RESEARCH ARTICLE

Staphylococcus epidermidis in feedings and feces of preterm neonates

Laura Moles1,*, Marta Gómez1,2, Elena Moroder1, Gerardo Bustos3,4,5, Ana Melgar3,4, Rosa del Campo6, Juan M. Rodríguez1

1 Department of Nutrition and Food Science, Complutense University of Madrid, Madrid, Spain, 2 Pediatrics Service, Hospital Francesc de Borja, Gandía, Valencia, Spain, 3 Neonatology Service, University Hospital 12 de Octubre, Madrid, Spain, 4 Red de Salud Materno-Infantil y del Desarrollo (SAMID), Carlos III Institute, Madrid, Spain, 5 Department of Public Health, Mother and Child, Complutense University of Madrid, Madrid, Spain, 6 Microbiology and Parasitology Service, University Hospital Ramón y Cajal, Ramón y Cajal Health Research Institute, Madrid, Spain

* lawita21@hotmail.com

Abstract

Staphylococcus epidermidis has emerged as the leading agent causing neonatal late-onset sepsis in preterm neonates; although the severity of the episodes caused by this species is often underestimated, it might exert relevant short- and long-term detrimental effects on neonatal outcomes. In this context, the objective of this study was to characterize a collection of S. epidermidis strains obtained from meconium and feces of preterm infants, and to assess the potential role of the enteral feeding tubes as potential reservoirs for this microorganism. A total of 26 preterm infants were enrolled in the study. Meconium and fecal samples were collected weekly during their first month of life (n = 92). Feeding samples were collected after their pass through the enteral feeding tubes (n = 84).

S. epidermidis was present in the fecal samples of all the infants in at least one sampling time at concentrations ranging from 6.5 to 7.8 log10 CFU/g. Initially, 344 isolates were obtained and pulsed-field gel electrophoresis (PFGE) profiling allowed the reduction of the collection to 101 strains. Among them, multilocus sequence typing (MLST) profiling showed the presence of 32 different sequence types (ST). Globally, most of the STs clustered in hospital-adapted high-risk clones and belonged to clonal complexes (CC) associated to the hospital environment, such as CC2. The virulence gene most commonly detected among the strains was altE. High resistance rates to macrolides and aminoglycosides were detected and 64% of the strains harboured the mecA gene, which was codified in SCCmec types.

Introduction

Staphylococcus epidermidis is probably the most ubiquitous microorganism in human skin and mucosal surfaces [1,2]. This species seems to play a beneficial role in infants by inhibiting...
virulent pathogens and educating the innate immune system [3]. Several studies have shown that 
*S. epidermidis* is the predominant bacteria in colostrum and milk from healthy women 
[4–10] and there is a mother-to-infant transmission through breastfeeding [11]; in fact, its 
presence is known to be a differential trait of the fecal microbiota of breast-fed infants when 
compared to that of formula-fed ones [10, 12–16], being already present in the first meconium 
obtained from both term and preterm breastfed neonates [17, 18]; however, coagulase-negative 
staphylococci (CNS) have received a marginal attention regarding their role in the early 
colonization of the infant gut in contrast to lactobacilli, bifidobacteria and other gut-associated 
strict anaerobes.

The commensal or beneficial role of *S. epidermidis* in healthy hosts, including infants, 
may dramatically change in preterm neonates. Several factors in the neonatal intensive care 
unit (NICU), such as the widespread use of medical devices (catheters, enteral feeding tubes, 
mechanical ventilation . . .) where they can rapidly form thick biofilms, the selective pressure 
due to antibiotics or the immunocompromised status of the host, can explain the conversion 
of *S. epidermidis* from a symbiont member of the human microbiota to a very relevant oppor-
tunistic infectious agent [3]. Under such circumstances, this species has emerged as the pre-
dominant agent causing neonatal late-onset sepsis (LOS) in very low birth weight (VLBW) 
preterm neonates both in developed and developing countries [19–23]. Generally, LOS by 
CNS is associated to lower mortality rates when compared to other LOS agents [24]. However, 
beyond sepsis, *S. epidermidis* is associated with several neonatal morbidities, such as broncho-
pulmonary dysplasia, white matter injury, necrotizing enterocolitis and retinopathy of prema-
turity, which exert short- and long-term detrimental effects on neonatal outcomes [25, 26].

In a previous work, we studied the microbiota of feces from preterm neonates by using 
both culture-dependent and -independent techniques [18]. The dominant species found were 
*S. epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Serratia 
marcescens*, *Klebsiella pneumoniae* and *Escherichia coli*. Subsequently, most of the dominant 
species were characterized and a high proportion of antibiotic-resistant high-risk clones was 
detected during the NICU admittance [27]. However, although *S. epidermidis* was the main 
species taken in account to the percentage of carrying infants, isolates from this species were 
initially excluded from the first analysis due to the high number of isolates and because they 
seemed to have a particularly wide genetic diversity. In this context, the objective of this study 
was to characterize a collection of *S. epidermidis* strains isolated from meconium and feces of 
preterm infants.

**Material and methods**

**Patients and sampling**

Twenty-six preterm infants (gestational age <32 weeks and/or weight <1,500 g) born between 
October 2009 and June 2010 in the University Hospital Doce de Octubre (Madrid, Spain) were 
included in the study. Preterm infants with malformations, metabolic diseases or severe condi-
tions were excluded. The local ethic committee (CEIm Hospital Universitario 12 de Octubre) 
approved the study (reference 09/157), and parents signed informed consent. All infants were 
fed with human milk (pasteurized donor milk and/or their mother’s own milk [MOM]) and, 
occasionally, with preterm formula. The fortifier FM 85 (Nestlé, Vevey, Switzerland) was used 
from approximately the fourth week of life. The high heterogeneity in feeding patterns pre-
vented the formation of well-defined feeding groups.

First spontaneously evacuated meconium and fecal samples were collected weekly during the 
first month of life. Feeding samples were collected after their pass through the enteral feeding 
tubes from either the syringe or the extension set connected with the orogastric or nasogastric
feeding tube. Extension sets were routinely replaced and discarded every 24 h, thus during this period different feed types could pass through the same tube. In contrast, the nasogastric feeding tubes were remained inserted for up to 4 days. Contamination of feeding tubes and the connector with the extension set was reviewed and confirmed in a previous work [28]. Preparation of the feedings previous to their pass through the enteral feeding system was as follows: (a) MOM was extracted using an electric pump and stored either refrigerated (5˚C) for maximum of 24 h, or frozen (-20˚C) up to 3 months; (b) donor milk was pasteurized (62.5˚C, 30 min) and stored frozen (-20˚C) after collection up to 3 months; and (c) commercial sterilized formula milk was ready-to-use in individual doses. All feedings were incubated for 10 to 15 min at 37˚C to 40˚C before their administration. After collection, the samples were immediately stored at −80˚C until analysis.

**Culture-based analysis, bacterial identification and genotyping**

Adequate dilutions of meconium, feces and feeding samples obtained during the NICU stay were spread onto Columbia Nalidixic Acid (CNA, BioMérieux) agar plates. Plates were aerobically incubated at 37˚C for up to 48 h. After bacterial counting, at least one representative of each colony morphology were analyzed by optical microscopy and those isolates compatible with staphylococcal morphology were identified by species-specific PCR based on the *dnaJ* genes with primers J-StGen (5’-TGGCCAAAAGAGACTATTATGA-3’), J-StEpi (5’-CCACCAAAGCCCTTGACTT-3’) [10]. Bacterial identification was also confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry in a Vitek-MS instrument (BioMérieux).

In order to avoid repeated isolates, DNA from the *S. epidermidis* isolated colonies was extracted following the protocol of Ruiz-Barba et al. [29] and submitted to randomly amplified polymorphic DNA PCR (RAPD-PCR) using the primer OPL5 (5’-ACGCAGGCAC-3’) as described by Rodas et al. [30]. At least a representative isolate from each RAPD profile per sample was selected to perform PFGE in a CHEF DR II apparatus (Bio-Rad, Birmingham, UK). *Sma*I-digested fragments were separated with the electrophoresis conditions of 5 to 15 s for 10 h and 15 to 60 s for 13 h. A dendrogram analysis of PFGE profiles was performed using the UPGMA method based on Dice similarity index and the INFO QUEST software (BioLine). Finally, the *S. epidermidis* MLST scheme was applied for PFGE-unrelated strains of *S. epidermidis* (http://www.mlst.net). Genetic diversity was represented by the Minimum Spanning Tree algorithm using the Phyloviz software (http://www.phyloviz.net).

**Screening for potential virulence and antibiotic resistant determinants**

Assessment of genes encoding virulence factors was carried out with the same set of strains that were submitted to MLST analysis. The tested genes included *icaD*, *fbe* and *altE* (which encode proteins involved in adhesion and biofilm production) [31] and *mecA* (resistance to methicillin) [32]. The staphylococcal cassette chromosome *mec* (SCCmec) was determined by using a typing procedure involving a PCR amplification (*ccrB* genes) followed by restriction fragment length polymorphism (RFLP) analysis using endonucleases *Hinf*I and *Bsm*I [32]. SCCmec elements are classified into types by a hierarchical system, considering types I, II and III have a hospital-acquired origin, whereas type IV is associated to the community.

**Antimicrobial susceptibility testing**

All the *S. epidermidis* strains submitted to MLST analysis were also tested for antibiotic susceptibility testing. Susceptibility to penicillin, oxacillin, gentamicin, tobramycin, levofloxacin, erythromycin, clindamycin, linezolid, daptomycin, teicoplanin, vancomycin, phosphomycin,
fusidic acid, mupirocin, rifampicin and cotrimoxazol was assessed in a MicroScan WalkAway (Beckman Coulter) compact equipment. The breakpoints were selected according to the guidelines of the Clinical and Laboratory Standards Institute [33].

Statistical analysis
The statistical analysis was performed using R 2.15.3 (R-project, http://www.r-project.org). Data not normally distributed were represented as medians and interquartile ranges (Q1 and Q3); on the other hand, normally distributed data were expressed as means and 95% confidence interval (95% CI). The Kruskal-Wallis test was used to evaluate the differences between sampling times in non-normal data. Fisher’s exact test was used to compare proportions. In all cases, P values of <0.05 were considered to be significant.

Results
Characteristics of premature infants
Demographic and clinical data of the infants are provided in Table 1. Mean gestational age and birth weight were 27.7 weeks and 1,167 g, respectively. Most of them (92%) received antibiotic treatment during their hospital stay. Some of them (42%) received a combination of ampicillin and gentamicin as a prophylactic antibiotherapy while these and other antimicrobials (ampicillin B, amikacin, doxacillin, erythromycin, fluconazole, fluocytosine, meropenem, micafungin, teicoplanin, vancomycin) were occasionally used against a confirmed or suspected infection. Approximately half of the infants (54%) were born by Cesarean section and, consequently, their mothers received prophylactic or presurgical antibiotics per established clinical protocol. Respiratory disorders were common among our population since 21 of the newborns required continuous positive airway pressure (CPAP), 20 of them needed oxygen therapy and 16 of them required the use of mechanical ventilation, with median durations of 9, 32 and 8.5 days, respectively. Nearly all infants required parenteral nutrition at birth for a median duration of 7.5 days while all of them were fed with enteral feeding tubes for a mean period of 59 days. Median values for NICU and hospital stays reached 42 and 64 days, respectively. 27% of the infants suffered at least one sepsis episode during their NICU admittance. *S. epidermidis* was identified in the 71% of the sepsis episodes and affected to 19% of the infants, either alone or together with other microorganisms.

*S. epidermidis* from feces and feeding systems
The microbial culture of fecal samples yielded *S. epidermidis* colonies in all infants, although not in all samples. *S. epidermidis* was frequently detected in the meconium samples (38%), although its presence notably increased in feces collected at day 7 after birth (89%). Subsequently, its fecal presence decreased progressively until day 28, when only 27% of the infants harbored it (Table 2).

In the positive samples, the *S. epidermidis* counts varied between 6.5 and 7.8 log\(_{10}\) CFU/g over the studied period. In order to evaluate the effect of antibiotherapy specific to Gram-positive bacteria on the *S. epidermidis* counts over time, samples were subdivided in those that were collected after an antibiotic treatment or not. In this case, *S. epidermidis* counts varied from 5.0 to 7.7 log\(_{10}\) CFU/g (p.value 0.000) and 6.4 and 8.0 in samples collected when antibiotics were administered in the previous days or not respectively (Table 2). An increase in the concentration values from meconium to day 7 feces was observed; then, the concentrations decreased slowly and rose again at the end of the first month of life of the infants. When fecal bacterial counts were related to demographic and clinical data, *S. epidermidis* colonization
Table 1. Demographic data and clinical characteristics of the preterm infants participating this study.

| Characteristics          | Infants | Gestational age (wk)\(^a\) | Gender\(^b\) | Birth weight (g)\(^a\) | Delivery mode\(^b\) | Antibiotherapy (days)\(^b\) | Bronchopulmonary dysplasia\(^b\) | Chorioamnionitis\(^b\) | Sepsis\(^b\) | Parenteral nutrition, n = 22 (days)\(^\ast\) | Enteral feeding tube (days)\(^a\) | Mechanical ventilation, n = 16 (days)\(^\ast\) | CPAP, n = 21 (days)\(^\ast\) | Oxigenotherapy, n = 20 (days)\(^\ast\) | NICUs (days)\(^\ast\) | Hospital stay (days)\(^\ast\) |
|--------------------------|---------|----------------------------|--------------|-------------------------|----------------------|-----------------------------|-------------------------------|------------------------|--------------|-----------------------------------------------|-------------------------------|-----------------------------------------------|-----------------------------|-----------------------------------------------|-----------------------------|------------------------|
|                          | 26      | 27.7 (26.6;28.7)           | Male: 13 (50%) | Female: 13 (50%)        | Vaginal: 12 (46%)   | No: 2 (8%)                  | No: 15 (58%)                  | No: 24 (92%)         | Total: 7 (27%) | 7.5 (5–12.8)\(^\ast\)                          | 59 (46;72)                      | 8.5 (1–35.5)\(^\ast\)                      | 9 (5–45)\(^\ast\)            | 32 (2–84)\(^\ast\)                           | 42 (18.5–77.8)\(^\ast\)     | 64 (41–90)\(^\ast\)                          |
|                          |         |                            |              |                         | Cesarean section: 14 (54%) | Yes: 24 (92%)             | Yes: 11 (42%)              | Yes: 2 (8%)        | Caused by S. epidermidis: 5 (19%) |                          |                         |                             |                           |                          |                             |
| Values expressed as:     |         |                            |              |                         |                       |                             |                             |                        |                          | Mean (95% CI);                          |                             |                             |                             |                              |                             |                             |

\(^a\)Mean (95% CI);

\(^b\)n (%);

\(^\ast\)Median (IQR).

https://doi.org/10.1371/journal.pone.0227823.t001

Table 2. Isolation and quantification (log\(_{10}\) cfu/g) of S. epidermidis in meconium and fecal samples the recruited infants.

| Antibiotics previous to sample collection | Meconium (n = 16) | 1st week feces (n = 18) | 2nd week feces (n = 23) | 3rd week feces (n = 24) | 4th week feces (n = 11) | P value |
|------------------------------------------|-------------------|------------------------|------------------------|------------------------|------------------------|---------|
| Total                                    |                   |                        |                        |                        |                        |         |
| n (%)                                    | 6 (38%)           | 16 (89%)               | 13 (57%)               | 12 (50%)               | 3 (27%)                |         |
| Microbial Counts Median (IQR)            | 6.80 (4.80–8.80)  | 7.85 (7.00–8.38)       | 6.70 (6.30–7.30)       | 6.50 (5.93–7.53)       | 7.70 (7.65–8.50)       | 0.098\(^\ast\) |
| YES                                      |                   |                        |                        |                        |                        |         |
| n (%)                                    | 3 (50%)           | 15 (94%)               | 1 (8%)                 | 3 (25%)                | 0 (0%)                 |         |
| Microbial Counts Median (IQR)            | 6.60 (4.65–8.10)  | 7.70 (7.02–8.45)       | 5.00 (5.00–5.00)       | 7.65 (7.18–7.91)       | -                      | 0.000\(^\ast\) |
| NO                                       |                   |                        |                        |                        |                        |         |
| n (%)                                    | 3 (50%)           | 1 (6%)                 | 12 (92%)               | 9 (75%)                | 3 (100%)               |         |
| Microbial Counts Median (IQR)            | 7.93 (5.66–8.70)  | 8.00 (8.00–8.00)       | 7.13 (6.62–7.37)       | 6.39 (5.62–7.53)       | 7.54 (6.09–9.19)       | 0.225\(^\ast\) |

\(^\ast\)Friedman test.

https://doi.org/10.1371/journal.pone.0227823.t002
tended to be higher in those infants with longer NICU stay, requiring more days of CPAP or mechanical ventilation, and born by Cesarean section, although the values did not reach a statistically-significant level. Interestingly, the *S. epidermidis* bacterial counts were slightly lower in those infants suffering sepsis caused by *S. epidermidis*, however it is important to remark that these infants were treated with antibiotics (combinations of Teicoplanin with Vancomycin, Eritromycin, Gentamicin, Meropenem and/or Amikacin) at the moment of sample collection (Table 3).

In relation to the feeding samples after their pass through the enteral feeding system, *S. epidermidis* was present in 84% of the MOM samples while the detection frequency was significantly lower in donor milk and formula samples (42% and 14%, respectively). *S. epidermidis* counts were also considerably higher in MOM samples (Table 4).

Table 3. Relationships between *S. epidermidis* colonization (counts expressed as log₁₀ CFU/g) and the main clinical and demographic data.

| Parameter                        | Positive samples and counts | P value |
|----------------------------------|-----------------------------|---------|
|                                  | n (%) | Microbial Counts Median (IQR) |       |
| Gestational age                  |       |                                |        |
| < 28 weeks                       | 16 (62%) | 7.91 (6.47–8.35) | 0.958  |
| > 28 weeks                       | 10 (38%) | 7.51 (6.61–8.56) |       |
| Birth weight                     |       |                                |        |
| < 1000 g                         | 11 (42%) | 7.98 (7.03–8.37) | 0.391  |
| > 1000 g                         | 15 (58%) | 7.46 (6.18–8.48) |       |
| Delivery mode                    |       |                                |        |
| Vaginal                          | 12 (46%) | 7.47 (6.67–7.94) | 0.291  |
| Cesarean section                 | 14 (54%) | 8.31 (6.20–8.71) |       |
| Antibiotherapy (days)            |       |                                |        |
| No Ab or <3 days                 | 10 (38%) | 7.46 (6.61–8.12) | 0.399  |
| >3 days                          | 16 (62%) | 7.95 (6.47–8.66) |       |
| Mechanical ventilation           |       |                                |        |
| YES                              | 16 (62%) | 7.95 (7.20–8.50) | 0.246  |
| NO                               | 10 (38%) | 7.08 (5.98–8.13) |       |
| CPAP                             |       |                                |        |
| < 7 days                         | 13 (50%) | 7.36 (5.78–8.32) | 0.238  |
| > 7 days                         | 13 (50%) | 7.98 (7.45–8.42) |       |
| Parenteral nutrition             |       |                                |        |
| < 7 days                         | 15 (58%) | 7.57 (6.64–8.68) | 0.736  |
| > 7 days                         | 11 (42%) | 7.92 (6.24–8.31) |       |
| Enteral feeding tubes            |       |                                |        |
| < 45 days                        | 11 (42%) | 7.46 (6.70–8.48) | 0.835  |
| > 45 days                        | 15 (58%) | 7.92 (5.74–8.37) |       |
| CVN                              |       |                                |        |
| < 7 days                         | 13 (50%) | 7.57 (6.70–8.64) | 0.700  |
| > 7 days                         | 13 (50%) | 7.90 (5.78–8.32) |       |
| NICU stay                        |       |                                |        |
| < 45 days                        | 15 (58%) | 7.45 (6.18–8.11) | 0.119  |
| > 45 days                        | 11 (42%) | 8.30 (7.64–8.57) |       |
| Hospital stay                    |       |                                |        |
| < 60 days                        | 13 (50%) | 7.46 (6.70–8.64) | 0.959  |
| > 60 days                        | 13 (50%) | 7.92 (5.78–8.32) |       |
| Predominance of MOM feedings     |       |                                |        |
| No                               | 5 (19%) | 7.90 (7.57–8.30) | 0.625  |
| Yes                              | 21 (81%) | 7.46 (5.78–8.42) |       |
| *S. epidermidis* Sepsis          |       |                                |        |
| No                               | 21 (81%) | 7.90 (6.70–8.64) | 0.228  |
| Yes                              | 5 (19%) | 6.70 (5.78–7.92) |       |
| Sampling time                    |       |                                |        |
| Meconium (n = 16)                | 6 (38%) | 6.80 (4.80–8.80) | 0.098* |
| 1st week feces (n = 18)          | 16 (89%) | 7.85 (7.00–8.38) |       |
| 2nd week feces (n = 23)          | 13 (57%) | 6.70 (6.30–7.30) |       |
| 3rd week feces (n = 24)          | 12 (50%) | 6.50 (5.93–7.53) |       |
| 4th week feces (n = 11)          | 3 (27%) | 7.70 (7.65–8.50) |       |

*Friedman test.

https://doi.org/10.1371/journal.pone.0227823.t003
Genetic diversity

Initially, 344 *S. epidermidis* isolates were obtained from 92 fecal and 84 feeding samples. After RAPD analysis, one representative of each RAPD profile per sample was selected for further analysis and, subsequently, 173 isolates were submitted to PFGE analysis. PFGE profiling allowed the selection of 101 strains, including one representative of each different PFGE profile per infant. This final collection was genetically characterized using the MLST scheme.

The 101 strains were distributed in 32 different sequence types (ST). The STs more frequently isolated from feces were ST69 (8 infants), ST425 (6 infants), ST59 (5 infants), and STs 2 and 51 (4 infants each), while those more widespread among the feeding samples were ST425 (9 samples), and STs 2, 32, 59 and 69 (5 samples each). Globally, the dominant STs in fecal samples were also majoritarian in the feeding ones. According to MLST profiling, the highest genetic diversity was related to MOM samples (30 different STs from 169 isolates), whereas in the fecal samples 12 different STs were identified from 103 isolates; in addition, up to 38% of the MOM samples contained 3 different ST strains while this percentage was lower (23%) in the fecal samples (Fig 1). MLST typing also revealed the transference or sharing of strains, such as...
as ST59 in infants 1 and 3, ST35 in infants 2 and 11, ST69 in infants 5, 10, 13 and 14, ST32 in infants 4 and 14, or ST278 in infants 19 and 24, between fecal and feeding samples (Fig 1).

Globally, 75% of STs detected in this study belonged to hospital-adapted high-risk clones although this percentage decreases to 53% when only milk samples are considered (Fig 1). Infants suffering a bacteremia episode caused by *S. epidermidis* were colonized by, at least, one strain of *S. epidermidis* belonged to high risk clones (Fig 1), with the exception of infant 24. From the 32 STs detected 13 belonged to clonal complexes (CC) associated to the hospital environment, as CC2, CC5 and CC6 (Fig 2). However, 7 STs had not been previously described; the new STs 589–591, 597 and 598 were assigned to five of them. The remaining two novel STs presented undescribed mutations on some of the studied alleles and their code assignment is still pending. The non-previously reported STs were mostly from MOM and only two of them (ST598 and STNew2), were also detected in fecal samples.

![MLST genetic diversity using minimum spanning tree algorithm](https://doi.org/10.1371/journal.pone.0227823.g002)

*Fig 2. MLST genetic diversity using minimum spanning tree algorithm.* Each circle represents a different MLST clone; size depends on the number of isolates in the group. Clone number is indicated above circle; Circles are colored depending on the frequency of the clone in the analyzed samples: black represent frequency in feces, light grey in MOM, dark grey in donor milk and white in formula milk samples. Clones considered to be of high risk are highlighted in bold. Genetically related sequence types (STs) are connected by grey lines.
Presence of potential virulence genes and antimicrobial susceptibility

The results of the screening for potential virulence genes and antimicrobial susceptibility are presented in Fig 3. The genes most commonly detected in the isolated strains were *altE* (98%), involved in adhesion and biofilm production, and *mecA* (55%), implicated in methicillin resistance. Type III (28.4%) and IV (71.6%) SCC*mec* were identified in 16 and 29 isolates from fecal samples and 14 and 19 isolates from the enteral feeding system respectively.

Regarding antibiotic resistance, most of the *S. epidermidis* strains exhibited resistance to macrolides (70% to erythromycin and 56% to clindamycin), tetracycline (72%), methicillin (64%), aminoglycosides (62% for amikacin and tobramycin and 54% for gentamicin), and teicoplanin (22%) whereas almost all remained susceptible to vancomycin, tigecycline, daptomycin, quinolones and linezolid. A single isolate grouped on the ST2 lineage was resistant to all tested antibiotic including linezolid, daptomycin, fosfomycin, and cotrimoxazol. Globally, the strains with the highest virulence potential were those belonging to the STs 280, 2, 425, 190, 51 and 69, which were also resistant to oxacillin and clindamycin, and showed different resistance levels to gentamycin, tobramycin and mupirocin (Fig 3).

### Fig 3. Profiles of antibiotic susceptibility and virulence factors in the different ST clones.

At least one different sequence types (ST) per infant is represented in each line. Results are represented in function of frequency. Black squares means 100% of the isolates were resistant to an antibiotic or present a virulence determinant, while black and grey striped boxes means 75–99%, grey squares 50–74%, grey and white striped squares 25–49% and white boxes 0–24%. Black bars in the left side of the figure represent the percentage of virulence genes present in each ST while the grey bars on the right side of the figure shows the percentage of antibiotic resistances per ST. OXA: Oxacillin; GM: Gentamicin; TOB: Tobramycin; LVX: Levofloxacin; ERY: Erythromycin; CLI: Clindamycin; LNZ: Linezolid; DAP: Daptomycin; TEIC: Teicoplanin; VAN: Vancomycin; TGC: Tigecycline; FOS: Fosfomycin; FUS: Fusidic Acid; MUP: Mupirocin; RIF: Rifampicin; SXT: Trimethoprim-sulphamethoxazole.

![Fig 3](https://doi.org/10.1371/journal.pone.0227823.g003)
Discussion
In this study we use phenotyping and genotyping techniques to determine the diversity and virulence of S. epidermidis isolates obtained from fecal samples of preterm infants during their hospital stay and from the different types of feeds (MOM, donor milk and adapted formula) collected after their pass through the enteral feeding tubes.

S. epidermidis was relatively frequent in meconium samples (~40%), but the detection frequency notably increased (~90%) in the fecal samples collected 7 days after birth. A pioneering study using a culture-dependent approach revealed that S. epidermidis was often present in aseptically-collected meconium from term neonates and suggested that gut colonization may start in utero during late pregnancy under physiological conditions [10]. Although the issue is still controversial and the subject of an intense scientific debate [34, 35], the results of subsequent studies that applied culture-dependent techniques to study meconium microbiota were similar, both in term [36–39] and preterm neonates [18]. The decrease in the fecal CNS populations after the first weeks of life has been previously reported [40–44].

In relation to the feeding samples, MOM contain their own microbiota while donor milk and preterm formula are usually sterile before their administration through the enteral feeding tubes. Studies carried in the last years have shown that human milk contains a site-specific microbiota and represents a continuous supply of commensal and potentially probiotic bacteria to the infant gut [5]. Most culture-dependent and independent studies on the human milk microbiota have found that CNS, and particularly S. epidermidis, is the dominant species in this biological fluid [5, 8–10, 45–48]. Once in the infant gut, these bacteria drive the assembly of a healthy infant gut microbiota [49] and may play several functions, contributing to the infant metabolism, protection against infections, immunomodulation or neuromodulation [5].

The different milk types were subsequently administered through enteral feeding tubes which are well-suited devices for the growth and enrichment of high risk hospital-associated clones, which subsequently act as reservoirs of such clones [28, 50–52]. In preterm neonates, gastric pH values are closer to neutrality while the pylorus is more relaxed than in adults; this allows the entry of gut bacteria to the gastric compartment. Later, the widespread NICUs practice of aspirating and measuring the residual gastric content before administrating the next feeding leads to the contamination of the upper parts of the enteral feeding systems, including the external parts, with the high risk clones that characterize the preterm gut microbiota. Since feeding tubes are usually placed in the digestive tract of neonates for 24–72 h and are kept at a temperature optimal for the growth of many facultative anaerobic bacteria (CNS, enterobacteria ...), such microorganisms have enough time to form thick mix biofilms in the internal surface of the tubes [28]. This may explain the presence of S. epidermidis in a percentage of donor milk and infant formula samples after their pass through such feeding systems; however, other sources, including healthcare workers and family members, can not be discarded and such possibility should be addressed in future studies.

The results of this study indicate that S. epidermidis population was genetically more diverse among MOM samples than among the fecal samples; this fact may reflect the coexistence of strains from both the human milk microbiota and the reservoir (enteral feeding tubes) when MOM passes through the enteral feeding system. Other contamination sources, such as endotracheal intubation or the hands of healthcare workers cannot be discarded. Later, the NICU environment may exert a role in selecting only those human milk strains which genome flexibility allow them to colonize the gastrointestinal tract of preterm neonates during their stay in the NICUs; as a result, the diversity of S. epidermidis strains is narrower in the feces of the infants. It must be highlighted that S. epidermidis is a bacterial species suited with an
extraordinary genetic flexibility, and can employ a multitude of mechanisms to become adapted to the changing environment [3, 53]. A study comparing 200 S. epidermidis strains isolated from women with lactational mastitis with 105 isolates from milk of healthy women showed that the number of strains that contained the biofilm-related icaD gene and that showed resistance to oxacillin, erythromycin, clindamycin and mupirocin was significantly higher among the strains isolated from the mastitis milk [31]. However, the same study also revealed that potentially pathogenic strains could also be isolated from some samples from healthy women. Another study confirmed that human milk from healthy women may be a reservoir of strains containing potential virulence factor and displaying (multi)resistance against clinically-relevant antibiotics [54]. In this context, most of the clones found in this work were precisely those enriched in virulence factors and antibiotic resistances (ST2, ST51 and ST425), showing higher adaptation to the NICU environment and, therefore, an enhanced capacity for persistence and dissemination. More specifically, a high proportion of the S. epidermidis strains exhibited resistance to β-lactams, macrolides and aminoglycosides. In addition, almost all the methicillin-resistant isolates were also biofilm producers and predominantly carried cassettes type III and IV, which is in agreement with previous reports [3, 20, 55–57]. Both antibiotic (multi)resistance and biofilm production capacity are common characteristics in strains (mainly CC2 ones) adapted to hospital environments [58, 59].

As previously stated [3], S. epidermidis strains may play quite different roles (from “guarding angel to pathogenic devil”) in the neonatal setting. A better knowledge of the predominant S. epidermidis clones (virulence factors, mechanism of biofilm formation in medical devices, mechanisms of immune-evasion, antibiotic resistances, specific sensitivity to phages, synergism or antagonism with other members of the preterm gut microbiota, CRISP systems . . .) that are particularly prevalent in NICUs is required in order to improve their control and, as a consequence, preterm health outcomes at the short and long terms. Microbiome-based strategies, including the restoration of the “safe” CNS population present in MOM when a neonate must be fed with pasteurized donor milk or formula have already been suggested [60]. CNS selected on the basis of a strain-by-strain rigorous safety assessment can be particularly useful to reduce the acquisition of undesired pathogens by infants, including preterm neonates, exposed to NICUs environments. It has been proposed that S. epidermidis and other CNS may have a probiotic function by preventing colonization of the host by more severe pathogens, such as S. aureus [2]. In fact, a future strategy to eradicate S. aureus from the mucosal surfaces has already been postulated based on their in vitro inhibition by S. epidermidis [61, 62] A better knowledge of NICU-associated S. epidermidis strains may help to devise strategies to avoid their conversion from symbiont to pathobiont microorganisms.

Acknowledgments
The dedicated collaboration of the study participants and their families is greatly appreciated.

Author Contributions

Conceptualization: Laura Moles, Gerardo Bustos, Rosa del Campo, Juan M. Rodríguez.

Data curation: Gerardo Bustos, Ana Melgar.

Formal analysis: Laura Moles, Marta Gómez, Elena Moroder.

Funding acquisition: Juan M. Rodríguez.

Investigation: Laura Moles, Juan M. Rodríguez.

Project administration: Juan M. Rodríguez.
References

1. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al. Topographical and temporal diversity of the human skin. Science. 2009; 324(5931):1190–2. https://doi.org/10.1126/science.1171700 PMID: 19478181

2. Otto M. Staphylococcus epidermidis—The "accidental" pathogen. Nat Rev Microbiol. 2009; 7(8):555–67. https://doi.org/10.1038/nrmicro2182 PMID: 19609257

3. Dong Y, Speer CP. The role of Staphylococcus epidermidis in neonatal sepsis: Guarding angel or pathogenic devil? Int J Med Microbiol. 2014; 304(5–6):513–20. https://doi.org/10.1016/j.ijmm.2014.04.013 PMID: 24881963

4. Altuntas EG. Isolation, identification and characterization of Staphylococcus epidermidis in human milk. Food Sci Technol. 2015; 60(1):36–41. https://doi.org/10.1016/j.lwt.2014.07.012

5. Heikkila MP, Saris PEJ. Inhibition of Staphylococcus aureus by the commensal bacteria of human milk. J Appl Microbiol. 2003; 95(3):471–8. https://doi.org/10.1046/j.1365-2672.2003.02002.x PMID: 12911694

6. Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, et al. The human milk microbiota: Origin and potential roles in health and disease. Pharmacol Res. 2013; 69(1):1–10. https://doi.org/10.1016/j.phrs.2012.09.001 PMID: 22974824

7. Damasceno QS, Souza JP, Nicoli JR, Paula RL, Assis GB, Figueiredo HC, et al. Evaluation of Potential Probiotics Isolated from Human Milk and Colostrum. Probiotics Antimicrob Proteins. 2017; 9(4):371–9. https://doi.org/10.1007/s12602-017-9270-1 PMID: 28374172

8. Martín Rocío, Heilig HGHJ, Zoetendal EG, Jimenez E. Cultivation-independent assessment of the bacterial diversity of breast milk among healthy women. Res Microbiol. 2007; 158:31–7. https://doi.org/10.1016/j.resmic.2006.11.004 PMID: 17224259

9. Jiménez E, Delgado S, Ferna L, Rodri JM. Assessment of the bacterial diversity of human colostrum and screening of staphylococcal and enterococcal populations for potential virulence factors. Res Microbiol. 2008; 159:595–601. https://doi.org/10.1016/j.resmic.2008.09.001 PMID: 18845249

10. Jiménez E, Delgado S, Maldonado A, Arroyo R, Albújar M, García N, et al. Staphylococcus epidermidis: A differential trait of the fecal microbiota of breast-fed infants. 2008; 11:1–11.

11. Martín V, Maldonado-Barragán A, Moles L, Rodriguez-Baños M, Campo R Del, Fernández L, et al. Sharing of bacterial strains between breast milk and infant feces. J Hum Lact. 2012; 28(1):36–44. https://doi.org/10.1177/08903344114124729 PMID: 22267318

12. Adlerberth I, Lindberg E, Åberg N, Hesselmar B, Saalman R, Strannegård IL, et al. Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: An effect of hygienic lifestyle? Pediatr Res. 2006; 59(1):96–101. https://doi.org/10.1203/pdr.0000191137.12774.b2 PMID: 16980405

13. Sakata H, Yoshio H, Fujita K. Development of the intestinal flora in very low birth weight infants compared to normal full-term newborns. 1985;186–90.

14. Lundequist B, Nord CE, Winberg J. The Composition of the Faecal Microflora in Breastfed and Bottle Fed Infants from Birth to Eight Weeks. Acta Paediatr Scand. 1985; 74(5):45–51. https://doi.org/10.1111/j.1651-2227.1985.tb10919.x PMID: 3984727

15. Borderon J, Lionnet C, Rondeau C, Suc A, Laugier J, Gold F. Current aspects of the fecal flora of the newborn without antibiotic therapy during the first 7 days of life: Enterobacteriaceae, enterococci, staphylococci. Pathol Biol. 1996; 44(5):416–22. PMID: 8758487

16. Balmer SE, Wharton BA. Diet and faecal flora in the newborn: breast milk and infant formula. Arch Dis Child. 1989; 64:1672–7.

17. Jiménez E, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, et al. Is meconium from healthy newborns actually sterile? Res Microbiol. 2008; 159(3):187–93. https://doi.org/10.1016/j.resmic.2007.12.007 PMID: 18281199
18. Moles L, Gómez M, Heilig H, Bustos G, Fuentes S, de Vos W, et al. Bacterial Diversity in Meconium of Preterm Neonates and Evolution of Their Fecal Microbiota during the First Month of Life. PLoS One. 2013; 8(6).

19. Otto M. Molecular basis of Staphylococcus epidermidis infections. Semin Immunopathol. 2012; 34(2):201–14. https://doi.org/10.1007/s00281-011-0296-2 PMID: 22095240

20. Du X, Zhu Y, Song Y, Li T, Luo T, Sun G, et al. Molecular Analysis of Staphylococcus epidermidis Strains Isolated from Community and Hospital Environments in China. PLoS One. 2013; 8(5).

21. Fill Malfertheiner S, Wendt S, Layer F, Weigl M, Seelbach-Göbel B, König W, et al. Prevalence, clonality, and pathogenicity of Staphylococcus epidermidis isolates in newborn feces. Eur J Clin Microbiol Infect Dis. 2017; 36(10):1955–64. https://doi.org/10.1007/s10096-017-3019-3 PMID: 28560472

22. Adams-Chapman I c, Stoll BJ. Prevention of nosocomial infections in the neonatal intensive care unit. Curr Opin Pediatr. 2002; 14(2):157–64. https://doi.org/10.1097/00008480-200204000-00003 PMID: 11981284

23. Hammoud MS, Al-Ta’ar A, Thalib L, Al-Sweih N, Pathan S, Isaacs D. Incidence, aetiology and resistance of late-onset neonatal sepsis: A five-year prospective study. J Paediatr Child Health. 2012; 48(7):604–9. https://doi.org/10.1111/j.1440-1754.2012.02432.x PMID: 22404730

24. Tsai M, Hsu J, Chu S, Lien R. Incidence, Clinical Characteristics and Risk Factors for Adverse Outcome in Neonates With Late-onset Sepsis. 2014; 33(1):7–13.

25. Dong Y, Speer CP, Glaser K. Beyond sepsis: Staphylococcus epidermidis is an underestimated but significant contributor to neonatal morbidity. 2018; 9(1):621–33.

26. Stoll BJ, Hansen NI, Adams-Chapman I, Hintz SR, Vohr B, Higgins RD. Neurodevelopmental and Growth Impairment Among Extremely Low-Birth-Weight Infants With Neonatal Infection. 2004; 48(7):2357–65.

27. Moles L, Gómez M, Jiménez E, Fernández L, Bustos G, Chaves F, et al. Preterm infant gut colonization in the neonatal ICU and complete restoration 2 years later. Clin Microbiol Infect. 2015; 21(10):936.e1–936.e10. https://doi.org/10.1016/j.cmi.2015.06.003 PMID: 26086569

28. Gómez M, Moles L, Melgar A, Ureta N, Bustos G, Fernández L, et al. Early gut colonization of preterm infants: Effect of enteral feeding tubes. J Pediatr Gastroenterol Nutr. 2016; 62(6):893–900. https://doi.org/10.1097/MPG.0000000000001104 PMID: 26741949

29. Ruiz-barbá JA, Maldonado A, Jiménez-díaz RW. Small-scale total DNA extraction from bacteria and yeast for PCR applications. 2005; 347:333–5.

30. Rodas AM, Ferrer S, Pardo I. Polyphasic study of wine Lactobacillus strains: taxonomic implications. 2005; 55:197–207.

31. Delgado S, Arroyo R, Jiménez E, Marin ML, Fernández L, Rodríguez JM. Staphylococcus epidermidis strains isolated from breast milk of women suffering infectious mastitis: potential virulence traits and resistance to antibiotics. 2009; 11:1–11.

32. Zhang K, Mcclure J, Elsayed S, Louie T, Conly JM. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome mec Types I to V in Methicillin-Resistant Staphylococcus aureus. 2005; 43(10):5026–33.

33. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement [Internet]. Vol. 31, MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports / Centers for Disease Control. 2011. M100-S21.

34. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. 2017; 81(4):1–67.

35. Perez-muñoz ME, Arrieta M, Ramer-tait AE, Walter J. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome. 2017;1–19.

36. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep. 2016; 6(October):1–13. https://doi.org/10.1038/srep32129 PMID: 27001291

37. Heida FH, Van Zoonen AGJF, Hulscher JBF, Te Kiefte BJC, Wessels R, Kooi EMW, et al. A necrotizing enterocolitis-associated gut microbiota is present in the meconium: Results of a prospective study. Clin Infect Dis. 2016; 62(7):863–70. https://doi.org/10.1093/cid/ciw016 PMID: 26787171

38. Nagpal R, Tsuji H, Takahashi T, Kawashima K, Nagata S, Nomoto K, et al. Sensitive quantitative analysis of the meconium bacterial microbiota in healthy term infants born vaginally or by cesarean section. Front Microbiol. 2016; 7(DEC):1–9.

39. Gosalbes MJ, Llop S, Valles Y, Moya A, Ballester F, Francino MP. Meconium microbiota types dominated by lactic acid or enteric bacteria are differentially associated with maternal eczema and respiratory problems in infants. Clin Exp Allergy. 2012; 43(2):198–211.
Jimeñez E, De Andrés J, Manrique M, Pareja-Tobes P, Tobes R, Martínez-Blanch JF, et al. Metage-
Mshviladzé M, Neu J, Shuster J, Theriaque D, Li N, Mai V. Intestinal microbial ecology in prematu-
Hunt KM, Foster JA, Forney LJ, Schu UME, Beck DL, Fox LK, et al. Characterization of the Diversity
Cacho NT, Harrison NA, Parker LA, Padgett KA, Lemas DJ, Marcial GE, et al. Personalization of the
Bačkhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy
Schwiertz A, Gruhl B, Löbnitz M, Michel P, Radke M, Blaut M. Development of the Intestinal Bacteri-
Hurrell E, Kucerová E, Loughlin M, Caubilla-Barroso J, Hilton A, Armstrong R, et al. Neonatal enteral
Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome
Smith B, Bode S, Skov TH, Mirsepassi H, Greisen G, Krogfelt KA. Investigation of the early intestinal
Magne F, Ab M, Morville P, Pochart P, Suau A. Low species diversity and high interindividual variability

40. LaTuga MS, Ellis JC, Cotton CM, Goldberg RN, Wynn JL, Jackson RB, et al. Beyond bacteria: A study of the enteric microbial consortium in extremely low birth weight infants. PLoS One. 2011; 6(12):1–10.

41. Magne F, Ab M, Morville P, Pochart P, Suau A. Low species diversity and high interindividual variability in faeces of preterm infants as revealed by sequences of 16S rRNA genes and PCR-temporal temperature gradient gel electrophoresis profiles. 2006; 57:128–38.

42. Schwietz A, Gruhl B, Löbnitz M, Michel P, Radke M, Blaut M. Development of the Intestinal Bacterial Composition in Hospitalized Preterm Infants in Comparison with Full-Term Infants. 2003; 54(3):393–9.

43. Smith B, Bodé S, Skov TH, Mirsepassi H, Greisen G, Krogfelt KA. Investigation of the early intestinal microflora in premature infants with/without necrotizing enterocolitis using two different methods. 2012; 71(1):115–20.

44. Mshviladzé M, Neu J, Shuster J, Theriaque D, Li N, Mai V. Intestinal microbial ecology in premature infants assessed using non culture based technique. 2010; 156(1):20–5.

45. Jiménez E, De Andrés J, Manrique M, Pareja-Tobes P, Tobes R, Martínez-Blanch JF, et al. Metagenomic analysis of milk of healthy and mastitis-suffering women. J Hum Lact. 2015; 31(3):406–15. https://doi.org/10.1177/0890344415585078 PMID: 25948578

46. Hunt KM, Foster JA, Forney LJ, Schu UME, Beck DL, Fox LK, et al. Characterization of the Diversity and Temporal Stability of Bacterial Communities in Human Milk. 2011; 6(6):1–8.

47. Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery 1–4. 2012; (August):544–51.

48. Jost T, Lacroix C, Braegger C, Chassard C. Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. Br J Nutr. 2013; 110:1253–62. https://doi.org/10.1017/S000711451200597 PMID: 23507238

49. Bäckhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. Cell Host Microbe. 2012; 12(5):611–22. https://doi.org/10.1016/j.chom.2012.10.012 PMID: 23159051

50. Hurrell E, Kucerová E, Loughlin M, Caubilla-Barron J, Hilton A, Armstrong R, et al. Neonatal enteral feeding tubes as loci for colonization by members of the Enterobacteriaceae. BMC Infect Dis. 2009; 9:146. https://doi.org/10.1186/1471-2334-9-146 PMID: 19723318

51. Moles L, Gómez M, Jiménez E, Bustos G, de Andrés J, Melgar A, et al. Bacterial Diversity of the Gastric Content of Preterm Infants during Their First Month of Life at the Hospital. Front Nutr. 2017; 4(April). https://doi.org/10.3389/fnut.2017.00012/full

52. Ogrodzki P, Cheung CS, Saad M, Dahmani K, Coxill R, Liang H, et al. Rapid in situ imaging and whole genome sequencing of biofilm in neonatal feeding tubes: A clinical proof of concept. Sci Rep. 2017; (July):1–8. https://doi.org/10.1038/s41598-017-15769-9 PMID: 29162873

53. Lepainteur M, Royer G, Bourrel AS, Romain O, Duport C, Doucet-populaire F, et al. Prevalence of resistance to antiseptics and mupirocin among invasive coagulase-negative staphylococci from very preterm neonates in NICU: the creeping threat? J Hosp Infect. 2013; 83(4):333–6. https://doi.org/10.1016/j.jhin.2012.11.025 PMID: 23414707

54. Begovic´ B, J B, P obradovic M, V K, L J, K M, et al. Genotypic diversity and virulent factors of Staphylococcus epidermidis isolated from human breast milk Milan Koji c. 2013; 168:77–83.

55. Villari P, Sarnataro C, Iacuzio L. Molecular Epidemiology of Staphylococcus epidermidis in a Neonatal Intensive Care Unit over a Three-Year Period 2000; 38(5):1740–6.

56. Cabrera-Contreras R, Morelos-Ramírez R, Galicia-Camacho AN, Melendez-Herrada E. Antibiotic resistance and biofilm production in Staphylococcus epidermidis strains, isolated from a tertiary care hospital in Mexico City. ISRN Microbiol. 2013;918921. https://doi.org/10.1155/2013/918921 PMID: 23724338

57. Khan MMA, Faiz A, Ashshi AM. Clinically significant coagulase-negative staphylococci in Human Milk from Mothers of Preterm Compared with Term Neonates. J Hum Lact. 2017; 33(2):329–40. https://doi.org/10.1177/0890344417691505 PMID: 28418807

58. Conlan S, Mijares LA, Becker J, Blakesley RW, Bouffard GG, Brooks S, et al. Staphylococcus epidermidis pan-genome sequence analysis reveals diversity of skin commensal and hospital infection-associated isolates. Genome Biol. 2012; 13(7):R64. https://doi.org/10.1186/gb-2012-13-7-r64 PMID: 22830599

59. Soeorg H, Metsvaht T, Eelma¨ e I, Metsvaht HK, Treumuth S, Merila M, et al. Coagulase-Negative Staphylococci in Human Milk from Mothers of Preterm Compared with Term Neonates. J Hum Lact. 2017; 33(2):329–40. https://doi.org/10.1177/0890344417691505 PMID: 28418807

60. Cacho NT, Harrison NA, Parker LA, Padgett KA, Lemas DJ, Marcial GE, et al. Personalization of the Microbiota of Donor Human Milk with Mother ’ s Own Milk. 2017; 8(August):1–12.
61. Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, et al. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. Nature. 2010; 465(7296):346–9. https://doi.org/10.1038/nature09074 PMID: 20485435

62. Park B, Iwase T, Liu GY. Intranasal Application of *S. epidermidis* Prevents Colonization by Methicillin-Resistant *Staphylococcus aureus* in Mice. 2011; 6(10):6–10.