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ZOONOTIC AND CONDITIONALLY PATHOGENIC MICROORGANISMS IN FISH: THE GAP BETWEEN REGULATORY CONTROL AND ACTUAL CONTAMINATION LEVELS

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Ukraine's integration into the European Union requires the adaptation of national documents to EU standards and regulations. One of the key priorities is ensuring the safety and quality of food products, which directly affects public health. Fish and fish products possess high biological value due to their optimal amino acid composition. Despite their nutritional benefits, fish can pose risks due to contamination with pathogenic microorganisms. In Ukraine, the monitoring system for fish products does not fully address risks related to farming, processing, and environmental contamination. Antibiotic resistance among microorganisms in fish products has become a global concern. To ensure the safety of fish products, it is essential to identify the actual species composition of microorganisms contaminating fish and fish products.

The aim of our in-depth microbiological research was to isolate and identify bacterial pathogens, determine their actual species diversity in fish and fish product samples, and conduct a quantitative comparative analysis of our findings against results from monitoring and routine testing to assess real risks of contamination by conditionally pathogenic and zoonotic microorganisms.

The research was conducted at the bacteriological department of the State Scientific Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Expertise (SSRILDVSE) in Kyiv and the Department of Microbiology and Virology at Bila Tserkva National Agrarian University (BNAU). Monitoring studies of fish and fish product samples for non-compliance were carried out in accordance with the orders of the State Service of Ukraine on Food Safety and Consumer Protection No. 641 and No. 889, approving the national monitoring plans for 2023 and 2024 respectively.

These monitoring tests were limited by a pre-established plan regarding the number of samples and the list of fish processing enterprises participating in the program. According to current regulatory documents, microbiological non-compliance in fish and fish products is assessed only by detecting *Salmonella* and *Listeria monocytogenes*.

Routine testing methods are based on the internal documentation of fish processing enterprises, including approved technical specifications and

methodological guidelines. Routine tests are conducted upon request by producers and typically target indicators such as total viable counts, coliforms, *Staphylococcus aureus*, *Salmonella*, *Listeria monocytogenes*, and sulfite-reducing clostridia, though the scope may be further limited by the producers.

Thus, both monitoring and routine testing do not provide a complete picture of the species composition of microorganisms present in fish and fish product samples, including pathogenic ones. Our in-depth studies involved direct inoculation from enrichment media (selected based on the target microorganisms), followed by cultivation at 37±1.0°C for 24 hours and subsequent plating on selective media for detecting *Escherichia coli*, *Staphylococcus spp*., *Listeria spp*., *Enterococcus spp*., *Bacillus spp*., *Proteus spp*., and sulfite-reducing bacteria.

All tests were performed in accordance with national and international standards, following methodologies regulated by documents such as DSTU 8534:2015, DSTU ISO 7251:2006, ISO 6579-1:2017, ISO 11290-1:2017, and others. The results of our in-depth studies of fish and fish product samples revealed a concerning picture, as zoonotic pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* were isolated from significantly more samples compared to monitoring and routine testing.

Moreover, our research identified new microbial species that had not been detected through routine testing methods. Analysis of the findings showed that a substantial portion of bacterial contaminants remained undetected in monitoring and routine examinations due to limitations in current regulatory protocols. As a result, bacterial contaminants identified through our advanced research – including *Escherichia coli*, *Enterococcus faecalis*, and *Proteus vulgaris* – persisted in fish raw materials and finished products.

Quantitative indicators of microbial isolates from our studies exceeded those of routine testing by a factor of 5.7, highlighting a serious gap in detection. This situation poses real risks of infection for animals, poultry, and humans from conditionally pathogenic and zoonotic microorganisms. An even greater concern is the lack of monitoring for antibiotic-resistant strains among the microorganisms isolated from fish and fish products. Given that fish and fish products are integral to the food chain under the One Health concept, the issue is further exacerbated by risks to safety and quality. According to results from the State Monitoring Program, only 1 isolate of *Listeria monocytogenes* (0.3%) was found among 215 samples.

In contrast, routine testing of 337 samples yielded 22 isolates (6.5%), while our in-depth research identified 125 isolates (37.1%) from the same number of samples, indicating a significantly higher level of contamination.