Is the drinking water free of antibiotic resistant bacteria? A study case of Ouro Preto – MG (Brazil)

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Highlights:

- Despite the high concentration of heterotrophic bacteria, raw water had a low ARB content;
- In chlorine-treated water was observed the highest absolute and relative concentration of ARBs;
- Fountain waters showed very low concentrations of ARBs.

Keywords: antibiotic resistance; total heterotrophic bacteria; water quality.

INTRODUCTION

Given the current pandemic scenario experienced by humanity, concern about diseases caused by emerging pathogens has been gaining notoriety. In this issue, antibiotic resistance stands out, which recently may have been driven by the excessive use of medications used to treat COVID-19, such as the antibiotic azithromycin (OPAS, 2022a; OPAS, 2022b).

Water is a potential vehicle for transmitting pathogens and, consequently, antibiotic resistant bacteria (ARB) to the population (WHO, 2017). Therefore, if it is not offered in adequate quantity and quality, by current drinking standards, it can pose risks to human health and become a possible reservoir of ARBs. Thus, the operation and conditions of water supply systems, from the collection point in the source, through water treatment plants (WTPs), to distribution networks, play a crucial role in controlling the spread of resistance to antibiotics (Tarazi et al., 2021; Chowdhury et al., 2021; Siedlecka et al., 2021).

Therefore, the present study sought to evaluate, together with usual parameters, the occurrence of bacteria resistant to the azithromycin (BR-AZI) and meropenem (BR-MER) antibiotics, plus an estimate of total heterotrophic bacteria (THB) in four treated and untreated water supplies in the city of Ouro Preto (Minas Gerais - Brazil).

METHODOLOGY

The sampling points chosen were: raw water (Passa Dez stream) - RW; treated water (water collected at a point in the city's distribution system) - TW; water from the Palácio D'Ouro fountain – F1W; water from the fountain at Feira de Pedra Sabão – F2W.

Sample campaigns were carried out in February/24, May/24, June/24, and August/24. All samples were characterized regarding the parameters pH, turbidity, and residual chlorine (treated water sample only). Additionally, the presence of Total Coliforms (TC) and *Escherichia coli* were also analyzed using the presence/absence method (APHA/AWWA/WEF, 2005).

The methodology for ARB determination were based on count colony forming units' quantification (CFU) of total heterotrophic bacteria (THB), ARB resistant do azithromycin (ARB-AZI) and resistant do meropenem (ARB-MER). Water samples were the filter membrane technique (for less concentrated samples) and spread plate, for more concentrated samples. In the case of the filtering membrane technique, different filtration volumes were tested throughout the sampling campaigns, in order to reach a viable number of CFU between 30 to 300. The R-2A medium was used. For both ARB-AZI and ARB-MER, a concentration of 10 mg L^{-1} of antibiotic was used on the plates. The following incubation conditions were used for all samplings: 30 °C - 24h.

RESULTS AND CONCLUSIONS

No major variations in water's parameters were noted, according to Table 1. The pH ranged from 6.49 to 8.62, with samples from FW2 tending to have slightly lower pH than others. For turbidity, RW presented higher values (1.51 to 3.81 NTU), as expected for surface waters, while the other points presented values < 1.75 NTU, except for F2W in Jun/24 (2.63). Residual chlorine varied from 0.16 to 0.60 mg Cl_2 L^{-1} in TW. Considering the Brazilian potability standard, the treated water was within the limits established for pH and residual chlorine (expect for Aug/24) (Brazil, 2021).

The presence of total coliforms was detected in most of the samples, except in F2W. Actually, for this fountain both sampling campaigns revealed the absence of *E. coli* and ARB (Fig. 1b and 1c), and very low levels of THB (< 0.01 CFU mL^{-1} – Fig. 1a), indicating that this water source is well preserved.

For F1W, despite being coliform positive in all samples and presenting some levels of THB (4 to 10 CFU mL^{-1} – Fig. 1a), ARB-MER were absent, while ARB-AZI concentrations were lower to 0.4 CFU mL^{-1} for most samplings, except in Aug/24, when these reached 0.77 and 2.87 CFU mL^{-1}, respectively.

On the other hand, TW samples, which were collected from the distribution network, revealed wide variability in microbiological data. The presence of total coliforms was noted only in the Feb/24 collection, concomitantly with the highest concentration of THB (13,480 CFU mL^{-1}) and ARBs (83% for AZI and 10% for MER – Fig. 1d), results in agreement with Sahoo et al. (2012). Interestingly, in this collection the residual chlorine concentration was 0.59 mg Cl_2 L^{-1}, indicating the presence of chlorine-tolerant bacteria at this point, but not necessarily pathogenic. Therefore, there may have been a problem that affected the integrity of the supply network or even due to heavy rains that occurred prior to collection. However, in the other sampling campaigns, total coliforms were absent, while THB was less than 1.26 CFU mL^{-1} and ARBs less than 0.3 CFU mL^{-1}.

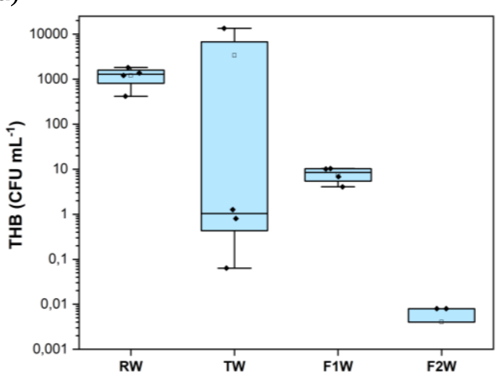
Less contamination was observed in the watercourse supplied to the water treatment and distribution company (RW samples). Despite being coliform positive and presenting turbidity levels, these samples also showed THB around 1,202 CFU mL^{-1}, but less than 2% of them were ARB resistant to MER and < 19% resistant to AZI, similar to reported by Hu et al. (2021).

Given this, it is worth highlighting that ARBs were detected in the treated water (0% to 83% for AZI and 0% to 38% for MER), indicating that the chlorination step may have selected bacteria resistant to azithromycin and meropenem, even in the presence of residual chlorine content and low turbidity (which could serve as a "harbor" for these organisms).

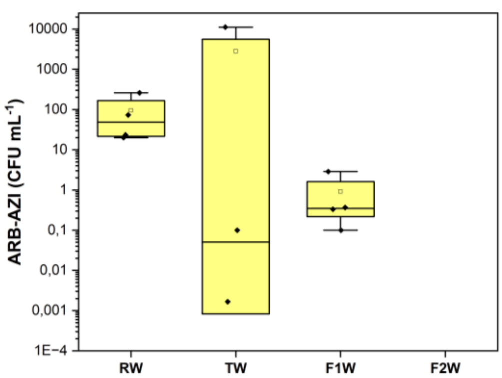
	RW	TW	F1W	F2W
Turbidity (NTU)	2.26 ± 1.05	1.03 ± 0.22	1.07 ± 0.47	1.77 ± 0.62
pH	7.99 ± 0.90	7.66 ± 0.82	7.64 ± 0.62	6.94 ± 0.32
Residual Chlorine (mgCl$_2$ L^{-1})	n.a.	0.48 ± 0.22	n.a.	n.a.

Table 1: Monitoring of physical-chemical configurations of water samples, for the four sampling campaigns (n=4) n.a. = not analyzed

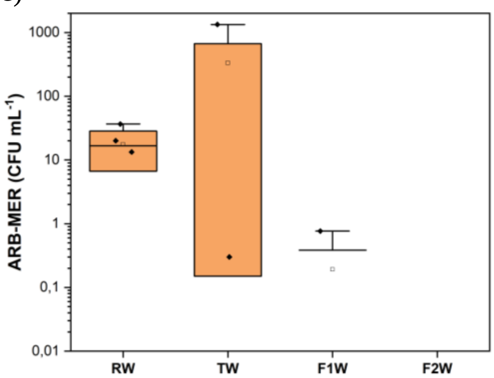
a)



b)



c)



d)

		Feb/24	May/24	jun/24	Aug/24
ARB AZI (%)	RW	1,66	4,0	5,6	19
	TW	83	13	0	2,6
	F1W	1,47	9	3,2	28
ARB MER (%)	RW	1,66	2,0	0	0,97
	TW	10	38	0	0
	F1W	0	0	0	8

Figure 1: Variation in the concentration of total heterotrophic bacteria - THB (a), azithromycin-resistant bacteria – ARB-AZI (b), meropenem-resistant bacteria – ARB-MER (c), and relative abundance (%) of resistant bacteria (d) for the four sampling campaigns (n=4). For F2W, no ARBs were detected in any of the sampling campaigns (d).

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