Antibiotic Resistance:
Contribution of Hospital Effluents to the spread of ATBR in environment

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The emergence and dissemination of ATBR genes follow ATB production and use.

Where and how does the dissemination take place?

Increasing abundance of ATBR genes in soils

Relative abundances of ATBR genes in archived soils collected from 1940 to 2008.

Progressive enrichment of ATBR gene

Transfer in the environment or accumulation?

Evidence of transfers between human pathogenic bacteria and environmental bacteria

They “provide evidence for recent exchange of antibiotic resistance genes between environmental bacteria and clinical pathogens”

They “describe multidrug-resistant soil bacteria containing resistance cassettes against five classes of antibiotics (β-lactams, aminoglycosides, amphenicols, sulfonamides, and tetracyclines) that have perfect nucleotide identity to genes from diverse human pathogens”
First evidence

Evidence for a Complex Relationship between Antibiotics and Antibiotic-Resistant *Escherichia Coli*: From Medical Center Patients to a Receiving Environment

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“Along the continuum, the occurrence of *E. coli* resistant to antibiotics and those carrying class 1 integrons decreased in water samples”

“At the retirement home, in the medical center,…, the highest occurrences of antibiotic resistance were in classes of quinolones, sulfonamides, tetracyclines, and penicillins, mainly due to the presence of multiple antibiotic-resistance genes on class 1 integrons.”

“…antibiotic-resistant *E. coli* is acquired in the gastro-intestinal tract of patients treated over the long-term, that these resistant strains and their corresponding genes spread out in waters, and that those genes could be accumulated in high levels in aquatic environments”
Problematic

- Can the presence of ATBR germs be « easily » detected?
- Is there any additional risk of spread of ATBR genes with the discharge of an hospital effluent compare to an urban effluent?
- Is there any risk of genes transfer in the continuum hospital – WWTP - environment ?
- What are the impacts of advanced processes on ATBR ?
• Can the presence of ATR germs be « easily » detected?

→ Involved in multi-resistance of a wide diversity of pathogenic bacterial species
→ Located on mobile DNA elements
→ 130 ATBR gene cassettes described

*int*l genes present on all integrons = qPCR targets

MARKER OF ANTIBIORESISTANCE

Integrons as platforms of ATBR gene capture
Integron quantification

Filtration (0.45µm) in triplicate

24h flow proportionate samples were collected on 3 ≠ days for each site

Day 1
Day 2
Day 3

Filtration (0.45µm) in triplicate

Total DNA extraction
PowerWater™ DNA isolation kit (MoBio Laboratories Inc.)

Quantification of class 1 MRI (qPCR targeting the integrase gene)

Quantification of the 16S RNA-encoding DNA gene = bacterial concentration estimation

Relative abundance

\[
\frac{\text{[class 1 MRI]}}{\text{[bacteria]}}
\]
Case: Same bacterial concentration

[Integron] = 5
[Bacteria] = 15
RA = 0.2

[Integron] = 26
[Bacteria] = 15
RA = 1.7

Case: Same RA

[Bacteria] = 15
[Integron] = 5
RA = 1.7

Case: Same integron concentration

[Integron] = 5
[Bacteria] = 15
RA = 0.2

[Integron] = 5
[Bacteria] = 15
RA = 1.7

[Bacteria] = 4
[Integron] = 1
RA = 0.25
Continuum – Hospital-WWTP-river: Sampling

- Germany
- Luxembourg
- Netherlands

- Switzerland
- UK (Scotland)

Long-care facilities

- UK (Scotland)

Hospital effluent

Effluent

Urban effluent

WWTP influent

Drinking water

WWTP effluent

River

Pond
• Is there any additional risk of spread of ATBR genes with the discharge of an hospital effluent compare to an urban effluent?
Concentration of integrons

Integron concentration per litre

10^7
10^8
10^9
10^10
10^11
10^12

Concentration of integrons reported values for wastewater

General hospital
Urban effluent
Natural water

reported values for river waters with anthropogenic influence

reported values for river waters

*The urban effluents contain the total urban area including the hospital effluents.

**Pond waters

Legend:
- Green: General hospital
- Orange: Geriatric & hospital effluent
- Yellow: Urban effluent
- Blue: Natural water

No pills
Pharmaceutical Input and Elimination from Local Sources
Relative abundance of integrons in wastewaters

- General hospital
- Geriatric & hospital effluent
- Urban effluent
- Natural water

*The urban effluents contain the total urban area including the hospital effluents.
**Pond waters
## Antibiotic resistance genes harbored by integrons

<table>
<thead>
<tr>
<th>Resistances encoded</th>
<th>Gene name</th>
<th>Hospital effluent</th>
<th>Urban effluent</th>
<th>WWTP influent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td><em>aadA</em> alleles</td>
<td>10</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td><em>aadB</em></td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>aacA</em> alleles</td>
<td>25</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td><strong>Beta-lactams</strong></td>
<td><em>blaOXA</em> alleles</td>
<td>26</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>blaBEL-1</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>blaGES-1</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Trimethoprim</strong></td>
<td><em>dfrA</em> alleles</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>dfrB</em> alleles</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Quaternary ammonium compounds</strong></td>
<td><em>qac</em> alleles</td>
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<td>2</td>
<td>6</td>
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<tr>
<td><strong>chloramphenicol</strong></td>
<td><em>cmlA1</em></td>
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<td>0</td>
<td>0</td>
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<td><strong>erythromycin</strong></td>
<td><em>ereA1</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>other</td>
<td>12</td>
<td>11</td>
<td>4</td>
</tr>
</tbody>
</table>
Antibiotic resistance genes harbored by integrons: % of similarity

The influent from the WWTP is more influenced by the urban effluent than the hospital effluent.
Message

• Concentrations of integrons are equivalent in hospital and in urban effluents
• Concentrations of integrons in the wastewaters are higher than in natural waters.
• The relative abundance in hospital effluents is specifically more important.
• No evidence of impact of HE due to the dilution in the sewage but occurrence of mATBR

![Diagram](image)
Message

• There is a (high) diversity of antibiotic resistance genes in HE and UE.
• Resistance to different antibiotics and resistances to specific ATB used in hospital spread out across the environment.
• ...but dilution of hospital antibiotic resistance genes
• ...and no evidence of gene transfer between environmental and clinical bacteria.
• What are the impacts of advanced processes on ATBR?
Processes tested

**Hospital**

**MBR (Ø 0.03µm)**

**O₃**

**PAC**

**Netherlands**

**Hospital**

**DN**

**MBR (Ø 0.4µm)**

**O₃**

**Luxembourg**
Processes tested

MBR (Ø 0.04μm)

O₃

PAC

Germany

O₃

SF

Hospital

SF
Reduction rate of integrons and bacterial concentrations with membrane processes

Integrons

Bacteria

General hospital
Urban effluent
Natural water
Processes efficiencies

Hospital

MBR (Ø 0.03µm) → O₃ → PAC

RI: ↓ 5 log
RA: ↓ 100x
[B]: ↓ 3 log

RI: ↓ <1log
RA: NS
[B]: ↓ <1log

RI: ↑ 1log
RA: NS
[B]: NS

Netherlands

DN → MBR (Ø 0.4µm)

O₃

RI: ↓ 1.8 log
RA: ↓ 15x
[B]: ↓ <1log

Luxembourg

RI: ↓ 2.4 log
↓ 1.3x
[B]: ↓ 2.3log
Message

• Membrane processes reduce the bacterial concentrations (especially UF)
• Bacteria concentrations in the treated effluents are equivalent to those required for bath waters
• The of integron concentrations were reduced up to 5 log (equivalent in treated effluent to bath water quality)
• The Relative Abundance decreased after the processes = Risk ↓ ↓
Take home conclusions

• Integron concentrations are equivalent in hospital or urban effluents but the relative abundance in hospital effluent is specifically more important. The intrinsic hazard of HE can be considered as higher.

• No evidence of the effect of a selective pressure due to the presence of ATB in effluent, leading to occurrence ATBR bacteria in water?

• The concentration of integrons and the relative abundance was strongly reduced after membrane reactor; we can consider that the risk decreases