



BRIEF REPORTS

Genetic patterns of *Streptococcus uberis* isolated from bovine mastitis



Elina B. Reinoso, Mirta C. Lasagno, Liliana M. Odierno*

Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina

Received 24 August 2014; accepted 9 February 2015

Available online 7 April 2015

KEYWORDS

Streptococcus uberis;
Bovine mastitis;
Pulsed-field gel
electrophoresis;
Virulence

Abstract The aim of this study was to evaluate the genotypic relationships among 40 *Streptococcus uberis* isolated from bovine mastitis by using pulsed-field gel electrophoresis (PFGE). Additionally, the association between PFGE patterns and virulence profiles was investigated. The isolates exhibited 17 PFGE patterns. Different strains were found within and among herds; however, a low number of isolates within the same herd shared an identical PFGE type. No association between PFGE patterns and virulence profiles was found. However, the detection of specific strains in some herds could indicate that some strains are more virulent than others. Further research needs to be undertaken to elucidate new virulence-associated genes that might contribute to the capability of these strains to produce infection.

© 2014 Asociación Argentina de Microbiología. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

PALABRAS CLAVE

Streptococcus uberis;
Mastitis bovina;
Electroforesis de
campos pulsantes;
Virulencia

Perfiles genéticos de *Streptococcus uberis* aislados de mastitis bovina

Resumen El objetivo del presente estudio fue evaluar las relaciones genotípicas entre 40 *Streptococcus uberis* aislados de mastitis bovina mediante la técnica de electroforesis de campos pulsantes (*pulsed-field gel electrophoresis* [PFGE]). Además, se investigó la asociación entre los patrones de PFGE y los perfiles de virulencia. Los aislamientos mostraron 17 patrones de PFGE. Se encontraron diferentes cepas dentro de los tambos y en los distintos tambos, y un bajo número de aislamientos dentro del mismo tambo compartieron un perfil idéntico de PFGE. No se encontró ninguna asociación entre los patrones de PFGE y los perfiles de virulencia. Sin embargo, la detección de cepas particulares en algunos tambos podría indicar

* Corresponding author.

E-mail address: lodierno@exa.unrc.edu.ar (L.M. Odierno).

que algunas de ellas son más virulentas que otras. Sería importante avanzar en las investigaciones para identificar nuevos genes relacionados con la virulencia que podrían contribuir a la capacidad infecciosa de estas cepas.

© 2014 Asociación Argentina de Microbiología. Publicado por Elsevier España, S.L.U. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Although an increasing prevalence of *Streptococcus uberis* mastitis has been reported throughout the world, it has not been conclusively proven that certain clones with enhanced virulence are responsible for mastitis. In an attempt to differentiate *S. uberis* strains, different typing methods have been developed. Pulsed-Field Gel Electrophoresis (PFGE) is considered a reference technique due to its high discriminatory power and reproducibility. Previous reports have shown evidence of heterogeneity in the *S. uberis* population using various typing schemes. However, the extent of this is not well defined and its significance to disease pathogenesis has not been determined. Genomic typing of *S. uberis* was conducted on a number of samples from different countries^{1–3,5,6,11}. Our research group is pioneer in working on molecular typing studies of bovine isolates of *S. uberis* by PFGE in South America⁴. As yet, there have been no reports on the association between PFGE patterns and virulence profiles among *S. uberis* isolates from mastitis in Argentina. The aim of this study was to evaluate the genetic relationships among 40 *S. uberis* isolates by PFGE and to determine whether certain PFGE patterns are associated with the most frequent virulence profiles. A total of 78 *S. uberis* isolates were collected from bovine subclinical mastitis from 17 herds located in the central region of Argentina in a previous study⁷.

In the present study, forty isolates selected among 78 *S. uberis* previously characterized⁷ were used. The distribution of virulence-associated genes as *cfu*, *gapC*, *hasA*, *hasB*, *hasC*, *lbp*, *oppF*, *pauA/B*, *skc*, *sua* was also determined in the mentioned study. The present work included 15 isolates belonging to the 10 most frequent virulence profiles and 25 isolates belonging to 23 virulence profiles less frequently detected, as previously described⁷. PFGE typing with *SmaI* was performed as described by Lasagno *et al.*⁴ Briefly, DNA fragments were separated using CHEF-DRII (Bio-Rad) with TBE as running buffer. Electrophoresis was carried out at a voltage gradient of 6 V/cm for 20 h (14 °C) with pulse times increasing linearly from 1 to 20 s. Lambda ladder PFGE markers (50–1000 kb; Promega) were included as molecular size standards. On completion of the assay, the gel was stained with 0.5 µg/ml of ethidium bromide and banding patterns were visualized using the MiniBisPRO gel documentation system (Micro Photonics Inc., Allentown, USA). Patterns obtained were subjected to the guidelines for interpretation of PFGE based on differences in banding patterns posed by Tenover *et al.*⁹ A cutoff value of 80% of genetic similarity among isolates was chosen for discrimination between distinct clusters. Dendrograms were generated by using the Dice coefficients and clustering was done by the unweighted-pair group method with arithmetic averages (UPGMA). Associations between PFGE patterns and virulence

Table 1 PFGE pattern distribution of *Streptococcus uberis* isolated on each dairy farm.

Farm number	PFGE patterns
I	A
II	A
III	A, A, F, G
IV	A
V	A, A, A, G
VI	A, A, C, C, E, G, H, I, P
VII	A, B, C, Q
VIII	A
IX	A
X	H
XI	C, K
XII	C, M
XIII	C, O
XIV	C, D
XV	J
XVI	L, L, Q
XVII	N

genes were examined by the chi-square test. The obtained PFGE patterns yielded 7–10 distinctive fragments from 23 to 340 kb size range. The assays were repeated twice. Among the 40 isolates, 17 different PFGE patterns (A–Q) were observed. DNA restriction with *SmaI* displayed both identical and non-identical PFGE patterns. To determine the degree of similarity of the 17 different PFGE patterns, the 40 isolates were examined by dendrogram analysis. All the isolates were divided into 10 major groups, using a cutoff value of 80% (Fig. 1). Pattern A was the most common, comprising 13 isolates (32.5%) distributed among 9 herds. A similar result was observed for pattern C, which clustered seven isolates (17.5%) distributed among 6 herds. The remaining 15 patterns were less frequent and clustered from 1 to 3 isolates. No evidence of an identical PFGE pattern in different herds was reported by most of other PFGE-based *S. uberis* epidemiological studies^{1,5,10,11}. Table 1 shows the PFGE pattern distribution of the 40 *S. uberis* isolates on each dairy farm. Different strains were found within and among herds. Of the 17 herds only herd VI had nine isolates, which were clustered in 7 PFGE patterns. Pulsotypes A and C comprised 2 and 2 isolates, respectively (Table 1). Within herds VII, XI, XII, XIII and XIV no common PFGE patterns were found. The results obtained in this study show that *S. uberis* is a highly heterogeneous species with multiple PFGE patterns present in a single dairy herd, suggesting

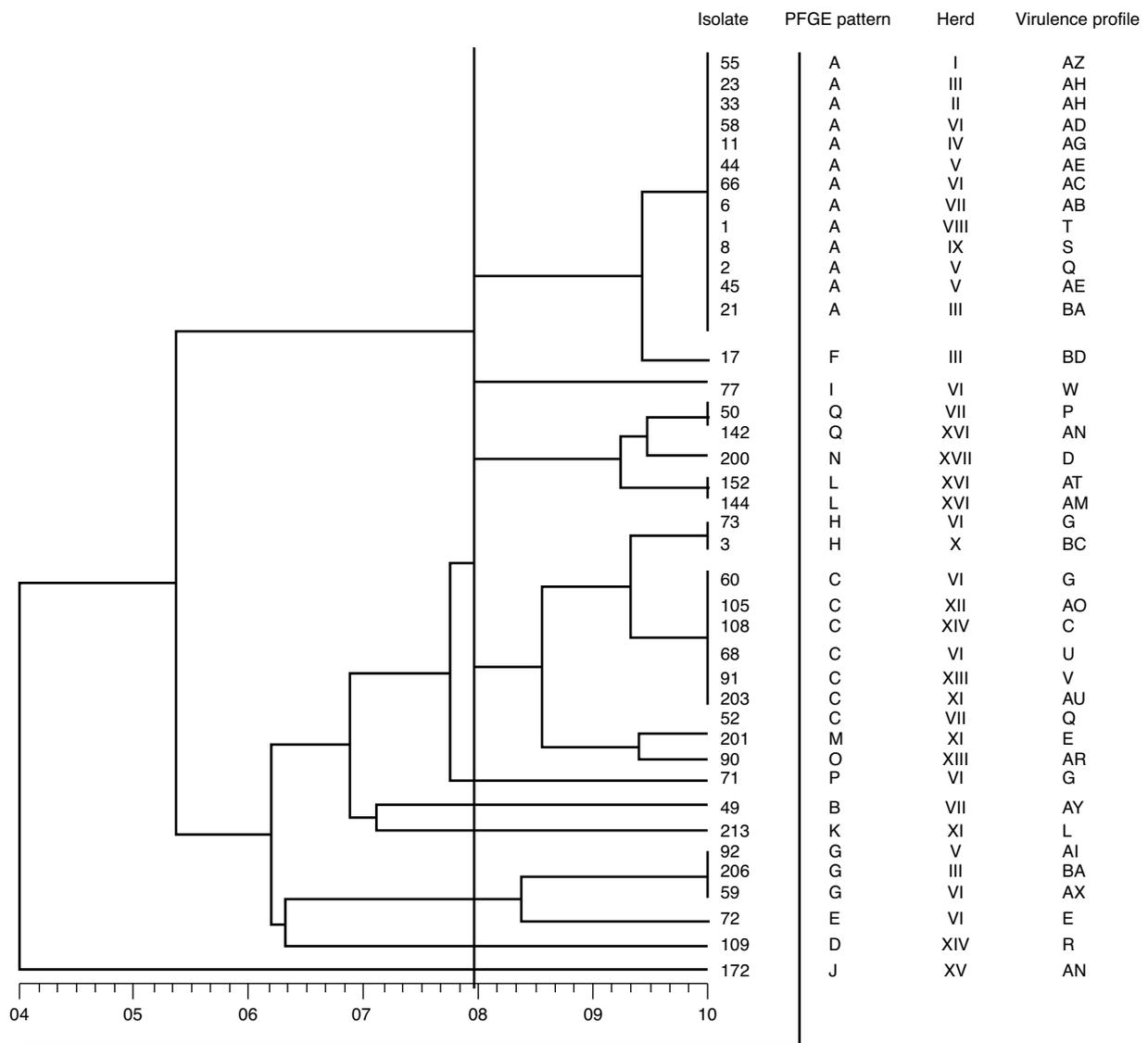


Figure 1 Dendrogram of similarity among the observed PFGE patterns of *SmI* digested DNAs from *Streptococcus uberis* isolates.

that the species is behaving as an opportunistic pathogen and might cause mammary gland infection due to the environmental contamination of the gland. Similar results were demonstrated in previous studies^{1-3,5,6,11} conducted on cow milk samples from dairy herds located in different regions. Identical results have been recently reported for the first time in Argentina by our research group⁴. In the present study there were three isolates of identical PFGE types (pulsotype A) recovered from herd V, suggesting that this clone is predominant in this herd. Although the number of isolates of *S. uberis* examined in each herd was too small in some cases to draw firm conclusions, these data suggest the presence of a single clone that was transmitted either between cows or acquired from a common source. They support the findings from previous studies^{1,5,10,11}, which reported that these events seemed to be uncommon. Phuektes *et al.*⁶ also reported the occurrence of a genetically diverse population; however, the authors demonstrated that two strains predominated at higher prevalence (>20%) in two of the four herds. A study carried out by Shome *et al.*⁸ was aimed

to investigate the virulence characteristics of streptococci from bovine milk and to assess the molecular epidemiology and population structure of the Indian isolates using multi-locus sequence typing (MLST) and PFGE. The comparative analysis of MLST and PFGE results indicated that isolates belonging to the same sequence type exhibited similar or related PFGE patterns for both *S. agalactiae* and *S. uberis* although restricted to a specific herd. From our results, it can be inferred that specific strains belonging to PFGE patterns A and C in some herds could have some advantages of infecting likely due to a special combination of virulence factors. In a previous report we examined 11 virulence-associated genes by PCR⁷. We found 58 different virulence profiles, and 33 (42.3%) *S. uberis* isolates belonged to the 12 most frequent profiles. The diversity of virulent profiles of selected isolates does not allow to draw firm conclusions on the diversity of virulent genes detected among the PFGE patterns. However, the data show the presence of *hasC*, *sua*, *cfu*, and *gapC* genes in most of the strains belonging to patterns A and C. Our data reveal that most of the isolates

(62.5%) had different virulence profiles. In this sense, A and C PFGE patterns grouped 50% of the isolates and showed 11 and 7 different virulence profiles, respectively. A possible relationship between virulence profiles and PFGE patterns was examined by the chi-square test and no association was found ($p > 0.05$). In a previous study, Wang *et al.*¹¹ did not report the possible existence of clones with enhanced virulence; however, these authors speculated that the great genetic diversity of *S. uberis* may indicate that virulence is not associated with any specific molecular type. Considering that no association was found between the virulence profiles and PFGE patterns in this study, four isolates were the exception. *S. uberis* strains 44 and 45 isolated from herd V and designated as the same strain by PFGE (pattern A) had a common virulence profile (AE). Additionally, only two strains (33 and 23) isolated from different herds (II and III) sharing the same PFGE pattern (A) were found to have the same virulence profile (AH). The present study describes the *S. uberis* genotypes responsible for the mastitis cases and shows that no association between virulence profiles and PFGE patterns was found among *S. uberis* isolates recovered from subclinical mastitis. Although there is no predominant PFGE pattern, the existence of specific strains in some herds belonging to more frequent patterns, could indicate that some strains may be more virulent than others. Further research needs to be undertaken to elucidate new virulence-associated genes that might contribute to the capability of these strains to produce infection.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Douglas VL, Fenwick SG, Pfeiffer DU, Williamson NB, Holmes CW. Genomic typing of *Streptococcus uberis* from cases of mastitis, in New Zealand dairy cows, using pulse field gel electrophoresis. *Vet Microbiol.* 2000;75:27–41.
2. Jayarao BM, Gillespie BE, Lewis MJ, Dowlen HH, Oliver SP. Epidemiology of *Streptococcus uberis* intramammary infections in a dairy herd. *J Vet Med B.* 1999;46:433–42.
3. Khan IU, Hassan AA, Abdulmawjood A, Lämmler C, Wolter W, Zschöck M. Identification and epidemiological characterization of *Streptococcus uberis* isolated from bovine mastitis using conventional and molecular methods. *J Vet Sci.* 2003;4: 213–23.
4. Lasagno MC, Reinoso EB, Dieser SA, Calvino LF, Buzzola F, Vissio C, Bogni CI, Odierno LM. Phenotypic and genotypic characterization of *Streptococcus uberis* isolated from bovine subclinical mastitis in Argentinean dairy farms. *Rev Argent Microbiol.* 2011;43:212–7.
5. Mc Dougall S, Parkinson TJ, Leyland M, Anniss FM, Fenwick SG. Duration of infection and strain variation in *Streptococcus uberis* isolated from cows's milk. *J Dairy Sci.* 2004;87:2062–72.
6. Phuektes P, Mansell PD, Dyson RS, Hooper ND, Dick JS, Brownling GF. Molecular epidemiology of *Streptococcus uberis* isolates from dairy cows with mastitis. *J Clin Microbiol.* 2001;39: 1460–6.
7. Reinoso EB, Lasagno MC, Dieser SA, Odierno LM. Distribution of virulence-associated genes in *Streptococcus uberis* isolated from bovine mastitis. *FEMS Microbiol Lett.* 2011;318:183–8.
8. Shome BR, Bhuvana M, Mitra SD, Krithiga N, Shome R, Velu D, Banerjee A, Barbuddhe SB, Prabhudas K, Rahman H. Molecular characterization of *Streptococcus agalactiae* and *Streptococcus uberis* isolates from bovine milk. *Trop Anim Health Prod.* 2012;44:1981–92.
9. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed field gel electrophoresis: criteria for bacterial strains typing. *J Clin Microbiol.* 1995;33:2233–9.
10. Tomita T, Meehan B, Wongkattiya N, Malmo J, Pullinger G, Leigh J, Deighton M. Identification of *Streptococcus uberis* multilocus sequence types highly associated with mastitis. *Appl Environ Microbiol.* 2007;74:114–24.
11. Wang SM, Deighton M, Capstick JA, Gerraty N. Epidemiological typing of bovine streptococci by pulsed-field gel electrophoresis. *Epidemiol Infect.* 1999;123:317–24.