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ORIGINAL ARTICLE

Occurrence of 28 Human and Veterinary Antibiotics Residues in Waters, North-Eastern Tunisia by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract
Drug residues are now ubiquitous in the environment due to the extensive and widespread use for animal and human health care. Current technologies do not allow to completely remove drug residues during waste water treatment process. Consequently, drug residues are widespread in the aquatic environment and present thus potential impacts on biodiversity, ecosystem functioning, and public health. As a result, their residues have attracted particular attention from government and research community. High concentrations of antibiotic residues detected in the environment have prompted scientists to advocate for the implementation of various laws around the world. This work aims to contribute to the lack of information on this emerging and essential subject. Twenty-eight antibiotics including five tetracyclines, two macrolides, seven fluoroquinolones, and seven sulfonamides were analyzed in the input and output of two wastewater treatment plans (WWTP) in Tunis, in Northern Tunisia, in a tap and well water. The quantification was performed using online SPE-LC-MS/MS after the sample's extraction/purification with offline SPE. At least 19 drugs were present at the quantifiable level. In the input and output of WWTP, drug residues were determined at high level with the Σ28drugs can be up to 239.8 ± 47.1 ng/L and 139.4 ± 31.9 ng/L, respectively. One WWTP was not operational during the sampling. The concentration level in the output was similar to which found in the input (no elimination process). Another WWTP allowed eliminating only 48% of the Σ28drugs. As a result, the Σ28drugs were detected at 109.5 ± 25.5 and 36.8 ± 8.7 ng/L respectively in well and tap water.

Keywords Antibiotics · wastewater · hospital center · off-line and online SPE-LC-MS-MS · Tunisia

1 Introduction

For several decades, organic micro-pollutants such as hydrocarbons, pesticides, and PCBs have been studied. However, there are lack data in the literature on drug residues in the natural environment on a worldwide scale. Drug residues used in human and animal health care can be quickly introduced into the aquatic environment via wastewater treatment plants (WWTPs) and other processes, including leaching from agricultural soil where there is a practice of spreading with sewage sludge and pig manure or poultry. Indeed, WWPT or lagoons cannot eliminate these compounds from wastewater or agricultural effluent1- 8,

Antibiotic use is increasing in both developed and developing countries worldwide. It is estimated that 100,000 to 200,000 tons of antibiotics are consumed globally, which

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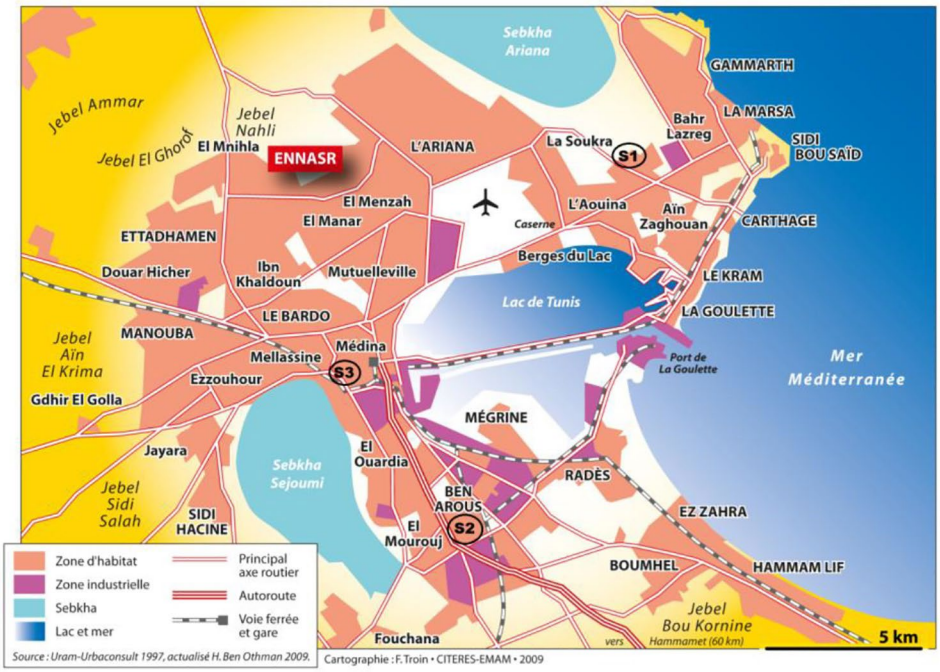

Fig. 1 Location of three studied sites located in Ariana, Ben Arous and Tunis, northern of Tunisia (Uram-Urbaconsult 1997, actualized by Ben Othmen, 2009).

has resulted in their frequent detection in wastewater treatment plants' (WWTPs) effluent, surface waters, and sediments around the world, resulting in the development of antibiotic resistance in the environment2-3. In Africa, 1800 active molecules are marketed. Tunisia is one of the big consumers of drugs. Drug residues are found ubiquitously today in hospital, urban and industrial effluents, as well as livestock manure, WWPT outputs, and surface water7, 9–12. These releases have a non-negligible environmental risk because drugs were initially produced to be biologically active. Indeed, although the effects of pharmaceutical products are studied through safety and toxicology studies, their potential effects on the environment are still unknown and have thus become subjects of interest to the scientific community12. A recent study showed that drug residues could cause endocrine disruption, changes in behavior, and genetic responses13. It was also shown that surface water, groundwater, and drinking water contain hormones from contraceptive treatments, anticancer drugs, anti-inflammatory and antibiotics7, 14–18. The pollution caused in aquatic environments by using human and veterinary pharmaceutical residues has become the source of severe concerns and requires studies to provide appropriate solutions. This work aim to study the occurrence of 28 antibiotics in the input and output of WWTP, in tap and well water collected from Northern Tunisia.

2 Materials and Methods

2.1 Chemicals and Materials

All reagents used were of analytical grade with > 95% of purity. The twenty-eight human and veterinary products were purchased from Sigma Aldrich except for gemfibrozil (GEM), carbamazepine (CBZ) and sulfamethoxazole (SMA) from Dr Ehrenstorfer (Augsburg, Germany). Carbamazepine-d10, sulfamethoxazol-d4 and trimethoprim-d3 were used as internal standards and were purchased from Dr Ehrenstorfer (Augsburg, Germany). All chemicals used in the analysis were purchased from VWR Prolabo and analytical and HPLC grades as ultra-pure water. The cartridges used for SPE were Oasis HLB (200 mg/6 mL) obtained from Waters (Milford MA, USA). Standard mixtures were prepared daily by appropriate dilution of the stock standard solutions using the same solvents as for stock solutions. The standard stock solutions can be preserved for one month[7]. This study prepared standard solutions weekly from commercial standards to avoid false positives or overestimated results. Glassware was first washed with diluted nitric acid, rinsed with distilled water, and then dried in an oven at 180°C for 2 h prior to use.

2.2 Study Sites and Sampling

The samples were collected from the Tunis capital, northern of Tunisia (Fig. 1). Even these areas present industrial and agricultural activities; only few data is available for

drug residues. (i) The first WWTP is Chotrana (S1) located in Route Sidi Salah km 5, Chotrana 1 Ariana. Chotrana WWTP was operational in 1986 and was dimensioned with the biological process with the treatment capacity of 40000 kg/BOD5/day[18] or 78000 m^3/day. Aerated pond combined with digestion techniques were used for the treatment. The volume of treated water is 20million m^3/year and it is the biggest WWTP of the Tunis capital. It receives mainly domestic effluents which represent 87%; industrial and touristic activities represented 12% and 1% respectively. (ii) The second WWTP is located in Médina Jadida-Ben Arous (S2). It was operational for the first time in 1982 with the capacity to treat 37500 m^3/day (9million m^3/year) of wastewater. However, it was non-operational during the sampling campaign. Ben Arous is the largest industrial city in Tunis and particularly pharmaceutical industry. Ben Arous WWTP received the industrial effluent including effluent from pharmaceutical industry.

These WWTPs receive various types of wastewater and apply different treatment techniques. Ben Arous WWTP receives exclusively industrial wastewater (100%). Activated sludge combined with chemical treatment was applied for Ben Arous WWTP. Sub-samples were first collected, mixed thoroughly then divided into three samples and finally stored at -5°C in the WWTP. (iii) Bab Saadoun health center (S3) regroups six centers and hospitals; it is the most important health care center in Tunisia. The sampling campaign was conducted in early spring 2016 from 12/03/2016 to 25/03/2016. All the samples were collected with intelligent sampling (10 days) using 2.5 L bottles and immediately capped with Teflon-lined lid.

2.3 Sample Preparation and Extraction

Water samples were directly filtered back to the laboratory using previously calcinated 0.7 µm Whatman glass microfiber filters. Filtered waters were firstly spiked with internal standards (Carbamazepine-d10, sulfamethoxazol-d4 and trimethoprim-d3). Spiked samples were extracted using solid phase extraction (SPE) technique according to the method developed previously by Gros[19]. The procedural blank was performed in triplicate for each set of samples. Briefly, the cartridge was firstly conditioned with 6 mL of methanol followed by 6 mL of water HPLC grade (previously acidified at pH 2.5) with a flow rate of 1 mL/min. The samples were then passed through the cartridges at a flow rate of 1 mL/min using a vacuum SPE manifold. After sample loading, the cartridge was washed with 6 mL of HPLC grade water. The cartridges were then dried under a nitrogen flow for 30 min using Supelco vacuum manifold (Sigma-Aldrich). Finally, the targeted drug residues were eluted with 6 ml of methanol at a flow rate of 1 mL/min.

2.4 On-line SPE-LC-MS/MS Analysis

The analysis was performed using an off-line SPE combined with an on-line SPE-LC-MS/MS according to the method developed by Tlili[7]. 28 drugs were analyzed and their therapeutic groups, abbreviation, molecular weight (MW), retention time (RT), parent ion, fragment ions, method yield and limit of detection (LOD) of each selected drug residues were presented in Table 1.

3 Results and Discussions

3.1 Occurrence of the 28 Drug Residues in the Different Water Matrices

The concentration of the 28 selected compounds was investigated in each sample and in triplicate. The individual concentration varied depending on the sample's origin. The highest level of Σ28Drugs was quantified at Chotrana WWTP input and followed by Ben Arous effluent at respectively 239.8 ± 47.1 ng/L and 199.7 ± 52.7 ng/L. These concentrations were two times lower than those detected in Arras WWTP (470.9 ± 149.2 ng/L) and Fresnoy aerated lagoon (470.9 ± 136.9 ng/L) in Northern France. Tlili[4], Oke[1] and Vieno[20] have reported high levels for fluoroquinolones in Spain, France, Germany and Belgium, where the concentration of ciprofloxacin and ofloxacin were in the range of 1-100 µg/L and 0.5 ng/L -10 µg/L respectively. Among the 28 drugs, CTC, MIN, DAN, CAR and GEM were quantified dominants (Table 2). However, OFL was < LOQ for all samples and NOR was detected only in Ben Arous WWTP effluent at 6.6 ± 2.8 ng/L. These results are the same order to which reported by Tahrani et al (2018)[43]. DIC was detected only in the untreated wastewater (Fig. 2). Among the 28 drug residues, 15 drugs were detected in the tap water of Tunis Hospital Center, 18 in the well water of Tunis Health Center[44], 24 in Ben Arous WWTP, 25 in the output of Chotrana WWTP and 22 in the input of Chotrana WWTP.

High levels of drug residues were found in the output of WWTPs which might indicate that the wastewater treatment processes used in the selection WWTPs were not appropriate for removing satisfactory the antibiotics. Indeed, these WWTPs consists of pretreatment, primary settling and activated sludge 40–42. In view of our results, others treatment process should be added to improve the efficiency of the treatment. A similar observation was also reported in the literature with the references herein 10,11, 18,39,40. Moreover, Ben Arous WWTP was not operational during the sampling. Therefore, the contamination levels found in the input were found in the output and then in the receiving aquatic environment. While WWTP Chotrana could eliminate 61%,

Table 1 Therapeutic groups, abbreviation, molecular weight (M_W), retention time (RT), parent ion, fragment ions, method yield and limit of detection (LOD) of each drug residues

Therapeutic group	Name	Abbre-viation	MW (g/mol)	RT (mn)	Parent ion (m/z)	Fragment ions (m/z)	Yield (%)*	LOD (ng/L)*
Tetracyclin Antibiotics	Chlortetracycline	CTC	382.88	8.72	479.1	462.0 ; 443.8	43	0.06
	Tetracycline	TC	444.43	6.08	445.1	409.9; 427.0	90	0.06
	Doxycycline	DOX	444.43	9.86	445.0	427.9; 153.8	69	0.06
	Minocycline	MIN	457.47	4.86	457.0	439.8; 175.2	78	0.06
	Oxytetracycline	OTC	460.43	6.82	461.1	425.9; 443.1	50	0.15
Fluoroquinolone Antibiotics	Difloxacine	DIF	399.39	7.10	400.0	381.9; 355.9	48	0.06
	Enrofloxacine	ENR	359.39	6.73	360.0	342.0; 315.9	49	0.06
	Norfloxacine	NOR	319.33	6.43	320.0	301.9; 275.9	50	0.15
	Ofloxacine	OFL	361.36	6.19	362.0	317.9; 260.8	48	0.15
	Orbifloxacine	ORB	395.37	6.91	396.0	351.9; 294.9	55	0.15
	Ciprofloxacine	CIP	331.34	6.47	332.0	313.9; 230.8	52	0.06
	Danofloxacine	DAN	357.39	6.65	358.0	339.7; 313.9	50	0.06
Sulfonamid antibiotics	Sulfabenzamide	SBZ	276.32	8.20	276.9	155.8; 108.0	100	0.06
	Sulfadiazine	SDZ	250.27	4.81	250.9	155.8; 108.0	82	0.9
	Sulfadimethoxine	SDMX	310.32	8.76	310.9	155.9; 108.0	75	0.06
	Sulfamerazine	SMZ	264.30	5.66	264.9	155.9; 171.8	79	0.06
	Sulfamethoxazole	SMA	253.27	7.03	253.9	155.7; 92.0	93	0.06
	Sulfanilamide	SN	172.20	2.78	172.9	155.8; 108.0	58	0.06
	Sulfathiazole	STZ	277.29	5.28	255.9	155.9; 108.0	68	0.06
bacteriostatic antibiotic	Trimethoprim	TRI	290.31	5.77	291.0	229.9; 260.9	94	0.06
	Florfenicol	Ff	358.21	6.96	355.9	335.9; 185.0	73	0.36
	Monensin	MON	692.85	19.33	693.3	675.3; 461.1	46	0.15
Macrolide Antibiotics	Tylosine	TYL	916.10	12.18	916.6	173.7; 772.3	64	0.06
	Erythromycin	ERY	733.92	20.75	733.0	720.6; 426.1	47	0.06
Lipid regulators	Gemfibrozil	GEM	250.33	19.92	294.1	121.1; 120.0	75	0.15
Insecticid	Dicyclanil	DIC	190.20	4.36	190.9	149.9; 162.8	73	0.15
ß-lactamantibiotic	Ampicilline	AMP	349.40	11.37	350.0	105.9; 113.9	52	0.15
Antiepileptic	Carbamazepine	CAR	236.26	11.48	236.9	193.8; 191.8	79	0.06

*The detail analysis conditions were reported in Tlili et al. (2016) with reference herein

58% and 43% respectively for CT, FQ and SN. The elimination yield of Chotrana WWTP were determined in average of 48% was higher than which found for WWTP Arras in northern France with only 20%[4, 5, 18]. Higher removal efficiency detected for the Chotrana WWTP was possibly due to the aerated pond combined with digestion at higher temperature 2–36, 42.

Among the 28 selected drugs, 15 have been detected in the tap water collected near the Tunis health center. However, their concentrations were detected at lower level than which detected in the input/output of WWTP and the well water selected in this work. Individual concentration varied from < LOQ to 6.8 ± 1.9 ng/L. Twenty of the 28 selected were detected in the well water, usually used for irrigation and domestic activities. Individual concentration varied from < LOQ to 16.7 ± 2.1 ng/L for the ∑28Drugs of 109.5 ± 25.5 ng/L (Fig. 2). Similar results have been reported int the literature 3,10, 22, 23,37,38,41.

Figure 2 shows the sum of TQs, FQs, SNs, and the other nine compounds in input and output of WWTPs, in well and tap water. The highest concentration of TQs was detected at Ben Arous WWTP where other drug residues were also present at a significant level. Additionally, this WWTP was not operational which indicates that the totality of these pollutants could be rejected in the surface water of the receiving natural environment. TQs and FQs were found at high level in well water and were similar to which found in the output of Chotrana WWTP.

3.2 Individual Concentration of Tetracyclines

Among the 5 TCs, the CTC and MIN were dominants in the Chotrana and Ben Arous WWTP (Fig. 3). CTC was detected around 40 ng/L in both the input of Chotrana and Ben Arous WWTPs. However, MIN was detected at the higher level (> 40 ng/L) in Ben Arous WWTP and it was 3 times higher than Chotrana. The DOX, OTC and TC were determined with the low average which did not exceed 10 ng/L. The OTC and DOX were detected at < LOQ value in output Chotrana WWTP which indicated the good elimination by the existing process. These results are similar to which reported by Tarhana et al[41]. For TC, the concentration was

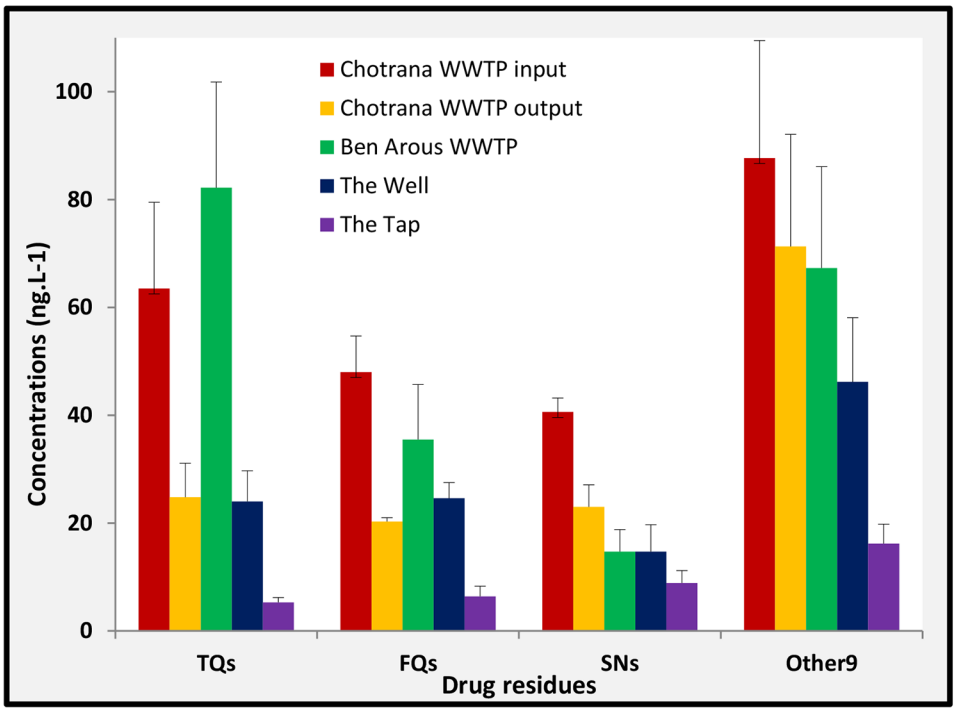

Fig. 2 Concentrations of the sum of TQs, FQs, SNs and the other nine compounds in input and output of WWTP, in well and tap water

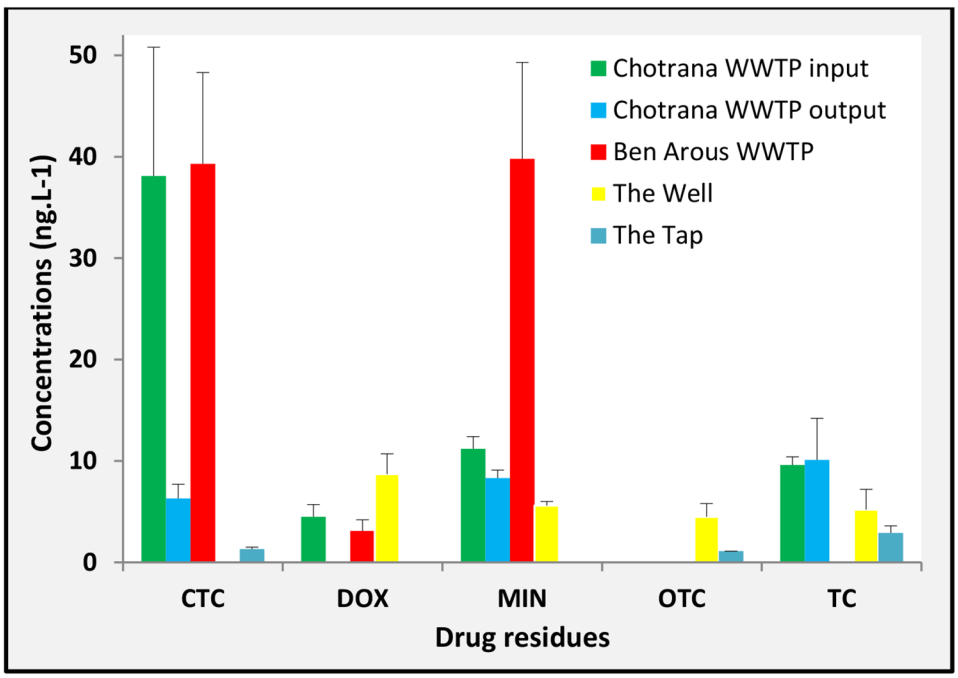

Fig. 3 Concentrations of Tetracyclines in the different sites

detected in each sample except for the Ben Arous WWTP (Fig. 3). This may be due to the fact that TC could be transformed to epitetracycline, epioxytetracycline and other products[3,4, 21, 39,40,49].

3.3 Individual Concentration of Sulfonilamides

SNs concentration (STZ, SDMX, SDZ, SBZ, STZ, SMX and SN) were lower than TCs concentrations. SDMX and SDZ were dominants in Chotrana input and output. SNs were detected in the input and output of WWTP and tap and well water (Fig. 4). Their present could be harmful to human health and need to reinforce the treatment for drinking

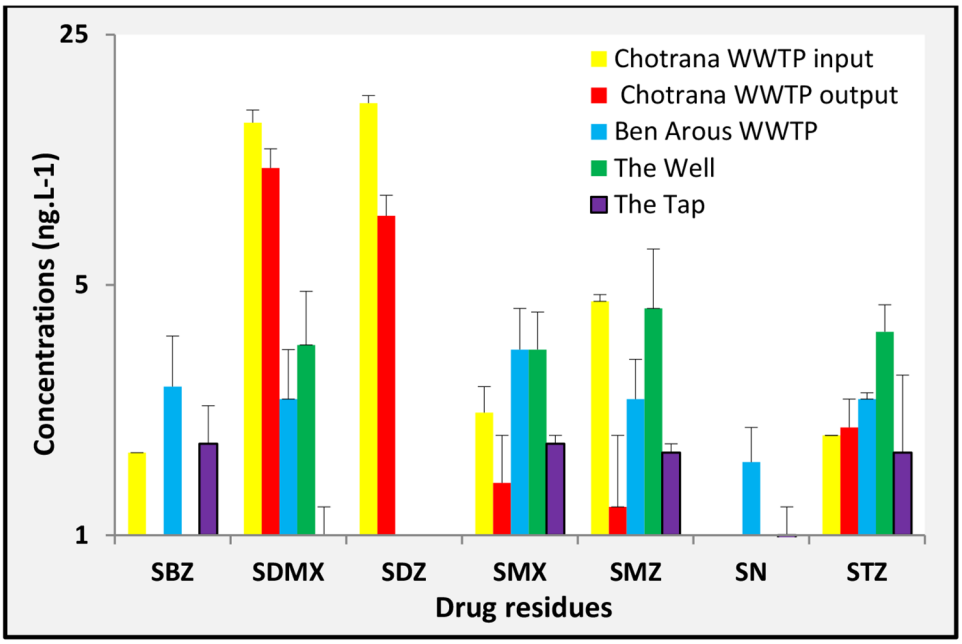

Fig. 4 Concentrations of Sulfonilamides in the different studied sites

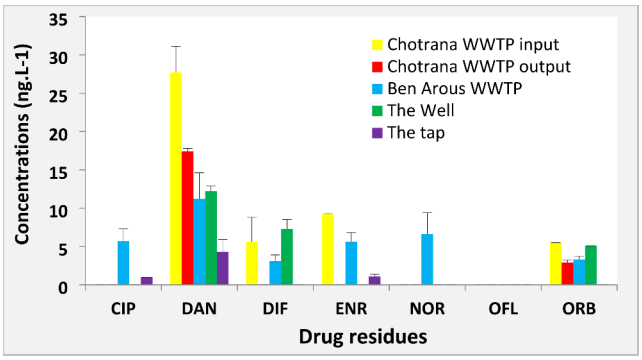

Fig. 5 Concentrations of FQs in the different studied sites

water[22]. SDMX is detected in all the samples with high concentrations ranging from 1 ng/L in the tap of Rabta hospital to 14.2 ng/L in Chotrana WWTP input. This ubiquitous may be due to its wide use in human medicine for the treatment of bacterial infections[5,23,42]. The Chotrana WWTP processing system removed the majority of SNs with a removal rate of 100% for SBZ, 70% for SMZ and 50% for SDZ. High elimination rates were observed for SNs, up to 100%. A similar observation was reported in the literature[27–29, 40,49].

3.4 Individual Concentration of Fluoroquinolones

FQs (CIP, DAN, DIF, ENR, NOR, OFL and ORB) commonly used as veterinary medicine in the past were found from < LOD to few dozen ng/L depending on compounds and the matrix. FQs were used as veterinary drugs therapeutic purposes. However, their high concentration can cause problem for human and environment due to their potential carcinogenic and possible development of antibiotic resistance in animals[30,40]. All the FQs except OFL were detected in Ben Arous WWTP effluent ranging from 3.1 ng/L for DIF until 11.2 ng/L for DAN.

The DIF and ENR were not detected in the effluent (Fig. 5), probably due to their presences in low concentrations or completely removed in the sewer system before arriving at the WWTP[31,40]. It was found that they can be partly removed by sorption and photo degradation[32,48]. The highest concentration was detected for DAN with the concentration ranging from 4.3 ng/L in the tap of Rabta hospital to 27.7 ng/L in Chotrana WWTP input. 63% of DAN has been eliminated by Chotrana WWTP. A similar finding was reported in the literature[39–42].

3.5 Nine Other Drugs

Table 2 shows that among the nine other antibiotics, CAR was detected dominant with concentration can be up to 40.8 ng/L in Ben Arous WWTP. It was detected in all the samples with high levels. Indeed, CAR was used as an anti-convulsion drug mainly for the treatment of epilepsy. It is one of the most frequently detected compounds in surface waters[12, 16, 33–35 ,37–39, 45]. The Chotrana WWTP could eliminated DIC, TYL and AMP with good efficiency. Their concentration detected in the output was < LOD. Their non-quantifiable level in the WWTP may be due to its presence in low concentrations respectively 5.3, 1.8, and 1.5 ng/L, or they were completely removed by the treatment system [8, 28, 37]. For TRI, 45% has been eliminated by Chotrana WWTP; 26.4 ng/L was detected in the input versus 14.5 ng/L in the output. While Florfenicol is an aminoglycoside and high heterogeneity of its concentration was observed

Table 2 Concentrations of the rest of antibiotics in the different studied sites

Concentration of selected drug residue (ng/L), n≥3					
Drug residues	**Chotrana WWTP input**	**Chotrana WWTP output**	**Ben Arous WWTP input = output***	**Well of Tunis Hospital Center**	**Tap of Tunis Hospital Center**
AMP	1.5 ± 0.5	< 0.5	1.2 ± 0.5	0.5 ± 0.0	< 0.5
CAR	29.1 ± 5.7	22.1 ± 8.1	40.8 ± 8.7	12.4 ± 2.5	3.0 ± 0.8
DIC	5.3 ± 1.6	< 0.5	6.2 ± 0.8	< 0.5	< 0.5
ERY	< 0.2	< 0.2	1.5 ± 0.1	6.2 ± 4.9	< 0.2
Ff	7.6 ± 1.9	6.1 ± 28	3.0 ± 1.7	3.7 ± 1.3	< 1.2
GEM	17.9 ± 3.4	11.4 ± 4.7	3.5 ± 4.1	4.0 ± 0.8	6.8 ± 1.9
MON	10.0 ± 4.2	5.3 ± 1.4	1.4 ± 0.1	1.5 ± 0.2	< 0.5
TRI	14.5 ± 4.2	26.4 ± 3.8	8.6 ± 2.7	16.7 ± 2.1	6.4 ± 0.9
TYL	1.8 ± 0.3	< 0.2	1.1 ± 0.1	1.2 ± 0.1	< 0.2
$\sum_9$Other	**87.7 ± 21.8**	**71.3 ± 20.8**	**67.3 ± 18.8**	**46.2. ± 11.9**	**16.2 ± 3.6**

Table 3 The calculated value of environmental risk quotient (RQ)

Drug residues	MEC_{max} (g/L)	PNEC (g/L)	RQ	Classification	References
CTC	39.3 ± 9.0 10^{-9}	0.3×10^{-6}	0.13	Medium risk	Chen et al., 2022 [46]
CAR	40.8 ± 8.7 10^{-9}	0.1×10^{-6}	0.81	Medium risk	Heye et al., 2019 [45]
DIC	6.2 ± 0.8 10^{-9}	0.1×10^{-6}	0.62	Medium risk	Jahnel et al., 2006 [44]
DAN	27.7 ± 3.4 10^{-9}	5.92×10^{-6}	0.04	Low risk	Grung et al., 2007 [47]

between samples. The highest concentration of Florfenicol was detected at 7.6 ng/L in the input of Chotrana WWTP. The elimination yield was calculated at < 50% for Chotrana WWTP. These findings are consistent with those reported by Tahrani et al[40].

3.6 Ecological Risk for Antibiotics in Water Samples

In order to evaluate the potential risk related to the presence of micropollutants in the aquatic environment, the European Union has defined the environmental risk quotient (RQ). The ecological risk quotient can be calculated by dividing the maximum measured environmental concentration (MECmax) by the predicted no-effectconcentration (PNEC) as follow:

3.7 RQ = MECmax/PNEC

The ecological risk can be classified into four levels: insignificant risk (RQ < 0.01), low risk (0.01 < RQ < 0.1), medium risk (0.1 < RQ < 1) and high risk (RQ > 1). These classification levels were used to evaluate the ecological risk of the antibiotics in our sample and results were presented in the Table 3.

4 Conclusion

This work presents the contamination level of 28 drug residues in two WWTPs, one tape water and one well water. The samples were extracted using off-line SPE then analyzed by on-line SPE-LC- MS/MS. The results showed that the treatment processes used in the two selected WWTPs were non-appropriates to eliminate the selected antibiotics. Low elimination was observed for the most drugs selected in this research. The results showed also high contamination level of drug residues in tape and well water. CTC, MIN, SDMX, SDZ, DAN, TRI, and CAR were detected dominant in the effluents. More intensive research is needed to better understand how these drugs disperse into the environment and reach groundwater. Their fate and transport in the aquatic environment should also be study to evaluate the global risk of drugs residues. Moreover, to reduce the contamination level in the aquatic environment, it is necessary to develop cost-effective technologies to remove drug residues from wastewaters and contaminated sites to ensure the need of the future generation.

Table 4 Individual concentrations of the target compounds in different water matrices

Concentration of selected drug residue (ng/L), n≥3					
Drug residues	**Chotrana WWTP input**	**Chotrana WWTP output**	**Ben Arous WWTP input = output***	**Well of Tunis Hospital Center**	**Tap of Tunis Hospital Center**
CTC	38.1 ± 12.7	6.3 ± 1.4	39.3 ± 9.0	< 0.2	1.3 ± 0.2
DOX	4.5 ± 1.2	< 0.2	3.1 ± 1.1	8.7 ± 2.0	< 0.2
MIN	11.2 ± 1.3	8.3 ± 0.8	39.8 ± 9.5	5.6 ± 0.4	< 0.2
OTC	<LOQ0.5	< 0.5	< 0.5	4.5 ± 1.3	1.1 ± 0.0
TC	9.6 ± 0.8	10.1 ± 4.1	< 0.2	5.2 ± 2.0	2.9 ± 0.7
∑TQs	**63.5 ± 16.0**	**24.8 ± 6.3**	**82.2 ± 19.6**	**24 ± 5.7**	**5.3 ± 0.9**
CIP	< 0.2	< 0.2	5.7 ± 1.6	< 0.2	1.0 ± 0.0
DAN	27.7 ± 3.4	17.4 ± 0.4	11.2 ± 3.4	12.2 ± 0.7	4.3 ± 1.6
DIF	5.6 ± 3.2	< 0.2	3.1 ± 0.8	7.3 ± 1.2	< 0.2
ENR	9.2 ± 0.1	< 0.2	5.6 ± 1.2	< 0.2	1.1 ± 0.3
NOR	< 0.5	< 0.5	6.6 ± 2.8	<0.5	< 0.5
OFL	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
ORB	5.5 ± 0.0	2.9 ± 0.3	3.3 ± 0.4	5.1 ± 1.0	< 0.5
∑FQs	**48 ± 6.7**	**20.3 ± 0.7**	**35.5 ± 10.2**	**24.6 ± 2.9**	**6.4 ± 1.9**
SBZ	1.7 ± 0.0	< 0.2	2.6 ± 1.0	< 0.2	1.8 ± 0.5
SDMX	14.2 ± 1.2	10.6 ± 1.4	2.4 ± 0.9	3.4 ± 1.4	1.0 ± 0.2
SDZ	16.1 ± 0.8	7.8 ± 1.1	< 0.2	< 0.2	< 0.2
SMX	2.2 ± 0.4	1.4 ± 0.5	3.3 ± 1.0	3.3 ± 0.9	1.8 ± 0.1
SMZ	4.5 ± 0.2	1.2 ± 0.7	2.4 ± 0.7	4.3 ± 2.0	1.7 ± 0.1
SN	< 0.2	< 0.2	1.6 ± 0.4	< 0.2	0.9 ± 0.3
STZ	1.9 ± 0.0	2.0 ± 0.4	2.4 ± 0.1	3.7 ± 0.7	1.7 ± 1.1
∑SNs	**40.6 ± 2.6**	**23 ± 4.1**	**14.7 ± 4.1**	**14.7 ± 5.0**	**8.9 ± 2.3**
AMP	1.5 ± 0.5	< 0.5	1.2 ± 0.5	0.5 ± 0.0	< 0.5
CAR	29.1 ± 5.7	22.1 ± 8.1	40.8 ± 8.7	12.4 ± 2.5	3.0 ± 0.8
DIC	5.3 ± 1.6	< 0.5	6.2 ± 0.8	< 0.5	< 0.5
ERY	< 0.2	< 0.2	1.5 ± 0.1	6.2 ± 4.9	< 0.2
Ff	7.6 ± 1.9	6.1 ± 28	3.0 ± 1.7	3.7 ± 1.3	< 1.2
GEM	17.9 ± 3.4	11.4 ± 4.7	3.5 ± 4.1	4.0 ± 0.8	6.8 ± 1.9
MON	10.0 ± 4.2	5.3 ± 1.4	1.4 ± 0.1	1.5 ± 0.2	< 0.5
TRI	14.5 ± 4.2	26.4 ± 3.8	8.6 ± 2.7	16.7 ± 2.1	6.4 ± 0.9
TYL	1.8 ± 0.3	< 0.2	1.1 ± 0.1	1.2 ± 0.1	< 0.2
$\sum_{9}$Other	**87.7 ± 21.8**	**71.3 ± 20.8**	**67.3 ± 18.8**	**46.2.±11.9**	**16.2 ± 3.6**
$\sum_{28}$Drugs	**239.8 ± 47.1**	**139.4 ± 31.9**	**199.7 ± 52.7**	**109.5 ± 25.5**	**36.8 ± 8.7**

* Ben Arous WWTP was non-operational and thus the concentration in the input = output

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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