



HAL
open science

Tracking antimicrobial resistance indicator genes in wild flatfish from the English Channel and the North Sea area: a One Health concern

Erwan Bourdonnais, Cédric Le Bris, Thomas Brauge, Graziella Midelet

► To cite this version:

Erwan Bourdonnais, Cédric Le Bris, Thomas Brauge, Graziella Midelet. Tracking antimicrobial resistance indicator genes in wild flatfish from the English Channel and the North Sea area: a One Health concern. *Environmental Pollution*, 2024, 343, pp.123274. 10.1016/j.envpol.2023.123274 . hal-04384404

HAL Id: hal-04384404

<https://hal.science/hal-04384404>

Submitted on 10 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

1 Tracking antimicrobial resistance indicator genes in wild flatfish from the English Channel 2 and the North Sea area: a One Health concern

3 Erwan Bourdonnais^{1,2}, Cédric Le Bris², Thomas Brauge¹ & Graziella Midelet¹

4 ¹ANSES, Laboratoire de Sécurité des Aliments, Unité Bactériologie et Parasitologie des Produits de la
5 Pêche et de l'Aquaculture, Boulogne-sur-Mer, France

6 ²Univ. du Littoral Côte d'Opale, UMR 1158 BioEcoAgro, Institut Charles Viollette, Unité Sous Contrat
7 ANSES, INRAe, Univ. Artois, Univ. Lille, Univ. de Picardie Jules Verne, Univ. de Liège, Junia, Boulogne-
8 sur-Mer, France

9 **Corresponding author at:** ANSES, Laboratoire de Sécurité des Aliments, Unité Bactériologie et
10 Parasitologie des Produits de la Pêche et de l'Aquaculture, Boulogne-sur-Mer, France.

11 *E-mail address:* thomas.brauge@anses.fr (T. Brauge)

12 **Keywords:** Antimicrobial resistance, indicator genes, flatfish, English Channel, North Sea, anthropic
13 pressure

14 Highlights

- 15 • Wild flatfish from the English Channel/North Sea are carriers of AMR
- 16 • *sul1/int1* were the predominant AMR indicator genes in the fish samples
- 17 • The contamination of fish by AMR was higher near major European ports
- 18 • There is a potential risk of AMR transmission to humans through consumption of fish

19 Abstract

20 Antimicrobial resistance (AMR) is a burgeoning environmental concern demanding a comprehensive
21 One Health investigation to thwart its transmission to animals and humans, ensuring food safety.
22 Seafood, housing bacterial AMR, poses a direct threat to consumer health, amplifying the risk of
23 hospitalization, invasive infections, and death due to compromised antimicrobial treatments. The
24 associated antimicrobial resistance genes (ARGs) in diverse marine species can amass and transmit
25 through various pathways, including surface contact, respiration, and feeding within food webs. Our
26 research, focused on the English Channel and North Sea, pivotal economic areas, specifically explores
27 the occurrence of four proposed AMR indicator genes (*tet(A)*, *blaTEM*, *sul1*, and *int1*) in a benthic food
28 web. Analyzing 350 flatfish samples' skin, gills, and gut, our quantitative PCR (qPCR) results disclosed
29 an overall prevalence of 71.4% for AMR indicator genes. Notably, *sul1* and *int1* genes exhibited higher
30 detection in fish skin, reaching a prevalence of 47.5%, compared to gills and gut samples. Proximity to
31 major European ports (Le Havre, Dunkirk, Rotterdam) correlated with increased AMR gene frequencies
32 in fish, suggesting these ports' potential role in AMR spread in marine environments. We observed a
33 broad dispersion of indicator genes in the English Channel and the North Sea, influenced by sea
34 currents, maritime traffic, and flatfish movements. In conclusion, *sul1* and *int1* genes emerge as robust
35 indicators of AMR contamination in the marine environment, evident in seawater and species
36 representing a benthic food web. Further studies are imperative to delineate marine species' role in
37 accumulating and transmitting AMR to humans via seafood consumption. This research sheds light on
38 the urgent need for a concerted effort in comprehending and mitigating AMR risks in marine
39 ecosystems within the context of One Health.

40 1. Introduction

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

89 the spread of AMR is understudied. Due to the diversity of ARGs in different environmental
90 compartments such as soil and water, monitoring individual genes is time-consuming and costly. The
91 multiplicity of methods for detecting and quantifying ARGs, coupled with different sampling and
92 sample processing techniques, makes it difficult to compare results obtained in different studies.
93 Harmonization of ARG analysis methods and the development of indicators of antimicrobial resistance
94 contamination are therefore necessary if results are to be complementary. The need for such genetic
95 indicators for monitoring and surveillance of antimicrobial resistance is a well-known issue. By
96 definition, an indicator gene should be relatively simple to monitor, provide information on global
97 resistance dynamics, indicate anthropogenic pressure and can be coupled with the search for ARGs to
98 study transfer dynamics (ANSES 2020). Tracking indicator genes rather than indicator bacteria helps to
99 compensate for the rapid loss of cultivability of bacteria in the environment, and to monitor the large-
100 scale spatio-temporal fate of antimicrobial resistance. Among clinically relevant ARGs often associated
101 with mobile genetic elements, the *tet(A)*, *bla_{TEM}*, *sul1* and *int11* genes have been proposed as potential
102 indicators for environmental AMR contamination (Berendonk et al. 2015) and have already been
103 identified in coastal and offshore seawaters (Zhang et al. 2020; Bourdonnais et al. 2022a; Han et al.
104 2022), but also associated with various biotic surfaces such as fish in the Mediterranean Sea (Brahmi
105 et al. 2018), bivalve mollusks collected near the Norwegian coast (Grevskott et al. 2017) and even
106 plankton collected from a river in China (Xue et al. 2021). Given this prevalence in the marine
107 environment and in various marine organisms, these four genes could be suitable indicators for
108 assessing AMR contamination of the marine environment. In the present study, we assessed the
109 prevalence and frequency of the *tet(A)*, *bla_{TEM}*, *sul1* and *int11* AMR indicator genes associated with
110 flatfish samples (skin, gills, guts) in the English Channel and the North Sea to determine whether these
111 marine species are vectors for the dissemination of AMR in the marine environment, and to identify
112 the potential sources of contamination in this environment. This work has been positioned within of
113 our entire research project on the AMR in the marine environment. We determined the occurrence of
114 the four genes *tet(A)*, *bla_{TEM}*, *sul1* and *int11*, proposed as AMR indicator genes, in a benthic food web
115 in the English Channel and North Sea. The sampling plan was carried out considering the position of
116 marine species in the water column and in relation to their consumption of lower trophic level marine
117 species. Due to the complexity of food webs, we decided to simplify the membership of the marine
118 species analyzed in this study to a defined trophic level based on the study by Giraldo et al. (2017)
119 carried out in the English Channel. The results of analyses of near-surface samples of open sea water
120 were presented in the work of Bourdonnais et al. (2022a) and constituted level 0 of the food web.
121 Phytoplankton belonged to level 1 and consisted of plant organisms and particulate organic matter.
122 Zooplankton, mainly copepods, represented level 2 of the food web (Bourdonnais et al. 2022b). Level
123 3 consisted of *Aequipecten opercularis* (white scallop) (Bourdonnais et al. 2022b) and, two species of
124 benthic flatfish: *Limanda limanda* (dab) and *Pleuronectes platessa* (plaice). Both flounder and plaice
125 feed on unicellular organisms and zooplankton such as copepods in the larval and juvenile stages, and
126 on crustaceans and bivalve molluscs as adults. Given the diversity in size and stage of development of
127 the species caught, we have chosen to consider them as belonging to trophic level 3. Plaice and
128 flounders were not differentiated in this study due to their morphological, behavioral and dietary
129 similarities, and also to have a more significant number of samples. We have therefore designated
130 them as "flatfish".

131

132 2. Material and methods

133 2.1. Sampling and animal ethics

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

144 2.2. Sample preparation

145 In the laboratory, flatfish individuals were thawed at room temperature. For this study, we focused on
146 the skin, gills and guts of the fish, which host an important and diversified microbiota due to significant
147 mucus secretion (Merrifield et Rodiles 2015). A dry sponge (3M, Saint Paul, USA) was soaked with 10
148 mL of sterile physiological water and then swabbed on the blind and ocular sides of the fish to collect
149 the mucus present on the fish skin. Solutions were recovered from 10 individuals from the same
150 sampling stations, pooled together and diluted by half with sterile physiological water. Similarly, the
151 gills and guts samples collected from the same 10 individuals were pooled together, diluted by half
152 with sterile physiological water and homogenized for 1 min with a Stomacher grinder (Biomérieux,
153 Marcy-l'Etoile, France). All these samples were supplemented with 20 % of glycerol and stored at -20
154 °C until total DNA extraction.

155 2.3. DNA extraction

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

164 2.4. Quantification of the AMR indicator genes by qPCR

165 In this study, the *tet(A)* (tetracycline resistance), *bla_{TEM}* (β-lactam resistance), *sul1* (sulfonamide
166 resistance) and *int11* (class 1 integron-integrase) AMR indicator genes were quantified by qPCR.
167 Supplementary Table 1 contains the primers and the amplification conditions used. The qPCR reactions
168 were performed as previously described in our previous study (Bourdonnais et al. 2022).

169 2.5. Data analysis

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

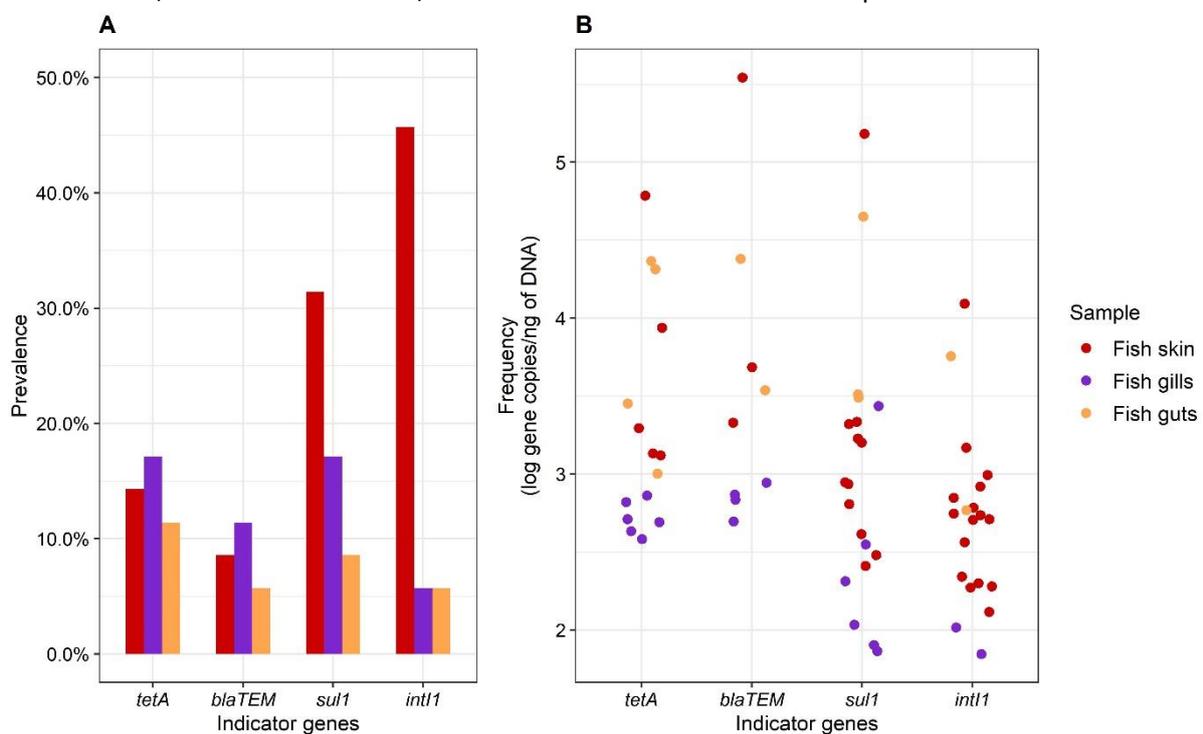
176 designed in part with RStudio Software version 1.4.1717 (RStudio, Inc., Boston, United States) using
177 the ggmap package.

178 3. Results and discussion

179 3.1. Occurrence of the AMR indicator genes in the flatfish

180 The four *tet(A)*, *bla_{TEM}*, *sul1* and *int11* indicator genes were detected in each sample type (skin, gills
181 and guts). The overall prevalence of the indicator genes, defined as the number of pooled fish
182 samples for which at least one gene was quantified, was 71.4 %. The *sul1* and *int11* genes had both a
183 higher prevalence in skin samples (31.4 and 45.7 %, respectively) compared to gills (17.1 and 5.7 %)
184 and guts (8.6 and 5.7 %) samples (Fig. 1A). We observed less than 17.1 % prevalence of the *tet(A)* and
185 *bla_{TEM}* genes in all fish samples. Among all sample types analyzed, the prevalence and frequency for
186 all four AMR indicator genes were lowest in the fish gut samples (Fig. 1A and 1B). The frequency of
187 the indicator genes in the flatfish skin, gill and gut samples ranged from 1.8 to 5.5 log copies/ng of
188 DNA (Fig. 1B). In the skin and gill samples, the *sul1* and *int11* genes were in the same order of
189 frequencies and mainly ranged from 2.0 to 3.5 log copies/ng of DNA and were above 3 log copies/ng
190 of DNA for the *tet(A)* and *bla_{TEM}* genes. To our knowledge, these four AMR indicator genes have not
191 been targeted in the marine environment. However, other genes encoding the same AMR have been
192 identified in aquatic organisms. Some research had revealed the occurrence of the *bla_{DHA}*, *tetM*,
193 *mphA* and *vgaB* genes in the skin and fillets of farmed rainbow trout (*Oncorhynchus mykiss*)
194 individuals and were quantified up to 2×10^{-1} gene copies/16S rDNA copy (Helsens et al. 2020).
195 Indeed, fish skin is characterized by mucus secretion that constitutes an immune barrier colonized by
196 different commensal or opportunistic bacterial species belonging mainly to the Proteobacteria,
197 Firmicutes and Acidobacteria phyla which may carry ARGs (Minniti et al. 2017). The intestinal cells of
198 fish also secrete mucus to protect them from aggression and fish gut is thus defined by a high
199 bacterial diversity that may be associated with ARGs. In fact, high abundances of aminoglycoside, β -
200 lactam and tetracycline resistance genes have been observed in the intestine of wild soles
201 (*Cynoglossus semilaevis*) from Bohai Bay in northeast China (Jia et al. 2022). Similarly to our results,
202 ARGs such as *sul1* and *bla_{TEM}* were quantified from intestinal mucus samples of carp (*Cyprinus*
203 *carpio*), barbel (*Luciobarbus graellsii*) and trout (*Salmo trutta*) in Spain (Marti et al. 2018). In addition,
204 the fish gills are a key organ for respiration and exchange with the environment and are subject to
205 the accumulation of environmental contaminants such as ARGs through the filtration of large
206 volumes of water. In the Mediterranean Sea, several strains of Enterobacteriaceae have been
207 isolated from gills and intestines of sardines (*Sardina pilchardus*), salema (*Sardina pilchardus*) and
208 horse mackerel (*Trachurus trachurus*) with a 23 % prevalence of the *bla_{TEM}* gene and 17 % of the *sul1*
209 gene among the 64 isolates (Brahmi et al. 2018) which was consistent with our results on flatfish
210 samples. In our entire research project on the AMR in the marine environment, the analysis of
211 enriched and non-enriched seawater (Bourdonnais et al. 2022a) and benthic food web samples
212 (phytoplankton and zooplankton communities (Bourdonnais et al. 2022b), *Aequipecten opercularis*
213 (scallop) (Bourdonnais et al. 2022b), and flatfish) revealed a total prevalence of 81.4% of the four
214 antimicrobial resistance indicator genes in the English Channel and North Sea, with a prevalence of
215 39.5% for the *tet(A)* gene, 36.7% for the *bla_{TEM}* gene, 57.1% for the *sul1* gene and 61.0% for the
216 *int11* gene. The *sul1/int11* genes were the most quantified indicators in seawater, phytoplankton,
217 zooplankton, bivalve mollusc and flatfish samples. The *tet(A)/bla_{TEM}* genes were poorly quantified in
218 the samples, but had a high prevalence in sample enrichments, notably gills and flatfish viscera.
219 Seabed fauna such as flatfish is particularly exposed to skin AMR contamination because of direct
220 contact with sediments that constitute a major reservoir of ARGs (Chen et al. 2013). ARGs can also
221 accumulate in the gills as fish filter large volumes of seawater containing ARGs as we have shown in a
222 previous study (Bourdonnais et al. 2022a), and also into their gut by consuming contaminated lower
223 trophic level marine species such as plankton and bivalve mollusks (Bourdonnais et al. 2022b), also
224 potential carriers of AMR (Xue et al. 2021; Silva et al. 2018). Due to the migratory flows of flatfish
225 and their movement favored by marine currents, these marine species can contribute to the

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



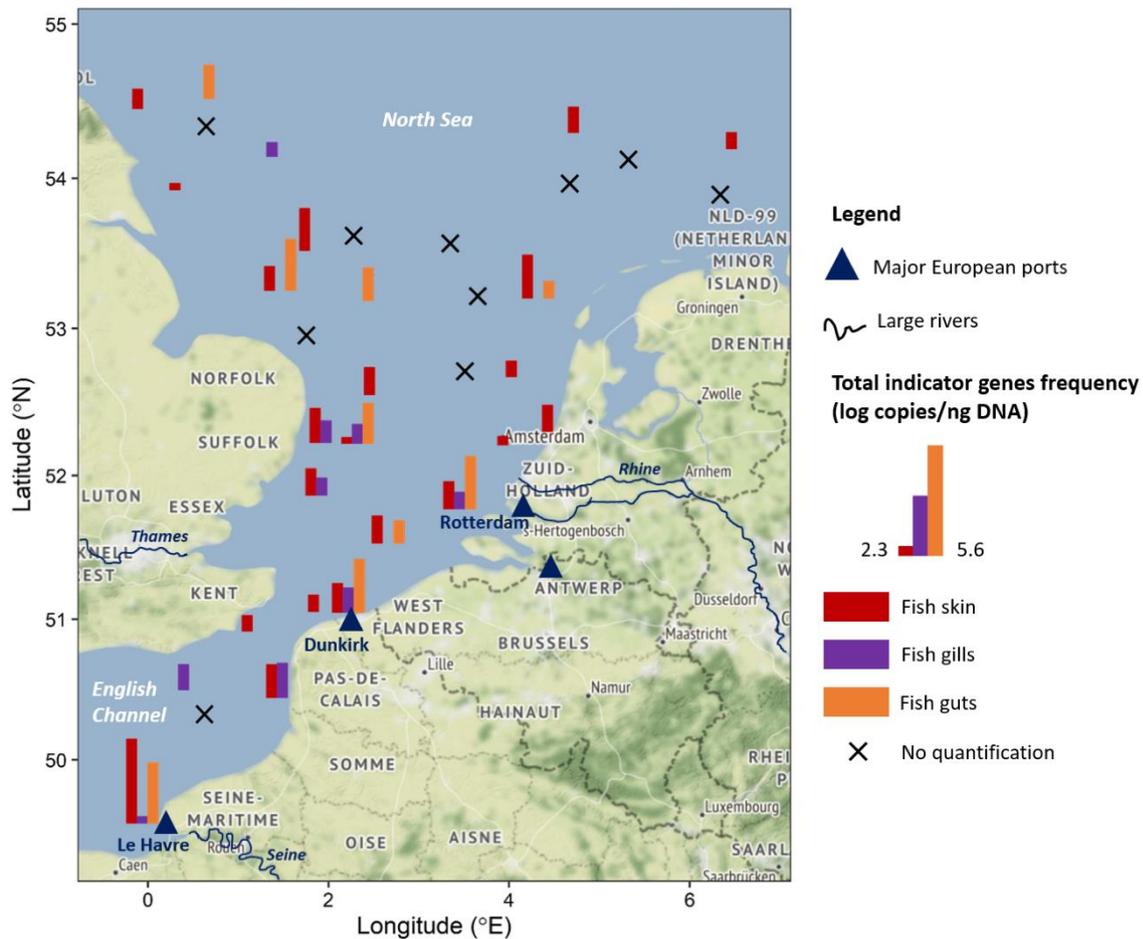
241
 242 Figure 1 : Prevalence (A) and frequency (B) of the *tet(A)*, *bla_{TEM}*, *sul1* and *int11* AMR indicator genes in the different samples
 243 (skin, gills, guts) from pooled flatfish.

244 3.2. Identification of anthropogenic pressures in the English Channel and the North Sea

245 We postulated that the frequency of the AMR indicator genes, observed in fish samples, could reflect
 246 the presence of anthropogenic inputs, i.e. sources of contamination of the marine environment. The
 247 total frequency of the indicator genes, corresponding to the number of copies of the four *tet(A)*, *bla_{TEM}*,
 248 *sul1* and *int11* genes per ng of DNA, was plotted on a map of the study area (the English Channel and
 249 the North Sea) for each flatfish sample type to better identify the potential link with anthropogenic
 250 sources of contamination (Fig. 2). A more significant contamination of fish with AMR, as reflected by
 251 the quantification of the total indicator genes in all three sample types (skin, gills, guts), was observed
 252 in the English Channel and the North Sea near European ports, namely Le Havre (France), Dunkirk
 253 (France) and Rotterdam (Netherlands). In addition to these port activities, two of the three areas are
 254 subject to the effluents from large rivers such as the Seine in Le Havre port and the Rhine in Rotterdam

255 port. The contamination of these rivers by AMR has been reported in previous studies such as the
256 presence of multi-resistant *E. coli* strains associated with a high prevalence of the *int11* gene in the
257 Seine and high abundances of the *int11*, *sul1*, *ermB* and *tetM* genes in the Rhine (Laroche et al. 2009;
258 Paulus, Hornstra, et Medema 2020). We also observed high contamination of flatfish near the East
259 Coast of England, an area characterized by a cluster of wind farms. Indeed, offshore wind farms can
260 act as artificial reefs attracting marine species such as fish and crustaceans (Degraer et al. 2020). These
261 species attract higher trophic level marine species that consume them, such as mammals and birds,
262 which may also be a vector of contamination by AMR in the marine environment. A study conducted
263 in the North Sea highlighted the attraction of some bird species such as gulls to these wind platforms
264 (Vanermen et al. 2015) while the feces of marine birds were found to be an important vector of
265 contaminations with ARGs such as *bla*, *sul* and *tet* genes (Poeta et al. 2008). Finally, we noticed a
266 scattered contamination of flatfish in the North Sea, near the coasts, but also in the open sea. This
267 observation may reflect an important dissemination of AMR in marine environment favored by the
268 migration of flatfish over long distances and facilitated by deep ocean currents.

269 There are two possible pathways of wild flatfish contamination with ARGs. The first is characterized by
270 direct contact with ARGs or ARG-carrying bacteria in free form in the water column and originating
271 from effluents from land-based sources. The second is based on a transfer of ARGs from the marine
272 sediments, which are known to be a reservoir of AMR, resulting from the sedimentation of the ARGs
273 following the discharges into the marine environment (Chen et al. 2013). ARGs could therefore be
274 associated with the skin of flatfish through close contact with contaminated sediment or seawater, but
275 also the gills as fish filter large volumes of seawater through brachial respiration, seawater being also
276 a reservoir of ARGs. Indeed, we showed in a previous study that surface waters of the English Channel
277 and the North Sea were an important carrier of the *sul1* and *int11* AMR indicator genes, detected in
278 approximately half the seawater samples collected, with abundances of up to 3.6 gene copies/ml
279 seawater (Bourdonnais et al. 2022a). However, we observed a higher level of AMR contamination in
280 flatfish collected near the ports while seawater contamination was higher off the West Coast of the
281 Netherlands. These results indicate that ARGs disseminate in seawater at the surface but also in the
282 seabed associated with flatfish. ARGs released into the marine environment can also accumulate in
283 lower trophic level marine species such as plankton that flatfish feed on and whose role in AMR
284 transport is still understudied compared to freshwater environments (Xue et al. 2021). This
285 accumulation of ARGs in the digestive system of wild fish thus represents a risk of dissemination in the
286 environment with release via feces and transmission between individuals through close contact.
287 Furthermore, all these results lead us to believe that the *sul1* and *int11* genes would be suitable
288 indicators for assessing AMR contamination of the marine ecosystem, their quantification allowing us
289 to identify sources of contamination while assessing the potential for AMR transfer via integrons.
290 Indeed, the class 1 integrase is associated with integrons, genetic structures that can transport and
291 exchange ARGs between different bacteria, and is therefore an important component in the
292 dissemination of AMR in bacterial populations and the environment. Previous studies have
293 demonstrated a correlation between the *int11* gene and various measures of human impact in aquatic
294 environments, making this gene an ideal candidate to be an indicator gene for AMR for the marine
295 environment (Gillings et al. 2015; Pruden, Arabi, et Storteboom 2012). The *sul1* gene encoding
296 sulfonamide resistance is very often associated with these class 1 integrons, thus easily transferable
297 within bacterial communities and potentially leading to the emergence of multidrug resistant bacterial
298 strains. It is important to pursue studies and to monitor AMR not only in seawater, but also in various
299 wild marine species, which correspond to two essential dimensions of the One Health perspective, in
300 order to improve our understanding of the risk caused by AMR to human health, the third component
301 of the One Health framework.



303

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

306 4. Conclusion

307 In summary, we found that the four *tet(A)*, *bla_{TEM}*, *sul1* and *int11* AMR indicator genes were present in
 308 the skin, gills and gut of wild flatfish with an overall prevalence of 71.4 %. The *sul1* and *int11* genes
 309 were more prevalent in skin samples than in gills and guts, and the frequency of all four genes were
 310 lowest in the fish gut samples. In our entire project on the research of the four *tet(A)*, *bla_{TEM}*, *sul1* and
 311 *int11* AMR indicator genes in the marine environment, we described 81.4% of the four antimicrobial
 312 resistance indicator genes in the English Channel and North Sea, with a prevalence of 39.5% for the
 313 *tet(A)* gene, 36.7% for the *bla_{TEM}* gene, 57.1% for the *sul1* gene and 61.0% for the *int11* gene. The
 314 *sul1/int11* genes were the most quantified indicators. The prevalence and abundance of these genes
 315 varied not only according to the nature of the sample, but also according to the sampling area, due to
 316 the different anthropogenic impacts associated with high gene dissemination. Greater contamination
 317 of samples was observed in the Eastern Channel, an area considered to be a veritable freeway of the
 318 sea due to intense maritime traffic and involving various discharges into the marine environment, as
 319 well as at the mouth of the Thames, which is subject to river effluents. Seawater samples were more
 320 contaminated with antimicrobial resistance indicator genes near the West Coast of the Netherlands,
 321 an area characterized by the presence of offshore wind and oil platforms. The *sul1* and *int11* genes are
 322 good indicators of marine environmental contamination. Marine fish and environment, two major
 323 components of the One Health framework, are important vectors of AMR. They pose a risk of

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

327 **Funding**

328 This study was supported by a doctoral fellowship from Région Hauts-de-France and Pôle
329 Métropolitain de la Côte d'Opale (PMCO).

330 **CRedit authorship contribution statement**

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

335 **Declaration of Competing Interest**

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

338 **Acknowledgements**

339 We thank the Ifremer institute for allowing us to be part of the IBTS oceanographic campaign to collect
340 the flatfish samples analyzed in this study. The graphical abstract was carried out on Biorender.com
341 under the agreement number OW25BKRVVG.

342 **References**

- 343 ANSES. 2020. « Antibiorésistance et environnement : état et causes possibles de la contamination
344 des milieux en France ». <https://www.anses.fr/fr/system/files/EAUX2016SA0252Ra.pdf>
- 345 Barraud, O., M. C. Baclet, F. Denis, et M. C. Ploy. 2010. « Quantitative Multiplex Real-Time PCR for
346 Detecting Class 1, 2 and 3 Integrons ». *The Journal of Antimicrobial Chemotherapy* 65 (8): 1642-45.
347 <https://doi.org/10.1093/jac/dkq167>.
- 348 Berendonk, Thomas U., Célia M. Manaia, Christophe Merlin, Despo Fatta-Kassinos, Eddie Cytryn,
349 Fiona Walsh, Helmut Bürgmann, et al. 2015. « Tackling antibiotic resistance: the environmental
350 framework ». *Nature Reviews Microbiology* 13 (5): 310-17. <https://doi.org/10.1038/nrmicro3439>.
- 351 Bibbal, D., V. Dupouy, J. P. Ferré, P. L. Toutain, O. Fayet, M. F. Prère, et A. Bousquet-Mélou. 2007.
352 « Impact of Three Ampicillin Dosage Regimens on Selection of Ampicillin Resistance in
353 Enterobacteriaceae and Excretion of blaTEM Genes in Swine Feces. » *Applied and Environmental*
354 *Microbiology* 73 (15): 4785-90. <https://doi.org/10.1128/AEM.00252-07>.
- 355 Bodor, Attila, Naila Bounedjoun, György Erik Vincze, Ágnes Erdeiné Kis, Krisztián Laczi, Gábor Bende,
356 Árpád Szilágyi, Tamás Kovács, Katalin Perei, et Gábor Rákhely. 2020. « Challenges of unculturable
357 bacteria: environmental perspectives ». *Reviews in Environmental Science and Bio/Technology* 19 (1):
358 1-22. <https://doi.org/10.1007/s11157-020-09522-4>.
- 359 Bourdonnais, Erwan, Darina Colcanap, Cédric Le Bris, Thomas Brauge, et Graziella Midelet. 2022a.
360 « Occurrence of Indicator Genes of Antimicrobial Resistance Contamination in the English Channel
361 and North Sea Sectors and Interactions With Environmental Variables ». *Frontiers in Microbiology* 13.
362 <https://www.frontiersin.org/articles/10.3389/fmicb.2022.883081>.

363 Bourdonnais, Erwan. 2022b. « Étude des gènes de résistance aux antibiotiques d'intérêt clinique au
364 sein d'un réseau trophique marin ». <https://theses.hal.science/tel-04000329>.

365 Brahmi, Soumia, Abdelaziz Touati, Catherine Dunyach-Remy, Albert Sotto, Alix Pantel, et Jean-
366 Philippe Lavigne. 2018. « High Prevalence of Extended-Spectrum β -Lactamase-Producing
367 Enterobacteriaceae in Wild Fish from the Mediterranean Sea in Algeria. » *Microbial Drug Resistance*
368 (*Larchmont, N.Y.*) 24 (3): 290-98. <https://doi.org/10.1089/mdr.2017.0149>.

369 Chen, Baowei, Ying Yang, Ximei Liang, Ke Yu, Tong Zhang, et Xiangdong Li. 2013. « Metagenomic
370 Profiles of Antibiotic Resistance Genes (ARGs) between Human Impacted Estuary and Deep Ocean
371 Sediments. » *Environmental Science & Technology* 47 (22): 12753-60.
372 <https://doi.org/10.1021/es403818e>.

373 Degraer, Steven, Drew Carey, Joop Coolen, Zoe Hutchison, Francis Kerckhof, Bob Rumes, et Jan
374 Vanaverbeke. 2020. « Offshore Wind Farm Artificial Reefs Affect Ecosystem Structure and
375 Functioning: A Synthesis ». *Oceanography* 33 (décembre): 48-57.
376 <https://doi.org/10.5670/oceanog.2020.405>.

377 EUMOFA. 2021. « European Market Observatory for Fisheries and Aquaculture Products - The UE fish
378 market, édition 2021 ». https://www.eumofa.eu/documents/20178/477018/EN_The+EU+fish+market_2021.pdf

380 FAO. 2020. « The State of World Fisheries and Aquaculture 2020: Sustainability in action. Rome ». <https://doi.org/10.4060/ca9229en>.

382 Gillings, Michael R., William H. Gaze, Amy Pruden, Kornelia Smalla, James M. Tiedje, et Yong-Guan
383 Zhu. 2015. « Using the Class 1 Integron-Integrase Gene as a Proxy for Anthropogenic Pollution. » *The*
384 *ISME Journal* 9 (6): 1269-79. <https://doi.org/10.1038/ismej.2014.226>.

385 Giraldo, C., Ernande, B., Cresson, P., Kopp, D., Cachera, M., Travers-Trolet, M. et Lefebvre, S. 2017.
386 « Depth gradient in the resource use of a fish community from a semi-enclosed sea ». *Limnology and*
387 *Oceanography*, 62, 2213-2226.

388 Grevskott, Didrik H., Cecilie S. Svanevik, Marianne Sunde, Astrid L. Wester, et Bjørn T. Lunestad.
389 2017. « Marine Bivalve Mollusks As Possible Indicators of Multidrug-Resistant Escherichia Coli and
390 Other Species of the Enterobacteriaceae Family. » *Frontiers in Microbiology* 8: 24.
391 <https://doi.org/10.3389/fmicb.2017.00024>.

392 Han, Ying, Yikai Hu, Hexin Wen, Jiayue Wu, Yuanwang Liu, Yingchao Zhang, et Hao Wu. 2022.
393 « Occurrence and driving mechanism of antibiotic resistance genes in marine recreational water
394 around Qinhuangdao, China ». *Frontiers in Marine Science* 9: 976438.

395 Helsen, Nicolas, Ségolène Calvez, Hervé Prevost, Agnès Bouju-Albert, Aurélien Maillet, Albert
396 Rossero, Dominique Hurtaud-Pessel, Monique Zagorec, et Catherine Magras. 2020. « Antibiotic
397 Resistance Genes and Bacterial Communities of Farmed Rainbow Trout Fillets (*Oncorhynchus*
398 *Mykiss*). » *Frontiers in Microbiology* 11: 590902. <https://doi.org/10.3389/fmicb.2020.590902>.

399 Heuer, Holger, et Kornelia Smalla. 2007. « Manure and Sulfadiazine Synergistically Increased Bacterial
400 Antibiotic Resistance in Soil over at Least Two Months. » *Environmental Microbiology* 9 (3): 657-66.
401 <https://doi.org/10.1111/j.1462-2920.2006.01185.x>.

402 Hocquet, Didier, Pierre Maris, Sandrine Baron, Delphine Bibbal, Olivier Chesneau, Christophe Dagot,
403 Marisa Haenni, Jérôme Labanowski, Fabrice Martin-Laurent, et Sylvie Nazaret. 2020.

404 « Antibiorésistance et environnement-État et causes possibles de la contamination des milieux en
405 France par les antibiotiques, les bactéries résistantes aux antibiotiques et les supports génétiques de
406 la résistance aux antibiotiques ». Anses.

407 ICES. 2015. « Manual for the International Bottom Trawl Surveys. Revision IX. »

408 Jia, Lei, Hao Liu, Na Zhao, Qixia Deng, Chunhua Zhu, et Bo Zhang. 2022. « Distribution and Transfer
409 of Antibiotic Resistance Genes in Coastal Aquatic Ecosystems of Bohai Bay ». *Water* 14 (6).
410 <https://doi.org/10.3390/w14060938>.

411 Laroche, Emilie, Barbara Pawlak, Thierry Berthe, David Skurnik, et Fabienne Petit. 2009. « Occurrence
412 of Antibiotic Resistance and Class 1, 2 and 3 Integrons in Escherichia Coli Isolated from a Densely
413 Populated Estuary (Seine, France). » *FEMS Microbiology Ecology* 68 (1): 118-30.
414 <https://doi.org/10.1111/j.1574-6941.2009.00655.x>.

415 Marti, Elisabet, Belinda Huerta, Sara Rodríguez-Mozaz, Damià Barceló, Rafael Marcé, et Jose Luis
416 Balcázar. 2018. « Abundance of Antibiotic Resistance Genes and Bacterial Community Composition in
417 Wild Freshwater Fish Species. » *Chemosphere* 196 (avril): 115-19.
418 <https://doi.org/10.1016/j.chemosphere.2017.12.108>.

419 Merrifield, Daniel L., et Ana Rodiles. 2015. « The fish microbiome and its interactions with mucosal
420 tissues ». In *Mucosal health in aquaculture*, 273-95. Elsevier.

421 Minniti, Giusi, Live Heldal Hagen, Davide Porcellato, Sven Martin Jørgensen, Phillip B. Pope, et Gustav
422 Vaaje-Kolstad. 2017. « The Skin-Mucus Microbial Community of Farmed Atlantic Salmon (*Salmo*
423 *Salar*). » *Frontiers in Microbiology* 8: 2043. <https://doi.org/10.3389/fmicb.2017.02043>.

424 Murray, Christopher JL, Kevin Shunji Ikuta, Fablina Sharara, Lucien Swetschinski, Gisela Robles
425 Aguilar, Authia Gray, Chieh Han, Catherine Bisignano, Puja Rao, et Eve Wool. 2022. « Global burden
426 of bacterial antimicrobial resistance in 2019: a systematic analysis ». *The Lancet* 399 (10325): 629-55.

427 Muziasari, W. I., Pitkänen, L. K., Sørnum, H., Stedtfeld, R. D., Tiedje, J. M. et Virta, M. 2017. « The
428 resistome of farmed fish feces contributes to the enrichment of antibiotic resistance genes in
429 sediments below Baltic Sea fish farms ». *Frontiers in Microbiology*, 7.

430 Ng, L. K., I. Martin, M. Alfa, et M. Mulvey. 2001. « Multiplex PCR for the Detection of Tetracycline
431 Resistant Genes. » *Molecular and Cellular Probes* 15 (4): 209-15.
432 <https://doi.org/10.1006/mcpr.2001.0363>.

433 Paul, Christophe, Zhanna Bayrychenko, Thomas Junier, Sevasti Filippidou, Karin Beck, Matthieu
434 Bueche, Gilbert Greub, Helmut Bürgmann, et Pilar Junier. 2018. « Dissemination of Antibiotic
435 Resistance Genes Associated with the Sporobiota in Sediments Impacted by Wastewater. » *PeerJ* 6:
436 e4989. <https://doi.org/10.7717/peerj.4989>.

437 Paulus, Gabriela K., Luc M. Hornstra, et Gertjan Medema. 2020. « International Tempo-Spatial Study
438 of Antibiotic Resistance Genes across the Rhine River Using Newly Developed Multiplex qPCR
439 Assays. » *The Science of the Total Environment* 706 (mars): 135733.
440 <https://doi.org/10.1016/j.scitotenv.2019.135733>.

441 Poeta, Patricia, Hajer Radhouani, Gilberto Igrejas, Alexandre Gonçalves, Carlos Carvalho, Jorge
442 Rodrigues, Laura Vinué, Sergio Somalo, et Carmen Torres. 2008. « Seagulls of the Berlengas Natural
443 Reserve of Portugal as Carriers of Fecal Escherichia Coli Harboring CTX-M and TEM Extended-
444 Spectrum Beta-Lactamases. » *Applied and Environmental Microbiology* 74 (23): 7439-41.

445 <https://doi.org/10.1128/AEM.00949-08>.

446 Pruden, Amy, Mazdak Arabi, et Heather N. Storteboom. 2012. « Correlation between Upstream
447 Human Activities and Riverine Antibiotic Resistance Genes. » *Environmental Science & Technology* 46
448 (21): 11541-49. <https://doi.org/10.1021/es302657r>.

449 Silva, Irana Paim, Camila de Souza Carneiro, Margarete Alice Fontes Saraiva, Thiago Alves Santos
450 de Oliveira, Oscarina Viana de Sousa, et Norma Suely Evangelista-Barreto. 2018. « Antimicrobial
451 resistance and potential virulence of *Vibrio parahaemolyticus* isolated from water and bivalve
452 mollusks from Bahia, Brazil ». *Marine Pollution Bulletin* 131 (juin): 757-62.
453 <https://doi.org/10.1016/j.marpolbul.2018.05.007>.

454 Vanermen, Nicolas, Thierry Onkelinx, Wouter Courtens, Marc Van de walle, Hilbran Verstraete, et
455 Eric W. M. Stienen. 2015. « Seabird avoidance and attraction at an offshore wind farm in the Belgian
456 part of the North Sea ». *Hydrobiologia* 756 (1): 51-61. <https://doi.org/10.1007/s10750-014-2088-x>.

457 WHO. 2022. « Antimicrobial resistance surveillance in Europe 2022–2020 data ».

458 Xue, Xue, Lihong Wang, Haoran Xing, Yu Zhao, Xiangju Li, Gaoxue Wang, et Zaizhao Wang. 2021.
459 « Characteristics of Phytoplankton-Zooplankton Communities and the Roles in the Transmission of
460 Antibiotic Resistance Genes under the Pressure of River Contamination. » *The Science of the Total
461 Environment* 780 (août): 146452. <https://doi.org/10.1016/j.scitotenv.2021.146452>.

462 Zhang, Shengcen, Jiangqing Huang, Zhichang Zhao, Yingping Cao, et Bin Li. 2020. « Hospital
463 Wastewater as a Reservoir for Antibiotic Resistance Genes: A Meta-Analysis. » *Frontiers in Public
464 Health* 8: 574968. <https://doi.org/10.3389/fpubh.2020.574968>.

465 Ziarati, Mina, Mohammad Jalil Zorriehzahra, Fatemeh Hassantabar, Zibandeh Mehrabi, Manish
466 Dhawan, Khan Sharun, Talha Bin Emran, Kuldeep Dhama, Wanpen Chaicumpa, et Shokoofeh Shamsi.
467 2022. « Zoonotic Diseases of Fish and Their Prevention and Control. » *The Veterinary Quarterly* 42
468 (1): 95-118. <https://doi.org/10.1080/01652176.2022.2080298>.

469
470

471 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
<i>tet(A)</i>	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		
<i>bla_{TEM}</i>	TTCCTGTTTTGCTCACCCAG	(Bibbal et al. 2007)	
	CTCAAGGATCTTACCGCTGTTG		
<i>sul1</i>	CCGTTGGCCTTCCTGTAAAG	(Heuer et Smalla 2007)	
	TTGCCGATCGCGTGAAGT		
	(FAM)CAGCGAGCCTTGCGGCGG(TAMRA)		
<i>intl1</i>	GCCTTGATGTTACCCGAGAG	(Barraud et al. 2010)	
	GATCGGTGCGAATGCGTGT		
	(6-FAM)ATTCCTGGCCGTGGTTCTGGGTTTT(BHQ1)		

472