

ANTIMICROBIAL AGENTS AND RESISTANCE

Transferable tetracycline resistance in *Listeria monocytogenes* from food in Italy

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Mechanisms of tetracycline resistance were investigated in two recent *Listeria monocytogenes* isolates from food, with *L. innocua* 52P tet^r as a control. Tetracycline resistance was transferred conjugatively from all three strains to *L. ivanovii* and from one isolate and the control to *Enterococcus faecalis*. Molecular analysis demonstrated a chromosomal location for the tet determinant, which was identified as tetM in all cases. These studies are the first to show that *L. monocytogenes* from food could be a source of tetracycline resistance genes able to spread to other micro-organisms.

Introduction

Listeria monocytogenes can cause severe infections in man, including septicaemia, meningitis, meningo-encephalitis and abortion. Food-borne transmission is the main route of acquisition [1–5]. *L. monocytogenes* is generally quite susceptible to antibiotics [6] but, recently, resistant strains have been reported from foodstuffs and man [7–15]. A previous study determined the susceptibility of 148 *L. monocytogenes* isolates from food to antibacterial agents commonly used in human and veterinary medicine [16]. Two isolates, independently obtained from poultry and fresh meat [16], were resistant to tetracycline, flumequine, lincomycin and fosfomycin. This study examined the basis of their tetracycline resistance.

Materials and methods

Bacterial strains

Three tetracycline-resistant strains were used: the *L. monocytogenes* isolates 266 and 286 [16], which also showed resistance to fosfomycin, lincomycin and flumequine, and *L. innocua* 52P [13] as a control. *L. ivanovii* CIP 7842 and *Enterococcus faecalis* JH2-2 were used as conjugation recipients; both were already resistant to fusidic acid and rifampicin (fus^r rif^r), but

were susceptible to tetracycline (tet^s) [13]. Mutational resistance to fusidic acid (fus^r) and rifampicin (rif^r) was maintained as described below.

Media and culture conditions

Bacteria were routinely grown on Tryptic Soy Agar (TSA; Oxoid). Recipient strains were cultured in Tryptic Soy Broth (TSB; Oxoid) and TSA containing rifampicin 100 mg/L and fusidic acid 50 mg/L. This double selective pressure maintained the resistance of the rif^r, fus^r derivatives used as recipients in conjugation experiments.

Conjugation

The mating method of Poyart-Salmeron *et al.* [12] was used, with some modifications. Overnight cultures of the donor strains grown in TSB containing tetracycline 5 mg/L and recipients grown in TSB with fusidic acid 50 mg/L plus rifampicin 100 mg/L, were diluted 1 in 100 in TSB and mixed in a 1:1 ratio. A 200- μ l sample of the mating mixture was spread on a 0.45- μ m pore nitrocellulose membrane filter (Millipore, Molsheim, France) which was placed on TSA and incubated at 37°C overnight. The filter was washed and vortex-mixed in TSB, which was then diluted 1000-fold and plated on TSA containing antibiotics. Transconjugants were selected on TSA with rifampicin 30 mg/L, fusidic acid 20 mg/L and tetracycline 10 mg/L. Transfer frequencies were expressed as the numbers of transconjugants per donor cell.

Preparation of genomic and plasmid DNA

Genomic DNA was isolated from donor strains with Qiagen RNA/DNA Kits (Qiagen, Valencia, CA, USA); PCR amplification for *tet* genes was performed with the methods and primers of Aminov *et al.* [17]. Plasmid DNA was extracted with both Qiagen Mini and Maxi Preps, according to the manufacturer's instructions, and by the modified method of Kado and Liu [18] as described in McLauchlin *et al.* [19].

PFGE of macrorestricted genomic DNA

Genomic DNA for pulsed-field gel electrophoresis was prepared by the method of Moore and Datta [20], then digested overnight at 25°C with 20 units of *Sma*I (Boehringer, Germany). Electrophoresis of the resulting fragments was performed in agarose 1% gels for 20 h with a contour-clamped homogeneous electric field electrophoresis (CHEF) apparatus (model DRII; Bio-Rad Laboratories, Hercules, CA, USA) at 200 V, with a pulse time of 1–9 s.

Southern blotting and hybridisation

*Sma*I-digested and undigested genomic DNA samples were electrophoresed through agarose 1% gels, then transferred to nylon membranes (Zeta-Probe, BioRad) by an alkaline transfer procedure with a vacuum blotter (Model 785, BioRad); this DNA was then hybridised under stringent conditions. The probe was the DNA fragment of the *tetM* gene amplified by PCR from *L. monocytogenes* 266, with primers TetM-FW and TetM-RV [17]. This PCR product was purified, by direct extraction from an agarose gel, with a NucleoSpin Extraction Kit (Clontech, Palo Alto, CA, USA), then labelled with alkaline phosphatase by the ECL Direct Labelling and Detection System RPN 300 (Amersham Pharmacia, Little Chalfont, Buckinghamshire).

Results and discussion

L. monocytogenes strains 266 and 286 and *L. innocua* 52P conjugatively transferred tetracycline resistance to *L. ivanovii* 7842 at frequencies of 1.9×10^{-6} , 4×10^{-6} and 6×10^{-6} , respectively. Only *L. monocytogenes* 286 and *L. innocua* 52P transferred resistance to *E. faecalis* JH2-2, with frequencies of 3×10^{-7} and 2.4×10^{-7} , respectively. The recipient strains were all resistant to fosfomycin, flumequine and lincomycin, so it was not possible to check whether these resistance traits were also transferred. The frequencies of transfer for *L. innocua* 52P were consistent with previously reported results [13]. No plasmids were detected in the donors or the transconjugants.

Primers to detect the various classes of *tet* genes were used [17]. Only *tetM* was found, being present in all three strains and their transconjugants (Fig. 1). The size

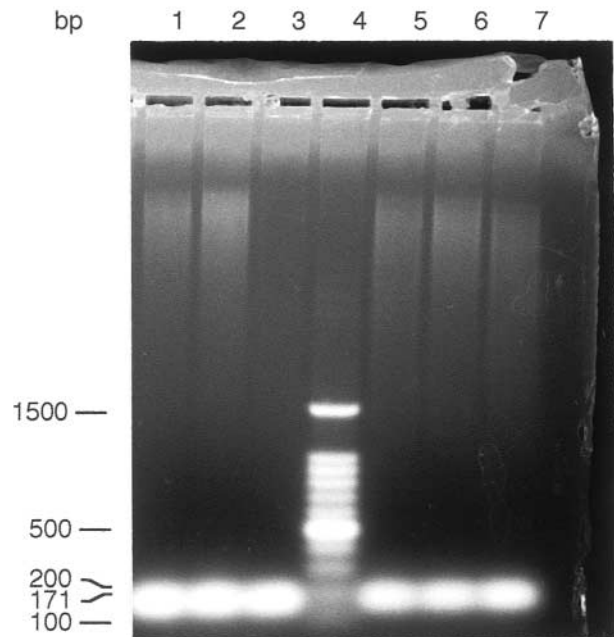


Fig. 1. Amplification of *tetM* fragments. Lane 1, *L. innocua* 52P; 2, *L. monocytogenes* 266; 3, *L. monocytogenes* 286; 4, 100-bp DNA Ladder (Promega); 5, 6 and 7, *L. ivanovii* CIP 7842 transconjugants of strains 266, 286 and 52P, respectively.

of the fragment amplified (171 bp) was consistent with that anticipated for *tetM* [17]. DNA-DNA hybridisation showed that *tetM* was located on undigested chromosomal DNA (Fig. 2); hybridisation analysis with DNA that had been digested with *Sma*I and separated by PFGE (Fig. 3) located the *tetM* gene on a 145-kb

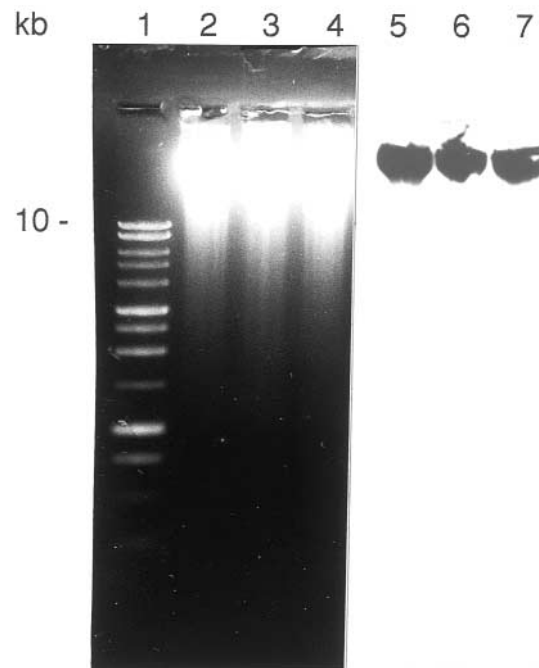


Fig. 2. Electrophoresis and hybridisation of total DNA: lane 1, 1-kb DNA ladder (Promega); 2, *L. innocua* 52P; 3, *L. monocytogenes* 266; 4, *L. monocytogenes* 286. Hybridisation of the *tetM* gene probe: lane 5, *L. innocua* 52P; 6, *L. monocytogenes* 266; 7, *L. monocytogenes* 286.

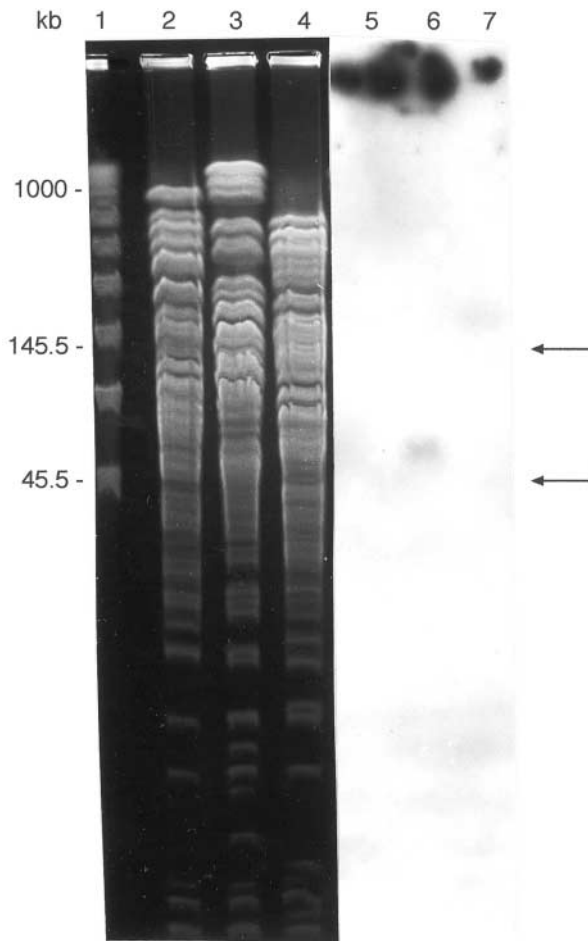


Fig. 3. *Sma*I digests separated by PFGE: lane 1, λ ladder PFG Marker (New England Biolabs); 2, *L. innocua* 52P; 3, *L. monocytogenes* 266; 4, *L. monocytogenes* 286. Hybridisation of the blotted gel with a *tetM* gene probe: lane 5, *L. innocua* 52P; 6, *L. monocytogenes* 266; 7, *L. monocytogenes* 286.

fragment in *L. innocua* 52P and *L. monocytogenes* 286 and on a 45.5-kb fragment in digests of *L. monocytogenes* 266.

Listeria spp. are common in the environment and in the gastrointestinal tracts of animals and man. The identification of conjugatively mobile *tetM* determinants in these organisms is a cause for concern, as resistance might transfer both to and from other gut bacteria, including enterococci and streptococci [21]. The transfer of tetracycline resistance between *L. monocytogenes* and *E. faecalis* confirms that enterococci might act as a reservoir of genes for *L. monocytogenes*, a finding consistent with other recent data on inducible transfer of resistance in the gastrointestinal tract [13, 22–24].

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