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Tracking antimicrobial resistance indicator genes in wild flatfish from the English Channel and the North Sea area: A one health concern

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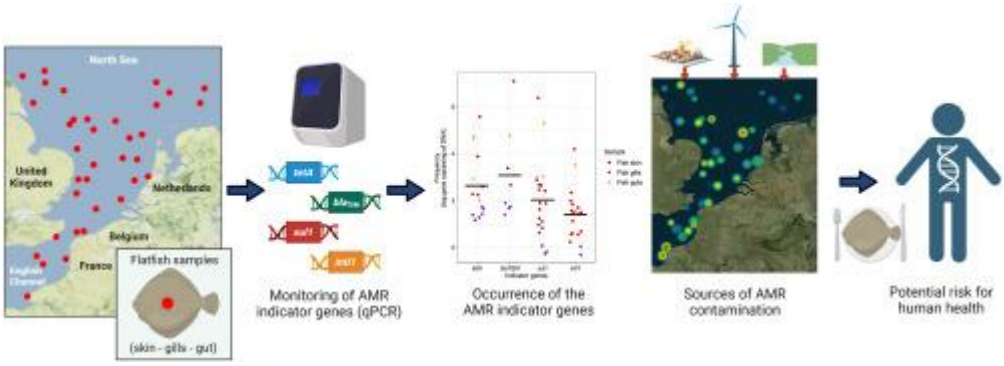
Abstract :

Antimicrobial resistance (AMR) is a burgeoning environmental concern demanding a comprehensive One Health investigation to thwart its transmission to animals and humans, ensuring food safety. Seafood, housing bacterial AMR, poses a direct threat to consumer health, amplifying the risk of hospitalization, invasive infections, and death due to compromised antimicrobial treatments. The associated antimicrobial resistance genes (ARGs) in diverse marine species can amass and transmit through various pathways, including surface contact, respiration, and feeding within food webs. Our research, focused on the English Channel and North Sea, pivotal economic areas, specifically explores the occurrence of four proposed AMR indicator genes (tet(A), blaTEM, sul1, and intI1) in a benthic food web. Analyzing 350 flatfish samples' skin, gills, and gut, our quantitative PCR (qPCR) results disclosed an overall prevalence of 71.4% for AMR indicator genes. Notably, sul1 and intI1 genes exhibited higher detection in fish skin, reaching a prevalence of 47.5%, compared to gills and gut samples. Proximity to major European ports (Le Havre, Dunkirk, Rotterdam) correlated with increased AMR gene frequencies in fish, suggesting these ports' potential role in AMR spread in marine environments. We observed a broad dispersion of indicator genes in the English Channel and the North Sea, influenced by sea currents, maritime traffic, and flatfish movements. In conclusion, sul1 and intI1 genes emerge as robust indicators of AMR contamination in the marine environment, evident in seawater and species representing a benthic food web. Further studies are imperative to delineate marine species' role in accumulating and transmitting AMR to humans via seafood consumption. This research sheds light on the urgent need for a concerted effort in comprehending and mitigating AMR risks in marine ecosystems within the context of One Health.

Graphical abstract



Highlights

► Wild flatfish from the English Channel/North Sea are carriers of AMR. ► *Sul1*/*intI1* were the predominant AMR indicator genes in the fish samples. ► The contamination of fish by AMR was higher near major European ports. ► There is a potential risk of AMR transmission to humans through consumption of fish.

Keywords : Antimicrobial resistance, Indicator genes, Flatfish, English channel, North sea, Anthropic pressure

1. Introduction

Antimicrobial resistance (AMR) is a global issue that clearly symbolizes the One Health concept, affecting humans, animals and the environment. Related to bacterial AMR are, more specifically, antimicrobial resistance genes (ARGs), which can be carried by both pathogenic and non-pathogenic bacteria, associated with bacteriophage DNA or in the form of free DNA. This constitutes an indirect risk for human health, as these resistances will constitute a genetic pool from which bacteria will be able to pick up these resistances and potentially transfer them to pathogenic bacteria. The transfer of AMR to human commensal bacteria can increase the reservoir of resistance constituted by the microbiota. These resistances can then spread to the environment. Indeed, AMR has become a major cause of death worldwide leading to 1.27 million deaths in 2019 (Murray et al. 2022). According to the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO), more than 670,000 infections are attributable to bacteria resistant to antimicrobials and around 33,000 people die from direct consequences each year in Europe (WHO 2022). If no action is taken to combat AMR, this number could rise over the next few years. In parallel, aquatic products (fisheries and aquaculture) are increasingly consumed worldwide, bringing health benefits to consumers. Consumption of aquatic products was 20.5 kg/capita worldwide in 2018, and 24.0 kg/capita in the European Union (EU) in 2019 (FAO, 2020, EUMOFA, 2021). But this consumption can also be a source of collective foodborne. According to the Centers for Disease Control and Prevention (CDC), nearly 48 million foodborne illnesses occur worldwide each year, resulting in 128,000 hospitalizations and 3,000 deaths. The presence of bacterial AMR and ARGs associated with aquatic products represents two main risks to consumer health. In the event of foodborne infection, treatment with antibiotics may prove less effective, or even lead to therapeutic impasse. What's more, these antimicrobial resistances can be transferred to the consumer's resident or transient flora, forming a gene pool that can spread in the environment. Contamination of aquatic products by bacterial AMR and ARGs can occur during handling or processing, but also directly in the marine environment. ARGs associated with different marine species can accumulate through surface contact with seawater or sediments, respiration or feeding via food webs. It has been suggested that the transmission of ARGs from animals to humans takes place via the food chain. Indeed, marine animals can be naturally colonized by bacteria in their skin, gastrointestinal tract, gills, exoskeleton and even shell in the case of bivalve molluscs. This colonization will depend on several factors, such as the position of the animals in the water column. The bacterial flora of benthic species such as flatfish and molluscs mainly reflects sediment-associated bacteria, while that of pelagic species reflects free bacteria in the water column. The concept of having AMR indicator genes available for monitoring and surveillance of AMR in the environment is crucial, given that only 1% of the bacterial flora in the environment is cultivable (Bodor et al. 2020). In fact, ARGs associated with marine food web and in particular wild marine species are poorly described in the litterature. Muziasari *et al.* (2017) described the *sul1, tet32, tetM, tetO, tetW, aadA1* and *aadA2* genes in the fish gut samples of rainbow trout (*Oncorhynchus mykiss*) and lavaret (*Coregonus lavaretus*) reared in open cage systems in the northern Baltic Sea and the presence of *emrB* gene was highest in skin and gill samples, an efflux pump and implicated in multiresistance to antibiotics. The marine environment is particularly pointed as the final receiving compartment supplied by anthropogenic inputs from land and sea activities such as river and marine traffic effluents. In particular, the English Channel and the North Sea, two seas located in the Northwest of Europe, are subject to effluents from the many countries bordering them such as France, Belgium, the United Kingdom, the Netherlands, Germany, Norway and Denmark. These areas are also characterized by significant marine traffic due to fishing activities, shipping and the presence of several offshore platforms, which can also cause direct contamination by the various effluents discharged into the sea. These anthropogenic activities may lead to the contamination of the marine environment by AMR thus representing a risk to human health through the consumption of seafood products. However, unlike the role of farm animals and humans, the role of the marine environment and marine wild animals in

the spread of AMR is understudied. Due to the diversity of ARGs in different environmental compartments such as soil and water, monitoring individual genes is time-consuming and costly. The multiplicity of methods for detecting and quantifying ARGs, coupled with different sampling and sample processing techniques, makes it difficult to compare results obtained in different studies. Harmonization of ARG analysis methods and the development of indicators of antimicrobial resistance contamination are therefore necessary if results are to be complementary. The need for such genetic indicators for monitoring and surveillance of antimicrobial resistance is a well-known issue. By definition, an indicator gene should be relatively simple to monitor, provide information on global resistance dynamics, indicate anthropogenic pressure and can be coupled with the search for ARGs to study transfer dynamics (ANSES 2020). Tracking indicator genes rather than indicator bacteria helps to compensate for the rapid loss of cultivability of bacteria in the environment, and to monitor the large-scale spatio-temporal fate of antimicrobial resistance. Among clinically relevant ARGs often associated with mobile genetic elements, the *tet(A)*, *bla*$_{TEM}$, *sul1* and *intI1* genes have been proposed as potential indicators for environmental AMR contamination (Berendonk et al. 2015) and have already been identified in coastal and offshore seawaters (Zhang et al. 2020; Bourdonnais et al. 2022a; Han et al. 2022), but also associated with various biotic surfaces such as fish in the Mediterranean Sea (Brahmi et al. 2018), bivalve mollusks collected near the Norwegian coast (Grevskott et al. 2017) and even plankton collected from a river in China (Xue et al. 2021). Given this prevalence in the marine environment and in various marine organisms, these four genes could be suitable indicators for assessing AMR contamination of the marine environment. In the present study, we assessed the prevalence and frequency of the *tet(A), bla*$_{TEM}$, *sul1* and *intI1* AMR indicator genes associated with flatfish samples (skin, gills, guts) in the English Channel and the North Sea to determine whether these marine species are vectors for the dissemination of AMR in the marine environment, and to identify the potential sources of contamination in this environment. This work has been positioned within of our entire research project on the AMR in the marine environment. We determined the occurrence of the four genes *tet(A)*, *bla*$_{TEM}$, *sul1* and *intI1,* proposed as AMR indicator genes, in a benthic food web in the English Channel and North Sea. The sampling plan was carried out considering the position of marine species in the water column and in relation to their consumption of lower trophic level marine species. Due to the complexity of food webs, we decided to simplify the membership of the marine species analyzed in this study to a defined trophic level based on the study by Giraldo et al. (2017) carried out in the English Channel. The results of analyses of near-surface samples of open sea water were presented in the work of Bourdonnais et al. (2022a) and constituted level 0 of the food web. Phytoplankton belonged to level 1 and consisted of plant organisms and particulate organic matter. Zooplankton, mainly copepods, represented level 2 of the food web (Bourdonnais et al. 2022b). Level 3 consisted of *Aequipecten opercularis* (white scallop) (Bourdonnais et al. 2022b) and, two species of benthic flatfish: *Limanda limanda* (dab) and *Pleuronectes platessa* (plaice). Both flounder and plaice feed on unicellular organisms and zooplankton such as copepods in the larval and juvenile stages, and on crustaceans and bivalve molluscs as adults. Given the diversity in size and stage of development of the species caught, we have chosen to consider them as belonging to trophic level 3. Plaice and flounders were not differentiated in this study due to their morphological, behavioral and dietary similarities, and also to have a more significant number of samples. We have therefore designated them as "flatfish".

2. Material and methods

2.1. Sampling and animal ethics

The flatfish samples (n = 350) were collected at 35 sampling stations (10 samples per station) in the English Channel and the North Sea during the International Bottom Trawl Survey (IBTS) oceanographic campaign in January 2020. Plaice (*Pleuronectes platessa*) and common dab (*Limanda limanda*) individuals were sampled with a 36/47 Grande Ouverture Verticale (GOV) bottom trawl towed during daylight for 30 minutes and at a constant speed of 4 knots according to the standard IBTS protocol (ICES 2015). Following trawling, the samples were identified, placed in airtight bags and stored in a cold room at -20 °C on board the ship, before their transfer to the laboratory at -20 °C. Protocols of all surveys are currently being evaluated by the French research institute for exploitation of the sea (Ifremer) and validated by the ICES IBTS International Group (ICES 2015). Moreover, survey's PIs received training about animal well-being and ethics.

2.2. Sample preparation

In the laboratory, flatfish individuals were thawed at room temperature. For this study, we focused on the skin, gills and guts of the fish, which host an important and diversified microbiota due to significant mucus secretion (Merrifield et Rodiles 2015). A dry sponge (3M, Saint Paul, USA) was soaked with 10 mL of sterile physiological water and then swabbed on the blind and ocular sides of the fish to collect the mucus present on the fish skin. Solutions were recovered from 10 individuals from the same sampling stations, pooled together and diluted by half with sterile physiological water. Similarly, the gills and guts samples collected from the same 10 individuals were pooled together, diluted by half with sterile physiological water and homogenized for 1 min with a Stomacher grinder (Biomérieux, Marcy-l'Etoile, France). All these samples were supplemented with 20 % of glycerol and stored at -20 °C until total DNA extraction.

2.3. DNA extraction

Total DNA was extracted from 1 mL of each sample preparation with the DNeasy® PowerBiofilm® kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with minor modifications. Briefly, the samples were centrifuged for 5 min at 10,000 × g and the pellets were suspended in 400 µL of MBL lysis buffer and 100 µL of FB buffer. The solutions were transferred to Beadtubes and then we followed the manufacturer's protocol until the DNA elution step. For this step, DNA was eluted by adding 50 µL of EB buffer on the membrane twice. The DNA concentration was analyzed with a DS-11 spectrophotometer (Denovix, Wilmington, USA) and diluted 10-fold with nuclease-free water before performing gene quantification by quantitative PCR (qPCR).

2.4. Quantification of the AMR indicator genes by qPCR

In this study, the *tet(A)* (tetracycline resistance), bla_{TEM} (β-lactam resistance), *sul1* (sulfonamide resistance) and *intI1* (class 1 integron-integrase) AMR indicator genes were quantified by qPCR. Supplementary Table 1 contains the primers and the amplification conditions used. The qPCR reactions were performed as previously described in our previous study (Bourdonnais et al. 2022).

2.5. Data analysis

We validated the qPCR results according to the NF T90-471:2015-06 standard (amplification efficiency between 75 and 125 %, coefficient of determination $R^2 > 0.99$ and no quantification of the negative control). The number of gene copies was calculated by Cq values and standard curves. The total indicator genes frequency was calculated by normalizing the sum of the number of gene copies with the amount of extracted DNA, expressed in log copies/ng of DNA (Paul et al. 2018). The map representing the total indicator genes frequencies in the English Channel and the North Sea was

designed in part with RStudio Software version 1.4.1717 (RStudio, Inc., Boston, United States) using the ggmap package.

3. Results and discussion

3.1. Occurrence of the AMR indicator genes in the flatfish

The four *tet(A), bla*$_{TEM}$, *sul1* and *intI1* indicator genes were detected in each sample type (skin, gills and guts). The overall prevalence of the indicator genes, defined as the number of pooled fish samples for which at least one gene was quantified, was 71.4 %. The *sul1* and *intI1* genes had both a higher prevalence in skin samples (31.4 and 45.7 %, respectively) compared to gills (17.1 and 5.7 %) and guts (8.6 and 5.7 %) samples (Fig. 1A). We observed less than 17.1 % prevalence of the *tet(A)* and *bla*$_{TEM}$ genes in all fish samples. Among all sample types analyzed, the prevalence and frequency for all four AMR indicator genes were lowest in the fish gut samples (Fig. 1A and 1B). The frequency of the indicator genes in the flatfish skin, gill and gut samples ranged from 1.8 to 5.5 log copies/ng of DNA (Fig. 1B). In the skin and gill samples, the *sul1* and *intI1* genes were in the same order of frequencies and mainly ranged from 2.0 to 3.5 log copies/ng of DNA and were above 3 log copies/ng of DNA for the *tet(A)* and *bla*$_{TEM}$ genes. To our knowledge, these four AMR indicator genes have not been targeted in the marine environment. However, other genes encoding the same AMR have been identified in aquatic organisms. Some research had revealed the occurrence of the *bla*$_{DHA}$, *tetM, mphA* and *vgaB* genes in the skin and fillets of farmed rainbow trout (*Oncorhynchus mykiss*) individuals and were quantified up to 2×10^{-1} gene copies/16S rDNA copy (Helsens et al. 2020). Indeed, fish skin is characterized by mucus secretion that constitutes an immune barrier colonized by different commensal or opportunistic bacterial species belonging mainly to the Proteobacteria, Firmicutes and Acidobacteria phyla which may carry ARGs (Minniti et al. 2017). The intestinal cells of fish also secrete mucus to protect them from aggression and fish gut is thus defined by a high bacterial diversity that may be associated with ARGs. In fact, high abundances of aminoglycoside, β-lactam and tetracycline resistance genes have been observed in the intestine of wild soles (*Cynoglossus semilaevis*) from Bohai Bay in northeast China (Jia et al. 2022). Similarly to our results, ARGs such as *sul1* and *bla*$_{TEM}$ were quantified from intestinal mucus samples of carp (*Cyprinus carpio*), barbel (*Luciobarbus graellsii*) and trout (*Salmo trutta*) in Spain (Marti et al. 2018). In addition, the fish gills are a key organ for respiration and exchange with the environment and are subject to the accumulation of environmental contaminants such as ARGs through the filtration of large volumes of water. In the Mediterranean Sea, several strains of Enterobacteriaceae have been isolated from gills and intestines of sardines (*Sardina pilchardus*), salema (*Sardina pilchardus*) and horse mackerel (*Trachurus trachurus*) with a 23 % prevalence of the *bla*$_{TEM}$ gene and 17 % of the *sul1* gene among the 64 isolates (Brahmi et al. 2018) which was consistent with our results on flatfish samples. In our entire research project on the AMR in the marine environment, the analysis of enriched and non-enriched seawater (Bourdonnais et al. 2022a) and benthic food web samples (phytoplankton and zooplankton communities (Bourdonnais et al. 2022b), *Aequipecten opercularis* (scallop) (Bourdonnais et al. 2022b),and fatfish) revealed a total prevalence of 81.4% of the four antimicrobial resistance indicator genes in the English Channel and North Sea, with a prevalence of 39.5% for the *tet(A)* gene, 36.7% for the *blaTEM* gene, 57.1% for the *sul1* gene and 61.0% for the *intI1* gene. The *sul1/intI1* genes were the most quantified indicators in seawater, phytoplankton, zooplankton, bivalve mollusc and flatfish samples. The *tet(A)/blaTEM* genes were poorly quantified in the samples, but had a high prevalence in sample enrichments, notably gills and flatfish viscera. Seabed fauna such as flatfish is particularly exposed to skin AMR contamination because of direct contact with sediments that constitute a major reservoir of ARGs (Chen et al. 2013). ARGs can also accumulate in the gills as fish filter large volumes of seawater containing ARGs as we have shown in a previous study (Bourdonnais et al. 2022a), and also into their gut by consuming contaminated lower trophic level marine species such as plankton and bivalve mollusks (Bourdonnais et al. 2022b), also potential carriers of AMR (Xue et al. 2021; Silva et al. 2018). Due to the migratory flows of flatfish and their movement favored by marine currents, these marine species can contribute to the

dissemination of AMR in the marine environment, even over long distances. As they live on the seabed, flatfish contribute to the spread of AMR by releasing excrement that may contain bacteria and ARGs, enriching marine sediments with AMR. As a result of their resuspension by seabed movements, there is a risk of contamination of other living benthic or even pelagic organisms. The presence of AMR associated with wild fish can affect marine biodiversity, notably by promoting the proliferation of resistant bacteria to the detriment of other non-resistant organisms, representing a risk to environmental health. This also represents a human health risk if these fish are consumed raw or undercooked, with the risk of transmission of pathogenic antimicrobial-resistant bacteria to humans via the food chain. Indeed, bacteria or ARGs occurring on fish skin, in their gills or intestines can interact with fish flesh during handling, potentially posing a human health problem through consumption (Ziarati et al. 2022). The presence of ARGs in non-pathogenic bacteria also poses a risk, as these genes can be transferred to human commensal flora. This can increase the reservoir of resistance constituted by the microbiota, which will then diffuse into the marine environment, the final receptor of effluents contaminated by man, thus forming a cycle of AMR between the environment, humans and animals, as referred to the One Health concept.

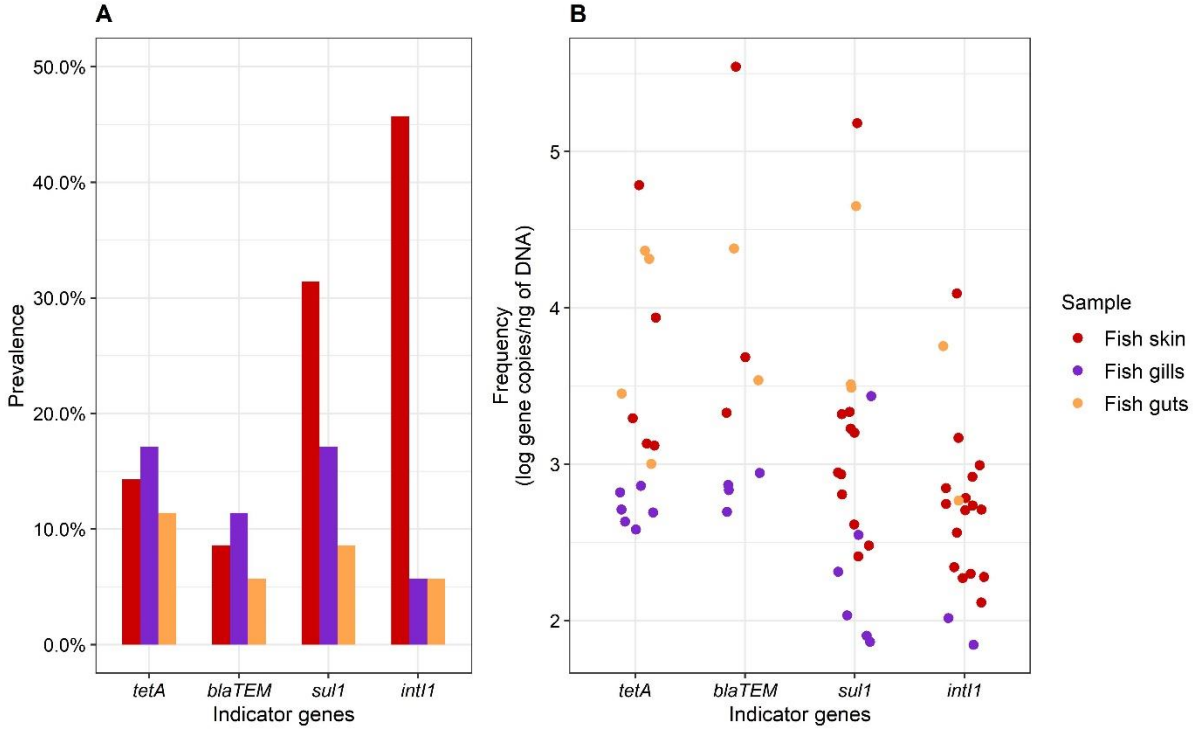


Figure 1 : Prevalence (A) and frequency (B) of the *tet(A)*, *bla*$_{TEM}$, *sul1* and *intI1* AMR indicator genes in the different samples (skin, gills, guts) from pooled flatfish.

3.2. Identification of anthropogenic pressures in the English Channel and the North Sea

We postulated that the frequency of the AMR indicator genes, observed in fish samples, could reflect the presence of anthropogenic inputs, i.e. sources of contamination of the marine environment. The total frequency of the indicator genes, corresponding to the number of copies of the four *tet(A)*, *bla*$_{TEM}$, *sul1* and *intI1* genes per ng of DNA, was plotted on a map of the study area (the English Channel and the North Sea) for each flatfish sample type to better identify the potential link with anthropogenic sources of contamination (Fig. 2). A more significant contamination of fish with AMR, as reflected by the quantification of the total indicator genes in all three sample types (skin, gills, guts), was observed in the English Channel and the North Sea near European ports, namely Le Havre (France), Dunkirk (France) and Rotterdam (Netherlands). In addition to these port activities, two of the three areas are subject to the effluents from large rivers such as the Seine in Le Havre port and the Rhine in Rotterdam

port. The contamination of these rivers by AMR has been reported in previous studies such as the presence of multi-resistant *E. coli* strains associated with a high prevalence of the *intI1* gene in the Seine and high abundances of the *intI1*, *sul1, ermB* and *tetM* genes in the Rhine (Laroche et al. 2009; Paulus, Hornstra, et Medema 2020). We also observed high contamination of flatfish near the East Coast of England, an area characterized by a cluster of wind farms. Indeed, offshore wind farms can act as artificial reefs attracting marine species such as fish and crustaceans (Degraer et al. 2020). These species attract higher trophic level marine species that consume them, such as mammals and birds, which may also be a vector of contamination by AMR in the marine environment. A study conducted in the North Sea highlighted the attraction of some bird species such as gulls to these wind platforms (Vanermen et al. 2015) while the feces of marine birds were found to be an important vector of contaminations with ARGs such as *bla, sul* and *tet* genes (Poeta et al. 2008). Finally, we noticed a scattered contamination of flatfish in the North Sea, near the coasts, but also in the open sea. This observation may reflect an important dissemination of AMR in marine environment favored by the migration of flatfish over long distances and facilitated by deep ocean currents.

There are two possible pathways of wild flatfish contamination with ARGs. The first is characterized by direct contact with ARGs or ARG-carrying bacteria in free form in the water column and originating from effluents from land-based sources. The second is based on a transfer of ARGs from the marine sediments, which are known to be a reservoir of AMR, resulting from the sedimentation of the ARGs following the discharges into the marine environment (Chen et al. 2013). ARGs could therefore be associated with the skin of flatfish through close contact with contaminated sediment or seawater, but also the gills as fish filter large volumes of seawater through brachial respiration, seawater being also a reservoir of ARGs. Indeed, we showed in a previous study that surface waters of the English Channel and the North Sea were an important carrier of the *sul1* and *intI1* AMR indicator genes, detected in approximately half the seawater samples collected, with abundances of up to 3.6 gene copies/ml seawater (Bourdonnais et al. 2022a). However, we observed a higher level of AMR contamination in flatfish collected near the ports while seawater contamination was higher off the West Coast of the Netherlands. These results indicate that ARGs disseminate in seawater at the surface but also in the seabed associated with flatfish. ARGs released into the marine environment can also accumulate in lower trophic level marine species such as plankton that flatfish feed on and whose role in AMR transport is still understudied compared to freshwater environments (Xue et al. 2021). This accumulation of ARGs in the digestive system of wild fish thus represents a risk of dissemination in the environment with release via feces and transmission between individuals through close contact. Furthermore, all these results lead us to believe that the *sul1* and *intI1* genes would be suitable indicators for assessing AMR contamination of the marine ecosystem, their quantification allowing us to identify sources of contamination while assessing the potential for AMR transfer via integrons. Indeed, the class 1 integrase is associated with integrons, genetic structures that can transport and exchange ARGs between different bacteria, and is therefore an important component in the dissemination of AMR in bacterial populations and the environment. Previous studies have demonstrated a correlation between the *intI1* gene and various measures of human impact in aquatic environments, making this gene an ideal candidate to be an indicator gene for AMR for the marine environment (Gillings et al. 2015; Pruden, Arabi, et Storteboom 2012). The *sul1* gene encoding sulfonamide resistance is very often associated with these class 1 integrons, thus easily transferable within bacterial communities and potentially leading to the emergence of multidrug resistant bacterial strains. It is important to pursue studies and to monitor AMR not only in seawater, but also in various wild marine species, which correspond to two essential dimensions of the One Health perspective, in order to improve our understanding of the risk caused by AMR to human health, the third component of the One Health framework.

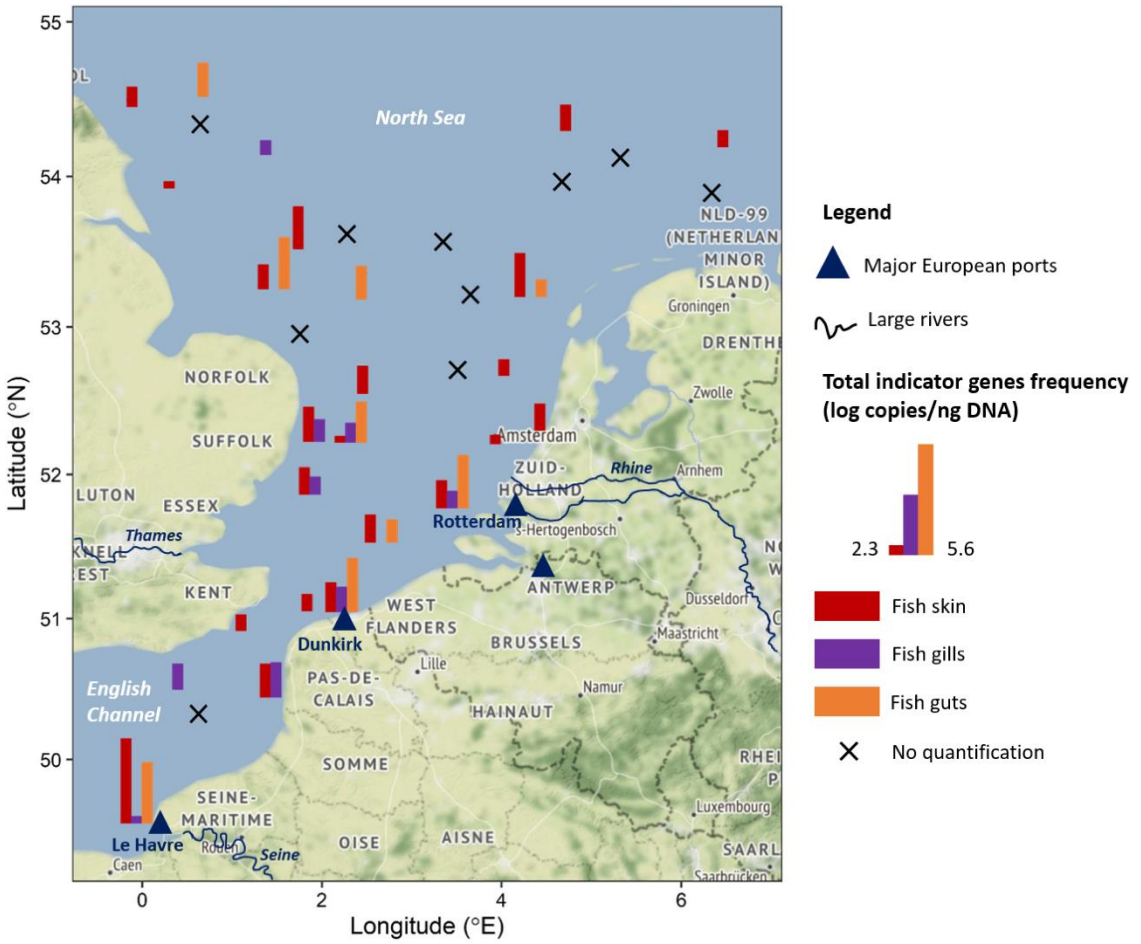


Figure 2 : Map indicating the total AMR indicator genes frequency in the flatfish samples from the English Channel and the North Sea associated with potential sources of pollution.

4. Conclusion

In summary, we found that the four *tet(A), bla*$_{TEM}$, *sul1* and *intI1* AMR indicator genes were present in the skin, gills and gut of wild flatfish with an overall prevalence of 71.4 %. The *sul1* and *intI1* genes were more prevalent in skin samples than in gills and guts, and the frequency of all four genes were lowest in the fish gut samples. In our entire project on the research of the four *tet(A), bla*$_{TEM}$, *sul1* and *intI1* AMR indicator genes in the marine environment, we described 81.4% of the four antimicrobial resistance indicator genes in the English Channel and North Sea, with a prevalence of 39.5% for the *tet(A)* gene, 36.7% for the *blaTEM* gene, 57.1% for the *sul1* gene and 61.0% for the *intI1* gene. The *sul1/intI1* genes were the most quantified indicators. The prevalence and abundance of these genes varied not only according to the nature of the sample, but also according to the sampling area, due to the different anthropogenic impacts associated with high gene dissemination. Greater contamination of samples was observed in the Eastern Channel, an area considered to be a veritable freeway of the sea due to intense maritime traffic and involving various discharges into the marine environment, as well as at the mouth of the Thames, which is subject to river effluents. Seawater samples were more contaminated with antimicrobial resistance indicator genes near the West Coast of the Netherlands, an area characterized by the presence of offshore wind and oil platforms. The *sul1* and *intI1* genes are good indicators of marine environmental contamination. Marine fish and environment, two major components of the One Health framework, are important vectors of AMR. They pose a risk of

transmission to humans, the third component of the One Health framework. It is important to improve and standardize the monitoring of AMR in the marine environment to better characterize its role in the spread and transmission of resistance to humans through the consumption of fishery products.

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CRediT authorship contribution statement

Erwan Bourdonnais: Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft. **Cédric Le Bris**: Supervision, Validation, Writing - review & editing. **Thomas Brauge**: Conceptualization, Supervision, Validation, Writing - review & editing. **Graziella Midelet**: Conceptualization, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Table 1 : qPCR primers and probes used for quantification of the four AMR indicator genes.

Genes	Primer and probe sequences (5' - 3')	References	Amplification conditions
tet(A)	GCTACATCCTGCTTGCCTTC	(Ng et al. 2001)	95 °C - 5 min (1 cycle); 95 °C - 10 s, 60 °C - 30 s, 72 °C - 5 s (45 cycles)
	CATAGATCGCCGTGAAGAGG		
bla_{TEM}	TTCCTGTTTTTGCTCACCCAG	(Bibbal et al. 2007)	
	CTCAAGGATCTTACCGCTGTTG		
sul1	CCGTTGGCCTTCCTGTAAAG	(Heuer et Smalla 2007)	95 °C - 5 min (1 cycle); 95 °C - 10 s, 60 °C - 1 min, 72 °C - 1 s (45 cycles)
	TTGCCGATCGCGTGAAGT		
	(FAM)CAGCGAGCCTTGCGGCGG(TAMRA)		
intI1	GCCTTGATGTTACCCGAGAG	(Barraud et al. 2010)	
	GATCGGTCGAATGCGTGT		
	(6-FAM)ATTCCTGGCCGTGGTTCTGGGTTTT(BHQ1)		