Isolation of Mycobacteria from Frozen Fish Destined for Human Consumption

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Nontuberculous mycobacteria (NTM) are transmitted to humans from the environment, including through ingestion of food. They have been isolated from beef, pork, lamb (19), milk and other dairy products (8, 16, 18, 21, 22, 23), water (1, 7, 10, 20), vegetables (broccoli, spinach, and lettuce) (24), fruit (cherries, pomegranates, and apples) (24), preserves and brine (19), herbs (basil and parsley) (24), oysters (18), and fish such as Pacific salmon (2) and Channa striatus (4). The objectives of all of these studies were to show the presence of mycobacteria in food samples and analyze these possible sources of human infection or colonization.

Mycobacteria can survive under environmental conditions that are intolerable for most other bacterial genera, including temperatures below 0°C. Strains are known that have remained viable in nutrient broth at temperatures below −70°C for years (11, 13). This may be due to the specific properties of their cell walls, such as high lipid content and therefore hydrophobicity, which renders them resistant to changes in environmental conditions (14).

Due to the association between mycobacteria and a variety of different aqueous environments (3, 5, 9, 15), it seems reasonable to believe that these organisms may occur in frozen foods, including fish, which are widely consumed by humans. When fish is frozen in order to preserve quality, microorganisms are inevitably included.

Our objective was to find out whether frozen fish contains NTM from which humans could be colonized.

Samples of frozen fish were obtained under sterile conditions inside a refrigeration chamber (−18 to −22°C) in a wholesale market from which these products are distributed to shops for retail sale and human consumption.

All of the fish had been gutted and frozen, with the head and caudal fin removed, and cut longitudinally into boneless fillets in the case of S. solea, boneless steaks in the case of M. morhua, and transverse slices in the central region of the body including the central bone in the case of M. merluccius, L. piscatorius, and G. blacodes. All of the samples included the skin except those of S. solea. The weights of the pieces varied from 50 to 200 g. For transportation from the wholesale market to the laboratory, isothermal bags were used.

In the laboratory, the samples were defrosted at 4°C for 24 h, after which time the water resulting from defrosting (DW) was separated from the solid food (SS) to be processed independently. The volume of DW obtained was 5 ml in the case of S. solea and 15 ml for the other species. A 25-g piece of the SS of fish was used to prepare a 1/10 dilution in tryptone medium, and this was homogenized in a Masticator (IUL) for 90 s. A volume of 25 ml was then filtered through sterile gauze. The volumes of the DW and SS of each sample were centrifuged at 2,400 × g for 20 min, and the sediments obtained were decontaminated using the sodium lauryl sulfate method (16). They were then inoculated onto Löwenstein-Jensen medium (Biomérieux, Lyon, France; reference no. 41699) for incubation at temperatures of 4, 25, 37, and 45°C for 2 months, and readings were taken weekly. The presence of mycobacteria in the positive cultures was confirmed by the Ziehl-Neelsen staining method.

The isolated mycobacteria were identified by biochemical tests and growth at different temperatures by the methods of Kent and Kubica (12) and also by hybridization of nucleic acids with probes (ACCURO PROBE System; Gen-Probe Inc., San Diego, Calif.) specific for Mycobacterium avium complex and M. gordonae. When these tests proved inconclusive, we used the PCR restriction fragment length polymorphism analysis (PRA) technique of Telenti et al. (17) with the restriction enzymes BsrEI and HaeIII. The band pattern of the PRA was interpreted with the LANE MANAGER computerized system (TDI). The results were analyzed using the algorithm of Devallois et al. (6) for the differentiation of mycobacterial species.

NTM were isolated from 29 of the 100 independently analyzed samples of DW and SS. By species of fish, considering 20 samples (DW and SS) of each species, the isolates were as follows: 1 from S. solea (5%), 1 from M. merluccius (5%), 8 from G. morhua (40%), 9 from G. blacodes (45%), and 10 from L. piscatorius (50%). The DW was positive in 38% (19 of 50) of the cases, and the SS was positive in 20% (10 of 50) of the cases. Two mixed cultures developed (both of them in samples of DW), making a total of 31 mycobacterial isolates.

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the following should be taken into consideration: (i) that although fish is usually cooked prior to consumption in our Western culture, this is not the case worldwide; (ii) that other frozen produce is often consumed raw; and (iii) that the commercial routes of food can result in variation in the species that are habitually isolated from patients, depending on the geographical area of origin.

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REFERENCES