ASK network for antimicrobial resistance in seafood as common ground for knowledge exchange

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ASK network for antimicrobial resistance in seafood as common ground for knowledge exchange and risk assessment

Abstract

The project entitled “AMR in seafood as common ground for knowledge exchange and risk assessment” (ASK) has been conducted with five partner organizations (Anses, IMR, Cefas, DTU, IZSUM) that expressed interest to join and build a consortium according to their capacity and technical expertise in the field of AMR in seafood. As there was no consensus established yet on what, where and how to undertake AMR surveillance in seafood, the objective of ASK was to i) share between partners, knowledge and expertise on antimicrobial resistance (AMR) in seafood, ii) propose approaches of methodology and guidelines for collecting AMR data in seafood, iii) unravel knowledge gaps for conducting an accurate risk assessment analysis (RA), including the data needed for identification and characterization of the hazard. In order to approach these three main objectives, a workshop was organized to gather a group of pathfinder experts, comprising risk assessors, microbiologists and molecular biologists.

The prevalence of AMR bacteria in seafood is of increasing importance as aquaculture production and seafood consumption are growing worldwide. Despite the fact that current data origin from studies that were non-consistent in methods, multidrug-resistant bacteria of clinical importance have been identified in seafood. This included bivalves from several countries, but in particular seafood imported from Southeast Asian countries showed high occurrence of AMR, and should be investigated further. Regarding the aim to estimate the risk for consumers, two main hazards can be identified – the direct hazard posed by ingestion of seafood contaminated by zoonotic, antimicrobial-resistant bacteria, and the indirect one posed by the presence of AMR determinants in commensal bacteria of seafood, that may be transferred to the microbiota of consumers including potentially pathogenic isolates. To estimate the risk for consumers, seafood must be sampled as close to the consumer as possible and sampling in term of type and number of samples should be representative of consumption of the human population. In the frame of a future implementation of monitoring, systematic random sampling at retail may be the best choice. The outcome from the ASK project highlights the need to maintain such a network to provide more standardized data on AMR in seafood in order to make a well-founded statement on the situation in this sector. Such baseline monitoring would allow risk assessment analysis at the relevant consumer stage. Future data from seafood should be compared to human data, and other animal and environmental sectors in the frame of One Health approach.

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1- Introduction

1-1 Context

Tackling antimicrobial resistance (AMR) in animals, food and humans is a key priority for the European Food Safety Authority (EFSA), as well as public health authorities and different Agencies at National level. Current antimicrobial resistance (AMR) monitoring programs and risk assessment studies on food as a potential transmission route for AMR focus on terrestrial food-producing animals, whereas the available knowledge on AMR in seafood, is limited. Bacteria of terrestrial origin, including those conferring AMR, reach the aquatic environments through runoff from land, faeces from wild animals and birds, or through sewage systems. Furthermore, use of antimicrobials in aquaculture can directly select for AMR bacteria that reach consumers via foodborne transfer. Any exposed food products harvested from aquatic environments may therefore be vectors for transmission and re-transmission of AMR back to humans, and risk assessment should be performed. In most countries, systematic surveillance of AMR in the aquatic environment or in seafood is only at the pilot study level or is lacking. Therefore, the knowledge in this field lags behind what is known for the human and veterinary sectors. Global seafood production is growing each year, and although international surveillance programs on key pathogens exist for some seafood items, AMR surveillance has not been implemented as it has for the zoonosis monitoring plans in terrestrial food animals.

As the European Commission pointed out in the 2017 report “A European One Health Action Plan against Antimicrobial resistance “, knowledge gaps exist in terms of the contribution of the environment to the AMR crisis in humans and there is need to determine appropriate indicator bacteria species for measuring occurrence and temporal trends of AMR in the marine sector (https://ec.europa.eu/health/amr/sites/amr/files/amr).The EFSA Emerging Risks Exchange Network (EREN) group recently emphasised the emergence of AMR in imported fish and seafood products (EFSA, 2016). Moreover, the technical specifications released by EFSA are currently under review in order to account for new scientific developments and data collection requirements, and to enlarge the scope of the AMR monitoring to allow for the collection of molecular typing data. Development of a rationale for AMR monitoring in seafood and reflection on potential methodological approaches for risk assessments were the key focus areas for knowledge exchanges among ASK partners.

The ASK project supports the definition of AMR described in the European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017: “AMR is the ability of microorganisms, such as bacteria, to become increasingly resistant to an antimicrobial to which they were previously susceptible” (EFSA, 2019). AMR can be acquired by mutations of genes encoded by the bacterial chromosome or by transfer of AMR genes encoded by mobile genetic element (MGE) such as plasmids and transposons. Acquisition of AMR could be facilitated by different factors, including use of antimicrobials in human and veterinary medicine and in agriculture, which may also be dependent on hygiene conditions and practices in healthcare settings or in the food chain.

Experts participating in the workshop highlighted that AMR can be considered in three different ways:

1- In a clinical perspective, whereby resistance is the ability of a bacterium to survive antimicrobial therapy.
2- In microbiological terms, whereby resistance is the property of a bacterium to survive at higher antimicrobial concentrations compared to other members of its species.

3- In genetic terms, whereby resistance is defined by the presence of mutations and/or genes mediating reduced antimicrobial susceptibility.

These definitions are of great importance for further risk assessment considerations and hazard identification.

1-2 Presentation of ASK project

This project entitled “AMR in Seafood as common ground for Knowledge exchange and risk assessment” (ASK) was designed in response to the EFSA call for Partnering Grants with an aim of knowledge exchange between partners. This was a 15 month-project and core partners were IMR (Norway), IZSUM (Italy), CEFAS (UK), DTU-Food (Denmark) and ANSES as coordinator. The project partners express a strong willingness to assess and address contribution of seafood to the global burden of AMR to human health, answering to the three main objectives defined by the project:

- Sharing knowledge and expertise on antimicrobial resistance (AMR) in seafood.
- Definition of methodology and guidelines for data collection and AMR monitoring in seafood.
- Identification of knowledge gaps for conducting an accurate risk assessment analysis (RA), including the data needed for the identification and characterization of the hazard.

The ASK project aimed to provide:

1) Knowledge and expertise on AMR in seafood.
2) A summary of gaps in knowledge that need to be to addressed in future research and monitoring plan to enable adequate assessment of the risk of AMR bacteria in seafood.

The project focused mainly on AMR in seafood from the marine sector, including both capture fishery and aquaculture species of both national and imported origin. Seafood can be harvested/grown in different areas with a wide variety of salinities, such as freshwater, brackish coastal areas, open sea and estuarine areas. There is not a clear separation between what is grown in marine and brackish environments, and this further complicates what could be considered as coming from the marine sector. For the purpose of surveillance and risk assessment in this project, species from the freshwater (limnic) environment were not specifically considered. However, resistance among freshwater bacteria were reported and consideration of these sector should be included in future projects.

The use of antimicrobials (ATB) in aquaculture selects for antimicrobial-resistant bacteria which are carried by farmed fish (Higuera-Llantén S. et al, 2018), and could reach humans either via direct contact or via foodborne transmission. However, the use of antimicrobials in fish is not, most likely, the only factor determining occurrence of resistant bacteria in fish. Several antimicrobial resistance genes (ARG) occur naturally in the environment, likely as an evolutionary consequence to allow survival in presence of naturally produced antibiotics, and thus the environment represents a reservoir of ARGs that fish bacteria can uptake and potentially transfer to humans. Furthermore, use of other chemicals, such as biocides is known to co-select for AMR bacteria and biocide use is common in seafood production and processing facilities (Joint FAO/WHO expert meeting reports, 2018). Otherwise, resistance genes to biocides or heavy metals have been described in bacteria carrying ARG, (Sellera et al, 2018, Mcintosh et al, 2008).
In summary, all steps of the seafood chain, i.e. production, processing and consumption (Figure 1), could potentially affect the prevalence of AMR bacteria.

The ASK project aimed to take into consideration several questions related to sampling strategies, definitions of indicator bacteria and relevant bacterial species targets, and AST methods. The World Health Organisation (WHO) Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) guidance considered surveillance on antimicrobial resistance in cross-sectorial fields: food, humans, animals and the environment (WHO, 2017). The European Commission pointed out in "A European One Health Action Plan against Antimicrobial resistance" 2017 report, along with WHO and FAO reports that knowledge gaps in terms of role of environment, the need for determining indicator bacteria in the marine sector (https://ec.europa.eu/health/amr/sites/amr/files/amr_action_plan_2017_en.pdf).
Regarding risk assessment, questions were raised about the relevance, need and availability of data. An assessment of AMR bacteria in the food chain has been achieved in Norway and could serve as basis for future risk assessment analysis (VKM, 2015).

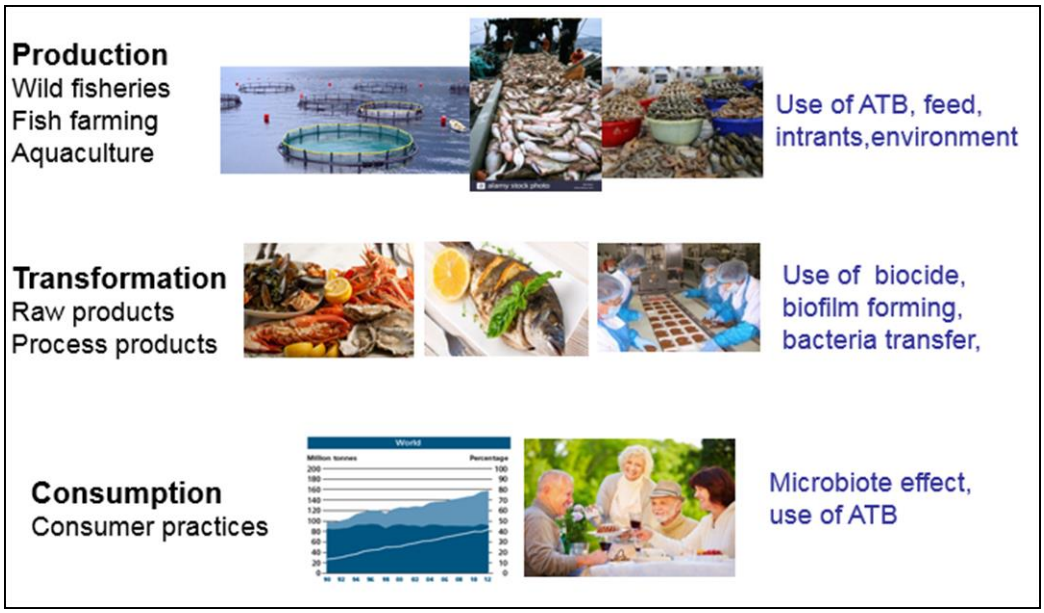

Figure1: The main steps of the seafood chain and potential factors contributing to occurrence of antimicrobial resistant bacteria, ATB: Antimicrobials

Currently, there are no regulations to monitor the presence of antimicrobial resistance in seafood despite this commodity can be contaminated by AMR bacteria from different sources. This underpins the importance of need to assess occurrence and characterize AMR in seafood. Rationale and methodological considerations for AMR monitoring in seafood as well as data needed for risk assessment analysis are discussed in this report.

2- Methodology of working

A kick-off meeting was held at EFSA offices in Parma, Italy in January 2018. During this, the project partners discussed how to identify the hazards of interest, the role of certain environmental factors, antimicrobial use, farming conditions and food processing, how resistance is being disseminated and links with other food sectors, since multiple factors impact on the prevalence of AMR in the environment. Large amounts of partially-treated urban wastewater are discharged in the sea, and studies have shown that this increases the abundance of AMR genes in the marine environment, and hence, the oceans are a reservoir

for AMR genes. Humans could be exposed to AMR bacteria or to AMR genes from the marine environment via seafood, aquaculture production and through recreational activity.

Within the framework of the ASK project, the first step for sharing knowledge was to produce an inventory of data on AMR in seafood currently available in the different partner organizations. Data from several studies carried out in different institutes were collected by circulating an Excel-based knowledge sharing table (KST) amongst the project partners. This data table was intended to serve as a basis for sharing knowledge and further information to support upcoming ASK activity. It provided an overview of current studies and monitoring undertaken by different partners. The fully completed KST table is presented in Annex 1.

The ASK project partners decided that the appropriate way to fully exchange knowledge and expertise regarding AMR in seafood was through a dedicated workshop gathering experts from the ASK core partners, together with invited expert scientist that are well-recognized for their knowledge on the subject. The workshop agenda was structured into three different sessions. The first session was informed by the KST and dealt with existing data and methods used by ASK members. The second session focused on the risk assessment approach and the third session addressed aims and methods for AMR monitoring.

The ASK workshop was held at the Anses headquarters in Maisons-Alfort, Paris, France on 24 and 25th October 2018. It was a highly successful workshop divided in three sessions: “Sharing existing data on AMR in seafood”, “Towards a risk assessment approach” and “A perspective of surveillance” according to the agenda.

The objective of the ASK workshop was to gather participants with interdisciplinary scientific expertise in the field of AMR, seafood microbiology and classification, molecular microbiology, epidemiology and surveillance, risk assessment and sociology, for enabling multiple views and sharing opinions on issues of AMR in seafood. Moreover, a working group had been set up at EFSA in order to revise and harmonise monitoring protocols for AMR in food animals and food products; therefore, it was expected that this workshop could provide some inputs for this field and contribute to provide recommendations about scientific challenges to address in the forthcoming years.

The final report aims to return insights based on the ASK workshop contributions from the 33 participating ASK partners and experts. (Annex 2).

3- Sharing existing data

The section about sharing existing data aimed to provide an overview of the work that has been done by the consortium members in terms of methodology, seafood items and targeted bacteria for AMR research. All members completed a template listing their main investigations to date (Annex 1. Information was obtained on twelve investigations performed on seafood in Norway, Italy, France, Tunisia, Spain, Poland, Scotland and the UK, as well as some imported seafood of Asian and South American origin. One study pertained to ornamental fish. The table was used to plan the line-up for the workshop session. The session had an introductory talk about AMR in the marine environment. Thereafter, theme-wise talks were held by the consortium members, organized either as single bacterial target species or as multiple bacterial species approaches, all related to seafood. The session ended with a general discussion. The knowledge shared is summarized below, which is a synthesis of already

existing AMR data across the workshop talks and discussions, the knowledge sharing table and relevant literature.

3-1 Methodological approaches for AMR detection

Among the studies presented during the workshop, AMR determination performed by the consortium members was in most cases based on culture-dependent methods, either with or without selective antimicrobials in the chosen agar plates for the detection of target bacterial species. Antimicrobial susceptibility tests (AST) were mainly performed by the disk diffusion method on Mueller Hinton agar applying EUCAST or CLSI standards and interpretation protocols, if available for the targeted bacteria. In two investigations, AST was performed by minimum inhibition concentration (MIC) determination according to EUCAST or CLSI guidelines and using the EUVSEC plates which contains the mandatory antimicrobials for AMR monitoring in *Escherichia coli* and *Salmonella* from food animals and food across EU (Decision 2013/652/EU).

The choice of method for AST of bacteria from aquatic organisms is challenging, because standards are not yet developed for most of the antimicrobials and bacterial species relevant in this context. Most importantly, the bacterial growth rate will be reduced by the temperature and low levels of salt in the standard media used for AST of bacteria from livestock animals and poultry, potentially resulting in larger inhibition zone diameters and /or lower MIC which could lead to misclassification of resistant bacteria as susceptible. Furthermore, if the media were to be amended with additional salt, some antibiotics will bind to the salt and become less potent, resulting in smaller inhibition zone diameters and/or higher MIC and the possibility of overestimating resistance. For *Aeromonas* spp., recent publications have developed a robust framework to begin developing AMR interpretive criteria (Baron et al., 2017, Smith et al, 2012) and interpretive criteria for *Aeromonas* spp. have been included in EUCAST (V 9.0), but only for cefepime, ceftazidime, aztreonam, ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole (The European Committee on Antimicrobial Susceptibility Testing, 2019). For *Vibrio* spp., ongoing work related to establishing epidemiological cut-off (ECOFF) values has been initiated after discussion and networking during the ASK workshop.

One of the latest developments for AMR detection is metagenomics which does not require any a priori knowledge of target species or any specific AST protocol. Metagenomics consists of direct analysis of all DNA contained in a sample and has great advantages due to the absence of selection bias linked, for example, to cultivation. Briefly, DNA is extracted directly from the sample and prepared into libraries for sequencing. The sequence data obtained can be analysed by using different bioinformatics tools. AMR genes are then identified by mapping the reads to databases of known AMR genes, and bacterial genomes are also identified by a similar approach using public databases for read mapping. Metagenomics offer the possibility to store data virtually indefinitely, which allows re-analysis when new research questions and new scientific knowledge arise. However, the enormous amounts of data generated could become an issue due to cost of storage and computer capacity. This methodology has been applied in the framework of an EU-funded project EFFORT for pig and poultry faecal samples (http://www.effort-against-amr.eu/) (Munk et al, 2018). Currently the same method has been applied to analyse the AMR gene content, or “resistome”, of metagenomics DNA from faecal/gut material of veal calves, turkey and rainbow trout. The main limitation arising during analysis of fish samples was the presence of host DNA that could not be removed. Most likely, this should be tackled at the sample storage and DNA extraction steps and studies to standardise these procedures for fish samples are currently needed. Such metagenomics analyses are important and will become more useful in the future with improvements in

scientific and technical knowledge. However, awareness should be brought to special needs to increase quality and diversity of marine samples (Tarnecki et al, 2017).

3-2 Data on AMR in seafood species and /or marine samples

AMR data examined here are coming from a large diversity of sampling context in term of nature, source and geographical location of seafood, fish or marine samples.

The main group of seafood that has been investigated by the consortium are marine bivalves, where 1814 samples/batch samples were reported as having been examined with respect to AMR (Figure 2) (Annex 1). These filter-feeding organisms are widely used as sentinels of contamination in the marine environment as they concentrate particles from the surrounding water. The majority (1280) of these samples were bivalves in the genus *Mytilus,* which comprise different species of mussels and are commercially important and commonly consumed in specific European countries. Furthermore, 248 samples of scallops and 147 samples of oysters represent groups of bivalves that are consumed raw or only lightly cooked and therefore may act as direct transmission vehicles of AMR bacteria to the consumer. Other bivalves were less frequently examined, though the complete sample list comprised 14 different species. Most of the included samples were from ongoing national surveillance programs for faecal contamination in bivalves, where monitoring of *Escherichia coli* is one of the parameters. Bivalve species that are already sampled for established surveillance programs have been identified as important candidates for surveillance of AMR in seafood and the marine environment.

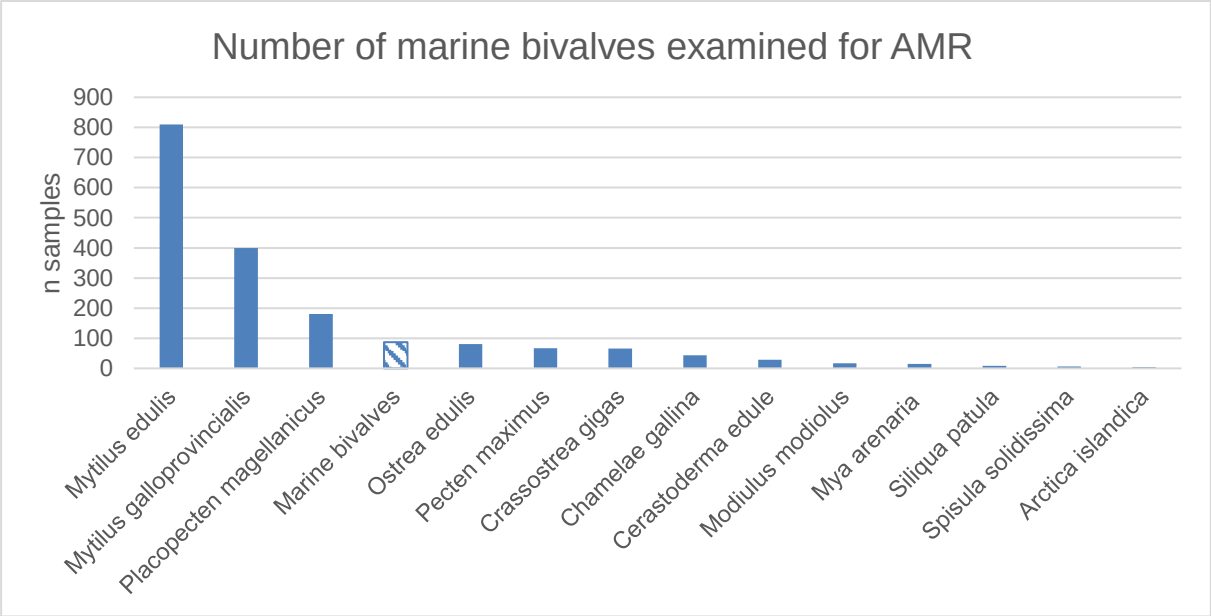

Figure 2: **Overview of the number of examined marine bivalve samples, by species. The bar "Marine bivalves" include samples where the species is not specified.**

Two investigations concerned farmed salmonid fish (n=234) from intensive aquaculture production, where usage of antimicrobials and prevalence of AMR have been a focus for decades. In contrast to many farmed fish species, the development of effective vaccines against the most important bacterial infections in salmonids has led to a sharp decrease in the application of antibiotics. In Norwegian aquaculture, salmonid production has increased from approximately 60 thousand metric tonnes in 1987 to around 1.3 million tonnes in 2018, whereas the use of antibiotics in production has decreased by 98 % during the same period (NORM-VET 2016).

The French RESAPTH network consists of 71 public and private volunteer labs that send sample and isolated bacteria from diagnostic specimens of diseased animals through routine veterinary activity. The data is submitted automatically, hence there is no control over the frequency and timing of sampling. The system monitors AMR in pathogenic bacteria from animals and characterizes the resistance mechanisms for comparison with those occurring in bacteria from humans. Out of 55 000 samples collected since 2016, only 174 were for AST of bacteria from seafood, with two labs providing 90% of these. Available data were mainly from farmed rainbow trout, and *Aeromonas* was isolated in 60% of samples.

In two investigations, AMR resistant bacteria were as detected in imported seafood (Briet et al, 2018. Oyelade et al, 2018). Crustaceans were particularly identified as carriers of AMR and represent a potential transmission vehicle, not only to consumers and, but a source of AMR spreading also between countries.

One investigation in the UK examined cold water (n=33) and warm water (n=94) ornamental fish such as goldfish, guppy, platy and tetra. These animals are shipped in water to which antimicrobials are added in order to suppress potential pathogens during transport. These fish were imported to UK from Singapore, Guyana and Columbia, and may be out in garden ponds or released elsewhere in the environment, possibly representing a transition route for any AMR bacteria they confer.

3-3 AMR in selected bacterial species

Culture-based AMR detection can focus on testing of both indicator and pathogenic bacteria, including the zoonotic bacteria responsible for infections transmitted between animals and humans either via environmental exposure or via ingestion of contaminated foodstuffs. Any detection of AMR in pathogenic bacteria is of particular concern, as this might compromise the effective treatment of infections in humans. Indicator bacteria are species, usually from commensal microbiota, that are routinely present in higher numbers than pathogens and therefore more readily detected in routine surveillance. These are selected as representatives for the target source of concern. The indicator bacteria should be present in high stable concentration in the source, be easily detected, and always present whenever pathogens from this source are present. The most common example is for the use of *E. coli* as an indicator of faecal contamination. In the case of AMR indicator bacteria, guidelines for AMR detection and criteria for AMR interpretation must be established under standardized guidelines such CLSI and/or EUCAST.

Faecal indicator bacteria: *Escherichia coli*

AMR monitoring in intestinal bacteria in both human and livestock/poultry sources relies on methods that have been standardised by international organizations such as EUCAST and CLSI with regards to experimental conditions, inoculum concentrations, choice of antibiotics and concentrations, and interpretive breakpoints. Hence, AMR testing of indicator organisms in seafood could be initiated following EU and ISO standards. Faecal indicator bacteria may be present in seafood either as a result of contamination of the marine environment by sewage discharges or run-off from land, or following poor hygienic conditions during handling. In filter-feeding bivalve molluscs, it has been shown that faecal indicator bacteria may arise from multiple sources, including *E. coli* of human-associated phylotypes and MLST profiles (Vignaroli et al., 2016, Grevskott et al., 2017). Additionally, environmental parameters such as salinity, tidal movement, rainfall, temperature, turbidity, bacterial distribution and dilution (Rees et al., 2015, Suzuki et al., 2018), and rate of digestion and elimination in the bivalves

themselves (Ottaviani et al., 2015, Leoni et al, 2017, Suzuki et al., 2018), will impact how long any given *E. coli* strain would be detectable.

AMR testing of *E. coli* from bivalve samples was performed in the Norwegian AMR monitoring program, where susceptibility to 24 antimicrobials was tested. In total, 75 of 199 (38%) *E. coli* isolates showed resistance to at least one antimicrobial, including most frequently resistance to extended-spectrum penicillins (83%), aminoglycosides (16%), trimethoprim (13%) and sulfonamides (11%). Whole genome sequencing (WGS) of two of the multidrug-resistant (MDR) isolates showed the presence of *bla*$_{\text{CTX-M}}$ genes, hence the isolates were extended-spectrum beta-lactamase producers (ESBL) (Grevskott et al., 2017). ESBLs confer resistance to critically important antimicrobial in human medicine and thus the possibility of zoonotic transfer of ESBLs constitutes a public health risk. In a second, and more comprehensive selective screening for *E.coli*, low levels of resistance (4.2%) were found among *E. coli* obtained on plates without antimicrobials. Among the 390 bivalve samples examined by selective methods with antimicrobials, one colony per positive sample was tested and 50, 13 and no isolates were resistant to ciprofloxacin, 3rd generation cephalosporins and carbapenems, respectively (NORM-VET, 2016).

In a French-Tunisian collaboration study, marine bivalves from the retail market were screened for ESBL- and carbapenemase-producing *E. coli*, and high occurrence (80%) of AMR *E. coli* including *bla*$_{\text{CTX-M-15}}$ ESBL-positive isolates was detected. Three different sequence types (ST) were identified, ST-617, ST-410 and ST-131, where ST-131 is usually associated with clinical infections and might contribute to the global dissemination of the *bla*$_{\text{CTX-M-15}}$ ESBL gene. Furthermore, the finding of the same *E. coli* plasmid type, F31:A4:B1, in many of the isolated strains raised the question about the possibility of *bla*$_{\text{CTX-M-15}}$-positive plasmid transfer within mussel isolates (Mani et al., 2017, Mani et al., 2018).

In the UK, bivalve molluscs (n=106) submitted for analysis under both the food hygiene monitoring programme and small research studies were examined for *E. coli* for subsequent examination for AMR. In total, 83 (78.3%) were susceptible to all of the antimicrobials tested, two isolates (1.9%) were resistant to more than nine antimicrobials. These were confirmed ESBL, one harboured *bla*$_{\text{CTX-M-15}}$ and the other *bla*$_{\text{CTX-M-27}}$. The number of isolates with reduced susceptibility to the other antimicrobials tested was as follows: tetracycline, 15 (14.2%); ampicillin, 12 (11.3%); sulphamethoxazole, 9 (8.5%); ciprofloxacin and trimethoprim, 7 each (6.6% each); nalidixic acid, 5 (4.7%); chloramphenicol, 4 (3.8%); and azithromycin 2 (1.9%). No resistance to colistin, gentamicin, and meropenem was observed. WGS of these isolates is ongoing.

Marine bacteria: *Vibrio* and *Aeromonas*

One investigation of twenty-one and four *Vibrio cholerae* non-O1/non-O139 from Italian and imported seafood, respectively found no positive isolates for the cholera toxin production or colonization factor. One isolate from imported seafood was positive for the heat-stable enterotoxin gene *stn*/*sto*. Among twelve antimicrobials used for AST, resistance towards ampicillin (24%), nalidixic acid (12%) and gentamicin (8%) was found.
All *Vibrio parahaemolyticus* are intrinsically resistant towards penicillins, including ampicillin and amoxicillin. In an investigation on 87 isolates from bivalves in Italy, no isolates showed resistance to chloramphenicol and tetracycline, and <10 % showed resistance to oxolinic acid, nalidixic acid, nitrofurantoin, trimethoprim/sulfamethoxazole, oxytetracycline and ciprofloxacin.

In France, 384 *V. parahaemolyticus* isolates from seafood were screened, and most (>80 %) showed no acquired resistance towards the antimicrobials tested. The most frequently resistance detected was for tetracycline (56 strains) and 2.5 % of the collection were resistant to at least three different classes of antimicrobials. One isolate from imported shrimp was resistant to nine antimicrobials, and was shown carrying the $bla_{\text{NDM-1}}$ gene (New Delhi metallo-ß-lactamase-1) conferring resistance to carbapenems, which are among the critically important antimicrobials for human medicine (Briet et al, 2018). This was the first report of $bla_{\text{NDM-1}}$ in *V. parahaemolyticus*. A recent publication has also shown presence of this gene in *Vibrio vulnificus* (Oyelade et. al., 2018).

Aeromonas is common in aquatic environments, frequently detected in both freshwater and wastewater, and includes numerous zoonotic species. The genus *Aeromonas* is interesting to study for AMR because its members are naturally competent (i.e. they can acquire exogenous DNA directly from their environment) and thus may represent important intermediates in the spread of ARG from aquatic environments. Previous studies have found widespread AMR in *Aeromonas* environmental strains (Odeyemi et al. 2017). *Aeromonas* species have been isolated from fish and shellfish. However, only a small number of food-borne outbreaks has been documented. The role of this pathogen in seafood remains unclear and requires more systematic study.

In 2009, UK undertook a study analysing the AMR profiles of *Aeromonas* spp isolated from ornamental fish and their carriage water (Verner-Jeffreys, et al., 2009). Clear differences were observed in the AMR profiles of *Aeromonas* isolated from cold water and warm water fish species. Although resistance to one or two antimicrobials were observed in over half of the cold-water isolates, half of the warm-water isolates were resistant to seven or more antimicrobials and several strains demonstrated resistance to over 20 antimicrobials. A single isolate obtained from a Singapore guppy was resistant to 28 antimicrobials. Tetracycline resistance was found to be common among all the screened isolates and tolerance to quinolones and fluoroquinolones was widespread in isolates from warm water species. Most of the bacterial isolates tested were susceptible to third- and fourth-generation cephalosporins. PCR testing identified that half of the isolates tested were positive for class 1 integrases. The gene for resistance to florfenicol and chloramphenicol, *floR*, was detected in three of the fish isolates and in 18 out of 21 carriage water microbial community samples. Other resistance genes detected included *tet* (D), *tet*(E), *tet* (G), bla_{TEM1}, bla_{OXA7}, *dfrA1*, *dfr12*, *dfr13*, *aadA1*, *aadA2*, *intl1*, *sul1*,*qnrS2*, *orfC*, *arr2*, *VatE*, *ant21a*, *catB8*, *strA*, *strB*, *aaa6lb*, and IncA/C. IncA/C plasmids have also been found in *A. salmonicida* in North America, where they were found to show significant homology to the *Salmonella enterica* IncA/C plasmid pSN254 (McIntosh, et al., 2008). This data suggested that ornamental fish and their carriage water might act as a reservoir for both multidrug-resistant bacteria.

Other AMR-indicator candidate species

A pilot study analysing possible marine AMR-indicators applied non-selective and antimicrobial selective screening at 25 °C for AST of multiple species originating from marine bivalves. Among 247 isolates, *Pseudomonas* spp. (63), *Vibrio* spp. (24), *Bacillus* spp. (18), *Staphylococcus* spp. (17), *Stenotrophomonas* spp. (16), *Acinetobacter* spp. (9), *Aeromonas* spp. (9), *Paenibacillus amylolyticus* (8), Flavobacteriaceae (7), Enterobacteriaceae (6), *Shewanella* spp. (6) and *Arthrobacter* spp. (1) were identified. Resistance was most prevalent in *Stenotrophomonas* spp. and *Pseudomonas* spp., in which resistance towards twelve different antimicrobials was found for several of the isolates, including resistance to critically important antimicrobials (Svanevik et al., 2018). The identified *Vibrio* species belonged to *V.*

anguillarum, V. aestuarianus, and *V. alginolyticus*, and 29 % of these were MDR with the most prevalent resistance phenotypes being ampicillin (83 %), amoxicillin (70 %), cefotaxime (54 %), ceftazidime (26 %), mecillinam (30 %) which could be explained by intrinsic resistance for *V. anguillarum* (Pedersen et al., 1995), trimethoprim resistance were observed for 17 %.

3-4 Highlights

The AMR studies presented during this session were performed on different seafood samples at different period of time and focused on different target bacteria using different methodologies. The results are therefore not comparable and could not be considered as AMR monitoring data. The session identified the potential presence of AMR bacteria isolated in seafood, fish and their environment. For future monitoring, the following knowledge gaps need to be addressed:

- identify relevant marine/aquatic AMR indicator organism(s)
- establish a standard AST protocol for marine bacteria
- focus on imported seafood for AMR screening particularly from South-East Asia
- frequent screening for AMR *E. coli* in marine bivalves through national surveillance programmes.

4- Perspective of AMR surveillance in seafood

The session concerning surveillance aimed to provide a structured perspective on available information and knowledge gaps that need to be addressed for establishing AMR surveillance in seafood. The urgent need for AMR surveillance in seafood is linked to the fact that production of seafood for consumption, including capture fisheries and aquaculture, is growing globally on a yearly basis (https://ourworldindata.org/). Seafood differ from other major food production systems, since no standardized AMR surveillance exists, despite several studies showing that such commodities can be contaminated by various antimicrobial resistant bacteria, including both human and zoonotic pathogens. A large proportion of the seafood consumed in the EU is imported from non-EU countries (http://www.eumofa.eu/) and it has been repeatedly shown that imported seafood can harbor genes conferring resistance to last resort antimicrobials such as carbapenems (Rubin et al., 2014; Morrison et al., 2015; Janecko et al., 2016; Mangat et al., 2016; Roschanski et al., 2017; Brouwer et al., 2018; Lee et al., 2018). Thus, seafood consumers in EU may be exposed to AMR genes that would otherwise mainly be restricted to clinical environments or would not even be present within EU. Hence particular focus should be put on products imported from high risk countries or regions, such as South East Asia.

The parameters having critical impact on the effectiveness of an AMR surveillance program are the sampling design and the methodological approaches. Sampling schemes for AMR monitoring in seafood should take into account the objectives of the surveillance as well as the sources to be sampled. Seafood includes extremely diverse organisms characterized by different lifestyles and habitats, which influence the microbiota and thereby the occurrence of antimicrobial-resistant bacteria. In addition, seafood consumption patterns differ widely across EU countries and consequently consumers are exposed to different seafood species with their own specific microbiota. Methods to detect and characterize AMR in bacteria range from classical microbiology to molecular methods, with each method varying in its comparability and usefulness based on the availability of standards, the necessary trained personnel and laboratory capacity, and the information yielded.

The following paragraphs summarize all these aspects as an outcome of the work performed during the ASK project and the workshop discussions.

4-1 Aims of AMR surveillance in seafood

Surveillance *'means the systematic ongoing collection, collation, and analysis of information (…) and the timely dissemination of information so that action can be take'* (OIE, 2018). Therefore, in the context of surveillance, information is gathered to support action. For this reason, objective setting represents a key phase in designing a surveillance system. Surveillance of AMR in seafood can pursue different aims, as detailed below:

1a. **Minimize the risk of AMR for the consumer**. The exposure to AMR from seafood may occur directly, by zoonotic resistant bacteria, or indirectly, by transferable resistance genes. Direct transmission occurs through the ingestion of seafood contaminated by pathogens able to cause disease in humans (e.g. *Salmonella* and *Vibrio* species). The presence of antibiotic-resistance in zoonotic bacteria from seafood is well-documented in literature (Elbashir et al, 2018).
The samples and matrices for AMR analysis should be selected according to EN ISO 6887-3:2017, which describes the specific rules for the preparation of fish and fishery products for microbiological examination (EN ISO 6887-3:2017).
Indirect transmission is caused by the dissemination of resistance genes from commensal bacteria in seafood to human pathogens and it is generally mediated by plasmids (Heuer et al, 2009). Gene transfer from commensal bacteria to human pathogens may occur at different stages, from primary production to the human gut (Heuer et al, 2009). The samples for AMR analysis should target the organs/tissues where the probability of detecting AMR is higher and this also depends on the bacterial species targeted as indicator. In bivalves, the same criteria listed above (EN ISO 6887-3:2017) can be followed. In farmed salmons and shrimps, the detection of AMR could be carried out starting from gut content /faeces (Higuera-Llannten et al, 2018; Su et al, 2018) and for shrimps the whole body could also be sampled (Yano et al, 2015). However, the parts of the body used for the analysis are often not reported in published papers, determining a lack of information in this area for the majority of the seafood species.

1b. Reducing the contamination of the marine environment. The spread of antibiotic resistant bacteria in the marine environment is a cause of concern, since humans may be exposed to AMR during recreational activities (Leonard et al, 2015). AMR bacteria in seafood can be used as an indicator of the AMR contamination of the coastal environment (Sellera et al, 2018). This information may be used to prioritize mitigation measures and to estimate their impact over time. It should be noticed that bivalves collect and concentrate bacteria of different origins and spend the whole of their life living in the same area, allowing to investigate spatial clustering or temporal trends of AMR in the environment (Grevskott et al, 2017). For sampling, the same criteria described in point 1a for indirect transmission are valid.

2a. **Reducing the improper use of antibiotics in the fish industry**. The administration of antibiotic to fish is carried out by flock treatment, using medicated feed or adding the antibiotics to the water (Heuer et al, 2009). As a consequence, the use of antibiotics in aquaculture exerts a selective pressure on bacteria hosted in fish and in the wider environment. To reduce this pressure, policies aimed at reducing the improper use of antibiotics in fisheries are urgently needed. A surveillance plan providing data on AMR in bacteria collected in primary production can identify changes in the pattern of AMR occurrence over time and geographically. This will

make possible to estimate the impact of intervention measures and mitigation adopted by the fish industry.

2b.**Drive the antibiotic use in fisheries.** Antibiotic resistance in pathogens is one of the main drivers of antibiotic consumption in farmed animals. Data on AMR in fish pathogens will help practitioners in selecting the appropriate molecule, finally reducing the improper use of antibiotics in the sector. At the same time, surveillance systems can provide an early detection of emerging resistant pathogens, allowing the adoption of measures to reduce their spread among fisheries. Sampling could be based on isolates from cases of diseases occurred in farmed fish through a passive surveillance system. However, since underreporting constitutes a common limitation of this system, appropriate measures should be put in place to ensure the representativeness of the sample.

The surveillance plan should be designed taking its main objectives into account. In this scenario, a proposal of the core elements of the plan (e.g. the type of surveillance and the sampling strategy) for each aim described above is detailed below in Table 1.

Table 1. Core elements of the surveillance plan

	Description	**What we want to measure**	**The sample should be representative of**	**Sampling strategy**	**Samples should be taken at**	**Matrices (examples)**
Aim 1a	Estimate the direct risk for the consumer	Foodborne exposure of the consumer	Sampling should be calculated to be representative of consumers (e.g. human population)	Representative random sampling	Retail	According to EN ISO 6887-3:2017
	Estimate the indirect risk for the consumer					Faeces/gut content or other matrices to be defined. In bivalves, according to EN ISO 6887-3:2017
Aim 1b	AMR contamination of the marine environment	Environmental exposure of people	Sampling should be representative of the production sites	Representative random sampling/ Risk-based/ Sentinel surveillance	Primary production	In bivalves, according to EN ISO 6887-3:2017 . Faeces/gut content or other matrices to be defined.
Aim 2a	AMR in farmed fish	AMR in relation to Antibiotic consumption	Sampling should be representative of the farmed fish population	Representative random sampling	Primary production	Faeces/gut content or other matrices to be defined.

Aim 2b	AMR in fish pathogens	Susceptibility of fish pathogens to AM	Sampling should be representative of the diseases linked to antibiotic consumption	Passive surveillance	Laboratory submissions	Isolates from cases of diseases occurred in farmed fish

4-2 Classification of seafood in an AMR surveillance context

In an AMR surveillance context, it was necessary to consider fish and shellfish beyond the classic taxonomic classification. It was observed that the taxonomical organization of species cannot be combined in the same classification structure related to the environment where fish and shellfish live. Therefore, the classification in 50 groups created for the FoodEx2 (food classification and description system by EFSA) was not considered optimal in this framework. Fish and shellfish were re-classified based on their habitat and feeding style, which could have relevant impact on AMR occurrence. The combination of these two points led to creation of four main groups. Some groups could be split in two or three subgroups; similarly, some subgroups could be split in two further subgroups.
The four main groups were:

1. filter-feeding animals (shellfish)
2. coastal animals
3. oceanic animals
4. aquaculture animals

Filter feeding animals are listed in Annex 3, with the potential target bacterial species for monitoring. These animals stand apart because they are able to concentrate bacteria from the water in which they live. In addition, they are not generally mobile and live the majority of their lifecycle in the same area. Thus, they are able to give information not only about the risk for the consumer but also about risks to harvesters, processors and recreational water users as well as information about other forms of coastal contamination, such as those from chemical, pharmaceutical, and microplastic pollution. Filter-feeding shellfish can be further split into animals living on the seabed (benthic) and animals born or living further above the seabed in the water column. The benthic shellfish could be further split into sessile and non-sessile animals.

Coastal fish are fish living in the neritic zone (0-200m depth) for most of their life. They can give information similar to that of the filter-feeding animals, but with some differences. These differences are due to the migration of fish over the course of a day, season or lifetime. Numerically it is the most important group in terms of amount of catch.

Oceanic fish live far from the coast in deep water. Some of these animals migrate over great distances, making it difficult to understand the source of any bacteria which can be collected from them.

Both coastal and oceanic fish can be divided into two subgroups, benthic or demersal fishes (living on the bottom of the sea) and pelagic fish (swimming in the water column).

The fourth group is the aquaculture animals. This group of animals is different from the others because they are human fed and because the bacterial strains from this group can be selected by the use of antibiotics in their production.

4-3 Bacterial targets for AMR surveillance in seafood

Different targets can be used to estimate AMR in seafood with differences related to the characteristics of the targets, the information that they can provide, the availability of standardised and harmonised protocols for bacterial isolation and AMR testing, and the costs of analysis. Depending on the aim of AMR surveillance in seafood, different possible bacterial targets can be considered (Table 2).

Aim 1a: Targeting zoonotic bacteria allows for the estimation of risk for the consumer and measurement of the foodborne exposure. Zoonotic bacteria such as *Salmonella* and *Campylobacter* are widely monitored in terrestrial food-producing animals and humans. However, marine animals and seafood are not reservoirs of these zoonotic pathogens which therefore may not be the right target for surveillance. Other bacterial species such as *Arcobacter*, *Shigella*, *Vibrio*, *Yersinia enterocolitica*, *Plesiomonas shigelloides* and *Listeria* could be considered, and for some of these genera/species there are available harmonised protocols for isolation, identification and AST. Notably, *Vibrio* species are autochthonous of the marine environment and they could be used to assess AMR since there are validated and harmonised protocols for the isolation, identification and AST of the species pathogenic to humans.

Aim 1b: For studying AMR contamination, bacterial targets should include autochthonous species/phylotypes indigenous to the marine environment. Marine bacteria could include *Vibrio* spp. and *Aeromonas* spp. as well as other bacterial species (for example *Shewanella* spp, *Pseudomonas* spp.). However, there are not validated and harmonised protocols for the isolation and identification of *Vibrio* species that are not pathogenic for humans neither an international standard for *Aeromonas* in seafood or for other marine bacteria. For AMR contamination in some species of animals consumed as seafood, *E*. *coli*, a good indicator of faecal contamination, could be considered. Moreover, *E*. *coli* is already a part of national surveillance plans for seafood such as bivalve molluscs and validated protocols are available to assess antibiotic susceptibility in this species. Finally, antimicrobial resistance genes could be directly detected in the sample, overcoming the need of culture and isolation. A detailed discussion of ARG detection in the context of AMR surveillance in seafood is included below in section 4-4 related to methodology.

Aim 2a: For monitoring AMR related to antimicrobials use in farmed fish, the same marine bacteria described above (aim 1b) could be considered. Similarly, ARG detection could be an alternative or complementary option. Since *E. coli* is a faecal indicator, it should not be relevant to monitor AMR in *E. coli* in this context.

Aim 2b: examples of fish pathogenic bacteria are several *Vibrio* species, such as *V. anguillarum*, *V. ordalii*, *V. harvey* for prawn and fish, *V. vulnificus*, V*. (aliivibrio) salmonicida*, *V. parahaemolitycus* for prawn and *V. splendidus* for oysters. *Photobacterium damselae piscida, P. damselae damselae, Aeromonas* such as *A. salmonicida,Edwarsiella* such as *E. tarda, E. piscicida, Pseudomonas* such as *P. anguilliseptica, Lactococcus and Streptococcus* such as *L. garviae* and *Streptococcus iniae* (in tuna) are also pathogenic for fish and could be isolated in the framework of veterinary clinical investigations.

Table 2: Bacterial targets according to sampling strategy

	Description	Sampling	Bacteria / AMR genes targets
Aim 1a	Estimate the risk for the consumer	Retail	Human pathogenic bacteria
Aim 1b	AMR contamination of the marine environment	Primary production	Bacterial species relevant for the marine environment / *E.coli* indicator/ AMR genes
Aim 2a	AMR in farmed fish	Primary production	Bacterial species relevant for the marine environment/ AMR genes
Aim 2b	AMR in fish pathogen	Veterinary sample	Fish pathogenic bacteria

4-4 Methods for AMR surveillance in seafood

Harmonised AMR surveillance relies on use of standardised methods to produce results that are comparable across sources, geographical areas and time. Standardisation of methods for AMR surveillance is lacking for many of the bacterial species and sample matrices relevant for seafood. Historically AMR surveillance has been performed nearly exclusively by phenotypic methods, but it is nowadays widely recognized that genotypic methods convey essential information to complement or even replace phenotypic methods. The methodological workflow for AMR surveillance in seafood starts by the definition of a target (Figure 3).
The target can be represented by bacterial isolates of selected species and/or by specific sample matrices (e.g. skin, gut content, etc.).

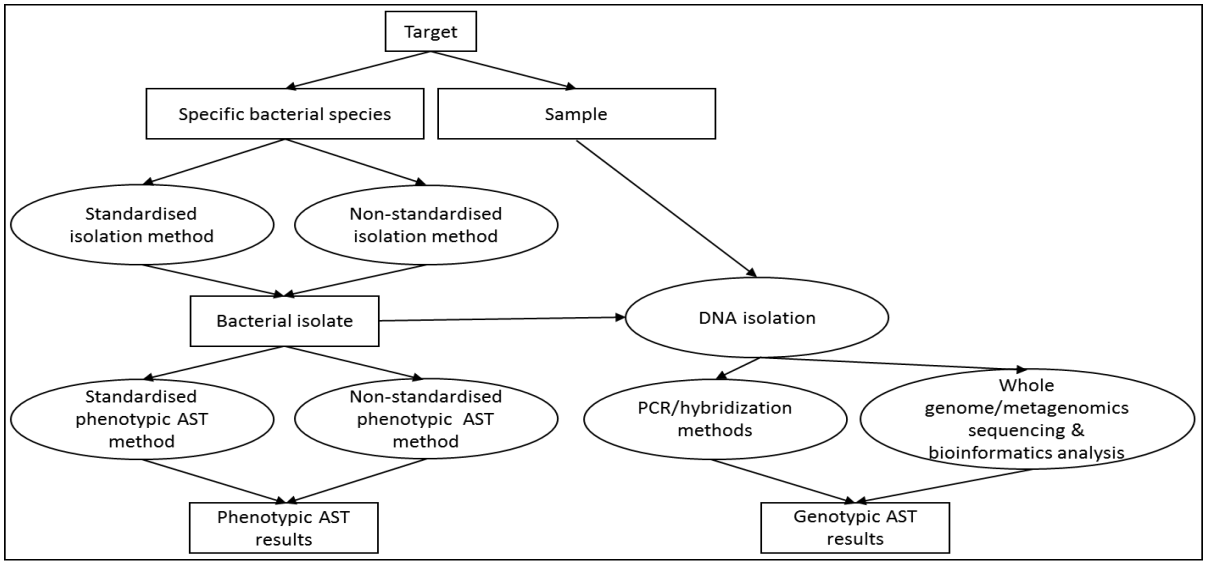

Figure 3: Schematic workflow for antimicrobial resistance monitoring in seafood by phenotypic and genotypic methods AST, antimicrobial susceptiblity testing

In the case of AMR surveillance based on bacterial isolates, the steps from sample to bacterial isolation are common to both phenotypic and genotypic methods. These steps may be or may not be standardised based on the bacterial target chosen. Among the bacterial species that could represent valuable targets for AMR surveillance in seafood, standard methods are available for isolation of *Escherichia coli* (ISO 16649-3:2015 and corrected on 15-12-2016),

Salmonella sp. (ISO 6579-1:2017), *Shigella* sp. (ISO 21567:2004) and Enterobacteriaceae (ISO 21528-2:2017) in general, *Campylobacter* sp. (ISO 10272-1:2017), *Listeria monocytogenes* (ISO 11290-1:2017), *Vibrio parahaemolyticus*, *V. vulnificus*, *V. cholerae* (ISO 21872-1:2017) and *Yersinia enterocolitica* (ISO 10273:2017). However, for other bacterial species potentially relevant for AMR surveillance in seafood, including *Arcobacter butzleri*, *A. cryaerophilus*, *A. skirrowii*, *Aeromonas* sp., *Enterococcus* sp., *Pseudomonas* sp. and *Shewanella* sp., no standardised isolation procedures exist. However, standardisation of bacterial isolation procedures is critical to provide comparable data on occurrence of AMR. Bacterial isolation can be performed after enrichment or directly from the sample, and the many possible variations in these steps may lead to contrasting results. For example, in case of initial relative low abundance of the target bacterium in a sample, enrichment procedures may be better suited compared to direct isolation from the sample which may provide false negative results. Nevertheless, enrichment procedures could enhance the growth of one bacteria clone within a targeted species or one species among the bacteria community.

After bacterial isolation, the workflow differs based on phenotypic and genotypic methods (Figure 3). By following the phenotypic method workflow, bacterial isolates are tested for antimicrobial susceptibility testing using standard methods such as broth micro-dilution and disc diffusion, though established guidelines and interpretive criteria exist only for selected bacterial species. To this extent, the most updated guidelines for antimicrobial susceptibility testing of bacteria isolated from aquatic animals have been published by the Clinical and Laboratory Standards Institute (CLSI) in 2014 (VET04-A2). These guidelines include standardised test conditions for quite a few bacterial species that are potentially relevant for AMR surveillance in seafood.

By following the genotypic method workflow, DNA is extracted and can then be subjected either to detection of specific AMR genes by PCR and hybridization techniques or to whole genome sequencing (WGS). Obtained data can then be analysed using different bio-informatics pipelines. No standardised approaches exist for this workflow and generally different laboratories apply their own established protocols. Nevertheless, a working group has been set up in the frame of ISO in order to harmonize WGS protocols and bio-informatic procedures (Ellington et al, 2017) and a large European research project (COMPARE) is working on this, together with similar initiatives at national levels in different countries worldwide.

Finally, the metagenomics workflow, whereby DNA is extracted directly from a sample and bio-informatics analysis is used to determine presence and abundance of AMR genes, represents the most recent tool for AMR surveillance. However, metagenomics methods have been applied to AMR detection in seafood only in pilot projects and thus are in need of standardisation before being widely used for harmonised AMR surveillance.

Phenotypic and genotypic methods for antimicrobial susceptibility testing have advantages and disadvantages. Phenotypic methods provide information about antimicrobial susceptibility and resistance but have limited discriminatory power, and thereby have limited value for epidemiological purposes. On the contrary, genotypic methods provide information which, especially in the case of whole genome sequencing of single isolates and, in a foreseeable future, of metagenomics, can support cluster analysis and thus reveal possible AMR transmission pathways. Furthermore, genotypic methods may reveal occurrence of virtually all known AMR genes in an isolate or in a sample, and sequence data can be stored and interrogated further in the future as knowledge on genetic bases of AMR develops. A potential disadvantage of genotypic methods is that presence of an AMR gene does not necessarily implies expression of such gene and thus AMR, however this can be considered of secondary importance for surveillance purposes.

There is no single method that can perfectly fit all situations in which AMR detection is relevant, and it is therefore crucial to define the specific objective of AMR surveillance in seafood to be able to choose the best method or combination of methods given the circumstances.

4-5 Highlights

The session about perspectives on surveillance identified the following common issues and needs for AMR surveillance in seafood:

- different sampling strategies, sample matrixes, and bacterial species should probably be selected based on the aim of AMR surveillance
- limited information about AMR in bacteria from bivalves, coastal animal species and farmed fish is available, and data gaps exist for species belonging to other seafood categories. Available studies highlighted the difficulty of selecting specific bacterial species as indicators of AMR as prevalence may vary according to seafood category and geographical distribution. Thus, there is a need to investigate further on the potential usefulness of environmental bacterial species present in seafood as indicators for AMR
- technical obstacles towards the development of a program for AMR monitoring in seafood are the lack of standardized methods for: i) isolation, ii) species identification, iii) antimicrobial susceptibility testing (AST) and/or interpretation of AST results for many bacterial species occurring in seafood. Thus, there is an urgent need of method standardization to establish harmonized AMR monitoring in seafood
- metagenomic approaches for AMR surveillance in seafood should take into account that the environment is a reservoir of resistance genes and thus different ARGs pose a different public health hazard.

5- Risk assessment approach

Risk assessment is one of the three components of risk analysis (Regulation (EC) n° 178/2002). Risk assessment (RA) provides a scientific basis for appropriate risk analysis, i.e. the assessment, communication and management to reduce, eliminate or prevent negative public health impacts. In the context of AMR, risk assessment considers different types of factors related to the mechanisms of resistance to the anti-microbial under consideration, to the selection of resistant bacteria in the food chain and in the environment in general, to the human exposure to resistant bacteria, and the consequences to human health.

5-1 Objectives of AMR risk assessment

The risk assessment (RA) objectives are multiples, e.g.:

- Assess the potential for human risk associated with exposure to a known resistant pathogen,
- Determine critical points for control,
- Determine specific treatment processes to reduce, remove, or inactivate various pathogens,
- Predict the consequences of various management options for reducing risk;
- Identify and prioritize research needs,
- Assist in epidemiological investigations.

5-2 Hazard identification

The first step is identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods (Codex Alimentarius). In relation to AMR, this step identifies the antimicrobial-resistant bacteria or resistance determinants therein that could be associated with human illness and are selected due to the use of the concerned antimicrobial substance in the target animal species (https://www.ema.europa.eu) . Resistance may develop both in bacteria that are zoonotic or commensal bacteria in animals that could pass resistance determinants to other bacteria that are pathogenic in humans.

In AMR risk assessment, hazard identification, one of the main encountered challenges by assessors is to agree whether the hazard of interest should be the AMR gene or the pathogenic bacteria holding the AMR gene (Codex Alimentarius, 2011). In other words, whether one should decide if AMR should be considered as a direct or indirect hazard.
This step is crucial because it defines the agent that causes the adverse effect in humans. It still remains to be further discussed whether the hazard should be the gene, the resistant pathogen or another parameter/criterion. The agent causing the adverse effect in humans is neither the antimicrobial substance nor the mechanism of resistance nor the commensal bacteria that transfers resistance to pathogenic bacteria. Resistance genes may not be the direct hazard of interest as they are not harmful in themselves yet they indirectly exert an adverse effect if transferred to a micro-organism that causes harm. The direct cause of the adverse effect observable in humans is the resistant pathogenic bacteria. It is therefore appropriate to define as the hazard the infectious agent that causes the adverse effect in humans. The antimicrobial substance, its use, resistance mechanism, commensal bacteria are factors that play a role in the emergence of resistance and its release in in the food chain or more generally in the environment, but are not considered direct hazards.

There will be a need to further describe whether isolated genes in the environment play a stronger role in the spreading and amplification of AMR rather that genes that could directly interact with the microbiota close to the ‘target’ of the pathogenicity (i.e. human or animal beings) leading pathogen bacteria to gain resistance. The role of AMR in opportunistic bacteria still need clarification. The spreading/ possible amplification through the environment (which is a reservoir of major interest for the understanding of epidemiological patterns linked to AMR) could be addressed as part of the epidemiological pattern, or as risk factors rather than as being the hazard itself.

5-3 Exposure assessment - consumption data in seafood -

The objective of exposure assessment is to estimate the probability of being exposed to AMR microorganisms or determinant. The exposure may be via food or other sources if relevant.

It includes describing the hazard sources or reservoirs and exposure pathways, as well as the relevant factors that may impact the prevalence and concentration of the AMR microorganism or determinant along the farm-to-fork continuum. The hazard may enter at any stage of the food chain. AMR microorganisms or determinants may be introduced into the environment prior to fishing or harvesting, or at any stage of processing or more generally during any handling of fish or seafood products. Data are needed to better describe the probability of the hazard transfer from one step to the next step of the food chain, as well as the growth potential

and survival capacity of AMR microorganisms in the production environment (including primary production) and in the processed or non-processed fish or seafood products (figure 3).

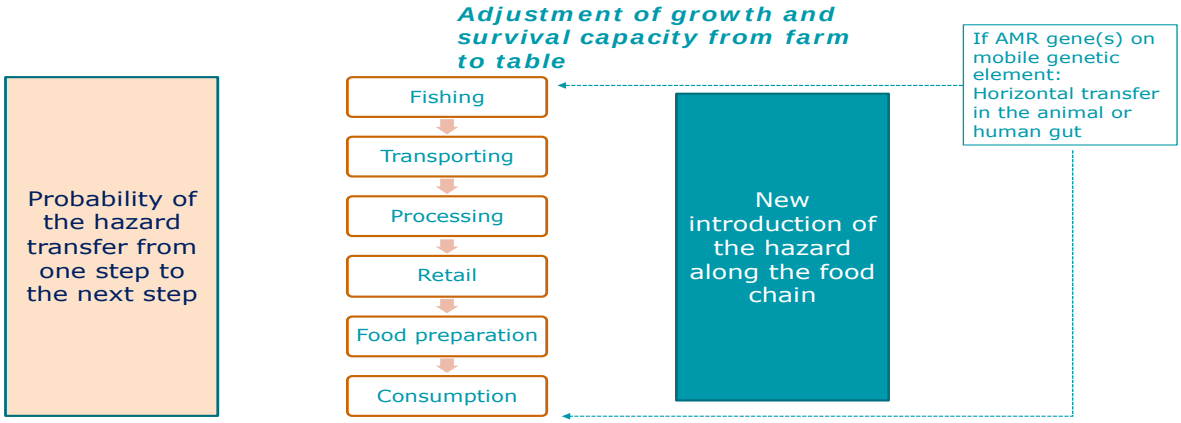

Figure 3: Exposure Assessment

AMR risk assessments have the potential to provide better and new types of information when WGS data are effectively integrated. For example, WGS data have the potential to characterize the relative importance of sources or reservoirs (attribution) of the AMR microorganisms and to identify strains that circulate widely and those that do not. WGS data also have the potential to provide information about how pathogen strains and sources are linked (or not) and how these sources and links persist and evolve over time and space. The benefit of WGS in risk assessment study of foodborne antimicrobial resistance is well highlighted in a recent article (Collineau et al, 2019).

In the situation where the risk assessment focuses on the AMR genes (indirect hazard). The horizontal transfer of AMR genes should be part the exposure assessment mainly to better understand the exposure pathways. The absence of quantitative data makes the modeling of horizontal transfer of AMR genes difficult to integrate in the risk assessment step. Indeed, quantitative data are needed to estimate the probability of AMR genes transfer under various physiological and environment conditions that might vary significantly between the different stages of the food chain.

The exposure can be estimated using data on the occurrence of a resistant pathogen in relevant food (prevalence and concentration) and on the consumption patterns (food handling, preparation and consumption). Consumption data for seafood cannot be derived from the general diet studies and have to be specifically collected. Seafood consumers represent in some countries a small proportion of the general population but may include individuals with high consumption, so it is important to design specific studies to assess the quantities consumed**.** In France, an ongoing study (CONSOMER study) on final phase will give original results with regard to this point.
Questions regarding occurrence data will not be elaborated in this part as this was presented in the section dedicated to surveillance.

5-4 Hazard Characterization

The aim of the hazard characterization step is to translate the levels of exposure to the hazard into a probability of one or more adverse health outcomes in humans. Where possible, the

hazard characterization comprises the establishment of a dose–response model, meaning a mathematical link between the exposure and the probability of observing the adverse outcomes. Different adverse outcomes may be considered such as infection, illness or death, as well as consequences associated to AMR resistant pathogen, including treatment failure, increased severity or duration of disease and death. The biological foundation for dose-response models originates from major steps in the disease process: exposure, infection, illness and consequences (recovery, sequel or death). The final outcome is the result from the interactions between the pathogen, the host and the food matrix. In general, due to the potential for microorganisms to grow within the host, it is assumed that a single viable infectious pathogenic organism is able to induce infection ("single-hit concept"). Mathematically, there is always a non-zero-probability of infection or illness when a host is exposed to infectious pathogenic organism. The minimal infectious dose is accordingly one single microorganism and the probability at this dose is in general very small.

Dose response relationships are established through the integration of different types of data such as foodborne outbreak data, experimental animal data, or in vitro data. These different types of data make it possible to explore the variability of host susceptibility to pathogens, the variability of virulence between bacterial strains and also the impact of the food matrix on the ability of bacteria to overcome different biological barriers.
It is therefore important to generate data specific to resistant microorganisms to explore the possibility of an increase in survival capacity or the virulence of resistant strains compared to non-resistant strains. The dose response models could further be adjusted strains in a way that strains with higher capacity of survival and virulence will require lower doses to cause infection or disease. Nevertheless, in the case of indirect hazard there are still huge difficulties in establishing the relationship between the exposure to the determinant of AMR and the observation of an adverse effect.

5-5 Strategy for conducting risk assessment

A consensus was established on the fact that it is advisable to favor a realistic approach and not to consider too large a scope for conducting RA, not all situations of exposure can be encompassed at the same time in a ‘full RA’. Priorities in order to address the main resistant pathogens are required. No position was taken on this point but the nature of the resistance itself needs consideration, critical antibiotics for instance could constitute a priority. Although they may not always reflect the development of resistance, the sales of antibiotics in animal productions can also be an indicator for setting priorities. Following the standardized risk assessment (RA) flowchart, from hazard identification and characterization to exposure calculations and finally risk characterization, some major questions were raised that showed the specificity of risk assessment with regard to AMR in food and the lack of standardized RA methods to address the question. However, a guideline on the assessment of the risk to public health from antimicrobial resistance due to the use of an antimicrobial veterinary medicinal product in food-producing animals has recently been published by the European Medicines Agency and could serve as a basis of reference to undertake a qualitative approach of the risk. Pending is the crucial question of the quantification of the problem that risk managers are facing and trying to tackle: classical tools used in RA are still valid for the calculations of the detrimental action of resistant pathogens and dose/response patterns seem to be applicable too but if sufficient fit-for-purpose data are available.

5-5 Highlights

In conclusion, it appeared that resistant pathogens seem to be the direct hazard itself with regard to RA perspectives. There is still a need to further describe a possible role of non-pathogens that could acquire new adverse properties. Reservoirs in a broad sense encompassing the environment and the internal microbiota of target-human/animal beings, are important with regard to epidemiological considerations.
We should bear in mind that RA is undertaken in order to help risk managers and to give a response to a public health issue, meaning we need to determine on common grounds what the issue actually is. It appears to be above all a potential risk to human health.

6- Summary of outcomes

6.1 Summary of knowledge gaps

One of the main objectives of the ASK project was to highlight the knowledge gaps on AMR in seafood that need to be addressed. The following points below were considered in a consensual way by the ASK group as key-actions:

Regarding AMR surveillance:

- Identification of appropriate AMR indicator organism(s)
- Standardization of AST protocols for aquatic bacteria
- Identification of relevant target samples
- Choice of appropriate phenotypic and/or genotypic analytical method

Regarding AMR bacteria selection, spreading and risk assessment:

- Role of imported seafood for AMR transmission
- Role of farming conditions: use of antimicrobials and biocides, feed additives
- In natural capture: role of AMR reservoir from marine environment
- The impact of the process and transformation
- The volume and practices of seafood consumption
- The prevalence of AMR bacteria and ARG in seafood

6.2 Recommendations and challenges in the forthcoming years.

The ASK partners have conducted several studies on AMR in seafood and the marine environment which should be of interest for the EFSA working group. Nevertheless, the high diversity in term of samples, targeted bacteria and methodology hampers the possibility to provide definitive statements and conclusions regarding the prevalence of AMR or AMR genes in the marine sector. It is necessary to define accurately the objective of the AMR surveillance in seafood by considering the circumstances discussed in chapter 4 of this report. A monitoring program could be well-defined in term of sampling, targeted bacteria, analytical methods in accordance with the EFSA working group in the forthcoming months. Some practical considerations should be taken into account, such as the availability of standard methods for isolation of target bacteria as well as the possibility to supplement the AMR monitoring program already implemented in EU. In this context, a baseline study of AMR in seafood could be defined with standardized methodology in several European countries, similar to what has been set up for MRSA in pork for instance (EFSA, 2009). Then, yearly monitoring could be

adjusted according to the situation, outcomes and possibility of mitigation measures in different Member states.

One crucial point would be monitoring of AMR in seafood products imported from countries where farming practices are not strictly controlled. This would create a link with the already ongoing work of international organization such as WHO and FAO.

Risk assessment analysis needs to define exactly what is the hazard, and several options and possibilities have been suggested in chapter 5 of this report. Therefore, risk assessment could be also undertaken using the data obtained from such further monitoring and other consolidated data of consumption, high differences could be observed according to the regional practices of consumers.

7- Conclusion

The ASK project was built with the aim to exchange and share knowledge and visions on AMR in seafood, where the organization of the workshop was the main activity for knowledge exchange. By gathering all the workshop presentations, discussions and thoughts, the ASK project unravelled some insights on existing data and methods, risk assessment approach and various aspects of methodologies for surveillance and monitoring.

The work presented by the partners on AMR in seafood was comprehensive, though diverse and not easily comparable. Several partners identified imported seafood products with MDR bacteria harbouring high-risk AMR genes not previously found in European seafood. Hence, imported seafood from South East Asia is a key area of concern and since there are EU regulations for imported seafood from third countries, there should be monitoring for certain resistant bacteria, as well as for residues of antimicrobial agents.

There is a need for a clear definition of the objective for AMR surveillance; at which stage and which samples should be monitored and how to define and standardize bacteria isolation and AMR testing, both by phenotypic and genotypic approaches. We also need to identify bacterial targets, including some specific for marine sources and different from terrestrial animal sectors. If the aim is to estimate the risk for consumers, two main risks can be identified – the direct risk posed by ingestion of zoonotic bacteria carrying AMR in seafood, and the indirect risk posed by the presence of AMR determinants in commensal bacteria of seafood, that could be transferred to the microbiota of consumers including pathogenic bacteria. To estimate the risk for consumers, seafood must be sampled as close to the consumer as possible and sampling should be representative of the consumption of human population, though the data around this are not easily available. A systematic random sampling at retail may be adapted to the purpose. Assessment of the occurrence of AMR in seafood can allow the evaluation of temporal and geographical trends and risk factors. Regarding geographical distribution, the impact of processing needs to be accounted for, as measured AMR prevalence may not be representative of the site of production. A sensitivity analysis could be used to create mitigation strategies, and the long-term impact of the countermeasures can be assessed.

Finally, there is a need to maintain a network of ASK partners in the forthcoming years and provide more standardized data on AMR in seafood in order to make a solid assessment of the situation in this sector. Such monitoring could be defined as a baseline survey and could allow performance of a risk assessment analysis at the relevant consumer stage. Future data from seafood should be compared to human data, and other animal and environmental sectors for a One Health approach.

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ASK Project –Final Report

"AMR in seafood as common ground for knowledge exchange and risk assessment"

draft of final report

APPENDICES

- Annex 1 -

Institute	Sampling purpose (official control/project) Year/duration	Seafood species	Habitat and/or Country	Methodology for sampling and determination of resistance	Antimicrobial agents	Bacterial species	Comments	Reference
Institute of Marine Research (IMR/HI)	Official control: National shellfish monitoring programme for *E. coli* /2015	Marine bivalves: Blue mussels (M. edulis) n=447, flat oysters (O. edulis) n=40, great scallops (P. maximus) n=39, carpet shells (M. arenaria) n=12, horse mussels (M. modiolus) n=11	Marine environment along the Norwegian coast	Culture method: MPN for *E. coli*. Further identification of bacteria by MALDI TOF MS Susceptibility test: Disk diffusion (EUCAST). Conjugation. MLST. WGS.	Ampicillin, amoxicillin, moxicillin/clavulanic acid, mecillinam, iperacillin/tazobactam, chloramphenicol, ciprofloxacin, levofloxacin, nalidixic acid, norfloxacin, nitrofurantoin, gentamicin, tobramycin, streptomycin, kanamycin, trimethoprim, trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime, doxycycline, tetracycline, colistinsulfate, imipenem, meropenem	*E. coli* (n=180), Klebsiella sp. (n=11), Other Enterobacteriaceae (n=9)	Beta-lactamase producing *E. coli* isolates; in particular ampicillin resistance.	Grevskott et al. 2017
Institute of Marine Research (IMR/HI) in collaboration with the National veterinary institute	Official control: National Shellfish monitoring programme for E. coli / National surveillance on antimicrobials in veterinary and food/2016	Marine bivalves (n=388): blue mussels (*M.edulis) n=310, flat oysters (O. edulis) n=38, pacific oysters (Crassostrea gigas) n=great scallops (P. maximus) n=26, soft-shell clam (M. arenaria) n=2, horse mussels (M. modiolus) n=6, ocean quahog (Arctica islandica) n= 3*	Marine environment along the Norwegian coast, 59 harvest areas	Culture method:MacConkey without antibiotics, MacConkey 1 µg/ml CTX, MacConkey 2/ml µg CTZ, MacConkey 0.06 µg/ml CIP, CARBA plates and OXA-48 plates according to EURL-protocol (Isolation of ESBL, AmpC and carbapenemase producing *E. coli* from fresh meat). Identified on MALDI-TOF-MS. Susceptibility test: Sensititre (TREK) and EUCAST. WGS.	Tetracycline, tigecyclin, chloramphenicol, ampicillin, cefotaxime, ceftazidime, meropenem, sulfalmethoxazole, trimetoprim, azithromycin, gentamicin, ciprofloxacin, nalidixic acid, colistin	*E. coli* (n=391), Cephalosporin resistant *E. coli* (n=13), Quinolone resistant *E. coli* (n=52)	Susceptibility for all antibiotics were found in 91.6 % of the isolates. Resistance to tetracycline were most frequent. Cephalosporine resistant *E.coli* (3.3%) harboured one or several of the $bla_{CTX-M-15}$, bla_{CMY-2} and bla_{TEM-1} genes. Quinolone resistant *E. coli* (13.3 %). Five of these were also ESBL harbouring bla_{CTX-M} gr. 1 and/or bla_{CMY-2} genes. No carbapenemase producing *E. coli* was detected	NORM-VET report 2016, published 2017.
Institute of Marine Research (IMR/HI)	Project for the Norwegian Environmental agency, 2017	Marine bivalves (n=26): blue mussels (*M.edulis) n=18, flat oysters (O. edulis) n=2, pacific oysters (Crassostrea gigas) (n= 1) great scallops (P. maximus) n=2, soft-shell clam (M. arenaria) n= 1, common cockle (Cerastoderma edule) n=1, ocean quahog (Arctica islandica) n=1*	Marine environment along the Norwegian coast, 26 harvest areas classified as low, medium and high anthropogenic influence	Culture method: Non-species selective Mueller Hinton (MH) agar without antibiotics, and with MH with 50 µg/ml AMP, MH 2 µg/ml , MH 0.06 µg/ml CIP, or MH 10 µg/ml IPM. Identified on MALDI-TOF-MS. 10-20 isolates per harvest area, totally, n=252 Susceptibility test: Disk diffusion (EUCAST). MIC-test on MH-agar plates for heavy metal resistance	Ampicillin, amoxicillin, mecillinam, piperacillin/tazobactam, chloramphenicol, ciprofloxacin, levofloxacin, nalidixic acid, nitrofurantoin, gentamicin, tobramycin, kanamycin, trimethoprim, trimethoprim/sulfamethoxazole, cefotaxime, ceftazidime, doxycycline, tetracycline, imipenem, meropenem, erythromycin, vancomycin. Heavy metals: copper, zinc and cadmium	Multiple species detected belonging to: Acinetobacter (9), Aeromonas (9), Arthrobacter (1), Bacillus (19), Chrysobacterium (6), Erwinia (1), Ewingella (1), Paenibacillus (8), Pseudomonas (63), Rhodococcus (2) Serratia (2), Shewanella (6), Staphylococcus (17), Stenotrophomonas (16), Vibrio (25)	Preliminary results: Non-selective (n=75): 28% susceptible to all antibiotics, 33 % MDR. No resistance towards nalidixic acid, ciprofloxacin, doxycycline, tetracycline, meropenem or levofloxacin. Correlation between: copper resistance and resistance to vancomycin, ampicillin and amoxicillin. Zinc resistance and resistance to imipenem. Cadmium resistance and resistance to doxycycline and trimethoprim/sulfamethoxazole	HI-report: "Screening for antibiotic resistant bacteria in marine bivalves", published in Norwegian, 2018.
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche	Official control: National shellfish monitoring programme for *E. coli*/Period of the study April 2013 to July 2014	Marine bivalves: Striped venus clams (Chamelea gallina) (n=43)	Marine environment along the central Adriatic coast of Italy, 7 natural beds	Culture method: MPN for E. coli. 4 to 10 presumptive colonies of E. coli were selected, mostly from samples with MPN values >230 MPN/100g. Further identification o E. coli by phylogroups multiplex-PCR which distinguishes E. coli sensu stricto Susceptibility	Ampicillin, gentamicin, ciprofloxacin, tetracycline, chloramphenicol, nalidixic acid,trimethoprim/sulphamethoxazole, and streptomycin	*E. coli (n=141)*	47 strains (33.3%) were resistant to at least one drug and 16 (11%) were MDR (i.e. resistant to 3 or more antibiotics). Resistance to tetracycline (25.5%) was the most frequent and was followed by resistance to ampicillin (17%) and	Vignaroli et al., 2016 NGS unpublished
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche	Research project funded by the Italian Ministry of Health/period 2009–2011	Marine bivalves (n=87)	Marine environment, several sites along the Italian coastal waters authorised for harvesting: 33 from the south Adriatic Sea, 19(21.8%) from the central Tyrrhenian Sea and 35 (40.2%) from the central Adriatic Sea	Culture method; Identified by biochemical tests and PCR for *toxR* gene. Susceptibility test: Disk diffusion (CLSI where available). For Beta-lactam-resistant strains, Beta-lactamase production determined by the chromogenic cephalosporin substrate method using the nitrocefin test. Detection of blaTEM, blaPSE-1, blaCARB-4 and blaOXA-30 by PCR. Class 1 integron and ICEs by PCR.	Ampicillin, amoxicillin, cefotaxime, cefalothin, cefalexin, colistin sulphate, polymyxin B, nalidixic acid, oxolinic acid, ciprofloxacin, trimethoprim/sulphamethoxazole, nitrofurantoin, erythromycin, chloramphenicol, streptomycin, kanamycin, neomycin, oxytetracycline, tetracycline, doxycycline	V. parahaemolyticus (n=87)	All isolates were susceptible to chloramphenicol and doxycycline and resistant to ampicillin and amoxicillin. >90% of isolates showed susceptibility to oxolinic acid, nalidixic acid, nitrofuran-toin, trimethoprim/sulfamethoxazole oxytetracycline,ciprofloxacin and 88% to tetracycline .No strains resistant to tetracycline, oxytetracycline and SXT although inter-mediate zone sizes were observed in 8%, 11%, 3%, respectively. None of the -lactam-resistant strains positive to PCR for blaTEM, blaPSE-1, blaCARB-4and blaOXA-30 or for beta-lactamase activity. All strains, PCR-negative for class 1integron and ICEs.	Ottaviani et al., 2013
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche	Research project funded by the Italian Ministry of Health/period 2003-2014	Seafood (n=25)	Marine environment along the Italian coast for 21/25 (84%); Asian countries 4 (16%)	Culture method; Identified by biochemical tests and PCR of 16S-23S rRNA intergenic spacer regions of *V. cholerae*. Susceptibility test: Disk diffusion (CLSI where available)	Ampicillin, amoxicillin- β-clavulanic acid, cefotaxime, meropenem , nalidixic acid, ciprofloxacin, cloramphenicol, polymyxins, colistin sulphate, macrolide, erythromycin, streptomycin, gentamicin, kanamicin, tetracycline, sulphonamide, trimethoprim + sulfamethoxazole	*V. cholerae* nonO1 nonO139 (n=25)	All strains were susceptible to ciprofloxacin and more than 90% to cloramphenicol and cefotaxime. Towards, AMC, K, S, CN, E, MER resistance was found in 100% (25/25) for CT, 24% (6/25) for AMP, 12% (3/25) for NA, 4% (1/25) for AMC, K, S, and MER, 8% (2/25) for CN. For E 0% (0/25), of the isolates were resistant, however, 92% of the isolates showed an intermediate profile (23/25). Four strains were multi-drug resistant (resistant to more than two different classes of antibiotics). Of these, the strain that showed AMP/CT/MER multidrug resistant pattern also had stn/sto gene and was isolated from seafood originating from Asia	Ottaviani et al., 2018

ANSES	RESAPATH Network 2016 (annual data). Monitoring isolates from animal diseases or pathologies, (Anses Lyon)	Farmed fish (N= 174). (rainbow trout, brown trout, salmon, bass…)		Antibiograms (disk diffusion method) sent from partner laboratories (eventually isolates too), After removal of shell debris, bivalves were dried, disinfected (70% ethanol), opened using a sterilized scalpel, and disposed in tubes containing peptone salt broth for 24 h at 37°C. Overnight cultures were streaked on selective MacConkey agar plates containing 4 mg/liter efotaxime, and one colony per morphology per plate was picked up. Antibiograms (disk diffusion method)	Ampicillin, amoxicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefalotin, cefuroxime, cefoxitin, cefepime, aztreonam, cefotaxime, ceftazidime, ceftiofur, cefquinome, ticarcillin, ticarcillin /clavulanic acid, meropenem, chloramphenicol, florfenicol, nalidixic acid, ofloxacin, enrofloxacin, gentamicin, tobramycin, streptomycin, kanamycin, netilmicin, amikacin, apramycin, trimethoprim, sulfonamides, tetracycline, colistin,	Aeromonas salmonicida (N=104), Aeromonas (N=21), Vibrio (N=19), Yersinia ruckeri (N=14)	Resistant isolates were collected on selective plates. One carbapenemase producing E. coli KPC-3 was detected. A dominant CTX-M-15 epidemiology was observed in different species of	Mani *et al*, 2017
ANSES	Work performed in the frame of a PhD thesis (Anses Lyon)	*Mytilus Galloprovincialis (N=400), Crassostrea gigas (N=36), Placopecten magellanicus (N=181)*	Marine environment along the Tunisian coast Madagascar, South-East Asia, Africa, Central America	Antibiograms by disc diffusion on Mueller Hinton on pure culture isolates	Ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefalotin, cefoxitin, cefotaxime, ceftazidime, ticarcillin /clavulanic acid, chloramphenicol, nalidixic acid, gentamicin, streptomycin, trimethoprim, sulfonamides, tetracycline, colistin , ciprofloxacin, azithromycin, témocillin	*E. coli,K. pneumonaie,A. baumannii,C. freundii*	*Enterobacteriacea*	Mani *et al*, 2018
ANSES	Collection of Vibrio isolates sent from partner labs for identification to French reference lab (Boulogne sur Mer) 2012-2016	Shrimps, Fish, Unknown sources				Vibrio parahaemolyticus (N=248)	17% resistant to at least one antibiotic out of which tetracycline, cefalotine, sulphonamide, nalidixic acid, azithromycin, chloramphenicol, streptomycin One MDR strain with 9 co-resistances and insertion sequence carrying NDM-1 gene.	Briet A*et al*, 2018
Technical University of Denmark (DTU)	EFFORT project. EFFORT: Ecology from Farm to Fork of microbial drug resistance and transmission (EU financed project)	Rainbow trout (Oncorhynchus mykiss) (n=60)	Fish aquaculture in Spain, France, Poland. 20 fish from each country.	Harvesting of fish, extraction of feces from fish intestinal content. Total fecal DNA is extracted. Metagenomic sequencing using Illumina NextSeq (~ 35M PE reads / sample). AMR content is determined in silico using MGmapper against the ResFinder database.	Virtually all. See AMR genes included in the ResFinder database.	Virtually all. Method is sample-centric and does not depend on bacterial isolation.		Ongoing work. Method similar to Munk et. Al (2018). http://rdcu.be/3ora
Centre for Environment, Fisheries & Aquaculture Science (Cefas)	Official control: Scottish shellfish monitoring programme for *E. coli (N=89)*. Some UK research samples (N=17)	Marine bivalves: Pacific oysters (N=28), Native oysters (N=1), Blue mussels (N=35), Razor clams (N=8), Surf clams (N=6), Common cockles (N=28)	Marine environment along the Scottish coast and the UK South Coast	Culture method: MPN for E. coli. Blue colonies TBX medium, Oxidase negative. Susceptibility test MIC (TREK sensititre) EUCAST. WGS	All isolates were tested againstsulfamethoxazole, trimethoprim, ciprofloxacin, tetracycline, meropenem, azithromycin, nalidixic acid, cefotaxime, chloramphenicol, tigecycline, ceftazidime, colistin, ampicillin and gentamicin. Those isolates suggestive of ESBL were further tested against; cefoxitin, ertapenem, imipenem, cefepime, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, and temocillin	*E. coli*	A total of 83 isolates showed no resistance to any of the antibiotics tested. The most common microbiological resistance phenotypes observed were to tetracycline and ampicillin. Two had an extended-spectrum beta-lactamase (ESBL) resistance; one harboured blaCTX-M15 and the other blaCTX-M27. 50 isolates still to be WGS.	
Centre for Environment, Fisheries & Aquaculture Science (Cefas)	Examination of ornamental fish and carriage water for antibiotic tolerant *Aeromonas* spp. (N=127 isolates)	Ornamental fish only: Cold Water (koi, goldfish) N=33; Warm water ornamentals (guppy, platy, tetra, etc) N=94	UK (koi and golfish only); warm water fish were imported from Singapore, Guyana, and Columbia	Culture method: Whole fish homogenate in PBS seeded onto Aeromonas media, subcultures confimed using phenotypic testing and API20NE, partial 16S rRNA gene sequencing. Susceptibility test disk diffusion (Abtek Biologicals) and MIC (Trek Sensititre), broth microdilution CLSI. PCR detection of AR genes, Class 1 integrons and Inc A/C and IncN plasmids.	Oxytetracycline, tetracycline, flumequine, oxolinic acid, enrofloxacin, ciprofloxacin, ofloxacin, gatifloxacin, chloramphenicol, florfenicol, amikacin, gentamicin, neomycin, spectinomycin, stretomycin, tobramycin, sulfamethoxazole/trimethoprim, furazolidone, amoxicillin, cefazolin, cephalothin, cefotetan sodium, ceftiofur, ceftazidime,ceftriaxone, moxalactam, cefpodoxime, piperacillin, cefepime, aztreonam, imipenam, meropenem, nitrofurantoin	*Aeromonas* spp	47/94 of warmwater isolates individually tolerant to >/= 15 antibiotics. Tolerance to antibiotics from more structural classes found in *Aeromonas* isolated from tropical species	Verner-Jeffreys, et al. 2009

- Annex 2-

Workshop EFSA ASK

Organisator : Anne BRISABOIS
- 24 & 25 of October 2018 -

*Participants (*ASK participants)*

	Name	First name	Organization
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6.	DUBUISSON	Carine	ANSES/DER
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8.	GRASTILLEUR*	Charlotte	ANSES/DER
9.	HAENNI	Marisa	ANSES/Lyon
10.	MADEC	Jean-Yves	ANSES/Lyon
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14.	URBAN	Delphine	ANSES/ANMV
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18.	BADAU	Estera	Université de la Sorbonne
19.	BORTOLAIA*	Valéria	DTU
20.	CHAMBERS	Edel	CEFAS
21.	ENDRESEN STORESUND	Julia	IMR
22.	ESTEVE	Jacques	Sté Ubiqus
23.	HERVIO-HEATH	Dominique	IFREMER
24.	HJERTAKER GREVSKOTT	Didirk	IMR
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26.	LEONI*	Francesca	IZSUM
27.	LUNESTAD*	Bjorn Tore	IMR
28.	MAGISTRALI*	Chiara	IZSUM
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31	RÖDER	Timo	DTU
32	SMITH	Peter	National University of Ireland
33	SVANEVIK*	Cecilie	IMR

- Annex 3 -

Linking seafood and bacteria target for AMR surveillance
Aim 1: Foodborne and environmental exposure of AMR bacteria
Aim 2: AMR in farmed fish

	Seafood species	Most popular species on the market	Aim	Bacterial target
Filter feeding	1.Crassostrea/Ostrea 2.Mytilus/Perna/Modiolus 3.Microcosmus/Pyura 4.Pollicipes 5.Acantochardia/ Cerastoderma/ Anarda 6.Ensis/Solen 7.Ruditapes, 8.Chamelea, 9.Mya 10.Venus verrucosa 11.Callista 12.Donax 13.Glycimeris 14.Arca noae 15.Pecten 16.Chlamys/Aequipecten	Mytilus Pecten/ Chlamys/ Aequipecten	**1-2**	*Escherichia coli* *Enterobacteriaceae* *Salmonella* *Vibrio (V. parahaemolyticus, V. vulnificus, nonO1 nonO139 V. cholerae, Vibrio spp.* such as *V. alginolyticus* and others) *Campylobacter* spp. *(C. jejuni, C. lari subsp. Concheus, Campylobacter peloridis)* *Arcobacter* spp. *(A. butzleri, A. cryaerophilus; others A. cloacae, A. defluvii, A. ellisii including marine species)* *Listeria* spp.? *Enterococcus* spp. *Pseudomonas* spp. *Shigella* *Aeromonas hydrophila* *Plesiomonas shigelloides* *Clostridium perfringens*

	Seafood species	Most popular species on the market	Aim	Bacterial target
Costal	1.Acipenser/Huso 2.Lepidopus/Aphanopus 3.Oncorhynchus/Salmo 4.Dicentrarchus 5.Umbrina 6.Sciaena 7.Argyrosomus 8.Dentex 9.Sparus 10.Pagellus 11.Pagrus 12.Spondyliosoma 13.Sarpa 14.Lithognathus 15.Diplodus 16.Oblada 17.Mullus 18.Gadus 19.Pollachius 20.Melanogrammus 21.Merlangius 22.Chelidonichthys 23.Epinephelus 24.Trachurus 25.Zeus 26.Squalus 27.Scyliorhinus 28.Mustelus 29.Raja 30.Sepia 31.Loligo 32.Totadores/Illex 33.Uranoscopus 34.Trachinus 35.Murena 36.Conger 37.Scorpaena 38.Arnoglossus 39.Pleuronectes 40.Solea/Microstomus 41.Psetta 42.Plathichthys/Limanda	Gadus Pollachius Clupea Sardina Merluccius Loligo/ Totadores/ Illex/Sepia Penaeus/ Palaemon/ Pleoticus/ Metapenaeus/ Crangon	**1-2**	*E. coli* *Vibrio* spp. *Enterobacteriaceae* species Oncorhynchus/Salmo: *Listeria* Penaeus/ Palaemon/Pleoticus/ Metapenaeus/ Crangon : *Vibrio parahaemolyticus, Vibrio vulnficus, V. cholerae* nonO1 nonO139, *Vibrio* spp. (*V. alginolyticus* and others)