SUMMARY

Infectious Potential and Antimicrobial Susceptibility of *Listeria* spp. Strains Isolated from Fish

Contamination of food with bacteria of the genus *Listeria* poses a significant problem in terms of food safety and public health, particularly in the context of unheated food products. Among *Listeria* spp., *Listeria monocytogenes* is of particular importance, as it is the causative agent of listeriosis – a zoonotic disease characterized by high rates of hospitalization and mortality in high-risk groups. Fish and fish products may constitute a significant link in the transmission of *Listeria* spp. within the food chain, as the aquatic environment is conducive to their survival and spread. At the same time, there is growing concern regarding the phenomenon of antimicrobial resistance observed in bacteria, including *Listeria* spp., which may lead to reduced effectiveness of infection treatment and facilitate the spread of resistance genes amongst microorganisms.

Data on the prevalence of antimicrobial resistance, virulence potential and cytotoxic properties of *Listeria* spp. bacteria isolated from raw fish available on the Polish market remain limited. The aim of this study was to comprehensively characterize *Listeria* spp. isolates obtained from raw fish sourced from various aquatic environments in northern Poland, including an assessment of prevalence, antimicrobial resistance profiles along with the detection of resistance genes, virulence genes, serogroup affiliation, biofilm-forming ability and cytotoxic activity. The study aimed to assess the risks associated with the presence of *Listeria* bacteria in raw fish and to determine their susceptibility to antimicrobial agents, as well as characteristics that may influence their survival and ability to colonies food production environments.

The study material consisted of 750 samples of raw fish sourced from various aquatic environments in northern Poland, including freshwater bodies with both flowing and standing water, as well as the Baltic Sea. Isolation of *Listeria* spp. was carried out in accordance with the procedure described in standard PN-EN ISO 11290-1:2017-07: Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp. – Part 1: Detection method. The isolates obtained were subjected to species identification, followed by detailed phenotypic and molecular analysis. Antimicrobial susceptibility was assessed using the disc diffusion method with a panel of antimicrobial agents representing various therapeutic groups. Concurrently, an analysis of the presence of genes associated with antimicrobial resistance was carried out using polymerase chain reaction (PCR). To determine the pathogenic potential of the strains under investigation, an analysis was

carried out to detect the presence of virulence genes, including key determinants associated with invasion, intracellular survival and the spread of the bacteria within the host. For *L. monocytogenes* isolates, genotyping was also carried out to determine the serogroup affiliation of these strains, which is of epidemiological significance. The potential for colonizing the food production environment was assessed on the basis of biofilm-forming ability using qualitative and quantitative methods, together with the detection of the gene responsible for biofilm formation (*luxS*). The study was supplemented by an assessment of the cytotoxic potential of *Listeria* spp. isolates *in vitro* using eukaryotic cell lines. This multi-faceted research approach enabled a comprehensive characterization of the studied *Listeria* spp. population.

As a result of the analyses carried out, a total of 104 *Listeria* spp. isolates were identified, representing a prevalence of 13.9%. Among the strains examined, *L. seeligeri* (34.6%) and *L. welshimeri* (28.8%) were the most common, whilst *L. monocytogenes* and *L. innocua* accounted for 23.1% and 13.5% of all isolates, respectively. Phenotypic assessment of antimicrobial susceptibility revealed widespread resistance to oxacillin, observed in all isolates tested. Very high levels of resistance were also found against cefotaxime, clindamycin, cefoxitin, rifampicin and trimethoprim with sulfamethoxazole. A particularly worrying finding was the presence of multidrug resistance in almost all isolates tested, with all *L. monocytogenes* isolates meeting the criteria for multidrug-resistant strains. Isolates of this species were characterized by complete resistance to meropenem, cefoxitin, cefotaxime, rifampicin and trimethoprim-sulfamethoxazole, and also exhibited high levels of resistance to ciprofloxacin, clindamycin, tetracycline, erythromycin, benzylpenicillin and nitrofurantoin. Molecular analysis confirmed the presence of genes associated with antimicrobial resistance, the most frequently identified of which were *blaTEM*, as well as *ampC* and *ereB*. The results obtained indicate a widespread prevalence of resistance traits amongst *Listeria* bacteria associated with aquatic environments and fish products. At the same time, the high prevalence of multidrug-resistant strains suggests the possibility of ongoing selective pressure in the aquatic environment and the potential role of these ecosystems in the spread of resistance determinants. The study also provided data on the species diversity of *Listeria* populations present in raw fish, indicating that, alongside the pathogenic species, species traditionally considered non-pathogenic or opportunistic also play a significant role.

An analysis of virulence potential revealed marked differences between the species studied. *L. monocytogenes* isolates were characterized by the presence of numerous virulence determinants associated with the pathogenesis of infections. Most strains possessed the

complete Listeria Pathogenicity Island 1 (LIPI-1), and all isolates were classified into serogroups 1/2a, 1/2c, 3a and 3c. Among *L. monocytogenes* strains, the *plcA*, *hlyA*, *mpl*, *inlA*, *inlC* and *inlJ* genes were commonly present, whilst the *prfA* and *actA* genes were identified in the vast majority of isolates. The *iap* gene was also present with high frequency. The other *Listeria* species exhibited the presence of only selected virulence genes, mainly *plcA* and *mpl*, and did not possess the full set of determinants characteristic of *L. monocytogenes*. The virulence potential of *L. monocytogenes* isolates was further confirmed at the phenotypic level, as cytotoxic activity against four eukaryotic cell lines was observed exclusively in this species. Strains *of L. innocua*, *L. seeligeri* and *L. welshimeri* did not exhibit cytotoxic activity despite the presence of individual virulence-associated genes.

The ability to form biofilm was a characteristic of most of the isolates studied. In quantitative studies, more than two-thirds of the isolates demonstrated the ability to form biofilm, indicating that this property is widespread amongst *Listeria* bacteria present in raw fish. Analysis of *the luxS* gene revealed its presence in only some of the isolates; however, no significant correlation was found between the presence of this gene and the ability to form biofilm.

The results obtained indicate that raw fish may be a source of *L. monocytogenes* strains that possess, simultaneously, numerous virulence determinants, a high level of antimicrobial resistance, and the ability to persist in the environment through biofilm formation. At the same time, the presence of other *Listeria* species possessing selected genes associated with virulence and environmental adaptability highlights the need to analyze the entire genus *Listeria* in food safety research. The results of our own research have provided a comprehensive characterization of *Listeria* spp. populations isolated from raw fish in northern Poland, including an assessment of their prevalence, antimicrobial resistance, virulence potential, cytotoxic activity and ability to form biofilms.