Contamination in hospital

Wastewater & Waste

Pharmafilter

Platform for a Cleaner Environment

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The outbreak of the COVID-19 pandemic directed global attention towards monitoring viruses and other infectious pathogens in hospital wastewater and waste, and yet this effluent continues to impose a serious and escalating threat to public health.

The increased use of medicines and specific drugs administered to patients in the hospital environment is already a major contributing further to antimicrobial resistance. Antibiotics are now entering sewage and wastewater in more significant quantities, resulting in ever-increasing damage to the environment and public health. Just as Alexander Fleming predicted in the middle of the last century, the declining effectiveness of antibiotics in treating common illnesses because of antimicrobial resistance (AMR), is now an increasing concern.

The presence of various recalcitrant organics and pharmaceutically active compounds in hospital wastewater also imparts a complex pollution load to water resources and ecosystems. In their day-to-day business, hospitals discharge wastewater containing large amounts of pharmaceuticals, metabolites and cytotoxins as well as pathogenic contamination. Many of the micropollutants present in hospital wastewater are highly toxic and what's more, their strong chemical compounds often find their way, untreated, into the public infrastructure, where they remain biologically active.

The interaction between these discharges, local wastewater treatment plants and their broader environment, is an area of growing concern, particularly in the context of eco-toxicity and antibiotic-resistant bacteria. Due to technical and economic constraints, it is not possible to remove this contamination at a receiving wastewater treatment plant because of the specialist removal techniques required. Since pharmaceuticals are, by design, intended to interact with living organisms, even very low environmental concentrations are a concern.

In recent years, hospital effluent has been the object of rigorous study and research with a growing appreciation of its unique character and impact on the environment and public health. Many of the studies cited in this paper reveal the urgent need to change the way we manage and treat hospital waste:

- In European countries, such as Ireland, France, Sweden, Spain, Netherlands, and Poland, 3.8–13.6% of the *E. coli* present in hospital effluents are found to be resistant to antibiotics [59].
- A high prevalence of vancomycin-resistant enterococci was observed in hospital effluents of the United Kingdom [64], [65], [66]. Fluoroquinolone-resistant *E. coli* in European countries was found to increase from 25% to 50% between 2002 and 2007 alone.
- Pharmaceutically active compounds such as diclofenac and ciprofloxacin are found at concentrations that are detrimental to human beings upon exposure [19].

There is also growing evidence that the disposal of untreated healthcare wastes in landfills can lead to the contamination of drinking, surface, and ground waters. Healthcare waste, from sharps, PPE, nappies and items contaminated with blood or body fluids, contain potentially harmful microorganisms that can infect hospital patients, health workers and the general public.

Hospitals play a pivotal role in the well-being of humanity and facilitate research in the field of medical advancement. However, many hospitals are now significantly challenged by rising antibiotic resistance, hospital-acquired infection, staff shortages, capital, and operational funding pressures. The emergence of technology to treat hospital wastewater can address this threat to public health at its very source. In deploying an onsite waste and wastewater treatment system, hospitals can reduce micropollutants, pharmaceutical and biological, to below the detectable limit, removing this input to the environment, and addressing the impact of antimicrobial-resistant genes before they enter and thrive in the wider environment.

Circumstances for change are now upon us in how we manage Healthcare Waste and Wastewater (HWW). The leading exponent of effective hospital wastewater treatment is the Pharmafilter system, which delivers an integrated approach to healthcare-generated wastes and wastewater, achieving better outcomes for the hospital, its patients, staff and the environment.

Water consumption and effluent generation from hospitals

Hospitals require large amounts of water for their proper functioning for various health care facilities. The resulting waste imposes a serious hazard to human health when inappropriately handled and disposed to the hydrosphere [27].

According to the World Health Organisation (WHO) guidelines for the proper functioning of healthcare facilities, 40–60 L/day of water is required for every inpatient. Operating theatres require around 100 L/intervention. The amount of water required for patients dealing with severe acute respiratory syndrome or viral hemorrhagic fever is around 100–400 L/patient/day [28], [29].

In turn, this consumption of water by hospitals leads to the generation of large volumes of wastewater [30]. The amount of wastewater depends on the capacity or the number of beds available in the hospital, the type and size of the healthcare facility, technical facilities available, services provided (laundry, kitchen, air-conditioning), and in-house water management facilities, among other factors [31].

Kumari et al. [1] reported that the wastewater generated by developing countries varies from 200 to 400 L/capita/day, while in developed countries, it varies from 400 to 1200 L/capita/day. To give a specific example: a hospital in Portugal, which has more than 30 clinical facilities and a capacity of 1120 beds, discharged 1000^3 of wastewater daily [7]. While an average of 30.8 L/patient/day and 54.5 L/bed/day was estimated during the sampling and analysis of two healthcare centres in Ghana [31].

On average, the hospitals in developed countries such as the United States, Germany, Italy, Spain, Denmark, Netherlands, and Portugal generate around $411m^3$ of wastewater daily, which amounts to around 730 L/patient/day.

In comparison, the average wastewater generated by hospitals from developing or semi-developed countries, such as India, Iran, Brazil, Ethiopia, Ghana, and Nepal, is around $290m^3$/hospital/day and 250 L/patient/day, which is significantly less than that of the developed countries. As per a report in 2008, the amount of wastewater generated by 19,712 hospitals in China is 1.29×10^6 m^3/day, i.e., 65 m^3/hospital/day [32].

The amount of wastewater generated by hospitals becomes a cause for concern when they are not discharged according to the standards and guidelines set by various organizations, such as the EPA, WHO, etc. [1]. Usually, hospital effluents are discharged into sewer systems before they are treated at municipal sewage treatment plants [21]. However, most sewage treatment plants are simply not designed to tackle bio-medical waste and persistent organic compounds, such as pharmaceutically active compounds (PhACs), personal care products, etc. [19]. Furthermore, there have been reports that many hospitals in developing countries, such as Algeria, Congo, Nepal, Pakistan, Bangladesh, and Vietnam discharge their effluent into drainage systems, rivers, and lakes without any pre-treatment [21], despite the very complex character of this effluent.

Characterising Hospital Wastewater

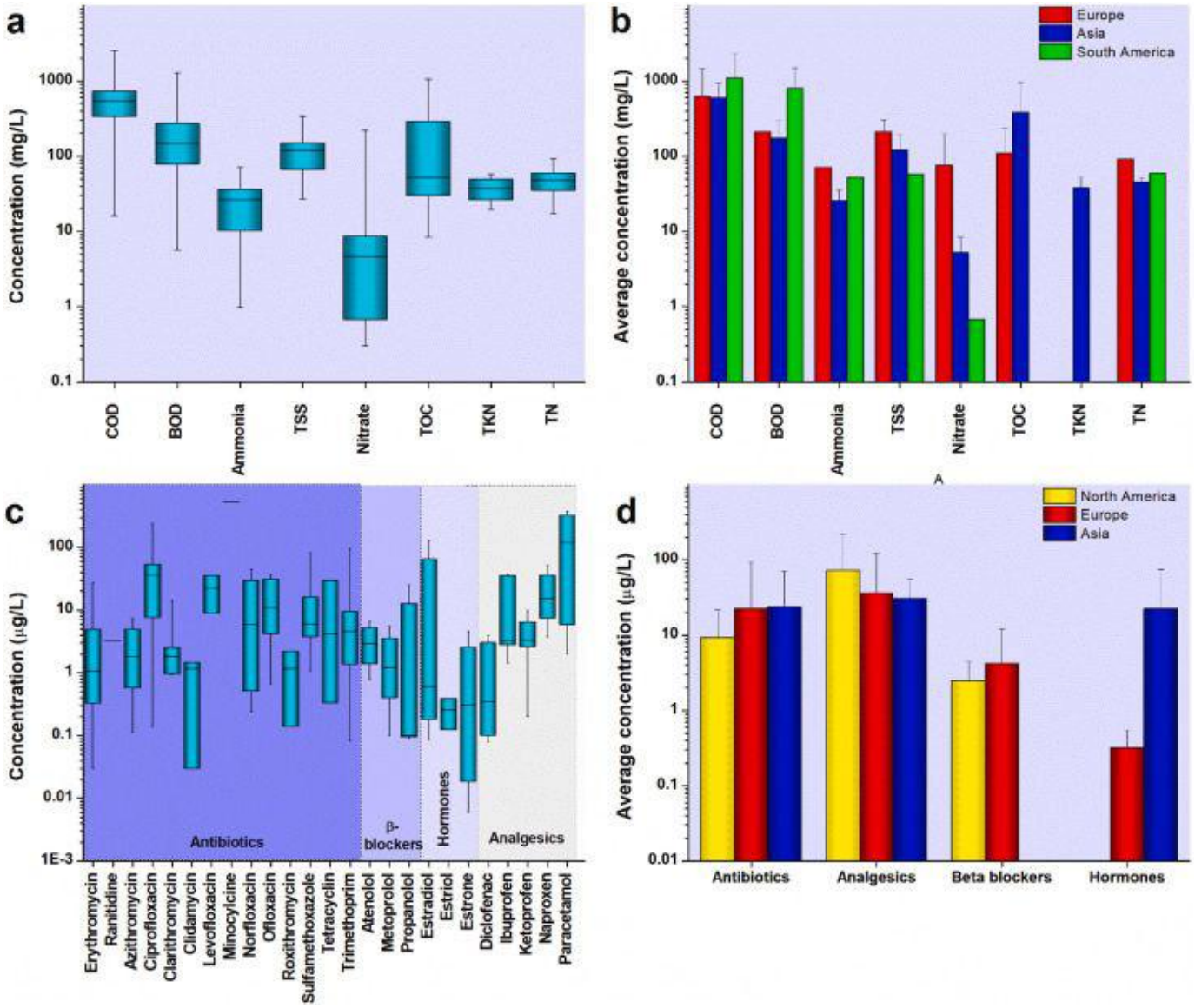
Hospital wastewater is characterised by the presence of various emerging contaminants, such as PhACs, microorganisms including antibiotic-resistant bacteria (ARB), antibiotic-resistant genes (ARG), and persistent viruses. [9], [10], [11], [12]. Generally, HWW has a high biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia, and nitrogen content, and their concentration is higher compared to domestic wastewater [13], [14].

BOD represents the amount of oxygen consumed by microorganisms to decompose organic matter under aerobic conditions at a specific temperature and duration of time, while COD is the amount of oxygen equivalents consumed in the chemical oxidation of organic matter by a strong oxidant [15], [16].

Hence, BOD can be referred to as the biodegradable fraction of wastewater, while COD is the measure of both biodegradable and non-biodegradable organic compounds. The ratio of BOD and COD of wastewater is referred to as the biodegradability index [16], [17]. The biodegradability index of HWW is lower than that of municipal wastewater, making them difficult to treat by conventional biological systems [13], [14], [18].

Many of the recalcitrant organic compounds present in HWW, such as PhACs, are highly toxic with very low drinking water equivalent limit (DWEL) values, making them a considerable threat to the environment [19]. Viruses, ARB, and ARG continue to survive even after the treatment of HWW, and their release into the aquatic ecosystem [6], [20].

To illustrate, the component-specific and continent-wise concentration of various HWW parameters has been depicted in Fig. 1a and b, on the next page. As we can see, the effluent coming out of different hospitals is rich in PhACs, and microorganisms, and it is also characterized by high COD, BOD, ammonia, nitrate, total nitrogen (TN), suspended solids (TSS), total organic carbon (TOC), total Kjeldahl Nitrogen (TKN).



Qualitative analysis of the medical waste of 10 hospitals sampled indicated that liquid waste had a 16.70% contribution to hazardous–infectious waste [33]. Broadly speaking, discharge from hospitals can be classified into four categories, i.e., blackwater, greywater, stormwater, and specific discharges. Blackwater or sewage mainly comprises the faecal matter and urine coming out of the toilets in hospital wards, which accounts for the major portion of the BOD of the wastewater [34]. Blackwater is rich in various kinds of microorganisms since the faecal matter is the primary source of microorganisms in wastewater. Apart from being pathogenic, these microorganisms may also have developed antimicrobial or antibiotic resistance [35]. The faecal matter and urine also contain unmetabolized PhACs, which had been administered to the patients during treatment [26], [36]. Greywater or 'sullage' is the water coming out of washing, bathing, laundry, and other processes like rinsing of X-Ray films, and disinfection.

This water contains recalcitrant compounds such as surfactants, detergents, and other cytotoxic or genotoxic agents and radioactive elements [1]. Stormwater is usually lost through the drain or groundwater percolation [34]. The wastewater generated from activities pertaining to laboratory work, such as research and diagnosis, the radiology department, is classified under specific discharges. This wastewater contains highly toxic substances, such as disinfectants, detergents, acids, alkalis, pharmaceutical residues, solvents, and X-ray contrast media. These substances are highly toxic and persistent and stay in the aqueous environment even after conventional treatment processes [1], [26], [36]. The effluents coming out of hospitals contain toxic heavy metals, such as Cd, Cu, Ni, Hg, Sn, etc. [1], [26]. In the pages that follow, we assess the scale of the environmental degradation and threat to public health that results from this complex pollution.

02 CONTAMINANTS IN HOSPITAL WASTEWATER

1. Pharmaceutically Active Compounds

Emerging contaminants, such as PhACs, are prevalent in hospital effluent because of their excessive use in medical facilities, and their component-specific and continent-wide occurrence in hospital effluent has been depicted in Fig. 1c and d, respectively. Furthermore, the concentration of analgesics, antibiotics, β-blockers, hormones etc. in hospital effluent is much higher compared to their concentrations in domestic wastewater [19], [46], [47], [48].

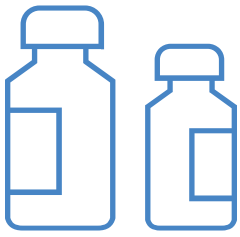
Although there are hundreds of PhACs detected in HWW, those PhACs which are most commonly detected and whose concentrations are such that they may pose a threat to the environment, have been considered in a broad range of studies.

Analgesics, such as acetaminophen, diclofenac, ketoprofen, ibuprofen and naproxen, were frequently detected in hospital effluents [19], [36], [46], [47], [48]. The average concentration of analgesics in hospital discharge was found to be more in North America, as compared to Asia and Europe. The concentration of acetaminophen was found to be 374 μg/L and accounted for 45% of the total PhAC average concentration in hospital effluent in the US [36]. Langford and Thomas [49] reported 325 μg/L of acetaminophen in hospital effluents in Norway. Ibuprofen was found in the range of 2.8–36.5 μg/L in various hospital effluents in the US, Italy, Spain, and Norway [23], [36], [42], [49].

Diclofenac was found in concentrations ranging from 0.5 μg/L to 3 μg/L in HWW of Norway which was higher than the DWEL (0.2 μg/L to 0.3 μg/L) [19], [49]. Prasertkulsak et al. [50] reported around 3.8 μg/L of diclofenac in the hospital effluent of Thailand. Ciprofloxacin, sulfamethoxazole, trimethoprim, norfloxacin, and ofloxacin were the most frequently reported antibiotics in hospital effluents [19]. The average concentration of antibiotics in the hospital effluents of Asia and Europe was found to be in the same range (Fig. 1d).

Researchers reported norfloxacin (29.6 μg/L), sulfamethoxazole (81 μg/L), and ciprofloxacin (237 μg/L) in various hospital effluents of India [48], [51]. Ciprofloxacin concentrations in some HWW samples of India (>200 μg/L) and Portugal (38.6 μg/L) were considerably higher than the acceptable DWEL values [19], [48], [51]. Traces of sulfamethoxazole were found in some hospital effluent in Portugal (8.7 μg/L) and the US (2.2 μg/L) [36], [52]. Ofloxacin, levofloxacin, erythromycin, and azithromycin were among other antibiotics found in various hospital effluents [19], [36], [46], [47], [48]. Atenolol, metoprolol, and propranolol were the most common β-blockers found in the hospital effluents of North America and Europe. Propranolol was detected in the range of 10 μg/L to 25 μg/L in a hospital effluent of Oslo, Norway [49]. Stimulants, such as caffeine, were found in the range of 53 μg/L to 325 μg/L in hospital discharges in the USA [36].

Hormones, such as estriol, estradiol, and estrone were detected in the range of 0.1 μg/L to 0.9 μg/L in hospital effluent of Iran, Korea, Belgium, and Norway [53], [54], [55], [56]. Prasertkulsak et al. [50] reported 128 μg/L of estradiol in the effluent of a hospital in Thailand. PhACs, such as carbamazepine, metformin, theobromine, theophylline, and gabapentin, were also common in hospital effluents of the US [36]. Ultimately, HWW was found to host a wide variety of PhACs and their metabolites, with analgesics and antibiotics being the most prevalent. Although the concentration of these compounds is not very high, they are highly toxic to biotic components of the environment. Most of the PhACs found in hospital effluent were at concentrations higher than the predicted no-effect concentration (PNEC) values, while a few PhACs, such as diclofenac and ciprofloxacin were found at concentrations higher than the DWEL values indicating a detrimental effect on human beings upon exposure [19].



The concentration of analgesics, antibiotics, β-blockers, hormones in hospital effluent has been found to be much higher compared to their concentrations in domestic wastewater.

Key Study: Pharmaceuticals in the Baltic Sea Region - emissions, consumption and environmental risks (2020)

Helene Ek Henning, Ieva Putna-Nīmane, Radoslaw Kalinowski, Noora Perkola, Aleksandra Bogusz, Anete Kublina, Egge Haiba, Ieva Bārda, Ieva Karkovska, Jan Schütz, Jukka Mehtonen, Katri Siimes, Kristina Nyhlén, Laura Dzintare, Lauri Äystö, Lauris Siņics, Mailis Laht, Mari Lehtonen, Michael Stapf, Pernilla Stridh, Rita Poikāne, Sabina Hoppe, Terhi Lehtinen, Vallo Kõrgma, Ville Junttila och Ülle Leisk.

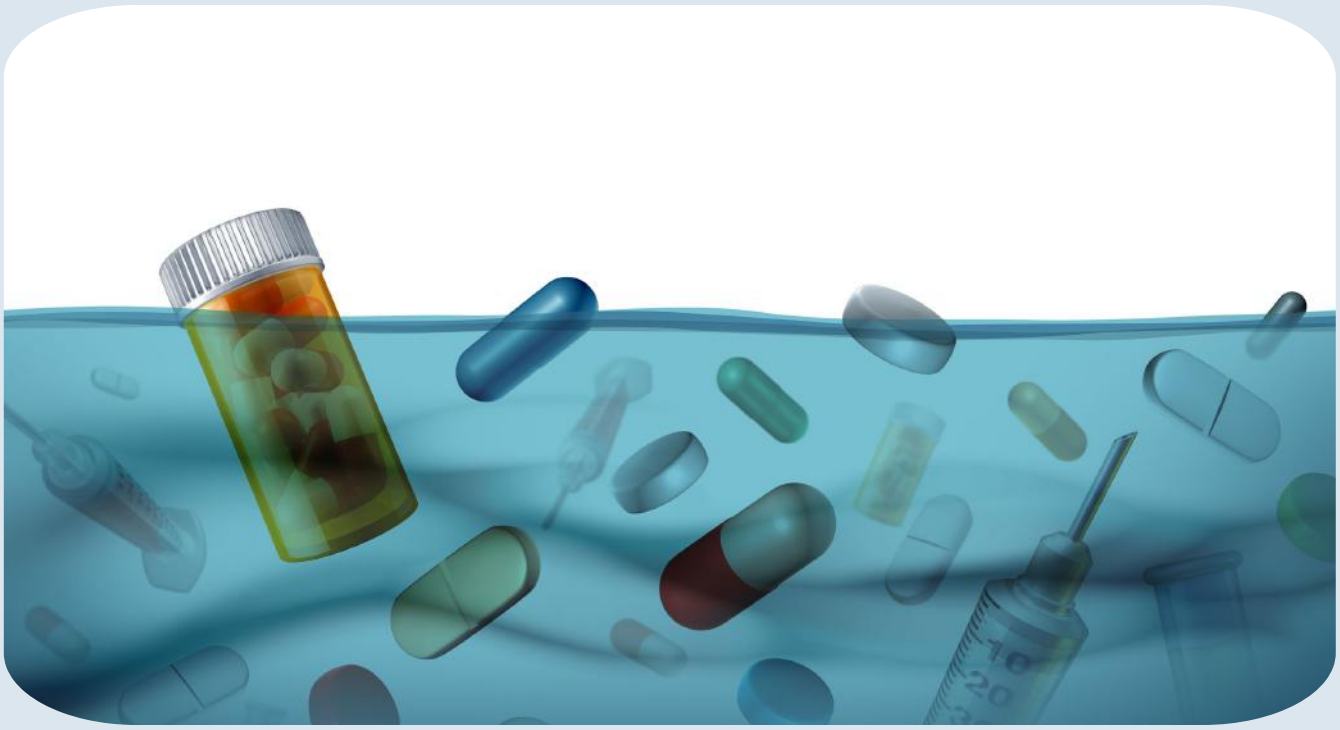
This study highlights results from a three-year project, *Clear Waters from Pharmaceuticals* (CWPharma), funded by the EU's Interreg Baltic Sea Region Programme. The overall aim of the project was to decrease the emissions and adverse effects of pharmaceuticals in the Baltic Sea region.

It addresses pollution caused by pharmaceuticals as an emerging problem due to the potential risks to ecosystems and humans, and concluded that *"the widespread and, in some places, high prevalence of APIs in the environment shows that immediate measures are needed to reduce the risk of negative environmental effects and the development of antibiotic resistance*." As identified by e.g. UNESCO and HELCOM (2017), and European Commission (2019), there are still data gaps with regard to the consumption of pharmaceuticals, environmental levels and emissions from various sources. This report focused on filling in some of these data gaps.

Environmental levels and sources of active pharmaceutical ingredients (APIs) were studied in selected river basin districts of Vantaa in Finland, Pärnu in Estonia, Lielupe and Daugava in Latvia, Vistula in Poland, Warnow-Peene in Germany and Motala ström in Sweden.

The measured concentrations of about 80 APIs were compared with ecotoxicological data to assess environmental risks. The APIs were selected based on analytical capacity, high consumption volumes, identified data gaps and potential environmental risks. The report also covered a compilation of human and veterinary consumption of the selected APIs.

The conclusion so far highly recommends upstream onsite treatment of healthcare wastewater, as cited in the Nordic Centre for Healthcare Sustainability stakeholders review.



2. Bacteria

Hospital effluents are a host to numerous bacteria and pathogenic microorganisms, such as *Escherichia coli* (E. coli), *Enterococci*, thermotolerant coliform, faecal coliform, etc. Liu et al. [32] reported around 2.40×10^6 to 1.19×10^{12} number/mL of bacteria and 9.0×10^4 to 2.38×10^{10} number/mL of coliform in HWW of Guangzhou, China. Other hospital effluents in China accounted for 9.9×10^3 to 1×10^7 PFU/L of bacteria and 16,000–10^8 PFU/L of faecal coliform [32]. Berto et al. [43] reported a total coliform concentration of 2×10^8 MPN/100 mL and a thermotolerant coliform concentration of 1.6×10^8 MPN/100 mL in Brazil. Beier et al. [57] reported E. coli, faecal coliform, and enterococci in the range of 10^3 to 10^6 MPN/100 mL.

In a hospital effluent in France, the *E. coli* concentration varied from 8.3×10^4 CFU/mL to 3×10^5 CFU/mL [58], [59]. In some hospital effluents in Sweden, the E. coli concentration was found to be in the range of 2.6×10^4 and 5.5×10^4 CFU/mL [9]. *E. coli* concentration of 5.4×10^6 CFU/mL was found in an HWW of Ireland [60]. ***HIQA*** et al. [59] reported enterococci concentrations of 6.5×10^6 MPN/100 mL and 1.4×10^6 MPN/100 mL in certain hospital effluents of France and the United Kingdom, respectively.

In the wastewater of six hospitals located in India, the the concentration of total coliform ranged from 0.92×10^3 to 2.4×10^3 MPN/100 mL, and faecal coliform ranged from 1.8×10^1 to 3.2×10^2 MPN/100 mL [18]. Although these microorganisms are present in significant numbers, the eminent danger lies in the presence of resistant bacteria, such as Proteus Vulgaris, Pseudomonas aeruginosa, vancomycin-resistant enterococci, mycobacteria, etc. and resistant strains (Enterobacter sakazakii, Extended-Spectrum Beta-Lactamase (ESBL)-producing-strains) [59], [61].

"The percentage of ESBLE in hospital wastewater is much higher compared to that in urban wastewater and the discharge of wastewater treatment plants, partly due to the large amounts of antibiotics, disinfectants etc, which boosts resistance among these bacterial populations. Unlike normal *E. coli*, these ESBLEs produce infections in human beings that can no longer be treated by ordinary antibiotics."

The resistance can be intrinsic or developed due to spontaneous mutations. Intrinsic resistance belongs to those microorganisms which can prevent the antibiotic from entering their cell wall [62]. ESBLs are a type of enzyme that is produced by certain bacteria, such as ESBL-producing *E. coli*, which makes them resistant to antibiotics.

In European countries, such as Ireland, France, Sweden, Spain, Netherlands, and Poland, 3.8–13.6% of the E. coli present in hospital effluents were found to be ESBLE [59]. Chagas et al. [5] reported that out of 7.4×10^3 CFU/mL of coliforms found in HWWs of Brazil, 38.6% were ESBL-producing coliforms.

The percentage of ESBLE present in HWW was much higher compared to that in urban wastewater and discharge of wastewater treatment plants (WWTP) [59]. This was because HWW contains large amounts of antibiotics, disinfectants, etc., making the ESBL-producing microorganisms resistant to them. Unlike normal *E. coli*, these ESBLEs are producing infections in human beings that can no longer be treated by ordinary antibiotics [59].

Pseudomonas aeruginosa, for example, is a multidrug-resistant pathogen found in HWW as a result of mutations or gene transfer. They can be found in water media with sufficient dissolved oxygen (DO) [59]. They can acquire resistance to multiple classes of antibiotics, thereby making infections caused by such microorganisms more complex. They have been found in HWW in the range of 4×10^3 CFU/mL, out of which 76% of them were resistant to one or more classes of antibiotics [63].

Enterococci, a very common bacteria usually found in the gastrointestinal tracts of humans and animals, have also exhibited resistance to antibiotics, and their prevalence has increased in the last few decades. In an HWW of France, 6.5×10^6 CFU/mL of enterococci were detected, out of which almost all were resistant to amoxicillin [59]. A high prevalence of vancomycin-resistant enterococci was observed in hospital effluents of the United Kingdom and Portugal [64], [65], [66]. Fluoroquinolone-resistant *E. coli* in European countries was found to increase from 25% to 50% between 2002 and 2007. Among others, Acinetobacter baumannii and Staphylococcus aureus also have shown the capacity to develop resistance to antimicrobials, such as methicillin [67]. Different phyla in HWW, such as proteobacteria, planctomycetes, nitrospirae, caldithrix, chlorobi, and acidobacteria were found to be resistant to various antibiotics, among which tetracycline was the most common [68].

3. Viruses

There are more than 120 identified human enteric viruses, among which the enteroviruses (polio-, echo- and coxsackieviruses), adenoviruses, hepatitis A, rotaviruses, and human caliciviruses (noroviruses) are the most prevalent in HWW [69].

The presence of viruses in HWW is a cause for major environmental and public health concerns. The outbreak of severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) in 2019–20 that led to the global pandemic is just the most recent example of the threat that viruses possess.

Viruses are known to be highly stable even under adverse conditions. Rotavirus A, norovirus, hepatitis virus, human adenovirus, etc. have been detected in effluents from HWW treatment plants in Brazil [6]. Liu et al. [32] found that viruses may persist in HWW even if *E. coli* concentration is less than 50 PFU/100 mL, as they are more tolerant compared to *E. coli*. Ibrahim et al. [70] detected human adenovirus in the effluent of two HWW treatment units. Caudovirales, Myoviridae, Podoviridae, and Siphoviridae were commonly detected in HWWs of Israel [20], [70]. Wang et al. [71], [73] found SARS-coronavirus in HWW of China, which was persistent for up to two days at 20 °C and 14 days at 4 °C, while Gundy et al. [72] also found that there was a 99.9% reduction of the coronavirus after 12 days when the water temperature was 23 °C.

The SARS-CoV-2 virus has been extensively studied all over the world. Since faecal shedding of SARS-CoV-2 RNA is widely reported, researchers investigated the presence of such RNA in municipal WWTPs of Spain and detected them in untreated water [74]. The SARS-CoV-2 RNA was present in 11% of the secondary treated water samples. Wang et al. [75] confirmed the presence of SARS-CoV-2 RNA in the sewage samples of the hospital of Zhejiang University, China. In another study, SARS-CoV-2 RNA was found in high concentrations of $0.5–18.7 \times 10^3$ copies/L in the septic tanks of Wuchang Cabin Hospital, Wuhan, China [76].

This RNA persisted even after disinfection by sodium hypochlorite. The reason behind the high level of persistence may be because the virus was embedded in the stool particles. However, when the dose of sodium hypochlorite was increased, no RNA was found, but high levels of disinfection by-products were detected [76]. Ahmed et al. [77] detected 1.9–12 copies/100 mL of SARS-CoV-2 RNA in wastewater samples in Australia. In Ahmedabad, India, Kumar et al. [78] tested water samples from a WWTP receiving effluent from a hospital treating COVID-19 patients.

"Viruses in HWW are persistent in adverse conditions. During wastewater treatment, they may settle on the suspended matter present in the wastewater and become highly stable."

Three genes of SARS-CoV-2, i.e., ORF1ab, N, and S genes, were detected in the influent of the WWTP. The number of copies of RNA detected increased by ten times over a period of 20 days, during which the number of COVID-19 patients also increased by two times in Ahmedabad. A similar correlation between the number of SARS-CoV-2 RNA and the number of COVID-19 patients was also observed in Australia, China, and France [76], [77], [78], [79]. Kitajima et al. [80] reported 2×10^5 and 3×10^4 copies/L SARS-CoV-2 RNA copies/L in the wastewater of Massachusetts, and Bozeman, Montana, respectively, while wastewater in France accounted for 10^5 to 10^6 SARS-CoV-2 RNA copies/L. Haramoto et al. [82] reported 2.4×10^3 copies/L of SARS-CoV-2 RNA in the wastewater of Japan.

The frequency of detection of SARS-CoV-2 RNA in the wastewater and hospital effluent samples was correlated with the percentage of people (per country) affected by the virus. The person's correlation coefficient was calculated to be 0.79, which indicated a strong positive trend [83]. The results suggested that the occurrence of SARS-CoV-2 RNA in wastewater was directly related to the percentage of people infected. Furthermore, proper wastewater monitoring can help in identifying areas with COVID-19-infected people.

Although most viruses are known to be highly stable even in adverse environmental conditions, SARS-CoV-2 was unstable in the presence of disinfectants and at a temperature higher than 20 °C [71], [84]. However, these viruses can survive when they get enveloped inside faecal particles or suspended solids. Furthermore, the entrapped virus in sewage can generate virus-laden aerosols during wastewater flushing and provide an air-borne route for the virus to transmit [85], [86].

Transmission of Pathogens

01

Viruses and bacteria enter the body via water, food or air and cause infection.

Asymptomatic patients and superspreaders enter hospital.

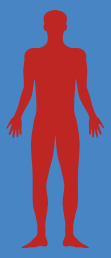
02

Patients consume pharmaceuticals, such as antivirals and antibiotics and the unmetabolised PhAcs, and mutated virus strains and bacteria are excreted through urine, stool, cough.

01

03

02



Contaminated surfaces.

The suspended matter in hospital wastewater support the ARB, ARG, and viruses, thereby giving them more stability, increasing the risk of hospital acquired infections.

Key Study: Hospital effluent: a reservoir for carbapenemase-producing Enterobacterals (2019)

Niamh Cahill, Louise O'Connor, Bláthnaid Mahon, Áine Varley, Elaine McGrath, Phelim Ryam, Martin Cormican, Carina Brehony, Keith A Jolley, Martin C Maiden, Sylvain Brisse, Dearbháile Morris

In Ireland, and most of Europe, hospital effluents are released into the urban wastewater system without any specific measurement of antibiotic levels or antibiotic-resistant bacteria, and without any pre-treatment. There are concerns that the release of these contaminants into urban wastewater may result in downstream exposure to antibiotics and contribute to the growing problem of antibiotic resistance.

This 2019 study aimed to examine hospital effluent and municipal wastewater in an urban area in Ireland for Carbapenemase-producing Enterobacterales. These represent a significant health threat as some strains are resistant to almost all available antibiotics. Samples of hospital effluent (n = 5), and municipal wastewater before (n = 5) and after (n = 4) the hospital effluent stream joined the municipal wastewater stream were collected over a nine-week period (May-June 2017).

All samples were examined for CPE by direct plating onto Brilliance CRE agar. Isolates were selected for susceptibility testing to 15 antimicrobial agents in accordance with EUCAST criteria. Where relevant, isolates were tested for carbapenemase-encoding genes by real-time PCR. CPE were detected in five samples of hospital effluent, one sample of pre-hospital wastewater and three samples of post-hospital wastewater.

The findings suggest hospital effluent is a major contributor to CPE in municipal wastewater and that monitoring of hospital effluent for CPE could have important applications in the detection and risk management of unrecognised dissemination of CPE in both the healthcare setting and the environment. The table below shows the antimicrobial susceptibility profiles of CPE *Enterobacterales* isolated.

Isolate ID	Sampling Type	Species	Carbapenemase	Cefpodoxime	Cefotaxime	Cefoxotin	Ceftazidime	Ampicillin	Streptomycin	Kanamycin	Gentamicin	Chloramphenicol	Trimpethoprim	Tetracycline	Ertapenem	Meropenem	Nalidixic Acid	Ciprofloxacin
NC3	Hospital Effluent	*Enterobacter cloacae complex*	OXA-48	R	R	R	R	R	I	R	I	S	S	S	R	R	R	R
NC7	Hospital Effluent	*Enterobacter cloacae complex*	IMP	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
NC8	Hospital Effluent	*Klebsiella pneumoniae*	OXA-48	R	R	R	R	R	R	R	R	S	R	R	R	R	I	R
NC16	Hospital Effluent	*Enterobacter cloacae complex*	VIM	R	R	R	R	R	I	I	I	S	R	R	R	R	I	R
NC17	Hospital Effluent	*Citrobacter freundii*	KPC	R	R	R	R	R	S	I	R	S	R	R	R	R	R	R
NC20	Hospital Effluent	*Enterobacter cloacae complex*	OXA-48	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
NC21A	Hospital Effluent	*Enterobacter cloacae complex*	IMP & OXA-48	R	R	R	R	R	S	R	I	S	S	R	R	R	R	R
NC21B	Hospital Effluent	*Enterobacter cloacae complex*	IMP & OXA-48	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
NC22	Hospital Effluent	*Enterobacter cloacae complex*	OXA-48	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
NC28	Pre-Hospital Wastewater	*Escherichia coli*	NDM	R	R	R	R	R	I	R	S	R	R	R	R	R	R	R
NC37	Post-Hospital Wastewater	*Klebsiella pneumoniae*	IMP	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
NC38	Post-Hospital Wastewater	*Citrobacter freundii*	KPC	R	R	R	R	R	S	I	R	S	R	R	R	R	R	R
NC46	Hospital Effluent	*Citrobacter freundii*	KPC	R	R	R	R	R	S	I	R	S	R	R	R	R	R	R
NC49	Hospital Effluent	*Citrobacter freundii*	OXA-48	R	R	R	R	R	I	S	S	S	R	S	R	I	R	R
NC54	Hospital Effluent	*Klebsiella pneumoniae*	IMP	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
NC66	Post-Hospital Wastewater	*Klebsiella oxytoca*	VIM	R	R	R	R	R	R	R	R	S	R	S	I	I	I	R
NC67	Post-Hospital Wastewater	*Citrobacter freundii*	KPC	R	R	R	R	R	S	I	R	S	R	R	R	R	R	R

03 THE CHALLENGE OF HEALTHCARE WASTE

The Challenge of Healthcare Waste

Healthcare is a rapidly growing industry as medical treatments become more sophisticated, and more in demand, due to the increasing incidence of chronic disease. The industry, globally, is creating more waste than ever before and, as such, there is a growing need to treat and dispose of this waste.

Healthcare waste (HCW) disposal includes a multitude of disposal methods, including incineration, landfilling and chemical treatments. These rudimentary methods and their growing use present their own problems that negatively impact both the environment and, in turn, damage public health. Since the quality of our environment affects public health, the healthcare sector should perform respective actions to preserve it [1].

The healthcare sector remains one of the largest industries globally: health expenditures are calculated at around 10 per cent of the global economic output [2], and it has an important role in damaging and degrading the natural environment due to the 24/7 operation, their rising energy consumption [3], and the huge amounts of healthcare/medical wastes they produce [4]. Even though the healthcare sector is considered to be greener than other industries worldwide [5], its global carbon footprint is found to be about 4.4 per cent of the world's total greenhouse emissions [6–8] and is expected to triple by 2050, reaching six gigatons a year [9].

Climate change has come to be recognised as one of the most crucial threats to our species in the last few decades, and many researchers, academics, experts, and other interested parties have expressed their concerns and given considerable attention to this matter [10], [11].

The World Health Organisation (WHO) has shown extreme concern regarding the high consumption of resources and the impact of climate change and the environment on healthcare providers [12]. It is noteworthy that a study by the WHO in 2021 stated that 25% of total deaths worldwide were due to an unhealthy work and living environment.

The Lancet has echoed these concerns, stating that this is one of the biggest global health threats of the 21st century, and to this end, the lives and well-being of billions of people are at an increased risk. In a recent study, Lancet [15] stated that pollution (i.e., air pollution, water pollution, toxic occupational hazards etc.) was responsible for one in six deaths (almost 9 million premature deaths worldwide).

In 2015, the United Nations published the *"Transforming our World: the 2030 Agenda for Sustainable Development"*, which sets 17 Sustainable Development Goals regarding (among others) the preservation and protection of the natural resources and our planet, the environmental degradation, and the inequalities within and among countries [16,17].

The Glasgow Climate Pact, where representatives from almost 200 countries met in November 2021 at the UN Climate Change Conference (CON26), agreed on the future strengthening of mitigation measures and new 2030 emissions targets (reducing methane emissions) and boosted efforts to deal with climate impacts [18,19]. Other factors that are contributing to the bulk of healthcare waste include the population ageing, the ever-increasing rates of chronic diseases, high healthcare costs, misallocation of financial resources combined with the inefficiency of health services and wastage of low-value healthcare, the pressures created by new medical technologies and drugs, lack of evidence to support reforms, etc. [13, 20–23].

Environmental Impact

Health systems are essential to the preservation of social health and well-being and are key factors in economic growth. It is becoming clear that appropriate organisational changes, health policy reformation, operational procedures, and management models should be made to further the sustainability of health systems and the health sector in general (sustainable healthcare). The WHO [24, 25] defines Environmentally Sustainable Health as a system that "*improves, maintains or restores health, while minimising negative impacts on the environment and leveraging opportunities to restore and improve it, to the benefit of the health and well-being of current and future generations*".

According to Namany et al. [26], limited resources and unsustainable consumption could result in ecological collapse and resource exhaustion, so it is imperative to have total resource efficiency to be sustainable: consuming fewer resources and producing less waste but, at the same time, offering the same quality of services in the healthcare sector [27]. Researchers have shown that healthcare providers have embraced and implemented various sustainability environmental practices, such as the adoption and use of green energy and the management and reduction of medical wastes [28–31]; specific pollution control (emissions to air, land, and water) [32–36]; practices for the protection, conservation and restoration of natural resources [5,37]; reuse/recycling, repair, and refurbishment of medical products [38,39]; efficient usage of resources [40]; sustainable procurement, etc. [1].

Therefore, healthcare providers, hospitals and healthcare systems, in general, need to alter their overall strategy in terms of sustainable development, with a clear mandate to manage environmental risks, adopt the proper environmental and sustainable management policies and training, and efficiently implement other initiatives to improve their reputation, reduce their operational costs and improve profitability, increase their staff's satisfaction and retention, manage potential risks and comply with the legal framework in which they operate, and present a more eco-friendly and socially responsible image to its interested parties [41–44].

It is becoming more important for each healthcare system to have an effective and sustainable medical waste management system to protect public health. It is essential to minimise and adequately manage HCWs and hazardous chemicals through the implementation of proper waste management methodologies. We need to promote efficient management of resources and sustainable procurements, introduce and monitor specific KPIs, reduce the health systems' emissions of greenhouse gases and air pollution, engage the health workforce, and implement the respective tools to minimise the threats to the environment [7, 13, 21–23].

Hospitals generate an enormous amount of waste: an average of 1,200 to 2,000 kilos per bed per year, but on average, only 15 to 20 per cent of all this waste is recycled. In the picture on page 16, we see artist and nurse Maria Koijck lying among the waste of her recent treatment for breast cancer. This is waste that was generated from one operation and four days of recovery. *'As the amount of waste produced globally is growing faster than the infrastructure to cope with it, half of the world's population is at risk from exposure to poor healthcare waste management,*" according to the European Public Alliance [89].

Managing Healthcare Waste

The priority is to reduce the impact of healthcare and improve the safety of patients, staff and the wider environment. Healthcare waste contains potentially harmful microorganisms that can infect hospital patients, health workers and the general public. The key potential hazards may include drug-resistant microorganisms which spread from health facilities into the environment.

Adverse health outcomes associated with healthcare waste and by-products also include sharps-inflicted injuries; toxic exposure to pharmaceutical products, in particular, antibiotics and cytotoxic drugs released into the surrounding environment, and substances such as mercury or dioxins during the handling or incineration of healthcare wastes; chemical burns arising in the context of disinfection, sterilisation or waste treatment activities; air pollution arising as a result of the release of particulate matter during medical waste incineration; thermal injuries occurring in conjunction with open burning and the operation of medical waste incinerators; and radiation burns.

Environmental Impact

Treatment and disposal of healthcare waste may also pose health risks indirectly through the release of pathogens and toxic pollutants into the environment.

The disposal of untreated healthcare wastes in landfills can lead to the contamination of drinking, surface, and ground waters if those landfills are not properly constructed. Moreover, the treatment of healthcare wastes with chemical disinfectants can result in the release of chemical substances into the environment if those substances are not handled, stored and disposed of in an environmentally sound manner.

> **“High-income countries generate on average up to 0.5 kg of hazardous waste per hospital bed per day; while low-income countries generate on average 0.2 kg.”**

Incineration of waste has been widely practised, but inadequate incineration or the incineration of unsuitable materials results in the release of pollutants into the air and the generation of ash residue. Incinerated materials containing or treated with chlorine can generate dioxins and furans, which are human carcinogens and have been associated with a range of adverse health effects. Incineration of heavy metals or materials with high metal content (in particular lead, mercury and cadmium) can lead to the spread of toxic metals in the environment.

Only modern incinerators operating at 850-1100°C and fitted with special gas-cleaning equipment can comply with the international emission standards for dioxins and furans. Alternatives to incineration such as autoclaving, microwaving, and steam treatment integrated with internal mixing, which minimises the formation and release of chemicals or hazardous emissions should be considered in settings where there are sufficient resources to operate and maintain such systems and dispose of the treated waste.

Waste Management: reasons for failure

Lack of awareness about the health hazards related to Healthcare waste, inadequate training in proper waste management, absence of waste management and disposal systems, insufficient financial and human resources and the low priority given to the topic are the most common problems connected with healthcare waste. Many countries either do not have appropriate regulations or do not enforce them.

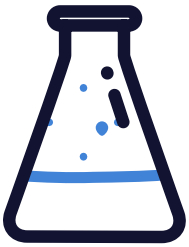
Healthcare Risk Waste (HCRW) also poses a growing risk to public health due to its potentially infectious nature (see page 18) and includes items contaminated with blood or body fluids, contaminated waste from patients with transmissible infectious diseases and other healthcare infectious waste.

The major sources of healthcare waste in hospitals and other health facilities include laboratories and research centres, mortuary and autopsy centres, animal research and testing laboratories, blood banks and collection services and nursing homes for the elderly.

High-income countries generate on average up to 0.5 kg of hazardous waste per hospital bed per day; while low-income countries generate an average of 0.2 kg. However, healthcare waste is often not separated into hazardous or non-hazardous wastes in low-income countries making the real quantity of hazardous waste much higher.

Waste is a vector for infection in hospitals

Pharmaceutical waste: expired, unused and contaminated drugs and vaccines.

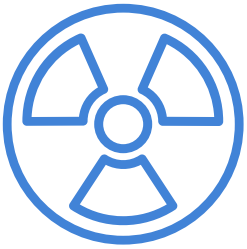


Chemical waste: for example solvents and reagents used for laboratory preparations, disinfectants, sterilants and heavy metals contained in medical devices (e.g. mercury in broken thermometers) and batteries.

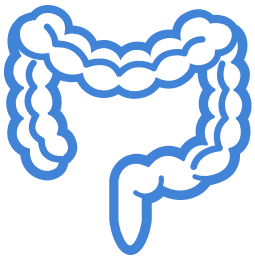
Non-hazardous or general waste: waste that does not pose any particular biological, chemical, radioactive or physical hazard.

Sharps waste: syringes, needles, disposable scalpels and blades.

Infectious waste: waste contaminated with blood and other bodily fluids (e.g. from discarded diagnostic samples), cultures and stocks of infectious agents from laboratory work (e.g. waste from autopsies and infected animals from laboratories), or waste from patients with infections (e.g. swabs, bandages and disposable medical devices).



Radioactive waste: such as products contaminated by radionuclides including radioactive diagnostic material or radiotherapeutic materials; and Cytotoxic waste: waste containing substances with genotoxic properties (i.e. highly hazardous substances thatare, mutagenic, teratogenic orcarcinogenic), such as cytotoxic drugs used in cancer treatment and their metabolites.



Pathological waste: human tissues, organs or fluids, body parts and contaminated animal carcasses.

Key Study: Hospital-Acquired Infections and Waste Contact (2017)

Mainul Haque, Massimo Sartelli, Judy McKimm, and Muhamad Abu Bakar

Healthcare-associated infections (HCAIs) are infections that occur while receiving health care, developed in a hospital or other healthcare facility that first appear 48 hours or more after hospital admission, or within 30 days after having received health care. The US Centers for Disease Control and Prevention identifies that nearly 1.7 million hospitalized patients annually acquire HCAIs while being treated for other health issues and that more than 98,000 patients (one in 17) die due to these.

When these infections occur in hospitals, they lead to prolonged stays, and disability, and impose a great economic burden on the hospital and society. Frequently prevalent infections include central line-associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonia. Nosocomial pathogens include bacteria, viruses and fungal parasites. According to WHO estimates, approximately 15% of all hospitalised patients suffer from these infections.

During hospitalisation, a patient is exposed to pathogens through different sources: including the environment, healthcare staff, and other infected patients. Transmission of these infections should be restricted for prevention. This paper stresses that hospital waste can serve as a potential source of pathogens and about 20%–25% of hospital waste is termed as hazardous.

Nosocomial infections can be controlled by practising infection control programs, by keeping a check on antimicrobial use and its resistance, and by adopting an antibiotic control policy. Efficient surveillance systems can play their part at national and international levels. Efforts are required by all stakeholders to prevent and control nosocomial infections. HCAIs are already a major safety concern for both healthcare providers and patients and they continue to escalate at an alarming rate, especially in emerging economies, with infection rates 3–20 times higher than in high-income countries.

04 THE THREAT OF ANTIMICROBIAL RESISTANT BACTERIA

The Threat of Antimicrobial Resistance

The fight against AMR is one of the most urgent stories of our time. 1.27 million people died in 2019 because of AMR (more than HIV, Malaria, and TB) and there is a growing consensus that this number will rise significantly over the next decade, with an elevated risk of an outbreak from multi-drug resistant bacteria and viruses.

Antibiotics are very much victims of their own success. The ability to treat infections, many of which were once life-threatening is one of the greatest advances in medicine. In turn, this ability enabled many other advances (in surgery and other therapeutics for example) as the risk of infection had largely been mitigated.

The success in treating infection led to the widespread use of antibiotics and the dual problems of overuse and misuse of these incredibly beneficial medicines. Use at this scale led to diminished effectiveness of antibiotics and the rise of resistant bacterial infections that our healthcare institutions are now encountering.

This cycle of resistance is now a critical issue for healthcare. Antibiotics of last resort are administered to treat patients where infections do not respond to other treatments. They are usually administered only in hospitals to treat other antibiotic-resistant infections, from where they find their way into the municipal wastewater system.

These resistant bacteria then multiply and/or exchange drug resistance ability on a genetic level. This has been shown to occur in water works, sewage treatment plants and other public water sources. As evidence builds that hospital wastewater contains a higher concentration of these antimicrobial bacteria, there is a growing understanding that hospital wastewater has unique characteristics and impacts on municipal and public infrastructure.

Antimicrobial resistance in hospital wastewater

A 2019 study of AMR in hospital wastewater in Scotland is instructive. The study aimed to use metagenomics to investigate whether the abundance of resistance genes in hospital wastewater reflects clinical activity within the hospital.

Hospital wastewater was collected over a 24-h period in June 2017, from multiple collection points representing different specialities within a tertiary hospital site in Scotland, and simultaneously from community sewage works. High throughput shotgun sequencing was done using Illumina HiSeq4000. Sequence reads were assigned taxonomically and to the AMR genes in the ResFinder database.

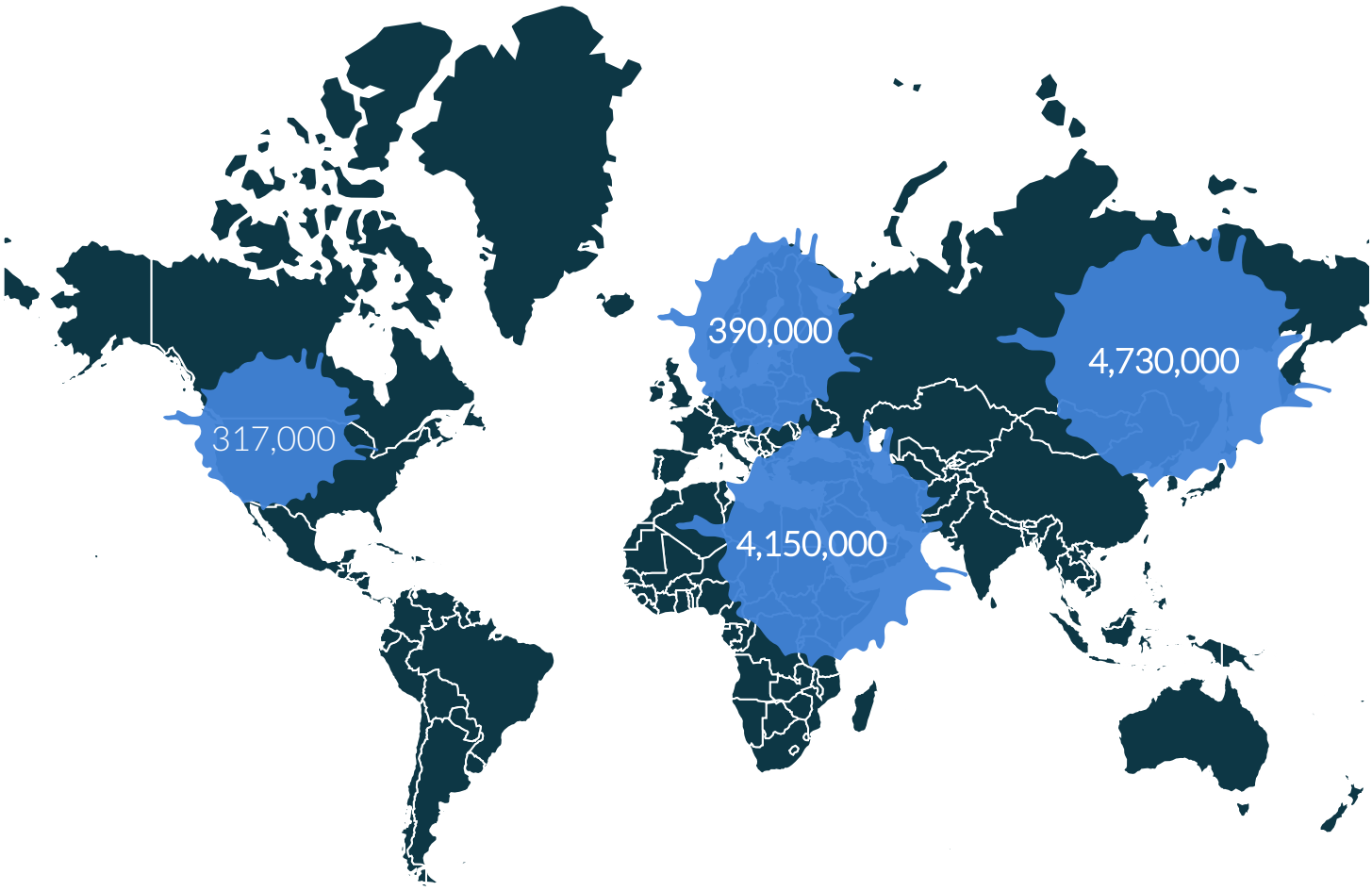
The measured AMR gene abundances were correlated to hospital antimicrobial usage in defined daily doses per 100 occupied bed-days, mean patient length of stay in the hospital, mean patient age per hospital collection point, and resistance levels in clinical isolates.

Their findings: 1047 bacterial genera and 174 different AMR genes were detected across all samples. Microbiota composition and AMR gene abundance and diversity varied between each collection point and AMR gene abundance was higher in hospital wastewater than in community influent. Moreover, the composition of AMR genes correlated with microbiota composition (Procrustes analysis, p=0·002). Increased antimicrobial consumption at a class level was associated with higher AMR gene abundance within that class in hospital wastewater (incidence rate ratio 2·80, 95% CI 1·2–6·5, p=0·016).

Prolonged mean patient length of stay was associated with higher total AMR gene abundance in hospital wastewater (2·05, 1·39–3·01, p=0·0003). No overall association was found between resistance in clinical isolates at an antimicrobial class level and AMR gene abundance in hospital wastewater. No overall association was found between resistance in clinical isolates at an antimicrobial class level and AMR gene abundance in hospital wastewater. The study found that antimicrobial usage is a major driver of AMR gene outflow from hospitals into the sewage environment. The positive relationship between the length of stay and AMR gene abundance is consistent with prolonged admission being a risk factor for carriage and infection with resistant microorganisms. The findings show that hospital wastewater does reflect inpatient activity, and the key studies that follow on pages 23 to 34, outline several urgent and recommended solutions to this escalating crisis.

Impact of AMR

Deaths attributable to AMR by 2050



1.27 million people died in 2019 because of AMR (more than HIV, Malaria, TB). There is a growing consensus that this number will rise significantly over the next decade, reaching the 10 million deaths, predicted by Lord Jim O'Neill in his 2014 review, far sooner than we expected.

Key Study: Hospital Effluents and Wastewater Treatment Plants: A Source of Oxytetracycline and Antimicrobial-resistant Bacteria in Seafood (2021)

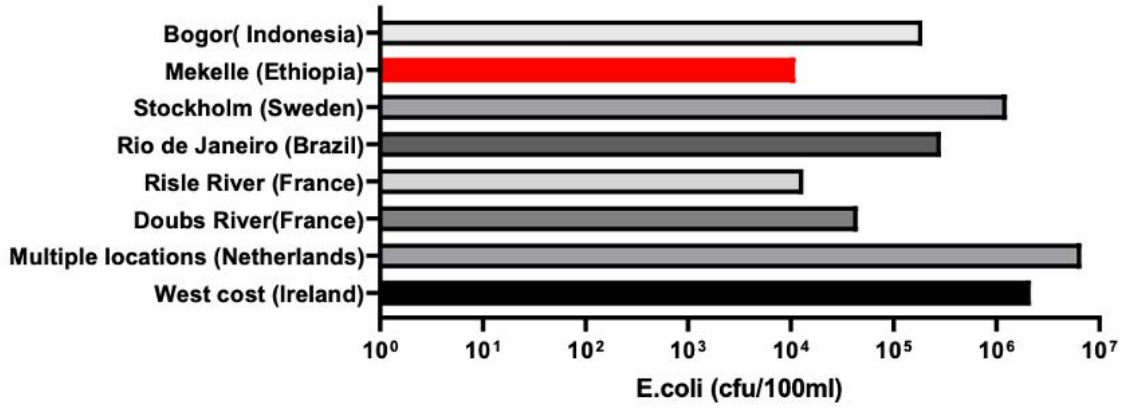
Bozena McCarthy, Samuel Obeng Apori

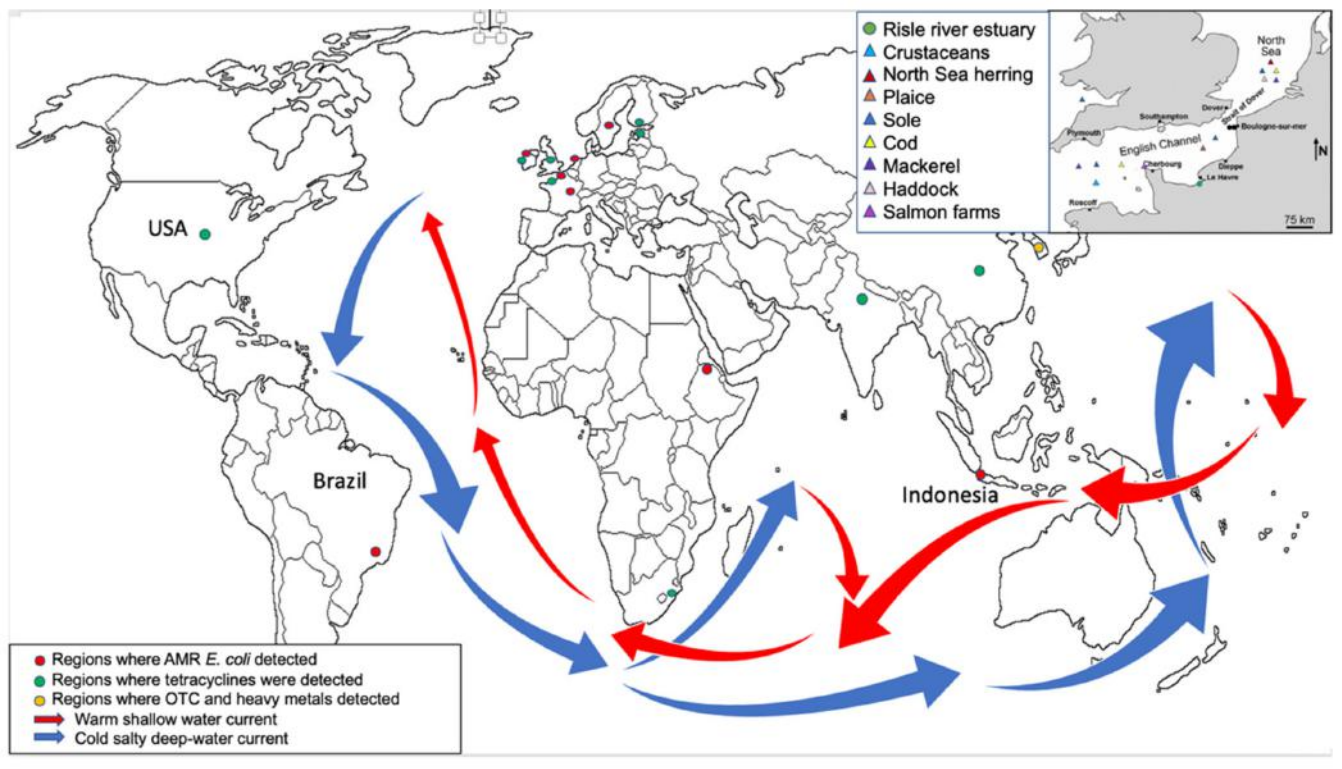
This study employed a data review on the presence and aggregation of oxytetracycline (OTC) and resistance (AMR) bacteria in wastewater treatment plants (WWTPs), and the distribution of the contaminated effluent with the aid of shallow and deep ocean currents.

The study aimed to determine the fate of OTC, and AMR bacteria in seafood, and demonstrate a relationship between AMR levels and human health. The review included: (1) OTC, (2) AMR bacteria, and (3) heavy metals in 16 aquatic environments and their relationship. Few publications describe OCT in surface waters. Although OTC and other tetracyclines were found in 10 countries in relatively low concentrations, the continuous water mass movement poses a contamination risk for mariculture and aquaculture.

10 locations were showing AMR bacteria in treated and untreated hospital effluent. Special effort was made to define the geographical distribution of OTC, AMR bacteria, and heavy metals detected in WWTPs to show the likely dissemination in the aquatic environment. The presence of OTC in surface waters in Asia, the USA, and Europe, can potentially impact seafood globally with the aid of ocean currents. Moreover, low concentrations of heavy metals exert environmental pressure and contribute to AMR dissemination. Recommended solutions are (1) quantitative analysis of OTC, heavy metals, and AMR bacteria to define their main sources, (2) employing effective technologies in urban and industrial wastewater treatment, and (3) selecting appropriate modelling from Global Ocean Observing System to predict the OTC, heavy metals, and AMR bacteria distribution.

A bar chart was constructed to illustrate the AMR concentration from different countries (Figure 1).





Conclusions: Hospital and WWTP effluent are indeed a source of antimicrobials and AMR bacteria in the aquatic environment. The use of antimicrobial agents, namely OTC and other tetracyclines in the treatment and prevention of pathogenic infection in humans and animals may be responsible for the accelerated spread of bacterial resistance in the aquatic environment.

The study highlights the importance of further research into the path of AMR bacteria in aquaculture and aquatic environments and its effects on human health. The OTC and ESBL-producing *E. coli* were studied in untreated HWW in 18 regions around the world. The OTC was detected in WWTPs in Asia, the USA and Europe. The lowest levels of AMR *E. coli* were observed in Northern France. The highest numbers of resistant *E.coli* were detected in the Netherlands in the North Sea region. Research to date contributed to an understanding of OTC and AMR *E. coli* existence and its spread in the aquatic environment.

The presence of OTC and low concentration of heavy metals affect development of AMR genes. The OTC from Asia, the USA and Europe has the potential to impact AMR bacterial and seafood globally due to continuous water mass movements assisted by ocean currents.

These findings emphasise the need for urgent, coordinated national and international interventions to limit the use of antimicrobials, and limit the global spread of AMR.

The proposed strategies also include the reduction of waste including industrial runoff, correct waste disposal, reduction in discharge of chemicals from pharmaceutical plants and the employment of effective technologies in hospital, urban and industrial wastewater treatment. Furthermore, the authors emphasize the need for appropriate modelling from Global Ocean Observing System to predict the OTC, heavy metals and AMR bacteria distribution.

Key Study: Patterns of Abundance of AMR Genes in Hospital Wastewater Vary by Specific Antimicrobial family (2021)

Perry MR, Lepper HC, McNally L, Wee BA, Munk P, Warr A, Moore B, Kalima P

This study used metagenomics to study how hospital clinical activity impacts antimicrobial resistance genes (ARGs) abundance in hospital wastewater.

Microbiota and ARG composition varied between CPs and overall ARG abundance was higher in hospital wastewater than in community influent. ARG and microbiota compositions were correlated (Procrustes analysis, p=0.014). Total antimicrobial usage was not associated with higher ARG abundance in wastewater. However, there was a small positive association between resistance genes and antimicrobial usage matched to ARG phenotype (IRR 1.11, CI 1.06–1.16, p<0.001).

Furthermore, analyzing carbapenem and vancomycin resistance separately indicated that counts of ARGs to these antimicrobials were positively associated with their increased usage [carbapenem rate ratio (RR) 1.91, 95% CI 1.01–3.72, p=0.07, and vancomycin RR 10.25, CI 2.32–49.10, p<0.01].

Overall, ARG abundance within hospital wastewater did not reflect resistance patterns in clinical isolates from concurrent hospital inpatients.

However, for clinical isolates of the family Enterococcaceae and Staphylococcaceaethere, there was a positive relationship with wastewater ARG abundance [odds ratio (OR) 1.62, CI 1.33–2.00, p<0.001, and OR 1.65, CI 1.21–2.30, p=0.006 respectively].

Conclusion: The study found that the relationship between hospital wastewater ARGs and antimicrobial usage or clinical isolate resistance varies by specific antimicrobial and bacterial family studied. One explanation is that relationships observed from multiple departments within a single hospital site will be detectable only for ARGs against parenteral antimicrobials uniquely used in the hospital setting. This work highlights that using metagenomics to identify the full range of ARGs in hospital wastewater is a useful surveillance tool to monitor hospital ARG carriage and outflow and guide environmental policy on AMR.

Key Study: The impact of on-site hospital wastewater treatment on the downstream communal wastewater system in terms of antibiotics and antibiotic resistance genes 2019)

Gabriela K. Paulus, Luc M. Hornstra, Jaroslav Slobodnik, Nikolaos Thomaidisd, Gertjan Medema,

Abstract

This study quantified antibiotic and antibiotic resistance gene (ARG) concentrations in hospital and communal wastewaters as well as the influents and effluents of the receiving urban wastewater treatment plants (UWWTP) in two Dutch cities. In only one city, hospital wastewater was treated on-site using advanced technologies, including membrane bioreactor treatment (MBR), ozonation, granulated activated carbon (GAC) and UV treatment.

On-site hospital wastewater (HWW) treatment reduced gene presence of hospital-related antibiotic resistance genes and antibiotic concentrations in the receiving urban wastewater treatment plant. These findings support the need for on-site treatment of high-risk point sources of antibiotic resistance genes.

13 antibiotic resistance genes, Integrase Class 1 and 16S rRNA concentrations were quantified using multiplex quantitative real-time PCR (qPCR) assays and the presence and/or concentration of 711 antibiotics were analyzed.

Hospital wastewater contained approximately 25% more antibiotics and gene concentrations between 0.4 log to 1.8-fold higher than communal wastewater (CWW). blaKPC and vanA could be identified as hospital-related genes and were reduced to under the limit of detection (LOD) during on-site treatment.

Advanced on-site treatment removed between 0.5 and 3.6-fold more genes than conventional biological urban wastewater treatment (activated sludge). Advanced on-site treatment was able to eliminate 12 out of 19 detected antibiotics, while urban wastewater treatment eliminated up to 1 (out of 21 detected).

Different advanced treatment technologies were able to target different pollutants to varying extents, making sequential alignment more effective. MBR treatment was most efficient in antibiotic resistance gene reduction and ozonation in antibiotic reduction.

bla_{KPC} could only be detected in the influent of the urban wastewater treatment plant receiving untreated hospital wastewater. Similarly, vanA was only consistently detected in this treatment plant. These results indicate a positive effect of on-site treatment of hospital wastewater on the communal sewage system.

1. Introduction

Antibiotic Resistance (AR) is a growing global threat which will require worldwide joint efforts to be conquered (ECDC/EMEA Joint Technical Report: The bacterial challenge, 2009; ECDC strategic multiannual programme 2014–2020, 2014).

Hospitals have been in the focus of AR research as one of the high-risk point sources of antibiotics (Brown et al., 2006; Kümmerer, 2001; Lien et al., 2016) and ARGs (Berendonk et al., 2015; Harris et al., 2010; Harris et al., 2013a,b Harris et al., 2013a,b; Lien et al., 2016; Rowe et al., 2017).

Although, the release of untreated HWW might be posing a hazard to the environment and human health, there are still few studies investigating the release and direct impact of HWW into the environment or communal sewage system (Czekalski et al., 2014; Wang et al., 2018a). Due to this gap in information, regulations for the treatment of HWW are absent in most countries (Aukidy et al., 2017; Liu et al., 2010; Rodriguez-Mozaz et al., 2015).

The release of untreated HWW could increase ARG prevalence in environmental water bodies. Antibiotic-resistant bacteria were shown to survive in the HWW, in the UWWTPs and, subsequently, in the UWWTP effluent (Thompson et al., 2013).

The risk potential of HWW is further increased by the fact that hospitals use last-resort antibiotics (e.g. piperacillin and vancomycin) more frequently and thus their ARG profiles might be different when compared to other wastewaters (Kümmerer and Henninger, 2003). Overall, conventional wastewater treatment renders limited results in terms of antibiotic and ARG removal and might even increase the concentration of certain ARGs (Berendonk et al., 2015; Luo et al., 2014; Narciso-Da-Rocha et al., 2014; Szczepanowski et al., 2009; Szekeres et al., 2017).

The present study investigates the impact and efficiency of antibiotic, ARG and bacterial removal of advanced on-site treatment compared to urban wastewater treatment. The effect of different advanced treatment steps and their impact on the downstream urban wastewater system are studied. ARG occurrence and concentrations in HWWs and CWWsin the Netherlands are compared to identify potential differences.

To this end, genes conferring resistance to aminoglycosides (aph(III)a), β-Lactam Antibiotics (*bla*$_{KPC}$, *bla*$_{SHV}$, *bla*$_{OXA}$, *mec*A), macrolides (*erm*B, *erm*F), quinolones (*qnr*S), sulfonamides (*sul*1), tetracyclines (*tet*B, *tet*M) and vancomycines (*van*A, *van*B) as well as a class 1 Integrase (*intI1*) were screened for and quantified. A total of 711 antibiotics were investigated, out of which 41 were quantified and 670 were screened for presence in the samples. Further, correlations between antibiotic and ARG concentrations were studied.

2. Materials and methods

2.1. Sampling

Samples were taken from two cities in the Netherlands, namely Delft (location 1) and Nieuwegein (location 2). At location 1 HWW was treated on-site. The following samples were taken from each location: hospital wastewater, communal sewage (at a location not impacted by HWW), on-site hospital wastewater treatment plant (Pharmafilter, location 1 only) and samples from the receiving UWWTPs.

Two sampling rounds were conducted at all sampling locations with at least 6 months in between sampling rounds. The first sampling round took place in spring and the second in winter.

2.1.1. Hospitals

Samples were taken from combined HWW at location 1 (H1) and location 2 (H2). All samples were composite samples (12h-composites – 1st sampling round; 8h-composites – 2nd sampling round). Both hospitals contained wards that typically have high antibiotic use.

2.1.2. Communal WW

CWW samples were taken from the urban sewage system, which was accessed by street manholes. Samples were taken at a location at which the sewage system was not impacted by HWW. Samples were combined grab samples consisting of at least 3 subsamples taken approx. 3h apart, which were pooled together before analysis.

2.1.3. Urban WWTPs

Samples were taken from two UWWTPs: 1) W1 (location 1) and 2) W2 (location 2).

W1 (built in 2006): The treatment plant processes a quantity of water which compares to a population equivalent of 1.260.000 (PE) and has an average in-and-outflow of 180.000 m^3/d. W1 receives CWW including wastewater from H1. Wastewater treatment consists of primary and secondary treatment, including influent screening (6mm bars), primary sedimentation, biological (activated sludge) treatment, final clarification and biological phosphorus removal.

W2 (built in 1975 and renovated in 2010): The treatment plant has a volume capacity which compares to 144.000 PE and an average in and outflow of 25.700 m^3/d. W2 receives CWW including wastewater from H2. Wastewater treatment consists of primary and secondary, including influent screening, primary sedimentation, biological (activated sludge) treatment and biological Nitrogen and phosphorus removal. 24h-composite samples (taken by automatized composite samplers) were obtained from each UWWTP (influent and effluent wastewater).

2.1.4. Pharmafilter

HWW at location 1 was treated on-site by an installation called the Pharmafilter. Pharmafilter treatment consists of 4 sequentially aligned treatment steps (see Fig. 1 on the following page): Membrane Bioreactor (Microfiltration) (MBR), Ozonation (Ozon), Granulated Activated Carbon (GAC), UV Treatment (UV). 24h-composite samples were taken after each treatment step, as well as from the MBR-Sludge.

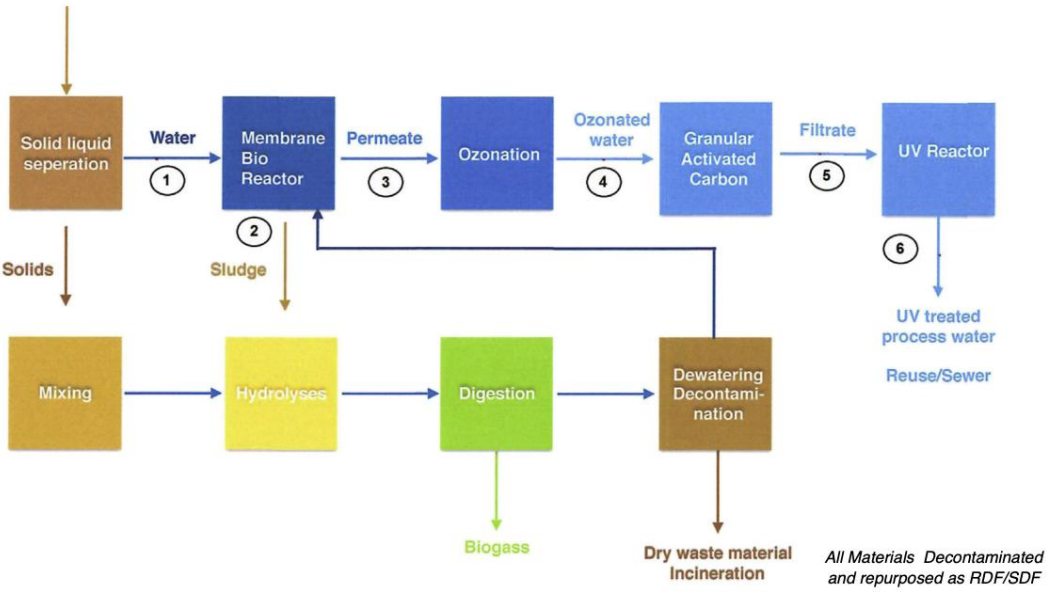


Fig. 1. Pharmafilter Installation and Process Steps; samples taken (1)–(6): (1) Untreated HWW, (2) Sludge, (3) MBR, (4) Ozonation, (5) GAC, (6) UV Treatment/ Effluent.

2.2. Sample preparation

Samples were processed immediately after arrival at the laboratory.

2.2.1. Biological analysis

Samples were filtered using 0.22-μm-pore-size polycarbonate tracketch filter membranes (Sartorius). DNA was extracted from the filters using DNeasy PowerSoil Kit (QIAGEN Benelux B.V).

Extraction was performed according to manufacturer instructions, with one exception. An internal control (IC) plasmid was added to the samples (concentration: 2.5×10^4 gene copies/μL) to quantify the DNA loss caused by the extraction process (Wullings et al., 2007). Extraction blanks yielded negative results. DNA loss was corrected for, based on IC concentrations measured by qPCR.

2.2.2. Chemical analysis

The sample preparation protocol involved clean-up and 4000x preconcentration on an Atlantic HLB-M Disk, using a HORIZON SPE-DEX 4790 (USA) with a 47mm disk holder. Conditioning and extraction programs used for the preparation of the wastewater samples can be found in the SI.

The extract was evaporated using a gentle stream of nitrogen and was reconstituted with 250 μl of 50:50 methanol: water mixture for instrumental analysis. Before analysis, extracts were filtered through RC syringe filters of 4 mm diameter and 0.2 μm pore size (Phenomenex, USA).

2.3. ARG detection and quantification – biological analysis

2.3.1. Multiplex qPCR assays

DNA extracts were stored at −20°C prior to qPCR analysis. All qPCR assays were performed at least twice using technical duplicates each time. 16S rRNA was quantified using a SYBR Green qPCR assay. The following genes were quantified by qPCR: *aph*(III)a, bla_{KPC}, bla_{OXA}, bla_{SHV}, *erm*B, *erm*F, *intI 1*, *mec*A, *qnr*S, *sul*1, *tet*B, *tet*M, *van*A and *van*B. Standards, a positive and a negative control were included in every assay to confirm multiplex qPCR quality. Standards were made up of 5 subsequent dilutions with concentrations ranging from 2.5×10^4 to 2.5×10^0 gene copies/μL. Multiplex qPCR assays were performed using the iQ™ Multiplex Powermix (Bio Rad, München, DE) and qPCR reactions were performed using a CFX96™ Real-Time PCR Detection System (Bio Rad, München, DE). CFX96™ Real-Time PCR Detection System data was interpreted by CFX Manager.

2.3.2. Data analysis

Python 3.6.0 (Hunter, 2007; Mckinney, 2010) executed in Jupyter Notebooks was used to clean and analyze raw data, to calculate descriptive statistics and correlations and to create data visualizations. R version 3.5.0 was used to perform inferential statistical analysis. Significant differences between experiments and/or measurements were detected by employing paired or unpaired Student's t-Tests, or Welch's t-Tests for the case that the sample variances were not comparable and data transformation was not possible.

Two samples/measurements were defined to be significantly different from each other for $p < 0.05$. Correlations between antibiotic and ARG concentrations were calculated using Pearson's rank correlation coefficient.

An ARG and antibiotic were considered correlated for $R2 > 0.5$, $p < 0.05$ and if there were ≥ 4 common data points available. Relatedness with values of $0.5 < R2 < 0.7$ was considered a 'moderate correlation', while $R2 > 0.7$ was considered a 'strong correlation'.

2.4. Antibiotic detection and quantification – chemical analysis

2.4.1. Instrumental analysis

Instrumental analysis was performed with a Thermo UHPLC Accela system connected to a TSQ Quantum Access triple quadrupole mass spectrometer from Thermo Electron Corporation (San Jose, CA, USA) equipped with an electrospray ionization source (Thermo IonMAX) in positive mode.

Chromatographic separation was achieved on an Atlantis T3 C18 (100 mm × 2.1 mm, 3 μm) column from Waters Corporation (Milford, MS, USA) at a constant flow rate of 100 μL/min. The mobile phase, the gradient elution programs and the ESI parameters are presented.

Identification and quantification were performed under selected reaction monitoring (SRM) mode, recording the transitions between the precursor ion and the two most abundant product ions for each target analyte, thus achieving 4 identification points per compound (2002/657/EC).

SRM transitions for each compound were optimized by infusion of standard solutions at a mean concentration of 1 mg/L.

To assure that as many antibiotics as possible were captured, extracts were also injected in a UHPLC-QTOFMS system, equipped with a UHPLC apparatus (Dionex UltiMate 3000 RSLC, Thermo Fisher Scientific, Dreieich, Germany), coupled to the QTOF-MS mass analyzer (Maxis Impact, Bruker Daltonics, Bremen, Germany).

Chromatographic separation was performed on an Acclaim RSLC C18 column (2.1 × 100 mm, 2.2 μm) from Thermo Fisher Scientific (Dreieich, Germany) preceded by a guard column of the same packaging material, kept at 30 °C.

3. Results and discussion

3.1. Antibiotics and ARGs in the urban WW cycle

3.1.1. Hospital wastewater had a higher prevalence and concentrations of antibiotics and ARGs than communal wastewater

HWW samples showed 0.4–1.8-fold higher relative ARG concentrations than CWW samples. No ARGs were observed in significantly higher concentrations in CWW samples. Similarly, absolute ARG concentrations (meaning: ARG concentrations per mL sample) which significantly differed from each other between HWW and CWW samples showed between 0.8 and 2.3-fold increase in HWW samples. The higher ARG pollution of HWWs suggests higher incidences of AR and can potentially suggest multi-drug-resistant bacteria, as has been found previously in several studies (Amador et al., 2015; Magalhães et al., 2016; Vaz-Moreira et al., 2015) or a larger proportion of resistant organisms compared to CWW.

bla$_{KPC}$ and *van*A were not found in any of the analyzed CWW samples, suggesting that these genes are hospital-related ARGs and that occurrences at other locations of the urban wastewater cycle originate from healthcare facilities. *Van*A has previously been suggested as an indicator gene to monitor the AR of anthropogenic origins in the environment (Narciso-Da-Rocha et al., 2014). VanA and *bla*$_{KPC}$ have repeatedly been detected in HWW (Chagas et al., 2011; Cuzon et al., 2011; Gootz et al., 2009; Hu et al., 2012; Iversen et al., 2002; Mascini and Bonten, 2005; Novais et al., 2005; Sahlström et al., 2009; Zhang et al., 2012).

Occurrences of *bla*$_{KPC}$ in the environment were only recently and rarely shown (Sellera et al., 2017). Some of these occurrences could be traced back to hospital-associated bacterial strains (Cerdeira et al., 2017).

The assumption of the association of HWW with *bla*$_{KPC}$ and *van*A is strengthened by previous findings that these genes are more prevalent in hospitals which use more carbapenems (Nasri et al., 2017) or vancomycin (Iversen et al., 2002).

VanA, is found downstream of hospital sewage release with a higher prevalence (Novais et al., 2005). The potential risks of these specific genes would be exacerbated by the possibility to be transferred horizontally between strains. At least in the case of *bla*$_{KPC}$, transconjugants were detected after horizontal gene transfer (HGT) (Hu et al., 2012), suggesting a heightened transfer risk potential of this gene.

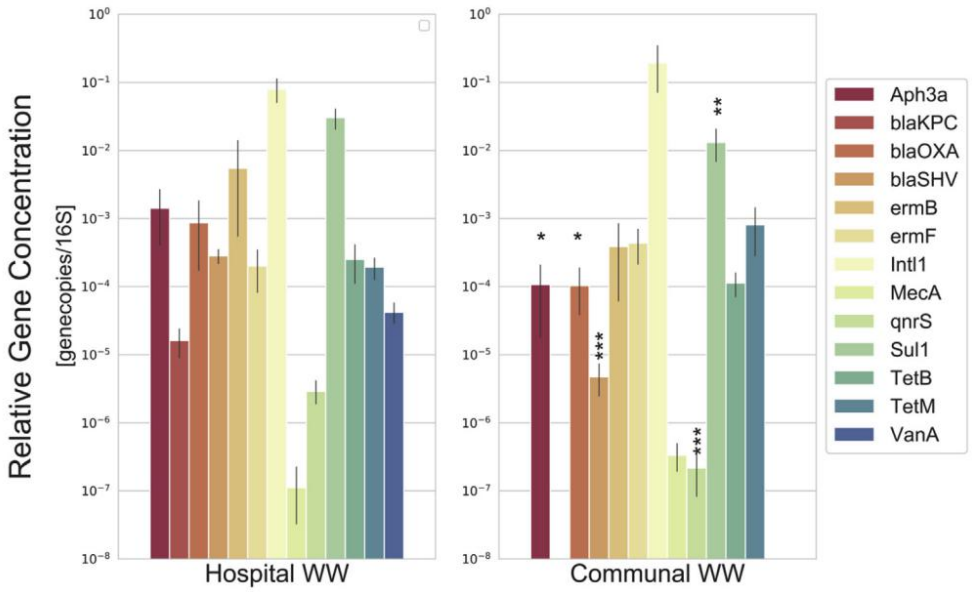


Fig. 2. Relative Gene Concentrations in Hospital and Communal Wastewater Samples; *values mean ± std of all four hospital or communal WW samples combined; * = ARG concentrations significantly ($p \leq 0.05$) lower in CWW, ** = ARG concentrations significantly ($p \leq 0.01$) lower in CWW, *** = ARG concentrations significantly ($p \leq 0.001$) lower in CWW.*

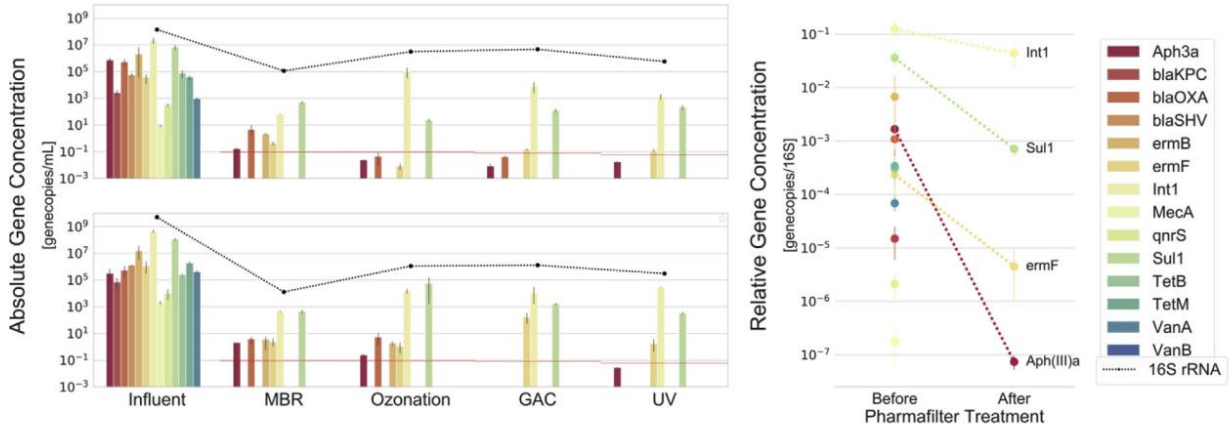


Fig. 3. Gene Reduction During Pharmafilter Treatment; left: impact of different treatment steps (1st (top) and 2nd (bottom) sampling rounds), dotted black line – 16S rRNA concentration; right: overall impact of Pharmafilter on HWW; red line – LOQ. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

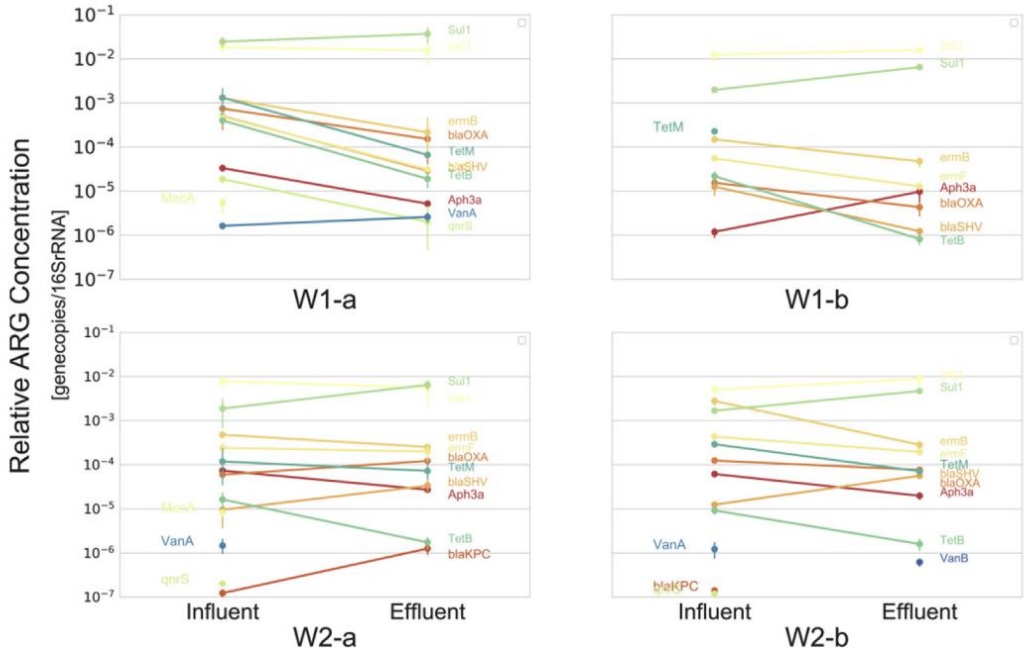


Figure 4. Change in Relative Gene Concentration During Communal Wastewater Treatment in W1 and W2 at Different Times, (a – 1st sampling round in spring, b – 2nd sampling round in winter).

Ciprofloxacin (2706 ng/L – H1, 3752 ng/L - H2) and sulfamethoxazole (367 ng/L - H1, 269 ng/L - H2) were detected at concentration levels of up to several orders of magnitude higher than in CWW samples. Metronidazole with a frequency of detection of 92% across all samples, reached concentrations as high as 4 ng/L (H1) and 7500 ng/L (H2). While antibiotic concentrations in HWWs can vary widely, concentrations within the same dimensions have been previously recorded, with ciprofloxacin, sulfamethoxazole and metrondazole frequently being detected (Baquero et al., 2008; Brenner et al., 2011; Lien et al., 2016). Some antibiotics could only be detected in HWW (Fluconazole, Sulfaclozine, Trimethoprim) or were detected in HWW with disproportionally higher concentrations than in CWW (Sulfamethoxazole, Ciprofloxacin; both detected at concentrations over 2-fold higher in HWWs). Ampicillin and Amoxicillin, on the other hand, were only detected in CWW. These findings are consistent with previous reports, that β-lactam antibiotics are largely used inside and outside hospitals with Amoxicillin being one of the most frequently used antibiotics for outpatient prescription (Durkin et al., 2018; Hicks et al., 2015; European Centre For Disease Prevention And Control, 2018). Quinolones and sulfonamides are more frequently used in hospitals than for outbound patients in the Netherlands (European Centre For Disease Prevention And Control, 2018).

3.2. Reduction of antibiotics and ARGs during communal and on-site treatment

3.2.1. On-site treatment eliminated antibiotics and ARGs efficiently

Bla$_{KPC}$, *bla*$_{SHV}$, *mec*A, *qnr*S, *tet*B, *tet*M and *van*A were reduced < LOD from HWW during MBR treatment (Fig. 3). The following genes could not be detected in the MBR permeate but were detected in the MBR sludge: blaSHV, tetB, tetM and vanA. No genes were consistently eliminated during the ozonation treatment step. GAC treatment showed some variation between the two sampling rounds, with some genes being significantly reduced or increased. IntI1 and sul1 were consistently detected in the highest and second-highest concentration, respectively. Overall changes in gene concentrations showed high consistency between the two sampling rounds. All detected genes were significantly reduced in absolute concentration and most also in relative concentration during the Pharmafilter treatment.

9 out of 13 initially detected ARGs in HWW were reduced < LOD during Pharmafilter treatment, including *bla*$_{KPC}$, *bla*$_{OXA}$, *bla*$_{SHV}$, *erm*B, *mec*A, *qnr*S, *tet*, *tet*M and *van*A. *Aph*(III)a, *erm*F, *intI1* and *sul*1 stayed detectable but were significantly reduced. Notably, the bacterial load increased during ozonation treatment. This can be explained by hydraulic retention times up to 2h before ozonation, during which the microbial community has time to adjust to the new conditions and propagate.

Pharmafilter treatment reduced ciprofloxacin from 2706 ng/L to 62 ng/L. Sulfamethoxazole was reduced from 367 ng/L to 0.9 ng/L (Fig. 5). Ozonation was the crucial treatment step for the elimination. MBR treatment seems to release cleavage forms of certain types of antibiotics thus increasing concentrations of certain antibiotics such as metronidazole, which is increased from 4 ng/L to 1203 ng/L during this step. The same trend was observed for other compounds: sulfamethoxazole (concentration difference after MBR treatment: +96%), ofloxacin (+110%), fluconazole (+289%) and erythromycin (17-fold difference). In some cases, concentrations of pharmaceutical residues appear to increase through MBR treatment, a documented phenomenon (Snyder et al., 2007), and might be explained by the cleavage of conjugated residues. For example, sulfamethoxazole can be generated during treatment by cleavage of its human metabolite N4-acetylsulfamethoxazole in WWTPs (Radjenović et al., 2009). Moreover, it is known that antibiotics are absorbed onto the negatively charged surface of sewage sludge through ionic interactions. In case of malfunction of membranes or poor maintenance it is possible that desorption phenomena may happen (Radjenović et al., 2009).

3.2.2. Urban wastewater treatment plants show low efficiency in antibiotic reduction and varying efficiency in ARG reduction

ARG concentrations did not uniformly show significant decrease during urban wastewater treatment. Significant gene reductions varied between 0.5 ± 0.1 (aph(III)a) to > 2.2-fold (vanA) in UWWTPs. ARG reduction efficiency varied between the two UWWTPs.

Significant changes in relative gene concentration were uniformly reductions in W1, while three ARGs significantly increased in concentration in W2. Genes which did not significantly decrease in concentration were intI1 and blaSHV (during both sampling rounds), blaKPC, blaOXA and sul1 (during the 2nd sampling round). On the other hand only one ARG was reduced < LOD in W1; mecA and tetM during the 1st and 2nd sampling round,respectively; while 3 ARGs were reduced < LOD in W2; qnrS and vanA (in both sampling rounds) and mecA (1st round) or blaKPC (2nd round). In W1 50–67% of present ARGs could be significantly reduced, while only 23–36% of present ARGs were significantly reduced in W2. A large proportion of genes did not show a significant change in relative concentration after treatment at W2. IntI1 is the only gene that does not show any significant changes in relative concentration in any of the different WWTPs and sampling rounds (see Fig. 4). Previous studies showed that secondary wastewater treatment decreased half of 78 detected ARGs by < 94% in concentration (Yang et al., 2014), while tertiary treatment has been found to retain 2%–50% of ARG raw influent concentrations (Mao et al., 2015).

Generally, UWWTPs were shown to have varying effects on ARG concentrations depending on wastewater treatment conditions and the type of ARG, even for wastewater treatment plants with tertiary treatment steps (Du et al., 2014; Rodriguez-Mozaz et al., 2015). Conventional urban wastewater treatment was not capable of removing ciprofloxacin effectively (removal efficiency: 41% for W1, 39% for W2). Similarly, sulfamethoxazole could not be eliminated effectively (removal efficiency: 25% for W1, 19% for W2). Both investigated UWWTPs fail to remove most of the detected antibiotics. Other antibiotics with poor removal efficiency were ofloxacin, trimethoprim, clarithromycin, sulfachloropyridazine, fluconazole, azithromycin, erythromycin and lincomycin (Fig. 5 on next page). The low biodegradability of many antibiotics might explain inefficient antibiotic removal (Kümmerer et al., 2000). Conventional urban wastewater treatment might therefore not be the most efficient method to reduce antibiotic and ARG concentrations from CWW, contaminated with HWW, prior to release into the environment. Due to substantial fluctuations in antibiotic and ARG concentrations and CWW quality, the resulting effluent will be of variable quality with unknown environmental impact.

3.3. Advanced on-site treatment is more efficient and constant than regular urban wastewater treatment

While relative ARG concentrations did not uniformly decrease in UWWTPs and increased for approximately 10–30% of all ARGs detected, all relative ARG concentrations were consistently significantly reduced during the Pharmafilter process (Fig. 3). Only intI1 was not consistently significantly removed during Pharmafilter treatment.

Pharmafilter treatment reduced approx. 70% of all detected ARGs to < LOD, while regular urban wastewater treatment reduced between 10% (W1) and 22% (W2) of detected ARGs to < LOD. Furthermore, the reduction of ARG concentrations, of genes which were still quantifiable after the respective treatments, was 0.5–4.4-fold during Pharmafilter treatment and 0.5–2.2-fold during UWWT. Pharmafilter reduces individual genes with efficiencies between 0.5-fold (intI1) to more than 3.6-fold (ermB) higher than that of UWWTPs. This discrepancy in efficiency is further increased considering that UWWTPs could increase certain ARG concentrations more than 1-fold. The increased ability of the Pharmafilter treatment compared to urban wastewater treatment is, with high probability, due to several interconnected factors: Conventional wastewater treatment has a limited capacity to remove resistance genes (Bouki et al., 2013; NarcisoDa-Rocha et al., 2014; Szekeres et al., 2017) while advanced wastewater treatment (including MBR, Ozone and UV treatment) has been shown to have a better efficiency (Zhang et al., 2015; Zhuang et al., 2015).

The sequential set-up of the Pharmafilter treatment steps seems to be of importance, as single treatment steps, seem to have the potential to increase the relative ARG concentrations when applied alone (Mao et al., 2015; Sousa et al., 2017).

This study showed similar findings. While MBR seems to be the single most effective treatment step to eliminate ARGs, only 7 out of 13 detected ARGs were reduced < LOD during this step. Two ARGs (blaOXA, ermB) were reduced < LOD, aph (III)a was significantly reduced in concentration in the subsequent treatment steps. The subsequent treatment steps, therefore, accounted for approximately 1/4 of the overall removal efficiency of the Pharmafilter. UV treatment had the least positive impact. Each of these advanced treatment types have their benefits and disadvantages (Aukidy et al., 2017) with ARG removal efficiency strongly depending on the type of ARGs present, the quality of wastewater influent and the applied treatment processes (Barancheshme and Munir, 2018; Sun et al., 2016). The high efficiency of MBR treatment is likely to be due largely to size exclusion, thus filtering out ARG-carrying microorganisms (Visvanathan et al., 2000; Judd, 2010). MBRs have been shown to develop characteristic communities, which differ from the influent community (Judd, 2010). Subsequent partial detachment of microorganisms from this characteristic community might explain why some ARGs are eliminated to a higher extent than others during this treatment step.

Further, antibiotics and other pharmaceutical compounds which might exert selective pressure and increase the HGT of ARGs (BengtssonPalme and Larsson, 2015; Wintersdorff et al., 2016; Xiong et al., 2015) are thoroughly eliminated by the Pharmafilter process. Correlations between antibiotic and ARG concentrations have been shown (Li et al., 2015b; Mao et al., 2015). Elevated concentrations of β-lactam antibiotics, glycopeptides and trimethoprim were detected in untreated HWW (Szekeres et al., 2017). In contrast to the Pharmafilter, UWWTPs were shown to eliminate a much lower percentage of chemicals, including antibiotics. Elimination of antibiotics can be as low as 20% for sulfamethoxazole, 69% for trimethoprim and 70% for ofloxacin (Brown et al., 2006). Correlations between antibiotic and ARG concentrations were detected during the present study. Of the 41 quantified antibiotics, concentrations of two antibiotics correlated strongly with ARG concentration ((rifaximin, metronidazole), two correlated moderately (azithromycin and norfloxacin) and ciprofloxacin correlated moderately to strongly (depending on the ARG). Antibiotics correlated with different numbers of ARG (azithromycin (3), rifaximin (7), metronidazole (6), ciprofloxacin (3), and norfloxacin (5). While most correlations were observed between unrelated antibiotic-ARG pairs, azithromycin (a macrolide) and ermF (R2 = 0.66), ciprofloxacin (a fluoroquinolone) and qnrS (R2 = 0.56) and norfloxacin (a quinolone) and qnrS (R2 = 0.64) correlated moderately.

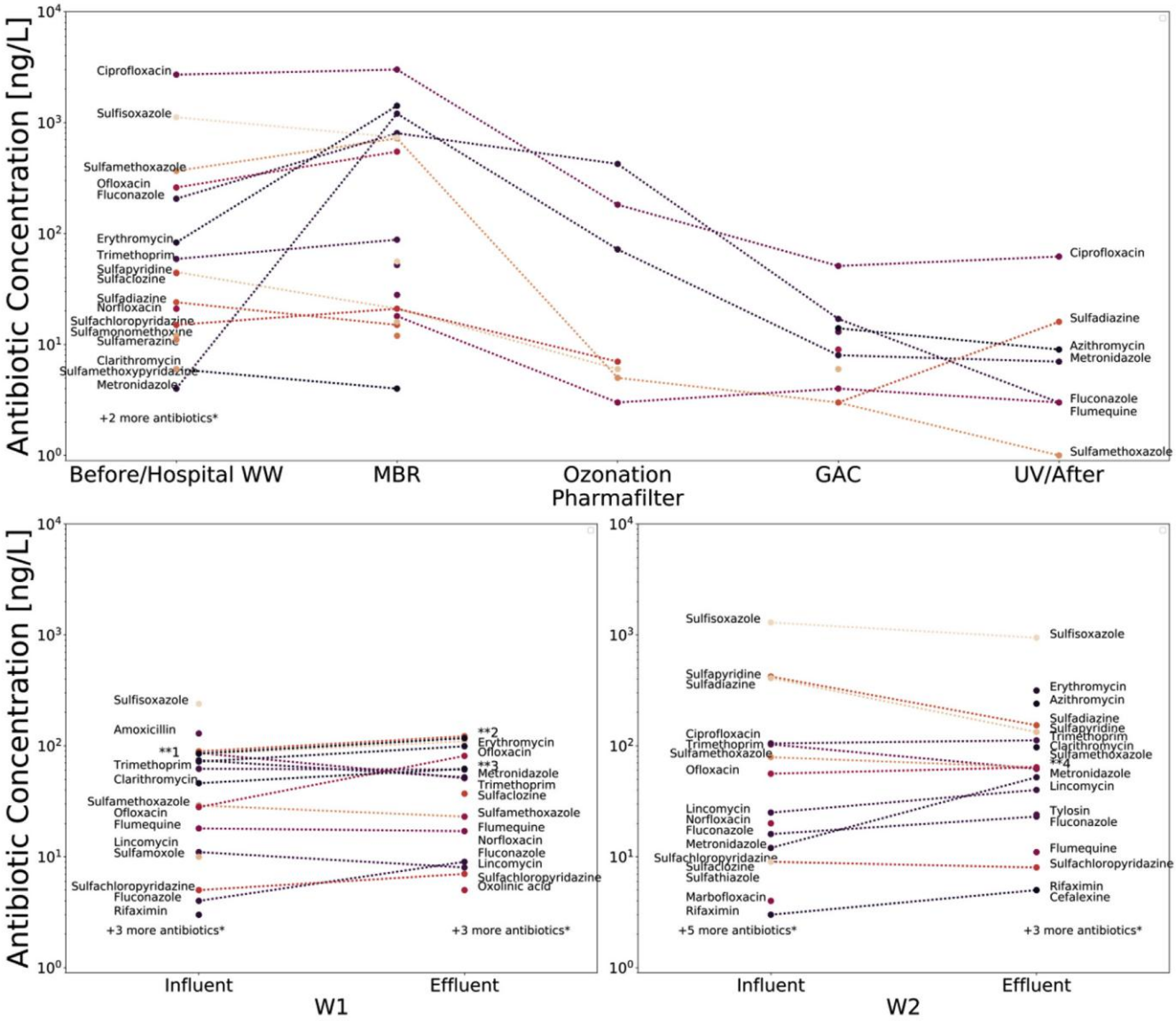


Fig. 5. Antibiotic Concentrations Compared During Pharmafilter Process and During Conventional Urban Wastewater Treatment; * - number of antibiotics detected but not quantified; **1 – Azithromycin, Erythromycin, Metronidazole, Ciprofloxacin, Sulfadiazine, Sulfapyridine (72–89 ng/L); **2 – Azithromycin, Sulfapyridine, Sulfadiazine (110–120 ng/L); **3 – Ciprofloxacin, Clarithromycin (51–62 ng/L); **4 – Ciprofloxacin, Ofloxacin (62-62 ng/L).

Interestingly, rifaximin and metronidazole concentrations correlated with ARG concentrations of a large number of unrelated ARGs. This could indicate that the selective pressure of antibiotics on unrelated ARGs might be a larger problem than selective pressure on related ARGs. Antibiotics like metronidazole which do not largely cause resistance (Otte et al., 2017; Regnath et al., 2017) might then have a larger impact on AR. Another explanation for these correlations could be co-selection. Co-selection of related and unrelated genes can be caused by co-occurrence on plasmids or other mobile genetic elements (Baker-Austin et al., 2006; Di Cesare et al., 2016; Stepanauskas et al., 2006; Gaze et al., 2011; Seiler and Berendonk, 2012; Li et al., 2015a). Finally, (non-antibiotic) pharmaceuticals which have not been investigated but are largely present in wastewaters, could be further driving HGT thus increasing AR (Hegstad et al., 2010; Wang et al., 2018b).

It is to be noted that correlation does not necessarily imply causation and that further research will be needed to conclude if one of the described mechanisms is responsible for the observed correlations. Nevertheless, these observations are of interest, in case of future research can find similar relationships between the respective antibiotics and ARGs.

3.4. The impact of pharmafilter treatment on ARG concentration in hospital wastewater effluents and the urban wastewater system

On-site wastewater treatment with the Pharmafilter reduces the number of quantified ARG present in hospital wastewater discharge from 13 to 4 and the number of quantified antibiotics from 17 to 7. ARG concentrations of the four genes still detectable after treatment are reduced between 0.5 ± 0.9 to > 3.8-fold.

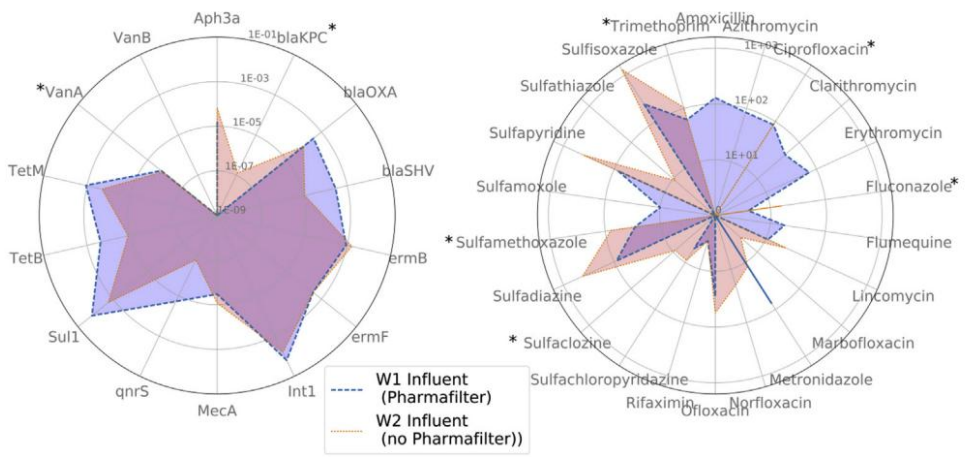


Fig. 6. Antibiotic and ARG Presence and Concentration in UWWTP Influents; left - ARG concentrations in Influents; right – antibiotic concentrations in Influents; * - hospital-related antibiotics or ARGs found in HWW of both location (H1 and H2) at comparable concentrations.

Similarly, relative gene concentrations are reduced for genes detectable after treatment. IntI1 has been identified as a measurement of HGT potential and gene acquisition (Narciso-da-Rocha et al., 2014) and it has been proposed as an indicator of anthropogenic pollution (Gillings et al., 2015). IntI1 was found to show the highest relative concentrations of all genes in all analyzed HWW samples. A study had previously found that high antibiotic concentrations increase intI1 rearrangement, thus increasing the likelihood of HGT (Barraud and Ploy, 2015). The overall discharge of ARGs concentrations, including intI1, from HWW to the communal sewage system is therefore greatly reduced by Pharmafilter treatment, decreasing the potential for HGT events induced by this otherwise high pollution point source for ARGs.

There are indications of a positive impact of the Pharmafilter treatment on the downstream urban wastewater system and, as a consequence, a benefit in terms of downstream environmental pollution. A lower number of genes was detected in influent samples of W1 (receiving treated HWW) than of W2 (receiving untreated HWW). Interestingly, hospital-related genes (not found in CWW) eliminated during Pharmafilter treatment could rarely be detected in W1. blaKPC could not be detected in W1 samples and vanA could only be detected during one of the two sampling rounds (Fig. 6). Both genes were consistently detected in W2 samples (Fig. 6). Similar results could be found for hospital-related antibiotics, which were consistently detected at elevated concentrations in W2, with concentrations up to > 5-fold higher (Fig. 6). Antibiotics only detected in W1 influent (amoxicillin, azithromycin clarithromycin, erythromycin, flumequine and sulfamoxole) were not detected in treated HWW (location 1) and must therefore originate from other sources. Antibiotics detected only in W2 influent (marbofloxacin, norfloxacin and sulfathiazole) were similarly only detected in H2, with the exception of norfloxacin, which was also detected in CWW, albeit at low concentrations.

4. Conclusion

On-site treatment was substantially more efficient in reducing antibiotic and ARG concentrations than UWWTPs. On-site treatment of HWW did also reduce UWWTP influent loads with hospital-related pollutants. IntI1 concentrations were reduced to a considerably larger extent, which could subsequently reduce HGT potential in wastewater.

Combining these findings with elevated levels of antibiotics and ARGs in HWWs (compared to CWW), on-site treatment of HWWs with sequentially aligned advanced treatment technologies is an important step to decrease the risk potential of HWWs and to decrease the impact of wastewater effluents on the environment and subsequently on human health. Alternatively, upgrading existing UWWTPs to include more advanced treatment technologies could mimic the benefits of onsite wastewater treatment of high-risk point sources.

Pharmafilter treatment results in the reduction of pharmaceuticals, including antibiotics, in the treated wastewater. Correlations between antibiotic and ARG concentrations suggest potential interactions between these two factors. This reduction could further decrease HGT events as potential sources of selective pressure are diminished, especially for last-resort antibiotics frequently used in hospitals.

Summarizing it can be said that on-site treatment of high-risk wastewater sources was proven to be highly advantageous in regard to antibiotic and ARG reduction. Legislative guidelines and requirements would be conducive to creating incentives and increasing practical implementation of on-site wastewater treatment.

Please see Appendix 2 for a full list of the sources referenced in this paper.

05 TREATING WASTE & WASTEWATER AT SOURCE

Pharmafilter

Removes pharmaceuticals, bacteria and viruses from hospital generated effluents

Decontamination of general, infectious, healthcare and hospital risk waste onsite

Purifies wastewater for re-use

Treating Waste and Wastewater at Source

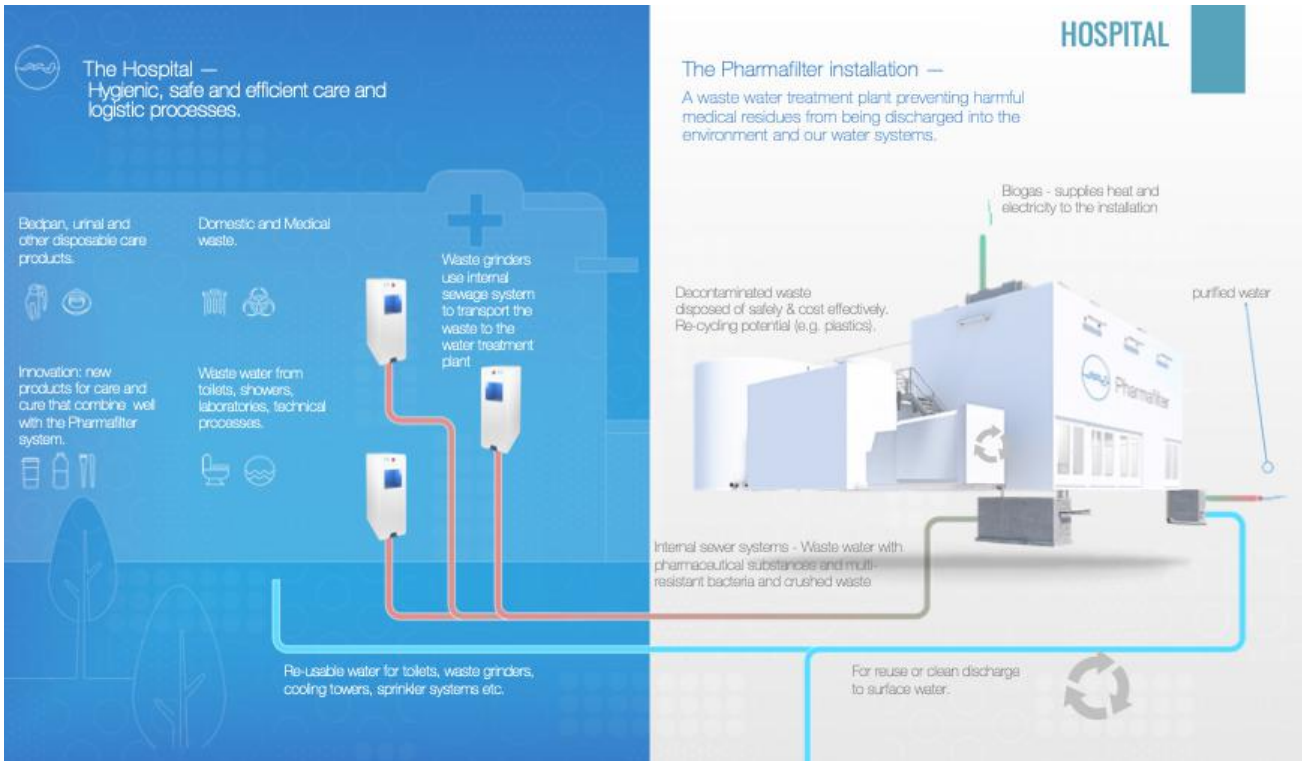
The Pharmafilter system is an on-site solution to hospital-generated wastes and wastewater. The system automates the transport and treatment of wastes that arise on wards (or other nominated areas of the hospital) by introducing intelligent shredders to process solid waste (i.e. risk waste, sharps, lab/pharmacy waste, household refuse, food waste, disposable bed pans, urinals and PPE), and uses the existing sewer network, or a dedicated pipeline, to transport this waste to an onsite treatment plant. Here the solid wastes are separated and decontaminated, and liquid wastes are purified of any micropollutants below a detectable limit.

The resulting output is dry, decontaminated inorganic material (plastics, metals etc) for recycling or repurposing, and purified clean water suitable for reuse as grey water or discharge to public sewer. This is vital as up to 90% of orally administered pharmaceuticals in hospitals are excreted into wastewater as active substances in the faeces and urine of patients [86], [87]. This enables pharmaceuticals and their metabolites to be released into the aquatic ecosystem through hospital effluents. Since pharmaceuticals are, by design, intended to interact with living organisms, even low environmental concentrations are a concern.

While conventional medicines are frequently consumed in the community, more specialised pharmaceutical products, e.g., cytostatic drugs, (restricted) antibiotics, and X-ray contrast agents, are principally administered in hospitals and clinics. In implementing the Pharmafilter system, the partner hospital dramatically reduces its environmental footprint in terms of reduced waste transport (local and international) and carbon emissions, removal of all chemical, pharmaceutical and biological micro-pollutants in hospital wastewater (including AMR - antimicrobial-resistant pathogens) and their subsequent dissemination through public infrastructure into the broader environment.

Decontaminating waste onsite

This is an integrated approach to waste and wastewater that radically improves hygiene, the working environment and therefore outcomes. The highly engineered Pharmafilter shredders (called a "Tonto", pictured below), replace the hospital's bedpan washers or existing paper macerators. Tonto units can also be strategically located in A&E, operating theatres, wards, or any other areas generating waste.



Currently, waste in hospitals is separated into various categories, transported through the hospital and stored in advance of transport off-site for pre-treatment and/or disposal. This activity requires significant staffing hours, physical infrastructure, and recording (sorting rooms, internal and external storage, lifts etc.) The reduction in manual transport of these materials through the hospital and the use of single-use bedpans and urinals result in several benefits to the hospital - not least in relation to patient and staff safety.

Purifying Wastewater

The main engineering component of the system is a small treatment plant, which connects to a hospital's sewerage mains. The plant captures all wastes introduced to the system via the Tonto units, sinks, toilets, showers and drains, and then efficiently decontaminates and sterilises the waste and wastewater, removing all pharmaceuticals, micropollutants, and contaminants.

The plant combines a waste biodigester with an integrated wastewater decontamination/recycling unit. It comprises of 4no primary units and 2no holding tanks for digestion treatment. After the solid and liquid waste has been separated, the wastewater flows through a biological reactor where air is introduced and where bacteria remove the organic substances thoroughly (see Flow Diagram on page 39).

The water is then further purified using ozonisation and filtration through activated carbon. In this way, the values of the classic wastewater purification parameters are reduced to the same levels as in a conventional sewage system. The clean water can then be recycled for reuse, such as flushing toilets or the Tontos. Critically, the removal of these contaminants at the source is far more desirable than decontamination at existing municipal facilities where the associated costs would be prohibitive. The treatment unit is designed to meet the complete decontamination requirements of the specific wastes generated in hospitals. It is a purpose-built system with none of the gaps or potential failures of more general designs or methods. This allows the hospital to eliminate the hazardous element of all waste exported offsite. After this process, the hazardous material is neither transported through the local community nor shipped out of the country, as is currently the case with some healthcare-risk wastes. This particular supply chain (like others) is experiencing structural and legislative changes that create local and international bottlenecks.

Since 2008, Pharmafilter has operated in the Netherlands as a complete, integrated waste and wastewater management solution. In Figures 1 to 4 on the following pages, we present the key outcomes for a partner hospital in terms of the removal of micropollutants, bacteria, viruses and other active compounds.

Pharmafilter Outcomes

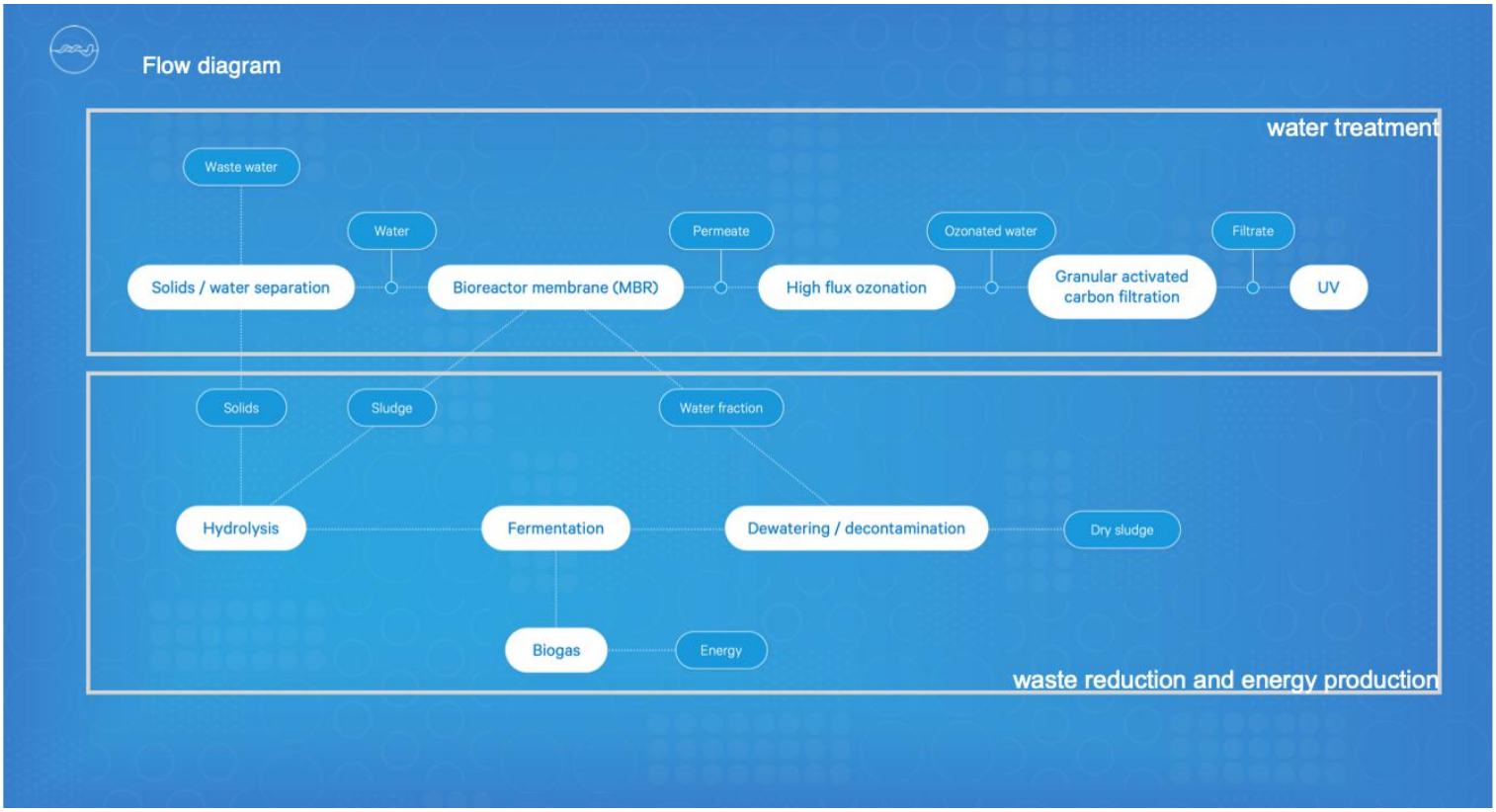


Fig 1: Removal after purification

Paramater	Influent	Removal after MBR, Ozone and Active Coal
COD	1235 mg/l	99.8%
BOD	205 mg/l	99.6%
Total P	32 mg/l	83.8%
Total N	125 mg/l	88.1%
Ammonium as N	37 mg/l	99.8%
Kjeldahl Nitrogen as N	114 mg/l	99.8%

Fig 2: Removal after purification

Paramater	Measured	Ozone	Active Coal
Medicine	94 / 27	99.8%	0
X-Ray Contrast Media	9 / 2-3	99.6%	0
Personal Care Products	11 / 3	83.8%	0
AR-, ER, GR-, PR - Calux	4 / 3-4	88.1%	0
Antibiotic Resistance	Project Interreg	Complete Removal	0

Fig 3: Microbiological parameters

Paramater	Influent	Effluent
Medicine	40,000 - 680,000	0
E. coli	40,000 - 80,000	0
Thermotolerant coliforms 44°C	30,000 - 480,000	0
CFU 36°C	330,000 - 3,100,000	30 - 2,000
CFU 22°C	600,000 - 4,000,000	60 - 1,400
Faecal streptococci	40,000 - 190,000	0
Pseudomonas aeruginosa	500 - 400,000	0
Enterococci	15,500 - 400,000	0
Legionella	< 250 - 500	< 100

Fig 4: Pharmaceutical Paramaters

Paramater	Influent	Effluent
17-a-ethynil	<1.50	<0.50
Aminoantipyrine	<0.05	<0.05
Azithromycin	<0.05	<0.05
Bezafibrate	<0.01	<0.01
Carbamazepine	5	<0.01
Chloramphenicol	<0.04	<0.01
Clarithromycin	60	<0.05
Clofibrate	<0.08	<0.02
Clofarabine	<0.02	<0.01
Cloxacillin	<0.01	<0.01
Caffeine	<1500	<0.05
Cyclophosphamide	22	<0.01
Dapsone	<0.15	<0.05
Diclofenac	10	<0.01
Dicloxacillin	<0.03	<0.01
Erythromycin	<0.15	<0.01
Phenazone	<0.02	<0.01
Fenofibrate	<0.02	<0.01
Fenoprofen	<0.01	<0.01
Fenoterol	<0.10	<0.01
Furazolidone	<0.60	<0.10
Gemfibrozil	92	<0.01
Ibuprofen	14	<0.01
Indomethacin	<0.15	<0.02
Ketoprofen	<0.03	<0.01
Lidocaine	27	<0.01
Lincomycin	<0.02	<0.01
Metoprolol	39	<0.01

Paramater	Influent	Effluent
Monesin	<0.04	<0.01
Nafcillin	<0.03	<0.01
Naproxen	59	<0.02
Oestrone	<0.25	<0.05
Oleandomycin	<0.02	<0.02
Oxacilline	<0.01	<0.01
Pentoxifylline	<0.02	<0.01
Primidone	<0.15	<0.01
Progesterone	<0.08	<0.01
Propranolol	<0.70	<0.01
Roxithromycin	<0.20	<0.01
Sotalol	64	<0.05
Spiramycin	<0.50	<0.05
Sulfapyridine	<0.1	<0.1
Sulfadimethoxine	<0.01	<0.01
Sulfadimidine	<0.05	<0.05
Sulfadimethoxazole	25	<0.01
Sulfaquinoxaline	<0.05	<0.05
Trimethoprim	35	<0.02
Amidotrizoic	3	<0.01
Iohexol	19	<0.01
Iomeprol	<0.01	<0.01
Iopamidol	<0.05	<0.01
Iopanoic acid	<0.01	<0.01
Ioxaglic acid	<0.1	<0.1
Ioxitalamic acid	15	<0.01
AR Calux (ext. lab)	66	<0.31
ER Calux (ext.lab)	69	<0.04

Performance verified in the Netherlands by Eurofins Omegam and system developed in conjunction with STOWA, the Dutch institute for advanced water research. The system, it's operation and performance, has been licensed in Ireland by the Environmental Protection Agency.

Independent Study of Pharmafilter System by SLR Consulting

"Pharmafilter Group Holdings Ltd are in the advanced stages of planning for the installation of a wastewater treatment unit in Dublin. The body who are providing financial support for the installation has requested that a review of the wastewater treatment technology is undertaken by a suitably qualified third party.

The developers of the Pharmafilter System claim that it processes and treats the waste and wastewater produced by a medical facility or hospital. The system incorporates an inward shredder which deals with general waste and clinical waste and is connected to the existing sewerage system. The material is flushed through the existing sewerage system to the Pharmafilter system where the material is treated and processed through several processes including thermophilic digestion.

The Pharmafilter system is designed to:

- Substantially reduce waste and eliminate associated waste management costs;
- Decontaminate and recycle water used by the hospital;
- Process and decontaminate all hospital-originating hazardous substances/materials, with none entering the environment;
- Automate the transport and decontamination of hospital arising waste material;
- Enable cleaner, safer more efficient working practices with the potential to render many parts of existing source segregation waste management procedures obsolete
- Create a platform to develop new approaches for reducing the risk of hospital-acquired infections; and
- Prevent the discharge of anti-microbial, pharmaceuticals and all other healthcare-specific contaminant micro-pollutants and pathogens in hospital wastewater to public infrastructure.

Following a site visit, an initial report was designed to document: Appropriateness of design, size and operational redundancy of both the Tonto and main Pharmafilter system; Review of the system operation and operational track record; Review of outputs (water, emissions and solid waste) including waste classification of same within an Irish legislative context; Review Operation & Maintenance provision and availability of spare parts; and Review of proposed operational oversight & monitoring of the system.

Following acceptance of this initial report, further work will be undertaken to establish: Considerations for deployment in Ireland compared to the Netherlands; Compliance with planning permission for Irish reference hospital an environmental permit submission; Review of Capex, OPEX and lifecycle assumptions; Biogas yield assumption; Considerations for integration with existing hospital sewage system; and EPC and O&M replacement analysis.

Key Findings

The Pharmafilter Treatment system comprises the Tonto shredding units and the Pharmafilter Treatment Plant. The shredding units are readily installed in areas previously occupied by bed pan washing units, operate quietly, and safely and create no nuisance odours. The simple operation of the shredding units means nursing staff spend less time undertaking tasks associated with the disposal of waste and are less likely to come into contact with such waste, therefore reducing the risk of cross-contamination.

The Pharmafilter Treatment Plant is a very well-designed plant which because of its modular construction can easily be extended if necessary. The same modular construction allows for easy replacement of treatment process units and operational and maintenance tasks can be undertaken quickly and safely. The inclusion of the SCADA system enhances the ease of those tasks, as the plant can be monitored from anywhere where an internet connection is available, and in the event of process issues a range of alarms are transmitted to appropriate personnel by the SCADA system. The quality of treated effluent produced by the Pharmafilter Treatment Plant is excellent, particularly concerning its ability to reduce the concentrations of medicinal substances discharged from the hospital: a benefit we feel will be of great interest to the Environmental Protection Agency in the Republic of Ireland. We understand that this treated effluent is typically discharged to sewer, but with the effluent of such quality, there are likely to be options for reusing the effluent as grey water.

SLR's understanding of how the process works suggest that robust design and operating procedures are likely to be in place that limits the likelihood that overflow to the public sewer will be necessary from the plant, however, it should be confirmed that suitable contingency arrangements are in place with sewer operators if overflow to the sewer is necessary.

The fact that this high degree of treatment is obtained without creating noise or odour nuisances, in a plant which can be operated in a very safe manner, is a testament to the design of the Pharmafilter system. The Environmental Protection Agency in the Republic of Ireland apply the same European Union waste characterisation protocols as their colleagues in the Netherlands therefore the same route of disposal for the dry waste material produced by the Pharmafilter Treatment Plant should be appropriate for similar systems installed in Ireland."

07 Key Environmental REGULATION

Pharmafiter's Social Responsibility

The Pharmafilter installation maintains a presence on a hospital site and in the local community for the long term. It is, therefore, proper and in line with good corporate governance that Corporate Social Responsibility be an integral consideration in the context of the systems construction, operation, and interactions with the hospital and local community based on “good neighbour” and responsible corporate citizenship principles.

As a component of the States' building stock, the acute hospital system is a significant consumer of energy and an emitter of contaminated waste and wastewater. Throughout the EU, governments are required to reduce, or if possible remove these emissions and verify the degree to which this has been achieved.

The EU is actively promoting the bio-circular economy and developing incentives for member states to advance their economies in line with these policy objectives. The Pharmafilter system enables healthcare systems to participate in the bio-circular economy and advance their service within these evolving frameworks in a manner that improves all aspects of the system’s operation.

The use of the Pharmafilter system promotes better hygiene, diminishes the risk of hospital-acquired infection and makes the hospital a safer, more pleasant place to be treated, work or visit. It simplifies the management and related administration required by current methods relating to hand hygiene, source segregation of waste and related reporting. In that manner Corporate Social and Environmental data is provided with key, verifiable metrics (in real-time) on which to populate that aspect of the hospital's reporting to the relevant government department, which in turn is empowered to avail of environmental impact reduction data from the acute hospital sector. This is an aspect of corporate governance that is gaining importance in each reporting cycle.

A Pharmafilter installation requires local authority consent, discharge permits from the local water authority and a licence from the EPA. These permissions are required to build maintain and operate the system. Pharmafilter Group Holding’s approach to all stakeholders (statutory, NGO and residential) has always been one of positive engagement. Interested parties, by way of a client offer, are invited to an operational plant (the Netherlands) to assess the obvious concerns of odour, noise, technical effectiveness and local impact.

These visits have consistently allayed those concerns as the system is a dramatic improvement from conventional waste and wastewater treatment that positively impacts patient care, staff and visitor safety while reducing the impact the hospital has on its neighbouring community.

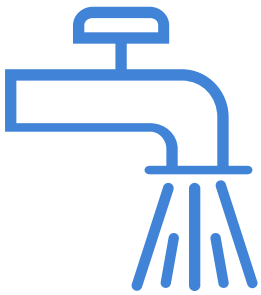
All of these permissions have been acquired by the company for its Irish operations. These will place certain limitations on the degree to which the systems construction and operation may generally impact the locality and the environment (notwithstanding a reduced impact compared to a “do nothing” scenario). Pharmafilter, of course, assigns to upholding the conditions set out in these permissions as a function of the company's standard operating procedures.

In implementing the Pharmafilter system, the hospital collapses its environmental footprint, removes the transport of hazardous waste through the local community (and beyond) and eliminates the discharge of environmentally harmful substances and pathogens into the broader environment. By taking these procedures in-house” the client hospital ensures the treatment required to remove the specific pollutants generated by a hospital is targeted and fit for purpose (which is not the case with municipal waste water treatment plants). Another benefit of the system’s deployment is the reduction in heavy goods traffic by up to 70%, depending on usage. In doing so, the hospital aligns itself more closely with three of the main pillars in EU waste, water and environmental policy, namely;

• *The Proximity Principle* -This requires Member States to treat the waste generated within that state as close to the point of generationasa possible

• *The Self-Sufficiency Principle* - all Member States are expected to be self-sufficient with regards to their waste treatment infrastructure.

• *The EU Green Deal* - within the EU Green Deal the target is to have “Zero Pollution” entering water bodies (the key targets are summarised on the next page). This goes well beyond previously measured parameters to include micropollutants amongst which pharmaceuticals, certain antibiotics and antimicrobial genes are to be targeted for removal. These new measures are to be implemented under a progressive element of the Water Framework Directive which will continue to evolve.



It's important to note that the ability to comply with these frameworks was and is considerably challenged by the SARS-CoV-2 pandemic due to methodology and capacity.

The Pharmafilter system provides the ability to maintain these services even under stressed or emergency circumstances without interruption while maintaining improved hygienic work practices.

In this manner, the client hospital is insulated from future regulatory changes as ALL hospital-specific pollutants are guaranteed to be removed entirely from the hospital's effluent for the duration of the operational contract. Indeed the system's operational and maintenance program allows for the continuous upgrading of the system to the most up-to-date, efficient processes throughout the lifetime of the contract.

Beyond the regulatory imperatives, the release of pharmaceuticals and/or their metabolites into infrastructure is deeply flawed as the receiving plant is not only incapable of removing them but in some cases amplifies their impact.

By design, pharmaceuticals interrupt, disrupt, augment, mutate or destroy biological organisms. Municipal wastewater treatment plants are biological processes containing viruses, (useful) bacteria and enzymes that are available to be affected by mutated (AMR) bacteria, viruses and altered by the presence of pharmaceuticals, even at low loads.

It is therefore not in the interest of healthcare to allow these substances into systems that are not capable of their removal. Over time this diminishes the efficacy of these drugs and is shown to have a significant impact on the environment and human health. The alternative is to develop other drugs that may not be as cost-effective with unknown environmental impacts while maintaining the known impacts due to the relationship between hospital sewage and municipal wastewater treatment plants.

The Pharmafilter system exceeds "mitigation" in terms of these emissions and due to the elimination of these inputs, public health, the taxpayer, the environment and society as a whole benefit. In that context, CSR reporting is greatly enhanced through regulated, demonstrable outcomes.

Improving protection of human health and ecosystems by:

- Controlling 25 new pollutants - pesticides, pharmaceuticals and industrial chemicals including a group of PFAS, the "forever chemicals"
- Reducing the maximum concentration values for several pollutants in surface and groundwater in line with recent science
- Developing a common methodology to measure and monitor microplastics and antimicrobial resistance genes in water

Making application of rules easier by:

- Improving and simplifying data collection on existing and emerging pollutants
- Faster updates of pollutants lists to keep pace with scientific developments

Key Environmental Regulation

The business environment that Pharmafilter operates within is regulated in a manner that is best described as "end to end". Both Pharmafilter's operation and the client hospital's clinical function are conducted under the oversight of governmental departments and/or agencies that ensure these services operate as mandated and users' safety is protected. As the main function of any client hospital is clinical, the operators (clinicians) are required to demonstrate fitness to practice on an ongoing basis. In turn, suppliers are expected/required to support this competency by demonstrating their own. In the case of publicly funded hospitals, there is the added requirement to demonstrate the correct use of public funds and that any risks in the use of those monies are properly mitigated. Whether a supplier of agency staff, equipment/systems or medicines, the supplier is required to demonstrate that they are in full compliance with the relevant overarching standards. This requirement applies to the Pharmafilter system.

Background

The specific requirements for regulation of the Pharmafilter system derive from the Waste Framework Directive 2008/98/EC with due regard to the Water Framework Directive 2000/60/EC. Within these frameworks, subcategories of waste and wastewater are identified and interventions are required based on the classification of that subcategory. In the context of the Pharmafilter system, the critical category relates to the hazardous waste element of the system's normal functioning. That hazardous waste comprises special healthcare risk waste (eg. cytotoxic cancer treatments) and other hazardous chemicals, pharmaceuticals and infectious or potentially infectious healthcare risk waste.

These two documents set out the requirements that EU member states are to observe in terms of the management and improvement of these two categories: waste and water. Member states have adopted these directives into national legislation. This adaptation may result in some local deviation but the legal framework reverts to the European source document. It is national legislation that sets out how activities in these categories are to be regulated locally.

Industrial Emissions Directive License (IED)

Pharmafilter is a unique, patented and successful methodology for the treatment of healthcare risk waste involving the mixing of hazardous hospital waste with other wastes and wastewater that from a legal perspective are classified as non-hazardous; this mixing process creates an additional hazardous waste element.

The Irish EPA has adjudicated the Pharmafiter system and the working method requires that an IED license be applied for and secured in advance of operations as the total volume is deemed to exceed the threshold where less detailed oversight and regulatory permissions would apply (eg; waste permit). Generally speaking, the mixing of waste is discouraged. There are, however, provisions within the Waste Framework Directive (2008/98 EC) which is the legal reference document that allows for such mixing of waste under certain circumstances (where risks to the environment and human health have been accounted for). The threshold at which those circumstances are deemed to have been met is achieved through the licensing dialogue and the "Best Available Techniques" (or BAT) criteria. The Pharmafilter system and the techniques deployed within meet BAT criteria. Moreover, the system eliminates the risks to the environment and human health associated with conventional waste management methods and the discharge of untreated hospital effluent to public infrastructure.

The Pharmafilter system has secured the award of an IED licence from the Irish EPA for its operation in Ireland. This is an important precedent as the license award in Ireland is proven to be in line with EU-wide regulations. There remains close alignment about this specific regulatory background between the EU and the UK and in that context, the UK Environment Agency may wish to refer to the determination of a neighbouring competent authority in its considerations.

The Following waste related processes are authorised (under BAT):

- Blending and mixing of hazardous and non-hazardous waste before further treatment activities
- Wastewater treatment and associated processes
- Activated sludge treatment by membrane bioreactor
- Ozonation
- Active carbon filtration
- Ultraviolet treatment
- Anaerobic Digestion of waste and associated processes including Waste pretreatment and preparation for anaerobic digestion, Digestate Treatment, Biogas combustion in a combined heat and power plant and biogas boiler, Thermal decontamination of solids, Storage of waste outputs of waste treatment,
- General waste handling, transfer and associated processes including Storage of waste pending transfer, Processes for the management and mitigation of environmental emissions.

Key Environmental Regulation

The following waste categories can be deposited in the Tonto Shredders:

Non-Hazardous waste

- 18 01 01 Sharps (except 18 01 03)
- 18 01 07 Chemicals other than those in 18 01 06
- 18 01 09 Medicines (except for those in 18 01 08
- 20 01 08 Biodegradable kitchen and canteen waste
- 20 03 01 Mixed municipal waste
- 20 03 99 Municipal waste not otherwise specified

Hazardous waste

- 18 01 03 Waste collection and disposal is subject to special requirements to prevent infection
- 18 01 08 Cytotoxic and cytostatic medicines
- 18 01 06 Chemicals consisting of/or containing hazardous substances

Health and Safety

The Pharmafilter system enables a working method, which simplifies waste processes, handling and logistics. As a consequence, opportunities for human error or oversight are reduced or in many cases removed. In relation to clinical waste, inappropriate disposal or failure to segregate results not only in that waste being inappropriately treated but also poses a cross-contamination or infection risk moment.

Clinical waste is defined as: ". . . any waste which consists wholly or partly of human or animal tissue, blood or other body fluids, excretions, drugs or other pharmaceutical products, swabs or dressings, syringes, needles or other sharp instruments, being waste which unless rendered safe may prove hazardous to any person coming into contact with it; and...." any other waste arising from medical, nursing, dental, veterinary, pharmaceutical or similar practice, investigation, treatment, care, teaching or research, or the collection of blood for transfusion, being waste which may cause infection to any person coming into contact with it."

It can be divided into two broad categories: (1) any healthcare waste which poses a risk of infection (and therefore by definition possesses the hazardous property H9 Infectious); and (2) certain healthcare wastes which pose a chemical hazard (for example one of H1 to H8, H10 to H15); medicines and medicinally-contaminated waste containing a pharmaceutically-active agent. (NICE Clinical Guidelines 2012).

The Phamafilter system automates the transport of waste from the ward and processing/decontaminating all wastes uniformly introduced to the system. By doing so, human contact with these processes and therefore opportunities for human error are eliminated. Contact with infectious or hazardous wastes and cross-contamination (both inside and outside the hospital) is dramatically reduced. Reducing human contact with these materials also aligns work practices more closely with Health and Safety guidelines, where;

Standard precautions require all healthcare employees to (a) Assume that every person is potentially infected or colonised with an organism that could be transmitted in the healthcare setting, (b) Apply a set of work practices to blood, all bodily fluids except sweat, mucous membranes and non-intact skin. These work practices should include:

- Hand hygiene
- Use of personal protective equipment
- Management of spillages of blood and bodily fluids
- Appropriate patient placement
- Management of sharps
- Safe injection practices
- Respiratory hygiene and cough etiquette
- Management of needle-stick injuries
- Management of waste
- Management of laundry
- Decontamination of reusable medical equipment
- Decontamination of the environment

Moreover EU health and safety legislation sets a hierarchy for exposure control measures (in relation to bioagents in the workplace) to be applied if a risk assessment reveals risks; namely:

- Elimination of the hazard by changing the process or product is at the top of the hierarchy. If elimination is not possible, then the dangerous substances or the process should be substituted with another, non-hazardous or less dangerous one.

- Where the risks to workers are not prevented, control measures should be implemented to remove or reduce the risks to workers' health.

Again, in deploying the Pharmafiter System, hospital management is improving operational safety, efficiency, resilience and future-proofing their organisation from an ever-evolving regulatory landscape while meaningfully supporting the hospital's Environmental, Social and Corporate Governance requirements.

Financials

The Pharmafilter System provides a solution to a critical challenge encountered daily in a modern hospital; that is, the treatment of medical and hazardous waste - which has become even more demanding with increasing volumes due to pandemics, ageing populations and increased healthcare treatment expectations from the Public. In the UK, this has been exacerbated by the existing service contractors in Belgium and China withdrawing from the marketplace. Pharmafilter Group Holdings (PGH) addresses this need with its onsite treatment process.

The opportunity to provide and deploy this sophisticated system on a long-term service contract to hospitals is an influential solution that is cost-neutral to the hospital after taking into account the following benefits:

- Hospital acquires a customised, proven and comprehensive treatment service for their Clinical, Human and Food waste, much of which is termed Healthcare Risk Waste;
- Operational responsibility remains with PGH (to cover operations, component replacement, performance standards and continuity);
- Service is paid from the operational budget on a cost-neutral basis / cost-benefit basis and interest rates are hedged to provide risk management, value and certainty;
- Reduction in waste handling and transport services (also incineration effects, carbon savings, reduced HCRW volumes and transport costs);
- Environmental and economic benefits from reuse of treated water (up to 70% of treated water can be reused generating savings on fresh water in and sewer water costs out) and residual Biogas generated in the process;
- Reduced “Hospital Acquired Infections” due to the reduced Hand Contact moments, avoidance of storage and shorter transport routes for hazardous waste;
- Reduced risk of AMR from the powerful drugs prescribed and present in wastewater and preventing its spread into the Community from treatment on-site; and
- Greater Staff and Patient safety and health benefits.

The Business Case

These various and obvious benefits are generated by the Pharmafilter system and calculated by observing the volumes of Waste and Waste Water generated at each Hospital (on a cubic meter per hour).

A Metric is applied to each particular saving and the sum of these reflects the total potential saving to the Hospital from optimising the use of the PF system.

Specific Savings include;

- Staff productivity increases, savings on Needle Stick injuries, Portering costs; Reduction in HAI at hospital level and AMR in the Community;
- Reduced cost and volumes of HCRW and its transport and incineration costs;
- Reduced Water In and Out costs and reuse of large volumes of treated water;
- Reduced sorting of Medical and Human Waste and damage to hospital infrastructure.

This information is gathered and collated by the Hospital upon which each Business Case is based. The fundamental rationale of the Business Case is to assess the level of Savings available annually to the hospital by installing the PF system, to fix at the outset - the cost of spiralling operating expenses (staff, services, components, interest rates and inflation) at prices set at Financial Close. These are controlled by the PF Service and indexed to the long-term inflation levels and replace the hospital’s current exposure to the vagaries of annual uncontrolled cost increases from the many changing environmental circumstances.

The PF Service then provides the following benefits to the Hospital Client:

- Provides a Cost Effective Waste and Waste Water Treatment system at neutral cost or potential savings of 10-30% pa on current metrics;

- Reduces the cost and volumes of HCRW at the hospital, providing a safer working environment;

- Provides a full Component Life Cycle Analysis for critical elements of the PF Plant and replaces these as needed over time from reserves retained from the Service Fee (MMRA);

- Project Manages the Design, Build, Installation and Commissioning of the PF Plant for the Client and procurement of the supporting Professional and Manufacturer Warranties and Indemnities;

- Takes full Operational and Maintenance responsibility for the PF Plant thus providing continuity (no service interruption), high levels of performance and service value, and peace of mind to Hospital and Clinical management, at a managed increase on current spiralling costs; and funded by MMRA;

- PGFH provides the Capital Funding for the Plant System including review, analysis, construction, installation, operation and performance of the Plant during its Contracted life (25 years);

- After the Service Fee, PF provides both Savings to the hospital's Operating Budget and obviates impact on its Capex Budget - which can be targeted to adding to new Theatres, Buildings, Rooms and Beds and other capital projects;

- The PF asset can also be reflected on the hospital's Balance Sheet and flexible arrangements can be agreed upon to purchase the PF Plant at some future date and at a predefined time when Plant has been updated, value assessed and a transition deal agreed upon.

- It is inherent in such a purchase that to maintain the demanding performance standards, that any such Sale is conditional upon a Revised Operating Contract Agreement for such term as the Plant remains viable.

The Stages of Procuring a Pharmafilter Service

It can take quite some time to procure and secure a Pharmafilter Service for each Hospital Client as it is a complex piece of engineering focused on treating and removing dangerous pathogens from Hospital Waste Streams, and which entity operates in a highly regulated environment.

So Pharmafilter builds the following programme with each Client to secure a Pharmafilter System for its Hospital:

- Prequalifies under a PQQ qualification questionnaire;

- Meets Public Procurement Qualifying Standards and Criteria;

- Applies with Client for an IED (Industrial Emissions License) to the EPA;

- Secures Planning Consent for the PF Plant with the Client Hospital;

- Analyses the Volume metrics being generated at the hospital and its future needs;

- Builds the Business Case and agrees the level of Savings potential from these Volume Metrics;

- Designs a stand alone SPV to house the PF Plant for each Hospital Client and which is 100% owned by Pharmafilter Group Holdings (PFGH);

- Completes the long Term PF/Client Service Contract with PFGH – usually for 25 years;

- Arranges the Funding required to cover Preliminary Costs (of assessment, surveys, professional fees etc.) and Construction and Operating Costs. All fees expended by the Hospital in relation to the Pharmafilter Project can be refunded at Financial Close to ensure that Client is not out of pocket for Project Costs.

The PHARMAFILTER SYSTEM will be provided on an As a Service basis to the Hospital, and funded from the Savings capable of being generated at each site.

Full Turnkey Solution and Lifetime Maintenance Contract.

BUSINESS ALL-STARS PHARMAFILTER GROUP HOLDINGS 2022 ACCREDITED

BUSINESS ALL-STARS aibf ACCREDITED AIBF Global Innovation 2022 PHARMAFILTER SYSTEM

For more information on Pharmafilter please visit: www.pharmafilter.nl/en/

To learn how the Pharmafilter platform contributes to a more efficient hospital and a cleaner environment, please contact us:

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