pharmaceutical Compounds and PCP
Compounds Are Frequently detectable by
Analytical Chemistry**

Low Level and Intermittent Contamination

Limitations of Analytical Chemistry

Limits of Detection of Target Compounds in Complex Matrices

Can only detect if it is present when you sample

Approach: Look For Biological Impacts of Low Level or Intermittent Contamination

Biomarkers – Fresh Water

Pharmaceuticals -- Oestrogens

Feminisation of Fish Gonads

A. Fogarty (AIT)

ITS NOT JUST US

Kummerer K J Antimicrob Chemother 2003;52:5-7

Kummerer K Clin Microbiol Infect 2003;9:1203-1214

Espiagres E Int J of Hyg & Env Health 2006;209:103-107

da Silva MF FEMS Microbiol Ecol 2007;60:166-176

Blanch AR J Appl Microbiol 2003;94:994-1002

Reinthaler FF Water Research 2003;37:1685-1690

Sahlstrom S Letters in Appl Microbiol 2006;43:46-52

Kummerer K J Antimicrob Chemother 2004;54:311-320

Chitnis V Water Research 2004; 38:441-447

Isidori M Science of the Total Environment 2005;346:87-89

Emmanuel E Environment International 2005;31:399-406

Emerging Solutions

**Novel Biological Methods to Detect Effect of
Compounds**

Microbial Source Tracking

Biomarkers To Monitor Pharmaceuticals In Environment

Selected compounds to bioconcentrate in mussels (filter feeders)

Concern in shell fish available for human consumption

Opportunity to Detect Presence of Low Level /Intermittent Contamination Through Biological Impact on Mussels

- (Brian Quinn GMIT)

Biomarkers To Monitor Pharmaceuticals In Environment

Use proteomics and genomics to identify compound/group associated biological impacts

Apply those techniques to detect those biological changes in mussels collected from specific sites

Development of a small scale toxicity test kit

- (Brian Quinn GMIT)

Biomarkers To Monitor Pharmaceuticals In Environment

AMR- Bacteria Could Be Regarded as a BioMarker of Antimicrobial Contamination

MICROBIAL SOURCE TRACKING

CONCEPT

Identify find something different about microorganisms in faeces of humans/animals

&

Between different animals species

Use that difference as a marker in pollution incidents

MICROBIAL SOURCE TRACKING – FINDING A DIFFERENCE

HOW ABOUT THE INDICATOR BACTERIA ?

E. coli

Enterococcus spp

Clostridium perfringens

PRESENT IN ALL FAECES

(THAT IS WHY THEY ARE GOOD INDICATORS)

MICROBIAL SOURCE TRACKING – FINDING A DIFFERENCE

A More Detailed Look At the Indicator Bacteria

E. coli/Enterococcus ratio ?

Phylogenetic Differences Between E. coli From Animals and Humans ?

NON-LIBRARY METHODS BACTERIA

INDICATOR BACTERIA

E. coli

Enterococcus spp.

Clostridium perfringens

ARE A SMALL MINORITY OF BACTERIA IN FAECES

NON-LIBRARY METHODS

Strict Anaerobes

BECAUSE DIFFICULT TO GROW

DIVERSITY OF BACTEROIDALES IN HUMANS IS NOT FULLY DEFINED

EVEN LESS WELL DEFINED IN ANIMAL FAECES

DIFFICULT TO DEVELOP A TEST FOR WATER/ENVIRONMENT

**NEW- MICROBIOLOGY
NOT DEPENDENT ON GROWTH**

***AMPLIFY A TARGET GENE FROM FAECES
(16SrRNA)***

CLONE AMPLIFIED FRAGMENTS

DETERMINE DNA SEQUENCE

**NEW- MICROBIOLOGY
NOT DEPENDENT ON GROWTH**

***COMPARE DNA SEQUENCES WITH DNA
DATABASE***

***LOOK FOR COMPLETE OR PARTIAL MATCH TO
KNOW BACTERIA 16SrRNA GENE***

***IS THE BACTERIAL GENE SPECIFIC FOR A
PARTICULAR ANIMAL?***

NUIG – EPA FUNDED PROJECT

IDENTIFIED NOVEL RUMINANT ASSOCIATED SEQUENCES

DEVELOPED DNA AMPLIFICATION ASSAYS TO TARGET THE NOVEL RUMINANT SPECIFIC SEQUENCES

SELECTED ASSAYS REPORTED AS HUMAN SPECIFIC
S. Dorai-Raj

What Does it All Mean?

Human Health Impact

Persistence, dispersal, biological accumulation & human exposure

To Antimicrobial Agents

To Antimicrobial Resistant Bacteria

Genetic Determinants of Bacterial Resistance

To Other Pharmaceuticals

To Nanoparticles

Too Early to Tell

WHAT W.H.O. SAYS

WHO SAFE MANAGEMENT OF WASTES FROM HEALTHCARE ACTIVITIES
(1999)

Discharge of Hospital wastewater to sewers is acceptable only if the following criteria are met

“public sewers are connected to efficiently operated wastewater treatment plants that ensure at least 95% removal of bacteria, the sludge resulting from treatment is subjected to anaerobic digestion, excreta from patients being treated with cytotoxic drugs can be collected and adequately treated (as for other cytotoxic waste), and the waste management system of the hospital or **health-care institution ensures the absence of** significant quantities of toxic chemicals, pharmaceuticals, radionuclides, cytotoxic drugs and **antibiotics in the discharged effluent”**

MORE FROM W.H.O.

WHO SAFE MANAGEMENT OF WASTES FROM HEALTHCARE ACTIVITIES (1999)

Chapter 16: Minimal programmes for health-care waste management

16.5.2 Pharmaceutical waste

“Small quantities of pharmaceutical waste, such as outdated drugs (**except cytotoxics and antibiotics**), may also be discharged to the sewer but should not be discharged into natural waters (rivers, lakes, etc.).”

Conclusions and Questions

Do Hospitals Need Integrated Pollution Control Systems and Certification ?

Conclusions and Questions

IRELAND

NEEDS TO DO BETTER WITH WATER

IMPROVE SOURCE PROTECTION

IMPROVED MONITORING

IMPROVED TREATMENT

Conclusions and Questions

IRELAND

NEEDS TO DO BETTER WITH WATER

Immediate & Direct Human Health Impact

Water Borne Infection

Water Borne Chemical Contaminants

The Wider View

What is the Impact of What We Are Doing On Biodiversity?

Impact of Discharges on Biodiversity

**We Know Very Little about Microbial Biodiversity
We Know Even Less About What We are Doing to It**

NOTE: *E. coli* Counts Fall Down Stream Of Hospital?

Acquatic Environments Are Complex Ecosystems

Microscopic – Macroscopic Are Interdependent

Human Impact on Biodiversity

Its not Just Loss of Species

Generation of Novel Biological Entities

GiSMO's

Genetically Self Modified Microorganisms

We Mess With the Environment
Create The Selective Pressure

They Will Do the Genetic Modification

CONCLUSIONS

the only sustainable way to good health is to
sustain health

(health care systems are becoming unsustainable)

the only way to sustain health is to sustain the
environment

ACKNOWLEDGEMENTS

ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL CHANGE INSTITUTE
ENTERPRISE IRELAND

Sandra Galvin
Dearbhaile Morris
Donna Kilmartin
Siobhan Dorai-Raj
Fiona Brennan (Teagasc)
Andrew Barber (GUH)
Niall Geraghty (NUIG)
Leon Barron (DCU)
Brian Quinn (GMIT)

Additional Information

Irish Waste Water & Environment Seminar 2009

http://www.esaiweb.org/esai_coll.php

(Brian Quinn GMIT)

ACKNOWLEDGEMENTS

LOCAL AUTHORITIES

HOSPITAL MANAGERS

ENVIRONMENTAL HEALTH OFFICERS



Enhancing Human Health Through Improved Water Quality