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Genotypic analysis of *Staphylococcus aureus* from milk of dairy cows with mastitis in Argentina

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SUMMARY

Staphylococcus aureus is the most prevalent pathogen causing mastitis of dairy ruminants. This study was developed to ascertain the genotypes and genealogical relationship among strains isolated from milk of bovines with mastitis in Argentina. Molecular epidemiological analysis of *S. aureus* was performed on 112 isolates from 21 districts. Clonality was assessed by *Sma*I pulsed-field gel electrophoresis (PFGE) typing, automated *Eco*RI ribotyping and restriction enzyme analysis of plasmid (REAP) DNA profiles. A total of 22 band patterns distributed in four clusters were found by *Sma*I PFGE analysis. The similarity of clusters 2, 3 and 4 with cluster 1 was 0·73, 0·69 and 0·33, respectively, and 101 of 112 isolates belonged in cluster 1. PFGE band patterns from 42 isolates within cluster 1 were indistinguishable from each other (type A). The second largest group of isolates with indistinguishable PFGE band patterns was subtype A11, which was composed of 19 isolates. Automated ribotyping assigned the 112 isolates into 13 ribotypes. Among these, the most prevalent ribotypes I and VI were composed of 49 and 35 isolates respectively. Although there was certain correspondence between PFGE genotypes and ribotypes, further discrimination was achieved by combining both methods. REAP DNA profile analysis was useful to provide even further discrimination between isolates with identical PFGE genotype and ribotype. The most prevalent *S. aureus* strains A/I and A11/VI were widely distributed in the country and were not restricted to individual nearby locations. Prevalence of these two strains varied consecutively within a period of 8 years. Whether the shift in type prevalence was due to selection of a phenotypic trait remains undisclosed.

INTRODUCTION

Bovine mastitis has been singled out as the most significant cause of economic loss to the dairy industry. These losses are primarily due to lower milk yields, reduced milk quality and higher production costs [1]. Although several bacterial pathogens can cause the disease, *Staphylococcus aureus* has emerged as the most important one and, once it is established in the milking animal, it is very difficult to eradicate [2]. Despite the use of a variety of antimicrobial agents, antibiotic therapy appears to be often ineffective [3]. Numerous methods have been utilized for epidemiological identification and comparison of *S. aureus* isolates from human and animal staphylo-

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coccal infections. More traditional biochemical and physiological typing methods have been superseded in the past decade by molecular genetic procedures [4]. Among these, pulsed-field gel electrophoresis (PFGE) of macrorestriction DNA fragments, random amplified polymorphic DNA polymerase chain reaction analysis, ribotyping, binary typing and polymorphism analysis of certain genes have been used to ascertain clonality of *S. aureus* isolates from veterinary settings [5–10].

It has been hypothesized that a better knowledge of infective strain distribution in dairy herds might help in formulating strategies to reduce infection spread [11]. Molecular epidemiological studies on bovine *S. aureus* isolates have shown that a large number of types are involved in bovine mastitis etiology worldwide and that certain types appear to predominate within geographical regions [6, 12–14]. Most molecular epidemiological studies on bovine *S. aureus* isolates have been conducted on isolates from Europe or North America, whereas data from South America are scant [15]. This study was aimed at evaluating clonal relationships between *S. aureus* isolates from milk of bovines with mastitis in Argentina. To this purpose, the validity of *Sma*I PFGE typing and automated ribotyping was ascertained.

MATERIALS AND METHODS

Bacterial isolates and cultures

One-hundred and twelve *S. aureus* were isolated from milk of cows with clinical mastitis between 1989 and 1997. Milk samples were obtained from herds located in 21 districts of Argentina. The nearest distance between herds in two adjacent districts was 32 km and the greatest distance between herds was approximately 1200 km. The isolates included in this study are representative of bovine mastitis in Argentina as a whole since they were obtained from the major dairy regions of this country. *S. aureus* was identified by a standard procedure of the microbiology laboratory [16]. Isolates were stored in brain–heart infusion (Difco) medium with 20% glycerol at -20 °C until used. *S. aureus* NCTC 8325 obtained from a commercial source was included as quality control strain.

PFGE

Staphylococcus aureus isolates were identified by PFGE by means of the CHEF DR-III system (Bio–Rad, Hercules, CA, USA) using a standard protocol [17]. Briefly, *S. aureus* was cultured and plugs were prepared. DNA was digested with *Sma*I and DNA fragments were resolved by electrophoresis in 0·8% agarose gels run in 0·5 × TBE buffer over 18 h at 6 V/cm, 13 °C. The included angle was 120° and initial and final switch times were 1 s and 30 s, respectively. *S. aureus* 8325 was included in each gel as quality control. After electrophoresis the gels were stained with 2 μg/ml ethidium bromide and scanned with the BioRad Gel Doc system using the Molecular Analyst Software (Bio–Rad, Hercules, CA, USA). For the final analysis band relative positions were established on thermal paper prints of the gels and compared with those generated with λ Ladder DNA concatemers (New England Biolabs, Beverly, MA, USA). To evaluate the clonal relationship between isolates the criteria by Tenover and coworkers [18] were applied. PFGE patterns differing in seven or more bands were recorded as PFGE types and identified with a capital letter, whereas those differing in 2–6 bands were recorded as different subtypes of the pattern with the highest prevalence and identified with a capital letter (type) followed by an arabic number.

Automated ribotyping

Isolates were identified by *Eco*RI ribotyping using the automated RiboPrint system (Qualicon, Wilmington, DE, USA). Ribotyping was performed according to the standard protocol recommended by the vendor (RiboPrinter, Microbial Characterization System, Ch 2: Operations user's guide, Qualicon). *S. aureus* was grown overnight in brain–heart infusion agar (Difco), and then suspended in sample preparation buffer and heated at 80 °C over 10 min in the heat treatment station. Samples were run in the aforementioned system and each band pattern defined and compared with an existing identification database.

Restriction enzyme analysis of plasmid (REAP) DNA profiles

Plasmid DNA was isolated by alkaline lysis according to Bimboim and Doly [19], previous treatment of bacteria with lysozyme and lysostaphin (Sigma Chemical Co., St Louis, MO, USA). For REAP, DNA was digested with *Eco*RI as recommended by the vendor (Promega Corp., Madison, WI, USA) and

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transferred into wells in a 0·7% agarose gel in TBE buffer. Electrophoresis was run 4 h at 90 V in tris–borate buffer and gels were stained and photographed. The number of bands was established on Polaroid prints and the molecular size of each band was estimated by comparison with those generated with the 1 kb DNA ladder molecular weight marker (Promega). Isolates yielding band profiles with differences in one band were considered to belong in different genotype groups.

Numerical analysis and discriminatory power

The similarity between PFGE types was evaluated by the Dice coefficient [20]. The resultant matrix was analysed by the unweighted pair group method of analysis (UPGMA) [21].

RESULTS

Molecular epidemiological analysis of milk *S. aureus* isolates was performed on 112 isolates from 21 districts. A total of 22 band patterns (including types and subtypes) in the range from 485 to 48·5 kb were found by *Sma*I PFGE analysis. A dendrogram that included all patterns was constructed on the basis of the levels of similarity (Fig. 1). A cut-off point of 80% was considered to define four clusters, namely 1, 2, 3 and 4. Each cluster included types A, B, C and D, respectively. Similarity of clusters 2, 3, and 4 with cluster 1 was 0·73, 0·69 and 0·33, respectively. *Sma*I PFGE band analysis revealed that 101 of 112 isolates (90%) belonged into cluster 1, according to the criteria of Tenover et al. [18]. PFGE band patterns from 42 isolates within cluster 1 were indistinguishable from each other, and were thus classified as type A. The second largest group of isolates with indistinguishable PFGE band patterns was subtype A11, which was composed of 19 isolates. Band patterns of type A and subtype A11 isolates differed in four bands (Fig. 2). The remaining 40 cluster 1 isolates were evenly distributed in 14 subtype groups of smaller size. Eight of 112 isolates were grouped within PFGE cluster 3 and discriminated into type C (2 isolates) subtypes C1, C2 and C3 isolates (2, 3 and 1 isolate respectively). Finally, there was one isolate within cluster 2 (type B) and there were two isolates within cluster 4 (type D).

A total of 112 *S. aureus* isolates were typed by means of the Riboprinter automated microbial characterization system, as described in Materials and Methods. The band pattern from each isolate was compared with those of an existing identification database. Species identification by conventional biochemical testing and by automated ribotyping

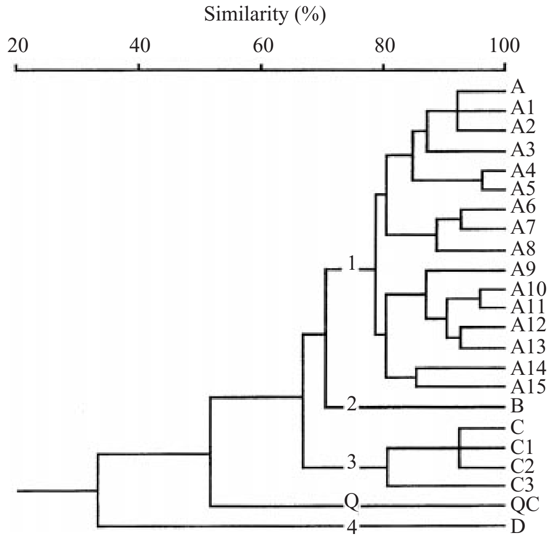

Fig. 1. Clonal relationships of 112 *S. aureus* isolates established with *Sma*I PFGE analysis. The scale indicates the percent of similarity according to the Dice coefficient within this set of strains. Clusters were labelled with Arabic numbers and Q is the cluster containing quality control strain NTCC 8325. Type A and subtype A11 were predominant within cluster 1.

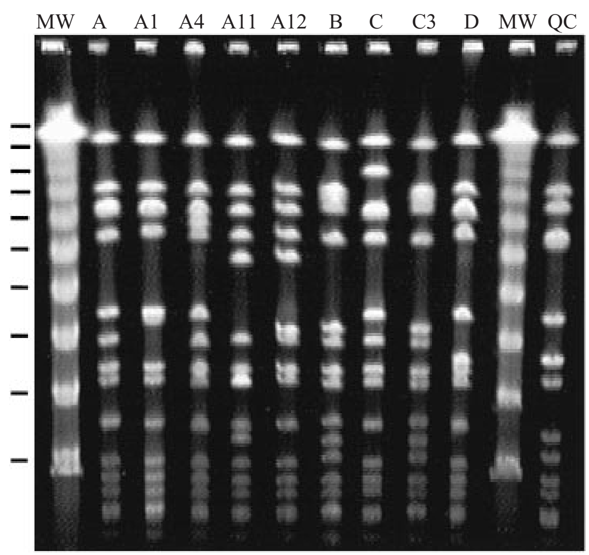

Fig. 2. PFGE band patterns of *S. aureus* bovine isolates from Argentina. Representative genotypes are shown in each lane. Type A and subtype A11 were the most prevalent ones within the period under scrutiny. Lanes MW are the λ ladder marker (position of each band is shown on the left-hand side. 1018·5–48·5 kb). Lane Q is the *S. aureus* control strain 8325–4.

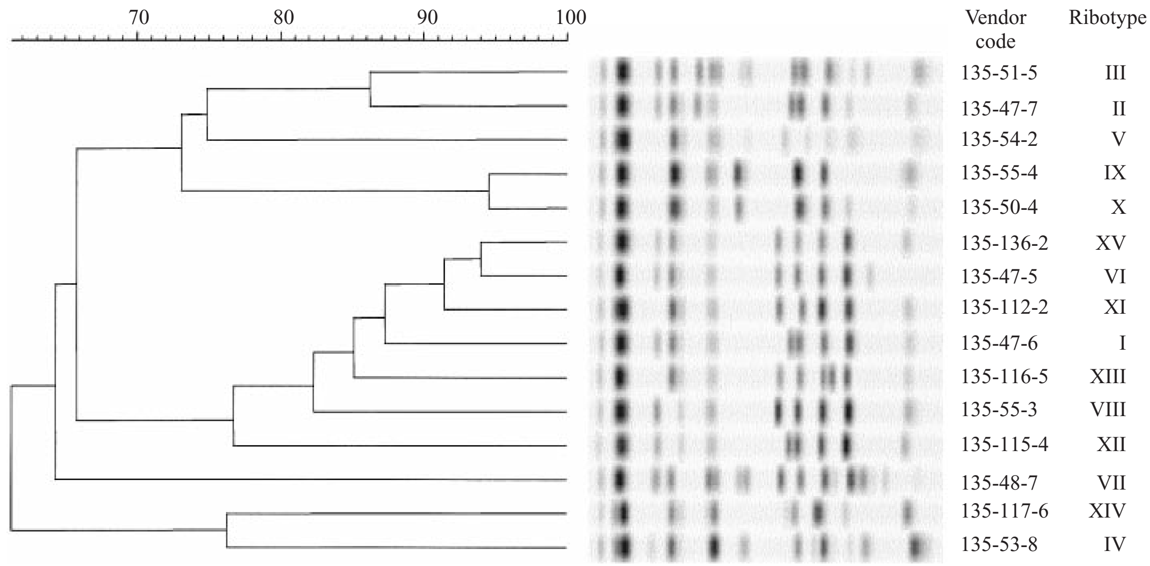

Fig. 3. Clonal relationships of 112 *S. aureus* isolates established with *Eco*RI automated ribotyping. The scale indicates the percent of similarity within this set of strains. Ribotypes I and VI were the predominent ones in this study. Control strain *S. aureus* 8325–4 exhibited ribotype X and an internal control strain derived from the same background showed ribotype IX. The remaining 13 ribotype patterns were found in bovine clinical isolates.

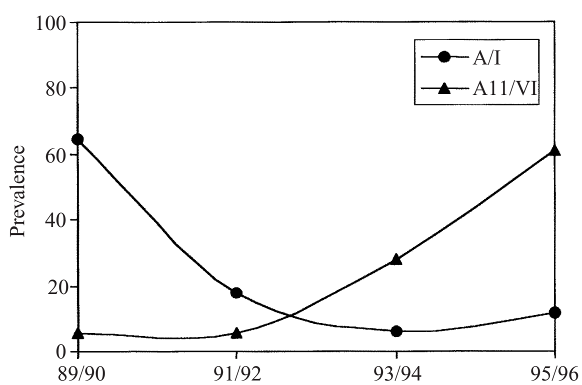

Fig. 4. Variation in prevalence of genotypes A/I and A11/VI during 1989–97. Each point represents percentages of A/I or A11/VI *S. aureus* isolates from the total number of isolates in 2-year periods.

rendered coincident results. The *S. aureus* isolates included in this study were assigned by the system to 13 ribotypes. Among these, the most prevalent ribotypes I and VI were composed of 49 and 35 isolates respectively. The remaining 28 isolates were evenly distributed in 11 groups of smaller size (Fig. 3).

Although there was certain correspondence between types obtained by PFGE analysis and ribotyping, further discrimination was achieved by combining both methods. On one hand, the 42 PFGE type A isolates were discriminated into four groups composed of 29, 7, 3 and 3 isolates according to their ribotypes (I, II, III and XII, respectively). Such discrimination, however, was not achieved when PFGE subtype A11 (19 isolates) were analysed by ribotyping. In fact, from 19 A11 isolates, 18 were ribotype VI, and the remaining A11 isolate was identified as ribotype VII. On the other hand, PFGE analysis was useful to provide further discrimination of the two largest groups composed of ribotype I and VI *S. aureus* isolates. There was certain correspondence between PFGE type A and ribotype I isolates. Indeed, 29 of 49 ribotype I isolates were PFGE type A. The remaining 20 isolates were discriminated into seven ribogroups composed of 1–5 isolates. Similarly, 18 of 35 ribotypes VI isolates belonged in PFGE subtype A11, and the remaining 17 isolates were discriminated into 10 groups of smaller size.

The most prevalent *S. aureus* strain A/I (as defined by PFGE typing and ribotyping) was widely distributed in the country and was not restricted to individual nearby locations. Indeed 29 isolates of strain A/I were found in 15 of 21 locations investigated. The second most prevalent genotype A11/VI was also widely distributed throughout distant locations in the country. Considerable strain variation was found from herd to herd within districts. Ten different PFGE/ribotype strains were isolated from the La Vacherie district from a total of 21 isolates, whereas 13 different strains were found in district Brandsen from a total of 17 isolates. Identification of six isolates obtained within a period of 2 months from district Brandsen revealed that these isolates corresponded to six different PFGE/ribotype strains. Similarly, six PFGE/ribotype strains were identified among seven isolates obtained within a 4-month period from herds in the La Vacherie district.

Prevalence of strains A/I and A11/VI varied considerably throughout the period under scrutiny, when all locations were considered together (Fig. 4). The same trend was also seen when isolates from two distant locations (17 from Brandsen and 21 from La Vacherie, districts located nearly 1100 km apart) were studied in more detail. The results of our study revealed that strain A/I was present from 1989 (high prevalence) to 1994 (low prevalence) in La Vacherie and from 1989 (high prevalence) to 1996 (low prevalence) in Brandsen. The second most prevalent strain (A11/VI) was isolated from 11 different locations from 1990 (low prevalence) to 1996 (high prevalence).

Combined use of *Sma*I PFGE and *Eco*RI ribotyping analyses permitted definition of two predominant clones, namely A/I and A11/VI. In order to test whether REAP was a method powerful enough to achieve further discrimination, two groups of A/I and A11/VI selected isolates respectively were investigated. Our experiments showed that all 11 A11/VI isolates obtained within a 5-month period from eight districts carried plasmids. These plasmids yielded 11 different REAP DNA profiles. Each profile consisted of 3–11 bands in the 9500–650 bp range (Fig. 5). It must be noted that three of these A11/VI isolates were obtained from different herds within the same district and yielded three different REAP DNA profiles. Evaluation of 14 A/I isolates obtained within a 4-month period from nine locations permitted identification of six REAP DNA profiles, which did not overlap with those found among A11/VI isolates. These profiles exhibited 2–11 bands in the range 18000–900 bp (Fig. 5) and one of the A/I isolates carried no plasmids. Two groups of 3 and 2 A/I isolates, respectively, from two districts (La Vacherie and Tandil) were discriminated by REAP DNA profile analysis. Conversely, two isolates from Brandsen (REAP type P15) and two isolates Bolívar (REAP type P14), respectively, were not discriminated from each other. Overall, the results showed that REAP DNA profile analysis was useful to provide further discrimination between isolates of identical *Sma*I PFGE genotype and ribotype.

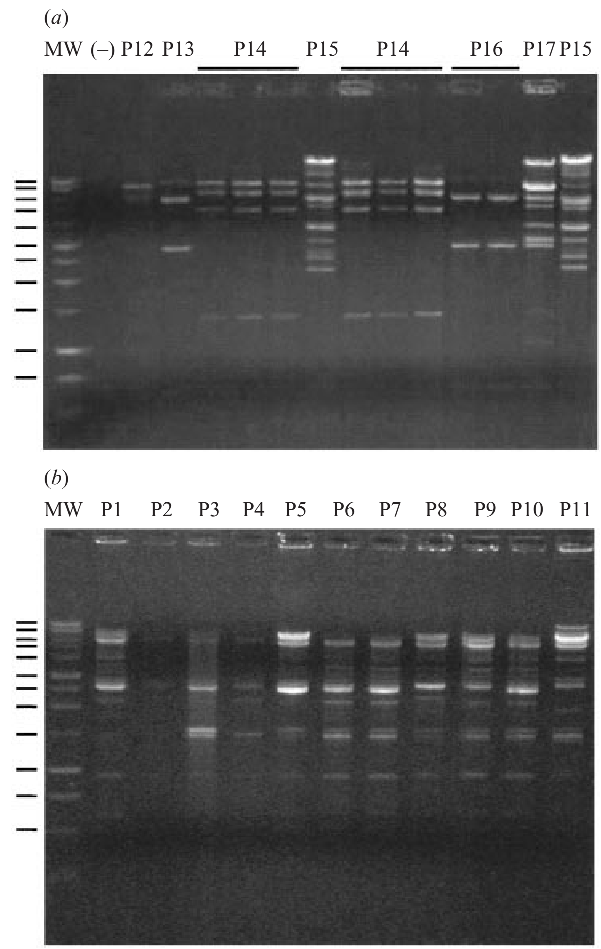

Fig. 5. Restriction enzyme (*Eco*RI) analysis of plasmid DNA profiles from selected *S. aureus* bovine isolates. REAP profiles are indicated on top of each panel. Panel *a* depicts profiles from 14 genotype A/I isolates obtained within a 4-month period. Lane (–) on panel *a* represents an isolate with no plasmids. Panel *b* depicts profiles from 11 genotypes A11/VI isolates obtained within a 5-month period. Lane MW in both panels is the 1-kb ladder (position of each band is shown on the left-hand side, 10–1 kb).

DISCUSSION

This study shows that there is a prevalent *Sma*I macrorestriction cluster in Argentina that included 90% of the *S. aureus* bovine isolates examined. Predominance of a reduced number of *S. aureus* types, as assessed by phenotypic and genotypic methods, was also observed in previous studies [11, 12, 22]. The nature of the process leading to selection of limited number of clones infecting dairy remains unclear. According to our results, strains falling within the main cluster were isolated throughout the full length of the study. Moreover, genotype A/I isolates were also found within the entire timeframe of the study, albeit at very low prevalence by the end of the period under scrutiny. Although REAP revealed marked differences in plasmid profiles among A/I isolates, our experiments suggested that, as seen through *Sma*I PFGE typing and automated ribotyping, the *S. aureus* genome may have suffered only minor changes throughout the study. In a study on different staphylococcal species, Pantucek et al. [23] concluded that there was considerable variability in *S. aureus*. The discrepancy between studies on this subject may be attributed to the different type of samples utilized. In our study, *S. aureus* isolates represent the country as a whole over several years and, for this reason, it is therefore conceivable that a *S. aureus* strain could have disseminated throughout the region and that only minimal genetic changes occurred over many years in Argentina. Whether cluster 1 strains disseminated throughout Argentina from a single source remains unknown. Further studies should be performed to ascertain whether *S. aureus* cluster 1 strains can be found in other regions outside Argentina.

There were predominant clones (A/I and A11/VI) in Argentina that in our study accounted for 41% of *S. aureus* isolates. In a previous study, Fitzgerald et al. [24] have shown that a few specialized clones may be responsible for cases of bovine mastitis and that these clones may have broad geographic distribution. It can be speculated that isolates with identical genotype may have a trait that give them an advantage over strains to survive in the environment, colonize the bovine udder and/or cause apparent disease. A previous study has shown that *S. aureus* from cows with mastitis are most refractory to killing by polymorphonuclear leukocytes [25], suggesting that a certain trait might be associated to pathogenicity in bovines. Furthermore, there is previous evidence that a limited number of *S. aureus* clones may be associated with the expression of methicillin-resistance [26], a characteristic that can be segregated under artificially applied selective pressure. Whether there is a virulence factor linked to *S. aureus* strains A/I and A11/VI is not known and deserves to be investigated.

Interestingly, investigation of *S. aureus* isolates obtained within a period of 8 years revealed a peak of prevalence of A/I strain isolates followed by a marked decrease in the prevalence of that genotype and a simultaneous increase in the prevalence of subtype A11 strains. Genetic dissimilarities between type A and subtype A11 isolates determined a 4-band difference in PFGE band patterns. According to Tenover et al. [18], isolates bearing these genotypes are possibly related and one may have derived from the other after two genetic events. The decrease in genotype A/I strains and the concomitant increase in A11/VI strain prevalences occurred in distant sites simultaneously. It can be speculated that the shift in prevalent types may have been due to use of antibiotics on diseased cows, a factor that poses selective pressure on bacterial populations [27]. Sixty-four percent of *S. aureus* isolates from mastitic bovines in Argentina are resistant to at least one currently used antibiotic [28]. Whether there was a shift in antibiotic resistance patterns of *S. aureus* from bovines in Argentina within the period 1989–97 is not known.

Analysis of plasmid DNA digested with endonuclease *Eco*RI has been used to epidemiologically investigate *S. aureus* isolates [29]. Although the relative stability of staphylococcal plasmids has often been debated, the method has been extensively utilized [8]. Indeed, REAP DNA profiling was useful in solving epidemiological problems concerning strains that are assigned to the same type by other typing techniques [30]. In our study, the method was utilized to investigate isolates obtained within a maximum period of 5 months. No isolate beyond this timeframe was compared to avoid differences attributable to plasmid instability. In fact, acquisition of a plasmid and selection of a given REAP type in response to, for instance, introduction of a new therapeutic scheme to diseased cows is a process that may take several months within a given area. From this study, it is suggested that collection of samples within a short timeframe makes REAP profiles an acceptable method to discriminate isolates with identical PFGE genotype and ribotype.

Both analysis of PFGE band patterns and automated ribotyping may be suitable methods to screen genealogical relatedness of *S. aureus* isolates from bovine milk. Both methods as performed in this study, displayed similar good levels of discrimination. An issue that has to be considered is that these methods utilize different criteria for data interpretation. Whereas the strict criteria by Tenover et al. [18] were utilized to assess the relatedness among isolates studied by PFGE typing, automated ribotyping utilizes a customized software that assigns each band pattern to a ribotype. Each band pattern is then compared with others in an existing identification database [31] and those that are statistically indistinguishable are clustered in the same ribogroup. The use of other criteria on non-automated ribotyping band patterns has led in the past to the conclusion that the method may not be useful for *S. aureus* [8].

Utilized as suggested by the vendor, automated ribotyping was as useful as PFGE typing for the purpose of this study. The choice for one or the other method may in the end depend upon equipment availability and/or cost. One feature of automated ribotyping is that the method can additionally provide confirmation of species identification. Whatever the screening method chosen it remains clear that thorough epidemiological assessment of *S. aureus* isolates should be performed with both methods, or any of these methods followed by other additional procedures.

In conclusion, a limited number of clones defined by combined use of *Sma*I PFGE analysis and automated ribotyping were found dispersed around distant locations in Argentina. Prevalence of two strains varied consecutively within a period of 8 years. Further discrimination of these clones was achieved by analysis of plasmid DNA digested with *Eco*RI. Whether the shift in type prevalence was due to selection of phenotypic trait remains undisclosed.

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REFERENCES

1. Yancey RJ. Vaccines and diagnostic methods for bovine mastitis: facts and fiction. Adv Vet Med 1999; **41**: 257–73.
2. Nickerson SC, Owens WE, Bodie RL. Mastitis in dairy heifers: initial studies on prevalence and control. J Dairy Sci 1995; **78**: 1607–18.
3. Pyörälä S, Pyörälä E. Efficacy of bovine clinical mastitis therapy during lactation. Proceedings of the XVII Nordic Veterinary Congress. Reykyavik, Iceland, 26–29 July 1994.
4. Tenover FC, Arbeit RD, Goering RV, and the Molecular Typing Working Group of the Society for Healthcare Epidemiology of America. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Infect Control Hosp Epidemiol 1997; **18**: 426–39.
5. Dalla Pozza MC, Ricci A, Vicenzoni G. Protein A gene polymorphism analysis in *Staphylococcus aureus* strains isolated from bovine subclinical mastitis. J Dairy Res 1999; **66**: 449–53.
6. Raimundo O, Deighton M, Capstick J, Gerraty N. Molecular typing of *Staphylococcus aureus* of bovine origin by polymorphisms of the coagulase gene. Vet Microbiol 1999; **66**: 275–84.
7. Rivas A, Gonzalez RN, Wiedmann M, et al. Diversity of *Streptococcus agalactiae* and *Staphylococcus aureus* ribotype recovered from New York dairy herds. Am J Vet Res 1997; **58**: 482–7.
8. Su C, Kanevsky I, Jayarao BM, Sordillo LM. Phylogenetic relationships of *Staphylococcus aureus* from bovine mastitis based on coagulase gene polymorphism. Vet Microbiol 2000; **71**: 53–8.
9. Tenover FC, Arbeit RD, Archer G, et al. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. J Clin Microbiol 1994; **32**: 407–15.
10. Van Leeuwen W, Verbrugh H, van der Velden J, van Leeuwen N, Heck M, van Belkum A. Validation of binary typing for *Staphylococcus aureus* strains. J Clin Microbiol 1999; **37**: 664–74.
11. Kapur V, Sischo WM, Greer RS, Whittam TS, Musser JM. Molecular population genetic analysis of *Staphylococcus aureus* recovered from cows. J Clin Microbiol 1995; **33**: 376–80.
12. Aarestrup FM, Wegener HC, Jensen NE, et al. A study of phage and ribotype patterns of *Staphylococcus aureus* isolated from bovine mastitis in the Nordic countries. Acta Vet Scand 1997; **38**: 243–52.
13. Myllys V, Ridell J, Bjorkroth J, Biese I, Pyörälä S. Persistence in bovine mastitis of *Staphylococcus aureus* clones as assessed by random amplified polymorphic DNA analysis, ribotyping and biotyping. Vet Microbiol 1997; **51**: 245–51.
14. Zadoks R, Van Leeuwen W, Barkema H, et al. Application of pulsed-field electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular analysis of bovine and human *Staphylococcus aureus* isolates. Infect Immun 2000; **38**: 1931–9.
15. Lange C, Cardoso M, Senczek D, Schwarz S. Molecular subtyping of *Staphylococcus aureus* isolates from cases of bovine mastitis in Brazil. Vet Microbiol 1999; **67**: 127–41.
16. Kloos WE, Bannerman TL. *Staphylococcus* and *Micrococcus*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology. Washington DC: ASM Press, 1995: 282–98.
17. Maslow JN, Slutsky AM, Arbeit RA. Application of pulsed-field gel electrophoresis to molecular epidemiology. In: Persing DH, Smith TF, Tenover FC, White TJ, eds. Diagnostic molecular microbiology. Principles and application. Washington DC: ASM Press, 1993: 563–72.
18. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting

chromosomal DNA restriction patterns produced by pulsed-filed gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.
19. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 1979; **7**: 1513–23.
20. Dice LR. Measures of the amount of ecological association between species. Ecology 1945; **26**: 297–302.
21. Sneath PHA, Sokal RR. Numerical Taxonomy. San Francisco: W. H. Freeman & Co., 1973.
22. Musser JM, Selander RK. Genetic analysis of natural populations of *Staphylococcus aureus*. In: Novick RP, ed. Molecular biology of the *Staphylococcus*. New Tork: VCH Publishers, 1990; **59**: 67.
23. Pantucek R, Gotz F, Doskar J, Rosypal S. Genetic variability of *Staphylococcus aureus* and the other coagulase-positive *Staphylococcus* species estimated by macrorestriction analysis using pulsed-field gel electrophoresis. Int J Syst Bacteriol 1996; **46**: 216–22.
24. Fitzgerald JR, Meaney WJ, Hartigan PJ, Smyth CJ, Kapur V. Fine-structure molecular epidemiological analysis of *Staphylococcus aureus* recovered from cows. Epidemiol Infect 1997; **119**: 261–9.
25. Aarestrup FM, Scott NL, Sordillo LM. Ability of *Staphylococcus aureus* to resist neutrophil bactericidal activity and phagocytosis. Infect Immun 1994; **62**: 5679–82.
26. Kreiswirth B, Komblum J, Arbeit RD, et al. Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. Science 1993; **259**: 227–30.
27. Wright BE. A biochemical mechanism of nonrandom mutations and evolution. J Bacteriol 2000; **182**: 2993–3001.
28. Gentilini E, Denamiel G, Llorente P, Godaly S, Rebuelto M, DeGregorio O. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Argentina. J Dairy Sci 2000; **83**: 1224–7.
29. Zuccarelli AJ, Roy I, Harding P, Couperus JJ. Diversity and stability of restriction enzyme profiles of plasmid DNA from methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 1990; **28**: 97–102.
30. Aarestrup FM, Wegener HC, Rosdahl VT. Evaluation of phenotypic and genotypic methods for epidemiological typing of *Staphylococcus aureus* isolates from bovine mastitis in Denmark. Vet Microbiol 1995; **45**: 139–50.
31. Bruce J. Automated system rapidly identifies and characterizes microorganisms in food. Food Technol 1996; **50**: 77–81.