Veterinary Research Communications, 27 Suppl. 1 (2003) 335–338

Application of Biomolecular Methods to *Staphylococcus aureus* Strains from Dairy Cows

V. Cuteri, P. Mezzasoma and C. Valente*
Department of Technology and Biotechnology of Animal Production, Faculty of Veterinary Medicine, University of Perugia, Perugia, Italy
**Correspondence: Dipartimento di Tecnologie e Biotecnologie delle Produzioni Animali, Sezione Malattie Infettive, Facoltà di Medicina Veterinaria, Via San Costanzo,4, 06126 Perugia, Italy*
E-mail:valente@unipg.it

Keywords: dairy cow, infectious disease, mammary gland, *Staphylococcus aureus*

Abbreviations: MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; PCR, polymerase chain reaction; PGE, pulsed-field gel electrophoresis

INTRODUCTION

Staphylococcus aureus is usually responsible for mastitis in dairy cows, sheep and goats as well as being responsible for different pathologies in many animal species (Valente *et al*., 1999). During therapeutic treatments using methicillin, resistant microrganisms can develop (Barber, 1961); methicillin-resistant strains of *S. aureus* (MRSA), have been reported for various animal species (Devriese, 1975; Tomlin *et al*., 1999). Resistance to methicillin is determined by the gene *mecA*, which encodes a penicillin-binding protein (PBP2a) that has low affinity for beta-lactamic compounds. Resistance to methicillin and oxacillin, a similar antibiotic used in the treatment of mastitis, is often associated with analogous behaviour against a wide range of antibiotics compromising therapeutic treatments.

The aim of this study was to isolate strains of *S. aureus* from the milk of dairy cows, to verify the presence of MRSA strains and to submit such strains to chromosomic DNA molecular study using pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

Cultures

In the period between 1996 and 1999, 2550 milk samples were taken from clinically healthy dairy cows. The milk was spread on nutrient agar with 5% sheep blood, mannitol salt agar and MacConkey agar (Oxoid, Milan, Italy) and incubated at 37°C

for 48 h. Strains were isolated as follows: 223 strains of *S. aureus* (8.74%); 385 coagulase negative *Staphylococcus* spp. (15.10%); 65 *Streptococcus* spp. (2.54%); and 94 microrganisms belonging to different species (3.68%). The bacteria were identified using biochemical and serological tests (Murray *et al.*, 1995), in particular for *S. aureus* with coagulase and DNAse tests, API Staph (BioMèrieux, France) and Sceptor System (Bekton Dickinson, UK). *S. aureus* ATCC 25923 was used in each test as reference strain.

Methicillin resistance

The resistance to this antibiotic was evaluated using the Sceptor System and with the E-Test (Oxoid, Milan). The strains of *S. aureus* were considered resistant (MRSA) if they showed an inhibition identical or superior to 16 μg/ml (MIC) whereas, otherwise they were considered susceptible (MSSA).

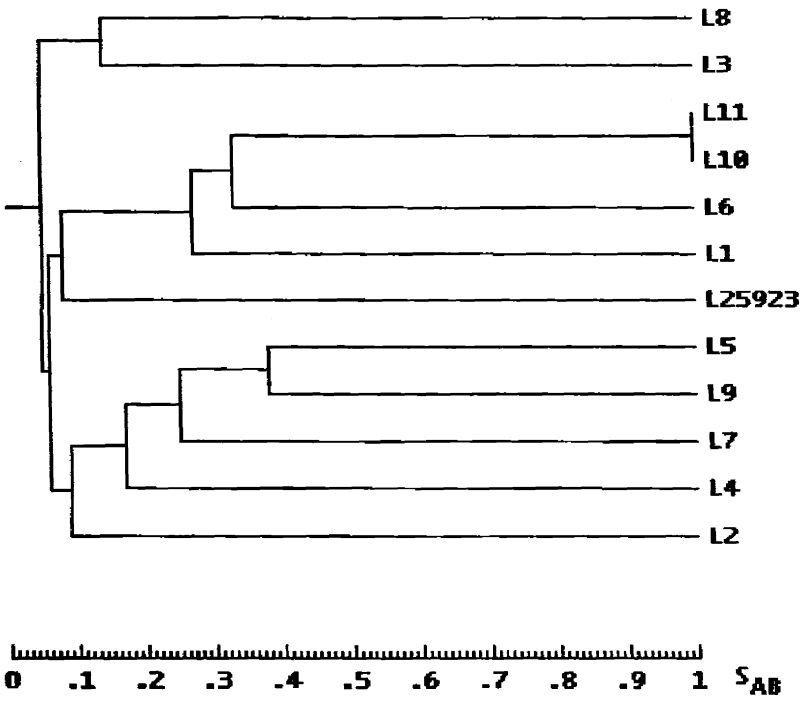

Figure 1. Dendogram of similarity of methicillin-resistant *Staphylococcus aureus*

MecA gene identification

The presence of the gene was determined by PCR after DNA extraction using a QI Amp tissue kit (Quiagen, Hilden, Germany) and it was amplified using the primers *mecA1* (5′-AAA ATC GAT GGT AAA GGT TGG C) and *mecA2* (5′-AGT TCT GCA GTA CCG GAT TTG C), which highlighted a 533 bp PCR product.

The DNA was amplified following the method of Ungeheur and colleagues (1994).

Electrophoresis of DNA was carried out in 2% agarose gel, stained with ethidium-bromide and the gel was photographed for UV visualization.

Pulsed-field gel electrophoresis (PFGE)

Among the 223 isolated *S. aureus* strains, 20 MRSA and 10 MSSA strains were randomly chosen. The strains were characterized by the macrorestriction method with *Sma*1 (Celbio, Milan, Italy) and resolved using the pulsed electrophoresis CHEF Mapper System (Biorad Laboratories, Hercules, CA, USA). The electrophoretic patterns were acquired with a camera (Gel-Doc 1000, Biorad) and processed using the Molecular Analyst Finger Printing Software (Biorad) to produce a dendrogram of similarity.

The similarity coefficients (S_{AB}) were calculated directly using the software: 0 indicated that when patterns were not correlated and 1 when they were strictly correlated.

RESULTS AND DISCUSSION

Of the 233 *S. aureus* strains 32 (14.34%) were resistant to methicillin. This high level of resistance is probably explained by the increased use of beta-lactamic antibiotics in treatment of mastitis.

For the 20 randomly chosen strains the *mecA* gene responsible for the methicillin resistance was identified using PCR, confirming the antibiogram result, while for the 10 MSSA strains the result was always negative.

Following the PFGE results, the study of genomic similarity of MRSA strains revealed by the dendrograms (Figures 1 and 2), showed that two strains were indistinguishable and two were strictly correlated, while the 16 remaining strains showed no correlations. There was no genomic similarity between the 20 MRSA and 10 MSSA strains. Two of the MRSA strains proved to be genetically identical, although they come from animals of different breeds. This is in agreement with results obtained in human pathology, where MRSA strains, isolated in different years and in different countries, were found to be indistinguishable (Scott, 1988).

The molecular study of MRSA strains thus reveals a therapeutic problem and shows the necessity to continue monitoring to determine the epidemiological map.

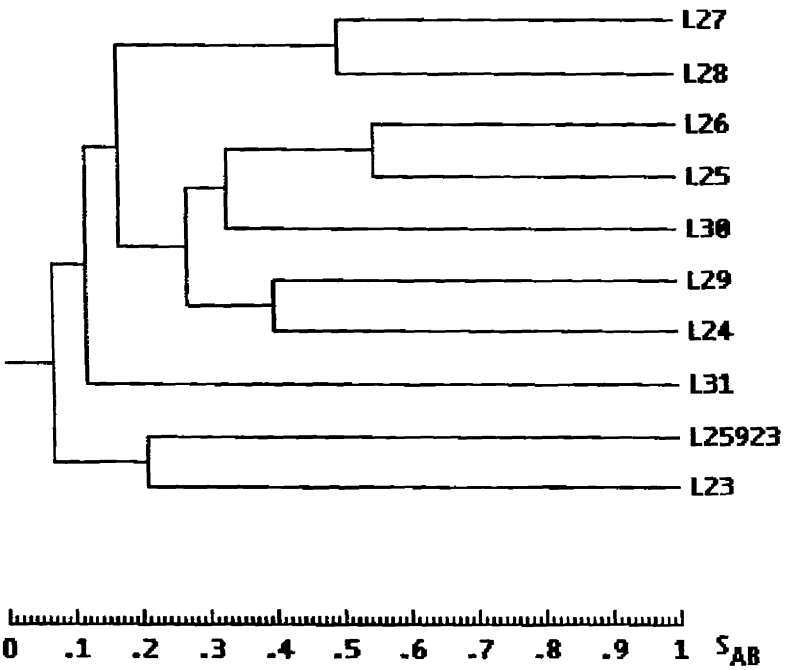

Figure 2. Dendogram of similarity of methicillin-resistant *Staphylococcus aureus*

REFERENCES

Barber, M., 1961. Methicillin-resistant staphylococci. *Journal of Clinical Pathology*, **14**, 385–393

Devriese, L.A., 1975. Epidemiology of methicillin-resistant *Staphylococcus aureus* in dairy herds. *Research in Veterinary Science*, **19**, 23–27

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H. (eds), 1995. In: *Manual of Clinical Microbiology*, 6th edn, (American Society for Microbiology Press, Washington DC), 190–208

Scott, G.M., Thompson, R., Malone-Lee, J. and Ridgeway, G. L., 1988. Cross-infection between animals and man: possible feline transmission of *Staphylococcus aureus* infection in humans. *Journal of Hospital Infection*, **12**, 29–34

Tomlin, J., Pead, M.J., Lloyd, D.H., Howell, S., Hartmann, F., Jackson, H.A. and Muir, P., 1999. Methicillin-resistant *Staphylococcus aureus* infections in 11 dogs. *The Veterinary Record*, **16**, 60–64

Ungeheuer, J., Wilms, S. and Sietzen, W., 1994. *MecA*-Gen bei Staphylokokken – vereinfachter Nachweis mit der PCR. *Chemotherapy Journal*, 1, 31–35

Valente, C., Cuteri, V., Quondam Giandomenico, R., Bernardini, D., Dalvit, P. and Vescera, F., 1999. Preliminary study on Concanavalin A in whey to evaluate the health status of the mammary gland. *Italian Journal of Food Science*, **11**(1), 67–73