Original Research Article

Effect of aqueous chlorhexidine on skin colonization with gram negative bacteria in preterm infants admitted in NICU: a blinded RCT

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INTRODUCTION

In neonates the dermis and the epidermis are not fully developed at birth. In healthy term infants, postnatally the skin maturation process is facilitated by colonization of symbiotic, nonpathogenic bacteria.¹ But lack of maturation processes in preterm infants predispose them to colonization which are different from that of term infants. In preterm infants, CoNS account for 80% of the total flora predominantly in the navel, skin folds, buttocks and soles. Subsequent colonisation by the transient flora is variable and depends on the neonate’s environment. Enterococci, Enterobacteriaceae, Acinetobacter spp., Pseudomonas aeruginosa have been isolated from the skin of these preterm neonates admitted in NICU and subjected to various invasive interventions like mechanical ventilation, peripheral and central venous access, use of parental nutrition and antimicrobials.² Microorganisms implicated in the etiology of nosocomial sepsis are those that colonize the skin, the gastrointestinal tract, the mucous membranes, and the monitoring and support devices in NICU.³ These organisms become

ABSTRACT

Background: Aqueous chlorhexidine applied repeatedly may predispose to increased gram negative bacterial colonization in preterm skin due to its higher bactericidal action against gram positive bacteria and absence of alcohol. The objective was to study the difference in rates of gram negative bacterial colonization in skin swabs taken from axilla and groin on day 7 after multiple applications of 0.5% aqueous chlorhexidine and placebo (sterile water).

Methods: Double blinded, randomised controlled trial recruiting preterm infants(28-34weeks) weighing ≥1000grams, stratified(28-31:32-34week) and randomly allocated to receive multiple body cleansing every 48hours starting from 6hours within birth during first postnatal week. Intervention group received cleansing with 0.5%chlorhexidine wipes and controls cleansed with similar looking sterile water wipes. Comparison of Proportions of swabs showing Gram negative bacterial growth in swabs taken from axilla and groin on day 7 of life after 3 cleansings (primary), at recruitment, 24 and 48 hours after first cleansing (secondary) were outcomes measured.

Results: Of 137 eligible neonates, 120 enrolled and 59 infants received chlorhexidine cleansing and 61 received sterile water. At the end of first week, the rate of skin colonization with gram negative bacteria after multiple applications of 0.5% aqueous chlorhexidine was comparable with sterile water cleansing in both axilla (40.9% vs 51.1%, p=0.432) and groin (60.7% vs 54.3%, p=0.592). There was no difference in the rate of gram negative bacterial colonization in axilla and groin skin at 24 and 48 hours after single application also.

Conclusions: Aqueous chlorhexidine even after multiple cleansings at 48hours intervals soon after birth has not predisposed to colonization with gram negative bacterial in preterm infants admitted in NICU.

Keywords: Aqueous chlorhexidine, Gram negative bacteria, Multiple cleansing, Preterm neonates, Skin colonization
opportunistic pathogens resulting in neonatal sepsis. So, skin disinfection is one of the best methods to prevent infections in preterm population. Isopropyl alcohol, povidone iodine and chlorhexidine are the best choices available for skin disinfection.\textsuperscript{4} Chlorhexidine (CHX) is a powerful, broad-spectrum antimicrobial antiseptic. It is effective against gram positive bacteria, somewhat less active against gram negative. CHX has significant residual activity and addition of alcohol results in significantly greater residual activity than alcohol alone. Its antimicrobial activity is slower than that of alcohol.\textsuperscript{5,7}

Systematic reviews and metanalysis on this topic concluded that topical chlorhexidine application reduced infections and mortality in settings with high sepsis rates, in community settings and when used on to prevent umbilical colonisation. CHX is highly bactericidal against gram positive organisms which are the commonly implicated aetiology causing infections in these settings. But such reduction was not possible in NICU or low sepsis prevalence settings where gram negative organisms are predominantly implicated.

The efficacy of Alcohol preparations of chlorhexidine (in 70% isopropyl alcohol) was similar or more when compared against that of povidone iodine and without side effects of thyroid dysfunction in various studies.\textsuperscript{8} But these preparations seem to produce erythema and burns when applied to the skin of VLBW infants.\textsuperscript{9} Hence aqueous preparations of chlorhexidine were used in preterm population. But these preparations have fared less when compared with Povidone iodine in skin disinfection probably due to absence of gram negative bactericidal action of alcohol.\textsuperscript{8} Adult ICUs have witnessed decrease in sepsis rate using daily bathing with chlorhexidine impregnated cloths, the effect and safety of which are yet to be attempted so far in neonates.\textsuperscript{9} But attempting this may decrease colonisation by the normal protective gram positive bacterial flora due to the predominant gram positive bactericidal action of aqueous CHX and may also increase pathogenic colonisation with gram negative bacteria from the NICU environment. The aqueous preparations without alcohol may also favour this. The increased colonisation with gram negative bacteria may predispose to increase in sepsis in NICUs unlike adult ICUs.\textsuperscript{10} Therefore, we designed a clinical trial to resolve this equipoise. We hypothesised that the multiple applications of aqueous chlorhexidine in preterm infants admitted in NICUs may increase the pathogenic colonisation with gram negative bacteria compared to placebo. Hence this clinical trial was undertaken with an objective to study the difference in rates of skin colonisation after multiple applications of 0.5% aqueous CHX in preterm infants in a NICU compared to placebo (sterile water).

**METHODS**

This study was conducted over 6 months in a level III NICU of a teaching hospital in South India after our Institutional ethical committee approved the protocol. Preterm infants (28-34 weeks) weighing ≥1000 gms admitted within 6 hours of birth and haemodynamically stable were eligible. Infants with major congenital malformations and epidermal defects involving >5% of the body surface area were excluded. We enrolled neonates after obtaining informed written consent from one of the parents or legally acceptable guardians. The baseline characteristics of the enrolled neonates and their maternal characteristics were recorded in the proforma. We allocated subjects by stratified block randomization. We stratified infants by gestational age into Stratum A (28-31 weeks) and Stratum B (32-34 weeks). Random sequence was generated online from a website for each stratum using even numbered, permuted blocks of varying sizes randomly with a 1:1 allocation ratio.

Both wipes of either 0.5% Chlorhexidine or sterile water were identically prepared and sealed by the same manufacturer. Neither the clinicians nor the research team could make out any differences between the wipes. We packed four individually sealed wipes in one zipped packet. All sealed wipes in a single packet were of either 0.5% Chlorhexidine or sterile water. The zipped packets were serially numbered and arranged in the generated sequence. We used wipes from one zipped packet allocated for each consecutively recruited infant in sequence and thereafter subsequently from the same packet for repeated applications. We cleansed the infants at 0, 48 and 96-hour after recruitment during the study. Principal investigator/duty staff wiped the infant skin (except face and scalp) from neck till soles using single wipe for each cleansing.

Each single wipe was divided into five portions and one portion was used to wipe each part of the body namely neck and chest, abdomen, perineum, upper and lower limbs. Fourth wipe was a reserve. Prior training on a mannequin ensured consistency of the procedure. Clinical team took decisions about the day to day management of the neonate. They were not a part of the study and were blinded to the intervention. We did not provide routine skin care to the enrolled infants during this period of the study (Figure 1). Authors collected skin swabs from the right axilla and groin regions by rubbing the sterile swab five times both horizontally and vertically rotating 360 degrees in the sampling area, placed it in transport medium (Trypticase soy broth) and sent for analysis.

Ensuring proper dispersion of contents, inoculum of 0.01 ml was placed onto sheep blood agar and MacConkey agar media using calibrated loop and incubated at 37 degree celsius for 18 to 24 hours. When the swabs showed growth on culture they were smeared, and gram staining was done to determine whether gram negative organisms were present in the growth. Our outcome was the number of swabs showing gram negative bacterial growth among the swabs which showed positive bacterial growth on culture collected from axilla and groin on Day 7 after the multiple applications of 0.5% aqueous
chlorhexidine vs sterile water at regular intervals of 48 hours. We also measured the rate of gram negative bacterial colonisation in swabs collected from axilla and groin at recruitment (baseline) and 24 and 48 hours after the first cleansing during the study. During the study period we also recorded details on the postnatal risk factors for skin colonisation in both the study groups. Based on day 7 skin colonization of 90% and 64.4% in control and intervention groups respectively in our pilot study with 90% power and significance level at 0.05, we needed 60 infants in each group for the trial. We used descriptive statistics to describe baseline variables.

Authors compared categorical variables by Chi square test; normally distributed variables by Student’s t test. The P value of less than 0.05 was considered statistically significant. We used statistical software package SPSS version 16.0 for analysis.

RESULTS
We enrolled 120 infants excluding 17 for reasons like congenital malformations (4), skin defects (1), delay in recruitment (9) and non-availability of the Principal investigator (3). Among the 120 enrolled infants, we randomly allocated 59 infants to receive 0.5% Chlorhexidine and 61 infants to receive sterile water cleansing. Demographic characteristics of the mothers

![Study Flow Chart](image_url)

Figure 1: Study flow chart.
and the infants were comparable in both the intervention and the control groups. (Table 1 and Table 2).

| Table 1: Baseline maternal characteristics. |
|-------------------------------------------|
| **Variable**                             | Chlorhexidine (N=59) n (%) | Sterile water (N=61) n (%) | P value |
| Maternal Age (years)*                    | 25±(4.5)                   | 24.6±(4.4)                 | 0.631   |
| Literacy rate                            | 27 (45.8)                  | 31 (50.9)                  | 0.710   |
| No. of ANC visits                        | 3.7 (1.1)                  | 4.0 (1.2)                  | 0.199   |
| AN steroids coverage                     | Nil                        | Partial                   | 0.574   |
|                                           | 27 (45.8)                  | 12 (20.3)                 |         |
|                                           | Complete                   | 20 (33.9)                 |         |
| Mode of delivery                         | Normal vaginal             | Instrumental              | 0.099   |
|                                           | 30 (50.8)                  | 8 (13.6)                  |         |
|                                           | LSCS                       | 21 (35.6)                 |         |
| ROM duration (hrs) (median, IQR)         | 8 (4; 25)                  | 17.5 (6; 44.3)            | 0.296   |
| Maternal infections                      | 4 (6.8)                    | 2 (3.3)                   | 0.645   |
| Mothers administered Intra partum antibiotic prophylaxis | 13 (22) | 12 (19.7) | 0.925 |

*mean (S.D), AN: Antenatal, ROM: Rupture of membranes.

| Table 2: Baseline neonatal characteristics. |
|-------------------------------------------|
| **Variable**                             | Chlorhexidine (N=59) n (%) | Sterile water (N=61) n (%) | P value |
| Gestational age weeks *                  | 32.5 (1.8)                 | 32.5 (1.8)                 | 0.875   |
| Birth weight (gms)*                      | 1691.7 (403.7)             | 1766 (425.7)               | 0.325   |
| Age at recruitment (mins)*              | 197.7 (96.4)               | 167.8 (98.8)               | 0.096   |
| Males                                   | 25 (42.5)                  | 33 (54.1)                  | 0.270   |
| Multiple births                         | 17 (28.8)                  | 12 (19.7)                  | 0.339   |
| Vernix                                   | 42 (71.2)                  | 36 (59.0)                  | 0.228   |
| Neomates resuscitated at birth          | 12 (20.3)                  | 10 (16.4)                  | 0.598   |

*Median (interquartile ranges) in days

The postnatal risk factors in the infants which predispose and influence skin colonisation like mechanical ventilation, ionotropic support, insertion and duration of peripheral and central venous access, surfactant administration, during their NICU stay were recorded during the study period. They were found to be equally distributed in both the study groups (p>0.05) (Table 3). During the study period due to high mortality in the preterm neonates we lost 4 infants in the chlorhexidine group and 5 infants in the control group (Table 5). Inspite of taking adequate precautions, due to unavoidable circumstances a few swabs got lost during the process of transportation to the microbiology lab. In the control group swabs taken from 3 patients at 24 hours, 2 patients at 48 hours were missing. In the chlorhexidine group swabs taken from 3 patients at 48 hours were missing (Table 4). These were handled as missing data.

| Table 3: Postnatal risk factors for skin colonization. |
|------------------------------------------------------|
| **Variable**                                         | Chlorhexidine (N=59) N (%) | Sterile water (N=61) N (%) | P value |
| Mechanical ventilation                               | 16 (27.1)                  | 24 (39.3)                  | 0.220   |
| Ionotropic support                                   | 10 (17.2)                  | 15 (24.6)                  | 0.420   |
| Intravenous access                                   | 36 (61.0)                  | 43 (70.5)                  | 0.812   |
| IV access duration*                                   | 8 (5-15.6)                 | 7 (5; 15)                  | 0.774   |
| PICC line insertion                                  | 12 (20.3)                  | 12 (19.6)                  | 0.891   |
| PICC line duration*                                   | 6.5 (5-8)                  | 6.5 (5; 8)                 | 0.887   |
| Umbilical line access                                | 3 (5.08)                   | 4 (6.55)                   | 0.963   |
| UVC line duration*                                   | 4 (3-5)                    | 4 (3.2-5.5)                | 0.857   |
| Surfactant therapy                                   | 10 (16.9)                  | 17 (27.9)                  | 0.225   |

*Median (interquartile ranges) in days

Hence primary outcome was available for 111 patients. (55 infants in the intervention and 56 infants in the control group) (Table 5). Inspite of taking adequate precautions, due to unavoidable circumstances a few swabs got lost during the process of transportation to the microbiology lab. In the control group swabs taken from 3 patients at 24 hours, 2 patients at 48 hours were missing. In the chlorhexidine group swabs taken from 3 patients at 48 hours were missing (Table 4). These were handled as missing data.
Primary outcome

On Day 7 swabs taken from axilla: Out of 55 skin swabs taken 22 swabs showed bacterial growth. In these, 9 swabs were gram stain negative (40.9%).

In the control group out of 56 swabs sent from axilla, 45 swabs showed bacterial growth. In these, 23 were gram stain negative (51.1%). The difference in axillary skin colonization with gram negative organisms between the intervention and the control groups was not statistically significant (p=0.432) (Figure 2).

Secondary outcomes

On Day 7 swabs taken from groin: Out of 55 skin swabs taken, 28 swabs showed bacterial growth. In these, 17 swabs were gram stain negative (60.7%) in the intervention group.

Table 4. Distribution of patients and swabs analysed in the study.

| Time    | Chlorhexidine (N=59) | Sterile water(N=61) |
|---------|----------------------|---------------------|
|         | No. of Infants | Axilla swabs | Groin swabs | No. of Infants | Axilla swabs | Groin swabs |
| Baseline| 59          | 59          | 59          | 61          | 61          | 61          |
| 24 hours| 58          | 58          | 58          | 60          | 57          | 57          |
| 48 hours| 58          | 55          | 55          | 60          | 58          | 58          |
| Day 7   | 55          | 55          | 55          | 56          | 56          | 56          |

Table 5: Gram Negative organism colonisation in culture positive swabs.

| Site/ time | Chlorhexidine | Placebo | P value |
|------------|---------------|---------|---------|
|            | Culture positive swabs (N) | Gram negative growth N % | Culture positive swabs (N) | Gram negative growth N % |
| Axilla- baseline | 38          | 17 (44.7) | 27          | 9 (33.3) | 0.355 |
| Axilla-24 hours | 37          | 22 (59.5) | 43          | 29 (67.4) | 0.459 |
| Axilla-48 hours | 31          | 18 (58.1) | 49          | 35 (71.4) | 0.218 |
| Axilla- day 7 | 22          | 9 (40.9)  | 45          | 23 (51.1) | 0.432 |
| Groin- baseline | 35          | 11 (31.4) | 21          | 9 (42.9)  | 0.405 |
| Groin-24 hours | 33          | 19 (57.6) | 44          | 33 (75.0) | 0.106 |
| Groin- 48 hours | 34          | 24 (70.6) | 50          | 32 (64.0) | 0.530 |
| Groin- Day 7 | 28          | 17 (60.7) | 46          | 25 (54.3) | 0.592 |

Figure 2: Comparison of rates of colonisation with gram negative bacteria.
In the control group out of 56 swabs sent, 46 swabs showed bacterial growth. In these, 25 were gram stain negative (54.3%). The difference in groin skin colonization of gram negative organisms between the intervention and the control groups was not statistically significant (p=0.592).

At recruitment (baseline) the rate of colonisation with gram negative bacteria in the intervention group (44.7%) and that of control group (33.3%) were not different (p=0.355) in axilla. Similarly, at groin colonization was not different between the groups at baseline (31.4% vs 42.9%, p=0.405). At 24 hours after the first cleansing the rate of gram negative bacterial colonization was not different between the intervention and the control groups in axilla (59.5% vs 67.4%, p=0.459) as well as groin (57.6% vs 75.0%, p=0.106). At 48 hours after the first cleansing there was no statistically significant difference in the rates of gram negative bacterial colonization among the study groups in axilla (58.1% vs 71.4%, p=0.218) and groin (70.6% vs 64.0%, p=0.530) (Table 5).

**DISCUSSION**

In NICUs infants are continuously at risk of pathogenic microbial colonization from their environment.2 Skin disinfection at regular intervals during this period may decrease colonisation and nosocomial sepsis rather than a single application.8,11 Though the efficacy of chlorhexidine increases with higher concentrations, 0.5 to 1% has been considered safe for use in very low birth weight preterm infants.8 In this population alcohol preparations have caused adverse skin reactions suggesting replacement with aqueous preparations.12,13 Recently meta-analysis and systematic reviews have not compared in subgroups benefits of alcohol vs aqueous chlorhexidine preparations.6,7 Though the aqueous preparations are safe in terms of skin toxicity, in the absence of alcohol it may not actively protect against gram negative bacterial colonisation.14

Many studies which reported efficacy of chlorhexidine on skin colonization have also looked at the type of colonization. Skin colonization may not always mean infection. The balance tips causing ill effects when the protective normal bacterial flora gets replaced with pathogenic flora acquired from NICU. This may throw insight to understand the differential benefits of chlorhexidine in community vs hospital settings, settings with high sepsis prevalence vs low sepsis prevalence and success of topical cord application to reduce infections and mortality.15 Hence, we attempted to look at the type of colonization in preterm infants after multiple applications of aqueous chlorhexidine in VLBW infants who have a constant predisposition to develop nosocomial sepsis from pathogenic colonization in NICU.

Present study was undertaken in Level III NICU catering to inborn preterm neonates who were admitted soon after birth. They were recruited early before their skin colonization started (less than 6 hours). We cleansed infants at 48-hour intervals and studied their colonization rates at the end first week. We did not find a difference in their rate of skin colonization with gram negative bacteria after multiple whole-body cleansing using aqueous preparations compared to our standard practice of sterile water on day 7. We measured the rate of skin colonization with gram negative bacteria at multiple time points (24 and 48 hours) after single application of the intervention also to capture the change in patterns over time during the study. We could not find any difference in the gram-negative bacteria colonization rates at both 24 hours and 48 hours after single application also. The pattern of change in the rates of gram negative bacteria colonization over time in the intervention and the control groups were random and no trend could be observed.

His Hwang et al studied the pattern of skin colonization after daily applications of chlorhexidine compared with normal saline at the exit site of peritoneal dialysis catheters at 6 and 12 months. Though the intervention produced a reduction in the MRSA colonization over time there was no difference in gram negative bacterial colonization either at 6 or 12 months.16

Among the studies which reported on the effect of chlorhexidine on bacterial skin colonization, the study by Jeeva Sankar et al in 2009 reported the colonization rates with Gram-negative pathogens were comparable between the 0.25 % aqueous chlorhexidine cleansing, normal saline cleansing and no cleansing groups at both 24 and 72h after the single cleansing.10 This was similar to present study results.

The rates of colonization with gram negative organisms were high in present study at both the axilla (40-60 %) and groin (60-70%) at all time points during the study. But the rates of gram negative organisms’ colonization in the Delhi study ranged between 10-20% and no gram-negative organisms in the chlorhexidine group. The rates were probably low because of strict adherence to aseptic hygienic practices among health care personnel in NICU in that apex teaching hospital of our country. This is supported by low rates of baseline skin colonization observed there compared to our setup.

Because the NICU practices influence neonatal colonization we measured and recorded all the risk factors predisposing to bacterial skin colonization in the enrolled infants during the study period unlike others.9,10,17,18 A similar study was done in Bangladesh (2007) attempting to study the extended cleansing effects of 0.25% chlorhexidine. Their skin colonization rates seem comparable to be to us. They identified species like Acinetobacter, Klebsiella, Staphylococcus and Streptococcus as common ones. There was no significant group difference in the baseline pathogen-specific culture rates across all sites and pathogens.19

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*Anitha MB et al. Int J Contemp Pediatr. 2018 Sep;5(5):1767-1774*
The Kathmandu study done in 2008 compared the efficacy of chlorhexidine of different concentrations (0.25%, 0.5% and 1%). They also had identified the microbial flora and quantified them. At baseline, gram-positive organisms were more commonly identified (267/513, 52%) than gram-negative organisms (117/513, 23%); there were also 122 (24%) swabs positive for both gram-positive and gram-negative organisms. This ratio did not change substantially across the three time periods (before cleansing, 2 hours, or 24 hours). There was only slight evidence that the impact of cleansing concentration on colonization varied by gram positive/negative status.18

**Strengths and limitations**

Present study was the first one to attempt multiple applications of aqueous chlorhexidine in preterm neonatal population and compare the gram negative bacterial colonization. Many of the previous studies have been done in term infants who have better maturation process of skin and hence differ by patter of skin colonization too. The study done exclusively in VLBW preterm infants was a pilot study sampling only 20 preterm infants. Present study had a good number of preterm infants enrolled and studied. It was a well-designed blinded randomized controlled trial with a rigorous methodology. Risk factors for the outcome (gram negative colonization) during the NICU interventions post randomization were measured and compared. Though the primary outcome was at the end of multiple applications colonization was studied at intervals after single application also enabling comparison with the previous studies employing single cleansing.

Though our sample population was large compared to previous studies and the required sample size was enrolled and randomized in the study, the high mortality in our NICUs in this group of preterm population precluded us from reaching the required numbers for our primary outcome. When recruiting patients, the sample size must be arrived with due consideration to their high mortality rates.

We did not type the gram-negative organisms because we believed colonization with any of the gram-negative organism was pathogenic unlike the gram-positive CoNs normal flora. We had not assayed the serum levels of chlorhexidine in spite of repeated applications in preterm neonates to ensure safety. There are no established values for what a safe concentration of chlorhexidine in the blood is.15,8,19,20

However, we limited our applications to three in the study since the much-feared neurotoxicity in the hexachlorophene usage through systemic absorption from the skin was reported after four or more applications.21 The alcohol that is known to potentiate the absorption of topically applied chlorhexidine was also avoided in present study. We skipped the areas like face and scalp which are highly vascular and would increase the chances of systemic absorption.18 Even then to document its safety with a full proof a long term neuro developmental follow up of the infants may be necessary. None of the studies in the literature also have attempted to study this aspect of its safety profile.

**CONCLUSION**

At the end first week, the rate of skin colonization with gram negative bacteria after multiple applications of 0.5% aqueous chlorhexidine at 48hour interval was comparable with whole body cleansing using sterile water in both axilla (40.9% vs 51.1%, p=0.432) and groin (60.7% vs 54.3%, p=0.592).

There was no difference in the rate of gram negative bacteria colonization in axilla at 24hours (59.5% vs 67.4%, p=0.459) and 48 hours (58.1% vs 71.4%, p=0.218) after single application also. Similar effect seen in groin at 24 hours (57.6% vs 75%, p=0.106) and at 48 hours (70.6% vs 64.0%, p=0.530) also. Thus, aqueous chlorhexidine even after multiple cleanings at intervals soon after birth has not predisposed to skin colonization with gram negative bacteria in preterm infants admitted in NICU.

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**Ethical approval:** The study was approved by the Institutional Ethics Committee

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