A peer-reviewed version of this preprint was published in PeerJ on 3 January 2018.

View the peer-reviewed version (peerj.com/articles/4197), which is the preferred citable publication unless you specifically need to cite this preprint.

Madueño L, Paul C, Junier T, Bayrychenko Z, Filippidou S, Beck K, Greub G, Bürgmann H, Junier P. 2018. A historical legacy of antibiotic utilization on bacterial seed banks in sediments. PeerJ 6:e4197
https://doi.org/10.7717/peerj.4197
A historical legacy of antibiotic utilization on bacterial seed banks in sediments

Laura Madueño 1, Christophe Paul 1, Thomas Junier 2, Zhanna Bayrychenko 1, Sevasti Filippidou 1, Karin Beck 3, Gilbert Greub 4, Helmut Bürgmann 3, Pilar Junier Corresp. 1

1 Laboratory of Microbiology, Institute of Biology, University of Neuchatel, Neuchâtel, NE, Switzerland
2 Vital-IT group, Swiss Institute of Bioinformatics, Lausanne, Switzerland
3 Surface Waters - Research and Management, Swiss Federal Institute of Technology, Zurich, Kastanienbaum, Switzerland
4 Institute of Microbiology, University Hospital of the University of Lausanne, Lausanne, Switzerland

Corresponding Author: Pilar Junier
Email address: pilar.junier@unine.ch

The introduction of antibiotics for both medical and non-medical purposes has had a positive effect in human welfare and agricultural output in the past century. However, there is also an important legacy in the use and disposal of antimicrobial agents in natural ecosystems. This historical legacy was investigated by quantifying two antibiotic resistance genes (ARG) conferring resistance to tetracycline (tet(W)) and sulfonamide (sul1) in bacterial seed bank DNA in sediments. The industrial introduction of antibiotics caused an abrupt increase in the total abundance of tet(W) and a steady increase in sul1. The abrupt change in tet(W) corresponded to an increase in relative abundance from ca. 1960 that peaked around 1976. This pattern of accumulation was highly correlated with the abundance of specific members of the seed bank community belonging to the Phylum Firmicutes. In contrast, the relative abundance of sul1 increased after 1976. This correlated with a taxonomically broad spectrum of bacteria, reflecting sul1 dissemination through horizontal gene transfer. The accumulation patterns of both ARGs correspond to the temporal scale of medical antibiotic use. Our results show that the bacterial seed bank can be used to look back at the historical usage of antibiotics and resistance prevalence.
A historical legacy of antibiotic utilization on bacterial seed banks in sediments

Short title: Antibacterial legacy in seed banks

Laura Madueño1*, Christophe Paul1*, Thomas Junier1-2*, Zhanna Bayrychenko1, Sevasti Filippidou1, Karin Beck3, Gilbert Greub4, Helmut Bürgmann3 and Pilar Junier1&

1Laboratory of Microbiology, Institute of Biology, University of Neuchâtel. Rue Emile-Argand 11, CH-2000, Neuchâtel, Switzerland
2Vital-IT group, Swiss Institute of Bioinformatics, Lausanne, Switzerland
3Eawag, Swiss Federal Institute of Aquatic Science and Technology, 6047 Kastanienbaum, Switzerland
4Institute of Microbiology, University of Lausanne and University Hospital (CHUV); Lausanne, Switzerland

*These authors contributed equally to this work.
&corresponding author: pilar.junier@unine.ch

Abstract
The introduction of antibiotics for both medical and non-medical purposes has had a positive effect in human welfare and agricultural output in the past century. However, there is also an important legacy in the use and disposal of antimicrobial agents in natural ecosystems. This historical legacy was investigated by quantifying two antibiotic resistance genes (ARG) conferring resistance to tetracycline (tet(W)) and sulfonamide (sul1) in bacterial seed bank DNA in sediments. The industrial introduction of antibiotics caused an abrupt increase in the total
abundance of \textit{tet}(W) and a steady increase in \textit{sul}1. The abrupt change in \textit{tet}(W) corresponded to an increase in relative abundance from ca. 1960 that peaked around 1976. This pattern of accumulation was highly correlated with the abundance of specific members of the seed bank community belonging to the Phylum Firmicutes. In contrast, the relative abundance of \textit{sul}1 increased after 1976. This correlated with a taxonomically broad spectrum of bacteria, reflecting \textit{sul}1 dissemination through horizontal gene transfer. The accumulation patterns of both ARGs correspond to the temporal scale of medical antibiotic use. Our results show that the bacterial seed bank can be used to look back at the historical usage of antibiotics and resistance prevalence.

\textbf{Introduction}

The use of antibiotics to treat infectious diseases represents one of the major scientific achievements of the 20\textsuperscript{th} century. Millions of lives have been saved since the introduction of antibiotics into general medical practice for the treatment of a large range of bacterial infections, as well as other medical procedures (Marti et al 2014). After the initial use of antibiotics in medicine, the utilization of antibiotics to increase agricultural productivity has become a common practice (Carlet et al 2011). Although the positive effect of the so-called antibiotic era on human welfare is not disputed, increased awareness of the risks posed by poor antibiotic stewardship mitigates this success. Nowadays, it is becoming clear that disposal of antibiotics in natural ecosystems can have far-reaching consequences. Recent studies on antibiotics and the emergence of resistance suggest that the function of antibiotics in nature cannot be explained solely within the paradigm of chemical weapon in which these compounds have been used since their industrial production (Aminov 2009, Aminov 2010). Instead, antibiotics and determinants of resistance are a fundamental component of the ecology of microbial ecosystems. Most of the
antibiotics used today are chemical derivatives of small bioactive molecules that perform a multitude of functions in nature (Taylor et al. 2011). Therefore, in evolutionary terms, industrialized production, use, and disposal of antibiotics is a relatively recent phenomenon that has presumably exerted a selective pressure for pathogens to acquire and further hone naturally occurring antibiotic resistance systems (Taylor et al. 2011). This phenomenon has given rise to increasing rates of antibiotic resistance, a problem that threatens health care systems worldwide (Wright 2010). Therefore, understanding the historical effect of antibiotic use on the natural reservoirs of ARGs is essential to develop a management strategy to reduce current and future risks.

ARGs were clearly present in microbial communities before the antibiotic era as shown by phylogenetic analysis of genes conferring resistance to different classes of antibiotics (Aminov and Mackie 2007). Given the presumed role of human activity in the levels of resistance in the environment, one can thus expect an increasing abundance of such genes in the past century. However, direct evidence for this is currently restricted to a limited number of studies. For example, soil archives from two regions in Europe clearly demonstrate a link between the history of antibiotic use and the increase in the abundance of various genes conferring resistance to a large range of antibiotics (Graham et al. 2016, Knapp et al. 2010). Furthermore, the analysis of soil records also demonstrated the interconnection between the medical and non-medical use of antibiotics, as well as the effect of changes in policy towards a more strict stewardship (Graham et al. 2016).

Besides soils, aquatic ecosystems have been identified as a key ecological component driving the emergence, spread, and persistence of antibiotic resistance (Taylor et al. 2011). Lake sediments are a major concern because they are a main environmental end-point not only for bacteria, but
also for ARGs and antimicrobial agents (Kümmerer 2009). The high numbers of cells in sediments made resuspended sediment material a highly likely source of resistance determinants. At the same time, lake sediments are natural environmental archives. Thus, the study of the sedimentary record might provide insights into the historical legacy of the antibiotic era and the accumulation of ARG in the environment. Attempts to use DNA extracted from sediments to investigate antibiotic resistance in aquatic systems have been made (Thevenon et al 2012), but suffer from uncertainty regarding the preservation of the environmental signal in the sediments. Sediment microbial communities are strongly shaped by the redox gradients experienced during early diagenesis, and it is therefore unclear how much of the originally resistant community, or of their resistance determinants, is preserved in deeper sediment layers, or how this relationship is affected by environmental factors. The use of microbial seed banks preserved in the sedimentary record as a proxy offers a likely solution to these problems. The seed bank can be broadly defined as a reservoir of dormant cells that can potentially be resuscitated under favorable environmental conditions (Lennon and Jones 2011). One of the defining features of dormant cells is their reduced metabolic activity (Driks 2002), decreasing the uncertainty generated by environmental changes during sediment diagenesis (Vuillemin et al 2016). In addition, dormant cells are more resistant to degradation than their actively growing counterparts (Abecasis et al 2013). We have used the latter property to develop a specific extraction method to enrich DNA from spores as an example of dormant cell forms (Wunderlin et al 2014b, Wunderlin et al 2016). With this approach we have previously shown that one particular group of bacteria capable of dormancy (endospore-forming Firmicutes) can be used as paleoecological biomarkers of the impact of lake eutrophication on microbial communities in sediments (Wunderlin et al 2014a). Using the same selective method we investigated if the
historical antibiotic usage has affected the levels of ARG found in the natural seed bank bacterial community.

**Material and Methods**

**Site description and sampling**

A sediment core was retrieved with a gravity corer (UWITEC, Mondstein, Au) in August 2011 in an inactive canyon (C1) on the eastern side of the Rhone delta in Lake Geneva (Switzerland) (CAN01, coordinates 559901-139859, 79 m depth, 105 cm). This core has previously been dated ($^{137}$Cs and magnetic susceptibility dating) and validated for paleoecology (Wunderlin et al 2014a).

**DNA extraction**

DNA from the seed bank was obtained using an indirect extraction method. The extraction of cells from sediments was performed as previously described (Wunderlin et al 2013). The cells extracted from 3 g of wet sediment were filtered onto two different 0.2 um pore-size nitrocellulose filters (Merck Millipore, Darmstadt, Germany). A treatment to separate seed bank from vegetative cells was performed on the biomass collected on nitrocellulose filters, as previously described (Wunderlin et al 2014b, Wunderlin et al 2016). One filter (1.5 grams of sediment) per sample was used for the treatment. The first step consisted of the lysis of vegetative cells by heat, enzymatic agents (lysozyme) and chemicals (Tris-EDTA, NaOH, SDS). Further DNase digestion was used to destroy any traces of free DNA. DNA was extracted from the pre-treated filters using a modified protocol with the FastDNA®SPIN kit for soil (MP Biomedicals, USA)(Wunderlin et al 2013), in which the lysing matrix was submitted to two successive bead-beating steps. Supernatants from each bead-beating step were treated separately.
downstream according to manufacturer’s instructions. DNA extracts were pooled by precipitation with 0.3 M Na-acetate and ethanol (99 %), stored at -20°C overnight and centrifuged for 1h at 21,460 x g and 4°C. Supernatant was removed and the pellet was washed with 1 volume of 70% ethanol and centrifuged for 30 min at 21,460 x g and 4°C. Supernatant was removed and the residual ethanol was allowed to evaporate at room temperature. DNA was re-suspended in 50 µl of PCR-grade water. Total DNA was quantified using Qubit® dsDNA HS Assay Kit on a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).

**Quantitative PCR on tet(W) and sul1 genes**

Quantitative Taqman®-PCR on sul1 and tet(W) genes was performed in 384-well plates using a LightCycler®480 Instrument II (Roche, Switzerland). For sul1, the primers used were qSUL653f and qSUL719r with tpSUL1 probe (Heuer and Smalla 2007). The reaction mix for sul1 consisted of 2 µL of DNA template (between 0.08 and 1.39 ng/µL), 0.025 µM of each primer, 0.25 µM of TaqMan probe and 1 x TaqMan® Fast Universal PCR Master Mix (Applied Biosystems, USA). Total reaction volume of 10 µL was reached with PCR-grade water. For tet(W), the primers used were tetW-F and tetW-R with tetW-S probe (Walsh et al 2011). The reaction mix for tet(W) consisted of 2 µL of DNA template, 0.025 µM of each primer, 0.1 µM of TaqMan probe and 1 x TaqMan® Fast Universal PCR Master Mix (Applied Biosystems, USA). Total reaction volume of 10 µL was reached with PCR-grade water. The qPCR program was the same for both genes and started with a hold at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s and annealing/elongation at 60°C for 1 min. The qPCR assays were performed in technical triplicates on samples, standards and negative controls. The negative controls consisted of PCR blanks with only the reaction mix and of PCR blanks containing the mix and 2 µL of PCR-grade water. Standard curves were prepared from serial 10-fold dilutions of plasmid DNA containing
the respective target gene in a range of $5 \times 10^7$ to 50 gene copies. For sul1, control plasmids and standard curves were prepared as previously described (Heuer and Smalla 2007). For tet(W), standard curves were prepared as previously described (Walsh et al 2011). The effect of inhibitors on amplification was tested for all the samples and for both genes. All samples were spiked with $10^4$ copies of plasmid DNA containing the tet(W) or the sul1 gene and amplified together with the same set of non-spiked samples and control DNA and the results indicated that inhibition was negligible.

**Sequencing and data analysis**

Purified DNA extracts were sent to Fasteris (Geneva, Switzerland) for 16S rRNA amplicon sequencing using Illumina MiSeq platform (Illumina, San Diego, USA), generating 250 bp paired-end reads. The hypervariable V3-V4 region was targeted using universal primers Bakt_341F (5’-CCTACGGGNGGCWGCAG-3’) and Bakt_805R (5’-GACTACHVGGGTATCTAATCC-3’) (Herlemann et al 2011). Analysis of the dataset was made using Mothur (Schloss et al 2009) following the standard MiSeq SOP(Kozich et al 2013). The SILVA reference database (Quast et al 2013) was used for the alignment of amplicons and the taxonomic assignment of representative OTUs. After quality filtering and removal of chimeras, a total of 2’837’393 amplicons was obtained (625’339 unique sequences). Singletons were removed prior to the clustering into OTUs. The number of singletons in the dataset was 560’158. Clustering of the 2’277’235 remaining sequences (65’181 unique sequences) was made using a threshold of 97% identity. Finally, 11’802 OTUs constitute the dataset. The generated datasets were submitted to NCBI under the Bioproject accession number PRJNA396276.

**Statistical and multivariate analyses**
Community and statistical analyses were performed using R version 3.4.0 (R Team 2014) and the phyloseq and vegan packages (McMurdie and Holmes 2013, Oksanen et al 2017). Pairwise correlations between OTUs relative abundance and ARGs frequency were calculated using Spearman's rank correlation coefficient. Seed bank community was analyzed by principal coordinates analysis (PCoA), based on Bray-Curtis dissimilarity and Hellinger transformation of the OTUs table (community matrix). Environmental parameters and ARGs abundance/frequency were standardized and passively fitted to the ordination. Only significant parameters were displayed (p<0.05).

Results

Quantification of ARGs in seed bank communities from sediment samples

Seed bank DNA was extracted from a sediment core previously validated for paleoecology covering approximately the last hundred years of sediment accumulation in Lake Geneva (Wunderlin et al 2014a). ARG in seed bank DNA was measured by quantifying the number of copies of genes conferring resistance to tetracycline (tet(W) gene) and sulfonamide (sul1 gene), two commonly reported antibiotics detected in environmental settings (Davies and Davies 2010). The detection of ARGs in the seed bank DNA changed beginning in 1960 (tet(W)) and 1970 (sul1). However, the accumulation pattern was different for the two ARGs. In the case of tet(W), the total abundance of the gene (copies/g of sediment) increased by an order of magnitude since 1965 compared to the values obtained from 1920 to 1960 (Supplementary Figure 1). Moreover, the relative abundance of this ARG (gene copies/ng of DNA) in the seed bank DNA increased from 1961 to 1975 (Figure 1). In the case of sul1, a steady increase of this ARG abundance was observed after 1970 (Supplementary Figure 1). The relative abundance of sul1 in seed bank
DNA increased from the same period, followed by a decline and a more recent increase after the
year ca. 2000 (Figure 1). The specific timeframe in which enrichment in ARG counts per ng of
DNA was observed concerned mainly the seed bank DNA, as opposed to the total bacterial
community. In addition, we could detect ARGs using a lower initial concentration of DNA for
the seed bank community (2 ng of DNA) compared to the total community (10-15 ng of DNA).
This further suggests a preferential enrichment of ARGs in seed bank bacteria compared to the
overall environmental background.

Characterization of the seed bank communities

Previous studies in Lake Geneva have shown a dramatic effect of human activity on the
nutritional status of the lake. The lake became eutrophic between 1954 and 1986, and this
modified the proportion of some members of the bacterial community in sediments (Wunderlin
et al 2014a). Eutrophication is partly related to the same human activities that also shaped the
antibiotic era (for example, increased agricultural and livestock output and population pressure).
Since changes in microbial community composition as well as the spread of ARG within
populations can influence the record of antibiotic resistance, it was important to analyze seed
bank community composition alongside ARG quantification. Representatives of six major
bacterial phyla (Proteobacteria, Firmicutes, Actinobacteria, Planctomycetes, Chlamydiae, and
Chloroflexi) were the main components of the bacterial seed bank community in sediments
(Supplementary Figure 2; Figure 2A). The overall community analysis revealed similarities in
the community composition in samples with higher relative abundance of either tet(W) or sul1
(Figure 2B). For the former, a significant contribution of OTUs associated to the Phylum
Firmicutes was observed, while in the case of sul1 no particular bacterial group was correlated with increased accumulation.

In order to understand more clearly the relationship between ARG enrichment and seed bank bacterial community, we next studied if the relative abundance of certain OTUs was correlated with ARG levels. For this, we calculated the correlation coefficient between the relative abundance of each OTU and the ARG relative abundance at different depths. Correlation coefficients were plotted as a continuum to analyze the overall response of the community (Figure 3A). In the case of tet(W) most of the non-Firmicutes seed bank community was not correlated with increased ARG relative abundance over time (most correlation coefficients were close to 0; Figure 3A; dashed line). However, when the analysis is made only for representatives of the Phylum Firmicutes, the distribution shifted significantly towards positive correlations (comparison of the distribution for the total and Firmicutes communities; $t = 16.52$, df $= 6171.6$, $p$-value $< 2.2e-16$; Figure 3A; solid line). This analysis confirmed the results of the total community analysis (Figure 2B). We investigated further the ten most positively correlated OTUs. Nine out of the ten operational taxonomic units (OTUs) positively correlated with tet(W) relative abundance belong to Firmicutes (Table 1). The origin and ecology of bacteria related to those OTUs suggests an equal contribution of bacteria from an environmental origin, mainly cellulose-degrading anaerobic bacteria such as *Anaerobacterium* (Horino et al 2014) (OTU00093 and OTU00528), *Clostridium* (Hernandez-Eugenio et al 2002, Miller et al 2011, Zhilina et al 2005) (OTU00262, OTU00084, and OTU02280), and *Acetivibrio* (Patel et al 1980) (OTU00908); and from human (or animal) intestinal origin such as *Ruminococcus* (Cann et al 2016, Chassard et al 2012, Crost et al 2016) (OTU01612 and OTU01577). The OTUs positively correlated to tet(W) represented a minor fraction of the bacterial seed bank community even for
those samples with the highest ARG abundance (relative OTU abundance not higher than 5%; Figure 3B).

The same analysis performed on sul1 showed a larger fraction of the community positively correlated to relative ARG abundance (Figure 3A), but in contrast to tet(W) this is not specifically significant for Firmicutes only. Instead, the 10 most positively correlated OTUs belonged to diverse phylogenetic groups (Actinobacteria, Chloroflexi, Firmicutes, Proteobacteria, Verrucomicrobia, and Planctomycetes) (Table 1). OTUs correlated positively with sul1 abundance represented only minor fractions of the seed bank community (Figure 3A). Interestingly, the correlation coefficients are higher for tet(W) than for sul1, suggesting a stronger relationship of particular OTUs with the former.

Even though the analysis of the total community already suggests that the effect of increased relative abundance of ARG is independent from the generalized effect of eutrophication, we performed the same correlation analysis between relative OTU abundance and iron and manganese concentrations in sediments. Iron and manganese can be used as a proxy for redox conditions in the water column (Corella et al 2012, Koinig et al 2003) and their concentration correlates with eutrophication in Lake Geneva (Wunderlin et al 2014a). The results show no overlap between the overall effect of eutrophication and the specific effect of ARG abundance in terms of the most correlated OTUs (Supplementary Figure 3).

Discussion

Lake Geneva is one of the largest lakes in Europe and constitutes a major reservoir of drinking water. The composition of bacterial communities (Haller et al 2011, Sauvain et al 2014), as well as the presence of toxic metals (Pote et al 2008), micropollutants (Bonvin et al 2011), and ARGs
(Czekalski et al. 2012, Czekalski et al. 2014, Devarajan et al. 2015), has been monitored regularly in its water column and sediments. All these studies have demonstrated the role of human activity in the transfer of contaminants (including antibiotics) into sediments. All these preliminary studies made of Lake Geneva an ideal model system to validate the use of the seed bank bacterial community as a proxy to the effect of the historical use of antibiotics on the abundance of ARG in the environment. Our results show that studying the bacterial seed bank community in sediments of Lake Geneva shows the historical increase in ARG abundance. There was a clear link between seed bank taxonomy and accumulation of tet(W). This taxonomy-specific effect has been well documented in the case of tetracycline (Roberts and Schwarz 2016).

Tetracycline is a class of broad-spectrum antibiotics active against a wide range of Gram-positive and Gram-negative bacteria, including some atypical pathogens such as *Mycoplasma* and *Chlamydia*, and even eukaryotic parasites. This antibiotic class was isolated from *Streptomyces* spp. between 1947 and 1950, constituting one of the earliest classes of antibiotics described (Roberts and Schwarz 2016). In the USA, tetracycline became extensively used in production of livestock between 1950s and 1970s and remains today the second most commonly used antibiotic in agriculture (Roberts and Schwarz 2016). The situation in Switzerland is similar, according to a recent report from the Swiss Federal Office of Public Health indicating that tetracycline (together with penicillin) is the second most sold antibiotic product, after sulfonamides (FOPH 2016). In Switzerland, the current use of tetracycline is mainly restricted to non-medical applications, with a reported consumption below 1% in hospitals (according to data covering the period from 2004 to 2015) and close to 11% in outpatient settings (FOPH 2016). In Switzerland the principal medical use of tetracycline was reported for the period of 1955 to 1970 (Table 2), but has since reduced dramatically following the use of amoxicillin-clavulanate for
skin and soft-tissue infections and the increased use of cotromixazole (a combination of sulfonamides and trimethoprim) for uncomplicated urinary tract infections, which represent the two most common bacterial infections encountered in outpatient clinics and private medical practice. This medical historical use fits well with the observed peak of relative accumulation of tet(W) in the seed bank DNA in the late 1970s, and would suggest a primarily medical origin to this ARG for this period of time.

Tetracycline binds to the elongating ribosome, affecting translation, and therefore resistance can be acquired through diverse mechanisms (Davies and Davies 2010, Roberts and Schwarz 2016). tet(W) confers resistance through ribosomal protection and although the ancestral source of the gene is unknown, it has been reported in both Gram-positive and Gram-negative bacteria (Roberts and Schwarz 2016). Even though our analysis cannot determine the origin of tet(W) in sediments, it clearly suggests that the accumulation of this gene in the seed bank during the medical use of this antibiotic is highly correlated with changes in the abundance of Firmicutes. One potential explanation for the link between medical use of tetracycline and tet(W) in Firmicutes is the fact that the human gut microbiome can serve as a reservoir of ARGs, and in particular to genes conferring resistance to tetracycline (de Vries et al 2011, van Schaik 2015). A recent analysis of the human gut microbiome suggests that Firmicutes are highly prevalent (Browne et al 2016, Dethlefsen et al 2007). More importantly, a recent study suggests that sporulation is a widespread characteristic of the human microbiome (Browne et al 2016), and it is precisely these dormant forms that can contribute to the seed bank in human-impacted ecosystems. However, linking tet(W) abundance and the human microbiome must not be seen as a confirmation of the relationship between medical antibiotic use and increase of ARGs levels in the environment. For example, a recent study monitoring the effect of tetracycline on the
The performance of anaerobic digestors used in wastewater treatment has shown a highly significant increase in the relative abundance of spore-forming Firmicutes after treatment with a concentration of 20 mg/L of tetracycline (Xiong et al. 2017). Overall the data suggest that antibiotics such as tetracycline can select for specific groups of Firmicutes.

The industrial introduction of sulfonamide was an entirely different effect to that of tetracycline. Sulfonamide drugs were also among the earliest antibiotics discovered. However, in contrast to tetracycline, sulfonamide and its derivatives were obtained by systematic screening of chemically synthesized compounds. The legacy of mass production of sulfonamide is reflected in one of the most broadly disseminated case of drug resistance, both in terms of prevalence and taxonomy (Aminov 2010). Resistance to this class of antibiotic is almost universally associated to genetic mobile elements that confer a fitness advantage to the receptor bacteria as shown in the case of non-pathogenic *Escherichia coli* (Enne et al. 2004). The abundance of *sul*1 may thus be indicative of a dissemination trend of certain widespread mobile genetic elements (e.g. class-1 integrons) (Gillings 2014, Skold 1976, Skold 2000) that may well carry other resistance elements.

Horizontal gene transfer mediated by mobile genetic elements is considered a major pathway of ARG dissemination in aquatic environments (Berglund 2015). This particular mechanism of ARG dissemination overcomes taxonomic barriers, probably explaining the wide taxonomic spectrum of bacterial seed bank groups related to *sul*1 quantification in the sediments.

Changes in guidelines to reduce usage of penicillin derivatives (such as co-amoxicillin) for uncomplicated urinary tract infection in favor of cotrimoxazole (Sulfamethoxazole-Trimethoprim combination) may partially explain the common occurrence of *sul*1 resistance gene in the seed bank DNA especially after 2005 (Table 2). At this time medical guidelines changed given the high rate of resistance of *E. coli* (90% of the etiology of cystitis in healthy adult female humans).
to penicillin derivatives, leading to the reintroduction of sulfonamides. Indeed, the resistance rate
of *E coli* to amoxicillin and to amoxicillin-clavulanate respectively reached 52% and 23% of the
isolates tested at the Lausanne University Hospital Diagnostic Laboratory in 2016 (4581 strains).

**Conclusions**

Previous studies on the historical legacy of the antibiotic era have come to contradictory
conclusions. On the one hand, they show the recent effect of human activity on ARGs in the
environments (Graham et al 2016, Knapp et al 2010, Thevenon et al 2012), and suggest that
reducing non-therapeutic antibiotic use can reduce some of the environmental ARG legacy. On
the other hand, the results show that this is not universally applicable to all antibiotic classes and
that policies intended to reduce non-therapeutic use can have undesirable consequences (Graham
et al 2016). Results for the accumulation of beta-lactamase genes in soils suggest that soil
accumulation reflected a broader expansion of antibiotic use across society, implying that
development of resistance in clinical and agricultural systems is mutually influential (Graham et
al 2016). Our data adds valuable information to the debate regarding the long-term effect of the
antibiotic era as we show that ARGs also affect a fraction of the microbial community that will
certainly outlast many of these policies: the seed bank bacterial community. This opens up a new
debate, concerning the potential long-term effect of these dormant, persistent ARG-contaminated
cellular structures and their potential for further spreading of ARGs in the environment.
Importantly however, we here by provide a proof of concept for a new way to study the historical
development of resistance that is applicable to many geographic regions and resistance
determinants and that does not rely on human archiving of environmental samples.
References

Abecasis AB, Serrano M, Alves R, Quintais L, Pereira-Leal JB, Henriques AO (2013). A genomic signature and the identification of new sporulation genes. *J Bacteriol* **195**: 2101–2115.

Aminov RI, Mackie RI (2007). Evolution and ecology of antibiotic resistance genes. *FEMS Microbiol Lett* **271**: 147-161.

Aminov RI (2009). The role of antibiotics and antibiotic resistance in nature. *Environ Microbiol* **11**: 2970-2988.

Aminov RI (2010). A brief history of the antibiotic era: lessons learned and challenges for the future. *Front Microbiol* **1**: 134.

Berglund B (2015). Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. *Infect Ecol Epidemiol* **5**: 28564.

Bonvin F, Rutler R, Chevre N, Halder J, Kohn T (2011). Spatial and Temporal Presence of a Wastewater-Derived Micropollutant Plume in Lake Geneva. *Environmental Science & Technology* **45**: 4702-4709.

Browne HP, Forster SC, Anonye BO, Kumar N, Neville BA, Stares MD et al (2016). Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature* **533**: 543-546.

Cann I, Bernardi RC, Mackie RI (2016). Cellulose degradation in the human gut: Ruminococcus champanellensis expands the cellulosome paradigm. *Environ Microbiol* **18**: 307-310.

Carlet J, Collignon P, Goldmann D, Goossens H, Gyssens IC, Harbarth S et al (2011). Society's failure to protect a precious resource: antibiotics. *Lancet* **378**: 369-371.

Chassard C, Delmas E, Robert C, Lawson PA, Bernalier-Donnadille A (2012). Ruminococcus champanellensis sp. nov., a cellulose-degrading bacterium from human gut microbiota. *Int J Syst Evol Microbiol* **62**: 138-143.

Clatworthy AE, Pierson E, Hung DT (2007). Targeting virulence: a new paradigm for antimicrobial therapy. *Nat Chem Biol* **3**: 541-548.

Corella JP, Brauer A, Mangili C, Rull V, Vegas-Vilarrubia T, Morellon M et al (2012). The 1.5-ka varved record of Lake Montcortes (southern Pyrenees, NE Spain). *Quaternary Res* **78**: 323-332.
Crost EH, Tailford LE, Monestier M, Swarbreck D, Henrissat B, Crossman LC et al (2016). The mucin-degradation strategy of Ruminococcus gnavus: The importance of intramolecular trans-sialidases. *Gut Microbes* 7: 302-312.

Czekalski N, Berthold T, Caucci S, Egli A, Burgmann H (2012). Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into lake geneva, Switzerland. *Front Microbiol* 3: 106.

Czekalski N, Gascon Diez E, Burgmann H (2014). Wastewater as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. *ISME J* 8: 1381-1390.

Davies J, Davies D (2010). Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 74: 417-433.

de Vries LE, Vallès Y, Agersø Y, Vaishampayan PA, García-Montaner A, Kuehl JV et al (2011). The Gut as Reservoir of Antibiotic Resistance: Microbial Diversity of Tetracycline Resistance in Mother and Infant. *PLOS ONE* 6: e21644.

Dethlefsen L, McFall-Ngai M, Relman DA (2007). An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* 449: 811-818.

Devarajan N, Laffite A, Graham ND, Meijer M, Prabakar K, Mubedi JI et al (2015). Accumulation of clinically relevant antibiotic-resistance genes, bacterial load, and metals in freshwater lake sediments in Central Europe. *Environ Sci Technol* 49: 6528-6537.

Driks A (2002). Overview: Development in bacteria: Spore formation in Bacillus subtilis. *Cell Mol Life Sci* 59: 389–391.

Enne VI, Bennett PM, Livermore DM, Hall LM (2004). Enhancement of host fitness by the sul2-coding plasmid p9123 in the absence of selective pressure. *J Antimicrob Chemother* 53: 958-963.

FOPH (2016). Swiss Antibiotic Resistance Report 2016. Usage of Antibiotics and Occurrence of Antibiotic Resistance in Bacteria from Humans and Animals in Switzerland. In: Heim HACD (ed). Federal Office of Public Health. p 176.

Gillings MR (2014). Integrons: past, present, and future. *Microbiol Mol Biol Rev* 78: 257-277.

Graham DW, Knapp CW, Christensen BT, McCluskey S, Dolfing J (2016). Appearance of β-lactam Resistance Genes in Agricultural Soils and Clinical Isolates over the 20th Century. *Scientific Reports* 6: 21550.

Haller L, Tonolla M, Zopfi J, Peduzzi R, Wildi W, Pote J (2011). Composition of bacterial and archaeal communities in freshwater sediments with different contamination levels (Lake Geneva, Switzerland). *Water Res* 45: 1213-1228.
431 Herlemann DP, Labrenz M, Jurgens K, Bertilsson S, Waniek JJ, Andersson AF (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J 5: 1571-1579.

435 Hernandez-Eugenio G, Fardeau ML, Cayol JL, Patel BK, Thomas P, Macarie H et al (2002). Clostridium thiosulfatireducens sp. nov., a proteolytic, thiosulfate- and sulfur-reducing bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. Int J Syst Evol Microbiol 52: 1461-1468.

439 Heuer H, Smalla K (2007). Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. Environ Microbiol 9: 657-666.

442 Horino H, Fujita T, Tonouchi A (2014). Description of Anaerobacterium chartisolvens gen. nov., sp. nov., an obligately anaerobic bacterium from Clostridium rRNA cluster III isolated from soil of a Japanese rice field, and reclassification of Bacteroides cellulosolvens Murray et al. 1984 as Pseudobacteroides cellulosolvens gen. nov., comb. nov. Int J Syst Evol Microbiol 64: 1296-1303.

448 Knapp CW, Dolfing J, Ehlert PAI, Graham DW (2010). Evidence of Increasing Antibiotic Resistance Gene Abundances in Archived Soils since 1940. Environmental Science & Technology 44: 580-587.

450 Koinig KA, Shotyk W, Lotter AF, Ohlendorf C, Sturm M (2003). 9000 years of geochemical evolution of lithogenic major and trace elements in the sediment of an alpine lake - the role of climate, vegetation, and land-use history. J Paleolimnol 30: 307-320.

456 Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79: 5112-5120.

459 Kümmerer K (2009). Antibiotics in the aquatic environment – A review – Part I. Chemosphere 75: 417-434.

463 Lennon JT, Jones SE (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol 9: 119-130.

466 Marti E, Variatza E, Balcazar JL (2014). The role of aquatic ecosystems as reservoirs of antibiotic resistance. Trends Microbiol 22: 36-41.

469 McMurdie PJ, Holmes S (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8: e61217.

472 Miller DA, Suen G, Bruce D, Copeland A, Cheng JF, Detter C et al (2011). Complete genome sequence of the cellulose-degrading bacterium Cellulosilyticum lentocellum. J Bacteriol 193: 2357-2358.
Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D et al (2017). vegan: Community Ecology Package. R package version 2.4-3.

Patel GB, Khan AW, Agnew BJ, Colvin JR (1980). Isolation and Characterization of an Anaerobic, Cellulolytic Microorganism, Acetivibrio cellulolyticus gen. nov., sp. nov.†. *International Journal of Systematic and Evolutionary Microbiology* **30**: 179-185.

Pote J, Haller L, Loizeau JL, Garcia Bravo A, Sastre V, Wildi W (2008). Effects of a sewage treatment plant outlet pipe extension on the distribution of contaminants in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. *Bioresour Technol* **99**: 7122-7131.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P et al (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**: D590-596.

Roberts MC, Schwarz S (2016). Tetracycline and Phenicol Resistance Genes and Mechanisms: Importance for Agriculture, the Environment, and Humans. *J Environ Qual* **45**: 576-592.

Sauvain L, Bueche M, Junier T, Masson M, Wunderlin T, Kohler-Milleret R et al (2014). Bacterial communities in trace metal contaminated lake sediments are dominated by endospore-forming bacteria. *Aquat Sci* **76**: S33-S46.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB et al (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537-7541.

Skold O (1976). R-factor-mediated resistance to sulfonamides by a plasmid-borne, drug-resistant dihydropteroate synthase. *Antimicrob Agents Chemother* **9**: 49-54.

Skold O (2000). Sulfonamide resistance: mechanisms and trends. *Drug Resist Updat* **3**: 155-160.

Taylor NG, Verner-Jeffreys DW, Baker-Austin C (2011). Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? *Trends Ecol Evol* **26**: 278-284.

Team RC (2014). *R: A Language and Environment for Statistical Computing*.

Thevenon F, Adatte T, Wildi W, Pote J (2012). Antibiotic resistant bacteria/genes dissemination in lacustrine sediments highly increased following cultural eutrophication of Lake Geneva (Switzerland). *Chemosphere* **86**: 468-476.

Torok E, Moran E, Cooke F (2009). *Oxford Handbook of Infections Diseases and Microbiology*. Oxford University Press: Oxford.

van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ (2011). Acquired antibiotic resistance genes: an overview. *Front Microbiol* **2**: 203.
van Schaik W (2015). The human gut resistome. *Philos Trans R Soc Lond B Biol Sci* **370**: 20140087.

Vuillemin A, Ariztegui D, Leavitt PR, Bunting L, the PASADO Science Team (2016). Recording of climate and diagenesis through sedimentary DNA and fossil pigments at Laguna Potrok Aike, Argentina. *Biogeosciences* **13**: 2475–2492.

Walsh F, Ingenfeld A, Zampicolli M, Hilber-Bodmer M, Frey JE, Duffy B (2011). Real-time PCR methods for quantitative monitoring of streptomycin and tetracycline resistance genes in agricultural ecosystems. *J Microbiol Methods* **86**: 150-155.

Wright GD (2010). Antibiotic resistance in the environment: a link to the clinic? *Curr Opin Microbiol* **13**: 589-594.

Wunderlin T, Junier T, Roussel-Delif L, Jeanneret N, Junier P (2013). Stage 0 sporulation gene A as a molecular marker to study diversity of endospore-forming Firmicutes. *Environ Microbiol Rep* **5**: 911-924.

Wunderlin T, Corella J, Junier T, Bueche M, Loizeau J-L, Girardclos Sp et al (2014a). Endospore-forming bacteria as new proxies to assess impact of eutrophication in Lake Geneva (Switzerland, AlFrance). *Aquat Sci* **76**: 103-116.

Wunderlin T, Junier T, Roussel-Delif L, Jeanneret N, Junier P (2014b). Endospore-enriched sequencing approach reveals unprecedented diversity of Firmicutes in sediments. *Environ Microbiol Rep* **6**: 631-639.

Wunderlin T, Junier T, Paul C, Jeanneret N, Junier P (2016). Physical Isolation of Endospores from Environmental Samples by Targeted Lysis of Vegetative Cells. *J Vis Exp*.

Xiong Y, Harb M, Hong PY (2017). Performance and microbial community variations of anaerobic digesters under increasing tetracycline concentrations. *Appl Microbiol Biotechnol*.

Zhilina TN, Kevbrin VV, Turova TP, Lysenko AM, Kostrikina NA, Zavarzin GA (2005). [Clostridium alkalicellum sp. nov., an obligately alkaliphilic cellulolytic bacterium from a soda lake in the Baikal region]. *Mikrobiologija* **74**: 642-653.

**Acknowledgments**

Swiss National Science Foundation (Interdisciplinary grant CR23I2_162810) for the funding of SF and TJ.

**Conflict of interest**

The authors declare no conflict of interest or competing financial interest.
Tables & Figures legends

Table 1. Correlation analysis between individual OTUs and relative abundance of tet(W) and sul1. Top 10 most positively and negatively correlated OTUs. For tet(W) gene, mostly OTUs belonging to Firmicutes have been correlated to tet(W) abundance. In contrast, for sul1, OTUs correlated to sul1 abundance belong to many phyla.

Table 2. Summary of antibiotic discovery, use and year in which resistance was documented. Temporal scale showing the respective period when a new antibiotic has been discovered, main period of clinical usage and the approximate year when a first resistance to that compound has been documented. The table is partially adapted from multiple sources (Clatworthy et al 2007, Torok et al 2009, van Hoek et al 2011), including national and international guidelines, as well as personal communication with Swiss and French doctors.

Figure 1. Tetracycline and Sulfonamide resistance in total bacterial community and in the seed bank over time. Relative abundance (gene copies/ng of extracted DNA) of two genes conferring resistance to the antibiotics tetracycline (tet(W)) and sulfonamide (sul1) in sediment samples covering the period between 1920 and 2010 in Lake Geneva, Switzerland. Quantification was made in DNA extracted from the seed bank (SB DNA) and total microbial community (total DNA).

Figure 2. Seed bank community composition in sediments from Lake Geneva. A. Contribution (relative abundance) of individual genera from the six most abundant bacterial phyla present in the sediment samples. B. Principal coordinates analysis (PCoA) of the seed bank bacterial community showing the effect of lake eutrophication (Axis 1; vector depth) and the accumulation of ARG (vector tet(W) and sul1).

Figure 3. Correlation of specific OTUs to the relative abundance of ARGS in sediments. A. Spearman correlation coefficients calculated for the relative abundance of each individual OTU and ARG frequency at different depths. The correlation coefficients were plotted as a continuum for the non-Firmicutes seed bank community (dashed line) or the OTUs belonging to Firmicutes only (solid line). B. Relative abundance of the ten most positively OTUs correlated with the relative abundance of each individual ARG.
Correlation analysis between individual OTUs and relative abundance of \textit{tet(W)} and \textit{sul1}.

Top 10 most positively and negatively correlated OTUs. For \textit{tet(W)} gene, mostly OTUs belonging to Firmicutes have been correlated to \textit{tet(W)} abundance. In contrast, for \textit{sul1}, OTUs correlated to \textit{sul1} abundance belong to many phyla.
**Tables**

**Table 1.** Correlation analysis between individual OTUs and relative abundance of tet(W) and sul1. Top 10 most positively and negatively correlated OTUs.

| Gene | OTU    | Phylum     | Genus              | Correlation coefficient |
|------|--------|------------|--------------------|-------------------------|
| tet(W) | Otu00093 | Firmicutes | *Anaerobacterium*   | 0.7890                  |
|       | Otu01612 | Firmicutes | *Lachnolosstridium*| 0.7391                  |
|       | Otu00262 | Firmicutes | Clostridiaceae unclassified | 0.7136                  |
|       | Otu00528 | Firmicutes | *Clostridium* unclassified | 0.6990                  |
|       | Otu01577 | Firmicutes | *Ruminococcus* 1 | 0.6791                  |
|       | Otu00084 | Firmicutes | Ruminococcaceae unclassified | 0.6722                  |
|       | Otu00908 | Firmicutes | Ruminococcaceae unclassified | 0.6684                  |
|       | Otu02280 | Firmicutes | *Epulopiscium*     | 0.6684                  |
|       | Otu01131 | Verrucomicrobia | Verrucomicrobia unclassified | 0.6659                  |
|       | Otu00529 | Firmicutes | *Geobacillus*      | 0.6652                  |
| sul1 | Otu00318 | Actinobacteria | *Mycobacterium* | 0.6656                  |
|       | Otu00382 | Chloroflexi | Caldilineaceae unclassified | 0.6517                  |
|       | Otu00975 | Firmicutes | *Ruminiclostridium* 1 | 0.6479                  |
|       | Otu03004 | Firmicutes | *Symbiobacterium* | 0.6341                  |
|       | Otu03302 | Actinobacteria | Actinobacteria unclassified | 0.6195                  |
|       | Otu00155 | Proteobacteria | *Hypomicrobium* | 0.6176                  |
|       | Otu00604 | Verrucomicrobia | Verrucomicrobia unclassified | 0.6170                  |
|       | Otu00853 | Acidobacteria | Subgroup 6 unclassified | 0.6103                  |
|       | Otu02777 | Actinobacteria | *Tessaracoccus* | 0.6095                  |
|       | Otu01652 | Planctomycetes | Plactomycetaceae unclassified | 0.6092                  |
Table 2 (on next page)

Summary of antibiotic discovery, use and year in which resistance was documented.

Temporal scale showing the respective period when a new antibiotic has been discovered, main period of clinical usage and the approximate year when a first resistance to that compound has been documented. The table is partially adapted from multiple sources (Clatworthy et al 2007, Torok et al 2009, van Hoek et al 2011), including national and international guidelines, as well as personal communication with Swiss and French doctors.
Table 2. Temporal scale showing the respective period when a new antibiotic has been discovered, main period of clinical usage and the approximate year when a first resistance to that compound has been documented. The table is partially adapted from multiple sources (Clatworthy et al 2007, Torok et al 2009, van Hoek et al 2011), including national and international guidelines, as well as personal communication with Swiss and French doctors.

| Antibiotics (class) | Discovery (year) | Period of usage | Resistance (year) |
|---------------------|------------------|-----------------|-------------------|
| Sulfonamides        | 1930             | 1940-1960       | 1940              |
|                     |                  | 1970-1985<sup>c</sup> |                   |
|                     |                  | 2005-2017<sup>c</sup> |                   |
| Tetracycline        | 1948             | 1955-1970       | 1953              |
| Penicillin          | 1929             | 1930-1970       | 1947              |
|                     |                  | 2005-2017<sup>d</sup> |                   |
| Methicillin         | 1960             | 1960-2017       | 1962              |
| Ampicillin          | 1962             | 1965-2017<sup>e</sup> | 1974              |
| Cephalosporins      | 1960-1970<sup>f</sup> | 1965-2017<sup>g</sup> | 1970              |
| Vancomycine         | 1957             | 1970-1995       | 1988              |
|                     |                  | 2000-2017<sup>h</sup> |                   |
| Streptomycin        | 1943             | 1946-1960       | 1958              |
| (Aminoglycosides)   |                  | 1980-2000       |                   |
| Chloramphenicol     | 1947             | 1950-1970       | 1958              |
| Erythromycin        | 1952             | 1995-2010<sup>i</sup> | 1988              |
| (Macrolides)        |                  |                 |                   |
| Norfloxacin (Quinolones) | 1979            | 1986-1995<sup>j</sup> | 1981              |
Ciprofloxacin (Quinolones) | 1987 | 1990-2005<sup>k</sup> | 1988
---|---|---|---
Linezolid | 2000 | 2010-2015 | 2004
Daptomycin | 2004 | 2012-2017 | 2005
Clindamycin (Lincosamides) | 1960 | 1960-1975<sup>l</sup> | 1964

<sup>a</sup>Estimates made for Europe; in some sub-Saharan countries, due to the difficulties of access some antibiotics (such as chloramphenicol and streptomycin) are still largely in use.

<sup>b</sup>Approximate date, mainly adapted from (Clatworthy et al 2007, van Hoek et al 2011). The year of resistance documentation is often much earlier than the year of the description of the mechanism leading to a resistance phenotype. For example for aminoglycosides, the first identified resistance mechanism was the decreased permeability, which was initially described only <i>in vitro</i>.

<sup>c</sup>With the availability of cotrimoxazole since 1968, there has been an increased use of cotrimoxazole until about 1985, when 2<sup>nd</sup> generation quinolones (such as ciprofloxacin) have been largely available.

<sup>d</sup>Recent increase in use of penicillin instead of methicillin or cephalosporins for susceptible strains in order to attempt to reduce selection pressure due to overuse of antibiotics.

<sup>e</sup>Including its use in combination with clavulanate.

<sup>f</sup>Successive discovery of first-, second- and third generation of cephalosporins.

<sup>g</sup>There is still a wide use of cephalosporins in Switzerland nowadays, mainly ceftriaxone for the treatment of severe infections due to Gram negative bacteremia, including <i>E. coli</i> bacteremia, which represents the most common cause of bacteremia (mainly in the setting of urosepsis).
After a first wide use of vancomycin in initial empirical therapy, especially for severe infections such as endocarditis, bacteremia and fever in neutropenic subjects, the use of vancomycin slightly decreased due to concern about emerging resistance in enterococci; use of vancomycin then again increased due to surge in prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and then due to common vancomycin-resistant enterococci (VRE).

Larger usage of macrolides when clarithromycin and azithromycin have been made available, especially for the empirical treatment of lower respiratory tract infections (in combination with cephalosporins, when severe)

Norfloxacin was largely replaced by ciprofloxacin as first line treatment for urinary tract infection from 1995 onwards, due to concern regarding antibiotic resistance and improved efficacy of ciprofloxacin for complicated urinary tract infections; in 2008, the European Medicines Agency recommended to avoid using oral norfloxacin for treatment of urinary infections (http://www.docguide.com/emea-restricts-use-oral-norfloxacin-drugs-utis)

Ciprofloxacin largely replaced norfloxacin for urinary tract infection from 1995 to 2005; in addition from 1990 to 2000, ciprofloxacin was largely used for the empirical treatment of lower respiratory tract infections (LRTI); then due to increased concern about resistance, cotrimoxazole was proposed as first-line empirical antibiotic treatment for uncomplicated urinary tract infection and macrolides replaced quinolones in the treatment of LRTI. This change for a decreasing usage of quinolones was also triggered by the decreased rate of susceptible Gram-negative bacilli to quinolones, which decreased from about 90% in 1990 to about 65-70% in 2000 in USA and in Europe.

Mainly used to treat staphylococcal infections from 1965 to 1975; however, its usage has declined much due to documentation of resistance and due to possible increased risk of post-
antibiotic colitis due to the broad antimicrobial effect of clindamycin on anaerobes, which constitute more than 90% of the intestinal microbiota.
Figure 1 (on next page)

Tetracycline and Sulfonamide resistance in total bacterial community and in the seed bank over time.

Relative abundance (gene copies/ng of extracted DNA) of two genes conferring resistance to the antibiotics tetracycline (tet(W)) and sulfonamide (su/1) in sediment samples covering the period between 1920 and 2010 in Lake Geneva, Switzerland. Quantification was made in DNA extracted from the seed bank (SB DNA) and total microbial community (total DNA).
Figure 2 (on next page)

Seed bank community composition in sediments from Lake Geneva.

A. Contribution (relative abundance) of individual genera from the six most abundant bacterial phyla present in the sediment samples. B. Principal coordinates analysis (PCoA) of the seed bank bacterial community showing the effect of lake eutrophication (Axis 1; vector depth) and the accumulation of ARG (vector tet(W) and sul1).
Figure 3 (on next page)

Correlation of specific OTUs to the relative abundance of ARGs in sediments.

A. Spearman correlation coefficients calculated for the relative abundance of each individual OTU and ARG frequency at different depths. The correlation coefficients were plotted as a continuum for the non-Firmicutes seed bank community (dashed line) or the OTUs belonging to Firmicutes only (solid line). B. Relative abundance of the ten most positively OTUs correlated with the relative abundance of each individual ARG.
