International Journal of Food Microbiology 430 (2025) 111063

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Imported seafood is a reservoir of *Enterobacteriaceae* carrying CTX-M-encoding genes of high clinical relevance

Ryan Pearce [a,b], Mattia Pirolo [b], Nicole B. Goecke [b], Valeria Toppi [b,c], Liam Good [a], Javier Guitian [a], Luca Guardabassi [a,b,*]

[a] Department of Pathobiology and Population Sciences, Royal Veterinary College, NW1 0TU London, United Kingdom
[b] Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark
[c] Department of Veterinary Medicine, University of Perugia, 06126 Perugia, Italy

ARTICLE INFO

Keywords:
Antimicrobial resistance
Extended-spectrum β-lactamase
Aquaculture
Co-selection

ABSTRACT

We determined the frequency, genotypes, phenotypes, and mobility of extended-spectrum β-lactamase (ESBL)-encoding genes in *Enterobacteriaceae* isolated from retail seafood products. Overall, 288 samples of fresh shrimps, catfish and seabass imported from Asia were collected from three supermarket chains in the UK (96 each). After enrichment in MacConkey broth supplemented with cefotaxime, total DNA was screened for the presence of CTX-M, SHV and TEM by real-time PCR. Positive samples were cultured on ESBL selective media and presumptive ESBL-producing isolates were confirmed by PCR and identified to the species level by MALDI-TOF-MS. CTX-M-positive isolates were further characterized by whole genome sequencing (WGS), antimicrobial susceptibility testing, and conjugation experiments. Approximately one in thirteen (7.6 %) seafood products were contaminated with ESBL-producing *Enterobacteriaceae*. WGS analysis revealed the presence of CTX-M-15 ($n = 7$), CTX-M-27 (n = 7), and CTX-M-55 (n = 7), CTX-M-14 ($n = 4$) among *Enterobacteriaceae* isolated from shrimp ($n = 21$) and catfish (n = 4), and FONA-6 in two *Serratia fonticola* isolates from seabass. The higher rate of contamination in shrimp could be due to post-harvest contamination due to human handling or washing practices during processing. Half ($n = 13$) of the CTX-M-producing isolates transferred $bla_{CTX\text{-}M}$ to laboratory *E. coli* via IncA/C ($n = 6$), IncX2 ($n = 4$), IncFIIK ($n = 1$) or non-typeable plasmids ($n = 2$). All plasmids contained additional resistance genes conferring resistance to antimicrobials used in aquaculture, indicating possible co-selection through the use these antimicrobials. The frequent occurrence of CTX-M-encoding genes of high clinical relevance in imported seafood, particularly shrimp, often on transferrable plasmids, underscores the need for ESBL surveillance on traded seafood, alongside quantitative risk assessment studies aimed at evaluating the potential health risks for consumers who are exposed to these bacteria via consumption of raw seafood.

1. Introduction

Extended-spectrum β-lactamases (ESBLs) are enzymes produced by Gram-negative bacteria that confer resistance to third and fourth generation cephalosporins, which are critically important antimicrobials according to the World Health Organization (WHO, 2017). Since the introduction of third generation cephalosporins in the 1980s and fourth generation cephalosporins in 1994, resistance to these drug classes has increased (Paterson and Bonomo, 2005). Among ESBLs, the CTX-M variants have emerged as the dominant enzyme in *Enterobacteriaceae*, with CTX-M-15 being the most prevalent in human infections globally, followed by CTX-M-14 and CTX-M-27 (Castanheira et al., 2021). Although human-to-human transmission remains the primary mode of ESBL dissemination in high-income countries (Perestrelo et al., 2022), there is growing concern about the potential risks associated with these resistance determinants in food and possible zoonotic transmission via contaminated food products (Castanheira et al., 2021).

In the last two decades seafood consumption has experienced a surge in popularity, with global consumption rising by 58 % from 100 million tonnes in the year 2000 to 158 million tonnes at the end of 2019 (FAO, 2022). As hubs of production emerge and supply products globally, the potential for food to be the vehicle of microbiological or chemical hazards on a large-scale increase (Antunes, 2023). Despite the economic significance of this food industry, the prevalence of ESBL-producing

* Corresponding author at: Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark.
E-mail address: lg@sund.ku.dk (L. Guardabassi).

https://doi.org/10.1016/j.ijfoodmicro.2025.111063
Received 28 August 2024; Received in revised form 7 January 2025; Accepted 10 January 2025
Available online 13 January 2025

bacterial contamination in imported seafood products sold has not been comprehensively investigated. A recent source attribution study in the Netherlands revealed seafood as one of the most important non-human source of community-acquired carriage of ESBL-producing *E. coli* (Mughini-Gras et al., 2019). To further investigate this transmission route, we conducted a cross-sectional survey to determine the frequency, genotypes, phenotypes, and mobility of ESBL-encoding genes in imported seafood products to which the United Kingdoms (UK) consumers are exposed to.

2. Material and methods

2.1. Sample collection and enrichment

Between October 5th 2021, and November 24th 2021, we collected 288 seafood products sold raw at retail from three (A, B and, C) UK wide retail supermarket chains in London and the UK at large. Three types of seafood were sampled: farmed basa/catfish (*Pangasius hypophthalmus*) fillets imported from Vietnam, farmed sea bass (*Dicentrarchus labrax*) fillets imported from Turkey and farmed shrimp without shell and head (n = 32 *Litopenaeus vannamei*, n = 32 *Penaeus monodon* and, n = 32 *Penaeus vannamei*) imported from Vietnam. These countries where chosen as the sample origins as they represent the largest exporters of these products to the UK at the time of sample collection (Tridge, 2019). The sampling consisted of 96 samples of each seafood type, including 32 samples from each sampling location per sample type. Sample size requirements were calculated based on an expected ESBL prevalence of 50 %, with a 10 % margin of error and 95 % confidence level, using the method described by Cochran (1977). Metadata for samples is provided in Table S1 in supplemental material. Samples were transported in coolers with ice packs to the testing laboratory. Surface bacteria from the seafood samples were collected using an adapted ISO 6887-2:2017 method (ISO, 2017). Briefly, 5 g of each sample was incubated in 20 mL of buffered peptone water (Thermo scientific, USA) under agitation (240 rpm) at 37 °C for 18–24 h. One mL samples were then transferred to 15 mL of MacConkey broth in a 50 mL tube (Sigma-Aldrich, Milwaukee, USA) for selective enrichment of *Enterobacteriaceae*. After incubation under agitation (240 rpm) at 37 °C for 18–24 h, aliquots (2 mL) of the enriched cultures were stored at −80 °C in 20 % glycerol and subsequently shipped to the University of Copenhagen for further analysis.

2.2. Detection of resistance genes via high-throughput real-time PCR

A high-throughput real-time PCR was employed for detection and typing of ESBL genes. These included CTX-M group 1, 2, 8, 9 and 25, in addition to SHV and TEM. Primer (forward and reverse) and probe sequences for each assay are provided in Table S2 in supplemental material. Total DNA was extracted using the Qiagen DNeasy PowerFood Microbial Kit (Qiagen, Germany) following the manufacturer's instructions from the samples received from the UK. For each sample, a pre-sample mix was prepared as follows: 3 μL of TaqMan Gene Expression Master Mix (Applied Biosystems, Foster city, USA), 0.3 μL of 20× Sample loading reagent (Standard BioTools, South San Francisco, USA) and 2.7 μL of extracted DNA. Mix of primers and probe was prepared for each PCR assay (containing 33 μM of each primer and 10 μM of probe) and 3 μL assay-mix was mixed with 3 μL 2× Assay loading reagent (Standard BioTools). HT-PCR was performed in a BioMark 48.48DA IFC (Standard BioTools) combining 48 samples with 48 assays for 2304 individual and simultaneous PCR reactions. The 48.48DA IFC was primed in the IFC controller MX (Standard BioTools) prior to loading of sample-mix and assay-mix. Sample-mix (5 μL), and assay-mix (5 μL) was dispensed into inlets on the 48.48DA IFC, which was again placed in the MX controller for loading and mixing of the 48 samples and 48 assays for 55 min. The 48.48DA IFC was then placed in the HT-PCR instrument BioMark HD (Standard BioTools) for thermal cycling with the following conditions: 15 min at 95 °C, followed by 40 cycles at 94 °C for 10 s, at 54 °C for 30 s and 72 °C for 10 s. In each chip run, positive and negative PCR controls were included. Data (Cq-values and amplification curves) was acquired on the BioMark HD system and analysed using the HT-PCR Analysis software 4.8.1 (Standard BioTools). Initially, the primer and probe sets used in the high-throughput real-time PCR analysis were validated on known bacteria strains and ten-fold serial dilutions were run in order to analyse the sensitivity and amplification efficiency of the PCR assays. The repeatability of the PCR assays was evaluated by running the positive PCR controls on several separate PCR runs (n = 11).

2.3. Isolation and identification of ESBL-producing bacteria

ESBL selective culture was performed on samples positive to at least one ESBL-encoding gene post detection of resistance genes via high-throughput real-time PCR, using a method for ESBL detection in foods adapted from the UK Food Standards Agency (FSA) (AHVLA, 2011). Briefly, 50 μL of the MacConkey enrichment broth were transferred in 5 mL of MacConkey broth supplemented with 1 μL/mL cefotaxime and incubated with agitation (240 rpm) at 37 °C for 18–24 h. One microliter of the enrichment culture was plated on brilliance ESBL selective agar plate (Oxoid, USA). For each sample, colonies displaying unique morphology were sub-cultured on selective plates. Each strain was then screened for the presence of ESBL genes by conventional PCR as previously described (Hasman et al., 2005), including testing for CTX-M, SHV, TEM, CMY-1, CMY-2, OXA-1, and OXA-2. Species identification was performed on PCR-positive isolates by matrix assisted laser deionisation time of flight mass spectrometry (MALDI-TOF MS, Vitek MS, bioMerieux).

2.4. Bacterial conjugation and plasmid-based replicon typing

Mobility of $bla_{CTX\text{-}M}$ from CTX-M producing isolates to rifampicin-resistant *Escherichia coli* K12 was tested by in vitro conjugation, as previously described (Subramani et al., 2023). Briefly, both donor and recipient strains were grown in Luria Bertani (LB) broth up to an OD600 = 0.5 on a Geneysis30 spectrophotometer (ThermoScientific), followed by centrifugation at 3000g for 3 min and resuspension of the pellet in fresh LB medium. Bacterial conjugation was performed on LB agar plates with filters (0.22 μM, Millipore, Copenhagen, Denmark) at 37 °C. Donor and recipient were mixed in a 1:1 ratio to a final volume of 100 μL on the filters. After overnight incubation at 37 °C, bacterial material was washed off from the filters by vertexing using 0.9 % isotonic NaCl and 100 μL of bacterial suspensions were plated on MacConkey agar plates containing 1 μg/L cefotaxime and 50 μg/L rifampicin for transconjugant selection. Transfer of $bla_{CTX\text{-}M}$ genes was confirmed in the presumptive transconjugants by PCR as previously described (Monstein et al., 2007). Plasmid replicon types transconjugants were determined using the PCR-Based Replicon Typing (PBRT) 2.0 Kit (Diatheva, Fano, Italy) following the manufacturer's guidelines (Carattoli et al., 2005).

2.5. Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MIC) were determined by broth microdilution using the GN3F plates and COMPGN1F plates (ThermoFisher Scientific, USA) on ESBL-producing strains and transconjugants, respectively. Transconjugants were additionally screened for resistance to nalidixic acid via MIC by broth microdilution. MIC results were interpreted according to EUCAST guidelines (EUCAST, 2022) using the Sensititre vison digital MIC viewer. Strains classified as intermediate resistant (now renamed 'susceptible, increased exposure') were included in the same group as susceptible when reporting percentages of resistant strains.

2.6. Whole genome sequencing and bioinformatics analysis

Whole-genome sequencing (WGS) was performed on CTX-M-positive

isolates. DNA was extracted using the Maxwell RSC Cultured Cells DNA Kit (Promega, Wisconsin, USA) and the Maxwell RSC platform (Promega) following the manufacturer's instructions. DNA libraries were constructed using the Nextera XT library preparation kit (Illumina, California, USA) following the manufacturer's sequencing protocol on MiSeq (Illumina). Illumina raw sequencing reads were assembled using SPAdes Genome Assembler (v.3.13.1) (Bankevich et al., 2012), and quality was checked using QUAST (v.5.0.2) (provided in Table S3 in supplemental material) (Gurevich et al., 2013). The assemblies were annotated using Prokka (Seemann, 2014). Assembled genomes were screened for resistance determinants using ABRicate v1.0.1 (https://github.com/tseemann/abricate) against the ResFinder database (Florensa et al., 2022). Raw sequences were additionally analysed using PubMLST to accurately identify each bacterial genera and species (Jolley et al., 2018). The sequence types (ST) of ESBL-positive strains belonging to species for which a multilocus sequence typing (MSLT) database is available were determined using MLST 2.0 (Larsen et al., 2012). For strains with an MLST scheme available without previously being defined, the online tools provided by the Pasteur institute and PubMLST (Jolley et al., 2018) were used to assign novel strain types to *Klebsiella pneumoniae* and *Citrobacter freundii* strains respectively.

2.7. Statistical analysis

The proportion of ESBL-positive samples was calculated across all samples and stratified by retailer and product type, with exact 95 % confidence intervals obtained using the Clopper-Pearson method. The association between retailer, product type, and the presence of ESBL in a sample was evaluated using logistic regression models. The impact of interaction between retailer and product type was examined by comparing models with and without interaction terms using the likelihood ratio test. All statistical analyses were conducted in R (version 4.3.0).

3. Results

3.1. Genotypic detection of β-lactamase-encoding genes

Real-time PCR screening revealed the occurrence of at least one of the three main β-lactamase gene classes associated with ESBL (namely $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV}) in 225 (78.1 %, 95 % CI:73.4 %–82.9 %) seafood samples. Overall, SHV (found in $n = 181$ samples) was more common than TEM ($n = 131$ samples) and CTX-M ($n = 75$ samples, 33 % of samples carrying at least one of the three ESBL gene classes). SHV β-lactamases were more common in catfish (84.4 %, 95 % CI:75.5 %–91 %) than in shrimp (62.5 %, 95 % CI:52 %–72.2 %) and seabass (41.7 %, 95 % CI:31.7 %–52.2 %), whereas TEM and CTX-M β-lactamases occurred more frequently in shrimp (64.5 %, 95 % CI: 54.2 %–74.1 % and 57.3 %, 95 % CI: 46.8 %–67.3 %, respectively) compared to catfish (54.2 %, 95 % CI: 43.7 %–64.4 % and 11.5 %, 95 % CI: 5.9 %–19.6 %, respectively) and seabass (17.7 %, 95 % CI: 10.7 %–26.8 % and 9.4 %, 95 % CI: 4.4 %–17.1 %, respectively). Co-occurrence of at least two CTX-M alleles was observed in 21 (9.3 %) samples. There was co-occurrence of CTX-M groups 1, 9, 8 and/or 25 in the collected seafood samples with an overall prevalence of CTX-M groups 1 (58.6 %) and 9 (53.3 %), followed by groups 8 (22.7 %) and 25 (1.3 %). A detailed overview of the association between retailer, product type, and the presence of ESBL encoding alleles in samples without interaction is presented in Table 1, as it provides more stable and interpretable estimates. However, caution is warranted in the interpretation, as the effects of sample type and retailer may not be consistent across all combinations. Additionally, the proportions of ESBL-positive samples by retailer and product type, along with their 95 % confidence intervals, are presented in Fig. 1.

3.2. Isolation and characterization of ESBL-producing bacteria

Among the 225 seafood samples testing positive for $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV}, 66 (22.9 %) were culturable on ESBL selective media, resulting in 77 isolates. From the 77 isolates, 28 isolates originating from 22 (7.6 %) samples were positive to CTX-M via PCR. Four CTX-M-producing isolates were additionally positive for SHV. Shrimp showed the highest frequency of CTX-M contamination, accounting for 78.6 % ($n = 22$) of the isolates, followed by catfish with 14.3 % ($n = 4$) and seabass with 7.1 % (n = 2). MALDI-TOF identified most of these 28 isolates as *Enterobacteriaceae* ($n = 26$), including *Klebsiella pneumoniae* ($n = 7$), *Atlantibacter hermannii* (formerly *Escherichia hermannii*) ($n = 5$), *Citrobacter* spp. (n = 5), *Enterobacter* spp. (n = 5), *Proteus vulgaris* (n = 2), *Escherichia coli* ($n = 1$) and, *Morganella morganii* (n = 1) (Table 2). The remaining two isolates were *Serratia fonticola* belonging to the family of *Yersiniaceae*.

According to EUCAST breakpoints, the 28 CTX-M PCR-positive isolates displayed higher levels of resistance to cefopodoxime (100.0 %) and ceftriaxone (92.9 %) as compared to ceftazidime (10.7 %) and cefepime (3.6 %). Resistance to non-β-lactam antimicrobial classes was infrequent (0–28.9 %) (Table 3). Resistance to carbapenems, β-lactamase inhibitor combinations (i.e., ampicillin/sulbactam and ticarcillin/

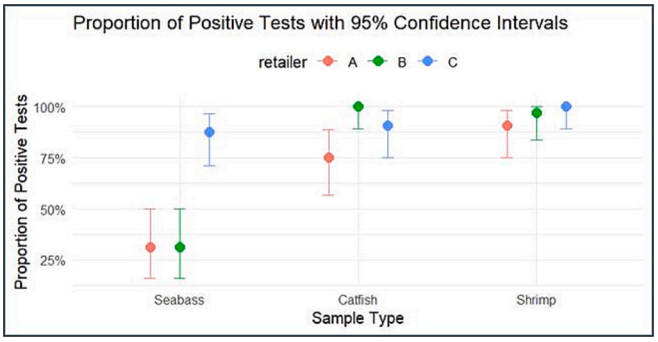

Fig. 1. Proportions of ESBL-positive samples by retailer and product type, and 95 % confidence intervals. Results from a survey of seafood products on retail in the UK ($n = 32$ samples of each product by retailer).

Table 1
Results of logistic regression analysis of ESBL presence on retailer and product type ($n = 96$ samples of three different seafood products purchased from three different retails in the UK between October 5th 2021, and November 24th 2021).

Variable	Estimate (β)	Standard error (SE)	Odds ratio (OR)	95 % CI for OR	*P*-value
Retailer					
A (reference)	–	–	1	–	–
B	0.7677	0.4003	2.155	0.983–4.722	0.055
C	2.4971	0.5168	12.148	4.412–33.449	<0.001
Product type					
Seabass (reference)	–	–	1	–	–
Catfish	2.3638	0.4175	10.632	4.691–24.098	<0.001
Shrimp	3.4977	0.5790	33.040	10.622–102.770	<0.001

Table 2
Species, sequence type (ST), source, and antimicrobial resistance gene (ARG) profiles of 27 ESBL-producing strains isolated from seafood.

ID	Source	Species	ST	ESBL ARG (s)	Non-ESBL β-lactam ARG (s)	Other ARG(s)
SH1	Shrimp	*Enterobacter hormaechei*	ND[a]	$bla_{CTX\text{-}M\text{-}55}$	$bla_{ATC\text{-}19}$	*oqxA5*, *oqxB9*, *mph(A)*, *qnrS1*, *aph(3')-Ia*
SH2	Shrimp	*Klebsiella pneumoniae*	ST1727[b]	$bla_{CTX\text{-}M\text{-}27}$	$bla_{LAP\text{-}2}$, $bla_{SHV\text{-}11}$	*oqxA5*, *oqxB26*, *fosA6*, *dfrA14*, *qnrS1*, *aac(3)-Iid*
SH3	Shrimp	*Proteus columbae*	ND	$bla_{CTX\text{-}M\text{-}15}$	–	*sul2*, *dfrA23*, *hugA*, *aph(3'')-Ib*, *aph(6)-Id*, *tet(A)*
SH4	Shrimp	*Klebsiella pneumoniae*	ST7164	$bla_{CTX\text{-}M\text{-}15}$	$bla_{OKP\text{-}A\text{-}3}$	*sul2*, *dfrA23*, *oqxA10*, *oqxB9*, *fosA_gen*, *aph(3'')-Ib*, *aph(6)-Id*, *tet(A)*
SH5	Shrimp	*Escherichia coli*	ST13773	$bla_{CTX\text{-}M\text{-}15}$	*EC-8*	*sul2*, *dfrA23*, *aph(6)-Id*, *aph(3'')-Ib*, *tet(A)*
SH6	Shrimp	*Atlantibacter hermannii*	ND	$bla_{CTX\text{-}M\text{-}15}$	$bla_{HER\text{-}1}$	*sul2*, *catA1*, *dfrA23*, *qnrS1*, *aph(3'')-Ib*, *aph(6)-Id*, *tet(A)*
SH7	Shrimp	*Citrobacter freundii*	ST1260	$bla_{CTX\text{-}M\text{-}55}$	$bla_{CMY\text{-}48}$	*qnrS13*
SH8	Shrimp	*Morganella morganii*	ND	$bla_{CTX\text{-}M\text{-}27}$	$bla_{DHA\text{-}20}$, $bla_{LAP\text{-}2}$	*catA2*, *qnrS1*, *qnrD1*
SH9	Shrimp	*Enterobacter mori*	ND	$bla_{CTX\text{-}M\text{-}14}$	$bla_{ACT\text{-}38}$, $bla_{LAP\text{-}2}$	*oqxB9*, *oqxA10*, *fosA*, *mcr-9.1*, *qnrE1*, *qnrS1*, *aac(3)-Iid*
SH10	Shrimp	*Enterobacter hormaechei*	ND	$bla_{CTX\text{-}M\text{-}55}$	$bla_{ACT\text{-}19}$	*oqxB9*, *oqxA5*, *sul3*, *dfrA1*, *sul1*, *qnrS1*, *tet(A)*
SH11	Shrimp	*Klebsiella pneumoniae*	ST34	$bla_{CTX\text{-}M\text{-}27}$	$bla_{SHV\text{-}26}$	*oqxB12*, *oqxA9*, *fosA5*, *qnrS1*, *aac(3)-Iid*
SH12	Shrimp	*Enterobacter hormaechei*	ND	$bla_{CTX\text{-}M\text{-}15}$	$bla_{ACT\text{-}17}$	*oqxB9*, *oqxA9*, *fosA*, *qnrS1*
SH13	Shrimp	*Atlantibacter hermannii*	ND	$bla_{CTX\text{-}M\text{-}27}$	$bla_{HER\text{-}1}$, $bla_{LAP\text{-}2}$	*catA1*, *dfrA14*, *qnrS1*, *tet(C)*
SH14	Shrimp	*Klebsiella pneumoniae*	ST7165	$bla_{CTX\text{-}M\text{-}27}$	$bla_{OKP\text{-}A\text{-}5}$, $bla_{LAP\text{-}2}$	*dfrA12*, *sul1*, *sul2*, *floR*, *fosA6*, *oqxA10*, *oqxB11*, *qnrS1*, *aadA2*, *aph(3'')-Ib*, *aph(6)-Id*, *tet(A)*
SH15	Shrimp	*Atlantibacter hermannii*	ND	$bla_{CTX\text{-}M\text{-}55}$	$bla_{HER\text{-}3}$	*catA1*, *catA2*, *qnrS13*
SH16	Shrimp	*Atlantibacter hermannii*	ND	$bla_{CTX\text{-}M\text{-}27}$	$bla_{LAP\text{-}2}$, $bla_{HER\text{-}5}$	*dfrA14*, *catA1*, *qnrS1*, *tet(C)*
SH17	Shrimp	*Klebsiella pneumoniae*	ST5594	$bla_{CTX\text{-}M\text{-}15}$	$bla_{OKP\text{-}B\text{-}2}$	*sul2*, *dfrA23*, *oqxA10*, *oqxB11*, *qnrS1*, *fosA_gen*, *aph(3'')-Ib*, *aph(6)-Id*, *tet(A)*
SH18	Shrimp	*Proteus terrae*	ND	$bla_{CTX\text{-}M\text{-}15}$	–	*hugA*, *dfrA23*, *sul2*, *qnrD1*, *aph(6)-Id*, *aph(3'')-Ib*, *tet(A)*
SH20	Shrimp	*Citrobacter freundii*	ST19	$bla_{CTX\text{-}M\text{-}55}$	$bla_{CMY\text{-}152}$	*catA2*, *qnrS13*
SH21	Shrimp	*Citrobacter braakii*	ND	$bla_{CTX\text{-}M\text{-}55}$	$bla_{CMY\text{-}74}$	*qnrS13*
SH22	Shrimp	*Citrobacter braakii*	ND	$bla_{CTX\text{-}M\text{-}55}$	$bla_{CMY\text{-}74}$	*qnrS13*
TU1	Catfish	*Klebsiella pneumoniae*	ST2702	$bla_{CTX\text{-}M\text{-}14}$	$bla_{SHV\text{-}110}$	*fosA_gen*, *qnrS1*, *aph(6)-Id*, *aph(3'')-Ib*
TU2	Catfish	*Enterobacter sichuanensis*	ND	$bla_{CTX\text{-}M\text{-}14}$	$bla_{LAP\text{-}2}$, $bla_{ACT\text{-}50}$	*fosA*, *oqxA6*, *oqxB17*, *mcr-10.1*, *qnrE1*, *qnrS1*, *aac(3)-Iid*
TU3	Catfish	*Klebsiella pneumoniae*	ST2702	$bla_{CTX\text{-}M\text{-}14}$	$bla_{SHV\text{-}110}$	*fosA_gen*, *qnrS1*, *aph(3'')-Ib*, *aph(6)-Id*, *aac(3)-Iid*
TU4	Catfish	*Atlantibacter hermannii*	ND	$bla_{CTX\text{-}M\text{-}27}$	$bla_{LAP\text{-}2}$, $bla_{HER\text{-}1}$	*dfrA14*, *catA1*, *qnrS1*, *tet(C)*
SB1	Seabass	*Serratia fonticola*	ND	$bla_{FONA\text{-}6}$	–	–
SB2	Seabass	*Serratia fonticola*	ND	$bla_{FONA\text{-}6}$	–	–

[a] ND indicates strains without an MLST scheme available.
[b] ST Indicates strains with available MLST schemes.

clavulanic acid), fluoroquinolones, amikacin and tigecycline was not detected. All isolates were classified as MDR, none as XDR or PDR.

WGS of the 28 CTX-M positive strains by PCR confirmed the presence of $bla_{CTX\text{-}M}$ in 25 strains, including $bla_{CTX\text{-}M\text{-}15}$ ($n = 7$), $bla_{CTX\text{-}M\text{-}27}$ (n = 7), $bla_{CTX\text{-}M\text{-}55}$ (n = 7), and $bla_{CTX\text{-}M\text{-}14}$ ($n = 4$), with one strain that was excluded from further analysis due to contamination. In addition to CTX-M, four *K. pneumoniae* strains carried SHV genes, namely $bla_{SHV\text{-}11}$, $bla_{SHV\text{-}26}$ and $bla_{SHV\text{-}110}$ (Table 2). For the two *S. fonticola* isolates, the ESBL gene FONA-6 was identified instead of CTX-M. Blast analysis of CTX-M primers used in the conventional PCR on the two genomes revealed an amplicon of the expected length (593 bp) within the FONA-6 sequence (position 223 to 816), indicating false positive detection of CTX-M by PCR.

Notably, we identified two *Enterobacter* strains, namely *Enterobacter mori* and *Enterobacter sichuanensis*, which carried a colistin resistance *mcr*-9.1 homologue (100 % coverage and 91 % nucleotide identity) and *mcr*-10.1, respectively, in combination with CTX-M-14. The complete resistance gene profiles of the sequenced isolates are presented in Table 2.

Ten strains belonged to species for which an MLST database is available. Of these, three strains were assigned to new STs, including *K. pneumoniae* ST7164 and ST7165 and *C. freundii* ST1260. The remaining five *K. pneumoniae* isolated from shrimp belonged to ST34 ($n = 1$), ST1727 (n = 1), and ST5594 (n = 1), while two *K. pneumoniae* isolated from catfish belonged to ST2702. The remaining two typeable strains were *E. coli* ST13773 and *C. freundii* ST19, all isolated from shrimp (Table 2).

3.3. *In vitro* conjugative transfer of $bla_{CTX\text{-}M}$

Conjugative transfer of ESBL-encoding genes to *E. coli* K12 was detected in 13 of the 27 ESBL-producing strains confirmed by WGS, all originating from shrimps (Table 4). PBRT analysis and antimicrobial susceptibility testing of the 13 transconjugants revealed co-transfer of CTX-M-15, tetracycline and trimethoprim/sulfamethoxazole resistance mediated by IncA/C ($n = 6$); transfer of CTX-M-55 ($n = 3$) or CTX-M-15 (n = 1) mediated by IncX2 with co-transfer of resistance to nalidixic acid; and co-transfer of CTX-M-27, tetracycline and trimethoprim/sulfamethoxazole resistance mediated by IncFIIK (n = 1). The two non-typeable plasmids transferred CTX-M-27 and CTX-M-55 in association with tetracycline and chlorphenicol resistance, respectively. While the occurrence of IncX2 plasmids was restricted to the genus *Citrobacter*, IncA/C was shared across six species belonging to four genera within *Enterobacteriaceae* (Table 4).

4. Discussion

This survey provides new knowledge on frequency, genotypes, phenotypes, and mobility of ESBL-producing bacteria isolated from retail seafood products imported from Asia. Approximately one in 13 products was contaminated with CTX-M-producing *Enterobacteriaceae*, especially shrimps imported from Vietnam. Our results show strong evidence of an association between type of product and ESBL presence, with catfish and especially shrimp samples more likely to be contaminated than seabass samples. There is also strong evidence of association between ESBL contamination and retailer. However, it is not possible to ascertain from our results whether the differences are due to a "retailer effect" or reflect variability in frequency of contamination between specific suppliers or batches. Comparison of models with and without the interaction term demonstrated a better fit for the model with interaction ($P = 0.003$, likelihood ratio test). However, the inclusion of interaction terms resulted in very wide confidence intervals and

Table 3
Prevalence of antimicrobial resistance in 27 ESBL-producing strains isolated from seafood.

Antimicrobial class	Antimicrobial subclass	Antimicrobial	No. of resistant strains (%)
β-Lactam	Penicillins	AMP	27 (100.0)
	Extended spectrum cephalosporins (3rd and 4th generation)	POD	27 (100.0)
		TAZ	3 (11.1)
		AXO	26 (92.6)
		FEP	1 (3.7)
	Cephamycin's	FOX	8 (29.6)
	Non-extended spectrum cephalosporins (1st and 2nd generation)	FAZ	27 (100.0)
		FUR	27 (100.0)
		CEP	27 (100.0)
	Monobactams	AZT	10 (37.0)
	Carbapenems	MERO	0
		ERT	0
	β-lactam + β-lactamase inhibitor combinations	A/S2	0
		P/T4	0
		TIM2	0
Aminoglycosides		AMI	0
		GEN	4 (14.8)
		TOB	1 (3.7)
Tetracyclines		TET	5 (18.5)
		TGC	0
Fluoroquinolones		CIP	0
Folate pathway inhibitors		SXT	8 (29.6)
Multidrug resistance			27 (100.0)

Abbreviations: A/S2, ampicillin/sulbactam; AMI, amikacin; AMP, ampicillin; AXO, ceftriaxone; AZT, aztreonam; CEP, cephalothin; CIP, ciprofloxacin; ERP, ertapenem; FAZ, cefazolin; FEP, cefepime; FOX, cefoxitin; FUR, cefuroxime; GEN, gentamicin; MERO, meropenem; P/T4, piperacillin/tazobactam constant 4; POD, cefpodoxime; SXT, trimethoprim/sulfamethoxazole; TAZ, ceftazidime; TET, tetracycline; TGC, tigecycline; TIM2, ticarcillin/clavulanic acid constant 2; TOB, tobramycin.

inestimable odds ratios for some combinations due to sparse data and complete separation in certain categories, where all catfish samples from retailer B and all shrimp samples from retailer C were ESBL positive. Half of the isolates displayed the ability to transfer $bla_{CTX\text{-}M}$ to *E. coli* under laboratory and nearly one third belonged to major opportunistic pathogenic species such as *K. pneumoniae* and *E. coli*. Some of the clonal lineages revealed by MLST have been previously associated with human infections, namely *K. pneumoniae* ST34 (Chen et al., 2020; Liao et al., 2020; Okanda et al., 2021) and ST1727 (Sidjabat et al., 2015; Fasciana et al., 2019), and *C. freundii* ST19 (Yao et al., 2021; Sommer et al., 2024). Although the STs identified do not represent high-risk clonal lineages, the mobility of CTX-M-encoding genes poses a potential risk of transferring these genes to the commensal microbiota of consumers, particularly depending on how seafood is handled and consumed. The risk of foodborne transmission is real if we consider the growing trend to consume raw and undercooked seafood products (Weissfeld, 2014) Despite this risk, seafood has received less attention compared to other animal-derived food products in national and regional surveillance programmes of antimicrobial resistance (Mateus et al., 2016). At the time of writing the UKs Food Standards Agency has commissioned a project to survey antimicrobial resistance in domestically produced Atlantic Salmon, which will be completed by May 2025 (FSA, 2024).

The high prevalence of ESBL contamination in seafood products corroborates the results of our recent systematic review which indicated that approximately one in five retail seafood products are contaminated with ESBL-producing bacteria globally (Pearce et al., 2023). The review focused on studies performed on retail seafood collected in Asian markets, whereas the present survey is one of the few reporting the prevalence of ESBL contamination in different seafood products imported to Europe and the first conducted in the UK. Among the seven European studies selected by this review, ESBL contamination in retail seafood products collected from Europe ranged from 0.3 % to 16.5 % (De Greef and Mounton, 2017; DANMAP, 2018; De Greeff and Mounton, 2018; Vitas et al., 2018; Vu et al., 2018; De Greeff et al., 2019; Silva et al., 2019). The wide range of contamination rates is likely attributable to the diversity of seafood products analysed across these studies as well as methodological differences between the studies. Our study provides strong evidence of an association between product type and ESBL presence, with catfish and especially shrimp samples being significantly more likely to be contaminated compared to seabass. The influence of seafood type on ESBL contamination rates is exemplified in the study by Vitas et al., where a high prevalence (19.4 %) of ESBL-producing *Enterobacteriaceae* was reported in sushi samples containing raw fish (salmon, tuna, shrimp, and swordfish), while no ESBL producers were detected in other raw fish (trout, salmon, and panga/catfish) obtained from the same retail market in Spain (Vitas et al., 2018). Another study on raw fish from large supermarkets and fish markets in northern Portugal (Silva et al., 2019) reported a relatively lower incidence of ESBL-contaminated sushi/sashimi samples (0.5 %) compared to our findings. Altogether, these data imply that the risk of ESBL contamination in seafood depends on the origin, type and human manipulation during processing, as well as the methodology used for ESBL detection.

The prevalence and distribution of ESBL types are known to vary across different host species and geographical regions (Ewers et al., 2012). A single recent study estimated the prevalence of CTX-M-producing *E. coli* carriage in the UK community to be 11 %, with London alone estimated at 17 % (Day et al., 2019). In line with previous

Table 4
Results of the conjugation experiments based on PCR-based replicon typing (PBRT) and antimicrobial susceptibility testing of 13 donor *Enterobacteriaceae* strains and the relative transconjugants obtained using *E. coli* K12 as the recipient strain.

ID	Source	Species	CTX-M variant	PBRT in donor	PBRT in transconjugant	Co-transfer of non-β-lactam resistance
SH3	Shrimp	*Proteus columbae*	CTX-M-15	IncA/C	IncA/C	TET, SXT
SH4	Shrimp	*Klebsiella pneumoniae*	CTX-M-15	IncIγ, IncA/C, IncR	IncA/C	TET, SXT
SH5	Shrimp	*Escherichia coli*	CTX-M-15	IncA/C	IncA/C	TET, SXT
SH6	Shrimp	*Atlantibacter hermannii*	CTX-M-15	IncA/C, IncR	IncA/C	TET, SXT
SH7	Shrimp	*Citrobacter freundii*	CTX-M-15	IncA/C, IncU, IncX2, IncFIB(KQ)	IncX2	NAL
SH13	Shrimp	*Atlantibacter hermannii*	CTX-M-27	None detected	Non-typable	TET
SH14	Shrimp	*Klebsiella pneumoniae*	CTX-M-27	IncIγ, IncFIIK, InxFIIB(KN)	IncFIIK	TET, SXT
SH15	Shrimp	*Atlantibacter hermannii*	CTX-M-55	IncX1, IncR	Non-typable	CHL, NAL
SH17	Shrimp	*Klebsiella pneumoniae*	CTX-M-15	IncA/C	IncA/C	TET, SXT
SH18	Shrimp	*Proteus terrae*	CTX-M-15	IncA/C	IncA/C	TET, SXT
SH20	Shrimp	*Citrobacter freundii*	CTX-M-55	IncX2	IncX2	NAL
SH21	Shrimp	*Citrobacter braakii*	CTX-M-55	IncX2	IncX2	NAL
SH22	Shrimp	*Citrobacter braakii*	CTX-M-55	IncX2	IncX2	NAL

Abbreviations: CHL, chloramphenicol; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; NAL, nalidixic acid.

research indicating a lack of epidemiological linkage of ESBL genes between livestock and people in the general population (Dorado-García et al., 2018), this study also showed that ESBL types in retail food products and slurry from dairy farms, mainly CTX-M-1, differed from those occurring in human carriers and bacteraemia patients in the UK, namely CTX-M-15, CTX-M-27 and CTX-M-14 (Day et al., 2019). The present survey substantiates the findings of our review (Pearce et al., 2023), affirming that seafood imported from Asia, unlike other animal-derived food products, is a potential reservoir for the spread of these clinically important ESBL types. However, this general trend is contradicted by specific studies, such as Silva-Bea et al. (2024), which found overlapping populations of CTX-M-15-producing *K. pneumoniae* in poultry meat and clinical samples in Northern Spain.

CTX-M-15 was transferable by conjugation in all strains carrying this variant, usually in conjunction with transfer of IncA/C plasmids. IncA/C plasmids are known to have broad host range and have been associated with β-lactam resistance genes of clinical interest, such as bla_{CMY} and bla_{NDM} (Hancock et al., 2017). The second most common plasmid replicon type, IncX2, which was associated with both CTX-M-55 and CTX-M-15, include conjugative plasmids with narrow host range within Enterobacteriaceae. This replicon type has been predominantly associated with fluoroquinolone resistance genes, which confer low-level resistance, especially qnrS (Liu et al., 2017), consistent with our findings. Notably, all plasmids carried one or two resistance genes in addition to $bla_{CTX\text{-}M}$, indicating that their dissemination could be driven by exposure to antimicrobials commonly used in fish and shrimp farming in Vietnam, mainly tetracyclines, sulphonamides and phenicols (Luu et al., 2021).

FONA-6 detected in *S. fonticola* is a relatively uncommon ESBL that was first described in 2020 in association with this bacterial species isolated from imported chicken meat in Japan (Tanimoto et al., 2021). A new variant of this ESBL (FONA-7) was also reported in migratory birds (Fuentes-Castillo et al., 2021). It appears that FONA-encoding genes are located on the chromosome of *S. fonticola* and are not transferred by plasmid transfer or other mobile genetic elements. Presently, there is no information available regarding the presence of this new emerging ESBL in other bacterial species, including human or animal clinical isolates.

Shrimp samples exhibited the highest proportion (22.9 %) of samples contaminated with CTX-M-producing *Enterobacteriaceae*. A Vietnamese study on domestically produced raw retail shrimp recently reported a similar prevalence of ESBL contamination (19.6 %) (Le et al., 2021). The relatively higher frequency of ESBL contamination in headless shrimp without shell, as opposed to fillets from catfish produced in the same water body (Mekong River basin), might be linked to differences in the production systems of these two seafood products. These differences include variations in the ability to eliminate bacteria originating from pre-harvest stages, as well as different risks of post-harvest contamination due to human handling or washing practices.

The origins of ESBL-producing *Enterobacteriaceae* found on these seafood products remain uncertain. ESBL contamination could originate from faecal contamination occurring in the aquaculture environment where seafood is harvested (pre-harvest) or during processing (post-harvest). A human origin is plausible, given the high levels of faecal contamination of the Mekong basin (Nakhle et al., 2021), along with the elevated rates of ESBL carriage in the Vietnamese community, which are associated with the same ESBL types found in these products (i.e., CTX-M-15, CTX-M27 and CTX-M-55) (Singh et al., 2021). The occurrence of ESBL-producing *Enterobacteriaceae* in this aquatic environment could be enhanced by the usage of antimicrobials in Vietnamese aquaculture, which accounts for 5 % of antimicrobial consumption in this sector in the Asia-Pacific region (Schar et al., 2020). The co-resistance patterns revealed by this survey in both the strains and the plasmids carrying $bla_{CTX\text{-}M}$ evidence the potential for ESBL co-selection by antimicrobials used in aquaculture.

The prevalence of presumptive ESBL-encoding genes detected by genotypic screening of total DNA extracted from the samples was markedly higher (78 %) than that observed by selective isolation (22.9 %). This discrepancy may be at least in part attributable to the PCR methodology, which allows detection of genes in bacteria which are either non-culturable or in cell-free DNA. Additionally, the primers used for detection of generic SHV and TEM are not specific for ESBL variants of these β-lactamases. Indeed, none of the three SHV variants detected in this survey (SHV-11, SHV-26 and SHV-110) are classified as ESBL (Liakopoulos et al., 2016).

We acknowledge some limitations of our study. The autumn sample collection period does not account for possible seasonal variation in seafood contamination with ESBL-producing bacteria. Differences in contamination between retailers may reflect variability in suppliers or batches, which our results cannot fully discern. We studied products purchased in three different supermarket chains. While they are among the largest UK retailers, our sampling strategy does not ensure representativeness. However, it provides a reasonable reflection of raw seafood products available to UK consumers, though caution is warranted when extrapolating the results to the broader seafood market. Furthermore, we studied only three seafood types imported from two countries. While these products are among the most widely consumed farmed seafood in the UK and are primarily imported from these countries, they do not capture the full diversity of seafood products available in the market. Despite these limitations, our study provides valuable insights into the presence of ESBL-producing bacteria in high-volume imported seafood products. Additionally, methods based on sample homogenization might have facilitated bacterial detachment from seafood surfaces, potentially leading to higher contamination rates. Also, the use of an enrichment selective procedure, which was used to enhance ESBL detection, altered the bacterial composition of the samples, potentially affecting the proportions of ESBL-producing species and ESBL types. The use of cefotaxime in the enrichment may have led to an underestimation of the occurrence of SHV because this third-generation cephalosporin favours detection of CTX-M producers, whereas ceftazidime is more effective for detecting SHV producers (Liakopoulos et al., 2016). This may explain why SHV-12, which has been previously reported as one of the predominant ESBL variants in seafood (Sousa et al., 2011; Roschanski et al., 2017; Vitas et al., 2018), was not detected in our survey. Moreover, our selective enrichment approach hindered quantification of ESBL-producing bacteria, which is critical information necessary for assessing the health risks to consumers.

In conclusion, this survey provides valuable insights into the prevalence, type and transferability of ESBL-encoding genes in seafood products imported to the UK. The CTX-M types identified in shrimp and to a lesser extent catfish imported from Vietnam, namely CTX-M-15, CTX-M-14, CTX-M-27, and CTX-M-55, hold significant clinical importance and differ from those occurring in other food products of animal origin. While the source of these ESBL-producing *Enterobacteriaceae* in shrimp products is still uncertain and could potentially be linked to post-harvest contamination, the presence of ESBL-encoding genes on plasmids alongside other resistance genes implies a possible co-selection by antimicrobials frequently employed in aquaculture. Our findings support the hypothesis that the global dissemination of these CTX-M variants could be facilitated by seafood trade, highlighting the potential health risks for individuals consuming raw seafood. ESBL surveillance on traded seafood and quantitative risk assessment studies are warranted to gain a deeper understanding of the magnitude of this phenomenon on a global scale and its potential implications for human health.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2025.111063.

CRediT authorship contribution statement

Ryan Pearce: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Mattia Pirolo:** Writing – review & editing, Methodology, Investigation, Data curation. **Nicole B. Goecke:**

Methodology, Investigation. **Valeria Toppi:** Investigation. **Liam Good:** Funding acquisition. **Javier Guitian:** Funding acquisition, Formal analysis. **Luca Guardabassi:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

Acknowledgements

This research was supported by the University of Copenhagen and the Royal Veterinary College through a BBSRC PhD grant on the London Interdisciplinary Biosciences Consortium Doctoral Training Partnership (LIDo). Grant code: "BB/M009513/1".

Data availability

Whole genome sequencing (WGS) data for the 28 sequenced isolates were submitted to NCBI Sequence Read Archive (SRA) under BioProject PRJNA1114648.

References

Animal Health and Veterinary Laboratories Agency (AHVLA), 2011. Evaluation and Recommendation of a Screening Protocol for the Detection of Extended Spectrum Beta Lactamases of *Enterobacteriaceae* in Food, 1st of March 2011. Food Standards Agency (FSA). https://www.food.gov.uk/research/foodborne-disease/development-of-a-screening-protocol-for-esbls-in-food (accessed 04.06.2024).

Antunes, L., 2023. Tackling Antimicrobial Resistance: From Science to Pharmaceuticals Policy. European parliamentary research service.

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., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (5), 455. https://doi.org/10.1089/CMB.2012.0021.

Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K.L., Threlfall, E.J., 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods 63 (3), 219–228. https://doi.org/10.1016/J.MIMET.2005.03.018.

Castanheira, M., Simner, P.J., Bradford, P.A., 2021. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology, and detection. JAC Antimicrob Resist 3 (3). https://doi.org/10.1093/JACAMR/DLAB092.

Chen, Q., Zhou, J., Wu, S., Yang, Y., Yu, D., Wang, X., Wu, M., 2020. Characterization of the IncX3 plasmid producing *blaNDM–7* from *Klebsiella pneumoniae* ST34. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.01885.

Cochran, W.G., 1977. Cochran 1977 Sampling Techniques Third Edition.pdf. John Wiley & Sons. https://www.wiley.com/en-us/Sampling+Techniques%2C+3rd+Edition-p-9780471162407.

DANMAP, 2018. Danmap, Danish Programme for Surveillance of Antimicrobial Consumption and Resistance in Bacteria From Food Animals, Food, and Humans. https://www.danmap.org/.

Day, M.J., Hopkins, K.L., Wareham, D.W., Toleman, M.A., Elviss, N., Randall, L., Teale, C., Cleary, P., Wiuff, C., Doumith, M., Ellington, M.J., Woodford, N., Livermore, D.M., 2019. Extended-spectrum β-lactamase-producing *Escherichia coli* in human-derived and food chain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. Lancet Infect. Dis. 19 (12), 1325–1335. https://doi.org/10.1016/S1473-3099(19)30273-7.

De Greef, S.C., Mounton, J.W., 2017. NethMap 2017: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands/MARAN 2017: Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2016 | RIVM. https://www.rivm.nl/publicaties/nethmap-2017-consumption-of-antimicrobial-agents-and-antimicrobial-resistance-among.

De Greeff, S.C., Mounton, J.W., 2018. NethMap 2018: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands/MARAN 2018: Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2017 | RIVM. https://www.rivm.nl/publicaties/nethmap-2018-consumption-of-antimicrobial-agents-and-antimicrobial-resistance-among.

De Greeff, Mounton, J.W., Heoing, A.F., Verduin, C.M., 2019. NethMap 2019: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands/MARAN 2019: Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2018 | RIVM. https://www.rivm.nl/publicaties/nethmap-2019-consumption-of-antimicrobial-agents-and-antimicrobial-resistance-among.

Dorado-García, A., Smid, J.H., Van Pelt, W., Bonten, M.J.M., Fluit, A.C., Van Den Bunt, G., Wagenaar, J.A., Hordijk, J., Dierikx, C.M., Veldman, K.T., De Koeijer, A., Dohmen, W., Schmitt, H., Liakopoulos, A., Pacholewicz, E., Lam, T.J.G.M., Velthuis, A.G., Heuvelink, A., Gonggrijp, M.A., Heederik, D.J.J., 2018. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food, and the environment: a pooled analysis. J. Antimicrob. Chemother. 73 (2), 339–347. https://doi.org/10.1093/JAC/DKX397.

EUCAST, 2022. Clinical Breakpoints and Dosing of Antibiotics. European Committee on Antimicrobial Susceptibility Testing. https://www.eucast.org/clinical_breakpoints (accessed 04.06.2024).

Ewers, C., Bethe, A., Semmler, T., Guenther, S., Wieler, L.H., 2012. Extended-spectrum β-lactamase-producing and *AmpC*-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 18 (7), 646–655. https://doi.org/10.1111/J.1469-0691.2012.03850.X.

Fasciana, T., Gentile, B., Aquilina, M., Ciammaruconi, A., Mascarella, C., Anselmo, A., Fortunato, A., Fillo, S., Petralito, G., Lista, F., Giammanco, A., 2019. Co-existence of virulence factors and antibiotic resistance in new *Klebsiella pneumoniae* clones emerging in south of Italy. BMC Infect. Dis. 19 (1), 928. https://doi.org/10.1186/s12879-019-4565-3.

Florensa, A.F., Kaas, R.S., Clausen, P.T.L.C., Aytan-Aktug, D., Aarestrup, F.M., 2022. ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microb. Genom. 8 (1), 2023. https://doi.org/10.1099/MGEN.0.000748.

Food and Agriculture Organization (FAO), 2022. The state of world Fisheries and aquaculture 2022. In: In the State of World Fisheries and Aquaculture 2022. FAO. https://doi.org/10.4060/cc0461en.

Food standards Agency (FSA), 2024. A survey of AMR *E. coli* and *Listeria* spp. on raw, prepacked, farmed salmon fillets on retail sale in the UK (FS900350). Food Standards Agency. Retrieved March 1, 2024, from. https://www.food.gov.uk/research/antimicrobial-resistance/a-survey-of-amr-e-coli-and-listeria-spp-on-raw-prepacked-farmed-salmon-fillets-on-retail-sale-in-the-uk-fs900350 (accessed 04.06.2024).

Fuentes-Castillo, D., Power, P., Cerdeira, L., Cardenas-Arias, A., Moura, Q., Oliveira, F.A., Levy, C.E., Gutkind, G., Catão-Dias, J.L., Lincopan, N., 2021. FONA-7, a novel extended-spectrum β-lactamase variant of the FONA family identified in *Serratia fonticola*. Microb. Drug Resist. 27 (5), 585–589. https://doi.org/10.1089/mdr.2020.0061.

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: quality assessment tool for genome assemblies. J. Bioinform. (Oxford, England) 29 (8), 1072–1075. https://doi.org/10.1093/BIOINFORMATICS/BTT086.

Hancock, S.J., Phan, M.D., Peters, K.M., Forde, B.M., Chong, T.M., Yin, W.F., Chan, K.G., Paterson, D.L., Walsh, T.R., Beatson, S.A., Schembri, M.A., 2017. Identification of IncA/C plasmid replication and maintenance genes and development of a plasmid multilocus sequence typing scheme. Antimicrob. Agents Chemother. 61 (2). https://doi.org/10.1128/AAC.01740-16.

Hasman, H., Mevius, D., Veldman, K., Olesen, I., Aarestrup, F.M., 2005. β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in the Netherlands. J. Antimicrob. Chemother. 56 (1), 115–121. https://doi.org/10.1093/JAC/DKI190.

ISO, 2017. ISO 6887-4:2017Microbiology of the food chain — Preparation of test samples, initial suspension, and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products, 2017, p. 5. https://www.iso.org/standard/63336.html (accessed 04.06.2024).

Jolley, K.A., Bray, J.E., Maiden, M.C.J., 2018. Open-access bacterial population genomics: BIGSdb software, the Pub MLST.org website and their applications. Wellcome Open Res 3. https://doi.org/10.12688/WELLCOMEOPENRES.14826.1.

Larsen, M.V., Cosentino, S., Rasmussen, S., Friis, C., Hasman, H., Marvig, R.L., Jelsbak, L., Sicheritz-Pontén, T., Ussery, D.W., Aarestrup, F.M., Lund, O., 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J. Clin. Microbiol. 50 (4), 1355–1361. https://doi.org/10.1128/JCM.06094-11.

Le, P.Q., Awasthi, S.P., Hatanaka, N., Hinenoya, A., Hassan, J., Ombarak, R.A., Iguchi, A., Tran, N.T.T., Dao, K.V.T., Vien, M.Q., Le, H.X., Do, H.T., Yamamoto, Y., Yamasaki, S., 2021. Prevalence of mobile colistin resistance (mcr) genes in extended-spectrum β-lactamase-producing *Escherichia coli* isolated from retail raw foods in Nha Trang, Vietnam. Int. J. Food Microbiol. 346, 109164. https://doi.org/10.1016/j.ijfoodmicro.2021.109164.

Liakopoulos, A., Mevius, D., Ceccarelli, D., 2016. A review of SHV extended-spectrum β-lactamases: neglected yet ubiquitous. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01374.

Liao, W., Li, D., Liu, F., Du, F., Long, D., Zhang, W., Liu, Y., 2020. Distribution of integrons and phylogenetic groups among highly virulent serotypes of *Klebsiella pneumoniae* in a Chinese tertiary hospital. J. Glob. Antimicrob. Resist. 21, 278–284. https://doi.org/10.1016/j.jgar.2019.11.016.

Liu, X., Steele, J.C., Meng, X.Z., 2017. Usage, residue, and human health risk of antibiotics in Chinese aquaculture: a review. Environ. Pollut. 223, 161–169. https://doi.org/10.1016/j.envpol.2017.01.003.

Luu, Q.H., Nguyen, T.B.T., Nguyen, T.L.A., Do, T.T.T., Dao, T.H.T., Padungtod, P., 2021. Antibiotics use in fish and shrimp farms in Vietnam. Aquacult. Rep. 20, 100711. https://doi.org/10.1016/j.aqrep.2021.100711.

Mateus, A., Takahashi, E.A., Elkholly, D.A., Crotta, M., Ekiri, A., Wylie, C., Guinat, C., Patricio, M., Marshall, L., Ferreira, J.P., Stärk, K., Guitian, J., College, R.V., 2016. A systematic review to assess the significance of the food chain in the context of antimicrobial resistance (AMR) with particular reference to pork and poultry meat, dairy products, seafood and fresh produce on retail sale in the UK. https://www.food.gov.uk/sites/default/files/media/document/amr-systematic-review-final-report-2016.pdf.

Monstein, H.J., Östholm-Balkhed, Å., Nilsson, M.V., Nilsson, M., Dornbusch, K., Nilsson, L.E., 2007. Multiplex PCR amplification assay for the detection of blaSHV,

blaTEM and blaCTX-M genes in *Enterobacteriaceae*. AMPIS 115 (12), 1400–1408. https://doi.org/10.1111/J.1600-0463.2007.00722.X.

Mughini-Gras, L., Dorado-García, A., van Duijkeren, E., van den Bunt, G., Dierikx, C.M., Bonten, M.J.M., Bootsma, M.C.J., Schmitt, H., Hald, T., Evers, E.G., de Koeijer, A., van Pelt, W., Franz, E., Mevius, D.J., Heederik, D.J.J., 2019. Attributable sources of community-acquired carriage of *Escherichia coli* containing β-lactam antibiotic resistance genes: a population-based modelling study. Lancet Planet Health 3 (8), e357–e369. https://doi.org/10.1016/S2542-5196(19)30130-5.

Nakhle, P., Ribolzi, O., Boithias, L., Rattanavong, S., Auda, Y., Sayavong, S., Zimmermann, R., Soulileuth, B., Pando, A., Thammahacksa, C., Rochelle-Newall, E. J., Santini, W., Martinez, J.-M., Gratiot, N., Pierret, A., 2021. Effects of hydrological regime and land use on in-stream *Escherichia coli* concentration in the Mekong basin, Lao PDR. Sci. Rep. 11 (1), 3460. https://doi.org/10.1038/s41598-021-82891-0.

Okanda, T., Haque, A., Koshikawa, T., Islam, A., Huda, Q., Takemura, H., Matsumoto, T., Nakamura, S., 2021. Characteristics of Carbapenemase-producing *Klebsiella pneumoniae* isolated in the intensive care unit of the largest tertiary Hospital in Bangladesh. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.612020.

Paterson, D.L., Bonomo, R.A., 2005. Extended-spectrum beta-lactamases: a clinical update. Microbiol. Rev. 18 (4), 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005.

Pearce, R., Conrady, B., Guardabassi, L., 2023. Prevalence and types of extended-spectrum β-lactamase-producing bacteria in retail seafood. Foods 12 (16), 3033. https://doi.org/10.3390/FOODS12163033. Vol. 12, Page 3033.

Perestrelo, S., Carreira, G.C., Valentin, L., Fischer, J., Pfeifer, Y., Werner, G., Schmiedel, J., Falgenhauer, L., Imirzalioglu, C., Chakraborty, T., Käsbohrer, A., 2022. Comparison of approaches for source attribution of ESBL-producing *Escherichia coli* in Germany. PloS One 17 (7). https://doi.org/10.1371/JOURNAL.PONE.0271317.

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). https://doi.org/10.2807/1560-7917.ES.2017.22.43.17-00032.

Schar, D., Klein, E.Y., Laxminarayan, R., Gilbert, M., Van Boeckel, T.P., 2020. Global trends in antimicrobial use in aquaculture. Sci. Rep. 10 (1), 1–9. https://doi.org/10.1038/s41598-020-78849-3.

Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. J. Bioinform. 30 (14), 2068–2069. https://doi.org/10.1093/BIOINFORMATICS/BTU153.

Sidjabat, H.E., Townell, N., Nimmo, G.R., George, N.M., Robson, J., Vohra, R., Davis, L., Heney, C., Paterson, D.L., 2015. Dominance of IMP-4-producing *Enterobacter cloacae* among carbapenemase-producing *Enterobacteriaceae* in Australia. Antimicrob. Agents Chemother. 59 (7), 4059–4066. https://doi.org/10.1128/AAC.04378-14.

Silva, V., Nunes, J., Gomes, A., Capita, R., Alonso-Calleja, C., Pereira, J.E., Torres, C., Igrejas, G., Poeta, P., 2019. Detection of antibiotic resistance in *Escherichia coli* strains: can fish commonly used in raw preparations such as sushi and sashimi constitute a public health problem? J. Food Prot. 82 (7), 1130–1134. https://doi.org/10.4315/0362-028X.JFP-18-575.

Silva-Bea, S., Romero, M., Parga, A., Fernández, J., Mora, A., Otero, A., 2024. Comparative analysis of multidrug-resistant *Klebsiella pneumoniae* strains of food and human origin reveals overlapping populations. Int. J. Food Microbiol. 413, 110605. https://doi.org/10.1016/J.IJFOODMICRO.2024.110605.

Singh, S.R., Teo, A.K.J., Prem, K., Ong, R.T.H., Ashley, E.A., van Doorn, H.R., Limmathurotsakul, D., Turner, P., Hsu, L.Y., 2021. Epidemiology of extended-spectrum beta-lactamase and carbapenemase-producing Enterobacterales in the greater Mekong subregion: a systematic-review and meta-analysis of risk factors associated with extended-spectrum beta-lactamase and carbapenemase isolations. Front. Microbiol. 12, 695027. https://doi.org/10.3389/fmicb.2021.695027.

Sommer, J., Reiter, H., Sattler, J., Cacace, E., Eisfeld, J., Gatermann, S., Hamprecht, A., Göttig, S., 2024. Emergence of OXA-48-like producing *Citrobacter* species, Germany, 2011 to 2022. Eurosurveillance 29 (15). https://doi.org/10.2807/1560-7917.ES.2024.29.15.2300528.

Sousa, M., Torres, C., Barros, J., Somalo, S., Igrejas, G., Poeta, P., 2011. Gilthead seabream (*Sparus aurata*) as carriers of SHV-12 and TEM-52 extended-spectrum beta-lactamases-containing *Escherichia coli* isolates. Foodborne Pathog. Dis. 8 (10), 1139–1141. https://doi.org/10.1089/fpd.2011.0866.

Subramani, P., Menichincheri, G., Pirolo, M., Arcari, G., Kudirkiene, E., Polani, R., Carattoli, A., Damborg, P., Guardabassi, L., 2023. Genetic background of neomycin resistance in clinical *Escherichia coli* isolated from Danish pig farms. Appl. Environ. Microbiol. 89 (10). https://doi.org/10.1128/aem.00559-23.

Tanimoto, K., Nomura, T., Hashimoto, Y., Hirakawa, H., Watanabe, H., Tomita, H., 2021. Isolation of *Serratia fonticola* producing FONA, a minor extended-spectrum β-lactamase (ESBL), from imported chicken meat in Japan. Jpn. J. Infect. Dis. 74 (1), 79–81. https://doi.org/10.7883/YOKEN.JJID.2020.114.

Tridge, 2019. Smarter global sourcing - Tridge [online] Available at: https://www.tridge.com/.

Vitas, A.I., Naik, D., Pérez-Etayo, L., González, D., 2018. Increased exposure to extended-spectrum β-lactamase-producing multidrug-resistant *Enterobacteriaceae* through the consumption of chicken and sushi products. Int. J. Food Microbiol. 269, 80–86. https://doi.org/10.1016/j.ijfoodmicro.2018.01.026.

Vu, T.T.T., Alter, T., Roesler, U., Roschanski, N., Huehn, S., 2018. Investigation of extended-spectrum and AmpC b-lactamase-producing *Enterobacteriaceae* from retail seafood in Berlin, Germany. J. Food Prot. 81 (7), 1079–1086. https://doi.org/10.4315/0362-028X.JFP-18-029.

Weissfeld, A.S., 2014. Infections from eating raw or undercooked seafood. Clin. Microbiol. Newsl. 36 (3), 17–21. https://doi.org/10.1016/j.clinmicnews.2014.01.004.

World Health Organization (WHO), 2017. Critically Important Antimicrobials for Human Medicine – 5th Rev. Geneva.

Yao, Y., Falgenhauer, L., Falgenhauer, J., Hauri, A.M., Heinmüller, P., Domann, E., Chakraborty, T., Imirzalioglu, C., 2021. Carbapenem-resistant *Citrobacter* spp. as an emerging concern in the hospital-setting: results from a genome-based regional surveillance study. Front. Cell. Infect. Microbiol. 11. https://doi.org/10.3389/fcimb.2021.744431.