Wastewater as a point source of antibiotic resistance genes in the sediment of a freshwater lake

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ABSTRACT

Antibiotic resistance genes (ARG) are currently discussed as emerging environmental contaminants. Hospital and municipal sewage are important sources of ARG for the receiving freshwater bodies. We investigated the spatial distribution of different ARG (sul1, sul2, tet(B), tet(M), tet(W) & qnrA) in freshwater lake sediments in the vicinity of a point source of treated wastewater. ARG contamination of Vidy Bay, Lake Geneva, Switzerland was quantified using real-time PCR and compared to total mercury (THg), a frequently particle-bound inorganic contaminant with known natural background levels. 2-D mapping of the investigated contaminants in lake sediments with geostatistical tools revealed total and relative abundance of ARG in close proximity of the sewage discharge point were up to 200-fold above levels measured at a remote reference site (center of the lake) and decreased exponentially with distance. Similar trends were observed in the spatial distribution of different ARG, whereas distributions of ARG and THg were only moderately correlated, indicating differences in the transport and fate of these pollutants, or additional sources of ARG contamination. The spatial pattern of ARG contamination and supporting data suggest that deposition of particle-associated wastewater bacteria rather than co-selection by e.g. heavy metals was the main cause of sediment ARG contamination.
Introduction

Since their introduction into medical practice in the 1930s, antibiotics have saved millions of lives but the increasing numbers of bacterial pathogens that are becoming resistant to antibiotics is a growing cause of concern. Research initially focused on clinical settings as the main site of the spread and evolution of antibiotic resistance. Over the course of the last two decades, however, researchers have increasingly broadened their focus to include the environment as a source of resistance genes and as a site of antibiotic resistance evolution (Kümmerer 2004). The existence of a natural environmental resistance gene pool, which includes all known clinical resistance mechanisms, has been discovered (Aminov 2009, D’Costa et al 2006). Consequently, bacterial pathogens are assumed to acquire resistance traits from the environment via horizontal gene transfer (Aminov 2009, Martinez 2009a). Moreover, various studies have demonstrated that wastewater and animal waste contain large numbers of resistant bacteria which can pass through the wastewater treatment plant (WWTP) and reach the receiving water bodies (Jury et al 2011, Kümmerer 2004, Rizzo et al 2013, Teuber 2001, Zurfluh et al 2013). There is growing concern that continuous discharge of these contaminants may, at least locally, lead to an increase in the natural resistance background levels and thus enhance the likelihood of ARG being transferred back to human and animal commensals or even pathogens (Cantas et al 2013, Kim and Aga 2007, Martinez 2009b). In the long-term this “environmental loop” could contribute to the spread of resistance in pathogens and undermine the effectiveness of current and future antibiotics.

Lake Geneva is the largest freshwater body in Western Europe and receives treated wastewaters from the surrounding cities. The largest WWTP is located in Lausanne and discharges treated wastewater and occasionally bypass water into Vidy Bay. The bay’s sediments have been reported to be heavily polluted by fecal indicator bacteria, heavy metals, nitrogen, and phosphorus (Poté et al 2008). Micropollutants, including antibiotics (Bonvin et al 2011), as well as multiresistant bacteria and ARG pass through the WWTP and are
subsequently discharged in considerable amounts into Vidy Bay (Czekalski et al 2012). Low levels of multiresistant bacteria and ARG were found even 3.2 km from the WWTP discharge pipe, where lake water is pumped for drinking water production (Czekalski et al 2012). However, it remained unclear whether this represented contamination or the (unknown) natural resistance background level of Lake Geneva.

Molecular tools, such as PCR and quantitative real-time PCR, have been developed for many ARG (Heuer and Smalla 2007, Walsh et al 2011). A limited number of studies have applied molecular methods to aquatic environments. Primarily, rivers impacted by different contamination sources have been considered (Graham et al 2010, Pei et al 2006, Pruden et al 2006). In these systems, the horizontal transport of contaminants is directional and constrained by the river channel and therefore relatively well defined. This is also true for most of the sampling points in the study of LaPara et al. (2011), which followed the input of ARG with wastewater into the St. Louis river and transport into Lake Superior. In freshwater lakes complex transport processes prevail, dominated e.g. by temporally variable wind regimes, currents, and stratification. Despite the high relevance of lakes as sources of drinking water, food, and as a place for recreational activities, the dynamics of antibiotic resistance contamination in lakes that are receiving waters for municipal wastewater has not been thoroughly investigated. It is currently unknown, e.g. at which spatial scales a local source of antibiotic resistant bacteria affects water and sediment in a receiving lake.

The present study aims to characterize the spatial impact of Lausanne’s WWTP discharge on the concentration of several plasmid-mediated ARG in the lake sediment. The tested ARG confer resistance to different classes of broad-spectrum antibiotics (sulfonamides: sul1, sul2, tetracyclines: tet(M), tet(B), tet(W)) and fluoroquinolones: qnrA), which differ in their origin (e.g. natural vs. synthetic), time of introduction, resistance mechanisms and clinical relevance. Sulfonamides were the first synthetic antibacterials. Tetracyclines, isolated from *Streptomyces*, followed in 1948. Fluoroquinolones were introduced during the 1970s and are,
of the three substance classes, currently the most frequently used in clinics. ARG were compared to an already well-studied WWTP-released contaminant, total mercury (THg), for which background levels in Lake Geneva are known from previous studies (Arboille et al 1989, Poté et al 2008). Mapping of the two-dimensional spatial pattern of the investigated ARG and THg was carried out using a geographic information system (ArcGIS). Further data on the microbial community structure in wastewater and sediment samples and sediment properties were considered in order to interpret the observed spatial trends of ARG in Vidy bay sediments.

Materials and methods

Study site description and sampling campaigns. For a detailed description of the Vidy Bay study site, please refer to Czekalski et al. (2012). Lausanne (population 214 000) is the biggest city in the Lake Geneva area. The city has no pharmaceutical industries but several health care centers. The largest is the Centre Hospitalier Universitaire Vaudois, an important site of antibiotic consumption, which discharges ~ 410 m³ day⁻¹ of raw wastewater into the municipal sewage system (Blanc 2010). The conventional WWTP of Lausanne treats the bulk of the wastewater it receives with a chemical phosphate removal process and an aerobic biological treatment, followed by clarification. Sludge is recycled or incinerated. Only part of the water receives a more elaborate biological denitrification treatment (Figure S1). Disinfection is not performed. Treated wastewater and occasionally untreated sewer overflow (bypass) is discharged at an average rate of 86.631 m³ day⁻¹ (1–3 m³ s⁻¹ up to 5-6 m³ s⁻¹ at low and peak flow, respectively) (Vioget et al 2011). The effluent pipe discharges the treated water 750 m offshore at a depth of 35 m into the Vidy Bay of Lake Geneva (Figure 1, sampling point STEP (“station d’éguration”)). The rivers Chamberonne and Flon (sampling points CHAM and FLON) form part of the city’s combined sewer overflow system and also discharge into the bay (1-3 m³ s⁻¹, Goldscheider et al., 2007). The main form of agricultural
land-use in their catchment areas is vineyards. Intensive animal farming is not prevalent. About 3.2 km south-west from STEP lake water is pumped for drinking water production (sampling point DWP).

Short sediment cores were taken at 22 sites during two sampling campaigns in August 2011 (Figure 1). Sediment cores were retrieved as described previously (Czekalski et al 2012). Sediment cores were transported to the laboratory within 8 hours and stored at 4°C for a maximum of 48 hours until processing. Three subsamples of 0.5 g (wet weight) from the sediment surface layer (0-3 cm) of each core were transferred to sterile 2-ml screw cap tubes. After shock-freezing in liquid nitrogen, the tubes were stored at -80°C until DNA extraction. For a subset of sites, additional subsamples of surface sediment were taken, immediately preserved by freezing at -20°C and freeze-dried to analyze total mercury (THg), grain size, and organic matter content.

Details on additional water samples taken in 2009 and 2010 from Lausanne’s sewage system and lake water in Vidy Bay that were analyzed by plate counts of resistant bacteria and qPCR assays are given in the Supplementary Information (SI).

**DNA extraction.** DNA from the three subsamples from each sampling site was extracted, quantified and quality-controlled as described in Czekalski et al. (2012) Briefly, cells were lysed using bead-beating and freeze-thaw cycles and DNA was purified using a phenol-chloroform extraction protocol. Extraction blanks omitting sediment were prepared to check for contamination sources. DNA quality was checked via agarose gel electrophoresis and UV-absorbance (Nanodrop) and quantified using the Quant-iT PicoGreen® DNA quantification kit (Invitrogen, Basel, Switzerland). Extraction blanks yielded negative results. DNA pellets were resuspended in 50-100 μl of TE-buffer and stored at -20°C until further analysis.
qPCR assays performed with sediment, lake, and wastewater DNA extracts. Six different ARG (sul1, sul2, tet(B), tet(M), tet(W) and qnrA) and bacterial 16S rRNA gene fragments were quantified using published protocols, primers and probes (Table S1). Standard curves were prepared from serial tenfold dilutions of plasmid DNA containing the respective target gene in a range of $3 \times 10^6$ to 30 gene copies. The preparation of control plasmids and standard curves for sul1 and sul2 was as described previously (Heuer and Smalla 2007, Heuer et al 2008). For tet(B), tet(M), tet(W) and qnrA standard curves were prepared from cloned PCR products as described for the 16S rRNA gene in Czekalski et al. (2012). qPCR assays for sul, tet and 16S rRNA genes were performed in 96 well plates using TaqMan® Environmental Master Mix 2.0 (Life Technologies Corp., Applied Biosystems, Carlsbad, CA, USA), which is optimized for samples with confirmed or expected presence of PCR inhibitors. qnrA was amplified using QuantiTect SYBR Green PCR chemistry (Qiagen, Hombrechtikon, Switzerland). In brief: each 25 μl reaction contained 1× of either Environmental or SYBR green Master Mix, primers, probe, and MgCl2 according to the published methods (Table S1) and 5 μl of template DNA or standard. All qPCR assays were performed in technical triplicates on each extract, standard and negative controls (no template control (NTC): salmon sperm DNA, 10 ng μl$^{-1}$ in order to control for specificity, PCR blanks: nuclease free water, and extraction blanks: see above). qPCR reactions were performed on a 7500 Fast Real-Time PCR System (Applied Biosystems) and analyzed using default settings. All negative controls resulted either in no amplification or a higher threshold cycle (Ct) than the most diluted quantification standard. A sample was considered to be below limit of detection (LOD) for a target gene if 2 or more out of 3 technical replicates were negative or if sample Ct values were $\geq$ Ct of negative controls. Samples above LOD were considered to be below the limit of quantification when the standard deviation of Ct values of
methodological triplicates was > 0.5 AND their Ct value was higher than the Ct of the most
dilated standard whose standard deviation of Ct values was ≤ 0.5.

For each PCR assay, the qPCR efficiency was calculated from the slope of the standard
curve \( E = 10^{\left(-\frac{1}{\text{slope}}\right)} \), Table S2). For a subset of 7 samples and for one target gene (sul2) the
effect of inhibitors in the sample matrix on amplification in qPCR assays was evaluated
directly. Each of the samples was spiked with \( 10^5 \) copies of quantification standard DNA and
amplified together with the same set of non-spiked samples and control DNA. The presence
of inhibitors was evaluated according to Pei et al. (2006) by calculating the suppression factor
\( S \). \( S \) varied between 1.1 and 1.4 indicating that inhibition was negligible (Table S3).

**ARISA-PCR and sequencing.** 1:10 dilutions of DNA extracts were subjected to automated
ribosomal intergenic spacer analysis (ARISA) using general bacterial primers (Table S1) and
PCR conditions according to Yannarell et al. (2003) with slight modifications (Bürgmann et
al 2011). 10 ng \( \mu l^{-1} \) of *E. coli* DNA served as a positive control, 10 ng \( \mu l^{-1} \) of salmon sperm
DNA and nuclease free water served as NTC and PCR blank, respectively

Samples were run on an ABI 3130xl capillary sequencer as described previously
(Bürgmann et al 2011). Data analysis was performed using Gene Mapper® Software version
4.0 (Applied Biosystems) considering only peaks with sizes between 350 and 1250 bp and a
minimum peak height of 125 fluorescence units. ARISA data were subjected to the automatic
size of 2.5 bp.

**Hg analysis.** THg concentrations were determined by cold vapor atomic absorption
spectrophotometry (CV-AAS) after dry mineralization and pre-concentration of Hg by means
of amalgamation on a gold trap (Száková et al 2004), using an automatic mercury analyzer
Altec, Model AMA 254) under the following conditions: typical sample mass 50-100 mg, drying time 45s, decomposition time 150s, waiting time (necessary for quantitative trapping of released mercury on the gold amalgamator) 45 s. All analyses were run in duplicates. The relative error was usually ±5% and always under ±10% (Roos-Barraclough et al 2002). The limit of detection is 0.01 ng and the working range between 0.05 to 600 ng (Yang and Pan 2007). Concentrations obtained for repeated analyses of certified reference materials (CRMs) never exceeded the published range of concentration (0.091±0.008 μg g⁻¹ for MESS-3).

**Sediment characterization and nutrient content.** The sediment grain size was measured using a Coulter® LS-100 laser diffractometer (Beckman Coulter, Fullerton, CA, USA), following 5-min ultrasonic dispersal in de-ionized water according to the method of Loizeau et al. (1994).

The sediment total organic matter content (TOM) was estimated from loss on ignition at 550 °C for 1 h in a laboratory oven (Nabertherm – LE14/11).

**Data analysis, visualization and statistics.** Total copy numbers of all target genes were normalized to sediment sample wet weight (g⁻¹ wwt). This is referred to as ARG concentration from here on. Additionally, ARG copy numbers were normalized to 16S rRNA gene copy numbers and reported as percentages, as an indicator of the relative abundance of resistance genes within the bacterial population. The number copies of the 16S rRNA genes per cell varies among bacterial species and may thus vary between samples, but keeping this caveat in mind we refer to this measure as “ARG abundance” from here on. Interpolation maps were created with ArcGIS (ESRI®ArcMap 10, Redlands, CA) with the Geostatistical Analyst extension, using the Inverse Distance Weighted (IDW) interpolation algorithm with power functions from 1-10 and a smoothing factor of 0.2.

In order to evaluate similarity of the distribution patterns between resistance genes and between resistance genes and Hg concentrations, as well as between ARG and distance from
the contamination source, correlation and regression analysis were performed in MS Excel 2010, using the Data Analysis Tool.

Community similarity analysis was performed based on Hellinger-transformed ARISA peak area data in R version 2.14.2 (R Development Core Team, 2011), using the BiodiversityR and vegan packages (Jari Oksanen 2012, Kindt 2005). Principle coordinates analysis (PCoA) was performed using the Bray distance metric. ARG abundance and environmental variables were passively fitted to community PCoA data and significance of fit was analyzed by means of permutation analysis (n=1000).

**Results and discussion**

**Spatial trends in ARG abundance.** A central goal of this study was to characterize the spatial impact of Lausanne’s WWTP discharge pipe on the contamination of Vidy Bay sediments with different ARG. *sul* genes were detected at all and *tet* genes at several of the sampled locations, whereas *qnrA* was always below detection. ARG abundance generally decreased from *sul1* over *sul2*, *tet(w)*, *tet(M)* to *tet(B)* (Figure 2). The prevalence of ARG in Vidy bay sediments thus reflects their abundance in Lausanne’s wastewater: *qnrA* was detected in low amounts and only in hospital sewage whereas *sul* and *tet* were more abundant in both WWTP influent and effluent samples, with *sul1* abundance always being highest (Figure S5). This abundance pattern may be related to the longer history of clinical application of sulfonamides and tetracyclines. *Sul* and *tet* genes have previously been found to be quite abundant in sewage and receiving waters (Pruden et al 2006) and in livestock waste sludge (Zhang et al 2013). Plasmid-mediated quinolone resistance encoded by *qnrA* was first reported in 1998 (Martinez-Martinez et al 1998), which may explain its low abundance in Lausanne’s wastewater system. Other quinolone resistance mechanisms were likewise infrequent, as shown by low colony counts on Ofloxacin- and Norfloxacin-amended agar
plates inoculated with samples from Lausanne’s sewage and wastewater treatment system (Figures S6 & S7).

For all detected ARG, highest gene concentrations and abundances occurred at sites in close proximity to the WWTP discharge (Figure 2), especially at sites STEP, EG2, and NC4 (Figure S2, S3). At site EG2 a maximum of \(2.2 \times 10^9\) (\(sul\)) and of \(1.5 \times 10^6\) (\(tet(B)\)) ARG copies g\(^{-1}\) wwt were determined (Figure S2). Abundance of \(sul\) at this site reached 12% (Figure S3). ARG concentration and abundance of both \(sul\) and \(tet\) genes were also high at sites NC1, NC2, NC3, NC5, and EG3 (Figure S2, S3), which are all located within 350 m of the STEP coordinate (Figure 1). Only \(sul\) was also detected at comparatively high concentration and abundance at more remote sites (EG4, NC7, NC9 and NC14).

The relationship of ARG with distance from the WWTP discharge pipe was not linear (Pearson’s \(r \sim -0.17\) for ARG concentration and \(r \sim -0.36\) for ARG abundance, Table S4, S5), but ARG abundance followed an exponential decay function (Figure 2). The decreasing trend appeared to be intact across the entire range of distances sampled. ARG concentration trends were similar since 16S rRNA gene copy numbers did not show a clear distance-related trend (Figure S4). However, for each ARG the \(R^2\) was higher for abundance data compared to concentration data. A possible explanation would be sample-to-sample variations in PCR inhibition in spite of our best efforts to minimize such effects. We concluded that ARG abundance is slightly more robust for the purpose of our analysis. The \(R^2\) for ARG abundance varied between 0.48 and 0.68, indicating that considerable amounts of variation are not explained by distance (Figure 2).

We therefore used spatial mapping to investigate whether deposition patterns deviate from a simple distance relationship, indicating directional transport (Figure 3 and S5). The maps clearly show the accumulation of ARG around the WWTP discharge pipe. Designating a \(sul\) abundance > 1% as “contaminated”, Figure 3a indicates an area of at least \(~0.3\) km\(^2\) has been
impacted. All sampling points within this area are hereafter referred to as ARG contaminated sediments.

ARG maps indicated a strong decrease in ARG abundance and concentration towards the deep lake (EG2 to EG9) and towards the south east (sampling locations EG14 and EG15). Ct values obtained for EG6, EG9, EG15, and the center of the lake (reference point SHL2) were either below the LOQ (sul genes) or even below LOD (tet genes). In contrast, our data indicated a less dramatic decrease in ARG levels along the transect following the shore line in south-western direction (NC5-NC13, Figure 2 a-e and Figure 3 a-e).

Finally, apart from site NC14 (close to the Chamberonne river - CHAM), samples taken close to shore (NC15, EG1) showed low levels of ARG.

These observations indicate a directionality of the pollutant transport in the water column of Vidy Bay. Hydrodynamic transport in lakes is often complex. Currents are influenced by changing wind regimes, stratification, bathymetry, and vary in direction and speed with depth (Righetti et al 2011, Wuest and Lorke 2003). For Vidy Bay it has recently been shown that near-shore gyres can occur (which enhance the retention time of water and pollutants in the bay) and that neither of two dominant wind regimes produce a consistent current pattern (Razmi et al, unpublished data). However, westward shore-parallel currents can disrupt gyre formation and move water from near the STEP site to the water column above the DWP, with a dilution factor of about 100 (Razmi et al., unpublished data). In previous studies, tracer released from the WWTP during holomixis reached surface waters at the DWP site (NC13) within 48 hours, whereas no tracers were detected when thermal stratification precluded upwelling (Goldscheider et al 2007, Wildi and Rossi 1997). In the case of particle-bound contaminants the contamination record in sediments is expected to integrate over such temporally variable transport processes.
Contamination effects on the microbial community. Analysis of bacterial community similarity (Figure 4a) showed a clearly distinct community in sites close to the discharge pipe. This confirms previous clone library based observations from Vidy Bay (Haller et al 2011). The communities at the most strongly ARG contaminated sites STEP and EG2 were highly similar to some untreated wastewater communities. Fitting of ARG abundances and environmental variables to the sediment community composition showed that these community changes correlated with indicators of contamination such as ARG abundance, THg, and TOM (Figure 4b). In contrast, particle composition parameters appeared either unrelated to the distinction between contaminated and uncontaminated sites (silt) or were not significantly correlated (clay, p>0.05). Vectors fitted for sul2 and tet(B) indicated a slightly different distribution within the microbial community compared to the other ARG. Overall, community analysis indicated that changes in ARG abundance were related to changes in community composition, which can be explained by deposition of bacteria originating in wastewater. However, an impact of confounding factors like e.g. heavy metal pollution cannot be ruled out by this analysis.

Comparison of ARG and Hg contamination. The spatial trends in ARG distribution were compared to THg levels (Figure 2 and 3). Hg is a pollutant that is known to be released from Lausanne’s WWTP (Loizeau et al 2004). Background concentrations in Lake Geneva sediments are ~0.2 mg kg\textsuperscript{-1}. Although the chemistry and transport behavior of Hg is complex, particulate transport is dominant in most systems (Glass et al 1990, Wang et al 2004). Assuming that particle-associated bacteria or bacterial aggregates released from the WWTP are in turn primarily responsible for ARG deposition in the sediment, we expected a similar pollution pattern for THg and ARG. However, ARG dynamics may be affected by bacterial mortality, e.g. through predation, UV radiation, or starvation. This could lead to a more restricted distribution compared to the heavy metal. On the other hand, released resistant
bacteria could proliferate or spread ARG to the indigenous community via horizontal gene
transfer. If the latter mechanisms dominate, a diffuse and more widespread pattern of ARG
concentrations compared to THg would be expected. Differences as well as similarities in the
spatial patterns of both contaminants may thus be informative.

Compared to previous studies (8 mg kg\(^{-1}\) sediment (Poté et al 2008)) a relatively low
maximum THg concentration of 1.3 mg kg\(^{-1}\) sediment was measured during our survey (EG2,
compare Figure 2f), indicating reduced recent deposition. We found elevated THg-levels to be
highest around the STEP site but above background levels up to 600 m away in direction of
the deep lake (EG4), 535 m away in the south-western direction (NC6), and 877 m in the
south-eastern direction (EG14, Figure 2f).

The similarity of the vectors of THg and ARG abundances fitted to community data in
Figure 4b indicated a close link, which could either be deposition from a common source or
because the heavy metal is a constraining factor of community composition. Nevertheless,
there are notable differences in the spatial distribution, as shown by relatively low \(R^2\) of linear
regressions of THg and ARG concentration (\(R^2 = 0.32-0.41\), \(p<0.05\), Table S6) and
abundance (\(R^2 = 0.21-0.63\), \(p<0.01\), except for \(sul2\) with \(p=0.05\), Table S7). The steeper slope
of ARG abundance with distance compared to THg (Figure2) indicates restrictions on ARG
dissemination compared to THg. However, the south-western transect deviated from this
general observation. Here, ARG levels decreased more slowly whereas sediments were
classified as unimpacted in terms of THg (Figure 3f).

Finally, low levels of THg were determined at near-shore sites (0.04 and 0.05 mg kg\(^{-1}\) for
EG1 and NC14, respectively), most likely related to the comparatively large grain sizes and
low organic matter content (Table S8) of sediments deposited in this area by the
Chamberonne and Flon rivers. In contrast, site NC14 near the Chamberonne river mouth
showed elevated ARG levels (Figure 3 a, b, and f). Previous studies monitoring pollutant
transport in the water column (Bonvin et al 2011, Goldscheider et al 2007, Haldimann 2009,
document that at least under certain circumstances site NC13 might be affected by contaminants released from the WWTP, including ARG (Czekalski et al 2012). However, the Chamberonne river is known as a discontinuous source of fecal bacteria (Haldimann 2009, Haller et al 2009), whereas its THg load is too low to explain elevated concentrations measured in sediments of Vidy Bay (Howa and Vernet 1988). Our data shows it to be a likely source of resistant bacteria and ARG contamination (compare Figure S5, S6 f and S7 f). Low ARG levels detected at locations close to the shore further east (EG1 and NC15) would support the hypothesis that the Chamberonne plume is partly responsible for the extension of the ARG contamination in the south-western direction. This is also supported by current measurements in Vidy Bay in 2010, which revealed 45% of the currents moving west-southwest (Razmi et al., unpublished data).

Direct and indirect causes of ARG contamination. Independently of transport, sediment properties might also influence the natural abundance of ARG and the further fate of deposited allochthonous ARG. Sediments containing a high fraction of clay minerals can bind DNA after cell death and protect it from degradation by DNase (Aardema et al 1983). In the sampled sediments the proportion of clay was quite low (0.01-3.92%, Table S8). ARG have previously been detected in extracellular DNA in livestock waste sludge, but were one to two orders of magnitude less frequent than the intracellular fraction, and both fractions showed similar dynamics (Zhang et al 2013). Therefore we assume that the detected ARG reflect the ARG in the viable bacterial biomass in our samples.

It has been demonstrated that heavy metals can cross- or co-select for antibiotic resistance genes (Berg et al 2010) as the same mechanisms often underlie both resistances, or resistance genes are located on the same mobile genetic element (Baker-Austin et al 2006, Knapp et al 2011). As sediments in Vidy Bay are contaminated not only with Hg but also with other heavy metals in excess of background levels (Poté et al 2008), it could be argued that the
observed elevated ARG levels are caused by heavy metal pollution rather than bacterial deposition. In a study carried out along a section of the Almendares River, which is affected by different urban and industrial contamination sources, good correlations were demonstrated for sediment ARG (tet and β-lactamases) and copper levels (Graham et al 2010). Other heavy metals (Pb, Co, Zn) and ARG also partly correlated with ARG, but neither Hg nor sul genes were considered in this study.

However, for the present study we conclude that the available evidence favors the hypothesis that the observed ARG levels are mainly a result of direct deposition of resistant bacteria rather than being due to co-selection by heavy metals: a) The correlations of ARG levels with THg were relatively poor. The observed correlation can be explained by the parallel release of both types of contaminants from the WWTP. b) The impact of the WWTP discharge on the deposition of fecal indicator bacteria and contrasting bacterial community composition has been shown previously (Haller et al 2011). c) Our analysis of community similarity using ARISA (Figure 4) confirmed a distinct community composition of ARG contaminated sediments in the vicinity of the discharge pipe which, furthermore, exhibits a high similarity to sewage communities (Fig. 4a), supporting the notion that bacteria from these environments are deposited in the sediments. d) A more rapid decrease of ARG abundance with distance compared to THg (Figure 2) is best explained by different dynamics during transport or deposition. Finally, e) we found highly similar patterns of ARG abundance in wastewater / sewage and sediment (Figure S4). Nevertheless, the role of the heavy metal contamination in Vidy Bay for selection or persistence of ARG warrants further study.

**Implications:** We present detailed results on the spatial distribution of different ARG in sediments of a freshwater lake which can be convincingly linked to the inflow of municipal wastewater. Our results demonstrate that the combination of qPCR and geostatistical tools is a suitable approach to quantitatively detect and assess the dissemination of ARG from local
sources. The release of wastewater into Vidy Bay has resulted in a high ARG accumulation in sediments in the vicinity of the contamination source, but a diffuse zone of elevated ARG concentrations extends further west towards the location of the drinking water pumping station, indicating that transport takes place over considerable distances. Uptake of ARG via drinking water is considered a risk for human health as horizontal transfer to pathogens may occur. Treatment and quality control of waste and drinking water should be reconsidered with this in mind. In Switzerland and many other European countries the majority of wastewater treatment plants operate without a final disinfection treatment, and many rely on basic biological treatment. As modernization of many of these facilities (including Lausanne’s WWTP) is imminent or already under way it will be important to consider modern procedures for disinfection and contaminant removal that may be able to remove ARG from waste and drinking water (Dodd 2012). Effective ARG and pollutant removal may help to break the cycle of ARG exchange between the human and the aquatic environment. The available technologies should be studied with regards to both their effectiveness in reducing the load of resistant bacteria. It should be kept in mind, however, that chemical disinfection methods may themselves cause an undesirable selection for antibiotic resistance. Even wastewater receiving elaborate treatment and disinfection has been shown to be a source of ARG contamination (LaPara et al 2011).

Supplementary information is available at ISMEJ's website.

Conflict of Interest
The authors declare no conflict of interest.

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**Titles and legends to figures**

Figure 1: Map of Vidy Bay, indicating its location in Lake Geneva, 22 sites from which sediments were sampled, and the two river mouths of the Flon and Chamberonne (CHAM). The WWTP discharge pipe is indicated by a dashed line starting at Lausanne’s WWTP to the point of discharge, the STEP site. Bathymetric data provided by Anh-Dao Le thi and Walter Wildi, Institut F.A. Forel. Maps used with permission of swisstopo (Art. 30 GeoIV): PK50©2007, 2005©swisstopo.

Figure 2: Log-log plot of ARG abundance (ARG copy numbers normalized to bacterial 16S rRNA gene copy numbers) versus distance from the STEP site. STEP was arbitrarily given a distance of ½ the distance to the next closest site to allow it to be included in the analysis. Lines with $R^2$ values are for fitted functions of type $y = a \cdot x^b$.

Figure 3: Spatial interpolation maps showing the distributions of ARG abundance (ARG copy numbers normalized to bacterial 16S rRNA gene copy numbers) and total mercury (THg) in Vidy Bay sediments. a) sul1, b) sul2, c) tet(W), d) tet(M), e) tet(B), f) total mercury (THg) in mg kg$^{-1}$.

Figure 4: a) Principle coordinates analysis of ARISA community profiles from sediment and water. Both biological and technical replicates are included. Dispersion ellipses are shown for sediment sampling sites near the WWTP discharge pipe and for untreated hospital and municipal wastewater (Sewage) b). Principle coordinates analysis of ARISA community profiles from selected sediments with passively fitted environmental variables and ARG abundance. For this analysis technical replicates were averaged, omitting peaks only present in one replicate. Samples were selected to match ARG and sediment data availability.
Symbols indicate samples grouped according to sample type and location. (n.s.) – not significant (p>0.05).