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Molecular Characterization of Listeria monocytogenes from Fresh Fish and Fish Products

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Abstract : Listeria monocytogenes is an important human and animal pathogen that causes foodborne outbreaks. The bacteria may be present in different types of food: cheese, raw vegetables, sliced meat products and vacuum-packed sausages, poultry, meat, fish. The most common method, which has been used for the investigation of genetic diversity of L. monocytogenes, is PFGE. This technique is reliable and reproducible and established as gold standard for typing of L. monocytogenes. The aim of the study was characterization by molecular serotyping and PFGE analysis of L. monocytogenes strains isolated from fresh fish and fish products in Poland. A total of 301 samples, including fresh fish (n = 129) and fish products (n = 172) were, collected between January 2014 and March 2016. The bacteria were detected using the ISO 11290-1 standard method. Molecular serotyping was performed with PCR. The isolates were tested with the PFGE method according to the protocol developed by the European Union Reference Laboratory for L. monocytogenes with some modifications. Based on the PFGE profiles, two dendrograms were generated for strains digested separately with two restriction enzymes: AscI and ApaI. Analysis of the fingerprint profiles was performed using Bionumerics software version 6.6 (Applied Maths, Belgium). The 95% of similarity was applied to differentiate the PFGE pulsotypes. The study revealed that 57 of 301 (18.9%) samples were positive for L. monocytogenes. The bacteria were identified in 29 (50.9%) ready-to-eat fish products and in 28 (49.1%) fresh fish. It was found that 40 (70.2%) strains were of serotype 1/2a, 14 (24.6%) 1/2b, two (4.3%) 4b and one (1.8%) 1/2c. Serotypes 1/2a, 1/2b, and 4b were presented with the same frequency in both categories of food, whereas serotype 1/2c was detected only in fresh fish. The PFGE analysis with AscI demonstrated 43 different pulsotypes; among them 33 (76.7%) were represented by only one strain. The remaining 10 profiles contained more than one isolate. Among them 8 pulsotypes comprised of two L. monocytogenes isolates, one profile of three isolates and one restriction type of 5 strains. In case of ApaI typing, the PFGE analysis showed 27 different pulsotypes including 17 (63.0%) types represented by only one strain. Ten (37.0%) clusters contained more than one strain among which four profiles covered two strains; three had three isolates, one with five strains, one with eight strains and one with ten isolates. It was observed that the isolates assigned to the same PFGE type were usually of the same serotype (1/2a or 1/2b). The majority of the clusters had strains of both sources (fresh fish and fish products) isolated at different time. Most of the strains grouped in one cluster of the AscI restriction was assigned to the same groups in ApaI investigation. In conclusion, PFGE used in the study showed a high genetic diversity among L. monocytogenes. The strains were grouped into varied clonal clusters, which may suggest different sources of contamination. The results demonstrated that 1/2a serotype was the most common among isolates from fresh fish and fish products in Poland.

Keywords : Listeria monocytogenes, molecular characteristic, PFGE, serotyping
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