



Review

Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review

L. Rizzo ^{a,*}, C. Manaia ^b, C. Merlin ^c, T. Schwartz ^d, C. Dagot ^e, M.C. Ploy ^f, I. Michael ^g, D. Fatta-Kassinos ^g

^a Department of Civil Engineering, University of Salerno, 84084, Fisciano (SA), Italy

^b CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal

^c Laboratoire de Chimie Physique et Microbiologie pour l'Environnement (LCPME), UMR 7564 CNRS-Université de Lorraine, 15 Avenue du Charmois, 54500 Vandoeuvre-les-Nancy, France

^d Department of Microbiology at Interfaces, Karlsruhe Institute of Technology (KIT) – Northern Campus, Institute of Functional Interfaces (IFI), D-76344 Eggenstein-Leopoldshafen, Germany

^e Université de Limoges, EA4330, GRESE, ENSIL, F87000, Limoges, France

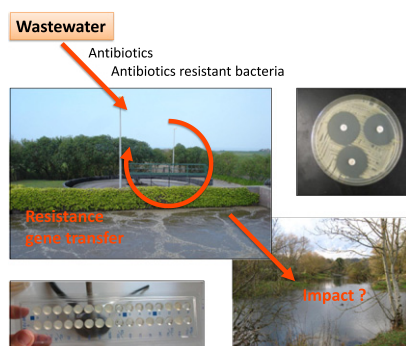
^f Université de Limoges, INSERM, UMR S1092, F-87000, Limoges, France

^g Nireas, International Water Research Centre, University of Cyprus, 1 Panepistimiou Avenue, P.O Box 20537, 1678 Nicosia, Cyprus

HIGHLIGHTS

- ▶ UWTPs may positively affect ARB spread and selection as well as ARG transfer.
- ▶ Resistance integrons may be used to characterize ARG transfer.
- ▶ High trough technologies are a useful complementation of PCR technologies.
- ▶ Biological process effect on ARB and ARG transfer should be further investigated.
- ▶ Advanced treatments/disinfection effect should be further investigated too.

GRAPHICAL ABSTRACT



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ABSTRACT

Urban wastewater treatment plants (UWTPs) are among the main sources of antibiotics' release into the environment. The occurrence of antibiotics may promote the selection of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB), which shade health risks to humans and animals. In this paper the fate of ARB and ARGs in UWTPs, focusing on different processes/technologies (i.e., biological processes, advanced treatment technologies and disinfection), was critically reviewed. The mechanisms by which biological processes influence the development/selection of ARB and ARGs transfer are still poorly understood. Advanced treatment technologies and disinfection process are regarded as a major tool to control the spread of ARB into the environment. In spite of intense efforts made over the last years to bring solutions to control antibiotic resistance spread in the environment, there are still important gaps to fill in. In particular, it is important to: (i) improve risk assessment studies in order to allow accurate estimates about the maximal abundance of ARB in UWTPs effluents that would not pose risks for human and environmental health; (ii) understand the factors and mechanisms that drive antibiotic resistance maintenance and selection in wastewater habitats. The final objective is to implement wastewater treatment technologies capable of assuring the production of UWTPs effluents with an acceptable level of ARB.

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* Corresponding author. Tel.: +39 089 969334; fax: +39 089 969620.

E-mail address: l.rizzo@unisa.it (L. Rizzo).

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1. Introduction

The intensive use of antibiotics for human, veterinary and agricultural purposes, results in their continuous release into the environment (Batt et al., 2006; Brown et al., 2006; Díaz-Cruz et al., 2003; Kümmerer, 2009). The main concern for the release of antibiotics into the environment is related to the development of antibiotic resistance genes (ARGs) and bacteria (ARB), which reduce the therapeutic potential against human and animal pathogens (Kemper, 2008; X.X. Zhang et al., 2009b). The increasing interest of the scientific community and international institutions/organization for this problem is respectively testified by the number of publications reviewed in this paper and internationally/scientifically relevant initiatives, such as research projects (e.g., PHARMAS) and networking (e.g., COST Action DARE).

Effluents from urban wastewater treatment plants (UWTPs) are suspected to be among the main anthropogenic sources for antibiotics (no maximum contaminant levels have been set by EU and other international institutions/organizations), ARGs and ARB spread into the environment (Ferreira da Silva et al., 2006; Figueira et al., 2011a; Kümmerer, 2009; Lupo et al., 2012). The biological treatment process creates an environment potentially suitable for resistance development and spread because bacteria are continuously mixed with antibiotics at sub-inhibitory concentrations (Auerbach et al., 2007; Davies et al., 2006; Ferreira da Silva et al., 2006). The knowledge regarding the effects of sub-inhibitory concentrations of anti-microbials and their effects on environmental bacteria, is scarce and contradictory especially with respect to resistance (Kümmerer, 2009).

ARBs are mostly studied in bacteria belonging to the common indicators of faecal contamination, namely coliforms and enterococci (Araújo et al., 2010; Boczek et al., 2007; Figueira et al., 2011a; Martins da Costa et al., 2006; Reinthaler et al., 2003; Sabate et al., 2008). The search for agents associated with human “difficult-to-treat infections” such as methicillin resistant *Staphylococcus aureus*, vancomycin resistant *Enterococcus* spp., and Gram-negative bacteria (enterobacteria, pseudomonads, acinetobacter) resistant to fluoroquinolones, carbapenems and producers of extended spectrum beta-lactamase, has also been addressed in such studies. Although at low percentages, when compared to what is observed in the clinical settings, antibiotic resistance profiles are often detected in wastewaters and recreational waters; a fact that may represent a relevant public health issue (Araújo et al., 2010; Soge et al., 2009). However, the current knowledge on the prevalence and types of antibiotic resistance in the environment is barely sufficient. In contrast to what has been done over the last years in the standardization

and organization of antibiotic resistance data of clinical origin (ECDC, 2012; EUCAST, 2011), information regarding resistance of environmental bacteria is still very fragmented.

UWTPs typically include different processes (e.g., mechanical, biological, physical, chemical and physical–chemical) which may affect the fate of antibiotics, ARB and ARGs in different ways and consequently the development and spread of resistance into the environment. Accordingly, the aim of the present paper is to critically review the fate of ARB and ARGs in UWTPs. In particular, the effect of different processes/technologies, namely biological processes, advanced treatment technologies and disinfection, is addressed. Moreover, methods for the characterization/evaluation of ARB, ARGs and genes transfer are presented and discussed in a comprehensive way to facilitate the understanding of the effect of wastewater treatment processes/technologies on ARB and ARGs.

2. Tools to assess antibiotic resistance in UWTPs

The relationship existing between antibiotic consumption and the emergence and development of resistances is now well documented (Davies, 2007). If the acquisition of mutations widely contributes to bacterial adaptation, the exchange and reshuffling of genetic material between bacteria involving mobile genetic elements are the main contributors explaining the rapid dissemination of antibiotic resistances (Courvalin, 2008). Identifying hotspots of possible ARG dissemination starts by determining the relative occurrence of ARB or ARG in the wastewater network. At this stage, two main approaches are followed, either culture-based or molecular-based, each of them exhibiting specific advantages and drawbacks. Lately the molecular-based approaches have been extended to the detection of genetic structures involved in ARG capture, namely integrons, enhancing the understanding of ARB and ARG dynamics in complex anthropogenic environments. Despite the recent progresses, demonstrating ARG transfer in UWTP remains a difficult task that should be studied on a case-by-case basis. Before addressing the effect of wastewater treatment processes on ARB and ARGs, the methods and tools for characterizing antibiotic resistance and genes transfer in the aquatic environment are presented first.

2.1. Antibiotic resistance characterization by cultivation-based methods

Antibiotic resistance testing is highly standardized worldwide, enabling laboratories to assist clinicians in bacterial infection therapy

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