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Effects of erythromycin, trimethoprim and clindamycin on attached microbial communities from an effluent dominated prairie stream

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ABSTRACT

In this study, differing metrics were utilized to measure effects of erythromycin (ER), trimethoprim (TR) and clindamycin (CL) on the structure and function of attached Wascana Creek, SK microbial communities. All three test antibiotics, especially ER, affected community structure and function of biofilms grown in rotating annular reactors. Biofilm thickness, bacterial biomass, and lectin binding biovolume (exopolymeric substances) were consistently less in ER treated biofilms when compared to the control. As well negative effects on protozoan numbers, and carbon utilization were detected. Finally, PCA analyses of DGGE results indicated that bacterial community diversity in ER exposed biofilms was always different from the control. ER exhibited toxic effects even at lower concentrations. Observations on TR and CL exposed biofilms indicated that bacterial biomass, lectin binding biovolume and carbon utilization were negatively affected as well. In terms of bacterial community diversity, however, CL exposed biofilms tended to group with the control while TR grouped with nutrient additions suggesting both nutritive and toxic effects. This study results represent an important step in understanding antibiotic effects, especially ER, on aquatic microbial communities. And because ER is so ubiquitous in receiving water bodies worldwide, the Wascana study results suggest the possibility of ecosystem disturbance elsewhere.

Capsule abstract: Erythromycin (ER) is ubiquitous in waterbodies receiving sewage effluent. Structure and function of microbial communities from an effluent dominated stream were negatively affected by ER, at realistic concentrations.

1. Introduction

In Canada, systemic anti-infectives are among the top prescribed medications (Morgan et. al., 2005) while in Europe, 13,500 tons of antibiotics are used each year with 65% for humans and the remaining 35% for animals (Christensen et al., 2006). Despite variation in excretion rates and types of metabolites, some antibiotics are excreted from humans and animals relatively unchanged. Further, many human antibiotics are not fully removed during sewage treatment. Consequently, surface waters worldwide receiving treated sewage effluent and animal waste contain antibiotics in the ng/L to μg/L range. Of these antibiotics, erythromycin (ER) and trimethoprim (TR), appear to be ubiquitous in receiving water bodies. A recent British investigation, for example, revealed average ER and TR concentrations of 159 ng/l and 12 ng/l, respectively, 1 km downstream of five sewage treatment plants (STPs) (Ashton et al., 2004). Maximum erythromycin-H_2O concentration (metabolite) in a United States survey of 139 streams was 1.7 μg/l (Kolpin et al., 2002) while in Italy, median ER concentrations in the Lambro and Po Rivers were 4.5 and 3.2 ng/l, respectively (Zuccato et al., 2006). Because they are so commonly found in receiving water bodies, many scientific reviews rank ciprofloxacin, TR, ER and sulfamethazine of particular concern (Johnson et al., 2015).

The presence of antibiotics in aquatic ecosystems is of concern for a number of reasons. Most antibiotics interact with a biological target (membranes, enzymes) shared by humans, animals, plants (e.g. plastids) and bacteria (Brain et al., 2008). They act at relatively low concentrations, need time to achieve effects and resist biodegradation (Jones et al., 2004). Proliferation of antibiotic resistance has been linked to chronic bacterial exposure (Eggen et al., 2004; Fent et al., 2006; Gullberg et al., 2011) and high nutrient conditions typifying water bodies receiving treated effluent may exacerbate such proliferation (Castiglioni et al., 2008). Low antibiotic concentrations may also stimulate or depress bacterial gene expression at the transcriptional level at concentrations significantly lower than the minimum inhibitory concentration (MIC)

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(Goh et al., 2002). Finally, their constant addition to aquatic ecosystems at rates exceeding transformation means not only that they are considered pseudo-persistent (Daughton and Ternes, 1999) but that aquatic organisms are chronically exposed.

Not a great deal, however, is known regarding such chronic effects as most research has been directed towards single species toxicity with acute exposures. Although these controlled studies have figured largely in risk assessment, their results lack environmental realism and extrapolation to communities at the ecosystem level is difficult. Community-based effects assessments, however, offer the potential of evaluating ecosystem level effects at relevant contaminant concentrations. Ideal candidates for community level effects studies are microorganisms. Within rivers, streams and other aquatic habitats they not only serve as an important food source for benthic invertebrates and protozoans, they are also major players in biogeochemical nutrient cycling and organic matter biodegradation. Due to their short life cycles, toxic induced community succession (TIS) can be observed in the laboratory over relatively brief time scales (Backhaus et al., 2011). Microbial communities, therefore, are potentially excellent indicators of changes in ecosystem health.

The purpose of this study was to investigate effects of three antibiotics, ER, TR and clindamycin (CL) on the structure, development, functioning and biodiversity of biofilm communities from an effluent dominated creek (Wascana Creek) in southern Saskatchewan, Canada using a laboratory approach. Previous seasonal monitoring studies here indicated that ER, CL, and TR were all present in ng/L and sometimes μg/L concentrations. With regard to their toxicity, single species research has revealed that a dosage of 1 mg/L ER inhibits *Synechocystis sp*. and *Lemna minor* growth by 70% and 20% (Pomati et al., 2004). Other studies indicated that the median inhibition concentration (IC50) of TR to *Pseudokirchneriella subcapitata* was 1000 μg/L (Yang et al., 2008). According to hazard quotients (HQ) generated from earlier Wascana Creek research, ER and TR were present at concentrations which could present a risk to aquatic organisms (Waiser et al., 2011b). Although CL did not generate a hazard quotient indicative of risk, it was added to this study due to its presence on all sampling dates and its persistence downstream of the STP. It was hoped that such community based research would play an important role not only in establishing effects of these antibiotics but in structuring future environmental risk assessments for Wascana Creek and perhaps other similarly affected aquatic ecosystems (c.f. Backhaus et al., 2011).

2. Materials and methods

2.1. Study area

Wascana Creek receives tertiary treated sewage effluent from the city of Regina, SK sewage treatment plant. From late October through to March most flow in the creek is treated sewage effluent. Wascana Creek is considered hypereutrophic with average seasonal total phosphorus (TP) concentration 0.24 mg/L, soluble reactive phosphorus (SRP) 0.09 mg/L, ammonia-N (NH_3) 0.04 mg/L and total dissolved nitrogen (TDN) 0.79 mg/L (Waiser et al., 2011a).

2.1.1. Attached microbial communities; rotating annular reactor experiments

Biofilms recruited from Wascana Creek (algae, bacteria and exopolysaccharide) were grown for 8 weeks under controlled conditions in rotating annular biofilm reactors as described by Lawrence et al. (2004). Wascana creek water was collected in August 2006 and November 2006 at a site approximately 30 km upstream of the sewage treatment plant. Previous monitoring studies indicated that none of the study *anti*biotics were present here (Waiser et al., 2011b). Creek waster served as the microbial inocula as well as a source of carbon (C), nitrogen (N) and phosphorus (P).

2.2. Experimental design

The August biofilm experiment (BEXP1) tested the effects of the three antibiotics, each at 4 μg/L (nominal concentrations), on creek biofilms. Control reactors with no additions were run at the same time. The November experiment (BEXP2) repeated these three treatments but also added 1, 2, 3 and 4 μg/L of ER (nominal concentrations) to the investigation. Each treatment and control had three identical replicate reactors randomly assigned to it in a block. Antibiotics were continuously added to the reactors using peristaltic pumps. Nutrient controls were also run where the molar equivalent of the antibiotic treatment was added as carbon (glucose) and nitrogen (ammonium chloride) (cf Lawrence et al., 2012). These treatments are designed to test whether the impact of the contaminant is more similar to degradation and utilization or toxicity. These nutrient controls are designated as CL control, ER control and TR control respectively.

The central rotating shaft of each bioreactor contained 12 identical polycarbonate slides on which the biofilms grew. At the end of the experiment, slides were randomly selected, removed and in some cases small subsections cut so that biofilms on these sections (coupons $\sim 1\ cm^2$) could be subjected to microscopic examination and other assays.

2.3. Algal biomass (Chl a)

Biomass on each 1×10 cm coupon (one from each replicate) was scraped into a 50-mL centrifuge tube containing 10 mL of 90% ethanol. Chl *a* was extracted coupons in 90% boiling ethanol and analysed fluorometrically using a Turner Designs Model 10-AU digital fluorometer (Turner Designs, Sunnyvale, CA) (Waiser and Robarts, 1997).

2.4. Bacterial production (BP)

One coupon from each replicate was used to estimate BP. Each coupon was placed in a plastic Petri dish containing 10 mL of 0.2 μm filter sterilized creek water. Approximately 30 nM of ^3H labeled thymidine (TdR –Perkin Elmer, Waltham, MA) was added to each dish and dishes swirled. One coupon from each treatment was placed into a corresponding 'killed' control dish to which 500 μL of formalin had been previously added. Samples were incubated for thirty minutes, at the end of which time live samples were killed with 500 μL of formalin. Bacterial DNA was extracted and bacterial production was estimated using [methyl]-^3H thymidine (TdR) incorporation into bacterial DNA according to established methods (Robarts and Wicks, 1989).

2.5. Confocal laser scanning microscopy and image analysis

Coupons with biofilms attached were mounted in small petri dishes using Dow Corning #3140 acid-free silicone coating (WPI, Inc., Sarasota, Fla.). Bacteria were then stained with a fluorescent nucleic acid stain (SYTO 9) (excitation wavelength, 488 nm [ex 488]; emission wavelength, 522–532 nm [em 522/32]). Biofilms were observed using a Bio-Rad MRC 1024 confocal laser scanning microscope (Zeiss, Jena, Germany) attached to a Microphot SA microscope (Nikon, Tokyo, Japan). Two water submersible lenses, a 63×0.9 numerical aperture (Zeiss, Jena, Germany) and a 40×0.55 numerical aperture (Nikon) were used for biofilm examination. Laser scanning microscopy imaging was done at five positions on five randomly chosen transects traversing each biofilm coupon.