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From tide to table: A whole-year, coastal-wide surveillance of antimicrobial resistance in *Escherichia coli* from marine bivalves

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ABSTRACT

This work is the first of its kind to report a whole-year and coastal-wide surveillance of antimicrobial resistance (AMR) of *Escherichia coli* with samples from the EU imposed Norwegian surveillance programme for marine bivalves. In total, 390 bivalve samples collected from January to December in 2016 at 59 different harvest locations, were examined. The occurrence of resistant *E. coli* in relation to the concentration of *E. coli* was also analysed. From each sample with *E. coli* ($n = 261$), one isolate was susceptibility tested against a panel of 14 antimicrobials from ten classes. The occurrence of resistance to at least one antimicrobial was 8.4 %. Resistance to tetracycline was most commonly detected (5.7 %), followed by resistance to ampicillin (4.6 %) and sulfamethoxazole (3.1 %). The occurrence of extended spectrum cephalosporin (ESC)-resistant *E. coli*, quinolone-resistant *E. coli* (QREC) and carbapenem-resistant Enterobacteriaceae (CRE) were detected through selective screening in 3.3 %, 12.8 % and none of the samples, respectively. Among the ESC-resistant *E. coli*, the $bla_{\text{CTX-M-15}}$ gene was detected in nine isolates, where two isolates also carried the $bla_{\text{CMY-42}}$ gene, followed by $bla_{\text{CTX-M-3}}$ in two and $bla_{\text{CTX-M-1}}$ in one. One isolate was resistant to ESC due to the n.-42C>T mutation in the AmpC gene. Only the presence of QREC clustered significantly ($p < 0.013$) in space including nine harvest locations. An increased risk (OR 9.4) of detecting ESC-resistant *E. coli* or QREC was found for samples with *E. coli* concentrations above the threshold of Class A for direct distribution to the market (i.e. 230 *E. coli*/100 g). However, five of the ESC-resistant *E. coli* and 26 of the QREC positive samples, had levels of *E. coli* below the threshold, thus from areas cleared for sale. Among the 17 ESC-resistant *E. coli* subjected to whole genome sequencing, two originated from two samples of great scallops and two samples of flat oysters, which are often consumed raw or lightly processed. One of these isolates belonged to the high-risk clone sequence type 131 and carried a plasmid born *senB* gene encoding the *Shigella* enterotoxin 2 (ShET2) attributed to cause watery diarrhoea in infections caused by Enteroinvasive *E. coli* (EIEC). Thus, our study shows that there is a potential risk for transmission of resistant and pathogenic *E. coli* to the consumers from these products.

1. Introduction

Antimicrobial resistance (AMR) is one of the main global health related challenges, and treatment failure of previously treatable infections are increasingly reported (WHO, 2022). Resistant bacteria may be transmitted to humans through multiple routes, from human carriers (Ulstad et al., 2016), from wild, farmed or pet animals (Nielsen et al., 2018), by international travel (Kennedy and Collignon, 2010; Peng et al., 2021), and through the food chain (Caniça et al., 2019). A One Health approach focus on the nexus of these compartments, and the role of the environmental dimension is becoming more important (Bengtsson-Palme et al., 2018; European Food Safety Authority, 2021; Huijbers et al., 2015; Larsson et al., 2018). This includes the links between terrestrial and marine environments, and seafood (Elbashir et al., 2018; Grevskott et al., 2017; Taylor et al., 2011). Even though antimicrobial resistant bacteria do exist among the indigenous marine microbiota, as reported for several *Vibrio* sp. (Håkonsholm et al., 2020), the transmission of antimicrobial resistant bacteria from terrestrial sources, such as sewage discharge, sewer overflows and runoffs from streets and farmyards, are of high concern for seafood safety.

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Being suspension feeders clearing on average 70 l of water per day retaining particles with viruses and bacteria attached (Cranford et al., 2011), marine bivalves are often suspected for being the transmitting vehicle in foodborne outbreaks (Potasman et al., 2002). Furthermore, recent reviews have identified such bivalves as microbial ticking bombs (Kijewska et al., 2023) with high prevalence risk of carrying antimicrobial resistant bacteria (Albini et al., 2022). Colistin-resistant bacteria have been reported from clams and scampi (Slettemeås et al., 2017; Valdez et al., 2022), carbapenem-resistant bacteria from shrimps and squids (Mangat et al., 2016; Morrison and Rubin, 2015), and bacteria resistant to extended spectrum cephalosporins (ESC) from fish and clams (Dib et al., 2018; Roschanski et al., 2017; Vignaroli et al., 2016). All examples of resistance of importance in human medicine and thereby occurrence of these in marine bivalves pose a possible health threat to consumers.

To ensure food safety, the EU legislation impose a surveillance programme for live bivalve molluscs where *Escherichia coli* is used as an indicator organism for faecal contamination (European Commission, 2019). All areas used for bivalve production or harvest have to be routinely tested by standardized methods (ISO 16649-3, 2005). According to the level of *E. coli* in a minimum of ten batch samples of bivalves collected over a whole year, the harvest locations are classified into three classes. Class A areas have 80 % of the bivalve samples below 230 *E. coli*/100 g and no samples over 700 *E. coli*/100 g. Bivalves from Class A areas can be used directly for human consumption without depuration, whereas the industry often practice some level of depuration regardless of classification. Class B areas have 90 % of the bivalve samples below 4600 *E. coli*/100 g and the remaining 10 % below 46,000 *E. coli*/100 g. Bivalves from these areas must be set for depuration in clean water or in a Class A area, or sufficiently heat treated before introduced to the market. In Class C areas, the bivalves must not exceed 46,000 *E. coli*/100 g, and bivalves from these areas must be relayed for more than two months in clean water, then reanalysed or heat treated before entering the market. Any area with bivalves having *E. coli* levels exceeding 46,000 *E. coli*/100 g must not be subjected to production and harvest for consumption is prohibited.

WHO rate members of the Enterobacteriaceae family as one of the leading causative agents of foodborne infections, and also list these species as part of the top three where there is a critical need for new antimicrobials due to substantial emergence of AMR (WHO, 2017). Especially *E. coli* receives considerable attention as it cause a large burden of disease (ECDC and WHO, 2022) and show rapid development of resistance to ESC, fluoroquinolones, carbapenems and colistin (EFSA, 2016). Norway is a country with restricted use of antimicrobials in human and veterinary medicine and annual surveillance show low levels of antimicrobial resistance compared to most other European countries (NORM/NORM-VET 2020 and 2021).

The global seafood consumption is increasing, and this is especially pronounced for raw or semi-raw delicacies such as scallops and oysters. Any seafood product containing bacteria resistant to antimicrobials represents a potential of transfer to humans during food handling and preparation, or directly through consumption. Thus, the objective of this study was to describe and assess the association between the occurrence of AMR in *E. coli* in marine bivalves and the concentration of *E. coli*, as well as the harvest locations and time of sampling. Samples were collected through the EU imposed Norwegian surveillance programme for bivalve molluscs and examined through the Norwegian surveillance programme on AMR in the veterinary sector (NORM-VET). To the best of our knowledge, this work is the first to document resistance patterns among *E. coli* in marine bivalves from a whole-year coastal-wide sampling.

2. Material and methods

2.1. Sampling

Samples in this study were obtained from the Norwegian surveillance programme for monitoring *E. coli* in marine bivalves in 2016 (Duinker et al., 2017; European Commission, 2004). The bivalve collection comprised 390 batch samples collected at 59 farmed bivalve production or harvest locations, scattered along the Norwegian coast where production occur (Fig. 1). The number of examined batch samples from each location varied between one and 19 samples, with a median of six. The examined species included 312 batch samples of blue mussels (*Mytilus edulis*), 38 batch samples of flat oysters (*Ostrea edulis*), 26 batch samples of great scallops (*Pecten maximus*), six batch samples of northern horse mussels (*Modiolus modiolus*), three batch samples of pacific oysters (*Crassostrea gigas*), three batch samples of ocean quahogs (*Arctica islandica*), and two batch samples of soft-shell clams (*Mya arenaria*).

2.2. E. coli quantification

All samples were quantitatively assessed for *E. coli* applying the standard Most Probable Number (MPN) method providing concentrations as MPN/100 g (ISO 16649-3, 2005) under the auspices of the Norwegian surveillance programme for monitoring *E. coli* in marine bivalves (Duinker et al., 2017). The limit of quantification was 18 *E. coli*/100 g.

2.3. Qualitative non-selective and selective isolation of E. coli

The methods used for isolation of *E. coli* and screening of specific resistances, as well as the susceptibility testing, were performed with the qualitative methods used for the routine monitoring of resistance in food and animals as performed in NORM-VET (NORM/NORM-VET 2016, 2017). In short, 25 g of bivalve soft tissue and mantel water (10–15 individuals) were homogenised (2 min) prior to the addition of 225 ml of buffered peptone water (bioMérieux Marcy-l'Étoile, France) followed by a new round of homogenisation (30 s). The homogenate was enriched at 37 ± 1 °C for 20 ± 2 h. After incubation, a loop-full (10 μl) from the enrichment broth was transferred to the following plates to detect: 1) *E. coli* on plain MacConkey agar (BD Biosciences, Le Pont de Claire, France), 2) ESC-resistant *E. coli* on MacConkey agar with 1 ml/lcefotaxime and MacConkey agar with 2 mg/l ceftazidime, 3) QREC on MacConkey agar with 0.06 mg/l ciprofloxacin. All plates were incubated at 44 ± 0.5 °C for 20 ± 2 h. Additionally, to detect carbapenem-resistant *E. coli,* a loop-full (10 μl) from the enrichment was transferred to CHROMID CARBA and CHROMID OXA-48 (bioMérieux) and incubated at 37 ± 0.5 °C for 20 ± 2 h. One randomly isolated *E. coli* from each positive plate was grown into pure culture and stored in Microbank™ tubes (Pro-Lab Diagnostics, Toronto, Canada) at −80 °C.

2.4. Antimicrobial resistance testing

All obtained isolates were subjected to antimicrobial susceptibility testing using a broth microdilution method obtaining minimum inhibitory concentration (MIC) for 14 antimicrobial agents using plates from Sensititre® (TREK Diagnostic Systems LTD, East Grinstead, United Kingdom). The panel contained ampicillin, tetracycline, chloramphenicol, sulfamethoxazole, trimethoprim, gentamicin, nalidixic acid, ciprofloxacin, cefotaxime, ceftazidime, colistin, meropenem, azithromycin and tigecycline. *E. coli* ATCC 25922 was used as susceptible quality control strain, whereas resistant control strains included *E. coli* CCUG 37382, *E. coli* K8-1 (ESBL), *E. coli* K5-20 (AmpC), *E. coli* 2012-60-1176-27 (*mcr-1)* and *E. coli* KP37 (*mcr-2)*. Wild-type isolates were differentiated from non-wild-type isolates according to EUCAST epidemiological cut-off values (ECOFFs, accessed 07.08.2023) from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) when

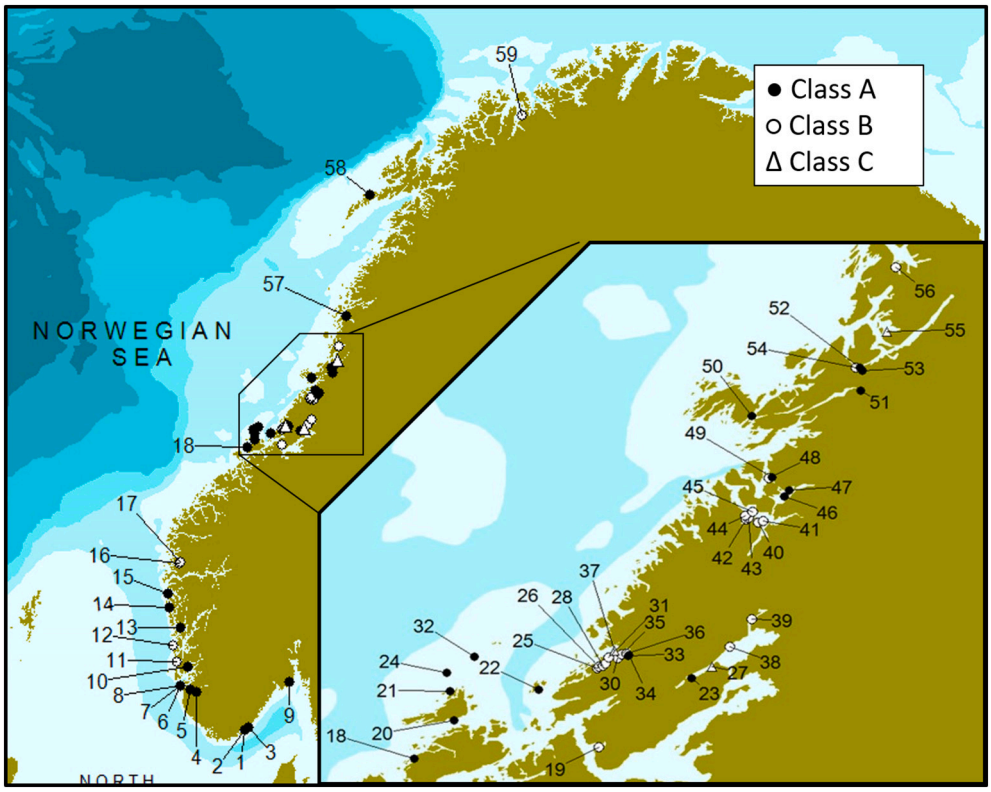

Fig. 1. Locations along the Norwegian coast from where batch samples of marine bivalve molluscs were collected. All locations were included in the Norwegian surveillance programme for *E. coli* in marine bivalves. The production sites are numbered 1–59 and classified according to the EU directive as: Class A: 80 % <230 *E. coli*/100 g and no samples >700 *E. coli*/100 g, Class B: 90 % < 4600 *E. coli*/100 g and 10 % < 46,000 *E. coli*/100 g, and Class C: > 46,000 *E. coli*.

available. Isolates categorised as non-wild-type are referred to as resistant.

2.5. Genomic analyses

The ESC-resistant *E. coli* isolates were further characterized using whole genome sequencing (WGS). For WGS, DNA was extracted using QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) and sequenced using an Illumina® MiSeq (Illumina, San Diego, California, USA). The sequence data were quality controlled by adapter and quality trimming using Trimmomatic (Bolger et al., 2014), and assembled using SPAdes v3.11.0 (Bankevich et al., 2012) using the "–careful" parameter and a contigs cut-off of "500". For the quality checking and assembly procedure, the Bifrost pipeline developed at the Norwegian Veterinary Institute (NVI) was applied (doi:https://doi.org/10.5281/zenodo.4043861). Assemblies or paired end reads were subjected to analysis using ResFinder V.4.1 for both acquired genes and chromosomal point mutations using the online tool at the Centre for Genomic Epidemiology web site (accessed 07.03.2023, https://cge.cbs.dtu.dk/services/ResFinder/). The reads were further subjected for multi-locus sequence typing (MLST) and virulence gene detection using the MLST software with PubMLST typing schemes (https://github.com/tseemann/mlst) and the Virulence_Finder v.1.0.0 pipeline (Joensen et al., 2014; Malberg Tetzschner et al., 2020), respectively. Both pipelines have been implemented on NVIs IRIDA platform (www.irida.ca). The reads are deposited to the European Nucleotide Archive (ENA) under study accession number PRJEB65091 (submission in progress).

2.6. Data sources and data management

Data and susceptibility test results on *E. coli* from bivalve samples from the NORM-VET programme 2016, were extracted from the internal recording system of the Norwegian Veterinary Institute and merged with the corresponding Norwegian surveillance programme for monitoring *E. coli* in marine bivalves' data from the internal recording system of the Institute of Marine Research. The latter including data on concentration of *E. coli* (MPN/100 g) and geographical locations of the sampled harvest locations. Data management was performed in R version 3.4.2 (R CoreTeam, 2019) and SAS SAS-PC system version 9.4 for Windows (SAS Institute inc., Cary, NC, USA).

2.7. Statistical analyses

Statistical analysis was performed in SAS-PC System® v 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). The 95 % confidence intervals were calculated by the exact binomial test using R version 3.6.1 for Windows (R Development Core Team, 2019).

The Odds Ratio (OR) of detecting ESC-resistant *E. coli* and QREC in bivalves with concentrations of *E. coli* above the Class A threshold (> 230 MPN/100 g) was calculated by contingency tables and Fisher's exact test performed in GraphPad Prism 9.1.1 (©1992–2021 Graph Pad Software, LLC).

We used SaTScan v 9.6 (SatScan.org) and performed a retrospective Space and Space-Time analysis scanning for clusters with high rates using the Bernoulli model (Kulldorff, 1997) to analyse for solely spatial or spatio-temporal clustering of the occurrence of ESC-resistant *E. coli* and QREC, respectively. The spatial window allowed a maximum spatial size of clusters to include 20 % of the population at risk and the window shape elliptic was chosen. For the temporal window, the time aggregation unit was set to month with a length of one. The default settings were used for the rest of the parameters. Identified spatial or spatio-temporal clusters with *P*-value below 0.05 were considered statistically significant.

3. Results

3.1. Detection and quantification of E. coli

Among the 390 examined bivalve samples, *E. coli* was detected by the qualititative method in 261 samples (67 %). By the the quantitative method, 209 (54 %) samples contained *E. coli*, whereas only 58 (15 %) of the samples had concentrations of *E. coli* above the Class A threshold (>230 *E. coli*/100 g) (Supplementary Table 1). The highest median values among the samples were detected during January, July, August, September and December (Fig. 2). The highest detected concentration was 18,000 MPN/100 g.

3.2. Occurrence of antimicrobial resistant E. coli

In total, 91.6 % [95 % CI: 87.5–94.6] of the 261 *E. coli* isolates, obtained through the non-selective method used in NORM-VET, were susceptible to all assessed antimicrobial classes. Altogether, 4.2 % [95 % CI: 2.1–7.4] of the isolates were resistant to one antimicrobial class, 2.7 % [95 % CI: 1.1–5.4] to two, 0.8 % [95 % CI: 0.1–2.7] to three or more antimicrobial classes as presented in Fig. 3. Resistance to tetracycline (5.7 %) was most frequently observed, followed by resistance to ampicillin (4.6 %) and sulfamethoxazole (3.1 %) (Supplementary Table 2). None of these *E. coli* isolates [95 % CI: 0.0–1.4 %] were resistant to cephalosporins, whereas quinolone resistance was identified in 0.8 % [95 % CI: 0.1–2.7] of the isolates.

3.3. Occurrence of and genetic profiles of the ESC-resistant E. coli and QREC

By selective screening, ESC-resistant *E. coli* were detected in 13 of the 390 samples (3.3 % [95 % CI: 1.8–5.6 %]) (Table 1). One isolate showed resistance due to the n.-42C > T mutation in the promoter and attenuator regions for the chromosomally located AmpC gene. Nine isolates showed resistance due to the plasmid encoded resistance gene $bla_{\text{CTX-M-15}}$. Two of these isolates carried additionally $bla_{\text{CMY-42}}$ and three additionally carried $bla_{\text{TEM-1B}}$. Two isolates showed resistance due to the plasmid encoded resistance gene $bla_{\text{CTX-M-3}}$ where one of them also carried the $bla_{\text{TEM-1B}}$ gene. The last isolate carried the gene $bla_{\text{CTX-M-1}}$ gene together with a $bla_{\text{TEM-210}}$ gene. None of the isolates showed resistance to meropenem.

In total 50 QREC (12.8 % [95 % CI: 9.6–16.5 %]) were detected through selective screening of the 390 samples. Among these isolates, 16 were only resistant to quinolones (32.0 %), whereas 12 were resistant to

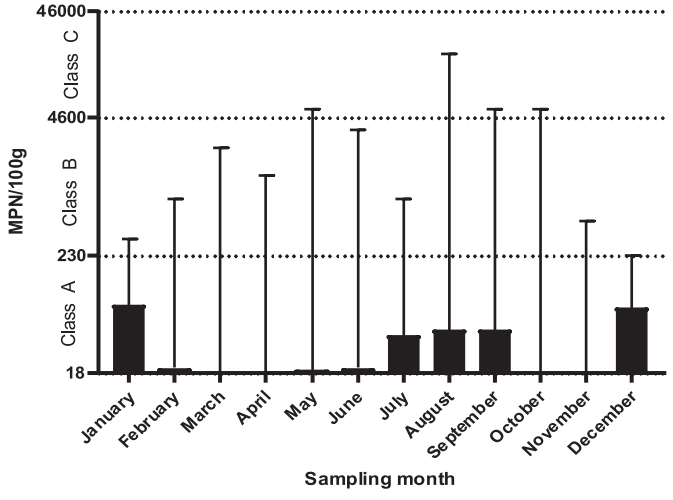

Fig. 2. Median values with range of MPN/100 g *E. coli* in marine bivalves. *N* = 390.

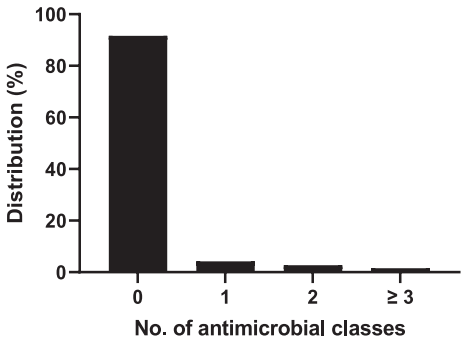

Fig. 3. Distribution of the 261 *E. coli* isolates originating from bivalves that showed resistance to none, one, two or three or more classes of antimicrobials.

one additional antimicrobial class (24.0 %, mainly ampicillin). The remaining 22 isolates were resistant to two more additional antimicrobial classes (44.0 %, mainly to ampicillin, sulfamethoxazole, trimethoprim, and tetracycline). Five of these QREC isolates showed additional resistance to ESCs, and for four of them, this was due to presence of $bla_{\text{CTX-M-15}}$, where two of the isolates also harboured $bla_{\text{CMY-42}}$. The last isolate was not subjected to WGS and ESC-resistant mechanisms was not detected by PCR. This isolate had a cefotaxime MIC of 0.5 μg/ml and ceftazidime MIC of ≤0.5 μg/ml, and was therefore regarded as sensitive.

Genomic analysis of the 17 confirmed ESC-resistant isolates from selective screening for both ESC-resistant *E. coli* and QREC, showed that isolates originating from the same sample were identical in terms of sequence type (ST) (Table 1) and identified virulence genes (Supplementary Table 3). In two samples sampled at the same time from locations relatively close, the isolates also appeared similar. The other isolates from different samples had all unique STs. Two isolates belonged to the high-risk clone ST131.

In-depth analysis of virulence genes is beyond the scope of this study. However, it is worth mentioning that four isolates carried a *senB* gene encoding the *Shigella* enterotoxin 2 (ShET2) and two other isolates carried an *astA* gene encoding an enteroaggregative heat-stable toxin (Supplementary Table 3).

3.4. Correlations between the occurrence of antimicrobial resistant E. coli, and E. coli concentrations, harvest locations and time of sampling

Eight of the 13 ESC-resistant *E. coli* isolates and 26 of the 50 QREC isolates were detected in bivalves with levels of *E. coli* above the Class A threshold. This shows a correlation between elevated concentrations of *E. coli* and the detection of ESC-resistant *E. coli* or QREC, with OR of 10.23 ($p < 0.0001$) and 10 ($p < 0.0001$), respectively.

No significant spatial or spatio-temporal clusters of ESC-resistant *E. coli* were detected. A significant ($p = 0.013$) spatial cluster of QREC was detected including the harvest locations 27, 38, 39, 40, 41, 42, 43, 44 and 45. The proportion of QREC within this area was 34.0 % constituting a relative risk of 3.17 to detect QREC within this cluster as compared to outside this cluster. The median concentrations of *E. coli* were 300 MPN/100 g ($n = 23$) within this cluster and 20 MPN/100 g ($n = 377$) outside of the cluster, and the OR for samples to be categorised as Class B were 11.42.

3.5. Bivalves intended for raw or lightly processed consumption

Among the 26 batch samples of great scallops, 12 were positive for *E. coli.* All samples were categorised as Class A. One of these samples was positive for ESC-resistant *E. coli* and one was positive for both ESC-resistant *E. coli* and QREC (Table 1). A third sample was positive for QREC without any other resistance traits detected (Supplementary Table 2).

A total of 23 of the 38 batch samples of flat oysters were positive for

Table 1
Overview of ESC-resistant *E. coli* organised according to classification by *E. coli* concentrations, and including information about species, location, sampling month, detection method, MLST profile, phenotypic resistance profile, acquired genes and genes with point mutations explaining their ESC resistance.

Class	MPN/100g	Species	Location	Month	Detection	MLST	Phenotype	Acquired genes	Point mutations	Sample ID
A	<18	Great scallops	50	April	ESC	131	AMP, CTX, CFT, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$ + $bla_{TEM\text{-}1B}$		2016–538
	<18	Blue mussels	41	October	ESC	5041	AMP, CTX, CFT	$bla_{CTX\text{-}M\text{-}1}$ + $bla_{TEM\text{-}210}$		2016–1647
	68	Flat oysters	6	August	ESC	201	AMP, CTX, CFT		ampC promoter n.-42C>T[a]	2016–1242
	110	Great scallops	7	September	ESC	38	AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$		2016–1423
					QREC	38	AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$		
	130	Ocean quahog	14	February	ESC	3268	TET, AMP, CTX, CFT, CIP	$bla_{CTX\text{-}M\text{-}15}$		2016–147
B	310	Blue mussels	19	May	ESC	120	CHL, AMP, CTX, SMX, TMP	$bla_{CTX\text{-}M\text{-}3}$ + $bla_{TEM\text{-}1B}$		2016–878
	330	Blue mussels	45	January	ESC	1193	TET, AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$ + $bla_{TEM\text{-}1B}$		2016–65
	460	Blue mussels	39	April	ESC	925	AMP, CTX, CFT	$bla_{CTX\text{-}M\text{-}3}$ + $bla_{TEM\text{-}3}$		2016–563
	490	Blue mussels	44	April	ESC	617	TET, AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$ + $bla_{CMY\text{-}42}$		2016–565
					QREC	617	TET, AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$ + $bla_{CMY\text{-}42}$		
	790	Blue mussels	17	August	ESC	43	AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$		2016–1240
					QREC	43	AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$		
	790	Blue mussels	29	September	ESC	131	AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$ + $bla_{TEM\text{-}1}$		2016–1397
	1300	Blue mussels	41	April	ESC	617	TET, AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$ + $bla_{CMY\text{-}42}$		2016–566
					QREC	617	TET, AMP, CTX, CFT, SMX, TMP, CIP, NAL	$bla_{CTX\text{-}M\text{-}15}$ + $bla_{CMY\text{-}42}$		
C	>18,000	Flat oysters	11	August	ESC	8207	AMP, CTX, CFT, CIP	$bla_{CTX\text{-}M\text{-}15}$		2016–1329

TET: tetracycline, AMP: ampicillin, CTX: cefotaxime, CFT: ceftazidime, SMX: sulfamethoxazole, TMP: trimethoprim, CIP: ciprofloxacin, NAL: nalidixic acid, ND: no data.
[a] Overexpression of chromosomally located *ampC* due to mutation in the promoter and attenuator regions.

E. coli, where 18 categorised into Class A, four within Class B, and one in Class C. One of the Class A samples of flat oysters was positive for ESC-resistant *E. coli*, whereas another was positive for QREC. The Class C sample of flat oysters contained extremely high levels of *E. coli* (>18,000 MPN/100 g) and harboured both ESC-resistant *E. coli* and QREC (Table 1). The *E. coli* isolates from this sample were likely three different strains as their phenotypic resistance profiles differed. The QREC expressed additional resistance to tetracycline, chloramphenicol, ampicillin, sulfamethoxazole and nalidixic acid, but did not show resistance towards any of the ESCs. The ESC-resistant isolate expressed only additional resistance to ciprofloxacin and harboured the $bla_{CTX\text{-}M\text{-}15}$ and *qnrS1* genes. The *E. coli* isolate from the non-selective method was fully susceptible to all antimicrobials tested for.

4. Discussion

Overall, we detected a low prevalence of AMR in marine bivalves. We found that both ESC-resistant *E. coli* (3.3 %) and QREC (12.8 %) could be detected, however only in low frequencies and mainly by selective methods. Among the 261 *E. coli* detected by a non-selective method, only 0.8 % were resistant to quinolones, and none to ESC. Grevskott et al. (2017) examined marine bivalves from similar harvest areas and found *E. coli* resistant to ESC among ~2 % and to quinolones among ~1 % of the samples examined by the standardized MPN-method ($n = 549$). In Portugal, *E. coli* resistant to ESC was detected in ~1 % of marine bivalve samples ($n = 522$) (Freire et al., 2023). Many other studies have reported findings of ESC-resistant *E. coli* and QREC from marine bivalves, such as in Venus Clams from Italy (Vignaroli et al., 2016) and Kuwait (Al-Sarawi et al., 2018), and bivalves from Brazil and the United States of America (Miotto et al., 2019). However, differences in study design, enrichment methodology and especially how many isolates picked from the same sample, makes it difficult to compare the results, underpinning the importance of separate protocols when monitoring AMR (Apostolakos et al., 2020). Also, geographical differences is expected as occurrence in bivalves is depending on levels of contamination in the nearby marine environment.

In the present study, a spatial cluster effect was identified for QREC for one geographical area with high marine bivalve production. *E. coli* might reach the marine environment through several routes, such as run-offs from land and faecal droppings from birds and marine mammals, and previous studies have demonstrated that bivalves can harbour *E. coli* from multiple sources, including human phylotypes (Vignaroli et al., 2016) and strains with MLST-profiles similar to isolates from human infections (Grevskott et al., 2017).

Given the low numbers of ESC-resistant *E. coli* in the present study, any harvest area cluster effect could not be detected. However, the majority of the ESC-resistant *E. coli* isolates carried the plasmid mediated $bla_{CTX\text{-}M\text{-}15}$ gene, though belonging to several different STs. The $bla_{CTX\text{-}M\text{-}15}$ gene has previously been described as the predominant CTX-M type in ESC-resistant *E. coli* causing human infections in Norway (Gladstone et al., 2021). This was also indicated by Grevskott et al. investigating sewage in the Bergen city area (Grevskott et al., 2021). The $bla_{CTX\text{-}M\text{-}15}$ gene has also been detected in ESC-resistant *E. coli* from animals in Norway, both in clinical isolates from dogs and cats and in isolates from healthy production animals, i.e. from turkey in 2022 (NORM/NORM-VET 2022, in press) and pigs during 2017–2021 (NORM/NORM-VET 2021), indicating that both animals and humans must be regarded as possible original sources for the bivalve

contamination. The pig isolates did, however, belong to other STs (i.e. ST10, ST 58 and ST164) than the bivalve isolates. Furthermore, one of the $bla_{CTX\text{-}M\text{-}15}$ bivalve isolates, belonged to ST38, a ST found to be dominating among ESC-resistant *E. coli* in broilers in Norway (Mo et al., 2023). In contrast to the ST38 isolates in the present study, Mo et al. showed that the ESC resistance in the Norwegian ST38 broiler isolates was due to presence of the $bla_{CMY\text{-}2}$ gene, indicating other sources than broilers for the occurrence of ST38 in bivalves in the present study. However, the overall occurrence of ESC-resistant *E. coli* due to plasmid-mediated genes is still very low in Norway with <5 % in healthy carriers (Ulstad et al., 2016), 5.8 % (blood) and 3.1 % (urine) in clinical human samples, and < 2 % in pigs (NORM/NORM-VET 2020, 2021; NORM/NORM-VET 2021, 2022).

No temporal correlation for detection of ESC-resistant *E. coli* or QREC could be shown in the present study. However, an increased detection rate of ESC-resistant *E. coli* and QREC was seen for samples with elevated concentrations of *E. coli*, and thus months with higher median concentrations, such as July, August and September, as well as December and January, should have increased attention. This corresponds well with the study of Lunestad et al., summarising data from 2007 to 2012 from the same surveillance programme, where elevated concentrations of *E. coli* in bivalves were found in June, July, October and also in December (Lunestad et al., 2015). In Italy, the late summer months were found to be associated with low levels of *E. coli* in bivalves, while elevated levels were found in February (Vignaroli et al., 2016). This support that geography and climate differences are important factors influencing the *E. coli* concentrations in bivalves. Other factors, such as sea current, bacterial distribution and dilution (Rees et al., 2015), as well as bivalve digestion and decomposition rates, which are found to both retain (Ottaviani et al., 2015) and eliminate bacteria (Suzuki et al., 2018), will impact how long the *E. coli* would sustain in the bivalves. These are all factors that might influence and contribute to the explanation of the observed cluster effect.

The anatomical and physiological differences between different bivalve species might also impact on how long the bacteria are retained. Blue mussels are found in the tidal zone, whereas flat oysters and scallops spend most of their time on the sea bed (Hovgaard et al., 2001). Furthermore, how these species are prepared is also important when assessing their risk as pathogenic vehicles. Unlike blue mussels, which are prepared by high temperature steaming, scallops are opened by hand, and the muscle and row is eaten raw or lightly preserved, without any heat treatment. Oysters are opened by hand as well, and in most cases, the whole soft tissue, including the digestive gland, is eaten raw. In total seven out of 64 (11 %) of the examined samples of great scallops and flat oysters contained either ESC-resistant *E. coli*, QREC or both, and five (8 %) of these samples were classified as Class A and cleared for sale without any additional depuration and entering the food chain contaminated.

Noteworthy, two isolates belonged to ST131 (Table 1), where one originated from a sample of great scallops with no quantifiable levels of *E. coli* and hence cleared for sale (2016–538). The ST 131 is known as a global spread high-risk clone causing infections in humans (Stoesser et al., 2016). Furthermore, the ST131 isolates carried a plasmid born *senB* (Supplementary Table 3) gene encoding the *Shigella* enterotoxin 2 protein (ShET2) attributed to cause watery diarrhoea in infections caused by Enteroinvasive *E. coli* (EIEC) (Bona et al., 2019; Pakbin et al., 2021). Another isolate, belonging to ST201, carried an *astA* gene encoding an enteroaggregative heat-stable toxin (EAST1) that is associated with several enteric pathogenic *E. coli* (Pakbin 2021). This isolate was detected from a sample of flat oysters (2016-1242). As for the ST131 isolates, the ST201 isolate was detected from a class A categorised sample with low levels of *E. coli*. The *astA* gene was also detected in an ST 8207 isolate that was detected from a sample of flat oysters with extreme numbers of *E. coli* (> 18,000 MPN/100 g) (2016-1329). Consequently, bivalves from this area could only be sold after purification in clean water for at least two months, before performing reanalysis or sufficient heat treatment.

Any person handling scallops containing ESC-resistant *E. coli* or QREC would be at risk to be exposed to these bacteria. There is a well-documented increased risk of virus infections after ingestion of undercooked bivalves (Elbashir et al., 2018), and the presence of any ESC-resistant *E. coli* or QREC adds to the challenge of ensuring food safety of such raw or lightly preserved delicacies.

5. Conclusion

Overall, this study showed that there is a low prevalence of AMR *E. coli* in marine bivalves in Norway, with indications that both geographical factors and increased concentrations of *E. coli* have an effect on detection of ESC-resistant *E. coli* or QREC. Still, ESC-resistant *E. coli* isolates linked to human and animal infeciontions could be found in bivalves cleared for sale without the need for any depuration or heat treatment, including great scallops and flat oysters. These bivalves are often consumed raw or only lightly processed, thereby constituting an increased risk for transmission of AMR and pathogenic *E. coli* to humans.

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CRediT authorship contribution statement

Cecilie Smith Svanevik: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization, Project administration. **Madelaine Norström:** Methodology, Data curation, Formal analysis, Writing – review & editing. **Bjørn Tore Lunestad:** Conceptualization, Methodology, Writing – review & editing. **Jannice Schau Slettemeås:** Formal analysis, Methodology, Investigation, Validation, Writing – review & editing. **Anne Margrete Urdahl:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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References

Albini, E., Orso, M., Cozzolino, F., Sacchini, L., Leoni, F., Magistrali, C.F., 2022. A systematic review and meta-analysis on antimicrobial resistance in marine bivalves. Front. Microbiol. 13, 1040568 https://doi.org/10.3389/fmicb.2022.1040568.

Al-Sarawi, H.A., Jha, A.N., Baker-Austin, C., Al-Sarawi, M.A., Lyons, B.P., 2018. Baseline screening for the presence of antimicrobial resistance in *E. coli* isolated from Kuwait's marine environment. Mar. Pollut. Bull. 129 (2), 893–898. https://doi.org/10.1016/j.marpolbul.2017.10.044.

Apostolakos, I., Mughini-Gras, L., Fasolato, L., Piccirillo, A., 2020. Impact of selective and non-selective media on prevalence and genetic makeup of ESBL/pAmpC-producing *Escherichia coli* in the broiler production pyramid. Vet. Microbiol. 240, 108536.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (5), 455–477.

Bengtsson-Palme, J., Kristiansson, E., Larsson, D.G.J., 2018. Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol. Rev. 42 (1) https://doi.org/10.1093/femsre/fux053 (fux053-fux053).

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120.

Bona, M., Medeiros, P.H., Santos, A.K., Freitas, T., Prata, M., Veras, H., Amaral, M., Oliveira, D., Havt, A., Lima, A.Â., 2019. Virulence-related genes are associated with clinical and nutritional outcomes of *Shigella*/Enteroinvasive *Escherichia coli* pathotype infection in children from Brazilian semiarid region: a community case-control study. Int. J. Med. Microbiol. 309 (2), 151–158.

Caniça, M., Manageiro, V., Abriouel, H., Moran-Gilad, J., Franz, C.M.A.P., 2019. Antibiotic resistance in foodborne bacteria. Trends Food Sci. Technol. 84, 41–44. https://doi.org/10.1016/j.tifs.2018.08.001.

Cranford, P.J., Ward, J.E., Shumway, S.E., 2011. Bivalve filter feeding: variability and limits of the aquaculture biofilter. Shellf. Aquacult. Environ. 81–124.

Dib, A.L., Agabou, A., Chahed, A., Kurekci, C., Moreno, E., Espigares, M., Espigares, E., 2018. Isolation, molecular characterization and antimicrobial resistance of enterobacteriaceae isolated from fish and seafood. Food Control 88, 54–60. https://doi.org/10.1016/j.foodcont.2018.01.005.

Duinker, A., Roiha, I.S., Lunestad, B.T., Sanden, M., 2017. Nasjonalt tilsynsprogram for produksjon av skjell og andre bløtdyr –prøver analyser i 2016:Kjemiske forurensende stoffer og mikroorganismer (Report, Issue). https://www.hi.no/resources/2016-Mattilsynets-overvaking-og-kartleggingsprogrammer-skjell-og-andre-blotdyr.pdf.

Regulation (EC) No. 854/2004 of the European Parliament and of the Council Laying Down Specific Rules for the Organisation of Official Controls on Products of Animal Origin Intended for Human Consumption 854/2004 (Vol. 854/2004, pp. 83–127) The European Parliament and the Council of the European Union, Brussels, Belgium, (2004).

Elbashir, S., Parveen, S., Schwarz, J., Rippen, T., Jahncke, M., DePaola, A., 2018. Seafood pathogens and information on antimicrobial resistance: a review. Food Microbiol. 70, 85–93. https://doi.org/10.1016/j.fm.2017.09.011.

Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls (Text with EEA relevance.), (2019). https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32019R0627.

European Centre for Disease Prevention and Control, & World Health Organization, 2022. Surveillance of antimicrobial resistance in Europe, 2021 data. https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2021-data.

European Food Safety Authority, 2016. EUSR on AMR in zoonotic and indicator bacteria from humans, animals and food 2014 (EFSA Journal, Issue). http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4380/abstract.

European Food Safety Authority, 2021. Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain (1831-4732). In: EFSA Journal, Issue.

Freire, S., Grilo, T., Rodrigues, B., Oliveira, R., Esteves, C., Marques, A., Poirel, L., Aires-de-Sousa, M., 2023. ESBL- and carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* among bivalves from Portuguese shellfish production areas. Microorganisms 11 (2), 415. https://www.mdpi.com/2076-2607/11/2/415.

Gladstone, R.A., McNally, A., Pöntinen, A.K., Tonkin-Hill, G., Lees, J.A., Skytén, K., Cléon, F., Christensen, M.O., Haldorsen, B.C., Bye, K.K., 2021. Emergence and dissemination of antimicrobial resistance in *Escherichia coli* causing bloodstream infections in Norway in 2002–17: a nationwide, longitudinal, microbial population genomic study. Lancet Microb. 2 (7), e331–e341.

Grevskott, D.H., Svanevik, C.S., Sunde, M., Wester, A., Lunestad, B.T., 2017. Marine bivalve mollusks as possible indicators of multidrug-resistant *Escherichia coli* and other species of the Enterobacteriaceae family [original research]. Front. Microbiol. 8 (24) https://doi.org/10.3389/fmicb.2017.00024.

Grevskott, D.H., Ghavidel, F.Z., Svanevik, C.S., Marathe, N.P., 2021. Resistance profiles and diversity of β-lactamases in *Escherichia coli* strains isolated from city-scale sewage surveillance in Bergen, Norway mimic clinical prevalence. Ecotoxicol. Environ. Saf. 226, 112788.

Håkonsholm, F., Lunestad, B.T., Aguirre Sánchez, J.R., Martinez-Urtaza, J., Marathe, N. P., Svanevik, C.S., 2020. Vibrios from the Norwegian marine environment: characterization of associated antibiotic resistance and virulence genes. Microbiologyopen 9 (9), e1093.

Hovgaard, P., Mortensen, S., Strand, Ø., 2001. Skjell: biologi og dyrking. Kystnæringen forl. og bokklubb.

Huijbers, P.M., Blaak, H., de Jong, M.C., Graat, E.A., Vandenbroucke-Grauls, C.M., de Roda Husman, A.M., 2015. Role of the environment in the transmission of antimicrobial resistance to humans: a review. Environ. Sci. Technol. 49 (20), 11993–12004.

ISO 16649-3, 2005. Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide. In: Geneva, Switserland: International Organization for Standardization (ISO).

Joensen, K.G., Scheutz, F., Lund, O., Hasman, H., Kaas, R.S., Nielsen, E.M., Aarestrup, F. M., 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. J. Clin. Microbiol. 52 (5), 1501–1510.

Kennedy, K., Collignon, P., 2010. Colonisation with *Escherichia coli* resistant to "critically important" antibiotics: a high risk for international travellers. Eur. J. Clin. Microbiol. Infect. Dis. 29 https://doi.org/10.1007/s10096-010-1031-y.

Kijewska, A., Koroza, A., Grudlewska-Buda, K., Kijewski, T., Wiktorczyk-Kapischke, N., Zorena, K., Skowron, K., 2023. Molluscs - A ticking microbial bomb. Front. Microbiol. 13, 1061223 https://doi.org/10.3389/fmicb.2022.1061223.

Kulldorff, M., 1997. A spatial scan statistic. Commun. Stat. Theor. Methods 26 (6), 1481–1496.

Larsson, D.G.J., Andremont, A., Bengtsson-Palme, J., Brandt, K.K., de Roda Husman, A. M., Fagerstedt, P., Fick, J., Flach, C.-F., Gaze, W.H., Kuroda, M., Kvint, K., Laxminarayan, R., Manaia, C.M., Nielsen, K.M., Plant, L., Ploy, M.-C., Segovia, C., Simonet, P., Smalla, K., Wernersson, A.-S., 2018. Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. Environ. Int. 117, 132–138. https://doi.org/10.1016/j.envint.2018.04.041.

Lunestad, B.T., Frantzen, S., Svanevik, C.S., Roiha, I.S., Duinker, A., 2015. Time trends in the prevalence of *Escherichia coli* and enterococci in bivalves harvested in Norway during 2007–2012. Food Control 60, 289–295.

Malberg Tetzschner, A.M., Johnson, J.R., Johnston, B.D., Lund, O., Scheutz, F., 2020. In silico genotyping of *Escherichia coli* isolates for extraintestinal virulence genes by use of whole-genome sequencing data. J. Clin. Microbiol. 58 (10) https://doi.org/10.1128/jcm.01269-01220.

Mangat, C.S., Boyd, D., Janecko, N., Martz, S.-L., Desruisseau, A., Carpenter, M., Reid-Smith, R.J., Mulvey, M.R., 2016. Characterization of VCC-1, a novel Ambler Class A carbapenemase from *Vibrio cholerae* isolated from imported retail shrimp sold in Canada. Antimicrob. Agents Chemother. https://doi.org/10.1128/aac.02812-15.

Mo, S.S., Fiskebeck, E.Z., Slettemeås, J.S., Lagesen, K., Nilsson, O., Naseer, U., Jørgensen, S.B., Thorsteinsdottir, T.R., Sunde, M., 2023. *Escherichia coli* multilocus sequence type 38 from humans and broiler production represent distinct monophyletic groups. Front. Microbiol. 14, 1173287.

Morrison, B.J., Rubin, J.E., 2015. Carbapenemase producing bacteria in the food supply escaping detection. PLoS One 10 (5), e0126717.

Nielsen, K.M., Gjøen, T., Asare, N.Y.O., Lunestad, B.T., Ytrehus, B., Yazdankhah, S.P., Godfroid, J., Jelmert, A., Klein, J., Okoli, A.S., 2018. Antimicrobial resistance in wildlife potential for dissemination. In: Opinion of the Panel on Microbial Ecology of the Norwegian Scientific Committee for Food and Environment. VKM Report.

NORM/NORM-VET 2016, 2017. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway https://www.vetinst.no/en/surveillance-programmes/norm-norm-vet-report.

NORM/NORM-VET 2020, 2021. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. https://www.vetinst.no/en/surveillance-programmes/norm-norm-vet-report.

NORM/NORM-VET 2021, 2022. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. https://www.vetinst.no/en/surveillance-programmes/norm-norm-vet-report.

Ottaviani, D., Chierichetti, S., Rocchegiani, E., Santarelli, S., Leoni, F., 2015. Bioaccumulation of *Escherichia coli* O157: H7 in *Mytilus galloprovincialis* under experimental conditions. Aquac. Res. 48 (1), 356–360. https://doi.org/10.1111/are.12852.

Pakbin, B., Brück, W.M., Rossen, J.W.A., 2021. Virulence factors of enteric pathogenic Escherichia coli: a review. Int. J. Mol. Sci. 22 (18), 9922. https://www.mdpi.com/1422-0067/22/18/9922.

Peng, Y., Liang, S.S., Poonsuk, K., On, H., Li, S.W., Maurin, M.M.P., Chan, C.H., Chan, C. L., Sin, Z.Y., Tun, H.M., 2021. Role of gut microbiota in travel-related acquisition of extended spectrum beta-lactamase-producing Enterobacteriaceae. J. Travel Med. 28 (3), taab022 https://doi.org/10.1093/jtm/taab022.

Potasman, I., Paz, A., Odeh, M., 2002. Infectious outbreaks associated with bivalve shellfish consumption: a worldwide perspective. Clin. Infect. Dis. 35 (8), 921–928. https://doi.org/10.1086/342330.

Rees, E.E., Davidson, J., Fairbrother, J.M., St. Hilaire, S., Saab, M., McClure, J.T., 2015. Occurrence and antimicrobial resistance of *Escherichia coli* in oysters and mussels from Atlantic Canada. Foodborne Pathog. Dis. 12 (2), 164–169.

Roschanski, N., Guenther, S., Vu, T.T.T., Fischer, J., Semmler, T., Huehn, S., Alter, T., Roesler, U., 2017. VIM-1 carbapenemase-producing *Escherichia coli* isolated from retail seafood, Germany 2016. Eurosurveillance 22 (43), 17-00032.

Slettemeås, J.S., Urdahl, A.M., Mo, S.S., Johannessen, G.S., Grave, K., Norström, M., Steinbakk, M., Sunde, M., 2017. Imported food and feed as contributors to the introduction of plasmid-mediated colistin-resistant Enterobacteriaceae to a 'low prevalence' country. J. Antimicrob. Chemother. 72 (9), 2675–2677. https://doi.org/10.1093/jac/dkx161.

Stoesser, N., Sheppard, A.E., Pankhurst, L., De Maio, N., Moore, C.E., Sebra, R., Turner, P., Anson, L.W., Kasarskis, A., Batty, E.M., 2016. Evolutionary history of the global emergence of the *Escherichia coli* epidemic clone ST131. mBio 7 (2). https://doi.org/10.1128/mbio.02162-02115.

Suzuki, S., Makihara, N., Kadoya, A., 2018. Tetracycline resistance gene tet(M) of a marine bacterial strain is not accumulated in bivalves from seawater in clam tank experiment and mussel monitoring. Sci. Total Environ. 634, 181–187. https://doi.org/10.1016/j.scitotenv.2018.03.305.

Taylor, N.G.H., Verner-Jeffreys, D.W., Baker-Austin, C., 2011. Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? Trends Ecol. Evol. 26 (6), 278–284. https://doi.org/10.1016/j.tree.2011.03.004.

Ulstad, C.R., Solheim, M., Berg, S., Lindbæk, M., Dahle, U.R., Wester, A.L., 2016. Carriage of ESBL/AmpC-producing or ciprofloxacin non-susceptible *Escherichia coli* and *Klebsiella* spp. in healty people in Norway. Antimicrob. Resist. Infect. Control 5 (1), 57. https://doi.org/10.1186/s13756-016-0156-x.

Valdez, C., Costa, C., Simões, M., de Carvalho, C., Baptista, T., Campos, M.J., 2022. Detection of mcr-1 gene in undefined *Vibrio* species isolated from clams. Microorganisms 10 (2). https://doi.org/10.3390/microorganisms10020394.

Vignaroli, C., Di Sante, L., Leoni, F., Chierichetti, S., Ottaviani, D., Citterio, B., Biavasco, F., 2016. Multidrug-resistant and epidemic clones of *Escherichia coli* from natural beds of Venus clam. Food Microbiol. 59, 1–6. https://doi.org/10.1016/j.fm.2016.05.003.

World Health Organization, 2017. Global Priority List of Antibiotic-Resistant bacteria to guide research, discovery, and development of new antibiotics. Retrieved 01.04 from. http://remed.org/wp-content/uploads/2017/03/lobal-priority-list-of-antibiotic-resistant-bacteria-2017.pdf.

World Health Organization, 2022. Antimicrobial Resistance Surveillance in Europe 2022–2020 Data.