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Determination of the contamination by azole antimycotics in hospital and house sewage - a pilot project for the city of Dresden

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Medicininal compounds and their metabolites are known to end up in sewerage and may slip through the cleaning process. Azole antimycotics are frequently used in hospitals, in particular for patients with cancer or immunosuppression. The aim of the study was to determine whether measurable azole antimycotic concentrations were introducted in the sewarage drain of an acute care hospital with special interest in oncology and hematology and the extent of removal of antimycotics by the sewerage treatment plant. For this, the concentrations of three commonly used azole antimycotics were measured in the effluent of the sewerage drain at the University Hospital Dresden, as well as in the influent and effluent of the main sewerage treatment plant of the city. To extrapolate the theoretical influent to the sewerage treatment plant, prescription from the region`s main health insurance the AOK Sachsen and the hospital consumption data were used. Measurable concentrations were obtained for fluconazole and ketoconazole in the influent and effluent of the sewerage treatment plant. Voriconazole's concentrations were under the lower limit of quantification. To determine the azole clearance of the treatment plant a sludge sample was investigated. Sufficient clearance was detected for ketoconazole but not for fluconazole. The consumption and prescription rates were collected and correlated with the measured concentrations. In result, only fluconazole's concentrations provided a good match with the prescription and consumption data.

1. Introduction

Little is known about the degree of contamination of aquatic and soil environment as a consequence of drug use. Previous studies have shown that agricultural and pharmaceutical azoles are present in the environment, including lakes and rivers (Roberts and Thomas 2006; Peschka et al. 2007; Kahle et al. 2008; Van De Steene and Lambert 2008). Brilante et al. (2016) reported a higher rate of *Candida* isolates in the surface water close to domestics and found a resistance rate of 35 %. Today, the resistance rate to antifungals is low in comparison to antibiotics. Nevertheless, especially immunocompromised patients are more prone to infections than other patients and contamination of water with resistant strains of fungi may be a relevant trigger of fungal infections and treatment may be complicated due to cross-resistance between azole antimycotics (Muller et al. 2000; Mosquera and Denning 2002; Panackal et al. 2006; Kanafani and Perfect 2008). To estimate the hazard of the potential vicious cycle for both the environment and the patients, the concentrations of three azole antimycotics were determined in the influent and effluent of the sewerage treatment plant (STP) Dresden Kaditz, and their reduction specified.

2. Investigations and results

2.1. Azole antimycotic concentrations in the sewerage drain system at the University Hospital Dresden

The four-hourly pooled samples of the sewerage drain system at University Hospital Dresden (UHD) showed an intraday variation of fluconazole. The maximum peak was observed at twenty hours each, with 289 ng/L at 4 h, 462 ng/L at 24 h, and 437 ng/L at 44 h. Within the 52 h monitoring period, the concentrations ranged between 14 and 462 ng/L (Fig. 1).

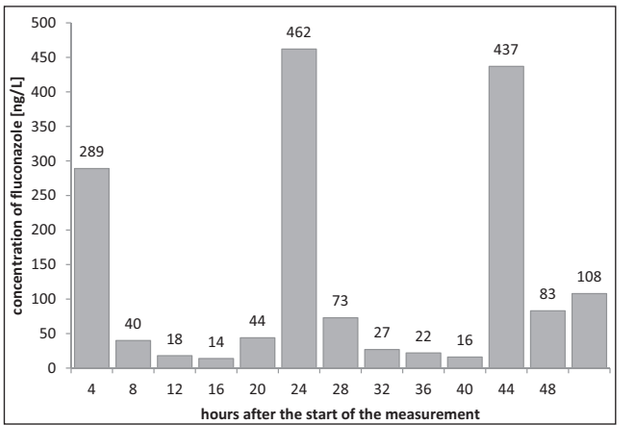

Fig. 1: Fluconazole concentrations within the 4-hourly pooled samples in the sewerage drain system at UHD, start of the measurement 12 pm on the 02.08.2012.

Ketoconazole showed a maximum concentration of 47 ng/L within the first four hours of sample drawing and declined thereafter. In total, the concentrations ranged between 6 and 17 ng/L without a second peak (Fig. 2).
The voriconazole results are not shown, as the concentrations were under the lower limit of quantification (LLoQ - 50 ng/L) (Gurke et al. 2015)

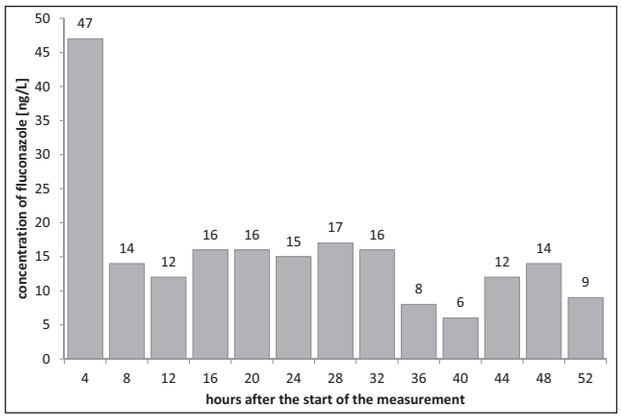

Fig. 2: Ketoconazole concentrations within the 4-hourly pooled samples in the sewerage drain system at UHD, start of the measurement 12 pm on the 02.08.2012.

2.2. Azole antimycotic concentrations in the sewage treatment plant (STP) of the city of Dresden

The concentration analysis of the two different azole antimycotics fluconazole and ketoconazole in the influent of the STP showed different minimal and maximal concentrations over the year (Tables 1 and 2). The lowest mean concentration of fluconazole was observed in June with 38 ng/L. The month was characterized by an extreme weather event with massive rainfall and flood water in Dresden. The highest mean concentration was observed in September with 167 ng/L. Overall, there was a trend towards higher concentrations within the second half of the year. Ketoconazole's concentrations showed a minimum in March and a maximum in May with mean concentrations of 18 ng/L and 348 ng/L, respectively.

Table 1: Fluconazole mean concentration per month measured in the influent and effluent of the STP in 2013 (n=317 days)

Month (Number of sampling days per month)	Fluconazole concentration		Ratio effluent/ influent
	Influent (mean [ng/L] ± SD)	Effluent (mean [ng/L] ± SD)	
January (18/31)	100 (±36)	107 (±34)	1.07
February (24/28)	98 (±21)	103 (±18)	1.05
March (26/31)	87 (±19)	90 (±18)	1.03
April (30/30)	92 (±16)	100 (±17)	1.09
May (30/31)	97 (±32)	97 (±29)	1.00
June (27/30)	38 (±19)	45 (±28)	1.18
July (31/31)	115 (±53)	110 (±30)	0.96
August (29/31)	151 (±37)	131 (±42)	0.87
September (30/30)	167 (±28)	153 (±64)	0.92
October (30/31)	166 (±45)	164 (±43)	0.99
November (29/30)	151 (±25)	158 (±35)	1.05
December (13/31)	163 (±25)	163 (±28)	1.00
Total concentration (n=317 days)	37.573 ng/L	37.277 ng/L	0.99

Table 2: Ketoconazole mean concentration per month measured in the influent and effluent of the STP for 2013 (n=251 days)

Month (Number of sampling days per month)	Ketoconazole concentration		Ratio effluent/ influent
	Influent (mean [ng/L] ± SD)	Effluent (mean [ng/L] ± SD)	
January (18/31)	76 (±84)	211 (±294)	2.78
February (24/28)	294 (±306)	59 (±86)	0.20
March (23/31)	18 (±35)	70 (±135)	3.89
April (5/30)	123 (±30)	48 (±57)	0.39
May (13/31)	348 (±179)	95 (±52)	0.27
June (22/30)	201 (±131)	58 (±47)	0.29
July (28/31)	23 (±32)	12 (±25)	0.52
August (23/31)	48 (±55)	35 (±39)	0.73
September (30/30)	57 (±64)	30 (±41)	0.53
October (26/31)	44 (±28)	17 (±21)	0.39
November (27/30)	46 (±60)	75 (±114)	1.63
December (12/31)	93 (±108)	26 (±26)	0.28
Total concentration (n=251 days)	25.355 ng/L	14.345 ng/L	0.57

Data for voriconazole are not shown, as the absolute voriconazole concentrations measured were frequently below 10 ng/L, and the LLoQ of voriconazole was determined to be 50 ng/L.

Furthermore, the effluent of the STP was analysed to determine the degree of elimination through the STP procedure. The effluent:influent ratio for fluconazole ranged from 0.87 to 1.18 and for ketoconazole from 0.2 to 3.89. A sludge sample of the STP was collected on July 17th, 2013, to quantify the concentrations of fluconazole and ketoconazole. In result, the sludge sample provided a ketoconazole concentration of 35.9 µg/kg in the dry mass. Fluconazole was not detectable.

2.3. Prescription data

The consumption and prescription rate of the three azol antimycotics for the City of Dresden were collected for the years 2010–2013 representing the inpatient presciptions by three hospitals in Dresden (UHD, Hospital Dresden Friedrichstadt HDF and Hospital Dresden Neustadt HDN) and the outpatient prescriptions of the main health insurance AOK-Sachsen. The UHD was identified being the main consumer of fluconazole (range: 21.600–26.115 defined daily doses, DDD), followed by the outpatient prescriptions (range: 7.153–8.861 DDD), the HDF (range: 3.035–5.165 DDD), and the HDN (range: 1.195–2.010 DDD) (Table 3).

Over the last years, the use of ketoconazole in hospitals decreased. This trend was reflected by our data, there was no data in the HDF for 2013 and in the HDN for 2011–2013. Otherwise annual data were available and the amount prescribed to outpatients (range topical: 284–559 DDD) was higher than the uptake in the UHD (range topical: 200–500 DDD) (Table 3).

The distribution of voriconazole consumption was equivalent: the main consumer was the UHD (range: 4.288–5.775 DDD) with a trend towards lower prescription volumes in 2012 (4.975 DDD), and 2013 (4.288 DDD) compared with 2011 (5.775 DDD). The outpatient and the HDF consumption were lower ranging from 813 to 1.198 DDD and from 378 to 1.140 DDD, respectively. Finally,

the HDN had a consumption ranging from 20 to 118 DDD for 2010–2013 (Table 3).

Table 3: Annual consumption and prescription rates of fluconazole, ketoconazole, and voriconazole between 2010 and 2013

		DDD			
		2010	2011	2012	2013
Fluconazole	UHD	26.115	21.850	21.600	21.610
	outpatient	7.283	8.861	7.153	8.248
	HDF	5.165	4.205	3.035	4.385
	HDN	2.010	1.430	1.560	1.195
Ketoconazole topical	UHD	200	500	267	267
	outpatient	559	430	284	469
	HDF	0	0	0	0
	HDN	0	0	0	0
Ketoconazole systemical	UHD	0	80	20	0
	outpatient	0	0	0	0
	HDF	40	40	20	0
	HDN	20	0	0	0
Voriconazole	UHD	5.398	5.775	4.975	4.288
	outpatient	813	1.198	932	769
	HDF	1.140	378	938	685
	HDN	118	78	20	58

2.4. Theoretically expected influent concentrations in the STP of the city of Dresden

The theoretically expected influent concentrations of the azol antimycotics fluconazole and ketoconazole were derived using the monthly consumption and prescription rates of 2013. The waste water input of the City of Dresden to the STP was 75 % under the condition that the AOK-Sachsen insured 40 % of Dresden residents. Additionally the theoretically expected influent concentrations of unmetabolised drugs were based on results from *in vivo* pharmacokinetics (Brammer et al. 1991).

The fluconazole concentrations (monthly mean) of the influent ranged from 38.3 to 167.0 ng/L; the corresponding extrapolated concentrations ranged from 65.5 to 145.5 ng/L (Fig. 3). For ketoconazole, the measured concentration range was 18.3–347.9 ng/L (mean/month), but the estimated concentration range was 0.1–12.5 ng/L (Fig. 4). Figure 3 shows some agreement for fluconazole, nevertheless a correlation could not be achieved for one of the azol antimycotics.

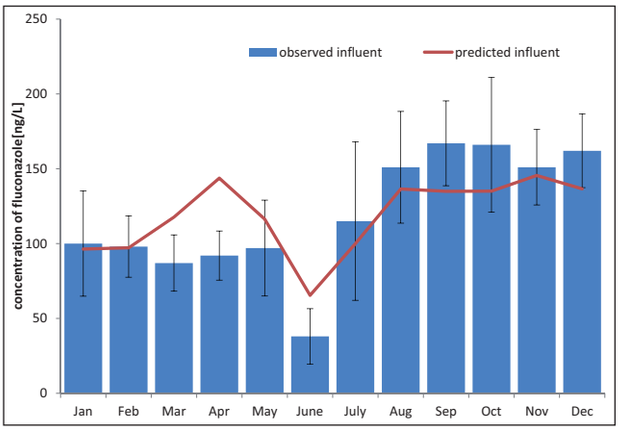

Fig. 3: Monthly means of the measured and the expected influent concentration based on the prescription and consumption rate of fluconazole of three Dresden hospitals and the AOK Sachsen dataset in 2013. Error bars represent the standard deviation.

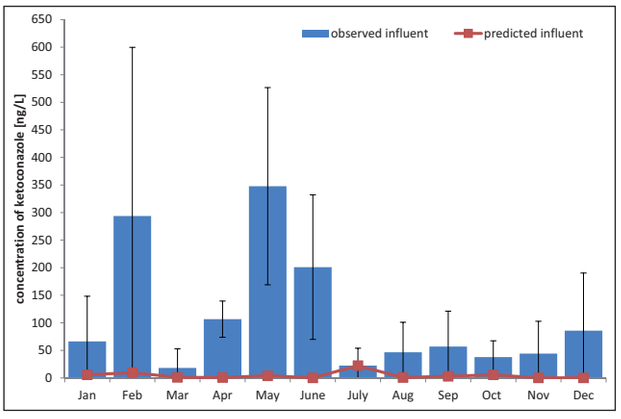

Fig. 4: Monthly means of the measured and the expected influent concentration based on the prescription and consumption rate of ketoconazole of three Dresden hospitals and the AOK Sachsen dataset in 2013. Error bars represent the standard deviation.

3. Discussion

The measurements of the azole concentrations in the sewerage drain system of the UHD, showed a high intraday variability for fluconazole reflecting the daily administration of the drug. The same intraday variability was not observed for ketoconazole. Instead, an even excretion pattern was observed within the 52 h observation period, with the exception of one measurement, accounted as an outlier. It showed a concentration of 47 ng/L, which was a factor of 2.8 higher than the other concentrations determined.

This difference could be explained by the topical use of ketoconazole and the partly removal by washing off the treated body-surface area. For the first time, the concentrations of fluconazole and ketoconazole were directly measured in a hospital sewerage drain system and provide additional knowledge to the publications on influent and effluent STP measurements (Lindberg et al. 2010; Peng et al. 2012).

We determined the influent and effluent concentrations of the three azole antimycotics at the STP Dresden Kaditz, too. The results of fluconazole and ketoconazole are comparable with previous publications (Lindberg et al. 2010; Peng et al. 2012). The degree of reduction obtained through the STP corresponds to literature data, particularly for fluconazole. Peng et al. (2012) reported that 95 % of the fluconazole remained in the effluent, in our study we found 99 %. They also observed a reduction down to 20 % for ketoconazole, we observed only 57 % (Peng et al. 2012). The differences could be due to a different treatment of the sewage, however, the publication of Peng et al. did not provide more details on the STP and how it works.

The measured concentrations of voriconazole were under the LLoQ - 50 ng/L. Nevertheless, a trend was observed (data not shown) so we can expect similar results as for fluconazol. The recovery rate within the effluent was 100 % and voriconazole was not detectable in the sludge. In order to improve the measurement accuracy further research should focus on dihydroxy-voriconazole. This metabolite of voriconazole is found in the urine to 14.63 % in comparison with the unchanged voriconazole with only 1.24 % (Geist et al. 2013).

The analysis of the consumption data of the three azol antimycotics showed that the UHD is the main prescriber in Dresden. In fact, it is the largest hospital in Dresden with the highest number of beds (capacity: UHD 1295, HDF 881, HDN 572) and, in addition, it is a center for oncological and haematological diseases in the region. Therefore, it is not astonishing that this hospital uses more azole antimycotics for prophylaxis and treatment of patients with invasive fungal infections than the other hospitals.

The extrapolation of the expected influent of the determined azole antimycotics was found to be the the closest for fluconazole. The prediction of the fluconazole load to the STP influent seems to be possible based on a more precise dataset like prescribed daily

doses. The expected influent concentrations of ketoconazole based on our estimations was undervalued probably because of the unknown contribution of over-the-counter products containing ketoconazole like shampoos. In result, the measured concentrations were higher as the assumed ones. Higher concentrations of ketonazole were detected in the winter months, which seems plausible in the context of the indication dandruff treatment.

In summary, fluconazole and ketoconazole are detectable in the sewerage drain of the hospital and STP and correlate to a certain extent to medicine usage data. At present, from the detected azole antimycotics only ketoconazole is partially removed by the STP and, thus, is uncontrollably released into the aquatic and soil environment. The absolute measured concentrations were low with a maximum mean per month of 167 ng/L or 348 ng/L for fluconazole or ketoconazole, respectively.

Fluconazole is not degraded by the current STP methods, as it is detected to almost 100 % in the effluent and ketoconazole is deposited in the sludge and is thus released to agriculture fields after STP treatment. The effect on the ecosystem is unpredictable. Peng et al. (2012) showed a higher reduction rate for ketoconazole than the STP achieved in the City of Dresden, a closer examination of the differences between the STP procedure would be of interest. Nevertheless, a more effective drug removal was already shown to be possible by introducing a tertiary sewage treatment, exemplarly for fluconanzole of 27 % up to more than 64 % (Margot et al. 2013). It should be officially debated to introduce this in the STPs Although the concentrations are low, an adaptation of fungi cannot be ruled out. Published data have already highlighted that fungi are able to pass on resistance genes (Ma et al. 2010). In consequence, there is growing risk of spreading resistence in the aquatic and soil environment, which in turn, could affect higher rates of severe systemic *Candida* infections.

4. Experimental

In the period of 2.8.2012 (12.00) until 4.8.2012, four-hourly pooled effluent samples were collected by an automated sampler in a shared sewerage drain of the clinic area for a total of 52 h. This sewerage drain was a merge of the emergency unit, neurology surgery, the clinic for visceral, thoracic and vascular surgery and was selected because it has continuous water flow and is shared solely among those clinics.

Between January and December 2013 a day-to-day pooled 24-hourly sample from the influent and effluent were collected at the STP Dresden Kaditz, Germany. In total, 317 and 251 corresponding influent/effluent samples were analysed each for fluconazole and ketoconazole, respectively. Furthermore, a sludge sample was taken from the STP on the 17.07.2013.

The sample collection, preparation and LC-MS analysis were done as previously described (Rossmann et al. 2014; Gurke et al. 2015).

Conflicts of interest: None reported.

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