Chapter 7
Potential dissemination of ARB and ARG into soil through the use of treated wastewater for agricultural irrigation –is it a true cause for concern?

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Keywords

Antibiotic resistance; New Delhi metallo-beta-lactamase; Horizontal gene transfer; solar inactivation; bacteriophage

Abstract

Resistance to antibiotics is increasingly being recognized as an emerging contaminant posing great risks to effective treatment of infections and to public health. Pristine soils or even soils that predate the antibiotic era naturally contain ARB and ARG. This book chapter explores the native resistome of soils, and collates information on whether soil perturbation through wastewater reuse can lead to accumulation of ARB and ARGs in agricultural soils. Special emphasis was given to ARGs, particularly the bla_{NDM} gene that confers resistance against carbapenem. The fate and persistence of these emerging ARGs have not been studied in depth, however this book chapter reviews available information on other ARGs to gain insight into the possibility of horizontal gene transfer events in wastewater-irrigated soils and plant surfaces and tissues. Lastly, this book chapter visits solar irradiation and bacteriophage treatment as intervention options to limit dissemination of emerging contaminant threats.

7.1 Introduction

Antibiotic resistance is increasingly being recognized as an emerging contaminant, threatening effective treatment of infections and carrying a great risk to public health. Anthropogenic activities such as the rise in antibiotic use for medical and agricultural purposes are considered a major cause for escalating the threat.

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In all cases of usage, antibiotic end up in sewage waters at sub-therapeutic levels, that is, in concentrations not high enough to kill bacteria but instead impose a selective pressure to favor the occurrence of antibiotic resistant bacteria (ARB) with their associated antibiotic resistance genes (ARGs) (Pruden et al. 2013). In recent years, WWTPs have been shown to be potential hotspots for ARB and antibiotic resistance genes (ARGs) propagation (Rizzo et al. 2013). Despite undergoing treatment, the treated municipal wastewater can still contain a significant amount of ARB and ARGs. This problem is of particular concern in water-scarce countries with pressing needs to reuse the treated wastewater. Reuse of treated wastewater effluents might impose a potential risk to the public health if ARB and ARGs accumulate in the agricultural soils.

Soils, however, also inherently contain a baseline abundance of ARB and ARGs. It is therefore required to account for how much of the ARB and ARGs in agricultural soils are truly contributed by wastewater during irrigation events, and also which of these ARB and ARGs are potential new threats of concern.

This book chapter aims to address the underlying question of whether the use of treated wastewater for agricultural irrigation can lead to potential dissemination of ARG and ARB. To achieve this aim, the chapter starts by first stating that pristine soils or even soils that predate the antibiotic era naturally contain ARB and ARG. Findings from earlier studies are collated to provide both sides of the argument on whether wastewater reuse can lead to accumulation of ARB and ARGs in agricultural soils. Emphasis is made on the emerging ARGs, particularly the bla_{NDM} gene that confers resistance against carbapenem. Carbapenem is an antibiotic typically used as a last line of defense against gram-negative bacterial infections (Walther-Rasmussen, Høiby 2007). Bacterial pathogens possessing the bla_{NDM} gene are hence associated with patient morbidity and mortality rates. The fate and persistence of emerging ARGs (e.g. bla_{NDM}) are not studied in-depth but the chapter reviews insights that have been gained from studies involving other types of ARGs to discern if horizontal gene transfers are likely in a wastewater-irrigated soil matrix. Finally, the chapter discusses several intervention strategies, namely solar irradiation and phage treatment that can potentially be applied in the agricultural setting to combat against emerging ARB and ARG threats.

7.2 Pristine environments harbor ARBs and ARGs

Natural environments are thought to be the origin of most antibiotic resistance genes and serve as reservoirs for antibiotic resistance (D'costa et al. 2006, Wright 2007). Soil environments are a particularly significant reservoir as they are one of the richest habitats for microbial diversity and abundance (Cytryn 2013). In one study, a majority of the 93 bacterial colonies isolated from a cave that had been secluded for over 4 million years were revealed to be multi-drug resistant. These bacterial isolates demonstrated resistance to a wide range of structurally different antibiotics, including the last-resort antibiotic daptomycin (Bhullar et al. 2012). However, resistance patterns showed relatively little resistance to new classes of synthetic antibiotics

compared to natural antibiotics. In another study on deep terrestrial subsurface soil samples, 153 bacterial isolates were tested against 13 antibiotics and results found 70% of these isolates to be resistant to more than one antibiotic, with over 35 isolates resistant to five or more antibiotics (Brown, Balkwill 2009). Most frequently noted resistance was against nalidixic acid, mupirocin, or ampicillin, and to lesser extents, against ciprofloxacin, tetracycline, neomycin and chloramphenicol. Resistance against rifampin, streptomycin, kanamycin, vancomycin, erythromycin and gentamicin was also detected.

7.2.1 Overview of range of antibiotic resistance classes in pristine environments/soil

Given the presence of ARB in pristine environments, detection of ARGs is expected too. An analysis of the ARGs distribution in glacier environments showed a widespread distribution of ARGs in samples from various glaciers in Central Asia, North and South America, Greenland and Africa (Segawa et al. 2013). Reported ARGs included $bla_{\text{TEM-1}}$, *tetW, aac(3), aacC* and *strA* and even metallo beta-lactamase gene (bla_{IMP}), encompassing ARGS of both clinical and agricultural origins. In another study, soil DNA was cloned into vectors, and expressed for the insert genes. It was determined that at least nine clones were resistant to aminoglycosides and one to tetracycline (Riesenfeld et al. 2004). Aminoglycoside resistance genes sequences were further analyzed and six of them resembled genes that expresses 6'-N-aminoglycoside acetyltransferase [AAC(6')] enzymes. All but one of the aminoglycoside resistance genes encode amino acid sequences that are considerably different (< 60% identity) from any previously reported sequences. This indicates that natural soil environments are not only reservoirs for common ARGs, but are also reservoirs for genetically diverse and novel ARGs. Another study used functional metagenomics to study remote Alaskan soils and revealed the presence of diverse beta-lactamases, namely Ambler classes A, C, D (active site serine beta-lactamases) and B (metallo beta-lactamase) (Allen et al. 2009). Class A beta-lactamase were recovered from *Burkholderia pseudomallei*, *Pseudomonas luteola* and *Yersinia entercolitica*, and these recovered beta-lactamases were distantly related to the clinically relevant CTX-M family. The lone representative of the class D beta-lactamases was linked with a class C beta-lactamase as part of a single open reading frame harboring two full-length genes, making the study one of the first to report a bifunctional beta-lactamase. Class D causes resistance to amoxicillin, ampicillin and carbenicillin, while class C causes resistance to cephalexin. Class B beta-lactamase in the Alaskan soils fell into one of the three subgroups of known metallo beta-lactamases, but were more closely related to the ancestral beta-lactamases than beta-lactamases isolated in clinical settings. They, however, remained capable of conferring resistance on *E. coli* despite this evolutionary distance, demonstrating that resistance genes residing in the environmental reservoir do pose a threat to human health, especially if they are horizontally transferred to pathogens.

7.2.2 ARGs predate the use of antibiotics

Pristine environments are those subjected to minimal perturbation by human activities, but they might still be indirectly subjected to unknown anthropogenic contamination due to weather elements and animal migration. For an assessment of samples free from modern anthropogenic activities and antibiotic influences, insight can be gained from examining pre-antibiotic era bacterial isolates. Retrospective studies have found ARGs in bacterial isolates sampled prior to 1950, with some of the detected resistance elements being able to be conjugatively transferred (Hughes, Datta 1983, Smith 1967). Metagenomic analysis of ancient 30,000-year-old DNA from permafrost sediments also identified a highly diverse collection of genes encoding resistance to beta-lactam, tetracycline and glycopeptide antibiotics, and confirmed the similarity of a complete *van*A gene to modern variants (D'Costa et al. 2011). These results showed that ARGs exist naturally in the environment even prior to extreme selection pressure imposed by rampant antibiotic use. ARGs appear to facilitate bacterial survival in the natural environment and may be co-selected for by environmental factors like solar radiation or the presence of heavy metals and other toxic compounds in soil (Piepersberg et al. 1988, Nies 2003, Poole 2005). It may also be possible that ARGs occur consequentially from symbiotic relationships shared among different microorganisms. For instance, to defend against antibiotic-producing *Streptomyces*, other bacterial species may have co-evolved resistance against the corresponding antibiotics (D'Costa et al. 2011, Allen et al. 2010).

7.2.3 Baseline abundance of ARGs in soil

Despite the vast information related to the diversity of ARBs and ARGs that could be recovered from pristine soils or from soils predating the antibiotic era, little information is available on the baseline abundance of ARGs in such samples. Wang and coworkers utilized qPCR arrays to report the relative abundance of a wide diversity of ARGs. Their results found seven ARGs (bla_{TEM}, bla_{SFO}, bla_{FOX}, *cph*A, *mex*F, *opr*D, and *opr*J) that were frequently and evenly represented across all samples, suggesting that the examined Antarctic region is a reservoir for these genes (Wang et al. 2016). However, the study only provides relative abundance of genes and not absolute copy numbers. Instead, such information can be inferred from another study that used qPCR to quantify values of different classes of ARGs in agricultural soil samples collected over the 1940s, 1960s, 1970s, 1980s and 2000s (Knapp et al. 2009)(Knapp 2010). The study revealed that some genes increased in abundance with time. Specifically, genes *tet*Q, *tet*O, *tet*M, $bla_{\text{TEM-1}}$ were among ARGs with the highest rates of increment, coinciding with the increase in industrial antibiotic production in the 1950s and the increased use of related antibiotics (i.e., tetracycline and beta-lactam) in the recent years (Van Boeckel et al. 2014). To illustrate, at one of the study's sampling sites (Site C), *tet*Q, *tet*O and $bla_{\text{TEM-1}}$ had abundance of $10^{-3.49}$, $10^{-5.47}$ and $10^{-1.85}$ copies, respectively, per gram of dry soil collected in 1942. The abundance of *tet*Q, *tet*O and $bla_{\text{TEM-1}}$ increased to $10^{-2.62}$, $10^{-2.65}$ and $10^{-0.50}$ copies, respectively,

per gram of dry soil collected in 1975. Although this study only examined archived soils collected from the Netherlands and may not be representative of baseline ARG abundance on a global scale, knowledge of the baseline ARG abundance would allow one to infer the required ARG fluxes from anthropogenic sources to significantly perturb the baseline ARG abundance. This would suggest that irrigation with municipal wastewater and/or manure application, both of which inherently contain high abundances of ARBs and ARGs (Da Silva et al. 2006, Munir et al. 2011, Zhu et al. 2013, Munir, Xagoraraki 2011), may be potential contributors of ARGs and ARB to the soils. The following subsection therefore aims to further review potential impact on soils arising from manure application and wastewater irrigation.

7.3 Antibiotic resistance genes in anthropologically perturbed soils

Numerous studies showed that application/irrigation of manure and wastewater can lead to potential detrimental impacts on soils. Soils applied with dairy or swine manure were compared to inorganically-fertilized soils (Marti et al. 2013), and results showed enrichment of ARB and increment of ARGs abundances in manure-applied soils. However, there was no coherent corresponding increase in abundances enumerated from vegetables grown in the soils. Heuer and coworkers applied manure-containing sulfadiazine, an antibiotic typically used on livestock, to soils and compared the abundance of sulfonamide resistance genes against the non-treated soils. Their findings showed an increase in sulfonamide resistance gene numbers compared to non-treated soils, and that sulfonamide resistance genes continued to be detected more than two months after manure application (Heuer, Smalla 2007). When sulfadiazine-supplemented manure was applied repeatedly to soils, sulfonamide resistance gene abundances accumulated within the soil samples (Heuer et al. 2011). Although such studies demonstrate an increase in ARGs as a result of manure application, the increase could be due to a higher nutrient input that subsequently changed the microbial community and enriched for bacterial populations that inherently possess the associated ARGs. To address this, a separate study examined soils treated with a single application of manure derived from cows which had not received any antibiotics treatment (Udikovic-Kolic et al. 2014). For over 130 days, the ARG abundances in manure-applied soils were compared to that detected in soils adjusted to the same nutrient input load (i.e., nitrogen, phosphorus, potassium) levels with inorganic fertilizer (i.e., controls). It was reported that manure-applied soils contained a higher abundance of beta-lactam-resistant bacteria with *bla*$_{CEP}$ (i.e., cephalothin) resistance genes. The increase in abundance for this gene was linked to the enrichment of beta-lactamase-harboring resident soil bacteria. A further identification showed an increase in abundance for *Pseudomonas* spp. and *Janthinobacterium* sp.; both known to harbor beta-lactamases. This suggests that increases in the abundance of ARG and ARB after manure application can possibly be accounted for by the influx of contaminants associated with the manure. On the same note, a qPCR-based assessment on tetracycline resistance genes and integrase genes

found that manure application caused gene abundances to increase by 6-fold (Hong et al. 2013). These genes remained above background levels for up to 16 months. Through 16S rRNA gene analysis, the study found that soil samples collected before and after manure application did not change significantly, suggesting that the increase in the ARGs was due to the manure application, possibly arising from the influx of these ARGs from the animal feces, and not due to changes in microbial communities.

The impact on soils arising from wastewater irrigation seems to differ from manure application depending on the extent of wastewater treatment received. A recent study analyzed soils that were continuously irrigated with untreated wastewater for 100 years (Dalkmann et al. 2012). Compared to control soils, sulfonamide resistance gene copy numbers increased when normalized to either 16S rRNA genes or per gram of dry soil. The wastewater-irrigated soils were also noted to have increased total number of 16S rRNA gene copies, and this long-term increase in biomass correlated to the increase in absolute concentration of resistance genes in soils.

In contrast, negligible or insignificant detrimental impact was observed when wastewater was first treated prior irrigation. To exemplify, a comparison between treated-wastewater-irrigated and freshwater-irrigated soils found that ARB and ARG levels were on the whole identical or sometimes even lower in treated-wastewater-irrigated soils (Negreanu et al. 2012). Their findings indicate that the high numbers of ARB that entered the soil did not compete successfully against the resident soil bacteria, and hence were unable to survive in the soil environment. Another study irrigated soil microcosms with secondary-treated (i.e., wastewater that received treatment in a biological activated sludge process), chlorinated, or dechlorinated effluents in a single irrigation event, and did not observe any significant changes in the ARG levels compared to microcosms irrigated with deionized water (Fahrenfeld et al. 2013). However, there were elevated levels of sulfonamide resistance genes in soils upon repeated irrigation with secondary-treated wastewater but not with the chlorinated and dechlorinated effluents. A follow-up study monitored abundances of ARB and ARGs in vegetables grown fields fertilized with digested biosolids or untreated municipal sewage sludge compared to inorganic fertilizer (Rahube et al. 2014). Results did not show that either treatment had a significant impact on viable coliform ARB, except in one instance where sewage sludge application increased the occurrence frequency of ARB from 46.4% to 79.2%. The PCR approach detected gene targets in both treated soils and vegetables grown in them that were not present in inorganically fertilized soils.

7.4 Performance of wastewater treatment plants

These combined reports emphasize the importance of treatments achieving sufficient microbial contaminant removal before wastewater is used to irrigate agricultural soils. A conventional municipal wastewater treatment plant (WWTP) is comprised of a primary clarifier that serves to provide sedimentation of settleable solid particulates from the raw wastewater (i.e., influent), followed by biological activated sludge

process. Within the activated sludge process, microorganisms serve to biodegrade the organic matter, hence reducing the organic and nutrient load. The wastewater generated from the biological activated sludge tank is then channeled to a secondary clarifier to separate the supernatant from the settleable solid particulates. Effluent generated at this point is typically referred to as the secondary-treated wastewater. In most WWTPs, chlorination is performed on the secondary treated wastewater to achieve an additional inactivation of microbial agents present. In most instances, secondary biological treatment processes can achieve satisfactory treatment with regards to fecal coliforms in wastewater, and generally are able to meet a discharge requirement that includes a permissible level of fecal coliforms in wastewater < 1000 CFU/100 mL for restricted irrigation or < 2.2 CFU/100 mL for unrestricted irrigation (Al-Jassim et al. 2015a, Al-Jasser 2011).

However, secondary treatment processes do not necessarily address specific classes of pathogens and/or emerging microbial contaminants like ARB and ARGs, which are more difficult to remove than fecal coliforms. To illustrate, an investigation of the performance of a full-scale wastewater treatment plant assessed influent, secondary-treated and chlorinated effluents using culture-based and molecular methods (Al-Jassim et al. 2015b). Results found that abundance of regulated contaminants like coliforms and fecal coliforms was effectively reduced and met quality standards for restricted irrigation. However, removal rates of emerging contaminants were lower and that proportions of pathogenic genera and multi-drug resistant bacteria increased over the treatment schematic. An assessment of the performance of a full-scale and a bench-scale membrane bioreactor for wastewater treatment found variable but never total removal of pathogens from the influent to the effluent, despite the use of microfiltration membranes (Harb, Hong 2016).

Given that a total removal of microbial agents is not likely to be achieved by most WWTPs, more care should be placed to ensure removal of emerging ARB and ARGs that do not constitute part of the baseline ARGs and ARB in soils, as discussed earlier. The *bla*$_{\text{NDM}}$ is an example of a gene that has thus far not been found to constitute part of the baseline ARGs in soils. It results in the production of New Delhi metallo-beta-lactamase (NDM), an enzyme that confers resistance to a wide spectrum of beta-lactams, including carbapenems. A study addressing the occurrence of *bla*$_{\text{NDM}}$ genes at different phases in two wastewater treatment facilities in northern China found that *bla*$_{\text{NDM}}$ genes were detected in the influent, effluent and chlorinated effluent, which in turn resulted in the discharge of significant levels of these genes to the environment (Luo et al. 2013). The findings from that study therefore indicate that *bla*$_{\text{NDM}}$ is an emerging contaminant of special concern when the treated wastewater is to be reused for agricultural irrigation.

7.4.1 New Delhi Metallo-beta-lactamase as an emerging contaminant of special concern

Carbapenems are beta-lactam antibiotics that have been used to combat severe gram-negative bacterial infections, and represent a last line of defense treatment (Walther-

Rasmussen, Høiby 2007). Hence, emergence and global spread of carbapenem resistance in bacteria that would render this last-resort treatment ineffective can be a cause of great concern to public health. Resistance is conferred through carbapenemases, a type of beta-lactamase enzymes categorized into Ambler classes B, A, C and D (Bush 2010). Class B carbapenemases are metallo-beta-lactamases, MBLs, that use bound zinc atoms in the active site to help ionize and coordinate a nucleophilic hydroxide ion to mediate hydrolysis, while class A, C and D carbapenemases are serine carbapenemases that use active site serine as a nucleophile (Bush 2010, Wang et al. 1999). The New Delhi Metallo-beta-lactamase is a broad-spectrum beta-lactamase that falls into Ambler class B, and is a novel MBL that was identified in 2009 in a Swedish patient of Indian origin who travelled to New Delhi, India, and acquired a carbapenem-resistant *K. pneumoniae* infection (Yong et al. 2009). MBL enzymes exhibit tendency to have a broad-spectrum substrate profile. Biochemical characterization of protein structure of variant NDM-1 has shown that it has an expansive active site with a unique electrostatic profile that leads to accommodation of a wide variety of substrate molecules (King, Strynadka 2011). Furthermore, the protein also exhibits a molecular profile that allows for broad-spectrum antibiotic substrate binding and product release, hence conferring a bacterium with NDM its unique trait of exhibiting broad-spectrum antibiotic resistance (King, Strynadka 2011).

Since discovery, *bla*$_{NDM}$-positive infections have been reported in nosocomial environments in numerous countries (including the United States, Canada, United Kingdom, Germany, Kenya, South Africa, Oman, Pakistan, Honk Kong, Japan, Australia and more) in all continents except Antarctica (Table 7.1). Besides the NDM-positive Enterobacteriaceae as shown in Table 7.1, *bla*$_{NDM}$ has also been detected in numerous, including virulent, bacterial species such as *Acinetobacter* spp., *Aeromonas caviae*, *Enterobacter cloacae*, *Pseudomonas* spp., and *Vibrio cholera* (Kumarasamy et al. 2010, Walsh et al. 2011), some of which are listed in Table 7.1. The encoding gene for *bla*$_{NDM}$ was initially detected in a 180-kb plasmid for *K. pneumoniae* and a 140-kb plasmid for *E. coli*, of which both were easily transferable and at a high frequency to susceptible *E. coli* J53 (Yong et al. 2009). Since then, the gene has been found in plasmids of various sizes (~50–300 kb) that belonged to different incompatibility (Inc) groups (A/C, FI/FII) (Table 7.1). In 2011, a variant of NDM-1 (designated NDM-2) that differed by a single amino acid was reported (Kaase et al. 2011). In 2013, a review paper reported that a series of further variants (designated NDM-3 to NDM-7) have been reported on the Lahey Clinic beta-lactamase website (http://www.lahey.org/Studies/) (Johnson, Woodford 2013). A subsequent search on the same database showed reports of NDM variants that include all the way to NDM-16, suggesting a rapid variation of NDM.

In addition to the wide substrate range and the rapid variation of NDM, the problem arising from NDM is further aggravated by a number of complications. These complications include a lack of standard routine phenotypic tests for MBL detection (Miriagou et al. 2010). A commonly used approach as of now is the use of EDTA as a chelator of zinc to detect loss of MBL activity. Consequently, high prevalence of

unrecognized asymptomatic carriers is probable, which would lead to an underestimation of the global dissemination of NDM-harboring bacteria. Given that the *bla*$_{NDM}$ gene is often encoded in plasmids that are of various types of incompatibility classes, this indicates to the possibility of horizontal gene transfer among many different types of gram-negative bacteria. Moreover, the scarcity of available effective antibiotics poses challenges to treatment, hence indicating a higher risk of morbidity or mortality for patients who are infected by NDM-positive pathogens.

7.4.2 New Delhi Metallo-beta-lactamase in wastewater

The presence of *bla*$_{NDM}$-positive isolates is not restricted to only nosocomial environment. Instead, *bla*$_{NDM}$-positive isolates have also been isolated from non-nosocomial environments. To illustrate, a *K. pneumoniae* carrying the *bla*$_{NDM-1}$ gene was isolated from river water in Hanoi, Vietnam (Isozumi et al. 2012). Various reports also implicate wastewaters as reservoirs for bacterial isolates carrying *bla*$_{NDM}$ genes. Bacterial species carrying NDM have been isolated from waste seepage and tap water sampled from New Delhi, India city center and surrounding areas (Walsh et al. 2011), and from untreated wastewater in Jeddah, Saudi Arabia (Mantilla-Calderon et al. 2016).

In one of these studies, in-depth genomic characterization of the *bla*$_{NDM}$-positive *E. coli* that was isolated from wastewater influent showed that this bacterium possessed a mosaic of traits representative of different *E. coli* pathotypes (Mantilla-Calderon et al. 2016). Furthermore, the isolate was demonstrated to internalize into mammalian cells, and has a genome encoding for various virulence traits. The non-chromosomal genome of this bacterium also includes at least one plasmid that encodes for the *bla*$_{NDM}$ gene, suggesting possible exchange of carbapenemase genes between this isolate with other competent recipients. Besides the presence of viable NDM-positive bacteria in wastewater, *bla*$_{NDM-1}$ genes were also shown to be present at significant numbers in municipal wastewaters, which include wastewater discharged from hospitals. Untreated hospital wastewater from two hospitals in Singapore contained 2.29 x 10^6 gene copies/mL of *bla*$_{NDM}$ and 4.08 x 10^7, 1.25 x 10^6, and 6.19 x 10^5 gene copies/mL, of genes *bla*$_{KPC}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$, respectively (Le et al. 2016). Another study that monitored *bla*$_{NDM-1}$ numbers in raw wastewater entering a WWTP in Saudi Arabia reported 3.4 x 10^4 ± 2.3 x 10^4 copies/m^3 (Mantilla-Calderon et al. 2016). Similarly, significant copy numbers of *bla*$_{NDM-1}$ persisted through several treatment units (including disinfection by chlorination) in two WWTPs in northern China (Luo et al. 2013). Levels present in the effluent discharged from both WWTPs were from 1.3 x 10^3 ± 2.3 x 10^2 to 1.4 x 10^3 ± 2.5 x 10^2 copies/mL, representing a range of 4.4 to 93.2%, respectively, of influent levels.

Collectively, the presence of viable NDM-positive bacteria and the ubiquitous detection of *bla*$_{NDM}$ genes reiterate causes for concern. This is especially in cases of wastewater release into the environment or application of these waters onto soils in agricultural settings.

7.5 Fate and persistence of ARB and ARGs

Although introduction of ARB and ARGs, particularly those that encode *bla*$_{NDM}$ genes, into soils via wastewater application might carry various risks, the full extent of the potential risks would need to be further elucidated by understanding the fate and persistence of these bacteria in the environment. Upon dissemination into the soil environment, ARGs can be adsorbed or degraded or taken up by competent cells. Similarly, ARBs can be adsorbed onto particulates or inactivated or internalized into other hosts. Hence, not all of the ARB and ARGs contributed by the wastewater into the soil matrix would remain available to impose potential public health risks. Conversely, if ARGs or ARB continue to persist or multiply in their copy numbers within the soil environment, the risks would be potentially exponentially amplified. The following subsection aims to elaborate on these various scenarios.

7.5.1 Horizontal gene transfer (HGT)

Horizontal gene transfer, HGT, is a mechanism for exchange of genetic material that can occur via transformation (i.e., uptake of naked DNA by bacteria) or conjugation (i.e., transfer mediated by cell-to-cell junctions and a pore through which DNA can pass) (Thomas, Nielsen 2005). It is now widely recognized that HGT is a major mechanism of bacteria adaptation to clinical antibiotic concentrations. This is even more evident when considering that the most potent ARGs in pathogens are often encoded on mobile genetic elements (Nesme, Simonet 2015, Stokes, Gillings 2011, Schlüter et al. 2007, Djordjevic et al. 2013). In the case of *bla*$_{NDM}$ genes, they are often found on plasmids belonging to different incompatibility groups that have a broad host range and can be replicated in different bacterial lineages (Table 7.1). The *bla*$_{NDM}$ genes are also often found on conjugative plasmids that possess all the genes required for their autonomous transfer (Nesme, Simonet 2015, Carattoli et al. 2012). Further highlighting the risk associated with HGT, many human pathogenic bacteria including representatives of the genera *Campylobacter*, *Haemophilus*, *Helicobacter*, *Neisseria*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* are naturally transformable (Lorenz, Wackernagel 1994). Soil environments present a large genetic diversity at small spatial scale, and ample opportunities for cell-to-cell contacts, cellular movement or activity. Soil matrices are therefore considered to be hotspots conducive for the exchange of genetic materials through HGT. A review by Elsas and Bailey names the plant rhizosphere and plant tissue, phyllosphere, manured soil, guts of soil animals, aquatic sediments, sewage and sludge environments as some of the most prominent hotspots (Van Elsas, Bailey 2002). Collectively, these environmental compartments may contribute to ARG dissemination between bacteria and eventually acquisition by pathogens (Nesme, Simonet 2015).

Natural transformation of naked DNA is dependent upon exposure of bacteria to extracellular DNA molecules in the environment. DNA can enter the environment through release from decomposing cells, disrupted cells and virus particles, or

excreted from living cells (Thomas, Nielsen 2005). This extracellular DNA may (i) persist by binding to soil minerals and humic substances, (ii) be degraded by microbial DNases and used as a nutrient for plant and microbial growth, or (iii) be incorporated into a bacterial genome as a possible source of genetic instructions (Levy-Booth et al. 2007). Extracellular DNA ranges at approximately 0.03-1 µg per g of material in soil and sediments (Ogram et al. 1987, Selenska, Klingmüller 1992), and in approximately 0.03 to 88 µg of dissolved DNA per liter of fresh and marine water (DeFlaun, Paul 1989, Karl, Bailiff 1989). Work estimating extracellular DNA in activated sludge found 4 to 52 mg per g of volatile suspended solids (VSS) in sludge collected from different wastewater treatment plants (Dominiak et al. 2011).

In the environment, various factors can affect transformation and success rate of recombination for this available extracellular DNA. One is that DNA adsorption to soil matrix is influenced by soil characteristics such as concentration of humic substances, soil mineralogy, cation concentration and soil pH (Levy-Booth et al. 2007). Work by Nielsen et al. found that cell lysates persisted for up to 4 days after incubation in sterile soil, and remained accessible for uptake by competent *Acinetobacter* sp. during this period. However, transformation activity was limited to 4-8 h in non-sterile soil because of DNA degradation, loss of DNA stability with temperature, and because DNA no longer maintained by cellular repair mechanisms decays faster (Nielsen et al. 1997a, Nielsen et al. 1997b, Nielsen et al. 2000). Nielsen et al provides a more detailed review of factors affecting stability of extracellular DNA (Nielsen et al. 2007), and readers of this chapter are encouraged to refer to that review paper for more details.

DNA degradation can also take place, resulting in fragmentation of long DNA to shorter sizes of approximately 400 bp. GC content affect DNA degradation kinetics. For example, DNA from high-GC-content gram-positive *Actinobacteria* was found to persist longer in frozen soil than DNA from low-GC-content gram-positive *Clostridiaceae* (Hofreiter et al. 2001). Although it has been acknowledged that long fragments may recombine more effectively compared to short linear DNA fragments of a few bp to less than 200 bp, such estimates may not be entirely accurate since recombination events resulting in nucleotide changes of only a few bp can be difficult to distinguish from genetic changes arising from sequential mutations. In this manner, HGT can hence be easily overlooked (Feil, Spratt 2001, Ikeda et al. 2004). Additionally, integration of foreign DNA into genome is influenced by a number of factors including competent cells availability and sequence homology between genomic DNA and foreign strand. This is particularly so if one were to consider that recombination typically occurs between chromosomal DNA and sequence that is less than 25% divergent (Matic et al. 1997, Vulić et al. 1997, de Vries et al. 2001, Majewski, Cohan 1998, Majewski et al. 2000).

Upon natural transformation, DNA may be integrated into the host's chromosome. Foreign DNA in the cytoplasm that is not integrated is degraded quickly by nucleases and enters the internal DNA metabolism cycle since the salvaged nucleoside can be used for the synthesis of nucleotide at a lower ATP cost. Different bacteria have different rates of DNA internalization and success of integration. Under

in-vitro conditions, DNA uptake occurred at rapid speeds of 100 bp per second and 60 bp per second in *S. pneumoniae* and *A. baylyi* competent bacteria, respectively (Palmen, Hellingwerf 1997, Méjean, Claverys 1993). Successful recombination of internalized DNA, also under optimal *in-vitro* conditions, has been reported at 0.1% of internalized DNA in *A. baylyi* and up to 25-50% of internalized DNA in *B. subtilis* and *S. pneumoniae* (Palmen, Hellingwerf 1997).

Besides natural transformation, conjugative transfer is a process more specifically linked to plasmid acquisition. Plasmids are autonomously replicating genetic elements that can remove the need for a foreign gene to integrate into the recipient chromosome to become established (Thomas, Nielsen 2005). Plasmid conjugation depends on the hosts, and thus the fate of conjugative plasmids depends on host fitness, efficiency of transfer to new hosts, and selective advantages and disadvantages conferred by the plasmids (Van Elsas et al. 2000, Fernandez-Astorga et al. 1992). Different plasmids also have different host ranges, with some exhibiting broader host range (e.g. IncA/C_2, IncL/M, IncN, IncP, IncQ and IncW incompatibility group plasmids (Novais et al. 2007, Götz et al. 1996)) while others exhibit narrower host range (e.g. IncF, IncH, IncI and IncX (Novais et al. 2007, Suzuki et al. 2010)).

Abiotic factors also affect conjugative plasmid transfer, and have been extensively reviewed by van Elsas et al (Van Elsas et al. 2000, Van Elsas, Bailey 2002). As examples, extreme pH and temperature values are detrimental to cells, while the presence of nutrients in wastewater and soil might enhance bacterial donor's activity. However, a study assessed conjugation in *E. coli* strains and found that conjugative plasmid transfer can take place within a wide range of conditions (Fernandez-Astorga et al. 1992). Conjugation was not affected in a wide range of pH (6-8.5), low nutrient levels (down to 1 mg of carbon per liter) and low temperatures (8 – 15 °C).

7.5.2 HGT on plant surfaces

Plant-associated bacteria have been observed frequently to form assemblages or biofilms. Biofilm formation can be due to passive processes like accumulation of bacterial cells as water moves along plant surfaces, or due to active bacterial attachment and production of exopolymeric substances (Morris, Monier 2003). An example is the genus *Pseudomonas*, which are ubiquitous in the terrestrial ecosystems, and are frequently found in association with plants (Espinosa-Urgel 2004). They aggregate at high cell densities, forming biofilms that are conducive for horizontal gene transfer and plasmid conjugation. Plant components like rhizosphere and phylloplane are hot spots for bacterial metabolic activity and HGT, as are other biofilm-supporting environments, with transconjugant to donor ratios (T/D) as high as 10^{-3} or even 10^{-1} for indigenous or foreign plasmids (Van Elsas, Bailey 2002, Lilley et al. 1994). This is in contrast to bulk environments such as bulk water and bulk soil where plasmid transfer efficacy is lower (T/D $<10^{-5}$) and usually requires nutrient enrichment (Sørensen, Jensen 1998). In other plant components, like the phytosphere, elevated transfer frequencies have generally been attributed to plant exudates

stimulating bacterial metabolic activity (Sørensen, Jensen 1998, Lilley et al. 1994). These observations suggest a likelihood that ARGs, if present in the wastewater that is to be used for agricultural irrigation, can be horizontally transferred to other bacterium attached on plant surfaces, as well as in the soil matrix.

To illustrate more specifically the potential risk from ARG presence in wastewater, the following analysis is presented. Past study has shown that the transformation frequency of antibiotic resistance in native populations of nonsterile sediments were approximately 3×10^{-9} when 10 µg of DNA were added to 1 cm^3 of sediment (ref). Given the abundance of $bla_{\text{NDM-1}}$ gene in WWTP discharge reported in China (approximately 1,374 copies per mL), and that $bla_{\text{NDM-1}}$ genes have been located on conjugative plasmids of size 126 kb (assumed average size based on *E. coli* and *K. pneumoniae* plasmids reported in Table 7.1), this would equate to an approximate amount of 0.19 pg/mL of extracellular plasmid DNA (based on Avogadro's constant of 6.022×10^{23} g/molecule). Thus, this would mean that up to 52 m^3 of treated wastewater would need to be irrigated in order to account for the required amount of DNA to cause a transformation frequency of 3×10^{-9} per cm^3 of sediment.

Hence, it is unlikely that a single event of reusing the treated wastewater would lead to any substantial concerns in terms of horizontal gene transfer. Even if the same plot of land is to be continuously exposed to treated wastewater, and if ARG continue to persist indefinitely and accumulate in the soils, this would equate to approximately less than one horizontal gene transfer event per cm^3 of soil when conditions are favorable for transformation. This estimate is made on the basis of an estimated cell number of 10^8 cells per g of soil (Raynaud, Nunan 2014). It is however to be pointed out that these calculated transformation events may not provide accurate estimate of the actual events as the assumed transformation rates did not take into account the variation in natural competence of different bacterial cells. Furthermore, it is likely that the transformation frequency may vary with different physicochemical factors like concentrations of ions, temperature, pH and natural organic matter content (Lorenz, Wackernagel 1994). Regardless, this estimation suggests that the contribution of ARGs by wastewater to agricultural soil may not be imposing that much of a concern, although it cannot be concluded whether the wide multitude of different types of ARGs in the same wastewater would collectively result in a concern or not.

7.5.3 Internalization of pathogens

Besides HGT of ARGs, pathogenic ARBs that may be present in the treated wastewater should also be assessed for their likelihood of internalizing into plants. There are various routes by which bacteria can enter plant tissues. Entry can occur through natural openings in the plant surface (stomata, lenticels, sites of lateral root emergence, etc.) and/or through sites of biological or physical damage (Brandl 2008, Itoh et al. 1998, Kroupitski et al. 2009). Following closure of the guard cells, internalized bacteria can be protected from various sanitizers (Gomes et al. 2009).

Bacteria may also be passively carried into the plant tissue with water (e.g. water used to soak seeds, irrigate plants, or to wash produce crops following harvest) (Deering et al. 2012). Bacteria can also be recovered from above-ground portions of the plant following exposure of the roots to water containing the pathogen, indicating that the bacteria can be taken up through the roots and move within the plant (Deering et al. 2012).

Bacteria of concern may actively infect and colonize plant tissues. It has been shown that certain plant pathogenic bacteria like *Pantoea agglomerans* and endophyte *K. pneumoniae* have been associated with opportunistic infections in animals, including humans (Holden et al. 2009). To illustrate further, recent studies on the plant pathogen *Pectobacterium atrosepticum* and the plant-associated *Klebsiella pneumoniae* were shown to share a remarkably high proportion of their genome with the human-pathogenic *K. pneumoniae* (Bell et al. 2004, Fouts et al. 2008, Toth et al. 2006, Holden et al. 2009). Many plant and animal pathogens share a common molecular mechanism, namely the Type III secretion system (TTSS), for attacking their host (Rahme et al. 1995, Staskawicz et al. 2001, Deering et al. 2012). Alternatively, wounding or destruction of living tissue in plants can be mediated by plant pathogen first, which in turn creates a microenvironment that is favorable to the survival and/or replication of human pathogens in the plant tissues.

Many studies have shown that both *Salmonella* spp. and *E. coli* O157:H7 can internalize within a variety of plant tissue types and that there are numerous factors that can influence the extent of internalization (Deering et al. 2012). Lettuce plants that were grown in manure amended with fluorescently marked *E. coli* O157:H7 were shown to harbor bacteria that had internalized into the plant tissue, including the edible parts of it (Holden et al. 2009, Solomon et al. 2002). Long-term persistence of *E. coli* O157:H7 in fresh produce has been demonstrated with carrots and onions grown in artificially contaminated manure compost (Islam et al. 2004). This study showed that the bacteria could be detected from carrots for up to 12 weeks after initial application and in onions for up to 9 weeks. A similar study showed that *S. enterica* could be detected in tomato plants harvested 7 weeks after the seeds were sown in soil artificially contaminated with the bacteria (Barak, Liang 2008).

7.6 Intervention strategies needed

Given that the presence of ARGs and ARBs in wastewater can be a potential cause of concern during long-term reuse events, this section aims to discuss several natural or low-cost intervention strategies to reduce ARB and ARG presence in wastewaters. The known effect and limitations of sunlight radiation are discussed, and the idea of water augmentation by bacteriophage therapy to improve ARB reduction is visited.

7.6.1 Solar inactivation

The biocidal effect of sunlight is attributed to the UV portion of its irradiance (wavelength ranges of UV are: 400–315nm for UV-A; 315–280nm for UV-B; and

280–100nm for UV-C (McGuigan et al. 2012)) that can result in photo-degradation by direct or indirect mechanisms. In direct photoinactivation, components like microbial genome and proteins absorb shorter wavelengths of sunlight radiation, and subsequently degrade (Boehm et al. 2009). Studies have shown that UV irradiation on growing *Escherichia coli* cultures results in DNA lesions where some of that light is absorbed by the pyrimidine rings of thymine and cytosine bases in the DNA. This leads to the formation of new bonds between adjacent pyrimidine bases, forming pyrimidine dimers (pairs connected by covalent bonds) (Goodsell 2001, McGuigan et al. 2012). These dimers prevent base-pairing with the complementary purines on the other strand of DNA, which changes the shape of the DNA molecule, in turn making it difficult for polymerases to move through the region of the dimer. The end result is a transient block on the essential processes of replication and transcription (Courcelle et al. 2001). In indirect photoinactivation, endogenous (e.g. porphyrins, flavins, quinones, NADH/NADPH, and others (Eisenstark 1987, Jagger 1981, Lloyd et al. 1990, McGuigan et al. 2012, Webb, Brown 1979)) or exogenous molecules (e.g. (humic substances and photosynthetic pigments like chlorophyll (Blough, Zepp 1995, McGuigan et al. 2012, Schwartz et al. 2003, Curtis et al. 1992)) may absorb UV light and subsequently damage other cellular material through generation of reactive oxygen species (ROS, examples include singlet oxygen, hydroxyl radicals or alkyl peroxyl radicals) (Pattison, Davies 2006, Santos et al. 2012).

The efficacy of solar photoinactivation on pathogenic waterborne bacteria and pathogen indicators has been variable in study reports. For instance, many studies found rapid inactivation of fecal indicator organisms within a few hours of exposure to natural sunlight, and it was reported that all of the classically defined waterborne pathogenic bacteria were readily amenable to 6 h of solar disinfection under suitable field conditions (Boyle et al. 2008, McGuigan et al. 1998, Ubomba-Jaswa et al. 2009, Wegelin et al. 1994, McGuigan et al. 2012). But on the other hand, numerous studies also reported fecal coliforms showing much slower inactivation rates, and that some indicator bacteria remained detectable after a full day of sunlight exposure (Oates et al. 2003, Rijal, Fujioka 2003, Fisher et al. 2008, Fisher et al. 2012, Sinton et al. 2002, Sommer et al. 1997). A study examined the use of effluent from a municipal WWTP, without and after solar disinfection, prior to its use as irrigation water for cultivated lettuce crops (Bichai et al. 2012). The effluent was from secondary treatment, i.e., after receiving a standard biological treatment (activated sludge) followed by sedimentation in settling ponds. Results of inactivation assays showed that solar disinfection processes can reduce bacterial concentrations from $>10^3$-10^4 *E. coli* CFU /mL in real WWTP effluent to <2 CFU/mL. Out of the 16 lettuce samples irrigated with untreated WWTP effluent (i.e., not treated with solar irradiation), 14 samples were contaminated and positive for the presence of *E. coli* 24 h after irrigation. On the other hand, out of 28 lettuce samples irrigated with solar-disinfected WWTP effluent, only two samples were positive, confirming improved safety of irrigation practices due to solar treatment. Positive presence of *E. coli* for one of the two lettuce samples was tracked back to a highly contaminated WWTP effluent with an initial *E. coli* concentration of $1.3x10^4$ CFU/mL vs. $2.4\text{-}3.8x10^3$ CFU/mL in all other wastewater

samples. The other positive sample is speculated to have had incomplete inactivation and/or microbial regrowth, as the wastewater effluent still contained organic carbon and nutrients that can be assimilated to allow for bacterial survival and replication during dark storage. Another study examined the effect of solar disinfection on two antibiotic-resistant *E. coli* isolates from a WWTP effluent (Rizzo et al. 2012). The inactivation rate observed during solar radiation test for both *E. coli* strains investigated, namely 60% and 40% removal after 180 min of irradiation, was quite low compared to previous works on similar inactivation of *E. coli* in confined systems (Malato et al. 2009, Dunlop et al. 2011). The differences might be explained by variation in experimental design, but more importantly, the antibiotic-resistant *E. coli* strains may have characteristics that affected their resistance to photo-inactivation, resulting in a lower inactivation rate.

In regards to the effect of solar-disinfection on ARGs, information on their inactivation kinetics upon exposure to solar irradiance is lacking, and this knowledge gap requires more in-depth and systematic future studies. Most available literature explores the use of UV-disinfection to reduce ARG loads within WWTPs. For example, one of the early studies was performed by McKinney and Pruden to explore the use of UV to dimerize ARGs, with the intention of first inactivating these genes prior to their discharge (McKinney, Pruden 2012b). Their findings revealed that this would require UV doses that are at least 1 order of magnitude higher than those required for inactivation of the associated host bacterial cells. Generally, about 200–400 mJ/cm^2 of UV dosage is required to result in 3–4 log damage to ARG. This UV dosage is slightly higher than the highest recommended UV dose of 186 mJ/cm^2 to achieve 4-log removal and/or inactivation of viruses (USEPA 2006). The study also found that certain ARGs like *tetA* and *ampC* were significantly harder to inactivate than *mecA* and *vanA*. To illustrate, a UV dose of 186 mJ/cm^2 would only achieve an inactivation of 1–2 log for *tetA* and *ampC*, while the same dose would have inactivated *mecA* and *vanA* by 3–4 log.

The inefficacy in reducing ARGs by UV is repeatedly shown in other studies. To illustrate, an independent study assessed ARG removal by UV (with UV transmittance of 45%, total power of 900 kW, and light intensity > 100 mJ/cm^2) in a WWTP using advanced treatment systems, and found no apparent decrease in *tetM*, *tetO*, *tetQ*, *tetW*, *sul1*, *sul2* and *intl1* genes in total extracted DNA from treated waste samples (Chen, Zhang 2013). In another study that used UV fluence of up to 249.5 mJ/cm^2 on secondary-treated municipal wastewater effluent samples, only 0.58-log removal of *tetX* gene was observed, with a less effective removal (at 0.36-0.4-log) of *sul1*, *tetG* and *intl1* genes (Zhang et al. 2015). Yet another study that assessed UV/H_2O_2 advanced oxidation processes for disinfection of sterile water spiked with *bla*$_{TEM}$ gene-carrying *E. coli* found that the treatment could inactivate the tested antibiotic-resistant *E. coli* strain, but did not significantly change the copy number per mL of *bla*$_{TEM}$ gene (Ferro et al. 2017). All these studies were in contrast with a study that assessed the effect of UV on secondary-treated municipal wastewater effluent samples and surprisingly found 3- and 1.9-log reduction of erythromycin and tetracycline resistance genes, respectively, by the time 5 mJ/cm^2 fluence is reached

(Guo et al. 2013). Another study found that UV/H_2O_2 disinfection achieved a reduction of 2.8–3.5 logs in copy numbers of *sul1*, *tetX*, and *tetG* from secondary-treated municipal wastewater effluent (Zhang et al. 2016). Little explanation was offered for these discrepancies in literature, and comparisons are made harder due to the differences in experimental designs, UV sources, tested water matrices and ARB/ARGs.

In application, the efficacy of solar photoinactivation can be subjected to various factors. These factors include atmospheric conditions like water vapor, CO_2, ozone and oxygen, in addition to pollutants in the atmosphere, which can scatter and absorb various portions of the light (McGuigan et al. 2012). Water quality parameters can have a big influence on efficacy of solar disinfection in water bodies with turbidity being one of the important factors, and dissolved solids such as iron can absorb UV light and decrease the UV transmittance (Jones et al. 2014). Exogenous photosensitizers naturally present in surface waters include humic acids and chlorophyll, both of which can absorb sunlight and then react with oxygen to produce ROS (Blough, Zepp 1995, McGuigan et al. 2012, Schwartz et al. 2003). Organic and inorganic matter present in water bodies can cause bacterial growth instead of inactivation (McGuigan et al. 2012), or may generate ROS upon sunlight irradiation (Corin et al. 1996, Rizzo et al. 2012). Water salinity and alkalinity also play a role in the end efficacy of solar irradiation. The presence of ions may help to retain bacterial integrity, and if ions are present in high concentrations, they could have a limiting effect on photo-inactivation. To illustrate, UV-A mediates its biological effects on bacteria by ROS like hydrogen peroxide and hydroxyl radicals. If bicarbonates HCO_3^- are present in water, they react with hydroxyl radicals producing $CO_3^{\bullet-}$, which has a slower reaction with organic molecules when compared to •O (Canonica et al. 2005, McGuigan et al. 2012). Also, HCO_3^- induces photo-absorption, which limits the amount of light reaching bacteria in water. Other anions such as phosphates, chloride and sulphates are shown to be absorbed by bacteria but do not illicit a direct effect on solar inactivation unless in the presence of a photo-catalyst such as titanium dioxide (McGuigan et al. 2012).

The total irradiance dose received by the bacteria influence the extent of photoinactivation damage, and there is evidence that the rate at which that dose is delivered is an equally important factor. Additionally, different portions of UV light also have different effects. UV-A radiation wavelengths bordering on visible light are not sufficiently energetic to directly modify DNA bases, but are able to induce cellular membrane damage through the production of reactive oxygen species (Khaengraeng, Reed 2005, Rizzo et al. 2012, McGuigan et al. 2012). UV-B and UV-C are the more germicidal portions of UV light and represent the most genotoxic wavebands of solar radiation reaching the Earth's surface, causing direct DNA damage by inducing the formation of DNA photoproducts (Pfeifer 1997, Rizzo et al. 2012) as well as indirectly through photosensitization processes (Bolton et al. 2010, Muela et al. 2002, Santos et al. 2012).

Lastly, bacteria may differ in their response to solar irradiance and their capacity to combat its effects. For example, bacteria with larger genome sizes were

observed to be more susceptible to UV damage, presumably because larger genomes offered more sites for UV damage (McKinney, Pruden 2012a).

7.6.2 Bacteriophages

A potential strategy that might be augmented into existing systems to alleviate ARB and ARG load is the use of bacteriophages as control agents. Bacteriophages are viruses that infect and lyse bacteria, and are categorized into virulent (or lytic) or temperate (lysogenic) bacteriophages (Withey et al. 2005). The two categories of viruses differ in their life cycles. During lytic infection, virulent phages inject their nucleic acid into the host cell after attachment. Expression of the phage genome directs the cellular machinery of the host to synthesize new phage capsule material. The resulting phage progeny are released by fatal cell lysis, enabling the lytic cycle to continue as new cells are infected. In contrast, during lysogenic infection, temperate phages' nucleic acid recombines with the host cell genome forming a dormant endogenous phage (known as a prophage). The prophage is reproduced in the host cell line and confers immunity from infection, and remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients. Subsequently, the prophages become active. At this point, they initiate the reproductive cycle, resulting in lysis of the host cell (Mason et al. 2011). Bacteriophages, or phages for short, have several characteristics that make them attractive options as therapeutic agents or agents of biocontrol (Jassim, Limoges 2014). Such characteristics include their effectiveness in killing their target bacteria (i.e., host specificity), adaptability, natural residence in the environment and the fact that they are self-replicating and self-limiting (Jassim, Limoges 2014, Sulakvelidze et al. 2001, Jassim et al. 2016).

Bacteriophages can be isolated from the environment. However, bacteriophage isolation is a time-consuming process, and it may be difficult to isolate the desired bacteriophages that demonstrate the right host specificity. Furthermore, bacteria may become desensitized to the isolated phages after long-term exposure, and would require repetition of the entire isolation process.

Alternatively, new synthetic phages can be programmed and used. Synthetic phages offer a powerful advantage in their potential to specifically target certain ARB of concern, functioning to sensitize bacteria to antibiotics and selectively killing ARB. A proof-of-concept study utilized temperate phages to deliver a functional CRISPR-associated (Cas) system, otherwise known as interspaced short palindromic repeats that are clustered regularly, into the genome of ARB (Yosef et al. 2015). The delivered CRISPR-Cas system destroyed antibiotic resistance-conferring plasmids via sequence-targeting DNA cleavage. In addition, the CRISPR-Cas system genetically modified lytic phages to kill only antibiotic-resistant bacteria while protecting antibiotic-sensitized bacteria. This linkage between antibiotic sensitization and protection from lytic phages was a key feature of the tested strategy.

Currently, phage treatment is utilized in a number of ways. Most notably, phage therapy is applied in medical settings for the treatment of ARB infections, in veterinary settings for the treatment and prevention of infections in animals, as well as

for the treatment of plants (Balogh et al. 2010, Jones et al. 2012). Other examples demonstrating the use of bacteriophages on larger scale include phage application for treatment and preservation of foods, phage treatment of aquaculture and fish, and in wastewater treatment (Araki 1986, Withey et al. 2005, Brockhurst et al. 2006, Goldman et al. 2009). When applied to wastewater treatment processes, phages have been proposed as an eco-friendly tool to control the abundance of filamentous bacteria, which pose bulking and foaming problems in wastewater activated sludge process (ASP) systems (Withey et al. 2005, Khan et al. 2002b, Khan et al. 2002a, Thomas et al. 2002, Weinbauer 2004, Petrovski et al. 2011a, b, Khairnar et al. 2014, Pal et al. 2014). During ASP for sewage treatment, sludge settles in tanks and the supernatant is drained off for further purification. This process is detrimentally affected by filamentous microbes, which because of their filamentous morphologies, have high surface area and low density, hence impeding settleability of biomass (Withey et al. 2005). *Sphaerotilus natans*, a filamentous bacteria, was targeted by specific phages isolated from sewage (Choi et al. 2011). Phage application was observed to reduce sludge volume and produced clearer supernatant after 12 h. In addition, the phages remained stable and active for over 9 months and tolerated temperature and pH fluctuations common to activated sludge processes (Jassim et al. 2016).

Regardless of the potentially promising results, the application of phages in wastewater treatment systems for control of ARB and ARGs is still in need of systematic and in-depth experimentation. Some of the challenges and obstacles in utilizing phage in wastewater treatment and/or application in a field scale are: (i) high concentrations of phages must be used for a successful application; (ii) use of polyvalent phages with broader host range could lead to the degradation of useful bacterial populations (e.g. nitrifying populations, phosphate accumulating bacteria etc); (iii) specific phages must be identified by WWTP operators to target specific undesired bacterial populations; (iv) microbial analysis of the system is a prerequisite to phage application as the bacterial population may vary between wastewater treatment plants (Jassim et al. 2016). As of now, there are no studies demonstrating successful phage application directly into the agricultural soil and/or in combination with other intervention strategies. Therefore it is likely that some other unique factors such as pH, temperature, multiplicity of infection (MOI, ratio of phage to bacterial particles), decay rates under solar irradiation and so on, could affect efficacies of bacteriophages in agricultural settings and would require further examination.

7.7 Concluding statement

This book chapter discussed the native and introduced resistomes of natural environments. Soil environments are rich natural reservoirs of ARGs, and are the source of many clinically relevant ARGs today, including undiscovered ARGs that may impose new health threats. Soils often receive high inputs of clinically relevant ARGs through manure and reused wastewater application. These ARGs, including novel ARGs of pressing concern such as the *bla*$_{NDM}$ genes/plasmids, confer a wide

range of antibiotic resistance, persist through WWTP schematics, and accumulate in soil environments. As soil and wastewater environments are conducive matrices for microbial interaction and horizontal gene transfer, the potential of ARG transfer to new and pathogenic bacteria poses great risks, and intervention strategies are necessary. Solar inactivation is a naturally available resource that has shown to reduce the numbers of ARB in water bodies, and can be further exploited to disinfect treated wastewater before irrigation. However, sunlight alone is much less effective in ARG removal, and bacteriophages offer a novel potential strategy to specifically target certain ARB and ARGs in water bodies. This tool still faces many obstacles before it can be applied effectively, and further investigation is required.

Table 7.1. List of *bla*$_{NDM}$-carrying plasmids of various sizes, Incompatibility groups and co-resistance isolated in Enterobacteriaceae from different sources

Country of Isolation	Source	Plasmid size (kb)	Inc Group	NDM variant	Co-resistance*	Reference
Escherichia coli						
Australia	Clinical	50	Untypable	NDM-1	NR	(Poirel et al. 2010b)
Canada	Clinical	75	Untypable	NDM-1	NR	(Peirano et al. 2011a)
Canada	Clinical	129	A/C	NDM-1	*bla*$_{CMY-6}$	(Mulvey et al. 2011)
Canada	Clinical	130	A/C	NDM-1	*bla*$_{CMY-6}$; *rmt*C	(Borgia et al. 2012)
China	Clinical	50	Untypable	NDM-1	NR	(Ho et al. 2012)
Denmark	Clinical	-	A/C	NDM-1	*bla*$_{CMY-4}$; *armA*	(Nielsen et al. 2012)
France	Clinical	120	FIA	NDM-4	*bla*$_{CTX-M-15}$; *bla*$_{OXA-1}$; *aac*A4	(Dortet 2012)
France	Clinical	150	A/C	NDM-1	*bla*$_{OXA-10}$; *bla*$_{CMY-16}$	(Denis et al. 2012, Poirel et al. 2011c)
France	Clinical	110	F	NDM-1	*bla*$_{OXA-1}$; and markers for kanamycin; gentamicin; tobramycin; trimethoprim; and sulfonamide resistance (genes not specified)	(Poirel et al. 2010a, Poirel et al. 2011c)
Hong Kong	Clinical	90	L/M	NDM-1	*bla*$_{TEM-1}$; *bla*$_{DHA-1}$; *aac*C2; *arm*A; *sul*1; *mel*; *mph*2	(Ho et al. 2011)
India	Clinical	120	F	NDM-4	*arm*A; and resistance to all aminoglycosides	(Nordmann et al. 2012, Poirel et al. 2011c)
India	Community-acquired	87	FII	NDM-1	*bla*$_{OXA-1}$; *aac*C2; *aac*C4; *aad*A2; *dfr*A12	(Bonnin et al. 2012)
India	Waste seepage	140	A/C	NDM-1	NR	(Walsh et al. 2011)
India	Waste seepage	140	A/C	NDM-1	NR	(Walsh et al. 2011)
India	Waste seepage	140	Untypable	NDM-1	NR	(Walsh et al. 2011)
Japan	Clinical	196	A/C	NDM-1	*bla*$_{TEM-1}$; *bla*$_{CMY-4}$; *aad*A2; *arm*A; *sul*1; *mel*; *mph*2; *dfr*A12	(Sekizuka et al. 2011)
New Zealand	Clinical	>100	Untypable	NDM-6	*rmt*C	(Williamson et al. 2012)

New Zealand	Clinical	>100	Untypable	NDM-1	NR	(Williamson et al. 2012)
New Zealand	Clinical	>100	Untypable	NDM-1	NR	(Williamson et al. 2012)
Poland	Clinical	90	FII	NDM-1	*aacA4*; *aacC2*	(Fiett et al. 2014)
Saudi Arabia	Wastewater	110	F	NDM-1	*rmt*C; *dhps*	(Mantilla-Calderon et al. 2016)
Spain	Clinical	300	HII	NDM-1	$bla_{\text{TEM-1}}$; $bla_{\text{CTX-M-15}}$; $bla_{\text{DHA-1}}$; *arm*A	(Sole et al. 2011)
Switzerland	Clinical	130	F	NDM-1	$bla_{\text{TEM-1}}$; *arm*A	(Poirel et al. 2011g)
UK	Clinical	>100	F	NDM-5	*aad*A5; *dfr*A17; *rmt*B	(Hornsey et al. 2011)
Klebsiella pneumoniae						
Australia	Clinical	70	Untypable	NDM-1	$bla_{\text{CMY-6}}$; *aac*-6`-1b; *rmt*C	(Sidjabat et al. 2011)
Canada	Clinical	102	A/C	NDM-1	$bla_{\text{CMY-6}}$	(Mulvey et al. 2011)
Canada	Clinical	120	FII	NDM-1	NR	(Peirano et al. 2011b)
Canada	Clinical	150	A/C	NDM-1	$bla_{\text{SHV-12}}$; *arm*A	(Tijet 2011, Peirano et al. 2011b)
Canada	Clinical	130	A/C	NDM-1	bla_{CMY-6}; *rmtC*	(Borgia et al. 2012)
China	Clinical	50	Untypable	NDM-1	NR	(Ho et al. 2012)
Croatia	Clinical	-	A/C	NDM-1	$bla_{\text{CTX-M-15}}$; $bla_{\text{CMY-16}}$; *qnr*A6	(Mazzariol et al. 2012)
France	NR	150	Untypable	NDM-1	*rmt*C	(Poirel et al. 2011c)
France	Clinical	270; 300	Untypable	NDM-1	$bla_{\text{CTX-M-15}}$; $bla_{\text{OXA-1}}$; *aac*(6`)-Ib-like; *arm*A; *qnr*B1	(Arpin et al. 2012)
France	Clinical	100	Untypable	NDM-1	NR	(Poirel et al. 2011c, Poirel et al. 2011d)
Guatemala	Clinical	-	Untypable	NDM-1	$bla_{\text{SHV-12}}$	(Pasteran et al. 2012)
India	Clinical	160	A/C	NDM-1	NR	(Kumarasamy, Kalyanasundaram 2012)

India	Clinical	180	Untypable	NDM-1	*arr*-2; *ere*C; *aad*A1; *cml*A7	(Yong et al. 2009)
India	Waste seepage	140	Untypable	NDM-1	NR	(Walsh et al. 2011)
Kenya	Clinical	120	A/C_2	NDM-1	*rmt*C	(Poirel et al. 2011e)
Mauritius	Clinical	120	A/C	NDM-1	bla_{CMY-6}; *rmt*C	(Poirel et al. 2012a)
Morocco	Clinical	250	Untypable	NDM-1	$bla_{CTX-M-15}$; bla_{OXA-1}	(Poirel et al. 2011b)
The Netherlands	Clinical	70	II	NDM-1	NR	(Halaby et al. 2012)
New Zealand	Clinical	>100	Untypable	NDM-1	NR	(Williamson et al. 2012)
Oman	Clinical	170	L/M	NDM-1	*arm*A	(Poirel et al. 2011a)
Oman	Clinical	170	Untypable	NDM-1	*arm*A	(Poirel et al. 2011a)
South Korea	Clinical	50; 60; 70; 100	N	NDM-1	NR	(Kim et al. 2012)
Spain	Clinical	120	FIB	NDM-1	NR	(Oteo et al. 2012)
Switzerland	Clinical	150	A/C	NDM-1	*rmt*A	(Poirel et al. 2011g)
Switzerland	Clinical	150	A/C	NDM-1	bla_{OXA-10}; bla_{CMY-16}; *qnr*A6	(Poirel et al. 2011g)
Turkey	Clinical	80	FIB	NDM-1	*rmt*B	(Poirel et al. 2012b)
Klebsiella oxytoca						
Taiwan	Clinical	-	Untypable	NDM-1	*arm*A; *aac*C2	(Lai et al. 2011)
Citrobacter freundii						
France	Clinical	65	Untypable	NDM-1	NR	(Poirel et al. 2011f)
India	Waste seepage	140	A/C	NDM-1	NR	(Walsh et al. 2011)
Proteus mirabilis						
Switzerland	Clinical	150	A/C	NDM-1	bla_{OXA-10}; bla_{CMY-16}; *arm*A	(Poirel et al. 2011g)
Providencia stuartii						
Afghanistan	Clinical	178	A/C	NDM-1	bla_{OXA-10}; *arm*A; *sul*1; *qnr*A1; *aac*(6`); *cml*A7	(McGann et al. 2012)
Shigella boydii						
India	Waste seepage	250	Untypable	NDM-1	NR	(Walsh et al. 2011)

*: Co-resistance indicates antibiotic resistance determinants reported in the same plasmid as the bla_{NDM} gene.
NR: not reported.

References

Al-Jasser AO (2011) Saudi wastewater reuse standards for agricultural irrigation: Riyadh treatment plants effluent compliance. Journal of King Saud University - Engineering Sciences 23 (1):1-8.

Al-Jassim N, Ansari MI, Harb M, Hong P-Y (2015a) Removal of bacterial contaminants and antibiotic resistance genes by conventional wastewater treatment processes in Saudi Arabia: Is the treated wastewater safe to reuse for agricultural irrigation? Water Research 2015 (73):277.

Al-Jassim N, Ansari MI, Harb M, Hong P-Y (2015b) Removal of bacterial contaminants and antibiotic resistance genes by conventional wastewater treatment processes in Saudi Arabia: Is the treated wastewater safe to reuse for agricultural irrigation? Water Research 73:277-290.

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J (2010) Call of the wild: antibiotic resistance genes in natural environments. Nature Reviews Microbiology 8 (4):251-259.

Allen HK, Moe LA, Rodbumrer J, Gaarder A, Handelsman J (2009) Functional metagenomics reveals diverse β-lactamases in a remote Alaskan soil. The ISME journal 3 (2):243-251.

Araki M (1986) Advanced slime control process with bacteriophage. Kogyo Yosui 332:25-30.

Arpin C, Noury P, Boraud D, Coulange L, Manetti A, Andre C, M'Zali F, Quentin C (2012) NDM-1-producing Klebsiella pneumoniae resistant to colistin in a French community patient without history of foreign travel. Antimicrobial Agents and Chemotherapy 56 (6):3432-3434.

Balogh B, Jones JB, Iriarte F, Momol M (2010) Phage therapy for plant disease control. Current Pharmaceutical Biotechnology 11 (1):48-57.

Barak JD, Liang AS (2008) Role of soil, crop debris, and a plant pathogen in Salmonella enterica contamination of tomato plants. PLoS One 3 (2):e1657.

Bell K, Sebaihia M, Pritchard L, Holden M, Hyman L, Holeva M, Thomson N, Bentley S, Churcher L, Mungall K (2004) Genome sequence of the enterobacterial phytopathogen Erwinia carotovora subsp. atroseptica and characterization of virulence factors. Proceedings of the National Academy of Sciences of the United States of America 101 (30):11105-11110.

Bhullar K, Waglechner N, Pawlowski A, Koteva K, Banks ED, Johnston MD, Barton HA, Wright GD (2012) Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One 7 (4):e34953.

Bichai F, Polo-López MI, Ibañez PF (2012) Solar disinfection of wastewater to reduce contamination of lettuce crops by Escherichia coli in reclaimed water irrigation. Water Research 46 (18):6040-6050.

Blough NV, Zepp RG (1995) Reactive oxygen species in natural waters. In: Active Oxygen in Chemistry. Springer, pp 280-333.

Boehm AB, Yamahara KM, Love DC, Peterson BM, McNeill K, Nelson KL (2009) Covariation and photoinactivation of traditional and novel indicator organisms and human viruses at a sewage-impacted marine beach. Environmental Science & Technology 43 (21):8046-8052.

Bolton NF, Cromar NJ, Hallsworth P, Fallowfield HJ (2010) A review of the factors affecting sunlight inactivation of micro-organisms in waste stabilisation ponds: preliminary results for enterococci. Water Science and Technology 61 (4):885-890.

Bonnin RA, Poirel L, Carattoli A, Nordmann P (2012) Characterization of an IncFII plasmid encoding NDM-1 from Escherichia coli ST131. PLoS One 7 (4):e34752.
Borgia S, Lastovetska O, Richardson D, Eshaghi A, Xiong J, Chung C, Baqi M, McGeer A, Ricci G, Sawicki R, Pantelidis R, Low DE, Patel SN, Melano RG (2012) Outbreak of carbapenem-resistant enterobacteriaceae containing blaNDM-1, Ontario, Canada. Clinical Infectious Diseases 55 (11):e109-117.
Boyle M, Sichel C, Fernandez-Ibanez P, Arias-Quiroz GB, Iriarte-Puna M, Mercado A, Ubomba-Jaswa E, McGuigan KG (2008) Bactericidal effect of solar water disinfection under real sunlight conditions. Applied Environmental Microbiology 74 (10):2997-3001.
Brandl M (2008) Plant lesions promote the rapid multiplication of Escherichia coli O157: H7 on postharvest lettuce. Applied and Environmental Microbiology 74 (17):5285-5289.
Brockhurst M, Buckling A, Rainey P (2006) Spatial heterogeneity and the stability of host-parasite coexistence. Journal of Evolutionary Biology 19 (2):374-379.
Brown MG, Balkwill DL (2009) Antibiotic resistance in bacteria isolated from the deep terrestrial subsurface. Microbial Ecology 57 (3):484.
Bush K (2010) Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Current Opinion in Microbiology 13 (5):558-564.
Canonica S, Kohn T, Mac M, Real FJ, Wirz J, von Gunten U (2005) Photosensitizer method to determine rate constants for the reaction of carbonate radical with organic compounds. Environmental Science & Technology 39 (23):9182-9188.
Carattoli A, Villa L, Poirel L, Bonnin RA, Nordmann P (2012) Evolution of IncA/C blaCMY-2-carrying plasmids by acquisition of the blaNDM-1 carbapenemase gene. Antimicrobial Agents and Chemotherapy 56 (2):783-786.
Chen H, Zhang M (2013) Effects of advanced treatment systems on the removal of antibiotic resistance genes in wastewater treatment plants from Hangzhou, China. Environmental Science & Technology 47 (15):8157-8163.
Choi J, Kotay SM, Goel R (2011) Bacteriophage-based biocontrol of biological sludge bulking in wastewater. Bioengineered Bugs 2 (4):214-217.
Corin N, Backlund P, Kulovaara M (1996) Degradation products formed during UV-irradiation of humic waters. Chemosphere 33 (2):245-255.
Courcelle J, Khodursky A, Peter B, Brown PO, Hanawalt PC (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient Escherichia coli. Genetics 158 (1):41-64.
Curtis TP, Mara DD, Silva SA (1992) Influence of pH, Oxygen, and Humic Substances on Ability of Sunlight To Damage Fecal Coliforms in Waste Stabilization Pond Water. Applied Environmental Microbiology 58 (4):1335-1343.
Cytryn E (2013) The soil resistome: the anthropogenic, the native, and the unknown. Soil Biology and Biochemistry 63:18-23.
D'costa VM, McGrann KM, Hughes DW, Wright GD (2006) Sampling the antibiotic resistome. Science 311 (5759):374-377.
D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R (2011) Antibiotic resistance is ancient. Nature 477 (7365):457-461.
Da Silva MF, Tiago I, Veríssimo A, Boaventura RA, Nunes OC, Manaia CM (2006) Antibiotic resistance of enterococci and related bacteria in an urban wastewater treatment plant. FEMS Microbiology Ecology 55 (2):322-329.
Dalkmann P, Broszat M, Siebe C, Willaschek E, Sakinc T, Huebner J, Amelung W, Grohmann E, Siemens J (2012) Accumulation of pharmaceuticals, Enterococcus, and resistance

genes in soils irrigated with wastewater for zero to 100 years in central Mexico. PLoS One 7 (9):e45397.
de Vries J, Meier P, Wackernagel W (2001) The natural transformation of the soil bacteria Pseudomonas stutzeri and Acinetobacter sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. FEMS Microbiology Letters 195 (2):211-215.
Deering AJ, Mauer LJ, Pruitt RE (2012) Internalization of E. coli O157: H7 and Salmonella spp. in plants: a review. Food Research International 45 (2):567-575.
DeFlaun MF, Paul JH (1989) Detection of exogenous gene sequences in dissolved DNA from aquatic environments. Microbial Ecology 18 (1):21-28.
Denis C, Poirel L, Carricajo A, Grattard F, Fascia P, Verhoeven P, Gay P, Nuti C, Nordmann P, Pozzetto B (2012) Nosocomial transmission of NDM-1-producing Escherichia coli within a non-endemic area in France. Clinical Microbiology and Infection 18 (5):E128-130.
Djordjevic SP, Stokes HW, Roy Chowdhury P (2013) Mobile elements, zoonotic pathogens and commensal bacteria: conduits for the delivery of resistance genes into humans, production animals and soil microbiota. Frontiers in Microbiology 4:86.
Dominiak DM, Nielsen JL, Nielsen PH (2011) Extracellular DNA is abundant and important for microcolony strength in mixed microbial biofilms. Environmental Microbiology 13 (3):710-721.
Dortet L (2012) New Delhi Metallo-β-Lactamase 4–producing Escherichia coli in Cameroon. Emerging Infectious Diseases 18 (9):1540.
Dunlop P, Ciavola M, Rizzo L, Byrne J (2011) Inactivation and injury assessment of Escherichia coli during solar and photocatalytic disinfection in LDPE bags. Chemosphere 85 (7):1160-1166.
Eisenstark A (1987) Mutagenic and lethal effects of near-ultraviolet radiation (290-400 nm) on bacteria and phage. Environmental and Molecular Mutagenesis 10 (3):317-337.
Espinosa-Urgel M (2004) Plant-associated Pseudomonas populations: molecular biology, DNA dynamics, and gene transfer. Plasmid 52 (3):139-150.
Fahrenfeld NL, Ma Y, O'Brien M, Pruden A (2013) Reclaimed water as a reservoir of antibiotic resistance genes: distribution system and irrigation implications. Frontiers in Microbiology 4:130.
Feil EJ, Spratt BG (2001) Recombination and the population structures of bacterial pathogens. Annual Reviews in Microbiology 55 (1):561-590.
Fernandez-Astorga A, Muela A, Cisterna R, Iriberri J, Barcina I (1992) Biotic and abiotic factors affecting plasmid transfer in Escherichia coli strains. Applied and Environmental Microbiology 58 (1):392-398.
Ferro G, Guarino F, Cicatelli A, Rizzo L (2017) β-lactams resistance gene quantification in an antibiotic resistant Escherichia coli water suspension treated by advanced oxidation with UV/H 2 O 2. Journal of Hazardous Materials 323:426-433.
Fiett J, Baraniak A, Izdebski R, Sitkiewicz I, Zabicka D, Meler A, Filczak K, Hryniewicz W, Gniadkowski M (2014) The first NDM metallo-beta-lactamase-producing Enterobacteriaceae isolate in Poland: evolution of IncFII-type plasmids carrying the bla(NDM-1) gene. Antimicrobial Agents and Chemotherapy 58 (2):1203-1207.
Fisher MB, Iriarte M, Nelson KL (2012) Solar water disinfection (SODIS) of Escherichia coli, Enterococcus spp., and MS2 coliphage: effects of additives and alternative container materials. Water Research 46 (6):1745-1754.

Fisher MB, Keenan CR, Nelson KL, Voelker BM (2008) Speeding up solar disinfection (SODIS): effects of hydrogen peroxide, temperature, pH, and copper plus ascorbate on the photoinactivation of E. coli. Journal of Water Health 6 (1):35-51.

Fouts DE, Tyler HL, DeBoy RT, Daugherty S, Ren Q, Badger JH, Durkin AS, Huot H, Shrivastava S, Kothari S (2008) Complete genome sequence of the N 2-fixing broad host range endophyte Klebsiella pneumoniae 342 and virulence predictions verified in mice. PLoS Genetics 4 (7):e1000141.

Goldman G, Starosvetsky J, Armon R (2009) Inhibition of biofilm formation on UF membrane by use of specific bacteriophages. Journal of Membrane Science 342 (1):145-152.

Gomes C, Da Silva P, Moreira RG, Castell-Perez E, Ellis EA, Pendleton M (2009) Understanding E. coli internalization in lettuce leaves for optimization of irradiation treatment. International Journal of Food Microbiology 135 (3):238-247.

Goodsell DS (2001) The molecular perspective: ultraviolet light and pyrimidine dimers. Oncologist 6 (3):298-299.

Götz A, Pukall R, Smit E, Tietze E, Prager R, Tschäpe H, Van Elsas J, Smalla K (1996) Detection and characterization of broad-host-range plasmids in environmental bacteria by PCR. Applied and Environmental Microbiology 62 (7):2621-2628.

Guo M-T, Yuan Q-B, Yang J (2013) Ultraviolet reduction of erythromycin and tetracycline resistant heterotrophic bacteria and their resistance genes in municipal wastewater. Chemosphere 93 (11):2864-2868.

Halaby T, Reuland AE, Al Naiemi N, Potron A, Savelkoul PH, Vandenbroucke-Grauls CM, Nordmann P (2012) A case of New Delhi metallo-beta-lactamase 1 (NDM-1)-producing Klebsiella pneumoniae with putative secondary transmission from the Balkan region in the Netherlands. Antimicrobial Agents and Chemotherapy 56 (5):2790-2791.

Harb M, Hong P-Y (2016) Molecular-based detection of potentially pathogenic bacteria in membrane bioreactor (MBR) systems treating municipal wastewater: a case study. Environmental Science and Pollution Research 24 (6):5370–5380.

Heuer H, Smalla K (2007) Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. Environmental Microbiology 9 (3):657-666.

Heuer H, Solehati Q, Zimmerling U, Kleineidam K, Schloter M, Müller T, Focks A, Thiele-Bruhn S, Smalla K (2011) Accumulation of sulfonamide resistance genes in arable soils due to repeated application of manure containing sulfadiazine. Applied and Environmental Microbiology 77 (7):2527-2530.

Ho PL, Li Z, Lai EL, Chiu SS, Cheng VC (2012) Emergence of NDM-1-producing Enterobacteriaceae in China. Journal of Antimicrobial Chemotherapy 67 (6):1553-1555.

Ho PL, Lo WU, Yeung MK, Lin CH, Chow KH, Ang I, Tong AH, Bao JY, Lok S, Lo JY (2011) Complete sequencing of pNDM-HK encoding NDM-1 carbapenemase from a multidrug-resistant Escherichia coli strain isolated in Hong Kong. PLoS One 6 (3):e17989.

Hofreiter M, Serre D, Poinar HN, Kuch M, Pääbo S (2001) Ancient DNA. Nature Reviews Genetics 2 (5):353-359.

Holden N, Pritchard L, Toth I (2009) Colonization outwith the colon: plants as an alternative environmental reservoir for human pathogenic enterobacteria. FEMS Microbiology Reviews 33 (4):689-703.

Hong P-Y, Yannarell AC, Dai Q, Ekizoglu M, Mackie RI (2013) Monitoring the perturbation of soil and groundwater microbial communities due to pig production activities. Applied and Environmental Microbiology 79 (8):2620-2629.

Hornsey M, Phee L, Wareham DW (2011) A novel variant, NDM-5, of the New Delhi metallo-beta-lactamase in a multidrug-resistant Escherichia coli ST648 isolate recovered from a patient in the United Kingdom. Antimicrobial Agents and Chemotherapy 55 (12):5952-5954.

Hughes VM, Datta N (1983) Conjugative plasmids in bacteria of the 'pre-antibiotic'era. Nature 302 (5910):725-726.

Ikeda H, Shiraishi K, Ogata Y (2004) Illegitimate recombination mediated by double-strand break and end-joining in Escherichia coli. Advances in Biophysics 38:3-20.

Islam M, Morgan J, Doyle MP, Jiang X (2004) Fate of Escherichia coli O157: H7 in manure compost-amended soil and on carrots and onions grown in an environmentally controlled growth chamber. Journal of Food Protection 67 (3):574-578.

Isozumi R, Yoshimatsu K, Yamashiro T, Hasebe F, Nguyen BM, Ngo TC, Yasuda SP, Koma T, Shimizu K, Arikawa J (2012) bla(NDM-1)-positive Klebsiella pneumoniae from environment, Vietnam. Emerging Infectious Diseases 18 (8):1383-1385.

Itoh Y, Sugita-Konishi Y, Kasuga F, Iwaki M, Hara-Kudo Y, Saito N, Noguchi Y, Konuma H, Kumagai S (1998) Enterohemorrhagic Escherichia coli O157: H7 present in radish sprouts. Applied and Environmental Microbiology 64 (4):1532-1535.

Jagger J (1981) Near-UV radiation effects on microorganisms. Photochem Photobiol 34 (6):761-768.

Jassim SA, Limoges RG (2014) Natural solution to antibiotic resistance: bacteriophages 'The Living Drugs'. World Journal of Microbiology and Biotechnology 30 (8):2153-2170.

Jassim SA, Limoges RG, El-Cheikh H (2016) Bacteriophage biocontrol in wastewater treatment. World Journal of Microbiology and Biotechnology 32 (4):1-10.

Johnson AP, Woodford N (2013) Global spread of antibiotic resistance: the example of New Delhi metallo-beta-lactamase (NDM)-mediated carbapenem resistance. Journal of Medical Microbiology 62 (Pt 4):499-513.

Jones JB, Vallad GE, Iriarte FB, Obradović A, Wernsing MH, Jackson LE, Balogh B, Hong JC, Momol MT (2012) Considerations for using bacteriophages for plant disease control. Bacteriophage 2 (4):e23857.

Jones LA, Worobo RW, Smart CD (2014) UV light inactivation of human and plant pathogens in unfiltered surface irrigation water. Applied Environmental Microbiology 80 (3):849-854.

Kaase M, Nordmann P, Wichelhaus TA, Gatermann SG, Bonnin RA, Poirel L (2011) NDM-2 carbapenemase in Acinetobacter baumannii from Egypt. Journal of Antimicrobial Chemotherapy 66 (6):1260-1262.

Karl DM, Bailiff MD (1989) The measurement and distribution of dissolved nucleic acids in aquatic environments. Limnology and Oceanography 34 (3):543-558.

Khaengraeng R, Reed RH (2005) Oxygen and photoinactivation of Escherichia coli in UVA and sunlight. Journal of Applied Microbiology 99 (1):39-50.

Khairnar K, Pal P, Chandekar RH, Paunikar WN (2014) Isolation and characterization of bacteriophages infecting nocardioforms in wastewater treatment plant. Biotechnology Research International 2014:151952.

Khan M, Satoh H, Mino T, Katayama H, Kurisu F, Matsuo T (2002a) Bacteriophage-host interaction in the enhanced biological phosphate removing activated sludge system. Water Science and Technology 46 (1-2):39-43.

Khan MA, Satoh H, Katayama H, Kurisu F, Mino T (2002b) Bacteriophages isolated from activated sludge processes and their polyvalency. Water Research 36 (13):3364-3370.

Kim MN, Yong D, An D, Chung HS, Woo JH, Lee K, Chong Y (2012) Nosocomial clustering of NDM-1-producing Klebsiella pneumoniae sequence type 340 strains in four patients at a South Korean tertiary care hospital. Journal of Clinical Microbiology 50 (4):1433-1436.

King D, Strynadka N (2011) Crystal structure of New Delhi metallo-beta-lactamase reveals molecular basis for antibiotic resistance. Protein Science 20 (9):1484-1491.

Knapp CW, Dolfing J, Ehlert PA, Graham DW (2009) Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environmental Science & Technology 44 (2):580-587.

Kroupitski Y, Golberg D, Belausov E, Pinto R, Swartzberg D, Granot D, Sela S (2009) Internalization of Salmonella enterica in leaves is induced by light and involves chemotaxis and penetration through open stomata. Applied and Environmental Microbiology 75 (19):6076-6086.

Kumarasamy K, Kalyanasundaram A (2012) Emergence of Klebsiella pneumoniae isolate co-producing NDM-1 with KPC-2 from India. Journal of Antimicrobial Chemotherapy 67 (1):243-244.

Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. The Lancet Infectious Diseases 10 (9):597-602.

Lai CC, Lin TL, Tseng SP, Huang YT, Wang JT, Chang SC, Teng LJ, Wang JT, Hsueh PR (2011) Pelvic abscess caused by New Delhi metallo-beta-lactamase-1-producing Klebsiella oxytoca in Taiwan in a patient who underwent renal transplantation in China. Diagnostic Microbiology and Infectious Disease 71 (4):474-475.

Le T-H, Ng C, Chen H, Yi XZ, Koh TH, Barkham TMS, Zhou Z, Gin KY-H (2016) Occurrences and Characterization of Antibiotic-Resistant Bacteria and Genetic Determinants of Hospital Wastewater in a Tropical Country. Antimicrobial Agents and Chemotherapy 60 (12):7449-7456.

Levy-Booth DJ, Campbell RG, Gulden RH, Hart MM, Powell JR, Klironomos JN, Pauls KP, Swanton CJ, Trevors JT, Dunfield KE (2007) Cycling of extracellular DNA in the soil environment. Soil Biology and Biochemistry 39 (12):2977-2991.

Lilley AK, Fry JC, Day MJ, Bailey MJ (1994) In situ transfer of an exogenously isolated plasmid between Pseudomonas spp. in sugar beet rhizosphere. Microbiology 140 (1):27-33.

Lloyd RE, Rinkenberger JL, Hug BA, Tuveson RW (1990) Growing Escherichia coli mutants deficient in riboflavin biosynthesis with non-limiting riboflavin results in sensitization to inactivation by broad-spectrum near-ultraviolet light (320-400 nm). Photochem Photobiol 52 (4):897-901.

Lorenz MG, Wackernagel W (1994) Bacterial gene transfer by natural genetic transformation in the environment. Microbiological Reviews 58 (3):563-602.

Luo Y, Yang F, Mathieu J, Mao D, Wang Q, Alvarez P (2013) Proliferation of multidrug-resistant New Delhi metallo-β-lactamase genes in municipal wastewater treatment plants in northern China. Environmental Science & Technology Letters 1 (1):26-30.

Majewski J, Cohan FM (1998) The effect of mismatch repair and heteroduplex formation on sexual isolation in Bacillus. Genetics 148 (1):13-18.

Majewski J, Zawadzki P, Pickerill P, Cohan FM, Dowson CG (2000) Barriers to genetic exchange between bacterial species: Streptococcus pneumoniae transformation. Journal of Bacteriology 182 (4):1016-1023.

Malato S, Fernández-Ibáñez P, Maldonado M, Blanco J, Gernjak W (2009) Decontamination and disinfection of water by solar photocatalysis: recent overview and trends. Catalysis Today 147 (1):1-59.

Mantilla-Calderon D, Jumat MR, Wang T, Ganesan P, Al-Jassim N, Hong P-Y (2016) Isolation and characterization of NDM-positive Escherichia coli from municipal wastewater in Jeddah, Saudi Arabia. Antimicrobial Agents and Chemotherapy 60 (9):5223-5231.

Marti R, Scott A, Tien Y-C, Murray R, Sabourin L, Zhang Y, Topp E (2013) Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. Applied and Environmental Microbiology 79 (18):5701-5709.

Mason KA, Losos JB, Singer SR, Peter H (2011) Biology. McGraw-Hill, New York. ISBN. p. 533.

Matic I, Radman M, Taddei F, Picard B, Doit C, Bingen E, Denamur E, Elion J (1997) Highly variable mutation rates in commensal and pathogenic Escherichia coli. Science 277 (5333):1833-1834.

Mazzariol A, Bosnjak Z, Ballarini P, Budimir A, Bedenic B, Kalenic S, Cornaglia G (2012) NDM-1-producing Klebsiella pneumoniae, Croatia. Emerging Infectious Diseases 18 (3):532-534.

McGann P, Hang J, Clifford RJ, Yang Y, Kwak YI, Kuschner RA, Lesho EP, Waterman PE (2012) Complete sequence of a novel 178-kilobase plasmid carrying bla(NDM-1) in a Providencia stuartii strain isolated in Afghanistan. Antimicrobial Agents and Chemotherapy 56 (4):1673-1679.

McGuigan KG, Conroy RM, Mosler HJ, du Preez M, Ubomba-Jaswa E, Fernandez-Ibanez P (2012) Solar water disinfection (SODIS): a review from bench-top to roof-top. Journal of Hazardous Materials 235-236:29-46.

McGuigan KG, Joyce TM, Conroy RM, Gillespie JB, Elmore-Meegan M (1998) Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. Journal of Applied Microbiology 84 (6):1138-1148.

McKinney CW, Pruden A (2012a) Ultraviolet disinfection of antibiotic resistant bacteria and their antibiotic resistance genes in water and wastewater. Environmental Science & Technology 46 (24):13393-13400.

McKinney CW, Pruden A (2012b) Ultraviolet disinfection of antibiotic resistant bacteria and their antibiotic resistance genes in water and wastewater. Environmental Science & Technology 46 (24):13393-13400.

Méjean V, Claverys J-P (1993) DNA processing during entry in transformation of Streptococcus pneumoniae. Journal of Biological Chemistry 268 (8):5594-5599.

Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske C, Gniadkowski M, Malamou-Lada E, Martinez-Martinez L, Navarro F, Nordmann P (2010) Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clinical Microbiology and Infection 16 (2):112-122.

Morris CE, Monier J-M (2003) The ecological significance of biofilm formation by plant-associated bacteria. Annual Review of Phytopathology 41 (1):429-453.

Muela A, Garcia-Bringas JM, Seco C, Arana I, Barcina I (2002) Participation of oxygen and role of exogenous and endogenous sensitizers in the photoinactivation of Escherichia coli by photosynthetically active radiation, UV-A and UV-B. Microbial Ecology 44 (4):354-364.

Mulvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA (2011) New Delhi metallo-beta-lactamase in Klebsiella pneumoniae and Escherichia coli, Canada. Emerging Infectious Diseases 17 (1):103-106.
Munir M, Wong K, Xagoraraki I (2011) Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Research 45 (2):681-693.
Munir M, Xagoraraki I (2011) Levels of antibiotic resistance genes in manure, biosolids, and fertilized soil. Journal of Environmental Quality 40 (1):248-255.
Negreanu Y, Pasternak Z, Jurkevitch E, Cytryn E (2012) Impact of treated wastewater irrigation on antibiotic resistance in agricultural soils. Environmental Science & Technology 46 (9):4800-4808.
Nesme J, Simonet P (2015) The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. Environmental Microbiology 17 (4):913-930.
Nielsen JB, Hansen F, Littauer P, Schonning K, Hammerum AM (2012) An NDM-1-producing Escherichia coli obtained in Denmark has a genetic profile similar to an NDM-1-producing E. coli isolate from the UK. Journal of Antimicrobial Chemotherapy 67 (8):2049-2051.
Nielsen KM, Bones AM, Van Elsas J (1997a) Induced Natural Transformation of Acinetobacter calcoaceticus in Soil Microcosms. Applied and Environmental Microbiology 63 (10):3972-3977.
Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D (2007) Release and persistence of extracellular DNA in the environment. Environmental Biosafety Research 6 (1-2):37-53.
Nielsen KM, van Elsas JD, Smalla K (2000) Transformation of Acinetobacter sp. strain BD413 (pFG4ΔnptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Applied and Environmental Microbiology 66 (3):1237-1242.
Nielsen KM, Van Weerelt M, Berg TN, Bones AM, Hagler AN, Van Elsas J (1997b) Natural transformation and availability of transforming DNA to Acinetobacter calcoaceticus in soil microcosms. Applied and Environmental Microbiology 63 (5):1945-1952.
Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiology Reviews 27 (2-3):313-339.
Nordmann P, Boulanger AE, Poirel L (2012) NDM-4 metallo-beta-lactamase with increased carbapenemase activity from Escherichia coli. Antimicrobial Agents and Chemotherapy 56 (4):2184-2186.
Novais Â, Cantón R, Moreira R, Peixe L, Baquero F, Coque TM (2007) Emergence and dissemination of Enterobacteriaceae isolates producing CTX-M-1-like enzymes in Spain are associated with IncFII (CTX-M-15) and broad-host-range (CTX-M-1,-3, and-32) plasmids. Antimicrobial Agents and chemotherapy 51 (2):796-799.
Oates PM, Shanahan P, Polz MF (2003) Solar disinfection (SODIS): simulation of solar radiation for global assessment and application for point-of-use water treatment in Haiti. Water Research 37 (1):47-54.
Ogram A, Sayler GS, Barkay T (1987) The extraction and purification of microbial DNA from sediments. Journal of Microbiological Methods 7 (2-3):57-66.
Oteo J, Domingo-Garcia D, Fernandez-Romero S, Saez D, Guiu A, Cuevas O, Lopez-Brea M, Campos J (2012) Abdominal abscess due to NDM-1-producing Klebsiella pneumoniae in Spain. Journal of Medical Microbiology 61 (Pt 6):864-867.

Pal P, Khairnar K, Paunikar W (2014) Causes and remedies for filamentous foaming in activated sludge treatment plant. Global Nest Journal 16 (4):762-772.
Palmen R, Hellingwerf KJ (1997) Uptake and processing of DNA by Acinetobacter calcoaceticus–areview. Gene 192 (1):179-190.
Pasteran F, Albornoz E, Faccone D, Gomez S, Valenzuela C, Morales M, Estrada P, Valenzuela L, Matheu J, Guerriero L, Arbizu E, Calderon Y, Ramon-Pardo P, Corso A (2012) Emergence of NDM-1-producing Klebsiella pneumoniae in Guatemala. Journal of Antimicrobial Chemotherapy 67 (7):1795-1797.
Pattison DI, Davies MJ (2006) Actions of ultraviolet light on cellular structures. In: Cancer: Cell Structures, Carcinogens and Genomic Instability. vol 96. Birkhäuser Basel, pp 131-157.
Peirano G, Ahmed-Bentley J, Woodford N, Pitout JD (2011a) New Delhi metallo-beta-lactamase from traveler returning to Canada. Emerging Infectious Diseases 17 (2):242-244.
Peirano G, Pillai DR, Pitondo-Silva A, Richardson D, Pitout JD (2011b) The characteristics of NDM-producing Klebsiella pneumoniae from Canada. Diagnostic Microbiology and Infectious Disease 71 (2):106-109.
Petrovski S, Seviour RJ, Tillett D (2011a) Characterization of the genome of the polyvalent lytic bacteriophage GTE2, which has potential for biocontrol of Gordonia-, Rhodococcus-, and Nocardia-stabilized foams in activated sludge plants. Applied and Environmental Microbiology 77 (12):3923-3929.
Petrovski S, Seviour RJ, Tillett D (2011b) Prevention of Gordonia and Nocardia stabilized foam formation by using bacteriophage GTE7. Applied and Environmental Microbiology 77 (21):7864-7867.
Pfeifer GP (1997) Formation and processing of UV photoproducts: effects of DNA sequence and chromatin environment. Photochem Photobiol 65 (2):270-283.
Piepersberg W, Distler J, Heinzel P, Perez-Gonzalez J-A (1988) Antibiotic resistance by modification: Many resistance genes could be derived from cellular control genes in actinomycetes-A hypothesis. Actinomycetologica 2 (2):83-98.
Poirel L, Al Maskari Z, Al Rashdi F, Bernabeu S, Nordmann P (2011a) NDM-1-producing Klebsiella pneumoniae isolated in the Sultanate of Oman. Journal of Antimicrobial Chemotherapy 66 (2):304-306.
Poirel L, Benouda A, Hays C, Nordmann P (2011b) Emergence of NDM-1-producing Klebsiella pneumoniae in Morocco. Journal of Antimicrobial Chemotherapy 66 (12):2781-2783.
Poirel L, Dortet L, Bernabeu S, Nordmann P (2011c) Genetic features of blaNDM-1-positive Enterobacteriaceae. Antimicrobial Agents and Chemotherapy 55 (11):5403-5407.
Poirel L, Fortineau N, Nordmann P (2011d) International transfer of NDM-1-producing Klebsiella pneumoniae from Iraq to France. Antimicrobial Agents and Chemotherapy 55 (4):1821-1822.
Poirel L, Hombrouck-Alet C, Freneaux C, Bernabeu S, Nordmann P (2010a) Global spread of New Delhi metallo-β-lactamase 1. The Lancet Infectious Diseases 10 (12):832.
Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P (2010b) Emergence of metallo-beta-lactamase NDM-1-producing multidrug-resistant Escherichia coli in Australia. Antimicrobial Agents and Chemotherapy 54 (11):4914-4916.
Poirel L, Lascols C, Bernabeu S, Nordmann P (2012a) NDM-1-producing Klebsiella pneumoniae in Mauritius. Antimicrobial Agents and Chemotherapy 56 (1):598-599.

Poirel L, Ozdamar M, Ocampo-Sosa AA, Turkoglu S, Ozer UG, Nordmann P (2012b) NDM-1-producing Klebsiella pneumoniae now in Turkey. Antimicrobial Agents and Chemotherapy 56 (5):2784-2785.
Poirel L, Revathi G, Bernabeu S, Nordmann P (2011e) Detection of NDM-1-producing Klebsiella pneumoniae in Kenya. Antimicrobial Agents and Chemotherapy 55 (2):934-936.
Poirel L, Ros A, Carricajo A, Berthelot P, Pozzetto B, Bernabeu S, Nordmann P (2011f) Extremely drug-resistant Citrobacter freundii isolate producing NDM-1 and other carbapenemases identified in a patient returning from India. Antimicrobial Agents and Chemotherapy 55 (1):447-448.
Poirel L, Schrenzel J, Cherkaoui A, Bernabeu S, Renzi G, Nordmann P (2011g) Molecular analysis of NDM-1-producing enterobacterial isolates from Geneva, Switzerland. Journal of Antimicrobial Chemotherapy 66 (8):1730-1733.
Poole K (2005) Efflux-mediated antimicrobial resistance. Journal of Antimicrobial Chemotherapy 56 (1):20-51.
Pruden A, Larsson DJ, Amézquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JM, Suzuki S, Silley P, Snape JR (2013) Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environmental Health Perspectives 121 (8):878.
Rahme LG, Stevens EJ, Wolfort SF, Shao J (1995) Common virulence factors for bacterial pathogenicity in plants and animals. Science 268 (5219):1899.
Rahube TO, Marti R, Scott A, Tien Y-C, Murray R, Sabourin L, Zhang Y, Duenk P, Lapen DR, Topp E (2014) Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. Applied and environmental microbiology 80 (22):6898-6907.
Raynaud X, Nunan N (2014) Spatial ecology of bacteria at the microscale in soil. PLoS One 9 (1):e87217.
Riesenfeld CS, Goodman RM, Handelsman J (2004) Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. Environmental Microbiology 6 (9):981-989.
Rijal GK, Fujioka RS (2003) Use of reflectors to enhance the synergistic effects of solar heating and solar wavelengths to disinfect drinking water sources. Water Science and Technology 48 (11-12):481-488.
Rizzo L, Fiorentino A, Anselmo A (2012) Effect of solar radiation on multidrug resistant E. coli strains and antibiotic mixture photodegradation in wastewater polluted stream. Science of the Total Environment 427:263-268.
Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Science of the Total Environment 447:345-360.
Santos AL, Gomes NC, Henriques I, Almeida A, Correia A, Cunha A (2012) Contribution of reactive oxygen species to UV-B-induced damage in bacteria. Journal of Photochemistry and Photobiology B: Biology 117:40-46.
Schlüter A, Szczepanowski R, Pühler A, Top EM (2007) Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. FEMS microbiology reviews 31 (4):449-477.

Schwartz T, Kohnen W, Jansen B, Obst U (2003) Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol Ecol 43 (3):325-335.
Segawa T, Takeuchi N, Rivera A, Yamada A, Yoshimura Y, Barcaza G, Shinbori K, Motoyama H, Kohshima S, Ushida K (2013) Distribution of antibiotic resistance genes in glacier environments. Environmental Microbiology Reports 5 (1):127-134.
Sekizuka T, Matsui M, Yamane K, Takeuchi F, Ohnishi M, Hishinuma A, Arakawa Y, Kuroda M (2011) Complete sequencing of the bla(NDM-1)-positive IncA/C plasmid from Escherichia coli ST38 isolate suggests a possible origin from plant pathogens. PLoS One 6 (9):e25334.
Selenska S, Klingmüller W (1992) Direct recovery and molecular analysis of DNA and RNA from soil. Microbial Releases: Viruses, Bacteria, Fungi 1 (1):41-46.
Sidjabat H, Nimmo GR, Walsh TR, Binotto E, Htin A, Hayashi Y, Li J, Nation RL, George N, Paterson DL (2011) Carbapenem resistance in Klebsiella pneumoniae due to the New Delhi Metallo-beta-lactamase. Clinical Infectious Diseases 52 (4):481-484.
Sinton LW, Hall CH, Lynch PA, Davies-Colley RJ (2002) Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. Applied Environmental Microbiology 68 (3):1122-1131.
Smith DH (1967) R factor infection of Escherichia coli lyophilized in 1946. Journal of Bacteriology 94 (6):2071.
Sole M, Pitart C, Roca I, Fabrega A, Salvador P, Munoz L, Oliveira I, Gascon J, Marco F, Vila J (2011) First description of an Escherichia coli strain producing NDM-1 carbapenemase in Spain. Antimicrobial Agents and Chemotherapy 55 (9):4402-4404.
Solomon EB, Yaron S, Matthews KR (2002) Transmission of Escherichia coli O157: H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Applied and environmental microbiology 68 (1):397-400.
Sommer B, Marino A, Solarte Y, Salas M, Dierolf C, Valiente C, Mora D, Rechsteiner R, Setter P, Wirojanagud W (1997) SODIS- an emerging water treatment process. AQUA(OXFORD) 46 (3):127-137.
Sørensen SJ, Jensen LE (1998) Transfer of plasmid RP4 in the spermosphere and rhizosphere of barley seedling. Antonie van Leeuwenhoek 73 (1):69-77.
Staskawicz BJ, Mudgett MB, Dangl JL, Galan JE (2001) Common and contrasting themes of plant and animal diseases. Science 292 (5525):2285-2289.
Stokes HW, Gillings MR (2011) Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS microbiology reviews 35 (5):790-819.
Sulakvelidze A, Alavidze Z, Morris JG (2001) Bacteriophage therapy. Antimicrobial Agents and Chemotherapy 45 (3):649-659.
Suzuki H, Yano H, Brown CJ, Top EM (2010) Predicting plasmid promiscuity based on genomic signature. Journal of Bacteriology 192 (22):6045-6055.
Thomas CM, Nielsen KM (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature Reviews Microbiology 3 (9):711-721.
Thomas J, Soddell JA, Kurtböke D (2002) Fighting foam with phages? Water Science and Technology 46 (1-2):511-518.
Tijet N (2011) New Delhi Metallo-β-Lactamase, Ontario, Canada. Emerging Infectious Diseases 17 (2):306.

Toth IK, Pritchard L, Birch PR (2006) Comparative genomics reveals what makes an enterobacterial plant pathogen. Annual Review of Phytopathology 44:305-336.
Ubomba-Jaswa E, Navntoft C, Polo-Lopez MI, Fernandez-Ibanez P, McGuigan KG (2009) Solar disinfection of drinking water (SODIS): an investigation of the effect of UV-A dose on inactivation efficiency. Photochemical & Photobiological Sciences 8 (5):587-595.
Udikovic-Kolic N, Wichmann F, Broderick NA, Handelsman J (2014) Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. Proceedings of the National Academy of Sciences 111 (42):15202-15207.
USEPA (2006) Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule https://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=600006T3.txt. Date last accessed: 4 May 2017
Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R (2014) Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. The Lancet Infectious Diseases 14 (8):742-750.
Van Elsas J, Fry J, Hirsch P, Molin S (2000) Ecology of plasmid transfer and spread. The Horizontal Gene Pool; Bacterial Plasmids and Gene Spread. Harwood Academic Publishers. 175-206.
Van Elsas JD, Bailey MJ (2002) The ecology of transfer of mobile genetic elements. FEMS Microbiology Ecology 42 (2):187-197.
Vulić M, Dionisio F, Taddei F, Radman M (1997) Molecular keys to speciation: DNA polymorphism and the control of genetic exchange in enterobacteria. Proceedings of the National Academy of Sciences 94 (18):9763-9767.
Walsh TR, Weeks J, Livermore DM, Toleman MA (2011) Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. The Lancet infectious diseases 11 (5):355-362.
Walther-Rasmussen J, Høiby N (2007) Class A carbapenemases. Journal of Antimicrobial Chemotherapy 60 (3):470-482.
Wang F, Stedtfeld RD, Kim O-S, Chai B, Yang L, Stedtfeld TM, Hong SG, Kim D, Lim HS, Hashsham SA (2016) Influence of Soil Characteristics and Proximity to Antarctic Research Stations on Abundance of Antibiotic Resistance Genes in Soils. Environmental Science & Technology 50 (23):12621-12629.
Wang Z, Fast W, Valentine AM, Benkovic SJ (1999) Metallo-β-lactamase: structure and mechanism. Current Opinion in Chemical Biology 3 (5):614-622.
Webb RB, Brown MS (1979) Action spectra for oxygen-dependent and independent inactivation of Escherichia coli WP2s from 254 to 460 nm. Photochemistry and Photobiology 29 (2):407-409.
Wegelin M, Canonica S, Mechsner K, Fleischmann T, Pesaro F, Metzler A (1994) Solar water disinfection: scope of the process and analysis of radiation experiments. Aqua 43 (4):154-169.
Weinbauer MG (2004) Ecology of prokaryotic viruses. FEMS Microbiology Reviews 28 (2):127-181.
Williamson DA, Sidjabat HE, Freeman JT, Roberts SA, Silvey A, Woodhouse R, Mowat E, Dyet K, Paterson DL, Blackmore T, Burns A, Heffernan H (2012) Identification and molecular characterisation of New Delhi metallo-beta-lactamase-1 (NDM-1)- and NDM-6-producing Enterobacteriaceae from New Zealand hospitals. International Journal of Antimicrobial Agents 39 (6):529-533.

Withey S, Cartmell E, Avery L, Stephenson T (2005) Bacteriophages—potential for application in wastewater treatment processes. Science of the Total Environment 339 (1):1-18.

Wright GD (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. Nature Reviews Microbiology 5 (3):175-186.

Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR (2009) Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrobial Agents and chemotherapy 53 (12):5046-5054.

Yosef I, Manor M, Kiro R, Qimron U (2015) Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. Proceedings of the National Academy of Sciences 112 (23):7267-7272.

Zhang Y, Zhuang Y, Geng J, Ren H, Xu K, Ding L (2016) Reduction of antibiotic resistance genes in municipal wastewater effluent by advanced oxidation processes. Science of the Total Environment 550:184-191.

Zhang Y, Zhuang Y, Geng J, Ren H, Zhang Y, Ding L, Xu K (2015) Inactivation of antibiotic resistance genes in municipal wastewater effluent by chlorination and sequential UV/chlorination disinfection. Science of the Total Environment 512:125-132.

Zhu Y-G, Johnson TA, Su J-Q, Qiao M, Guo G-X, Stedtfeld RD, Hashsham SA, Tiedje JM (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proceedings of the National Academy of Sciences 110 (9):3435-3440.