Mastitis is a common disease during lactation, with a prevalence of 3%–33% of lactating mothers [1, 2]. This inflammation of ≥1 lobule of the mammary gland usually has an infectious origin [3] involving staphylococci, streptococci, and/or corynebacteria [2]. Traditionally, *Staphylococcus aureus* has been considered to be the main etiological agent of acute mastitis, although *Staphylococcus epidermidis* is emerging as the leading cause of chronic mastitis in both human and veterinary medicine [4–7]. Multidrug resistance and/or the formation of biofilms are very common among clinical isolates of these 2 staphylococcal species. This explains why mastitis is difficult to treat with antibiotics and why it constitutes one of the main reasons to cease breastfeeding [2]. In this context, the development of new strategies based on probiotics, as alternatives or complements to antibiotic therapy for the management of mastitis, is particularly appealing.

In previous studies, we isolated potentially probiotic lactobacilli strains from the milk of healthy mothers [8–10]. Oral administration of either of 2 strains, *Lactobacillus salivarius* CECT5713 and *Lactobacillus gasseri* CECT5714, was an effective alternative for treating staphylococcal mastitis in cases in which previous antibiotic therapy had been unsuccessful [11]. The aim of the present study was to evaluate the efficacy of oral administration of each of 2 lactobacilli strains isolated from breast milk, *Lactobacillus fermentum* CECT5716 and *L. salivarius* CECT5713, for treating lactational
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>Day 0</th>
<th>Group B</th>
<th>Day 1</th>
<th>Group C</th>
<th>Day 1</th>
<th>p&lt;sub&gt;bc&lt;/sub&gt;</th>
<th>Group A</th>
<th>Day 21</th>
<th>Group B</th>
<th>Day 21</th>
<th>Group C&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>4.35 ± 0.57</td>
<td>127</td>
<td>4.47 ± 0.53</td>
<td>101</td>
<td>4.39 ± 0.41</td>
<td>140</td>
<td>2.61 ± 0.64</td>
<td>127</td>
<td>2.33 ± 0.90</td>
<td>101</td>
<td>3.28 ± 1.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>92</td>
<td>4.18 ± 0.70</td>
<td>88</td>
<td>4.30 ± 0.59</td>
<td>76</td>
<td>4.21 ± 0.52</td>
<td>336</td>
<td>2.62 ± 0.49</td>
<td>80</td>
<td>2.52 ± 0.42</td>
<td>76</td>
<td>3.31 ± 0.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>67</td>
<td>3.83 ± 0.55</td>
<td>55</td>
<td>4.06 ± 0.67</td>
<td>30</td>
<td>3.95 ± 0.54</td>
<td>108</td>
<td>4.21 ± 0.50</td>
<td>40</td>
<td>2.26 ± 0.55</td>
<td>25</td>
<td>2.97 ± 0.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>36</td>
<td>3.96 ± 0.47</td>
<td>36</td>
<td>4.07 ± 0.51</td>
<td>35</td>
<td>4.12 ± 0.45</td>
<td>162</td>
<td>3.23 ± 0.37</td>
<td>28</td>
<td>2.29 ± 0.48</td>
<td>31</td>
<td>3.14 ± 0.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>4</td>
<td>4.39 ± 0.56</td>
<td>7</td>
<td>4.08 ± 0.59</td>
<td>4</td>
<td>3.71 ± 0.33</td>
<td>18</td>
<td>2.23 ± 0.60</td>
<td>5</td>
<td>2.09 ± 0.47</td>
<td>3</td>
<td>3.12 ± 1.09</td>
<td></td>
</tr>
<tr>
<td>Rothia spp</td>
<td>2</td>
<td>3.24 ± 0.08</td>
<td>10</td>
<td>3.87 ± 0.58</td>
<td>5</td>
<td>3.48 ± 0.42</td>
<td>0</td>
<td>2.04 ± 0.24</td>
<td>2</td>
<td>2.27 ± 0.04</td>
<td>2</td>
<td>2.39 ± 0.99</td>
<td></td>
</tr>
<tr>
<td>Corynebacterium spp</td>
<td>5</td>
<td>3.65 ± 0.60</td>
<td>2</td>
<td>4.64 ± 0.51</td>
<td>6</td>
<td>3.86 ± 0.50</td>
<td>5</td>
<td>1.94 ± 0.25</td>
<td>2</td>
<td>2.27 ± 0.04</td>
<td>5</td>
<td>2.39 ± 0.99</td>
<td></td>
</tr>
<tr>
<td>Breast pain score</td>
<td>124</td>
<td>2.35 ± 1.28</td>
<td>127</td>
<td>2.16 ± 1.28</td>
<td>101</td>
<td>2.01 ± 1.09</td>
<td>185</td>
<td>8.68 ± 1.06</td>
<td>127</td>
<td>8.61 ± 1.25</td>
<td>101</td>
<td>5.81 ± 2.50</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NOTE. Data are expressed as log<sub>10</sub> 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.

<sup>a</sup> 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; α = 0.05).

<sup>b</sup> Kruskal-Wallis test, α = 0.05.

mastitis in a higher number of women and to compare such an approach with the antibiotic therapy that is usually prescribed 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 >4 log<sub>10</sub> colony-forming units (CFU)/mL, and milk leukocyte count >6 log<sub>10</sub> 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 Clí nico 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 ~9 log<sub>10</sub> 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. The 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 <i>dsr</i> genes with primers J-StGen (<i>S. aureus</i>) and J-StAur (<i>S. epidermidis</i>) [9]. The primer pair J-StGen and J-StEpi results in a 249 bp species-specific fragment, and the primer pair J-StGen and J-StEpi results in a 249 bp <i>S. epidermidis</i> species-specific fragment [11]. Identification of streptococci was performed by partial amplification (488 bp) and sequencing of the gene tuf with primers TufStrep-1 (<i>S. gordonii</i>) and TufStrep-2 (<i>S. epidermidis</i>) [13]. Identification of the potential <i>Strepto-
Lactobacillus salivarius CECT5713 and L. fermentum were submitted to pulsed-field gel electrophoresis (PFGE) genotyping as previously described [11]. Their profiles were compared with those of L. salivarius DSM 20492, L. fermentum CECT4063, L. salivarius DSM 20492, L. fermentum CECT285, L. fermentum CECT4007, and 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 logarithmic 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-

**Antibiotics vs Probiotics for Mastitis**

- **Staphylococcus epidermidis**
- **Staphylococcus aureus**
- **Streptococcus mitis**

**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′-ATCCAC- CGTAAAGAATG-3′) and SAL285R (5′-TATCTACCTGCTCTTGG- TAG-3′). Parallel, a fragment (192 bp) was amplified from L. fermentum CECT5716 genomic DNA with primers Lfer-3 (5′-ACTAAGTCTGATCTACGAG-3′) and Lfer-4 (5′-TTCACTGCTCAAGTATCAG-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 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′-AGAGTTTGATCCTGGCT- CAG-3′) and mlb16 (5′-GGCTGCTGCGCAGTAGTTAG-3′) [15]. Their identity was determined 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′-ATCCAC- CGTAAAGAATG-3′) and SAL285R (5′-TATCTACCTGCTCTTGG- TAG-3′). Parallel, a fragment (192 bp) was amplified from L. fermentum CECT5716 genomic DNA with primers Lfer-3 (5′-ACTAAGTCTGATCTACGAG-3′) and Lfer-4 (5′-TTCACTGCTCAAGTATCAG-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 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 logarithmic 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-

**Figure 1.** Box and whisker plots showing changes in bacterial count (total, Staphylococcus epidermidis, Staphylococcus aureus, and Streptococcus mitis) of breast milk samples and changes in breast pain score reported by the participants after probiotic (Lactobacillus fermentum CECT5716 in group A and Lactobacillus salivarius CECT5713 in group B) or antibiotic (group C) treatment. Differences in the changes experienced for each group were evaluated with nonparametric multiple comparison tests and are shown with horizontal lines inside each graph (*P<.01; **P<.001). The horizontal line in the middle of each box represents the median, while the top and bottom borders of the box represent the 75% and 25% percentiles, respectively. The mean is represented as a cross, and the outliers as individual points outside the boxes. Breast pain score ranged from 0 (extremely painful) to 10 (no pain).
Other bacterial species were identified in S. mitis (43%) and S. epidermidis (isolated from 73% of the women), and S. aureus (from 30%) were the dominant species in the milk 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.

### 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.

Other analyses were performed using the software package SAS, version 9.1 (SAS Institute).

### 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>Reduction in bacterial counts(^b)</th>
<th>Change in breast pain score(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Mean ± SD</td>
<td>n Mean ± SD</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>39 –1.22 ± 0.84</td>
<td>39 4.67 ± 1.90</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>23 –0.55 ± 0.56</td>
<td>23 2.61 ± 2.52</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>19 –2.50 ± 1.21</td>
<td>19 1.50 ± 2.15</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>18 –0.27 ± 0.41</td>
<td>18 2.61 ± 2.52</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2 2 –0.50 ± 0.59</td>
<td>2 1.50 ± 2.15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>101 –1.15 ± 0.84</td>
<td>101 4.67 ± 1.90</td>
</tr>
</tbody>
</table>

### 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 67 (54.0)</td>
</tr>
<tr>
<td>Lactobacillus salivarius CECT5713</td>
<td>127 68 (53.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>251 135 (53.8)</td>
</tr>
<tr>
<td>Antibiotic</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>39 0 (0)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>23 0 (0)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>19 0 (0)</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>18 0 (0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2 0 (0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>101 0 (0)</td>
</tr>
</tbody>
</table>

\(^a\) 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).

\(^b\) 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 (BioMérieux).

\(^c\) \(\chi^2 = 0.91, P = .340\)

\(^d\) \(\chi^2 = 27.08, P < .001\)

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

\(\chi^2\) value was set at .05. All analyses were performed using the software package SAS, version 9.1 (SAS Institute).
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_{10} CFU/mL for groups A and B, and 1.5 log_{10} 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 Smal-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 CECT2285 (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).
L. fermentum,
ported flatulence associated with the ingestion of the probiotic than the corresponding rate in the probiotic groups ( ). Mastitis in the antibiotic group (30.7%) was significantly higher belonged to the antibiotic group. The rate of recurrence of ( ). Finally, 9 (5.6%) of the women of the group A re-
the rest with cloxacillin ( ) or amoxicillin-clavulanic acid reported in the probiotic groups. Most of the vaginal candidiasis ( ) who decided to stop breastfeeding during the trial
1), whereas the evolution was variable among those assigned to either probiotic group (Table 2; Figure 2). In fact, all the women assigned to either probiotic group (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 ( $\rho = -0.764$) ($P < .001$).

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 ($\chi^2 = 27.08$, $P < .001$), 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%; $\chi^2 = 0.91$, $P = .340$) (Table 3).

Some of the women who were receiving antibiotics (9 [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. fermentum, although all of them completed the trial period.

**DISCUSSION**

In previous studies, we isolated some lactobacilli strains from human milk, including L. 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. aureus [20]. Recently, a pilot trial highlighted the potential of L. salivarius CECT5713 and L. 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. 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 in bovine and human mastitis [4–7]. In fact, inoculation of S. epidermidis strains isolated from human mastitis into the mammary glands of lactating mice leads to clinical and histological signs of mastitis [30]. A streptococcal species (S. mitis) was also commonly isolated from milk of women with mastitis in this study. The S. mitis group contains 11 species that have been traditionally considered to be prototypes of commensals of the digestive and upper respiratory tracts, along with one of the leading human pathogens (Streptococcus pneumoniae). However, in recent years, it has become evident that the pathogenic potential of S. mitis has been underrated [14, 31].

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