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Prevalence and antimicrobial resistance profile of *Listeria* spp. isolated from raw fish

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Abstract

Background *Listeria* spp. contamination in food, particularly antimicrobial-resistant strains, poses an escalating concern for public health. This study investigated the prevalence and antimicrobial resistance profiles of *Listeria* spp. isolated from raw fish collected from diverse sources in Northern Poland.

Methods A total of 750 raw fish samples were collected from standing freshwater tanks, flowing freshwater reservoirs, and a saltwater reservoir. Isolation was performed following the standard protocol, which describes the horizontal method for detecting *Listeria spp*., including *Listeria monocytogenes*. The antimicrobial resistance profiles of isolated strains were performed using the disk diffusion method. Antimicrobial resistance genes were identified using PCR, targeting 11 genes associated with resistance to β-lactams, macrolides, glycopeptides, and sulfonamides. Statistical analyses included Phi correlation coefficients, hierarchical clustering, and logistic regression to examine associations between phenotypic resistance patterns and antimicrobial resistance genes.

Results *Listeria* spp. was isolated from 13.9% of samples (104 positive samples), comprising *L. seeligeri* (34.6%), *L. welshimeri* (28.8%), *L. monocytogenes* (23.1%), and *L. innocua* (13.5%). Phenotypic antimicrobial susceptibility testing revealed universal resistance to oxacillin (100%) across all *Listeria* spp. isolates. High resistance levels were also observed for cefotaxime (97.1%), cefoxitin (92.3%), rifampicin (92.3%), clindamycin (96.2%), and trimethoprim-sulfamethoxazole (91.3%). Alarmingly, 98.1% of all *Listeria* spp. isolates exhibited multidrug resistance (MDR), reaching 100% MDR among *L. monocytogenes* isolates. Specifically, *L. monocytogenes* isolates exhibited complete resistance to meropenem, cefoxitin, cefotaxime, rifampicin, and trimethoprim-sulfamethoxazole, and significant resistance to ciprofloxacin (91.7%), clindamycin (83.3%), tetracycline (75.0%), erythromycin (75.0%), benzylpenicillin (70.8%), and nitrofurantoin (70.8%). Molecular analysis identified *blaTEM* (100%), *ampC* (37.5%), and *ereB* (37.5%) as the most prevalent antimicrobial resistance genes in *L. monocytogenes*.

Conclusions The exceptionally high prevalence of multidrug-resistant *Listeria* spp., particularly *L. monocytogenes*, in raw fish underscores a critical public health risk, suggesting the urgent need for ongoing surveillance and robust risk mitigation strategies in aquaculture and seafood processing. The elevated antimicrobial resistance levels may also indicate aquatic environmental contamination, warranting further investigation into the sources and broader ecological implications of antimicrobial resistance in these ecosystems.

Keywords Aquaculture, *Listeria monocytogenes*, Multidrug resistance, Raw fish, Seafood safety

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Background

Listeria species (spp.), a genus of Gram-positive, facultative anaerobic bacteria, currently encompasses 21 recognized species [1, 2]. These bacteria are ubiquitous, inhabiting diverse environmental niches ranging from soil and water to various food sources, including raw and processed meats, dairy, and seafood [1, 2]. Among these species, *L. monocytogenes* and *L. ivanovii* are distinguished as historically recognized pathogens. *L. monocytogenes* is particularly significant as a zoonotic pathogen, capable of causing listeriosis in both humans and animals, while *L. ivanovii* primarily affects ruminants and is only rarely associated with human disease [3]. While species such as *L. seeligeri*, *L. welshimeri*, and *L. innocua* were initially considered non-pathogenic, emerging evidence links them to sporadic human listeriosis cases, suggesting a broader and perhaps underappreciated pathogenic potential within the genus [4].

L. monocytogenes warrants particular attention as a major foodborne pathogen and the causative agent of listeriosis, a disease with significant public health implications. This bacterium exhibits a widespread distribution, being commonly isolated from diverse sources including human and animal populations, as well as a wide array of food products, especially those of animal origin [1, 5]. Food-producing animals act as key reservoirs, facilitating the transmission of *L. monocytogenes* to humans primarily through the consumption of contaminated food [1, 6, 7]. Despite its environmental ubiquity, the primary route of human infection is overwhelmingly foodborne, with raw or inadequately processed foods representing the most significant sources of exposure.

Listeriosis presents a considerable public health challenge, especially within industrialized nations, primarily due to its elevated fatality rate, particularly among vulnerable demographics including neonates, pregnant women, those with compromised immune systems, and the elderly. Clinical manifestations of listeriosis are diverse and severe, ranging from septicemia and meningitis to adverse pregnancy outcomes such as fetal death and premature birth [8]. Compounding this public health concern is the reported rise in listeriosis incidence across Europe. The 2023 European Food Safety Authority (EFSA) report highlights this increasing trend, noting that *L. monocytogenes* infections were the fifth most frequently reported zoonosis in 2022, with a 15.9% increase in case notification rates compared to 2021. Alarmingly, the EFSA report also underscores that *L. monocytogenes* infections exhibit the highest rates of hospitalization and case-fatality among reported zoonoses, with a case-fatality rate reaching 18.1% in 2022 [9].

While *Listeria* spp. is generally considered susceptible to a broad spectrum of antimicrobials, the emergence of resistant strains of *L. monocytogenes* is an escalating concern. These resistant strains are increasingly identified in food, environmental matrices, and clinical cases of listeriosis [10–12]. Standard treatment protocols for listeriosis typically involve a combination therapy of beta-lactam antibiotics, such as ampicillin or penicillin, with an aminoglycoside, often gentamicin. In cases of beta-lactam allergies or for pregnant women, alternative therapeutic options may include vancomycin, erythromycin, or trimethoprim-sulfamethoxazole. However, it is crucial to recognize that resistance patterns exhibit significant geographic variability, influenced by local antimicrobial usage practices, thereby highlighting the imperative for continuous surveillance of antimicrobial susceptibility in *Listeria* spp. across diverse regions [1]. Such vigilant monitoring is essential for preserving the effectiveness of current treatment regimens and ensuring ongoing public health protection.

Antimicrobial resistance (AMR) in *Listeria* spp. arises through various mechanisms, notably the acquisition of mobile genetic elements, including self-transferable plasmids, mobilizable plasmids, and conjugative transposons. Furthermore, resistance can also develop via point mutations within genes encoding antimicrobial target sites [1]. Consequently, monitoring the occurrence of antimicrobial resistance genes (AMR genes) represents a crucial strategy for tracking and understanding the evolution of AMR in *Listeria* spp. Such genetic monitoring can provide early warnings of emerging resistance trends and complement phenotypic surveillance efforts.

Given the significant role of food contamination in *L. monocytogenes* transmission, and the particular risk associated with raw and processed fish products, continuous monitoring of both prevalence and AMR in seafood is of paramount importance. Numerous studies have indeed linked listeriosis outbreaks to contaminated fish products, underscoring the significant public health hazard posed throughout the fish supply chain, from aquaculture to retail [13, 14]. Despite this recognized risk, a notable gap exists in the literature: to date, no study has specifically addressed the risk factors associated with *Listeria* spp. contamination in fish within Poland.

Therefore, this study was designed with two primary objectives: first, to assess the prevalence of *Listeria* spp. and *L. monocytogenes* in raw fish sourced from diverse aquatic environments in Northern Poland; and second, to meticulously characterize the AMR profiles of the isolated strains, with a specific focus on identifying genes associated with resistance to antimicrobials commonly employed in the clinical treatment of listeriosis in both, humans and animals. By addressing these objectives, our study aims to contribute valuable data to the understanding of *Listeria* ecology and AMR in a geographically under-examined region, informing future risk

assessments and public health interventions related to seafood safety.

Methods

Study area and sample collection

The study was conducted in northern Poland from January 2020 to December 2022. A total of 750 dead raw fish samples were collected from three different environments: standing freshwater tanks (250 samples), flowing freshwater reservoirs (250 samples), and a saltwater reservoir (250 samples) (Table 1; Fig. 1). All sampling material came from five local fish markets located in northern Poland or local fish farm (Szwaderki, Poland). The standing freshwater samples consisted entirely of crucian carp (*Carassius carassius*), while the flowing freshwater samples included 100 crucian carp, 75 sturgeon (*Acipenseridae*), and 75 trout (*Oncorhynchus mykiss*). The saltwater samples comprised 250 herrings (*Clupea harengus*). Each sample was individually placed in a sterile plastic bag and transported to the laboratory under cooled conditions to ensure preservation.

Phenotypic isolation and identification of *Listeria* spp. from fish samples

Isolation was performed following the detection protocol outlined in part 1 of PN-EN ISO 11290-1:2017-07, which describes detecting methods of *L. monocytogenes* and other *Listeria* spp [15]. Initially, 25 ml of each sample was added to 225 ml of Half-Fraser broth medium (Oxoid, Basingstoke, UK) and incubated aerobically at 30 °C for 24 ± 2 h. Subsequently, 0.1 ml of this culture was transferred to 10 ml of Fraser broth medium (Oxoid, Basingstoke, UK) and further incubated at 37 °C for 24 ± 2 h. Parallel isolations were performed using Ottaviano Agosti ALOA agar (Oxoid, Basingstoke, UK) and Palcam agar (Oxoid, Basingstoke, UK). After 24 to 48 h of incubation at 37 °C, colonies were assessed for phenotypic characteristics typical of *Listeria* spp.

Three colonies with typical *Listeria* morphology were selected from each plate, represented three isolated strains, and plated on TSEYA agar (Oxoid, Basingstoke, UK), followed by an 18 ± 2-hour incubation. The resulting bacterial colonies were first examined by Gram staining for morphological confirmation. Species-level identification of Listeria isolates was then confirmed using the Microbact Listeria 12 L biochemical identification kit (Oxoid, Basingstoke, UK), which tests for esculin hydrolysis, hemolysis, and carbohydrate utilization. All procedures were performed according to the manufacturer's instructions. The isolated strains were then stored at -80 °C in 20% glycerol for future analysis.

Table 1 Distribution of Raw fish samples across different aquatic environments

Environment	Fish type	No. of samples
Standing freshwater	Crucian carp	250
Flowing freshwater	Crucian carp	100
	Sturgeon*	75
	Trout	75
Saltwater	Herring	250
		Total = 750

* fish from fish farm

Antimicrobial susceptibility testing of *Listeria* spp. isolated from fish

The antimicrobial resistance profiles of *Listeria* strains were analyzed using the disk diffusion method, following the guidelines set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [16] and the Clinical and Laboratory Standards Institute (CLSI) [17]. For each selected colony, bacterial suspensions were prepared in physiological saline (BioMaxima, Lublin, Poland) to a density equivalent to 0.5 McFarland. The suspensions were uniformly spread onto Mueller-Hinton Agar (Oxoid, Basingstoke, UK), supplemented with 5% defibrinated horse blood (BioMaxima, Lublin, Poland) and 20 mg/L β-NAD (MH-F) (Oxoid, Basingstoke, UK).

A total of 17 different antimicrobial discs, representing 13 antibiotic groups (Fig. 2), were applied to the agar plates. The tested antimicrobials included ampicillin (2 µg), oxacillin (1 µg), meropenem (10 µg), benzylpenicillin (1 unit), cefoxitin (30 µg), cefotaxime (30 µg), gentamycin (10 µg), erythromycin (15 µg), rifampicin (5 µg), vancomycin (30 µg), trimethoprim-sulfamethoxazole (1.25–23.75 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), nitrofurantoin (100 µg), and clindamycin (2 µg).

The plates were incubated aerobically at 37 °C for 18 to 24 ± 2 h. Zones of inhibition were measured and interpreted according to EUCAST standards for *L. monocytogenes* (ampicillin, benzylpenicillin, meropenem, erythromycin and trimethoprim-sulfamethoxazole), *Staphylococcus* spp. (cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, gentamycin, oxacillin, rifampicin and tetracycline) and *Enterococcus* spp. (vancomycin) [16]. The CLSI [17] guidelines were followed for cefotaxime and for enrofloxacin resistance in *Staphylococcus* spp.

Strains that exhibited resistance to at least one antimicrobial drug in more than three antimicrobial classes were classified as multidrug-resistant (MDR) [18]. Quality control for the antimicrobial susceptibility testing was ensured by using reference strains: *Streptococcus pneumoniae* ATCC 49,619 (ATCC, Manassas, VA, USA), *Enterococcus faecalis* ATCC 29,212 (ATCC, Manassas, VA, USA), and *Staphylococcus aureus* ATCC 29,213 (ATCC, Manassas, VA, USA). Each test was performed

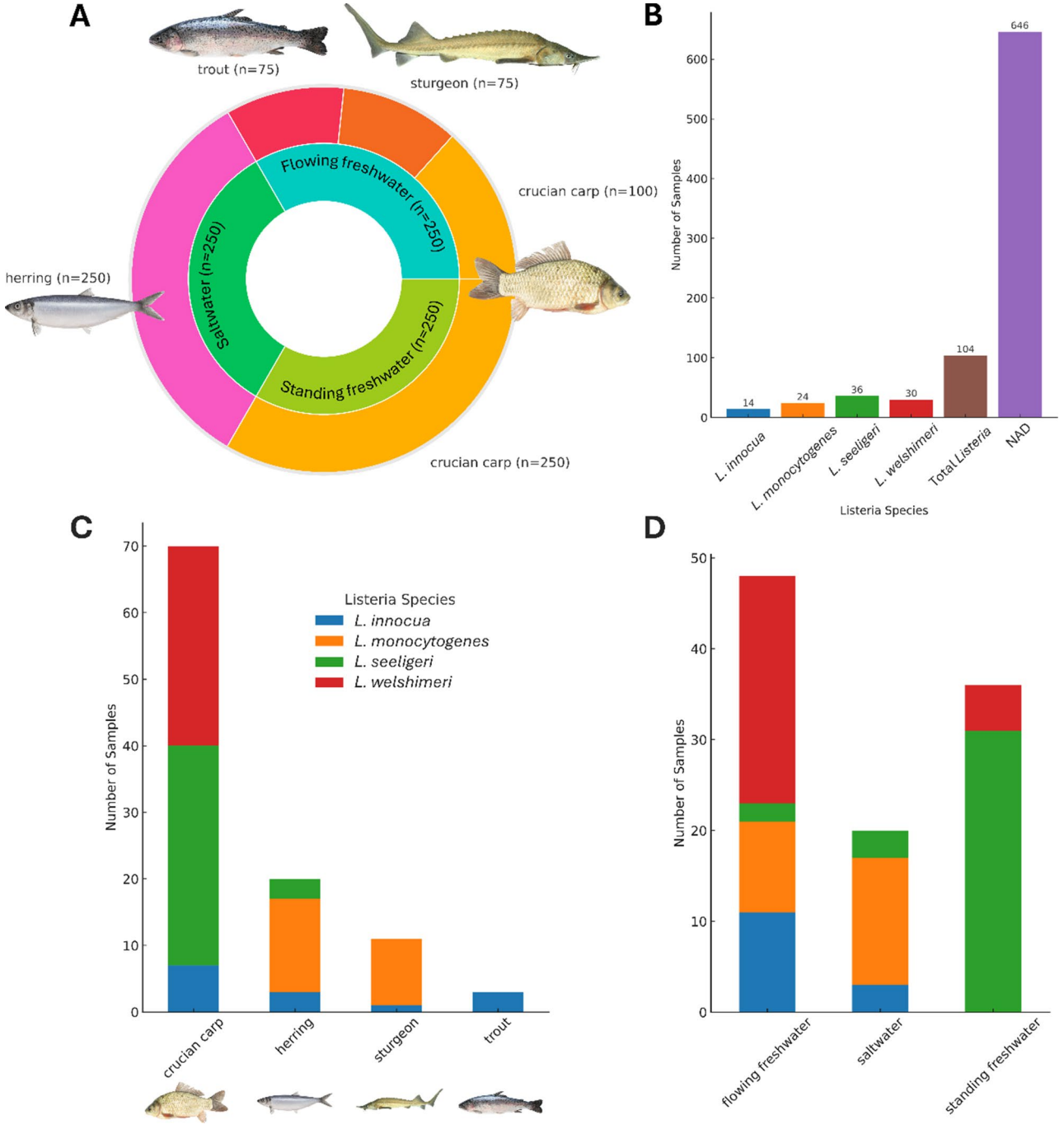

Fig. 1 Distribution of *Listeria* spp. isolates by sample origin and species. Figure provides a overview of the distribution of *Listeria* spp. isolates obtained from raw fish samples across different aquatic environments and fish types. (**A**) A donut chart illustrates the distribution of the 750 raw fish samples collected, with the inner ring representing the aquatic environment: saltwater, flowing freshwater, standing freshwater, and the outer ring segments further detailing the types of fish sampled within each environment: crucian carp, herring, sturgeon, and trout. The sample size (n) for each environment and fish type is indicated within the chart segments, alongside representative images of each fish species. (**B**) A bar chart presents the total counts of each *Listeria* spp. isolated (*L. innocua*, *L. monocytogenes*, *L. seeligeri*, *L. welshimeri*) and a summary category for the total number *Listeria* spp., NAD- Not Assigned to Listeria spp. Detection (**C**) A stacked bar chart details the distribution of *Listeria* spp. across the four fish types: crucian carp, herring, sturgeon, and trout, displayed on the x-axis with representative images. The y-axis indicates the number of samples, with color-coded stacked bars representing the count of each *Listeria* spp. isolated from each fish type, as detailed in the legend. (**D**) Another stacked bar chart shows the distribution of *Listeria* spp. across the three aquatic environments: flowing freshwater, saltwater, and standing freshwater. The y-axis denotes the number of samples, with stacked, color-coded bars indicating the counts of each *Listeria* spp. isolated from each environment, as specified in the legend

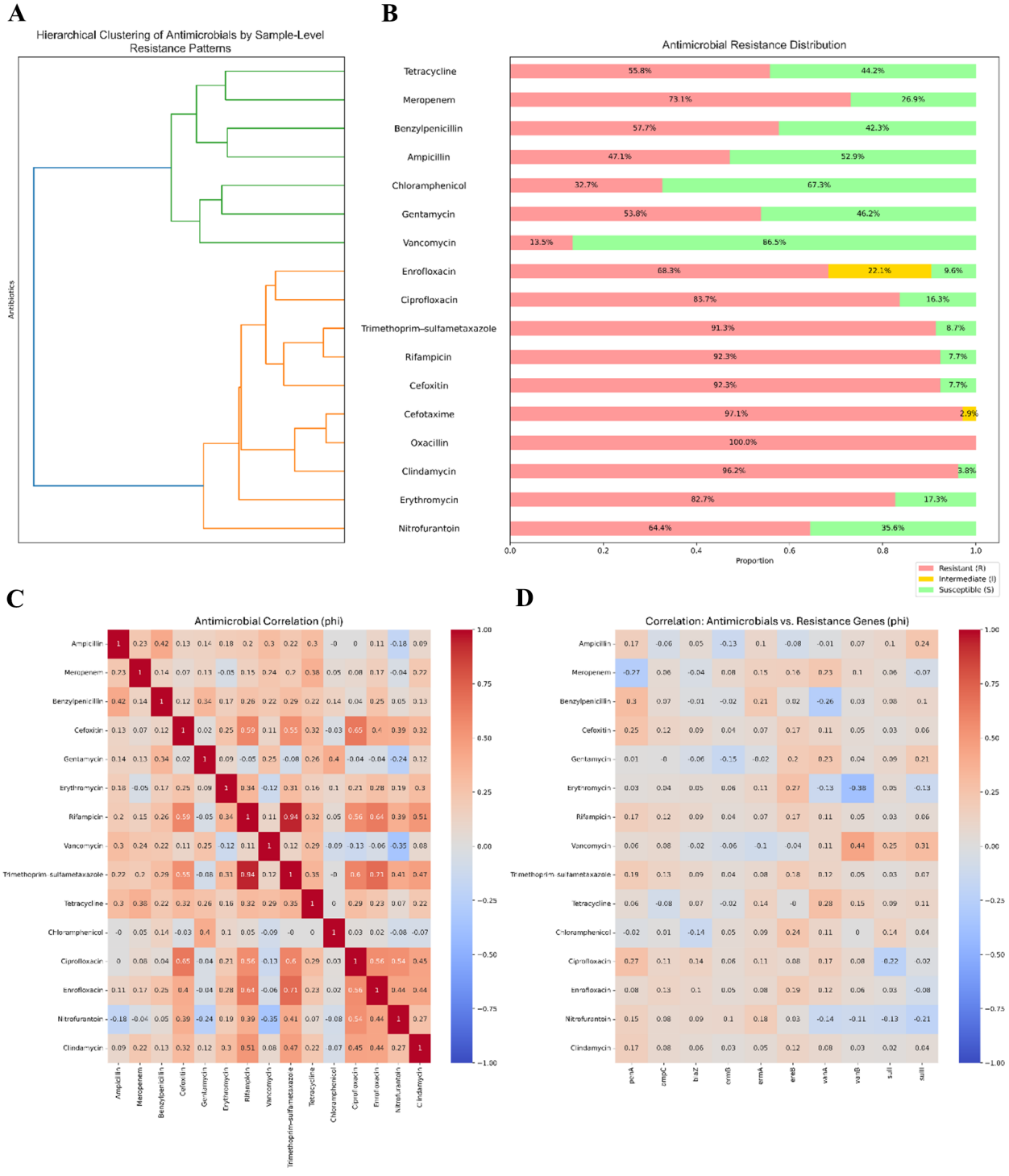

Fig. 2 Phenotypic antimicrobial resistance distribution and correlation analyses of *Listeria* spp. isolates from raw fish. (**A**) A dendrogram on the left illustrates the hierarchical clustering of antimicrobials based on the resistance profiles observed across the *Listeria* spp. isolates. The clustering, performed using Euclidean distance and Ward's linkage, groups antimicrobials with similar resistance patterns. (**B**) A horizontal stacked bar chart on the right displays the antibiotic resistance distribution for each of the 17 tested antimicrobials. Each bar is segmented and color-coded to show the proportion of isolates classified as Resistant (red), Intermediate (yellow), and Susceptible (green) according to EUCAST and CLSI breakpoints [16, 17]. The percentage values for each category are indicated within the bar segments. (**C**) A heatmap at the bottom left presents the Phi correlation matrix for antimicrobial resistance profiles. (**D**) A heatmap at the bottom right shows the Phi correlation matrix for antimicrobial resistance (antimicrobials listed on the y-axis) versus the presence of antimicrobial resistance genes (genes listed on the x-axis)

in triplicate to ensure reproducibility and reliability of results.

DNA isolation and PCR confirmation of *Listeria* spp. isolates

Genomic DNA was extracted from the bacterial isolates using a commercial kit (A&A Biotechnology, Gdynia, Poland), with lysozyme and mutanolysin for cell lysis. Briefly, 1 ml of overnight bacterial culture was centrifuged, and the pellet was suspended in a lysis solution containing lysozyme (A&A Biotechnology, Gdynia, Poland), mutanolysin (Sigma-Aldrich, St. Louis, MO, USA), and proteinase K (A&A Biotechnology, Gdynia, Poland). The mixture was incubated at 50 °C for 30 min, followed by vortexing and centrifugation to isolate DNA. The extracted DNA was stored at -20 °C until use. DNA concentration and purity were measured using a BioSpectrometer (Eppendorf, Hamburg, Germany) at 260 nm, with samples deemed suitable for use when DNA concentration was between 80 ng/µl and 180 ng/µl.

Polymerase Chain Reaction (PCR) was employed to confirm the identity of the isolates, targeting the *prs* gene for *Listeria* spp. identification and species-specific genes such as *lmo1030* for *L. monocytogenes*, *namA* for *L. ivanovii*, and others. The primers used are detailed in Table 2. PCR reactions were performed using 2 µl of DNA, 12.5 µl of MixPCR solution (A&A Biotechnology, Gdynia, Poland), which included $MgCl_2$ at 3 mM, dNTPs at 0.2 mM, primers at 0.5 µM, 1.25 U of Taq DNA polymerase (A&A Biotechnology, Gdynia, Poland), and sterile water to make a final volume of 25 µl.

The reactions included positive controls (reference strains of *Listeria* spp.: *L. monocytogenes* ATCC 13932, *L. ivanovii* ATCC 19119, *L. innocua* ATCC 33090, *L. welshimeri* ATCC 35897 and *L. seeligeri* ATCC 35967) and negative controls (samples without DNA). The PCR cycles were programmed as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing for 30 s, and elongation at 72 °C for 45 s. A final elongation step was carried out at 72 °C for 5 min. PCR products were analyzed using 2.0% agarose gel electrophoresis (Sigma-Aldrich, St. Louis, MO, USA), stained with SimplySafe (EURx, Gdańsk, Poland). Gel visualization was performed with a Gel Doc EZ System (Bio-Rad, Hercules, CA, USA), and PCR products were compared to a Perfect Plus 1 kb DNA Ladder (EURx, Gdańsk, Poland) for size estimation.

Molecular detection of antimicrobial resistance genes in *Listeria* spp. from raw fish

Antibiotic resistance genes were identified using PCR, targeting 11 genes associated with resistance to four antibiotic groups: β-lactams, macrolides, glycopeptides, and sulfonamides. The β-lactam resistance genes assessed included *penA*, *ampC*, *blaTEM*, and *blaZ*. For macrolide resistance, the genes *ermB*, *ermA*, and *ereB* were analyzed. Glycopeptide resistance was evaluated through the detection of *vanA* and *vanB*, while sulfonamide resistance was assessed by screening for *sulI* and *sulII*.

The procedure for genomic DNA extraction, concentration measurement, and purity assessment was conducted similarly to the method described in the DNA isolation and PCR confirmation of *Listeria* spp. isolates section. Briefly, genomic DNA was extracted from the bacterial isolates using a commercial kit (A&A Biotechnology, Gdynia, Poland) and prepared for PCR analysis. PCR reactions were performed using MixPCR solution (A&A Biotechnology, Gdynia, Poland), which included $MgCl_2$, dNTPs, primers, and Taq DNA polymerase. Positive controls (reference strains) and negative controls (samples without DNA) were included to ensure the

Table 2 Primers used for PCR-Based identification of *Listeria* spp

Species	Gene	Primer	Sequences (5′-3′)	PCR product size (bp)	Anneling temterature	Reference
Listeria spp.	*prs*	prs-F	GCTGAAGAGATTGCGAAAGAAG	370	58°	[42]
		prs-R	CAAAGAAACCTTGGATTTGCGG			
Listeria spp.	*spp*	spp-F	CTGGCACGGACTTCCACTTAC	116	55°	[43]
		spp-R	TTTGAGCACCGATGATGATTT			
L. monocytogenes	*Glycosyl hydrolase*	lm-F	GTTCGTCGGTCCGTGGTA	583	55°	[44]
		lm-R	TTGGCAAGCAAGCAGTTCA			
L. ivanovii	*namA*	iv-F	CGAATTCCTTATTCACTTGAGC	463	53°	[45]
		iv-R	GGTGCTGCGAACTTAACTCA			
L. innocua	*lin0464*	in-F	CGCATTTATCGCCAAAACTC	749	54°	[46]
		in-R	TCGTGACATAGACGCGATTG			
L. seeligeri	*lmo0333*	se-F	GTACCTGCTGGGAGTACATA	673	52°	[47]
		se-R	CTGTCTCCATATCCGTACAG			
L. welshimeri	*scrA*	we-F	CGTGGCACAATAGCAATCTG	281	52°	[47]
		we-R	GACATGCCTGCTGAACTAGA			
L. grayi	*Oxidoreductase*	gr-F	GCGGATAAAGGTGTTCGGGTCAA	201	61°	[47]
		gr-R	ATTTGCTATCGTCCGAGGCTAGG			

accuracy of the analysis. The PCR cycling conditions, product visualization using 2.0% agarose gel electrophoresis (Sigma-Aldrich, St. Louis, MO, USA), and size estimation using the Perfect Plus 1 kb DNA Ladder (EURx, Gdańsk, Poland) followed the same procedure outlined in the PCR confirmation section. The primers used for detecting the antimicrobial resistance genes are presented in Table 3.

Statistical analysis

The dataset analyzed in this study comprised 750 fish samples, from which 104 positive samples of *Listeria* isolates were obtained. Each isolate was characterized by species identification, fish type, and environmental origin, providing a comprehensive context for assessing antimicrobial resistance patterns. Susceptibility profiles were determined for 17 antimicrobials from multiple classes, enabling a detailed evaluation of resistance trends.

To ensure a robust analysis, antimicrobial susceptibility was assessed in two ways. First, the data were categorized into three discrete groups: resistant (R), intermediate (I), and susceptible (S). Second, to simplify interpretation, the intermediate (I) category was merged with resistant (R), classifying isolates as either susceptible (0) or non-susceptible [1] (resistant/intermediate). Additionally, the presence or absence of key resistance genes was recorded as a binary variable (0/1), allowing for correlations between phenotypic resistance and genetic determinants.

To classify the antimicrobials based on their chemical structures, we applied hierarchical clustering using molecular fingerprints. Each antimicrobial's molecular structure was first converted into a SMILES representation. These fingerprints were used to compute Tanimoto similarity coefficients, which were subsequently transformed into distance values (1– Tanimoto) to enable clustering. Ward's linkage clustering was then applied to generate a dendrogram, allowing us to group antimicrobials based on structural similarities. The analysis included ampicillin, oxacillin, meropenem, benzylpenicillin, cefoxitin, cefotaxime, gentamicin, erythromycin, rifampicin, vancomycin, trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, ciprofloxacin, enrofloxacin, nitrofurantoin, and clindamycin. The resulting clustering led to the classification of antimicrobials into distinct structural groups, including penicillins (ampicillin, oxacillin, benzylpenicillin), carbapenems (meropenem), cephalosporins (cefoxitin, cefotaxime), aminoglycosides (gentamicin), macrolides (erythromycin), rifamycins (rifampicin), glycopeptides (vancomycin), sulfonamides (trimethoprim-sulfamethoxazole), tetracyclines (tetracycline), phenicols (chloramphenicol), fluoroquinolones (ciprofloxacin, enrofloxacin), nitrofurans (nitrofurantoin), and lincosamides (clindamycin). This approach enabled a data-driven division of antimicrobials into structurally similar groups, providing a more refined framework for analyzing antimicrobial resistance patterns and understanding potential

Table 3 Primer pairs used for amplification of antibiotic resistance gene in *Listeria* spp. Isolates

Group of antibiotics	Gene	Primer	Sequences (5'-3')	PCR product size (bp)	Annealing temperature	Reference
B-lactamas	*penA*	penA-F	ATCGAACAGGCGACGATGTC	500	52°	[48]
		penA-R	GATTAAGACGGTGTTTTACGG			
	ampC	ampC-F	TTCTATCAAMACTGGCARCC	550	50°	[49]
		ampC-R	CCYTTTTATGTACCCAYGA			
	bla_{TEM}	bla-F	TTTCGTGTCGCCCTTATTCC	690	60°	[49]
		bla-R	CCGGCTCCAGATTTATCAGC			
	blaZ	blaZ-F	ACTTCAACACCTGCTGCTTTC	490	60°	[49]
		blaZ-R	TGACCACTTTTATCAGCAACC			
Sulfonamides	*sulI*	sulI-F	GTGACGGTGTTCGGCATTCT	779	68°	[50]
		sulI-R	TCCGAGAAGGTGATTGCGCT			
	sulII	sulII-F	CGGCATCGTCAACATAACCT	721	66°	[50]
		sulII-R	TGTGCGGATGAAGTCAGCTC			
Macrolides	*ermB*	ermB-F	GAAAAGGTACTCAACCAAATA	639	52°	[48]
		ermB-R	AGTAACGGTACTTAAATTGTTTAC			
	ermA	ermA-F	AACACCCTGAACCCAAGGGACG	420	62°	[48]
		ermA-R	CTTCACATCCGGATTCGCTCGA			
	ereB	ereB-F	AGAAATGGAGGTTCATACTTACCA	546	53°	[48]
		ereB-R	CATATAATCATCACCAATGGCA			
Glycopeptides	*vanA*	vanA-F	CATGACGTATCGGTAAAATC	885	52°	[48]
		vanA-R	ACCGGGCAGRGTATTGAC			
	vanB	vanB-F	CATGATGTGTCGGTAAAATC	882	52°	[48]
		vanB-R	ACCGGGCAGRGTATTGAC			

cross-resistance mechanisms within these structurally related antimicrobial classes.

For correlation analyses, we calculated Phi correlation coefficients, which are valid for binary data, to assess associations (i) among antimicrobial resistance profiles and (ii) between antimicrobial resistance profiles and resistance genes. The correlations were presented in heatmap format to visualize both positive and negative relationships.

Chi-square tests of independence were performed to compare frequencies of MDR across different *Listeria* spp., and significant global results were further examined using Bonferroni-corrected post-hoc tests. Contingency tables were created to quantify how many isolates were resistant to specific numbers of antimicrobial groups, and these data were displayed using both stacked bar charts and kernel density (KDE)-enhanced plots.

Lastly, individual logistic regression models were fitted for each antimicrobial as the dependent variable (resistant vs. susceptible) to investigate the effects of species (one-hot encoded) and the presence of resistance genes. Model parameters were estimated using various optimization methods (BFGS, Newton, L-BFGS) to ensure robust convergence. Model fit and predictive performance were evaluated via confusion matrices (from which sensitivity, specificity, and accuracy were derived) and ROC curve analyses (AUC). An overall likelihood-ratio test (compared to a null model) determined each model's statistical significance, and terms with $p < 0.05$ were deemed significant.

All statistical procedures and data visualizations were conducted in Python (using libraries such as pandas, numpy, scipy, statsmodels, and matplotlib).

Results

Prevalence of *Listeria* spp. and *L. monocytogenes* in raw fish

A total of 104 bacterial isolates of *Listeria* spp. were recovered from 750 fish samples analyzed, yielding a prevalence rate of 13.9%. The isolates were found across various fish types (Table 4), with crucian carp (67.3%, $p < 0.05$) being the most frequent source, followed by herring (19.2%), sturgeon (10.6%), and trout (2.9%). *L. seeligeri* and *L. welshimeri* were the most frequently isolated species, comprising 34.6% and 28.8% of all isolates, respectively, and were predominantly detected in crucian carp and herring. *L. monocytogenes* accounted for 23.1% of the total isolates and were found exclusively in herring and farmed sturgeons. In terms of environmental distribution, 58.3% of *L. monocytogenes* originated from wild saltwater fish, herring, while 41.7% were from farmed sturgeons in flowing freshwater. *L. innocua*, although detected across all fish species, was the least frequently isolated species (13.5%) and was most frequently found in crucian carp (Fig. 1).

Antimicrobial resistance profile of *Listeria* spp. isolated from fish

AMR profiles are shown in Fig. 2. Additionally, Fig. 2 presents correlations between resistance to individual antimicrobials and the associations between AMR profiles and AMR genes, providing further insights into the genetic basis of resistance in *Listeria* spp. This study assessed the AMR profiles of 104 *Listeria* spp. strains isolated from raw fish in Polish aquaculture. Oxacillin exhibited the highest resistance rate (100.0%), followed by cefotaxime (97.1%), clindamycin (96.2%), cefoxitin (92.3%), rifampicin (92.3%), and trimethoprim-sulfamethoxazole (91.3%) (Table 5). The five antimicrobials with the lowest resistance rates were tetracycline (55.8%), gentamicin (53.8%), ampicillin (47.1%), chloramphenicol (32.7%), and vancomycin (13.5%). All strains displayed resistance to at least one antimicrobial agent. Intermediate resistance was observed in fluoroquinolones and cephalosporins. Specifically, 22.1% of strains exhibited intermediate resistance to enrofloxacin, and 2.9% showed intermediate resistance to cefotaxime.

Notably, 98.1% of *Listeria* spp. strains were classified as MDR [18]. The most common MDR pattern involved resistance to: penicillin, cephalosporins, lincosamides, rifamycin, sulfonamides and fluoroquinolones. The highest resistance rates were observed for cephalosporins (100.0%) and penicillins (100.0%), followed by lincosamides (96.2%), rifamycins (92.3%), and fluoroquinolones (92.3%). Resistance to sulfonamides (91.3%), macrolides (82.7%), and carbapenems (73.1%) was also prevalent. Lower resistance rates were noted for nitrofurans (64.4%),

Table 4 Distribution of isolates from Raw fish across different aquatic environments: counts of *Listeria* spp., *L. monocytogenes*, *L. innocua*, *L. seeligeri*, and *L. welshimeri* isolates

Environment	Sample	Listeria				
		n	monocytogenes	innocua	seligeri	welshimeri
saltwater	herring	20	14	3	3	0
flowing freshwater	sturgeon	11	10	1	0	0
	trout	3	0	3	0	0
	crucian	34	0	7	2	25
standing freshwater	crucian	36	0	0	31	5
total		104	24	14	36	30

Table 5 Distribution of antimicrobial resistance: counts of resistant *Listeria* spp., *L. monocytogenes*, *L. innocua*, *L. seeligeri*, and *L. welshimeri* isolates

Antimicrobial	Listeria spp	L. monocytogenes	L. innocua	L. seligeri	L. weshimeri
	n = 104	n = 24	n = 14	n = 36	n = 30
Ampicillin	45 (43.3%)	10 (41.7%)	7 (50.0%)	11 (30.6%)	17 (56.7%)
Oxacillin	104 (100.0%)	24 (100.0%)	14 (100.0%)	36 (100.0%)	30 (100.0%)
Meropenem	73 (70.2%)	24 (100.0%)	8 (57.1%)	24 (66.7%)	17 (56.7%)
Benzylpenicillin	60 (57.7%)	17 (70.8%)	10 (71.4%)	15 (41.7%)	18 (60.0%)
Cefoxitin	96 (92.3%)	24 (100.0%)	9 (64.3%)	36 (100.0%)	27 (90.0%)
Cefotaxime	102 (98.1%)	24 (100.0%)	12 (85.7%)	36 (100.0%)	30 (100.0%)
Gentamycin	56 (53.8%)	12 (50.0%)	7 (50.0%)	23 (63.9%)	14 (46.7%)
Erythromycin	86 (82.7%)	18 (75.0%)	11 (78.6%)	33 (91.7%)	24 (80.0%)
Rifampicin	96 (92.3%)	24 (100.0%)	13 (92.9%)	36 (100.0%)	23 (76.7%)
Vancomycin	12 (11.5%)	8 (33.3%)	1 (7.1%)	1 (2.8%)	2 (6.7%)
Trimethoprim-sulfamethoxazole	92 (88.5%)	24 (100.0%)	12 (85.7%)	36 (100.0%)	20 (66.7%)
Tetracycline	56 (53.8%)	18 (75.0%)	4 (28.6%)	20 (55.6%)	14 (46.7%)
Chloramphenicol	33 (31.7%)	6 (25.0%)	6 (42.9%)	16 (44.4%)	5 (16.7%)
Ciprofloxacin	86 (82.7%)	22 (91.7%)	8 (57.1%)	36 (100.0%)	20 (66.7%)
Enrofloxacin	70 (67.3%)	14 (58.3%)	5 (35.7%)	35 (97.2%)	16 (53.3%)
Nitrofurantoin	67 (64.4%)	17 (70.8%)	5 (35.7%)	27 (75.0%)	18 (60.0%)
Clindamycin	96 (92.3%)	20 (83.3%)	12 (85.7%)	36 (100.0%)	28 (93.3%)

tetracyclines (55.8%), and aminoglycosides (53.8%), while phenicols (32.7%) and glycopeptides (13.5%) showed the lowest resistance rates.

The distribution of MDR across *Listeria* spp. varied significantly, with *L. monocytogenes* exhibiting the highest resistance ($p < 0.05$). Among the isolates, 41.7% of *L. monocytogenes* strains displayed resistance to 10 antimicrobial categories, while 37.5% were resistant to 11 categories. This highlights *L. monocytogenes* as the most resistant species within the dataset. *L. seeligeri* also demonstrated a high degree of resistance, with 16.7% of strains exhibiting resistance to 8, 9, and 10 categories, while 36.11% were resistant to 11 categories.

In contrast, *L. welshimeri* exhibited a broader but less concentrated resistance pattern. Approximately 33.3% of *L. welshimeri* isolates showed resistance to 10 antimicrobial categories, while 16.7% displayed resistance to 8 categories. *L. innocua* presented a relatively moderate resistance profile, with 7.2% of strains resistant to 4–6 antimicrobial categories. However, 21.3% of isolates exhibited resistance to 11 categories, highlighting a subset of strains with a particularly high MDR burden.

When analyzing the overall resistance distribution in *Listeria* spp., it became evident that 87.5% of total strains exhibited resistance to at least 8 antimicrobial categories, emphasizing the widespread presence of MDR within the population. Lower MDR levels, ranging from resistance to 2–7 categories, were present in only 12.5% of strains, demonstrating that the majority of isolates had resistance patterns spanning multiple antimicrobial groups (Fig. 3). Additionally, logistic regression models were constructed for each antimicrobial to investigate the effect of *Listeria* spp. and the presence/absence of relevant resistance genes. These models are provided in Supplementary Figs. 1–15. It was not possible to build models for those antimicrobials displaying complete resistance across all isolates.

Antimicrobial resistance profile of *L. monocytogenes* isolated from fish

Table 5 presents the resistance profiles of the 24 *L. monocytogenes* isolates. All isolates (100.0%) exhibited resistance to oxacillin, meropenem, cefoxitin, cefotaxime, rifampicin, and trimethoprim-sulfamethoxazole in the disk diffusion test. Resistance to benzylpenicillin was observed in 70.8% of isolates, while 50.0% were resistant to gentamycin. A high proportion of isolates also displayed resistance to erythromycin (75.0%) and tetracycline (75.0%), indicating significant resistance to macrolides and tetracyclines. Ciprofloxacin resistance was found in 91.7% of isolates, suggesting that fluoroquinolone resistance is widespread within *L. monocytogenes.*

Resistance to enrofloxacin was observed in 58.3% of isolates, while 70.8% demonstrated resistance to nitrofurantoin. Clindamycin resistance was found in 83.3% of isolates, further highlighting the broad resistance spectrum of *L. monocytogenes* strains in this study. In contrast, lower resistance rates were noted for vancomycin (33.3%) and chloramphenicol (25.0%), suggesting that these antibiotics may still retain some efficacy against *L. monocytogenes*.

Intermediate resistance was observed for enrofloxacin in 58.3% of the isolates, indicating that while resistance is present, some isolates may still be partially susceptible.

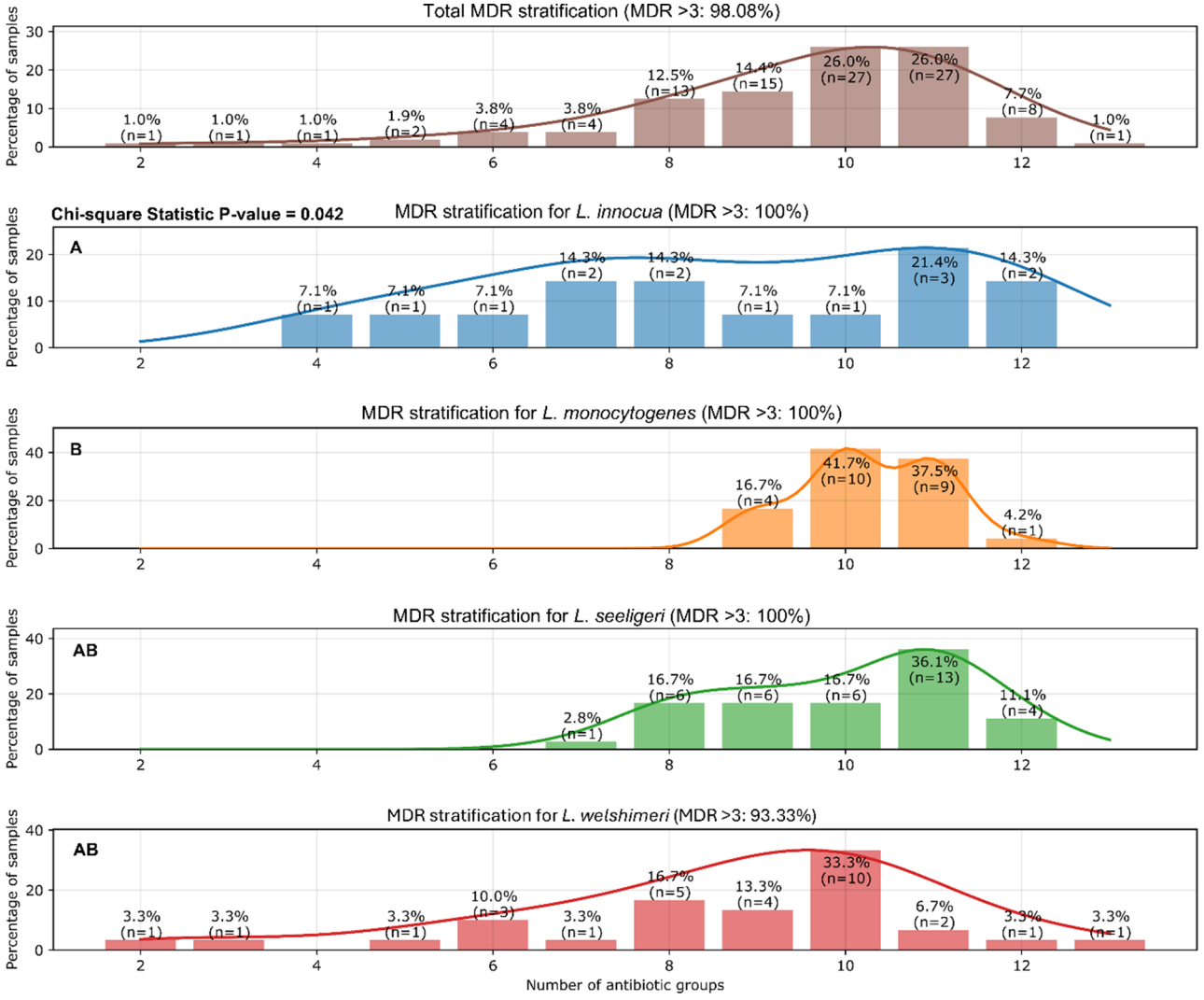

Fig. 3 Multidrug Resistance (MDR) stratification across *Listeria* spp. isolates from raw fish. Figure presents a stratified analysis of MDR among *Listeria* spp. isolates, categorized by species and overall MDR distribution, where multidrug resistance is defined as resistance to at least one antimicrobial drug in more than three antimicrobial classes. (**A**) Total MDR stratification across all *Listeria* spp. isolates. This panel displays a histogram overlaid with a Kernel Density Estimate (KDE) curve, providing an overview of the overall distribution of MDR levels in the entire dataset and summarizing MDR prevalence across all *Listeria* isolates. (**B**) MDR stratification for *L. innocua* isolates. (**C**) MDR stratification for *L. monocytogenes* isolates. (**D**) MDR stratification for *L. seeligeri* isolates. (**E**) MDR stratification for *L. welshimeri* isolates. In all subplots, the x-axis represents the number of antimicrobial groups to which isolates are resistant, and the y-axis indicates the percentage of samples at each resistance level. Each bar in the histograms is annotated with the percentage and count (n) of isolates at each resistance level. Different letters above the graphs indicate significantly different MDR distribution patterns among *Listeria* species ($p < 0.05$), as determined by post hoc chi-square analysis

Distribution of antimicrobial resistance genes among *Listeria* spp. and *L. monocytogenes* isolates

PCR screening for 11 genes related to resistance against four antimicrobial families (penicillins, macrolides, glycopeptides, and sulfonamides) was performed on the *Listeria* isolates. For penicillin resistance, the genes *penA*, *ampC*, *blaTEM*, and *blaZ* were detected in 16.7%, 37.5%, 100.0%, and 16.7% of penicillin-resistant *L. monocytogenes* isolates, respectively. The high prevalence of *blaTEM* across all isolates suggests a dominant mechanism of β-lactam resistance in this species.

Among macrolide-resistant isolates, *ermB* was present in 4.2%, while *ermA* was not detected in any isolate. The *ereB* gene, associated with erythromycin resistance, was found in 37.5% of *L. monocytogenes* isolates, indicating a moderate level of acquired macrolide resistance.

Of the glycopeptide-resistant isolates, *vanA* and *vanB* were each detected in 12.5% of the isolates, highlighting a subset of *L. monocytogenes* with potential resistance to vancomycin. Additionally, for sulfonamide resistance, the *sulI* gene was not detected, while *sulII* was identified in 8.3% of sulfonamide-resistant isolates, indicating that

alternative mechanisms of sulfonamide resistance may be present within this population.

Discussion

Globally, projections indicate a significant surge in seafood consumption, with estimates from Naylor et al. (2021) suggesting a potential 80% increase worldwide between 2015 and 2050 [19]. This anticipated rise in fish consumption underscores the escalating importance of ensuring seafood safety, as aquatic products are increasingly recognized as a significant vehicle for foodborne pathogens. Among the most significant pathogens, *Listeria* spp., and particularly *L. monocytogenes*, presents a substantial challenge to public health. *L. monocytogenes*, with its ability to thrive in refrigerated conditions and ready-to-eat (RTE) environments, poses a noteworthy zoonotic risk factor associated with fish and fish products. Given the pathogen's capacity to cause severe illness, especially in vulnerable populations, understanding and mitigating the risks associated with *Listeria* contamination in seafood is of paramount concern for safeguarding public health [13]. This context highlights the critical need for studies like this one, focusing on the prevalence and characteristics of *Listeria* in fish, to better inform risk management strategies within the expanding global seafood industry.

The primary aim of this study was to evaluate the occurrence and distribution of *Listeria* spp., with a specific focus on *L. monocytogenes*, in raw fish sourced from diverse aquatic environments in northern Poland. Our investigation revealed a *Listeria* spp. prevalence of 13.9% across the raw fish samples, with *L. monocytogenes* being identified in 3.2% of these samples. This level of prevalence is consistent with findings from prior research; for instance, a study in Ireland by Walsh (2001) documented *Listeria* spp. contamination in 28.6% of raw fish, with *L. innocua* being the predominant species, followed by *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri* [20]. Furthermore, our prevalence rates are also in line with the broader spectrum reported in other studies, which indicate *Listeria* spp. contamination ranging from 10.5 to 77.5% and *L. monocytogenes* specifically from 3.0 to 22.5% [12, 21–23]. It's important to note that the variability in *Listeria* spp. prevalence reported across different studies is likely multifactorial. These variations can stem from differences in methodological approaches, including diverse sampling techniques, geographic and environmental variables, seasonal influences, and the specific hygiene and handling practices prevalent during fish processing and storage [24–26]. As an example, higher prevalence rates in certain studies might reflect less stringent sanitary conditions in the studied environments or differences in the sensitivity of detection methods employed. Indeed, a comprehensive systematic review and meta-analysis by Zakrzewski et al. (2024) highlighted this variability, reporting a *L. monocytogenes* prevalence of 5.8% in raw fish compared to a notably higher 14.5% in RTE products [22]. Interestingly, this meta-analysis also pointed out a geographical trend, with higher *L. monocytogenes* prevalence in raw fish in high-income countries (15.0%) versus lower to middle-income countries (3.0%) [22]. Such nuanced findings underscore the complex interplay of factors influencing *Listeria* spp. contamination in seafood and the importance of contextualizing prevalence data within specific regional and methodological frameworks.

Many food processing plants choose to test for *Listeria* spp. as predictors for the presence of *L. monocytogenes*. Despite the widespread practice of using *Listeria* spp. as an indicator of *L. monocytogenes* presence, it is still not clear whether *Listeria* spp. reliably serve this role. In study of Tompkin et al. (2002) authors indicated that *Listeria* spp. in the environment were a reliable predicator of *L. monocytogenes* in the food products in ready-to-eat meat processing plants [27]. However, results of Alali et al. (2013) research were in general disagreement with previous studies. Authors estimated, based on systematically examine published data, that *Listeria* spp. was not a reliable predictor for the presence of *L. monocytogenes* in raw and ready-to-eat foods from the seafood processing plants [28]. In our study *L. monocytogenes* was identified in 3.2% of positive samples, while *L. seeligeri* and *L. welshimeri* were the most frequently isolated species. The low prevalence of *L. monocytogenes* suggests that identification of *Listeria* spp. in raw fish may be not sufficient as predictor for *L. monocytogenes* presence. However, control of *L. monocytogenes* contamination in seafood should be part of any food safety program.

Given that certain food processing methods for raw fish, such as cold-smoking, can unfortunately facilitate the proliferation of *L. monocytogenes* in fish products, minimizing the presence of this pathogen in raw fish is of paramount importance. However, it's crucial to recognize, as evidenced by multiple studies, that the contamination route of *L. monocytogenes* within seafood processing facilities is complex. Indeed, research has indicated that contamination of the final product is not always directly attributable to the raw fish itself [13]. For example, the findings of Chen et al. (2016) demonstrated an absence of *L. monocytogenes* contamination in transportation tanks, the aquaculture environment, and even in raw fish samples *prior* to processing in a RTE tilapia sashimi facility [29]. Instead, contamination was notably present on final products, workers' hands, and various facility surfaces. This pattern strongly suggests that inadequate cleaning and sanitation practices within the processing environment, rather than contamination originating from the aquaculture or transportation phases,

are frequently the primary drivers of *Listeria* contamination in seafood processing settings [29].

Considering the ubiquitous nature of *L. monocytogenes*, its capacity to form protective biofilms, and its ability to proliferate under stress conditions, its persistence across diverse aquaculture environments is not unexpected. Our study revealed that fish from flowing freshwater environments exhibited the highest prevalence of *Listeria* spp. (19.2%), followed by standing freshwater (14.4%) environments, whereas saltwater fish despite lowest over all contamination (8.0%) showed the highest contamination with *L. monocytogenes* (70.0%). Notably, *L. monocytogenes* was absent in fish from standing freshwater environments. These findings align with prior research indicating a greater incidence of *Listeria* spp. in freshwater fish compared to seawater fish [23]. The increased contamination in flowing freshwater settings may be attributed to factors such as agricultural runoff, which introduces various contaminants into water systems, or elevated levels of organic matter that promote bacterial growth. Indeed, Miettinen and Wirtanen (2006) previously established a significant influence of seasons and weather patterns on the presence of *Listeria* spp. in fish farms [24], while Fagerlund et al. (2022) observed a positive correlation between *L. monocytogenes* presence and rainfall [30]. Conversely, it's been noted that *L. monocytogenes* prevalence in raw fish is typically low—ranging from 0.0–10.0%—with contamination more likely in fish from waters heavily impacted by land runoff [24, 29, 31]. However, understanding the specific location of *L. monocytogenes* on the fish is crucial for developing and selecting appropriate transportation, handling, and processing methods to prevent contamination of fish flesh [24]. Furthermore, minimizing raw fish contamination is essential to prevent subsequent contamination of processing equipment and surfaces [24].

Expanding on the species-specific distribution, our study's findings, in conjunction with pooled data analysis, indicate varying contamination levels across different fish families. Notably, the highest levels of contamination were observed in *Pleuronectidae* and *Salmonidae*, with prevalence rates of 21.4% and 28.5%, respectively [22]. Zakrzewski et al. (2024) elaborate that *Pleuronectidae* encompasses significant flatfish species such as halibut and sole, typically inhabiting cold and temperate waters. In contrast, *Salmonidae*, which include salmon and trout, are commonly found in cold-water environments, predominantly in the Northern Hemisphere [22]. Furthermore, species-specific analysis within Zakrzewski et al. (2024) study revealed a 50.0% prevalence of *L. monocytogenes* in *Mugil cephalus*, the flathead gray mullet. This species, often found in coastal and surface waters of lakes, is also known to be a common habitat for *Listeria* [22]. Consequently, the contamination of processing facilities with *L. monocytogenes* may be linked to the presence of imported raw fish, as Zakrzewski et al. (2024) suggest that increased time between catch and sale can elevate detection rates [22]. Additionally, studies have reported that *Oncorhynchus mykiss* and *Salmo salar*, commonly known as rainbow trout/steelhead and Atlantic salmon, respectively, show notable prevalence rates of 36.9% and 30.3% [22]. In a focused study on farmed rainbow trout, Miettinen and Wirtanen (2005) identified the gills as the primary site of *L. monocytogenes* contamination, with less frequent detection on the skin and in the viscera [24]. The gills' structure and function, as described by Miettinen and Wirtanen (2005), create an environment conducive to bacterial proliferation, acting as the initial point of contamination as large water volumes filter through them [24].

Despite the fact that listeriosis linked to raw fish consumption is less frequently documented compared to other foodborne sources, several factors contribute to an underestimation of its true incidence. These include the typically low levels of *L. monocytogenes* present in seafood, heightened consumer awareness regarding the necessity of refrigeration for seafood products, and challenges in epidemiological tracking due to the extended incubation period of listeriosis. Furthermore, individuals at higher risk of listeriosis may consciously avoid consuming raw seafood, potentially skewing reported case numbers [13]. Notwithstanding this potential underreporting, continuous surveillance of antimicrobial susceptibility in *L. monocytogenes* from seafood remains crucial for public health. This is particularly highlighted when contrasting our findings with research conducted in Poland by Maćkiw et al. (2020), which, in RTE foods, revealed a concerningly high rate of ampicillin resistance (83.0%) [32]. Given that ampicillin is a primary antimicrobial drug for treating listeriosis, this level of resistance is significant. As Maćkiw and colleagues observed high ampicillin resistance especially in pork products [32], their work, alongside ours, underscores the considerable variability in antimicrobial resistance patterns across different food matrices. Such variations emphasize the critical need for localized and product-specific monitoring of antimicrobial resistance to inform effective public health strategies. In further comparison, our study's findings regarding ampicillin and penicillin resistance in *L. monocytogenes* are also mirrored in research from the United States by Hailu et al. (2021), where a notable proportion of isolates exhibited resistance to these antimicrobials [33]. This observation, however, contrasts with other studies that report lower levels of ampicillin resistance in *Listeria* [1, 10, 20, 26, 32]. These discrepancies across different geographical studies and food types again highlight the importance of continuous, region-specific

surveillance to accurately assess and manage the evolving threat of antimicrobial-resistant *L. monocytogenes*.

The detection of antimicrobial-resistant *L. monocytogenes* isolates in this study is consistent with a broader global trend of increasing antimicrobial resistance among foodborne pathogens [1, 10, 34]. Of particular concern is the resistance observed to antimicrobials like tetracycline and cephalosporins. These drugs are critical therapeutic alternatives, especially for treating listeriosis in patients with beta-lactam allergies or for pregnant women [19]. In our study, we observed notable resistance rates of 91.7% to ciprofloxacin, 75.0% to erythromycin, and 75.0% to tetracycline among *L. monocytogenes* isolates. These rates are elevated compared to some prior publications [35], yet are consistent with findings reported by Bouymajane (2021) and Rezai (2018) [10, 26], indicating a geographically variable but consistently present challenge of resistance to these antimicrobials.

Resistance to oxacillin, meropenem, rifampicin, clindamycin, cefoxitin, cefotaxime and trimethoprim-sulfamethoxazole was universally observed in our *L. monocytogenes* isolates, with all 24 isolates exhibiting resistance. Our findings regarding oxacillin resistance are in accordance with Haubert et al. (2016), although it's important to note that the general resistance prevalence in their study was significantly lower, at approximately 10.0% [36]. Conversely, our results diverge from Chen et al. (2010), who reported complete susceptibility of *L. monocytogenes* isolates to rifampicin and trimethoprim–sulfamethoxazole [29]. The high oxacillin resistance observed in our *Listeria* spp. isolates is also mirrored in the findings of Gomez et al. (2014) in RTE meat products [37], suggesting a broader trend of oxacillin resistance across different *Listeria* spp. and food matrices.

Our findings reveled high AMR of *L. monocytogenes* isolates for tested drugs in raw fish samples. This characteristic entails a significant risk of listeriosis infections in human, particularly in raw fish stored refrigerated and non-subjected to heat treatment before consumption [38]. Despite this, when comparing data about AMR of clinical strains of *L. monocytogenes* in human in Poland, isolates showed full sensitivity to tested antimicrobials (involved penicillin G, ampicillin, amoxicillin-clavulanic acid, meropenem, gentamicin, ciprofloxacin, tetracycline, vancomycin, chloramphenicol, erythromycin, and sulfamethoxazole-trimethoprim) [39]. However, in the study of Żurawik et al. (2024) only 8 clinical strains were tested, so the results should be interpretated with caution. if taken under consideration, a different pattern of results could be identified [39].

The presence of MDR *Listeria spp.* strains in our study signifies a substantial public health concern. Specifically, 98.1% of our *Listeria* spp. isolates were classified as MDR, with the most prevalent resistance profile including oxacillin, cefotaxime, and clindamycin resistance. This level of MDR prevalence contrasts with the findings of Walsh et al. (2001) in Ireland, who, in a study of retail foods, observed multidrug resistance far less frequently. In their study, only 0.6% of *L. monocytogenes* isolates exhibited resistance to multiple antimicrobials, compared to 19.5% of *L. innocua* isolates [20]. Notably, Walsh et al. (2001) reported no resistance in either *L. seeligeri* or *L. welshimeri* [20]. The relatively high MDR rates in our *Listeria* isolates, particularly in *L. monocytogenes*, underscore the potential for limited therapeutic options during listeriosis outbreaks caused by such strains. Our findings resonate with those of Hailu et al. (2021), who also reported that all *Listeria* isolates in their study exhibited resistance to at least one antimicrobial drug, although the specific MDR patterns differed, involving ampicillin and penicillin resistance [33].

A genomic analysis by Hanes and Huang (2022) identified *fosX*, *lin*, *abc-f*, and *tet(M)* as the four most prevalent AMR genes in *L. monocytogenes*, followed by *tet(M)* and *vanC*, *vanR*, *vanS*, *vanT*, and *vanXY-C*, which occurred at a greater frequency [11]. Hanes and Huang (2022) underscored the critical importance of continued surveillance for AMR in *Listeria* spp. and *L. monocytogenes*, along with monitoring the prevalence of AMR genes [11]. In light of the current treatment guidelines for severe listeriosis, which recommend ampicillin, either alone or with gentamicin, our analysis gains particular relevance. Our findings demonstrate the presence of AMR genes, such as *ampC*, conferring ampicillin resistance in *L. monocytogenes* isolates. While *ampC* was detected in a subset of our *Listeria* spp. isolates, the current frequency of its occurrence warrants close monitoring to determine if it could serve as an early indicator of increasing ampicillin resistance and a broader rise in AMR prevalence within *Listeria* spp. This study, therefore, lays a crucial baseline for ongoing surveillance of AMR genes in *L. monocytogenes*.

Intrinsic resistance to antimicrobials is a trait universally found within the genome of a number of bacterial species, including *L. monocytogenes*, and is a serious therapeutic problem. This naturally occurring phenomenon is independent of antimicrobial selective pressure. According to data, *L. monocytogenes* isolated in human clinical cases was intrinsically resistant to broad spectrum cephalosporin antibiotics [40]. According to current knowledge, the best results of human listeriosis therapy rely on the use of bacteriostatic antibiotics from the group of penicillins, such as ampicillin or penicillin G. because of their bacteriostatic action on *L. monocytogenes* in conjunction with gentamicin to enhance the bactericidal effect of therapy [40]. However, intrinsic resistance of *L. monocytogenes* to cephalosporins may be significant in clinical settings as members of b-lactams

family, used frequently for sepsis therapy of unknown etiology [40]. Although, the expression of genes involved in the intrinsic resistance were not performed, in our study the high AMR of cephalosporins was noted. Analysis of the regulation of the expression of genes involved in the intrinsic resistance of *L. monocytogenes* highlights the need of high complexity of control of the intrinsic resistance phenotype.

Interestingly, a subset of our *L. monocytogenes* isolates exhibited phenotypic multidrug resistance yet low correlation between the specific antimicrobial resistance genes evaluated in this study and resistance established using the disk diffusion method. This observation suggests that alternative resistance mechanisms, beyond the genes screened, may be operative, or that other, as-yet-uncharacterized genes are involved in the acquisition of antimicrobial resistance in these isolates. Multidrug-resistant *L. monocytogenes* isolates, regardless of the underlying mechanism, can act as a reservoir of resistance genes. Considering the established presence of mobile genetic elements in *Listeria*, further research is warranted to investigate the potential for horizontal transferability of the antimicrobial resistance genes identified in this study, and other resistance determinants, to both commensal and pathogenic bacteria within the food production environment.

In a comparative context, studies conducted in Poland by Zakrzewski et al. (2024) and in Italy by Conter et al. (2009) have reported varying levels of antimicrobial resistance in *L. monocytogenes* isolates from fish [22, 41]. While our findings indicate a higher prevalence of certain resistant strains, the overall resistance patterns observed in our study are consistent with the broader global concern regarding antimicrobial resistance in foodborne pathogens.

It is important to acknowledge several limitations inherent in our study design. Firstly, our research was geographically constrained to Northern Poland, and it is plausible that antibiotic resistance patterns in *Listeria* spp. may exhibit significant regional variations across Poland and in other countries. Secondly, from a methodological standpoint, our reliance on disc diffusion for antimicrobial resistance testing, while a widely used and informative method, is less granular than state-of-the-art microdilution techniques. Similarly, the use of capsular gene PCR for serotyping, although effective for lineage classification, offers less discriminatory power compared to whole-genome sequencing-based serotyping methods. Compounding these limitations, the absence of Next-Generation Sequencing (NGS) in our study represents a further constraint. While our budget limitations precluded the application of NGS, this technology would have offered a considerably more detailed and comprehensive genetic characterization of the isolates, enhancing our understanding of virulence and resistance factors. Consequently, our findings, while providing valuable insights into *Listeria* spp. prevalence and AMR in raw fish in Northern Poland, should be interpreted cautiously, and over-extrapolation of the results should be avoided given these recognized limitations.

Conclusion

In conclusion, our study provides a detailed assessment of *Listeria* spp. prevalence and antimicrobial resistance in raw fish from Northern Poland, revealing a nuanced picture of both risks and areas of relative safety. While the overall prevalence of *L. monocytogenes* in raw fish was found to be relatively low, the alarmingly high levels of MDR observed among these isolates raise significant public health concerns, particularly regarding potential human listeriosis cases with limited treatment options. The pervasive resistance to clinically relevant antimicrobials, including tetracycline, cephalosporins, and fluoroquinolones, alongside the complete resistance to oxacillin, meropenem, rifampicin, and trimethoprim-sulfamethoxazole in *L. monocytogenes*, underscores the urgency of this issue. Furthermore, the identification of *blaTEM*, *ampC*, and *ereB* as prevalent AMR genes in these isolates provides a molecular basis for the observed phenotypic resistance, highlighting potential mechanisms of resistance dissemination. The elevated levels of AMR detected may also serve as an indicator of broader aquatic environmental pollution in Northern Poland, especially within the Baltic Sea region, with potential implications extending to other Baltic countries. These collective findings underscore the critical necessity for continuous, comprehensive monitoring of both *L. monocytogenes* prevalence and the evolving landscape of antimicrobial resistance in seafood.

Abbreviations

AMR	Antimicrobial Resistance
AMR	genes Antimicrobial Resistance Genes
CLSI	Clinical and Laboratory Standards Institute
EFSA	European Food Safety Authority
EUCAST	European Committee on Antimicrobial Susceptibility Testing
I	Intermediate
KDE	Kernel Density Enhanced plot
L.	innocua Listeria innocua
L.	ivanovii Listeria ivanovii
L.	monocytogenes Listeria monocytogenes
L.	seeligeri Listeria seeligeri
L.	welshimeri Listeria welshimeri
Listeria spp	Listeria species
MDR	Multidrug-Resistant
NGS	Next-Generation Sequencing
PCR	Polymerase Chain Reaction
R	Resistant
RTE	Ready-To-Eat
S	Susceptible

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04792-y.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

Supplementary Material 12

Supplementary Material 13

Supplementary Material 14

Supplementary Material 15

Supplementary Material 16

Supplementary Material 17

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Data availability
None of the data was deposited in an official repository. All the data obtained in the present research are presented in this manuscript. The data that support the study findings are available from the authors upon request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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