GENOTYPING OF *Staphylococcus aureus* ISOLATED FROM DAIRY HERDS IN MEXICO

Genotipificacion de *Staphylococcus aureus* aislado de vacas lecheras en México

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ABSTRACT

In the present work, *Staphyloccous aureus* field strains were isolated from 27 mastitic cows representing 12 dairy herds. This was selected of almost 3,000 field strains of mastitic cows. The strains were subjected to different Polymerase Chain Reaction (PCR) to detect the toxin encoding genes SEA, SEB, SEC, SED, SEE, SEG, SEJ and TST genes. The investigated strains were then subjected to fingerprinting by the means of Pulse Field Gel Electrophoresis (PFGE). The screening for the previously mentioned toxin encoding genes revealed the absence of all toxin encoding genes with the exception of SEI which could be detected in a single strain. Meanwhile, the data obtained through the PFGE analysis indicated the close relationship of *S. aureus* field strains responsible for the induction of mastitis in western Mexico.

Key words: Bovine mastitis, *Staphylococcus aureus*, molecular biology, enterotoxins, patogenic bacterius, genotipic relationship.

RESUMEN

En la presente investigación fueron aisladas cepas de campo de *Staphyloccous aureus* de 27 vacas con mastitis, que representan a 12 hatos lecheros; las cepas de campo se seleccionaron de un total de casi 3.000 cepas aisladas. Las cepas fueron sujetas a las pruebas de Reacción en Cadena con Polimerasa (PCR) para detectar las toxinas codificadas por los genes SEA, SEB, SEC, SED, SEE, SEG, SEJ y el TST. Las cepas fueron investigadas mediante la técnica de fingerprinting, con la electroforesis de campos pulsátiles (PFGE). El monitoreo de los genes que codifican las toxinas fue negativo, con excepción de la SEI, la cual fue detectada en una cepa. Es muy significativo hacer notar que los resultados obtenidos mediante el análisis de PFGE indican que hay una relación estrecha entre las cepas de campo de *S. aureus* responsables de la mastitis de las vacas lecheras en el Occidente de México.

Palabras clave: Mastitis bovina, *Staphylococcus aureus*, ganado lechero, biología molecular, enterotoxinas, bacterias patógenas, relaciones genotípicas.

INTRODUCTION

Mastitis is one of the main problems facing dairy industry. Economic loses due to clinical mastitis were estimated to be about 35 Billion US Dollars per year [4, 45]. One of the major causative agents of mastitis is the *Staphylococcus aureus* which is responsible for most cases of mastitis worldwide. It is considered to be the most important intra mammary infection in dairy herds [18] and was detected in 25-30% of mastitic cows [40]. S. aureus could also be cultured from 20-60% of subclinical mastitic milk samples [8, 9]. The *S. aureus* incidents proved in Jalisco -–5.8% of the whole milk samples– was more frequently proved in herds with a low cell count in the collective milk and less frequently proved in those herds with a high cell count in the collective milk [46]. For disease induction, *S. aureus* developed different virulence factors including the production of a large variety of toxins such as ETA, ETB, TSST and the enterotoxins SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER and SEU [16, 21, 23, 30, 32, 33, 36, 48] . Larsen et al. [20] examined 414 bovine *S. aureus* strains. Only one of them held genes for the production of SEC and TSST-1. Out of 166 bovine *S. au-*

Recibido: 04 / 02 / 2011. Aceptado: 07 / 04 / 2011.

reus isolates from Korea, examined by Lim et al. [22], 32 isolates held the SEA gene, three isolates the SEB gene, and only one the SEC gene. Also Zschöck et al. [51] examined by means of the Polymerase Chain Reaction (PCR) a sum of 533 *S. aureus* mastitis isolates concerning the occurrence of the SEC gene and the TSST-1 gene. It showed a prevalence of 9.3%. In another examination of 104 strains from German cattle farms Zshöck et al. [52] could prove that 58.7% held one or a combination of the new enterotoxins SEG - SEJ. Out of these positive strains 60.7% additionally produced one of the classical enterotoxins SEA, SEE and TSST-1.

The contamination of milk and milk products leading to enterotoxicosis is not caused by the primary contamination of the suffering animal but by secondary contamination of milk through infected wounds and eczema on the hands of the personnel [26]. In contrast, other authors suppose that an animal origin, namely the milk of infected animals, is the main source for enterotoxins forming *Staphylococci* [6, 7, 19].

In raw milk, the forming of toxins is impeded by the natural flora of mainly proteolytic and saccharolytic bacteria with a short generation time. But there are special circumstances when residues of anti-infective are still in the milk which impedes the development of the natural milk flora. They enable resistant enterotoxin forming *Staphylococci* to proliferate [41, 49, 50]. In Mexico, which produces 1.9% of the global milk production, a special attention to mastitis is given [28]. With the exception of SEI, the screening for various enterotoxin genes could not detect any of them among *S. aureus* population [11].

It was found that significant genetic differences exist between *S.aureus* isolates responsible for the induction of clinical mastitis and those causing subclinical mastitis. The ability of certain isolate to develop certain forms of the disease depends on the available combinations of the genes encoding such virulence factors [11, 13, 37].

The aim of the present work was to investigate the role of enterotoxins in native *S. aureus* field isolates of Mexico in disease induction and to study the genetic relationship among field isolates.

MATERIALS AND METHODS

Isolation of bacterial strains. In the present work, 27 *S. aureus* field strains were isolated from 12 different herds suffering from mastitis in the Jalisco State, Mexico. The farms represent all territories of Jalisco in order to offer a regional epidemiological map for the causative agents – genotypes of mastitis in the State.

Biochemical identification. In addition to colony morphology and type of hemolysis produced, suspected colonies were extra identified by Catalase Test [5] and Tube Coagulase Test [15]. The results obtained by the biochemical identification were confirmed by the application of species specific PCR.

Molecular identification – PCR. For molecular confirmation of the results obtained through the biochemical identification, the *S. aureus* species specific primer pair Staur4 – Staur6, was used, Staur4 (5'- ACG GAG TTA CAA AGG ACG AC -3') and reverse primer genStaur6 (5'-AGC TCA GCC TTA ACG AGT AC-3'). For this purpose, the suspected colonies were picked up, subcultured on blood agar and were incubated (Incubator Heraeus 50L, Germany) overnight at 37°C. For the extraction of bacterial DNA, 5-10 colonies were suspended in 100µlLTE buffer before adding 5µL lysostaphin (1.8 U/µL) (Sigma, Deisenhofen, Germany). Subsequently, the mixtures were incubated at 37°C for 1 h to enhance cell wall lysis. Followed by the addition of 10 µl proteinase K (14.8 mg/mL, Roche, Mannheim, Germany), the tubes were then re-incubated at 56°C for 2 h. The samples were then boiled for 10 min in order to inactivate the proteinase K. Finally, the mixtures were centrifuged (Centrifuge Sigma 1-14, Germany), (1 min, 10,000 g), and the supernatant was frozen (freezer Liebherr, Germany) at –20°C till being used as a template for the PCR reactions, as a negative control *S. epidermidis* was entrained in line 1, which did not produce an amplificate.

Oligonucleotide primers and thermocycler programs. In the present work, the PCR was used to confirm the colonies identity and to detect their virulence profiles based on the availability of SEA, SEB, SEC, SED, SEE, SEG, SEJ and TSST-1 encoding genes.

The reaction mixture (20 µL) contained 12.9 µL distilled water, 0.7 µL of both forward and reverse primers (10 pmol/µL), 0.4 µL of the dNTP (Biozym, Germany), 2 µL of 10X PCR buffer (Sigma, Germany), 1.2 µL of $MgCl_2$ (25 mmol, Sigma), 0.1 µL of Taq DNA polymerase (5 U/µL, Sigma). Finally, the template DNA (2 µL) was added separately in each reaction tube. The reaction types were then put into the thermocycler (T3 Thermocycler, Biometra, Germany). The used primer sequences and programmes are listed in TABLE I. The PCR products were visualized in a 2% agarose gel (Roth, Karlsruhe, Germany) in 35 ml TBE buffer (40 mmol/L Tris, 1% [v/v] acetic acid, 1 mmol/L EDTA). The gels were loaded by 8µL of the PCR product mixed with 3 µL sample buffer (50% glycerine, 50 mmol/L EDTA, 0.25% bromphenole-blue). In addition to the samples, a marker was added to the gel (GeneRuler^{TM} DNA Ladder Mix, MBI Fermentas, St. Leon-Rot; 100Bp DNA Ladder, Gibco/BRL, Eggenstein; DNA length standard VIII, Roche, Mannheim; DNA length standard VI, Roche). The gels were subjected to electrophoretic separation for 1 h at 100 V. Before being stained with Ethidium Bromide for 20 min (5 µg/mL, Sigma, Deisenhofen, Germany). The gels were evaluated under UV light (gel documentation system Image Master, VDS, Pharmacia Biotech, Freiburg, Germany).

Macrorestriction analysis of genomic DNA (Pulsed field gel electrophoresis- PFGE). For this aim, the genomic DNA of 27 isolates was prepared according to a modified method [11, 24, 43]. The 27 isolates were selected as they

TABLE I
PULSE FIELD GEL ELECTROPHORESIS (PFGE) CONDITIONS

	1st run	2nd run
voltage	5 V/cm	6 V/cm
initial switching time	0.1 sec	9.0 sec
final switching time	11.0 sec	40.0 sec
duration	8 h	17 h

showed 27 different virulence profiles concerning the toxin genes SEA, SEB, SEC, SED, SEE, SEG, SEJ, and TSST-.1.

In general, 1-4 isolates were selected per herd. The pure cultures of the investigated isolates were spread on blood agar, before being inoculated into 40 mL THB and incubated for 24 h at 37°C under aerobics conditions. This was followed by the centrifugation of the cultures for 10 min at 4,000 g. The pellets were then harvest and washed in a TE buffer (10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA) twice. After short re-centrifugation, the pellet was resuspended in 0.25 mL TE-buffer and set photometrically at a wavelength of 620 nm (Spectronic 20, Bausch and Lomb, New York) on 5% transmission.

Four agarose blocks per culture were prepared. The bacteria suspensions were warmed to 55°C (Block heater, Stuart, SHB 130 10, Poland). From which 200 µL were mixed thoroughly with 200 µL of an 1% low melting, low gelling in Cert agarose (Biozym Diagnostik, Germany). The mixture was then pipetted into a stamped depression (100µL, BioRad, Munich, Germany). The lysis of the bacteria cell wall was performed in 200µL lysis buffer (6 mmol/L Tris [pH 7.6], 1 mol/L NaCl, 10 mmol/L EDTA [pH 7.6], 0.5% Brij-58 (polyoxyethylene 20 cetyl ether (Sigma, Deisendorf, Germany), 0.2% natriumdodecylsulfate (Sigma), 0.5% sodium lauroylsarcosine (Sigma), adding 5 mg/mL lysozyme (Sigma) and 0.5 U/mL lysostaphine (Sigma) overnight at 37°C.The lytic enzymes were then deactivated by the addition of proteinase K (final concentration 0.5µg/mL; Boehringer, Germany) followed by overnight incubation at 56°C. After washing the blocks twice with 200 µL TE buffer, they were incubated for 30 min at room temperature. The blocks were again washed with 200µL TE buffer twice before adding phenylmethylsulfonylfluoride (final concentration 1.0 mmol; Sigma). The blocks were then incubated for 1 h at 56°C for the inactivation of proteinase K. Followed by a double washing step with 200 µL TE buffer and finally the blocks were incubated for 30 min at room temperature. For the digestion of the genomic DNA with restriction enzymes, digestion with *SmaI* restriction endonuclease (Promega, Mannheim, Germany) was applied. The restriction enzyme buffer (per block) composed of Aqua distilled (158 µL), 20µL of buffer J (Promega), 20µL of bovine serum albumin (10 x BSA, Promega) and finally 2µL of the restriction enzyme *(SmaI)*.

The agarose blocks were incubated for 5h at 25°C. For the separation of the fragments, the agarose blocks were bedded in a 1% agarose gel (1.1g agarose in 110 mL 0.5 x TBE buffer (45mmol/L Tris, 45 mmol/L borate, 1.0 mmol/L EDTA, pH 8.0). After placing the blocks into the slots of the gel, 1% agarose gel was used to close the slots. This was followed by the electrophoresis in 2 L 0.5x TBE buffer in a Chef-DR II electrophoresis apparatus followed (Bio-Rad, Munich, Germany) at 14°C. The used program was: voltage (5 V/cm, 6 V/cm), initial switching time (0.1 sec, 9.0 sec), final switching time (11.0 sec, 40.0 sec) and the duration (8 h, 17 h) for the first and second runs, respectively. As a marker, the γ ladder PFGE marker (Bio-Rad) was used. Afterwards, the gel was stained.

Evaluation of the PFGE pattern. The electrophoretic band patterns delivered through PFGE were evaluated visually by two independent persons. According to Tenover et al. [42] isolates were not differentiated and belonged to an identical macro-restriction type, when their band patterns completely corresponded. When two patterns had the same number of bands and differed only in one band, it was judged as a loss or absorption of DNA. Isolates with those band patterns were classified as subclones of the corresponding PFGE genotype. The same was considered for those isolates which showed a difference in two or three bands, due to an inclusion or loss of an interface for the restriction enzyme. As far as isolates, which were classified as subclones of one PFGE genotype, were related epidemiologically, i. e. originated in one herd, the patterns were categorised as identical. In case of a larger difference of the band patterns the isolates were categorised as different and classified into different PFGE genotypes.

RESULTS AND DISCUSSION

Different bacterial pathogens were isolated form quarter foremilk samples. The TABLE II shows the complete results in quarters, cows and herds. All investigated *S. aureus* cultures were positive in the tube coagulase test, and the cultures that showed different biochemical pattern were not included. The partial amplification of the species specific 23S rRNA gene with the oligonucleotide primer Staur4 and Staur6 delivered in all isolates an universal amplicon size of 1.270 base pairs (Bp) FIG. 1.

The screening for the previously mentioned toxin encoding genes revealed the absence of all toxin encoding genes with the exception of SEI which could be detected in a single strain.

Fiveteen different macrorestriction patterns could be differentiated by the macrorestriction analysis of the 27 *S. aureus* cultures and the subsequent separation of the fragments in the PFGE. Between 12 and 17 fragments per isolate could be depicted. In most of the cases a complete conformity of the patterns was found. In the farms 5 and 10 slight differences (one band, which moved less far during the electrophoresis) were proved. In farm 2, the isolate showed differences in two bands. Isolates related epidemiologically, which originated from one

TABLE II
RESULTS OF THE BACTERIOLOGICAL EXAMINATIONS OF QUARTER FOREMILK SAMPLES IN QUARTERS, COWS AND HERDS

Pathogen bacteria	Quarter prevalence	Cow prevalence	Herd prevalence
CNS	464 (15.4%)	295 (15.4%)	33 (100%)
Corynebact. spp.	417 (13.9%)	227 (30.2%)	30 (90.1%)
S. agalactiae	200 (6.6%)	111 (14.8%)	19 (57.6%)
S. aureus	175 (5.8%)	118 (15.7%)	22 (66.7%)
Coliform pathogens	123 (4.1%)	109 (14.5%)	27 (81.8%)
Streptococcus spp.	109 (3.6%)	95 (12.6%)	30 (90.1%)
Total	2979 (100%)	752 (100%)	33 (100%)

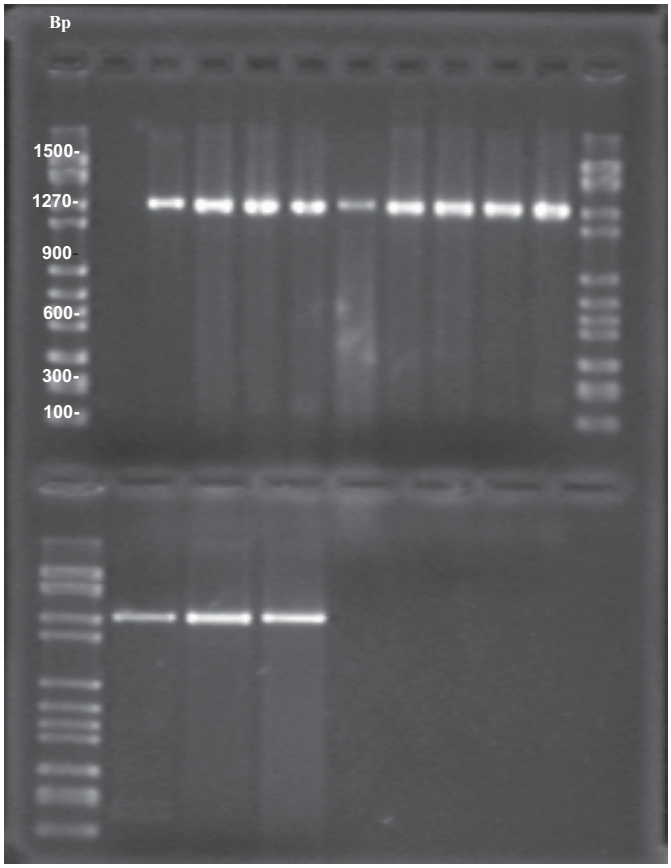

FIGURE 1. AMPLIFIED OF THE SPECIES SPECIFIC AREA OF THE 23S rRNA GENE OF *S. AUREUS* WITH AN UNIFORM AMPLIFICATE OF 1,270 BASE PAIRS. M = MOLECULAR WEIGHT MARKER. AS A NEGATIVE CONTROL *S. EPIDERMIDIS* WAS ENTRAINED IN LINE 1, WHICH DID NOT PRODUCE AN AMPLIFICATE.

single farm and differed only in 1-3 bands were valued as subclones of the corresponding PFGE genotype and signified in TABLE III as a/b. The remaining PFGE genotypes could be differentiated clearly by more than four deviant bands and were signified in TABLE III.

Notably, many of the macrorestriction types differed in only 1-3 bands between the examined farms. This leads to the conclusion of a close relationship between the isolates. Isolate VII differed from XXV and XXVIIc in only one single band, and from isolate XXIIa in only two bands. Isolate Xc and XIIc seemed to be identical and differed from isolate XXVIIb in only one single band. Also the isolates IXa and XXXI showed a difference in only one band comparing their band patterns (FIGS. 2, 3 and 4).

A total of 27 isolates of *S. aureus* obtained from 12 different herds suffering mastitis in Jalisco State in Mexico were analyzed by PCR to determine the presence of encoding genes for enterotoxins SEA, SEB, SEC, SED, SEE, SEG, SEJ and TST-1. All were negative except SEI, was detected in 1 strain (4.2%). Genes for the SEA, SEB, SEC, SEE, SEG, SEJ, and the TSST-1 could be proved in none of the examined strains.

This is not a surprising result compared to the findings of Puig de C et al. [34]. In 27 strains from Argentina they could not prove any enterotoxin or TSST-1 producing isolate. The worldwide geographic distribution of enterotoxin producing *S. aureus* strains is rather differing. Examinations in Denmark did not show any enterotoxins in the *S. aureus* strains, whereas in the other Scandinavian countries they could be proved rather frequently [44]. Eissa [10] found out, that obviously the egc (enterotoxin gene cluster) and the number of spa-gene-repeats act a part in the pathogenesis of mastitis caused by *S. aureus* in Hessian farms. Here generally, the phenotypic β-haemolysing field strains seem to be of decisive importance, for those field strains show a statistically higher significant virulence potential. Also, the number of spa-gene-repeats could serve as a marker, for they are positively associated with different virulence genes.

For the proof of epidemiologic relationship between the farms and within one farm, the macrorestriction analysis of the whole DNA of 27 *S. aureus* strains from 12 farms was applied. According to Kapur et al. [17] a more detailed knowledge about the distribution of infective strains in cattle herds is essential for the development of a strategy to reduce the spreading of infections. The examinations showed that the isolates of four farms were identical and showed no differences in the restriction patterns. The farm specific genotypes mostly showed a close relationship to the genotypes of other farms. Therefore the contagious character of mastitis pathogens and the dominating occurrence of certain *S. aureus* clones could be proved.

TABLE III
GENOTYPIC RELATIONSHIP OF *Staphylococcus aureus* ISOLATES IN BOVINE MASTITIS MILK SAMPLES IN DAIRY HERDS

Farm	number of examined isolates	Relationship of isolates	Isolate term	Relationship with other farms
1	2	identical	VII	with XXIIc, XXV XXVII
2	2	difference in two bands	IXa, IXb	IXa with XXXIa
3	2	difference in eight bands	Xc, Xd	Xc with XIIc and XXVIIb
4	2	difference in seven bands	XIIc, XIId	XIIc with Xc and XXVIIb
5	4	in each case isolates identical, but 1 band difference between the isolates	IXXa, IXXb	
6	1	-	XX	
7	3	2 isolates identic, the third isolate showed difference in 5 bands	XXIIc, XXId	XXIIc with VII, XXVIIb
8	2	identical	XXIII	
9	3	identical	XXV	with VII
10	2	difference in 1 band	XXVIIa, XXVIIb	XXVIIb with Xc and XIIc
11	3	identical	XXXI	XXXI with IXa
12	1	-	XXXIII	

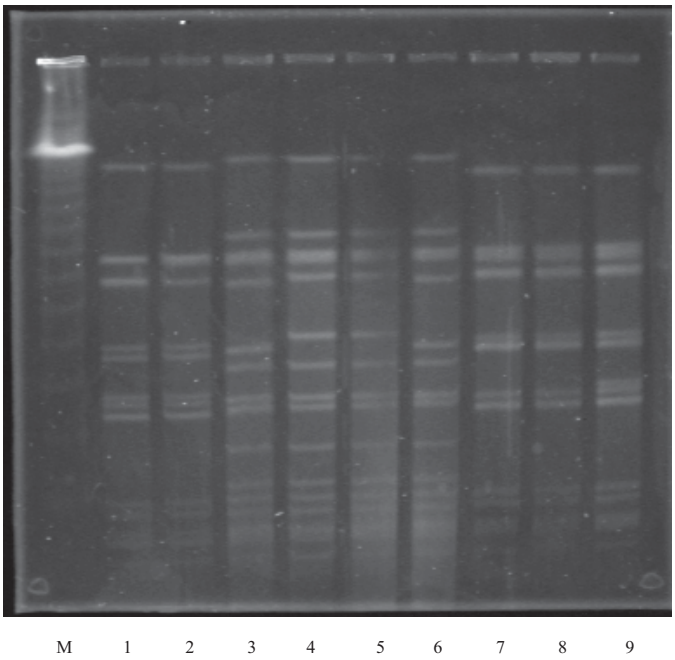

FIGURE 2. PFGE CHROMOSOMAL DNA RESTRICTION PATTERN OF 9 *S. AUREUS* ISOLATES AFTER DIGESTION WITH THE RESTRICTION ENZYME *SMAI* (1-2: ISOLATES FROM FARM 1; 3-6: ISOLATES FROM FARM 5; 7-9: ISOLATES FROM FARM 9).
M= molecular weight marker (low-range PFGE marker, 0.1-200 Kb, lambda ladder PFGE marker, 50-1,000 Kb, Sigma).

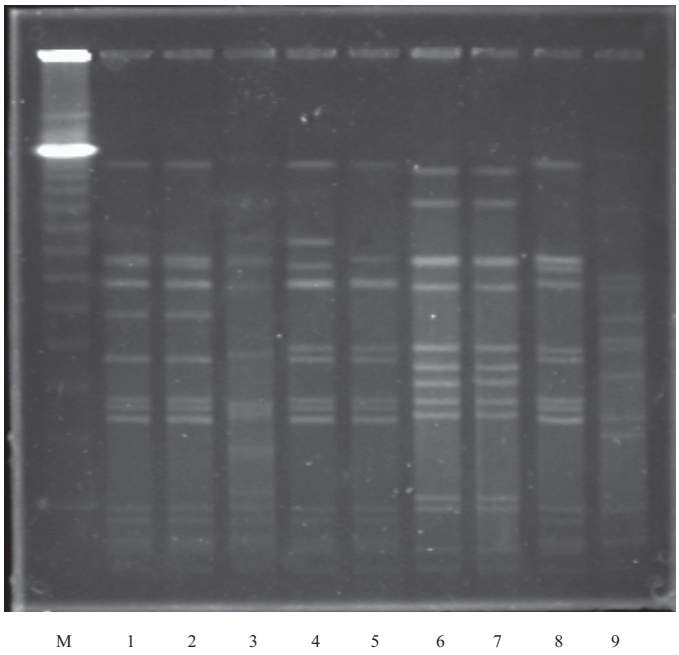

FIGURE 3. PFGE CHROMOSOMAL DNA RESTRICTION PATTERN OF 9 *S. AUREUS* ISOLATES AFTER DIGESTION WITH THE RESTRICTION ENZYME *SMAI*; (1-3: ISOLATES OF FARM 7; 4-5: ISOLATES OF FARM 10; 6-7: ISOLATES OF FARM 8; 8-9: ISOLATES OF FARM 4).
M= molecular weight marker (low-range PFGE marker, 0.1-200 Kb, lambda ladder PFGE marker, 50-1,000 Kb, Sigma).

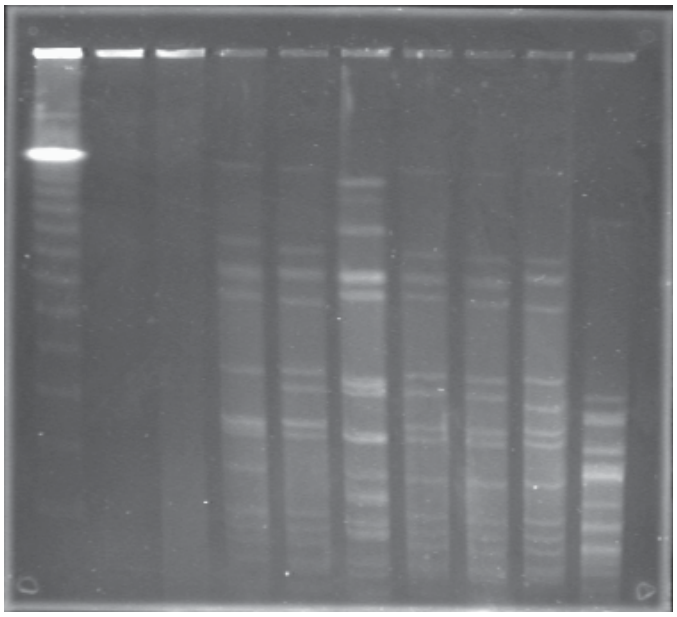

FIGURE 4. PFGE CHROMOSOMAL DNA RESTRICTION PATTERN OF 9 *S. AUREUS* ISOLATES AFTER DIGESTION WITH THE RESTRICTION ENZYME *SMAI*; (1-2: ISOLATES OF FARM 3 (DID NOT GO IN THIS RUN); 3-4: ISOLATES OF FARM 2; 5: ISOLATE OF FARM 6; 6-8: ISOLATES OF FARM 11; 9: ISOLATE FROM FARM 12).
M= molecular weight marker (low range PFGE marker, 0.1-200 Kb, Lambda ladder PFGE marker, 50-1,000 Kb, Sigma).

Also, for the lack of variations of band patterns within the farms it could suppose that in each population one single bacteria clone is responsible for the mastitis situation. This observation is confirmed with examinations by Annemüller et al. [2] and Zschöck et al. [52]. They proved the contagiosity of *S. aureus* mastitis by means of macrorestriction analysis. One single clone spreads within the herd. This clone is supposed to have a higher virulence for its risen contagiosity potential , its higher resistance against the defence mechanism of the host, and for the increased tendency to persist in the host. For example, it is known that isolates of the dominating genotype show an increased resistance against the bactericidal activity of the neutrophilic granulocytes [38].

Within farm 5 the patterns differed in one fragment. According to Goering [14] this result is possible but rather unlikely. Possibly the deletion of one DNA fragment wtih both flanking interfaces could lead to the loss of one single fragment in the PFGE pattern. The relative reliability of those genetic events makes suppose that most of the described single fragment differences are double fragment differences in fact, where one of the fragment is hidden behind another one of same size. In farm 10 the examined isolates showed deviations in form of two bands. According to Goering [14], this difference could be caused by insertion, respectively, deletion of one DNA sequence part outside a *SmaI* interface. Because of their slight differences to the original clone within the particular farm, these cultures should be valued as subclones of a macrorestriction type. As Zadok et al. [47] describe, the subclonal heterogeneity is conditioned by the temporal evolution. This phenomenon is described for human *S. aureus* strains [31]. The variation of the genetic content of chromosomes of *S. aureus* can be socialized with the loss of not essentially but clinically and epidemiologically relevant genes, e. g. virulent or resistant genes. This might explain the less frequent occurrence of certain clones [25, 35].

Among the farms 3, 4 and 7, the isolates showed a difference of more than 5 bands in the PFGE. According to Bannermann et al. [3], isolates with a difference in more than 3 bands are identified as different and not related strains, and therefore classified as different macro restriction types.

The majority of the genotypes proved on the 12 farms was found in several herds. This phenomenon occurred in other studies as well [1, 2, 12, 27, 47]. These authors proved a limited number of dominant *S. aureus* clones within a herd as well as among different diary cattle herds. This leads to the supposition that certain environmental variants are in advantage for intramammary infections. According to Aarestrup et al. [1] and Mullarky et al. [29] show a higher resistance against the immune system of the host organism. Fitzgerald et al. [12] describes that these clones are geographically far spread, enabled by special factors for an effective distribution in the bovine environment. A limited number of dominating types could be found in different herds, regions, countries, and even continents.

But usually, like in the farms examined for this studies, a certain genetic heterogenity of the strains within closed populations was proved [2, 12, 17, 26, 39]. Even in single animals or in one udder quarter different *S. aureus* clones sometimes could be found.

In conclusion, the represented data indicate the neglected role of enterotoxins in native *S. aureus* field isolates of Mexico which has an important impact on public health. It seems that a closely related *S. aureus* isolates are responsible for the induction of mastitis in western Mexico.

CONCLUSIONS

Twenty seven *S. aureus* field isolates strains of 12 herds in Jalisco State were someted to the partial amplification of the species specific 23S rRNA gene with the oligonucleotide primer Staur4 and Staur6, delivered in all isolates an universal amplicon size of 1.270 base pairs (Bp).

Of almost 3,000 isolated strains of *S. aureus,* 27 field strains were selected and subjected to different PCR, to detect the toxin encoding genes SEA, SEB, SEC, SEE, SEG, SEJ, and the TSST-1. The screening with PCR revealed the absence of all toxin encoding genes with the exception of SEI,

which could be detected in a single strain. The represented data indicate the neglected role of enterotoxins in native *S. aureus* field isolates of Mexico. The investigated strains were then subjected to fingerprinting by the means of PFGE. The data obtained through the PFGE analysis indicated the close relationship of *S. aureus* field strains responsible for the induction of mastitis in Mexico, which has an important impact on public health. It seems that certain closely related clones of *S. aureus* isolates are responsible for the induction of mastitis in western Mexico.

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