Since ancient times, humans have randomly disposed of waste into the environment, such as in rivers and cesspits. The industrial revolution of the late eighteenth and early nineteenth centuries was a period that saw increased disposal of toxic organic chemicals by direct release into the environment. Many of these toxic molecules had antimicrobial activity, and it can be assumed that microbes resistant to these toxins multiplied in such environments. As a modern example, one can cite the concentrations of heavy oils that were dumped near detection stations in the distant early warning line at the end of the Second World War. These sites are now excellent sources of bacteria with enhanced biodegradation capacities and have been extensively studied in recent years.

Following the discovery of the chemically synthesized sulphonamides and trimethoprim and the identification of dual resistance in 1969, the subsequent and most disastrous environmental pollution has come from the disposal of antibiotic production wastes in various forms. These discarded products were developed as food supplements for farm animals to promote weight gain in all aspects of animal and fish husbandry worldwide. The amounts of antibiotics and antibiotic wastes disposed in this way cannot be accurately identified. However, according to recent estimates by the Union of Concerned Scientists in the United States, antibiotic use for nontherapeutic purposes in three major livestock sectors (chickens, cattle, and swine) was about eight times more than the consumption for human medicine (Mellon et al., 2001).

In the past 50 years, we have seen the rapid evolution of a new plague—that of worldwide antibiotic resistance. Though not a disease in itself, antimicrobial resistance (AR) results in the failure to effectively prevent and treat many diseases, leading to widespread untreatable microbial infections and greatly increased morbidity and mortality: a plague of resistance genes (Davies and Davies, 2010). The global use of antibiotics at low cost, auto medication, and short duration of treatment has accelerated, extended, and expanded the spectra of resistance worldwide. The earth has been continuously bathed in a dilute solution of antibiotics for more than half a century.
Aquatic ecosystems have been identified as hotspots of resistance mechanisms (Rizzo et al., 2013). This is due to the large diversity of pathogenic and commensal microorganisms and the continuous discharge of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) into these environments. As part of aquatic ecosystems, urban wastewater treatment systems (collecting sanitary sewage, hospital effluents, and storm water runoff) possess all the components required to ensure the acquisition of all varieties of resistance genes. The antimicrobials present in wastewater due to incomplete degradation by humans and animals, disposal of unused drugs, and runoff losses from land application, together with environmental and pathogenic bacteria in nutrient-rich engineered systems, provide all the necessary requirements to support a breeding ground for horizontal gene transfer and the propagation of resistance genes (Davies and Davies, 2010; Ferreira da Silva et al., 2006; Kim and Aga, 2007; Lefkowitz and Duran, 2009).

Since 1890 with the building of the first biological wastewater treatment plant (WWTP) in Worcester, Massachusetts, advances in wastewater treatment technology have been improving the efficient removal of biodegradable organic pollutants. Currently, enhanced biological phosphorus removal processes have not only enabled the removal of traditional carbonaceous contaminants but also reduced phosphorus concentrations to very low levels (<0.1 mg/L) in the effluent discharge (Zuthi et al., 2013).

Over the past 15 years, increasing attention has shifted toward the identification and removal mechanisms of micropollutants from wastewater and sludge. Micropollutants are persistent organic or mineral substances such as pharmaceuticals and personal care products, detergents, and pesticides whose discharge, even at very low concentrations, is a constant growing environmental contamination (Luo et al., 2014).

Despite the evolution of wastewater treatment technologies from conventional to advanced treatment configurations, existing urban biological wastewater treatment systems are not designed to remove micropollutants and ARGs. Studies on antibiotics as emerging classes of micropollutants have confirmed the high frequency of antimicrobial resistant genotypes as well as ARB in wastewater treatment systems, including constructed wetlands and WWTPs (Martins da Costa et al., 2006; Kim et al., 2010; Volkmann et al., 2004; Luczkiewicz et al., 2010; Reintzaler et al., 2003).

In a landmark series of papers published between 2003 and 2009, Szczepanowski and colleagues presented the first extensive DNA sequence–based screening of a large set of known ARGs in samples of activated sludge and the final effluent of a WWTP in Bielefeld-Heepen, Germany. This comprehensive survey identified 140 different clinically relevant antimicrobial resistant genotypes and contaminants. From these investigations, it is evident that such treatment systems may play important roles in the development and assortment of multidrug-resistant (MDR) bacteria among complex populations.

The occurrence of ARB and ARGs in the two main by-products of wastewater treatment systems (biosolids and effluent discharge) has been reported frequently. Currently, effluent water quality standards, prior to discharge, are limited to controlling the concentrations of carbonaceous biochemical oxygen-demanding matter, suspended solids, total residual chlorine and un-ionized ammonia. There exist no regulatory guidelines to monitor and control the levels of ARGs in bacteria and extracellular DNA from lysed microbial cells in the effluent discharge. Accordingly, studies have reported that
antibiotic resistance determinants and MDR pathogens are transported from the effluent to the receiving water (Iwane et al., 2001; Galvin et al., 2010; Goñi-Urriza et al., 2000). For example, LaPara et al. (2011) showed that the quantities of three tetracycline resistance genes were significantly higher in a tertiary treated effluent discharge than in receiving water samples in the St. Louis River, Duluth-Superior Harbor, and Lake Superior, USA.

Despite the evidence for the occurrence of resistance genes in effluent discharge points, the overall impact of treated wastewater applications on irrigation processes is unclear. Some studies have observed an increase in soil microbial activity and biomass after irrigation by treated wastewater as shown by a shift in the composition of soil bacterial communities (Oved et al., 2001; Broszat et al., 2014). However, recent studies have observed no significant impact on AR in the wastewater-irrigated soil microbiome (Gatica and Cytryn, 2013; Negreanu et al., 2012).

The presence of ARB and ARGs in biosolids-amended soils is well documented (Brooks et al., 2006; Rahube et al., 2014). Biosolids are the treated and stabilized nutrient-rich organic residuals produced as a by-product of wastewater treatment and widely used as fertilizer to stimulate plant growth (Lu and Stoffella, 2012). Recent studies have demonstrated that complementary technologies such as aerobic digestion and lime stabilization can be used as approaches to reduce the quantities of ARGs in biosolids (Munir et al., 2011). However, ARG concentrations and corresponding decay rates can be variable depending on the application methods, biosolids treatment reactor design, storage conditions, the specific ARGs involved, and the frequency of biosolids application (Burch et al., 2013; Miller et al., 2014).

Although ARB and genes encoding antibiotic resistance have been commonly detected in wastewater and the by-products of treatment systems, the role of wastewater treatment processes in the dissemination of AR is not clear. In recent years, a number of studies have investigated the variables affecting the patterns of ARB and ARGs during treatment processes (Xia et al., 2012; Yuan et al., 2014). However, in spite of many studies indicating a contribution from treatment processes to the evolution, spread, and positive selection of antimicrobial resistant isolates, it has been shown that wastewater treatment process can act as efficient barriers to decrease the number of ARB and concentrations of ARGs (Gao et al., 2012; Duong et al., 2008; Nagulapally et al., 2009). The reasons for such discrepancies are the large number of variables in conditions such as influent source, input quality, treatment process configurations, and operating conditions.

Hospital wastewater is probably a major contributor to the spread of pathogenic MDR bacteria in WWTPs (Brown et al., 2006). Due to the presence of constant subinhibitory levels of broad-spectrum antimicrobials, hospital sewage creates a perfect situation for the exchange of ARGs and their combinations between clinical pathogens and environmental bacteria (Amador et al., 2015; Santoro et al., 2015). In this respect, the ratios of influent wastewater from institutions (including hospitals), blackwater (excreta, urine, and fecal sludge), graywater (kitchen and bathing wastewater), storm water, and other urban runoff sources are important determinants of the input quality, the frequency of detection of ARGs and pathogenic ARB, and the dissemination of antibiotics and AR from treatment plants (Harris et al., 2013).

Over the past few years, some European countries have constructed specialized WWTPs to provide separate treatment of hospital wastewater (HWW). With membrane
bioreactors as a pretreatment, ozonation, and powdered and granulated activated carbon have been proposed as the most attractive options to remove micropollutants from HWW (Beier et al., 2010; Beier et al., 2012; Kovalova et al., 2013). Very recently, Chonova and coworkers (2016) published a comparative study on the efficiency of the removal of antibiotics from parallel wastewater systems providing separate treatment of hospital and urban wastewater. Despite the higher concentration of antibiotics in the hospital influent as well as treated effluent, the results indicated increased removal efficiency of antibiotics during the separate treatment of HWW. It was also demonstrated that biofilm communities receiving hospital treated effluent had lower bacterial diversity and less developed biomass. Observations from this study confirm the adaptations of wastewater bacterial communities receiving HWW. With respect to the dedicated treatment of hospital waste, more studies are needed to reveal the mechanisms by which adapted biofilm microbial communities can be transferred to aquatic environments.

Advanced wastewater disinfection technologies such as ultraviolet radiation and ozonation are effective approaches to decrease the extent of ARB and levels of ARGs (Zhang et al., 2015). However, other research has observed higher survival rates of resistant strains compared to sensitive bacteria, selection of ARGs, and shifts in bacterial population in the effluent after advanced treatments (Lüddeke et al., 2015; McKinney and Pruden, 2012; Alexander et al., 2016; Hu et al., 2016). Variations in reports on the efficiency of advanced approaches to wastewater treatment in controlling AR may be due to underestimates of the roles of variable operating conditions.

Solids retention time (SRT) is a design and operational parameter that has a crucial impact on the performance of activated sludge processes. SRT or the mean cell residence time is defined as total solids mass present in the system divided by solids mass disposed of per day (Clara et al., 2005). As SRT controls the net growth rate of the entire system, it is the main factor influencing dominant composition of a wastewater microbial community (Benefield and Randall, 1980; Xia et al., 2012). As an example, Liu and Wang (2014) showed that the nitrite-oxidizing bacteria/ammonia-oxidizing bacteria ratio is significantly influenced by variations in SRT.

A recent approach to wastewater management minimizes sludge production through microbial predation and metabolic changes (Amanatidou et al., 2015). One of the key factors that influences bacterial ecosystem manipulation and reduces the excess production of sludge is operation of the system at high SRTs (Yoon et al., 2004; Li and Wu, 2014). However, the role of prolonged SRT on the composition of bacterial processes contributing to AR is not yet clear. Although antibiotic degradation is maximized by prolonged cell residence time, extended exposure of bacteria to antibiotics from the source may increase the potential for development of AR (Walston, 2013; Xia et al., 2012). Meanwhile, environmental concerns associated with transformation of antibiotics into other biologically active compounds during the extended SRT operations have not been considered in many cases. More detailed research is required to detect antimicrobial degradation products in the treatment process and to investigate the optimal SRT required to achieve the best ARG removal.

Another serious operating challenge in wastewater management is the control of filamentous bulking and foaming. Although filamentous microorganisms support the activated sludge floc formation, their overabundance in WWTPs causes considerable operational difficulties such as poor sludge settling and thickening (Cydzik-Kwiatkowska and Zielińska, 2016; Pal et al., 2014). Different strategies have been employed to control
foaming, including polymer addition, the application of disinfectants such as chlorine, and the use of foam-classifying selectors to skim and remove foaming bacteria (Parker et al., 2003). The use of bacteriophages to reduce the concentration of filamentous bacteria is one of the most promising environmentally friendly approaches to control foaming (Liu et al., 2015). Despite the role of foaming bacteria on the efficiency of the treatment process and the environmental risks associated with foam disposal or formation of undesirable chlorinated by-products, no studies of antibiotic resistance patterns in foam-causing bacteria have been reported. More detailed studies of the impact of chemical disinfectants on the susceptibility profiles of foam-causing bacteria and the survival and gene transfer after disposal of resistant foaming bacteria and their survival are required.

For more than a decade, culture-dependent approaches have been the most common methods to study antimicrobial resistance in WWTPs (Al-Bahry et al., 2009; Okoh and Igbinosa, 2010). In these studies, resistance profiles of pathogenic population subsets of bacterial communities downstream of the effluent discharge were studied (Lefkowitz and Duran, 2009; Akiyama and Savin, 2010; Zhang et al., 2009).

Culture-dependent methods have also been used to investigate the role of mobile genetic elements (MGEs) in the dissemination of antibiotic resistance genes in WWTPs. It has been shown that MGEs influence bacterial evolution, adaptation, and the roles that genetic elements play in the emergence, recombination, and propagation of antibiotic resistance (Jackson et al., 2011). Studies to date have documented the incidence of integron-associated ARG cassettes on MGEs such as plasmids in WWTP samples (Tennstedt et al., 2003; Koczura et al., 2012; Kotlarska et al., 2015; Ma et al., 2011). In this respect, understanding the correlation between the distribution of pathogenic bacteria and associated integron patterns will aid in clarifying resistance mechanisms in WWTPs.

In addition to resistance genes and associated elements, it is probable that virulence and biodegradation gene clusters are propagated in WWTPs. In a recent publication, Olaniran and colleagues (2015) detected four virulence-associated genes in Listeria and Aeromonas spp. isolated from treated effluents of two WWTPs and receiving waters in Durban, South Africa. This study emphasizes the need for more investigation of virulent bacteria found in WWTPs and the co-occurrence of virulence genes and ARGs.

Despite the advantages of culture-dependent techniques, including low cost and the potential to combine with other methods, the availability of culture-based methods for studies of environmental microbes gives a highly restricted view of microbial community structure in environmental ecosystems (Mahmod, 2014; Heidrich et al., 2016). Hence, culture-based approaches are not appropriate for comprehensive studies of the diversity and abundance of ARGs as well as the incidence of MGEs in WWTPs (Wang et al., 2013). To date, a variety of molecular approaches have been applied to study the relationships between wastewater microbial communities and treatment process performance (Shah, 2014; Jabari et al., 2016; Gómez-Villalba, 2006; Kim et al., 2013; McIlroy et al., 2015; Ju et al., 2014). A brief summary of the most commonly used molecular techniques is provided in Table 1.1.

The application of targeted (PCR- and/or microarray-based) and sequenced-based metagenomics provides more extensive and accurate assessments of the abundance of ARGs and the phylogenetic and functional diversity of wastewater resistome (Schmieder and Edwards, 2012; Parsley et al., 2010; Ma et al., 2016). As an example, Wang and
coworkers (2013) performed a metagenomic study of MGEs and ARGs in both anaerobic and aerobic sludge of a tannery WWTP in China. Metagenomic analyses showed that the taxonomic classification, as well as the abundance of functional genes in aerobic and anaerobic sludge microbial communities, was different. They also observed a high prevalence of insertion sequences (ISs) and integron-integrase genes highlighting the important role of MGEs in gene transfer in the tannery WWTP.

Recently, Li and coworkers (2015) compared the metagenomic libraries of total and plasmid DNA from influent, activated sludge and digested sludge of two WWTPs in Hong Kong. They observed that, compared to DNA metagenomes, plasmid metagenomes encoded significantly higher numbers of ARGs. This emphasizes the significant role of horizontal gene transfer in WWTPs. They also observed a prominent decrease in the number of ARGs in activated and digested sludge metagenomes compared to the influent metagenome.

Despite the many advantages of high-throughput shotgun sequencing methodologies to identify the structure of biological wastewater communities as well as WWTP resistomes, these approaches do not provide definitive relationships of ARGs to their host microorganisms. This may lead to varied correlations between WWTP resistome content and corresponding microbiome (Noyes et al., 2016). In addition, the scope of metagenomic read mapping approaches is limited to prior knowledge of resistance genes (i.e., through comparison of the sequence reads to known ARGs). In this respect, function-based metagenomics are more valuable approaches as they have the potential to identify novel ARGs and MGEs and to correlate resistance genes with the community structure. A functional metagenomics approach involves construction of metagenomic libraries through extraction of DNA, cloning DNA fragments, heterologous expression in surrogate hosts, and screening for specific activities (Lam et al., 2015).

In recent years, functional metagenomic studies of antibiotic resistance in environmental microbiomes such as soil and marine water have added considerably to our knowledge of the diversity of the natural gene pool of ARGs and revealed many unknown functions (Torres-Cortés et al., 2011; Donato et al., 2010; Schmieder and Edwards, 2012; Hatosy and Martiny, 2015). However, only limited studies have constructed functional metagenomic libraries from compartments of wastewater treatment processes and studied the diversity of ARGs and their host organisms (Amos et al., 2014; Uyaguari et al., 2011; Li et al., 2015). In a recent publication, Munck and coworkers (2015) combined

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<thead>
<tr>
<th>Partial Community Analysis Approaches</th>
<th>Whole Community Analysis Approaches</th>
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<tbody>
<tr>
<td>Denaturing gradient gel electrophoresis (DGGE)</td>
<td>Whole genome sequencing</td>
</tr>
<tr>
<td>Temperature gradient gel electrophoresis (TGGE)</td>
<td>Sequenced-based metagenomics</td>
</tr>
<tr>
<td>Single strand conformation polymorphism (SSCP)</td>
<td>G+C fractionation</td>
</tr>
<tr>
<td>Deoxyribonucleic acid (DNA) microarrays</td>
<td>Functional metagenomics</td>
</tr>
<tr>
<td>Real time-polymerase chain reaction (RT-PCR)</td>
<td>Metatranscriptomics</td>
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<tr>
<td>Fluorescence in situ hybridization (FISH)</td>
<td>Metaproteomics</td>
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metagenomic functional selections and deep metagenomic sequencing data to identify the diversity of ARGs in a core WWTP resistome in Denmark. They found that the core resistome consists of stably maintained and (mostly) novel ARGs that confer resistance to the 15 antimicrobials tested. They also showed that the WWTP microbial community is remarkably stable with a strong correlation between the resistome and the microbial composition and limited gene transfer with the human gut microbiota.

Recently, integrated “omics” analyses have provided an enhanced understanding of the species and their functions in wastewater microbial systems (Narayanasamy et al., 2015). As an example, Roume and coworkers (2015) showed that seasonal variations did not significantly affect the expression of enzymes involved in nitrogen metabolism in the anoxic tank of a biological WWTP in Luxembourg. However, in winter, when lipid accumulation was higher, they observed significant expression of enzymes involved in glycerolipid metabolism. As an integrated “omics” analysis identifies the links between genes encoding key biological functionalities and functionally important community members, it can be used to optimize the wastewater treatment processes. This can be done through enrichment of favourable microorganisms such as lipid-accumulating organisms as proposed by Roume and coworkers (2015).

There is much current research focused on gaining a better understanding of the role of wastewater treatment in propagation and selection of antimicrobial resistance. Current information suggests that WWTPs serve as a nexus between contaminants in human waste and the environment. However, there are still many gaps in our knowledge that need to be addressed to help understand whether WWTPs are a minor, major, or variable contributor to the worldwide problem of antibiotic resistance.

A potential approach to the global concern of AR is to generate truly novel antibiotics with narrow spectra of action that can be combined with inhibitors of AR function. There is no shortage of potential therapeutic agents in nature; there are many novel antibiotics to be discovered and current methodology comes nowhere near to exhausting the molecular richness of natural environments. Creative screening approaches that rely on properties such as signaling will likely lead to a constant supply of novel bioactive compounds by using bioinformatic-heterologous expression approaches (Donia and Fischbach, 2015). However, new antibiotics will have short useful lives unless there is strict control of their use. AR is an evolutionary response by microbes that has had drastic consequences for the human race. It is essential that studies of the origins of AR and their “natural” functions be a priority. A key component will be to understand how AR diversity is generated as a result of rapid gene transfer and turnover. Finally, it must be recognized that the worldwide plague of AR was entirely manmade and could/should have been prevented and/or contained by stricter control of the use of antibiotics. Without appropriate regulations and strict compliance, the evolution and dissemination of AR will never be prevented.

References


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