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The occurrence of antimicrobial substances in toilet, sink and shower drainpipes of clinical units: A neglected source of antibiotic residues

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ABSTRACT

Antibiotics represent one of the most important drug groups used in the management of bacterial infections in humans and animals. Due to the increasing problem of antibiotic resistance, assurance of the antibacterial effectiveness of these substances has moved into the focus of public health. The reduction in antibiotic residues in wastewater and the environment may play a decisive role in the development of increasing rates of antibiotic resistance. The present study examines the wastewater of 31 patient rooms of various German clinics for possible residues of antibiotics, as well as the wastewater of five private households as a reference.

To the best of our knowledge, this study shows for the first time that in hospitals with high antibiotic consumption rates, residues of these drugs can be regularly detected in toilets, sink siphons and shower drains at concentrations ranging from 0.02 $\mu g \cdot L^{-1}$ to a maximum of 79 $mg \cdot L^{-1}$. After complete flushing of the wastewater siphons, antibiotics are no longer detectable, but after temporal stagnation, the concentration of the active substances in the water phases of respective siphons increases again, suggesting that antibiotics persist through the washing process in biofilms. This study demonstrates that clinical wastewater systems offer further possibilities for the optimization of antibiotic resistance surveillance.

1. Introduction

The treatment of bacterial infections remains a serious and cost-intensive factor in modern healthcare (European Commission, without year; U.S. Department of Health and Human Services - Centers for Disease Control and Prevention, April 23, 2013). According to the US Center for Disease Control and Prevention (US-CDC), at least 2 million patients in the United States of America (USA) are infected with resistant bacteria every year. Moreover, at least 23,000 patients die each year as a result of such infections (U.S. Department of Health and Human Services - Centers for Disease Control and Prevention, April 23, 2013). Cases in the European Union (EU) and the European Economic

Abbreviations: AMOX, amoxicillin; AMP, ampicillin; AZI, azithromycin; CEFA, cefaclor; CEFO, cefotaxime; CEFT, ceftazidime; CEFU, cefuroxime; CIP, ciprofloxacin; CLA, clarithromycin; CLIN, clindamycin; CTC, chlortetracycline; CLOX, cloxacillin; DDD, defined daily doses; DICLOX, dicloxacillin; DOC, doxycycline; ERY, erythromycin; dh-ERY, dehydrato-erythromycin; ENRO, enrofloxacin; ESBL, extended-spectrum beta-lactamase; FLU, flucloxacillin; LOQ, limit of quantification; LIN, linezolid; GLASS, Global Antimicrobial Resistance Surveillance System; MERO, meropenem; METHI, methicillin; METRO, metronidazole; MEZLO, mezlocillin; MIC, minimal inhibition concentration; MOX, moxifloxacin; MDRO, multi drug resistant organisms; NAF, nafcillin; OFLOX, ofloxacin; OXA, oxacillin; OTC, oxytetracycline; PEN-G, penicillin G; PEN-V, penicillin V; PIP, piperacillin; PNEC, predicted no effect concentration; RDD, recommended daily dose; ROX, roxithromycin; STP, sewage treatment plant; SH, shower drain; SI, sink siphon; SPIR, spiramycin; SCP, sulfachlorpyridazine; SDZ, sulfadiazine; SDMX, sulfadimethoxine; SDMD, sulfadimidine; SDX, sulfadoxine; SEP, sulfaethoxypyridazine; SMZ, sulfamerazine; SMX, sulfamethoxazole; N4AcSMX, N4-Acetylsulfamethoxazole; SMP, sulfathiazole; STZ, sulfamethoxypyridazine; TC, tetracycline; TMP, trimethoprim; TYL, Tylosin; US-CDC, US Center for Disease Control and Prevention; WC, water closet; WHO, World Health Organization; VANC, vancomycin

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Area (EEA) are comparable with estimated 33,110 deaths from resistant bacteria in 2015, with mortality rates higher in the southeast than in the northwest (Cassini et al., 2018). Based on data from the World Health Organization (WHO) evaluated in the Global Antimicrobial Resistance Surveillance System (GLASS), *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Salmonella* spp. were the most frequently reported antibiotic resistant bacteria (World Health Organization, January 29, 2018).

In response, national and international antibiotic resistance surveillance programs have been established (Bundesministerium für Gesundheit et al., April 2018; Qiao et al., 2018; The White House Washington, March 2015). In addition, various international programs have been set up to evaluate and share national research data, meet harmonized standards and coordinate global action against the spread and development of antibiotic resistance (European Centre for Disease Prevention and Control, 2017; World Health Organization, 2015, 2017b).

Although the antimicrobial resistance situation in Germany has not increased in recent years, it should be mentioned that it is at a significantly higher level than in the 1990s (BVL & Paul-Ehrlich-Gesellschaft für Chemotherapie e.V., 2016). In particular, Vancomycin resistant Enterobacteriaceae (VRE), methicillin-resistant Staphylococcus (MRSA) and carbapenem-resistant Enterobacteriaceae (CRE) represent current dangers for the global health system (U.S. Department of Health and Human Services - Centers for Disease Control and Prevention, April 23, 2013). Therefore, numerous studies regarding the medical importance of VRE (Cetinkaya et al., 2000; O'Driscoll and Crank, 2015; Sievert et al., 2013), MRSA (Hassoun et al., 2017; Sievert et al., 2013) and CRE (Kizny Gordon et al., 2017; Sievert et al., 2013; Walsh, 2010) have been published in recent decades. Moreover, increased global commodity flow (Iark et al., 2018; Roca et al., 2015; Teubner, 1999) and inter-continental travel (European Centre for Disease Prevention and Control, 2018; Ostholm-Balkhed et al., 2013; Tängdén et al., 2010; Wiklund et al., 2015) constitute potential risk factors. A previous study has demonstrated that approximately one quarter of the travelers (inter-continental) examined were colonized with extended-spectrum beta-lactamase- (ESBL) producing *Escherichia coli* during their journey, with hotspots including the Middle East (e.g. Egypt) and Asia (especially India) (Tängdén et al., 2010). In addition, a recent study has shown the contamination of chicken meat with ESBL-producing *Escherichia coli* (CTX-M-44) in Brazil and the possible risk of export (Iark et al., 2018).

In this context, it is interesting to note that in Germany the total amount of defined daily doses (DDD) and the number of prescriptions of antibiotics remained constant between 2005 and 2014, whereas fluorochinolones and cephalosporins are still very high (sum of prescriptions in 2014: “reserve” cephalosporins = 7.4 million DDD; “reserve” fluorochinolones = 5.6 million DDD). The total amount of antibiotics used in Germany for human medicine is approximately 700–800 t (BVL & Paul-Ehrlich-Gesellschaft für Chemotherapie e.V., 2016).

In addition to human medicine, the usage of antibiotics in veterinary medicine is another important field of application that is important for the interpretation of the total consumption of antibacterial substances (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, July 23, 2018; BVL & Paul-Ehrlich-Gesellschaft für Chemotherapie e.V., 2016; European Commission, without year; Qiao et al., 2018). In the period from 2011 to 2017, the total quantity of antibiotics (data evaluated by DIMDI (German Institute for Medical Documentation and Information) fell from 1.706 t to 733 t (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, July 23, 2018). This corresponds with a percentage of antibiotics for veterinary applications of approximately 50% of total consumption. In the USA, it is estimated that around 80% of total consumption is used for animal fattening (European Commission, without year). In China a comparable proportion of antibiotics was found in livestock farming (52%), but with a significantly higher total amount of 92,000 t in 2013 (Qiao et al., 2018).

It is important to note that the human health system, the veterinary sector and the environment are interrelated (Feuerpfeil et al., 1999; Kümmerer, 2003; Roca et al., 2015; Westphal-Settele et al., 2018) and cannot be considered separately. Rather, the whole context must be contemplated, as described in the “One-Health” concept (World Health Organization, November 12, 2018).

Thus, for example, the decreasing total quantity of prescribed antibiotics in Germany in livestock sectors, the quantities of fluoroquinolones (9.9 t, +1.7%), cephalosporins of the 3rd generation (2.3 t, +0.2%), and the large amount of polymyxin (74 t), must be viewed critically (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, July 23, 2018), because these antibiotics, which are related to the “watch” and “reserve” group, should be restricted for treating serious or life-threating infections in human patients (World Health Organization, March 2017). In general a reduction or renouncement in antibiotics in intensive livestock farming, which are defined as “critically important antimicrobials” (e.g. vancomycin, meropenem, linezolid, ciprofloxacin, ampicillin and colistin) for human medicine by the WHO, should be considered (World Health Organization, 2017a).

A good example of a more restrictive usage of antibiotics in veterinary fields is the prohibition of colistin as a potential growth stimulator in China in 2017 (Qiao et al., 2018) and the general restriction of antimicrobial substances for growth promotion in the EU since 2006 (VO (EG) Nr. 1831/2003, December 30, 2005). Furthermore, the development of “Guidelines for careful handling of antibacterial veterinary medicinal products” in 2015 may help veterinarians to find a more effective and restrictive application of antibiotics (Bundestierärztekammer (BTK), 2015).

The reduction of the release of antibiotic residues, resistant genes and antibiotic resistant bacteria into the environment should be emphasized in the development of new wastewater treatment processes and a sufficient antibiotic resistant surveillance system. This can be explained by the fact that antibiotic residues are just partially metabolized, and excreted unchanged will enter the wastewater path (Faerber et al., 2003; Kümmerer, 2003, 2009). Recent studies have demonstrated that antibiotic genes (Rizzo et al., 2013; Xu et al., 2015; Yang et al., 2014), antibiotic-resistant bacteria (Feuerpfeil et al., 1999; Müller et al., 2018; Rizzo et al., 2013) and antibiotic residues (Chang et al., 2010; Feuerpfeil et al., 1999; Kümmerer, 2001) are incompletely eliminated in sewage treatment plants and enter the (aquatic) environment.

The fate of antibiotic residues varies with the respective substance class. Tetracyclines and fluoroquinolones have a high adsorption capacity (Golet et al., 2003; Kümmerer, 2003, 2009) and are removed from the water cycle with sewage sludge. B-lactams exhibit increased hydrolysis sensitivity regarding their β-lactam ring (Deshpande et al., 2004; Kümmerer, 2009). On the other hand, sulfonamides and macrolides tend to have a higher persistence within the wastewater treatment plant and can therefore be detected more frequently and in higher concentrations in surface waters (Faerber et al., 2003; Kümmerer, 2009).

For conventional sewage treatment plants (STP), it has been shown in former studies that a general reduction in bacteria by one to three log levels can be achieved (Koivunen and Heinonen-Tanski, 2005; Mandilara et al., 2006; Rechenburg et al., 2006; Schreiber et al., 2015). A similar reduction capacity can be achieved for antibiotic-resistant bacteria (publication in preparation).

A schematic overview of the potential clinical wastewater pathways of antibiotic residues in the aquatic environment is shown in Fig. 1.

Released into the environment, antibiotic residues may be a selection factor in aquatic matrices in favor of antibiotic-resistant bacteria (Baquero et al., 2008; Bengtsson-Palme and Larsson, 2016). Even small concentrations (< MIC, minimal inhibition concentration) of antibacterial substances can stimulate the transfer of resistance genes via horizontal gene transfer or promote the interaction of resistant

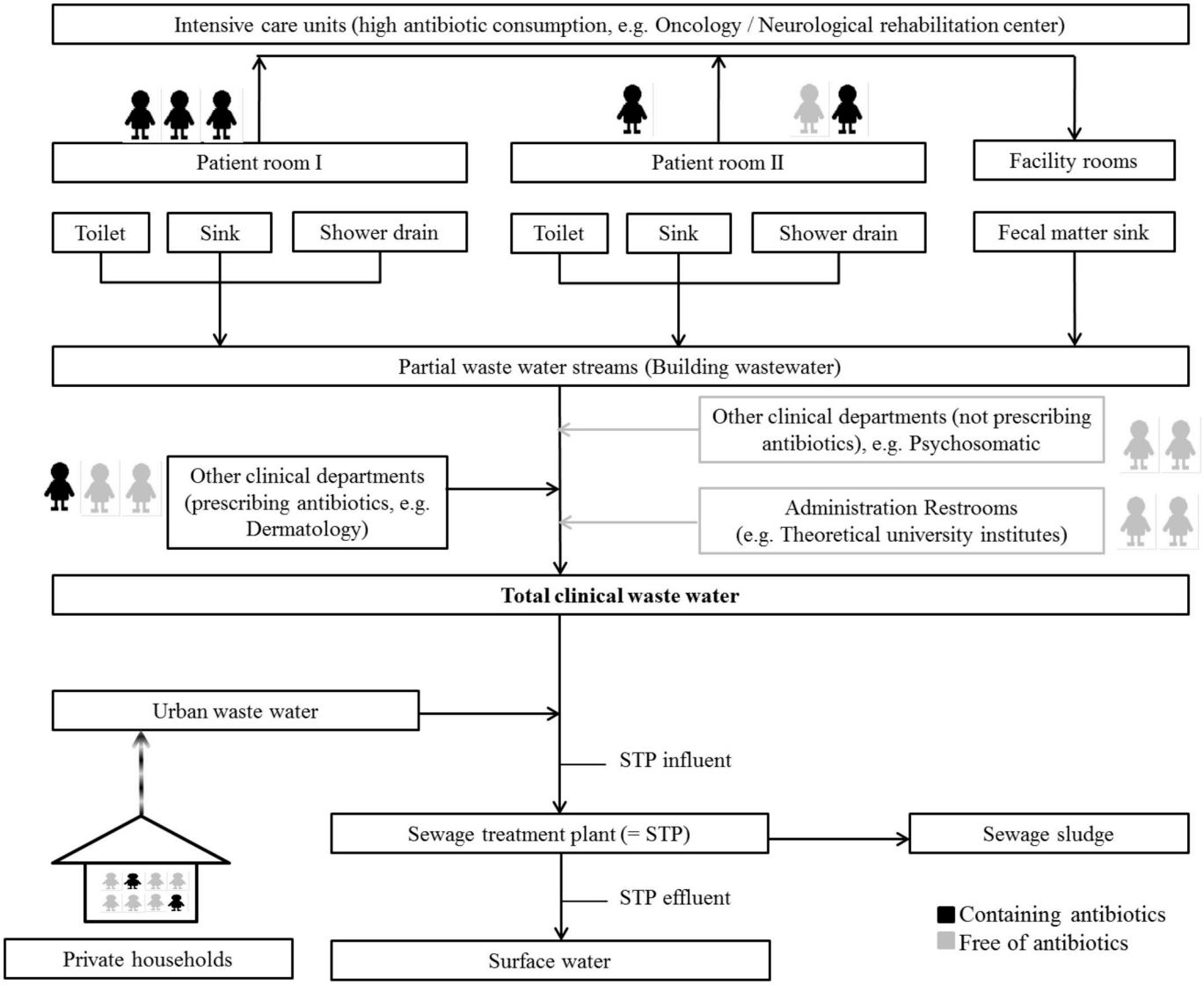

Fig. 1. Schematic overview representation of the clinical wastewater path (from the patient's room to the effluent of treated wastewater into surface water) and potential entry paths of antibiotic residues into the hospital wastewater and ultimately the aquatic environment. As expected, wastewater pathways free from antibiotics are shown in gray and wastewater pathways contaminated with antibiotics are shown in black.

wastewater bacteria with sensitive germs in the aquatic environment (Finley et al., 2013; Jutkina et al., 2018). Furthermore, it must be considered that certain environmental bacteria, like *Acinetobacter calcoaceticus*, are able to acquire genome fragments (which could include antibiotic resistance genes) (Palmen et al., 1993).

Antibiotics are detected more frequently and in higher concentrations in hospital wastewater than in municipal wastewater (Kümmerer, 2001; Watkinson et al., 2009). Some studies have already revealed high levels of contamination of total hospital wastewater with antibiotic residues through detection of ciprofloxacin (up to concentrations in the three digit $\mu g \cdot L^{-1}$ range) (Diwan et al., 2010; Kümmerer, 2003; Lindberg et al., 2004) or ampicillin (up to concentrations in the two digit $\mu g \cdot L^{-1}$ range) (Kümmerer, 2003).

In addition, clinical wastewater has been identified as a potential hotspot for antibiotic-resistant bacteria and genes (Koh et al., 2015; Müller et al., 2018; Picão et al., 2013; Schwartz et al., 2006; Simo Tchuinte et al., 2016; Zhang et al., 2014).

Therefore, an important approach to preventing the further development of resistance is the reduction of the input of these substances into the wastewater pathway. To clarify this pathway, the wastewater from various clinical areas in several German hospitals was examined in this study using a screening method by LC-MS/MS through injecting aqueous samples without complex sample preparation (filtration only). The aim was to examine the hospital wastewater more closely on the basis of its spectrum and its content of antibiotic residues, and to identify largely contaminated wastewater substreams.

2. Material and methods

2.1. Materials and chemicals

All chemicals used were purchased from VWR International GmbH (Langenfeld, Germany) and Carl Roth GmbH + Co. KG (Karlsruhe, Germany), and were HPLC-MS grade. The analytical standards were purchased from Sigma Aldrich (Taufkirchen, Germany), Biomol GmbH (Hamburg, Germany), Toronto Research Chemicals Canada (Toronto, Canada) and USP Reference Standard (Basel, Switzerland). The LC columns and micropore filters used were obtained from Macherey and Nagel (Düren, Germany).

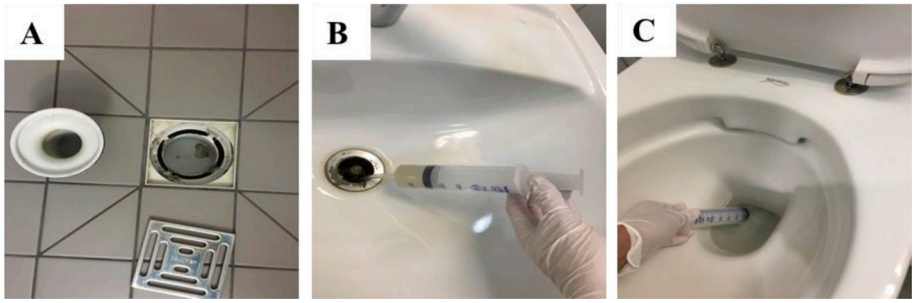

Fig. 2. Sampling spots in the oncology department: A) shower drainage B) sink and C) toilet.

2.2. Sampling sites

In the period from April 2017 to February 2018, the wastewater (defined as the aqueous phase in toilets, shower drains and washbasin siphons) of 31 patient rooms [shower drains (SH), toilet drains (WC) and washbasin siphons (SI)] in four clinical areas (oncology, neurological rehabilitation (regular ward and neuro-urology), dermatology and psychosomatics) was examined for antibiotic residues (see Fig. 2). Sanitary areas in a theoretical university institute (i.e., lecture halls, without any relations to patients) and in five private households without current use of antibiotics were investigated in the same way.

The oncology area investigated was characterized by a slightly increased amount of antibiotic consumption (recommended daily dose per 100 patient days = RDD·100 d^{-1}) in total 145.27 RDD·d^{-1}, in comparison to other hematological oncologies in Germany (based on data for 2017). For further information see Table S2.

In the case of oncology, the rooms were randomly selected to obtain a cross-section of the entire clinic (with a generally high and frequent use of antibiotics). The current administration of antibiotics was previously known (in 14 of 20 investigated rooms, see Table S1), but this did not determine whether a room was examined or not. In the case of dermatology and neurological rehabilitation, patient rooms with prior administration of antibiotics were targeted (see Table S1) in order to be compared with other clinics with a different medical specialization. Private households, the theoretical university institute and psychosomatics were selected as reference samples due to the lack of current antibiotic administration.

2.3. Sampling procedure

Sampling was undertaken in two phases (1. status quo, 2. stagnation) at several defined times (status quo, 0, 2, 4, 6, 12 and 24 h). The general sampling procedure is described below. Samples from WC were taken using a 50 mL disposable Omnifix® syringe from B. Braun Melsungen AG (Melsungen, Germany) directly from the water phase. For sampling the aqueous phase of SI and SH, additional inert tubes (length: approx. 32 cm) were used. The water was bottled, stored at 5 ± 2 °C and analyzed within 24 h.

To prevent possible falsifications of the results, contact or mobilization of the biofilm and other incrustations on the walls was strictly avoided during the sampling procedure.

2.3.1. Status quo sample

The “status quo” sample was a random grab sample (single sample) taken at a specific time in a patient's room without knowing when the last washbasin, shower or toilet was used or cleaned by the patient or staff. The systems were flushed with approximately 15 L of drinking water (antibiotic-free and filtered through inline water filters from Pall GmbH (Dreieich, Germany)) directly afterwards. (Note: preliminary tests had shown that approximately 15 L of rinsing volume were necessary so that no active substances could be detected in the water phase.)

2.3.2. Stagnation samples

When selecting the patient rooms for the stagnation tests, care was taken to ensure that the patient rooms could be locked for at least 24 h (or only the bathroom in the case of bedridden patients). This was the limiting factor for the possible number of patient rooms, which were examined (status quo sample and stagnation samples) immediately following discharge or during the hospital stay of bedridden patients. Immediately after rinsing the respective siphon, a t_0 sample (= 0 h stagnation) was drawn. At regular intervals (approx. 2 h, 4 h, 6 h, 12 h and 24 h) further stagnation samples (sample t_2-t_4, etc.) were taken analogous to the t_0 sample and analyzed as described above (one replicate per sample).

2.4. High-performance liquid chromatography

All samples were diluted 1:1 with a water-acetonitrile (95:5, v/v) mixture with 0.8 g/L Na_2-EDTA. This mixture was then cleaned using a water-wettable H-PTFE filter (0.45 µm pore size) and 20 µL were injected into the LC (direct injection). An Agilent LC-System 1290 II with a Nucleoshell RP18Plus® column 2 mm × 100 mm, 2.7 µm (Macherey & Nagel, Düren, Germany) was used to separate 48 different antibiotics. The total duration time was 15 min.

2.5. Mass spectrometry

The separated antibiotics were detected by a QTRAP® 6500 + mass spectrometer from AB Sciex GmbH (Darmstadt, Germany). The analytes were ionized by an electrospray ionization (= ESI) interface in positive mode with an ionization voltage of 5500 V. Scheduled multiple reaction monitoring (sMRM) mode was used to detect the two most intensive ion transitions of each antibiotic for identification and quantification.

3. Results

3.1. Status quo samples

3.1.1. Oncology

Between 2017 and 2018, a total of 20 patient rooms (aqueous phases of 19 SH, 20 WC and 20 SI) were examined for residues of antibiotics. The time of sampling varied between 08:00 a.m. and 04:00 p.m. (see Section II.3.1).

In the 19 SHs studied, at least one antibiotic was detected in each sample (percentage of frequency: SMX 100%, TMP 95%, N4AcSMX 84%). Residue concentrations ranged from 0.02 µg·L^{-1} to 3500 µg·L^{-1}, with PIP at 3500 µg·L^{-1}, N4AcSMX at 480 µg·L^{-1} and TMP at 260 µg·L^{-1} having the highest concentrations.

At least one antibiotic was also detected in 19 of the 20 SIs investigated (percentage of frequency: SMX 95%, TMP 90%, N4AcSMX 60%). The residue concentrations ranged from 0.04 µg·L-1 to 230 µg·L^{-1}. The highest concentrations were found for PIP (230 µg·L^{-1}), CIP (50 µg·L^{-1}) and CLA (20 µg·L^{-1}).

In total, 16 of 20 WC had antibiotic residues in the range of

0.02 µg·L^{-1} to 79,000 µg·L^{-1}, including SMX (percentage of frequency: 70%), TMP (65%) and N4AcSMX (70%). However, the highest values were found for CLA (1200 µg·L^{-1}) and PIP (79,000 µg·L^{-1}).

3.1.2. Neurological rehabilitation

Five additional patient rooms were sampled according to the same scheme in neurological rehabilitation (regular ward and neuro-urology).

In all WC (5), SI (4) and SH (5) tested, at least one antibiotic could be detected in ranges from 0.05 µg·L^{-1} to 1300 µg·L^{-1}. The number of different antibiotics was a maximum of nine (in SH) and a minimum of four (SI).

CIP and VANC (80% and 60%) could be detected most frequently in the shower drains (SI: 50% CIP and 75% PIP; WC: 60% PIP). The highest concentration detected was 1300 µg·L^{-1} MERO in a WC. All results for oncology and the neurological rehabilitation are presented in Table 2.

3.1.3. Dermatology

In the sanitary area of a single patient room, antibiotic residues in a range from 0.11 µg·L^{-1} to 39 µg·L^{-1} could be detected in all three samples. The WC showed the highest sum of antibiotic residues with 75.3 µg·L^{-1} (39 µg·L^{-1} CEFTA, 33 µg·L^{-1} CLIN and 3.30 µg·L^{-1} PIP). CLIN (1.80 µg·L^{-1}), CIP (3.80 µg·L^{-1}) and dh-ERY (0.11 µg·L^{-1}) were identified in SH. The SI showed the lowest antibiotic load; however, residues of CLIN and PIP were found there (0.35 µg·L^{-1} and 0.45 µg·L^{-1}, respectively).

3.1.4. Psychosomatics, university institute (without patient care) and private households

The final antibiotics were prescribed at least two months ago in the psychosomatics studied. None of the 18 samples (6 SH, 6 WC and 6 SI) contained antibiotic residues.

Outside the clinical areas, three two-person households, one one-person household and one four-person household, as well as a theoretical university institute, were examined. In all households studied, no antibiotics had been taken for more than a year; in the university institute this was not known due to the changing use of sanitary facilities by staff, students and visitors. None of the 17 sanitary units examined (8 WC, 6 SH, 7 SI) contained antibiotic residues. Therefore, stagnation tests were not carried out in all of these areas.

3.2. Stagnation tests

3.2.1. Oncology and dermatology

It was concluded that the frequent detection of antibiotics in the examined status quo samples may be due to insufficient flushing by the patient. Therefore, after rinsing the respective system with approximately 15 L of drinking water (see Section II.3.1), the t_0 samples were first taken. No active substances could be detected in 15 of 24 t_0 samples (sum of all sanitary units). The remaining nine samples, which exhibited small traces of antibiotics even after rinsing, were distributed over six DU (max. 1.30 µg·L^{-1} CIP, status quo: 31 µg·L^{-1} and rising up to 12 µg·L^{-1}), two WC (max. 2.30 µg·L^{-1} CIP, status quo: 98 µg·L^{-1} and rising up to 7.50 µg·L^{-1}) and one SI (= 0.28 µg·L^{-1} N4AcSMX, status quo: 3.00 µg·L^{-1} and rising during stagnation up to 2.00 µg·L^{-1}).

In order to clarify whether the siphon water may be re-contaminated by active substances adhering to the pipe system, so-called stagnation samples were taken at intervals in accordance with the above sampling scheme. In seven bathrooms in oncology and one bathroom in dermatology, the stagnation process was examined over a period of about 24 h.

In these stagnation samples (samples t_2, t_4, t_6 … t_{24}), it was shown that in the siphons of WC, SH and SI a renewed increase of the antibiotic concentrations in the previously rinsed systems could often be detected without patients influencing the system during these controlled periods.

In the stagnation waters, CIP, CLIN, CLA, PIP, LIN, METRO, SMX, TMP and VANC and the two metabolites dh-ERY and N4AcSMX could be found. The residue concentrations in the stagnation samples ranged from 0.02 µg·L^{-1} (SMX; accounted for 1.4% of the status quo value determined in this sample) to 12 µg·L^{-1} (CIP; accounted for 38.7% of the status quo value determined in this sample).

Fig. 3 and Fig. 4 show similar results (regardless of the type of sanitary unit) in the form of an increasing concentration curve, which, however, seems to partly depend on the analytes and other influencing variables (e.g. cleaning state, microbial siphon coating and time of last active substance application). Thus, Fig. 4 shows a clearly deviating stagnation behavior of CIP in the SH of dermatology.

The typical concentration curve was characterized by a steep increase in concentration within the first 2–5 h, followed by a flatter increase to a maximum residue concentration (within the first 10 h) and the achievement of an “equilibrium concentration” in which the antibiotic concentration does not change noticeably in the further course of the stagnation period. This re-occurrence was unexpected as there was no “external” supply of antibiotics during the stagnation periods, and the reason for this must be comtingent on the siphons themselves. Some stagnation curves of antibiotic residues found in the sanitary units of two patient rooms (oncology room M and dermatology) are exemplarily shown in Figs. 3 and 4.

4. Discussion

4.1. Antibiotic residues in U-bends (WC, SI and SH)

To the best of our knowledge, the results obtained in this study show for the first time the presence of antibiotic residues in SH, SI and WC in very high concentrations (up to a maximum of 79,000 µg·L^{-1}). Such residues could exert a selection pressure in the sewage system of the respective patient room in favor of resistant bacteria (Baquero et al., 2008; Bengtsson-Palme and Larsson, 2016; Finley et al., 2013; Jutkina et al., 2018).

A comparison of the different clinics demonstrated that the oncology and neurological rehabilitation clinics were frequently (and in some cases very heavily) contaminated with antibiotic residues in the sanitary units. In contrast, no residues of antibiotics could be detected in the psychosomatics clinic, where no antibiotics had been administered for at least two months. Accordingly, no residues could be found in the private households examined or in the sanitary facilities of the theoretical hospital institute. These results clearly show that the presence of antibiotic residues in WC, SI and SH correlate with high consumption levels in the respective clinics.

The increased concentrations in patient wastewater are consistent with various studies from recent years, which have repeatedly demonstrated high concentrations of antibiotic residues in the total wastewater of various hospitals (Chang et al., 2010; Diwan et al., 2010; Faerber et al., 2003; Lindberg et al., 2004; Oliveira et al., 2015; Ory et al., 2016; Watkinson et al., 2009). Furthermore, a correlation between the detected antibiotic residues and the antibiotics administered (pattern and amount) could be determined (Faerber et al., 2003; Oliveira et al., 2015; Ory et al., 2016; Watkinson et al., 2009).

In spite of the large number of detections of antibiotic residues in total hospital wastewater in recent decades, it is surprising that antibiotic residues can be detected in SI, WC and SH, because these should be antibiotic-free by regular flushing of the pipe system with drinking water, which must comply with the requirements of the German Drinking Water Ordinance (TrinkwV 2001, 21. Mai 2001).

The cause of antibiotic residues in WC is more explicable relative to the findings in SH and SI, as WC are exposed to a high concentration of antibiotic residues in the urine and feces. Potential sources of antibiotic residues in SI or SH could be the brushing of teeth, showering of the body or ejection of pulmonary secretions, as residues of PIP in saliva

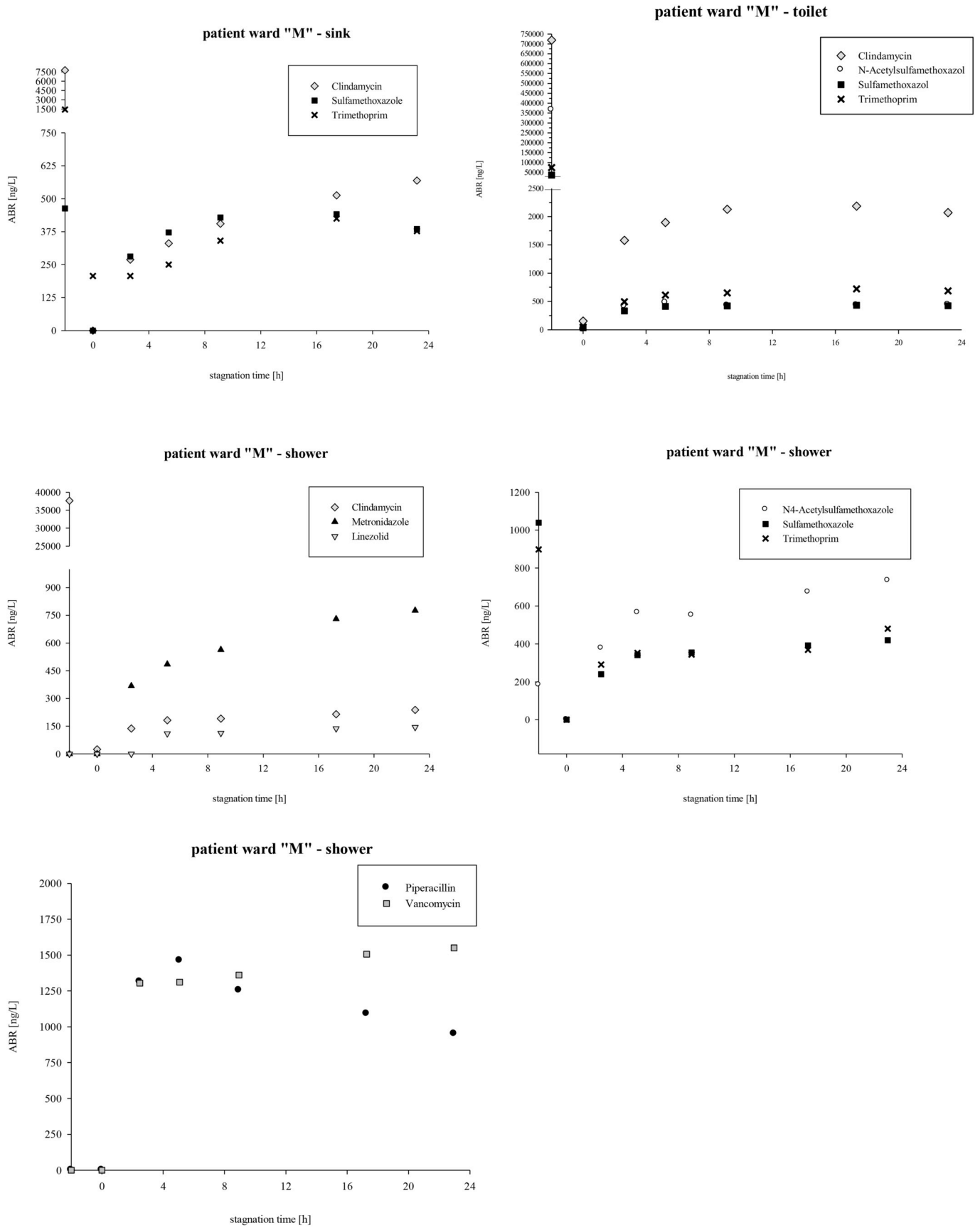

Fig. 3. Selection of typical stagnation curves for various antibiotics in different sanitation units in an oncological patient room (values on the Y-axis correspond to the status quo results for the particular antibiotic).

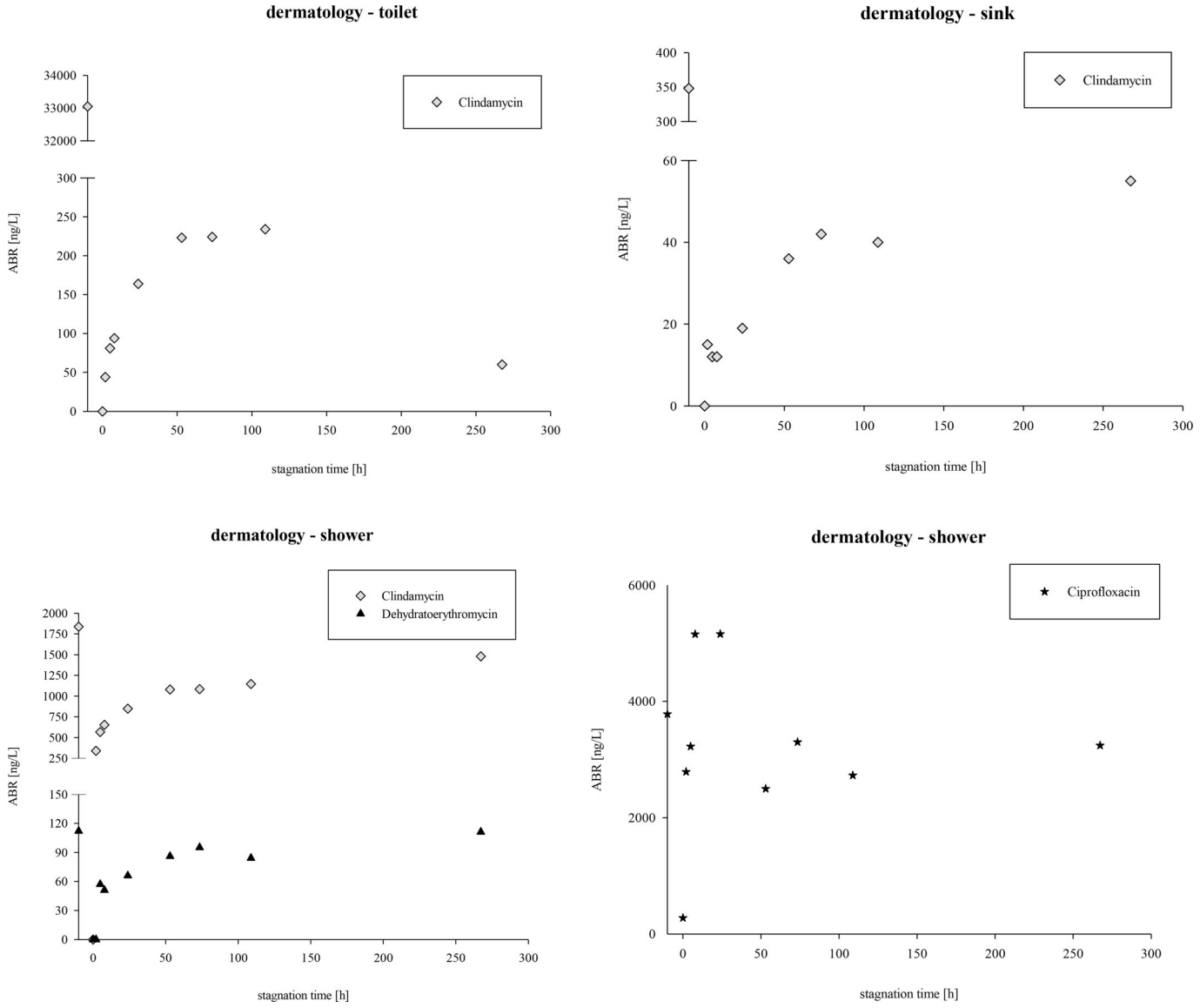

Fig. 4. Typical stagnation curves for various antibiotics in different sanitation units in a dermatological patient room (values on the Y-axis correspond to the status quo results for the particular antibiotic).

(Sagent Pharmaceuticals, 2013) or lactam antibiotics in sweat in general (Hoiby et al., 2000) have already been detected. Furthermore, improper urinating in SI and SH (based on reduced mobility) may represent another entry path. The disposal of patient wastewater into SI is also possible (Parkes and Hota, 2018).

Furthermore, this study may indicate an astonishing casuistry in dermatology. In this case, the patient was administered clindamycin (also PIP and CEFTA) immediately before sampling, which was detected in the shower. In addition, the patient defecated frequently during the inpatient stay and was therefore washed in the shower by the nursing staff, hence this path of entry of antibiotics into the SH must also be taken into account.

Even after more than 10 d of temporal stagnation (WC, SH and SI in the dermatology), significant residues of CLIN could be detected in SH (Fig. 4). This suggests that rinsing should have been conducted with a much larger amount of water. The other two antibiotics prescribed to the patient, PIP and CEFTA, were only detectable in WC and SI.

In addition to oncology, neurological rehabilitation was examined as another clinical area with high antibiotic prescription. The results of this clinic are in accordance with the findings of oncology in general, and show significant contamination of the siphon water with antibiotic residues (Table 2).

The detected antibiotic residues from oncology and dermatology are congruent with the general consumption of these clinics (Table S2) and the current room's antibiotic usage (received from the patient documentation, Table S1), respectively. A good example is the detection of PIP (20% positive samples, 0.12 $\mu g \cdot L^{-1}$ up to 79,000 $\mu g \cdot L^{-1}$), one of the most frequently used antibiotics in oncology (18.80 RDD·100 d^{-1}, c.f. Table S2). Furthermore, clindamycin and macrolides, LIN, carbapenems (especially MERO), PIP, narrow spectrum penicillins (especially AMPI), fluoroquinolones (especially CIP) and sulfonamides (SMX + TMP) are frequently used (RDD > 75th percentile).

In particular, the frequent evidence of SMX, TMP and CIP can be explained by the prescription practice in this clinic. As part of a general prevention of nosocomial infections, a combination of CIP and Cotrimoxazol® (TMP/SMX) is administered preventively in the first days of treatment. It is not surprising that CIP as well as SMX and TMP, which together constitute the medicine Cotrimoxazol® in a mixing ratio of 5:1, were most frequently detected in the sanitary units of oncology with 43%, 88% and 83%.

In accordance with these results, N4AcSMX (main metabolite of SMX) was found in approximately 71% of the sanitary units in

oncology. In general, 45–70% of the administered dose is excreted renally within 24 h, whereby approximately 20% is excreted as unchanged active SMX and 40% as N4AcSMX (Radke et al., 2009). This may explain the findings of SMX and N4AcSMX in WC, SH and SI via fecal entry.

However, there were also differences between the various clinics, most likely due to different prescription practices (Table 3). As a result, fewer and less common residues of SMX and TMP could generally be found. This can be explained by a lack of prevention therapy with Cotrimoxazol®. In addition, no Cotrimoxazol® was being administered in the sampled rooms at the time of sampling.

The antibiotic residues of MERO, PIP and VANC detected were consistent with the current room's antibiotic usage (antibiotic usage at the day of sampling or recently before, received from the patient documentations, Table S1). The antibiotics mentioned above are used for more serious infections (Harris et al., 2015; Isaac et al., 2017; Yang et al., 2015). This may owe to the fact that in the neurological rehabilitation of patients a treatment approach with a significantly longer inpatient stay is used, and thus a higher risk of colonization with multidrug resistant organisms (MDRO) exists (excluding other parameters like immune status, primary diseases or patient characteristics, e.g. age). Although oncological patients are more frequently treated in clinics, the length of stay is usually shorter. However, not all currently administered antibiotics could be detected in the siphons of the respective patient rooms (Table S1). This could be due to the fact that other influencing variables such as adsorption (fluoroquinolones), chelation (tetracyclines), photocatalytic degradation (e.g. fluoroquinolones, sulfonamides, tetracyclines), hydrolysis (β-lactam antibiotics) (Deshpande et al., 2004; Kümmerer, 2009) and of course the specific metabolization of the individual active substances (Kümmerer, 2009) play a role, in addition to the pure consumption figures of the examined clinics (see e.g. Table S2).

In accordance with the lack of detected β-lactams, a study from 2010 showed that in the wastewater of a clinic where fluoroquinolones (e.g. CIP) and the β-lactams ceftriaxone (126,700 mg·days^{-1}) and amoxicillin (19,150 mg·days^{-1}) accounted for the majority of prescriptions, residues of CIP could be detected in the up to three-digit µg·L^{-1} range, but no β-lactams could be detected (Diwan et al., 2010). This accords with the results of this study, as despite high β-lactam consumption rates (based on RDD·100^{-1}, c.f. Table S2), especially from MERO and AMP, lactams were only sporadically found in the sanitary units of oncology, see Table 2. This rarer detection of cephalosporins, penicillins and carbapenems (which all belong to the group of β-lactams) in oncology could be due to a reduced stability caused in the cleavage of the β-lactam ring (Deshpande et al., 2004; Kümmerer, 2009).

An exception is PIP, which was relatively common (20% in WC, 40% in SI) and partly with extremely high concentrations (up to 79,000 µg·L^{-1}). These results are plausible to the extent that PIP accounts for around 13% of the total prescriptions in oncology (based on RDD·100^{-1}, c.f. Table S2). However, this is countered by the fact that MERO, with about 14% of the antibiotic used mostly in the second line, was not found in any single oncology sample.

The comparison of the pharmacokinetics of PIP and MERO cannot explain the discrepancy between the antibiotic residues found and the prescription pattern, as the excretion pathways and rates are similar (Moon et al., 1997; Sörgel and Kinzig, 1993). On average, 50–70% of the PIP dose administered (related to the dose and other prescribed drugs) is excreted via urine and partially eliminated via feces (Sörgel and Kinzig, 1993). For comparison, MERO is excreted predominantly renally (about 72% of the dose as unchanged active substance and about 23% as inactive metabolite), as well as small amounts via feces (Moon et al., 1997).

A final explanation for this situation could not be found using the tests undertaken. However, it must always be considered that the status quo samples were random grab samples without prior knowledge of the room history. Thus, there is the possibility that the rooms examined were merely those in which MERO was not currently being used or had not been used in the recent past. Furthermore, residues of antibiotics could be detected that were not part of the antibiotics administered before the sanitary units were sampled. These results could be explained by the use of sinks or toilets by visitors or other patients who also received antibiotics. However, this does not explain the findings in SH. This provided the first indications of a kind of storage or accumulation of antibiotic residues in the control system or in the respective siphons (see Section IV.2).

For an initial interpretation of the residue concentrations found with regard to possible resistance developments, the status quo results found in this study were compared with PNEC and MIC values proposed in a recent study (Bengtsson-Palme and Larsson, 2016). The PNEC corresponds to the “predicted no effect concentrations for resistance” and the MIC to the “lowest minimal inhibitory concentration” observed for any species in the EUCAST database (Bengtsson-Palme and Larsson, 2016). It appears that in the vast majority of rooms (92%, total amount of rooms: 25), the residues of at least one antibiotic exceeded the PNEC (yellow spots) or even the MIC (red spots) (Fig. 5). Thus, by exceeding the PNEC over a long period of time, selection can be increased in favor of resistant organisms (Bengtsson-Palme and Larsson, 2016). Moreover, exceeding the MIC would mean a preference for already resistant organisms and a suppression of sensitive strains, thus providing a growth advantage for the resistant strains.

However, it should be noted that due to the high limit of quantification (LOQ) of CIP (200 ng·L^{-1}), no statements can be made regarding the influences or missing influences on resistance developments for negative samples or samples with a CIP content lower than the LOQ, since the LOQ is above the PNECs proposed by Bengtsson-Palme and Larsson (2016) of 64 ng·L^{-1}. Accordingly, for CIP only the samples with a concentration greater than the PNEC and the LOQ are highlighted in yellow, and greater than the MIC in red.

The SHs seem to have a particularly high or frequent load, as this is where most antibiotic residues were found in comparison to the three sanitary units. In addition, the sum of the SHs investigated yielded the highest number of antibiotic residues exceeding either MIC or PNEC. This may be due to insufficient flushing (too low flushing volume) of the SH after showering or alternatively to the lack of mechanical cleaning (as in toilets), as the shower drain is closed by a grid.

It is also worth mentioning that in the investigated WC (n = 20), 22 antibiotic residues exceeded the proposed MIC.

In the five rooms of neurological rehabilitation examined WC and SI seem to constitute potential hotspots, as no detected antibiotic residue in the SH exceeded the MIC. Nevertheless, the sum of SH also showed the most frequently found antibiotic residues. For a deeper comparison, however, more samples must be investigated.

Nevertheless, N4AcSMX should be considered in the evaluation of the results, even if this metabolite no longer has an antibacterial effect. Under certain conditions, a retransformation into the active starting antibiotic substance can occur (Radke et al., 2009). Therefore, occasionally extremely high concentrations of N4AcSMX, in spite of the lack of PNEC and antibacterial efficacy, should be taken into account in any risk assessment.

A further study (Sib et al., submitted) will be published soon, which will present the microbiological results of the investigated sanitary units (same status quo samples examined here) and compare the microbiological and chemical results.

4.2. The recurrence of antibiotic residues after water stagnancy

In addition to the general detection of antibiotic residues in patient siphons, this study has demonstrated for the first time that the residue content rises again after sufficient flushing of the system and temporal stagnation of the sanitary unit. Following adequate rinsing of the system, the antibiotic concentrations in the t_0-samples examined were

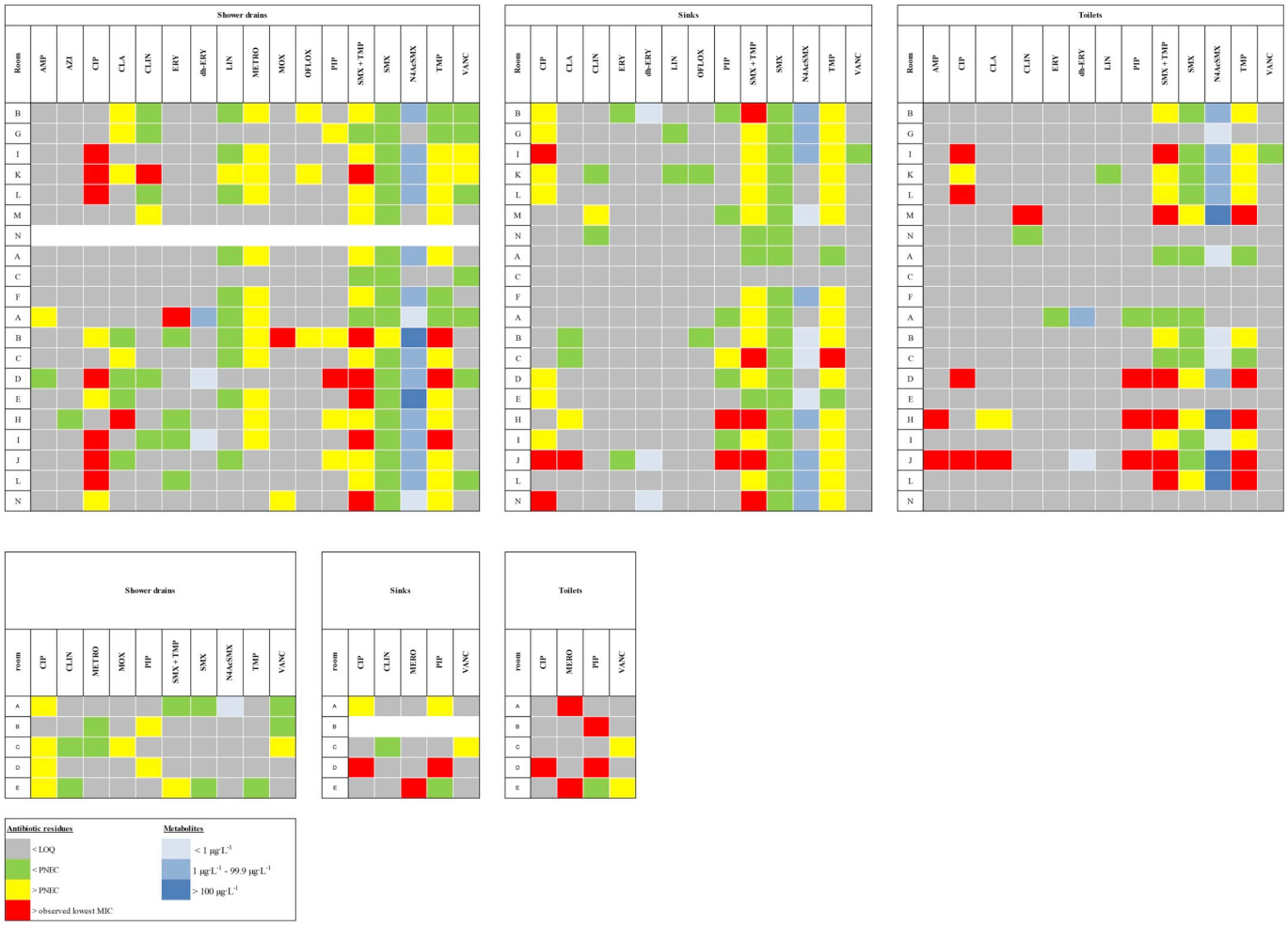

Fig. 5. Comparison of the detected antibiotic residue concentrations in several patient rooms (some rooms have multiple listings related to different sampling times) from this study with predicted PNECs and MICs (Bengtsson-Palme and Larsson, 2016) in light of a potential increase in resistance selection (first row = oncology, second row = neurological rehabilitation).

below the respective method detection limits (see Table 1).

The renewed increase in antibiotic concentrations during and after the stagnation period shows that there must be an intermediate storage of antibiotics, akin to that described for organic compounds (e.g., accumulation of nutrients or sorption of xenobiotics in biofilms) in previous publications (Flemming and Wingender, 2010), and a subsequent release of antibiotic substances into the water phase of sanitary units. However, the accumulation of antibiotic residues in the biofilm matrix of extracellular polymeric substances (Flemming and Wingender, 2010) appears to be the most plausible explanation, as the capability of antibiotics to penetrate through biofilms is already known (Anderl et al., 2000; Rodríguez-Marínez et al., 2007; Stewart and William Costerton, 2001; Singh et al., 2010; Stewart, 2015).

In addition, all systems investigated should have in common the presence of a biofilm typical for water pipes (about 95% of the biomass is located on the drain walls, and just 5% is in the water phase) (Flemming et al., 2002). Therefore, the biofilm matrix is a reservoir for antibiotic residues independent of the sanitary unit (WC, SI or SH). After flushing the system, antibiotic residues from the biofilm matrix may diffuse back into the respective waters, which could explain the noticeable increase in the concentration of antibiotics, since diffusion through biofilms has been described (Stewart, 1996, 1998).

In general there is a complex relationship between the structural properties of the individual active substances, the sorption capacity, the dose used, the period of administration, and potential biochemical reactions between antibiotics and the biofilm matrix, and therefore overall statements across substance classes are difficult to make (Stewart, 2015). Nevertheless, a comparable tendency for stagnation behavior in WC, SH and SI has been demonstrated in this study. Therefore, a recurrence of antibiotic residues during back diffusion

Table 1
List of analyzed antibiotics (Limit of quantification (= LOQ) [ng·L^{-1}]) sorted by their classes.

Penicillins			
AMOX (50)	AMP (200)	PEN-G (50)	CLOX (20)
DICLOX (20)	FLU (20)	METHI (10)	MEZLO (20)
NAF (20)	OXA (10)	PEN-V (20)	PIP (100)
Macrolids/lincosamides			
AZI (50)	CLA (50)	CLIN (20)	ERY (50)
dh-ERY (50)	ROX (100)	SPIR (100)	TYL (20)
Cephalosporines			
CEFA (50)	CEFO (50)	CEFT (100)	CEFU (200)
Tetracyclines			
CTC (200)	DOC (200)	OTC (200)	TC (200)
Fluorquinolones			
CIP (200)	ENR (200)	MOX (200)	OFLOX (200)
Carbapenems			
MERO (200)			
Sulfonamides/trimethoprim			
SCP (50)	SDZ (100)	SDMX (50)	SDMD (20)
SDX (50)	SEP (50)	SMZ (50)	SMX (20)
N4AcSMX (100)	SMP (10)	STZ (100)	TMP (20)
Others			
LIN (100)	VANC (100)	METRO (100)	

Table 2
Antibiotic residues in status quo samples of WC, SH and SI of an oncology and a neurological rehabilitation unit [μg·L^{-1}], as well as the frequency of each detected antibiotic (n [%]).

Antibiotics	Oncology								
	Toilet (n = 20)			Sink (n = 20)			Shower (n = 19)		
	min. [μg·L^{-1}]	max. [μg·L^{-1}]	frequency [%]	min. [μg·L^{-1}]	max. [μg·L^{-1}]	frequency [%]	min. [μg·L^{-1}]	max. [μg·L^{-1}]	frequency [%]
AMP	4.80	5.80	10	–	–	–	0.74	0.74	5
AZI	–	–	–	–	–	–	0.19	0.19	5
CIP	0.58	98	25	0.24	30	50	0.34	41	53
CLA	5.10	1200	10	0.07	20	20	0.06	73	47
CLIN	0.02	720	10	0.04	7.80	15	0.02	18	37
ERY	0.83	0.83	5	0.51	0.61	10	0.07	110	26
dh-ERY	0.05	1.03	10	0.21	0.44	15	0.08	64	16
LIN	2.30	2.30	5	0.10	3.90	10	0.10	23	58
MERO	–	–	–	–	–	–	–	–	–
METRO	–	–	–	–	–	–	0.15	7.70	63
MOXI	–	–	–	–	–	–	1.30	2.00	11
OFLOX	–	–	–	0.43	0.45	10	1.40	2.50	16
PIP	0.32	79,000	20	0.12	230	40	0.70	3500	26
SMX	0.02	36	70	0.08	6.10	95	0.05	20	100
N4AcSMX	0.27	700	70	0.38	8.30	60	0.26	480	84
TMP	0.17	110	65	0.12	19	90	0.08	260	95
VANC	0.10	0.10	5	0.13	0.13	5	0.17	26	47

Antibiotics	Neurological rehabilitation								
	Toilet (n = 5)			Sink (n = 4)			Shower (n = 5)		
	min. [μg·L^{-1}]	max. [μg·L^{-1}]	frequency [%]	min. [μg·L^{-1}]	max. [μg·L^{-1}]	frequency [%]	min. [μg·L^{-1}]	max. [μg·L^{-1}]	frequency [%]
AMP	–	–	–	–	–	–	–	–	–
AZI	–	–	–	–	–	–	–	–	–
CIP	50	50	20	1.40	2.40	50	0.27	0.85	80
CLA	–	–	–	–	–	0	–	–	–
CLIN	–	–	–	0.06	0.06	25	0.05	0.10	40
ERY	–	–	–	–	–	–	–	–	–
dh-ERY	–	–	–	–	–	–	–	–	–
LIN	–	–	–	–	–	–	–	–	–
MERO	94	1300	40	21	21	25	–	–	–
METRO	–	–	–	–	–	–	0.44	1.20	40
MOXI	–	–	–	–	–	–	0.51	0.51	20
OFLOX	–	–	–	–	–	–	–	–	–
PIP	0.11	370	60	0.16	120	75	1.80	3.00	40
SMX	–	–	–	–	–	–	0.06	0.07	40
N4AcSMX	–	–	–	–	–	–	0.20	0.20	20
TMP	–	–	–	–	–	–	0.45	0.45	20
VANC	10	56	40	12	12	25	0.23	100	60

Table 3
Contrast in detected antibiotics [$\mu g \cdot L^{-1}$] (sum of all antibiotics in all sanitary units) in status quo samples of the oncology and neurological rehabilitation clinics (sorted by antibiotic classes).

Sum of all detected antibiotics [$\mu g \cdot L^{-1}$] sorted by their class	Status quo - samples	
	Oncology (n = 59)	Neuro. Rehabilitation (n = 14)
Carbapenems	–	1515
Cephalosporins	–	–
Fluoroquinolones	328	54
Lincosamid antibiotics	826	0.20
Macrolid antibiotics	1405	–
Nitroimidazoles	19	1.64
Oxazolidinones	34	–
Penicillins	115,226	507
Glycopeptide antibiotics	57	179
Sulfonamides	3412	0.77
Tetracyclines	–	–

seems to be generally independent of the sanitary units.

In SH of room M (see Fig. 3), some antibiotics (e.g. PIP, VANC or CLIN) could be found during the stagnation, although the status quo sample was negative (related to these antibiotics). In this case, it should be noted that the status quo samples (beginning of stagnation experiments) are random grab samples and that the shower may have been flushed by patients or cleaning staff immediately before sampling. Thus, the previous residue concentration could be diluted (possibly below LOQ in the status quo sample) and diffuse back into the aqueous phase during the stagnation period. However, Fig. 3 shows that the saturation phase can take different lengths of time depending on the sanitary unit or analyte. Thus, the residue concentrations of SMX, CLIN or TMP are constant over the entire 24 h after reaching saturation. For PIP in SH of room M, however, a decrease in the residue concentration could already be observed after approximately 4–5 h, as seen in Fig. 3. In addition to the duration of the saturation phase, the different rooms and active substances also varied in terms of level of saturation concentration. These differences may be related to the time of the last application of the respective antibiotic. Depending on the length of this period, the antibiotic content could be reduced by degradation processes, rinsing processes or cleaning processes to such an extent that sufficient quantities of antibiotics can no longer diffuse back into the water (Stewart, 1998). A correlation between the status quo values and the detected residue concentrations in the saturation phase could not be determined.

Substance-specific factors should also be taken into account. The aforementioned decrease of PIP could be due to the already described high degradability of β-lactams (Deshpande et al., 2004; Kümmerer, 2009). This theory is accompanied by the fact that hydrolysis-stable antibiotics were found more frequently in the stagnation samples.

Furthermore, Fig. 4 shows that the stagnation course of CIP (SH, dermatology) did not follow the otherwise comparable rising pattern. A possible explanation for the different stagnation behavior of CIP could be the adsorption capacity of fluoroquinolones into sediments, soils and sewage sludge described in the literature (Golet et al., 2003; Kümmerer, 2003, 2009), caused in the characteristic amino and carboxylic acid group, and leading to the possibility of being positively charged as well as zwitter-ionic contingent on the pH value (at high basic pH values also being negative) (Kümmerer, 2009). Therefore a possible adsorption of CIP into the biofilm may theoretically impede both penetration into the biofilm and back diffusion from the biofilm into the aqueous phase after rinsing. Comparable results could be shown for aminoglycosides (positively charged), whose inward diffusion into a polyanionic alginate matrix at physiological pH was more retarded than the diffusion of β-Lactams (which should not be positively charged at physiological pH) (Hoyle et al., 1990). However, it must be mentioned that the penetration capacity also depends on the biofilm-forming bacteria (Stewart, 2015). In this context, the penetration of amikacin and CIP through biofilms (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were not affected in comparison with the affected penetration capacity of OXA, CEFO and VANC (Singh et al., 2010). In *Pseudomonas aeruginosa*, however, it could be shown that fluorochinolones (like CIP) can penetrate readily, while aminoglycosides (such as amikacin) are retarded (Al-Fattani and Douglas, 2004).

The theory of the intermediate storage of antibiotic residues in biofilm could further explain why some antibiotics were detected that were not part of the recently applied antibiotics (c.f. Table S1). It should also be mentioned that antibiotic residues could only be detected sporadically in the samples (status quo, t_0 … t_{24}). This could be due to the following reasons:

1) An increased amount of urine, excreta, etc. in the aqueous phase of the sanitary unit due to insufficient flushing by the patient or personnel.
2) The biofilm can be mobilized during each sampling while sucking in the aqueous phase through the syringe, scratching the biofilm with the sampling utensils, unscrewing the odor traps, removing the shower outlet grids, or inserting the sampling hose through the perforated basin covers.

In spite of divergent results, a general trend could be shown in the investigated stagnation samples (eight patient rooms) by a renewed increase in antibiotic residue concentrations after previous rinsing of the system in SH, SI and WC with a comparable course.

5. Conclusions

In summary, direct patient wastewater, in addition to the total hospital effluent already identified (Chang et al., 2010; Kümmerer, 2001; Lindberg et al., 2004; Ory et al., 2016; Watkinson et al., 2009), appears to be a point source for antibiotic residues and a potential medium for resistance development. Therefore, the wastewater system of hospitals represents a neglected regulatory reservoir for antibiotic residues in the direct exposure area for vulnerable patients, particularly in areas with high antibiotic consumption rates. Biofilm in the sanitary units of patient wards may serve as a reservoir for antibiotic enrichment and subsequent back diffusion into the water medium.

However, the wastewater systems of clinics and ward areas with frequent use of antibiotics are independent risk areas for the development of antimicrobial resistance and require additional measures to reduce antibiotic resistance.

Given that the role of biofilm associated resistance mechanisms (Mah and O'Toole, 2001; Stewart, 2002; Stewart and William Costerton, 2001) and the potential risk of infections related to sanitary units (Kizny Gordon et al., 2017; Parkes and Hota, 2018) are already known, future research should focus on prevention and practice-oriented cleaning measures, i.e., mechanical removal of biofilms in running water/wastewater systems, technical solutions (e.g., thermal disinfection, chemical disinfection, sanitary equipment design and inhibition of retrograde contamination) as well as organizational solutions (rinsing, dilution, etc.) to reduce antibiotic residue concentrations and prevent further selection pressure in favor of antibiotic-resistant bacteria.

Conflicts of interest

The authors declare no conflict of interest.

This study complies with the ethical guidelines of the Declaration of Helsinki by the “World Medical Association” from 1964. The ethics committee of the Medical Faculty of the University of Bonn was involved and approved the procedures and the publication of the results (reference no. 120/16).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2018.12.013.

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