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Genomic fingerprints of *Staphylococcus aureus* of bovine origin by polymerase chain reaction-based DNA fingerprinting

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SUMMARY

Staphylococcus aureus ($n = 75$) isolated from mammary secretions of cows with subclinical and clinical mastitis from several geographic locations in the USA were examined using polymerase chain reaction-based DNA fingerprinting. DNA fingerprints were produced using a synthetic oligonucleotide primer (5′GTAACGCC3′) to produce a distinct spectrum of amplified DNA fragments facilitating a high degree of resolution for differentiating *S. aureus* strains. PCR-based DNA fingerprinting grouped the 75 *S. aureus* isolates into 19 distinct profiles. The technique differentiated closely related strains within and between geographic locations. Findings suggest that certain types are found across geographic regions suggesting a common clonal type. Within herd data suggest heterogeneity among subclinical and clinical isolates of *S. aureus* strains. Compared to existing typing methods, PCR-based DNA fingerprinting is easy to perform and interpret. Use of PCR-based DNA fingerprinting may allow for a more detailed investigation of the epidemiology of *S. aureus* mastitis in dairy cows.

INTRODUCTION

Staphylococcus aureus is an infectious agent isolated frequently from chronic bovine mastitis [1]. However, *S. aureus* can persist for long periods in other body sites including the vagina, teat and skin lesions, the nares, and infected tonsils of cows [2]. *Staphylococcus aureus* intramammary infections in nulliparous heifers have also been reported [3] indicating that sources other than the lactating cow

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may serve as reservoirs of *S. aureus* in the dairy herd. Despite the availability of a variety of antibiotics and numerous reports on the sensitivity of *S. aureus* to antibiotics, success of treatment during lactation is still very low. Thus, identification and elimination of reservoirs of *S. aureus* on the farm could be of great economic importance.

Biotyping, serotyping, phage typing and plasmid pattern analysis have been used for epidemiological studies of *S. aureus* [4–7]. However, these methods are not without limitations. Using a set of 16 phages at routine test dilution resulted in 84–9% typability of *S. aureus* [7]. When the international basic set of phages were subsequently tested only 73·6% of *S. aureus* isolates were typeable [4]. Matthews and co-workers [5] recently reported on plasmid profile analysis for discriminating between isolates of *Staphylococcus* species and concluded that plasmid profile analysis was not an adequate method for differentiating isolates of *Staphylococcus* species recovered frequently from bovine milk.

DNA restriction endonuclease fingerprinting (REF) has been developed for many organisms including streptococci [8], campylobacter [9], and *Bacteroides* species [10]. DNA REF was shown recently to be a useful technique for subtyping staphylococci isolated frequently from mammary secretions of cows with subclinical mastitis [11]. REF analysis is simple to perform, however, comparison and evaluation of DNA digests can be difficult [11]. Recently, polymerase chain reaction-based DNA fingerprinting has been used to detect polymorphisms in strains of *Staphylococcus* species [12] and *Streptococcus* species [13, 14]. The technique involves amplification of template DNA directed by one or more short oligonucleotide primers to produce a distinct spectrum of amplified DNA fragments [12]. PCR-based DNA fingerprinting has also been used for subtyping strains of *Streptococcus uberis* [13]. The objective of the present study was to use PCR-based DNA fingerprinting to differentiate strains of *Staphylococcus aureus* isolated from mammary secretions of cows with clinical and subclinical mastitis from several geographic locations in the USA.

MATERIALS AND METHODS

Bacteria

Staphylococcus aureus ($n = 75$) isolated from mammary secretions of cows with subclinical and clinical mastitis were evaluated. Isolates were obtained from several geographic locations in the USA and included some isolates originally collected in the late 1960s. Upon arrival, cultures were transferred to trypticase soy broth (TSB), incubated at 37 °C overnight and checked for purity by streaking onto sheep blood agar. Isolates were stored at −80 °C in sterile skimmed milk and activated when required in the study.

Two strains obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) were used as control organisms: *S. aureus* ATCC 10832 and *S. aureus* ATCC 13709.

Isolation of DNA

A dense suspension of each isolate was made by transferring bacterial growth from blood agar plates into 100 μl of Tris (10 mM)–EDTA (5 mM), pH 7·8. After addition of 100 μl of lysostaphin (1 mg/μl) (ICN Biochemicals, Cleveland, OH,

USA), bacteria were incubated at 37 °C for 45 min. Lysis of cells was achieved by addition of 20 μl of sodium dodecyl sulphate (SDS) buffer (20% w/v) in Tris (50 mM)–EDTA (20 mM), pH 7·8 followed by 3 μl of proteinase K (20 mg/ml; Sigma Chemical Co., St Louis, MO, USA) and further incubation at 37 °C for 1 h. Protein was precipitated by addition of 200 μl saturated NaCl (5 M) followed by agitation for 15 s and was removed by centrifugation (7000 ***g***) for 15 min at 4 °C. The supernatant was subjected to phenol-chloroform (1:1) extraction followed by chloroform-isoamyl alcohol (24:1) extraction. Two volumes (800 μl) of cold 95% ethanol were added and the solution kept at −20 °C overnight. The resulting DNA precipitate was collected by centrifugation at 7000 ***g*** for 10 min at 4 °C, washed in 70% ethanol, and vacuum dried and resuspended in 30 μl of buffer (Tris [10 mM]–EDTA [1 mM]; pH 7·5).

PCR amplification

DNA amplification was done in a total volume of 30 μl containing 20 ng of template DNA, 2·5 μM of primer, 100 μM (each of the four) deoxynucleoside triphosphates (Boehringer Mannheim, Indianapolis, ID, USA), 2·5 U of *Taq* polymerase (Promega, Madison, WI, USA) and 3 μl of 10× *Taq* buffer. Each reaction mixture was overlaid with 30 μl of mineral oil and run for 35 cycles in a Ericomp Thermocycler (Ericomp Inc., San Diego, CA, USA). One cycle consisted of 90 s at 94 °C and 90 s at 30 °C. On completion of 35 cycles, a final 75 °C extension was used. The oligonucleotide primer, designated previously as primer 8·6 d [14], was synthesized by the Analytical Services Laboratory, The University of Tennessee, Knoxville and had the following sequence: 5′-GTAACGCC3′.

Gel electrophoresis and photography

Ten microlitres of PCR mixture were electrophoresed in 2% agarose in Tris-borate-EDTA buffer (0·09 M Tris base, 0·09 M sodium borate, 2·5 mM-EDTA, pH 8·3) in gels at 100 V for 3 h. After electrophoresis, gels were stained with ethidium bromide (1 μg/ml) and visualized under u.v. light and photographed with type 55 polaroid film. *Bst* NI-digested pBR322 DNA was used as a molecular weight market (New England Biolabs, Beverly, MA, USA).

PCR product analysis

A computer-integrated laser densitometer (Ultroscan XL, LKB Produkter AB, Bromma, Sweden) was used to scan along each track of the polaroid negative. Scans were stored and retrieved for the purpose of comparison and evaluation with the Gelscan XL version 2·0 software package (Pharmacia LKB Biotechnology, Uppsala, Sweden). The number of DNA fragments and sizes in base pairs were determined.

RESULTS

The 75 *S. aureus* strains produced 19 distinct profiles. The six most prevalent profiles are shown in Figure 1. Generally, strains were distinguished based on presence or absence of 1 or 2 PCR products. For instance, only type 4 strains (Fig. 1) had a 1370 bp PCR product. Densitometric scans (Fig. 2) are of PCR-based DNA fingerprint profiles shown in Figure 1. Densitometric analysis of PCR-based

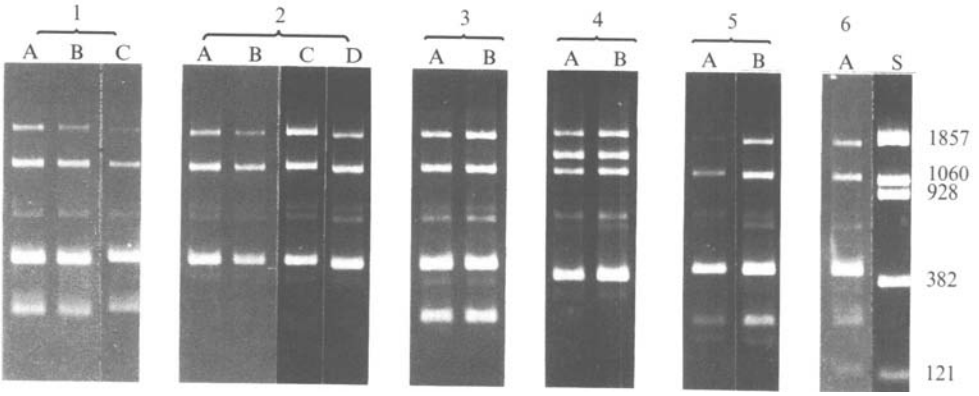

Fig. 1. Composite of *Staphylococcus aureus* types based on PCR-based DNA fingerprint profiles of isolates from several geographic locations in the US. Type 1, A and B, Kentucky; C, Wisconsin. Type 2, A, Ohio; B, Vermont; C and D, Idaho. Type 3, A and B, Idaho. Type 4, A and B, Washington. Type 5, A and B, Idaho. Type 6, A, Idaho. S, molecular size markers. Top to bottom: 1857 bp; 1060 bp; 928 bp; 383 bp; 121 bp; respectively.

DNA fingerprint profiles aided in determining molecular weight of PCR products and number of distinguishable fragments (Fig. 2). The size of PCR products ranged from 157 to 1760 bp. An 1100 bp product was found in approximately 99% of strains of *S. aureus* evaluated. Products of 465 and 1760 bp were found in > 86% of strains.

Distribution of strains of *Staphylococcus aureus* by PCR-based DNA fingerprint profile types is presented in Tables 1–4. Eighteen isolates from four geographic locations, including five isolates from the 1960s, were classified as type 1 based on PCR profiles (Table 1). Type 2 ($n = 21$) strains were predominantly from a herd in Washington state (Table 2). Types 3, 4, 5, 6, 8 and 9 (Tables 3, 4) were each composed of isolates from a single geographic location. Ten isolates had distinctive PCR profiles and were considered unique types (Table 4). Within a herd, PCR-based DNA fingerprint profiles from isolates obtained from cows with subclinical and clinical mastitis differed (Table 5). The 10 *S. aureus* isolates from mammary secretions of 10 cows produced four distinct PCR-based DNA fingerprint profiles. Isolates from mammary secretions of two cows (Table 5; cow 446 and cow 941) with clinical mastitis had different profile types which were isolated also from cows with subclinical mastitis.

DISCUSSION

Several methods are currently available for typing and subtyping bacteria. However, some techniques require individual reagents for each genus or species, some are useful for only a limited number of species, and some may not be suitable for all strains of a species [15]. Matthews and colleagues [11] reported on the use of REF of genomic DNA of *Staphylococcus* species for differentiating closely related and unrelated strains from bovine mammary secretions. They [11] indicated that the technique was easy to perform and reproducible, but differentiation of isolates was somewhat difficult due to the complexity of DNA REF patterns.

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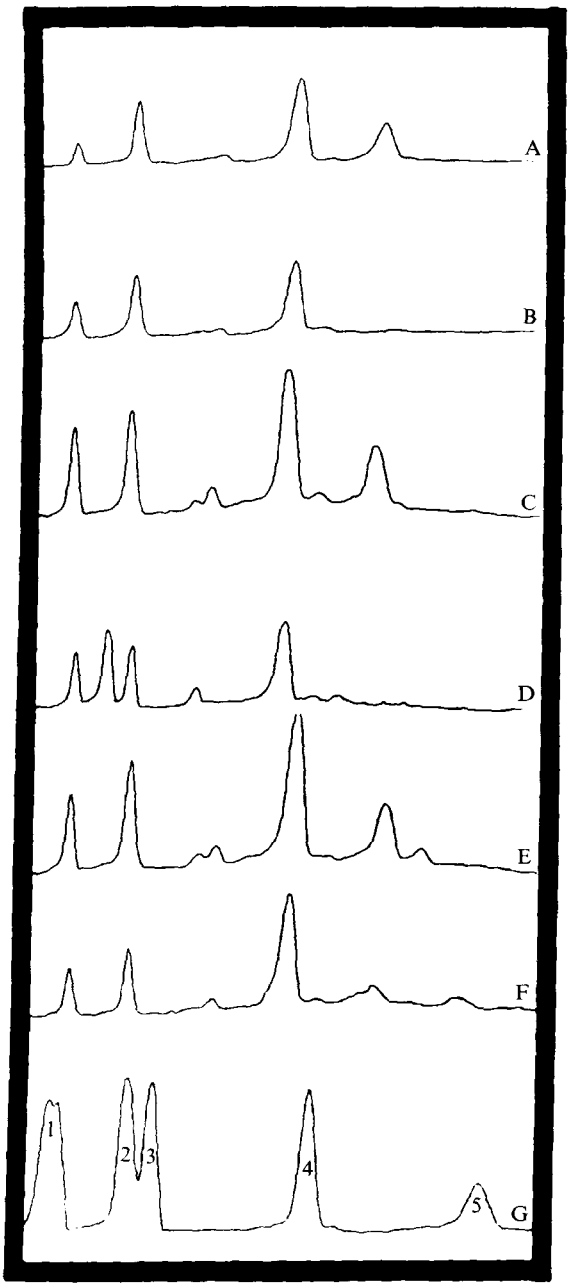

Fig. 2. Laser densitometric scans of PCR-based DNA fingerprint profiles of genomic DNA from *Staphylococcus aureus* showing different patterns of types 1–6. Scan A, type 1; B, type 2; C, type 3; D, type 4; E, type 5; F, Type 6; G, molecular size marker (1, 1857 bp; 2, 1060 bp; 3, 928 bp; 4, 383 bp; 5, 121 bp).

In the present study, PCR-based DNA fingerprinting using a short oligonucleotide primer produced simple banding patterns facilitating a high degree of resolution for differentiating strains of *S. aureus*. The 8·6 d primer has been used previously for differentiation and identification of members of the genera

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Table 1. *Type 1* Staphylococcus aureus *isolates by PCR-based DNA fingerprinting*

Culture no.	Origin*	Source†	Sample‡
1	TN	UTDH	hs
3	TN	UTDH	hs
4	TN	UTDH	hs
5	TN	UTDH	hs
6	TN	UTDH	hs
13	TN	UTDH	hs
14	TN	UTDH	hs
16	WA	CDH	hs
39	KY	UKDH	cln
41	WI	CDH	hs
43	KY	UKDH	hs
45	KY	UKDH	hs
48	WI	CDH	hs
51	KY	UKDH	1960s
53	KY	UKDH	1960s
54	KY	UKDH	1960s
55	KY	UKDH	1960s
56	KY	UKDH	1960s

* KY, Kentucky; TN, Tennessee; WA, Washington; WI, Wisconsin, USA.

† CDH, commercial dairy herd; UKDH, University of Kentucky dairy herd; UTDH, University of Tennessee dairy herd.

‡ cln, clinical episode; hs, herd survey; 1960s, isolates originally obtained in the late 1960s from cows in UKDH.

Table 2. *Type 2* Staphylococcus aureus *isolates by PCR-based DNA fingerprinting*

Culture no.	Origin*	Source†	Sample‡
7	TN	UTDH	hs
8	TN	UTDH	hs
9	TN	UTDH	hs
12	TN	UTDH	hs
20	WA	CDH	hs
21	WA	CDH	hs
23	WA	CDH	hs
25	WA	CDH	hs
26	WA	CDH	hs
27	WA	CDH	hs
28	WA	CDH	hs
29	WA	CDH	hs
30	WA	CDH	hs
36	WA	CDH	hs
37	WA	CDH	hs
38	WA	CDH	hs
46	OH	CDH	hs
47	VT	CDH	hs
70	ID	CDH	cln
74	ID	CDH	hs
76	ID	CDH	hs

* OH, Ohio; ID, Idaho; TN, Tennessee; VT, Vermont; WA, Washington, USA.

† CDH, commercial dairy herd; UTDH,University of Tennessee dairy herd.

‡ cln, clinical episode; hs, herd survey.

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Table 3. Staphylococcus aureus *types based on PCR-based DNA fingerprint profiles*

Culture no.	Origin*	Source†	Sample‡	Type§
57	ID	CDH	hs	3
58	ID	CDH	hs	3
59	ID	CDH	hs	3
60	ID	CDH	hs	3
61	ID	CDH	hs	3
62	ID	CDH	hs	3
69	ID	CDH	hs	3
72	ID	CDH	cln	3
18	WA	CDH	hs	4
19	WA	CDH	hs	4
32	WA	CDH	hs	4
33	WA	CDH	hs	4
34	WA	CDH	hs	4
65	ID	CDH	hs	5
66	ID	CDH	hs	5
68	ID	CDH	hs	5
71	ID	CDH	hs	5
75	ID	CDH	hs	5
67	ID	CDH	hs	6
73	ID	CDH	hs	6

* ID, Idaho; WA, Washington, USA.
† CDH, commercial dairy herd.
‡ cln, clinical episode; hs, herd survey.
§ Genomic DNA fingerprint type.

Table 4. Staphylococcus aureus *types based on PCR-based DNA fingerprint profiles*

Culture no.	Origin*	Source†	Sample‡	Type§
15	WA	CDH	hs	7
42	WI	CDH	hs	7
10	TN	UTDH	hs	8
11	TN	UTDH	hs	8
22	WA	CDH	hs	9
35	WA	CDH	hs	9
1	TN	UTDH	hs	10
9	TN	UTDH	hs	11
17	WA	CDH	hs	12
24	WA	CDH	hs	13
40	WI	CDH	hs	14
44	KY	UKDH	hs	15
49	KY	UKDH	hs	16
50	KY	UKDH	cln	17
63	ID	CDH	hs	18
64	ID	CDH	hs	19

* ID, Idaho; KY, Kentucky; TN, Tennessee; WA, Washington; WI, Wisconsin, USA.
† CDH, commercial dairy herd; UKDH, University of Kentucky dairy herd; UTDH, University of Tennessee dairy herd.
‡ cln, clinical episode; hs, herd survey.
§ Genomic DNA fingerprint type.

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Table 5. *PCR-based DNA fingerprint profiles of* Staphylococcus aureus *isolated from a herd in Idaho*

Cow no.	Q*	S†	Type‡
76	LF	HS	6
102	LR	HS	5
174	LF	HS	3
446	LR	cln	2
470	LR	HS	5
941	RR	cln	3
1046	RR	HS	6
5459	RF	HS	2
5815	RF	HS	5
5995	RF	HS	2

* Q, relative position of the quarter of the mammary gland: r, right: l, left: f, front: r, rear.
† S, sample: hs, herd survey: cln, clinical.
‡ Genomic DNA fingerprint type based on all isolates ($n = 75$) evaluated.

Streptococcus and *Enterococcus* [16]. Welsh and McClelland [12] showed that strains of five *Staphylococcus* species could be distinguished by comparing polymorphisms in genomic fingerprints generated by arbitrarily primed-polymerase chain reaction. *Staphylococcus haemolyticus* isolates were grouped into two different PCR patterns indicating the species has a distinct population structure of two distantly related sub-species or perhaps even species [12].

In the present study, 19 distinct PCR-based DNA fingerprint profiles were observed for the 75 isolates evaluated. Isolates from all geographic regions were grouped into types 1 and 2 (Tables 1, 2), which included 52% of isolates evaluated. This finding suggests that certain types are not confined to specific geographic regions. Ten isolates collected from 10 different cows from one herd in Idaho during a herd survey, including two episodes of clinical mastitis, belonged to four different types (Table 5). These data suggest heterogeneity among subclinical and clinical isolates of *S. aureus* strains within a herd. Of the four types, only one (type 2) was found in isolates from other regions in the USA. Therefore, prevalence of a particular type could be confined to a herd.

Most studies utilizing PCR-based DNA fingerprinting have concentrated on *Staphylococcus* species of human importance and have not utilized this technique for differentiation of veterinary isolates, in particular, *S. aureus* isolated from subclinical and clinical episodes of bovine mastitis. From an epidemiological standpoint, it is important to determine the origin of organisms involved in the aetiology of a disease. To control *S. aureus* intramammary infections, strategies must be initiated to block transmission to the host from reservoirs and vectors on the farm. Comparison of *S. aureus* isolates from body sites and mammary secretions of nulliparous, primiparous and multiparous cows, and sites on the farm may aid in identifying the source of *S. aureus* on the farm. Utilizing PCR-based DNA fingerprinting to type strains may also determine which *S. aureus* strains are the cause of clinical mastitis (Table 5). This may aid researchers investigating potential virulence factors and help to identify pathogenic and nonpathogenic strains of *S. aureus* isolated from bovine mammary secretions. In addition, identification by PCR-based DNA fingerprinting of a common type in the US (Tables 1, 2) may aid in the development of an effective *S. aureus* vaccine.

PCR-based DNA fingerprint analysis is simple to execute and interpret compared with other genotyping methods such as genomic restriction endonuclease finger-printing, DNA hybridization, ribotyping, and use of specific radioactive probes which require special handling and disposal. Results of the present study using PCR-based DNA fingerprinting to differentiate between strains of *S. aureus* are encouraging. Use of a computer-integrated scanning laser densitometer proved to be an excellent means of storing, retrieving, comparing and evaluating PCR-based DNA fingerprint profiles. In conclusion, PCR-based DNA fingerprint analysis appears to be a suitable method for differentiation of strains of *S. aureus* of bovine origin, and can also be used to draw epidemiological inferences.

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REFERENCES

1. Baumgartner A, Nicolet J, Eggimann M. Plasmid profiles of *Staphylococcus aureus* causing bovine mastitis. J Appl Bacteriol 1984; **56**: 159–63.
2. Matos JS, White DG, Harmon RJ, Langlois BE. Isolation of *Staphylococcus aureus* from sites other than the lactating mammary gland. J Dairy Sci 1991; **74**: 1544–9.
3. Trinidad P, Nickerson SC, Alley TK. Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. J Dairy Sci 1990; **73**: 107–14.
4. Carroll PJ, Francis PG. The basic phage set for typing bovine staphylococci. J Hyg 1985; **95**: 665–9.
5. Matthews KR, Jayarao BM, Oliver SP. Plasmid pattern analysis of *Staphylococcus* species isolated from bovine mammary secretions. J Dairy Sci 1992; **75**: 3318–23.
6. Matthews KR, Oliver SP, King SH. Comparison of Vitek Gram-positive identification system with API Staph-Trac system for species identification of staphylococci of bovine origin. J Clin Microbiol 1990; **28**: 1649–51.
7. Davidson I. A collaborative investigation of phages for typing bovine staphylococci. Bull World Health Org 1972; **46**: 81–98.
8. Jayarao BM, Oliver SP, Tagg JR, Matthews KR. Genotypic and phenotypic analysis of *Streptococcus uberis* isolated from bovine mammary secretions. Epidemiol Infect 1991; **107**: 543–55.
9. Kakoylannis CK, Winter PJ, Marshall BB. Identification of isolates of *Campylobacter coli* from animals and humans by bacterial restriction endonuclease DNA analysis (BRENDA). Appl Environ Microbiol 1984; **48**: 545–9.
10. Bradbury WE, Murray RGE, Mancini C, Morris VI. Bacterial chromosomal restriction endonuclease analysis of the homology of *Bacteroides* species. J Clin Microbiol 1985; **21**: 24–8.
11. Matthews KR, Jayarao BM, Oliver SP. Restriction endonuclease fingerprinting of genomic DNA of *Staphylococcus* species of bovine origin. Epidemiol Infect 1992; **109**: 59–68.
12. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 1990; **18**: 7213–8.
13. Jayarao BM, Bassam BJ, Caetano-Anolles G, Gresshoff PM, Oliver SP. Subtyping of *Streptococcus uberis* by DNA amplification fingerprinting. J Clin Microbiol 1992; **30**: 1347–50.
14. Jayarao BM, Dorè Jr. JE, Baumbach GA, Matthews KR, Oliver SP. Differentiation of *Streptococcus uberis* from *Streptococcus parauberis* by polymerase chain reaction and restriction fragment length polymorphism analysis of 16S ribosomal DNA. J Clin Microbiol 1991; **29**: 2774–8.

15. Bralkowska-Hobrzanska H, Jaskot D, Hammerberg O. Evaluation of restriction endonuclease fingerprinting of chromosomal DNA and plasmid profile analysis for characterization of multiresistant coagulase-negative staphylococci in bacteremic neonates. J Clin Microbiol 1990; **28**: 269–75.
16. Jayarao BM, Keane KA, Gillespie BE, Luther DA, Oliver SP. DNA fingerprinting: A new technique for identifying mastitis pathogens. Proc Natl Mastitis Counc 1993; **32**: 167–77.

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