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

MANOOCHEHER POURSHABAN, ANNA MARIA FERRINI, VERUSCKA MANNONI, BRUNELLO OLIVA* and PAOLO AURELI

Istituto Superiore di Sanità, Food Department, Viale Regina Elena, 299, 00161 Rome and *Department of Experimental Medicine, Microbiology Section, University of L’Aquila, L’Aquila, Italy

Mechanisms of tetracycline resistance were investigated in two recent *Listeria monocytogenes* isolates from food, with *L. innocua* 52P tet* 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, meningoencephalitis 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 (fus*) and rifampicin (rif*) [13]. Mutational resistance to fusidic acid (fus') and rifampicin (rif') 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*, fus* 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-μL sample of the mating mixture was spread on a 0.45-μm 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 and fusidic acid 20 mg/L and tetracycline 10 mg/L. Transfer frequencies were expressed as the numbers of transconjugants per donor cell.

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Corresponding author: Dr P. Aureli (e-mail: p.aureli@iss.it).
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 SmaI (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

SmaI-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 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 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 SmaI and separated by PFGE (Fig. 3) located the tetM gene on a 145-kb fragment.
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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References

22. François B, Charles M, Courvalin P. Conjugative transfer of tet(S) between strains of Enterococcus faecalis is associated with the exchange of large fragments of chromosomal DNA. Microbiology 1997; 143: 2145–2154.