Antimicrobial Resistance in Wastewater Treatment Processes
Antimicrobial Resistance in Wastewater Treatment Processes

Edited by Patricia L. Keen and Raphaël Fugère
The editors dedicate this book to the memory of Fred Koch.
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List of Contributors

**Diana S. Aga**
Department of Chemistry  
University at Buffalo, State University of New York  
Buffalo  
USA

**Denisse Archundia**
University Grenoble Alpes, IRD, CNRS  
IGE, Grenoble, France;  
Consejo Nacional de Ciencia y Tecnología (CONACYT)  
México, D.F., México;  
ERNO, Instituto de Geología  
Hermosillo, Sonora  
Mexico

**Fernando Baquero**
Departamento de Microbiología  
Hospital Universitario Ramón y Cajal  
Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS)  
Madrid  
Spain

**Thomas U. Berendonk**
Institute for Hydrobiology  
Technische Universität Dresden  
Dresden, Germany

**Melanie Broszat**
Division of Infectious Diseases  
University Medical Centre Freiburg  
Freiburg  
Germany

**Irene Bueno**
College of Veterinary Medicine  
University of Minnesota  
St. Paul  
USA

**Simon D. Costanzo**
University of Maryland  
Center for Environmental Science  
Cambridge  
USA

**Eddie Cytryn**
Institute of Soil, Water, and Environmental Sciences  
Agricultural Research Organization, Volcani Center  
Rishon Lezion  
Israel

**Julian Davies**
Department of Microbiology and Immunology  
University of British Columbia  
Vancouver  
Canada

**Thi Thuy Do**
Antimicrobial Resistance and Microbiome Research Group  
Department of Biology  
National University of Ireland Maynooth  
County Kildare  
Ireland
**Erica Donner**  
Future Industries Institute  
University of South Australia  
Mawson Lakes  
Australia

**Lisa M. Durso**  
Agroecosystem Management Research Unit  
Agricultural Research Service  
U.S. Department of Agriculture  
Lincoln, Nebraska  
USA

**Celine Duwig**  
University Grenoble Alpes  
IRD, CNRS, IGE  
Grenoble  
France

**Despo Fatta-Kassinos**  
Department of Civil and Environmental Engineering and Nireas International Water Research Centre  
School of Engineering  
University of Cyprus  
Nicosia  
Cyprus

**Gabriela Flores**  
University Grenoble Alpes, IRD, CNRS, IGE, Grenoble, France; MMAYA, Ministerio de Medio Ambiente y Agua (Ministry of Water and Environment of Bolivia)  
La Paz  
Bolivia

**Raphaël Fugère**  
Raphaël Fugère and Associates  
Lévis, Québec  
Canada

**Charles P. Gerba**  
University of Arizona  
Department of Soil, Water, and Environmental Science  
Tucson  
USA

**Elisabeth Grohmann**  
Division of Infectious Diseases  
University Medical Centre Freiburg  
Freiburg  
Germany

**Judith Isaac-Renton**  
University of British Columbia  
Vancouver  
Canada

**Popi Karaolia**  
Department of Civil and Environmental Engineering and Nireas International Water Research Centre  
School of Engineering  
University of Cyprus  
Nicosia  
Cyprus

**Antti Karkman**  
University of Gothenburg  
Göteborg  
Sweden

**Patricia L. Keen**  
Department of Civil Engineering  
University of British Columbia  
Vancouver  
New York Institute of Technology  
Vancouver  
Canada

**Frederic Lehembre**  
University Grenoble Alpes, IRD, CNRS, IGE  
Grenoble  
France

**Agnes V. MacDonald**  
Department of Sociology  
Simon Fraser University  
Burnaby, British Columbia  
Canada
Roberto B. M. Marano
Institute of Soil, Water, and Environmental Sciences
Agricultural Research Organization, Volcani Center
Rishon Lezion, Israel
Department of Agroecology and Plant Health
Robert H. Smith Faculty of Agriculture, Food, and Environment
The Hebrew University of Jerusalem
Rehovot
Israel

Jose Luis Martinez
Departamento de Biotecnología Microbiana
Centro Nacional de Biotecnología
Consejo Superior de Investigaciones Científicas
Madrid
Spain

Jean M.F. Martins
University Grenoble Alpes
IRD, CNRS, IGE
Grenoble
France

Jean E. McLain
University of Arizona
Water Resources Research Center
Department of Soil, Water, and Environmental Science
Tucson
USA

Stella Michael
Department of Civil and Environmental Engineering and Nireas International Water Research Centre
School of Engineering
University of Cyprus
Nicosia
Cyprus

Mehrnoush Mohammadali
Department of Civil Engineering
University of British Columbia
Vancouver
Canada

Marie-Christine Morel
University Grenoble Alpes, IRD, CNRS, IGE, Grenoble, France; CNAM, Laboratoire d’analyses chimiques et bio analyses
Paris
France

Rachel A. Mullen
Department of Chemistry
University at Buffalo, State University of New York
Buffalo
USA

Sinéad Murphy
Antimicrobial Resistance and Microbiome Research Group
Department of Biology
National University of Ireland Maynooth
County Kildare
Ireland

Johanna Muurinen
University of Helsinki
Helsinki
Finland

David M. Patrick
School of Population and Public Health
University of British Columbia
Vancouver
Canada

Marilyn C. Roberts
Department of Environmental and Occupational Health Sciences
School of Public Health
University of Washington
Seattle
USA
List of Contributors

Channah M. Rock
University of Arizona
Department of Soil, Water, and Environmental Science
Tucson
USA

Amy Millmier Schmidt
Biological Systems Engineering and Animal Science
University of Nebraska
Lincoln
USA

Randall S. Singer
College of Veterinary Medicine
University of Minnesota
St. Paul
USA

Randolph R. Singh
Department of Chemistry
University at Buffalo, State University of New York
Buffalo
USA

Sotirios Vasileiadis
Future Industries Institute
University of South Australia
Mawson Lakes
Australia

Marko Virta
University of Helsinki
Helsinki
Finland

Veiko Voolaid
Institute for Hydrobiology
Technische Universität Dresden
Dresden
Germany

Fiona Walsh
Antimicrobial Resistance and Microbiome Research Group
Department of Biology
National University of Ireland Maynooth
County Kildare
Ireland

Andrew J. Watkinson
University of Queensland
St. Lucia
Australia

Jessica Williams-Nguyen
Department of Epidemiology
School of Public Health
University of Washington
Seattle
USA

Ying Yang
Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering
School of Marine Sciences
Sun Yat-sen University
Guangzhou
China

Tong Zhang
Environmental Biotechnology Laboratory
Department of Civil Engineering
University of Hong Kong
Hong Kong
China
Antimicrobial resistance is arguably the greatest threat to worldwide human health. This book evaluates the roles of human water use, treatment, and conservation in the development and spread of antimicrobial resistance.

The collection of wastewater dates back to the Roman Empire when sewage, surface runoff, and drainage water were received in the Cloaca Maxima and flushed into the Tiber River by water transported from a vast network of aqueducts. By the Middle Ages, several major urban centers developed throughout Europe that included systems of open ditches and wooden, lead, or clay pipes designed for the disposal of sewage. Rapid increases in population densities in major European cities since that time demanded considerable improvements in water distribution systems and wastewater management in order to protect the public health of citizens. However, it was not until the mid-nineteenth century that many of these improvements were, in fact, realized. Canals dedicated to the transport of wastewater for direct discharge into rivers were constructed, although frequently, drinking water pumps were installed in close proximity to the wastewater removal systems. In essentially all situations, wastewater was ultimately returned to the environment in an untreated form.

Since 1850, the increased frequency of disease outbreaks, such as cholera and typhus, has required dedicated engineering efforts for the treatment of wastewater. Early examples of sewage treatment were simply the application of lime to cesspools, intended to reduce foul odors. During the same period, infectious disease epidemics were still believed to be transmitted through the human population by exposure to filth and foul smells and via person-to-person contact. While major advancements in the disciplines of sanitary engineering and health sciences were being made, it became increasingly clear that water played a critical role in the spread of infectious disease among the human population and that the safety of drinking water was compromised by any possibility of exposure to sewage. Water flushed waste through vast networks of ditches and underground sewers, then discharged in major rivers such as the Thames, the Seine, and the Danube. This rapid growth of urban centers was associated with persistent objectionable smells emanating from water courses that bisected large cities. This, in turn, reinforced political will to improve environmental conditions and led to pioneering technologies in sanitary engineering.

The engineering of technologies specific to the treatment of wastewater experienced a period of unprecedented growth beginning in the mid-nineteenth century. Coincidentally, regulations intended to protect the environment from the impacts of discharge of sewage into receiving waters began to appear more frequently in many
major urban centers throughout Europe (Cooper, 2001). The goal of wastewater
treatment was to ensure that effluent was sufficiently free of disease-causing entities
that it could be released without impacting the safety of drinking water that was
being employed for the human population. The secondary objective for improve-
ment in wastewater treatment was driven by the economic incentive linked to the
production of artificial guano (Cooper, 2001). Fertility of agricultural lands was
decreasin to such an extent that crop yields were diminishing while the human popu-
lation in urban centers was constantly growing. To counter this threat to food secu-
ritiy, bird droppings were being imported from South America to the United Kingdom
for use as agricultural fertilizer. Although land application of domestic waste had
been practiced since Roman times, large areas of land adjacent to major urban
centers were purchased and designated as “sewage farms.” These farms for the land
treatment of sewage needed increasing allotments of valuable land. They were sub-
ject to a number of weather-related complications and failed to achieve adequate
hygiene standards that would ensure the protection of the health of farm workers
and citizens at large. In this way, the intimate link between engineered systems for
wastewater treatment, agricultural food production, and public health was firmly
rooted throughout history.

Antimicrobial resistance in pathogenic organisms is a health risk that has been
increasing for the last half century. Domestic sewage contains microbes originating
from microbiomes of the human population resident in any community. Wastewater
treatment plants receive influent composed largely by domestic sewage and therefore
concentrate a vast and diverse collection of microbes in one location. Discharge of
effluent from wastewater treatment plants represents the most important source
of environmental contaminants, including those that are associated with development
of antimicrobial resistance in bacteria.

For some time, antibiotic compounds have been identified as emerging contaminants
and included in the category of pharmaceuticals and personal care products. Antibiotics
can retain their activity after excretion from human patients such that bacterial com-
munities in biological wastewater treatment systems are impacted by exposure to such
contaminants and this antibiotic activity could potentially persist if their removal is
incomplete following wastewater treatment. Improvements in instrumentation and
tools for the analyses of genes in complex environmental samples have enhanced the
ability to track mobile genetic elements of antimicrobial resistance through the waste-
water treatment process. Increasing evidence is being gathered to suggest that the
dynamic chemical, biological, and ecological conditions of operations in wastewater
treatment processes influence the abundance of antimicrobial resistance genes in the
effluents discharged to the environment after treatment. Because wastewater treatment
plants receive sewage composed of contributions from gut flora of healthy and sick
individuals, bacteria that are highly resistant to antibiotic therapy are increasingly
detected in wastewater treatment systems.

More than 50% of the world’s populations live in cities. Developing nations are wit-
tnessing a trend of accelerated urbanization that, in some cases, is accompanied by
increased health risks. Clean water, in terms of availability and safe quality, remains a
key concern in urban centers. Urban water cycles are now recognized as subsystems
where patterns of water use, wastewater treatment, and water reuse play major roles in
protection of public health.
Designed as a companion volume to Antimicrobial Resistance in the Environment (Wiley-Blackwell, 2012), this book is a multidisciplinary synthesis of topics related to antimicrobial resistance and wastewater treatment processes. Building on the increasing understanding of the central role of the environment in the development and spread of antimicrobial resistance, the book begins with five chapters that describe key issues from a more general perspective before focusing specifically on issues related to wastewater treatment processes. Detailed discussions concerning chemical analyses of antibiotics are included as well as comprehensive examinations of the features of experimental design that are particularly important in studies related to antimicrobial resistance. Advanced treatment strategies for mitigating the effects of factors that influence development and dissemination of antimicrobial resistance in the receiving environment are examined in detail. Several chapters discuss the ever-growing improvements in metagenomics, molecular methods, culture-based analyses, and gene sequencing capabilities, which are becoming popular for the examination of antimicrobial resistance in environmental samples, including those derived from wastewater.

We thank our fantastic team of contributing authors whom we are extremely pleased to regard as both our professional colleagues and our friends. Each chapter of this book has been crafted by some of the world’s leading authorities on the topic, in many cases together with early career researchers who continue to explore unanswered questions about risks linked to the development and spread of antimicrobial resistance. We thank the team at Wiley-Blackwell led by Mindy Okura-Marszycki and Kshitija Iyer for guiding us through the entire process of assembling this book from concept to completion. We extend our sincere gratitude to Philippe Raphanel for the use of the image from his wonderful painting on the cover of this book.

Patricia offers her thanks to colleagues from the Department of Civil Engineering at the University of British Columbia and also to her colleagues at the New York Institute of Technology, especially those individuals in the Energy Management Program at the Vancouver Campus, for their roles in making the development of this book a fun and rewarding experience. As well, she would like to express her sincere appreciation to Steve Clark for kindness, patience, and technical support throughout the book preparation process. Raphaël offers his thanks to René Fugère for his encouragement and support throughout all the phases that have led to the completion of this book. This book owes its existence to the inspiration of Julian Davies’ letter in Nature published in 2012. Patricia and Raphaël are deeply grateful to Julian for his willingness to share his inspiration, knowledge, friendship, and scholarly guidance, which have enabled us to complete this work.

Reference

La résistance aux antibiotiques est probablement la plus grande menace à la santé humaine. Le présent livre traitera essentiellement des rôles que jouent le traitement, l'utilisation, et la conservation de l'eau dans le développement et la propagation de la résistance aux antibiotiques.

Le premier exemples documenté de collecte des eaux usées daterait de l'empire Romain, où les eaux usées et les eaux pluviales étaient dirigées vers le Cloaca Maxima ("Grand égout collecteur") et rejetées dans le Tibre par un vaste réseau de conduites d'égout. Dès le Moyen Âge, l'Europe vit plusieurs centres urbains se développer, et ces derniers incluaient des systèmes de fossés, ainsi que des conduites de bois, plomb, ou argile, afin d'évacuer les eaux usées. La densification rapide des centres urbains européens à cette époque demanda des avancées majeures afin de protéger la santé publique. Malgré cela, ce n'est pas avant la mi-19ème siècle que la plupart de ces avancées significatives furent réalisées. Des conduites dédiées à la canalisation des eaux usées vers les rivières environnantes furent mises en place, quoique des pompes d'eau potable furent installées à proximité immédiate des points de rejet. La stratégie universelle de gestion des eaux usées et pluviales demeurait le rejet direct au milieu naturel.

Depuis 1850, la fréquence accrue d'épidémies telles que le choléra et le typhus ont requis une ingénière de systèmes de traitement des eaux usées. Ces mesures techniques étaient à l'origine assez sommaires, tel que le chaulage des fosses septiques afin d'atténuer les odeurs infectes qui s'en dégageaient. Au cours de la même période, la croyance populaire voulait que les épidémies étaient transmises via l'exposition à la "saleté", les mauvaises odeurs et les contacts physiques entre individus. Avec les avancées majeures dans les domaines du génie sanitaire et médical, il devint clair que l'eau jouait un rôle critique dans la propagation des maladies infectieuses au sein des populations humaines, et que l'eau potable était corrompue par tout contact avec des eaux usées. À cette époque, les eaux usées étaient quand même acheminées vers des rivières ou fleuves d'importance tels que la Tamise, la Seine, ou le Danube. Les cours d'eau traversant les grandes villes devinrent synonymes d'odeurs nauséabondes. Ceci eut pour effet de mener à une amélioration des conditions environnementales et le développement de technologies de pointes en génie sanitaire.

Le domaine du génie sanitaire a connu une période de croissance sans précédent vers la mi-19ème siècle. Conséquemment, la réglementation visant à protéger l'environnement de l'impact des rejets d'eaux usées dans les cours d'eau fit son apparition et se répandit à de nombreuses capitales européennes (Cooper, 2001). Le but visé par le traitement des eaux usées était de s'assurer que l'effluent était suffisamment exempt d'entités
causant des maladies pour être rejeté sans impact directement l’eau potable utilisée par la population. Le but secondaire du perfectionnement du traitement des eaux usées était essentiellement économique et relié à la production de guano artificiel (Cooper, 2001). À cette période, la fertilité des sols était en telle décroissance que les rendements agricoles étaient en forte baisse, alors que la population urbaine croissait sans cesse. Afin de combattre cette menace à la sécurité alimentaire, des excréments d’oiseau étaient importés d’Amérique du Sud vers la Grande-Bretagne et étaient utilisés comme fertilisants agricoles. Malgré le fait que l’épandage d’eaux usées domestiques ait été pratiqué depuis l’époque romaine, de grandes superficies de terrain adjacentes aux centres urbains majeurs furent acquis et désignés comme “fermes de traitement des eaux usées”. Ces fermes visant à traiter les eaux usées vinrent à requérir de plus en plus de surface de terrain, ce qui mit leur opération en péril. De plus, elles étaient sujet à un grand nombre de complications reliées à la météo et n’atteignirent jamais des standards d’hygiène suffisants pour permettre la protection des travailleurs agricoles ou des citoyens. C’est ainsi que le lien intime entre les systèmes de traitement des eaux usées, la production agricole et la santé publiques se souda.

La résistance aux antibiotiques des organismes pathogènes est un risque sanitaire qui ne fait que croître depuis un demi-siècle. Les eaux usées domestiques contiennent des bactéries provenant des microbiomes de la population composant toute communauté urbaine. Les usines d’épuration des eaux usées reçoivent un affluent composé essentiellement d’eaux usées domestiques et se trouvent donc à concentrer une population microbienne vaste et diversifiée en un seul endroit. Les rejets des usines de traitement des eaux usées représentent donc une des plus importantes sources de contaminants environnementaux, incluant ceux qui sont associés avec le développement de la résistance aux antibiotiques chez les bactéries.

Il y a quelques années, les composés antibiotiques ont été identifiés comme contaminants émergents et incorporés à la catégorie des produits de soins personnels et pharmaceutiques. Même après excrétion par des patients, les antibiotiques peuvent préserver leur potentiel biochimique à un point tel qu’ils peuvent avoir un impact significatif sur la diversité microbienne des systèmes de traitement des eaux usées. Cette activité pourrait même persister dans l’environnement advenant une dégradation incomplète dans le système de traitement des eaux usées. Des avancées récentes au niveau de l’instrumentation et des outils d’analyse génétique dans des échantillons environnementaux complexes ont permis de retracer les marqueurs génétiques de la résistance aux antibiotiques tout au long de la chaîne de traitement des eaux usées. Il devient de plus en plus clair que les conditions chimiques, biologiques et écologiques d’opération des procédés de traitement des eaux usées affectent directement la quantité de gènes de résistance aux antibiotiques rejetés à l’environnement après traitement. Étant donné que les usines de traitement des eaux usées recueillent des eaux usées dont une partie provient autant de la flore intestinale de personnes malades que de la flore intestinale de personnes en santé, les bactéries résistantes aux antibiotiques sont de plus en plus détectées à l’affluent de systèmes de traitement des eaux usées.

Plus de cinquante pourcent de la population mondiale vit maintenant en zone urbaine. Les pays en voie de développement voient se développer une tendance d’urbanisation galopante, assortie de risques accrus à la santé publique. L’eau potable - tant en quantité qu’en qualité - demeure une préoccupation de premier plan au sein des centres urbains. Le cycle urbain de l’eau est maintenant reconnu comme tel, et il devient clair que
l'utilisation de l'eau, le traitement des eaux usées et la réutilisation de l'eau jouent des rôles cruciaux dans la protection de la santé publique.

Conçu comme un complément au volume Antimicrobial Resistance in the Environment (Wiley-Blackwell, 2012), ce livre est une synthèse multidisciplinaire de sujets touchant à la résistance aux antibiotiques et aux procédés de traitement des eaux usées. Se basant sur la compréhension grandissante du rôle central de l'environnement dans le développement et la propagation de la résistance aux antibiotiques, le livre débute avec cinq chapitres qui décrivent les points saillants d'un point de vue généraliste avant de se concentrer sur les enjeux ayant trait aux procédés de traitement des eaux usées. Des discussions détaillées des analyses chimiques sont présentées, tout comme l'examen en profondeur des particularités des protocoles expérimentaux qui sont particulièrement cruciaux dans le domaine de la résistance aux antibiotiques. Plusieurs procédés de traitement avancé visant à réduire le développement et la dispersion de la résistance aux antibiotiques dans l'environnement sont examinés en détail. Plusieurs chapitres discutent des améliorations dans les domaines de la métagénomique, des méthodes moléculaires, des analyses basées sur les cultures bactériennes et des méthodes de séquençage génétique qui deviennent de plus en plus populaires pour la détermination de la résistance aux antibiotiques dans des échantillons environnementaux, incluant des échantillons d'eaux usées.

Nous remercions notre incroyable équipe d'auteurs-contributeurs que nous considérons comme des collègues professionnels et des amis. Chaque chapitre de ce livre a été rédigé par plusieurs références internationales sur le sujet, en collaboration avec des chercheurs en début de carrière, qui continuent à explorer les questions encore sans réponse des risques reliés au développement et à la propagation de la résistance aux antibiotiques. Nous remercions l'équipe à Wiley-Blackwell, dirigée par Mindy Okura-Marszycki and Kshitija Iyer, qui nous ont guidés tout au long du processus de rédaction, de l'idée conceptuelle jusqu'à la publication. Nous exprimons également notre profonde gratitude envers Philippe Raphanel qui nous a gracieusement permis d'utiliser une photographie d'une de ses peintures pour égayer la couverture du livre.

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Référence

Paris-born Canadian painter Philippe Raphanel has a deeply held passion for the natural environment, which is reflected in nearly all of his work. As a member of the Young Romantics, a Vancouver-based group of artists whose work in the mid-1980s signaled a distinct shift in contemporary painting, Philippe refined a unique visual language that consistently references beauty in nature. Having spent much of his life on the west coast of Canada, his paintings are imbued with a similar reverence for sensuality in nature as that also captured in works by Emily Carr, Gordon Smith, and members of the Group of Seven. His work constantly explores the eternal bond between humans and the environment.

As an artist, Philippe is one of very few individuals whose formative years included a direct link to the realm of microbiology. As a young man in Paris, Philippe was close to long-time family friends and well-known microbiologists Germaine Stanier Cohen-Bazire and Roger Stanier. Known for their pioneering research in ultrastructure and physiology of cyanobacteria, Germaine and Roger Stanier introduced Philippe to the natural beauty of the microbiological world during their time at the Pasteur Institute, and later they invited him to their summer home in British Columbia. That appreciation for nature at the cellular level and, of course, the friendship has lasted a lifetime.

Philippe has been recognized with multiple awards celebrating achievement in contemporary art throughout his career and he is currently a lecturer at the Emily Carr University of Arts and Design in Vancouver, BC. His paintings can be found in museums, public institutions, corporate collections, and private collections worldwide. We are very pleased that a segment of Philippe’s painting “Quick Sands” is presented on the cover of this book.

About the Cover Artist
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<td>ACs</td>
<td>antibiotic compounds</td>
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<tr>
<td>AOP</td>
<td>advanced oxidation process</td>
</tr>
<tr>
<td>AR</td>
<td>antibiotic/antimicrobial resistance</td>
</tr>
<tr>
<td>ARB</td>
<td>antibiotic/antimicrobial resistant bacteria</td>
</tr>
<tr>
<td>ARGs</td>
<td>antibiotic/antimicrobial resistance genes</td>
</tr>
<tr>
<td>BACI</td>
<td>before-after-control-impact</td>
</tr>
<tr>
<td>BHR</td>
<td>broad host range</td>
</tr>
<tr>
<td>CAS</td>
<td>conventional activated sludge</td>
</tr>
<tr>
<td>CEC</td>
<td>contaminant of emerging concern</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>CPO</td>
<td>carbapenamase-producing organisms</td>
</tr>
<tr>
<td>DAGs</td>
<td>directed acyclic graphs</td>
</tr>
<tr>
<td>DGGE</td>
<td>denaturing gradient gel electrophoresis</td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
</tr>
<tr>
<td>DS</td>
<td>dissolved solids</td>
</tr>
<tr>
<td>dsDNA</td>
<td>double strand DNA</td>
</tr>
<tr>
<td>ESBL</td>
<td>extended-spectrum beta-lactamase</td>
</tr>
<tr>
<td>FTICR</td>
<td>Fourier transform ion cyclotron resonance</td>
</tr>
<tr>
<td>GC</td>
<td>gene cassette</td>
</tr>
<tr>
<td>GTA</td>
<td>gene transfer agent</td>
</tr>
<tr>
<td>HBP</td>
<td>human bacterial pathogen</td>
</tr>
<tr>
<td>HGT</td>
<td>horizontal gene transfer</td>
</tr>
<tr>
<td>HRMS</td>
<td>high-resolution mass spectrometry</td>
</tr>
<tr>
<td>HRT</td>
<td>hydraulic retention time</td>
</tr>
<tr>
<td>HWW</td>
<td>hospital wastewater</td>
</tr>
<tr>
<td>Inc</td>
<td>plasmid incompatibility</td>
</tr>
<tr>
<td>IR</td>
<td>inverted repeat</td>
</tr>
<tr>
<td>IS</td>
<td>insertion sequence</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
</tr>
<tr>
<td>LCMS</td>
<td>liquid chromatography tandem mass spectrometry</td>
</tr>
<tr>
<td>LOD</td>
<td>limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>limit of quantification</td>
</tr>
<tr>
<td>MBR</td>
<td>membrane bioreactor</td>
</tr>
<tr>
<td>MDR</td>
<td>multidrug resistant</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>MECs</td>
<td>measured environmental concentrations</td>
</tr>
<tr>
<td>MGEs</td>
<td>mobile genetic elements</td>
</tr>
<tr>
<td>MIC</td>
<td>minimal inhibitory concentration</td>
</tr>
<tr>
<td>MLOQ</td>
<td>method limits of quantification</td>
</tr>
<tr>
<td>MLS</td>
<td>macrolides-lincosamides and streptogramin</td>
</tr>
<tr>
<td>MPN</td>
<td>most probable number</td>
</tr>
<tr>
<td>MRSA</td>
<td>methicillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MST</td>
<td>microbial source tracking</td>
</tr>
<tr>
<td>NOM</td>
<td>natural organic matter</td>
</tr>
<tr>
<td>NPS</td>
<td>nonpoint source</td>
</tr>
<tr>
<td>ORF</td>
<td>open reading frame</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PECO</td>
<td>population; exposure; comparator; outcome</td>
</tr>
<tr>
<td>PECs</td>
<td>predicted environmental concentrations</td>
</tr>
<tr>
<td>PS</td>
<td>point source</td>
</tr>
<tr>
<td>QACs</td>
<td>quaternary ammonium compounds</td>
</tr>
<tr>
<td>QMRA</td>
<td>quantitative microbial risk assessment</td>
</tr>
<tr>
<td>qPCR</td>
<td>quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>QqQ</td>
<td>triple quadrupole mass spectrometry</td>
</tr>
<tr>
<td>RIs</td>
<td>resistance integrons</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RCTs</td>
<td>randomized control trials</td>
</tr>
<tr>
<td>RWI</td>
<td>reclaimed water irrigated</td>
</tr>
<tr>
<td>SAT</td>
<td>soil aquifer treatment</td>
</tr>
<tr>
<td>SMX</td>
<td>sulfamethoxazole</td>
</tr>
<tr>
<td>SPE</td>
<td>solid phase extraction</td>
</tr>
<tr>
<td>SRT</td>
<td>solid retention time</td>
</tr>
<tr>
<td>SS</td>
<td>suspended solids</td>
</tr>
<tr>
<td>TIAC</td>
<td>total investigated antibiotic concentration</td>
</tr>
<tr>
<td>TMP</td>
<td>trimethoprim</td>
</tr>
<tr>
<td>ToF</td>
<td>MS time of flight mass spectrometry</td>
</tr>
<tr>
<td>TOC</td>
<td>total organic carbon</td>
</tr>
<tr>
<td>TPs</td>
<td>transformation products</td>
</tr>
<tr>
<td>TRACA</td>
<td>transposon-aided capture</td>
</tr>
<tr>
<td>TWW</td>
<td>treated wastewater</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>UWWTP</td>
<td>urban wastewater treatment plant</td>
</tr>
<tr>
<td>VRE</td>
<td>vancomycin resistant enterococci</td>
</tr>
<tr>
<td>WW</td>
<td>wastewater</td>
</tr>
<tr>
<td>WWTP</td>
<td>wastewater treatment plant</td>
</tr>
</tbody>
</table>
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; Reinthaler 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