Treatment of Infectious Mastitis during Lactation: Antibiotics versus Oral Administration of Lactobacilli Isolated from Breast Milk

Rebeca Arroyo, Virginia Martín, Antonio Maldonado, Esther Jiménez, Leónides Fernández, and Juan Miguel Rodríguez
Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Universidad Complutense de Madrid, Madrid, Spain

Background. Mastitis is a common infectious disease during lactation, and the main etiological agents are staphylococci, streptococci, and/or corynebacteria. The efficacy of oral administration of Lactobacillus fermentum CECT5716 or Lactobacillus salivarius CECT5713, two lactobacilli strains isolated from breast milk, to treat lactational mastitis was evaluated and was compared with the efficacy of antibiotic therapy.

Methods. In this study, 352 women with infectious mastitis were randomly assigned to 3 groups. Women in groups A (n = 124) and B (n = 127) ingested daily 9 log 10 colony-forming units (CFU) of L. fermentum CECT5716 or L. salivarius CECT5713, respectively, for 3 weeks, whereas those in group C (n = 101) received the antibiotic therapy prescribed in their respective primary care centers.

Results. On day 0, the mean bacterial counts in milk samples of the 3 groups were similar (4.35–4.47 log 10 CFU/mL), and lactobacilli could not be detected. On day 21, the mean bacterial counts in the probiotic groups (2.61 and 2.33 log 10 CFU/mL) were lower than that of the control group (3.28 log 10 CFU/mL). L. fermentum CECT5716 and L. salivarius CECT5713 were isolated from the milk samples of women in the probiotic groups A and B, respectively. Women assigned to the probiotic groups improved more and had lower recurrence of mastitis than those assigned to the antibiotic group.

Conclusions. The use of L. fermentum CECT5716 or L. salivarius CECT5713 appears to be an efficient alternative to the use of commonly prescribed antibiotics for the treatment of infectious mastitis during lactation.

ClinicalTrials.gov identifier. NCT00716183.
mastitis in a higher number of women and to compare such
an approach with the antibiotic therapy that is usually pre-
scribed to treat this condition.

MATERIALS AND METHODS

Design of the study and collection of the milk samples.
A total of 352 women with symptoms of mastitis participated in the study. All met the following criteria: breast inflammation, painful breastfeeding, milk bacterial count $\geq 4 \log_{10}$ colony-forming units (CFU)/mL, and milk leukocyte count $\geq 6 \log_{10}$ cells/mL. Many of the women ($n = 74$) presented fissures in the mammary areola and/or nipple. None of them ingested commercial probiotic foods or supplements during the study. Women with mammary abscesses, Raynaud syndrome, or any other mammary pathology were excluded. All volunteers gave written informed consent to the protocol, which was approved by the Ethical Committee of Hospital Clínico of Madrid (Spain). The study was registered in the ClinicalTrials.gov database (NCT00716183). The volunteers were randomly assigned to 3 groups (2 probiotic groups and 1 antibiotic group), and neither volunteers nor investigators knew the assignments during the investigation.

The study lasted 21 days, and during this period, the probiotic groups A ($n = 124$) and B ($n = 127$) consumed daily a capsule with 200 mg of a freeze-dried probiotic containing $\sim 9 \log_{10}$ CFU of L. fermentum CECT5716 [8] or L. salivarius CECT5713 [10]. Capsules were manufactured at the probiotic plant of Puleva Biotech (Granada, Spain) and were kept at 4°C throughout the study. Women of the antibiotic group (group C, $n = 101$) received the antibiotic treatment prescribed in their primary care centers. Breast milk samples were obtained from the volunteers at the beginning (day 0) and at the end (day 21) of the study, in accordance with a previously described procedure [11]. The evolution of the symptoms was evaluated at days 0 and 21 by midwives of their primary care centers. At both times, the volunteers were asked to score their breast pain from 0 (extremely painful) to 10 (no pain).

Count and identification of bacteria in the milk samples.
Samples were spread onto Baird-Parker, Columbia, MacConkey, and Sabouraud dextrose chloramphenicol agar plates (BioMérieux) for selective isolation and quantification of the main agents involved in infectious mastitis [12] and, parallel, onto agar plates of MRS (Oxoid) supplemented with L-cysteine (0.5 g/L) (MRS-Cys) for isolation of lactobacilli. The plates were incubated for 48 hours at 37°C in aerobic conditions, except for the MRS-Cys plates, which were incubated anaerobically (in 85% nitrogen, 10% hydrogen, and 5% carbon dioxide) in an anaerobic workstation (DW Scientific).

Bacteria isolated from milk were initially identified at the species level by classic morphological and biochemical tests. The identification of bacteria belonging to the S. epidermidis or S. aureus species was confirmed by a multiplex polymerase chain reaction (PCR) method based on dnaJ genes with primers J-StGen (5′-TGCGAAAGAGACTATTATGA-3′) and J-StAur (5′-GGATCTTGTTCGCGG-3′), and J-StEpi (5′-CCACCAAGCTTGACT-3′) in a iCycler thermocycler (Bio-Rad Laboratories). The primer pair J-StGen and J-StAur results in a 1278-bp fragment, and the primer pair J-StGen and J-StEpi results in a 249-bp S. epidermidis species-specific fragment [11]. Identification of streptococci was performed by partial amplification (488 bp) and sequencing of the gene tuf with primers TufStrep-1 (5′-GAAGATTTGAAGTTGGTTG-3′) and TufStrep-2 (5′-GCAGGATTTGCGAAGATGG-3′) [13]. Identification of the potential *Streptococcus* spp.

### Table 1. Bacterial Counts from Breast Milk and Breast Pain Score at the Beginning (Day 0) and the End (Day 21) of the Trial

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>pᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>127</td>
<td>101</td>
<td>101</td>
<td>101</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>4.47 ± 0.53</td>
<td>4.39 ± 0.41</td>
<td>4.21 ± 0.52</td>
<td>2.62 ± 0.49</td>
<td>2.52 ± 0.42</td>
<td>3.31 ± 0.82</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4.06 ± 0.67</td>
<td>3.95 ± 0.54</td>
<td>4.21 ± 0.50</td>
<td>2.26 ± 0.55</td>
<td>2.26 ± 0.55</td>
<td>2.97 ± 0.88</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>4.12 ± 0.45</td>
<td>3.23 ± 0.37</td>
<td>2.28 ± 0.48</td>
<td>3.14 ± 0.72</td>
<td>3.14 ± 0.72</td>
<td>3.14 ± 0.72</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>4.08 ± 0.59</td>
<td>3.71 ± 0.33</td>
<td>2.23 ± 0.60</td>
<td>3.12 ± 0.9</td>
<td>3.12 ± 0.9</td>
<td>3.12 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Rothia spp.</td>
<td>3.34 ± 0.08</td>
<td>3.97 ± 0.58</td>
<td>3.48 ± 0.42</td>
<td>2.04 ± 0.24</td>
<td>2.42 ± 0.67</td>
<td>2.42 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>3.86 ± 0.50</td>
<td>3.86 ± 0.50</td>
<td>3.86 ± 0.50</td>
<td>2.04 ± 0.24</td>
<td>2.39 ± 0.99</td>
<td>2.39 ± 0.99</td>
<td></td>
</tr>
<tr>
<td><strong>Breast pain score</strong></td>
<td>2.16 ± 1.28</td>
<td>2.16 ± 1.28</td>
<td>2.16 ± 1.28</td>
<td>2.16 ± 1.28</td>
<td>2.16 ± 1.28</td>
<td>2.16 ± 1.28</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are expressed as log_{10} colony-forming units/mL, unless otherwise indicated. Treatment for group A was Lactobacillus fermentum CECT5716; for group B, Lactobacillus salivarius CECT5713; and for group C, antibiotic. Breast pain score ranged from extremely painful (0) to no pain (10). n, no. of women in the group or having the listed bacterial species in their milk; SD, standard deviation.

ᵇ On day 21, group C differed significantly from group A and group B in counts for total bacteria, S. epidermidis, S. aureus, and S. mitis and in breast pain score (nonparametric multiple comparison test; P < .001).

Kruskal-Wallis test, α = 0.05.
For this purpose, 2 species-specific probes were designed on the basis of unique 16S rRNA sequences. In the case of *L. salivarius*, a fragment (210 bp) was amplified from *L. salivarius* CECT5713 genomic DNA with primers SAL91F (5′-ATTCCACGTAAAGAGT-3′) and SAL285R (5′-TATCATCCTTTGCTAG-3′). Parallel, a fragment (192 bp) was amplified from *L. fermentum* CECT5716 genomic DNA with primers Lfer-3 (5′-ACTAAGTCTAGTCTAGCA-3′) and Lfer-4 (5′-TCTACTGCTCAAGTAACATC-3′) [16]. The PCR conditions were as follows: 95°C for 2 minutes (1 cycle); 95°C for 30 seconds, 46°C (*L. salivarius*) or 55°C (*L. fermentum*) for 30 seconds, and 72°C for 45 seconds (40 cycles); and a final extension at 72°C for 4 minutes. Both PCR fragments were purified using the QIAquick PCR purification kit (Qiagen) and labeled using the Amersham ECL direct nucleic acid labelling and detection system (GE Healthcare).

Colonies obtained on MRS-Cys plates from milk samples (day 21) were spotted in a regular array on 2 sets of MRS-Cys replica plates. Then, nylon Hybond-N′ discs (GE Healthcare) were laid directly on the culture surfaces and were kept there for 1 minute. Both hybridization and detection were performed as previously described [11]. The identity of the isolates that gave a positive signal after colony hybridization was confirmed by 16S rRNA sequencing as described above.

*L. salivarius* and *L. fermentum* isolates were submitted to pulsed-field gel electrophoresis (PFGE) genotyping as previously described [11]. Their profiles were compared with those of *L. salivarius* CECT5713, *L. salivarius* CECT4062, *L. salivarius* CECT4063, *L. salivarius* DSM 20492, *L. fermentum* CECT5716, *L. fermentum* CECT285, *L. fermentum* CECT4007, and/or *L. fermentum*. The LMG 8900 Low Range PFG marker (New England BioLabs) was used as the molecular size standard.

**Statistical analysis.** Microbiological data, recorded as number of CFU per mL of milk, were transformed to log_{10} values before calculation of means and statistical analysis. The reported values of bacterial counts are the mean values of duplicate or triplicate determinations. The continuous variables “bacterial counts” and “breast pain score” were not normally distributed. Three bacterial species occurred in sufficient numbers of breast milk samples to allow statistical comparison between groups. Kruskal-Wallis tests were performed to determine statistically significant differences between the bacterial counts (total and main bacterial species) and between the breast pain scores at the beginning (day 0) and at the end (day 21) of the trial. The same approach was used to determine whether there were differences in the change of these variables among the 3 groups. When statistically significant differences were found, nonparametric multiple comparisons were performed to ascertain which pair of groups was different. The association of mastitis recurrence with the treatment was compared with the χ² test. The relationship between total bacterial count and breast pain score was analyzed using the Spearman rank cor-

*Streptococcus mitis* isolates was confirmed by testing optochin sensitivity and bile solubility [14] and by testing latex agglutination with the Slide Pneumo kit (BioMérieux).

The remaining isolates were identified by 16S rRNA sequencing with primers pbl16 (5′-AGAGTTTGATCCTGGCTCAG-3′) and mb16 (5′-GGCTGCTGGCACGTAGTTAG-3′) [15]. Their identity was determined on the basis of the highest scores (≥99%) among the sequences deposited in the European Molecular Biology Laboratory database, by means of the Basic Local Alignment Search Tool algorithm.

**Identification of L. salivarius CECT5713 and L. fermentum CECT5716 in the milk samples.** A DNA-DNA colony hybridization assay was developed to investigate whether oral administration of the lactobacilli led to their presence in milk. For this purpose, 2 species-specific probes were designed on the basis of unique 16S rRNA sequences. In the case of *L. salivarius*, a fragment (210 bp) was amplified from *L. salivarius* CECT5713 genomic DNA with primers SAL91F (5′-ATTCCACGTAAAGAGT-3′) and SAL285R (5′-TATCATCCTTTGCTAG-3′). Parallel, a fragment (192 bp) was amplified from *L. fermentum* CECT5716 genomic DNA with primers Lfer-3 (5′-ACTAAGTCTAGTCTAGCA-3′) and Lfer-4 (5′-TCTACTGCTCAAGTAACATC-3′) [16]. The PCR conditions were as follows: 95°C for 2 minutes (1 cycle); 95°C for 30 seconds, 46°C (*L. salivarius*) or 55°C (*L. fermentum*) for 30 seconds, and 72°C for 45 seconds (40 cycles); and a final extension at 72°C for 4 minutes. Both PCR fragments were purified using the QIAquick PCR purification kit (Qiagen) and labeled using the Amersham ECL direct nucleic acid labelling and detection system (GE Healthcare).

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Table 2. Reduction in Bacterial Counts in Breast Milk and Change in Breast Pain Score from Day 0 to Day 21, according to the Antibiotic Prescribed to Group C Women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Amoxicillin-clavulanic acid</th>
<th>Amoxicillin</th>
<th>Cotrimoxazole</th>
<th>Cloxacillin</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Reduction in bacterial counts**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>−1.22 ± 0.84</td>
<td>23</td>
<td>−0.55 ± 0.56</td>
<td>19</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>32</td>
<td>−1.15 ± 0.67</td>
<td>18</td>
<td>−0.50 ± 0.59</td>
<td>11</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10</td>
<td>−1.74 ± 1.28</td>
<td>12</td>
<td>−0.79 ± 0.59</td>
<td>6</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>15</td>
<td>−1.20 ± 0.94</td>
<td>4</td>
<td>−1.66 ± 1.67</td>
<td>6</td>
</tr>
<tr>
<td>Change in breast pain score†</td>
<td>39</td>
<td>4.67 ± 1.90</td>
<td>23</td>
<td>2.61 ± 2.52</td>
<td>19</td>
</tr>
</tbody>
</table>

NOTE. n, no. of women in the group or having the listed bacterial species in their milk; NA, not applicable.

a Kruskal-Wallis test, except for erythromycin data.
b Reduction in bacterial counts was calculated as Δ log_{10} colony-forming units per mL.
c Breast pain score ranged from extremely painful (0) to no pain (10), and change in breast pain score used 0 for no change.

Table 3. Additional Outcomes of the Study of Treatment of Infectious Mastitis during Lactation

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With detection of lactobacilli</td>
</tr>
<tr>
<td>Probiotic</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus fermentum CECT5716</td>
<td>124</td>
</tr>
<tr>
<td>Lactobacillus salivarius CECT5716</td>
<td>127</td>
</tr>
<tr>
<td>Total</td>
<td>251</td>
</tr>
<tr>
<td>Antibiotic</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>39</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>23</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>19</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>18</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
</tr>
</tbody>
</table>

* Recurrence was defined as a new episode of mastitis (clinical symptoms and bacterial concentration ≥ 4 log_{10} colony-forming units [CFU/mL]) in a follow-up period of 3 months after these parameters had reached physiologic values (no clinical symptoms and bacterial concentration < 3 log_{10} CFU/mL).
† Vaginal candidiasis was defined as the presence of clinical symptoms compatible with such condition, together with a dense population of Candida albicans in culture of vaginal exudates on Sabouraud dextrose chloramphenicol agar plates (BioMéieux).
‡ χ² = 0.91, P = .340
§ χ² = 27.08, P < .001.

The relation coefficient for nonparametric data. The significance level was set at .05. All analyses were performed using the software package SAS, version 9.1 (SAS Institute).

RESULTS

Bacterial counts in the milk samples. At day 0, the mean values of total bacterial count in milk were very similar in the 3 groups and ranged 4.35–4.47 log_{10} CFU/mL (Table 1). S. epidermidis (isolated from 73% of the women), S. aureus (from 43%), and S. mitis (from 30%) were the dominant species (Table 1). Other bacterial species were identified in <5% of the samples, and lactobacilli could not be detected in any sample.

On day 21, differences in the total bacterial counts of the 3 groups were found (Kruskal-Wallis, P < .001) (Table 1). The mean values of log_{10} total bacterial count in the probiotic groups (2.61 and 2.33 log_{10} CFU/mL for groups A and B, respectively) were significantly lower (P < .001) than the corresponding value in the antibiotic group (3.28 log_{10} CFU/mL). Mean reductions of 1.74 and 2.15 log_{10} cycles in the total bacterial count were observed in groups A and B, respectively, whereas in the antibiotic group the reduction was significantly lower (1.10 log_{10} cycle) (Figure 1). The distribution of the bacterial species in the milk samples on day 21 was similar to that observed on day 0. There were statistically significant differences in the bacterial counts of each dominant bacterial species (S. epidermidis, S. aureus, and S. mitis) in the 3 groups at the end of the trial.
Antibiotics vs Probiotics for Mastitis

Figure 2. Distribution of breast pain scores reported by participants at the beginning (day 0) and at the end (day 21) of the trial in the probiotic groups (group A, *Lactobacillus fermentum* CECT5716; and group B, *Lactobacillus salivarius* CECT5713) and in the antibiotic group (group C). Breast pain categories were 0–4, extremely painful; 5–7, discomfort; and 8–10, no pain.

(Kruskal-Wallis, *P* < .001), and they were always lower (*P* < .001) in the probiotic groups than in the antibiotic group (Table 1).

The highest reductions in the bacterial counts were found in group B (*L. salivarius*) (Figure 1). There was a statistically significant difference (*P* < .001) in the decrease of total bacterial and *S. epidermidis* bacterial counts between the 2 probiotic groups, although the women in both probiotic groups reported the same change in breast pain score (Figure 1). The highest bacterial count decrease was observed for *S. aureus* (2.3 and 2.4 log<sub>10</sub> CFU/mL for groups A and B, and 1.5 log<sub>10</sub> CFU/mL for the antibiotic group) (Figure 1).

The antibiotics prescribed to group C women were amoxicillin-clavulanic acid (38.6%), amoxicillin (22.8%), cotrimoxazole (18.8%), cloxacillin (17.8%), and erythromycin (2%) (Table 2). The effectiveness of these antibiotics in the reduction of bacterial counts differed significantly (Kruskall-Wallis, *P* < .001 for total bacteria and *S. epidermidis*, *P* = .005 for *S. aureus*).

Figure 3. Banding patterns determined by pulsed-field gel electrophoresis (PFGE) of SmaI-digested genomic DNA from *Lactobacillus salivarius* CECT5713 (lane 1), 2 milk isolates that hybridized with the *L. salivarius* probe in the colony hybridization assay (lanes 2 and 3), *L. salivarius* CECT4062 (lane 4), *L. salivarius* CECT4063 (lane 5), *L. salivarius* DSM 20492 (lane 6), *Lactobacillus fermentum* CECT5716 (lane 7), 2 milk isolates that hybridized with the *L. fermentum* probe in the hybridization assay (lanes 8 and 9), *L. fermentum* CECT1285 (lane 10), *L. fermentum* CECT4007 (lane 11), and *L. fermentum* LMG 8900 (lane 12). Lane L represents the Low Range PFG standard (New England BioLabs).
and $P = .018\text{ for }S.\text{ mitis}$. Cotrimoxazole lowered the mean bacterial count by $2.5\log_{10}$ cycles and was particularly effective against $S.\text{ aureus}$. Amoxicillin-clavulanic acid led to a $1.22\log_{10}$ cycles reduction of the mean bacterial count, whereas the efficacy of amoxicillin and cloxacillin was lower. The counts of the 2 women who received erythromycin did not change at the end of the study (Table 2). On day 21, lactobacilli could not be detected in samples from the antibiotic group, but they were isolated from more than half of the women in the probiotic groups (Table 3).

**Evolution of the clinical symptoms.** The mean score of breast pain reported by the women was similar at day 0 in the 3 groups, ranging 2.01–2.35 (Table 1). At day 21, the breast pain score had improved in most of the participants, but 11 women (11%) of the antibiotic group reported no change or felt slightly worse. There were statistically significant differences (Kruskal-Wallis, $P < .001$) between the breast pain scores in the probiotic groups (8.68 and 8.61) and the breast pain score in the antibiotic group (5.81) at day 21 (Table 1). The scores of breast pain in women assigned to group C varied depending on the antibiotic (Table 2) and were widely distributed at the end of the trial: 27 women reported an intense pain (score 0–4), 45 women improved but still reported discomfort for breastfeeding (5–7), and only 29 women recovered completely (8–10) (Figure 2). In contrast, most of the women of the probiotic groups (88% of group A and 85% of group B) had complete recovery at the end of the trial, whereas the rest (12% of group A and 14% of group B) reported slight breastfeeding discomfort. The breast pain score was strongly related to the value of total bacterial load in breast milk at both day 0 (Spearman $\rho = −0.750$) and day 21 ($\rho = −0.764\text{ } (P < .001)$).

Clinical symptoms disappeared or notably improved among most of the women assigned to either probiotic group (Table 1), whereas the evolution was variable among those assigned to the antibiotic group (Table 2; Figure 2). In fact, all the women ($n = 9$) who decided to stop breastfeeding during the trial belonged to the antibiotic group. The rate of recurrence of mastitis in the antibiotic group (30.7%) was significantly higher than the corresponding rate in the probiotic groups ($\chi^2 = 27.08\text{, }P < .001$), but there was no difference between the probiotic groups regarding this parameter (rate for group A, 10.5%, and rate for group B, 7.1%; $\chi^2 = 0.91\text{, }P = .340\text{ } (P > .05)\text{ (Table 3).}$ Some of the women who were receiving antibiotics ($9\text{ [8.9%]}$) developed vaginal candidiasis, whereas this effect was not reported in the probiotic groups. Most of the vaginal candidiasis cases were associated with the use of amoxicillin ($n = 5$) and the rest with cloxacillin ($n = 3$) or amoxicillin-clavulanic acid ($n = 1$). Finally, 9 (5.6%) of the women of the group A reported flatulence associated with the ingestion of the probiotic $L.\text{ fermentum,}$ although all of them completed the trial period.

**Detection of $L.\text{ salivarius CECT5713$ and L. fermentum CECT5716.** Lactobacilli were typified by the PFGE technique. The profiles revealed that all the $L.\text{ salivarius$ and $L. fermentum$ isolates detected by colony hybridization belonged to the strains CECT5713 and CECT5716, respectively (Figure 3).

**DISCUSSION**

In previous studies, we isolated some lactobacilli strains from human milk, including $L.\text{ salivarius CECT5713$ and L. fermentum CECT5716$ [8, 10]. These strains were particularly appealing as a probiotic alternative for the treatment of mastitis because of their origin, safety [17], and anti-infectious [18] and immunomodulatory [19] properties. It has already been shown that lactic acid bacteria isolated from human milk have the potential to prevent breast infection caused by $S.\text{ aureus}$ [20]. Recently, a pilot trial highlighted the potential of $L.\text{ salivarius CECT5713$ and $L.\text{ gasseri CECT5714,}$ 2 strains isolated from breast milk, for the treatment of staphylococcal mastitis [11]. After 30 days, probiotics reduced the mean staphylococcal counts by $\sim 2\log_{10}$ cycles, compared with the value achieved by the antibiotic group. At day 14, no clinical signs of mastitis were observed in women who were assigned to the probiotic group, whereas clinical signs persisted in the control group throughout the study.

In this study, probiotic treatment led to a $1.7–2.1\log_{10}$ cycle reduction in the bacterial count of the milk and to a rapid improvement of the condition. The final bacterial count was $\sim 2.5\log_{10}$ CFU/ml, an acceptable bacterial load in the milk of healthy women [2, 20]. After the probiotic treatment, $L.\text{ salivarius CECT5713$ and $L. fermentum CECT5716$ were detected in milk, but further studies are required to elucidate the pathways that lactobacilli may follow to colonize the mammary gland after oral ingestion.

The antibiotics prescribed to group C women differed significantly in effectiveness, both in the reduction of bacterial counts and in the improvement of the pain score. Although hypothetical, it is probable that a change of antibiotic yielded better results in those cases where treatment was ineffective after the first few days. In fact, cultures of milk samples (including antibiogram) in women with symptoms of mastitis seem to be essential for a more rational and efficient treatment of this condition. For example, staphylococci resistant to $\beta$-lactams are rapidly increasing at the community level [21–24], but such strains remain susceptible to multiple non-$\beta$-lactam antibiotics [25]. However, widespread antibiotic therapy is linked to the increasing rates of bacterial resistance, to molecular changes that may enhance the virulence and biofilm-forming ability of different microorganisms [26], and/or to a variety of adverse effects, including antibiotic-associated diarrhea and vaginal candidiasis [27]. Therefore, the use of probiotics con-
stitutes an attractive approach in the management of mastitis, as suggested by the results of this study.

The use of lactic acid bacteria to treat bovine mastitis has also been tested recently in 2 field trials and has been compared with the use of conventional antibiotic therapy [28, 29]. Results from both trials indicated that intramammary treatment with Lactococcus lactis DPC3147 was at least as efficacious as common antibiotic treatments. Flow cytometry assays demonstrated that live L. lactis can specifically trigger the mammary immune response to elicit polymorphonuclear leukocyte accumulation [29]. These results suggest that the mechanism responsible for this probiotic treatment of mastitis is associated with stimulation of the host intramammary immune system.

Staphylococci are the main etiologic agents of infectious mastitis during lactation. At the species level, S. aureus has been traditionally considered to be the most common agent; however, recent studies have revealed the increasing importance of S. epidermidis that live as an effective alternative to antibiotics for the treatment of mastitis into the mammary glands of lactating mice leads to clinical signs of mastitis [30]. A streptococcal species that have been traditionally considered to be prototypes of commensals of the digestive and upper respiratory tracts, S. mitis (and histological signs of mastitis [30]. A streptococcal species that traditionally considered to be the most common agent; however, recent studies have revealed the increasing importance of S. mitis has been underrated for such effects.

In conclusion, the results obtained in this study suggest that L. salivarius CECT 5713 and L. fermentum CECT5716 can be used as an effective alternative to antibiotics for the treatment of mastitis. Work is in progress to elucidate the mechanisms responsible for such effects.

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References


