

## Prevalence of amoxicillin resistance by Enterococci found in the hospital wastewater

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**Date of submission:** 11 Nov 2017, **Date of acceptance:** 01 Jan 2018

### ABSTRACT

Enterococci are found worldwide and are considered to be opportunistic pathogens. Antimicrobial resistance in these bacteria is increasing and is associated with their capability to sustain in the wastewater. We, therefore, aimed to determine the antimicrobial resistance of *Enterococcus* spp. that are isolated from the wastewater and clinical specimens. Isolation, identification, and determination of the antibiotic susceptibility patterns in the *Enterococcus* isolates were performed according to the standard procedures. The concentration of amoxicillin in the wastewater was determined by high-performance liquid chromatography (HPLC). A total of 20 Enterococci were isolated and identified in this study. Of all the *Enterococcus* spp. isolates, 85 and 70% were resistant to tetracycline and ciprofloxacin, respectively. Surprisingly, the antimicrobial susceptibility tests revealed that the wastewater isolates had a higher rate of resistance than the clinical isolates. Amoxicillin concentrations in both the teaching hospitals were analyzed to be 8.6 and 6.8 ppm, respectively. The results of this study revealed that the hospital wastewater, which mixes with the environment, usually comprises resistant Enterococci. The use of HPLC allows the detection of amoxicillin concentrations in the wastewater samples of the teaching hospitals.

**Keywords:** Amoxicillin resistance, Enterococci isolates, hospital wastewater

### Introduction

Antibiotic resistance is a rising concern that threatens the present treatment of infectious diseases,<sup>1</sup> in particular, among the immunocompromised patients. Frequent use of the antimicrobial agents in controlling and preventing infectious diseases may serve as a selective pressure for more resistance, and these agents may spread in the environment;<sup>2</sup> hospitals act as the key reservoirs for resistant bacteria. Dissemination of the resistant bacteria from the hospitals may occur in several ways

including hospital personnels, wastewater, and discharged patients.<sup>3</sup> An elevated level of antibiotics in the hospital environments like wastewater is considered favorable for the horizontal gene transfer of resistant genes among bacteria,<sup>4</sup> for example, genus *Enterococcus*.<sup>5</sup> These bacteria may develop resistance to several antibiotics including clindamycin, cephalosporins, and amino glycosides,<sup>6</sup> and they may act as opportunistic pathogens and are frequently associated with the nosocomial infections. Therapeutic compounds, in particular, antibiotics have raised increasing concerns over the last two decades as their environmental effects remain unclear.<sup>7</sup> Therefore, it is essential to monitor such pharmaceutical products in order to gain better information regarding their existence, their distribution in the environment, and their effects

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**Citation:** Kalantar E, Mohammadi S, Dehghanifard E, Ebrahimi R, Hatami A, Khodaei F. Prevalence of amoxicillin resistance by Enterococci found in the hospital wastewater. J Adv Environ Health Res 2017; 5(3): 192-195

on the bacteria when these microbes are exposed to the pharmaceutical compounds.<sup>8</sup> The pharmaceutical products present in the hospital wastewater can be quantified by HPLC, which is one of the most common technique used to determine the concentrations of such compounds in the environment.<sup>9</sup>

Our research aimed to determine the occurrence of *amoxicillin* resistance among the Enterococci isolates and the detection of *amoxicillin* in the hospital wastewater. The selection of amoxicillin was mainly based on the resistance pattern of the Enterococci isolates and the consumption of this antibiotic in the hospital. Although the Enterococci isolates were resistant to various antibiotics, due to financial issues we could not detect the other antibiotics in the hospital wastewater.

## Materials and Methods

### Wastewater sample collection

The wastewater samples were obtained from two teaching hospitals and one private hospital in Karaj, Iran. Samples were collected using sterile bottles and were preserved at 4 °C until they were processed. Following studies were conducted: isolation, identification, and determination of the antibiotic susceptibility patterns in *Enterococcus* isolates obtained from the clinical samples and the wastewater samples.

The clinical isolates were provided by the laboratory of each hospital. They were studied and their genus was confirmed; an antibiogram

was performed on each isolate according to the CLSI guidelines.<sup>10</sup>

The concentration of amoxicillin in the wastewater samples was determined using a Knauer high performance liquid chromatography (HPLC) prepared with a C18 column (250 mm × 4.6 mm × 5 μm) and a UV-PAD detector at 190 nm. Mixture of phosphate buffer (pH: 4.8) and methanol (ratio of 60:40) with a flow rate of 1 mL/min was considered as the mobile phase.

## Results and Discussion

A total of 20 *Enterococcus* spp. were isolated and identified in this study; 9 (45%) isolates belonged to the clinical specimens from 3 hospitals located in Karaj, Iran, and 11 (55%) strains were isolated from the hospital wastewater. All the *Enterococcus* isolates revealed resistance of 85 and 70% to tetracycline and ciprofloxacin, respectively. Surprisingly, the antimicrobial susceptibility tests revealed that the wastewater isolates had a higher rate of resistance than the clinical isolates, as listed in Table 1. Furthermore, some specific resistance patterns were common among the clinical and the wastewater isolates. The amoxicillin concentrations in the three hospital wastewater samples were measured by HPLC, and are listed in Table 2. In this study, the amoxicillin concentrations in both the teaching hospitals were analyzed to be 8.6 and 6.8 ppm, respectively.

Table 1. Percentages of antibiotic resistance for the Enterococcal isolates from clinical and hospital wastewater samples

Origin (n)	Interpretive Criteria	Antibiotic (percentage %)						
		Tet	Van	Amx	CTX	Amp	Ery	Cip
Clinical n=11	Susceptible	11.11	63.63	55.55	55.55	66.66	66.66	66.66
	Intermediate	22.22	27.27	22.22	33.33	0.00	22.22	22.22
	Resistant	66.66	09.09	22.22	11.11	33.33	11.11	11.11
Wastewater N=9	Susceptible	0.00	63.63	36.36	81.81	63.63	63.63	18.18
	Intermediate	09.09	27.27	18.18	09.09	09.09	18.18	09.09
	Resistant	90.90	09.09	45.45	09.09	27.27	18.18	72.72

Tet :Tetracycline; Van: Vancomycin; CTX; Co- trimaxsazol; Amx: Amoxicillin; Amp: Ampicillin; Ery: Erytromycin; Cip: Ciprofloxacin

Table 2. Determination of Amoxicillin concentration in hospital wastewater by HPLC

Hospitals	Amoxicillin concentration (ppm)
Teaching hospital A	8.6
Teaching hospital B	6.8
Private hospital	Not detected

In general, the human gut is known to have *Enterococcus* spp., which are commensals and are known to have virulence properties that aid them in causing infections in the immunocompromised population including the elderly people, children, pregnant women, and

long-term hospitalized patients.<sup>11</sup>

Antibiotic susceptibility of these isolates was studied using a panel of antibiotics, which are commonly used in the hospitals to treat various infectious diseases. Almost all the isolates were resistant to tetracycline, and to some extent amoxicillin. Several studies have revealed a higher incidence of such resistance among the Enterococci isolates worldwide.<sup>12</sup>

To a certain extent, the grade of similarity between the resistance patterns of the *Enterococcus* spp. isolated from the hospital wastewater and the clinical isolates suggest that the hospital wastewater may have contributed immensely to the resistances observed. Several investigators have revealed that wastewater can provide favorable environmental conditions needed for the antibiotic resistant gene transfer to the bacteria.<sup>13</sup> Environmental pollution is one of the key problems faced by various countries, in particular, Iran. Pharmaceuticals essentially contribute to the environmental pollutants. Their occurrence has been reported in natural waters, wastewater, sediments, and sludge. New studies reveal their occurrence in samples investigated worldwide.<sup>14,15</sup> The results of this study revealed that the hospital wastewater discharged to the environment comprised multiresistant Enterococci. The use of HPLC allows the reliable detection of amoxicillin presence in the wastewater samples of the teaching hospitals. Antibiotics in wastewater can emerge from defecation, direct disposal of expired drugs, and spilling these events could serve as an additional selective pressure on the bacteria in the wastewater. The amoxicillin concentrations in the wastewater measured in this study are comparable to or less than those observed in several countries.<sup>16,17</sup> Amoxicillin was not detected in the private hospital wastewaters analyzed in this study, which may be due to the newly established. These findings require interventions by the relevant experts, including health care managers, as these may have great public health implications on the health of humans as well as animals that are exposed to such environment.

#### Acknowledgments

The authors are thankful to the Alborz

University of Medical sciences for their financial support.

#### Ethics statement

The study protocol was accepted by the Medical Research and Ethics Committee of the Alborz university of Medical Science.

#### Conclusion of Interest

The authors declare that they have no competing interests.

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