2021-12-06

# Hospital Effluents and Wastewaters Treatment Plants: A Source of Oxytetracycline and Antimicrobial-resistant Bacteria in Seafood

Bozena McCarthy
*Technological University Dublin*

Samuel Obeng Apori
*Technological University Dublin*

Michelle Giltrap
*Technological University Dublin*, michelle.giltrap@tudublin.ie

*See next page for additional authors*



## Recommended Citation

McCarthy, B.; Apori, S.O.; Giltrap, M.; Bhat, A.; Curtin, J.; Tian, F. Hospital Effluents and Wastewater Treatment Plants: A Source of Oxytetracycline and Antimicrobial-Resistant Bacteria in Seafood. Sustainability 2021, 13, 13967. DOI: 10.3390/su132413967

Authors

Bozena McCarthy, Samuel Obeng Apori, Michelle Giltrap, Abhijnan Bhat, James Curtin, and Furong Tian

*Review*

# Hospital Effluents and Wastewaters Treatment Plants: A Source of Oxytetracycline and Antimicrobial-resistant Bacteria in Seafood

**Bozena McCarthy [1], Samuel Obeng Apori [1], Michelle Giltrap [1,2], Abhijnan Bhat [1,3], James Curtin [1,4] and Furong Tian [1,3,*]**

[1] School of Food Science and Environmental Health, College of Sciences and Health, Technological University Dublin, Dublin 7, Ireland.

[2] Radiation and Environmental Science Centre, FOCAS Research Institute, Kevin Street, D08 NF82, Ireland

[3] Nanolab, FOCAS Research Institute, Kevin Street, D08 NF82, Ireland

[4] College of Engineering and Building Engineering, Technological University Dublin, Dublin 7, Ireland.

* Correspondence: furong.tian@tudublin.ie

**Abstract:** The present study employs a data review on the presence and aggregation of oxytetracycline (OTC) and resistance (AMR) bacteria in wastewater treatment plants (WWTPs), and distribution of the contaminated effluent with the aid of shallow and deep ocean currents. The study aims to determine the fate of OTC, AMR bacteria in seafood, and demonstrate a relationship between AMR levels and human health. This review includes (1) OTC, (2) AMR bacteria, (3) heavy metals in 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. There are 10 locations showing AMR bacteria in treated and untreated hospital effluent. Special effort was made to define the geography distribution of OTC, AMR bacteria, and heavy metals detected in WWTPs to show the likely dissemination in aquatic environment. The presence of OTC in surface waters in Asia, 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) employ effective technologies in urban and industrial wastewater treatment, and (3) select appropriate modelling from Global Ocean Observing System to predict the OTC, heavy metals, and AMR bacteria distribution.

**Keywords:** AMR bacteria, *E. coli*, metals, oxytetracycline, wastewater, seafood, human health.

## 1. Introduction

## 2. Materials and Methods

### 2.1. Search Strategy

The systematic search and review processes were conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement criteria reported by [50]. For the present study, research articles were being searched on Google scholar (https://scholar.google.com/) and Scopus databases (https://www.scopus.com/home.uri) using the following search terms: "AMR and OTC successfully combined with "hospital treated and untreated wastewater" "seafood", "human health", "hospital effluents", "wastewater treatment plant", "seafood contamination". Scopus and scholar google were used for the review due to their largest dynamic reference information base explored for writings incorporating logical diaries, books and gathering procedures [51]. To further ensure that we had assembled a comprehensive list of studies, we asked researchers having the relevant knowledge on the topic to review and suggest additions to the keywords. The search was limited to scientific articles published between 2010 to the current date of conducting the review (2021) and yielded 100 research papers.

The literature search was limited to the following:

- Articles publication years were between 2010 and 2021;
- The keywords AMR and OTC successfully combined with "hospital treated and untreated wastewater" "seafood", "human health", "hospital effluents", "wastewater treatment plant," "seafood contamination in the title and abstract;
- The articles had to be scientific indexed papers only;
- Search was limited to research articles only;

Retrieved articles were imported using Zotero 2.03 and duplicate records were deleted and scrutinized. The results were screened against inclusion criteria i.e. articles that are not relevant to the studies. Full text of papers for all the articles that fitted into the inclusion criteria was retrieved. Articles were being excluded if :

- Published in languages other than English;
- Articles which only an abstract were available;
- Articles that are not related to the studies are also excluded.

### *2.2. Data extraction and reporting*

A standard, purpose-designed form was adopted and modified from Armah et al. (2014) for extraction of the following data from the paper [52]:

- Location, sample size (L), total samples, WWTP effluent, WWTP influent and AMR genes detected;
- Types of tetracycline antibiotic and region of the study;
- Results, including a mean antibiotic concentration in hospital effluents and wastewater treatment plants.

Most of the articles included in this review (>90%) provided measures of central tendency, that is, arithmetic measures and a few of them were usually accompanied by standard deviations (SDs). In this study, AMR *E. coli* data extracted from the papers was limited to cfu/mL values, and favoured over percentage to highlight precisely the bacteria cell count and distribution and construct bar chart (Figure 1). Therefore, only eight publications were related to AMR as a result of hospital WWTPs. Variance in sample size (between 0.05 to 1 L), the total number of samples (1-48), hospital effluent samples (0-24) and WWTP influent samples (between 0-24) were included in analysis at the same unit.

## 3. Results and Discussion

### *3.1. Levels of OTC and AMR bacteria in HWW*

Ten papers reported on research related to tetracyclines in WWTPS, as presented in Table 1. Only two papers shown tetracyclines and heavy metals in WWTPs ($n$=2). The average results of *E. coli* numbers are presented in section 3.2 AMR genes in bacterial genomes ($n$=8). AMR concentrations construct a bar chart is list at below, while the location of tetracyclines, heavy metals, and AMR bacterial are green, yellow, and red on the map, respectively in section 3.6 OTC and AMR distribution globally.

**Table 1.** Environmental concentration of antibiotics of the tetracycline family.

| Antibiotic | Mean antibiotic concentration (ng/L) | AMR bacteria or ARG | Matrix | Region | Reference |
|---|---|---|---|---|---|
| TC | N/D | Resistant *E. coil* 3264 cfu/100 mL | Hospital effluent | West coast, Ireland | [7] |
| TC | 10 | N/D | Hospital effluent | Risle river, Northern France | [27] |
| TC | 1.9 | N/D | WWTP municipal Effluent | Beijing, China | [53] |
| OTC | 3.8 | | | | |
| TC | N/D | *tetM* detected in 100% bacteria in all three locations<br>*tetC* detected in 80% bacteria Helsinki,<br>27% Tallin and 73% Tartu | WWTP effluent | Helsinki, Finland<br>Tallin, Estonia<br>Tartu, Estonia | [56] |
| OTC | $32.0 \times 10^7$ OTC | N/D | PWWTP Influent and effluent | North China | [54] |
| TC | $2.6 \times 10^6$ TC | | | | |
| TIG | N/D | *bla*$_{NDM1}$ | Seepage and tap water | New Deli, India | [35] |
| OTC | N/D | $4.8 \times 10^5$ cfu/100mL in river<br>$4.8 \times 10^6$ cfu/100mL in WWTP<br>*tetM* detected in surface water | River, WWTP and surface water | Coast of North-East South Africa | [55] |
| OTC | 70 – 1340 ng/L | N/D | Surface water | USA | [58] |

| | | | | | |
|---|---|---|---|---|---|
| OTC | up to 340 ng/L | N/D | Surface water | UK | [58] |
| OTC | 71700 ng/L | N/D | Runoff | England | [58] |

N/D – not determined; OTC – oxytetracycline; TC – tetracycline; TIG- tigecycline; WWTP – wastewater treatment plant; PWWTP – pharmaceutical wastewater treatment plant; ARG – antimicrobial resistance genes.

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

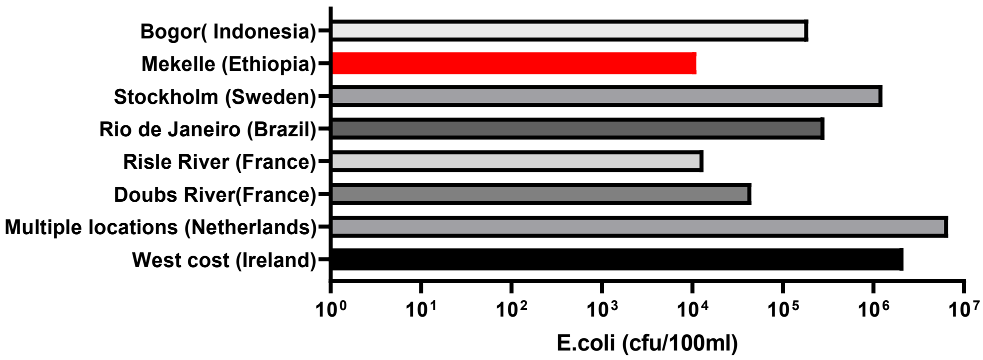


**Figure 1.** AMR *E. coli* levels in treated and untreated hospital effluent in different regions around the world. In black: untreated hospital effluent; in red: treated hospital effluent.

*3.2. AMR genes in bacterial genomes*

A study conducted in Rio de Janeiro (Brazil) found *E. coli* was present along with many other AMR bacteria such as *Pseudomonas* spp., *Enterobacter* and *Klebsiella* in hospital and WWTP treated effluent [68]. Among detected bacteria, resistant *Pseudomonas* spp. has been placed on the World Health Organisation (WHO) list of bacteria for which antibiotics are critically needed [60]. Notably, extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* such as *E. coli*, *Enterobacter* and *Klebsiella* are also a pathogenic bacterium highly resistant to many antibiotics [69].

The selection of eight papers for data mining is outlined in Table 2, AMR *E. coli* levels are clustered to define the most significant region among the eight regions around the world. All of the samples are obtained from WWTPs, which served at least one nearby hospital. Due to the lack of a standardised sampling method, the following limitations are noted. The heterogeneous nature of the sample, a varying number of samples drawn at a different time of the day, month and year are the main variables. All of the studies, except one, present the findings on resistant *E. coli* numbers in untreated hospital effluent. *E. coli* found is resistant to one or more antibiotics. AMR susceptibility testing methods found in the study include antimicrobial disk, Polymer Chain Reaction (PCR), Pulsed-Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST) and Check-Points CT101 microarray. All of the studies except for Stockholm, Sweden have employed Antimicrobial disk. In addition, the antibiotic resistance indicator bacteria (AREB) test has been employed in Stockholm, Sweden. Six papers that exploited additional approaches to gene detection are presented in Table 2; however, one study did not determine sample size.

**Table 2.** Procedures used in *E. coli* detection in hospital effluent and AMR genes detected

| Location | Sample size (L) | Total samples | WWTP Effluent | WWTP influent | AMR genes detected | Reference |
|---|---|---|---|---|---|---|
| West coast, Ireland | 1 | 44 | 17 | 0 | *blaCTX-M (blaCTX-M-28, blaCTX-M-3, blaCTX-M-61, blaCTX-M-15 blaCTX-M-14), blaTEM, blaSHV* | [7] |
| Netherlands (North Sea) | 1 | 5 | 5 | 0 | *blaOXA* | [24] |
| Doubs river, Besancon, Eastern France | N/D | 1 | C | 1 | *blaSHV* | [70] |
| Risle river, Northern France | 1 | 48 [1] | 24 [1] | 24 [1] | *blaTEM* | [27] |
| Rio de Janeiro, Brazil | 1 | 3 | 0 | 8 | *blaCTX-M* | [68] |
| Stockholm, Sweden | 0.05 | 6 | 6 | 0 | *(blaCTX–M group1, blaCTX–M group9, blaCTX–M group2)* | [77] |
| Mekelle, Ethiopia [2] | 0.125 & 0.25 | 20 | 20 | 0 | *blaSHV,* | [76] |
| Bogor, Indonesia | 0.25 | 1 | 0 | 1 | *blaTEM* | [78] |

[1] mean; [2] treated hospital effluent; N/D - not determined

Tetracyclines including OTC, TC and TIG were detected in ten regions. OTC was found in Beijing in China, North China, USA, UK and England. Municipal WWTP treated effluent contained 3.8 ng/L of OTC in China [53]. OTC of 32.0 x $10^7$ ng/L was found in North China in pharmaceutical WWTP (PWWTP) [54]. Findings revealed in South Africa suggested high dissemination of OTC resistance bacteria in the environment [55]. OTC of 4.8 x $10^5$ cfu/100mL resistant bacteria was found in a river near North-East South Africa [55]. WWTP located in the same region of South Africa obtained 4.8 x $10^6$ cfu/100mL of bacteria containing resistant genes. Moreover, t*etM* gene associated with OTC resistance was found throughout river water [55]. Surface water in USA had 70-1340 ng/L of OTC.

Similarly, in UK up to 340 ng/L and 71700 ng/L of OTC was found in surface water and runoff, respectively.

TC has been detected in six locations: Ireland, France, Finland, Estonia, China and North China. Hospital effluent in Irish West coast had 3264 cfu/100 mL of TC resistant *E. coil* [7]. Risle river in Northern France and WWTP municipal effluent in Beijing, China contained 10 and 1.9 ng/L TC, respectively [27, 53]. Significant findings in Helsinki, Finland, Tallin, Estonia and Tartu, Estonia were obtained. WWTP effluent contained *tetM* gene in 100% of bacteria in all four locations. Further, *tetC* gene was detected in 80, 27 and 73 % of bacteria in Helsinki, Tallin and Tartu, respectively [56]. In PWWTP in North China where the OTC was found, a 2.6 x $10^6$ cfu/100 mL of TC was present in the effluent [54]. In New Delhi, India, seepage and tap water contained bacteria with $bla_{NDM1}$ present associated with TIG, a member of the tetracyclines antibiotics family [57]. Borgi and Palma (2014) suggested that OT and doxycycline are found in concentrations below the limit of measuring the effects on bacteria and fish and shellfish. This poses a challenge in detecting tetracyclines in aquatic environments and hospital/municipal wastewaters [58].

20% of *E. coli* isolates from the human intestine, was found to be resistant to TC. However, administration of TC for 10 weeks (500-1000 mg/day) resulted in significantly increased resistance, to 96% [58]. Therefore, it is imperative to establish OTC connection with AMR bacteria and resistant genes.

Coagulase-negative staphylococci (CoNS) including *Staphylococcus epidermis* are commensal bacteria found on human skin. It is understood that *S. epidermis* is usually not associated with high morbidity in humans [59]. However, it can cause pneumonia in premature infants and post-surgery infections in older kids [60]. In Ethiopia, CoNS isolated from hospital effluent were found to be 100% resistant to penicillin and half of the isolates showed tolerance to Cefoxitin. ESBL-producing *S. aureus* was 100% antibiotic tolerant when found in treated effluent. As much as 77% and 33% of *S. aureus* isolates were resilient to penicillin and cefoxitin, respectively. Similarly, 100% resistance to ampicillin was detected in *Klebsiella* spp. and *Citrobacter* spp. [67]. In Turkey, 40.9% of blood infection in geriatric health care setting were caused by ESBL-producing and carbapenem-resistant *Klebsiella* [61].

In addition, vancomycin-resistant CoNS (VRCoNS) and vancomycin-resistant enterococci (VRE) are example of pathogens commonly found throughout hospitals and other healthcare facilities. Infections with high morbidity and mortality are associated with VRCoNS and VRE including skin and wound infection, urinary tract infections (UTI), blood infections, sepsis in infants and meningitis, among others [62].

Little data exist on recent prevalence of VRE and VRCoNS in HWW. This presents a problem in describing the magnitude of dissemination of these pathogens in HWW especially in developing countries. However, in Bahir Dar, Ethiopia, CoNS and VRE were found in patients' blood, urine and wounds, 12% and 34.61%, respectively [62]. In hospital in Iran, 33.4% prevalence of VRE was also found [63].

Hospital effluent was found to contain *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, both carbapenem-resistant, and vancomycin-resistant *Enterococcus faecium.* The levels of ARGs and antimicrobials was higher when compared with other sources [64].

Once reach peak in the bloodstream pharmaceuticals such as antibiotics are excreted by the kidneys in a form of urine. Antimicrobials may also be eliminated by the liver through excretion into bile and finally removed in faeces. However, urine remains the main path of antibiotic excretion [65].

In summary, besides *E. coli* there are other AMR bacteria present in healthcare facilities. These bacteria and ARGs may access HWW by excreted urine, other bodily fluids, and faeces [66, 67].

### *3.3. Bacterial resistance to antimicrobials*

ESBL-producing *E. coli* was found in untreated HWW in eight regions worldwide, with the lowest levels observed in Northern France. The highest numbers of resistant *E. coli* were detected in the Netherlands in the North Sea region (Figure 1). Wastewater pathway (hospital-WWTP-river Risle) was evaluated for resistant *E. coli* presence in Risle river in France. Although resistant *E. coli* levels decreased along the hospital-WWTP-river continuum, amoxicillin, ticarcillin and TC resistant strains remained [27]. Resistant *E. coli* presents in the river even after antibiotics are hydrolysed. River Risle eventually ends up in the English Channel, which serves as a large fishing farm. OTC is one of the most commonly used antibiotics used in fish farms. Moreover, OTC, chlortetracycline (CTC), and TC are difficult to remove in WWTPs [54].

In Rio de Janeiro in Brazil, bacterial population reduced upon high levels of chlorine treatment [68]. However, the number of certain resistant strains was reported higher after the treatment due to development of resistance genes [68, 70]. There are only three out of 127 hospitals in this region which have treated wastewater. Tetracyclines were found in the effluent of WWTPs [68]. It is indeed necessary to develop sufficient wastewater treatment in Brazil.

In Bogor, Indonesia, nearly all *E. coli* was amoxicillin and erythromycin-resistant [78]. Similarly, *E. coli* is resistant to penicillin in an aquatic environment in another location in Indonesia (Sumatra) [81]. Similar result was obtained in New Deli in India, where high levels of ARGs associated with resistance to TC, sulfonamide and β-lactam were detected in all stages in WWTP [57].

In Ethiopia, treated hospital effluent was exceedingly high in resistant *E. coli*. Besides, a study from Eastern Cape, South Africa, concluded that resistant bacteria rates are higher after chlorine treatment [82]. Study in Bangladesh determined tetracyclines resistance in HWW as a contributing factor in multidrug resistance phenomenon [83]. Recently, Chen et al. [71], investigated the transmission of ARGs between bacteria under a variety of light conditions. Their study findings revealed that antibiotic resistant strains *E. coli* DH5alpha and *E. coli* C600 have a stress responses to simulated sunlight and UV irradiation [71, 72]. Many studies have also shown that wastewater treatment with chlorination is ineffective in eradicating resistant bacteria in Ireland, Brazil, France, Poland, Austria, Switzerland, and China [7, 68, 70, 73, 74, 75, 79, 84, 85].

Moreover, a study from Eastern Cape, South Africa, illustrate that resistant bacteria rates are higher after chlorine treatment [82]. There are 19 TC resistant genes from wastewater used for urban agriculture in West and South Africa as reservoirs for antibacterial resistance dissemination. Further, more advanced and effective wastewater treatments methods to remove AMR bacteria are desirable [80, 84].

### *3.4. Heavy metals as AMR genes co-regulators*

Heavy metals are among the substances found to fuel AMR dissemination. These compounds are naturally found in the environment. In addition, urbanized areas and agriculture are a source of high heavy metal levels [86, 87].

Antibiotic resistance may occur through bacteria mutation and horizontal gene transfer (HGT). HGT is a series of processes, including conjugation, transformation and transduction. Conjugation is a transfer of genetic material, usually plasmid which requires two bacterial cells to be close to one another, and it can be intra-species and inter-species events. However, the latter takes place less regularly. The transformation is described as the uptake of unprotected DNA by the recipient bacteria cell. Transduction involves the use of bacteriophage viruses as vesicles to transport genes [87].

HGT may also be promoted by antibiotics and other microbial agents in levels below minimum inhibitory concentration (MIC) [88]. Moreover, heavy metals such as $Cu^{2+}$, $Zn^{2+}$, $Ag^{2+}$ and $Cd^{2+}$ may increase oxidative stress and genotoxicity in bacteria, causing the cell cycle to be disrupted, impairing DNA repair and replication [86]. In addition, heavy metals can cause bacterial mutation and transfer AMR genes through HGT. For example, *E. coli* was found to engage in the conjugative transmission of AMR genes to other genera [87].

Co-selection is a term describing a collection of multiple antibiotic resistance genes where one gene is expressed. Gene resistance to one antibiotic may be responsible for resistance to multiple antimicrobials. Moreover, bacteria may be resistant to antibiotics and heavy metals. Genes participating in this process are located in a mobile genetic element that can be transferred between bacteria. These include structures such as plasmids, transposons and integrons. However, a bacterial plasmid containing a certain number of genes, including AMR was usually the primary genetic structure involved in AMR dissemination across bacterial clusters [86].

Heavy metals affect AMR genes dissemination in the aquatic environments. When $Cu^{2+}$, $Zn^{2+}$ and $Cd^{2+}$ concentrations are above MIC, lower prevalence of conjugative transfers is observed. However, heavy metals are usually present in the natural environment at levels below MIC. Sub-lethal values of Cu, Zn, Cd, Cr, Pb, Ag and Hg involve intracellular ROS generation, increasing cell membrane permeability, inducing oxidative stress and the SOS response, and altering gene expression conjugative transfer [86].

### *3. 5 Connecting OTC, heavy metals and AMR bacteria*

OTC interactions with $Cu^{2+}$, $Zn^{2+}$ and, $Cd^{2+}$ in the aquatic environment were studied in China. OTC binds heavy metals through electron donation, which poses a significant environmental implication. Antibiotic and heavy metal complex exhibited higher toxicity than OTC and heavy metals alone. Moreover, OTC metal complex was associated with higher AMR genes in aquatic environments [89].

It was established that OTC and heavy metals such as $Zn2^{+}$ and $Pb^{2+}$ reside in soil. Due to the continuous movement of the water cycle in the environment, it was suggested that OTC metal complex can be absorbed by the soil and eventually access rivers and coastal waters [90].

Manila clam (Ruditapes philippinarum) was studied to present AMR bacteria and heavy metals in Korea. 42% of *Aeromonas* spp. isolates were resistant to OTC. Moreover, they were identified as a factor in developing *bla*TEM and *qnr*S resistance genes, among many others. Further, $Cu^{2+,}$ $Zn^{2+,}$ $Cd^{2+}$ and $Cr^{2+}$ were also present in *R. philippinarum* clam [91].

The reuse of water causes higher risk in Asian countries [21]. A high probability of AMR transmission exists in hospitals and the highest average relative abundances of *tetX* genes in China's south region [90, 92]. The *tetX* genes resistant to TC came from different sources, including aquaculture and agriculture. The *tet*-resistant gene was necessary to draw an OTC distribution, heavy metal, and AMR E. coli globally to understand the bacteria's fate and impact on seafood and human health [93].

### *3.6 OTC and AMR distribution globally*

Figure 2 represents regions where OTC-resistant *E. coli* and heavy metals were detected. North and South Atlantic currents were also outlined to illustrate how AMR is present in effluent travels around the Atlantic coasts. For example, AMR and ARGs in Rio de Janeiro effluent will arrive at Wester African coast, and eventually, end up in Central and North America, and then even further in Western Europe [94].

River Risle mouth is located at the English Channel, which serves as a large fish farm and trawls fishing location. OTC is one of the most used antibiotics in fish farms. According to the UK Sea and Fisheries Statistic report (2019), 21%, comparable with 31 thousand tons of shellfish, was sourced from English Channel. Fish such as plaice, sole, cod and mackerel are also found sin the Western and Eastern Channel (insert in Figure 2). Fish farms, including salmon in Brittany (France), are located along the Channel coasts [95, 96]. These findings are troubling as tetracyclines in Risle river may access seafood in the Channel and access human microbiota upon ingestion.

Furthermore, it was found that North Sea serves as a large fishing location for the UK population. Species of herring, sole, cod, haddock and mackerel are sourced in the North Sea region. Tetracyclines resistance was detected in Denmark in farmed fish and river fish in 29% and 6%, respectively [97]. Therefore, OTC released near North Sea Coasts may enter seafood and pose a risk to human health. Plaice and cod sourced from Eastern English Channel and French coastal shallow waters were tested for heavy metals. In cod cadmium, copper, manganese, and lead mean values (from three different locations) were found at 0.07, 9.06, 11.1 and 0.15 μg/g, respectively. In the dry weight of cod muscles, cadmium, copper, manganese, and lead were found to be 0.008, 1.23, 1.35 and 0.027 μg/g, respectively [98].

Similarly, in the dry weight of plaice liver, cadmium, copper, manganese and lead were detected at 0.26, 10.8, 5.7 and 0.24 μg/g, respectively. In the muscle 0.02, 1.425, 1.29 and 0.05 μg/g of Cd, Cu, Mn and Pb were observed, respectively. Comparing the above results with a study conducted in 1989, lead and cadmium values detected in the 2004 study were lower [98]. However, as previously mentioned in the present study, low concentrations of heavy metals contribute to AMR dissemination in the aquatic environment.

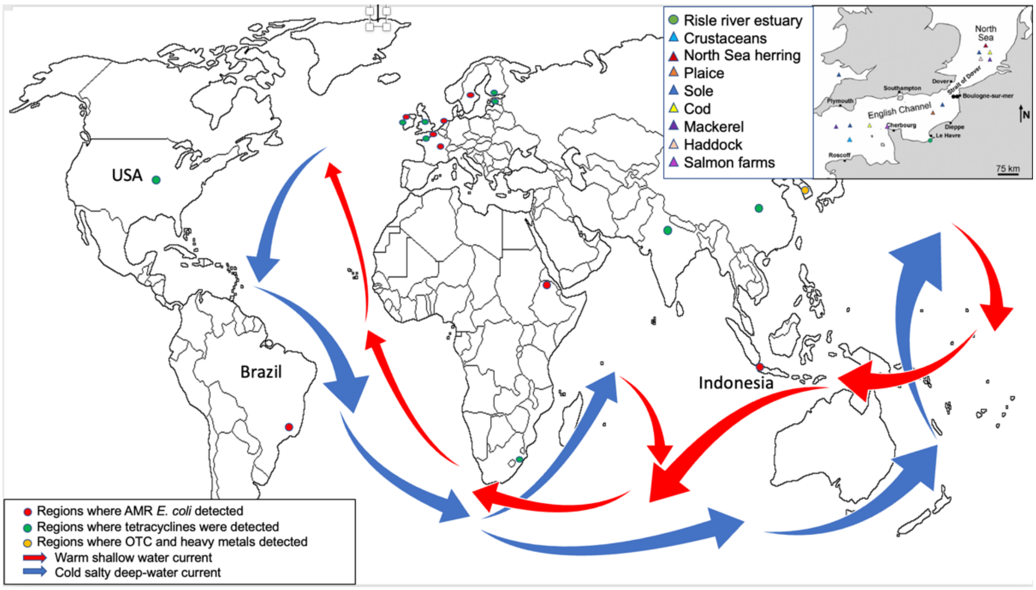


**Figure 2.** Illustration of regions where AMR *E. coli*, tetracyclines, and OTC and heavy metals distribution around the world, and a map of North and South Atlantic currents contributing to AMR spread. Insert shows the English Channel.

According to Reverter et al. (2020), warmer months may have influenced bacteria to replicate more efficiently than cold weather. Additionally, many variables such as global warming may have an impact on the AMR bacteria concentration [99]. Finally, tropical climate in Africa, Asia, and America continent may have impacted the AMR concentration and colder European countries. Ocean models play a large role in understanding the ocean's influence on weather and climate. Global Ocean Observing System and Integrated Ocean Observing System provide efficacious modelling methods. The optimise selection of modelling from the open accessed Observing System to fit each ocean geography will open a new chapter to predicting OCT, heavy metal and AMR distribution.

## 4. 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 accelerated spread of bacterial resistance in the aquatic environment. Present study highlights the importance of further research needed into the fate of

AMR bacteria in the aquaculture and aquatic environments, and its effects on human health.

The OTC and ESBL-producing *E. coli* was studied in untreated HWW in 18 regions around the world. The OTC were detected in WWTPs in Asia, USA and Europe. The lowest levels of AMR *E. coli* observed in Northern France. The highest numbers of resistant *E. coli* were detected in 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 have an effect on development of AMR genes. The OTC from Asia, USA and Europe has potation to impact AMR bacterial and seafood globally due to continuous water mass movements assisted by ocean currents.

Our 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 are: 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, we suggest an appropriate modelling from Global Ocean Observing System to predict the OTC, heavy metals and AMR bacteria distribution.

**Author Contributions:** Conceptualization, B.M., M.G. and F.T.; methodology, B.M. and M.G.; formal analysis, B.M. and M.G..; investigation, F.T.; resources, M.G. and F.T.; writing—original draft preparation, B.M., M.G. and F.T.; writing—review and editing, M.G.; B.M.; and A.B.; visualization, B.M.; F.T.; and A.B.; supervision, M.G.; and F.T. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest**.**

## References

1. Orias, F.; Perrodin, Y. Characterisation Of The Ecotoxicity Of Hospital Effluents: A Review. *Sci. Total Environ.* **2013**, *454-455*, 250-276. doi.org/10.1016/j.scitotenv.2013.02.064
2. Weissbrodt, D.; Kovalova, L.; Ort, C.; Pazhepurackel, V.; Moser, R.; Hollender, J.; Siegrist, H.; McArdell, C. Mass Flows of X-Ray Contrast Media and Cytostatics In Hospital Wastewater. *Environ. Sci. Technol.* **2009**, *43*, 4810-7. 10.1021/es8036725
3. Emmanuel, E.; Keck, G.; Blanchard, J.; Vermande, P.; Perrodin, Y. Toxicological Effects of Disinfections Using Sodium Hypochlorite on Aquatic Organisms and Its Contribution To AOX Formation In Hospital Wastewater. *Environ. Int.* **2004**, *30*, 891-900. 10.1016/j.envint.2004.02.004
4. Okoh, A.I.; Odjadjare, E.E.; Igbinosa, E.O.; Osode, A.N. Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. *Afr. J. Biotechnol.* **2007**, *6*, 2932-44. 10.5897/AJB2007.000-2462
5. Langford, K.H.; Thomas, K.V. Determination Of Pharmaceutical Compounds In Hospital Effluents And Their Contribution To Wastewater Treatment Works. *Environ. Int.* **2009**, *35*, 766-770. DOI: 10.1016/j.envint.2009.02.007
6. Verlicchi, P.; Galletti, A.; Petrovic, M.; Barceló, D. Hospital Effluents as A Source of Emerging Pollutants: An Overview of Micropollutants And Sustainable Treatment Options. *J. Hydrol.* **2010**, *389*, 416-428. doi.org/10.1016/j.jhydrol.2010.06.005

7. Galvin, S.; Boyle, F.; Hickey, P.; Vellinga, A.; Morris, D.; Cormican, M. Enumeration and Characterization of Antimicrobial-Resistant Escherichia Coli Bacteria in Effluent from Municipal, Hospital, And Secondary Treatment Facility Sources. *Appl. Environ. Microbiol.* **2010**, *76*, 4772-9. DOI**:** 10.1128/AEM.02898-09
8. Angulo, F.J.; Nargund, V.N.; Chiller, T.C. Evidence of An Association Between Use of Anti-Microbial Agents in Food Animals and Anti-Microbial Resistance Among Bacteria Isolated from Humans and The Human Health Consequences of Such Resistance. *J. Vet. Med. Series B* **2004**, *51*, 374-379. 10.1111/j.1439-0450.2004.00789.x
9. Antimicrobial Resistance. Fact Sheet 194. World Health Organization. Available online: http://www.who.int/mediacentre/factsheets/fs194/en/ (accessed on 31 July 2021).
10. The Medical Impact of the Use of Antimicrobials in Food Animals. Report of a WHO Meeting. Berlin, Germany, 13-17 October 1997. Available on: http://whqlibdoc.who.int/hq/1997/WHO_EMC_ZOO_97.4.pdf (accessed 31 July 2021).
11. de Kraker, M.E..; Stewardson, A.J.; Harbarth, S. Will 10 Million People Die A Year Due To Antimicrobial Resistance By 2050?. *PLoS Med.* **2016**, *13*. doi.org/10.1371/journal.pmed.1002184
12. Ekwanzala, M.D.; Dewar, J.B.; Kamika, I.; Momba, M.N. Tracking The Environmental Dissemination Of Carbapenem-Resistant Klebsiella Pneumoniae Using Whole Genome Sequencing. *Sci. Total Environ.* **2019**, *691*, 80-92. doi.org/10.1016/j.scitotenv.2019.06.533
13. Rose, W.E.; Rybak, M.J. Tigecycline: First of a New Class of Antimicrobial Agents. Pharmacotherapy **2006**, *26*, 1099–110. doi: 10.1592/phco.26.8.1099.
14. Mihciokur, H.; Oguz, M. Removal Of Oxytetracycline And Determining Its Biosorption Properties On Aerobic Granular Sludge. *Environ. Toxicol. Pharmacol.* **2016**, *46*, 174-182. 10.1016/j.etap.2016.07.017
15. Ayandiran, T.O.; Falgenhauer, L.; Schmiedel, J.; Chakraborty, T.; Ayeni, F.A. High Resistance To Tetracycline And Ciprofloxacin In Bacteria Isolated From Poultry Farms In Ibadan, Nigeria. *J. Infect. Dev. Ctries.* **2018**, *12*. 10.3855/jidc.9862
16. Economou, V.; Gousia, P. Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infec. Drug Resist.* **2015**, *8*, 49-61. DOI: 10.2147/IDR.S55778
17. Bogomolni, A.L.; Gast, R.J.; Ellis, J.C.; Dennett, M.; Pugliares, K.R.; Lentell, B.J.; Moore, M.J. Victims Or Vectors: A Survey Of Marine Vertebrate Zoonoses From Coastal Waters Of The Northwest Atlantic. *Dis. Aquat. Organ.* **2008**, *81*, 13-38. doi.org/10.3354/dao01936
18. Grevskott, D.H.; Svanevik, C.S.; Sunde, M.; Wester, A.L.; Lunestad, B.T. Marine Bivalve Mollusks As Possible Indicators Of Multidrug-Resistant Escherichia Coli And Other Species Of The Enterobacteriaceae Family. *Front. Microbiol.* **2017**, *8*. doi.org/10.3389/fmicb.2017.00024
19. Moller, T.S.; Overgaard, M.; Nielsen, S.S.; Bortolaia, V.; Sommer, M.O.; Guardabassi, L.; Olsen, J.E. Relation Between Tetr And Teta Expression in Tetracycline Resistant *Escherichia coli*. *BMC Microbiol.* **2016**, *16*. doi.org/10.1186/s12866-016-0649-z
20. D'Accolti, M.; Soffritti, I.; Mazzacane, S.; Caselli, E. Fighting AMR In The Healthcare Environment: Microbiome-Based Sanitation Approaches And Monitoring Tools. *Int. J. of Mol. Sci.* **2019**, *20,* 1535. doi.org/10.3390/ijms20071535
21. Girijan, S.K; Paul, R.; V.J., R.K.; Pillai, D. Investigating The Impact Of Hospital Antibiotic Usage On Aquatic Environment And Aquaculture Systems: A Molecular Study Of Quinolone Resistance In Escherichia Coli. *Sci. Total Environ.* **2020**, *748*, 141538. doi.org/10.1016/j.scitotenv.2020.141538
22. Jamborova, I.; Janecko, N.; Halova, D.; Sedmik, J.; Mezerova, K.; Papousek, I.; Kutilova, I.; Dolejska, M.; Cizek, A.; Literak, I. Molecular characterization of plasmid-mediated AmpC beta-lactamase- and extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae among corvids (Corvus brachyrhynchos and Corvus corax) roosting in Canada. *FEMS Microbiol. Ecol.* **2018**, *94*. 10.1093/femsec/fiy166
23. Lamba, M.; Graham, D.W.; Ahammad, S.Z. Hospital Wastewater Releases Of Carbapenem-Resistance Pathogens And Genes In Urban India. *Environ. Sci. Technol.* **2017**, *51*, 13906-13912. 10.1021/acs.est.7b03380

24. Blaak, H.; Lynch, G.; Italiaander, R.; Hamidjaja, R.A.; Schets, F.M.; de Roda Husman, A.M. Multidrug-Resistant And Extended Spectrum Beta-Lactamase-Producing Escherichia Coli In Dutch Surface Water And Wastewater. *PLoS ONE* **2015**, *10*. doi.org/10.1371/journal.pone.0127752
25. Agwuh, K.N.; MacGowan, A. Pharmacokinetics and Pharmacodynamics of The Tetracyclines Including Glycylcyclines. *J. Antimicrob. Chemother.* **2006**, *58*, 256-265. 10.1093/jac/dkl224
26. Rang, H.; Ritter, J.; Flower,R.; Henderson, G. *Rang&Dale's Pharmacology*, 8th ed.; Elsevier Churchill Livingstone: London, UK, 2016; p.619.
27. Oberlé, K.; Capdeville, M.J.; Berthe, T.; Budzinski, H.; Petit, F. Evidence For A Complex Relationship Between Antibiotics And Antibiotic-Resistantescherichia Coli: From Medical Center Patients To A Receiving Environment. *Environ. Sci. Technol*. **2012**, *46*, 1859-1868. doi.org/10.1021/es203399h
28. Chen, X.; Wang, J. Degradation Of Norfloxacin In Aqueous Solution By Ionizing Irradiation: Kinetics, Pathway And Biological Toxicity. *Chem. Eng. J.* **2020**, *395*, 125095. doi.org/10.1016/j.cej.2020.125095
29. Wang, J.; Zhuan, R. Degradation of antibiotics by advanced oxidation processes: An overview. *Sci. Total Environ.* **2020**, 701, 135023 doi.org/10. 1016/j.scitotenv.2019.135023
30. Paucar, N.; Kim, I.; Tanaka, H.; Sato, C. Ozone Treatment Process For The Removal Of Pharmaceuticals And Personal Care Products In Wastewater. *Ozone: Sci. Eng.* **2018**, *41*, 3-16.
31. Oh, J.; Salcedo, D.; Medriano, C.; Kim, S. Comparison Of Different Disinfection Processes In The Effective Removal Of Antibiotic-Resistant Bacteria And Genes. *J. Environ. Sci.* **2014**, *26*, 1238-1242. doi.org/10.1016/S1001-0742(13)60594-X
32. Chen, J.; Deng, W.; Liu, Y.; Hu, L.; He, L.; Zhao, J.; Wang, T.; Ying, G. Fate And Removal Of Antibiotics And Antibiotic Resistance Genes In Hybrid Constructed Wetlands. *Environ. Pollut.* **2019**, *249*, 894-903. doi.org/10.1016/j.envpol.2019.03.111
33. Furukawa, T.; Jikumaru, A.; Ueno, T.; Sei, K. Inactivation Effect Of Antibiotic-Resistant Gene Using Chlorine Disinfection. *Water* **2017**, *9*, 547. https://doi.org/10.3390/w9070547
34. Jia, S.; Wu, J.; Ye, L.; Zhao, F.; Li, T.; Zhang, X. Metagenomic Assembly Provides A Deep Insight Into The Antibiotic Resistome Alteration Induced By Drinking Water Chlorination And Its Correlations With Bacterial Host Changes. *J. Hazard. Mater.* **2019**, *379*, 120841. doi.org/10.1016/j.jhazmat.2019.120841
35. Destiani, R.; Templeton, M. Chlorination And Ultraviolet Disinfection Of Antibiotic-Resistant Bacteria And Antibiotic Resistance Genes In Drinking Water. *AIMS Environ. Sci.* **2019**, *6*, 222-241. 10.3934/environsci.2019.3.222
36. Majumder, A.; Gupta, A.; Ghosal, P.; Varma, M. A Review On Hospital Wastewater Treatment: A Special Emphasis On Occurrence And Removal Of Pharmaceutically Active Compounds, Resistant Microorganisms, And SARS-Cov-2. *J. Environ. Chem. Eng.* **2021**, *9*, 104812. doi.org/10.1016/j.jece.2020.104812
37. Kosma, C.; Lambropoulou, D.; Albanis, T. Occurrence And Removal Of Ppcps In Municipal And Hospital Wastewaters In Greece. *J. Hazard. Mater.* **2010**, *179*, 804-817. 10.1016/j.jhazmat.2010.03.075
38. Yuan, S.; Jiang, X.; Xia, X.; Zhang, H.; Zheng, S. Detection, Occurrence And Fate Of 22 Psychiatric Pharmaceuticals In Psychiatric Hospital And Municipal Wastewater Treatment Plants In Beijing, China. *Chemosphere* **2013**, *90*, 2520-2525. doi.org/10.1016/j.chemosphere.2012.10.089
39. Kappell, A.; Kimbell, L.; Seib, M.; Carey, D.; Choi, M.; Kalayil, T.; Fujimoto, M.; Zitomer, D.; McNamara, P. Removal Of Antibiotic Resistance Genes In An Anaerobic Membrane Bioreactor Treating Primary Clarifier Effluent At 20 °C. *Environ. Sci. Water Res. Technol.* **2018**, *4*, 1783-1793. 10.1039/C8EW00270C
40. Cheng, H.; Hong, P. Removal Of Antibiotic-Resistant Bacteria And Antibiotic Resistance Genes Affected By Varying Degrees Of Fouling On Anaerobic Microfiltration Membranes. *Environ. Sci. Technol.* **2017**, *51*, 12200-12209. doi.org/10.1021/acs.est.7b03798

41. Sui, Q.; Jiang, C.; Zhang, J.; Yu, D.; Chen, M.; Wang, Y.; Wei, Y. Does The Biological Treatment Or Membrane Separation Reduce The Antibiotic Resistance Genes From Swine Wastewater Through A Sequencing-Batch Membrane Bioreactor Treatment Process. *Environ. Int.* **2018**, *118*, 274-281. doi.org/10.1016/j.envint.2018.06.008
42. Bijlsma, L.; Pitarch, E.; Fonseca, E.; Ibanez, M.; Botero, A.; Claros, J.; Pastor, L.; Hernandez, F. Investigation Of Pharmaceuticals In A Conventional Wastewater Treatment Plant: Removal Efficiency, Seasonal Variation And Impact Of A Nearby Hospital. *J. Envin. Chem. Eng.* **2021**, *9*, 105548. doi.org/10.1016/j.jece.2021.105548
43. Yao, S.; Ye, J.; Yang, Q.; Hu, Y.; Zhang, T.; Jiang, L.; Munezero, S.; Lin, K.; Cui, C. Occurrence And Removal Of Antibiotics, Antibiotic Resistance Genes, And Bacterial Communities In Hospital Wastewater. *Environ. Sci. Pollut. Res.* **2021**, *28*, 57321-57333. doi.org/10.1007/s11356-021-14735-3
44. Herraiz-Carboné, M.; Cotillas, S.; Lacasa, E.; Sainz de Baranda, C.; Riquelme, E.; Cañizares, P.; Rodrigo, M.; Sáez, C. A Review On Disinfection Technologies For Controlling The Antibiotic Resistance Spread. *Sci. Total Environ.* **2021**, *797*, 149150. doi.org/10.1016/j.scitotenv.2021.149150
45. Grehs, B.; Lopes, A.; Moreira, N.; Fernandes, T.; Linton, M.; Silva, A.; Manaia, C.; Carissimi, E.; Nunes, O. Removal Of Microorganisms And Antibiotic Resistance Genes From Treated Urban Wastewater: A Comparison Between Aluminium Sulphate And Tannin Coagulants. *Water Res.* **2019**, *166*, 115056. doi.org/10.1016/j.watres.2019.115056
46. Mathur, P.; Sanyal, D.; Callahan, D.; Conlan, X.; Pfeffer, F. Treatment Technologies To Mitigate The Harmful Effects Of Recalcitrant Fluoroquinolone Antibiotics On The Environ- Ment And Human Health. *Environ. Pollut.* **2021**, *291*, 118233. doi.org/10.1016/j.envpol.2021.118233
47. Fu, Y.; Wang, F.; Sheng, H.; Xu, M.; Liang, Y.; Bian, Y.; Hashsham, S.; Jiang, X.; Tiedje, J. Enhanced Antibacterial Activity Of Magnetic Biochar Conjugated Quaternary Phosphonium Salt. *Carbon* **2020**, *163*, 360-369. doi.org/10.1016/j.carbon.2020.03.010
48. Umar, M.; Roddick, F.; Fan, L. Moving From The Traditional Paradigm Of Pathogen Inactivation To Controlling Antibiotic Resistance In Water - Role Of Ultraviolet Irradiation. *Sci. Total Environ.* **2019**, *662*, 923-939. doi.org/10.1016/j.scitotenv.2019.01.289
49. Wang, L.; Qiu, S.; Guo, J.; Ge, S. Light Irradiation Enables Rapid Start-Up Of Nitritation Through Suppressing Nxrb Gene Expression And Stimulating Ammonia-Oxidizing Bacteria. *Environ. Sci. Technol.* **2021**. doi.org/10.1021/acs.est.1c04174
50. Liberati, A.; Altman, D.J.; Tetzlaff, J.; Mulrow, C.; Gotzsche, P.C.; Ioannidis, J.P.; Clarke, M.; Devereaux, P.J.; Kleijnen, J.; Moher, D. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Healthcare Interventions: Explanation and Elaboration. *BMJ* **2009**, *339*, b2700. doi.org/10.1136/bmj.b2700
51. Al-Mutairi, K.A.; Yap, C.K. A Review of Heavy Metals in Coastal Surface Sediments from The Red Sea: Health-Ecological Risk Assessments. *Int. J. Environ. Res. Public Health* **2021**, *18*, 2798. 10.3390/ijerph18062798
52. Armah, F.A.; Quansah, R.; Luginaah, I. A Systematic Review of Heavy Metals of Anthropogenic Origin in Environmental Media and Biota in The Context of Gold Mining in Ghana. *Int. Sch. Res. Notices* **2014**, *2014*. 10.1155/2014/252148
53. Jia, A.; Xiao, Y.; Hu, J.; Asami, M.; Kunikane, S. Simultaneous Determination Of Tetracyclines And Their Degradation Products In Environmental Waters By Liquid Chromatography–Electrospray Tandem Mass Spectrometry. *J. Chromatogr. A* **2009**, *1216*, 4655-62. 10.1016/j.chroma.2009.03.073
54. Hou, J.; Wang, C.; Mao, D.; Luo, Y. The Occurrence And Fate Of Tetracyclines In Two Pharmaceutical Wastewater Treatment Plants Of Northern China. *Environ. Sci. Pollut. Res.* **2015**, *23*. doi.org/10.1007/s11356-015-5431-5
55. Suzuki, S.; Ogo, M.; Koike, T.; Takada, H.; Newman, B. Sulfonamide And Tetracycline Resistance Genes In Total- And Culturable-Bacterial Assemblages In South African Aquatic Environments. *Front. Microbiol.* **2015**, *6*, 796. 10.3389/fmicb.2015.00796
56. Laht, M.; Karkman, A.; Voolaid, V.; Ritz, C.; Tenson, T.; Virta, M.; Kisand, V. Abundances Of Tetracycline, Sulphonamide And Beta-Lactam Antibiotic Resistance Genes In Conventional Wastewater Treatment Plants (Wwtps) With Different Waste Load. *PLoS ONE* **2014**, *9*. doi: 10.1371/journal.pone.0103705

57. Walsh, T.R.; Weeks, J.; Livermore, D.M.; Toleman, M.A. Dissemination Of NDM-1 Positive Bacteria In The New Delhi Environment And Its Implications For Human Health: An Environmental Point Prevalence Study. *Lancet Infect. Dis.* **2011**, *11*,355-362. doi.org/10.1016/S1473-3099(11)70059-7
58. Borghi, A.A.; Palma, M.S. Tetracycline: Production, Waste Treatment And Environmental Impact Assessment. *Braz. J. Pharm. Sci.* **2014**, *50*. doi.org/10.1590/S1984-82502011000100003
59. Otto, M. Staphylococcus Epidermidis — The 'Accidental' Pathogen. *Nat. Rev. Microbiol.* **2009**, *7*, 555-567. 10.1038/nrmicro2182
60. Toltzis, P. 50 Years Ago In The Journal Of Pediatrics. *J. Pediatr.* **2018**, *197*, 56. doi.org/10.1016/j.jpeds.2017.11.052
61. Avcu, G.; Atıkan, B. Healthcare-Associated Infections At A Tertiary Level Pediatric Intensive Care Unit From Turkey. *J. Pediatr. Res.* **2021**, *8*, 246-250. 10.4274/jpr.galenos.2020.67299
62. Ashagrie, D.; Genet, C.; Abera, B. Vancomycin-Resistant Enterococci And Coagulase-Negative Staphylococci Prevalence Among Patients Attending At Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Ethiopia. *PLoS ONE* **2021**, *16*, e0249823. doi.org/10.1371/journal.pone.0249823
63. Hasanpour, F.; Neyestani, Z.; Arzanlou, M.; Moradi-Asl, E.; Sahebkar, A.; Khademi, F. Vancomycin-Resistant Enterococci In Iran: A Systematic Review And Meta-Analysis Of Non-Clinical Studies. *Gene Rep.* **2021**, *24*, 101265. doi.org/10.1016/j.genrep.2021.101265
64. Verlicchi, P. Trends, New Insights And Perspectives In The Treatment Of Hospital Effluents. *Curr. Opin. Environ. Sci. Health.* **2021**, *19*, 100217. doi.org/10.1016/j.coesh.2020.10.005
65. Kuriyama, T.; Karasawa, T.; Williams, D. Antimicrobial Chemotherapy. *Biofilms in Infection Prevention and Control* **2014**, 209-244. doi.org/10.1016/B978-0-12-397043-5.00013-X
66. Browne, H.; Neville, B.; Forster, S.; Lawley, T. Transmission Of The Gut Microbiota: Spreading Of Health. *Nat. Rev. Microbiol.* **2017**, *15*, 531-543. 10.1038/nrmicro.2017.50
67. Herraiz-Carboné, M.; Cotillas, S.; Lacasa, E.; Sainz de Baranda, C.; Riquelme, E.; Cañizares, P.; Rodrigo, M.; Sáez, C. Are We Correctly Targeting The Research On Disinfection Of Antibiotic-Resistant Bacteria (ARB)?. *J. Clean. Prod.* **2021**, *320*, 128865. doi.org/10.1016/j.jclepro.2021.128865
68. Chagas, T.P.; Seki, L.M.; Cury, J.C.; Oliveira, J.A.; Dávila, A.M.; Silva, D.M.; Asensi, M.D. Multiresistance, Beta-Lactamase-Encoding Genes And Bacterial Diversity In Hospital Wastewater In Rio De Janeiro, Brazil. *J. Appl. Microbiol.* **2011**, *111*, 572-581. doi.org/10.1111/j.1365-2672.2011.05072.x
69. WHO publishes list of bacteria for which new antibiotics are urgently needed. Available online: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (accessed on 26 May 2021).
70. Bréchet, C.; Plantin, J.; Sauget, M.; Thouverez, M.; Talon, D.; Cholley, P.; Guyeux, C.; Hocquet, D.; Bertrand, X. Wastewater Treatment Plants Release Large Amounts Of Extended-Spectrum B-Lactamase–Producing Escherichia Coli Into The Environment. *Clin. Infect. Dis.* **2014**, *58*, 1658-1665. doi.org/10.1093/cid/ciu190
71. Chen, X.; Yin, H.; Li, G.; Wang, W.; Wong, P.; Zhao, H.; An, T. Antibiotic-Resistance Gene Transfer In Antibiotic-Resistance Bacteria Under Different Light Irradiation: Implications From Oxidative Stress And Gene Expression. *Water Res.* **2019**, *149*, 282-291. doi.org/10.1016/j.watres.2018.11.019
72. Watts, J.; Schreier, H.; Lanska, L.; Hale, M. The Rising Tide Of Antimicrobial Resistance In Aquaculture: Sources, Sinks And Solutions. *Mar. Drugs* **2017**, *15*, 158. doi.org/10.3390/md15060158
73. Ng, C.; Gin, K.Y. Monitoring Antimicrobial Resistance Dissemination in Aquatic Systems. *Water* **2019**, *11*, 71. doi:10.3390/w11010071
74. Korzeniewska, E.; Korzeniewska, A.; Harnisz, M. Antibiotic resistant Escherichia coli in hospital and municipal sewage and their emission to the environment. *Ecotoxicol. Environ. Saf.* **2013**, *91*, 96-102. doi.org/10.1016/j.ecoenv.2013.01.014
75. Reinthaler, F.F.; Posch, J.; Feierl, G.; Wust, G.; Haas, D.; Ruckenbauer, G.; Mascher, F.; Marth, E. Antibiotic resistance of E. coli in sewage and sludge. *Water Res.* **2003**, 37, 1685-1690. doi.org/10.1016/S0043-1354(02)00569-9

76. Asfaw, T.; Negash, L.; Kahsay, A.; Weldu, Y. Antibiotic Resistant Bacteria From Treated And Untreated Hospital Wastewater At Ayder Referral Hospital, Mekelle, North Ethiopia. *Adv. Microbiol.* **2017**, *07*, 871-886. DOI: 10.4236/aim.2017.712067
77. Kwak, Y.K; Colque, P.; Byfors, S.; Giske, C.G.; Möllby, R.; Kühn, I. Surveillance Of Antimicrobial Resistance Among Escherichia Coli In Wastewater In Stockholm During 1 Year: Does It Reflect The Resistance Trends In The Society?. *Int. J. Antimicrob. Agents* **2015**, *4*, 25-32. doi.org/10.1016/j.ijantimicag.2014.09.016
78. Budiarti, S.; Lingga, R.; Rusmana, I.; Wahyudi, A.T. Antibiotics resistant *Escherichia coli* from hospital liquid waste. *J. Appl. Biol. Sci.* **2018**, *1*, 36-40.
79. Czekalski, N.; Berthold, T.; Caucci, S.; Egli, A.; Bürgmann, H. Increased Levels of Multiresistant Bacteria and Resistance Genes after Wastewater Treatment and Their Dissemination into Lake Geneva, Switzerland. *Front. Microbiol.* **2012**, *3*. doi.org/10.3389/fmicb.2012.00106
80. Adegoke, A.A.; Madu, C.E.; Aiyegoro, O.A.; Stenström, T.A.; Okoh, A.I. Antibiogram and beta-lactamase genes among cefotaxime resistant E. coli from wastewater treatment plant. *Antimicrob. Resist. Infect. Control* **2020**, *9*. doi.org/10.1186/s13756-020-0702-4
81. Verawaty, M.; Apriani, N.; Tarigan, L.R.; Apiran, E.T.; Laurenta, W.C.; Muharni. Antibiotics resistant Escherichia coli isolated from aquatic ecosystems in Palembang, South Sumatra, Indonesia. *Biodiversitas* **2020**, *21*. DOI: 10.13057/biodiv/d210113
82. Iweriebor, B.C.; Gaqavu, S.; Obi, L.C.; Nwodo, U.U.; Okoh, A.I. Antibiotic Susceptibilities of Enterococcus Species Isolated from Hospital and Domestic Wastewater Effluents in Alice, Eastern Cape Province of South Africa. *Int. J. Environ. Res. Public Health* **2015**, *12*, 4231-4246. doi.org/10.3390/ijerph120404231
83. Omar Faruk, S.M.; Masudul Azad, C.A.M.; Nayeem, U.K. Isolation of Cefixime Resistant Salmonella from Hospitals Waste and Profiling Multi-Drug Resistance Pattern of the Selected Isolates. *Int. Res. J. Biol. Sci.* **2014**, *3*, 86-92.
84. Yuan, Q.B.; Guo, M.T.; Yang, J. Fate of Antibiotic Resistant Bacteria and Genes during Wastewater Chlorination: Implication for Antibiotic Resistance Control. *PLoS ONE* **2015**, *1*. doi.org/10.1371/journal.pone.0119403.
85. Liu, J.; Du, J.; Yi, L. Ra Tracer-Based Study of Submarine Groundwater Discharge and Associated Nutrient Fluxes into the Bohai Sea, China: A Highly Human-Affected Marginal Sea. *J. Geophys. Res. Oceans* **2017**, *122*, 8646-8660. doi.org/10.1002/2017JC013095
86. Zhang, Y.; Gu, A.Z.; Cen, T.; Li, X.; He, M.; Li, D.; Chen, J. Sub-Inhibitory Concentrations Of Heavy Metals Facilitate The Horizontal Transfer Of Plasmid-Mediated Antibiotic Resistance Genes In Water Environment. *Environ. Pollut.* **2018**, *237*, 74-82. doi.org/10.1016/j.envpol.2018.01.032
87. Lu, J.; Wang, Y.; Jin, M.; Yuan, Z.; Bond, P.; Guo, J. Both Silver Ions And Silver Nanoparticles Facilitate The Horizontal Transfer Of Plasmid-Mediated Antibiotic Resistance Genes. *Water Res.* **2020**, *169*, 115229. doi.org/10.1016/j.watres.2019.115229
88. Mackulak, T.; Cverenkarova, K.; Vojs Stanova, A.; Feher, M.; Tamas, M.; Skulcova, A.; Gal, M.; Naumowicz, M.; Spalkova, V.; Birosova, L. Hospital Wastewater—Source Of Specific Micropollutants, Antibiotic-Resistant Microorganisms, Viruses, And Their Elimination. *Antibiotics* **2021**, *10*, 1070. 10.3390/antibiotics10091070
89. Zhang, Y.; Cai, X.; Lang, X.; Qiao, X.; Li, X.; Chen, J. Insights into aquatic toxicities of the antibiotics oxytetracycline and ciprofloxacin in the presence of metal: Complexation versus mixture. *Environ. Pollut.* **2012**, *166*, 48-56. doi.org/10.1016/j.envpol.2012.03.009
90. Gao, M.; Lv, M.; Han, M.; Song, W.; Wang, D. Avoidance behavior of *Eisenia fetida* in oxytetracycline- and heavy metal-contaminated soils. *Environ. Toxicol. Pharmacol.* **2016**, *47*, 119-123. doi.org/10.1016/j.etap.2016.09.011
91. Dahanayake, P.S.; Hossain, S.; Wickramanayake, M.V.; Heo, G.J. Antibiotic and heavy metal resistance genes in *Aeromonas* spp. isolated from marketed Manila Clam (*Ruditapes philippinarum*) in Korea. *Journal of Applied Microbiology* **2019**, *127*, 941-952. doi.org/10.1111/jam.14355
92. Zhang, H.; He, H.; Chen, S.; Huang, T.; Lu, K.; Zhang, Z.; Wang, R.; Zhang, X.; Li, H. Abundance of antibiotic resistance genes and their association with bacterial communities in activated sludge of wastewater treatment plants: Geographical distribution and network analysis. *J. Environ. Sci.* **2019**, *82*, 24-38. doi.org/10.1016/j.jes.2019.02.023

93. Obayashi, Y.; Kadoya, A.; Kataoka, N.; Kanda, K.; Bak, S.M.; Iwata, H.; Suzuki, S. Tetracycline Resistance Gene Profiles in Red Seabream (*Pagrus major)* Intestine and Rearing Water After Oxytetracycline Administration. *Front. Microbiol.* **2020**, *11*. doi.org/10.3389/fmicb.2020.01764
94. Meehl, G.A. Characteristics of Surface Current Flow Inferred from a Global Ocean Current Data Set. *J. Phys. Oceanogr.* **1982**, *12*, 538-555.
95. GOV.UK National Statistics Sea Fisheries Annual Statistics Report 2019. Available online: https://www.gov.uk/government/statistics/uk-sea-fisheries-annual-statistics-report-2019 (accessed on: 26 May 2021).
96. UK Parliament House of Commons Library. Available online: https://commonslibrary.parliament.uk/research-briefings/sn02788/ (accessed on 26 May 2021).
97. Spanggaard, B.; Jørgensen, F.; Gram, L.; Huss, H.H. Antibiotic resistance in bacteria isolated from three freshwater fish farms and an unpolluted stream in Denmark. *Aquaculture* **1993**, *115*, 195-207. doi.org/10.1016/0044-8486(93)90136-M
98. Henry, F.; Amara, R.; Courcot, L.; Lacouture, D.; Bertho, M.L. Heavy metals in four fish species from the French coast of the Eastern English Channel and Southern Bight of the North Sea. *Environ. Int.* **2004**, *30*, 675-683. doi.org/10.1016/j.envint.2003.12.007
99. Reverter, M.; Sarter, S.; Caruso, D.; Avarre, J.C.; Combe, M.; Pepey, E.; Pouyaud, L.; Vega-Heredía, S.; de Verdal, H.; Gozlan, R.E. Aquaculture at the crossroads of global warming and antimicrobial resistance. *Nat. Commun.* **2020**, *11*, 1870. doi.org/10.1038/s41467-020-15735-6