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Prevalence of *Listeria* species in camel sausages from retail markets in Aydin province in Turkey and RAPD analysis of *Listeria monocytogenes* isolates

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Samples were taken from 100 camel sausages from the different retail markets in Aydin province in the south-west of Turkey and they were tested for the presence of *Listeria* spp by biochemical methods. Samples were enriched using *Listeria* Enrichment Broth and they were inoculated onto *Listeria* Selective Agar. *Listeria monocytogenes* was isolated from nine samples (9%), *Listeria innocua* from 14 samples (14%) and *Listeria welshimeri* from two samples (2%). A 701bp fragment of listeriolysin O sequence for *L. monocytogenes* was amplified using specific primers by polymerase chain reaction (PCR) for confirmation of the identification. A random primer (OPA-11) was used in a random amplified polymorphic DNA (RAPD) assay. This detected five different band profiles amongst the *L. monocytogenes* isolates, indicating a relatively large amount of genetic heterogeneity amongst the nine isolates. The study has highlighted the need for improved strategies for food safety, in particular appropriate hygienic precautions to avoid contamination of sausage during the manufacturing process and appropriate preservation techniques during storage and transport, to prevent transmission of *Listeria* spp to consumers at home and abroad.

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Introduction

A wide variety of meats and meat products, including fermented sausages, can be contaminated with *Listeria* spp (Johnson *et al.*, 1990). *L. monocytogenes* is known to survive the commercial dry sausage manufacturing process (Incze, 1998). In Turkey, Ciftcioglu (1992) found *Listeria* spp in 11% of sausages, with *L. monocytogenes* in 2% and *L. innocua* in 8%. A subsequent study by Guven and Patir (1998) found *Listeria* spp in 16.3% of sausages, with *L. monocytogenes* in 11.9% and *L. innocua* in 11.3%.

Several molecular genotyping methods have been used to type *L. monocytogenes*, such as DNA restriction endonuclease analysis (Wesley and Ashton, 1991), multilocus enzyme electrophoresis (Bibb *et al.*, 1990), ribotyping (Baloga and Harlander, 1991) and pulsed-field gel electrophoresis (PFGE) (Autio *et al.*, 1999). However, these methods are not well suited for routine use in laboratories and are time-consuming. PFGE is very discriminative, but it is labour-intensive

and requires expensive apparatus (Franciosa *et al.*, 1998). RAPD typing is suitable for differentiation of the most commonly found serotypes and for screening large panels of strains (Byun *et al.*, 2001).

The aims of this study were to determine the prevalence of *Listeria* spp in camel sausages at different retail markets in Aydin province in the south-west of Turkey, to analyse genetic variability among *L. monocytogenes* isolates by RAPD using a random primer and to enquire whether there is a public health risk of acquiring listeriosis from consumption of camel sausage.

Materials and methods

Material

Samples were taken from 100 camel sausages obtained from the different retail markets in Aydin province, in the south-west of Turkey.

Detection of *Listeria* spp

For microbiological analysis, the sausage casing was removed aseptically. A 25g sample from each sausage was added to 225ml of *Listeria* Enrichment Broth (LEB, Oxoid) and homogenised in a stomacher (Interscience, 78860 St Nom-France) at high speed, for one minute at room temperature and incubated at 37°C for 24 hours (primary enrichment). Then, an aliquot of 0.1ml of the culture was transferred into tubes containing 10ml LEB. The tubes were incubated for 24 hours to 48 hours at 37°C (secondary enrichment). A loopful of each enrichment culture was streaked onto *Listeria* Selective Agar (LSA, Oxoid) and was incubated for 24 hours, at 37°C. The suspected

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colonies with a dark brown colour or black halo were transferred onto tryptic soy agar (TSA, Difco) and incubated for 24 hours, at 37°C. The isolates were identified using conventional methods: Gram staining; the Christie, Atkins, Munch-Petersen (CAMP) test; typical umbrella motility; and fermentation of mannitol, rhamnose and xylose (Seeliger *et al.*, 1986).

Extraction of DNA

A few colonies from cultures were transferred into an Eppendorf tube containing 300µl of distilled water and the tubes were vortexed. Lysis was accomplished by the addition of 300µl of TNES buffer (20mM Tris-HCl pH 8.0, 150mM NaCl, 10mM EDTA, 0.2% SDS) and 200µg/ml Proteinase K. The lysis mixture was incubated at 37°C for two hours and boiled for 30 minutes. Bacterial DNA was extracted by the phenol:chloroform:isoamylalcohol procedure. All purifications and PCR reactions used *L. monocytogenes* serovar 1/2a: CIP 104794/ATCC-35152 (obtained from Pasteur Institute) as the positive control and distilled water as the negative control.

Primers

Primers used in this study were designed by Border *et al.* (1990). The sequences of primer pairs were as follows: LM1 (5'- CCT AAG ACG CCA ATC GAA - 3') and LM2 (5'- AAG CGC TTG CAA CTG CTC - 3'). These primers amplify a 701 bp fragment on listeriolysin O sequence of *L. monocytogenes*.

PCR

PCR reactions were performed in a reaction mixture (50µl final volume) containing 5µl of 10 x PCR buffer (10mM Tris-HCl, pH 9.0, 50mM KCl, 0.1% Triton X-100), 5µl of 25mM MgCl₂, 250µM of each deoxynucleoside triphosphate, 2U of Taq DNA polymerase (Fermentas, Lithuania), 50pmol of each primer and 5µl of template sample DNA. Amplification was obtained with an initial denaturation step at 94°C for five minutes, followed by 45 cycles at 94°C for 30 seconds, 52°C for one minute and 72°C for one and a half minutes. The final cycle was at 72°C for seven minutes. For all experiments, a Touchdown Thermocycler (Hybaid, Middlesex, England) was used. Ten µl of amplified products were detected by electrophoresis in 1.5% agarose, in 1 X Tris-borate-EDTA buffer. Gels were stained with ethidium bromide (0.5µg/ml). The DNA fragments were visualised by UV illumination and photographed with Polaroid film. The molecular sizes of the PCR products were compared with a 100bp DNA ladder.

RAPD analysis

The RAPD reaction mixture was prepared in a total volume of 25µl consisting of 2.5µl of 10xPCR buffer (750mM Tris-HCl, 200mM [NH₄]₂SO₄, 0.1% Tween 20, pH 8.8), 3.5mM MgCl₂, 200µM of each deoxynucleoside triphosphates, 1.25U of Taq DNA polymerase (Fermentas, Lithuania), 1µM of OPA-11 primer (5'-CA AT CG CC GT-3'), 11µl of dsH₂O (sterile distilled water) and 2.5µl of template DNA. Each sample was amplified through 50 cycles of denaturation (one minute at 94°C), primer annealing (one minute at 37°C) and extension (one minute at 72°C). A last cycle of extension was applied at 72°C for 10 minutes. Twenty microlitres of product was resolved by electrophoresis on a 2% agarose gel in Tris-borate EDTA buffer and visualised by staining with 0.5µg/ml ethidium bromide for 30 minutes. The agarose gels were photographed under UV light. A 1kb DNA ladder (Promega, Maddison, USA) was used as a molecular size standard. RAPD assays were performed at least three times each to check reproducibility.

Results

Culture and PCR

Of the 100 camel sausage samples, nine (9%) contained *L. monocytogenes*, 14 (14%) contained *L. innocua* and 2 (2%) contained *L. welshimeri*. A 701bp fragment was amplified from all the tested *L. monocytogenes* strains identified by conventional procedures (Figure 1), but not from the negative control.

RAPD

RAPD typing using OPA-11 primer detected five different band profiles (a, b, c, d, e) on nine isolates of *L. monocytogenes* (Figure 2).

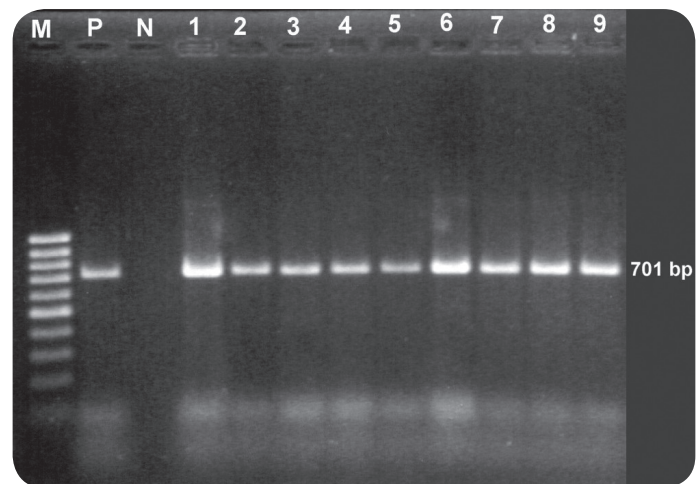


Figure 1: An agarose gel stained with ethidium bromide, with PCR products of *L. monocytogenes* isolates from sausages (M: 100 bp DNA ladder; P: positive control; N: negative control; lanes 1-9: *L. monocytogenes* isolates).

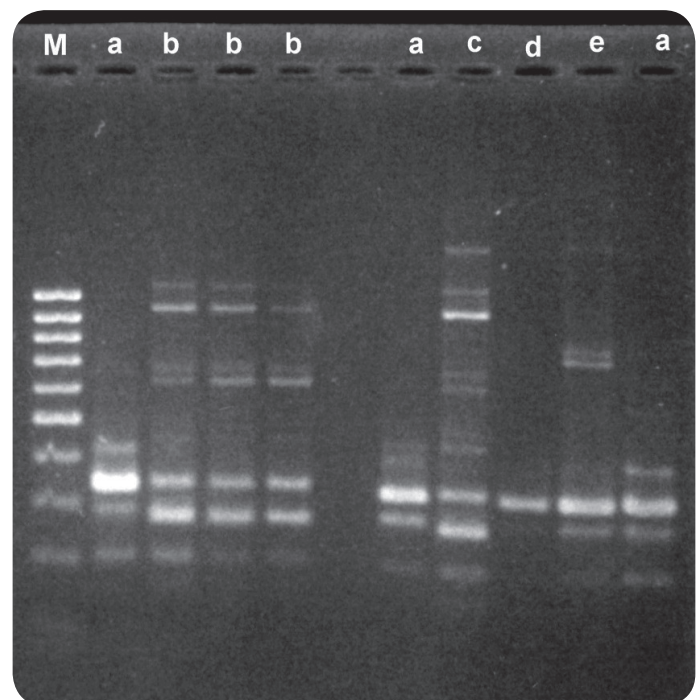


Figure 2: RAPD analysis of *L. monocytogenes* isolates from sausages (M: 100 bp DNA ladder; lanes a, b, c, d, e: profiles).

Discussion

Studies carried out in different parts of Turkey have found *Listeria* spp in 11% (Ciftcioglu, 1992) to 16.3% (Güven and Patir, 1998) of sausages. In some other countries prevalence has been reported to be considerably higher (Breuer and Schopfer, 1989; Farber et al., 1989). In the present study, *Listeria* spp were obtained from 25% of the sampled sausages. In this and in other studies, three *Listeria* species have been identified: *L. innocua*, *L. monocytogenes* and *L. welshimeri* (Breuer and Prandl, 1988; Schmidt et al., 1988; Güven and Patir, 1998; Paziak-Domanska et al., 1999; Uyttendaele et al., 1999). In the present study, all the isolates of *L. monocytogenes* were successfully typed by the RAPD method using only OPA-11 primer. One random primer was used and five different profiles were observed, thus indicating a large amount of genetic heterogeneity amongst the nine isolates of *L. monocytogenes*.

Overall, *L. innocua* has been isolated from sausage more frequently than have the other two species. For instance, Paziak-Domanska et al. (1999) recovered *Listeria* from 15 samples of sausage, *L. innocua* from 10 samples, *L. monocytogenes* from three samples and *L. welshimeri* from two samples. A similar trend was evident in the present study: *L. innocua* from 14 samples, *L. monocytogenes* from nine samples and *L. welshimeri* from two samples.

In summary, this study revealed the presence of *Listeria* spp in a significant proportion (25%) of samples of camel sausage on sale in different retail markets in Aydin province in the south-west of Turkey. It has indicated that a large amount of genetic heterogeneity exists among *L. monocytogenes* isolates from camel sausage. Thus, it has highlighted the need for improved strategies for food safety, in particular appropriate hygienic precautions to avoid contamination during the manufacturing process and appropriate preservation techniques during storage and transport to prevent transmission of *Listeria* spp to consumers at home and abroad.

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