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Identification and Quantification of Antibiotic Residues and Evaluation of Microbial Resistance to Antibiotics in Huatanay River Waters in Peru

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ABSTRACT

The Huatanay River in Cusco-Peru, is affected by wastewater discharges along its course. In order to evaluate this impact, we evaluate antibiotic residues and their impact on the increase of bacterial resistance in the city of Cusco treatment plant. For this purpose, water samples from the influent and effluent of the treatment plant were analyzed by chromatographic methods; additionally, sensitivity tests were performed with three bacterial strains (*Escherichia coli*, *Salmonella sp*., and *Klebsiella sp*.), which were isolated from the same place. Six antibiotic residues were identified (ceftriaxone, amoxicillin, trimethoprim, sulfamethoxazole, dicloxacillin, and lincomycin). Those found in the highest concentration were: amoxicillin (91495 and 0 µg/L) and lincomycin (33970 and 10800 µg/L) in the influent and effluent, respectively. There is more resistance in the effluent than the influent in the case of *E. coli* shows resistance in the effluent to cephalexin (30 µg) and azithromycin (15 µg). *Salmonella sp.* is resistant to amoxicillin (15 µg), dicloxacillin (1 µg), lincomycin (2 µg), ceftriaxone (30 µg), cephalexin (30 µg), and ciprofloxacin (5 µg). Finally, *Klebsiella sp.* is sensitive to ceftriaxone (30 µg), amoxicillin (15 µg), and cephalexin (30 µg). This confirms that the antibiotic residues contained in the wastewater of Cusco generate resistance in the isolated bacteria.

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INTRODUCTION

Antibiotics have long been considered miraculous compounds that save lives because they are useful against bacterial infections (Mohammad et al., 2021; Chabilan et al., 2022; Gozzo et al., 2023). However, these residues arrive in wastewater (Sarafraz et al., 2020; Gozzo et al., 2023) and from it to the environment, causing water quality degradation and affecting the entire biological community in these ecosystems (Almeida et al., 2017; Mohammad et al., 2021); even generating antibiotic-resistant bacteria and genes (Novo et al., 2013; Zhang et al., 2021; Gozzo et al., 2023). Additionally, contributed to spreading this bacteria and residues to aquatic ecosystems like rivers (Carvalho y Santos, 2016; Binh et al., 2018; Chabilan et al., 2022). These polluted waters are used for various activities, such as irrigation and aquaculture, and reach humans through water consumption (Lehutso et al., 2017). This represents a risk because

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*Corresponding Author Email: *fzirenav@unam.edu.pe* it affects the population's health (Carmona et al., 2017; Zhang et al., 2019; Pournamdari y Geramizadegan, 2023).

Likewise, in developed countries, discharges of antibiotic residues and antibiotic-resistant genes have been widely recorded and studied (Diwan et al., 2010), while in developing countries, this type of study is still scarce. In the area where the present study was carried out, there is only one study that analyzed wastewater samples, in which residues of several antibiotics were found in the WWTP of Cusco (Nieto-Juarez et al., 2021). The effluent from this plant is discharged directly into the Huatanay River, which flows through the city of Cusco, and there are also several illegal dumping points for domestic wastewater along its course, which degrades water quality. This river is a tributary of the Vilcanota River basin, which flows through several districts and population centers where tourism, agriculture, aquaculture, livestock, and commerce are the main activities (INGEMET, 2011). It receives treatment in a plant equivalent to 85% of the city's wastewater (Nieto-Juarez et al., 2021), which is then discharged back into the river, resulting in pollution.

For this reason, it is important to study antibiotic pollution in the rivers, because the rivers receive treated and untreated wastewater, which increases the concentration of the pollutant in river water (Sarafraz et al., 2020; Geramizadegan et al., 2022). This problem is generalized around the world, like Asian countries (Binh et al., 2018; Wang et al., 2018; Liu et al., 2021; Mohammad et al., 2021), Europe (Carvalho y Santos, 2016; Carmona et al., 2017; Gozzo et al., 2023), Africa (Lehutso et al., 2017) and América (Lopes et al., 2016). Unfortunately, these water sources are used for different activities by the surrounding communities, such as human consumption (Liu et al., 2021) and irrigation (Santiago et al., 2018), which represents a risk, because poor people usually use this water without any treatment, even for consumption for animals and persons.

This research aimed to identify and quantify the antibiotic residues in waters of the Huatanay Cusco river pre- and post-treatment of the WWTP, using the HPLC technique, and also to evaluate the bacterial sensitivity or resistance through microbiological analysis of three bacteria isolated from the treatment plant, *Escherichia coli*, *Salmonella* sp., and *Klebsiella* sp.

MATERIALS AND METHODS

Study area and sample collection

The study was conducted in the department of Cusco, province of Cusco, district of San Jeronimo, 20 km south of the city at 3345 meters above sea level, where the WWTP of San Jeronimo (Cusco) is located. The Huatanay River receives all the city's wastewater directly, so the treatment plant processes the equivalent of 85% of the city's wastewater, which enters the plant from the Huatanay River and is then discharged back into the same water source. The average inflow volume is 446 L/s (Nieto-Juarez et al., 2021). Samples were taken according to national regulations for surface water sampling (MINSA, 2007). The samples were taken in glass bottles of 500 ml capacity and then refrigerated with ice at 4 °C until arrival at the laboratory, in a time not exceeding 2 hours. A total of 20 samples were taken for the influent and 20 for the effluent, a total of 40 samples. To stabilize the samples, ascorbic acid was added at 1 %. The formula applied to identify the percentage of removal was as follows:

$$\text{Removal percentage } \% = \frac{(C_i - C_e)}{C_i} * 100 \qquad (1)$$

Where Ci is the concentration of antibiotics in the influent, Ce is the concentration in the effluent. (Nieto-Juarez et al., 2021).

Antibiotics and Chemicals

The chemicals and reagents used in the analyses, such as methanol, acetonitrile, formic acid, Na_2EDTA, citric acid monohydrate, orthophosphoric acid, sodium citrate dihydrate, and hydrochloric acid, were purchased from MERCK HPLC grade. H_3PO_4 from J.T. Baker. Antibiotic standards such as ciprofloxacin (CIP), amoxicillin (AMX), dicloxacillin (DCX), ceftriaxone (CFX), lincomycin (LCM), gentamicin (GEN), Cefalexin (CPN), and Azithromycin (AZM) were purchased from Sigma-Aldrich (Texas, USA). Individual standards were prepared in methanol at 1 mg mL^{-1}. The solutions were stored in the dark at −20 °C and prepared before to use. Working standard mixtures (1 g L^{-1}) whit, the six compounds were prepared in methanol and diluted to the appropriate concentration in methanol/water (30:70, v/v).

Chromatographic conditions

Antibiotic residues were analyzed on an Agilent 1200 series chromatograph equipped with a diode array detector (DAD), an automatic injector, a binary pump, a vacuum degasser, and a thermostated column compartment. An Agilent Zorbax XDB-C8 column (4.6 mm diameter, 75.0 mm long, particle size 3.5 µm, Pre-Column Zorbax Eclipse XDB-C18 4.6 x 12.5 mm x 5um at a constant temperature of 40°C was used. Regarding the mobile phase, it was used for phase A (ultrapure water with 0.1% phosphoric acid) and phase B (Acetonitrile) as follows: 0 % B (3 min), 0-15 % B (5 min), 15-50 % B at 12 min, 50-60 % B at 14 min, 60-70 % B at 15 min, 70-100 % B at 21 min at a flow rate of 0.6 ml/min. Detection was monitored at 202 nm and the injection volume was 10.0 µl. Data acquisition was performed using Chemstation V03.02 software.

Sample preparation and solid phase extraction

The method described by Benito-Peña et al. (2006) was used, and the samples were filtered through cotton filters. To avoid degradation, 1g of ascorbic acid was added and stored at 4 °C until analysis less than 24 hours after sampling. 100 mL of sample was transferred to a separation bulb together with 100 uL of H_3PO_4, and 0.5g of Ca SO_4 was homogenized, then mixed with 3ml of chloroform; the organic phase was discarded, and the aqueous phase was filtered under vacuum through a funnel with compressed cotton into a Kitasate flask. Subsequently, the solid phase extraction was started. For this, an Agilent vacuum pump with 10 positions was used. The Oasis HBL 3cc Vac Cartridge, 60 mg, were conditioned with 6 ml of methanol (Carmona et al., 2017), followed by 10 ml of 0.01% HCl, then loaded with 100 ml of the sample filtrate; at the end, 10 ml of 0.01% HCl was passed to wash, and then the retained analytes were eluted with 2 ml of acetonitrile and divided into two vials at 1 ml in each one (Fig. 1).

Samples analyses

Before performing the sampling analysis, calibration curves from 40, 50, 100, 200, and 400 ug/mL were prepared, and the correlation coefficient for all the compounds analyzed was ≥ 0.99

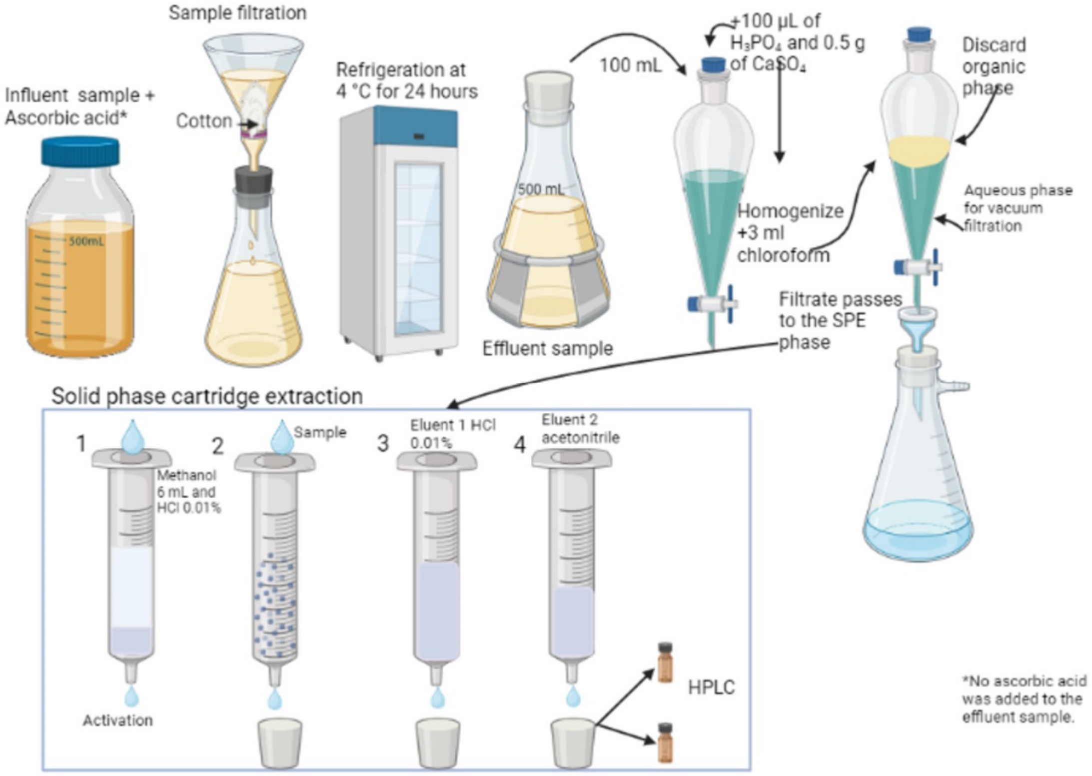
Physicochemical characteristics of wastewater

Measurements of physicochemical parameters were taken on the exact sampling dates of pre-and post-treatment wastewater, according to the protocols of the Cusco Water Treatment Plant (PTAR-Cusco). The variables considered were: BOD_5, thermo-tolerant coliforms, pH, temperature, conductivity, total solids, nitrites, nitrates, turbidity, and flow rate. These measurements were carried out using the techniques standardized by APHA and other standardized methods (APHA, 2017).

Bacterial resistance assessment

Sample preparation

For microbiological analysis, dilutions of 1/10 and 1/100 with sterile deionized water were performed on the heavily contaminated samples. Those not (effluent) were seeded undiluted in



*No ascorbic acid was added to the effluent sample.

Fig. 1. Methodological scheme of sample processing.

a series of tubes chosen according to the corresponding MPN (most probable number) table. They were considered positive if the medium was turbid and the colonies fermented lactose with gas production (more than 20% in Durham hoods placed in each tube with brila broth) (APHA, 2017).

Escherichia coli confirmatory tests

For this objective, the NCCLS (National Committee for Clinical Laboratory Standards) protocol was followed (Cavalieri et al., 2005), in which the bacteria were cultured on EMB (Eosin Methylene Blue) Agar medium for isolation and then proceeded to biochemical identification and confirmation tests for gram-negative bacteria. They were considered positive if the colonies fermented lactose with gas production (more than 20% in Durham hoods placed in each tube with brila broth) and the medium was turbid. The *Escherichia coli* confirmation test was positive when there was a growth of metallic green to black colonies. From the growth on EMB Agar, the remaining biochemical tests and staining were performed by subculturing on the following media: Tryptone water, Simmons citrate (sowing on the agar surface), and Gram stain (APHA, 2017).

Salmonella sp. confirmatory tests

The sample was received in peptonated water (0.1 %), pre-enrichment was performed to rehabilitate the *Salmonella* cells; then enrichment was performed in tetrathionate base broth (Merck) to increase the number of *Salmonella* bacteria and inhibit other microorganisms in the sample. The isolation was performed in Hektoen enteric agar (Merck), observing greenish blue to blue colonies with and without black centers, and Xylose Lysine Deoxycholate agar (Merck) where pink colonies with and without black centers were distinguished; both media allowed to see suspicious colonies of *Salmonella*; and finally, the biochemical identification that allowed to determine the metabolic activity of this bacterium, in Urea agar (Merck), iron lysine agar (LIA, Merck) and triple sugar iron agar (TSI, Merck). In addition, tubes were inoculated with indole acid sulfhydric acid medium for sulfide, indole, and motility (SIM Merck) (FDA, 2022).

Klebsiella sp. confirmatory tests

A roast from the first positive tube of the MPN test was taken and cultured on MacConkey agar medium for 24 hours at 35°C for isolation, looking for *Klebsiella* spp as a positive lactose-positive mucous colony with growth in extension. Subsequently, biochemical identification and confirmation tests for gram-negative bacteria were performed: triple sugar iron agar (TSI, Merck), lysine iron agar (LIA, Merck), and Urea agar (Merck). In addition, tubes were inoculated with indole acid sulfhydric acid medium for sulfide, indole, and motility (SIM Merck) (APHA, 2017).

Bacterial sensitivity test

In order to verify the antimicrobial susceptibility profile of the bacterial isolates, the standard Kirby-Bauer disk diffusion method was used (Cavalieri et al., 2005; Dires et al., 2018), which consists of the following steps: first, bacterial species are selected, isolated and identified, then the inoculum is prepared and standardized. Next, the plates are inoculated, and the selected antibiotic discs are added and incubated at 37±2 °C; then, the corresponding inhibition halos are measured, interpreting the result based on their sensitivity or resistance.

Statistical analysis

The Wilcoxon test was performed with the antibiotic concentration data in the influent and effluent since it did not meet the assumptions required to apply parametric analysis. In the case of bacteria isolated from the influent and effluent, the Student's t-test was applied after checking the assumptions required for the analysis. Data management and analysis were performed in Excel spreadsheet and R Studio version 4.0.0.

RESULTS AND DISCUSSION

Residues of antibiotics in the WWTP Cusco

Six antibiotics residues were found in the influent to the treatment plant (Table 1). Amoxicillin (91495 µg/L) and lincomycin (33970 µg/L) were the most abundant. Often in similar studies, it is found that of all the antibiotics evaluated, only some are found in the residues, mainly because they are only some of the most widely used by the population, and the other antibiotics are found in such minimal concentrations that they are undetectable (Diwan et al., 2010).

Amoxicillin is an antibiotic derived from penicillin (Bhattacharjee, 2016). In the samples analyzed, high concentrations were recorded in the influent (91495.0 µg/L), with an average value of 0 µg/L. Other studies found similar results for amoxicillin, as it is a predominant metabolite in wastewater samples (Diwan et al., 2010; Binh et al., 2018; Nieto-Juarez et al., 2021) due to its widespread use since it is an antibiotic with a broad spectrum of action, which acts on large Gram-positive and harmful bacteria (Bhattacharjee, 2016). Therefore, its use during the pandemic (study period) has also increased due to its efficiency in treating respiratory infections and their symptoms, even when it is self-medicated by the same population (Gonzalez-Zorn, 2021). Therefore, its residues are frequently found in wastewater samples. It is also worth mentioning that amoxicillin, due to its capacity to generate bacterial resistance, has been considered among the five antibiotics that must be monitored in aquatic ecosystems in the European Union in order to care for the integrity of water quality (Rodriguez-Mozaz et al., 2020). The fact that monitoring lists are generated, prioritizing some compounds, demonstrates the growing concern about the existence of these residues in the environment.

Table 1. Presence of antibiotics and Wilcoxon test analysis in wastewater treatment plant of Cusco city.

Antibiotics	Abbreviation	Source	Average µg/L	p-value	Average differences	Average removal %
Ceftriaxone	CFX	Influent Effluent	7940 590	9.53E-05	7350	93.3
Amoxicillin	AMX	Influent Effluent	91495 0	1.91E-06	91495	100
Trimethoprim	TMP	Influent Effluent	2815 0	9.46E-05	2815	100
Sulfamethoxazole	SMT	Influent Effluent	1195 0	0.000141	1195	100
Dicloxacillin	DCX	Influent Effluent	350 1795	9.26E-05	-1445	-229.7
Lincomycin	LMC	Influent Effluent	33970 10800	9.56E-05	23170	65.5

Concerning lincomycin, high concentrations were also recorded in the influent (33970 µg/L). This antibiotic inhibits protein synthesis by binding to the 50S ribosome, as do macrolides (Bhattacharjee, 2016). It is a frequently used antibiotic; therefore, its residues are also often found in wastewater, although in lower concentrations of 60.7±0.44 ng/L (Chen, 2014). In another study in which antibiotic residues were analyzed at the same study site, lincomycin was found (0.28 µg/L), a much lower concentration than that recorded in this study (Nieto-Juarez et al., 2021). Similarly, another study found low concentrations of this compound (100.33 and 150.08 ng/L) (Harrabi et al., 2018). This difference may be because the studies above were conducted before or at the pandemic's beginning. In contrast, the results of this study came from the pandemic period when antibiotics such as lincomycin were widely used to combat respiratory diseases such as pneumonia, a complication of COVID-19 (Forestieri et al., 2021).

As for ceftriaxone, an average of 7940 µg/L was found. In this regard, Gonzalez-Zorn (2021) found that the consumption of this antibiotic increased by 204% compared to 2020. This corroborates its increased consumption by the population in the context of the pandemic, which has contributed to the increased amount of residues in wastewater. Furthermore, even before COVID, this antibiotic was frequently found in wastewater samples in Latin America (Reichert et al., 2019). Trimethoprim was only found in the influent (2815 µg/L average). This antibiotic is used in aquaculture, livestock, hospitals, pharmaceutical manufacturing, and domestic consumption (Binh et al., 2018); therefore, its wide application is increasing even more. Similarly, sulfamethoxazole was found only in the influent (1195 µg/L average). These are higher concentrations than those found in the Titicaca basin, where sulfamethoxazole and trimethoprim were also found at 46 to 106 ng/L, respectively, in the dry season and 145 to 312 ng/L in the wet season (Archundia et al., 2017). In another study on wastewater samples from Tunisia, sulfamethoxazole was recorded at 126.70 ng/L (Harrabi et al., 2018), which shows that this antibiotic is widely used worldwide.

In the case of dicloxacillin, it was found at an average of 350 µg/L in the influent, while higher concentrations were found in the effluent, averaging 1795 µg/L. Factors that could explain this may include the removal of antibiotics adsorbed on the particulate material during sample processing and unaccounted hydraulic retention time during sampling. Physicochemical changes during the treatment process influence the adsorption of the antibiotics and therefore affect the partition ratio between the aqueous, suspended, and sediment phases and between the influent and effluent concentrations. Accumulating active pharmaceutical ingredients, biotic or abiotic dissolution, reverse transformation, and deconjugating metabolic products back to the original compounds can increase measured effluent concentrations (Haddad et al., 2015). It is also worth mentioning that the physicochemical treatments show limited removal percentages concerning dicloxacillin (Rivera-Gutiérrez et al., 2020).

Regarding the removal of antibiotic residues in the wastewater contained in the Huatanay River, the Wilcoxon test at 95% confidence showed that the wastewater treatment at the WWTP significantly influences the removal of ceftriaxone (p=0.000), eliminating 93.3% of this substance, amoxicillin (p=0.000) eliminating 100.0%, trimethoprim (p=0.000) eliminating 100%, sulfamethoxazole (p=0.002) eliminating 100% and lincomycin (p=0.000) eliminating 65.5% of the wastewater from the Huatanay river. Because five antibiotics were removed in good percentages, even three were removed at 100% (amoxicillin, trimethoprim, and sulfamethoxazole) (**Fig. 2**). However, in the case of dicloxacillin, the plant influenced its increase in the effluent, which is not new since similar results have been found in other studies about other antibiotics (Nieto-Juarez et al., 2021). This is mainly due to solid matrix effects (mainly ionization suppression), which would report lower concentrations in the influent and higher concentrations in the effluent (Lehutso et al., 2017).

Therefore, it became evident that wastewater treatment plants (WWTPs) are essential in eliminating antibiotics. However, additional tertiary treatment is required to eliminate these compounds (Carvalho y Santos, 2016). Since, as evidenced in this study, the existing treatment is insufficient to remove these compounds from the aquatic environment. Artificial wetlands may be a sustainable and economically viable alternative as their installation does not require high technology or costs (Chen, 2014; Maldonado et al., 2022).

In addition, before designing any treatment, it is necessary to consider the physicochemical characteristics of the sample since these will influence whether the antibiotic residues are deposited in the sediments or are more diluted in the water (Chabilan et al., 2022). Since biological and physicochemical processes govern the life cycle of antibiotics in the environment in soil-water systems, particularly stability, sorption, leaching, and degradation, which depend on the physicochemical properties of the antibiotics (Table 2), and other environmental factors (Table 2). Biological and physicochemical processes govern the life cycle of antibiotics in the environment in soil-water systems, particularly stability, sorption, leaching, and degradation,

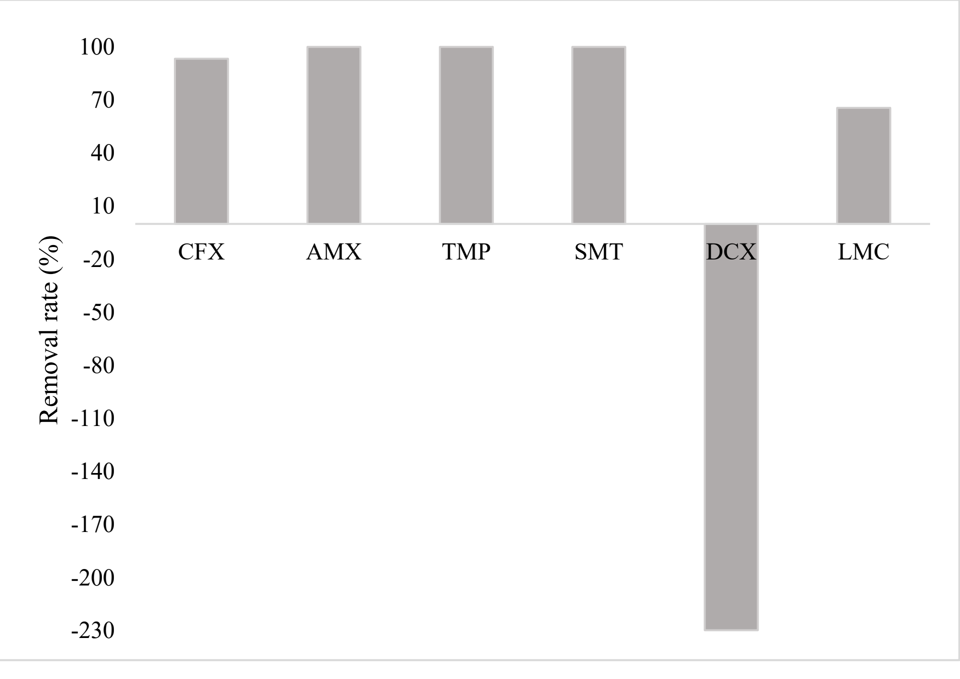


Fig. 2. The efficiency of the plant in the removal of antibiotics.

Table 2. Physicochemical characteristics of antibiotics found in wastewater samples from the city of Cusco treatment plant.

Antibiótic	**Molecular weight (g/mol)**	**Solubility (mg/L)**	**Log Kow**	**PKa**
Amoxicillin	365.41	3,430	0.87	3.2 and 11.7
Ceftriaxone	554.58	105		
Dicloxacillin	470.327			2.50–2.80
Lincomycin	406.538			7.6
Sulfamethoxazole	253.28	610	0.89	5.4
Trimethoprim	290.32			

Table 3. Susceptibility of *Escherichia coli* to antibiotics found in wastewater from the city of Cusco.

Antibiotics	Conc. µg	Source	Sensible		Intermedium		Resistant		Student's T (p-value)
			Freq.	%	Freq.	%	Freq.	%	
Amoxicillin	15	Influent	0	0	6	100	0	0	1
		Effluent	0	0	6	100	0	0	
Dicloxacillin	1	Influent	6	100	0	0	0	0	1
		Effluent	6	100	0	0	0	0	
Gentamicin	10	Influent	6	100	0	0	0	0	0.014
		Effluent	0	0	**6**	100	0	0	
Lincomycin	2	Influent	6	100	0	0	0	0	0.014
		Effluent	0	0	**6**	100	0	0	
Ceftriaxone	30	Influent	6	100	0	0	0	0	0.014
		Effluent	0	0	**6**	100	0	0	
Cefalexin	30	Influent	0	0	6	100	0	0	0.014
		Effluent	0	0	0	0	**6**	100	
Ciprofloxacin	5	Influent	6	100	0	0	0	0	1
		Effluent	6	100	0	0	0	0	
Azithromycin	15	Influent	0	0	6	100	0	0	0.014
		Effluent	0	0	0	0	**6**	100	

which depend on the physicochemical properties of the antibiotics (Table 2), and other environmental factors (Table 3) (Carvalho y Santos, 2016). The antibiotics with Positive log (Kow) values indicate lipophilic/hydrophobic compounds. A low and negative log (Kow) indicates hydrophilic compounds (Chabilan et al., 2022). Therefore, it is necessary to consider these characteristics when designing a treatment plant to be efficient in the removal process.

Variations in the antibiotic susceptibility of Escherichia coli strain

At 95% confidence of the Student's t-test, it is evident that the treatment of wastewater at the WWTPS does not significantly influence the microbial resistance of the strains isolated from the Huatanay river water against the antibiotics: amoxicillin, dicloxacillin, and ciprofloxacin ($p>0.05$) (Table 3).

Most antibiotic-resistant genes and bacteria contamination come from wastewater treatment plant effluents (Novo et al., 2013; Ben et al., 2019; Reichert et al., 2019). Therefore, these bacteria will be found in most rivers impacted by this type of discharge (Palhares et al., 2014; Loudermilk et al., 2022). *Escherichia coli* is widely used for sensitivity testing (Ben et al., 2019). Bacteria can develop resistance through various mechanisms, such as the TolC efflux pump in *E. coli* and enzyme modification of antibiotics and their metabolites (Kumar et al., 2019).

The results of this study showed that *E. coli* does not show resistance to antibiotics (amoxicillin, dicloxacillin, and ciprofloxacin). However, it is known that amoxicillin is widely studied for its capacity to generate bacterial resistance (Reichert et al., 2019). Likewise, in another study with E. coli, the multiresistant capacity of this bacterium was found to be 90.6% susceptible, 6% intermediate, and 3.4% resistant to amoxicillin+clavulanic acid (Larson et al., 2019). Additionally, Sakkas et al. (2019) found that *E. coli* showed resistance in 79.2% of the samples (n=24) to the antibiotics amoxicillin + clavulanic acid (20/10 µg). Thus, showing that amoxicillin can generate this *E. coli* resistance. Similarly, another study in which *E. coli* was isolated from hospital wastewater showed multi-resistance to antibiotics such as ampicillin (81.8%) and amoxicillin+clavulanic acid (72.7%) (Dires et al., 2018). For this reason, even in the European community, it has been included among the five antibiotics that must be monitored due to the environmental risks it represents (Rodriguez-Mozaz et al., 2020).

Similarly, other studies have shown that dicloxacillin generated bacterial resistance in *E. coli* at 2450 and 4000 µg/L (Rivera-Gutiérrez et al., 2020). This may be because the study's bacteria came from hospital effluent, which usually contains more antibiotics. In contrast, the bacteria in this study were isolated from the treatment plant, where the concentration of antibiotics is generally lower. Concerning amoxicillin, in this study, there was no evidence of resistance to the antibiotic at 15 µg. The fact that resistance has not yet been found in this study does not imply that there are no risks since it will probably be found in the future, as evidenced in other studies in which the sensitivity of *E. coli* to different antibiotics was evaluated, including amoxicillin, in the first study, it was found to be sensitive and intermediate.

Therefore, it is likely that, with constant contact with this residue, resistance to antibiotics may develop over a more extended period, generating a growing concern. On the other hand, it significantly influences the microbial resistance of the strains isolated from the Huatanay river water against the antibiotics: gentamicin, with a change to intermediate in the effluent and sensitivity in the influent samples. Similarly, a study showed that gentamicin (10 µg) in wastewater-disturbed *E. coli*, is considered sensitive (Lopes et al., 2016).

Regarding lincomycin: observed in the influent sensitivity and the effluent a change to intermediate; Ceftriaxone: observed in the influent sensitivity and the effluent a change to intermediate; cephalexin: observed in the influent to be intermediate and in the effluent a change to resistant. Finally, azithromycin: intermediate in the influent and a change to resistance in the effluent. Similarly, in a study in which the resistance of *E. coli* to different antibiotics was evaluated, it was found that *E. coli* was considered a multiresistant bacterium because it showed characteristics of susceptibility to antibiotics such as ampicillin, cefoxitin, Cefaclor Imipenem, Gentamicin, ciprofloxacin, Norfloxacin and chloramphenicol (sensitive) and for amoxicillin, cephalothin and streptomycin (intermediate), which shows that the evaluated area was contaminated with wastewater, from which this bacterium came as well as the antibiotic residues that generated this resistance (Lopes et al., 2016).

In addition, wastewater effluent can contaminate water resources with resistant bacteria. In this regard, in a study in which drinking water samples were analyzed in Cajamarca-Peru, *E. coli* resistant to antibiotics (tetracycline, ampicillin, sulfamethoxazole-trimethoprim, and nalidixic acid) were found (Larson et al., 2019). This shows that rural areas, where less contamination is expected, are also contaminated with this type of bacteria.

Variations in the antibiotic susceptibility of Salmonella sp. strain

Salmonella sp. is a species widely distributed in water contaminated with sewage, which has also developed resistance, mainly to amoxicillin, when in contact with antibiotic residues (Bhattacharjee, 2016). The results of this study show that the microbial resistance of the isolated strains of Salmonella sp in the waters of the Huatanay river to the antibiotics amoxicillin, dicloxacillin, lincomycin, ceftriaxone, cephalexin, and ciprofloxacin before treatment at the WWTP was 100% resistant (Table 4). Another study in which *Salmonella* was subjected to antibiotics such as ciprofloxacin showed that a concentration of 25 µg/l can have toxic effects

Table 4. Susceptibility of *Salmonella* sp. to antibiotics found in the sewage effluent of the city of Cusco.

Antibiotics	Conc. µg	Source	Sensible Freq.	Sensible %	Intermedium Freq.	Intermedium %	Resistant µg	Resistant %
Amoxicillin	15	Influent	0	0	0	0	6	100
		Effluent	0	0	0	0	0	0
Dicloxacillin	1	Influent	0	0	0	0	6	100
		Effluent	0	0	0	0	0	0
Gentamicin	10	Influent	0	0	6	100	0	0
		Effluent	0	0	0	0	0	0
Lincomycin	2	Influent	0	0	0	0	6	100
		Effluent	0	0	0	0	0	0
Ceftriaxone	30	Influent	0	0	0	0	6	100
		Effluent	0	0	0	0	0	0
Cefalexin	30	Influent	0	0	0	0	6	100
		Effluent	0	0	0	0	0	0
Ciprofloxacin	5	Influent	0	0	0	0	6	100
		Effluent	0	0	0	0	0	0
Azithromycin	15	Influent	6	100	0	0	0	0
		Effluent	0	0	0	0	0	0

on *Salmonella* (Ao et al., 2018) because it is a bactericidal antibiotic (Kumar et al., 2019).

Additionally, it should be noted that this species is also known as multiresistant due to its resistance response to several antibiotics, as evidenced by a study in which this species was isolated from animal excrement, of which 18% of the isolated samples had a multi-resistance response (Palhares et al., 2014). On the other hand, in another research in which *Salmonella* was isolated from hospital wastewater, it was found that this species could be multi-resistance to antibiotics such as ampicillin, doxycycline, erythromycin, ceftazidime, cefoxitin, and chloramphenicol (Dires et al., 2018). Likewise, in another study in which *Salmonella* was isolated from various wastewater effluents, the isolated bacteria showed resistance to numerous antibiotics, such as beta-lactamases, chloramphenicol, and quinolones (Masarikova et al., 2016). Also, in another investigation in which *Salmonella* was isolated from wastewater samples, it was found that *Salmonella* showed a multi-resistance capacity to antibiotics, for tetracycline (47.5% of the isolated bacteria) and sulfamethoxazole (38. 5%), followed by ampicillin (25.3%), streptomycin (17.6%), chloramphenicol (CHL, 15.4%), gentamicin (11.3%) and low resistance to norfloxacin (0.45%), ciprofloxacin (0.9%) and cefotaxime (0.9%) (Zhang et al., 2019). Similarly, another study in which 23 *Salmonella* strains resist one or more antibiotics compounds (Santiago et al., 2018) shows that this species can resist several antibiotics simultaneously.

Some aspects contribute to developing this resistance, the best known of which are two mechanisms. First, decrease the antibiotic concentration to a level below the Minimum Inhibitory Concentration (MIC) so that it has no significant inhibitory effect on the bacteria. Second, alter the target of the antibiotic so that it is no longer affected by the antibiotic (Bhattacharjee, 2016). Additionally, in *Salmonella,* integrons are genetic platforms that enable the bacteria to capture antibiotic resistance genes, as evidenced by Zhang et al. (2019), who found that bacteria with integrons developed more excellent bacterial resistance than those that did not. In addition, another study also found integrons in resistant bacteria (Masarikova et al., 2016). This shows that the presence of integrons is one of the mechanisms of bacterial resistance.

On the other hand, before treatment with the antibiotic gentamicin, the strain of *Salmonella* sp. is of intermediate sensitivity, and concerning azithromycin, it is sensitive; however, after effluent treatment, this strain was not isolated because it was not found in the effluent; therefore, it was not possible to evaluate if there were changes in the susceptibility to the antibiotics as mentioned above. The treatment of wastewater from the Huatanay River at the WWTP with chlorine before discharging the treated water back to the riverbed may have caused the absence of the *Salmonella* sp. strain in the effluent water samples analyzed. Therefore, this would be evidence of the effectiveness of the treatment plant's treatment concerning this bacterial strain.

Variations in the antibiotic susceptibility of Klebsiella sp. strain

Klebsiella is another bacterium frequently found in wastewater samples and sewage-contaminated sources, mainly contaminated with hospital wastewater (Loudermilk et al., 2022), and in addition, multi-resistance to antibiotics has been found (Ben et al., 2019; Kumar et al., 2019). At 95% confidence of the Student's t-test, it is evident that the wastewater treatment at the WWTPS significantly influences ($p<0.05$) the microbial susceptibility profile of the isolated strains of *Klebsiella* sp. from the Huatanay river water against the antibiotics analyzed (Table 5).

Concerning amoxicillin, the influent presents sensitivity and a change to resistance in the effluent. Similarly, in southern Brazil, it was found that the presence of amoxicillin in wastewater influenced the appearance of bacterial resistance (Lopes et al., 2016). Another study found that *K. pneumoniae* showed resistance in 75% of the samples with amoxicillin+clavulanic acid. (Sakkas et al., 2019), Amoxicillin is an antibiotic widely present in wastewater and causes resistance in *E. coli*, *Klebsiella*, and other enteric bacteria (Novo et al., 2013). In addition, *Klebsiella* lives in the environment with its capacity for resistance, which is why it can be found in aquatic ecosystems contaminated with wastewater, with this capacity for resistance (Palhares et al., 2014; Loudermilk et al., 2022).

Regarding the other antibiotics, dicloxacillin was sensitive in the influent and intermediate in the effluent. Moreover, concerning ceftriaxone and cephalexin, a change in resistance was found in the influent sensitivity and the effluent. On the other hand, there is no significant influence ($p>0.05$) on the microbial susceptibility of the isolated strains of *Klebsiella* sp. from Huatanay river water against the antibiotics: gentamicin, lincomycin, ciprofloxacin, and azithromycin.

As has been evidenced, this bacterium is a multiresistant bacterium, which is also corroborated by the current scientific information (Kumar et al., 2019). Species are found even in water samples with this antibiotic-resistant capacity (Betalactamases) (Larson et al., 2019). This species has even shown some resistance to fosfomycin, an antibiotic used to combat

Table 5. Susceptibility of *Klebsiella* sp. to antibiotics found in the wastewater effluent of the city of Cusco.

Antibiotics	Conc.	Source	Sensible		Intermedium		Resistant		Student's T (p-value)
	µg		Freq.	%	Freq.	%	Freq.	%	
Amoxicillin	15	Influent	6	100	0	0	0	0	0.001
		Effluent	0	0	0	0	6	100	
Dicloxacillin	1	Influent	6	100	0	0	0	0	0.001
		Effluent	0	0	6	100	0	0	
Gentamicin	10	Influent	6	100	0	0	0	0	1
		Effluent	6	100	0	0	0	0	
Lincomycin	2	Influent	6	100	0	0	0	0	1
		Effluent	6	100	0	0	0	0	
Ceftriaxone	30	Influent	6	100	0	0	0	0	0.001
		Effluent	0	0	0	0	6	100	
Cefalexin	30	Influent	6	100	0	0	0	0	0.001
		Effluent	0	0	0	0	6	100	
Ciprofloxacin	5	Influent	6	100	0	0	0	0	1
		Effluent	6	100	0	0	0	0	
Azithromycin	15	Influent	6	100	0	0	0	0	1
		Effluent	6	100	0	0	0	0	

Table 6. Physicochemical parameters of water quality at the Cusco treatment plant.

Parameters	Influent	Effluent	ECAs	Unit
DBO_5	537.25	307.75	10	mg/L
Thermotolerant coliforms	35500000	3132.5	0.0001	mg/L
pH	8.37	7.92	6,5 a 9,0	
Temperature	16.9	17.1	Δ 3	ºC
Conductivity	1721.3	1609.65	1 000	uS/cm
Total solids	1468.5	896.5	≤ 25	mg/L
Nitrites	0.024	0.2285		mg/L
Nitrates	2.095	2.115	13	mg/L
Turbid	502.6	30.65		NTU
Flow	754.875	637.265		L/s

bacteria that are multiresistant to antibiotics (Kumar et al. 2019); therefore, it poses risks to the environment and human health.

Wastewater is a primary source of antibiotic-resistant bacteria (Masarikova et al. 2016); *Escherichia coli*, *Salmonella* sp., and Streptomyces sp. bacteria are frequently found in this type of water, mainly if these are contaminated with hospital waste, as evidenced in this study.

Physicochemical parameters of the influent and effluent of the treatment plant

Concerning the physicochemical parameters of the plant, in general, the inlet parameters show poor water quality; however, in the effluent, an improvement in the physicochemical parameters is perceived. Peruvian environmental quality standards compared these parameters with the values considered for aquatic ecosystems (rivers) (MINAM, 2017). This information can be seen in Table 6, where the effluent parameters are still above the values considered in the Environmental quality standard of Perú (ECAs), although the quality improved in contrast to the effluent.

In studies evaluating the removal efficiency of wastewater treatment plants, in most cases, treatment plants improve wastewater quality (Lopes et al., 2016), although not those expected by the legislation (MINAM, 2017). As a result, the effluent water quality is still poor. For example, in the case of BOD5, although it does not meet the standards required by national laws (537.25 mg/L), it decreases significantly compared to the influent (307.75 mg/L). The same is true for thermotolerant coliforms, decreasing from 35500000 CFU in the influent to 3132.5 CFU in the effluent, which is somewhat acceptable to the environment, similarly, for conductivity and total solids. The only values that comply with national legislation are the nitrate and pH values in the influent and effluent. Therefore, in general, the treatment plant in the city of Cusco generates better water quality and removal of antibiotics compared to the existing treatment plants in Lima. (Nieto-Juarez et al., 2021).

CONCLUSION

The presence of antibiotic residues is evidenced in the wastewater effluent samples from the Cusco treatment plant, of which amoxicillin (91495 µg/L) and lincomycin (33970 µg/L) are the ones with the highest concentrations. At the same time, trimethoprim (2815 µg/L), sulfamethoxazole (1195 µg/L), and dicloxacillin (350 µg/L) are the ones that show the lowest concentrations. Only dicloxacillin (1795 µg/L) and lincomycin (10800 µg/L) were found in the effluent. Regarding bacterial resistance, *Escherichia coli* is resistant to cephalexin and azithromycin in the effluent. *Salmonella* sp. is resistant to amoxicillin, dicloxacillin, lincomycin, ceftriaxone, cephalexin, and ciprofloxacin. Finally, related *Klebsiella* sp. is sensitive to the effluent of amoxicillin, ceftriaxone, and cephalexin. This indicates that the contact time between bacteria and antibiotic residues in the treatment plant influences the capacity to generate bacterial resistance, representing risks to health and the environment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy, have been ultimately observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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REFERENCES

Almeida, C. M. R., Santos, F., Ferreira, A. C. F., Lourinha, I., Basto, M. C. P., & Mucha, A. P. (2017). Can veterinary antibiotics affect constructed wetlands performance during treatment of livestock wastewater?. *Ecol. Eng.* 102, 583-588. doi:10.1016/j.ecoleng.2017.02.035.

Ao, X., Liu, W., Sun, W., Cai, M., Ye, Z., Yang, C., ... & Li, C. (2018). Medium pressure UV-activated peroxymonosulfate for ciprofloxacin degradation: Kinetics, mechanism, & genotoxicity. *Chem. Eng. J.* 345, 87-97. doi:10.1016/j.cej.2018.03.133.

APHA (2017). *Standard Methods for the Examination of Water & Wastewater 23rd edition*. 23.ª ed. Washington D.C.

Archundia, D., Duwig, C., Lehembre, F., Chiron, S., Morel, M. C., Prado, B., ... & Martins, J. M. F. (2017). Antibiotic pollution in the Katari subcatchment of the Titicaca Lake: Major transformation products & occurrence of resistance genes. *Sci. Total Environ.* 576, 671-682. doi:10.1016/j.scitotenv.2016.10.129.

Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M. H., & Zheng, C. (2019). Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. *Environ. Res.* 169, 483-493. doi:10.1016/j.envres.2018.11.040.

Bhattacharjee, M. K. (2016). *Chemistry of Antibiotics & Related Drugs.* , ed. Springer Cham: Springer International Publishing doi:10.1007/978-3-319-40746-3.

Binh, V. N., Dang, N., Thi, N., Anh, K., Ky, L. X., & Thai, P. K. (2018). Antibiotics in the aquatic environment of Vietnam: Sources, concentrations, risk & control strategy. *Chemosphere* 197, 438-450. doi:10.1016/j.chemosphere.2018.01.061.

Carmona, E., Andreu, V., & Picó, Y. (2017). Multi-residue determination of 47 organic compounds in water, soil, sediment & fish—Turia River as case study. *J. Pharm. Biomed. Anal.* 146, 117-125. doi:10.1016/j.jpba.2017.08.014.

Carvalho, I. T., & Santos, L. (2016). Antibiotics in the aquatic environments: A review of the European scenario. *Environ. Int.* 94, 736-757. doi:10.1016/j.envint.2016.06.025.

Cavalieri, S. J., Harbeck, R. J., McCarter, Y. S., Ortez, J. H., Rankin, I. D., Sautter, R. L., ... & Spiegel, C. A. (2005). Manual de pruebas de susceptibilidad antimicrobiana. *Seattle: University of Washington.*

Available at: https://www.paho.org/hq/dmdocuments/2005/susceptibilidad-antimicrobiana-manual-pruebas-2005.pdf.

Chabilan, A., Landwehr, N., Horn, H., & Borowska, E. (2022). Impact of log(Kow) Value on the Extraction of Antibiotics from River Sediments with Pressurized Liquid Extraction. *Water (Switzerland)* 14, 2534. doi:10.3390/W14162534/S1.

Chen, J. (2014). Removal of antibiotics & antibiotic resistance genes in rural wastewater by an integrated constructed wetland. *Environ. Sci. Pollut. Res.* 22, 1794–1803. doi:10.1007/s11356-014-2800-4.

Dires, S., Birhanu, T., Ambelu, A., & Sahilu, G. (2018). Antibiotic resistant bacteria removal of subsurface flow constructed wetlands from hospital wastewater. *J. Environ. Chem. Eng.* 6, 4265-4272. doi:10.1016/j.jece.2018.06.034.

Diwan, V., Tamhankar, A. J., Khandal, R. K., Sen, S., Aggarwal, M., Marothi, Y., ... & Stålsby-Lundborg, C. (2010). Antibiotics & antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. *BMC public health*, *10*(1), 1-8. 10, 414. doi:10.1186/1471-2458-10-414.

FDA (2022). Bacteriological Analytical Manual (BAM). *FDA*. Available at: https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam [Accedido septiembre 13, 2022].

Forestieri, S., Pintus, R., Marcialis, M. A., Pintus, M. C., & Fanos, V. (2021). COVID-19 & developmental origins of health & disease. *Early Hum. Dev.* 155, 105322. doi:10.1016/j.earlhumdev.2021.105322.

Geramizadegan, A., Ghazanfari, D., & Amiri, A. (2022). Determination of amount herbicide toxic fenpyroximate in surface water by analysis molecularly imprinted solid phase extraction method & relative error assessment using artificial neural network model. *https://doi.org/10.1080/03067319.2021.2001465*, 1-18. doi:10.1080/03067319.2021.2001465.

Gonzalez-Zorn, B. (2021). Antibiotic use in the COVID-19 crisis in Spain. *Clin. Microbiol. Infect.* 27, 646-647. doi:10.1016/j.cmi.2020.09.055.

Gozzo, S., Moles, S., Kińska, K., Ormad, M. P., Mosteo, R., Gómez, J., ... & Szpunar, J. (2022). Screening for Antibiotics & Their Degradation Products in Surface & Wastewaters of the POCTEFA Territory by Solid-Phase Extraction-UPLC-Electrospray MS/MS. *Water (Switzerland)* 15. doi:10.3390/w15010014.

Haddad, T., Baginska, E., & Kümmerer, K. (2015). Transformation products of antibiotic & cytostatic drugs in the aquatic cycle that result from effluent treatment & abiotic/biotic reactions in the environment: An increasing challenge calling for higher emphasis on measures at the beginning of the pipe. *Water Res.* 72, 75-126. doi:10.1016/J.WATRES.2014.12.042.

Harrabi, M., Varela Della Giustina, S., Aloulou, F., Rodriguez-Mozaz, S., Barceló, D., & Elleuch, B. (2018). Analysis of multiclass antibiotic residues in urban wastewater in Tunisia. *Environ. Nanotechnology, Monit. Manag.* 10, 163-170. doi:10.1016/j.enmm.2018.05.006.

Hartinger, S. M., Medina-Pizzali, M. L., Salmon-Mulanovich, G., Larson, A. J., Pinedo-Bardales, M., Verastegui, H., ... & Mäusezahl, D. (2021). Antimicrobial resistance in humans, animals, water & household environs in rural andean peru: Exploring dissemination pathways through the one health lens. *Int. J. Environ. Res. Public Health* 18, 4604. doi:10.3390/IJERPH18094604/S1.

Kumar, M., Jaiswal, S., Sodhi, K. K., Shree, P., Singh, D. K., Agrawal, P. K., & Shukla, P. (2019). Antibiotics bioremediation: perspectives on its ecotoxicity & resistance. *Environ. Int.* 124, 448-461. doi:10.1016/j.envint.2018.12.065.

Larson, A., Hartinger, S. M., Riveros, M., Salmon-Mulanovich, G., Hattendorf, J., Verastegui, H., ... & Mäusezahl, D. (2019). Antibiotic-resistant Escherichia coli in drinking water samples from rural Andean households in Cajamarca, Peru. *Am.J. Trop. Med. Hyg.* 100, 1363-1368. doi:10.4269/ajtmh.18-0776.

Lehutso, R. F., Daso, A. P., & Okonkwo, J. O. (2017). Occurrence & environmental levels of triclosan & triclocarban in selected wastewater treatment plants in Gauteng Province, South Africa. *Emerg. Contam.* 3, 107-114. doi:10.1016/j.emcon.2017.07.001.

Liu, Y., Chen, Y., Feng, M., Chen, J., Shen, W., & Zhang, S. (2021). Occurrence of antibiotics & antibiotic resistance genes & their correlations in river-type drinking water source, China. *Environ. Sci. Pollut. Res.* 28, 42339-42352. doi:10.1007/s11356-021-13637-8.

Lopes, T. R., Costa, I. L., Periotto, F., & Pletsch, A. L. (2016). Antibiotic resistance in E. coli isolated in effluent from a wastewater treatment plant & sediments in receiver body. *Int. J. River Basin Manag.* 14, 441-445. doi:10.1080/15715124.2016.1201094.

Loudermilk, E. M., Kotay, S. M., Barry, K. E., Parikh, H. I., Colosi, L. M., & Mathers, A. J. (2022). Tracking Klebsiella pneumoniae carbapenemase gene as an indicator of antimicrobial resistance dissemination from a hospital to surface water via a municipal wastewater treatment plant. *Water*

Res. 213, 118151. doi:10.1016/j.watres.2022.118151.

Maldonado, I., Moreno, E. G., & Zirena, F. (2022). Application of duckweed (Lemna sp.) & water fern (Azolla sp.) in the removal of pharmaceutical residues in water : State of art focus on antibiotics. *Sci. Total Environ.* 838, 156565. doi:10.1016/j.scitotenv.2022.156565.

Masarikova, M., Manga, I., Cizek, A., Dolejska, M., Oravcova, V., Myskova, P., ... & Literak, I. (2016). Salmonella enterica resistant to antimicrobials in wastewater effluents & black-headed gulls in the Czech Republic, 2012. *Sci. Total Environ.* 542, 102-107. doi:10.1016/j.scitotenv.2015.10.069.

Masco, M. L. (2017). Determinación de contaminantes tensioactivos tipo ácido dodecilbenceno sulfónico lineal en aguas del Río Huatanay - Cusco. *Repos. Inst. UNA-Puno*, 1-125.

MINAM (2017). Estandares de Calidad Ambiental (ECA) para Agua. *El Peru.*, 6-9. Available at: http://www.minam.gob.pe/wp-content/uploads/2017/06/DS-004-2017-MINAM.pdf.

MINSA (2007). *Protocolo de monitoreo de la calidad sanitaria de los recursos hídricos superficiales*.

Mohammad, R. E. A., Elbashir, A. A., Karim, J., Yahaya, N., Rahim, N. Y., & Miskam, M. (2021). Adsorptive performances of magnetic graphene oxide adsorbent for the removal of fluoroquinolones in the Langat River Basin, Malaysia. *https://doi.org/10.1080/03067319.2021.1957464*, 1-21. doi:10.1080/03067319.2021.1957464.

Nieto-Juarez, J., Torres-Palma, R., Botero-Coy, A., & Hernández, F. (2021). Pharmaceuticals & environmental risk assessment in municipal wastewater treatment plants & rivers from Peru. *Environ. Int. J.* 155, 106674. doi:https://doi.org/10.1016/j.envint.2021.106674.

Novo, A., André, S., Viana, P., Nunes, O. C., & Manaia, C. M. (2013). Antibiotic resistance, Antimicrobial residues & bacterial community composition in urban wastewater. *Water Res.* 47, 1875-1887. doi:10.1016/j.watres.2013.01.010.

Palhares, J. C. P., Kich, J. D., Bessa, M. C., Biesus, L. L., Berno, L. G., & Triques, N. J. (2014). Salmonella & antimicrobial resistance in an animal-based agriculture river system. *Sci. Total Environ.* 472, 654-661. doi:10.1016/j.scitotenv.2013.11.052.

Pournamdari, E., & Geramizadegan, A. (2023). Assessment & Selective Extraction Megestrol Drug by Molecularly Imprinted Polymers Method in Human Fluid Samples Using Liquid Chromatography. *Int. J. Obstet. Anesth.* 10, 169-182. doi:10.1054/ijoa.2001.0838.

Reichert, G., Hilgert, S., Fuchs, S., & Azevedo, J. C. R. (2019). Emerging contaminants & antibiotic resistance in the different environmental matrices of Latin America. *Environ. Pollut.* 255, 113140. doi:10.1016/j.envpol.2019.113140.

Rivera-Gutiérrez, E., Ramírez-García, J. J., Pavón Romero, S. H., Rodríguez, M. M., Ramírez-Serrano, A., & Jiménez-Marin, A. (2020). Dicloxacillin Degradation with Free-Living Bacteria. *Water. Air. Soil Pollut.* 231, 1-13. doi:10.1007/s11270-020-4456-7.

Rodriguez-Mozaz, S., Vaz-Moreira, I., Della Giustina, S. V., Llorca, M., Barceló, D., Schubert, S., ... & Manaia, C. M. (2020). Antibiotic residues in final effluents of European wastewater treatment plants & their impact on the aquatic environment. *Environ. Int.* 140, 105733. doi:10.1016/j.envint.2020.105733.

Sakkas, H., Bozidis, P., Ilia, A., Mpekoulis, G., & Papadopoulou, C. (2019). Antimicrobial resistance in bacterial pathogens & detection of carbapenemases in Klebsiella pneumoniae isolates from hospital wastewater. *Antibiotics* 8, 1-12. doi:10.3390/antibiotics8030085.

Santiago, P., Jiménez-Belenguer, A., García-Hernández, J., Estellés, R. M., Pérez, M. H., López, M. A. C., ... & Moreno, Y. (2018). High prevalence of Salmonella spp. in wastewater reused for irrigation assessed by molecular methods. *Int. J. Hyg. Environ. Health* 221, 95-101. doi:10.1016/j.ijheh.2017.10.007.

Sarafraz, M., Ali, S., Sadani, M., Heidarinejad, Z., Bay, A., Fakhri, Y., & Mousavi Khaneghah, A. (2022). A global systematic, review-meta analysis & ecological risk assessment of ciprofloxacin in river water.*Int. J. Environ. Anal. Chem.* 102, 1-15. doi:10.1080/03067319.2020.1791330.

Wang, H., Qu, B., Liu, H., Ding, J., & Ren, N. (2018). Fast determination of β-endosulfan, α-hexachlorocyclohexane & pentachlorobenzene in the river water from northeast of China. *Int. J. Environ. Anal. Chem.* 98, 413-428. doi:10.1080/03067319.2018.1475563.

Zhang, C. M., Xu, L. M., Mou, X., Xu, H., Liu, J., Miao, Y. H., ... & Li, X. (2019). Characterization & evolution of antibiotic resistance of Salmonella in municipal wastewater treatment plants. *J. Environ. Manage.* 251, 109547. doi:10.1016/j.jenvman.2019.109547.

Zhang, Y., Qu, C., Qi, S., Zhang, Y., Mao, L., Liu, J., ... & Yang, D. (2021). Spatial-temporal variations & transport process of polycyclic aromatic hydrocarbons in Poyang Lake: Implication for dry–wet cycle impacts. *J. Geochemical Explor.* 226, 106738. doi:10.1016/j.gexplo.2021.106738.