Effect of Chlorhexidine 2% versus Alcohol 70% on Catheter - Related Bloodstream Infections in Neonates: Randomized Control Trial

Rania A. El-Farrash[1], Marwa Mamdouh Ashour[1], Marwa Adel Ahmed[2] and Yasmin A. Farid[1,*]

[1]*Pediatrics Department, Faculty of Medicine - Ain Shams University, Egypt*

[2]*Clinical Pharmacy Department, Faculty of Pharmacy, Ain Shams University, Egypt*

Abstract: *Background*: Central venous catheters (CVCs) are essential for neonatal intensive care but are strongly associated with catheter-related bloodstream infections (CRBSI), which significantly increase morbidity, mortality, and hospital stay. The optimal antiseptic for neonatal skin disinfection remains debated, with chlorhexidine-alcohol solutions increasingly recommended but still under investigation for safety and efficacy.

Objective: To compare the effectiveness of 2% chlorhexidine in 70% isopropyl alcohol versus 70% alcohol alone in reducing catheter-related bloodstream infections among neonates requiring CVCs.

Methods: A prospective randomized controlled trial was conducted on 100 neonates admitted to the NICU at Ain Shams University Hospital. Participants were randomly assigned to two groups: Group A (alcohol-only antisepsis) and Group B (chlorhexidine-alcohol antisepsis). Data collected included patient demographics, catheter type and duration, infection rates, blood culture results, inflammatory markers, and clinical outcomes.

Results: Group B (chlorhexidine-alcohol) demonstrated significantly lower rates of positive blood cultures (notably Klebsiella and E. coli) compared to Group A. The incidence of infection was highest in neonates with percutaneous central venous catheters (PCVCs) and umbilical venous catheters (UVCs), while peripherally inserted central catheters (PICCs) showed the lowest infection burden. Mean catheter duration was 9-11 days, with most infections occurring beyond the seventh day. Hemoglobin and hematocrit levels declined significantly over time in infected cases. Group B also required fewer escalations to second-line antibiotics.

Conclusions: Using 2% chlorhexidine in 70% isopropyl alcohol significantly reduced catheter-related bloodstream infections (CRBSIs) in neonates, compared to 70% alcohol alone. Peripherally inserted central catheters (PICCs) exhibited the lowest infection rates, highlighting the importance of both antiseptic selection and catheter type in neonatal infection prevention protocols.

Keywords: Neonates, catheter-related bloodstream infection, chlorhexidine, central venous catheter, antisepsis, PICC, NICU.

INTRODUCTION

Healthcare-associated infections (HAIs), particularly catheter-related bloodstream infections (CRBSIs), represent a critical concern in neonatal intensive care units (NICUs). Neonates-especially preterm and low-birth-weight infants-are at heightened risk due to their immature immune systems and the frequent need for invasive procedures such as central venous catheterization (CVC) [1]. Despite advancements in neonatal care and infection control measures, catheter-related bloodstream infections (CRBSIs) remain a major contributor to neonatal morbidity, extended hospitalization, increased healthcare expenditures, and mortality in severe cases. Due to this high burden, implementing effective preventive strategies has become a global health priority [2].

Catheter-related bloodstream infection (CRBSI) is confirmed when the catheter is shown to be the source of infection, as evidenced by the same organism in peripheral and catheter-drawn blood cultures or in catheter tip cultures. Diagnosis is supported by a≥2-hour earlier positivity in catheter-drawn blood (differential time to positivity), a ≥3:1 colony ratio, or>15 CFU from catheter tip culture. Other sources of infection must be excluded, and improvement after catheter removal supports the diagnosis [3, 4].

A key component of CRBSI prevention is the choice of antiseptic agent for skin preparation prior to CVC insertion and during maintenance. Chlorhexidine gluconate (CHG) in alcohol-based formulations, particularly 2% CHG in 70% isopropyl alcohol, has demonstrated superior and sustained antimicrobial activity in adult and pediatric populations compared to aqueous antiseptics or alcohol alone [3]. Consequently, alcohol-based chlorhexidine has become a recommended standard in many international infection

*Address correspondence to this author at the Pediatrics Department, Faculty of Medicine - Ain Shams University, Egypt;
E-mail: Yasmin_Aly_Farid@hotmail.com

E-ISSN: 1929-4247/26

control guidelines. However, its use in neonates has remained controversial, mainly due to safety concerns related to skin irritation, chemical burns, and systemic absorption, especially in preterm infants with underdeveloped skin barriers [5, 6].

Although effective in older patients, 2% chlorhexidine-alcohol has not been well studied in neonates. Randomized trials in this age group are scarce. Much of the available data is either retrospective, observational, or extrapolated from non-neonatal populations [7]. Although several studies indicate that chlorhexidine may reduce catheter colonization and bloodstream infections, few have directly compared it to alcohol-only antiseptics in controlled neonatal trials. This lack of robust, neonatal-specific evidence has led to considerable variability in practice across NICUs worldwide. Many units continue to use suboptimal antiseptics, potentially compromising infection prevention efforts, driven by concerns unsupported by strong evidence [8, 9].

Our study addresses this critical gap by directly comparing 2% chlorhexidine in 70% isopropyl alcohol with alcohol-only antisepsis in a randomized controlled trial in neonates. This design provides controlled, prospective evidence on both efficacy and safety. Furthermore, our research is novel in that it not only evaluates CRBSI rates but also correlates microbiological profiles, line types, antibiotic use, and clinical outcomes in a developing-country NICU context, where infection rates are often underreported.

Consequently, the aim of this study is to assess whether the use of 2% chlorhexidine in 70% isopropyl alcohol reduces catheter-related bloodstream infections compared with 70% isopropyl alcohol alone in neonates, and to explore how catheter type influences infection rates and outcomes. Through this, we aim to inform antiseptic protocols and improve neonatal care standards in resource-limited settings.

PATIENTS AND METHODS

After ethical committee approval and informed consent from the parents or guardians of the children, this study was conducted as a randomized controlled trial (RCT) and registered on ClinicalTrials.gov under the ID NCT06194396. The trial was carried out over a six-month period from May to November 2023 at the Neonatal Intensive Care Unit (NICU) of the Children's Hospital, Ain Shams University, Cairo, Egypt. All procedures were conducted in accordance with the principles of the Declaration of Helsinki. The study started after approval of the Research Ethics Committee, Faculty of Medicine, Ain Shams University (FMASU MS 325/2023).

Inclusion and Exclusion Criteria

Neonates were considered eligible for inclusion in the study if they met the following criteria: a gestational age of 28 weeks or more, and a clinical indication for the insertion of a central venous catheter (CVC).

Conversely, neonates were excluded if they had any skin conditions contraindicating the use of antiseptics, such as epidermolysis bullosa, or if they had a known allergy to either chlorhexidine or isopropyl alcohol. These exclusions were necessary to protect participants' safety and to avoid potential adverse dermatologic or systemic reactions associated with antiseptic exposure.

Randomization

Randomization was conducted using a computer-generated table of random numbers through the Simple Randomization Service by Sealed Envelope Ltd. (2017). Eligible neonates were allocated in a 1:1 ratio to receive either 70% alcohol or 2% chlorhexidine-70% isopropyl alcohol for skin disinfection prior to central venous line insertion. Once allocation was generated, it was not subject to change.

Allocation Concealment

Sequentially numbered, opaque, sealed envelopes enclosed the group assignment letter. These envelopes were stored securely and opened only on the day of catheter insertion by the study nurse, immediately prior to the procedure, to assign the disinfectant solution. Allocation concealment ensured that neither the parents, data collectors, nor outcome adjudicators were aware of group assignment. However, the operative team (neonatologist and nurse inserting the catheter) could not be blinded to the assigned solution due to the distinct smell and physical properties of the disinfectants.

Blinding

Parents, data collectors, and outcome adjudicators were blinded to the allocated group. The clinical team inserting the catheters could not be blinded due to the distinct smell and handling characteristics of the antiseptics; however, they were not involved in data analysis, minimizing potential bias.

Study Procedures: All participants were subjected to the following:

History Taking: including

A comprehensive clinical history was obtained for each neonate prior to inclusion in the study. The history included both neonatal and maternal factors. From the neonatal side, data were collected on gestational age, postnatal age, sex, and birth weight. Additionally, the mode of delivery (either vaginal or cesarean section) and the primary diagnosis or indication for NICU admission (such as respiratory distress, sepsis, or congenital anomalies) were documented.

From the maternal perspective, potential risk factors influencing neonatal infection susceptibility were noted. These included conditions like premature rupture of membranes (PROM), maternal hypertension, urinary tract infections (UTIs), diabetes mellitus, placenta previa, and maternal infections during pregnancy.

Clinical Examination

All neonates underwent thorough systematic clinical examination upon admission and throughout the study period. Particular attention was paid to signs of sepsis, respiratory distress, neurological status, hemodynamic stability, and peripheral perfusion. The clinical assessment also helped evaluate the need for ventilatory support and inotropic therapy, both of which were documented.

Investigations

To complement the clinical evaluation, a series of laboratory and microbiological investigations was conducted for all enrolled neonates. These included:

1. Complete Blood Count (CBC): A total of 1 mL venous blood sample was collected from each study participant by a trained laboratory technologist using the Vacutainer tube method in a lavender-top EDTA tube. Then, it was put in an automated hematology analyzer (Sysmex Corporation, Japan).

2. C-Reactive Protein (CRP): A total of 1ml venous blood sample was collected under all aseptic precautions from the dorsal vein by a clean puncture, avoiding bubbles and froth. The blood sample collected in a Red-top tube or gel-barrier tube was then placed in a CRP turbidimetry system (semi-automated Mindary BA-88A, China) for 3 minutes. Immunoassays and laser nephelometry are methods for quantifying CRP levels [10].

3. Blood Cultures: A total of 1-2ml venous blood sample was collected under strict aseptic precautions by a sterile puncture, avoiding bubbles and froth. Blood sample collected in a blood culture bottle. Bottles were continuously monitored for up to 7 days at 37°C using the BacT/Alert Viro detection system (BioMérieux, France) [11].

4. Catheter Tip Cultures: Upon catheter removal, 1 cm segments (proximal and tip) were cultured using semi-quantitative techniques. Identification was done via Gram staining and automated systems (Vitek-2, BioMérieux). Paired blood and catheter cultures were used to confirm catheter-related infections via molecular typing.

All microbiological samples were transported in sterile conditions and processed immediately in the hospital's microbiology laboratory. Identification of pathogens was performed using Gram staining, catalase testing, and automated systems such as VITEK-2.

Study Interventions

Antiseptic Groups

Participants were randomly assigned to one of two antiseptic protocols: Group A received alcohol only, and Group B received 2% chlorhexidine in 70% isopropyl alcohol. Both solutions were applied before catheter insertion and during routine maintenance. Their composition and handling were standardized.

Disinfectant Solutions

All neonates enrolled in the study required the insertion of a central venous catheter (CVC). Prior to catheter insertion and throughout routine catheter care, the skin was disinfected according to the assigned intervention group. Neonates were randomized into two groups. Group A received 70% alcohol (Shield Wall, Health Line Group, Egypt; production date March 2023), a transparent liquid with a pronounced odor and a boiling point of 78.3 °C. Group B received 2% chlorhexidine gluconate in 70% isopropyl alcohol (Germadine, BIOGUARD, Egypt; production date February 2023). This solution was transparent, had a pleasant odor, a pH of 7, a boiling point of 83 °C, and a specific gravity of 0.972 [12].

Skin Disinfection at Catheter Insertion

Study packs containing two bottles of the allocated antiseptic were stored in a locked, temperature-monitored cupboard. One bottle was used at the time of catheter insertion, and the other was retained for catheter removal. Approximately 2 mL of the assigned antiseptic was applied to the insertion site using sterile gauze for 10-20 seconds. To ensure safety, pooling of the solution was avoided, and any excess was gently removed. The skin was allowed to air dry completely for at least 30 seconds before proceeding with catheter insertion. During follow-up, the catheter insertion site was inspected daily, and antiseptic reapplication and dressing change were performed every 24 hours or earlier if the dressing became soiled or loose. Saline or water was not used to clean the insertion site unless catheterization failed.

Catheter Insertion

All procedures involved only the insertion of CVCs. Catheter insertion was performed using a standardized aseptic technique and established catheter-care bundles. All staff involved in the procedures were trained in catheter insertion and maintenance. Operators wore sterile gowns, gloves, and face masks to maintain asepsis and minimize contamination risk from the odor of the assigned antiseptic.

Types included:

- Peripherally inserted central catheters (PICCs): Polyurethane Premicath (VYGON, UK), 1Fr/28G.
- Umbilical venous catheter (UVC): VYGON, size 4Fr-8Fr based on weight.
- Percutaneous central venous catheter (PCVC): AMECATH (USA), 3-4 Fr, inserted via ultra-sound-guided technique. Ultrasound guidance (Mindary, China) was routinely used for PCVC placement, typically in the right external jugular vein. All staff involved in the procedures were trained in catheter insertion and maintenance.

Personnel used sterile gowns, masks, and gloves to maintain asepsis and avoid bias due to antiseptic odor. The decision to insert a CVC and the choice of catheter type were at the discretion of the attending clinical team.

Catheter Removal and Collection of Study Specimens

Catheters were removed based on clinical judgment or upon reaching maximum dwell time:

- PCVC: 3 weeks
- UVC: 7-14 days
- PICCs: 2-3 weeks

Removed catheters were processed for microbiological analysis, including colony count, Gram staining, and organism identification.

In some cases, removal was indicated earlier than planned because of complications, such as suspected sepsis, or once the maximum recommended dwell time of three weeks was reached [13]. The decision to remove the catheter was made by the attending clinical team.

At the time of catheter removal, two approximately 1 cm segments (proximal and tip) were collected for microbiological analysis. Proximal catheter segments were included because they have higher colonization rates than catheter tips, thereby improving the diagnostic yield of catheter colonization. The segments were placed in 1 ml of phosphate-buffered saline, transported immediately to the microbiology laboratory, and vortexed before further processing [14].

Microbiological assessment was performed according to standard protocols. Colony counts and bacterial identification were carried out after 48 hours of incubation, with agar plates incubated at 37 °C for 24 hours and re-incubated for an additional 24 hours if no growth was initially observed [15]. Manual total and semiquantitative aerobic colony counts were recorded [16]. Organism identification was achieved using Gram stain, catalase testing, pyrrolidonyl aminopeptidase activity, and latex agglutination (Pastorex Staph-Plus, BioRad, Redmond, WA) for Gram-positive organisms, or the automated Vitek-2 system (bioMérieux, Marcy-l'Étoile, France) for Gram-negative organisms [17].

Sample Size: The sample size was calculated using PASS 15 software, with a power set at 80% and an alpha of 0.05. Based on these parameters, it was estimated that 50 neonates per group would be required to detect a medium effect size difference (h = 0.5) between the two interventions. Accordingly, 100 neonates were included in the study, with 50 assigned to each group.

Statistical Analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM

SPSS) version 27. The quantitative data were presented as means, standard deviations, and ranges when parametric, and as medians and interquartile ranges (IQR) when nonparametric. Also, qualitative variables were presented as numbers and percentages.

The comparison between groups for qualitative data was performed using the *Chi-square test* and/or Fisher's *exact test* when the expected count in any cell was less than 5. The comparison between two independent groups with quantitative data and a parametric distribution was done using an independent *t-test,* while comparisons with nonparametric distributions were done using the *Mann-Whitney test.* The comparison between two paired groups regarding quantitative data and parametric distributions was done using the *Paired t-test,* while for non-parametric distributions, the *Wilcoxon Rank test was used.* The comparison between more than two groups regarding quantitative data and parametric distributions was performed using the *One-Way ANOVA test,* followed by post hoc analysis using the *LSD test,* whereas comparisons with nonparametric distributions were performed using the Kruskal-Wallis *test,* followed by post hoc analysis using the *Mann-Whitney test*. The confidence interval was set to 95%, and the accepted margin of error was 5%. So, the p-value was considered significant as follows:

P-value > 0.05: Non-significant (NS)

P-value < 0.05: Significant (S)

P-value < 0.01: Highly significant (HS).

RESULTS

The results of the present study are demonstrated in the following tables.

Baseline Characteristics

A total of 100 neonates were randomized in equal numbers. Both groups were comparable in gestational age, sex, and delivery mode. However, Group B (chlorhexidine-alcohol) had a significantly higher mean birth weight (2.22 ± 0.77 kg) than Group A (1.85 ± 0.64 kg, $p = 0.011$) (Table **1**).

Bloodstream Infections and Microbiological Findings

Group B demonstrated a significantly lower incidence of CRBSI (32%) compared to Group A (58%) ($p = 0.012$). The most common pathogens were Klebsiella and E. coli, which were significantly more frequent in Group A (Klebsiella: 48% vs. 24%, $p = 0.012$; E. coli: 22% vs. 6%, $p = 0.021$). Staphylococcus spp. and Acinetobacter were less commonly isolated (Table **2**).

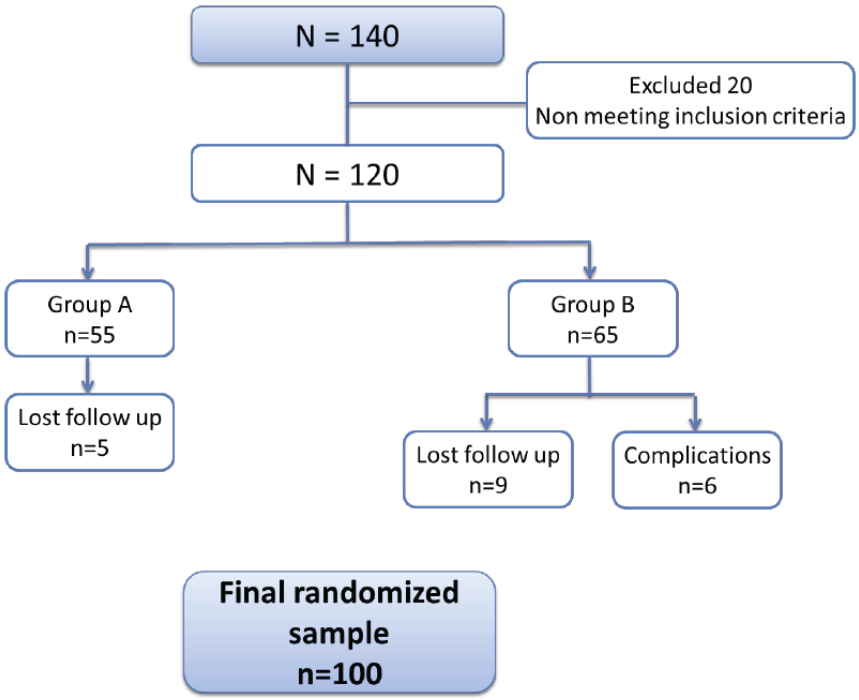

Figure 1: CONSORT Chart of the study groups.

Table 1: Demographic Data and Characteristics of the Studied Patients

		Total
		No. = 100
Gestational age	Preterm ≤ 36 wks	63 (63%)
	Full term ≥ 37 wks	37 (37%)
Postnatal age (days)	Median (IQR)	2 (1 - 4)
	Range	1 - 26
Gender	Male	55 (55%)
	Female	45 (45%)
Weight	Mean ± SD	2.04 ± 0.73
	Range	0.89 - 4
Mode of delivery	Cs	65 (65%)
	NVD	35 (35%)
Diagnosis	RD	78 (78%)
	Sepsis	29 (29%)
	Jaundice	1 (1%)
	Convulsion	2 (2%)
	Hematemesis	2 (2%)
	Congenital anomalies	4 (4%)
	IEM	5 (5%)
	CHD	2 (2%)
	Diarrhea	1 (1%)
	Anemia	1 (1%)
Maternal history		60 (60%)
	DM	6 (6%)
	HTN	12 (12%)
	UTI	10 (10%)
	PROM	36 (36%)
	ITP	2 (2%)
	Placenta previa	4 (4%)

IQR: inter-quartile range, SD: standard deviation, CS: cesarian section, NVD: normal vaginal delivery, IEM: inborn errors of metabolism, CHD: congenital heart disease, DM: diabetes mellitus, HTN: hypertension, UTI: urinary tract infection, PROM: premature rupture of membranes, ITP: idiopathic thrombocytopenic purpura.

Detailed pathogen distribution by catheter type and second culture positivity is provided in Supplementary Tables.

Catheter Tip Cultures by Catheter Type

Microbial growth on catheter tips varied by catheter type. Peripherally inserted central catheters (PICCs) had the lowest infection rates, with 52.9% showing no microbial growth. In contrast, umbilical venous catheters (UVCs) and percutaneous central venous catheters (PCVCs) were more frequently colonized. In PCVCs, Klebsiella was the predominant organism (40.8%), followed by *E. coli* (10.2%) and Staphylococcus (12.2%) (Table **3**).

Inflammatory and Hematologic Changes

In Group A, median CRP rose significantly post-insertion (13.7 mg/L), while Group B showed a milder elevation (5.6 mg/L, p = 0.012). Hemoglobin and hematocrit levels declined in both groups, but more

Table 2: Blood Culture 1 and Blood Culture 2 among the Studied Patients

		No. = 100
Blood culture 1	No growth	50 (50.0%)
	klebseilla	37 (37.0%)
	Acinobacter	2 (2.0%)
	E. coli	6 (6.0%)
	Staph	5 (5.0%)
Blood culture 2	No growth	38 (38.0%)
	klebseilla	36 (36.0%)
	Acinobacter	1 (1.0%)
	pseudomonous	1 (1.0%)
	E. coli	14 (14.0%)
	Staph	10 (10.0%)

Table 3: Cultures Taken from CVC Inserted in Neonates during their Stay in NICU

		No. = 100
UVC	No growth	14 (41.2%)
	klebseilla	14 (41.2%)
	E. coli	3 (8.8%)
	Staph	3 (8.8%)
PCVC	No growth	16 (32.7%)
	klebseilla	20 (40.8%)
	Acinobacter	2 (4.1%)
	E. coli	5 (10.2%)
	Staph	6 (12.2%)
PICC	No growth	9 (52.9%)
	klebseilla	2 (11.8%)
	Acinobacter	1 (5.9%)
	pseudomonous	1 (5.9%)
	E. coli	2 (11.8%)
	Staph	2 (11.8%)

UVC: umbilical venous catheter, PCVC: Percutaneous central venous catheter, PICC: peripherally inserted central catheter.

sharply in Group A ($p < 0.05$), indicating a stronger systemic inflammatory response in the alcohol-only group (Table **4**).

Expanded laboratory trends, including WBC and platelet counts, are detailed in the Supplementary Table.

Clinical Outcomes

Group B had a lower mortality rate (22%) than Group A (40%), though the difference narrowly missed statistical significance ($p = 0.052$). The median NICU stay was slightly longer in Group B (19 days vs. 14.5 days), likely due to longer survival (Table **5**).

Table 4: Comparison between Laboratory Data at of Baseline Samples and Follow-Up Samples among Neonates Enrolled in the Study

		Total	Total	Difference	Test value	P-value	Sig.
		1st visit	2nd visit	Mean ± SD			
HB	Mean ± SD	14.44 ± 3.55	12.53 ± 2.63	-1.91 ± 3.65	-5.226•	<0.001	HS
	Range	6.3 - 21.4	6.5 - 20.1	-1.91 ± 3.65	-5.226•	<0.001	
TLC	Mean ± SD	13.07 ± 6.86	13.91 ± 7.85	0.83 ± 9.15	0.909•	0.365	NS
	Range	2.8 - 32.2	2.5 - 41	0.83 ± 9.15	0.909•	0.365	
HCT	Mean ± SD	42.57 ± 11.13	36.74 ± 7.89	-5.83 ± 11.25	-5.186•	<0.001	HS
	Range	19.2 - 64	17.6 - 55.7	-5.83 ± 11.25	-5.186•	<0.001	
Platelets	Median (IQR)	213.5 (132 - 296.5)	218.5 (150.5 - 341)	30.01 ± 159.72	-1.178≠	0.239	NS
	Range	5 - 929	16 - 839	30.01 ± 159.72	-1.178≠	0.239	
CRP	Median (IQR)	4.75 (1.45 - 32.55)	8.45 (2.35 - 32.2)	-1.49 ± 49.80	-0.872≠	0.383	NS
	Range	0 - 246	0.1 - 235.5	-1.49 ± 49.80	-0.872≠	0.383	

P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant •: Paired t-test; ≠: Wilcoxon Signed Ranks test HB: hemoglobin, TLC: total leukocytic count, HCT: hematocrit, CRP: c-reactive protein, SD: standard deviation, IQR: inter-quartile range.

Table 5: Prognosis and Duration of Stay in NICU among the Studied Patients

		Total
		No. = 100
Prognosis	Survived	69 (69%)
	Died	31 (31%)
Duration of stay in NICU (days)	Median (IQR)	15 (12 - 20)
	Range	2 - 70

IQR: inter-quartile range NICU: neonatal intensive care unit.

Detailed data on ventilation support, antibiotic escalation, and catheter duration are available in Supplementary Tables.

The flow diagram illustrates the participant selection and allocation process in our randomized controlled trial. A total of 140 neonates were initially assessed for eligibility; 20 were excluded for not meeting the inclusion criteria, leaving 120 eligible participants. These were then randomized into two groups: Group A (n=55) received alcohol-only antisepsis, and Group B (n=65) received chlorhexidine-alcohol antisepsis. During the follow-up period, 5 neonates from Group A and 9 neonates from Group B were lost to follow-up. Additionally, 6 neonates in Group B developed complications that led to exclusion from final analysis. This process yielded a final randomized sample of 100 neonates, ensuring a robust comparative analysis of the two antiseptic protocols.

DISCUSSION

A critical aspect of preventing healthcare-associated infections lies in the choice of antiseptics used for skin preparation prior to catheter insertion and during maintenance. While 2% chlorhexidine in 70% isopropyl alcohol is widely recommended in adults due to its superior antimicrobial properties, its use in neonates remains controversial, mainly because of concerns about skin irritation and safety. As a result, many neonatal units continue to use alcohol-only preparations, despite limited evidence supporting their efficacy in reducing catheter-associated infections in this vulnerable population [6, 7].

This discrepancy highlights a significant gap in the literature: while adult and pediatric populations are well-represented in antiseptic research, data specific to neonates-particularly those comparing chlorhexidine-alcohol to alcohol alone-are sparse and inconclusive.

Furthermore, most available studies focus on older pediatric or adult ICU populations, and few provide randomized controlled evidence within the neonatal setting [18, 19].

Catheter-related bloodstream infections (CRBSIs) in neonates affect more than immediate health. They disrupt vital early-life processes-particularly feeding, nutrition, and growth-that are crucial for long-term neurodevelopment. In our study, neonates with CRBSIs demonstrated elevated inflammatory markers (e.g., CRP) and greater hemoglobin decline, both of which are known to interfere with metabolic homeostasis and nutrient utilization.

Moreover, systemic infections frequently lead to feeding intolerance, delayed initiation or interruption of enteral nutrition, and increased reliance on parenteral nutrition. These disruptions not only impair growth trajectories but also elevate the risk for catheter-associated complications and liver dysfunction. Infected neonates in our alcohol-only group (Group A) experienced more severe anemia and inflammation, likely contributing to poor feeding tolerance and slower weight gain.

Reducing CRBSI-as demonstrated by the 26% absolute risk reduction in Group B-has the potential to stabilize nutritional intake and reduce inflammatory burden. This is especially critical in preterm infants, where infection-induced inflammation is a known contributor to adverse neurodevelopmental outcomes, including white matter injury and cerebral palsy. Several studies have linked early systemic inflammation to long-term deficits in cognition and motor function [10, 18].

Furthermore, CRBSI increases the need for broad-spectrum antibiotics, which can disturb gut microbiota-an essential player in nutrient absorption, immune regulation, and neurodevelopment. By reducing infection rates, chlorhexidine-alcohol protocols may preserve gut integrity and microbial diversity, promoting better enteral nutrition and neuroimmune maturation.

CRBSI and Its Impact on Feeding, Nutrition, and Growth

Catheter-related bloodstream infections (CRBSIs) interrupt critical processes required for early neonatal growth, particularly by impairing feeding tolerance and nutrient assimilation. Infected neonates commonly develop feeding intolerance, necessitating the interruption or delay of enteral nutrition and increasing reliance on parenteral feeding. This shift not only limits optimal calorie and protein intake but also increases the risk of parenteral nutrition-associated complications such as cholestasis and liver dysfunction. In our study, neonates in the alcohol-only group exhibited significantly elevated CRP levels and more pronounced declines in hemoglobin and hematocrit, both markers of systemic inflammation. These physiological disturbances are known to interfere with nutrient transport, oxygen delivery, and metabolic homeostasis, leading to suboptimal weight gain and disruptions in growth trajectories.

Infection Reduction as a Pathway to Improved Neonatal Outcomes

The significant reduction in CRBSI observed in the chlorhexidine-alcohol group (26% absolute risk reduction) had multiple downstream benefits. Lower systemic inflammation, milder anemia, and a reduced need for escalation to broad-spectrum antibiotics were evident in this group, suggesting improved clinical stability. These benefits likely contributed to better feeding tolerance, fewer disruptions in nutritional plans, and reduced exposure to gut-disruptive agents such as meropenem or vancomycin. Moreover, with fewer interruptions in enteral feeding, neonates had a better chance of meeting caloric targets essential for early catch-up growth. Notably, although Group B had a slightly longer median NICU stay, this appears to reflect improved survival rather than prolonged illness, affording additional time for nutritional and hematologic recovery.

Implications for Developmental and Long-Term Health

Reducing neonatal infections, such as CRBSI, may have developmental implications that extend beyond hospital discharge. Early-life inflammation is a well-recognized risk factor for neurodevelopmental impairment, including cerebral palsy and cognitive delays-particularly among preterm infants. By lowering systemic inflammatory burden, the chlorhexidine-alcohol protocol may mitigate these risks. Furthermore, anemia of infection compromises brain oxygenation and may necessitate transfusions, each of which can influence neurologic outcomes. In our study, the chlorhexidine group demonstrated more stable hematologic profiles, suggesting better overall physiologic resilience. Although long-term neurodevelopmental follow-up was not within the scope of this study, the observed reductions in inflammation

and anemia provide a compelling rationale for future research exploring the developmental benefits of CRBSI prevention strategies.

Importantly, the chlorhexidine group in our study had lower infection severity and required fewer escalations to second-line antibiotics, suggesting not only a survival benefit but a qualitatively better recovery. A longer NICU stay observed in Group B likely reflects prolonged survival rather than morbidity, affording these neonates more time for nutritional rehabilitation and developmental catch-up.

Our study found a 50% blood culture positivity rate, predominantly due to multidrug-resistant Gram-negative organisms, including *Klebsiella* (37%) and *E. coli* (6%). This rate is considerably higher than previously reported by Clarke *et al*. [9] and Bakır *et al*. [20] likely due to differences in antiseptic protocols, catheter practices, and local microbial ecology. As noted by Helmi *et al*. [21], environmental exposure to Gram-negative organisms is more prominent in Egyptian NICUs, whereas Lin *et al*. [22] observed a predominance of Gram-positive pathogens in other settings, reflecting geographic variability in infection profiles.

Our study revealed a significant inflammatory response associated with catheter-related infections. In Group A (alcohol-only), CRP levels increased significantly from 4.5 to 13.7 mg/L (p = 0.012), while Group B (chlorhexidine-alcohol) showed no significant rise (p = 0.196), indicating a lower systemic inflammatory burden. Hemoglobin and hematocrit levels declined in both groups, but more sharply in Group A, reflecting the physiological impact of infection and the possible influence of antiseptic protocol. These findings align with previous studies that have identified CRP and neutrophilia as early markers of catheter-related sepsis [18, 22].

Antibiotic use was nearly universal: 51% of neonates received first-line antibiotics, while 42% required escalation to agents such as vancomycin or meropenem. Invasive ventilation was used in 85% of cases, with a higher frequency in Group A (63.5%), suggesting greater clinical severity. Inotrope use (dopamine/dobutamine) also reflected infection-associated hemodynamic compromise. High empirical antibiotic use is consistent with data from Helmi *et al*. and Lin *et al*. [21, 22], though our 42% escalation rate exceeds that of other studies using chlorhexidine [8]. Improved antiseptic protocols have been shown to reduce antibiotic use-supporting our findings in Group B [19].

Although the difference in mortality was not statistically significant (22% vs. 40%, p = 0.052), the 18% absolute reduction in the chlorhexidine group is clinically meaningful. Neonates in Group B also showed better inflammatory profiles and hematologic stability, which are important given that early-life inflammation is linked to poor neurodevelopmental outcomes, including cerebral palsy and cognitive delay [10, 18].

Less severe anemia in Group B may also improve brain oxygenation, reduce transfusion needs, and support better feeding tolerance and somatic growth-key determinants of neurodevelopment. The longer NICU stay in Group B likely reflects increased survival time for recovery and nutritional catch-up, rather than prolonged illness.

These findings reinforce the value of chlorhexidine-alcohol as a developmentally supportive and infection-reducing intervention, particularly in low-resource NICUs where advanced therapies may not be available. Future studies should assess the long-term developmental impact of CRBSI prevention.

Our overall mortality rate (31%) was higher than previously reported in other cohorts [8, 9, 20], likely due to higher rates of Gram-negative infections and limited intensive care resources. Prior studies have shown that wider use of PICCs and lower Gram-negative exposure are associated with reduced mortality, supporting the importance of both antiseptic strategy and line type [19, 22].

Our findings show that the type of central venous catheter significantly affects inflammatory and hematologic responses in neonates. PCVCs and UVCs were associated with higher CRP levels and greater drops in hemoglobin and hematocrit, suggesting stronger systemic inflammation and blood loss. In contrast, PICCs had the lowest infection burden and most stable lab profiles, indicating their relative safety for long-term use.

These results align with previous studies recommending PICCs for their lower infection risk and minimal systemic impact [18, 22]. UVCs, particularly in preterm infants, have been linked to higher infection rates and early-onset sepsis, supporting our findings [8, 20]. While Pinilla-González *et al*. noted that antiseptic

choice plays a major role in infection prevention, our data suggest that both catheter type and antiseptic protocol are independently important in minimizing CRBSI risk [19].

Clinical Implications for Neonatal Care

Our findings offer robust evidence that the use of 2% chlorhexidine in 70% alcohol significantly reduces catheter-related bloodstream infections (CRBSIs) among neonates. This translates into fewer cases of sepsis, reduced inflammation, lower antibiotic escalation, and improved survival. Importantly, these benefits were seen without added safety concerns, even among preterm infants. In high-risk NICU populations, such infection control gains can directly enhance growth, feeding stability, and developmental outcomes.

In low- and middle-income countries (LMICs), antiseptic protocols often rely on alcohol-only solutions due to cost concerns or lack of local guidelines. However, our data show that chlorhexidine-alcohol is not only more effective but feasible-it is already commercially available in many LMICs and requires no advanced storage or infrastructure. As such, transitioning to chlorhexidine-alcohol antisepsis is a cost-effective, high-impact intervention that can be rapidly adopted without large investments.

One of the key strengths of our study is its randomized controlled design, which enhances the reliability of the observed differences between the intervention (chlorhexidine-alcohol) and control (alcohol-only) groups. The study also provides detailed microbiological profiles, revealing Klebsiella as the dominant pathogen, thereby offering localized epidemiological insight. Another strength is the inclusion of multiple central line types (PCVC, PICC, UVC), enabling meaningful comparisons of infection rates and laboratory trends across access modalities. Furthermore, the study integrates clinical indicators (e.g., ventilation, inotrope need), culture results, and blood parameters to create a well-rounded clinical picture, making the results more translatable to bedside decision-making.

Despite its strengths, the study faces several important limitations. Firstly, the single-center scope limits generalizability, particularly in comparing outcomes across NICUs with different infection control infrastructures. The sample size of 100 patients, while adequate for a preliminary analysis, limits statistical power, especially when stratified by catheter type or organism. Laboratory inflammatory markers, such as CRP, were not consistently tracked over time, limiting our ability to dynamically assess infection progression. There was also limited discussion of antiseptic application techniques, dwell time protocols, or compliance with full CRBSI prevention bundles, which are known to independently influence infection rates. Lastly, the study did not include long-term neurodevelopmental follow-up of survivors, which could have added depth to outcome assessment.

CONCLUSION

Reducing CRBSI is not only a matter of infection control-it is a direct intervention to protect neonatal nutrition, growth, and development. In this trial, 2% chlorhexidine in 70% alcohol significantly reduced CRBSI rates, with associated improvements in inflammation, hematologic stability, and survival trends.

These benefits translate into better feeding tolerance, fewer nutritional interruptions, and improved growth trajectories, especially in preterm infants.

Chlorhexidine-alcohol is affordable, accessible, and easily integrated into existing NICU protocols, making it a practical solution for low-resource settings.

Take-home message: Chlorhexidine-based antisepsis is a simple, scalable strategy that safeguards not just lives-but also the growth and future development of vulnerable neonates.

POLICY AND INFECTION CONTROL RECOMMENDATIONS

Based on the outcomes of this trial, we propose the following actionable steps:

1. Adopt 2% chlorhexidine in 70% alcohol as the standard antiseptic for all central line insertions and maintenance in neonates, including preterm infants.

2. Integrate this protocol into existing catheter care bundles, supported by simple training for NICU staff on safe application techniques.

3. Prioritize the use of PICC lines where possible, as they showed the lowest infection risk in our cohort.

4. Implement serial monitoring of inflammatory markers (e.g., CRP) to enable early detection and intervention for suspected CRBSI.

5. Encourage antimicrobial stewardship by reducing unnecessary second-line antibiotic use through better prevention strategies.

LIST OF ABBREVIATIONS

CVC = Central Venous Catheter

PCVC = Percutaneous Central Venous Catheter

PICC = Peripherally Inserted Central Catheter

UVC = Umbilical Venous Catheter

NICU = Neonatal Intensive Care Unit

CHG = Chlorhexidine Gluconate

CRBSI = Catheter-Related Bloodstream Infection

CBC = Complete Blood Count

CRP = C-Reactive Protein

IQR = Interquartile Range

SD = Standard Deviation

SPSS = Statistical Package for the Social Sciences

ETHICAL CONSIDERATIONS

The study was approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MS 325/2023). The trial was registered at ClinicalTrials.gov (ID: NCT06194396). Written informed consent was obtained from the parents or legal guardians of all participating neonates. The study adhered to the principles of the Declaration of Helsinki, and all efforts were made to minimize risks, ensure participant safety, and preserve participants' dignity.

ACKNOWLEDGEMENT

The authors are grateful to the medical and nursing staff of the NICU at Children's Hospital, Ain Shams University, for their assistance with patient care and study procedures. Special thanks are extended to the participating families for their trust and cooperation.

AUTHOR CONTRIBUTIONS

Concept and design: Rania A. El-Farrash, Marwa Adel Ahmed; Yasmin A. Farid.

Acquisition, analysis, or interpretation of data: Marwa Mamdouh Ashour, Marwa Adel Ahmed, Yasmin A. Farid.

Drafting of the manuscript: Marwa Mamdouh Ashour, Yasmin A. Farid.

Critical review of the manuscript for important intellectual content: All authors.

Supervision: Rania A. El-Farrash.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest related to this work.

CONFIDENTIALITY OF DATA

All collected data were anonymized and coded before analysis to maintain participant confidentiality. No identifying patient information was disclosed or published. Access to the dataset was restricted to the study investigators.

REFERENCES

[1] Centers for Disease Control and Prevention (CDC). Guidelines for the prevention of intravascular catheter-related infections. Atlanta (GA): CDC; 2017. Available from: https://www.cdc.gov/infection-control/hcp/intravascular-catheter-related-infection

[2] Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, *et al*. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 2009; 49(1): 1-45. https://doi.org/10.1086/599376

[3] Centers for Disease Control and Prevention (CDC). National Healthcare Safety Network (NHSN) surveillance definitions for specific types of infections—bloodstream infection event (CLABSI and non-CLABSI). Atlanta (GA): CDC; 2023. Available from: https://www.cdc.gov/nhsn

[4] Kalu IC, Curless MS, Ponnampalavanar S, Milstone AM, Kamar AA. Strategies to reduce hospital-associated bloodstream infections in a limited resource setting: Preventing Infections in Neonates (PIN) collaborative. Antimicrob Stewardsh Healthc Epidemiol 2023; 3(1): e144. https://doi.org/10.1017/ash.2023.415

[5] Ayala MU, Almendral A, García IJ, Vergeli MR, Porrón R, Maura YL, *et al*. Central line-associated bloodstream infections (CLABSI) in pediatric and neonatal intensive care units: the VINCat program 2013-2022. Enferm Infecc Microbiol Clin 2025; 43(Suppl): S90-7. https://doi.org/10.1016/j.eimce.2024.09.014

[6] Kopsidas I, Collins M, Zaoutis T. Healthcare-associated infections—can we do better? Pediatr Infect Dis J 2021; 40(8): e305-9. https://doi.org/10.1097/INF.0000000000003203

[7] Rani U, Lewis LE, Chawla K, Naha A, Kumar P. Development and evaluation of a prevention bundle for neonatal healthcare-associated infections: an interventional study. F1000Res 2024; 12: 687. https://doi.org/10.12688/f1000research.132819.2

[8] Capasso A, Paonessa A, Ferrara T, Grasso F, Capasso L, Migliaro F, *et al*. Use of 2% chlorhexidine gluconate in 70% isopropyl alcohol for skin disinfection during central catheter insertion in premature neonates. J Vasc Access 2025; 26:

11297298251330941.
https://doi.org/10.1177/11297298251330941

[9] Clarke P, Soe A, Nichols A, Harizaj H, Webber MA, Linsell L, *et al*. 2% chlorhexidine gluconate aqueous versus 2% chlorhexidine gluconate in 70% isopropyl alcohol for skin disinfection prior to percutaneous central venous catheterisation: the ARCTIC randomised controlled feasibility trial. Arch Dis Child Fetal Neonatal Ed 2024; 109(2): 202-10.
https://doi.org/10.1136/archdischild-2023-325871

[10] Eschborn S, Weitkamp JH. Procalcitonin versus C-reactive protein: review of kinetics and performance for diagnosis of neonatal sepsis. J Perinatol 2019; 39(7): 893-903.
https://doi.org/10.1038/s41372-019-0363-4

[11] Menchinelli G, Liotti FM, Fiori B, De Angelis G, D'Inzeo T, Giordano L, *et al*. Corrigendum: *In vitro* evaluation of BACT/ALERT VIRTUO, BACT/ALERT 3D, and BACTEC FX automated blood culture systems for detection of microbial pathogens using simulated human blood samples. Front Microbiol 2019; 10: 221-238.
https://doi.org/10.3389/fmicb.2019.00221

[12] Payne V, Hall M, Prieto J. Care bundles to reduce central line-associated bloodstream infections in the neonatal unit: a systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed 2018; 103(5): F422-9.
https://doi.org/10.1136/archdischild-2017-313362

[13] Park S, Moon S, Pai H, Kim B. Appropriate duration of peripherally inserted central catheter maintenance to prevent central line-associated bloodstream infection. PLoS One 2020; 15(6): e0234966.
https://doi.org/10.1371/journal.pone.0234966

[14] Dramowski A, Pillay S, Bekker A, Abrahams I, Cotton MF, Coffin SE, *et al*. Impact of 1% chlorhexidine gluconate bathing and emollient application on bacterial pathogen colonization dynamics in hospitalized preterm neonates: a pilot clinical trial. E Clinical Medicine 2021; 37: 100983.
https://doi.org/10.1016/j.eclinm.2021.100946

[15] Farrell FD. Mechanical interactions in bacterial colonies and the surfing probability of beneficial mutations. J R Soc Interface 2017; 14(131): 20170073.
https://doi.org/10.1098/rsif.2017.0073

[16] Folgori L, Tersigni C, Hsia Y. The relationship between Gram-negative colonization and bloodstream infections in neonates: a systematic review and meta-analysis. Clin Microbiol Infect 2018; 24(3): 251-7.
https://doi.org/10.1016/j.cmi.2017.08.008

[17] Sharma A, Kulkarni S, Thukral A, Sankar MJ, Agarwal R, Deorari AK, *et al*. Aqueous chlorhexidine 1% versus 2% for neonatal skin antisepsis: a randomised non-inferiority trial. Arch Dis Child Fetal Neonatal Ed 2021; 106(6): 643-8.
https://doi.org/10.1136/archdischild-2020-321174

[18] Muller M, Bryant KA, Espinosa C, Jones JA, Quach C, Rindels JR, *et al*. SHEA Neonatal Intensive Care Unit (NICU) White Paper Series: practical approaches for the prevention of central-line-associated bloodstream infections. Infect Control Hosp Epidemiol 2023; 44(4): 550-64.
https://doi.org/10.1017/ice.2022.53

[19] Pinilla-González A, Pérez-Fiérrez L, Solaz-García A, Torrejón-Rodríguez L, Parra-Llorca A, Pérez-Oliver T, *et al*. Neonatal skin antisepsis with alcohol-based compared to aqueous 2% chlorhexidine in preterm infants and risk of catheter-related bloodstream infections. Arch Dis Child Fetal Neonatal Ed 2025 Feb 4. [Epub ahead of print]

[20] Bakır E, Sezer TA, Çavuşoğlu H, Parlakay AÖ, Gürsel O. Comparison of 2% chlorhexidine gluconate in 70% alcohol and 10% povidone-iodine used for port catheter dressing changes in pediatric hematology-oncology patients: a prospective observational study. Turk Arch Pediatr 2021; 56(5): 451-6.
https://doi.org/10.5152/TurkArchPediatr.2021.21035

[21] Helmi MA, Lai NM, Van Rostenberghe H, Ayub I, Mading E. Antiseptic solutions for skin preparation during central catheter insertion in neonates. Cochrane Database Syst Rev 2023; 5: CD013841.
https://doi.org/10.1002/14651858.CD013841.pub2

[22] Lin MR, Chang PJ, Hsu PC, Lin CS, Chiu CH, Chen CJ. Comparison of efficacy of 2% chlorhexidine gluconate-alcohol and 10% povidone-iodine-alcohol against catheter-related bloodstream infections and bacterial colonization at central venous catheter insertion sites. J Clin Med 2022; 11(8): 2242.
https://doi.org/10.3390/jcm11082242