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 tet^M 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 septicemia, 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^) but were susceptible to tetracycline (tet^) [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, 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 \textit{tet} genes was performed with the methods and primers of Aminov \textit{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 \textit{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 \textit{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 DR II; Bio-Rad Laboratories, Hercules, CA, USA) at 200 V, with a pulse time of 1–9 s.

Southern blotting and hybridisation

\textit{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 blower (Model 785, BioRad); this DNA was then hybridised under stringent conditions. The probe was the DNA fragment of the \textit{tetM} gene amplified by PCR from \textit{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

\textit{L. monocytogenes} strains 266 and 286 and \textit{L. innocua} 52P conjugatively transferred tetracycline resistance to \textit{L. ivanovii} 7842 at frequencies of $1 \times 10^{-6}$, $4 \times 10^{-6}$ and $6 \times 10^{-6}$, respectively. Only \textit{L. monocytogenes} 286 and \textit{L. innocua} 52P transferred resistance to \textit{E. faecalis} JH2-2, with frequencies of $3 \times 10^{-7}$ and $2.4 \times 10^{-7}$, 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 \textit{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 \textit{tet} genes were used [17]. Only \textit{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 \textit{tetM} [17]. DNA-DNA hybridisation showed that \textit{tetM} was located on undigested chromosomal DNA (Fig. 2); hybridisation analysis with DNA that had been digested with \textit{SmaI} and separated by PFGE (Fig. 3) located the \textit{tetM} gene on a 145-kb fragment.

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

\textbf{Fig. 2.} Electrophoresis and hybridisation of total DNA: lane 1, 1-kb DNA ladder (Promega); 2, \textit{L. innocua} 52P; 3, \textit{L. monocytogenes} 266; 4, \textit{L. monocytogenes} 286. Hybridisation of the \textit{tetM} gene probe: lane 5, \textit{L. innocua} 52P; 6, \textit{L. monocytogenes} 266; 7, \textit{L. monocytogenes} 286.
Fig. 3. Smal digests separated by PFGE: lane 1, λ ladder PFGE Marker (New England BioIabs); 2, L. innocua 52P; 3, L. monocytogenes 266; 4, L. monocytogenes 286. Hybridisation of the blotted gel with a tetM gene probe: lane 1, L. monocytogenes 286; 2, L. monocytogenes 286 (S) between strains of Enterococcus faecalis. Antimicrob Agents Chemother 1997; 41: 1134–1136.


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