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Short Communication

Prevalence, genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece

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ABSTRACT

A total of 210 food samples originating from milk products, ready-to-eat salads, raw meat and raw meat products purchased in ten open-air market places in Thessaloniki, Greece, were analyzed for the presence of *Listeria monocytogenes*. Thirty (14.3%) contained *L. monocytogenes* with the highest prevalence in raw meat (27.5%), raw meat products (18%) and cheese (8%). The strains were susceptible to 16 antimicrobials as determined by microbroth dilution, except one strain which displayed resistance to tetracycline (MIC > 32 μg/ml). This strain carried the tetracycline resistance gene *tet*(M). Pulsed-field gel electrophoresis (PFGE) revealed a low genetic diversity among the isolates, irrespective of their origin. This suggests that dominant *L. monocytogenes* clones are widespread in different food product types in open-air food markets in Greece. The high prevalence of *L. monocytogenes* in these products indicates that appropriate hygienic measures and periodic bacteriological controls are also necessary in open-air food markets to reduce contamination with food-borne pathogens. Greek specialties made with raw meat and raw milk may contain *L. monocytogenes* and should not be consumed by persons at risk.

1. Introduction

Listeria monocytogenes is the etiological agent of human and animal listeriosis. The public health importance of listeriosis is not always taken into account as the disease is relatively rare compared to other more commonly occurring food-borne diseases such as salmonellosis. However, listeriosis is fatal in about 30% of the human cases (De Valk et al., 2005).

Food is considered to be the main vector of the bacterium in epidemic and sporadic cases of systemic human listeriosis (Farber & Peterkin, 1991; Pinner et al., 1992). Meat, milk and vegetables represent important sources of contamination and *L. monocytogenes* can survive and multiply in raw products or ready-to-eat food made with such raw items, even if refrigerated. In Greece, the prevalence of *L. monocytogenes* in ready-to-eat meat products can reach up to 12% (Abrahim, Papa, Soultos, Ambrosiadis, & Antoniadis, 1998; Angelidis & Koutsoumanis, 2006), but the frequency of reported food-borne listeriosis in humans remains low (De Valk et al., 2005).

The effective treatment of listeriosis is usually based on the administration of ampicillin or penicillin plus gentamicin or the combination sulfonamides–trimethoprim (Safdar & Armstrong, 2003). The emergence of antibiotic resistant strains and their transfer to humans through the food chain may have public health consequences (Perreten, 2005). Thus, the antimicrobial susceptibility profile was determined for *L. monocytogenes* isolated from different food products sold in open-air markets in Northern Greece. The genetic relatedness of the *L. monocytogenes* isolates was also investigated by PFGE to determine whether specific clones are spread among Greek food specialties. The determination of the genetic profile of *L. monocytogenes* isolates present in food sold in open markets may also help to trace back future listeriosis outbreaks.

2. Materials and methods

2.1. Sampling and cultivation

In total, 210 food samples were collected from ten open-air market places from October 2005 to July 2006 in Thessaloniki, Greece. The analyzed products consisted of milk products, ready-to-eat salads, fish, raw meat (beef and poultry) and raw meat delicatessen which include products such as salami, bacon and other processed meat products (Table 1). All samples were aseptically collected early in the morning and transported in isothermal boxes within 3 h to the laboratory. *L. monocytogenes* was isolated according to EN ISO 11290-1. Briefly, food samples (25 g or 25 ml) were pre-enriched in 1:9 (w/w) half Frazer broth (Merck KGaA, Darmstadt, Germany) at 30 °C for 24 h. After enrichment (0.1 ml in 10 ml) in full Fraser broth at 37 °C for 24–48 h (Merck KGaA,

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Table 1
Prevalence of *Listeria monocytogenes* in different food products from Greek open-air markets

Food products	*n*	Frequency	PFGE profiles
Milk products	50	4 (8%)	
Pasteurised milk	10	0	
Yoghurt	10	0	
Hard cheese	10	0	
Feta cheese	10	0	
Soft cheese	10	4	III, IV, VI
Raw meat	40	11 (27.5%)	
Beef	20	4 (20%)	I, III, IV
Poultry	20	7 (35%)	I, III, VI, VIII
Meat products	50	9 (18%)	II, V, VI, VII
Fish	20	6 (30%)	II, III, VI, VIII
Salads	50	0	
Total	210	30 (14.3%)	I–VIII

Darmstadt, Germany), *Listeria* isolation was attempted on Oxford agar (BioMérieux, Marcy-l'Etoile, France) and Ottaviani Agosti agar (BioMérieux, Marcy-l'Etoile, France). Suspected colonies were purified and further identified as *L. monocytogenes* by CAMP-test and API *Listeria* system (BioMérieux, Marcy-l'Etoile, France). Moreover, the *lmo0733* gene which is specific to *L. monocytogenes* was amplified by PCR as described previously (Liu, Ainsworth, Austin, & Lawrence, 2004).

2.2. Antimicrobial susceptibility testing and PCR

MICs were determined by broth microdilution using custom Sensititre susceptibility plates (Trek Diagnostics Systems, East Grinstead, England, and MCS Diagnostics BV, Swalmen, The Netherlands) according to CLSI guidelines (Clinical, 2006b). The tetracycline resistance gene was detected by PCR using primers tet1 and tet2 which allow the amplification of either *tet*(M), *tet*(O) and *tet*(S) genes as previously described (Barbosa, Scott, & Flint, 1999). PCR were performed using *taq* polymerase (FIREPole® BioDyne, Tartu, Estonia) and an annealing temperature of 50 °C. PCR products were sequenced on ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA) using dRodamine-labeled terminators. Nucleotide sequences were aligned and compared to GenBank sequences using BLAST of the National Center for Biotechnology Information, Bethesda, MD (www.ncbi.nlm.nih.gov).

2.3. PFGE analysis

Pulsed-field gel electrophoresis (PFGE) was performed following the PulseNet standardized protocol for subtyping of *L. monocytogenes* (Graves & Swaminathan, 2001). DNA was digested with *Sma*I and separated at 12 °C in 2.0% AgaroseNA agar (Amersham Biosciences, Uppsala, Sweden) for 16 h at 6 V/cm with pulse time ramping from 4 to 40 s in a CHEF-DR II unit (Bio-Rad Laboratories, Richmond, CA). Lambda ladder with a size range of 0.13–194 kb (Low Range PFG Marker, New England Biolabs Inc.) was used as molecular weight standard. The DNA bands were visualized on a UV transilluminator and Polaroid photographs of the gels were scanned and images in tiff file format were imported into GelCompar II (Applied Maths, Kortrijk, Belgium). Degrees of similarity between isolates were calculated with 2.0% tolerance and 1.0% optimization by applying the band-based Dice similarity coefficient. Clustering analysis was performed with the unweighted pair group method (UPGMA), by average linkages.

3. Results and discussion

Out of 210 food samples, 30 (14.3%) were found to contain *L. monocytogenes*. Raw meat showed the highest prevalence of contamination (27.5%) with *L. monocytogenes* followed by meat products (18%) and cheese (8%). No *L. monocytogenes* was detected in ready-to-eat salads (Table 1). A high rate of *L. monocytogenes* in raw meat was also reported in France, Italy and Spain (Chasseignaux et al., 2001; Peccio, Autio, Korkeala, Rosmini, & Trevisani, 2003; Vitas, Sánchez, Aguado, & García-Jalón, 2007). Meat carcasses are often contaminated during slaughtering (Samelis & Metaxopoulos, 1999). Cooking of the raw meat before consumption significantly reduces the risk of food-borne listeriosis. On the other hand, meat products made with raw material represent a higher risk since they are directly consumed without preliminary heat treatment. The number of such raw meat products containing *L. monocytogenes* was alarmingly high (18%) in open-air market in Thessaloniki. A previous study already showed a high prevalence of *L. monocytogenes* in bacon (25%) from retail market in Thessaloniki (Angelidis & Koutsoumanis, 2006). *L. monocytogenes* was also isolated from soft cheese. It is known that raw milk products as well as raw meat products represent an important reservoir for *L. monocytogenes* (Loncarevic, Danielsson-Tham, & Tham, 1995) and these products have been incriminated in several outbreaks of listeriosis (De Valk et al., 2005).

The minimal inhibitory concentration (MIC) of antibiotics of the major classes of antibiotics used in veterinary and human medicine was determined for the *L. monocytogenes* isolates. CLSI resistance breakpoints are only available for penicillin and the combination sulfomethoxazole–trimethoprim (Clinical, 2006a) which are the drug of choice for the treatment of human listeriosis. No strains displayed resistance to these drugs. For the other antibiotics tested, no official breakpoints are available. The MICs of these antibiotics were low and monomodally distributed (Table 2), suggesting no acquired resistance to antibiotics. One strain, *L. monocytogenes* GL14 isolated from beef, displayed resistance to tetracycline with a MIC > 32 μg/ml. This strain carried the tetracycline resistance gene *tet*(M). Tetracycline resistance encoded by the *tet*(M) or *tet*(S) genes is the most common resistance trait found in *Listeria* species (Bertrand et al., 2005; Charpentier, Gerbaud, Jacquet, Rocourt, & Courvalin, 1995). A previous study from Greece already reported a low prevalence of acquired resistance in *Listeria* from sausages (Abrahim et al., 1998). However, multidrug resistance has emerged in a clinical isolate of *L. monocytogenes* in Greece causing meningitis in a neonate (Tsakris, Papa, Douboyas, & Antoniadis, 1997). The origin of this strain remains unknown. Serological and molecular techniques have been implemented in several countries to trace back *L. monocytogenes* involved in outbreaks (De Valk et al., 2005). The genetic fingerprint of the *L. monocytogenes* strains isolated in food from Greek markets was determined using PFGE (Graves & Swaminathan, 2001). Eight PFGE profiles with indistinguishable PFGE patterns were found revealing a low genetic diversity among isolates from different food sources (Fig. 1). Only 28% of the *L. monocytogenes* strains displayed a different PFGE profile. This contrasts with the high genetic diversity found among *L. monocytogenes* isolated from retail food in the USA with 73% of distinguishable PFGE profiles (Zhang et al., 2007), from different food products in Ireland (55%) (Corcoran et al., 2006) and from ready-to-eat food in Austria (53%) (Wagner, Auer, Trittremmel, Hein, & Schoder, 2007). Lower genomic diversity were reported when *L. monocytogenes* were isolated from the same food product type like smoked salmon (23%) (Corcoran et al., 2006) and retail broiler meat (20%) (Praakle-Amin, Hänninen, & Korkeala, 2006) where specific clones are predominant. However, similar strains can also be found in different food product types from different producers and countries (Autio et al., 2002). In our study, the same clones were found in distant related food products such as cheese, fish, beef and poultry. This suggests that a dominant clone is either widespread in this region of Greece or that contamination may have occurred at the market places. Specific clones may have contaminated different