



## Identification of antibiotic-resistant *Escherichia coli* isolated from a municipal wastewater treatment plant



Carlo Zanotto<sup>a</sup>, Massimiliano Bissa<sup>b,1</sup>, Elena Illiano<sup>b</sup>, Valeria Mezzanotte<sup>c</sup>,  
Francesca Marazzi<sup>c</sup>, Andrea Turolla<sup>d</sup>, Manuela Antonelli<sup>d</sup>, Carlo De Giuli Morghen<sup>e,\*\*</sup>,  
Antonia Radaelli<sup>b,f,\*</sup>

<sup>a</sup> Department of Medical Biotechnologies and Translational Medicine, University of Milan, Via Vanvitelli, 32, 20129, Milan, Italy

<sup>b</sup> Department of Pharmacological and Biomolecular Sciences, University of Milan, Via Balzaretti, 9, 20133, Milan, Italy

<sup>c</sup> Department of Environmental and Regional Sciences (DISAT), University of Milan Bicocca, Piazza della Scienza 1, 20126, Milan, Italy

<sup>d</sup> Environmental Section, Department of Civil and Environmental Engineering (DICA), Milan Polytechnic, Piazza Leonardo da Vinci 32, 20133, Milan, Italy

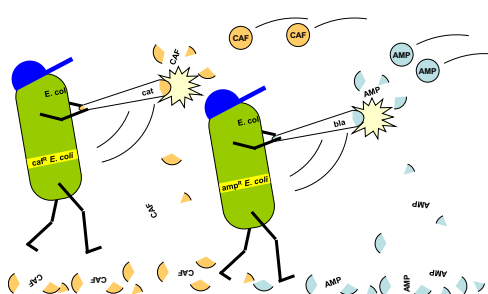
<sup>e</sup> Catholic University "Our Lady of Good Counsel", Rr. Dritan Hoxha, Tirana, Albania

<sup>f</sup> Cellular and Molecular Pharmacology Section, National Research Council (CNR) Institute of Neurosciences, University of Milan, Via Vanvitelli, 32, 20129, Milan, Italy

### HIGHLIGHTS

- AMP<sup>R</sup> *Escherichia coli* colonies are prevalent among AMP<sup>R</sup> coliforms.
- AMP<sup>R</sup> and CAF<sup>R</sup> *E. coli* show a 2-log decrease after the biological treatment.
- *Bla*<sup>+</sup> *E. coli* percentage increases after the biological treatment and filtration.
- AMP<sup>R</sup>/CAF<sup>R</sup> double-resistant *E. coli* colonies are mostly *bla*<sup>+</sup>.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 20 April 2016

Received in revised form

5 August 2016

Accepted 8 August 2016

Handling Editor: Shane Snyder

### ABSTRACT

The emergence and diffusion of antibiotic-resistant bacteria has been a major public health problem for many years now. In this study, antibiotic-resistance of coliforms and *Escherichia coli* were investigated after their isolation from samples collected in a municipal wastewater treatment plant in the Milan area (Italy) along different points of the treatment sequence: inflow to biological treatment; outflow from biological treatment following rapid sand filtration; and outflow from peracetic acid disinfection. The presence of *E. coli* that showed resistance to ampicillin (AMP) and chloramphenicol (CAF), used as representative antibiotics for the efficacy against Gram-positive and Gram-negative bacteria, was evaluated. After determining *E. coli* survival using increasing AMP and CAF concentrations, specific single-

\* Corresponding author. Department of Pharmacological and Biomolecular Sciences, University of Milan, Via Balzaretti, 9, 20133, Milan, Italy.

\*\* Corresponding author.

E-mail addresses: [carlo.zanotto@unimi.it](mailto:carlo.zanotto@unimi.it) (C. Zanotto), [massimiliano.bissa@nih.gov](mailto:massimiliano.bissa@nih.gov), [bissa.massimiliano@gmail.com](mailto:bissa.massimiliano@gmail.com) (M. Bissa), [elena.illiano@unimi.it](mailto:elena.illiano@unimi.it) (E. Illiano), [valeria.mezzanotte@unimib.it](mailto:valeria.mezzanotte@unimib.it) (V. Mezzanotte), [francesca.marazzi@unimib.it](mailto:francesca.marazzi@unimib.it) (F. Marazzi), [andrea.turolla@polimi.it](mailto:andrea.turolla@polimi.it) (A. Turolla), [manuela.antonelli@polimi.it](mailto:manuela.antonelli@polimi.it) (M. Antonelli), [carlo.degiulimorghen@unimi.it](mailto:carlo.degiulimorghen@unimi.it) (C. De Giuli Morghen), [antonia.radaelli@unimi.it](mailto:antonia.radaelli@unimi.it) (A. Radaelli).

<sup>1</sup> Present address: National Cancer Institute (NCI), National Institutes of Health (NIH), Basic Res Lab, AMRVS, Bethesda, MD, USA.

**Keywords:**

Antibiotic resistance  
WWTPs  
*Escherichia coli*  
*bla* and *cat* genes

resistant (AMP<sup>R</sup> or CAF<sup>R</sup>) and double-resistant (AMP<sup>R</sup>/CAF<sup>R</sup>) strains were identified among *E. coli* colonies, through amplification of the  $\beta$ -lactamase *Tem-1* (*bla*) and acetyl-transferase *catA1* (*cat*) gene sequences. While a limited number of CAF<sup>R</sup> bacteria was observed, most AMP<sup>R</sup> colonies showed the specific resistance genes to both antibiotics, which was mainly due to the presence of the *bla* gene sequence. The peracetic acid, used as disinfection agent, showed to be very effective in reducing bacteria at the negligible levels of less than 10 CFU/100 mL, compatible with those admitted for the irrigation use of treated waters.

© 2016 Published by Elsevier Ltd.

## 1. Introduction

Antimicrobial agents are successfully used to treat animal and human diseases. After discovering bacterial innate resistance, such as the one of Enterobacteria to  $\beta$ -lactams (Sykes and Matthew, 1976), the extensive and unnecessary use of antibiotics has caused the selection of antibiotic-resistant bacteria (ARBs) (O'Neill, 2016). Over time, although new antibiotics were developed to contrast the emergence of ARBs, these have not been able to block the selection of strains resistant to these new antibiotics (Keen and Montforts, 2012).

To this purpose, different molecular mechanisms have been described by which bacteria become antibiotic resistant. The mechanisms include genetic mutations and horizontal transfer by conjugative plasmids or transposons of mobile antibiotic resistance genes (ARGs) (Alanis, 2005), easily identified by modern technologies, that may allow the survival also at high antibiotic concentrations (Meredith et al., 2015).

Antibiotic resistance has not been completely explored in the environment (Marti et al., 2014), but the continuous release of antibiotics in wastewater discharges, and their possible outflow in recycled water for agricultural purposes, may impair autochthonous bacteria and freshwater ecosystems (Roose-Amsaleg and Laverman, 2015). Sub-inhibitory antibiotic concentrations were also recently found in environmental settings, as those present in the aquatic environment (Kümmerer, 2009a, 2009b), that may promote antibiotic resistance and select for ARBs (Chow et al., 2015; Gullberg et al., 2011).

Wastewater treatment plants (WWTP) can also be unable to effectively eliminate water contaminants (Pruden, 2014), which may be dangerous for human health and ecosystems (Richardson and Ternes, 2014). Many pharmaceuticals can persist in the environment and they are frequently detected in drinking water. Also, antibiotics are often administered for economic reasons in the production of food animals to prevent animal infectious diseases, thus increasing their possible assumption with the diet by human beings (Silbergeld et al., 2008).

It is also known that ARGs are not easily removed and ARBs may also increase inside WWTPs, since bacteria are often exposed to antibiotics in activated sludge (Proia et al., 2016; Rizzo et al., 2013), where microbial concentrations and diversity can also facilitate gene transfer (Zhang et al., 2009). Bacterial inactivation procedures may also be unable to deactivate intracellular genes (Dodd, 2012; Sharma et al., 2016), that can persist even after chlorination (Yuan et al., 2015) although the sequential use of chlorination and UV irradiation may improve ARG inactivation (Zhang et al., 2015).

WWTPs are therefore sites that need to be monitored, as they may be the recipients of waters that contain antimicrobials as well as human and animal metabolic waste (Kim and Aga, 2007) and they can provide a suitable environment for the spread of ARBs (Baquero et al., 2008; Marathe et al., 2013). Moreover, the fate of antibiotics and other pharmaceuticals can be strongly influenced

not only by WWTP biological/chemical procedures (Sharma et al., 2013), but also by the design and type of treatment system and sewer network as well as by the efficiency of disinfection (Azzellino et al., 2011; Mezzanotte et al., 2007).

The aim of the present study was to detect ampicillin-resistant (AMP<sup>R</sup>) and chloramphenicol-resistant (CAF<sup>R</sup>) *Escherichia coli* in the inflow and outflow from a municipal WWTP in the Milan area (Italy). AMP and CAF were used as representative antibiotics for the efficacy against Gram-positive and Gram-negative bacteria.

AMP and CAF are the commonly used antibiotics in clinic, breedings and research laboratories and their extensive use in both human and veterinary medicine can promote resistance inside WWTPs. Although AMP is more generally used, CAF is more specific for Enterobacteria such as *E. coli*, which is the reference standard indicator of faecal contamination and used to monitor the spread of ARBs in recycled waters (Watkinson et al., 2007).

Antibiotic resistance was first determined by analysing *E. coli* survival after spiking culture medium with increasing AMP and CAF concentrations.

The results of this study show that the biological process of this WWTP is effective in reducing ARB concentration. A prevalence of AMP<sup>R</sup> versus CAF<sup>R</sup> *E. coli* colonies was also observed, that can be explained by the larger use of  $\beta$ -lactam antibiotics. After disinfection, ARB removal is almost complete and residual bacteria are compatible with the irrigation use of treated waters.

## 2. Materials and methods

### 2.1. Wastewater treatment plant

The WWTP is located in an urban area of Milan (Italy), and it receives wastewater from the city (1,250,000 Inhabitant Equivalents, corresponding to 432,000 m<sup>3</sup>/day average inflow (Pizza, 2014), which includes that from many hospitals. The within-plant treatment scheme (Fig. 1) starts with pretreatments (screening, sand and oil removal). This step is followed by the biological treatment with activated sludge, including pre-denitrification and biological oxidation (8 h hydraulic retention time, 30 day sludge retention time). After secondary settling, the biologically treated effluent undergoes rapid sand filtration to improve the removal of suspended solids and phosphorus.

The final disinfection is based on peracetic acid (about 2 mg/L, 45 min contact time in dry weather) and aimed at complying with the microbiological limits for the reuse of treated wastewater for agricultural purposes: 10 *E. coli* colony forming units (CFU)/100 mL.

### 2.2. Wastewater sampling and physicochemical characterisation

Wastewater samples were collected five times from 19 September to 17 October, 2012, from three different points in the plant: at the inflow to the biological treatment (i.e., IN-BIO samples), at the outflow from sand filtration (i.e., OUT-BIO samples),

Download English Version:

<https://daneshyari.com/en/article/6306114>

Download Persian Version:

<https://daneshyari.com/article/6306114>

[Daneshyari.com](https://daneshyari.com)