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

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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.sco-

pus.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 *tetC* detected in 80% bacteria Helsinki, 27% Tallin and 73% Tartu	WWTP effluent	Helsinki, Finland Tallin, Estonia Tartu, Estonia	[56]
OTC	32.0 x 10^7 OTC	N/D	PWWTP Influent and effluent	North China	[54]
TC	2.6 x 10^6 TC				
TIG	N/D	*bla*$_{NDM1}$	Seepage and tap water	New Deli, India	[35]
OTC	N/D	4.8 x 10^5 cfu/100mL in river 4.8 x 10^6 cfu/100mL in WWTP *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).
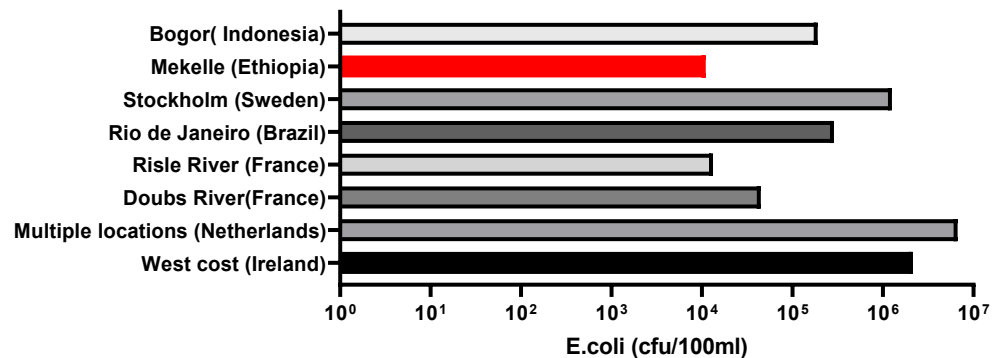

Figure 1. AMR *E. coli* levels in treated and untreated hospital effluent in different regions around the world. In black: untreated hospital effluent; in red: treated hospital effluent.

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 32.0×10^7 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 4.8×10^5 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 4.8×10^6 cfu/100mL of bacteria containing resistant genes. Moreover, *tetM* 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, Fin-
land, 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 2.6×10^6 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 asso-
ciated 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 iso-
lated 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 tol-
erant 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 enter-
ococci (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 espe-
cially 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.
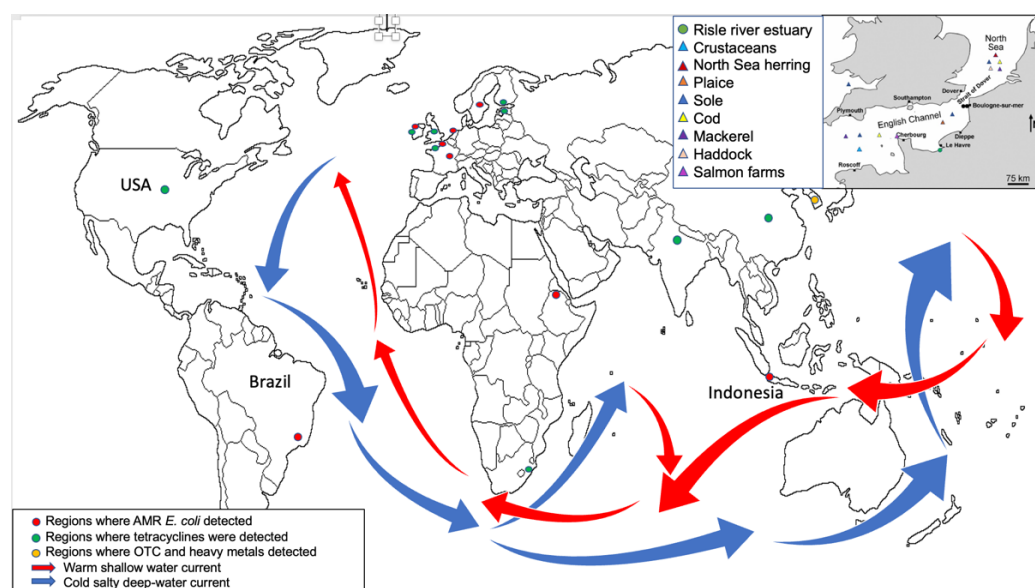

Figure 2. Illustration of regions where AMR *E. coli*, tetracyclines, and OTC and heavy metals distribution around the world, and a map of North and South Atlantic currents contributing to AMR spread. Insert shows the English Channel.

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.

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