Bacteriological screening of breast milk samples destined to direct milk donation: Prospective evaluation between 2007 and 2016

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Abstract

This study analyzes the bacteriological quality of breast milk samples destined to direct milk donation to preterm infants under 34 Gestational Weeks (GW) hospitalized in a neonatology and a neonatal intensive care unit of a French university hospital.

All samples of breast milk destined to direct milk donation between April 2007 and December 2016 were included. A sample was defined as compliant if its total flora was less than 10^6 Colony Forming Units per milliliter (CFU/mL) and in the absence of *Staphylococcus aureus* and other pathogens.

A total of 777 samples were taken from 629 mothers. The overall non-compliance rate for the initial sample was 21.3%; 63 samples (10.0%) had a total flora $\geq 10^6$ CFU/mL, 63 (10.0%) were contaminated by a pathogenic bacteria and 8 (1.3%) were non-compliant because of both. An increase of the non-compliance rate was observed between 2008 and 2016 (10.2% to 26.1%). The increase of the total flora non-compliance rate began in 2011, in link with the doubling of the number of samples taken, to reach a peak in 2013 then decreased in link with development of portable pump. No statistically significant difference of the presence of *S. aureus* in breast milk was observed. For the other pathogenic bacteria, the rate increased significantly in 2014.

The increase of the non-compliance rate could be explained by a decrease of best practices in milk collection. Education of mothers should be strengthened.

Keywords: Neonatal intensive care unit; preterm neonate; breast milk; *Staphylococcus aureus*; direct milk donation

Introduction

Many studies have shown the benefits of breastfeeding for the development of preterm infants during the first months of life (Armstrong and Reilly, 2002; Herrmann and Carroll, 2014; Howie et al., 1990; Kull et al., 2002). Picaud et al. (2018) showed that the precocity of breastfeeding is one of the keys to the success of breastfeeding a premature child. There are different types of breastfeeding: direct milk donation or fresh milk donation (milk directly given from the mother to her child hospitalized without pasteurization), mother's milk (milk given from the mother to her child hospitalized after pasteurization) and human milk (anonymous donor gives milk to a milk bank) (Ministère du Travail et des Affaires Sociales, 1997). Cong et al. (2016) showed that infants fed by mother's own breast milk had a significantly more diverse gut microbiome and higher abundance of *Clostridales* and *Lactobacillale* than those fed with human donor milk and or formula. Even if many bacteria contribute to the gut microbiome, some are pathogenic bacteria and can cause severe pathologies in these preterm children because of their immature immune system. Cases of sepsis were described with *Escherichia coli*, *Klebsiella pneumoniae* due to direct donation (Jawa et al., 2013; Widger et al., 2010) and more recently with *Cronobacter sakazakii* (Bowen et al., 2017). Therefore, this type of diet requires hygiene recommendations to guarantee the infants' safety.

Since 1997, direct milk donation from a mother to her child is authorized in France in the absence of communicable diseases: Human Immunodeficiency Virus (HIV), hepatitis B and C virus, Human T cell Leukemia Virus (HTLV) 1 and 2 (Ministère du Travail et des Affaires Sociales, 1997). In case of very premature infants, born before 32 weeks of Gestational Age (GA) or/ and very low birth weight infant, the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) advises against direct milk donation if the mother has a positive Cytomegalovirus (CMV) serology. Since 2005, ANSES

recommends analyzing at least one breast milk sample but there is no bacteriological threshold to define a non-compliant sample. When the collection has been refrigerated for more than 48 h and was not administered to the newborn, it is frozen and sent to the milk bank to be pasteurized (AFSSA, 2005).

The university hospital of this study has implemented breast milk direct donation for preterm children under 34 weeks of GA in 2007. The objective of this study was to analyze the bacteriological quality of breast milk samples destined to direct milk donation for preterm children in our institution between 2007 and 2016.

Methods

Setting

We conducted a prospective observational study in a 2200-bed French University Hospital. All samples of breast milk destined to direct milk donation collected between April 17, 2007, and December 31, 2016, were included in the study. During this period, the average number of deliveries per year was 2661 ± 411 and the average number of births per year was 2745 ± 422. On average, 26 ± 3.4 % of the children were hospitalized in a 36 -bed neonatology and neonatal intensive care units (NICU).

Study ethics approval was obtained on 30 March 2018 (CECIC Rhône-Alpes-Auvergne, Clermont-Ferrand, IRB 5891).

Breast milk collection

Before breast milk collection, mothers' mandatory serologies were checked to avoid virus transmission (HTLV, Hepatitis B and C, HIV, and CMV for preterms under 32 weeks of GA). Recommendations about inclusion and exclusion criteria have made available in paper format since 2007 and have made available by electronic means (inclusion in the documentation

management software) in 2011. Before milk collection, NICU nurses gave recommendations about everyday body and hand hygiene, and correct cleaning of breast pumps, equipment and surfaces. Before 2013, mothers could only collect their milk in a collection room with stationary pumps. Since 2013, mothers can collect their milk in this room or in their child's room with a portable pump. A hygiene recommendation sheet is posted in the breast milk collection room and above each portable pump. An information booklet in their language about breast milk collection is given to every mother before the first collection.

Bacteriological analyses of breast milk samples

French regulation recommends to analyze at least one breast milk sample during direct milk donation in the hospitalization even though there is no bacteriological threshold to define a non-compliant sample. As a consequence, we defined our own threshold. We have considered a sample to be compliant if its total flora is under 10^6 CFU/mL and in the absence of *Staphylococcus aureus* and pathogens (*Enterobacteriaceae*, *Enterococcus*…). Milk testing was done only once at birth for every infant, except for infants with a GA < 34 weeks, for whom milk was tested every 4 weeks until they reached 34 weeks of post conceptional age. If the sample presented total flora was $\geq 10^6$ CFU/mL, or pathogenic bacteria, direct milk donation was halted until the mother's education about milk collection was strengthened. If *S. aureus* was present, a decolonization treatment was given to the mother (chlorhexidine showers once a day for 5 days and intranasal mupirocin applications for 5 days) (Reagan et al., 1991). Mothers were not allowed to donate milk during the decolonization process. Milk was tested after the process to check if breastfeeding could start again.

If infants had under 34 weeks of GA and their mother wished to give their milk at the time of non-compliant result, sample were controlled as required. Mothers had more than one sample if it was a non compliant sample or when the GA was less than 34 weeks.

Mothers could also decide to stop direct milk donation, so if the initial sample was not compliant, there was no need to have other samples.

Samples of milk were seeded: 0.01 mL on blood agar and 0.01 mL on Chapman agar, and inoculated at 36°C for 48 hours with a first verification after 24 hours (Ministère du Travail et des Affaires Sociales, 1997).

For non-compliant breast milk samples, we checked whether the child had a bacteraemia with the bacteriology laboratory data until the hospital ‘s discharge of the newborn.

Statistical analysis

Qualitative variables were described by their number and their percentage and quantitative variables by their mean and their standard deviation. Comparison of qualitative variables was conducted with a Chi2 test if the conditions for application of this test were verified. Otherwise a Fisher's exact test was performed.

We analyzed breast milk samples of the mother of a single child (exclusion of samples from a mother of twins). Samples from mothers of twins are not independent. We excluded them to avoid confusion in the results. We decided to analyze periods according to the visual distribution of the non-compliance rate. We chose to exclude the 2007 tests because it was incomplete. The significance threshold was set at 0.05. The statistical analyses were performed with R version 3.3.2.

Results

A total of 808 direct milk donation samples were collected from 641 mothers between April 17, 2007, and December 31, 2016: 777 samples from mothers who gave birth to a single child and 31 from mothers of twins.

Among the 777 samples, 629 were initial samples and 148 were control samples (Figure 1). Of the 629 initial samples, 495 (78.7%) were compliant.

Among the 134 (21.3%) non-compliant samples, 63 (10.0%) had a total flora $\geq 10^6$ CFU/mL, 63 (10.0%) were only contaminated by a pathogenic bacteria and 8 (1.3%) were non-compliant because of both (Table 1). On the whole, *S. aureus* was identified in 58 (9.2%) samples. One was a methicillin-resistant *S. aureus*, 19 (3.0%) were $\geq 10^4$ CFU/mL. Other pathogens were found in 15 (2.4%) samples: mainly Gram-Negative *Bacilli* (*Klebsiella pneumoniae, Klebsiella oxytoca*, *Escherichia coli*, *Enterobacter cloacae*...), with one *E. coli* producing extended-spectrum beta-lactamases (ESBL).

We described in figure 2 the evolution of the number of samples analyzed and the proportion of non-compliant samples between 2007 and 2016. There was an increase in the number of samples analyzed in 2011.

According to figure 3, an increase of the non-compliance rate was observed between 2008 and 2016 (10.2% to 26.1%). The rate of non-compliance was stable until 2009, decreased in 2010, increased in 2011 and reach a peak in 2013 and then decreased. The evolution of the total flora non-compliance rate was similar. The presence of *S. aureus* in breast milk appeared stable over time. For the pathogenic bacteria presence (without *S. aureus*), the rate increased in 2014. The overall non-compliance rate was significantly higher between 2011-2013 and between 2014-2016 than between 2008-2010 (23.4% vs. 9.4%; p=0.001 and 29.2% vs.9.4%, p<0.001) (Table 2). This result was also observed for the total flora $\geq 10^6$CFU/mL (15.6% vs. 1.6%, p<0.001 and 14.4% vs. 1.6%; p<0.001). No statistically significant difference of the presence of *S. aureus* in breast milk was observed between 2008-2010, 2011-2013 and 2014-2016 (7.9%, 9.3% and 10.5% p = 0.7). For the pathogenic bacteria presence, the rate was significantly higher between 2014-2016 than between 2011-2013 (4.7% vs. 0.5%; p=0.008).

Forty-seven samples of breast milk were verified, 27 (57.4%) were not compliant. Among the 27 samples, 11 (40.7%) had a second verification and 5 were not compliant (Figure 1). Of note, one sample was verified 5 times.

Among 134 children with an initial non-compliant breast milk sample, 21 bacteraemia were recorded and 8 occurred after breast milk donation. Of those eight, four had bacteraemia due to coagulase negative *Staphylococcus.* The breast milk samples collected before bacteraemia were non-compliant due to an excess of the total flora. The four other children were already colonized by *S. aureus* and developed a bacteraemia with *S. aureus*, or had bacteraemia due to a different pathogen from the one of the milk sample.

Discussion

This study highlighted an increase in the overall non-compliance rate of breast milk destined to direct milk donation between 2008 and 2016 in our institution. A peak was observed in 2013. Rates of samples with total flora $\geq 10^6$ CFU/mL increased between 2008 and 2016.

The overall non-compliance rate was 21.3% in accordance with our strict criteria. The non-compliance rate was higher than the 11% non-compliance rate found in French milk banks before pasteurization (Dewitte et al., 2015) but criteria were not the same mainly for *S. aureus*. Dewitte et al. (2015) showed that 4.6% of the overall collected breast milk was discarded because of non-compliant total flora ($\geq 10^6$ CFU/mL).

In our study, the contamination by pathogenic bacteria (*S. aureus* and other pathogens) was 11.3%. This result is concordant with Picaud et al. (2018) and it is in the low range. Picaud et al. (2018) reported that approximately 10-40% of human milk samples collected from mothers in neonatal units were contaminated with pathogenic germs. This result is very reassuring.

In this University Hospital study, the evolution of the overall non-compliance rate followed closely the total flora non-compliance rate. Decrease in 2010 of the overall non-compliance rate could be explained by an outbreak of *Bacillus cereus* with several infections between June and December 2009. No breast milk samples have been identified *B. cereus* but all hygiene practices were reinforced during and after this outbreak. An increase of the overall non-compliance rate and the total flora non-compliance rate began in 2011 and was observed until 2013. We observed that the number of samples nearly doubled in 2011, probably in link with increasing availability of recommendations. Therefore, nurses could not be as often present as during the past years during milk collection which can be an explanation to the increase of the non-compliance rate. For example, we notice nowadays that mothers without supervision tend to use smartphones during milk collection, which is not the case when there is a staff member. Our hypothesis is that when being observed while pumping milk, mothers are more attentive to correct hygiene practices. The non-compliant total flora decreases from 25.4% in 2013 to 7.7% in 2016. This result could be linked to the implementation of portable breast milk pumps in 2013. Before portable pump implementation, only one breast milk collection room with three stationary pumps was available, where mothers expressed milk without any supervision of the hospital staff. Portable pumps enable to express milk in the child's room under supervision of the hospital staff. In the child's room, the nurse can check the mother's habits and give advice, unlike in the collection room with stationary pumps where advice for a better milk collection is given only once. In the Bowen et al. (2017) note to Centers for Disease Control, *Cronobacter sakazakii* (Gram Negative Bacillus) infection was recorded in a preterm infant fed with expressed maternal milk. The mother had expressed milk with her proper pump and a hospital bedside breast pump. This pathogen was found in the mother's pump which had been cleaned at home and probably contaminated by inadequate cleaning. Best practices to clean pumps are an important factor to avoid contamination. In

China, a screening program for breast milk found a 63% rate of contamination (contamination defined by any growth of pathogens including Gram negative bacteria, enterococci or *S. aureus*, or total bacterial count >10^5CFU/mL). Avoiding bathing after birth for one month is a Chinese tradition for mothers (Ng et al., 2004). Picaud et al. (2018) reported that some studies showed that collecting human milk in neonatal units significantly reduces the risk of microbial contamination in comparison to home collection. These studies highlight the importance of personal hygiene before breast milk collection. Education of the mother by a nurse or a neonatologist is important in avoiding contamination.

Our study reported a significant increase of pathogenic bacteria non-compliance rate in 2014, among which are mainly fecal pathogens. This increase may be linked to the increase of activity of the consultation called permanence of access to health care in 2013 due to an increase of human resources. This consultation is dedicated to people in precarious situations (homeless people, without social protection…). We found only two multidrug resistant bacteria; this result is reassuring in the context of the global emergence of multi-drug resistant bacteria. An epidemic *Enterobacteriaceae*-ESBL breast milk contamination was described in Japan (Nakamura et al., 2015).

The level of *S. aureus* in mother's milk was 9.2% and seems to be stable in our institution, ranging from 7.9% in 2008-2010, 9.3% in 2011-2013 to 10.5% in 2014-2016. Furthermore, 3.0% was ≥10^4 CFU/mL. In the lactarium study of Dewitte et al. (2015), 5.9% of the overall breast milk was discarded because of a non-compliance rate due to the presence of *S. aureus* ≥10^4 CFU/mL.

Nowadays, recommendations are not precise for the bacteriological quality evaluation of breast milk destined to direct milk donation. We did not find bacteraemia linked to direct milk donation. This result is reassuring. A recent review (Picaud et al., 2018) reported that infants who received direct milk donation were exposed to pathogenic bacteria without

systematically developing an infection, whereas there is a documented risk of severe infections in preterm infants with several bacteria (*Streptococcus B*, *S. aureus* and enterobacteria). The incidence of bacterial infections transmitted by direct milk donation is difficult to quantify. Picaud et al. (2018) indicated that premature infants fed with direct milk donation or pasteurized donor human milk had fewer infections than those fed with preterm formula. They proposed a strategy to reduce the risk of transmission of pathogenic bacteria to preterm infants born before 28 weeks of GA or with a birthweight below 1000g. They proposed for these preterm infants to perform a microbiological follow-up once a week until corrected GA ≥28 weeks, and to wait for the results before breastfeeding. For infants born after 28 weeks of GA, breastfeeding was performed without waiting for microbiological results, but mothers were monitored.

Contrary to the fact that some countries (South Africa, Sweden, and Switzerland) do not quantify total flora, we will pursue our policy of having no pathogenic bacteria and total flora quantification $<10^6$ CFU/mL (Dewitte et al., 2015) and we will include the strategy proposed by Picaud et al. (2018) for the Gestational Age of the preterm infants. These criteria guarantee the bacteriological quality of breast milk destined to direct milk donation.

Our study has several limitations. Firstly, milk collection practices were not audited during this period. Secondly, only one sample of the first milk collection was analyzed for compliance rate evaluation, and this sample did not necessarily reflect the bacteriological quality during all the breast feeding period. Thirdly, we cannot exclude that some unknown events could have affect the rate of non-compliance sample. Finally, this is a single center study.

Conclusions

The increase in the overall non-compliance rate of direct milk donation since 2007 should indicate the need to strengthen best practices for mothers, particularly hand hygiene with smartphone use. We are considering setting up direct milk donation only with portable breast pumps in the child's room. Hygiene recommendations by pediatric nurses could lead to better practices.

Conflict of Interest: All authors report no conflicts of interest relevant to this article.

Acknowledgments

We thank pediatric nurses who explained breast milk collection to the mothers, the laboratory technicians for the microbiological analysis, Dr Elodie Sellier and Marianne Jeannin for the delivery and birth data in the study hospital, Dr Jacqueline Shum and Dr Philippe Saviuc for their explanation and memory of events, and Alison and William Terreaux for their help to the English translation.

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TABLE 1: Description of the type of the non-compliance for the first breast milk samples

	Initial breast milk samples N = 629
Non-compliant breast milk samples	**134 (21.3%)**
Total flora ≥ 10^6CFU/mL	63 (10.0%)
Presence of pathogenic bacteria	63 (10.0%)
Total flora ≥ 10^6CFU/mL and presence of pathogenic bacteria	8 (1.3%)
Description of each non-compliance	
Total flora non-compliance	**71 (11.3%)**
Presence of pathogenic bacteria	**71 (11.3%)**
Presence of *S. aureus*	58 (9.2%)
which MRSA	1
which *S. aureus* ≥ 10^4 UFC/mL	19 (3.0%)
Presence of other pathogens	15 (2.4%)
Enterococcus faecalis	5
Klebsiella oxytoca	3
Escherichia coli	3
which ESBL	1
Enterobacter cloacae	2
Klebsiella pneumoniae	1
Enterobacter aerogenes	1

NOTE. CFU: Colony Forming Units; MRSA: Methicillin-resistant *Staphylococcus aureus*; ESBL: Extended-Spectrum Beta-Lactamases. Results are expressed by number and percentage.

TABLE 2: Comparison of the overall non-compliance, total flora, *Staphylococcus aureus* and other pathogens presence according to the 3 periods

	2008-2010		2011-2013		2014-2016	
	n	%	n	%	n	%
Breast milk samples non compliance						
Overall non compliance	12	9.4	48	23.4[a]	75	29.2[b,d]
Total flora≥ 10^6	2	1.6	32	15,6[a]	37	14.4[b,d]
Staphylococcus aureus presence	10	7.9	19	9.3	27	10.5
Pathogenic bacteria presence (without *Staphylococcus aureus*)	1	0.8	1	0.5	12	4.7[c,d]
Total of samples	127	100	205	100	257	100

NOTE.

n : number of samples, % : percents of samples

a: Significant *P* value between the 2008-2010 period versus 2011-2013 period

b: Significant *P* value between the 2008-2010 period versus 2014-2016 period

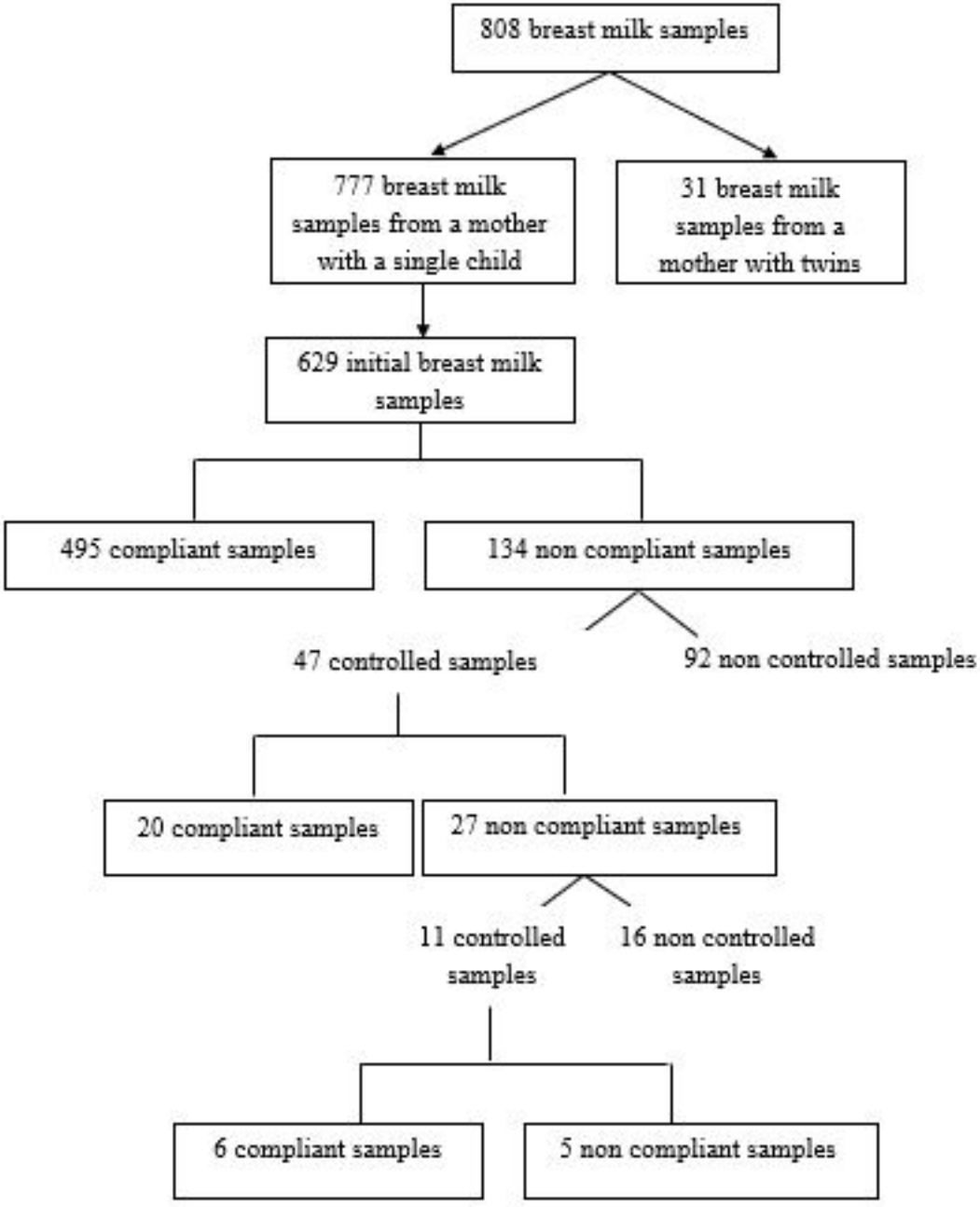
c: Significant *P* value between 2011-2013 period versus 2014-2016 period

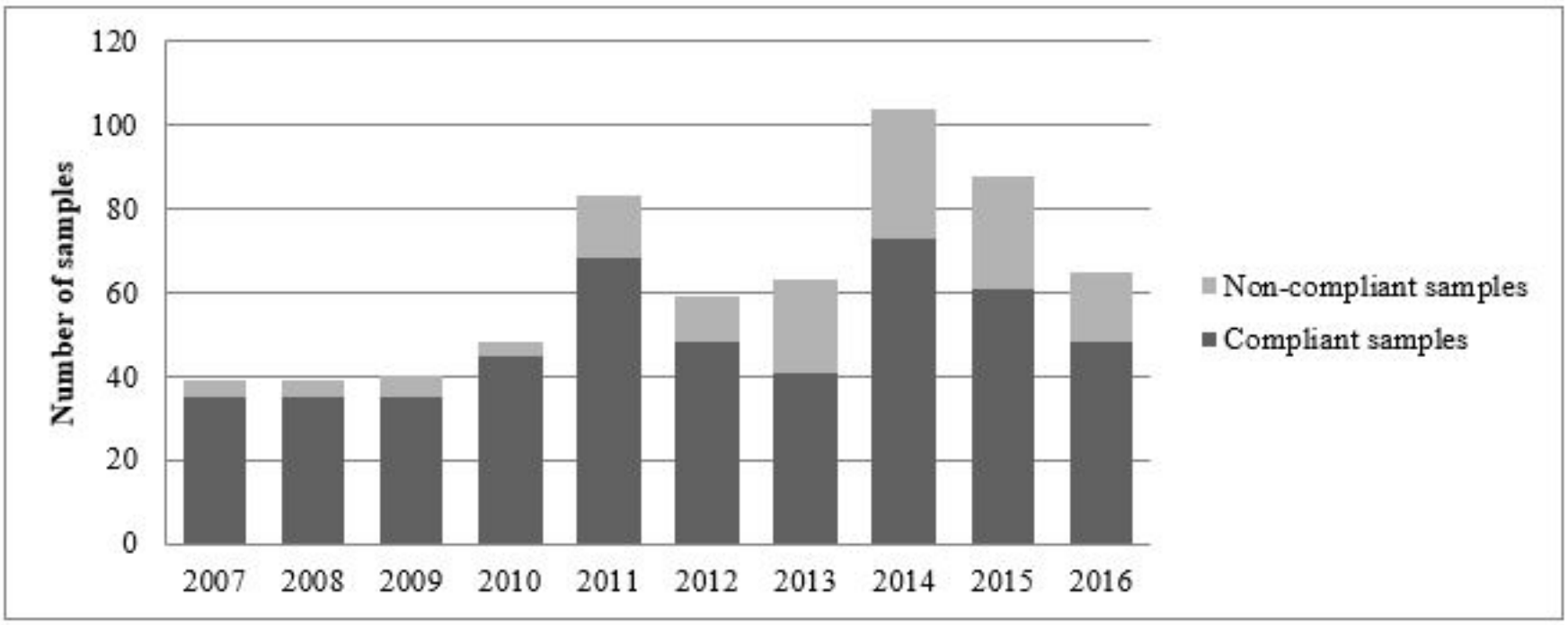
d: Significant *P* value between the 3 periods

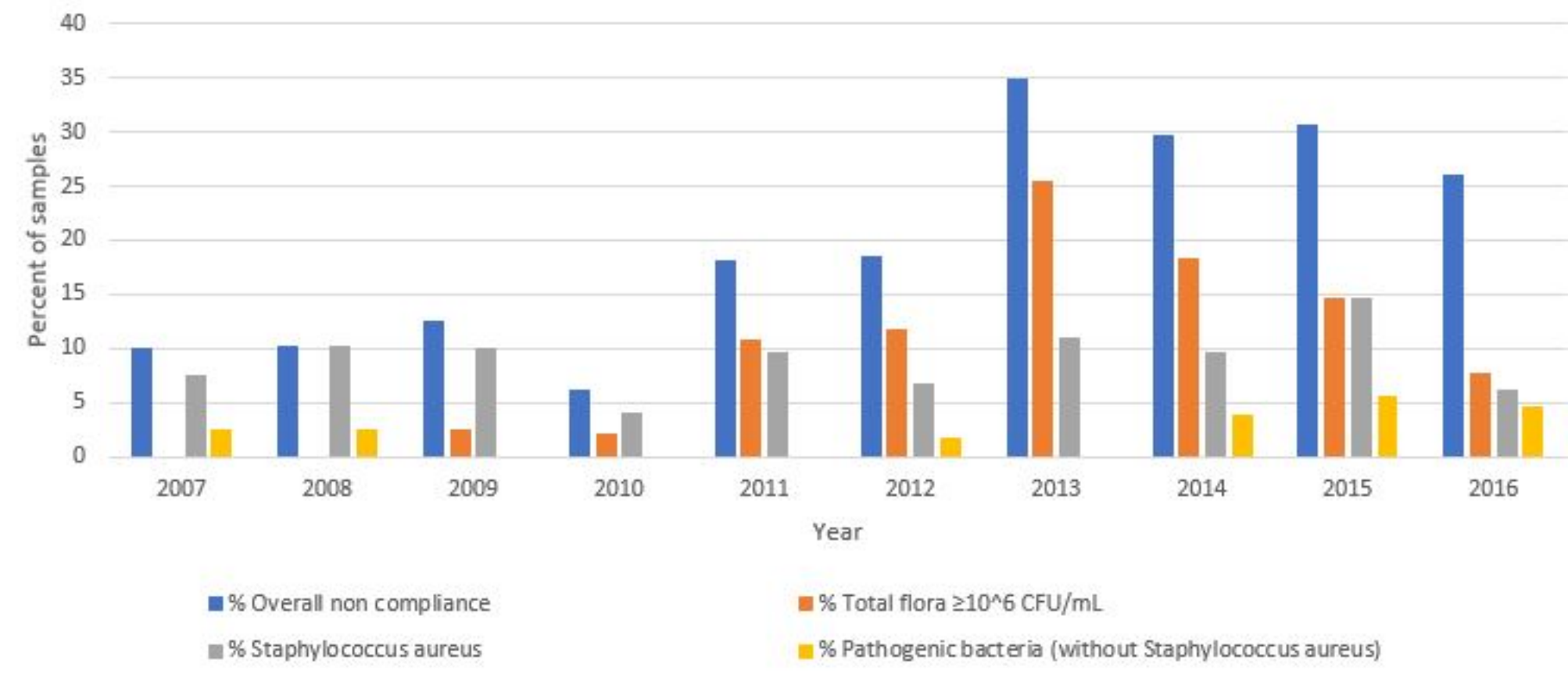
FIGURE 1: Compliance of breast milk samples destined to direct milk donation for a single child

FIGURE 2: Evolution of the number of samples analyzed and the proportion of non-compliant samples destined to direct milk donation between 2007 and 2016

FIGURE 3: Evolution of breast milk samples percentage with total flora $\geq 10^6$CFU/mL, with presence of *S. aureus*, with presence of pathogenic bacteria and the evolution of the overall non-compliance between 2007 and 2016


808 breast milk samples
777 breast milk samples from a mother with a single child
31 breast milk samples from a mother with twins
629 initial breast milk samples
495 compliant samples
134 non compliant samples
47 controlled samples
92 non controlled samples
20 compliant samples
27 non compliant samples
11 controlled samples
16 non controlled samples
6 compliant samples
5 non compliant samples


120
100
80
60
40
20
0
Number of samples
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Non-compliant samples
Compliant samples


40
35
30
25
20
15
10
5
0
Percent of samples
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Year
% Overall non compliance
% Total flora ≥10^6 CFU/mL
% Staphylococcus aureus
% Pathogenic bacteria (without Staphylococcus aureus)