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Vancomycin resistant enterococci: From the hospital effluent to the urban wastewater treatment plant

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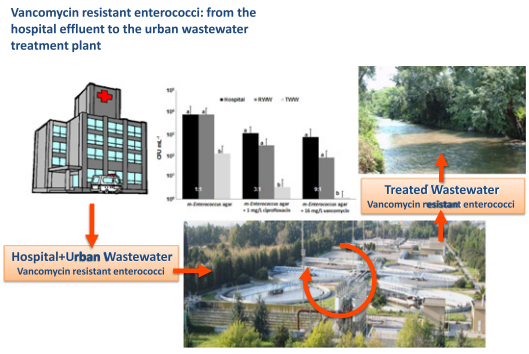
HIGHLIGHTS

- Total, vancomycin and ciprofloxacin resistant enterococci were isolated from hospital and urban wastewater.
- Hospital effluent and raw urban wastewater had identical counts of vancomycin resistant enterococci.
- Vancomycin resistant enterococci were significantly more prevalent in the hospital effluent than in the urban wastewater.
- Similar multidrug-resistance phenotypes were observed in isolates from patients, hospital effluent and urban wastewater.
- This study suggests that hospital effluents may contribute to spread vancomycin resistant enterococci to the environment.

GRAPHICAL ABSTRACT



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ABSTRACT

Vancomycin is an important antibiotic to treat serious nosocomial enterococci infections. Human activities, in particular those related with clinical practices performed in hospitals, can potentiate the transfer and selection of clinically-relevant resistant bacteria such as vancomycin resistant enterococci (VRE). Indeed, previous studies demonstrated the occurrence of VRE in urban wastewater treatment plants and related environments (e.g. sewage, rivers). In this study, the occurrence of VRE in a hospital effluent and in the receiving urban wastewater treatment plant was investigated. Vancomycin and ciprofloxacin resistant bacteria occurred in the hospital effluent and in raw municipal inflow at densities of 10^3 to 10^2 CFU mL^{-1}, being significantly more prevalent in the hospital effluent than in the urban wastewater. Most of the VRE isolated from the hospital effluent belonged to the species *Enterococcus faecalis* and *Enterococcus faecium* and presented multidrug-resistance phenotypes to ciprofloxacin, tetracycline, erythromycin, and high-level gentamicin.

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The same pattern was observed in clinical isolates and in enterococci isolated from the final effluent of the urban wastewater treatment plant. These results show that hospital effluents discharged into urban wastewater treatment plants may be a relevant source of resistance spread to the environment.

1. Introduction

Enterococcus is a genus of lactic acid bacteria, comprising more than 40 species, of which the most well-known representatives are *Enterococcus faecalis* and *Enterococcus faecium*. This genus comprises Gram-positive, fermentative and aerotolerant bacteria, that although not depending on the presence of oxygen to survive, are not inhibited in its presence. Due to these and other properties, enterococci are ubiquitous in nature and are frequently found in association with animals, both avian and mammals, and vegetables (Devriese et al., 1992; Klein, 2003). Their occurrence in food products is also common and expected, for instance, in some dairy products (Devriese et al., 1992; Klein, 2003). Enterococci are, nevertheless, indicators of faecal contamination of waters (Ashbolt et al., 2001; EU, 2006). Given the environmental distribution, the interaction with humans and other animals and their physiology, members of this genus play an important role on healthcare associated infections worldwide (Siegel et al., 2006; ECDC, 2012). According to the ECDC — European Centre for Disease Prevention and Control (ECDC) (2012), enterococci are among the most frequently isolated microorganisms in bloodstream and urinary tract infections acquired in intensive care units (ECDC, 2012).

Vancomycin is a glycopeptide antibiotic, which is assigned for the treatment of serious, life-threatening infections by Gram-positive bacteria, when treatment with other antibiotics has failed. The excessive use of this antibiotic, which has no significant effect on Gram-negative bacteria, caused the emergence of vancomycin resistant enterococci (VRE), with particular concern for the species *E. faecalis* and *E. faecium* (Bonten et al., 2001). According to the European Centre for Disease Prevention and Control, in the period 2005–2010, the highest average values of prevalence of vancomycin resistance in *E. faecalis* in European countries were observed in Ireland (2.1%), United Kingdom (2.1%), Italy (2.5%), Luxemburg (2.9%), Portugal (4.0%) and Greece (5.3%) (ECDC, 2012). Over the same period, vancomycin resistance was much more prevalent in *E. faecium*, with the leading countries being almost the same (12.0% Luxemburg, 18.8% Cyprus, 20.6% United Kingdom, 26.4% Portugal, 32.3% Greece, 35.2% Ireland). In 2009, in Europe, VRE accounted for 2.7% of the total enterococci associated with intensive care unit acquired infections (ECDC, 2012). In the United States of America, in 2003, that value was ten times higher (28.5%) (Siegel et al., 2006).

Urban wastewater treatment plants, because receive high loads of antibiotics, antibiotic resistant bacteria and genetic resistance determinants are among the main sources of antibiotic resistance into various environmental compartments worldwide (Manaia et al., 2012; Michael et al., 2013; Rizzo et al., 2013; Novo et al., 2013). VRE have been also detected in wastewaters in percentages up to 3% in Portugal and up to 7% in Poland (Martins da Costa et al., 2006; Luczkiewicz et al., 2010). Several other studies have demonstrated the occurrence of VRE or their antibiotic-resistance determinants in wastewater-related environments (e.g. sewage, rivers) (Sahlström et al., 2009; Novais et al., 2005; Araújo et al., 2010).

The knowledge on the modes and paths of antibiotic resistance dissemination in the environment is still scarce (Martinez, 2009; Manaia et al., 2012). However, it is assumed that human activities, in particular those related with antibiotics use, mainly in veterinary, agriculture and fish farm applications, as well as those related with human medicine can represent important sources of antibiotic resistance in the environment (Baquero et al., 2008; Martinez, 2009). In particular, given the amounts of antibiotics used and emitted, hospitals may represent critical points to potentiate the transfer and selection of clinically-relevant resistant bacteria or genetic determinants to the environment (Kummerer and Henninger, 2003). Such an assumption is particularly relevant when it refers to antibiotics used preferentially in hospitals, as is the case of vancomycin. In contrast to vancomycin, ciprofloxacin is also widely consumed in ambulatory and in the community. This study compared the fate of both antibiotic resistant populations, and was based on the hypotheses: 1) that hospital effluents may supply vancomycin resistant enterococci to the urban wastewater treatment plants receiving those effluents; 2) and that part of these bacteria or their resistance determinants can survive wastewater treatment and be released in the final effluent, increasing the risks of spread in the surrounding environment.

2. Materials and methods

2.1. Samples

This study examined wastewater samples from a hospital effluent, collected on the 20th of September, 19th of October, 28th of November of 2011, 9th of January 2012, and from the raw and treated wastewater of the urban treatment plant receiving that hospital effluent. Samples were collected on the 26th September, 18th the October and the 23th November of 2011. The hospital has a capacity of 1120 beds and covers a geographic region corresponding to 3 million people, housing more than 30 clinical specialities. The hospital effluent has an average flow of 1000 m^3/day, 630 mg L^{-1} COD, 250 mg L^{-1} BOD and 260 mg L^{-1} suspended solids. The receiving urban wastewater treatment plant, which serves a population equivalent to 200 000 inhabitants, registered an average monthly flow of 947 000 m^3/day, with average values of 919 mg L^{-1} COD, 558 mg L^{-1} BOD and 387 mg L^{-1} suspended solids in the raw inflow and 32 mg L^{-1} COD, 7 mg L^{-1} BOD and 9 mg L^{-1} suspended solids in the treated effluent, for the sampling period included in this study (source: wastewater treatment plant). The treatment comprises four stages — i) the preliminary treatment that involves the removal of solids and fats; ii) the primary treatment that corresponds to a sedimentation tank to remove settling suspended solids; iii) the secondary treatment that involves the removal of organic matter, including nitrogen and phosphorus, in a biological reactor composed of three zones — anoxic (de-nitrification process), aerated (nitrification) and endogenous (phosphorus removal), with recirculation of the mixed liquor from the aeration (nitrification) tank to anoxic zone, secondary decanters and sludge recirculation from the bottom of the decanter to the aeration tank; and iv) the tertiary treatment that involves sand filtration (to improve the removal of suspended solids and of attached microorganisms). Volumes of 1 L of urban wastewater, corresponding to 24 hour composite samples of raw and treated wastewater were collected simultaneously, in order to allow the comparison of average values of antibiotic resistance prevalence in the raw and in the treated wastewater. Spot hospital wastewater samples were collected by hospital staff according to the procedures used for routine monitoring sampling. All samples were transported refrigerated and analysed within 4 h.

2.2. Enumeration of presumptive culturable enterococci

Bacteria were enumerated on m-*Enterococcus* agar (20 g L^{-1} tryptose, 5 g L^{-1} yeast extract, 2 g L^{-1} dextrose, 4 g L^{-1} dipotassium