

Comparing the effect of Topical Application of Breast Milk, Chlorhexidine and Dry Cord Care Methods on Bacterial Colonization in Umbilical Cord of Preterm Neonates in NICU: a randomized clinical trialMozhgan Yaghoobi¹, Behjatossadat Bolandi², Mohammadhasan Namaei³, Gholamreza Faal⁴¹ School of Medicine, Birjand University of Medical Sciences, Birjand, Iran² MD in Pediatric Nephrology, Assistant Professor, Department of Pediatrics, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran³ MD, PhD, Professor of Microbiology, Birjand Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran⁴ MD in Neonatology, Assistant Professor, Department of Pediatrics, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran**Type of article:** Original**Abstract****Background:** Preterm neonates have a higher tendency in developing infections and their umbilical cord serves as a good environment for bacterial growth.**Objective:** The aim of this study was to compare the topical effects of breast milk, chlorhexidine and dry cord care methods on bacterial colonization of preterm neonates' umbilical cord in the Neonatal Intensive Care Unit (NICU)**Methods:** The trial was one-centered, single-blind, and in phase 2 of clinical trials from Vali-e-Asr Educational Hospital, Birjand, Iran from January to June 2017. A sample of 75 preterm infants were recruited by convenience sampling method and assigned into one of the three groups of drying, chlorhexidine, and milk by using a table of random numbers (n=25 per group). Bacterial colonization and its density were assessed in all groups during the first 12 to 24 hours of admission, and again after 72 hours. A total of 25 subjects were entered in each group and the data were analyzed by Chi-square tests (Fisher's exact test), Kruskal–Wallis analysis, Wilcoxon test, McNemar's test, and ANOVA.**Results:** The bacteria colonized in the newborns of groups I, II, and III before and after intervention were 64% and 36% (p=0.03), 52 and 20% (p=0.008) and 64 and 32 (p=0.02) respectively. After the intervention, colonized bacteria were significantly decreased in all three groups.**Conclusion:** Based on the results of this study, all three methods of drying approach, use of chlorhexidine, as well as breast milk were effective in controlling bacterial colonization in the umbilical cord of preterm neonates. Although there were no significant differences between the three groups, ultimately the use of these methods could be effective in reducing the need for antibiotics.**Trial registration:** Prior to the study, the protocol of study was registered at Iran's Clinical Trials Registry (<http://www.irct.ir>) (Registration ID: IRCT2017090517756N27).**Funding:** The present study was funded fully by Birjand University of Medical Sciences (grant number: 455078).**Keywords:** Infant, Premature, Umbilical cord, Milk, Human, Chlorhexidine**Abbreviations / Acronyms:****CFU:** Colony-Forming Unit; **NICU:** Neonatal Intensive Care Unit; **PBS:** Phosphate Buffered Saline; **WHO:** World Health Organization**Corresponding author:**

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1. Introduction

Preterm birth is one of the main causes of infant mortality. Preterm delivery complications in premature infants account for the greatest cause of direct death in infants and are responsible for 35% of the 3 million worldwide mortality rates in neonates (1). The umbilical cord is immediately necrotized after birth and provides a good environment for the growth of bacteria that causes infections in the neonate, especially in preterm neonates (2). Most factors that are colonized on the umbilical cord originate from the skin, the urethra and genital tract of the infant's mother, and most often include *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus* (3, 4). Although the ratio of umbilical cord infection on infectious death in infants is still unclear, cord infection regardless of epithelial infection is known to be one of the main pathways for the introduction of bacteria into the infant's body (2). Since the umbilical cord has direct access to the bloodstream, bacterial colonization or infection can bring about several complications including intra-abdominal abscess, periumbilical cellulitis, thrombophlebitis in port or amyloid veins, peritonitis, and intestinal ischemia (5).

The World Health Organization (WHO) has recommended the use of a dry cord approach to cord storage since 1998 (6). The drying approach involves keeping the umbilical cord clean and exposing it to fresh air, or at most, storing it within a clean cloth. In centers where the health status is poorly maintained, or the infection rate is high, the WHO recommends using chlorhexidine (5, 7). There have been controversies regarding the effect of chlorhexidine usage on newborn mortality when born in hospitals. In fact, the use of topical chlorhexidine is a safe treatment approach, although there have been reports of small amounts of the substance existing in neonatal blood after topical use (8). Another traditional method with this regard is to recommend breast milk which offers antibacterial properties and causes an early removal of the cord (9, 10). Considering that there had not been a study until now that compares the effects of local breast milk, chlorhexidine and dry storage method specifically in preterm neonates admitted to Neonatal Intensive Care Unit (NICU), and all related studies have been with regard to term infants and outpatients, the aim of this study was to compare the topical effects of breast milk, chlorhexidine and dry cord care methods on bacterial colonization of preterm neonates' umbilical cord in the NICU.

2. Material and Methods

2.1. Trial design

The trial is one-centered, single-blind, and in phase 2 of clinical trials from Vali-e-Asr Educational Hospital, Birjand, Iran from January to June 2017. A sample of 75 preterm infants were recruited by convenience sampling method and assigned into one of the three groups of drying, chlorhexidine, and milk by using a table of random numbers (n=25 per group). Data needed for this study were collected using a checklist containing treatment group, gender, delivery method, birth weight and bacterial density before and after treatment. The method for data collection was field-based with the use of interviews, examinations and laboratory tests. A trained nurse, who was blinded, collected the samples. Drug prescribing and drying was carried out by a qualified nurse in each work shift outside the research team. The researchers performed sampling as well as conducting microbiological tests.

2.2. Selection criteria

Inclusion criteria for this study were:

- 1) gestational age less than 37 weeks
- 2) need for admission in the neonatal intensive care unit
- 3) treatment with ampicillin and gentamicin antibiotics.

The following were set as the exclusion criteria:

- 1) dissatisfaction of the legal guardian(s) for participation
- 2) confirmed sepsis before bacterial culture
- 3) history of non-sterile childbirth
- 4) presence of anatomical malformation
- 5) history of perinatal asphyxia
- 6) presence of symptoms of omphalitis before the test
- 7) history of infections in the mother during pregnancy
- 8) prescribed antibiotics in the mother before delivery
- 9) prolonged rupture of fetal membranes
- 10) umbilical catheterization
- 11) any umbilical intervention during hospitalization; chorioamnionitis

2.3. Sample size

The sample size required for this study was calculated using the following formula and based on a study by Taffazoli et al. (11) and with a confidence level of 95% and $\alpha=0.05$ and $\beta=0.2$

$$n = Z_{1-\alpha}^2 [2P(1-P)]^{1/2} + Z_{1-\beta} [P_1(1-P_1) + P_2(1-P_2)]^{1/2} / (P_1-P_2)^2$$

2.4. Randomization and blinding

Infants were randomly assigned to one of three groups: 1, 2 and 3 (Figure 1). The study was single-blinded in that prescription or drying was performed by experienced nurses in various working shifts outside the research team. The researchers performed sampling as well as conducting microbiological tests.

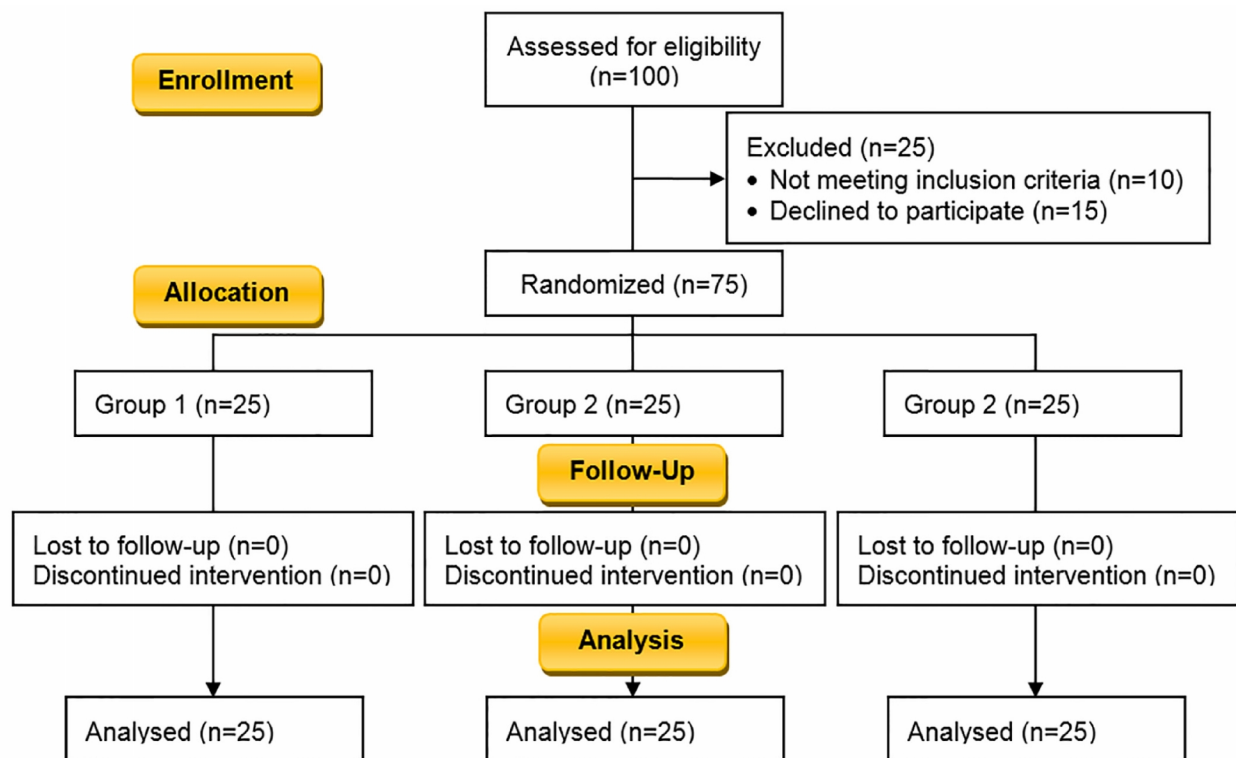


Figure 1. CONSORT Flow Diagram of the trial

2.5. Intervention and measurements

During the first 12 to 24 hours of admission, umbilical cord samples were collected from the neonates using a sterile swab; the wet sterilized swab was used to scrub the whole skin surface starting from the umbilical cord area with a radius of 1 cm. Next, the specimen was prepared (taking into account the mean diameter of the umbilical cord, 1.5 cm plus 1 centimeter around it) and transferred to the laboratory in a Phosphate Buffered Saline (PBS) medium tube containing 5cc phosphate buffer saline with pH 7. Serial dilutions were prepared in 0.1% of samples, then diluted 0.01, and then the 0.01 dilutions were transferred onto an agar culture medium and Eosin methylene blue. Plates were stored at 37 °C for 48 hours. Next, specific numbers of colonies were determined by isolation from the bacterial strain of the grown bacteria in the media using standard laboratory method. Infants were randomly assigned to one of three groups. In each group, 12 intervention sessions were performed at six-hour intervals. In the dry group, the cord was kept dry only with sterile gas. In the chlorhexidine group, 2-3 cc based on the size of the umbilical cord, the umbilical cord, and 1 centimeter of the surrounding area were kept moist every six hours with 0.2% of chlorhexidine mouthwash, made by the Health Laboratory of the World Health Organization. In the breast milk group, between 2-3 cc from each infant's mother's milk based on the size of the umbilical cord, the entire umbilical cord and 1 centimeter of the surrounding area, were impregnated with milk every six hours. After 72 hours, with a minimum of six hours interval from the last intervention, samples were collected again from the umbilical cord surface swab and re-cultured. The results of two cultured groups were compared in each group followed by comparison in the two other groups. In order to evaluate the average bacterial density, the number of live bacteria per unit area of the body was used, which is calculated from the number of bacteria grown on the

culture medium in the dilution image and divided by the sampling surface, which includes the total area of the scrubbed region minus the cord area average. The bacteria included different species of bacteria isolated from the sampling surface, which were identified by laboratory tests. In all groups, treatment began immediately after sampling was performed.

2.6. Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 21. Analytical analysis of qualitative data was performed by Chi-square test (Fisher's exact test) along with Kruskal-Wallis, Wilcoxon test, McNemar's test and ANOVA test. The significance level of p was considered at a significance level of $p \leq 0.05$.

2.7. Research ethics

All the procedures in this study were in conformity with the Declaration of Helsinki and were approved by the local Human Research Ethics Committee. Prior to the study, its protocol was approved by the Ethics Committee of Birjand University of Medical Sciences (Ir.bums.REC.1396.63) and registered at Iran's Clinical Trials Registry System (IRCT2017090517756N27).

3. Results

A total of 75 patients were enrolled in the study ($n=25$ per group). Comparison of frequency distribution of variables (infants' age, birth weight, gestational age and birth method) in three groups of study showed no significant differences (Table 1). The frequency of colonized bacteria in the umbilical cord before and after intervention shown in Table 2 and Fisher's exact test indicates no difference between the groups with this regard. Table 2 also indicates no significant difference in the comparison of the type of bacteria colonized in the umbilical cord of preterm neonates after using drying method, chlorhexidine, and breast milk. The most common bacteria colonized in Group 1 and Group 3 were Staphylococcus Epidermidis (16% in both groups) and for Group 2 was Staphylococcus Aureus (12%).

Table 1. Comparison of frequency distribution of variables in three groups of study

Variable		Group 1	Group 2	Group 3	Statistics
Gender; n (%)	Boy	13 (52)	12 (48)	14 (56)	$p=0.85^*$, $X^2=0.32$
	Girl	12 (48)	13 (52)	11 (44)	
Birth method; n (%)	Cesarean	19 (76)	19 (76)	19 (76)	$p=1^*$, $X^2=0.00$
	Vaginal	6 (24)	6 (24)	6 (24)	
Birth weight (g)		1899 \pm 463.96	1872.6 \pm 472.7	1896.2 \pm 537.9	$p=0.9^{**}$, $F=0.02$
Gestational age (week)		32.6 \pm 2.21	32.32 \pm 2.34	36.36 \pm 2.03	$p=0.9^{**}$, $F=0.2$

Group 1: drying method; Group 2: chlorhexidine; Group 3: breast milk. * The result of Chi-squared test was not significant at the level of $p \leq 0.05$. ** The ANOVA test was not significant at the level of $p \leq 0.05$

Table 2. Frequency of colonized bacteria in umbilical cord before and after intervention

Variables		Group 1	Group 2	Group 3	Statistics
Without growth; n (%)	Before	9 (36)	12 (48)	9 (36)	Before intervention: Fisher exact=7.2, $p=0.74$
	After	16 (64)	20 (80)	17 (68)	
Staphylococcus epidermidis; n (%)	Before	10 (40)	6 (24)	10 (40)	After intervention Fisher exact=7.2, $p=0.74$
	After	4 (16)	0 (0)	4 (16)	
Staphylococcus aureus; n (%)	Before	2 (8)	4 (16)	3 (12)	
	After	3 (12)	3 (12)	1 (4)	
Enterococcus; n (%)	Before	2 (8)	1 (4)	0(0)	
	After	1 (4)	0 (0)	0 (0)	
Escherichia coli; n (%)	Before	1 (4)	2 (8)	3 (12)	
	After	1 (4)	2 (8)	3 (12)	
Klebsiella; n (%)	Before	1 (4)	0 (0)	0 (0)	
	After	0 (0)	0 (0)	0 (0)	

Group 1: drying method; Group 2: chlorhexidine; Group 3: breast milk.

Table 3 presents the mean of colonization of bacterial density before and after intervention as follows: Group 1 (287.3 \pm 171.7 and 19.51 \pm 10.7), Group 2 (230.8 \pm 184.1 and 26.12 \pm 11.05), and Group 3 (533.4 \pm 395.9 and

32.04±18.03). A significant decrease was observable in all cases based on results of Wilcoxon test ($p < 0.001$). Comparison of bacterial density changes in Table 3 by Kruskal-Wallis test indicated that there was no significant difference between the three groups ($p = 0.4$). Determination of the frequency of colonization of bacteria in the umbilical cord of all preterm neonates was first investigated and intervention was done, as presented in Table 3, there was no difference between the groups ($p = 0.6$ and 0.4 , respectively). However, the growth of colonized bacteria in newborns in all three groups had a significant decrease. The number of colonized bacteria before and after the intervention on neonates in Group 1, Group 2, and Group 3 are as follows respectively: 64 and 36% ($p = 0.03$), 52 and 20% ($p = 0.008$), 64 and 32% ($p = 0.02$) and results of McNemar's test showed there are significance difference.

Table 3. Comparison of the average density of colonized bacteria (CFU/cm²) and growth of bacteria in the umbilical cord of preterm neonates before and after intervention

Groups	Before intervention		After intervention		Wilcoxon test	Mean changes in bacterial density (Mean ± SD)
	Median (CFU/cm ²)**	Mean ± SD	Median	Mean ± SD		
Group 1	118.47	287.3±171.7	0	19.51±10.7	$p < 0.001$, $Z = -3.5$	-283.52±161.07
Group 2	127.38	230.8±184.1	0	26.12±11.05	$p < 0.001$, $Z = -3.1$	-219.24±173.09
Group 3	150.31	533.4±395.9	0	32.04±18.03	$p < 0.001$, $Z = -3.5$	-507.46±377.93
Kruskal-Wallis Test*	$X^2 = 1.4$, $p = 0.4$		$X^2 = 1.3$, $p = 0.5$		-	$X^2 = 1.5$, $p = 0.4$
Growth of bacteria (CFU/cm ²) before and after intervention in three groups						
	Group 1		Group 2		Group 3	
Before intervention	16 (64%)		13 (52%)		16 (64%)	
After intervention	9 (36%)		5 (20%)		8 (32%)	
McNemar's test	$p = 0.03$		$p = 0.008$		$p = 0.02$	

CFU: Colony-Forming Unit; Group 1: drying method; Group 2: chlorhexidine; Group 3: breast milk.

*The result of McNemar's test was not significant at $p \leq 0.05$; **Colony-forming unit/square centimeter

4. Discussion

The WHO recommended clean dry cord care for infants born in health facilities and at home in 2013 (11). According to WHO recommendations, some factors such as topical antiseptic usage, infection and Cesarean section were responsible for delaying umbilical cord separation (6, 12). Most studies are conducted on well and term neonates, but our study was carried out on premature neonates who were admitted to the NICU. This is one of the most important differences in our study with other studies. This has made it difficult to compare with the results of other studies. In some studies, cord separation time has been identified as a useful criterion. However, it was decided that prevention of infection was the most important issue for premature infants, and for this reason we did not use the umbilical cord separation (9, 12, 13). We did not find an article with an emphasis on the type of bacteria and the number of colonies in searches made in scientific publications such as PubMed. But in a systematic review with high-quality evidence from three community cRCTs, it is suggested that the use of 4% CHX reduces neonatal mortality in community settings, more so if this intervention is used in regions where infection rates are high, and with a moderate quality evidence suggesting that 4% CHX greatly reduces morbidity especially when started within 24 hours of birth in community settings and low-quality evidence, it is suggested that applying an antimicrobial is no better than leaving the cord to dry when in a high-income or middle income hospital setting (14).

Our study focuses on the type of germs, not on prognosis. It was revealed to us that there was no statistically significant difference between groups in terms of the type of microbial growth that developed. the results of the present study stated that the most common bacteria colonized in Group 1 and Group 3 was Staphylococcus epidermidis (which are non-pathogenic and natural flora of skin), and for Group 2 was Staphylococcus aureus (which is a pathogen bacteria). In a clinical trial study by Abbaszadeh et al., a significant correlation was not found between signs of infection (discharge, redness and swelling) in both groups. No cases of granuloma formation and sepsis were found and none of the neonates needed to be hospitalized. In other words, they found that mother's milk could be as effective as chlorhexidine (broad-spectrum antibiotics) in reducing signs of infection of cord. This result is matched with a study by Ahmadvour-Kacho et al. Regarding the fact that redness and swelling can be a sign of the growth of microbes on the umbilical cord, it can be compared with our study, in which we also did not make a significant difference between the rate of microbial growth in both breast milk and chlorhexidine groups (9, 13). The study of Vural and Kisa, which compared cord care using topical breast milk, drying, and povidone-iodine on 150 healthy, full-term newborns could not find significant differences between the three groups in terms of omphalitis

occurrence. They suggested the cultural practice of applying human milk to the umbilical cord stump appears to have no adverse effects. Because omphalitis is associated with microbial infection, the findings of the study are consistent with our findings (10). Taffazoli et al. found the most common cultured organisms were *S. epidermidis*, *S. aureus*, *E. coli* and *Klebsiella pneumoniae* in the umbilical stump, there were significant differences between two groups in colonization rate (dry cord method vs breast milk method). We found the most common organisms are *Staphylococcus epidermidis*, *Staphylococcus epidermidis*, *Enterococcus*, *Escherichia coli*, and *Klebsiella* but we could not find significant differences between the three groups in colonization rate (dry cord method vs. breast milk method vs. chlorhexidine method) (15). The studies of Mahrous et al. and Taffazoli et al. are of a few studies that have shown that other methods aside from maintaining dryness and preservation method in sterile delivery conditions have indeed a significant effect on the reduction of pathogenic bacterial level (15, 16). Nevertheless, it should be noted that cord storage strategies, except for the use of chlorhexidine, have not been systematically evaluated so far, although it has been shown that the use of traditional methods such as local use of ash, breast milk, or herbal teas only provide a suitable environment for the growth of bacteria and have no clinical benefit (2). However, it is possible that using breast milk in the umbilical cord could lead to a decrease in the removal duration of the umbilical cord, and consequently lead to decreased chances of infection and bacterial colonization (8, 10).

Various therapeutic approaches other than the use of breast milk have been evaluated in terms of umbilical cord care. In a study by Evens et al. where umbilical cord care in premature newborns in the time of separation as well as bacterial colonization was investigated, neonates were randomly treated with alcohol or with dryness (17). Ozdemir et al., who evaluated the effect of different antiseptics on cord separation time, also showed that chlorhexidine application was the most effective agent in decreasing colonization, as it significantly increased cord separation time (18).

Finally, the results of the present study showed that there were no statistically significant differences between the average density and type of bacteria colonized in the umbilical cord of the three groups after the intervention. Of course, it should be noted that each of the methods of drying, use of chlorhexidine, as well as the use of breast milk were effective in controlling the bacterial colonization of the umbilical cord of the newborns. The significance of the present study was the evaluation of three main different methods for umbilical cord care in a sterile hospital condition, with a single-blind approach, which offers a uniqueness compared to other studies in the field. The limitations of this study however, would be the lack of evaluation of the effect of each method on the time of umbilical cord separation. The present study was conducted on preterm neonates born in sterile conditions and the results should not be generalized and applied to infants born at home.

5. Conclusions

The results of the present study indicate that after the intervention, colonized bacteria were significantly decreased in all three cord care groups (breast milk method, dry method and chlorhexidine method). The amount of reduction was not different between the groups. Based on the results of this study, all three methods of drying approach, use of chlorhexidine, as well as breast milk were effective in controlling bacterial colonization in the umbilical cord of preterm neonates. Although there were no significant differences between the three groups, ultimately, the use of these methods could be effective in reducing the need for antibiotics.

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Trial registration:

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

GF designed the study and performed the data analysis. All authors contributed in data acquisition. All authors contributed in drafting the manuscript and GF revised the manuscript. All authors are accountable for all aspects of the work

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