# Antibiotics and resistant bacteria in our waterways: should we be worried?

*My research has found antibiotics and antibiotic-resistant bacteria in the waterways of south-east Queensland and wastewaters targeted for recycling. These findings may have significant environmental and public health consequences and raise the issue of drug resistant pathogens in our environment.*

Andrew Watkinson
CRC for Water Quality and Treatment
National Research Centre for Environmental Toxicology- The University of Queensland

**Background and Rationale**

Over the last century much has changed with respect to how we view infectious disease. Historically, the bubonic plague, typhus, pneumonia, cholera and dysentery were a constant threat to humanity (1). However in modern society, particularly in developed countries, treatments are available to prevent these types of outbreaks. These treatments are primarily in the form of antibiotics, which are drugs that kill or inhibit microorganisms, such as bacteria.

Bacterial resistance to antibiotics is simply the persistence or growth of bacteria in the presence of antibiotics. The emergence of antibiotic resistant bacteria (ARB) virtually coincided with the introduction of antibiotics themselves. Increasing levels of ARB were combated through the modification of existing drugs and the development of new drugs, including the first fully synthetic antibiotics (the quinolones). However, the development and discovery of new antibiotics has slowed dramatically over the last few decades mostly due to the large cost in bringing them to market. This, combined with antibiotic overuse and poor management, means that we now find ourselves in the precarious situation where careful management of antibiotics is essential to ensure a new era of plague / epidemics are avoided. While much attention has been directed towards management of antibiotic use and monitoring the prevalence of bacterial resistance within the community, antibiotics and ARB in the aquatic environment have received comparatively little attention. Antibiotics released into the aquatic environment are of concern for the following reasons:

- Contamination of water used for drinking, irrigation and recreation
- Potential to accelerate widespread bacterial resistance to antibiotics
- Negative effect on important ecosystem bacteria (e.g. nitrogen cycle)

Global antibiotic consumption has been estimated to be between 100,000 to 200,000 tonnes per year (2). Australia uses approximately 700 tonnes of antibiotics annually, of which only 35% are used for human application. The remainder are used for agricultural production, mostly as growth-promoting or prophylactic agents, rather than for direct treatment of infection. Many antibiotics are not completely metabolised and are excreted unchanged via urine or faeces (3). Additionally, most antibiotics are water soluble and after excretion can be transported to the environment through either point or non-point sources including, for example, effluent from wastewater treatment plants, abattoirs and agricultural runoff.

**Study design, methodology and results:**

This project was designed to provide an understanding of the presence and fate of antibiotics and ARB in the waterways of south-east Queensland (SE Qld). To achieve this, my project aimed to:

- Establish methods for the detection of antibiotics and ARB's in water;
- Determine the efficiency of wastewater treatment plant processes to remove selected antibiotics;
- Survey surface waters of SE Qld for antibiotics and ARB;
- Assess the potential risks associated with the presence of antibiotics and ARB in the aquatic environment.

Table 1: Percent removal of total antibiotics through various wastewater treatment facilities in SE Queensland

| WWTP | Treatment Type | | Total Antibiotic $\mu g\ L^{-1}$ | % Antibiotic Removal |
|---|---|---|---|---|
| 1 | Acitvated Sludge (AS) | Influent | 2.01 | 80 |
| | | Effluent | 0.39 | |
| 2 | AS with Microfiltration/ Reverse Osmosis | Influent | 2.01 | 91 |
| | | Effluent | 0.18 | |
| 3 | Reed Bed with UV Disinfection | Influent | 27.38 | 99 |
| | | Effluent | 0.22 | |
| 4 | AS with Chlorination | Influent | 34.80 | 94 |
| | | Effluent | 2.04 | |
| 5 | Oxidation Ditch with UV Disinfection | Influent | 4.87 | 92 |
| | | Effluent | 0.38 | |

Methods to detect antibiotics in environmental matrices have only recently been established. The majority were developed in North America and Europe and are highly specific to certain antibiotic classes. In the initial stages of my research, through collaboration with the National Measurement Institute (Sydney), I developed and validated a method to detect a suite of key antibiotics in a range of water types (including wastewater, freshwater and saltwater). This is the first broad spectrum method to be established worldwide and has resulted in the first reported findings of antibiotics in SE Qld wastewater treatment plants (WWTPs) and waterways.

Table 2: Concentrations of total antibiotics in agricultural drainages and WWTP effluents

| | Total Antibiotic $\mu g\ L^{-1}$ Average | Max |
|---|---|---|
| Chicken Farm Drainages | 3.6 | 9.9 |
| Dairy Farm Drainages | 0.3 | 0.8 |
| WWTP Effluents | 0.7 | 2.3 |

My research has demonstrated that current practices utilised in wastewater treatment are highly effective in removing antibiotics, with between 80 and 99% antibiotic removal in treatment plants studied in SE Qld (Table 1). Despite this removal efficiency, antibiotics were still detected in effluents discharged from these WWTPs, including treatment plants undertaking wastewater recycling and reuse, though at low levels. A major objective in current SE Qld water management is the recycling of wastewater (e.g. sewage, grey water) for purposes such as irrigation, aquaculture and even drinking. The presence of antibiotics and ARB poses a potential health risk in waters targeted for this application. My research has also identified antibiotics in waters directly associated with animal production in SE Qld at levels comparable with those seen in wastewater effluents (Table 2).

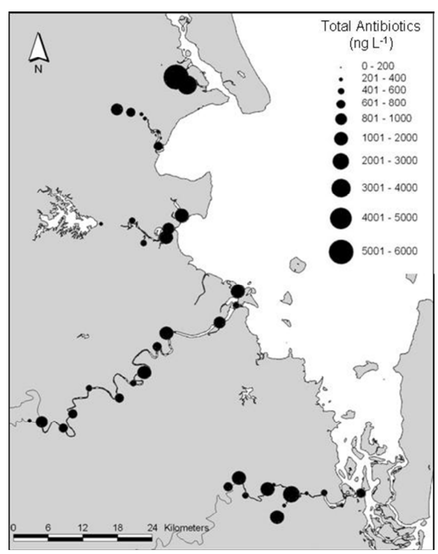

Figure 1: Occurrence of antibiotics in surface waters of SE Queensland

The next stage of my project was to investigate the presence of antibiotics in surface waters of SE Qld. Antibiotics were detected in these waters at relatively low concentrations in comparison to wastewater effluent (Figure 1). While the occurrence of antibiotics in SE Qld surface waters was found to be mostly associated with WWTP discharges, relatively high concentrations were also found in the proximity of intensive poultry production sites. The composition of antibiotics found in surface waters could possibly act as a tracer for their origin.

The fluoroquinolone drugs (e.g. ciprofloxacin and norfloxacin) are used solely for the treatment of infection in humans and thus are only likely to be found in WWTP discharges. Conversely, the ionophores (e.g. monensin and salinomycin) are specifically used in animal production and can be directly associated with agriculture. The proportion of these human-specific antibiotics in SE Qld surface waters was high, while the proportion of animal-specific antibiotics was low, indicating that SE Qld surface waters are influenced mostly by the discharge of antibiotics from WWTPs (Figure 2). Antibiotics were found ubiquitously in investigated surface waters, indicating they are well distributed through the investigated river systems. Additionally, most are not considered persistent, so their apparent ubiquitous presence could indicate a pseudo-persistence through their continuous introduction to surface waters from effluent discharges.

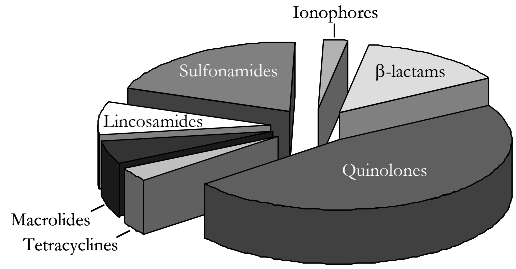


Figure 2: Proportion of antibiotic groups found in surface waters of SE Qld

Table 3: Antibiotic Resistance in *E.coli* and Faecal *Enterococci* isolated from wastewater treatment plants

| Antibiotic | % Resistant *E.coli* (n=44) | % Resistant Faecal *Enterococci* (n=42) |
|---|---|---|
| Ampicillin | 31 | 5 |
| Cephalexin | 52 | - |
| Naladixic Acid | 10 | - |
| Sulfamethoxazole | 31 | - |
| Gentamicin | 0 | 2 |
| Tetracycline | 12 | 7 |
| Erythromycin | - | 62 |
| Ciprofloxacin | - | 52 |
| Vancomycin | - | 10 |

Table 4: Incidence of multiple drug resistance in *E.coli* and Faecal *Enterococci* isolated from wastewater treatment plants

| Resistance Pattern | % Resistant *E.coli* (n=44) | % Resistant Faecal *Enterococci* (n=42) |
|---|---|---|
| Resistant 2 drugs | 29 | 12 |
| Resistant 3 drugs | 12 | 4 |
| Resistant 4 drugs | 5 | 0 |
| Resistant 5 drugs | 0 | 1 |

Concentrations of antibiotics found in SE Qld surface waters (ng $L^{-1}$) were at least six orders of magnitude below concentrations used in clinical therapy (typically mg $L^{-1}$) and at these levels are referred to as sub-inhibitory concentrations. The limited evidence available would suggest it is unlikely that these levels represent a direct acute risk to aquatic organisms. However, the potential for these low concentrations to promote bacterial resistance to antibiotics in the aquatic environment is unknown and remains the single greatest risk associated with their presence. In the clinical environment, techniques have long been established for the assessment of bacterial resistance. I applied these techniques initially to assess the presence of bacterial resistance within wastewater treatment plants and their effluents in SE Qld (Table 3). Antibiotic resistance was quite high in these facilities and incidences of multi-antibiotic resistance were also commonly observed, with some isolates exhibiting resistance to four or five different antibiotics (Table 4). Applying clinical techniques for assessing bacterial resistance to environmental studies proved to be extremely time-consuming and mostly impractical due to the complicated isolation processes involved.

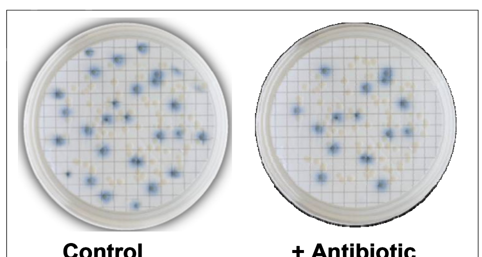


Figure 3: Chromogenic agar technique showing control growth vs antibiotic growth. Blue colonies are confirmed *E.coli*.

This led me to develop a novel approach in order to rigorously assess bacterial resistance. This was achieved by integrating a rapid environmental test for a specific bacterium (*Escherichia coli*) with a current clinical antibiotic-resistance testing protocol, neither of which have been documented in combination.

The rapid identification technique involves the use of chromogenic (colour-converting) and fluorogenic agar which identifies *E.coli* through reaction with an endemic enzyme. Specifically, the *E.coli* enzyme β-glucuronidase cleaves an included cromogen, IBDG, causing positive colonies to appear blue. Additionally, a second *E. coli* enzyme β-galactosidase cleaves an included flurogen, MUGal, which further confirms positive colonies under UV light. Resistance testing is achieved simultaneously by impregnating this agar with specific antibiotics (Figure 3) at known clinical concentrations that define resistance. The level of resistance is quantified by comparing the growth on agar impregnated with antibiotics and agar without antibiotics (control). This new method allows for rapid screening for antibiotic resistance, facilitating better site coverage through time and resource savings. This technique has been applied to assess the presence of bacterial resistance to antibiotics in the Brisbane River, with highest levels of resistance associated with wastewater treatment plant discharges (Table 5).

Table 5: Antibiotic Resistance of *E. coli* in the Brisbane River

| Influence at Site | *E.coli* / 100 mLs | % Resistant Ampicillin | Tetracycline | Sulfamethoxaxole | Ciprofloxacin |
|---|---|---|---|---|---|
| WWTP with disinfection | 11 | 0 | 0 | 0 | 0 |
| WWTP with disinfection | 114 | 47 | 24 | 63 | 0 |
| WWTP | 364 | 3 | 2 | 4 | 0 |
| WWTP | 3333 | 12 | 9 | 12 | 1 |
| Urban | 197 | 3 | 1 | 0 | 0 |
| Urban | 300 | 4 | 4 | 6 | 0 |
| Agriculture | 55 | 3 | 7 | 0 | 0 |
| Agriculture | 40 | 0 | 0 | 0 | 0 |
| Agriculture | 5 | 9 | 9 | 37 | 0 |

## Conclusions

My research has reported the first findings of antibiotics and antibiotic-resistant bacteria in the waterways of south- east Queensland. While it is unlikely that the levels of antibiotics found in WWTP effluents and surface waters pose a direct risk to human health, understanding their impact on the maintenance, development and transfer of antibiotic resistance in bacteria is critical for the regulation and use of our wastewaters. Outcomes from this research will aid in the development of future guidelines for treating and discharging waste and address risks associated with the presence of antibiotics and antibiotic resistant bacteria. This will not only apply to environmental management, but specifically to endeavours such as wastewater recycling, recreation and food production.

## References


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3 Hirsch, R.; Ternes, T. A.; Haberer, K.; Kratz, K. L. Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment*. 1999, *225*, 109-118.


## Watkinson Publications


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