Malaysian Journal of Medical Sciences, Vol. 8, No. 1, Januari 2001 (59-62)

ORIGINAL ARTICLE

PLASMID-MEDIATED STREPTOMYCIN RESISTANCE OF *LISTERIA MONOCYTOGENES*

Endang Purwati, Son Radu[*], Zaiton Hassan[**], Ooi Wai Ling and Raha Abdul Rahim

Fakultas Peternakan, Universitas Andalas, Kampus Limau Manis, Padang, Indonesia.
[*]Department of Biotechnology, [**]Department of Food Science,
Faculty of Food Science and Biotechnology, University Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.

A strain of streptomycin-resistant *Listeria monocytogenes* LM35 isolated from imported frozen beef was examined in this study. In conjugation studies, the *L. monocytogenes* LM35 strain harbouring two plasmids of 54, 3.0, 2.8 and 2.7 kilobase was used as the donor and streptomycin-sensitive and plasmidless *L. monocytogenes* LM65 and LM100 strains as the recipients. Streptomycin resistance was transferred to *L. monocytogenes* LM65 and LM100 strains at frequencies of 3.3×10^{-8} and 1.2×10^{-9} per input donor cells, respectively. In both occasions, we also observed the concomitant transfer of the donor's 54 kilobase plasmid. These results suggest that streptomycin resistance in *L. monocytogenes* LM35 was mediated by the 54 kilobase plasmid.

Key words : *Listeria monocytogenes*, plasmid, streptomycin, transfer

Submitted-27.6.2000, Revised Article-14.12.2000, Date Accepted-15.1.2001

Introduction

Listeria monocytogenes is a gram-positive opportunistic pathogen responsible for severe infections (septicemia, meningitis and meningoencephalitis) primarily in immunocompromised hosts, the elderly, neonates, and foetuses. Infections caused by *L. monocytogenes* are likely to be foodborne as the intestinal tract is the most probable site of invasion (1). *Listeria* contamination occurs in a wide range of foods such as dairy products, vegetables, raw fish, fermented sausage, meat and poultry (2-5). In Malaysia, beef is a popular food. Multi-antibiotic resistance plasmids encoding resistance to chloramphenicol, macrolide/lincosamide/streptogramin, tetracycline, erythromycin and streptomycin have been found in *Listeria monocytogenes* (6-7). To the best of our knowledge, there has been no report yet on plasmid-mediated antibiotic resistance among *Listeria monocytogenes* from food sources in Malaysia. In our previous study, we reported on the conjugative transfer of plasmid-mediated kanamycin resistance in *Listeria innocua* strain isolated from fermented fish (8). Thus, there is a need to assess the transferability of antimicrobial resistance of *Listeria monocytogenes* to establish the possible hazards to public health due to digestion of foodborne resistant strains.

The objective of the present study was to determine whether genetic information coding for streptomycin resistance in *L. monocytogenes* strain LM35 may be carried on conjugative R plasmid.

Materials and Methods

Bacterial conjugation

A streptomycin-resistant *Listeria monocytogenes* LM35 harbouring a 54 kilobase plasmid and three small plasmids of 2.7, 2.8 and 3.0 kilobase in sizes (donor strain, see Figure 1), and streptomycin-sensitive and plasmidless *L. monocytogenes* LM65 and LM100 (recipient strains)

isolated from imported frozen beef used in this study have been described previously (9).

Donor and recipient cells were grown to mid-log phase (10^7 cfu/ml) in tryptic soy broth (TSB) at 35°C. A 0.5 ml sample of the donor strains was added to 1.0 ml of the recipient sample on a tryptic soy agar (TSA) plate, and incubated overnight at 35°C. Bacteria were harvested from the TSA plate and a ten-fold serial dilutions of each mating mixtures in saline (0.85%) were spread on plates supplemented with 30 µg of streptomycin and tetracycline (*L. monocytogenes* LM65) or streptomycin and chloramphenicol (*L. monocytogenes* LM100) to which the recipients were resistant, respectively. Plate counts were performed for estimates of donor and recipient population on TSA plates containing antibiotic to which the donor or recipient strains were resistant, respectively. Colonies growing on this double-inhibitor-supplemented medium after 24 to 48 h of incubation at 35°C were scored as presumptive transconjugants, and the frequency of transfer was calculated as the number of transconjugants per initial number of donors. Ten or more transconjugants from each mating were picked and tested for their antibiotic resistance as described previously (8).

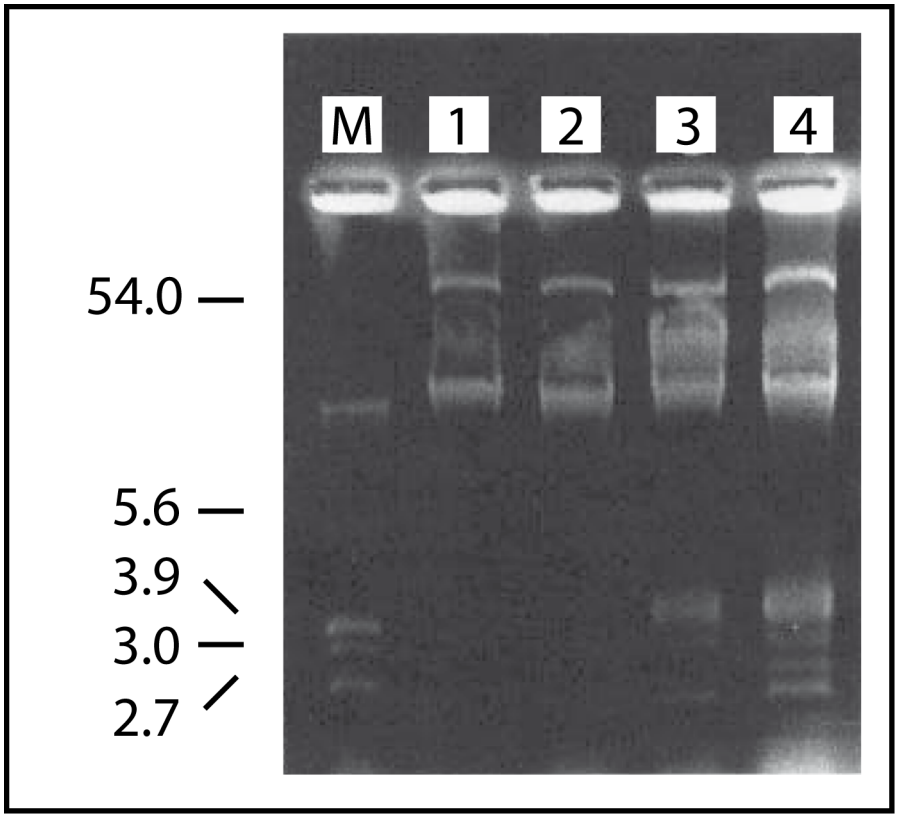
Plasmid isolation

Streptomycin-resistant transconjugants were screened for the presence of plasmid by the method of LeBlanc and Lee (10). Extracted plasmids were electrophoresed for 2 h at 35 mA on a 0.85% agarose gel in TBE buffer (89 mM Tris-base - 89 mM boric acid - 2.5 mM disodium RDTA) as described by Sambrook *et al*. (11). The approximate molecular mass of each plasmid was determined by comparison with plasmid of known molecular mass from *E. coli* V517 (12).

Results and discussion

The presence of streptomycin resistance in *Listeria monocytogenes* LM35 prompted us to investigate whether this strain could act as donor of streptomycin resistant in mating experiments with the streptomycin-sensitive *Listeria monocytogenes* LM65 and LM100 strains. In three independent experiments the *L. monocytogenes* LM35 strain was able to transfer streptomycin resistance to recipient

Figure 1: Agarose gel (0.85%) gel electrophoresis of plasmid DNA from *L. monocytogenes* strains and their respective transconjugants. Lanes: 1, transconjugant LM65; 2, transconjugant LM100; 3 and 4, donor L35; M, E.



listeriae. The frequencies of transfer, expressed as the number of transconjugants per donor colony forming unit (CFU), were 3.3 x 10^{-8} to *L. monocytogenes* LM65 and 1.2 x 10^{-9} to *L. monocytogenes* LM100. The 54 kilobase plasmid of the donor was detected in the streptomycin-resistant transconjugants (Figure 1). However, the 2.7, 2.8 and 3.0 kilobase plasmids were not transferred to the streptomycin-resistant transconjugants. Antibiotic resistance is often determined by genetic information of plasmid origin and that the correlation between antibiotic resistance and plasmid profile may indicate that the genetic information is plasmid-borne (13). Thus, it was apparent from the results obtained in this study that the streptomycin resistance phenotype of the *L. monocytogenes* LM35 strain was mediated by the 54 kilobase plasmid. However, it should be noted here that further evidence to support the finding on the conjugal transfer of the streptomycin resistance and the 54 kilobase plasmid can be obtain by conducting curing and hybridization experiments or cloning of the streptomycin resistance gene from the 54 kilobase plasmid.

On the basis of their studies of antibiotic resistance in *L. monocytogenes*, and more particularly the transferability of streptomycin resistant between *L. monocytogenes* and *E. faecalis*, Poyart-Salmeron *et al*. (14) suggested that enterococci might be a reservoir of resistance for *L. monocytogenes*. However, since the *L. monocytogenes* LM35 strain examined in this study harboured a self-transmissible plasmid, our results may suggest that *L. monocytogenes* could act as a reservoir of streptomycin resistance genes for intra- and intergeneric dissemination of antibiotic resistance. Transfer of resistance between *L. monocytogenes* and other bacterial species might occur in the gastrointestinal tract of domestic animals and man where these species may live. Since the intestinal tract represents the portal entry for *Listeria* strains (14-16) human infections caused by antibiotic-resistant *L. monocytogenes* from food may occur.

In conclusion, since listeriosis is often fatal even with antibiotic therapy, there is every reason to be concerned at reports of *Listeria monocytogenes* with plasmid-mediated antibiotic resistance as evidenced by the results obtained in this study. Thus food samples may need to be monitored for the emergence of resistant strains.

Acknowledgements

This study was supported by the Malaysian Government through the IRPA grant mechanism and University Putra Malaysia through the allocation of Seed Money grant No. 50522.

Correspondence :

Assoc. Prof. Dr. Son Radu,
Department of Biotechnology,
Faculty of Food Science and Biotechnology,
University Putra Malaysia, 43400 UPM Serdang,
Selangor, Malaysia.
Tel: +6 03 - 89486101 ext. 3446
Fax: +6 03 -89423552
Email: son@fsb.upm.edu.my

References

1. Kvenberg, J. E. Outbreaks of listeriosis/*Listeria*-contaminated foods. *Microbiol. Sci.* 1988; **5**: 355-58.
2. Gellin , B. G. and Broome, C. V. Listeriosis. *J. Am. Med. Assoc.* 1989; **261**: 1313-20.
3. Beuchat, L. R., Berang, M. E. and Brackett, R. E. Presence and public health implications of *Listeria monocytogenes* on vegetable. In: Miller, A. L., Smith, J. L. and Somkuti, G. A., eds. Foodborne listeriosis: Elsevier Science Publishers B. V. (Biomedical Division), Amsterdam, 1990: 175-81.
4. Carosella, J. M. Occurrence of *Listeria monocytogenes* in meat and poultry. In: Miller, A. L., Smith, J. L. and Somkuti, G. A., eds. *Foodborne listeriosis:* Elsevier Science Publishers B. V. (Biomedical Division), Amsterdam, 1990: 165-73.
5. Marth, E. H. and Ryser, E. T. Occurrence of *Listeria* in foods: milk and dairy foods. In: Miller, A. L., Smith, J. L. and Somkuti, G. A., eds. *Foodborne listeriosis:* Elsevier Science Publishers B. V. (Biomedical Division), Amsterdam, 1990: 151-64.
6. Hadon, K., Hachler, H., Schaffner, A. and Kayser, F. H. Genetic characterization of plasmid-encoded multiple antibiotic resistance in a strain of *Listeria monocytogenes* causing endocarditis. *Eur. J. Clin. Microbiol. Infect. Dis.* 1993; **12**: 928-37.
7. Marilyn, C. R., Facinelli, B., Giovanetti, E. and Varaldo, P. E. Transferable erythromycin resistance in *Listeria* spp. isolated from food. *Appl. Environ. Microbiol.* 1996; **62**: 269-70.
8. Endang, P., Son, R., Zaiton, H. and Rusul, G. Antimicrobial drug resistance and resistance factor transfer among *Listeria* species. *Asian Fish. Sci.* 1998; **11**: 261-70.

9. Endang, P., Son, R., Hassan, Z. and Rusul, G. Characterization of *Listeria monocytogenes* isolated from imported frozen beef: antimicrobial resistance, plasmid profiles and randomly amplification of polymorphic DNA analysis: *3rd UNESCO National Workshop on Promotion of Microbiology in Malaysia,* Bangi, Selangor, 1999: pp. 36.

10. LeBlanc, D. J. and Lee, L. N. Rapid screening procedure for detection of plasmids in streptococci. *J. Bacteriol*. 1979; **140**: 1112-15.

11. Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.

12. Macrina, F. L., Kopecko, D. J., Jones, K. R., Ayers, D. J. and Cowan, S. M. N. A multiple-plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. *Plasmids* 1978; **1**: 417-20.

13. Hinton, M., Kaukas, A. and Linton, A. H. The ecology of drug resistance in enteric bacteria. In Bateson, M., Benham, C. L. and Skinner, F. A. eds. Microorganisms in Agriculture. Society for Applied Bacteriology Symposium Series. *J. Appl. Bacteriol. Suppl*. (1986); **15**: 77S-92S.

14. Poyart-Salmeron, C., Carlier, C., Treu-Cuot, P., Coutieu, A. L. and Couvalin, P. Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. *Lancet* 1990; **335**: 1422-26.

15. Poyart-Salmeron, C., Trieu-Cuot, P, Carlier, C., MacGowan, A., McLauchlin, J. and Courvalin, P. Genetic basis of tetracycline resistance in clinical isolates of *Listeria monocytogenes*. *Antimicrob. Agents Chemother*. 1992; **36**: 463-66.

16. Farber, J. M. and Peterkin, P. I. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev*. 1991; **55**: 476-511.